PROCEEDING

ICOLIB

International Conference on Life Sciences and Biotechnology

EXPLORATION AND CONSERVATION OF BIODIVERSITY

The ICOLIB 2015 focuses on life sciences and biotechnology aspects to explore and conserve biodiversity by bringing together investigators from different fields such as health and medicine, agriculture, food technology and security, new and renewable energy, conservation and management including exploration of biodiversity

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International Conference on Life Sciences and Biotechnology (ICOLIB)

Exploration and Conservation of Biodiversity

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Preface from the Editor

The explosive development of the sciences and its expansion into other disciplines such as the Life Sciences field is yielding groundbreaking discoveries from novel genes and bio-products to cutting-edge nanotechnology, resulting in a transformed science landscape with profound global applications in understanding life, eradicating diseases, securing a more equitable food and water supply distribution as well as creating novel bio-industries and products.

Based on these phenomena above, the ICOLIB 2015 with theme "**Exploration and Conservation of Biodiversity**", provide an interdisciplinary platform of life sciences for researchers, academics, students, professionals, industries, and policy makers. This meeting also proposed to among scientists and professionals to stay at the leading edge of recent advances in life sciences and sustainability, act as a catalyst for further research, improve international collaboration while bridging the scientific and technological differences among scientists, and foster global health security.In order to disseminate to community more broadest, the articles were published as a proceeding.

The conference was organized by the Department of Biology, Faculty of mathematic and natural sciences, The University of Jember collaboration with the Flensburg University of Applied Sciences, Deutscher Akademischer Austauch Dienst (DAAD), Indonesian-German Network for Teaching, Training and Research Collaboration (IGN-TTRC), University of Kassel and IndoBIC (Indonesian Biotechnology Information Centre) The Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP). The conference participants from 5 countries and of which 9 lectures within the field health and medicine, agriculture, food technology and security, new and renewable energy, conservation and management including exploration of biodiversity. Presentation divided into plenary, oral and poster session. More than 150 researchers including students participated on this meeting.

On behalf of the organizing committee, i would like to thank all invited speakers and presenters for participating in the ICOLIB 2015 for giving valuable contribution to this conference. Also, acknowledgements are address to Rector University of Jember, Flensburg University of Applied Sciences, DAAD, Indonesian-German Network for Teaching, IGN-TTRC, University of Kassel and IndoBIC-SEAMO BIOTROP as well as all sponsors for the efforts. Finally, i would like to express deep appreciation to the member of the organizing committee for the good teamwork and the great effort to bring success to the conference.

Jember, September 2015

Kahar Muzakhar
Committee

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POSTER SESSION

Keynote Speakers

JUMPING DNA: REGULATION and APPLICATION in FUNCTIONAL GENE ANALYSIS

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Abstract

Mobile genetic elements like transposons and retrotransposons constitute large parts of most eukaryotic genomes. Since they are believed to be of viral origin, one of their intrinsic features is to change their position in the genome and/or to multiply. Though these properties contribute to variation and evolution, extensive transposition can lead to genome instability and hazardous mutations. We summarise here the mechanism that is used to keep the *Dictyostelium* retroelement DIRS-1 under control. In addition we show how the DIRS-1 control mechanism can be applied to knock down the expression of other endogens to investigate their biological functions.

Keywords: Transposons, retrotransposons, gene silencing, RNA interference, LTR, siRNAs, argonaute proteins.

Introduction

Transposons and retrotransposons are highly abundant elements in eukaryotic genomes. While transposons can be cut out and be reintegrated at other positions in the genome, retrotransposons are transcribed, a cDNA is synthesized by the reverse transcriptase encoded by the retroelement and the new copy is inserted into the genome. Both kinds of elements may thus be mutagenic by disrupting genes and by altering expression of neighboring genes by their promoters. On the other hand, transposition and retrotransposition have apparently contributed significantly to evolution (1).

Nevertheless, an excess of mobility is deleterious to organisms and the genetic costs of huge amounts of DNA that has to be replicated in every cell cycle, is substantial. Several diverse mechanisms have been developed to minimize the effects of mobile elements and at the same time maintain the evolutionary advantages of them.

One mechanism of "taming" mobile elements is apparently that they adopt secondary functions. In *Drosophila* for example, retrotransposons occupy the ends of chromosomes and have become part of the telomeres (2). In *Dictyostelium*, the retrotransposon DIRS-1 constitutes the centromeres of all chromosomes (3,4).

A second mechanism to avoid damage by mobile elements is the preference of specific integration sites. For example, several retroelements in yeast, *Dictyostelium* and others only insert at specific positions upstream or downstream of tRNA (5) genes and thus do not disrupt other genes .

Nevertheless, other measures are necessary to avoid abundant expression of transposon genes and proteins. In many cases, the DNA of the mobile elements is methylated which leads to heterochromatin formation and strongly reduced transcription. Another mechanism is gene silencing by RNA interference.

1. RNA interference

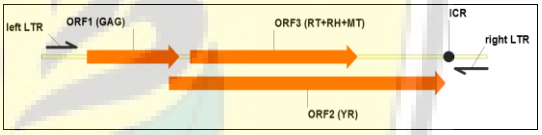

Fig. 1: Structure of the *Dictyostelium* DIRS-1 retrotransposon.

DIRS-1 consists of thre overlapping reading frames encoding a gag protein (Orf 1), a tyrosine recombinase (Orf 2) and a protein with reverse transcriptase, RNaseH and methyl-transferase domains (ORF 3). The coding genes are flanked by inverted long terminal repeats (LTRs). ICR is an "internal comple-mentary region" believed to be involved in completion of the element after reverse transcription.

RNA interference is based on siRNAs (small interfering RNAs) which are generated from double stranded RNA by a highly conserved biochemical pathway in eukaryotes (6), summarized in (7). Since some retrotransposons have inverted, long terminal repeats at their ends and since these serve as promoters for the retroviral genes, transcripts in both orientations are synthesized. These can form completely complementary double stranded structures that are then processed by the RNAi machinery. An enzyme denominated Dicer cleaves the long double strands into fragments of 21bp. These are then transferred to RISC (RNA induced silencing complex). One of the two 21nts strands is removed and the other one guides RISC to an mRNA

with complementary sequence. A protein within RISC which is termed Argonaute, has nuclease activity and cleaves the mRNA which is then subjected to degradation. Since one double stranded RNA generates many siRNAs and since one siRNA loaded RISC can cleave multiple mRNA targets, a substantial reduction of functional mRNAs is achieved.

In addition to cleavage, siRNAs can also serve as guides or primers for RNA dependent RNA polymerases (RdRP). These enzymes are encoded in the host genome and synthesize an RNA on an RNA template. siRNAs can thus serve to generate even more siRNAs.

2. The *Dictyostelium* retroelement DIRS-1

DIRS-1 is a retroelement with long terminal repeats and present in 16 complete copies in the genome (8). In addition, up to 200 incomplete copies are present. These are generated by the preference of DIRS-1 to integrate into its own sequences. DIRS-1 sequences are only found in the centromeres of the six chromosomes (4) and most likely serves for essential features since it is also associated with the centromere-specific histone CENH3.

A multitude of transcripts from DIRS-1 is detected in Northern blots but it is not clear if these are specific processing products or degradation products (9). Some of these transcripts are most likely generated by the partial copies in the genome. The left and the right LTR are strong promoters and apparently also contain transcription-stop and/or polyadenylation signals. Thus, transcripts in both orientations (sense and antisense) are synthesized.

Deep sequencing of small RNAs revealed that the vast majority of siRNAs in *Dictyostelium* is derived from DIRS-1 thus suggesting that the retroelement is controlled by RNAi (10).

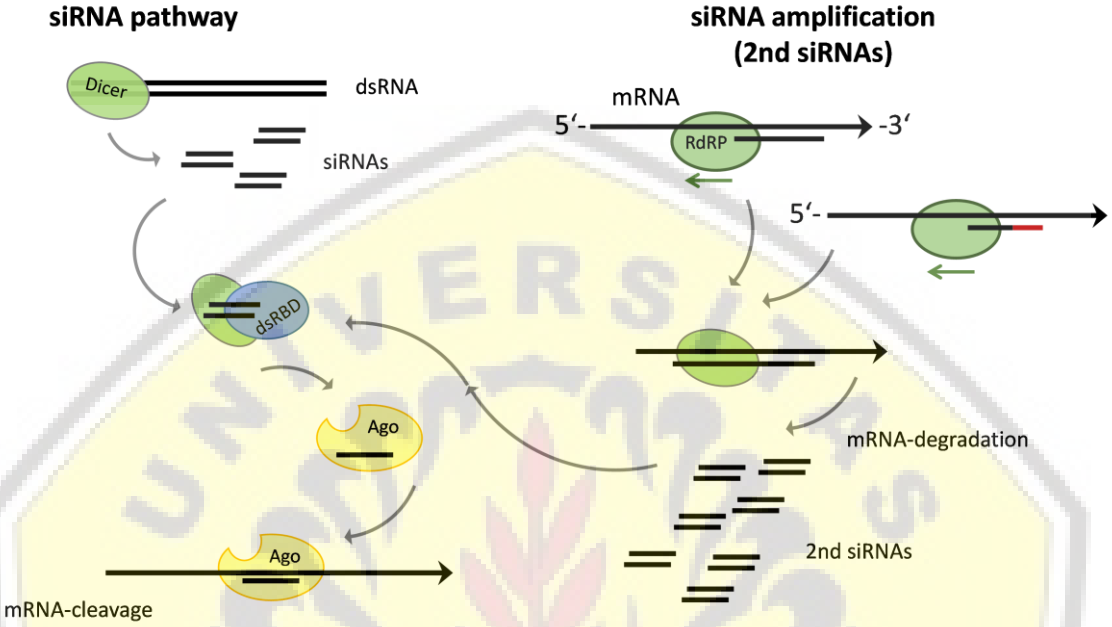

Fig. 2: siRNA pathway

Left: dsRNA from complementary transcripts (sense and anti-sense) is cleaved by Dicer to 21nts siRNAs. With the help of a dsRBD protein, one strand is transferred to an Argonaute protein and guides the Argonaute to a matching mRNA. There, the nuclease activity of the Argonaute cleaves the mRNA.

Right: siRNAs guided to an mRNA may also recruit an RNA dependent RNA polymerase (RdRP) which copies the mRNA and generates a dsRNA. This is a target for Dicer and processed to further secondary siRNAs (2^{nd} siRNAs). This amplification increases the pool of siRNAs substantially.

3. The *Dictyostelium* RNAi system

The RNAi system in *Dictyostelium* has been extensively investigated since 2001 (11,12). Most proteins involved in the gene silencing pathway have been identified and distinct functions for some could be assigned. The current, simplified model for the siRNA pathway is depicted in Fig. 2

The Argonaute A protein (agnA) is required for the generation of siRNAs since a knock-out of the gene substantially reduces the amount of siRNAs and increases the amount of full length mRNAs from DIRS-1(13). Similarly, a knock-out of the RNA dependent RNA polymerase gene rrpC reduces the amount of siRNAs (14). *Dictyostelium* has two genes encoding Dicer proteins. A knock-out of DrnB

affected the related micro RNA pathway but had no obvious consequences on siRNA production. . Unfortunately, a knock-out of the other Dicer gene, drnA could not be achieved but it was concluded that the DrnA protein is most likely responsible for the generation of siRNAs.

Malfunction of the RNAi system resulted in overexpression of DIRS-1 derived transcripts and the synthesis of DIRS-1 encoded proteins that were undetectable in the wild type strain. Recent results suggest that the mobility, i.e. transposition of the element is substantially increased and that possibly virus-like particles are produced (unpublished data).

4. A gene knock-down system based on DIRS-1

Given that the RNAi system could efficiently silence the DIRS-1 retrotransposon, it was investigated if this could be applied to reduce expression of endogenous genes. Apparently, the strong LTR promoters of DIRS-1 transcribed sufficient amounts of dsRNA to activate the RNAi system. If fragments of endogenous genes were inserted in between the LTRs to replace the DIRS-sequences, one could expect that siRNAs were generated and may target mRNAs of the corresponding endogene in trans. Consequently, the endogene would be silenced and phenotypes of reduced expression could be investigated.

The hypothesis was tested by inserting fragments of seven different genes in between the opposing LTR promoters and by introducing the constructs into *Dictyostelium* wild type cells. In all cases, siRNAs of the corresponding endogens were found and these were not detectable in the untransformed wild type. Furthermore, a reduction of the respective mRNAs was seen in Northern blots

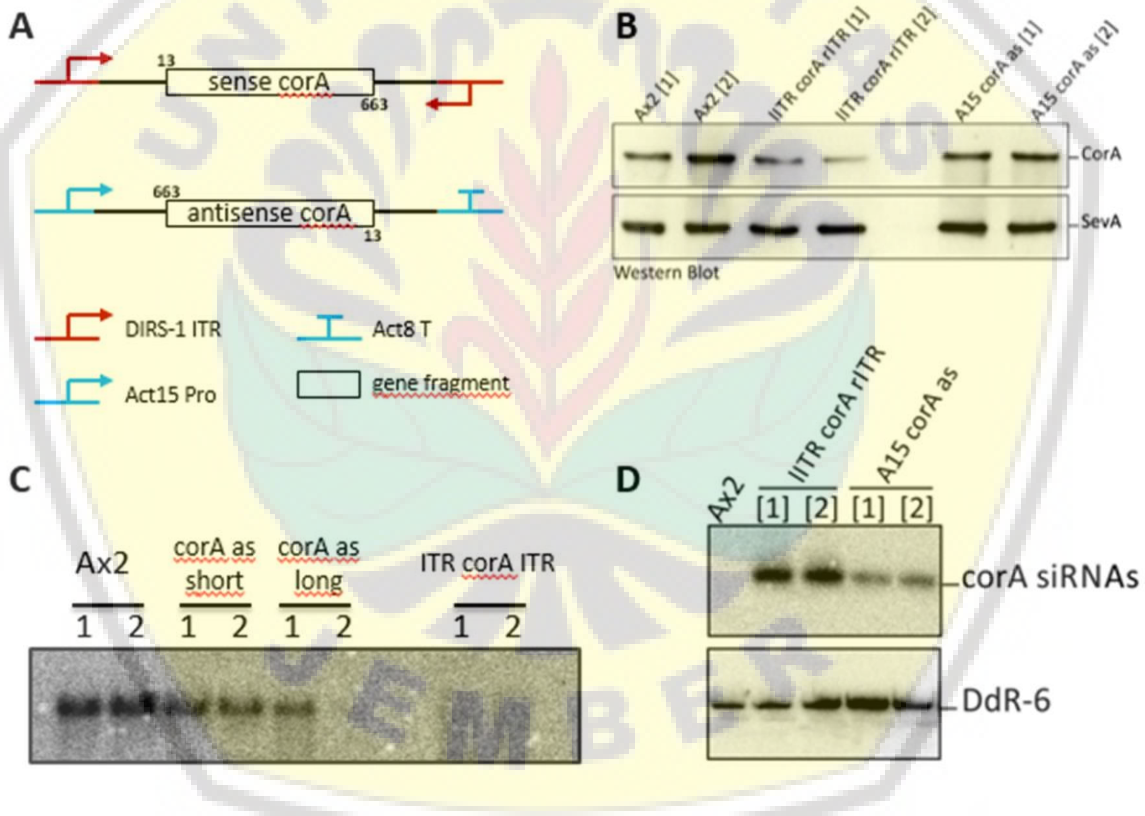

Fig. 3: Comparison between RNAi and antisense mediated gene silencing

A: Antisense and RNAi constructs directed against the corA gene are schematically depicted.

B: Western blots of cells transformed with the constructs shown in A and probed with antibodies directed against CorA and, as a loading control, against the protein SevA. Two independent samples are shown for the wild type (AX2), the RNAi construct (lLTR corA rLTR) and the antisense construct (A15 corA as).

C: Northern blot on corA mRNA levels from wild type (AX2) and from cells transformed with the antisense construct corA as short, corA as long and the RNAi construct ITR corA ITR. The corA long construct contains the full length corA coding region and may lead to somewhat better silencing (see text).
D: Northern blot on siRNAs from wild type (AX2) and two independent isolates from cells transformed with the RNAi and the antisense constructs. As a loading control, the blot was reprobed against the snoRNA DdR-6.

and where antibodies were available, a reduction on the protein level was also observed (15).

As a further control, the LTR constructs were also transformed into a strain where the agnA gene had been destroyed. Since AgnA is required for siRNA production, silencing did not work in this strain, demonstrating that in the wild type the knock-down strategy really involved the RNAi system.

In several cases it was checked if mutant phenotypes could be observed in the knock-down strains. In fact, for the mhcA gene and for corA, defects in cytokinesis, leading to multinucleate cells were found. These corresponded to the phenotypes previously observed in knock-out mutants of the same genes. Most interestingly, the casK gene in *Dictyostelium* could not be knocked out in previous attempts thus suggesting that the gene product was essential for viability. In contrast, the knock-down approach was successful resulting in cells with significantly slower growth. It is thus possible to investigate the function of essential genes in knock-down strains with strongly reduced expression levels.

One may ask the question if any opposing promoters flanking a gene fragment may have the same effect. This was tested with several promoter combinations and even though small amounts of siRNAs were sometimes detected, silencing was never as pronounced as with the LTR constructs. There could be two reasons for this observation: either the LTRs are extremely strong promoters and thus generate sufficient amounts of siRNAs which could not be achieved by other promoters, or the LTRs harbor some other features that specifically attract the RNAi machinery (15).

5. RNAi and antisense

In previous work, knock-downs have been achieved with some success by introducing genes or gene fragments in antisense orientation in respect to a promoter (16). Before the discovery of the RNAi mechanism, it was generally believed that hybrid formation between the mRNA and the antisense RNA would just inhibit translation, though other mechanisms had already been suggested (17). A comparison of antisense and RNAi constructs revealed that the former also resulted in the appearance of siRNAs but their amount was in general much lower than with the LTR constructs. Fig. 3 shows the results of such a comparison.

The same fragment of the corA gene was either inserted in between the opposing DIRS-1 LTRs or placed, in antisense orientation, behind the strong actin promoter. The constructs were introduced into *Dictyostelium* cells on an extrachromosomal expression vector as previously described (15). After selection on media containing the antibiotic G418, the resulting transformants were analysed by Western blot protein levels and by Northern blot for mRNA and siRNA levels.

The data show that, compared with the wild type, protein and mRNA levels are much more reduced with the RNAi system than with the antisense system. Consistantly, there are significantly less siRNAs produced by the antisense construct than by the RNAi construct. In addition, a longer antisense construct, containing the complete coding sequence of the corA gene was used. Possibly, longer antisense has a stronger silencing effect but ss can be seen in Fig. 3C (corA as long) the two independent clones display very different levels of mRNA. We have no explanation for this variability but this was never observed with RNAi constructs.

Since the production of primary siRNAs depends on the amount of available mRNA, 1st siRNAs maybe limiting in the case of antisense. In fact, previous work has shown that there appears to be a threshold level of siRNAs and silencing is only achieved when this threshold is exceeded (18). In contrast, the DIRS-1 derived RNAi constructs produce escessive and equal amounts of sense and antisense RNA and may therefore be more efficient.

Conclusions

The RNAi system in *Dictyostelium* can be efficiently employed to rapidly generate knock-down strains. The procedure is much faster than the construction and verification of knock-outs and first results may be achieved within three to four weeks. A disadvantage is that the degree of downregulation appears to depend on the target gene and may vary between 40% and 70%. Another shortcoming is that the system may not be applicable for knocking down specific genes of closely related copies of a gene family: the siRNAs may target all members of the family.

Acknowledgment

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For methods used in the experiments, please refer to (15)

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ENZYMES from INDONESIAN BIODIVERSITY: MOLECULAR CHARACTERIZATION and THEIR POTENTIAL APPLICATIONS

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Abstract

Indonesia is rich of biodiversity that offers resources of interesting enzymes. Many have important applications including in pharmaceutical field. Our laboratory works on a number of enzymes with various applications in pharmacy such as superoxide dismutase (SOD), nattokinase, Douchi Fibrinolytic Enzyme (DFE) and cyclodextrin glucanotransferase (CGTase). Two SODs are the focus of our research; *Staphycoccus equorum* and *Citrus limon* SODs. The first SOD displayed moderate thermostability, wide range of pH stability, stability in the presence of several denaturants and detergents and was showed to form dimer. The thermoflour experiment showed that dimerization is important for its stability and some ions improved its stability. An *in vitro* experiment using fibroblast cell line demonstrated that the SOD protected collagen degradation caused by UV exposure, indicating that it has potential as anti-aging agent. *Citrus limon* SOD was engineered to produce as several fusion proteins with different parts of gliadin molecules to facilitate its permeation through epithelial cells. Our preliminary data indicate that the gliadin molecule in SOD protein facilitates the permeation of protein through epithelial cells. Genes encoding nattokinase and DFE were obtained from Indonesian traditional fermented food by metagenomics approach. Both genes were found not to be identical with those in public sequences and expressed in *Escherichia coli*. The purified enzymes were proved to be active. CGTase from two Bacillus isolates were also characterized and the genes were partially identified using LASP-PCR. Indonesia as a country with high biodiversity, it is prospective to do research on enzymes to explore their potential applications.

Keywords: anti-aging, biodiversity, CGTase, DFE, enzymes, Indonesia, nattokinase, pharmaceutical, SOD.

BIOCONVERSION of RARE SUGARS by SUGAR ISOMERASE and EPIMERASE from MICROORGANISMS

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Abstract

The D-Lyxose isomerase gene from *Serratia proteamaculans* and cellobiose 2-epimerase gene from *Caldicellulosiruptor saccharolyticus* were cloned and expressed in *E. coli*, respectively. The recombinant D-lyxose isomerase and cellobiose 2-epimerase showed the highest activity at pH7.5, 40°C and pH7.5, 75°C, respectively. In the substrate specificities for monosaccharides, D-lyxose isomerase has enzyme activity for the aldoses with the C2 and C3 hydroxyl groups in the left-hand configuration. In the case of cellobiose 2-epimerase, the enzyme catalyzed the epimerization reactions of the aldoses harboring hydroxyl groups oriented in the right-hand configuration at the C2 position and the left-hand configuration at the C3 position.

Keywords: Rare sugar, Bioconversion, Substrate specificity, D-Lyxose isomerase, Cellobiose 2-epimerase

Introduction

Rare sugars are defined as monosaccharides and their derivatives that rarely exist in nature by International Society of Rare Sugars (ISRS). The rare sugars have been focused much interesting due to their possible applications as low-calorie sweeteners, bulking agents, antioxidants, inhibitors of microbial growth and glycosidases, nucleoside analogues, and immunosuppressants, etc.

Recently, the bioconversion of rare sugars by enzymes from microorganisms have been attracted much attention as an environmentally friendly rare sugars production method [3, 4, 5, 12, 13, 15, 18, 19]. Among various enzymes, enzymes related sugar metabolism such as sugar phosphate isomerases, sugar isomerases, and sugar epimerase have been suggested as biological resources for the bioconversion of rare sugars because they are involved interconversion of various types of monosaccharides in the sugar metabolism [1, 2, 6, 7, 8, 9, 10, 16, 17, 20].

In this study, the characterization of the two enzymes, D-lyxose isomerase and cellobiose 2-epimerase, have been studied for bioconversion of rare sugars by enzymatic method.

Materials and Methods

Bacterial strains, plasmid, and culture conditions

The genomic DNA from *Serratia proteamaculans*, *Caldicellulosiruptor saccharolyticus*, *Escherichia coli* ER2566, and pET-24a (+) were used as the sources of D-lyxose isomerase gene, cellobiose 2-epimerase gene, host cells, and expression vector, respectively. The recombinant *E. coli* for protein expression was cultivated in a 2-l flask containing 500 ml of Luria-Bertani (LB) medium and 20 μg ml^{-1} of kanamycin at 37°C with agitation at 200 rpm. When the optical density of the bacteria reached 0·5 at 600 nm, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0·1 mmol l^{-1} to induce D-lyxose isomerase and cellobiose 2-epimerase expression, and the culture was incubated with shaking at 150 rpm at 16°C for 16 h.

Cloning and gene expression

The gene encoding a putative D-lyxose isomerase and cellobiose 2-epimerase were amplified by PCR using *S. proteamaculans* and *C. saccharolyticus* genomic DNA as a template. The sequence of the oligonucleotide primers used for gene cloning was based on the DNA sequence of the putative enzyme from *S. proteamaculans* and *C. saccharolyticus*. The amplified DNA fragment obtained by PCR were subcloned into the same sites of pET-24a (+) plasmid. The resulting plasmid was transformed into *E. coli* ER2566 strain and grown on LB medium containing 20 μg ml^{-1} of kanamycin. The expression of the gene encoding the isomerase was determined by both SDS-PAGE and enzyme assay.

Enzyme purification

The grown cells were harvested from culture broth and resuspended in lysis buffer (pH 8·0) containing 50 mmol l^{-1} NaH_2PO_4 and 300 mmol l^{-1} NaCl. The resuspended cells were disrupted on ice using a sonicator. The supernatant of disrupted cells was applied onto a His-Trap HP chromatography column (Amersham Biosciences, Uppsala, Sweden) and eluted with a linear gradient from 10 to 250 mmol l^{-1} imidazole at a flow rate of 1 ml min^{-1}. The active fraction was collected and desalting using centricon at 4°C against 50 mmol l^{-1} *N*-(2-hyroxyethyl)piperazine-*N*′-(3-propanesulfonic acid) (EPPS) buffer (pH 7·5) and the resulting solution was used as a purified enzyme

Enzyme assay

Unless otherwise stated, the reaction was performed in 50 mM EPPS buffer (pH 7·5) containing 10 mM D-lyxose and 0·16 U/ml of enzyme in the presence of 1 mM Mn^{2+} at 40°C for 10 min. Cellobiose 2-epimerase was used without ethylenediaminetetraacetic acid (EDTA) treatment. The enzyme reactions were carried out in 50 mM PIPES buffer (pH 7.5) containing 10 mM substrate at 75°C for 20 min by adjusting the enzyme amount (0.4–400 U/ml). The reaction was stopped by the addition of HCl to the reaction mixture at a final concentration of 200 mM.

Molecular mass determination

The subunit molecular mass of the putative sugar isomerase was examined by SDS-PAGE under denaturing conditions, using the proteins of a pre-stained ladder (MBI Fermentas, Hanover, MD) as reference proteins. All protein bands were stained with Coomassie blue for visualisation. The molecular mass of the native enzyme was determined by gel filtration chromatography using a Sephacryl S-300 HR 16/60 preparative-grade column (Amersham Biosciences). The column was calibrated with blue aldolase (150 kDa), albumin (66 kDa), ovalbumin (43 kDa), and cymotrypsin (29 kDa) as reference proteins (Amersham Biosciences) and the molecular mass of the native enzyme was calculated by comparing with the migration length of reference proteins.

Effects of metal ions, pH, and temperature

To investigate the effect of metal ions on the putative sugar isomerase, enzyme assay was carried out after treatment with 1 mM ethylenediaminetetraacetic acid (EDTA) at 35°C for 1 h or after adding 1 mM of each metal ion such as Co^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, or Ba^{2+}.

To find optimal pH of the putative sugar isomerase, pH was varied from 6·5 to 8·5 using 50 mmol l^{-1} piperazine-*N*,*N*'-bis(2-ethanesulfonic acid) (PIPES) buffer (pH 6·5−7·5) and 50 mmol l^{-1} EPPS buffer (pH 7·5−8·5). The effects of temperature on the enzyme activity and enzyme stability were evaluated in 50 mmol l^{-1} EPPS or PIPES buffer (pH 7·5) at temperature ranging from 30 to 90°C.

Results and Discussion

Cloning, purification, and molecular enzymes

The gene 687 bp and 1,173 bp encoding a putative sugar isomerase and sugar epimerase were cloned and expressed in *E. coli*. The enzyme was purified as a soluble protein from crude extract by His-Trap affinity chromatography.

The molecular masses of the purified sugar isomerase and sugar epimerase analyzed by SDS-PAGE were approximately 27 kDa and 47 kDa (Fig. 1). Based on the masses of reference proteins, the native D-lyxose isomerase and cellobiose 2-epimerase existed as dimer and monomer by gel filtration chromatography, respectively (Fig. 2).

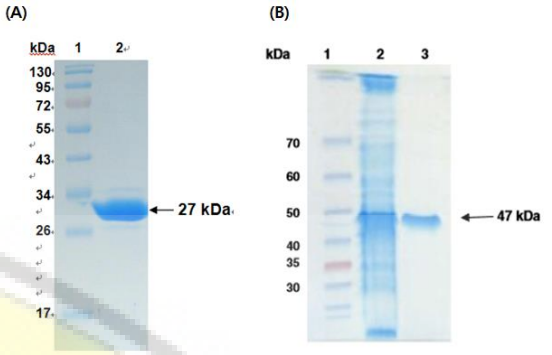

Fig. 1 SDS-PAGE analysis of D-lyxose isomerase from *S. proteamaculans* (A) and cellobiose 2-epimerase from *C. saccharolyticus*(B).

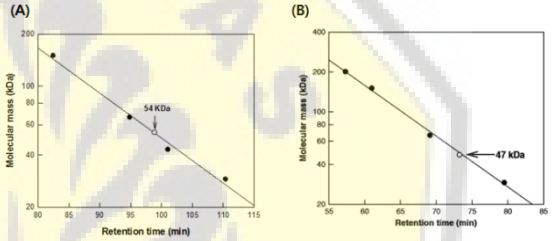

Fig. 2 Determination of the total molecular mass of D-lyxose isomerase from *S. proteamaculans* (A) and cellobiose 2-epimerase from *C. saccharolyticus*(B) by gel filtration chromatography.

Effects of metal ions, pH, and temperature on the activity of enzymes

In the experiment of effect of the metal ion, D-Lyxose isomerases from D-lyxose isomerase from *S. proteamaculans* was investigated as a Mn2+ dependent enzyume and and cellobiose 2-epimerase from *C. saccharolyticus* showed character as a metal ion independent enzyme.

The maximum activity of *S. proteamaculans* putative sugar isomerase was recorded at pH 7·5 and 40°C. And the cellobiose 2-epimerase from *C. saccharolyticus* showed the maximum activity at pH 7.5 and and 75°C.

Substrate specificity of enzymes

The specific activity of *S. proteamaculans* putative sugar isomerase for the aldose substrates followed the order D-lyxose > D-mannose > L-gulose > D-talose > L-ribose. For the ketose substrates, the activity followed the order D-xylulose > D-fructose > L-ribulose > D-tagatose > L-sorbose(Table 1). The substrate specificity of *C. saccharolyticus* cellobiose 2epimerase for monosaccharides was investigated with the D- and L-forms of all pentoses and hexoses. The enzyme converted the substrate aldoses to their

C2 epimers by the epimerization reaction. Among the substrate aldoses, the specific activity was highest for D-mannose, followed by Dlyxose > D-xylose > D-glucose > D-arabinose (Table 2).

Table 1. Specific activity of D-lyxose Isomerase from *S. proteamaculans*

Substrate		Product	Specific activity (μmol min⁻¹ mg⁻¹)
Aldose	D-Lyxose	D-Xylulose	10·0 ± 0·13
	D-Mannose	D-Fructose	5·42 ± 0·075
	L-Gulose	L-Sorbose	1·05 ± 0·013
	D-Talose	D-Tagatose	0·19 ± 0·004
	L-Ribose	L-Ribulose	0·14 ± 0·002
Ketose	D-Xylulose	D-Lyxose	26·2 ± 0·18
	D-Fructose	D -Mannose	1·41 ± 0·090
	L-Ribulose	L-Ribose	0·40 ± 0·002
	D-Tagatose	D-Talose	0·28 ± 0·007
	L-Sorbose	L-Talose	0·02 ± 0·0003

Table 2. Specific activity of cellobiose 2-epimerase from *C. saccharolyticus*

	Substrate	Product	Specific activity (μmol min⁻¹ mg⁻¹)
Aldose	D-Mannose	D-Glucose	150 ± 0.5
	D-Xylose	D-Lyxose	40 ± 0.7
	D-Lyxose	D-Xylose	60 ± 0.3
	D-Glucose	D-Mannose	35 ± 0.5
Ketose	D-Fructose	D-Glucose	9.5 ± 0.06

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MOLECULAR STUDY on DROUGHT STRESS RESPONSE to IMPROVE SUGARCANE PRODUCTIVITY by GENETIC TRANSFORMATION

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Abstract

Plants are frequently exposed to environmental stresses such as high-temperature, floods and drought that reduced the plant growth and yield. To survive and develop normally, plants adapt to the drought stress with various strategies include change in the genes expression and accumulation of organic compounds called compatible solute. Thus, understanding the biochemical and physiological is of immense importance to improve the plants yield and sustain agricultural production. Sugarcane is the most important agricultural crops for sugar production and the drought stress has an impact on the growth and yield. Observations on chlorophyll content, leaf area index and sugar productivity were reduced under drought treatments. However, sugar synthesis was increased concomitant with sugar accumulation during the drought stress. The increase is due to an activation of sucrose-phosphate synthase (SPS), a key enzyme for sucrose biosynthesis in sugarcane. Study on protein profile of drought-tolerant sugarcane leaves revealed that the drought-inducible protein with molecular mass of 22 kDa increased remarkably after drought-stress. Expression of the drought-inducible protein of 22 kDa was induced by drought stress as well as mannitol and ABA treatments. It was presumed that the protein function to adapt to drought stress in sugarcane leaves, but detail study on this protein remains to be elucidated. Genetic transformation is powerful tool and an important technique to create drought-tolerant sugarcane rather than conventional method of genetic hybridization. Recently, drought-tolerant sugarcane has been developed by introducing *betA* gene encoding for choline dehydrogenase (CDH) from *Rhizobium meliloti*. The CDH converts choline into betaine aldehyde to produce glycine betaine which was significantly accumulated in transgenic sugarcane during drought stress. Glycine betaine is a compatible solute thought to act as an osmoprotectant and helping the sugarcane acclimate to drought condition. Confined field trial showed that the glycine betaine contents highly elevated and the sugar production increased 10-30% under drought condition. This drought-tolerant sugarcane has been approved by the National Genetically Modified Product Biosafety Commission for the commercialization. In term of sucrose content, the genes encoding for sucrose-phosphate synthase (*SoSPS1*) and sucrose transporter protein (*SoSUT1*) have been overexpressed in sugarcane. Double over-expression of these two genes enhanced sucrose biosynthesis and translocation, thus significantly elevated sucrose content in sugarcane stem. The increased of sucrose plays important role on the capability of sugarcane adapt to drought condition, since sugars is common solute and used for osmotic adjustment. It will be interesting to postulate that higher sucrose accumulation might provide the transgenic sugarcane with has a character of high sugar yield as well as drought-tolerance sugarcane.

Keyword: sugarcane, *SoSPS1, SoSUT1,* genetic transformation, *Rhizobium meliloti***,** sucrose-phosphate synthase

CONTRIBUTION of INTEGRATED PEST MANAGEMENT in ENHANCING BIODIVERSITY in TROPICAL VEGETABLE PRODUCTION

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Abstract

Vegetables are high value crops with the potential to alleviate the poverty in tropical Asia and sub-Saharan Africa. However, their productivity is limited by biotic constraints including insect and mite pests. In an attempt to produce blemish-free vegetables, chemical pesticides are excessively and indiscriminately used by farmers in most developing countries. Such pesticide over-use not only poses risks to the health of vegetable producers and consumers, but also decimates biodiversity in the production system and interrupts ecosystem services. Development and adoption of integrated pest management (IPM) strategies as an alternative to dangerous chemical pesticides enhances biodiversity in tropical vegetable production systems. For instance, withholding chemical pesticides may allow the natural enemies of pest insects to proliferate. Adoption of IPM favors the performance of pollinators. Deployment of a 'dead-end' trap crop makes a pest insect highly vulnerable to its natural enemies. The effect of IPM technologies developed and promoted by AVRDC – The World Vegetable Center on the enhancement of local biodiversity in tropical Asia will be discussed in detail.

Keywords: vegetables, biological control, pheromones, bio-pesticides, trap crop, biodiversity

Introduction

Vegetables are important components in human diets and agricultural production systems in South and Southeast Asia. Although the vegetable cultivation area and productivity have increased tremendously in recent decades, average productivity remains lower in Asia compared to North America or Australia. Arthropod pests are one of the major limiting factors in vegetable production. Because vegetables are high value crops, farmers use large quantities of chemical pesticides to protect them, resulting in pesticide misuse. Such widespread pesticide misuse adversely affects the environment and causes a loss in biodiversity. It also affects the health of farmers, their families, and consumers. Integrated pest management (IPM) strategies are being promoted to reduce overall pesticide misuse in vegetable production systems in Asia. For instance, AVRDC – The World Vegetable Center has promoted the biological control of diamondback moth in brassicas, IPM for eggplant fruit and shoot borer, safer tomato production practices, etc. Wider adoption of these IPM strategies could make a significant contribution toward rebuilding biodiversity in a particular region. Strengthening biodiversity in a production system would enhance overall ecosystem services in a region. This review will compile the Center's impact on biodiversity in tropical Asia through implementation of selected IPM strategies.

Selected examples

The interplay between tomato fruit borer and its unusual host plant, tropical soda apple

Tropical soda apple (*Solanum viarum*) is a wild relative of cultivated eggplant. Eggplant fruit and shoot borer (*Leucinodes orbonalis*) is the major pest of eggplant in South and Southeast Asia. *S. viarum* possesses appreciable levels of resistance to *L. orbonalis*, and thus has been widely utilized in resistance breeding programs in Asia. In the late 1990s, AVRDC screened *S. viarum* germplasm for resistance to *L. orbonalis*. Although resistant accessions were identified, heavy loads of tomato fruit borer (*Helicoverpa armigera*) eggs and larvae were observed on foliage of *S. viarum* in spring 1998 [23]. However, *S. viarum* was never reported to be the host of this noctuid. A subsequent study also revealed that *H. armigera* overwhelmingly preferred to lay eggs on *S. viarum* over tomato, its normal host plant [7]. Since *S. viarum* showed susceptibility to *H. armigera*, the *L. orbonalis* resistant accessions were not utilized in insect resistance breeding programs. Nevertheless, AVRDC researchers turned their attention toward the interaction of *H. armigera* and *S. viarum*.

We found that the host plant volatiles, especially *n*-alkanes in *S. viarum*, could form the basis for host selection by *H. armigera* female moths [16]. In addition, the presence of feeding stimulants that triggered voracious feeding of *H. armigera* larvae on *S. viarum* also was confirmed [16].

Interestingly, the larval mortality and developmental period significantly increased, with subsequent reduction in pupal weight, when *H. armigera* larvae fed on *S. viarum* leaves [20]. Thus, a majority of *H. armigera* larvae were unable to complete their life-cycle successfully on *S. viarum*, which makes it a perfect 'dead-end' trap crop. However, the story does not stop at this point, because *S. viarum* seemed to have a positive impact on the third trophic level (natural enemies of insect pests) as well.

We also monitored the natural parasitism of *H. armigera* by a larval parasitoid, *Campoletis chlorideae,* on both tomato and *S. viarum* plants three to four times during the season at an interval of 15 days. The results confirmed a significantly higher parasitism of *H. armigera* on *S. viarum* (49.20%) than on tomato (36.20%) [15]. Thus we confirmed that a naturally occurring wild relative (*S. viarum*) can be exploited in the management of a noxious insect pest (*H. armigera*), not only as a direct tool (trap crop), but also to augment biological control by enhancing parasitoid activity.

Role of *Bacillus thuringiensis* in enhancing diamondback moth biological control

Diamondback moth (*Plutella xylostella*) is the most destructive insect pest on vegetable brassicas worldwide, sometimes causing complete crop losses. Pesticides have been the predominant control method for several decades [21], and chemical costs to control *P. xylostella* were estimated to be US$4-5 billion per year worldwide [24]. Efforts to introduce biocontrol agents to manage this pest also have a long history. One of the earliest parasitoid introductions occurred in Indonesia in 1928 [6]. A similar effort was made in 1936, when *Diadegma semiclausum*, an ichneumonid larval parasitoid, was introduced from England into New Zealand [22]. *D. semiclausum* was introduced from New Zealand into Indonesia in the early 1950s. However, due to the intensive use of chemical pesticides on vegetable brassicas, the beneficial effect of this parasitoid was not realized in tropical Asia until the mid-1980s. With the adoption of the microbial pesticide, *B. thuringiensis* (Bt), *D. semiclausum* established in several countries and exerted more than 70% parasitism on *P. xylostella* [22]. AVRDC took the lead in IPM for *P. xylostella* in Asia. The Center implemented a brassica IPM program under the Asian Vegetable Network (AVNET) from 1989-1992. It introduced parasitoids such as *D. semiclausum*, *Cotesia plutellae*, *Diadromus collaris,* and *Trichogrammatoidea bactrae* in Indonesia, Malaysia, the Philippines, and Thailand. Biopesticides such as *B. thuringiensis* complemented the action of these parasitoids [18]. However, *P. xylostella* still resurges as a major threat to brassica production wherever chemical pesticides are used extensively. Biological pesticides enhance the restoration of native parasitoids and help introduced parasitoids become established, thus building the biodiversity in a production system.

Establishment of a parasitoid of eggplant fruit and shoot borer in the absence of chemical pesticides

Eggplant (*Solanum melongena*) is one of the most important vegetables in South and Southeast Asia. Eggplant fruit and shoot borer (*L. orbonalis;* EFSB) is one of the most destructive pests on eggplant in this region. Yield reduction can be as high as 70% [4]. Farmers in the region rely exclusively on the application of chemical insecticides to combat EFSB, which has resulted in misuse of pesticides in an attempt to produce damage-free marketable fruits. While attempting to develop an integrated pest management strategy, it was observed that parasitoids emerged as a major control factor if pesticides were withheld for a particular period of time in South Asia. When eggplant was grown at Gannoruwa, Peradeniya, Sri Lanka for two years from January 2001 to December 2002 without pesticide application, *Trathala flavo-orbitalis* emerged as a major parasitoid [1]. The parasitoid was active year-round; parasitism ranged from 9.5% to almost 39%, which is substantial considering the fact that parasitism rarely exceeds 10% elsewhere. Similar parasitoid monitoring was carried out from August 2000 to August 2002 at several villages in Kheda and Anand districts in central Gujarat, India, where pesticide use in eggplant is only marginal (five to six sprays per season). Although *T. flavo-orbitalis* was the only parasitoid found in central Gujarat and its parasitism in some fields reached as high as 55%, in some neighboring areas it was below 10% [1].

In Bangladesh, the abundance of *T. flavo-orbitalis* was monitored in pesticide-sprayed and pesticide-free eggplant fields at three locations: Gazipur (pesticide spray at weekly intervals) in central Bangladesh; Jessore (farmers spray pesticides daily or every other day) in the southwest; and Rangamati (pesticide application once every two weeks) in the Raikhali Hills in the southeast, from July 2001 to June 2002. At the beginning of the study, the average number of *T. flavo-orbitalis* that emerged from field-collected EFSB larvae was 4.3 at Jessore and 7.0 at Gazipur. After 12 months of withholding pesticide use, the number of parasitoids increased to 40.7 and 61.7 at Jessore and Gazipur, respectively. Thus, it has been proven in South Asia that natural enemies proliferate in eggplant production systems if externalities such as pesticides are withdrawn. If this level of parasitism could be sustained over larger areas throughout the year, it would reduce the pest population on a sustainable basis and thus reduce the need for pesticide use in combating EFSB [1].

The tug of war between the generalist and specialist parasitoids of legume pod borer

Legume pod borer, *Maruca vitrata* (syn. *M. testulalis*) is the most serious insect pest of legumes in Asia and its distribution stretches from West Africa, to the West Indies and Americas, and to Fiji and Samoa in Oceania [13]. Exclusive reliance on and injudicious application of chemical insecticides to combat this insect have resulted in environmental degradation, decimation of natural enemies, pest resistance and resurgence [5]. We are attempting to develop alternative strategies that could reduce pesticide misuse in legume production systems.

M. vitrata has been reported to feed on 39 different host plant species including two non-leguminous hosts [13]. As a borer pest, its larvae usually feed on reproductive parts of the host plants. However, *M. vitrata* was observed to have a different feeding habit on *Sesbania cannabina*, which is grown as a green manure crop in Taiwan during the summer. It is interesting to note that *S. cannabina* was not recorded as a host of *M. vitrata* except in Taiwan [14]. In *S. cannabina*, the larva folds several leaflets, ties them with silken thread, and feeds on the foliage while remaining concealed inside [9]. Since this green manure crop is rarely sprayed with insecticides, a large number of natural enemies were found to attack *M. vitrata* larvae. [10] monitored the seasonal occurrence of these natural enemies and found that a braconid, *Apanteles taragamae*, accounted for an average of 92% of all parasitoid specimens reared from *M. vitrata* larvae and pupae. Its parasitism reached as high as 63% of *M. vitrata* larvae. However, *A. taragamae* is not a specific parasitoid of *M. vitrata*; rather it also attacks *Grapholita critica* on pigeon pea [12], *Opisina arenosella* on coconut [8], and *Diaphania indica* on cucurbits [11] in India. In our recent efforts to identify species-specific parasitoids of *M. vitrata*, we identified *Therophilus javanus* through exploratory surveys in Taiwan [19]. We also confirmed that parasitoids such as *T. javanus* dominate legume production systems and completely replaced the previously known dominant parasitoids such as *A. taragamae* in Taiwan. This confirms the dominance of the species-specific *T. javanus* over the generalist parasitoid, *A. taragamae*. Thus, several natural enemies appeared to dominate attacking a pest species, especially when it seemed to alter its feeding habits in a new cropping system. The generalist natural enemies, however, could not withstand the dominance put forth by species-specific natural enemies over time and space, and thus gradually were replaced.

How does bitter gourd IPM support honey bee pollinators?

Honey bees (*Apis mellifera*) are one of the most important pollinators of agricultural and horticultural crops. Cucurbitaceous vegetables require insect pollinators such as honey bees to transfer pollen from male to female flowers. Research has shown that pollination by honey bees increases fruit size, yield and quality of cucurbit crops. Among the cucurbits, bitter gourd (*Momordica charantia*) is widely cultivated and consumed in South, Southeast and East Asia for its nutritional and medicinal content. Insect pests are a serious problem in bitter gourd production, and various pest management practices have been adopted to suppress pest populations below economic injury level. Integrated pest management strategies and chemical pesticides are used to control pests on bitter gourd [2]. However, pest management practices may be harmful to beneficial insects, including honey bees.

In a field study, we evaluated the effects of an IPM strategy and the application of chemical pesticides on honey bee pollinators and yield of bitter gourd. The IPM strategy included yellow sticky traps with methyl eugenol lures and weekly spray applications of a biopesticide, *B. thuringiensis*. For the chemical pest management, malathion 50% EC was sprayed at weekly intervals. The results confirmed that the IPM strategy did not adversely affect the foraging activity of honey bees. However, malathion caused significant mortality of honey bees one day after application [3]. In addition, pollination by honey bees increased the number of bitter gourd fruit and also increased the yield to a maximum of 226%. Thus, adoption of IPM strategies not only suppresses the pest population, but also enhances the activities of pollinators, thus increasing the quantity as well as quality of cucurbits.

Conclusions

The above examples clearly indicate that chemical pesticides disrupt interactions between insect pests and their natural enemies. When this external pressure is withdrawn for a particular period, it favors a substantial increase in the population of natural enemies, which could at times provide sufficient control of the pest organisms on vegetable crops. Even if natural enemies are unable to provide adequate control, their performance can be easily complemented by other compatible factors such as bio-pesticides, sex pheromones, and trap crops. In addition to pest control due to increased activity of natural enemies, pollinators that would boost the crop yields are also increased in an ecosystem. Thus integrated pest management strategies that cause the least ecological disruption contribute positively to enhancing biodiversity.

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RISK ASSESSMENT of SILVER NANO PARTICLES

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Abstract

The antimicrobial properties of silver nanoparticles (AgNPs) have made these particles one of the most frequently utilized nanomaterials in consumer products; therefore, a comprehensive understanding of their toxicity is necessary. In particular, information about the uptake and size dependence of AgNPs is insufficient. In this study, we evaluated the size-dependent effects of AgNPs by treating the human LoVo cell line, an intestinal epithelium model, with spherical AgNPs of well-defined sizes (10, 20, 40, 60 and 100 nm). The cellular uptake was visualized by confocal laser scanning microscopy, and various cytotoxicity parameters were analyzed in a size- and dose-dependent manner. In addition, the cellular proteomic response to 20 and 100 nm AgNPs was investigated to increase the understanding of potential mechanisms of action. Our data indicated that cellular uptake and toxicity were regulated by size; smaller particles easily penetrated the cells, and 100 nm particles did not. It was hypothesized that this size-dependent effect resulted from the stimulation of a signaling cascade that generated ROS and inflammatory markers, leading to mitochondrial dysfunction and subsequently inducing apoptosis. By contrast, the toxicity of AgNPs, as measured by cell proliferation, was independent of particle size, indicating a differentially regulated, ROS-independent pathway.

Keyword: silver nanoparticles, toxicity, cytotoxicity, biotecnology

CONSERVATION of THREATENED TREE SPECIES on EX-MINE SITES in INDONESIA

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Abstract

Mining industry, generally, has been considered as the most destructive industry destroying forest and its biodiversity, changing landscape; and creating soil, water and air pollution. Concern on biodiversity loss due to mining is even higher in Indonesia due to its tropical rain forest the house of divers flora and fauna. Since 2000, efforts have been made to re-establish forest on ex-mine sites. Soil amelioration, including application of compost, fertilizer, and lime has been conducted to enhance the growth of fast growing species, especially *Acacia mangium* and *Falcataria moluccana* the two most popular species for mine reclamation. This has resulted on the establishment of monoculture exotic forests on ex-mine sites. In term of re-greening the ex-mine sites, planting those two species have fulfill the main purpose of mine reclamation programme. In 2009 the Ministry of Forestry of the Republic of Indonesia has release a new regulation that mining companies should use at least 40% of local climax species for their mine reclamation programmes. This paper will discuss about techniques and the potential of conserving threatened forest tree species on ex-mine sites in Indonesia.

Keywords: biodiversity, conservation, *Diospyros celebica*, forest, *Intsia* sp., mining, reclamation, tropical

STATUS and CHALLENGES of AGRICULTURE BIOTECHNOLOGY DEVELOPMENT in INDONESIA

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Abstract

Indonesia is among the few countries in the world that advocates the adoption of biotechnology in agriculture. Since the commercial application globally of Bt-corn in 1996, Indonesia has introduced it in South Sulawesi in early 2000. However, the initiative was partially aborted due to non-technical considerations. Today, aside from the 15 biotechnology products that have been approved for food safety in the past years, the Indonesian Biosafety Commission has recently approved two more biotechnology products, namely: drought- tolerant sugarcane and herbicide- tolerant corn for environment safety. These products, following the approval by Variety Release Commission, may soon be eligible to be cultivated in any farms in Indonesia for the benefit of farmers. Research and development in biotechnology to support agriculture in Indonesia has been done in various areas of life sciences by various public and private institutions. Among these include the production of Bird-Close 5.1, a reverse genetic vaccine to combat bird flu in poultry, the application of feed supplements and additives to improve the efficiency of poultry industry, and the introduction of the golden rice to combat vitamin A deficiency. More research and development works in plant, animal and industrial biotechnology are currently underway for application soon. Despite these developments, Indonesia has yet to address several challenges to fully reap the benefits of biotechnology application in agriculture. These challenges include : 1) strengthening partnership between public research institutions and private sectors, 2) integrating biotechnology in the national research and development agenda including provision of appropriate budget, 3) having clear, timely and favorable regulations and approval process, and 5) applying appropriate strategies in biotechnology information dissemination to various stakeholders.

Oral Session

ROLE of S100B PROTEIN as BIOMARKERS PRENATAL ISCHEMIC HYPOXIC

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Abstract

Hypoxic ischemic brain damage as a result of prenatal is a major cause of mortality and morbidity. Hypoxic ischemic prenatal abnormalities associated with the onset of cognitive, motor and behavioral. One cognitive ability is memory. Cerebral ischemic will lead to cell dysfunction can even cause death. S100β protein known as biomarker some brain cells damage. Biomarkers hypoxic ischemic brain damage due to prenatal needs to be further investigated Objective: to study the role of S100β protein induced prenatal hypoxic ischemic hypoxic. Methods: An experimental study with post-test design control design. Subjects were 18 *Rattus norvegicus* prenatal ischemic hypoxia-induced (by way of unilateral uterine artery ligation when the parent *Rattus norvegicus* pregnant 7 and 11 days). Independent variables: prenatal ischemic hypoxic. The dependent variable: S100β protein level. S100β proteins from blood plasma were measured using ELISA technique. Results: The protein S100β analyzed using ANOVA. The limit of significance was set at $p < 0.05$. The protein S100β increased although not statistically significant.. Conclusion The protein S100β increased in prenatal ischemic hypoxic.

Keywords: hypoxic, ischemic, proteins S100β, prenatal

Introduction

Brain damage caused by prenatal ischemic hipoxic is a major cause of mortality and morbidity. Prenatal ischemic hypoxic can cause motor disturbances, and cognitive behavior in children. The incidence of prenatal ischemic hypoxic about 2-4 per thousand births, of which about 60% of premature birth and birth to underweight [1,2]. The incidence of asphyxia neonatarum in Indonesia approximately 40 per 1,000 live births, the overall 110,000 newborns die each year due to asphyxia [3]. Incidence of perinatal morbidity due to intrauterine hypoxia and asphyxia has not changed significantly, although there were improvements in labor management. Perinatal morbidity reflects antenatal hazardous conditions [4].

Cerebral ischemia will lead to cell dysfunction can even cause death [5,6]. Proteins S100β is biomarkers include damage to brain cells. Necrotic damage glial cells and Schwann cells due to ischemia and disturbant membrane integrity due sitoktosik and vasogenic edema will cause the passage of proteins from the cytosol to the extracellular S100β. With specific biomarkers that the researchers expect an improvement in the diagnosis, degree of severity, course of the disease over time, the effects of therapy and prognosis of patients [7,8,9]. In addition to the factors mentioned above researchers directing focus to look for biomarkers that may be a prognostic factor that can complement prognostic factors were already relatively well established. Biomarkers hypoxic ischemic brain damage caused by prenatal needs to be further investigated. Researchers interested in studying the role of protein S100 β as sensitive and specific biomarkers of brain damage caused by prenatal ischemic hypoxia.

Methods

This type of research is experimental research. The research design used is post-control design. This research was conducted in the laboratory Biomedic, Muhammadiyah University of Yogyakarta. Prenatal ischemic hypoxic do with ligation uterine artery unilateral. Anaestetic uses ketamine 40 mg/kg body weight, intra muscular . Subjects were *Rattus norvegicus* Spraque Dawley strain. Subjects devided 3 groups: controle (from mothers not induction ischemic hypoxic), treatment group 1 (with induction prenatal ischaemic hypoxic at 7 days breeding), and treatment group 2 (with induction prenatal ischemic hypoxic at 11 days breeding). ELISA method uses to test Protein S100β in blood at 30 days ages. The results are analyzed using ANOVA test

Subjects were divided into 3 groups. The control group, induction of ischemic hypoxic gestation 7 (PI-7), and 11 (PI-11) days.

Table 1. Birth Weight Research Subjects

Kelompok	Minimum (gram)	Maximum (gram)	Mean (gram)	mean ± SD
Kontrol	6.5	7.1	6.7	6.7±0
PI-7	4.8	5.3	4.9	.6
PI-11	4.5	5.3	4.5	4.9±0 .5 4,.±0. 8

S100 β protein examination of the blood using rat S100β kit EILSA (MBS731676). ELISA test results for blood S100 β protein showed results that were not statistically significant (p>0.05)

Discussion

This study showed increasing S100 β protein that were not statistically significant (p>0.05). Research that has been carried by [10] of the influence of hypoxia prenatal to the development of the brain and due to short-term and long on rodensia show that there is delay the development of the ability sensory and motoris after one month postnatal [10].Cells damage started about 3 hours after induction ischemic hypoxic .The increasing number of apoptosis cells there have been three days after induction prenatal ischemic hypoxic and peaked 7 the last day. About 14-20 days after induction prenatal ischemic hypoxic, happened a lesion in brain tissue. [11] said the change in neuroanatomikal , functional and behavior in mice adult due to induction prenatal ischemic hypoxic [11]. Research that has been carried by [12] revealed that induction prenatal ischemic hypoxic at the age of ischemic hypoxic pregnancy 30-32 day on pigs cause imunoreaktivitas hif-1 in layers granular genitalia the cerebellum and obstructed [12].

Conclusion

Prenatal hypoxic ischemic causes an increase in S100B Protein although not statistically significant

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INCREASING ENVIRONMENTAL HEALTH THROUGH EARLY CHILDHOOD HEALTH EMPOWERMENT in APPLYING THE PHBS in GUMUKSARI 3 ELEMENTARY SCHOOL KALISAT JEMBER

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Abstract

The school environment is one of the important tool to foster and cultivate a clean and healthy living. But in fact most of the schools have not been able to implement a culture of behavior on clean and healthy one in Gumuksari 3 elementary school kalisat jember. Behavior on clean and healthy in indonesia called PHBS. Many factors cause this school can not apply a clean and healthy behavior, such as less supportive school environment, and less knowledge people in schools about clean and healthy living behavior. Activities that we have done that early childhood health empowerment with four phases namely preparation, implementation, evaluation and reporting of results of activities. In the implementation phase includes training of small doctors, adventure PHBS, PHBS challenge, and final challenge of PHBS. Output resulting in the empowerment of this is the formation of small physician trained in PHBS, knowledge and attitude of people in schools on clean and healthy life behavior that have increased, and the creation of a paperback book ISBN 978-602-9030-75-4. The advice we provide, include to facilitate small doctor in doing activities, schools need to increase health services, and programs of this kind should be given to all schools in Indonesia.

Keywords: Empowerment, PHBS, Environmental Health, Early Childhood, Elementary School

Introduction

PHBS in school is a set of behaviors practiced by students, teachers and the general school environment on the basis of consciousness as a result of learning, so that independently able to prevent disease, improve health, as well as play an active role in realizing a healthy environment. In Law Number 36 in 2009 article 79 on health, asserted that the "School Health" was held to enhance the ability of the learners in the healthy living healthy living environment so that learners can learn, grow and develop harmoniously and extended to become qualified human resources.

Background the need for applied PHBS in school that is due to the emergence ofvarious diseases that often strike children of school age (early age 6-10), generally deals with the PHBS. Elementary school is the initial stage to provide insight into behaving live clean and healthy. Because at the primary school stage prone to contract the disease. Jember health service data from 2013, a disease that often strikes early childhood is a disease of dengue fever and diarrhea diseases, causing death. So the need for special attention to early childhood in order to be able to independently prevent it self from the threat of various diseases.

In Jember district there is a primary school in the village of Gumuksari village, Krajan, Kalisat, a less noticed some important aspects, one of which resulted in students health often contract the disease and be able to have an effect on their learning achievements. The name of the elementary school is Gumuksari 3 Elementary School.

See the State Gumuksari 3 Elementary School, then he did training to create a clean and healthy life behavior as an attempt to let students know, willing and able to pull PHBS. With the PHBS in the school environment indicators: wash your hands with running water and SOAP wear, consume healthy snacks in the cafeteria of the school, using the toilets are clean and healthy, sports regularly and measurable, eradicate the mosquito larva, it is prohibited to smoke, weight and measure the height , throw away trash in its place.

So the goal, students are able to independently prevent them selves from the threat of disease, improve health for him self, able to improve learning achievements, as well as play an active role in realizing a healthier environment at school and also his family.

Method

Methods under taken include health empowerment with training interventions

PHBS, environmental observation and intervention of health care changes. Health empowermwnt was carried out for 5 months and through 4 stages namely, preparation, implementation, evaluation and reporting the results of activities. At the stage of preparation includes the mentoring team for PHBS deepening PKM-M PHBS and coordination and advocacy meetings with

the Board of teachers SDN Gumuksari 3. In the implementation phase includes training of small doctors, adventure PHBS, PHBS challenge, and final challenge of PHBS. Then, to the stage of the evaluation the evaluation is divided into an early stage, the stage of the process and the final stage. Next up is stage of reporting the results of activities. To test the success of the program used the approach reserved pretest and post test before he did, and after he did, training of PHBS.

Results And Discussion

Gumuksari 3 Elementary School has a number of students grades 1-6 of 114 students. Each class consists of, there are 24 student from grade 1, grade 2 are 15 students, there are 24 student from grade 3, grade 4 are 17 students, there are 18 student from grade 5, grade 6 are 16 students. Utensils cleanliness in everyclass was minimal, and no one finds the trash in area schools. The UKS (Health Student Unit) at Gumuksari Elementary 3 school which should be a means to improve the quality of education and learning achievements of learners by increasing clean and healthy lifestyles was not running as its function. So, arguably the UKS only a place not used or has no health workers operating the UKS. There is no consciousness for the students to behave in a clean and healthy life early on. The circumstances of the case is very troubling health schools and citizens can lower student achievement. For that, holding the health empowerment to early childhood.

Health empowerment was carried out for 5 months and through 4 stages namely, preparation, implementation, evaluation and reporting the results of activities. At the stage of preparation includes the mentoring team for PHBS deepening PKM M PHBS and coordination and advocacy meetings with the board of teachers SDN Gumuksari 3. In mentoring deepening PHBS produces 13 mentors are experts in the field of clean living and healthy behavior. Later, at the meeting of the coordination and advocacyteams PKM-M PHBS with Board teacher SDN Gumuksari 3 produce an agreement of cooperation in health empowerment program at SDN Gumuksari 3.

In the implementation phase includes training of small doctors, adventure PHBS, PHBS challenge, and final challenge of PHBS. On the training of doctors in small generating 24 small doctors drawn from representative grades 1-6. The small doctor has been trained in behave of life clean and healthy. Then, on an adventure involving PHBS 114 students who came from grades 1-6, this resulted in behavior change adventure students Gumuksari 3 Elementary School be know, willing and able to implement PHBS in everyday life. Further, PHBS challenge and monitoring of PHBS challenge are implemented to control the behavior of students in a clean and healthy life behaved while up lifting in living habits do behave in race clean and healthy. Last activity is final challenge of PHBS in the fifth month. On the activities of these final challenge of PHBS discussed activities in the months before.

Then, to the stage of the evaluation the evaluation is divided into an early stage, the stage of the process and the final stage. On the evaluation of the initial phase of the regatta is reserved pre test, evaluation of the process seen from the monitoring and evaluation of PHBS race end seen from the results of the post test and change the behavior of the students of Gumuksari 3 Elementary School. Next up is stage of reporting the results of activities.

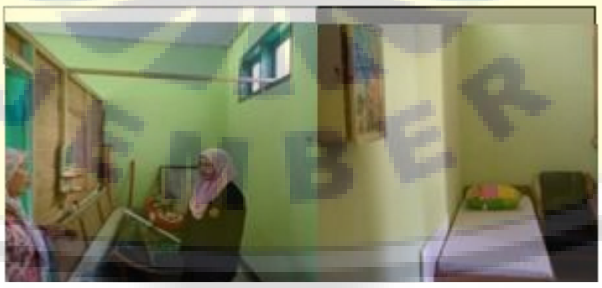

Figure 1. Changes to UKS at Gumuksari 3 Elementary School

To test the success of holding the approach reserved pre test and post test. Reserved pre test given before training takes place and the question of the post test is given at the end of the training, with the results of pre test and post test bellow.

Knowledge and Behavior of Students about Consume Healthy Snacks in the Cafeteria of the School

To consume healthy snacks is consuming nutrient-containing snacks such as calories,protein and vitamins, the place should be closed, and includes as well as the color of the food are not striking. Students knowledge about consume healthy snacks in the school cafetaria before before the holding of training is extremely low, amounting to: class 1 (7.6%), class 2 (19.6%), class 3 (24.4%), class 4 (29.2%), class 5(56%), and class 6 (47.0%) know about healthy snacks in the cafeteria of the school. So the students who know it is about 38.5% and 38.2% do not know. After the holding of the training, the number of students who knows about healthy snacks is increasing: class 1 (57.4%), class 2 (67%), class 3 (57.4%), class 4 (88.5%), class 5 (55.7%) and class 6 (88.2%). So the average of 136.8% who know and who don't know 13.6%.

Table 1. Knowledge and behavior of student of Gumuksari 3 elementary school about consume healthy snacks in the school's cafetaria

Did the students about healthy snacks in the school cafeteria?				
Class	Before Training		After Training	
	Know (%)	Do not know (%)	Know (%)	Do not know (%)
1	7.6	92.4	92.4	7.6
2	19.6	80.4	67	33
3	24.4	75.6	92.4	7.6
4	47.8	52.2	88.5	11.5
5	56	44	89.6	10.4
6	75.6	24.4	88.2	11.8
Average	38.5	61.5	86.4	13.6

Knowledge and Behavior of Student about Using Toilets Clean and Healthy

The occurrence of a wide range of causes of disease caused by environmental sanition conditions that are not supported by the clean and healthy lifestyles such as the development of vector diseases [3]. Adverse environmental conditions and not tackle PHBS led to the expansion of the vector of the disease because of the availability of the media causes the transmission of a variety of diseases especially diarrhea [1]. This condition is aggravated by the habit of disposing of large water and discard the water in the toilet is not small but in a river which can lead to the development of the disease vector, processing bad feces where flies can breed, making it easier for the spread of the bacteria *E-colli* [6]. Before the holding of the training, the number of students who use toilets while urinating and large as much as: class 1 (32.8%), class 2 (29.1%), class 3 (30.8%), class 4 (52.3%), class 5 (56%), and class 6 (47.0%). So average that use the toilets during urination and huge amounting to 32.4% and still throw shit and little water in the river of 47.8%. After the holding of the training, the number of students who use toilets while urinating and large as much as: class 1 (57.4%), class 2 (67%), class 3 (57.4%), class 4 (88.5%), class 5 (55.7%) and class 6 (81.9%). So the average of 53.0% are already using and not using 14.7%.

Table 2. Knowledge and behavior of students about using the toilet during urination and defecation

Have students use the toilets during urination and defecation?				
Class	Before Training		After Training	
	Already (%)	Not yet (%)	Already (%)	Not yet (%)
1	32.8	67.2	92.4	7.6
2	46.9	53.1	67	33
3	49.6	50.4	92.4	3.9
4	52.3	47.8	88.5	11.5
5	56	44	89.6	10.4
6	75.6	30.7	81.9	18.1
Average	52.2	47.8	85.3	14.7

Knowledge and Behavior of Student about Eradicating Mosquito Larva

Eradicating mosquito larva is an activity check and clean up places of shelter clean water that is in school in order to be freed from the mosquito larva [5]. Before the holding of the training, the number of students who knows about how to eradicate mosquito larva as much as: class 1 (7.6%), class 2 (12.9%), class 3 (16%), class 4 (21.4%), 5 th grade (44.8%) and class 6 (35.2%). So knowing the average 28.7% and 77.1% did not know. After the holding of the training, the number of students who knows about how to eradicate mosquito larva as much as: class 1 (88.2%), class 2 (73.7%), class 3 (96.6%), class 4 (51.3%), class 5 (55.7%) and class 6 (88.2%). So knowing the average 53.7% and 13.5% did not know.

Table 3. Knowledge and behavior of students about eradicating mosquito larva

Did the students about how to eradicate mosquito larva?				
Class	Before Training		After Training	
	Know (%)	Do not know (%)	Know (%)	Do not know (%)
1	7.6	92.4	7.6	92.4
2	12.9	87.1	12.9	87.1
3	16	84	16	84
4	34.4	65.2	34.4	65.2
5	44.8	55.2	44.8	55.2
6	56.7	43.3	56.7	43.3
Average	28.7	71.3	28.7	71.3

Knowledge and Behavior about Washing Hands with Running Water and using SOAP

Before the holding of the training, the number of students who do wash your hands before eating as muchas: class 1 (24.4%), class 2 (53.1%), class 3 (33.9%), class 4 (43.2%), 5th grade (72.8%), and class 6(81.9%).So average that has been doing hand-washing before eating 36.9% and 40.6% have not do. After the holding of the training, the number of students who do wash your hands before eating as much as: class 1 (57.4%), class 2 (93.8%), class 3 (96.6%), class 4 (58.7%), class 5 (95.2%), and class 6 (94.5%). So the average of 94.5% have already done and haven't done 5.5%.

Table 4. Knowledge and behavior of students of SDN Gumuksari 3 about washing hands with running water and using SOAP

Have students wash the hands before eating				
Class	Before Training		After Training	
	Already (%)	Not yet (%)	Already (%)	Not yet (%)
1	24.4	75.2	92.4	7.6
2	53.1	46.9	93.8	6.2
3	54.6	45.4	96.6	3.4
4	69.6	30.4	94.4	5.6
5	72.8	27.2	95.2	4.8
6	81.9	18.1	94.5	5.5
Average	59.4	40.6	94.5	5.5

Knowledge and Behavior of Student About throw away trash in its place

Very bad environmental conditions influence on increasing the development ofvectors in the environment, such as the environmental management of the trash is not good, the same litter provides media culture flies that can transmit disease diarrhea. Before the Holding of the training, the number of students who already dispose of trash in its place: class 1 (31.3%), class 2 (37.2%), class 3 (31.3%), class 4 (63.8%), class 5 (78%), and class 6 (81.9%). So average that's been throw away trash in place of 64.2% and who have not made of 35.8%. After the holding of the training, the number of students who already dispose of trash in place as much as: class 1 (96.6%), class 2 (50.0%), class 3 (96.6%), class 4 (58.7%), class 5 (95.2%), and class 6 (94.5%). So an already average 93% and yet dum on place 7%.

Table 5. Knowledge and behavior of students of regarding discard trash in its place

Have the students to throw away trash in its place?				
Class	Before Training		After Training	
	Already (%)	Not yet (%)	Already (%)	Not yet (%)
1	50.4	49.6	96.6	3.4
2	59.8	40.2	80.4	19.6
3	50.4	49.6	96.6	3.4
4	63.8	36.2	94.4	5.6
5	78.4	21.6	95.2	4.8
6	81.9	18.1	94.5	5.5
Average	64.2	35.8	93	7

Knowledge and Behavior of Students about the Dangers of Smoking

Before the holding of the training, the number of students who knows about the dangers of smoking as much as: class 1 (11.8%), class 2 (16.3%), class 3 (17.8%), class 4 (28.8%), class 5 grade (61.6%) and class 6 (43.1%). So on average, knowing the dangers of smoking 25.3% and 36.8% who do not know. After the holding of the training, the number of students who knows about the dangers of smoking as much as: class 1 (79.8%), class 2 (87.1%), class 3 (57.4%), class 4 (58.7%), class 5 (95.2%), and class 6 (94.5%). So the average of 90.6% who know and who doesn't know the 9.4%.

Table 6. Knowledge and behavior of students about concerning non smoking

The students know about the dangers of smoking?				
Class	Before Training		After Training	
	Know (%)	Do not know (%)	Know (%)	Do not know (%)
1	11.8	88.2	79.8	20.2
2	26.3	73.7	87.1	12.9
3	28.6	71.4	92.4	7.6
4	46.4	53.6	94.4	5.6
5	61.6	38.4	95.2	4.8
6	69.3	30.7	94.5	5.5
Average	40.7	59.3	90.6	9.4

Knowledge and Behavior of about Weight and Measure the Height

Before the holding of the training, the number of students who knows how to weightt and measure the height of: class 1 (11.8%), class 2 (19.6%), class 3 (42%), class 4 (32.6%), class 5 (41.8%) and class 6 (47.0%). So the average of students who knows are 27.8% and who did not know are 55.2% . After the holding of the training, the number of students

who knows how to weight and measure the height as much as: class 1 (57.4%), class 2 (67%), class 3 (57.4%), cl ass 4 (88.5%), class 5 (55.7%) and class 6 (88.2%). So the average of 136.8% who know and who don't know 13.6%.

Table 7. Knowledge and behavior of students about weight and measure the height

Can students weigh and measure the height?				
Class	Before Training		After Training	
	Could (%)	Couldn't (%)	Could (%)	Couldn't (%)
1	11.8	88.2	92.4	7.6
2	19.6	80.4	67	33
3	42	58	92.4	3.9
4	52.4	47.8	88.5	11.5
5	67.2	32.8	89.6	10.4
6	75.6	24.4	88.2	11.8
Average	44.8	55.2	86.4	13.6

Knowledge and Behavior of Students about Sports Regularly and Measurable

Before the holding of the training, the number of students who have exercise regularly and measured as much as: class 1 (57.4%), class 2 (93.8%), class 3 (57.4%), class 4 (92.8%), class 5 (95.2%), and class 6 (94.5%). So average that has been exercising regulary and measurable of 88.5% and 6.5% that haven't. After theholding of the training, the number of students who exercise regularly and measured as much as: class 1 (96.6%), class 2 (50.0%), class 3 (57.4%), class 4 (58.7%), class 5 (55.7%) and class 6 (88.2%). So knowing the average 56.1% and who don't know the 9.7%.

Table 8. Knowledge and behavior of students about sports that regular and measurable

Have the students work out regularly and measurable?				
Class	Before Training		After Training	
	Already (%)	Not yet (%)	Already (%)	Not yet (%)
1	96.6	7.6	92.4	3.4
2	80.4	6.2	93.8	19.6
3	92.4	7.6	92.4	7.6
4	94.4	7.2	92.8	5.6
5	89.6	4.8	95.2	10.4
6	88.2	5.5	94.5	11.8
Average	90.3	6.5	93.5	9.7

External Activities

External activities of the intensive training con ducted by students to early childhood to create a clean and healthy lifestyles. As some activities (training of small doctors, adventure PHBS, PHBS challenge, and final challenge of PHBS). Activities ("the formation of Health and empowerment of Small Doctor Early in an effort to realize a Healthy and clean Live Behavior in Gumuksari 3 Elementary School Kalisat Jember") are written in the form of scientific articles.

There are two additional outer form "Poster Handwashing with 7 steps" contained in every corners of the school to help students well and wash your hands properly and "paperback book clean and Healthy Lifestyles in schools" have earned number ISBN 978-590-9030-75-4, the book is distributed to the students of Gumuksari 3

Elementary School and sell to the public so that the circles are beneficial to many people in life behave clean and healthy.

Conclusion

By holding an intensive training to increase clean and healthy lifestyles in school residents, particularly students of Gumuksari 3 elementary school. The learning achievements of students, increasing the formation of small doctors have been trained to help students at the school in the conduct of life clean and healthy so that it is able to create a healthy school. After an intensive training was held for 5 months, there is a change in behavior on each indicator in the PHBS. The students of Gumuksari 3 elementary school getting used to implement clean and healthy living behaviours in everyday life both school surroundings as well as family surroundings.

Advice

1. It does training, an intensive monitoring and surveillance to early childhood in order to know, want and are able to independently maintain yourself from the threat of various diseases through healthy and clean life behavior.
2. The granting of motivation to each school about how important citizens behaveliving clean and healthy.
3. Formation of small doctor at each school to become an example and taught his friends in a clean and healthy life behaved as well as facilitate doctors in occupatio
4. The school will need to continue to improve health services.
5. The Program of this kind should be given to all schools on Indonesia.

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QUALITY EVALUATION and PREPARATION of VOLUNTARY ACCEPTANCE of JELLY CANDY KETEPENG CHINA LEAF EXTRACT *(Senna alata* L.*)* Roxb as ANTHELMINTHIC

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Abstract

Childhood is a time of growth, but because of the lack of awareness of good hygiene to one self or to the environment, so that from their very susceptible to viruses, bacteria, and various diseases from the outside which is caused by a worm. But because anthelminthic drugs on the market to day are less attractive to children, thus making the children tend to be reluctant to consume despite being infected with the worm. In connection with this, the researcher wants to make preparations jelly candies are made from extracts of leaves ketepeng china anthelminthic as well as knowing the quality of jelly sweets which include organoleptic test, water content, total plate count and acceptance of volunteers. This research was conducted in the laboratory of the Academy of Pharmacy Putra Indonesia Malang during the months of February to June 2013. The results show a fairly good organoleptic. On the water content of the test results obtained by the average percentage of 11,191% can be seen that the results meet the requirements SNI.3547.2: 2008. In the total plate count results obtained at 6,7 x 10^3, the result has met the standard. Acceptance of volunteer obtained an average percentage of 78%, which is included in the criteria for "good enough".

Keywords: Anthelminthic, ketepeng china leaf extract, jelly candy.

Introduction

Intestinal worm infections are transmitted through the ground one of them is round worms (Ascarieslumbricoides) These worms can lead to declining health conditions, nutrition, children's intelligence because it causes loss of carbohydrates and protein and blood loss, so the decline in the quality of human resources [1]

One study says that ketepeng china (*Senna alata* L.) Roxb be effective as anthelminthic been proven and tested inpreclinical content contained in ketepeng china which contain tannins.

Oral treatment for intestinal worms in children who have many in the market is identical with a bitter taste. So that preparations in the market less attractive to children, therefore the thought of making anew break through, which the researcher wants to make worming preparations in the form of jelly candies. Jelly candy is candy that have a common characteristic, namely chewy which varies froms light lysoftto moderately hard [2].

Research Methods

The design of this research, including experimental research by making a jelly preparation containing extracts ketepeng china based on a standard formula that has been done the previous observation [3]

Tools and materials

Tools used in the manufacture of jelly candy ketepeng china leaf extract is a measuring cup, thermometer, pans, stove, basin, and candy molds. Where as the necessary ingredients are extracts ketepeng china, water, citric acid, sodium propionate, glucose syrup, gelatin, stevia, and flavor.

Research stage

Steps being taken in this study is the first, preparatory stage includes populations, samples, location and time of the research, tools and materials, and calculate the material requirements. Second, the implementation phase consists of the manufacture of ketepeng china leaf extract and manufacture of jelly candies. Manufacture of ketepeng china leaf extract using infundation at temperatures 90 ° C for 15 minutes. Preparation of jelly sweets and evaluation of the stocks. These three data analysis

Table1. Formula Candy Jelly@Candy

Materials	Formula	Percentage %
leaf extract ketepeng china	6,07 g	30,31%
Glucose Syrup	6,07 g	30,31%

Materials	Formula	Percentage %
Sodium Propionate	0,06 g	0,29%
Stevia	3 g	14,98%
Gelatin	2 g	9,99%
Flavor	0,02 g	0,09%
Water	2,76 ml	13,78%

Research Result

Implementation of the research that was conducted in February until June 2013, observations of the quality of jelly sweets as anthelminthic include organoleptis can be seen in table 2 below.

Table2.Observations organoleptic

Organoleptis	Jelly candy
Form	Semi solid
Color	Brown transparant
Flavorful	Not flavorful
Flavor	Sweet cacao
Texture	Soft supple

Based on the table 2 it can be concluded that the results of the determination by organoleptic showed that the leaf extract ketepeng china jelly candy has the shape of a semi solid, transparent brown, not flavorful, sweet taste and soft texture cacao.

Quality Parameters

Further more, namely water content test. Observation results can be seen in Table 3.

Table 3. ResultsAssay TestWater

Trial	W_0	W_1	W_2	Water conten %
I	48, 861g	53, 855 g	53, 297g	11,173%
II	47, 518g	52, 594 g	52, 025g	11,209%
	Average			11,191%

Based on the calculation of the first experimental test of the water content and the second with an average of test results of 11.191% moisture content can be said that the test results of water content jelly extract ketepeng china meet the requirements of SNI.3547.2: 2008 to a maximum of 20.0%. The observation can be seen in Table 4 below.

Table 4. Test results Microbial Contamination

Dilution	Amount Colony		Result
	I	2	
10^1	94	80	$8,7 \times 10^2$
10^2	79	65	$7,2 \times 10^2$
10^3	74	71	$7,2 \times 10^4$
Average			$6,7 \times 10^3$

Based on the table 4. Indicates that the test microbial contamination on candy jelly extract ketepeng china meet the requirements that quality requirements jelly candy in accord ance with the SNI is $5x10^4$.

Results voluntary admission to the 25 panelists conducted through a questionnaire giving the percentage of each receipt is obtained overall volunteer who can be seen in chart 1 below.

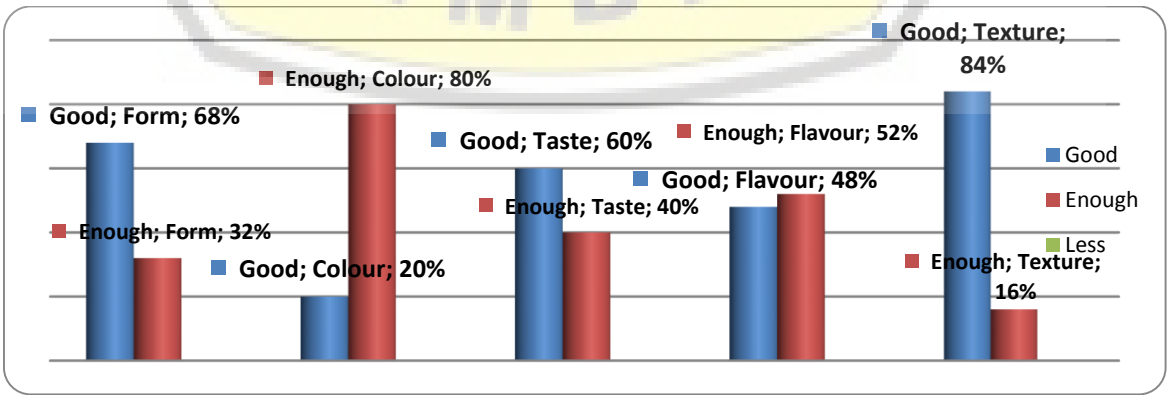

Figure 1. Volunteers acceptant presentation

Discussion

Jelly candy made from extracts of leaves of ketepeng china jelly candies are efficacious as anthelmintic. Jelly candy made in order to be accepted or not by the children, the quality evaluation and acceptance of volunteer preparation jelly candies.

Results of this research data analysis. Based on data from the questionnaire results of voluntary acceptance, to forma jelly candy leaf extract members ketepeng china good ratings as much as 68%. Voluntarily assume the form of a semi solid jelly candy this,it is appropriate from the elasticity of jelly candy when bitten.

Results of the volunteer reception parameters to extract jelly candy texture ketepeng china almost all volunteers like. This is because it has the texture of jelly sweetsa nd soft chewy texture when chewed. Gelatin concentration used greatly affect the elasticity and shape jelly candy produced, so with the appropriate concentration of gelatin jelly candy produced is not too soft and not to origid.

Overall proceeds from the volunteers showed that the leaf extract jelly candy ketepeng china included in the criteria quite well with the percentage of 78% and can be accepted by children. However,the short age is need forchange in the formulation so that the resulting jelly candies as expected.

In addition to organoleptic observation, testing water content and microbial contamination of total plate count ketepeng china leaf extract jelly candy.The test results of water content obtained an average of11.191% can be seen that the test results of water content jelly extract ketepeng china meet the requirements of SNI. 3547.2: 2008 to a maximum of 20.0%.Water content in food will determine the freshness and power of the durable food stuffs. High water levels caused easily bacteria,molds, and yeasts to proliferateso that there will be changes in food stuffs.

Microbial contamination testing results of total plate coun tjelly candy china ketepeng leaf extract showed a yield of $6,7x10^3$, the resultis in accord ance with the requirements of SNI. So that the bacteria contained in the jelly candies safe when consumed and tested on volunteers.

Conclusion

The results showed the determination of organoleptic candy jelly extract ketepeng china shows the corresponding results. The test results of water content and microbial contamination testing of total plate count jelly candy leaf extract preparation ketepeng china qualified according to ISO. 3547.2: 2008. From the summary of the volunteer reception obtained an average percentage of 78%, which is included in the criteria for"good enough".

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STRUCTURAL and FUNCTIONAL RECOVERY of B-CELLS PANCREAS in TYPE 1 DIABETES MELLITUS INDUCED MESENHYMAL STEM CELL CONDITIONED MEDIUM

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Abstract

This study aimed to investigate the role of human umbilical cord mesenchymal stem cell-derived conditioned medium (CM) on the recovery of β-cells pancreas in type 1 diabetic mellitus of rat. The 0.05 ml of CM induction was applied to the diabetic group of rats at the week 1, 2, 3 and 4. One week after second and fourth CM induction, insulin concentration was analyzed with ELISA. Pancreas tissues were stained with Hematoxillin-Eosin and immunohistochemical method for insulin. The studies showed the damage of pancreatic tissues after the injection of single dose alloxan. Microscopic observation detected the presence of small islet in diabetic group, 1 week after first 0.05 ml CM induction. The number and size of islet increased in agreement with the CM doses and time of inductions. Immunohistochemically, the presence of low intensity of insulin positive cell can be recognized at the spleenic and duodenal regions of pancreas, but not gastric region, 1 week after first and second 0.05 ml CM induction. The staining intensity and number of insulin-positive cells increase dramatically at 1 week after third and fourth 0.05 ml CM induction in all regions of pancreas. The data of insulin blood concentration showed clear differences between the second and the fourth induction of 0.05 ml CM. In conclusion, this study showed very strong evidence on the role of human umbilical cord mesenchymal stem cell-derived conditioned medium to recover β-cells pancreas damage in type 1 diabetic mellitus condition of rat (*Rattus norvegicus*), structurally and functionally.

Keywords: conditioned-medium, type 1 diabetes mellitus, β-cell pancreas, structure and function recovery

Introduction

Some studies revealed that stem cells induce tissue repair due to their ability to secrete trophic factors that exerted beneficial impact on the damaged tissue [1]. Various studies on stem cell-derived secreted factors showed that the secreted factor alone without the stem cell itself may induce tissue repair in various conditions that inflicted tissue/organ damage. The secreted factors can be found in the medium where the stem cells are cultured and called conditioned medium or CM [2].

The use of CM has several advantages compared to the use of stem cells, as CM can be manufactured, freeze-dried, packaged, and transported more easily. Moreover, as it is devoid of cells, there is no need to match the donor and the recipient to avoid rejection problems. Therefore, stem cell-derived conditioned medium have a promising prospect to be produced as pharmaceuticals for regenerative medicine and will be booming in the near future.

Conditioned medium contains various growth factors and tissue regenerative agents, which were secreted by the stem cells as shown by various studies [3, 4, 5, 6, 7]. Moreover, various studies reported the use of various kinds of stem cells and various methods to get the CM to cure various kinds of degenerative diseases in various animal models. Therefore, this study aimed to investigate the role of human umbilical cord mesenchymal stem cell-derived conditioned medium on the structural and functional recovery of β-cells pancreas in type 1 diabetic mellitus condition of rat (*Rattus norvegicus*).

Materials and Methods

Thirty male white rats (*Rattus norvegicus*) with average body weight of 150-250 gr were used in this study. They were provided with food and drink *ad libitum.* The rat samples were divided into 2 groups: control group and diabetic group. Conditioned Medium was prepared from the media culture at the passage 3 of human umbilical cord mesenchymal cells culture. Type 1 diabetes mellitus condition was made by single dose intra muscular injection of 125 mg per kg body weight of alloxan monohydrate.

The 0.05 ml conditioned medium induction was applied to the diabetic rat groups at week 1, 2, 3, and 4 with intra muscular injection. One week after second and fourth CM induction, the blood of rat samples were collected for insulin concentration level analysis with ELISA method. After blood

collection, the rat samples were euthanized, the pancreas were collected and fixed in Bouin's solution for 24 hr. The pancreas were divided into 3 regions (gastric, spleenic, and duodenal regions), processed for paraffin block tissues and cut serially in 5 µm of thickness. One serial slide of pancreas tissues were stained with Hematoxillin-Eosin for basic structure observation and the others were used to indicate the presence of insulin in the islets of Langerhans with N-Histofine® Simple Stain Rat MAX PO method of immunohistochemistry.

Results and Discussions

The present studies showed the decrease in the total number of islets after the injection of single dose 125 mg per kg body weight of alloxan as indicated by complete damage of all islets (Figs. 1A and 1B). The large numbers of exocrine acini was also damaged beyond recognition (Figs. 1A and 1B). Moreover, the plasma insulin concentration has been noted to decrease at the same time (Table 1).

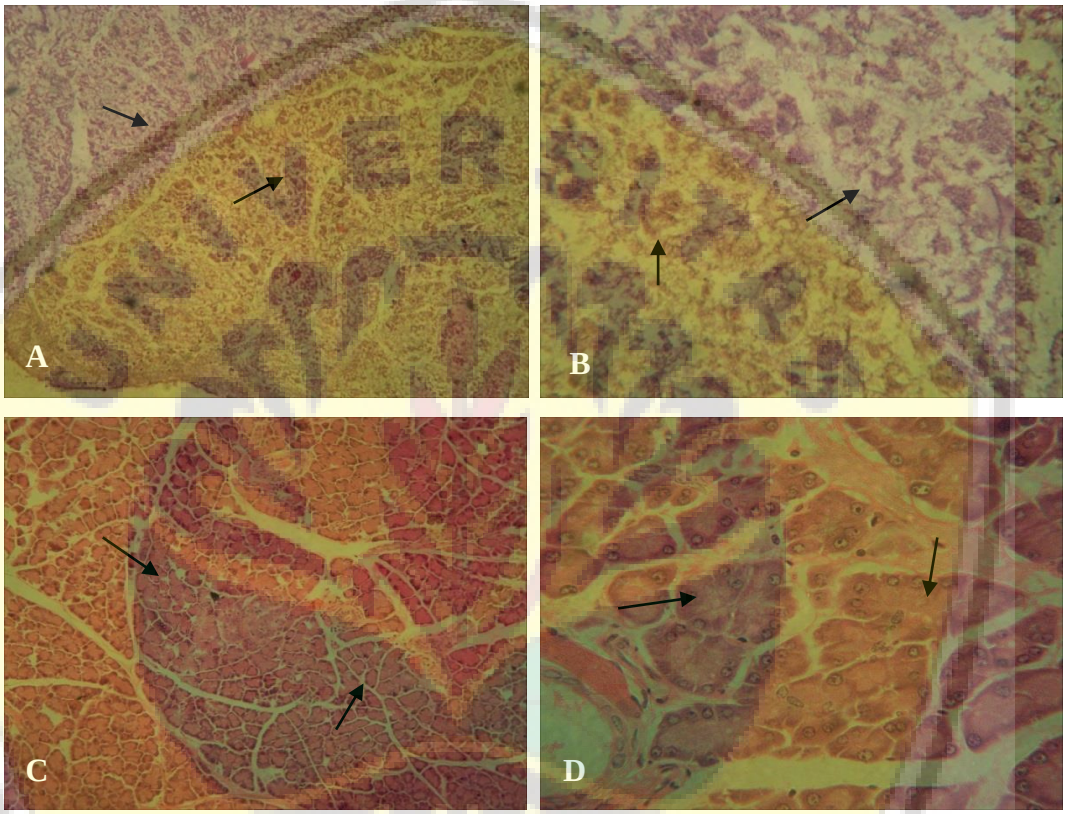

Figure 1. Histological structure of pancreatic tissues damage caused by alloxan injection and its recovery structure after 4 week without any treatments (HE; 130x and 520x). The decrease in total number of islets after the injection of single dose of alloxan as indicated by complete damage of all islets and exocrine acini in low (A) and high (B) magnifications. The recovery pancreas structure at week 4 after alloxan injection indicate normal exocrine acini, but no islets of Langerhans, in low (C) and high (D) magnifications. Arrows indicate exocrine acinus.

The presence of small islet in diabetic group was first detected at week 1 after first 0.05 ml CM induction in all regions of pancreas (Figs. 2A, 2B, 2C). The number and size of islet increase in agreement with the CM doses and time of treatments in all regions of pancreas (Fig. 2). Immunohistochemically, the presence of low intensity of insulin positive cells can be recognized in the splenic (Fig. 2B) and duodenal (Fig. 2C) regions of pancreas, but not gastric region (Fig. 2A), week 1 after first and second 0.05 ml CM induction. The intensity of staining and number of insulin-positive cells increase dramatically at week 1 after third (Figs. 2G, 2H, 2I) and fourth (Figs. 2J, 2K, 2L) 0.05 ml CM induction in all regions of pancreas.

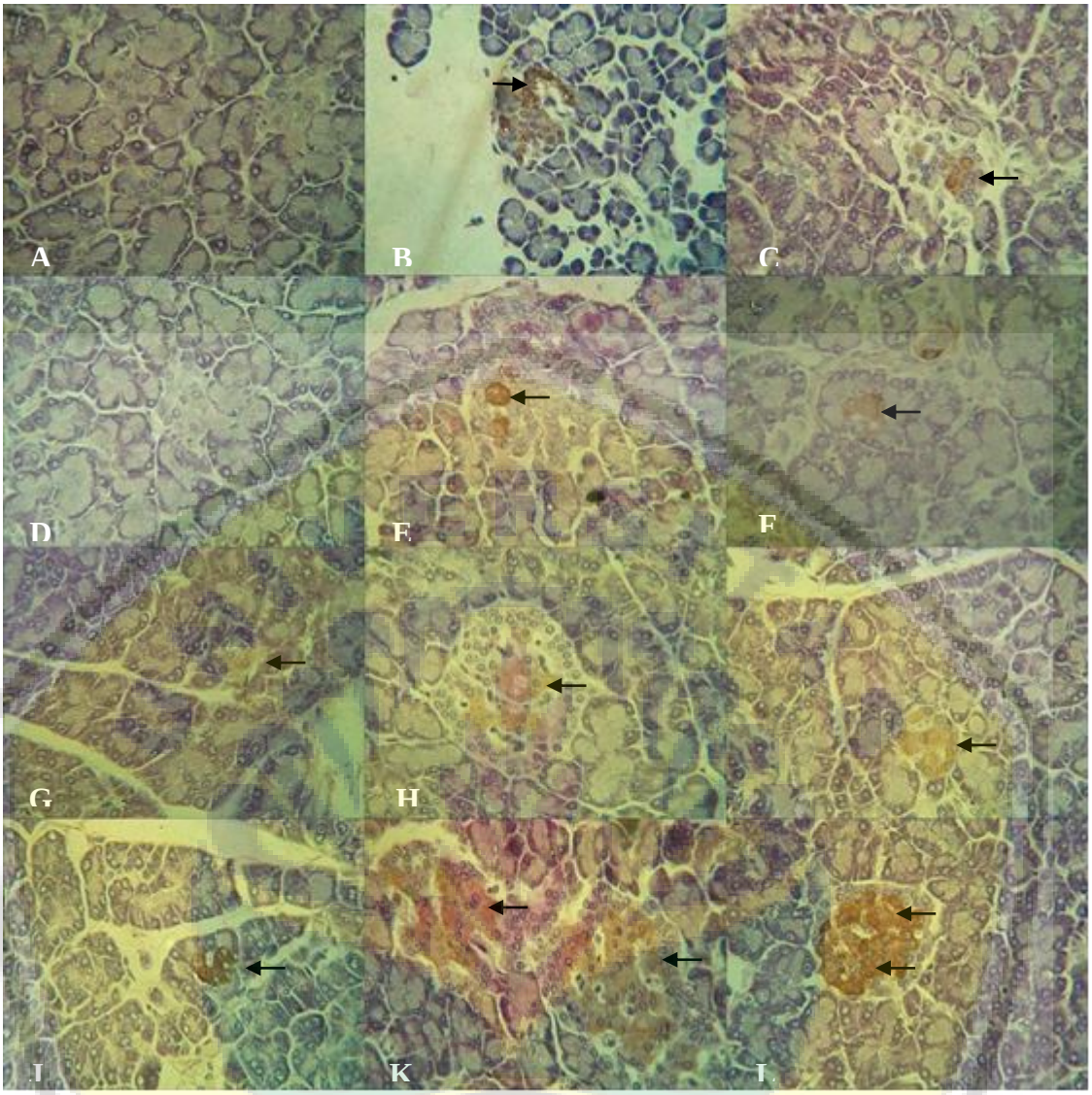

Figure 2. The structural and functional recovery of islets induced mesenchymal stem cell conditioned medium at week 1, 2, 3, 4 after single dose of 125 mg per kg body weight alloxan injection lead to type 1 diabetes mellitus (520x). The presence of small islets were already detected at week 1 after first 0.05 ml CM induction in all regions of pancreas. Insulin positive cells were initially recognized with low intensity in the splenic (B) and duodenal (C) regions of pancreas, but not gastric (A) region, week 1 after first induction of 0.05 ml CM. The similar feature of first induction was shown at second induction of CM (D, E, F). At week 1 after third induction of CM, the number of low intensity insulin positive cells increase and was detected in gastric (G), splenic (H), and duodenal (I) regions of pancreas. The intensity of staining and number of insulin-positive cells increase dramatically at week 1 after fourth 0.05 ml CM induction in all regions of pancreas (J, K, L). Arrows indicates of insulin positive β-cells in islets of Langerhans.

Table 1. The profile of insulin concentration (µIU/ml) in the blood of rat samples before or after alloxan injection and week 1 after second or fourth 0.05 ml CM induction of alloxan treated rats.

No.	Insulin Concentration in Blood Plasma (µIU/ml)			
	3 hours before alloxan injection	6 days after alloxan injection	Alloxan + 1 week after second 0.05 ml CM induction	Alloxan + 1 week after fourth 0.05 ml CM induction
1	78.67	21.64	19.30	48.20
2	117.73	6.01	31.01	61.48
3	107.58	17.73	45.08	89.61

In agreement with the results of immunohistochemical finding, the data of insulin plasma concentration showed clear differences between the second and the fourth induction of 0.05 ml CM (Table 1).

The number of people affected by Type 1 diabetes mellitus is approximately 20 million worldwide and is rapidly rising [8]. According to the International Diabetic Federation, there are 387 million diabetics worldwide, 9 million in Indonesia only, which keeps the country in rank seventh in the world at the moment. While exogenous administration of insulin is an effective treatment for acute hyperglycaemia in Type 1 diabetes mellitus, it does not prevent secondary complications [3] and can in some cases lead to hypoglycaemia [9]. Alternative therapeutic strategies include pancreas transplantation, islet transplantation, gene therapy, and cell stem therapy. Those kinds of therapies has many limitations, including limited availability of suitable donors, high cost and high rate of partial or total graft failure, toxicity of immunosuppressive drugs, glucotoxicity, and recurrence of auto immunity [9].

As alternative, it is important to develop the stem cells-derived conditioned medium that can recover structure and function of β-cells from islets destruction. This technique may not only improve the structural regeneration of β-cells but also induce and maintain its function to produce the insulin after islets destruction. The results of this study, however, showed very strong evidences on the role of human umbilical cord mesenchymal stem cell-derived conditioned medium in the recovery of β-cells pancreas damage in type 1 diabetic mellitus condition of rat (*Rattus norvegicus*), structurally and functionally.

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NANOMEDICINE: A "PANACEA" in MEDICINE

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Abstract

Recent advances in nanotechnology, materials science, and biotechnology have led to innovations in the field of nanomedicine. Improvements in the diagnosis and management of diseases and disorders are urgently needed, and it may now be possible to achieve marked improvements in both of these areas using nanomedicine. This scientific review focuses on the potential of nanomedicine, especially: nanoparticles and their benefits, nanoparticles for siRNA delivery, nanopores, nanodots, nanotheragnostic, nanodrugs and targeting mechanisms, aptamer nanomedicine.The fusion of different scientific fields is accelerating these developments, and these interdisciplinary efforts to have considerable ripple effects on various fields of research. The abilities of nanomedicine are huge, and nanotechnology could give medicine an entirely new outlook.

Keywords: nanomedicine, nanotechnology, nanoparticles, nanotheragnostic, aptamer nanomedicine.

Introduction

The first use of the distinguishing concepts in 'nanotechnology' (but predating use of that name) was in "There's Plenty of Room at the Bottom," a talk given by physicist Richard Feynman at an American Physical Society meeting at Caltech on December 29, 1959 [1].

Nanotechnology refers broadly to a field of applied science and technology whose unifying theme is the control of matter on the molecular level in scales smaller than 1 micrometer, normally 1 to 100 nanometers, and the fabrication of devices within that size range.2 Nanomedicine is the design and development of therapeutics and diagnostic tools distinguished by the nanoscopic scale of its delivery vehicles and diagnostic agents.3 In short, nanomedicine is an applied nanotechnology in the medical field.

Nanomedicine has already offered some new solutions, and many pharmaceutical companies are trying to develop targeted drug delivery using nanotechnology and already existing drugs. It has the potential to transform our approach to human health and disease. It also promises a transfigured portrait of better health care, health economics and personalized medicine. The ultimate goal is improved quality-of-life [4]

Advances in development of lipid-based nanomedicine, nanostructured drugs with effective site-targeting, nanoplatform, nano-imaging, nano-immunochemotherapy, nanopharmaceuticals, nanotheranostics and nano-drug delivery will drive the future development of nanomedicine, personalized medicine, and targeted therapy [5].

Nanoparticles (NPs)

NPs are particles, usually consist of lipid or polymer, typically less than 200 nm in diameter. NPs spatially localize through passive-active targeting and are capable of delivering drugs through epi-endothelial barriers [6].

Polyvalent decoration of a NP's surface with a ligand can ease binding to a biomarker which is specifically overrepresented in targeted cells and trigger receptor-mediated endocytosis, a process that has considerable implications for targeted delivery [7]. The ligands used to modify NPs include antibodies, engineered antibody fragments, proteins, peptides, small molecules, and aptamers [8].

Some of NPs are explained herein. Arginine–glycine–aspartate-grafted NPs can target avb3 integrin overexpressed by the tumor endothelium, and extravasate more efficiently and enter the tumor via the enhanced permeability and retention effect [9].

A nanomedicine made of pegylated chitosan NPs with conjugated anti-transferrin receptor antibodies can transport a blood–brain-barrier-impermeable caspase inhibitor to the brain [9, 10].

The preparation of solid lipid NPs coated with the mucoadhesive polymer chitosan for intestinal absorption of insulin [9, 10].

Use of nanocrystalline solid dispersions, PEG-PLGA NPs, proticles, nanoparticle precipitates, and liposomes for the treatment of pulmonary arterial hypertension [9].

Benefits of Nanoparticles

The development of nanoparticles for diagnostic and screening purposes, DNA sequencing using nanopores, manufacture of drug delivery systems and single-virus detection, the significance and

recent advances of gene/drug delivery to cancer cells, the molecular imaging and diagnosis of cancer by targeted functional nanoparticles, the development and potential applications of nanoscale platforms in medical diagnosis and treatment, the use of nanoparticles for stem cell tracking, differentiation, transplantation, and biosensing, magnetic nanoparticle- and quantum dot-based applications in stem cell and tissue engineering in humans (nano-regenerative medicine) [11].

Nanoparticles for siRNA Delivery

Some requisites of nanoparticles to enable siRNA delivery into the tumor, i.e.: very small (size no bigger than 1000 nm), biocompatible, biodegradable, lack of immunostimulatory properties, and be able to bypass rapid hepatic/renal clearance. Some of them are lipid complex (cationic liposomes, lipoplexes, etc), conjugated polymers (cholesterol, polymer-PEG, etc), cationic polymers (chitosan, atellocollagen, etc) [12]

Nanoparticles serving as delivery vehicles for siRNA present numerous advantages over naked siRNA delivery due to its ability to stabilize siRNA while delivering higher concentrations of siRNA directly into tumor sites. More importantly, some of these nanoparticles can be modifi ed with high affi nity ligands to specifi cally target siRNA directly into the tumor. These nanoparticles can serve to promote controlled release and when formulated correctly, they can provide a safe and reliable platform for siRNA delivery for treatment of cancer and other diseases [12]

Nanopores

The flow of DNA through nanopores can be used to discriminate low copy numbers of DNA, permitting very rapid genome sequencing. The first demonstration of this principle used an array of cylindrical gold nanotubules with inner diameters as small as 1.6 nanometres [13]. When the tubules were positively charged, positive ions were excluded and negative ions were transported through the membrane. In contrast, when the membrane was negatively charged, only positive ions passed through [14].

Nanodots

Fluorescent nanoparticles, such as 'quantum dots', PEBBLES (probes encapsulated by biologically localised embedding) and perfluorocarbon particles, potentially overcome these issues [15]. 'Quantum dot' nanocrystals, for instance, are manufactured to several nanometres in diameter with a nearly unlimited range of sharply defined colours [15, 16]. The particles are excitable using white light and can be linked to biomolecules to form long-lived sensitive probes. In principle, separate biological events can be monitored by simultaneously tagging different proteins or DNA sequences with nanodots of a specific colour [14].

Nanotheragnostic

Nanotheragnostic (theragnostic nanoparticles) or theragnostic nanomedicines are integrated nanoparticulate systems formulated to simultaneously diagnose, deliver targeted therapy, and monitor response to therapy [17].

Nanotheragnostic regimens are beneficial for treatment of cancer, cardiovascular diseases (i.e.: atherosclerosis, thrombosis) and a potential application in arthritic diseases (e.g. rheumatoid arthritis), age-related macular degeneration, atherosclerosis, neurodegenerative diseases, and psoriasis [18, 19].

The four basic elements that should be fulfilled in structure of nanotheragnostic are the biodegradable nanocarrier material (based on an organic matrix, an inorganic component, or hybrid materials), the imaging agent or signal emitter (unique optical, magnetic, or radioactive characteristics), the drug or therapeutic molecule, and modifications to the later element based on passive-active delivery strategies [20, 21].

Ideally, theragnostic nanotools would consist in a multimodality imaging technique combined with a multi-drug nanocarrier plus supplementary treatment strategies (e.g., hyperthermia, photodynamic therapy, and/or photothermal therapy). Nanotheragnostics and image-guided drug delivery are expected to enable "personalized" medicine [21, 22].

Nanodrugs and Targeting Mechanisms

Nanodrugs in cancerous tissue has uptake and accumulation. Both of them can occur through two mechanisms, i.e.: "passive targeting" and "active targeting". Passive targeting is based on both the minute size of drug carriers and the leaky neovasculature of the tumor. Passive accumulation at the tumor site is predicted to take place through Enhanced Permeability and Retention (EPR) effect. With the longer blood circulation time achieved by stealth modification (e.g., PEGylation), increased accumulation of NPs is possible through the EPR effect.[11, 23] EPR happens due to the increased vessel leakiness and impaired lymphatic function usually found in tumor tissue; this permits nanomaterials to penetrate and accumulate there [17, 24].

Active targeting of nanomaterials is being explored as a method to achieve spatial localization by intentionally homing NPs to active diseased sites while annihilating off-target adverse effects in normal tissue. It is attained by functionalization of their surface with bioactive molecules such as transferrin, engineered antibodies, enzymes and folic acid, which recognize and interact with cancer-

specific targets overexpressed on the surface of malignant cells [5, 25].

Active targeting to achieve effective nanomedicine accumulation in tumor tissue is debatable, while some experts endeavor to design innovative strategies for active tumor targeting [11, 17].

For active targeting, the most commonly used targeting moieties are monoclonal antibodies or antibody fragments, antigen binding fragments, and single chain variable fragments; the latter being preferable due to their reduced immunogenicity and high target specificity [5, 11]

Aptamer Nanomedicine

Aptamer nanomedicine is an emerging and promising class of therapeutics to address the challenges faced by recent cancer therapy. It may address limitations of other ligands for targeted therapy in oncology and highly compatible with combinational drug therapy. However, the approach would require a better understanding of carrier design, drug-loading efficiency, and drug-releasing mechanism [26, 27]

Small interfering RNA (siRNA), small hairpin RNA (shRNA), micro-RNA (miRNA), and antisense oligonucleotides are designed for knocking down a certain gene (deleting a gene function) to kill certain types of cells. In contrast, plasmid DNA or mRNA are used for transfection to deliver a certain gene (adding a gene function) to cure a disease. To date, most studies focus on the development of aptamer mediated siRNA, shRNA, or miRNA delivery systems for gene silencing applications. This is an emerging class of gene therapy particularly promising for cancer therapy [26, 27].

The antinucleolin aptamer, AS1411, for breast cancer cell targeting, coupled to this liposomal design, killed cancer cells with high specificity. This aptamer-doxorubicin liposome formulation inhibited breast tumor growth induced by estrogen as no significant growth of the tumor was observed in the group treated with the aptamer-doxorubicin liposome, while the size of the tumor in the control group increased 166% [4, 5, 11, 17].

Similar to drug delivery, the easiest strategy for aptamer-based nucleic acid delivery is to link the therapeutic nucleic acid directly to the aptamer. This is known as an aptamer-therapeutic nucleic acid chimera [11, 27].

The chimeras Chi-29b and GL21.T-let are additional examples of direct conjugation of the aptamer to a therapeutic nucleic acid. Chi-29b comprises an antimucin 1 (MUC1) aptamer and miRNA miR-29b for ovarian cancer treatment [11, 27].

Ultimately, it becomes clear that the critical steps for clinical translation of nanotherapeutics require further interdisciplinary and international effort, where the whole stakeholder community is involved from bench to bedside.

The era of nanomedicine is poised to mature in the next few decades; incorporating elements of personalized medicine, it will affect the therapeutic world in a powerful and permanent way.

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TOXICITY of MIXTURING of ANNONA SQUAMOSA SEED and PIPER BETLE LEAF EXTRACT for THE MORTALITY of *Aedes aegypti* LARVAE (Preliminary study)

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Abstract

In Indonesia, Dengue hemorrhagic fever (DHF) diseases become a national issue that must be covered. This disease is transmitted by the mosquito bite of *Aedes aegypti* L..The method for handle this problem now, only use of synthetic insecticides that make reistensi for mosquitoes. This is become the reason of this issue, so that the new insecticide must be found very soon. WHO has suggested to find out bioinsecticide that comes from environment to avoid the negative efffect. One of the alternative ways is the use of active substance of *Annona squamosa* that is mixed with active subtance from the green *Piper betle* leaf. *Annona squamosa* seed contain active substance annonain, squamosin, asimisin, alkaloid and resin that has larvaside characteristic, while the green *Piper betle* leaf contain flavonoid, eugenol, kavikol, tannin and adsiri oil. The objective of this research is to know the toxicity of *Annona squamosa* seed extract mixed with the green *Piper betle* leaf extract to the larva of *Aedes aegypti* L..The methode of this research is divided into two steps. The first step is an extraction step by using 96% ethanol and the scond step is toxicity test for 24 hours. The data will be analyzed by using probit analysis to determine LC_{50}, concentration that used are 1 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm and aquades as the control. This result of this research shows that Lethal Concentration (LC50) for a period of 24 hours amounted to 4.63641 ppm..the result of this preliminary study is very proper to be continued in the last test.The results of this research will be continued until get a new biopesticide.

Keywords: *Annona squamosa* seed, *Aedes aegypti*, the green *Piper betle* leaf.

Introduction

Aedes aegypti L. a mosquito that can act as vectors of various diseases including Dengue Hemorrhagic Fever (DHF). In the life cycle has a habit of breed (spawn) in places were flooded and are not directly related to the land, on tires that stagnant rainwater, cans and bottles, vases, birdbath, pieces of bamboo and others. In breaking the cycle of dengue mosquito breeding is mostly done by fogging are using chemical active ingredients. The active ingredients are no longer effective in controlling the vector as Aedes have shown resistance to some insecticides.In addition the use of these insecticides (chemical synthetic) can be bad for the environment [1].

Ingredients natural insecticide is the best substitute developed for vector control. Natural insecticide has several advantages including the price is affordable, easy to making and easy to decompose so it is safe for humans and even animals [2].

Several plants have been widely used as a natural insecticide such as *Piper betle*, carica, yam, musa paradiceae, and *Annona squamosa*. Based on previous research that has been developed is green leaf piper betle and seed of *Annona Squamosa*. Piper leaves containing phenol and compounds of derivatives such as kavikol, kavibetol, karvacol, eugenol, and allilpyrocatechol which can be used as larvasida [3]. Meanwhile, "Ref. [4] contain natural chemicals are contained in srikaya among others acetogenin, squamocin, bullatacin, annonacin, and neonnonacin that are larvasida".

Methods

On research conducted "Ref. [5] that at a concentration of 0.989% *Piper betle* leaf extract can kill larvae up to 100%". While the research conducted by "Ref. [4] showed that the lethal concentration 50 values of *Annona squomosa* seed extracts against *Aedes aegypti* L. mortality amounted to 14.71% or 14.71 ml / 100ml".

Result and Discussion

Based on the research that has been described above both research extracts of *Piper betle* L. seed extract and *Annona squamosa* L. have known each extract has an active substance that is toxic to larval mortality *Aedes aegypti* L. When two the different toxic compounds are mixed together, it is possible that a mixture of the active compounds are synergistic or antagonistic need to do research on "toxicity mixture leaf extract of *Piper betle* L. and seeds of *Annona squamosa* L. against larvae of *Aedes aegypti* L. mortality.

Annona squmosa seed are used comes from Situbondo that have been selected clean, no holes, intact, not moldy, and there are no caterpillars. While green *Piper betle* are used buy from Tanjung tradisional market Jember and have selected is not yellow, there are no caterpillars, deformed and torn.

The manufacture of *Annona squmosa* seed extract and green *Piper betle* leaf extract using ethanol 96%. The first stage of manufacture of the extract is selecting materials according to criteria of both *Annona squmosa* seeds and green *Piper betle*, after that wind dried until its weight is stable, polished by machine until it becomes a powder. Then, the resulting powder was added in macerated with 96% ethanol in the ratio 1: 4 for 3 days. The resulting liquid extract into rotary process with a Rotary Evaporator to separate the ethanol with extract so the resulting extract is viscous.

Annona squmosa seed extract contains active compounds that annonain, squamosin, resin, asimisin, saponins, tannins and alkaloids. While the green *Piper betle* leaf extract contains active compounds that eugenol, kavikol, saponins, tannins, flavonoids and essential oils.

Toxicity test consists of two stages, namely a preliminary test and final test. *Aedes aegypti* larvae were used for both the preliminary test and final test with 20 larvae. A preliminary test carried out to obtain the concentration of the extract mixture of leaf *Piper betle* L. with seeds of *Annona squamosa* L. seed, which can kill the larvae of *Aedes aegypti* by 10% and 90% of the total number of test larvae. This test is performed without repetition and the results are not analyzed. Concentration used for *Annona squamosa* L. seed extract is 1 ppm, 10 ppm, 20 ppm, 40 ppm and 60 ppm. The concentration of green *Piper betle* L. extract is 100 ppm, 400 ppm, 800 ppm, 1200 ppm and 1500 ppm. While the concentration of a mixture extract is 1 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm.

Determined the final test concentrations of some sort that will be used to look at the results of the preliminary test. Data that will be obtained from the final test will be performed analysis.

The Mixture toxicity of Extract of *Annona squamosa* seed and *Piper betle* green leaves against larvae of *Aedes aegypti* L. with a time of 24 hours uncovered.

Tabel 1. Parameter Estimates of Probit Analysis

		Standard	95,0% Normal CI	
Parameter	Estimate	Error	Lower	Upper
Shape	1,71569	0,127840	1,48257	1,98547
Scale	5,74060	0,250634	5,26980	6,25346

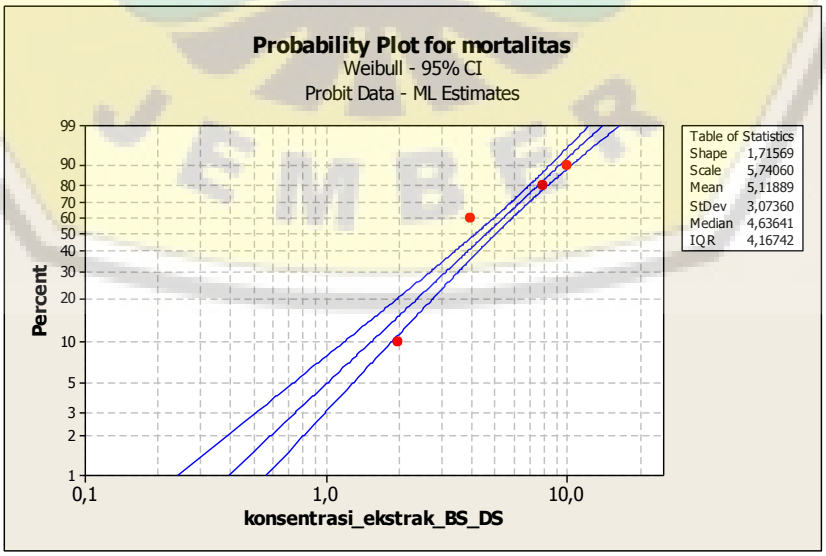

Figure 1. Probability Plot for mortality

Seed extract of *Annona squamosa* L. contains the active ingredient annonain, squamosin, asimisin, and a resin which has the ability as larvicidal [4]. Extracts of *Piper betle* L. green leaf also has an active ingredient as larvicidal namely saponins, tannins, alkaloids, flavonoids and essential oils [6].

Based on the above results, it is known that seed extract of *Annona squamosa* L.has a high toxicity at concentrations of 60 ppm has been able to kill 100% (LC50 = 10.3372 ppm). While the results for the extract of green leaf *Piper betle* L. show that has a low toxicity evidenced by high concentrations to kill 100% of the larvae used were 1600ppm (LC 50 = 606.802 ppm) toxicity mix green leaf extract of *Piper betle* L. with seed extract of *Annona squamosa* L. against larvae of *Aedes aegypti* L. is obtained 10 ppm have been able to kill 90% (LC 50 = 4.63641 ppm).

From the research that has been conducted shows that the active compounds contained in seed extract of *Annona squamosa* L. and the active compound in extract green leaf of *Piper betle* L. are woriking synergistic or mutually supportive, even blend extract with the same composition was made possible has established an active compound newly whose performance as larvicides have a high toxicity compared to the toxicity of *Annona squamosa* L extract and green *Piper betle* L. ie the concentration of 10 ppm has been able to kill all larvae test. The same mixture composition ratio ie 50:50 has been proven that the performance of the active compound between two such compounds can be blended well and working optimally. If we use the analogy that *Annona squamosa* L seed extract is used only 50% on the mix has been able to kill all larvae test at a concentration of 10 ppm, while the *Annona squamosa* L seed extract alone requires a concentration of 60 ppm to kill all larvae test. It is also common in *Piper betle* L. leaf extract than the *Piper betle* L leaf extract on the mixture, where in the concentration of 100 ppm in the extract of betel only able to kill one larvae test that is used while in the extract mixture requires only a concentration of 10 ppm has been able to kill all larvae test used. From this that reinforce the notion that the active compounds the mixture between two very synergistic and even possible has established a new active compounds which toxicity is very high.

Saponin is stomach poisoning or stomach poison for larvae of *Aedes aegypti*. Mechanism of saponins that can lower the surface tension of the mucosal lining of the digestive tract so that the larvae become corrosive digestive tract wall. Mechanisms of larval mortality associated with the function of saponin compound that can hinder eating larvae (antifedant), when these compounds into the body of larvae, the digestive apparatus will be disturbed. In addition, these compounds inhibit the taste receptors in the mouth area larvae. This resulted in the larvae failed to get a taste stimulus that can not recognize the food that the larvae die of starvation [7].

The working mechanism of saponin role as respiratory poison because when the larvae are already in a state of weakness because poisoned by way of direct contact or from digestion will reduce the ability of larvae to close spirakelnya when diving. Reduced ability will make the water can get into the spiracles. The influx of water to the spiracles will grow rapidly because the larvae continue to move without control. The presence of water in the respiratory tract will impede larvae in doing respiration, so the larvae die from lack of oxygen. In normal conditions, spiracles mosquito larvae is closed and only opened when the larvae perform air exchange [7].

The ability of tannin in killing mosquito larvae, caused by these compounds can inhibit the action of the enzyme and substrate removal (protein). Tannins can bind to lipids and proteins and are thought to bind to the protease enzyme that plays a role in catalyzing the protein into amino acids necessary for the growth of the larvae. With dependent enzyme by tannins, the work of the enzyme is inhibited, so that the process of cell metabolism can be disturbed and the larvae will be shortage of nutrients. As a result, the growth of the larvae become obstructed and if this process continues, it will have an impact on mortality of larvae [8].

Alkaloid is a compound that also acts as an insecticide. In addition to causing a bitter taste that inhibit the activity of eating, Alkaloids able to exhibit paralytic activity causes paralysis in insects, disrupt the central nervous system, the production of feces and urine production. In the nervous system of insects are enzin acetylcholinesterase (AChE) function to solve acetylcholine into choline, acetic acid and water. Excessive alkaloids are expected to inhibit AChE enzyme works which resulted in a buildup of acetylcholine, causing chaos in the delivery systems implus into muscle cells. This causes the message next message can not be forwarded, larvae undergo continuous spasms and eventual paralysis and this condition continues, causing death [8].

Flavonoids are compounds that play an important role in the pollination by insects. A number of flavonoids have a bitter taste to be rejected certain type of caterpillar. Rotenon a natural insecticide which is derived flavonoids, as well as insecticide rotenon also is toxic to fish. Rotenon work as a contact and stomach poison toxins that kill insects eat slowly until the activity stops (stop feeding action). How it works rotenon inhibit the respiratory enzyme, between NAD + (coenzyme involved in oxidation and reduction in the metabolic process) and coenzyme Q (coenzyme respiratory responsible for carrying electrons in the electron transport chain) resulting in respiratory failure [9].

While the compounds that are larvicides on srikaya seed extract is annonain, squamosin, asimisin, and resin. Where annonin and squamosin can invade tissues the nerves in the body of insects. Attacks on neural networks this may lead to insects become no appetite, or even not able to move so that the test insects shrivel and dry up. Annonain able to inhibit the transfer of electrons at one site by means

of blocking the enzyme NADH with ubiquinon bond in the electron transfer chain in the process of cell respiration and consequently the process of formation of metabolic energy becomes blocked so that dead larvae [10].

Contact poison insecticides absorption occurs largely in the cuticle. Active compounds will penetrate into the body of insects through parts are coated by a thin cuticle, such as inter-segment membranes, membrane joints at the base embelan and chemoreceptor on the tarsus [11]. The content of essential oil on sugar apple seed extract allegedly able to diffuse from the outer cuticle layer through deeper layers towards hemolimfa, following hemolimfa flow and spread throughout the body larvae test [12].

Based on the research results mixtures of various compounds that work to support each other as evidenced by the sharp increase in the toxicity of 10 ppm has been able to kill all the larvae were initially require 60 ppm for *Annona squamosa* L seed extract and 1600 ppm for *Piper betle* L. leaf extract.

Based on these results it is a mixture of *Annona squamosa* L seed extract and green *Piper betle* L. leaf promising for further investigation so that it can be used as a new biopesticide against larvae of Aedes aegypti L.

Conclusion

Based on the description of the results of research and data analysis has been done it can be concluded that the toxicity of a mixture of *Annona squamosa* L seed extract and green *Piper betle* L. leaf against larvae of *Aedes aegypti* L. can increase the mortality of larvae with Lethal Concentration (LC50) for a period of 24 hours amounted to 4.63641 uncovered ppm.

Suggestion

There needs to be further research on the addition of observation time during the toxicity test mixture s *Annona squamosa* L seed extract and green *Piper betle* L. leaf against larvae of Aedes aegypti L. in order to provide recommendations insecticide concentration Hayani more precise (optimal) as well as the need for further research to do granusi and formulation to be appointed as the new biopesticide.

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THE ANT PLANT (*Myrmecodia pendans*) INFUSE as AN ACUTE DIARRHEA MEDICINE

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Abstract

Diarrhea is the most deadly diseases in the world to the children. Diarrhea is usually gastrointestinal infections caused by viruses, bacteria, or parasites. Diarrhea infection can spread through dirty hands, contaminated food or water, pet, direct contact with feces. Tannin, contained in the Ant plant hypocotyl, was used as a traditional medicine to cure diarrhea. Therefore, the aims of the research were to prove the effectiveness of ant plant infuses on the diarrhea. This research used 25 rats that divided into 4 groups, as a Control (-) add with aquadest, Control (+) add Attapulgite normal dose, Dose I 0,45 grams.KgBW^{-1}, Dose II 0,9 grams.KgBW^{-1}, Dose III 1,8 gram.KgBW^{-1}. The data (frequencies of defecate, consistency of feces and the length of diarrhea) were analyzed by the Mann- Whitney test. The result showed that the ant plant infuses curing the acute diarrhea. And the effective effect for acute diarrhea was on dose 0.9 gramKgBW^{1}.

Key words: Diarhea, Ant Plant hypocotyl, attapulgite

Introduction

Diarrhea is diseases cause of the bad hygiene in the food and the environment. In the developed country, 2 million children were death by diarrhea [1]. Diarrhea in Indonesia is elevated from 374 per 1000 people in 2003 to 432 per 1000 people in 2006 [3]. During diarrhea, there was disturbing in the nutrient absorption, lead to malnutrition furthermore a big risk to growth failure of the child. Nutrient status on child is getting worse. Diarrhea in malnutrition, is more dangerous because higher frequencies, longer, and worse during diarrhea.

Regarding to MDG's to eradicate the child mortality up to 2/3 part in 1990-2015, the government stated the policy to reduce mortality and morbidity on diarrhea by conduct the diarrhea management in the health institution and the house as well. The basic diarrhea management should performed to prevent and to cure dehydration, by zinc and food supplement, to cure the cause and complication also educate the hygiene to people.
The curing effort including of symptom and causative therapy by modern medicine or traditional medicine. Medicine therapy that used to on diarrhea were rehydration therapy with loperamid or absorbent also diet management. For traditional medicine therapy as WHO reported that 60 % of Capital Country and 80 % of Developed country were use the traditional medicine. WHO also recommended the traditional therapy, including herbal medicine in the maintenance of public health, preventive and curative for some diseases.

The traditional therapy for diarrhea used bio active substances in plant, by Indonesian people. Abundant of Indonesian plant were used to cure diarrhea. Some plants that used as antidiarrhea because of tannin in it. Tannins have a function as an astringent or polyphenol to bind and to precipitate protein [6]. Astringent has a function to reduce diarrhea by shrinking the mucous membrane of intestinal [5]. One of the plants contained tannin is an ant plant (*Mymercodia pendens)*. Ant plants found in peninsula Malaysia to Philiphine, Cambodia, Sumatra, Kalimantan, Java, Papua, Cape York [6]. Ant plant contained flavonoide active, tannin, tocopherol, glicoside, vitamin, and minerals such as Ca, Na, K, P, Zn, Fe, Mg, and polyshacarida. The abundant of tannin and flavonoide leads to the taste similar with tea if boiled or as infusion, so that ant plant assumed able to cure the diarrhea.

The acute diarrhea without good management is the cause of child death. Ten to 15 % of acute diarrhea leads to bad nutritious status and death. This study was conducted to address the effectivity of ant plant to cure the acute diarrhea on rat.

Method

a.Grouping the subject.

Twenty five male rat of Wistar strain, ± 200 grams body weight, 2 months old, were divided into 5 groups. After a week aclimatitation , rats were added 3 ml oleum ricini to induce acute diarhhea. When the stool was juicy, the rat add some treats. Control (-) added by aquadest, control(+) added by attapulgite normal dose (54mg.KgBW^{-1}), Dose I 0.45 gr.KgBW^{-1} (ant plant infusion), Dose II 0.9 gr.KgBW^{-1}, Dose III 1.8 gr.KgBW^{-1}. The treatment was repeated 3 times every 6 hours. The observation for the number of recovered rat, the consistency of

stool and the frequency of defecate was conducted 4 times in 6 hours, 12 hours, 18 hours and 24 hours. The research was conducted at animal laboratory, Universitas Muhammadiyah Yogyakarta.

b. Infusion preparation

Five grams Ant plant simplicia was heated into100 ml water(90º C). After 15 minutes was cooled down.Water was filtered to seperate from the leaf. This infusa was 50 mg/ ml equal with 0.45 gr.KgBW^{-1}. The similar way to prepare 0.9 gr.KgBW^{-1}and 1.8 gr.KgBW^{-1}used 10 gr and 20 gr of ant plant simplicia.

c. Statistical analyses

Data are analyzed by Mann-Whitney test and statistical significance was set upon $p < 0.05$.

Result and Discussion

There were many functions of ant plant to human body.. The beneficial substance of the ant plant in example are flavonoid fraction, tocopherol and alpha-tocopherol, saponin. This study was reported that ant plant infusion able to reduce or to cure the diarrhea, as shown in table 1.The number of recovered rat after added ant plant infusion was higher than added aquadest or attapulgite normal dose. The table 1 also showed that the increasing recovered number of rat in line with the increasing of dose ant plant infusion, also in line with the time of adding the infusion. Chi Square test showed that there is no significant of the treatment (p=0.123), because after did treatment, rats were recovery to normal. But in this study we showed the acute diarrhea will better if add the ant plant infusion especially in first 6 hours.

Table 1. The Number of Recovered Mice

The observation		Control (-)	Control (+)	Dose I	Dose II	Dose III
		Number of rats	Number of rats	Number of rats	Number of rats	Number of rats
6 hrs	Recovered mice	0	1	2	2	2
	Diarrhea mice	5	4	3	3	3
12 hrs	Recovered mice	2	3	3	3	4
	Diarrhea mice	3	2	2	2	1
18 hrs	Recovered mice	4	4	5	5	5
	Diarrhea mice	1	1	0	0	0
24 hrs	Recovered mice	4	5	5	5	5
	Diarrhea mice	1	0	0	0	0

note : Control (-) : Add aquadest

Control (+) : Add Attapulgite (54 mg/KgBB)

Dose I : 0.45 gKgBW-1 Dose II : 0.9 gKgBW-1 Dose III: 1.8 gKgBW-1

The frequency of defecate showed that there were worse in the first 6 hours in Control (-), as shown in table 2. After did treatments with ant plant infusion the frequency of defecate were reduce and to be normal.

Table 2. The Frequency of Defecation

Duration	Frequency	Contol (-)	Control (+)	Dose I	Dose II	Dose III
		Number of Rats	Number of Rats	Number of Rats	Number of Rats	Number of Rats
6 hrs	Many times	5	3	0	0	0
	Moderate	0	2	3	3	3
	Normal	0	0	2	2	2
12 hrs	Many times	0	0	0	0	0
	Moderate	5	3	2	2	1
	Normal	0	2	3	3	4
18 hrs	Many times	0	0	0	0	0
	Moderate	2	1	1	0	0
	Normal	3	4	4	5	5

Duration	Frequency	Contol (-)	Control (+)	Dose I	Dose II	Dose III
		Number of Rats	Number of Rats	Number of Rats	Number of Rats	Number of Rats
24 hrs	Many times	0	0	0	0	0
	Moderate	1	0	0	0	0
	Normal	4	5	5	5	5

note : Control (-) : Add aquadest
Control (+) : Add Attapulgite (54 mgKgBW-1)
Dose I : 0.45 gKgBW^{-1} Dose II : 0.9 gKgBW-1 Dose III: 1.8 gKgBW-1

The increasing of dose ant plant infusion following by the reducing of the frequency of defecate also the longer of treatment following by the reducing of the frequency of defecate. The Mann Whitney test showed that there were significant different between control (-) to the Dose I, II, III in the 12 hours, ($P<0.05$) 18 hours ($p<0.05$). So, acute diarrhea would be better if added the ant plant infusion. As we showed in table 3 the consistency of stool, in the first 6 hours consistency of stool control (-) was juicy and slowly reduced the water in the 12 hours, so the consistency of stool became moderate. But in the first 6 hours, dose I, dose II and Dose III the consistency of stool were better as the increasing of dose. The Mann Whitney test showed $p< 0.05$ between control (-) and dose I, II, III was significant different.

Table 3.The Stool Consistency

Duration	Consistency of stool	Contol (-)	Control (+)	Dose I	Dose II	Dose III
		Number of Rats	Number of Rats	Number of Rats	Number of Rats	Number of Rats
6 hrs	juicy stool	5	5	3	2	2
	moderate aquos stool	0	0	2	3	3
	litle bit aquos stool	0	0	0	0	0
	normal	0	0	0	0	0
12 hrs	juicy stool	5	0	0	0	0
	moderate aquos stool	0	5	2	0	0
	litle bit aquos stool	0	0	3	5	5
	normal	0	0	0	0	0
16 hrs	juicy stool	0	0	0	0	0
	moderate aquos stool	0	0	0	0	0
	litle bit aquos stool	5	5	0	0	0
	normal	0	0	5	5	5
24 hrs	juicy stool	0	0	0	0	0
	moderate aquos stool	0	0	0	0	0
	litle bit aquos stool	2	5	5	0	0
	normal	3	0	0	5	5

note : Control (-) : Add aquadest
Control (+) : Add Attapulgite (54 mg KgBW-1)
Dose I : 0.45 gKgBW-1 Dose II : 0.9 gKgBW-1 Dose III: 1.8 gKgBW-1

So ant plant was able to cure the acute diarrhea, because the ant plant contained tannin. Tannin have an effect as an astringent/Chelator. The chelator able to shrink the intestinal mucose membrane leads to reduce the excretion of diarrhea and was able as an antibacterial and anti inflamation [5]. Tanin stimulate the water reabsorbtion in the intestinum [7]. Tanin damaged protein, result in tanat lead the protein resistance, following by reducing water excretion, accompanying by the increasing of water and NaCl reabsorbtion. [8]. Tannin in tea contained *epigallocatechin-3-gallate* (EGCG), polyphenol hidrofil, colorless and bitter.as an astringent able to curve diarrhea [9]. The EGCG also able to inhibit prostaglandin production that stimulated by oleum ricini. Flavonid in the ant plant also able to inhibit the intestinum motility and hydroelectric secretion by inhibite prostaglandin production. [10] reported that sago root (*Metroxylon sagu*) infusion contained tannin, Flavonoid and saponin , also able to cure the acute diarrhea. Tannin was able to inhibit the secretion of chloride that induced by forskolin and toxin. Tannin also inhibit protein transmembrane.

Ant plant contains several chemical compounds such as flavonoids, tannins, and polyphenols having antioxidant activity. Moreover, the extract of ant plant capable to inhibit the activity of xanthine oxidase and uric acid [11]. [12] proved that the flavonoid quercetin has a protective effect in gentamicin- induced nephrotoxic rats. The flavonoid compounds identified in ant plant, were kaempferol, luteoline, rutin, quercetin and apigenin.

Conclusion

The ant plant infuse was able to reduce the frequency of defecate, recovered the consistency of stool and cure the acute diarrhea. The effective dose of antplant infuse was 0.9 gKgBW-1.

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THE EFFECTS of DIFFERENT DILUENT MEDIUM on HUMAN SPERM VIABILITY

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Abstract

As a rule the human sperm storage is done at a minus temperature. This storage method require complex equipment and require very expensive cost. Because of that the other human sperm storage method must be finded. The objectives of this research are to find diluent media that can maintain the viability of human spermatozoa during storage period, to obtain spermatozoa storage technologies easily and inexpensively. Diluent media for human spermatozoa storage should be able to maintain human sperm viability as well as the fresh human sperm viability. The type of tis research is experimental research. There are three types of diluent medium namely Tris aminomethane, CEP-D, and sil-select-stock. The human sperm that is storage in these diluent medium is analyzed their viability every 6 hours. The results is obtained that three types of spermatozoa diluent media is used in this study could be used for the storage of human spermatozoa with different specifications. Diluent medium sil-select-stock is the best media for storage the human sperm. This medium can maintain viability pproximately 12 to 18 hours in the room condition. While the CEP-D is commonly is used as a diluent medium for animals spermatozoa can also be used for human spermatozoa diluent medium with the capacity is not as much as sil-select-stock. Tris aminomethane have no good result.

Keywords: viability, diluent medium, human sperm

Introduction

Human spermatozoa storage methods that is used in the day, is not easy and very expensive. The methods commonly use the minus temperature, because of that the storage methods of human sperm needs so the expensive and sophisticated equipment. Based on the fact, still is needed to find the efficient methods to human sperm storage.

Recently so many efforts to maintain the quality of spermatozoa that is stored in freezing conditions have done. One kind of effort is vitrification method that have success when widely applied in the egg cell of cow [6], horses [5] and sheep [3] The principle of this method is to increase the viscosity of the solution or diluents medium by increase cooling and warming rate within certain limits. The other way is adding or using cryoprotectant medium which will suppress the increasing of viscosity at low temperatures [8].

The other method to, minimize genetic material damage is done with the choice of diluent. On sperm cells, the choice of the appropriate diluent have purpose to supply the physical and the chemical requirements of the cell, also protects sperm from damage due to rapid cooling process, maintain osmotic pressure and electrolyte balance too, inhibit the growth of bacteria and prevent the harmful effects due to changes in pH. There is no criteria of diluent medium standard for cryopreservation of sperm cells of mammals but there are some common diluents medium that have used, namely: Tris, skim milk, lactose and so on.

The efforts in above have needed expensive cost, sophisticated equipment and special and high skills of practice, because of that the frozen storage method is still less practical. Therefore it is still necessary to find another method that is more practical but still maintain the genetic material stability of the spermatozoa.

One of the aspects that determine the survival of sperm quality during storage is the diluents medium where the sperm is stored, which is referred to as a diluting solution. Diluting solution commonly is used include egg yolk Tris (KT) 20% and Andromed which is produced by the producers. Both kinds of diluents are commonly used for frozen storage. In this study will be developed media (diluting solution for spermatozoa) that can maintain quality of sperm cell as long as possible in storage at cold temperatures. Quality of spermatozoa will be determined based on the viability and motility of spermatozoa during storage (the first year of this research) and the stability of the sperm cell genetic material (the second year of this research).

In the first year of this study will be studied three kinds of diluting solution or diluents media namely the tris-egg yolk, the silk and the CEPD. There are some reasons for the case, the tris-egg yolk and the CEPD are commonly is used as a diluent medium for storing of groups mammals sperm and the silk commonly is used as diluent media for storing human spermatozoa.

Methods

The research have conducted in Department of Biology, State University of Surabaya. This experimental research want to investigate the composition of diluting solution or diluent medium to storage the sperm in a cold temperature (the temperature of the refrigerator, is not the frozen temperature). The study is used three types of diluting solution namely the tris-egg yolk, the sil-select-stock and the CEP-D.

Human spermatozoa is obtained from the volunteers. Along with that is prepared three types of diluting solution. Spermatozoa that have collected is observed it's viability for use in further processing. These aspects is observed based on some parameters like the volume, the color, the pH, the sperm concentration, the viscosity and the viability. Furthermore, the spermatozoa that complied with, is washed immediately and is diluted in various compositions of diluent media which is prepared. And then the packaging step of spermatozoa and the last step is sperm saving in cold temperatures. During storage period is observed the viability of the spermatozoa every six hours by negrosin eosin staining and observation under a microscope in 400x magnification.

The Results and Discussion

The viability or the vitality of spermatozoa have observed with negrosin eosin staining. The dead spermatozoa will be full red color, while the living spermatozoa just look colorless. The performance of the spermatozoa after staining process can be seen in Figure 1.

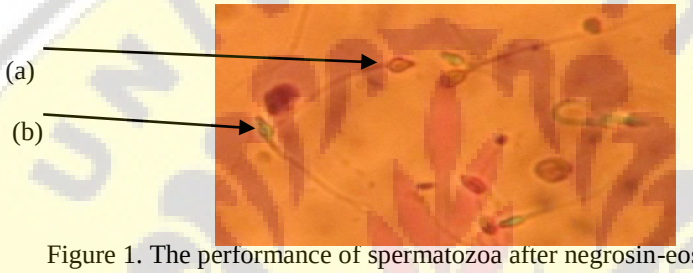

Figure 1. The performance of spermatozoa after negrosin-eosin staining process.
(a) The live spermatozoa (b) The dead spermatozoa

The determination of spermatozoa viability is based on the ratio of live and dead spermatozoa. The spermatozoa viability depend on the mechanism of ion pump into and out of the cell [2]. Observations of spermatozoa viability that have stained in negrosin eosin is performed by a microscope under 400x magnification. The viable spermatozoa had colorless performance due to the selective process to absorb the external materials including the color, while the dead spermatozoa had full color performance because of it's inability to select the external materials that come into the cell. The viable spermatozoa have the selective permeability of the membrane. On the other hand the dead spermatozoa have high membrane permeability [11]. The viability of spermatozoa is observed every 6 hours on each treatment. The observation of the spermatozoa viability as in Table

.

Table 1: The spermatozoa viability on the three kinds of diluents media

Treatment (The kinds of diluent media)	Number of experimental Unit	Viability percentage each 6 hours observation (%)				Average
		1	2	3	4	
P1 (SILK)	1	57.00	53.21	32.45	27.34	
	2	58.11	45.00	20.00	15.66	
	3	62.20	55.40	53.70	47.93	
	4	68.00	57.37	51.05	49.70	
	5	67.44	63.10	46.78	32.54	
	6	72.00	68.00	42.00	36.03	
	7	88.00	65.00	44.00	34.26	
	Average	**67.54**	**58.15**	**41.43**	**34.78**	**50.48**
P2 (TRIS-EGG YOLK)	1	46.00	26.00	22.00	15.87	
	2	41.00	43.39	31.00	25.71	
	3	40.82	37.48	20.00	14.54	
	4	42.27	38.00	25.00	20.12	
	5	60.34	54.25	46.36	32.35	
	6	58.00	51.00	44.58	30.56	
	7	64.22	59.91	44.56	41.25	
	Average	**50.38**	**44.29**	**33.36**	**25.77**	**38.45**
P3 (CEP-D)	1	50.20	43.00	31.57	27.76	
	2	51.36	32.43	32.01	28.78	
	3	60.00	57.37	49.87	49.56	
	4	64.70	60.00	49.00	46.74	
	5	59.33	58.51	47.10	33.75	
	6	60.86	56.14	49.12	38.81	
	7	68.00	61.00	45.32	43.20	
	Average	**59.21**	**52.64**	**43.43**	**38.37**	**48.41**

The results showed that silk and CEPD diluting solution can maintain the viability of spermatozoa that is stored in it. Viability of spermatozoa is highly depend on the stability of the cell membrane. Stable cell membrane will ensure normality metabolic processes that occur in it. In the silk medium contain HEPES buffer that can maintain the stability of the pH of the solution, even under conditions of changing temperature. This condition trigger the enzyme to work maximally. Because of that the spermatozoa viability can be maintained. Other components such as human serum and albumin that is contained in the silk medium was also very supportive to spermatozoa viability. This ingredient supplied the nutrient for the spermatozoa.

The CEPD medium is also a good buffer for spermatozoa. The medium contain ingredients that very useful to maintain the integrity of the cell membrane, to trigger the enzyme working maximally and to supply important nutrients to the spermatozoa. This condition in very important to maintain the spermatozoa viability. Opposite to the tris- egg yolk media, the spermatozoa that storage in it have the low viability.

Integrity and stability of the cell membrane is very important for the viability of the spermatozoa. The damage cell membrane cause membrane permeability increases. This condition will cause the necessary ingredients of cells that should be contained in the cell will be out freely. And the tocxic ingredients will be come in to the cell freely. Because of the cases the life of the spermatozoa will be disrupted [7].

There are several factors that caused the damage cell membranes. The presence of the dead spermatozoa will be toxic to the other still alive spermatozoa. The existence of these toxic substances will lead to increased levels of free radicals that can damage the integrity of the cell plasma membrane [9]. The change of temperature and pH during storage period can also raise the occurrence of lipid peroxidation (Conservation, 2011). Lipid peroxidation is the result of a chain reaction of free radicals with a lipid bilayer membrane, so that the cell membrane becomes damaged. This case is evidenced by fully colored of die spermatozoa because of damaged membrane. In the damaged membrane the dye can be absorbed and stored in it freely. Opposite on the alive cell, the dye will be absorbed by selective mechanism. The components that is contained in the diluent media must be able to prevent the destruction of the cell membrane.

Conclusion

The third type of diluents media is used in this research can be used for the storage of human spermatozoa with different specifications. The sil-select-stock medium is the best media to storage the human spermatozoa approximately 12 to 18 hours. While the CEP-D medium that commonly is used as a diluent medium of animals spermatozoa it can also be used for diluent medium for human spermatozoa with the carrying capacity is not much different from the sil-select-stock. The tris-egg yolk medium have given yield the lowest human spermatozoa viability.

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ANTIBACTERIAL ACTIVITY of *Pleurotus ostreatus Grey oyster* VARIETY AGAINST PATHOGEN BACTERIAL of *Salmonella typhi*

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Abstract

Salmonella typhi was one of pathogen microbial which can be prevented granting both synthetic or herbal antimicrobial substances, e.g from an *Pleurotus ostreatus* species *Grey oyster* variety that was one of an improvement strain from *Pleurotus ostreatus* and *Pleurotus sajorcaju* species. This research aimed to know the extract activities of *Pleurotus ostreatus Grey oyster* variety as antimicrobial agent against *Salmonella typhi*. This research used *True Experimental* with *Postest-Only Control Design* which were done by *in vitro* Diffusion methods. The materials which used were *Pleurotus ostreatus Grey oyster* variety and a panel microorganism of *Salmonella typhi*. This test was performed at six concentrations of each extract 1.0, 2.5, 5.0, 10.0, 15.0, and 20.0 mg/mL and ethanol control 500 mg/mL. The observation parameters was growth inhibitory diameters (DHP) of *Pleurotus ostreatus Grey oyster* variety extract as antimicrobial agent against *Salmonella typhi.* This finding of antibacterial activities by diffusion method was confirmed best inhibition zone in 2.5 mg/mL showed diameters about 22,62±0,990 mm. Parametric analyze of *One-way* ANOVA results test showed significant differences in inhibition zone diameters between groups. Test results of LSD and Duncan showed significant differences at 5.0 mg/mL, 20.0 mg/mL and K500.0 mg/mL to the others concentrations. From the statistical test results obtained a conclusion that the extract showed a good influence as antibacterial agent against *Salmonella typhi*.

Keywords : *Pleurotus ostreatus Grey oyster* variety, *Salmonella typhi*

Introduction

Salmonella typhi is a gram negative bacterium which caused *enteric fever* and pathogenic in humans. Approximately 420.000 deaths occur annually in Asia due to typhoid fever. Without treatment, case-fatality rates of infection are 10%. With appropiate antibiotic therapy treatment, case-fatality rates can be reduced to below 1%. Although the infection is treatable with antibiotics, treatment is complicated by growing resistance to widely available oral antibiotics in several areas of the world, including Southeast Asia [15].

The using of antibiotics to treat the disease which is caused due to bacterial infection, can cause some problems related to the toxic effects of drugs, drugs residues and the growing of resistance microbes. Antimicrobial resistance has become a global problem. Strategies to improve the current situation include research in finding new and innovative antimicrobials [4].

New antimicrobial production from bio-pharmacy (medicinal plants) is an alternative to consider for use [10]. This research use oyster mushroom because this species can grow widely in tropic and sub-tropic area, it is easily cultivated as well. There are approximately 40 oyster mushroom species and ranked in the second grade among the other important cultivated mushrooms in the world [2].

The oyster mushrooms contain pleuran compound which act as antitumor, lowering cholesterol levels and antioxidant. The presence of polysaccharide, especially β-d-glucan give positive effect as antitumor, anticancer, antivirus (icluding AIDS), lowering cholesterol levels, antifungal, antibacterial and can boost the immune system [3].

Due to oyster mushroom potential which give many benefits, many researchs are explored to create new oyster mushroom varieties, one of the results strain is *Grey oyster* variety which is improvement strain from *Pleurotus ostreatus* and *Pleurotus sajorcaju* [11]. This species has advantages in wider shape, thick cowls and duratibility outside of cooling machine (refrigerator) keep it fresh for up to 5 days [12].

According to the antibacterial activities testing of *Pleurotus ostreatus* and *Pleurotus sajorcaju*, both of the species showed as antimicrobial agent. *Pleurotus ostreatus* has been explored to against simple and multiple bacteria isolates of *Eschrichia coli, Staphylococcus epidermidis, S. Aureus* [1] and species of *Candida* that has been resistance of drugs [14]. *Pleurotus sajorcaju* has substansial that proved as antimicrobial, anti hypercholesterolemia, antioxidant and toxicity activities to Hep-2 cancer cell [9].

The antimicrobial exploration from *Pleurotus ostreatus Grey oyster* strain has not been exposed. Therefore this research is designed to test the antibacterial activity against phatogen microbe of *Salmonella typhi.*

Materials And Methods

Mushroom collection

Oyster mushrooms *Grey oyster* variety were collected from mushrooms warehouse of IKIP PGRI Jember, Indonesia working with Agathapratama-Group. Mushrooms were initially dried in an dehumified room, then further dried in an oven at ca. 40°C and finely powdered.

Preparation ef Extract

175 grams of mushrooms powder were extracted with 3,5 L of 96 % solvent of Ethanol using maceration apparatus [8]. The residue was filtered and concentrated to a dry mass in a rotary evaporator (rotavapor) at 40°C and 200 rpm. The fitrate thus obtained was used as mushroom extract. The concentrated extract was re-dissolved in aquadest which helped dissolved firstly by Twin solvent into 1.0 , 2.5 , 5.0 , 10.0 , 15.0 , 20.0 mg/mL extract concentration [13].

Test Microorganism

The Gram-negative bacteria which used were collected from Bio-Pharmacy Laboratory, Pharmacy Faculty of Jember University, Indonesia. The bacterial strains were maintained in NB (Nutrient Broth). For antibacterial test, organism were grown overnight in NB medium followed by incubation at 37°C [7].

Antimicrobial Susceptibility Testing

The antimicrobial activities were found by using agar diffusion technique in petriplates. 0.5 mL of the seeded broth containing 10^4 test organism was inoculated uniformly. Briefly 5 mL of each extract concentration was loaded on a sterile filter paper disc 14.20 mm in diameter and air dried. Indicator organisms were spread on *Nutrien Agar* plates with sterile effusion and the disc were placed on agar plates. After incubation for 48 hours at 37°C, a clear zone around a disc was evidence of antibacterial activity. Diameter of the zones of inhibition was measured in millimeters. Each test was prepared in quarduplicates and solvent of ethanol was used as a negative control [7].

Statistical Analysis

All experiments were conducted in quadruplicates and the parameters were given as means ± standard deviation using statistical package within Microsoft® Excel Version 2010. And parametric analysis of DHP on each groups using SPSS 17.0 Version to analyze the different inhibition zones between groups [6].

Results and Discussion

Inhibition zone of *Pleurotus ostreatus Grey oyster* extract and ethanol as control against *Salmonella typhi* showed various diameter (Figure 1). The extract of *Pleurotus ostreatus Grey oyster* in 2.5 mg/mL was more potent and revealed high zone (22.62±0,990 mm) formation against *Salmonella typhi*, whereas the other concentration of extract showed not so different in diameters. The results of present study revealed that *Pleurotus ostreatus Grey oyster* extract demonstrated higher antibacterial activity comparison of ethanol control

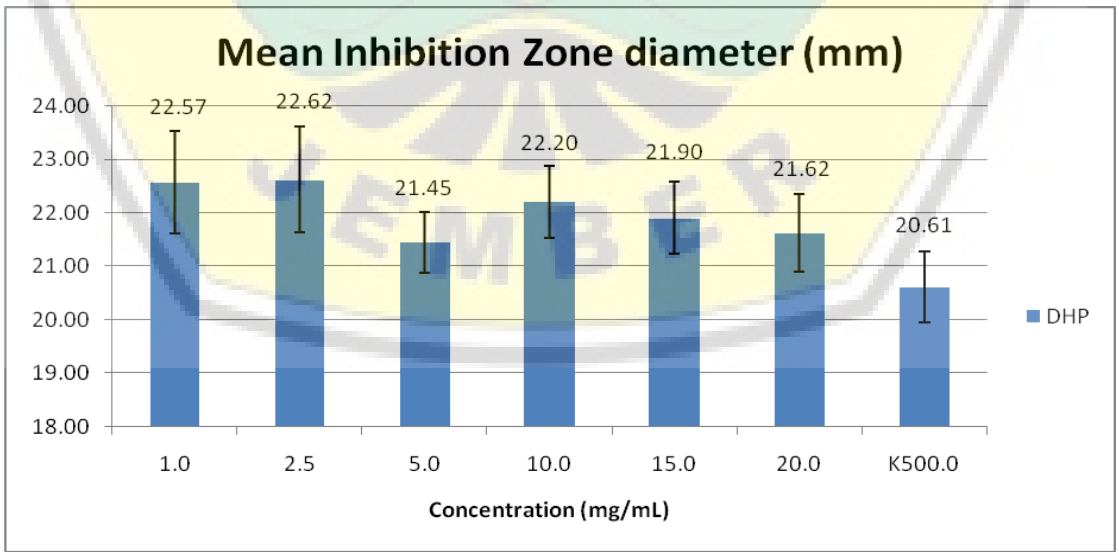

Figure 1. Means inhibition zone diameter (mm) of *Pleurotus ostreatus Grey oyster* variety against *Salmonella*

Statistical analysis with *One-way Anova*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.868	6	1.978	3.357	.018
Within Groups	12.372	21	.589		
Total	24.240	27			

Parametric analysis with *One-way Anova* shows Sig. value at 0.018>0.05 which was categorized there were significant different between groups of *Pleurotus ostreatus Grey oyster* extract against *Salmonella typhi.*

Post-hoc notation data of DHP *Salmonella typhi*

Concentration(mg/mL)	Means (mm)
1.0	22,57±0,964 [b]
2.5	22,62±0,990 [b]
5.0	21,45±0,570 [ab]
10.0	22,20±0,675 [b]
15.0	21,90±0,674 [b]
20.0	21,62±0,729 [ab]
K500.0	20,61±0,669 [a]

Post-hoc test results showed significant differences at 5.0 mg/mL, 20.0 mg/mL and K500.0 mg/mL to the others concentrations that indicated by differences subset coloumn which were described on alphabetical varians of means coloumn. According to the test results could be concluded that higher concentration of extract, diameter means decreases. This condition was caused the bacteria being resistance of *Pleurotus ostreatus Grey oyster* extract. The lowest means diameter showed at 5.0 mg/mL concentrations. An error occurred when test of antibacterial activity can also affect the formation of growth inhibitory zone.

Ethanol was taken as negative control for its selective, netral and non-toxic. Fungi and bacteria were difficult to grow on ethanol with a concentraton above 20%. The absorption of ethanol for simplicia compounds was good [5]. Ethanol control showed broad-spectrum (20,61±0,669 mm) at 500 mg/mL that aquivalent with 50% concentration. Compare to this, *Pleurotus ostreatus Grey oyster* extract also shows broad-spectrum antibacterial activity with a concentration below 20% which indicated its activities better though with low concentration.

Antibacterial activities could be classified based on the magnitude of the inhibitory zones [16].

Classificasions of Antibacterial activity

Antibacterial activity	Inhibitory zones (mm)
Weak	<5
Moderate	5-10
Strong	10-20
Antibacterial activity	Inhibitory zones (mm)
Very strong	>20

The *Pleurotus ostreatus Grey oyster* extract concentration ranging from 1.0 – 20.0 mg/mL has diameter means of the inhibitory zone 21.45 - 22.62 mm, so it belongs to very strong activity.

The effectivity of *Pleurotus ostreatus Grey oyster* extract as antibacterial suspected because of the glycosides compounds like lectins, polysaccharides, polysaccharide-peptides, polysaccharide-protein complex which have been isolated from its stem mushroom and these compounds have been found to have antioxidant, anticancer, antimicrobial, antidiabetic, anti hypercholestrolemic and immunomodulatory properties. The observed phenolic and tannin constituents of *P. ostreatus* may also elicit antibacterial activity as found in many medicinal plants with mechanisms of action characterized by cell memberane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhensin. Whereas, fungal cell wall are rich in non-starch polysaccharides, of which β-glucan are most interesting functional components and phenolic compounds such as protocatechuic acid, gallic acid, homogentisic acid, rutin, myrictin, chrysin, naringin, tocopherol like α-tocopherol and γ- tocopherol, ascorbic acid and β-carotene of each having their own outstanding medical effects [3]. In conclusion, bioactive compounds from *Pleurotus ostreatus Grey oyster* extracts could be used as an alternate to antibiotics, considering the side effect and escalating levels of antibiotic resistance among microorganism.

Conclusion

Pleurotus ostreatus Grey oyster extract demonstrated high antibacterial activity on *Salmonella typhi*. The extract was more effective than control ethanol to against pathogenic microorganism studied. Further work is needed to isolates the secondary metabolites and study of metabolic interchanges in bacterial metabolic pathways when applying this extract. It also need to use positive control of traditional antibiotic to evaluate extract efectivity along antibiotics, so the study can explore resistance bacteria. Study of extract toxic effects also needs to be done through toxicity test. The obtained results may be useful for evaluating substances of interest produced by these bacteria as antibacterial agents.

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THE EFFECT of EXTRACT THYMOQUINONE BLACK CUMIN SEED (*Nigella sativa*) of AGAINST NEUTROPHILS NUMBER in RATS SOCKET POST EXTRACTION with TRAUMATIC TISSUE

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Abstract

Tooth extraction may accompany by complications, such as tissue laseration, bone resorption, adjacent tooth mobility and dry socket. They caused inflammation or infection. Treatment of traumatic tissue usually given an anti-inflammatory drugs,but aspirin has many side effects. To alternate from side effects then used herbal remedies, one of which has a Black Cumin active ingredient is Thymoquinone. To look at the effect of the extract thymoquinone against neutrofphils number in rats socket post extraction with traumatic tissue. Methods: In this study used 12 rats that had been done tooth extraction and expansion of the wound. Furthermore, preparations were made in areas such revocation and stained using hematoxylin-eosin. There is a significant difference between the negative control group with the positive control group and the group extract thymoquinone, but there was no significant differences between the positive control group and the group thymoquinone extract. Data were analyzed using statististical test one-way anova. Extract Thymoquinone could reduce widely inflammation respons in rats socket post extraction with traumatic tissue and its that effect similar to aspirin.

Keywords: extraction, neutrophils, thymoquinone, traumatic

Introduction

Tooth extraction is an act of removing the tooth from the alveolar bone socket. Tooth extraction commonly caused by caries, periodontal disease, supernumerary teeth, impacted teeth (odontectomy), teeth that are no longer performed endodontic treatment, teeth involved cysts and tumors, and teeth that engage jaw fracture. Measures tooth extraction can be done also on healthy teeth with the aim of improving the malocclusion, for aesthetic reasons, and also the interests of orthodontic or prosthodontic treatment [1].

Complications due to tooth extraction are bleeding, hematoma, infection, fracture of crown or root, alveolar bone fracture, and nerve damage [2]. One of the complications after tooth extraction is tissue trauma due to the use of instruments that are less careful [3].

Management of complications of tooth extraction to prevent the occurrence of a severe infection and decrease pain required analgesic and anti-inflammatory medications. Medications commonly used to treat the pain is non-steroidal anti-inflammatory analgesic drugs [4].

The use of non-steroidal anti-inflammatory analgesic drugs has many side effects as the risk of gastrointestinal toxicity, impaired renal function and postoperative bleeding. Therefore, the use of these drugs be avoided in patients with a history of gastritis or peptic ulcer disease and hemophilia, should be cautious in patients with corticosteroids or anticoagulant drugs consumption. These medications include piroxicam, tenoxicam, indomethacin, and aspirin [5].

Lately, the experts have developed herbal medicines in therapeutic medicine. Herbal medicines or traditional may as primary therapy or as an alternative therapy [6].

Traditional medicine is relatively safe if used in the right way at the right dose with appropriate indications and rarely caused side effects. One of the traditional plant that recently began to gain attention is black cumin (*Nigella sativa L.*). Black cumin has been widely used as an anti-hypertension, liver tonic, diuretic, digestive, anti-diarrhea, analgesic, anti-bacterial and skin disorders. Beside that black cumin also used for anti-diabetic, anti-cancer, immunomodulatory, antimicrobial, anti-inflammatory, spasmolytic, and bronchodilators [7].

Thymoquinone (TQ) is the main bioactive components of black cumin. Thymoquinone have been reported to have anti-inflammatory effects were observed in several models of inflammation including the experimental encephalomyelitis, colitis, peritonitis, edema, and arthritis through suppression of inflammatory mediators like prostaglandins and leukotrienes [7]. Anti-inflammatory effect was confirmed by Al-Ghamdi in 2001 with rats using black cumin seeds and the result is the black cumin and TQ may inhibit edema in the legs of rats [8]. Another study cases of osteoporosis with inflammation reported that black cumin and TQ have anti-inflammatory effects by

inhibiting the pro-inflammatory cytokines like interleukin 1 and interleukin 6 [6].

Based on the previous data, the researchers are interested to research the effects of extracts thymoquinone black cumin seed to the number of neutrophils in rats socket post tooth extraction with traumatic tissue.

Research methods

The research was an experimental laboratory with Post test Only Control Group Design. The research located at Biomedical Laboratory Section of Physiology of the Faculty of Dentistry, University of Jember to treatment on rats and located at the Laboratory of Pathology Faculty of Dentistry, University of Jember to preparations and observations preparat in January-February 2015. Population in this study was wistar rats with the male sex. The number of samples obtained by using the formula of Daniel. The samples used were 12 rats and were selected based on criteria of sample.

Research sample criteria were male wistar rats, 150-200 grams of body weight, 2-3 months old, rats in a healthy state characterized by the active movement.

Research tools used weight scales rats, digital scales, latex gloves, measuring cups, stomach probes, dysposible syringe, excavators, sonde straight and half round, scalpel and blade, tweezers dentistry, rack slide, deck glass, glass objects, binocular microscope, equipment for the manufacture of tissue preparations, contra handpiece, round end bur, eyepiece micrometer, slide warmer.

Research materials used an extracts thymoquinone, an aspirin, a ketamine solution, sterile distilled water, cotton pellets, alcohol, formalin 10%, paraffin powder, xylol, meyer egg albumin, 10% formic acid, paint hematoxylin-eosin, glycerin, and entelan.

First research procedure was performed adaptation of rats for 7 days then grouped into 3 according to the criteria that have been determined. Then do tooth extraction on the left mandibular first molar of rats. After that drilling on the socket using a diamond round end to the base of the socket for one time. Furthermore, given the treatment in accordance with the group. In the group A was given as 2ml distilled water, in group B was given aspirin, and group C was given the extract Thymoquinone appropriate dosage. Then, 24 hours after extraction and taken the lower jaw.

The second is making preparations consisting of fixation, decalcification, dehydration, clearing, impregnation, planting in paraffin, slicing tissue, and tissue staining using Haematoxylin-eosin. Preparat were observed and calculated neutrophils with a microscope magnification of 1000x and using the eyepiece micrometer (1 cm^2) at 3 different locations then calculated the average for the observation and recorded the results.

Data have been obtained then tabulated and analyzed. Normality of the data test has been obtained in advance, using the Kolmogorov-Smirnov test. Then using Levene test for homogeneity of the data. The purpose of normality and homogeneity test as a requirement to be able to use parametric One-way Anova test.

Analysis of the data further to be used if the data were normally distributed and homogeneous, is using parametric One-way Anova. If the data is not normally distributed then using Kruskal Wallis test.

Result

The observation of neutrophil cells in rats after tooth extraction sockets were accompanied by tissue trauma using 1000x magnification microscope with the following picture:

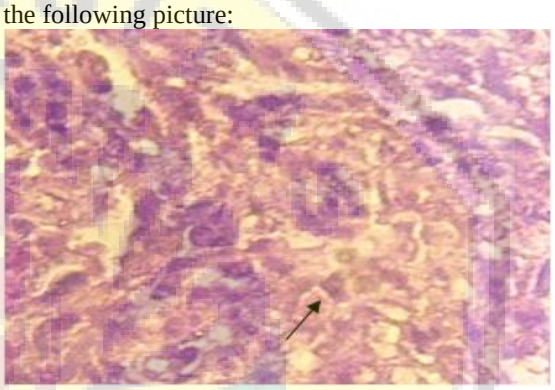

Figure 1. Cells neutrophils with hematoxilin-eosin staining 1000x magnification (arrows) in the negative control group

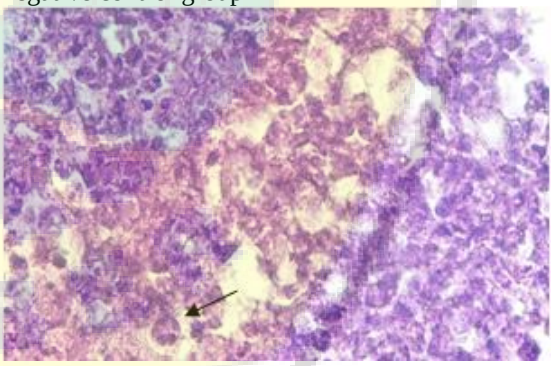

Figure 2. Cells neutrophils with hematoxilin-eosin staining 1000x magnification (arrows) in the positive control group

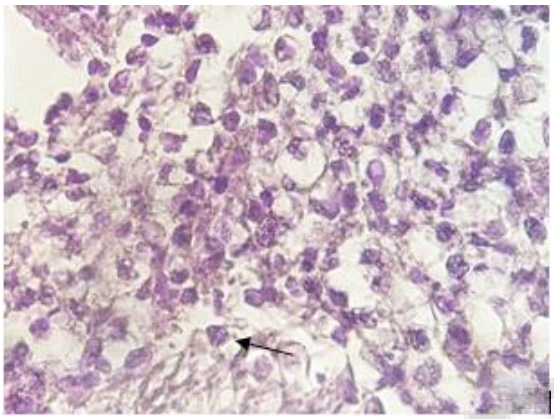

Figure 3. Cells neutrophils with hematoxilin-eosin staining 1000x magnification (arrows) in the treatment group the extract thymoquinone

The observation of the neutrophil count in rats after tooth extraction sockets with trauma network by using a microscope 1000x, the results obtained in Table 1.

Treatment	Neutrofil (average ±SD)
Negatif control	32 ± 6,21825
Positif control	23 ± 4,1633
Thymoquinone Extract	26,5 ± 4,79583

Table 1. The number of neutrophils in the tooth socket after tooth extraction with traumatic tissue

Results of the above data and then tested by computerized using the Kolmogorov-Smirnov test to determine the normal distribution of data. Kolmogorov-Smirnov test results obtained normal distribution of data.

Homogeneity test is then performed using the Levene test. Levene test results obtained probability values obtained 0,211 greater than 0.05 can be interpreted that the data is homogeneous. Furthermore, after it emerged that the data were normally distributed and homogeneous and then followed by one-way Anova test to determine the difference between groups. The test results obtained one-way Anova probability value of 0.038 ($p < 0.05$), which means there are significant differences between the treatment groups. To know the difference is then performed post-hoc LSD test.

The test results obtained post-hoc LSD probability value between group A (negative control) and B (positive control) is 0.025 ($p < 0.05$) means that there is a significant difference between group A (negative control) and B (positive control), while the probability value between group A (negative control) and C (treatment group) is 0.025 ($p < 0.05$) means that there is a significant difference between group A (negative control) and C (treatment group), while the probability values between group B (positive control) and C (treatment group) is 1.000 ($p > 0.05$) means that there is no significant difference between group B (positive control) and C (treatment group).

Discussion

The study compared the effects of extracts Thymoquinone with an aspirin as a positive control and with distilled water as a negative control, against the neutrophil count in rats after traumatic extraction sockets using 12 male Wistar rats were divided into three groups according to predetermined criteria.

The observation and analysis of data showed that the number of inflammatory cells in the negative control group or were given distilled water showed the highest number of neutrophils followed by groups of rats were given the extract, and the lowest is group were given an aspirin. The significant difference between negative and positive control group and negative control group to the treatment group were given the extract Thymoquinone, whereas the positive control group and the treatment group were given extracts of Thymoquinone no significant difference.

Significant differences between the control group and the treatment group were given extracts of Thymoquinone happened because Thymoquinone can inhibit arachidonic acid metabolism then inhibit the cyclooxygenase and lipoxygenase pathway [9]. After the cyclooxygenase pathway also inhibited the prostaglandin, as well as inhibition of leukotrienes in the lipoxygenase pathway. After the cyclooxygenase pathway inhibited the prostaglandin, as well as inhibition of leukotrienes in the lipoxygenase pathway. In inflammation, prostaglandin which have a role is prostosiklin (PGI2), PGD2, PGE2 and PGF2. Prostaglandins act as a vasodilator. Vasodilatation of blood vessels resulting in increased blood flow and increased vascular permeability that would bring blood cells to the area of infection or trauma. The white blood cells then attached to the wall using vascular endothelial adhesion molecules and will migrate toward the inflamed tissue [10, 11, 12].

Acute inflammation is the initial response of the body and the body's first line of defense against danger, include infection, trauma, tissue necrosis and foreign body. The acute inflammation commonly can be caused by a inflammatory reactions. The most important inflammatory cells are neutrophils [13].

Neutrophils have a very important function in acute inflammation. Neutrophils are the body's primary defense against pathogens, such as bacteria. Neutrophils accumulate in the center of the wound and will be immediately activated. Neutrophil intracellular fluid consisting of oxidative and non-oxidative can be activated simultaneously during phagocytosis to kill bacteria. Although the destruction of intracellular infectious agent occurs, released cytotoxic molecules into the extracellular environment can damage body tissue [11, 14].

The process of inflammation is actually one defense mechanism (homeostasis) to trauma, hemorrhage, entry of foreign objects into the body in a certain period of time, but if this process continues over time it would damage healthy tissue [15]. To limit excessive inflammatory response is needed anti-inflammatory drugs [16].

The probability value between a positive or a control group of rats that were given an aspirin in the treatment group were given extracts of Thymoquinone showed no significant difference. No significant difference is because aspirin has anti-inflammatory effects that are similar to Thymoquinone extract. Aspirin blocking the cyclooxygenase enzymes. In the right dose, aspirin will be able to reduce the formation of prostaglandins and thromboxane [17].

Results of this study can be seen the effect of extract thymoquinone black cumin seed (*Nigella sativa*) of against neutrophils number in rats socket post extraction with traumatic tissue. Thymoquinone can reduce the number of neutrophils in rats after tooth extraction sockets with traumatic tissue. Thymoquinone have anti-inflammatory power similar to aspirin and can be used as an alternative medicine for patients who have a poor risk when taking aspirin.

Conclusions and advice

The conclusion that can be drawn is Thymoquinone extract of black cumin (*Nigella sativa*) lowering effect of neutrophils in rats after tooth extraction sockets with traumatic tissue and has anti-inflammatory effect similar to aspirin.

Advice can be given that is necessary to conduct further research on the power of anti-inflammatory of the Thymoquinone extract of black cumin after tooth extraction accompanied by traumatic tissue using immunohistochemical methods to identify neutrophil more accurate and needs to be done further research on the effects thymoquinone black cumin towards healing wound after tooth extraction accompanied by tissue trauma observation time is longer.

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TRADITIONAL MEDICATION of OSING TRIBE in BANYUWANGI

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Abstract

Most Indonesian people use traditional medication to prevent and cure the disease. Tradisionl medication is closely linked to the culture of society as it exists in Osing tribe in Banyuwangi. This study aimed to explore the characteristics of traditional healers and patients, as well as procedures for traditional medication in Osing tribe. This study used a qualitative approach of phenomenology with depth interviews, participatory observation, documentary materials and browsing the internet material. This study located in 2 traditional village, involves five (5) informants who work as healers. Patients include infants to adult for symptoms of disease. Treatment using massage method, spells, and rajah; giving herbs are eaten or drunk; and do not use any special tools. Treatment using natural materials such as coconut oil, spices, water. The time required in a relatively short treatment is 15-20 minutes, while the time it takes patients to recover is one day to a week. The conclusion that the Osing tribe in Banyuwangi today still use traditional medication to cure the disease by the method of massage, spells, and rajah.

Keywords: traditional, medication, osing tribe.

Introduction

Traditional treatment is treatment with the drug, procedure and treatment, which refers to the experience and skills from generation to generation and is applied as a norm in the society [1]. Traditional treatment is usually performed by traditional healers or commonly called a *dukun*, recognized and utilized by the local community as a medium to achieve a cure. Traditional medicine typically uses traditional medicine in the form of materials or ingredients in the form of vegetation, animal elements, and minerals, which are considered efficacious been used for treatment based on experience [2].

Data from the Ministry of Health in 2007, found that someone who is sick will take action to treat themselves and act seek healing to both traditional and modern treatment facilities. The medical facilities including hospitals, physician practices, health center/sub health centers, other health care workers, as well as herbalists / traditional healers [3].

Traditional medicine has a close relationship with the culture of the society in the countries of the eastern hemisphere, including Indonesia. One of the tribes living in East Java Banyuwangi is Osing tribe who have different cultures, languages and traditions in terms of treatment. Treatment may include medication occult, prayer, and herbal treatment [4]. Osing society is a society that is unique, making numerous experts are interested in researching and writing about the study Osing both related, language, arts, oral literature, anthropology and other studies. Specific novelty in this study (which is different from previous studies) is that; studies relating to the previous community substance Osing more emphasis on the spoken language and literature is about black magic spells in [5,6], whereas this study emphasizes the traditional treatment methods that lead to healing which has been less attention.

Preliminary studies conducted by the researchers of the two informants who work as traditional healers who live in District Slippery as one of the areas of the tribe Osing stay in Banyuwangi. Based on interviews with informants can be seen that the practice of medicine they did include several methods: by using a healing prayer, healing using vein and nerve massage, healing by consuming natural ingredients, as well as healing disease and connective mate by using magic. Informants stated that the skills and abilities they have obtained from elder people in the region.

The problem in this study is the first one is how the characteristics of traditional healers and patients who use the treatment ?; Secondly, what and how procedures of traditional medicine in the Tribe Osing ?. Traditional medicine Osing tribe is very interesting and important to study because first, this treatment is part of the rich culture and local wisdom Nations Indonesia to be preserved; second, the results of research can enrich the study of traditional medicine so that further strengthen the foundation for treatment and health policy in Indonesia.

Method

This study used a qualitative approach using the phenomenology. The research location is set in the Osing traditional village in Banyuwangi; Kemiren and Olehsari. Based on the literature review found that the two villages are living territories Osing native tribe, while based on the testimony of key informants found that there are four traditional village Osing namely Kemiren, Olehsari, Boyolangu, and Alasmalang.

Data were obtained from from direct participation observation and interviews of the traditional healers; second. Data collection techniques used in this study include in-depth interviews (Indepth Interview), observation, and

documentation (recording and recording). Interviews were conducted using open-ended questions, which means the answers given by the informants are not limited to (not bound), so hopefully investigators gain a deep and accurate information regarding the method of treatment in the community Osing tribe Banyuwangi. Researchers act as an observer, which means being part of a group of researchers who studied, so the researchers will stay at the study site to observe the social interaction associated with traditional treatment Osing tribe. Recording the activities carried out to record the data obtained from the field directly, in the sense of all the data and information obtained in the field accurately recorded on the same day.

Data were collected through participatory observation, field notes and recording equipment, ditransikripsikan into the written record. The recording is used to record the activities of the communication process and interview to overcome the limitations researchers recorded directly in the process of communication in participatory observation and interviews. Data were unearthed and collected, then classified and subsequently held interpretation in the form of descriptive qualitative analysis.

Result and Analysis

a. The process of field work

Based on the literature review found that Banyuwangi has some areas that are home Osing tribe, one of which is a village in the district Kemiren Glagah. Researchers decided to come to the village of Kemiren as initial steps in data collection. Kemiren researchers came to the village and met with traditional leaders Osing which then became the first key informants in this study. Based on information from the head of customs in the village Osing Kemiren that there are four traditional village Osing, namely: Kemiren village, Village Olehsari, Boyolangu Village, and Village Alasmalang. Based on this information the researchers took the data in Kemiren village as the village first and then proceed to the village of Olehsari. Researchers completed data collection in each village sequentially, except if there is data that needs redeveloping the researchers came back to certain customary village as needed data.

b. Characteristics and description of informant

Investigators identified key informants based on information from key informants in each village. Key informants in the village kemiren initially there are 3 (three) members, namely TM, KT, and SK. 2 (two) informants successfully met, but an informant could not be found, namely SK, although researchers repeatedly came and waited at his residence. By main informant in the village of Sari there are three (3) members, namely JJ, MS, and SN. All key informants in the village of Sari By successfully met and the information unearthed by researchers.

The term is typical for traditional healers are very diverse in the various regions in Indonesia in accordance with the language and local culture. Traditional healers in the village of Sari By Kemiren and commonly called *dukun or wong pinter*. Ethnic Mentawai in West Sumatra used to refer to traditional healers by name sikerei [7], while the Bajo People in Kendari used to call with sandro [8]. The existence of traditional healers based on a social selection that is not obtained through formal education and only requires the recognition of local people [9].

Based on the data related to the characteristics and depth picture informant, that traditional healers in the village Kemiren and By Sari consists of genders male and female, aged adults to the elderly, educated highest Elementary School, and has been involved treatment in a relatively long time that is above 30 years. Characteristics of traditional healers is different in each society, there are certain very strict ethnic restrict a physician with gender, age, and specific experience, but there are also ethnic that are not strictly set.

Traditional healers in the village of Sari By Kemiren and treatment not acquire the ability of formal education, but of descent, dreams, DI, and learning from books and other traditional healers. According to the results of research conducted in Kaili Da'a Ethnic, North Mamuju that a traditional healers commonly called a topo tawui, acquire knowledge from dreams or family history. A topo tawui that previously did not have a family history as topo tawui usually get the science of dreams. Usually this dream only comes one time when it was midnight. topo tawui dream of meeting someone who tells how to perform the treatment along with several charms (Dowa) where the mantra healing every disease is different [10].

Research in Ethnic Rote Rote Ndau district revealed that there is a famous traditional healers ability to treat diseases associated with fractures and diseases of the stomach, called Oma Sr. Oma Sr get a gift when breastfeeding her first child, about 50 years ago. At that time, Oma Sr suddenly want to fast for ten days, although eventually only three days. On the third night, Oma Sr. got a sort of visual clue. After getting the gift, Oma Sr. met with the Americans who taught how to bear children, to show people that fractures and sprains, and a variety of modern medicine that is usually used by health workers [11]. Government policy related to the legality of the practice in line with the treatment of the phenomena, which permits the practice can be obtained by taking care of a letter of recommendation from the village and legal institutions, whereas age-related requirements, diplomas and experience are not included in the policy of the Department of Health [12].

c. Patient

Patients that can be treated by traditional healers in Kemiren and Oleh Sari are different, there is a remedy that can treat patients infancy to adulthood with various complaints of the disease, but there are also only accept only adult patients, or patients with certain complaints. Here are excerpts of the interview to the informant:

"... Yes all, all kinds of diseases" [TM]

"... Massaging sprain, massaging in order to become pregnant or massaging the man so he could 'upright', baby massage, and massage to tired also" [KT]

"... Medicine, babu body, itching, of fertility, cough ..." [JJ]

"... If the high blood pressure that bothered, it should be a doctor ... yes, otherwise it could" [SN].

Traditional healers can handle a variety of complaints diseases [8], but Healers who receive patients of a certain age or a specific complaint is consistent with the practice of medicine in the People Bajo, that in The Bajo in Kendari there is a remedy certain that only deal with women who have just given birth and the baby are commonly called as tungkenei [9].

d. Treatment

Traditional medicine in Kemiren and Oleh Sari used various ways that massage, herbal powders, *mantra* and *rajah*, giving herb to eat and drink or as a powder, and a prohibition against the act. Here are the results of interviews with informants:

"... Do not plant flowers dark colored, dark green it. Unusual planted should not be planted. It usually should not be planted by your descendants, so it still should not be "[MS]

"... How to cure it using the rajah" [SN]

All of this treatment method is commonly used in other ethnic communities in Indonesia, for example, the Baduy people who cure various medical and non- medical illnesses by means such as herbal medicines that surround the Bedouin village. Traditional healers do not just use the plants and animals alone but accompanied also *jampe-jampe* or incantation uttered a prayer of healing [13].

Massage method performed by Ethnic Sumab NTT, which is massaging the patient's body with varying movement, because between every shamans have a different way of massage. Basically the sequence is different movements with massages. Sort rather the rubbing motion vertically or horizontally, but with the pressure. While massage is continuously pressing movement in one part or several parts of the body. Usually this method will be complemented by the provision of medicinal herbs should be taken of patients, and it has not been mentioned as most herbalists secret [14].

The provision of medicinal herbs when sick or a family member is sick practiced by ethnic Mentawai in Mentawai District. Society will make a concoction of drugs from medicinal plants that can be found around the home or farm community. Medicinal plants obtained will be processed into drugs, both medicinal topical or external drug and a drug that can be taken for example when there are people who are sick hot or skyrocket, then one of the family members who know about the types of medicinal plants (sikerei simata) will find plant named Botbolo. The leaves having taken approximately 2 to 4 pieces and then crushed or crushed and then applied to the forehead [7].

e. Material

Traditional medicine in Kemiren and Oleh Sari used natural materials such as coconut oil, spices, white water. Coconut oil is used in massage methods and sequence, spices used in the method sequence, powders, and potions were drunk, while the water is used only for drinking only. Here are excerpts of an interview with the informant:

"... Herbs Java ... like turmeric acid, a key betel nut, ginger, yes more" [JJ]

"... Massage oil is coconut oil plus spices such as nutmeg, ginger, galangal, cloves, all mixed together, pulverized in advance, squeezed, and eucalyptus oil plus a few" [KT]

"... Yes, given a drink of water ... yes with spells you know, the tattoo as well and mix by using the middle finger or index finger" [SN]

"... Take the flowers near the door, mashed, then smeared" [MS]

Ethnic Dayak Ngaju in Kapuas has a treatment called as Danum tawar. Danum tawar treatment is a form of treatment that uses a water prayed media. Within the meaning of said Danum is water and tawar is fresh so the meaning of Danum tawar is used to neutralize the negative effects in the body [15].

The provision of medicinal herbs in the form of a mixture of spices carried Ethnic Sumba in NTT, although most shamans also objected to notify the mixing of potions they make. Confidentiality is actually they watch the meeting, although they have no objection to show an example of the potion. Their ability to dispensing drugs obtained through occult process delivered through a dream. As well as with their ability to cure the disease. Ability they sort of grace or revelation to the chosen people only. Herb that is used to sort the most frequently used is coconut flesh burnt to black, mixed with coconut milk, ginger, and tobacco. There is also a shaman who mix it with turmeric. This herb is used to massage the body ill patients [14]. Spices are also used in traditional medicine Sumba with sprayed or hawurut method. The way it works is by spitting herb in the form of betel, areca nut, lime, and sometimes coupled with ginger (red ginger). The concoction chewed by shamans then ejected to part of the sick body [14].

f. Medical tool

Traditional medicine in Kemiren and Oleh Sari didn't use any special tools in medicine, except the massage which is performed by the first informant who use stones to be used in a bath of coconut oil to massage. The existence of the stone is believed also by the Dayak ethnic community Kayanatn in Porcupine District of West Kalimantan know media treatment using objects obtained a shaman through dreams or given oelh inadvertently ancestral form of stone, valve, or the like. How to use the objects that are included in the water and then the water is prayed then the patient is asked to drink water [16].

g. Time Needed For Medical

Traditional medicine in Kemiren and Oleh Sari do the treatment within 15-20 minutes for each patient. Procession treatment include: admissions, listening to complaints, followed by treatment until completion. Simple procession in a relatively short time is also performed by shamans in Baduy Ethnic Lebak [13], the procession begins with the patient came and told him about the grievances felt. Further services provided depends on the patient's own request, whether treated with herbs and prayers.

This is different from the treatment in Kanayatn Dayak ethnic community in which one treatment called balenggang, performed within two days and two nights, involving a variety of equipment and animals such as dogs and pigs, and accompanied by a call to the spirits or ghosts [16].

Conclusion

Based on data collected by the researchers, it can be concluded while, namely:

1. Traditional Healers in the village of Sari Kemiren and By-sex male and female, aged to elderly adults, the highest educated elementary school, and has been involved in the treatment of a relatively long time that is above 30 years.
2. Patients treated by traditional healers in the village of Sari By Kemiren and is patient infancy to adulthood with various complaints diseases.
3. Traditional medicine in the village of Sari Kemiren and by using various ways that massage, spells and tattoo, giving concoction eaten or drunk.
4. Traditional medicine in the village of Sari Kemiren and by using natural materials such as coconut oil, spices, water.
5. Traditional medicine in the village of Sari Kemiren and by not using any special tools in the treatment.
6. The time required within a relatively short treatment is 15-20 minutes
7. The method of treatment in the community Osing tribe in the village of Sari By Kemiren and has many similarities with traditional treatment methods in other ethnic Indonesia healers in terms of characteristics, patient characteristics, treatment method, the materials used in medicine, medical instruments, and the time of treatment. Differences were found is on the language used in the phrase spells and prayers.

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ANTIHYPERLIPIDEMIC ACTIVITY of THE COMBINATION of *Guazuma ulmifolia* L. LEAVES and *Hibiscus sabdariffa* L. FLOWERS EXTRACT in RATS INDUCED by HIGH-FAT DIET

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Abstract

Hyperlipidemia is a lipid metabolism disorder characterized by increased plasma concentrations of triglyceride and cholesterol, which is a risk factor for cardiovascular disease. *Guazuma ulmifolia* L. leaves and *Hibiscus sabdariffa* L. flowers has been traditionally used to lose body weight and reduce body fat. A study showed that the extract of *Guazuma ulmifolia* L. leaves may decrease plasma concentration of cholesterol, and possibly through the mechanism of inhibition of HMG-CoA reductase, a key enzyme in cholesterol synthesis *de novo*. *Hibiscus sabdariffa* L. is a good inhibitor of pancreatic lipase. Combination of both extracts are expected to work synergistically to reduce the plasma concentration of triglyceride and cholesterol. This study aimed to examine the antihiperlipidemic activity of the combination *Guazuma ulmifolia* L. leaves extract (GuLE) and *Hibiscus sabdariffa* L. flowers extract (HsFE) in rats induced by high-fat diet. There are three combinations of extracts GuLE and HsFE, dose of 178.8 : 17.88; 59.6 : 53.64 and 119.2: 35.76 mg/kg body weight, and positive control using orlistat 0.9 mg/kg body weight. All combinations of extracts and positive control can reduce plasma concentrations of cholesterol ($P < 0.01$), triglycerides ($P < 0.01$), and LDL ($P < 0.01$). Only the combination of extracts GulE and HsFE dose of 119.2 : 35.76 mg/kg body weight increase significantly ($P < 0.05$) the plasma concentration of HDL. Furthermore, all combinations of extracts and positive control were able to lose body weight between 1.5% to 5%.

Keywords: antihyperlipidemic, *Guazuma ulmifolia*, leaf, *Hibiscus sabdariffa*, flower

Introduction

Hyperlipidemia is a lipid metabolism disorder characterized by increased plasma concentrations of triglyceride and cholesterol, which is a risk factor for cardiovascular disease. *Guazuma ulmifolia* L. leaves and *Hibiscus sabdariffa* L. flowers has been traditionally used to lose body weight and reduce body fat. A study showed that the extract of *Guazuma ulmifolia* L. leaves may decrease plasma concentration of cholesterol, and possibly through the mechanism of inhibition of HMG-CoA reductase, a key enzyme in cholesterol synthesis *de novo*. This leaves contain high levels of flavonoids and tannins [1], also contain other compounds, that is alkaloids, tannins, saponins, terpenoids, glycoside, and steroid [2]. *Hibiscus sabdariffa* L. flowers is a good inhibitor of pancreatic lipase. This flowers contain some of flavonoids that is anthocyanin, anthocyanidins and quercetin glycosides [3]. Combination of both extracts are expected to work synergistically to reduce the plasma concentration of triglyceride and cholesterol

1. Materials and Methods

Plant Material

The fresh *Guazuma ulmifolia* L. leaves and *Hibiscus sabdariffa* L. flowers were collected form "Taman Nasional Meru Betiri" Jember.

Preparation of Extracts

Powdered Guazuma ulmifolia L. leaves was macerated with 96% ethanol in maserator at room temperature. After 24 hours, it was filtered and the filtrate was concentrated in a vacuum using a rotary evaporator to obtain *Guazuma ulmifolia* L. leaves extract (GuLE). Powdered *Hibiscus sabdariffa* L. flowers was macerated with water at a temperature of 90 °C for 15 minutes, then it was cooled and filtered. The filtrate was concentrated using a freeze dryer to obtain *Hibiscus sabdariffa* L. flowers extract (HsFE).

Animals

Adult Wistar albino male rats, weighing 150-200 g were obtained from the institutional animal house. The animals were housed at room temperature for 7 days and were provided standard rat diet and water *ad libitum*.

High fat diet-induced hypelipidemia

The rats were fed with hyperlipidemia diet for 60 days to induce hyperlipidemic. The composition of hyperlipidemia diet was pellets (80%), lard (15%), and egg yolks (5%). The rats also were given propylthiouracil 0.01% in drinking water [4].

Experimental design

The rats were divided into five groups consisting of three animals each. The extracts and positive control orlistat were suspended in 0.1% w/v carboxymethyl cellulose (CMC) for oral administration.

Group I. Negative control or Hyperlipidemic control group, rats were administered with high fat diet for 60 days and continued for 10 days.

Group II. Positive control group, rats were administered with high fat diet for 60 days and the next 10 days accompanied by administration of Orlistat 0.9 mg/kg body weight.

Group III. The rats were administered with high fat diet for 60 days and the next 10 days accompanied by administration of combination of GuLE and HsFE extracts, dose of 178.8 : 17.88 mg/kg body weight.

Group IV. The rats were administered with high fat diet for 60 days and the next 10 days accompanied by administration of combination of GuLE and HsFE extracts, dose of 59.6 : 53.64 mg/kg body weight.

Group V. The rats were administered with high fat diet for 60 days and the next 10 days accompanied by administration of combination of GuLE and HsFE extracts, dose of 119.2: 35.76 mg/kg body weight.

The rats body weight were weighed at the days 1st, 7th, 15th, 30th, 45th, 60th, and 70th. The blood samples were collected from rats by retro orbital sinus puncture at the end of 60th day (pretreatment) and at the end of 70th day (posttreatment) of the experimental protocol, samples were immediately centrifuged and assayed. Plasma cholesterol, triglycerides, LDL and HDL were estimated using microlab-100®.

Statistical analysis

The data were statistical analized by Anova test and then by LSD test. Significance was accepted at $p \leq 0.05$.

Result

Administration of a high-fat diet and a 0.01% propylthiouracil in drinking water for 60 days increased the body weight of rats. Treatment with a combination of extracts GuLE and HsFE and also Orlistat for 10 days could reduce the rats body weight of of 1.5% - 5%. Group III showed the highest weight loss is 5% (Fig. 1).

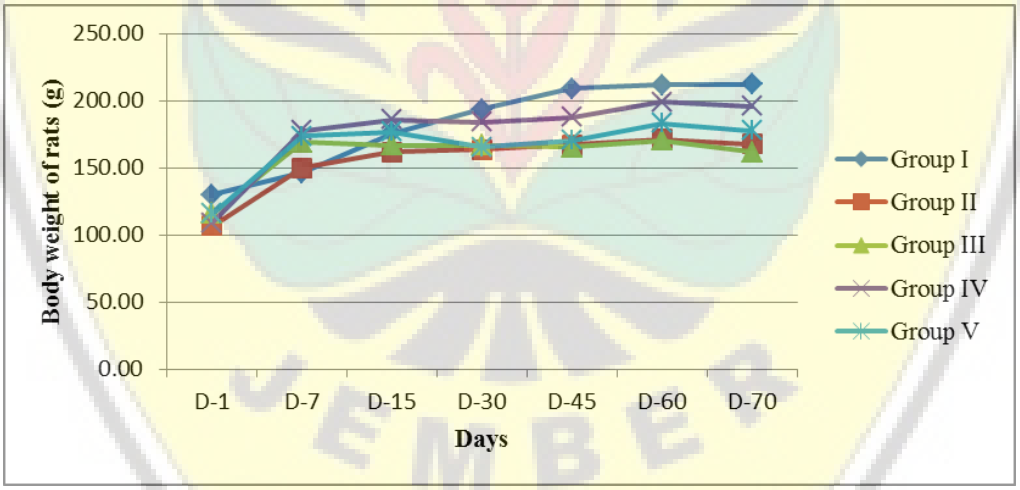

Figure 1. Perkembangan berat badan tikus

The treatment with all combination of GuLE and HsFE extracts and Orlistat showed significant reduction in plasma cholesterol (P<0,01) as compared with negative control (Fig. 2). Administration of all combination of GuLE and HsFE extracts and Orlistat also showed significant reduction (P<0,01) in plasma triglyceride and LDL (Fig. 3 and 4). Futher, only administration of combination of GuLE and HsFE extracts, dose of 119.2: 35.76 mg/kg body weight increased significantly (P <0.05) the plasma concentration of HDL (Fig. 5).

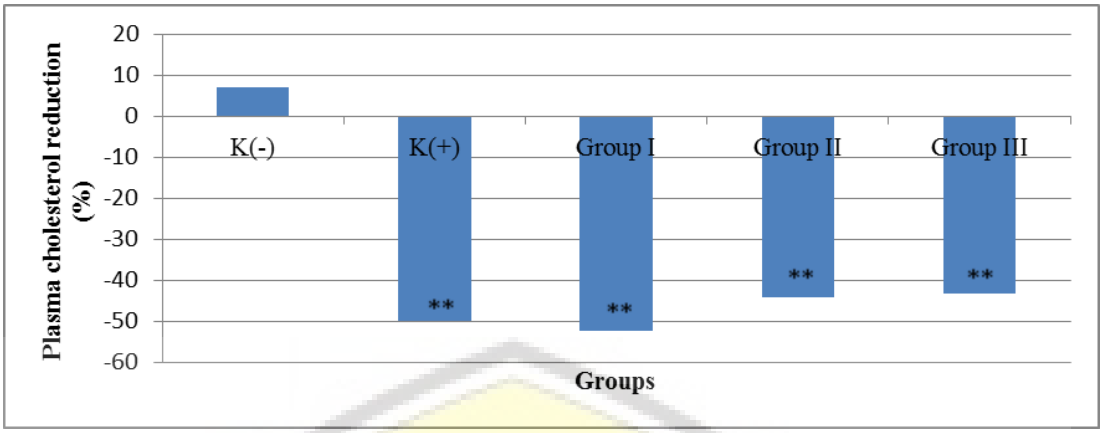

Figure 2. Effect of combination of GuLE and HsFE extracts on plasma cholesterol reduction. **P<0.01

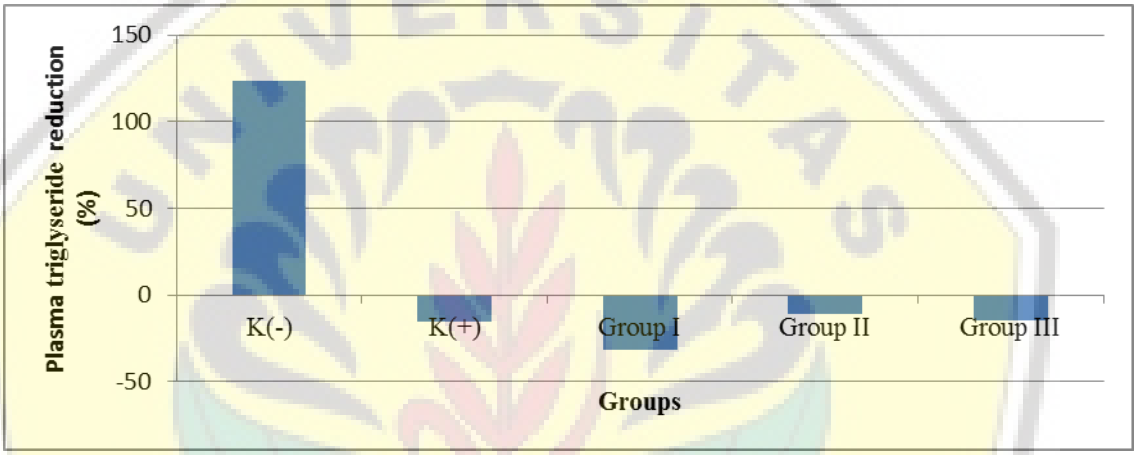

Figure 3. Effect of combination of GuLE and HsFE extracts on plasma triglyseride reduction. **P<0.01

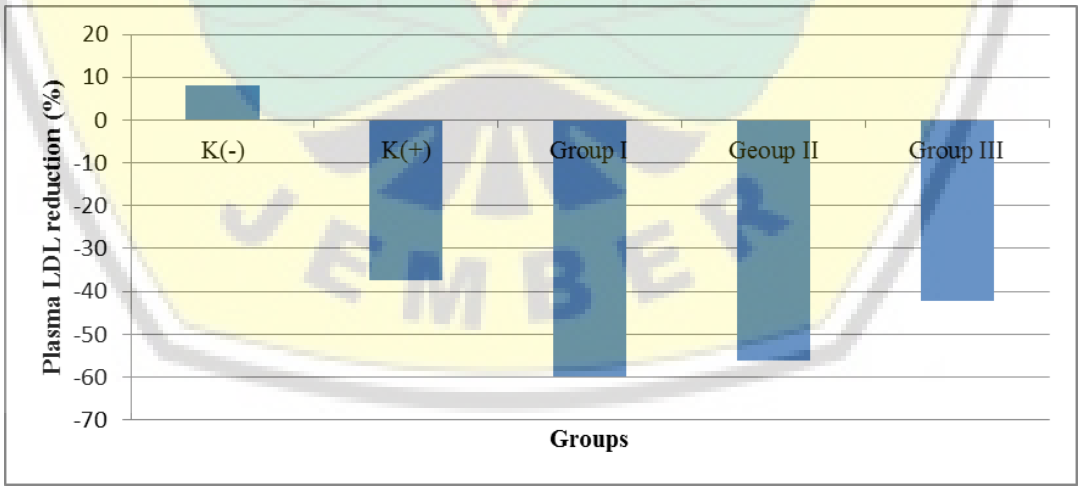

Figure 4. Effect of combination of GuLE and HsFE extracts on plasma LDL reduction. **P<0.01

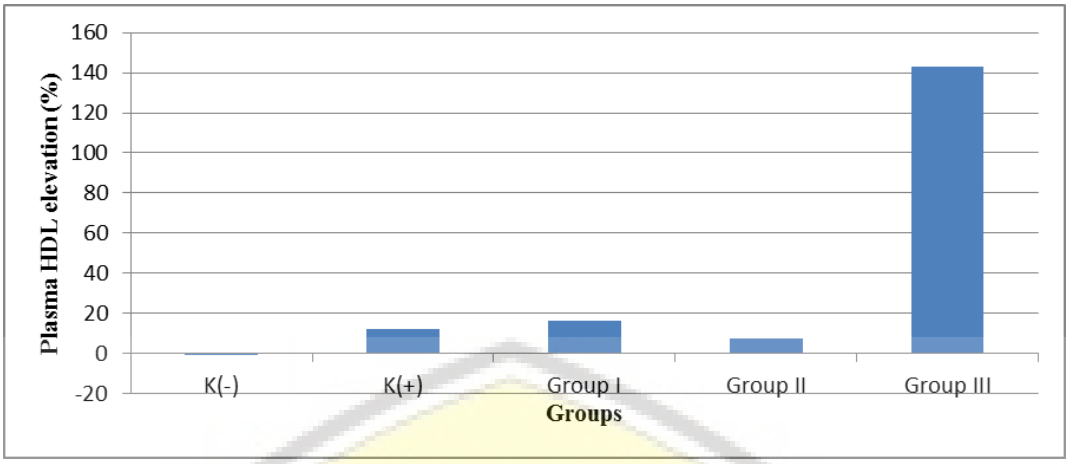

Figure 5. Effect of combination of GuLE and HsFE extracts on plasma LDL elevation. *P<0.05

Discussion

Hyperlipidemia is a condition of lipid levels, especially cholesterol and triglycerides, which rises above the normal range in the blood [5, 6, 7]. Hyperlipidemia include two categories, namely hypercholesterolemia characterized by elevated levels of cholesterol and hypertriglyceridemia characterized by elevated levels of triglycerides in the blood [8]. Hypercholesterolemia is a major cause of cardiovascular disease, as well as hypertriglyceridemia [9]. On the other hand, low plasma concentration of HDL is also responsible for coronary heart disease.

In this study, the effect of oral administration of combinations of extracts GuLE and HsFE, dose of 178.8 : 17.88; 59.6 : 53.64 and 119.2: 35.76 mg/kg body weight on experimentally induced hyperlipidemia in rats was investigated. All of this combinations demonstrated a significant reduction in the raised diet-induced levels of plasma concentration of cholesterol, triglyceride, and LDL. The lipids were absorbed in the gastrointestinal tract after hydrolyzed by pancreatic lipase into free fatty acids. Water extract of *Hibiscus sabdariffa* L. flowers showed the best antilipase activity among some Indonesian herbal anticholesterol [10]. Therefore, the triglycerides reduction by combinations of extracts GuLE and HsFE may be mediated by pancreatic lipase inhibition. *Guazuma ulmifolia* L. leaves contain flavonoid compounds, namely catechin, kaempherol, procyanidins and tiliroside [7]. A study showed that moreloflavon, a biflavonoid composed of naringenin and luteolin could inhibit HMG-CoA reductase. HMG-CoA reductase was a key enzyme in cholesterol synthesis *de novo*. The activity of HMG-CoA reductase was regulated by phosphorylation and dephosphorylation reactions. The phosphorylation of HMG-CoA reductase by c-AMP-dependent protein phosphokinase cause the enzyme was inactive. Coenzyme c-AMP was cleaved by c-AMP phosphodiesterase (c-AMP PDE), which could be inhibited by flavonoids. c-AMP PDE inhibition resulted the concentrations of c-AMP increased, phosphorylation of HMG-CoA reductase increased and cholesterol synthesis decreased [11]. Catechin, kaempherol, procyanidins and tiliroside in *Guazuma ulmifolia* L. leaves may be inhibited HMG-CoA reductase mediated by c-AMP PDE inhibition. Futhermore, only combinations of extracts GuLE and HsFE, dose of 119.2: 35.76 mg/kg body weight demonstrated a significant elevation of plasma concentration of HDL. This was also reduce risk coronary heart disease.

Conclusion

The combinations of extracts GuLE and HsFE, dose of 178.8 : 17.88; 59.6 : 53.64 and 119.2: 35.76 mg/kg body weight were able to reduce plasma concentrations of cholesterol, triglycerides, LDL, and were able to lose body weight. Only the combination of extracts GulE and HsFE dose of 119.2 : 35.76 mg/kg body weight increased significantly the plasma concentration of HDL.

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THE EFFECT of TOMATO (*Lycopersicum pyriforme*) EXTRACT on LIPID PEROXIDE and CELL DAMAGE of CARBONTETRACHLORIDE in RAT LIVER

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Abstract

Liver disease is the third in the world after aother tropic disease. CCl_4 will change free radical when in the cell, so it can damage cell liver when it stay in the liver. Tomato fruits has vitamin C, lcopene and β-caroten. Tomato juice can to prevent cell damage. It can prevent to increase of SGOT and SGPT on CCl_4 induction. But We didn't know that the extract of tomato coult prevent to increase cell damage and increase of MDA liver as result of induction by CCl_4. The research was conducted to study the effect of *tomato* extract in preventing liver cell damage caused by free radical produced in CCl_4 intoxication. Twenty four rats were randomly divided into 4 groups of 6 each. The duration of treatment was 7 days. Group I and IV served as positive and negative controls which given water 11 ml/kg/d. Group II and III respectively were given combination of vitamin C (8,19 mg/kg BW/day) and β-carotene 0,0798 mg/kg/d, and *tomato* extract (11 mg/kg/d). On day 8 , 055 mg coconut oil/g were given to group IV, while 0,55 mg/g CCl_4 were given to group I, II and III. Forty eight hours later all the rats we sacrificed. The liver was then removed to analyse the MDA level of the homogenate. Beside that, the liver were counted cell damage of 50 each and were counted every liver 5 times. The data collected were analized using analysis of variance and the defference were analysised by the least significant difference test (LSD) at 5 % level of confidence. The result showed that group I (rat is given CCl_4) had significantly highest level of the MDA, total of cell damage as compared to other groups (ie. Group II, III and IV). No significans difference were observed in the MDA levels and total cell damage between group III and IV, but no significans difference than goup II. Significans difference were observed in the MDA levels and total cell damage between gruop I and II. Dosis of tomato extract 11 gr/kg/BW/day is optimum dosis to preventive cell damage and lipid peroxide in liver rat what is induction by CCl_4.

Keywords : tomato, lipid peroxide, carbontetrachloride, lycopene, β-carotene

Introduction

One of cell damage is explanation by free radical. So that, human must prevent their cell from free radical. Free radical is product of what is built in reaction of process and metabolics of cell or from out of cell as CCl_4 [14].

One of materials of toxic chemistry what can built free radical is carbon tetraclorida (CCl_4). CCl_4 is chemistry materil in group of *chlorinated hydrocarbon.* Carbon tetraclorida by oral can cell damage in liver more seriously than another organ. In the body CCl_4 will product free radical and react with poly unsaturated fatty acid and will built lipid peroxide lipid in cell membran [1].

The over lipid peroxide will be affect to disorganization of cell membran as cause from change composition of polysaturated fatty acid fraction in phospolipid fraction so ratio poly saturated fatty aic and another fatty acid changed too. This condition will be cause to decrease the fluidity of mcell membran and the end it will give effect to structure and function of cell membran, and than cell will be dead.

In the body have some anti oxidant as enzim Superoxide Dismutase (SOD), catalase, and glutathion peroxidase (GSH Px) what could to prevent cell damage [2, 11, 14], and material from out of body what can prevent to free radical. The name is exogen antioxidant. Its include in this category are: vitamin E, vitamin C, beta-karoten dan vitamin A.

Tomato has vitamin C, beta-caroteen and lycopene. Extract of tomato can prevent to active metabolits $CCl_3\bullet$ or $CCl_3O_2\bullet$, so that covale band of $CCl_3\bullet$ or $CCl_3O_2\bullet$ with protein, lipid, and nucleotide no occur and no mutation so that damage of cell no occur. *Lycopene* is chemistry material in tomato. *Lycopene* can react with free radical and than free radical will stop to damage to cell. Lycopene is well than β–caroten as antioxidant [12].

The before study found that tomato has vitamin C and hight carotenoid. Biside of the study found that consumt extrac of tomato can prevent to increase SGOT and SGPT as consequence induction CCl_4. But We didn't know that the extract of tomato coult prevent to increase cell damage and increase of MDA liver as result of induction by CCl_4.

Purpose of study is: to study the effect of tomato *juice* in preventing liver cell damage caused by free radical produced in CCl_4 intoxication.

Methods

Study Design

Three step of study is:(1). Analysis duration of savings tomato to vitamin C and β-caroteen; (2). To found optimum dose of tomato as antioxidant in rat what is induce by CCl_4, (3). Comparative test between tomato extract with combination of vitamin C and β-caroteen Uji komparatif antar tomat dengan kombinasi vitamin C, dan β-karoten what has content equivalent with in tomato extract.

Material and Tool

Rat wistar age 3 months, 200 – 300 gram, CCl_4 , reagen KIT dari Roche, formalin 10%, alkohol 95%, alkohol absolut, xylol, toluena, parafin, entelan, Harris Hematoxylin and Eosin, Standar food standar konsentrat 511®, , spuit disposible syring 3 cc, scalpel, gunting, pot-pot plastik, tissue processor, tissue net, blok-blok jaringan, waterbath, microskop dan spekcrofotometer. Vitamin C, tomato, beta karoten and Reagen for MDA test.

Treatment of research consist of:

Group I as control (+) rat is not given treatment (only aquadest 11 ml/kg every day for a week and the eight day is given carbontetrachlorida 0,55 mg/g BW/day

Group II rat is given treatment combination vitamin C=8,19 g/kg BW/day and β-caroteen=0,0798 mg/kg BW/day and its given aquades untill 21 ml for a week and the eight day its given CCl_4 0,55 mg/g BW.

Group III rat is given treatment extract of tomato to rat 11 g/kg/day, for a week , and the eight day its given CCl_4 0,55 mg/g BW.

Group IV as control (-) rat is not given treatment (only aquadest 11 ml/kg every day for a week and the eight day is given oil of coconut 0,55 mg/g BW.

Malondialdehyde Assay

The MDA assay provides in vitro estimates of PUFA peroxidation. Determinations of MDA were made on freshly thawed serum by a modification of the Yagi method at the Genox Corporation. The serum sample was incubated for a hour at 95° C with thiobarbituric acid, after with a thiobarbituric acid MDA adduct was measured by absortion at530 nm. A standard curve for absorption and MDA concentration was generated, from lipid peroxidation was reported as micromoles of MDA equivalents. The run-to-run CV for the MDA assay at Genox was 6.5%.

Histology and immunohistochemistry.

Fromalin-fixed tissue samples were embedded in parafin and 5 µm section were cut. Replicate section were stained with hematoxylin and eosin (H and E) for evaluation of damage cell. All section were obtain from the left lateral lobe. The damage cell was estimated by evaluating the number of microscopic. Indicator of histological subject counted total of damage cell in every 50 cell.

Statistical analyses

Effect of tomato extract to decrease MDA and total of damage cell are examinated by histopatologic. Test ANOVA is used to know there are different or not, and than test LSD to know where are the treatment different.

Result

Tomato extract , MDA Test and Damage Cell

Table 3.1. Avereage of MDA Liver

Treatment	MDA Liver (ppm)						
	1	**2**	**3**	**4**	**5**	**6**	**rerata**
control (+)	0,716	0,695	0,750	0,710	0,710	0,720	**0.717 (a)**
Vit C + β-karoten	0,641	0,568	0,650	0,656	0,680	0,650	**0,641 (b)**
11 gr/kg/BW	0,426	0,474	0,410	0,425	0,509	0,490	**0,456 (c)**
control (-)	0,456	0,577	0,482	0,420	0,500	0,470	**0,484 (c)**

Keterangan : average what is followed the same letter show that no difference (p< 0,05)

Data of MDA liver show at table 3.1 and avereage of damage cell show attable 3.2

Tabel 3. 2 Average of damage cell of Liver in every 50 cell

Treatment	Average of Cell Damage						
	1	**2**	**3**	**4**	**5**	**6**	**Rerata**
Kontrol (+)	18,20	20,00	18,80	19,10	19,60	18,00	**18,95 (a)**
Vit C + β-karoten	12,10	12,50	12,20	11,90	11,80	12,40	**12,15 (b)**
11 gr/kg/BW	1,40	2,10	1,50	2,00	1,20	2,10	**1,72 (c)**
Kontrol (-)	1,00	2,80	1,80	2,10	2,50	2,20	**2,10 (c)**

Keterangan : average what is followed the same letter show that no difference ($p < 0,05$)

Discussion

Purpose this research to study the effect of tomato *juice* in preventing liver cell damage caused by free radical produced in CCl_4 intoxication. Beside that, to know difference level of effectivity of tomato juice to prevent hepatotoxic than to give combination vitamin C and beta caroteen in CCl_4 intoxication.

Carbon tetrachloride will be break down to become CCl_3• + Cl. CCl_3• will fuse with oxygen to built CCl_3O_2• (*trichlorometil peroxil*) . CCl3O2• is very reactive to bio mollecular as protein, lipid, carbohydrate and nucleotida. Carbon tetrachloride (CCl_4) is chemistry material what can affect damage cell in liver than another organ [19]. That is equal this research in before step. It show that 48 hour after induction CCl_4 sebanyak 0,55 gr/kg/BW/day by oral to affect to damage cell. It show MDA liver = 0.712 ppm, and total of damage cell in liver = 19,14 (38,28 %), in treatment what is given CCl_4.

The low of MDA liver in giving combination of β-karoten 0,0798 mg/kg/day + vitamin C 8,17 mg/kg/day because two material as antioxidant to oxidant (free radical) product of CCl_4 degradation in liver cell. Carbontetracorida will be degradation become CCl_3• + Cl. CCl_3• will be reacted with oxigen to built CCl_3O_2• (*trichlorometil peroxil*) what will reacted than CCl_3•. CCl_3O_2• is ver reactive to biomolecul as protein, lipid, carbohydrat and nucleotida. The end function biomolecular of biologics will disturbed. Free radical of CCl_3O_2• in liver cell will react with Poly Unsatured Fatty Acid (*PUFA*) to built lipid peroxide (Harahap IP., *dkk*, 1995; Timbrell J, 1994). Malondaildehyde (MDA) is aldehyde what product of process to cut a chain (*PUFA*) and it can use to determine level lipid peroxide.

No lipid peroxide will effect integrity of cell membrane no disturbed. But if integrity of cell membrane is disturbed so that enzyme GOT and GPT will go out . The damage of cell membrane Sebaliknya will effect to degeneration picnotis. It will not be happened if in the cell has vitamin C β-karoten and lycopene. Cell is normal

In giving juice of tomato 11 gr/kg BW/day for a week before CCl_4 induction has MDA lower than treatment combination β-caroteen and vitamin C , especially than rats without antioxidant, because in the juice of tomato has β-caroteen, vitamin C and lycopene. Lycopene can react with free radical to stop disturbed cell. To prevent the free radical, lycopene is well than β-caroteen (Giovannucci E., 1999). In step I of this reserach found another β-caroteen in tomato. Its lycopene.

The level of damage cell in giving juice of tomato 11 gr/kg BW/day for a week before CCl_4 induction, karena because in the juice of tomato has vitamin C, β-caroteen and lycopene what is optimal to prevent free radical from CCl_4 in the liver. After entrance in cell Carbontetrakcloride will be degradation to become CCl_3• + Cl. Free radical of CCl_3• will react with oxygen soon and will biult CCl_3O_2• (*trichlorometil peroxil*). CCl_3O_2• has very reeactive to biomolecul of protein, lipid, carbohydrat and nucleotida. Free radical of CCl_3O_2• in the liver will be react with Poly Unsaturated Fatty Acid (*PUFA*) to built lipid peroxide [15, 18].

Catching to free radical of CCl_3• and CCl_3O_2• by vitamin C, β-caroteen and lycopene in the liver cell will because no disturbed to biologics function of protein, lipid and Poly Unsaturated Fatty Acid *UFA*) no disturbed, so lipid peroxide no built too. No built of lipid peroxide will because integrity of cell membran no disturbed, so that the liver cell will be normal.

Conclusion

Tomato extract could to preventive cell damage and lipid peroxide in liver by activity of licopene, β-carotene and vitamin C. Tomato juice is well than combination of β-carotene and vitamin C to preventive cell damage and lipid peroxide in liver rat what is induction by CCl_4. Dosis of tomato juice 11 gr/kg/BW/day is optimum dose to preventive cell damage and lipid peroxide in liver rat what is induction by CCl_4.

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THE ROLE of WATER CLOVER (*Marsilia crenata*) on ESTROGEN LEVEL and UTERINE HISTOLOGY in RATS (*Rattus norvegicus*)

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Abstract

Water clover (*Marsilea crenata*) is contained with isoflavones, shown to mimic the role of the female hormone estrogen. Isoflavones binding to estrogen receptors that produce beneficial effects. However, research on the optimal dose of water clover use is still not much studied. The purpose of this study was to determine the effects of water clover extract to estrogen and how they affect the histology of uterine. This study used 30 female Wistar Rats (*Ratus norvegicus*) divided into six groups. Group I (P1) as a negative control group, group II (P2) as a positive control group. Group III, Group IV, Group V and Group VI (P3, P4, P5, P6) were given extract water clover concentrations given as follow (6.25%, 12.5%, 25%, 50%). The results was analized with one way ANOVA showed not significant ($p > 0.05$) between group, eventhough group P3, P4, P5 and P6 produces estrogen that is higher than the negative control (P1) and a positive control (P2). Histological features of the uterus in the positive control group showed endometrial lining thicker than the negative control group. Histology of the uterus in the treatment group P4, P5 and P6 are also seen endometrium thickening as in group P3. This research has proved that water clover extract consumption show a promising replacement estrogen hormone theraphy in the future. However there was needed a much futher research in order to found a proper dose for human consumption.

Keyword: Water Clover (*Marsilia crenata*), estrogen, uterine histology

Introduction

Water clover (*Marsilea crenata*) is a group of plants salviniales, living wild in aquatic environments such as ponds, paddy fields, lakes, and marshes. Water leaf clover round and consist of four strands child leaves [1]. Plant part used is the leaves and stalks are consumed by steaming. Many nutrients contained in clover, make it credible as a highly nutritious plant. According [10] which are as fever, cure high blood pressure and shortness of breath, a good remedy for people who snore and can be used as a gargle for sore throat pain sufferers.

[23] states that fresh clover plant content phytochemical that are reducing sugar, steroid, carbohydrate, and flavonoids. Flavonoids also have a function as an antibacterial, anti-inflammatory, antitumor, allergenic, and prevent osteoporosis. At this time the research on water clover is still not a done deal. One of the important information that the public is not known for their ability to increase estrogen levels in the body. Activity estrogenic isoflavones have been linked with a chemical structure similar to stilbesterol, which is used as an estrogenic drug [3]. Isoflavones can activate estrogen receptors in mammals, so it is often called isoflavone phytoestrogens [12].

In humans isoflavone compounds are widely used as medical action in postmenopausal women without any side effects. Menopause, ovariectomy, and reproductive system disorders (amenorrhea) can affect target cells resulting in decreased levels of estrogen in the body. Declining levels of estrogen can cause interference with urogenital system, cardiovascular disease, and osteoporosis [14]. In women hormone replacement therapy is often done. Hormone replacement therapy, among others, can be done with a synthetic hormone estrogen. However, the granting of these hormones in the long term have a variety of side effects, such as breast pain, vaginal bleeding, and trigger breast cancer [21].

Water clover is contained with isoflavones, shown to mimic the role of the female hormone estrogen. If the body to consume isoflavones, there will be the effect of binding isoflavones to estrogen receptors that produce beneficial effects, thereby reducing the symptoms of menopause, clover will reduce the clinical symptoms that appear before and while entering the stage of menopause and to improve the quality of bone to avoid osteoporosis [13]. However, research on the optimal dose of water clover use is still not much studied, it would require a study of estrogenic activity with various doses of water clover. The purpose of this study was to determine the effects of water clover extract to estrogen and how they affect the histology of uterine.

Materials and Methods

Materials

Water clover (*Marsilea crenata*) purchased from the local market in Sidoarjo, East Java, Indonesia. Rats supplied by the laboratory of molecular

biology, faculty of mathematics and natural sciences, University of Brawijaya. Phosphate buffered saline (PBS), Mycroginon (synthetic estrogen), ELISA Kit estradiol, Sephadex Gel for the separation of the isoflavone derivatives, histological Colouring agent for soft tissue, hormones PGF2α as estrus synchronization experimental animals.

Preparation *Marsilea crenata*

Water clover (*Marsilea crenata*) were analyzed by an independent institution with a certificate of determination by Materia Medica. Leaf clover is dried by the sun, dried using sunlight to avoid damage to the composition. Then mashed into simplicia use dishmeal, in order to obtain water clover leaf meal (WCLM) amount of 1 kg. Stock solution of WCLM (100%) the extraction of N Hexana of 1 kg of crude drugs. Stock dilution solution (100%) into a solution treatment (P3, P4, P5, P6) WCLM solution with a concentration of 6.25%, 12.5%, 25%, 50%.

Animal experiments

This study used 30 female Wistar Rats (Ratus norvegicus) 2 months old, 100-150 grams, divided into six groups each group consisting of 5 rats. Group I (P1) as a control group, rats given Phosphate buffered saline (PBS) orally. Group II (P2) as a positive control group, rats given Mycrogynon orally. Group III, Group IV, Group V and Group VI (P3, P4, P5, P6) is the treatment group were given water clover with different concentrations which 6.25%, 12.5%, 25%, 50%. All groups were injected PGF2 α for estrus synchronization before experimental. The treatment group, clover leaf extract water according to the concentration is given orally 2 ml per rats, every day for 14 days. the last period of the study, the animals were sacrificed in compliance standard Research Ethics guidelines. Necropsy performed to take a sample of uterine organs and blood samples. The whole animal has been in compliance with the guidelines declared by instutional animal care and use commite.

Data analysis

Blood estrogen hormone profile was observed quantitatively by using SPSS 16.0 for Windows with statistical analysis one way ANOVA. If there is a real difference multiple comparison test followed by Turkey test with $\alpha = 0.05$. Results of histological preparations ovaries were observed using a microscope with a comparison between control and treatment groups.

Results and Discussion

Hormone estrogen level

Levels of the hormone estrogen group I (P1) is a group of negative control, mean levels of estrogen is the lowest among the five groups other treatments 231.67±34.06 pg/ml, according to research conducted by [17] estrogen levels in normal rats is between 132-140 pg/ml of blood. Group II (P2) showed the average levels of the hormone estrogen amounted to 396.00±76.71 pg/ml. P2 group is a positive control group were given microginon orally. Microgynon is a synthetic estrogen hormone compounds are often used for hormone replacement therapy, containing 0.15 mg Levonorgestrel and 0.03 mg ethinylestradiol 7. In general microgynon also used in oral contraceptives, treatment of dysmenorrhea, irregular menstrual cycles, endometriosis, and hormonal sterility (ISO 2012). Estrogen levels in the treatment group giving water clover that Group III, Group IV, Group V, and Group VI (P3, P4, P5 and P6) sequentially which is 402.00±133.55 pg/ml, 408.33±176.69 pg/ml, 452.67±205.73 pg/ml, 394.33±191.11 pg/ml Table 1.

Table 1. the Result of hormone estrogen level (pg/ml)

Group	Estrogen (pg/ml) Mean±SD
P1 (Control)	231.67 ± 34.06
P2 (microgynon)	396.00 ± 76.71
P3 (6.25% extract)	402.00 ± 133.55
P4 (12.5% extract)	408.33 ± 176.69
P5 (25% extract)	452.67 ± 205.73
P6 (50% extract)	394.33 ± 191.11

Table 1. shows that the administration of clover extract with various doses to groups of P3, P4, P5 and P6 produces estrogen that is higher than the negative control (P1) and a positive control (P2). The test results in estrogen levels then performed statistical analysis using one way ANOVA and obtain measurement results are not significant ($p > 0.05$) between the treatment groups. When compared with the blood serum levels of rat model of menopause which showed the average 40.73 pg/ml [22], then the estrogen levels in this study was much higher up to 10 times ranged at 394.33 to 452.67 pg/ml. This means the water clover extract can be a substitute for hormone replacement estrogen promising. As research conducted by [16] that the clover extract 0.8 g plus 10 mg once daily of vitamin B1 can significantly increase the concentration of estrogen in postmenopausal women and delay the increase in the imbalance of bone remodeling in postmenopausal women.

The content of phytoestrogens in clover plant leaves has been investigated by [15] showed that the estrogen content of the juice of fresh clover was 538.0 pg/g, while the dried clover increased to 1068.0 pg/g. As phytoestrogens, isoflavones in the

plant has two important effects. First, when estrogen levels are high, phytoestrogens can stop the more potent form of estrogen produced by the body (by blocking the estrogen receptor) and can help prevent diseases driven by hormones, such as breast cancer. Secondly, when estrogen levels are low, such as in a state after menopause, phytoestrogens can replace the body's own estrogen, which can reduce hot flashes and protect bones.

Isoflavones are phytoestrogens that are part of this has important functions in the defense mechanisms of plants. Isoflavones are the active substances that contain estrogen hormone from plant material, water clover isoflavones contained shown to emulate the role of the female hormone estrogen. Estrogen binds to estrogen receptors as part of hormonal activity, causing a series of reactions that benefit the body. Phytoestrogens role in stabilizing the hormonal functions, namely by inhibiting the excessive estrogen activity that can induce cancer and also can substitute when the estrogen levels in the body is low. Phytoestrogens have a normalizing effect of hormones, not only inhibits the absorption of excessive estrogen, but also able to increase estrogen levels in women who are low.

Isoflavones bind to estrogen receptors in these organs, although less potent than estradiol 17β but at higher levels and recurrent circulation can cause potential effects. The estrogen receptor is blocked by phytoestrogens and can not be occupied by estrogen. Phytoestrogens after binding to the estrogen receptor, will cause a relatively weak estrogenic activity [19]. Although affinity for estrogen receptors, but not as high estradiol is able to cause estrogenic effects of phytoestrogens [18].

Uterine histology

Phytoestrogen affected organs include the ovaries, uterus, testis, prostate, and several other organs [19]. According to [4] uterus divided into 3 parts, which is the outermost layer (perimetrium), the middle layer (myometrium), and the mucosa lining (endometrium). Histology of the uterus in the negative control group showed endometrial lining inactive and compact containing blood vessels and connective tissue cells such as fibroblasts, macrophages and mast cells (Figure 1.A). Mucosa lining of the uterus (endometrium) in the inactive state, especially on the endometrial tissue, merely consists of tubular glands, fibroblastic stroma dense and thin blood vessels normally occur during anestrus or before puberty. After menopause at the age of non-productive animals are found lining mucosa of the uterus (endometrium) also experienced a state of inactive proved to be a process of proliferation and secretion, thinner form, and often accompanied by a cyst in the abdominal cavity with flattened or cuboidal cells, as well as the presence of fibrotic stroma.

Histological features of the uterus in the positive control group showed endometrial lining thicker than the negative control group. Histological features of treatment group showed superficial layers form at the functional zones consisting of loose connective tissue. During estrus would appear a large cavities were irregular were lack of fluid between cells in the functional zone called endometrial edema (Figure 1.B). This is consistent with [20] which says that the estrus phase were also called follicular phase, will have an enlarged uterus and swollen due to the accumulation of fluid under the influence of estrogen.

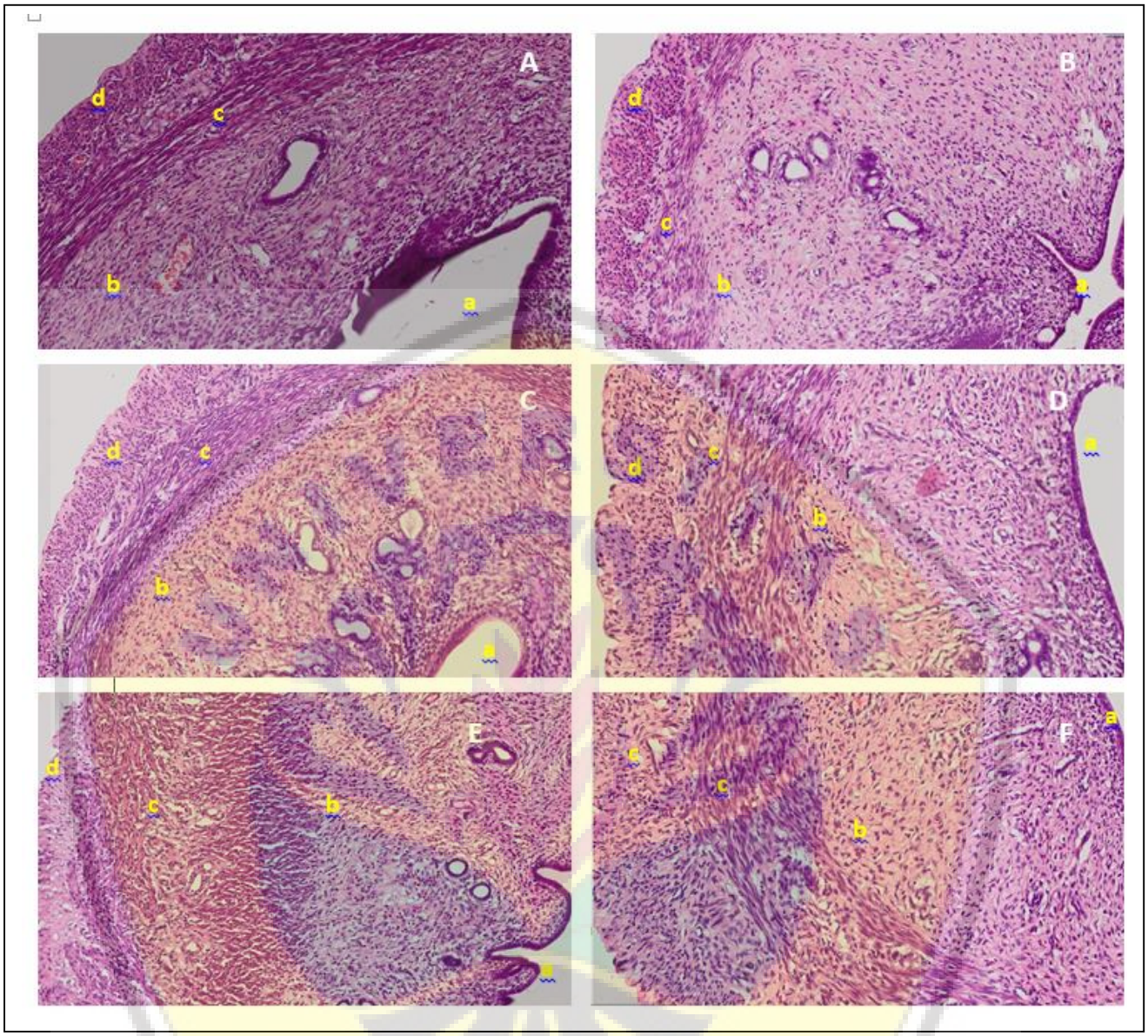

Figure 1. Photomicrograph uterine of rat (A) normal-control negative group; (B) microgynone-control positive group; (C) 6,25% extract-treatment group; (D) 12,5% extract-treatment group; (E) 25% extract-treatment group; (F) 50% extract-treatment group. (a) uterine lumen, (b) uterine glands, (c) longitudinal muscle layers (d) tunica serosa (H & E staining, 400x).

Uterine glands on histology also look wider, according [4] an increase in the content of estrogen in the blood can stimulate the growth of uterine glands.

Histological uterus is changed according to reproductive status, because of the influence of reproductive hormones such as progesterone and estrogen. Visible wall thickening of the endometrium or endometrial dilution according to estrus phase. Histology of the uterus in the treatment group P4, and P5 are also seen endometrium thickening as in group P3. In the estrus phase which occurred in the treatment group showed an increase of endometrium and myometrium mass in the form of hyperplasia and hypertrophy [11, 21]. Thus increased levels of estrogen in the estrus phase will also affect the weight of the uterus and ovaries. This shows that the water clover can increase uterine that seen from the changes in histology and this is also confirmed by examination of blood serum estrogen. Research [5] using soy beans that also has uterothrophic activity of phytoestrogens also seen an increase in the uterus mass.

Estrogen has two types of receptors that are estrogen receptor alpha (REα) and beta (REβ). α receptors present in organs ovary, breast, uterus, testis, pituitary, kidneys, epididymis, and adrenal whereas β receptors are found in organ ovary [6]. Phytoestrogen although not a hormone, but because its structure similar to estradiol may also occupying estrogen receptors and capable of causing the withdrawal effects of endogenous estrogen itself [9]. Phytoestrogens have a chemical structure similar to 17βestradiol, that can bind to the estrogen receptor that are ERα and ERβ. Phytoestrogens binding affinity to both receptors are not the same, phytoestrogens greater affinity for ERβ compared to ERα [19].

In the ovary, estrogen will occupying estrogen receptors α and β, while in the uterus will occupy the estrogen receptor α so that the ovaries and uterus will occurs a proliferation. This proliferration would lead to organ becomes heavier weights. The hormone estrogen causes development and the maintain secondary sex signs in women, such as breast, and are also involved in the thickening of the endometrium as well as in regulating the menstrual cycle. [7] suggest that estrogen causes a significant change in endometrial glands and consequently the size of the uterus increase two to three times more than before puberty.

Conclution

This research has proved that water clover extract consumption show a promising replacement estrogen hormone theraphy in the future. However there was needed a much futher research in order to found a proper dose for human consumption.

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EFFECT of CADMIUM (CD) ACCUMULATION on PROTEIN LEVELS of GILLS and KIDNEYS FRESHWATER MUSSEL *Elongaria orientalis* (LEA, 1840)

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Abstract

Cadmium (Cd) is wide-spread metals in aquatic ecosystem. It is one of non-essensial metals and toxic in low concentration. Aquatic animals uptake Cd through gills and digestive tract. Mussels are aquatic organism that capable to accumulate metals in their body. Cd in body interfere cell metabolism. Protein is an important macromolecule in metabolism process. The purpose of this research were to know the effects of Cd exposure on protein levels of gills and kidneys freshwater mussel *Elongaria orientalis*. Mussels are exposed Cd via water (20 µg L^{-1}) for 24 days and 12 days of depuration. Mussels were dissected into gills and kidneys on day 0, 1, 6, 12, 18, 24 (exposure) and day 30, 36 (depuration). Cd concentration was determined by using Atomic Absorbance Spectrophotometer (AAS), while protein levels were determined by Bradford methods. The result showed that pattern of Cd accumulation attend to increase in both of tissues within exposure time and attend to decrease within depuration time. Protein levels decrease during exposure time and attend to increase during depuration time.

Keywords: accumulation, cadmium, protein, freshwater mussel

Introduction

Cadmium (Cd) is an important heavy metal in electroplating, textile, plastic, and battery industries [2, 12]. Cd is non-essential metal, non-degradable, and toxic at low concentration [10,14]. Cd pollution in the freshwater ecosystem from the usage of Cd in many industrial activities will suffer biological system of aquatic orgasnism [8].

Freshwater mussels are an important organism in aquatic ecosystems [4]. As filter feeder, they are known to accumulate metals by direct transport of water across gills, and from ingestion of suspended particles and bottom sediments. They are sedentary, long-lived, widely distributed and tolerant of high trace metal concentration [1, 5, 15]. Metal accumulation in mussels can reflect the pollution status over long time periods, making them useful for biomonitoring [8, 15]. Cd in body is able to bind with -SH (sulfuhidril) groups on proteins, so it can interfere protein activities in various metabolic processes [9]. Proteins have an important role in biological processes in organism, such as enzymes that catalyzing biochemical reaction, transport and storage of molecules in and out or within cells, and have structural and mechanical function [8].

Physiological and biochemical responses by the organism are an initial parameter that can be used to determine the toxicity of a compound in the body [12]. Changes in the physiological response of an organism can affect the development and sustainability population of a species.

Gills and kidneys are vital organ in body. Gills play a role in respiration process, while kidneys play a role in excretion process [2]. This research used freshwater mussels (*Elongaria orientalis* Lea, 1840) as a model organism to investigate protein levels as one of the physiological responses of freshwater mussels to Cd exposure. The purposes of this research are to know the pattern of Cd accumulation and its effect on protein levels in gills and kidneys of freshwater mussel.

Materials and methods

Organism

About 60 freshwater mussels from unpolluted river, with shell lengths of 10-12 cm and weight of 100-200 g are bought to the laboratory and placed in 50 L aerated water in 100 L glass aquaria. During this period they are not fed (14 days); every day, half of the water is exchanged.

Experiment design

48 mussels are selected to match in size and divided into two groups consisting 24 mussels each. They are placed in two 100 L glass aquaria containing 50 L artificial pond water (APW). Two-third of the water is exchanged every second day; a complete change is conducted in every sixth days. A control group (1) is kept in APW. Another group (2) is exposed to 20 µg/L $CdSO_4$ (exposure period). Both of groups are fed with pellet (free Cd). On the day 24, the six mussels remaining in each group are transferred to APW (without Cd) for 12 days of depuration. For sampling, three mussels of each group are taken for analysis at 0, 1, 6, 12, 18, 24 (exposure) and at day 30 and 36 (depuration). The

mussels are dissected on ice into gills and kidneys. The tissues are washed with bidistilated water, dried using filter paper, placed in aluminum foil and weighed to obtain the wet weight (ww) and placed in oven (70^{o}C) until the weight constant and weighed again for dry weight (dw). For protein analysis, 0,1 gram of tissues are placed in 2mL micro tube at 80^{o}C [8, with modified]. Treatment of mussels conducted at Ecology and Conservation Laboratory, Biology Faculty, UGM in September-March 2014

Metal analysis

The dry samples (gills and kidneys) placed in furnish at a temperature of 700^{0}C for 3 hours of heating. Each sample is placed in 100 mL erlenmeyer glass. 5mL of mixture of (4:1) HNO3 (65%) and HCl (37%) are added to each glass. Samples were heated in hot plate (250^{o}C) until the solution becomes pure. The digested samples are diluted with bidistilated water to 10 mL. Cd concentrations are determined by Atomic Absorbance Spectrophotometer (AAS).

Protein analysis

Each 0.1 grams of sample was added by mixture of 25 mL lisozyme (50 mM) and 400 mL Tris-HCl buffer (50 mM, pH 7.5, containing protease inhibitor cocktail). Samples were centrifuged (4^{o}C,10.000 rpm) for 30 minutes. Protein levels were determined by Bradford methods. Each of 40 mL of supernatant was added with 1.000 mL Bradford solution. Absorbance of the sample was read at 595 nm with spectrophotometer. The concentrations were determined using bovine serum albumin as standard solution.

Data analysis

Data for total Cd are statistically evaluated by two-way analysis of variance (ANOVA). If significant differences are found, between exposures time are tested by Dunnet multiple comparison tests, between exposure pathways and controls using independent t-test.

Result and Discussion

Cadmium accumulation

Accumulation of cadmium (Cd) in the gills and kidneys of *Elongaria orientalis* are presented in Fig. 1. The pattern of accumulation in both tissues attends to increase within exposure time and attend to decrease within depuration time. The highest concentration of Cd in kidneys is reached 6,8-fold from initial concentration within exposure time (day 18), whereas the accumulation of Cd in gills are reached 15-fold from initial concentration in the end of exposure time (day 24). Cd enters the body through water or contaminated food. Cd accumulation in gills will be increased, and then passed into the digestive tract and process to the kidneys for elimination [2]. Gills are the site of action for heavy metal. It related with the function of gills as an organ of respiration. Gills are composed of branchial epithelium which is the place for active and passive transport in gas exchange, ion regulation, and maintain the acid-base balance. The large surface of gills enhances absorption of the metal [2, 13]. According to [5], the main target tissue of Cd exposure is kidneys. Kidneys are the the primary site of action of elimination process. Cd in the circulation system will undergo a process of filtration in the kidneys glomerolus and re-absorption process further in proximal tubular cells, and accumulate in lysosomes.

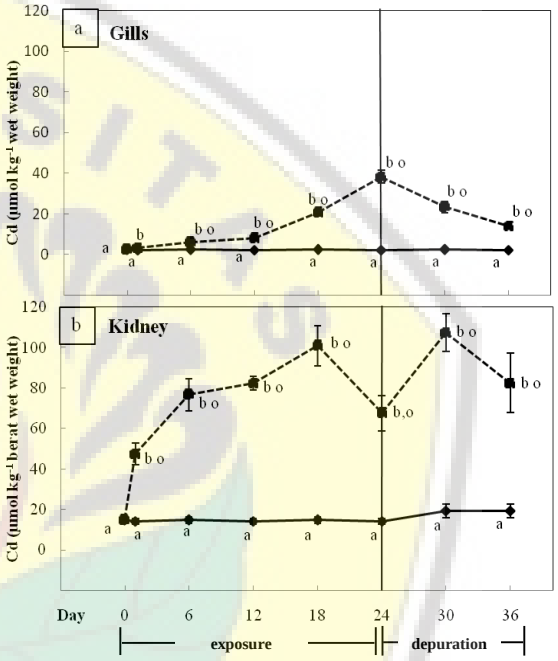

Figure 1. Cd accumulation in *Elongaria orientalis* : (a) gills; (b) kidneys (♦ = control group; ■ = treatment group). Significant differences in comparison to control within each group are indicated by º. The same letter indicates that differences of Cd concentration are not significant among groups at each time sampling.

Upon depuration, Cd accumulation in gills attends to decrease. According to [7], Cd concentration in kidneys is relative constant during depuration. As an excretion organ, kidneys is the place of Cd collection from various circulation pathways in body. It causes the Cd accumulation in kidneys increase on day 6 of depuration (day 30). At the end of depuration (day 36), although Cd accumulation in both of organ attend to decrease, but in gills and kidneys still remain Cd accumulation about 4-fold and 3,7-fold from initial concentration.

It means that *Elongaria orientalis* have capability to eliminate Cd, but the rate of elimination processes is slow. The rate of elimination is depending on the release of bond between the metal and the organ. Slow elimination is caused by the strong bond between the metal with the organ [8].

The slow rate of elimination due to strong bonds between Cd and -SH group making it difficult to break this bond during depuration. Metallothionein complex bond with cysteine residues (-SH) in lysosomes can reduce the toxicity of the metal and prevent metal interaction with cellular metabolism [13]. Comparison of absorption and elimination rate of Cd in gills and kidneys *Elongaria orientalis* showed in Table 1 and Table 2.

Table 1. Absorption rate of Cd in gills and kidneys *Elongaria orientalis*

No	Tissue	Absorption rate day- (µmol kg^{-1} ww per day)				
		1	6	12	18	24
1	Kidneys	32,87	5,81	0,97	3,09	5,58
2	Gills	3,37	0,54	0,32	2,10	2,96

Tabel 2. Elimination rate of Cd in gills and kidneys *Elongaria orientalis*

No	Tissue	Elimination rate (µmol kg^{-1} ww per day)	
		Day 6 depuration	Day 12 depuration
1	Kidneys	(8,70)	6,21
2	Gills	2,48	1,55

Effect of Cd accumulation on protein levels

Cd has long-half time, non-essential and non-dagradable material. These characteristics causes Cd accumulate in body easily and disturb many metabolic activities [2]. Cd in the body is able to bind with the sulfur and carboxyl group on a protein that inhibits the action of enzymes in the metabolic process [9]. Effect Cd on total protein levels in gills and kidneys of freshwater mussels *Elongaria orientalis* can be seen in Fig. 2.

Fig. 2 showed that total protein levels attend to decrease within exposure time. The decrease of protein levels has reported in *Mytilus edulis* after exposure to Cd (200 g / L) for 21 days [3, 11]. As long exposure time of toxicant in body cause increase of energy usage as respon to detoxify toxicant. Inspite of carbohydrate, protein is second macromoleculs that can serve energy. The high activity in protein degradation cause high rate catabolic procces to degrade protein in organ for energy harvesting. It cause decrease protein levels in organ.

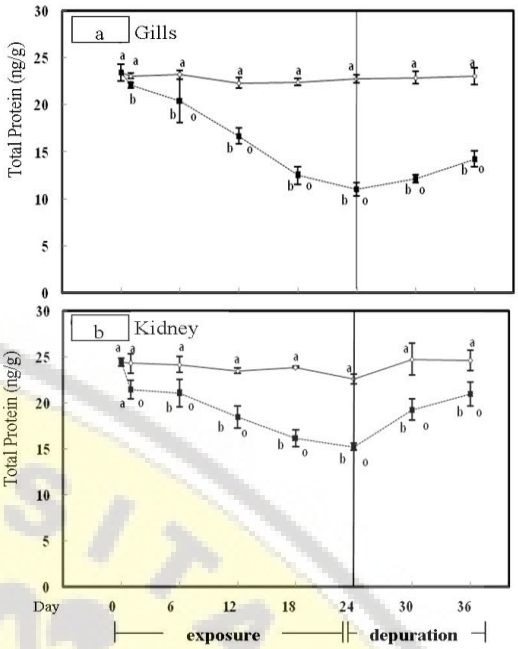

Figure 2. Total protein levels in *Elongaria orientalis* : (a) gills; (b) kidneys (Ket: ◊ = control group; ■ = treatment group). Significant differences in comparison to control within each group are indicated by °. The same letter indicates that differences of Cd concentration are not significant among groups at each time sampling.

Toxic metals such as Cd appear to use the transport pathways that exist for biologically essential metals. The transport of Cd does not require energy, therefore is not an active transport. It occurs by ion channels and carriers that involve interaction with sulfhydril groups. These processes apparently exist for the transport of essential metal such as calcium, copper, and zinc. Once inside the cell, Cd ions can interfere with the cell metabolism by mimicking the action of other divalent cations (especially of calcium) that are employed in activating or inhibiting the action of various enzyme [16]. Inhibition of various enzymes in metabolic process, including enzyme in protein metabolism, inhibit protein production and cause protein level decline.

Ca, Cu, and Zn are essential metals group. These trace element play an important role in activation various enzyme in biochemical reaction in body. Freshwater mussels require Ca for shell formation and for regulation of physiological processes. Cell must maintain Ca levels in relation to its almost universal importance for nerve conduction, as second messenger for cellular metabolisms and growth. Inhibition Ca uptake in cell can lead to biochemical and physiological alteration, such as interference with Ca homeostatic and increase in carbohydrate and protein catabolism [8]. It means that presence Cd in cell activated enzyme which

plays a role in protein degradation and it makes protein level decline.

Upon depuration time, total protein in both of organ attends to increase. It showed that cell have capability to respons Cd exposure as defense for toxicological effect. It also means that effects of Cd exposure (20 µg/L) can interfere protein metabolism. The Cd concentration (20 µg/L) cause reversible disruption.

Conclusions

Cd exposure via water in freshwater mussels (*Elongaria orientalis*) increase Cd accumulation in kidneys and gills. Gills and kidneys is an important organ in the absorption and elimination process. Decrease of Cd concentrations during depuration showed that the mussels are able to eliminate cadmium from the body, although it does not take place completely.

Cd exposure results decreases of protein levels in kidneys and gills freshwater mussels (*Elongaria orientalis*). But protein levels attend to increase during depuration time. It showed that in low concentration (20 µg/L), Cd disturb metabolism process and it can interfere of mussel's growth and development.

Acknowledgment

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EFFECTS of ANTIOXIDANT DIFFERENT in CEP DILUENT on SPERM QUALITY of BRAHMAN BULL DURING STORAGE at REFRIGERATOR TEMPERATURE

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Abstract

Spermatozoa storage at low and room temperature can not avoid from the presence of free radicals . Effort to minimize the effects of free radicals is with the addition of antioxidants in the diluent. The aim of this study was to study effect variaty of antioxidant in CEP diluent at refrigerator temperature. Fresh semen was obtained from brahman bull, diluted with CEP without antioxidant (control), CEP with vitamin E, CEP with vitamin E and vitamin C, and CEP with gliserol. Sperm motility was evaluated subjectively by ligth microscope with a magnification 200X at 37°C. Sperm viability was assessed by nigrosin/eosin (N/E) staining procedure. The result showed significant difference($p<0.05$) between control and treatment. Best motility and viability were the addition of viamin E and gliserol. Conclusion of the research was various of antioxidant had difference effect to sperm quality during storage.

Keyword: antioxidant, sperm quality, CEP diluent, brahman bull

Introduction

Technology of sperm storage is a series of artificial insemination (AI) process . The success of storage techniques can affect the success of the AI, because it can affect the quality of spermatozoa . Semen storage requires a diluent to maintain the quality of spermatozoa. [15] have developed CEP diluent for bull semen for storage at 4-5ºC, and it has been modified method by [6] that can maintain motility and viability of Limousin sperm during 8 days according Indonesian National Standard (SNI) for AI.

Spermatozoa storage at low temperatures can affect sperm quality due to occurrence of cold shock and the presence of free radicals [9] . The existence of free radicals (ROS) during storage can cause both lipid and protein oxidation membrane causing membrane integrity will be disturbed [12]. Seminal plasma protect spermatozoa of ROS with antioxidants , but in small quantities . If the amount exceeds the amount of antioxidants ROS ROS will then oxidize membrane components that causes a decrease in sperm quality . Therefore, in the diluent should be antioxidant additional to minimize the influence of ROS during the storage process. The addition of antioxidant in the diluent exerted a protective effect against lipid peroxidation, thereby preserving the metabolic activity and cellular viability of spermatozoa. The aim of this study is to examine the effect of different antioxidants (vitamin E, vitamin C and gliserol) in CEP diluent on the quality of spermatozoa during storage at a temperature of 4-5 ° C .

Material and Method

Reparation of extender with antioxidant addition

Chemicals to make extender CEP-2 is based on the research that have been developed by [15,16] with antibiotic and making method different (Ducha *et al*., 2012) consisting of NaCl 15 mmol/lt; KCl 7.0 mmol/lt; $CaCl_2(H_2O)_2$ 3.0 mmol/lt; $MgCl_2(H_2O)_6$ 3.0 mmol/lt; $NaHCO_3$ 11.9 mmol/lt; NaH_2PO_4 8.0 mmol/lt; KH_2PO_4 20.0 mmol/lt; fructose 55 mmol/lt; sorbitol 1.0 gr/lt; BSA 2.0 gr/lt; Tris 133.7 mmol/lt; penicillin 1000 IUI; streptomycin 1 gr; and citrate acid mmol/lt. Measuring of extender osmolarity used electric osmolarity with osmolarity approximately 250-325 mOsm, and pH 6-7. Extender sterilized by using milipore membrane with size of 0.22 μm and supplemented with vitamin E 2mM, vitamin C 0,5 mM, and gliserol 5%. Egg yolk addition was 20%.

Collection and Preparation of Semen

Fresh semen was collected from the AI Center in Singosari – Malang. The fresh semen was selected based on quality of spermatozoa that is fulfilling Indonesian National Standart (SNI) from fresh semen process to AI application. Several provisions from SNI are the individual motility should be at least 70%, the minimum mass motility should be 2+, and the abnormality and the viability should be at least 70%. Fresh semen were diluted in CEP extender with antioxidant (vitamin E, vitamin C, gliserol) and without antioxidant (control). Spermatozoa were stored at refrigerator temperature in darkness with 25 x 10^6 concentration.

Sperm Viability

Spermatozoa motility were assessed a drop of semen on slide warmer (37°C) under light microscope for the percentage of progressive motility. Spermatozoa in CEP-2 extender with and without egg yolk at day 0 and day 8 refrigerator storage) were taken using stick glass and placed on object glass, covered with cover glass and placed on the slide warmer at 37°C, then observed on the light microscope at a magnification of 400x [3; 4]. Evaluation of motility was done by two person that observed on progress if motility that compared with backwards motility and only rotated, based method of [7].

Sperm Motility

Method of observation for sperm viability uses eosin-negrosin staing. The advantages to this stain are that permanent slides can be made and the nigrosin provides a dark background for easier recognition of the non-stained, viable cells. Non-viable sperm have red or dark-pink heads and viable sperm have white or faintly-pink heads.

Data Analysis

Data in the form of percentage, it transformed into arcshine , and then test of homogenety and normality . If the data proved to be normally distributed and homogeneous , then the ANOVA test . Based on the results of ANOVA test if there was a difference of treatment , then tested further to determine differences between treatments used Duncan test.

Result Research

Motility

Result research of sperm motility during storage can be seen in Table 1. The motility of spermatozoa showed a significant difference ($p < 0.05$) in a variety of treatments , from the second day until the seventh day of storage . On day 0 , 1st and 2nd has not shown any differences in the various treatments . Best motility is in treatment vitamin E and glycerol after seven days of storage , with the motility that according with the standard ISO for AI, that is not less than 40 %.

Table 1. Sperm motility of brahman bull during storage at 4-5°C

Various of antioxidant in extender	Motility (%)							
	Day-0	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7
CEP (control)	58,83^{a} ± 0,84	55,83^{a} ± 0,83	52,50 ^{a} ± 1,43	48,33 ^{a} ± 1,66	44,17 ^{b} ± 0,96	41,67 ^{a} ± 1,67	38,33 ^{b} ± 0,85	35,00 ^{b} ± 0,00
CEP + vitamin E	56,67^{a} ± 0,83	55,00^{a} ± 0,00	50,00 ^{ab} ± 0,00	50,00 ^{a} ± 0,00	49,17 ^{a} ± 0,83	46,67 ^{a} ± 1,66	45,83 ^{a} ± 0,83	45,00 ^{a} ± 0,00
CEP + vitamin E + vit C	58,83^{a} ± 0,84	55,00^{a} ± 0,00	49,17 ^{b} ± 0,83	48,33 ^{a} ± 1,66	40,83 ^{b} ± 0,84	23,83 ^{b} ± 5,34	11,67 ^{c} ± 3,54	00,00 ^{c} ± 0,00
CEP + gliserol	58,75^{a} ± 0,84	55,83^{a} ± 0,00	51,67 ^{ab} ± 0,83	48,33 ^{a} ± 1,66	48,33 ^{a} ± 1,66	48,33 ^{a} ± 1,66	45,83 ^{a} ± 1,66	43,33 ^{a} ± 1,68

Viability

Table 2 showed the results of spermatozoa viability in various treatments . Viability of spermatozoa showed significant differences in the various treatment from the first day until the seventh day of storage . Best viability is the treatment of vitamin E and glycerol .

Table 2. Sperm viability of brahman bull during storage at 4-5°C

Various of antioxidant in extender	Viability (%) during storage							
	Day-0	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7
CEP (control)	80,76 ^{a} ± 1,06	70,94 ^{bc} ± 0,76	73,00 ^{ab} ± 0,59	69,54 ^{ab} ± 0,68	62,61 ^{b} ± 1,53	49,46 ^{c} ± 2,68	47,17 ^{b} ± 1,78	45,50 ^{b} ± 1,00
CEP + vitamin E	79,21 ^{a} ± 0,20	71,45 ^{c} ± 0,82	69,77 ^{b} ± 0,77	68,37 ^{b} ± 0,43	64,13 ^{ab} ± 0,31	60,58 ^{b} ± 1,31	58,44 ^{a} ± 0,47	56,10 ^{a} ± 0,11
CEP + vitamin E + vitamin C	79,89 ^{a} ± 0,59	76,37 ^{ab} ± 0,24	73,01 ^{ab} ± 0,60	70,07 ^{ab} ± 0,15	54,30 ^{c} ± 2,43	45,78 ^{c} ± 1,28	38,49 ^{c} ± 1,46	31,58 ^{c} ± 1,87
CEP + gliserol	80,63 ^{a} ± 0,89	77,61 ^{a} ± 1,34	73,63 ^{a} ± 1,03	70,65 ^{a} ± 1,10	69,55 ^{b} ± 1,48	68,64 ^{a} ± 1,10	62,43 ^{a} ± 1,03	56,60 ^{a} ± 1,19

Discussion

Data of sperm motility during storage at 4-5 ° C in a variety of treatments showed a significant difference , the best motility after seven days of storage were on the treatment of vitamin E and glycerol . The addition of vitamin E in the CEP diluent showed a good ability to capture free radicals , so that the motility of spermatozoa was well preserved . Results of this study was similar with the results research of [2], which showed vitamin E was able to maintain the motility of the buffalo bull sperm, also in Ovine sperm [14] .

During the storage process , spermatozoa can not avoid the presence of free radicals (ROS), as a result of the process of electron transport in the mitochondria that generate oxygen radicals [8] . The existence of free radicals can cause lipid peroxidation that can damage the sperm membrane . Free radicals are highly reactive molecules or atoms that have one or more unpaired electrons [1] .

The existence of ROS in semen can cause damage to cells when the oxidant exceeds the amount of antioxidants in the cells , resulting in excessive ROS generation , and when antioxidants can not neutralize (scavenge) free radicals are formed. The phenomenon is called oxidative stress (OS) , [13; 5]. Result of the oxidation of the membrane lipids by the partially reduced oxygen molecules such as superoxide, hydrogen peroxide and hydroxyl radicals. LPO due to the attacks by ROS, formed through the univalent reduction of oxygen, led to the impairment of sperm function, e.g. sperm motility, functional membrane integrity and fertility through oxidative stress and the production of cytotoxic aldehydes. Vitamin E is able

to capture free radicals and inhibit lipid peroxidation chain reaction on the membrane [1].

The addition of glycerol in the CEP diluent was able to maintain motility of brahman bull sperm during storage at temperature of 4-5 ° C . Glycerol is able to flex so that the cell membrane is not easily fragile . In the plasma membrane , glycerol will bind the cluster center membrane phospholipids thus overcoming instability and interact with the membrane to bind to proteins and glycoproteins that cause collected the intramembranous particles [11]

Data of sperm viability showed significant difference in the various treatments , the viability of the best is on the treatment of vitamin E and glycerol . These results suggested that vitamin E and glycerol able to protect spermatozoa from events peroxidation by free radicals . These results were similar to the results of research [14] that able to maintain the viability of ovine sperm . The existence of free radicals cause damage to the membrane , which can cause cell death , because it would disrupt the electrolyte balance inside and outside cells.

Conclusion

The conclusion from this study was different antioxidants have different effects on the sperm quality of brahman bull. Vitamin E and gliserol were the best antioxidant in maintaining the quality of spermatozoa during storage in CEP diluent.

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EFFECT of EMOTIONAL INTELLIGENCE and PHYSICAL INTELLIGENCE ATTITUDES TOWARDS HEALTHY LIVING LEATHER GLOVE FACTORY EMPLOYEES in YOGYAKARTA

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Abstract

Health of the body is determined by lifestyle (the behavior). A healthy lifestyle is influenced by the attitude of a person's thinking pattern of healthy living. Factors that influence the attitude of healthy living that experience of life, the environment, culture, beliefs, information media, the workplace, religion, place of residence and psychic. Stability of health conditions can be maintained preventively with the formation of healthy life attitude through various efforts, including in the form of an increase in emotional intelligence and physical intelligence. This study aimed to determine the effect of physical intelligence on healthy living attitudes leather gloves factory workers in Yogyakarta. The subjects of the study (respondents) comprises one control group and treatment group, each group a number of 10 employees. The control group and the treatment group tested levels of emotional intelligence as well as the physical intelligence and attitude of her life with the tool questionnaire before and after the implementation of the treatment. The treatment group were given education and training on emotional intelligence and physical intelligence during the fourth stage. Each stage lasts three sessions in meeting room Leather Glove Factory in Yogyakarta. Data were obtained when analyzed with One Sample Kolmogorov-Smirnov Test and Pair Sample Test showed a significant difference emotional intelligence scores ($p = 0.008$), physical intelligence ($p = 0.013$) as well as the attitude of healthy life ($p = 0.001$) after treatment education and training. The result of regression analysis with ANOVA showed significant effect ($p = 0.012$) emotional intelligence and physical intelligence on healthy living attitudes leather gloves factory workers in Yogyakarta. Increased emotional intelligence and physical intelligence affect the improvement of healthy living attitudes employee Leather Glove Factory in Yogyakarta.

Keywords: emotional intelligence; physical intelligence; healthy life attitude.

Introduction

The success of health care is very determined attitude to life. The attitude of healthy life is the driving force themselves good health to be able to maintain the stability of [1]. Healthy living attitudes contribute to direct action in the nutritional adequacy requirements, the activity of the body, breaks and behavior that becomes the basis of a healthy lifestyle [9]. Unhealthy attitudes result in poor attention to quality of life (balance the needs of nutrition, rest, physical activity, etc.). The achievement of a healthy livelihood needs influenced by self-management. The success of self-management one of which is supported by the quality factor of emotional intelligence ([11].

[8] research results, et.al., 2011 states that emotional intelligence is useful in lowering the level of expression of one's emotions and dependence themselves against others. Ability decreased expression of these emotions supported by the level of emotional intelligence in overcoming the challenges of her emotional turmoil [12]. [6] explains that emotional intelligence plays a role in providing the ability to self-knowledge, self-awareness, social awareness, empathy and good communication with others. Laila et.al. 2012 says that increasing emotional intelligence in patients able to reduce personality disorders and depression. Emotional intelligence also serves to control against pleasure, impulse, the originator of self-motivation, preventing the occurrence of frustration, develop a sense of empathy, directing one's thoughts and actions [4]. [15] research results, et. al. (2008) concluded that the program to improve emotional intelligence in patients with type 2 mellitus diebetes positive effect on improving the quality of life. Other research conducted by [7] at the upper secondary school students in Taiwan who were trained in emotional intelligence program positively correlated to the increased impetus for physical activity, mental health and ability in their social interactions. Social interaction in a work environment that is of sufficient quality to contribute the achievement of a healthy life. Achievement of healthy life requires patience capabilities supported by the level of emotional intelligence [3]. Maintenance of a healthy body that is also supported in the ability to maintain the balance of motion activity, [14] explains

that the habits of the elderly were accustom regular physical activity causes physical and mental quality to be better. Motion activity is measured and orderly management of calories your body properly. Management of these calories will regulate the breathing rhythm and diet so that needs oxygen for metabolism adhenosine formation triphosphate (ATP) is fulfilled properly [5]. This medical condition can also be maintained one of them according [13] in the presence of physical intelligence that could encourage efforts judgment based on the standard diet caloric needs his own body and is not affected to the whims of the type of food they consume. The ability to control this diet can suppress the emergence of a fault condition hiperkholesterolemia [2]. Based on these descriptions, it is important to do research on the Effect of Emotional Intelligence and Physical Intelligence Attitudes towards Healthy Living Employee Leather Glove Factory in Yogyakarta.

Materials and Methods

This type of research in the form of an experimental study is done by providing education and training to improve emotional intelligence. The study design with a Pre-Test Post-Test along with the control group (without provision of education and training of emotional intelligence).

Research subjects (respondents) for the control group consisted of 10 people and the treatment group (education and training) a number of 10 people. Determination of the research subject with purposive sampling technique that is with consideration of inclusion and exclusion criteria. Inclusion criteria such as the status of permanent employees at the factory in Yogyakarta leather gloves with a minimum term of 1 year. Exclusion criteria were permanent employees of the factory leather gloves in Yogyakarta who suffer from a mental disorder or illness in the care of a doctor.

The dependent variable in the form of healthy living attitudes of the employees of the factory leather gloves in Yogyakarta and independent variables form of emotional intelligence and physical intelligence employees leather gloves factory in Yogyakarta.

Data collected by questionnaire as a measure of emotional intelligence as well as the physical intelligence and attitude questionnaire healthy life. This questionnaire is done before and after treatment administration emotional intelligence training and education as well as physical intelligence. This questionnaire measuring instrument comprises 25 items of questions that have previously been tested by product moment validity and reliability testing with Cronbach Alpha.

The research was conducted in September-December 2014 in the meeting room Leather Glove Factory in Yogyakarta. Before starting treatment education and training, all respondents (control group and treatment group) were measured emotional intelligence and physical intelligence and healthy life attitude. Furthermore, education and training carried out periodically with 4 levels. Each period required 3 sessions with each session for 100 minutes. After completion of the provision of education and training, then all of the respondents (the control group and the treatment group) were measured emotional intelligence and physical intelligence and attitude of healthy life. The data were then tabulated and analyzed by One Sample Kolmogorov-Smirnov Test and Pair Sample Test.

Results

Table 1. Analysis of One Sample Kolmogorov Smirnov Test and Pair Sample Test, Emotional intelligence and physical intelligence Attitude Against Healthy Living

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	KE_sebelum - KE_setelah	-5.60000	5.18973	1.64114	-9.31251	-1.88749	-3.412	9	.008
Pair 2	KF_sebelum - KF_setelah	-8.60000	8.84685	2.79762	-14.92865	-2.27135	-3.074	9	.013
Pair 3	SKH_sebelum - SKH_setelah	-5.50000	3.47211	1.09798	-7.98380	-3.01620	-5.009	9	.001

Pair test sample test results (Table 1) shows that the average score of each emotional intelligence and value (p = 0.008), physical intelligence (p = 0.013) means that there are significant differences in average (pre-test to post-test) scores emotional intelligence as well as the physical intelligence. Analysis of differences in healthy life attitude scores with pre-test post test (Table 1) showed with p = 0.001, meaning

that there is a significant difference in the average scores of healthy living attitudes pre-test to post-test.

Data were analyzed by ANOVA after (Table 2) between emotional intelligence and physical intelligence attitude towards healthy life showed significant effect (P = 0.012). Anova Regression analysis showed an increase in emotional intelligence and physical intelligence significantly influence employee attitudes healthy life Leather Glove Factory in Yogyakarta.

Table 2. Regression ANOVA (b) Emotional Intelligence (EI) and Physical Intelligence (PI) Attitudes towards Healthy Living (AHL) Leather Glove Factory Employees In Yogyakarta.

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	723,785	2	361,892	8,762	,012(a)
	Residual	289,115	7	41,302		
	Total	1012,900	9			

a. Predictors: (Constant), PI after, EI_after education and training
b. Dependent Variable: AHL after education and training

Discussion

Pair test sample test (Table 1) showed a significant difference (p = 0.002) mean score of emotional intelligence as well as the average score of the attitude of healthy life (p = 0.010) after the treatment was given education and training in emotional intelligence. Regression analysis with ANOVA (Table 2) clarify the increase emotional intelligence, have a significant effect (p = 0.005) on the formation of healthy life attitudes leather gloves factory workers in Yogyakarta. Increased emotional intelligence provides the ability positive [16] the employees of the factory leather gloves in Yogyakarta to get to know ourselves as well as increased awareness of themselves so that they can understand the difference between desire and turmoil obey obey the need for a healthy life. The ability to manage turmoil desires of self is due to the important role of emotional intelligence [10]. This condition is in accordance with the opinion of Goleman (2007) that the achievement of self-awareness is supported by emotional intelligence that enabled the self-control against the wishes that it can be self-destructive behavior and the ability to direct the order to remain in a safe condition. Self-awareness role is to encourage employee factory in Yogyakarta leather glove toward the formation of attitudes to meet the needs of a healthy life. This healthy attitude contributes to action for healthy living. The results are consistent with the results of the study [16] that the program to improve emotional intelligence in patients with type 2 mellitus diebetes positive effect on improving the quality of life.

The combined increase in emotional intelligence and physical intelligence on these employees will strengthen the efforts of self-control as the two forms of intelligence that complement each other in giving consideration decision to act, this act Considerations in accordance explanation [13] on the functioning of physical intelligence.

Conclusion

The conclusion of this study is emotional intelligence and physical intelligence can be enhanced by the provision of education and training in stages and structured. Intelligence deficits improve physical and emotional intelligence significantly influence the improvement of healthy living attitudes employee Leather Glove Factory in Yogyakarta.

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THE EFFECT of BASES TOWARD DISINTEGRATION TIME of PHYTO-CAPSULES

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Abstract

The aim of the research was to determine the effect of bases toward the disintegration time of phyto-capsules, capsules originated from plant polysaccharides. Phyto-capsules were made from polysaccharides and sorbitol and 0.05 g of bases, MgO and $Ca(OH)_2$ of each. The seven capsules of each were determined the disintegration time using Erweka disintegrator. The mean of disintegration time of phyto-capsules with MgO and $Ca(OH)_2$ were 2 hours and 25 minutes ; 2 hours, respectively. The gelatin capsules with and without color showed the mean of disintegration time of 7'30" and 2'35", respectively.

Keywords: Phyto-capsule, Bases, Disintegration time

Introduction

Capsules are solid dosage form comprising a drug in a hard or soft shell soluble [1]. The compound that used as hard capsule is gelatin [1]. Phyto-capsule is capsule from plant, especially polysaccharide. To make the capsules more homogeneous on the surface and pores were added crosslinker. One of the parameter that used for capsule is disintegration time [1]. Disintegration time is the time requisite for dosage to break up in to granules or smaller particles under specific condition. Water is commonly used as disintegration a dosage form in Pharmacopoeias [2]. The capsule shell material was influence to in vitro disintegration time of the green tea extract capsule [3]. In this research the polysaccharides from seaweed was used as hard shell (Phyto-capsule) with adding MgO or $Ca(OH)_2$ as crosslinker.

Experiment

Polysaccharides were mixed with distilled water and added of sorbitol, 0.05 g of MgO and $Ca(OH)_2$ of each. The gel was heated in the waterbath for an hour until the viscosity in range 4000 -5000 cps. The gel was poured in the template and dipped using pinbar. Capsules were dried for three hours, and repealed, then kept in the jar. The capsules with and without plasticizers were determined the disintegration time using Erweka disintegrator at pH 6.0 and temperature was 37ºC and their compared with gelatin capsules.

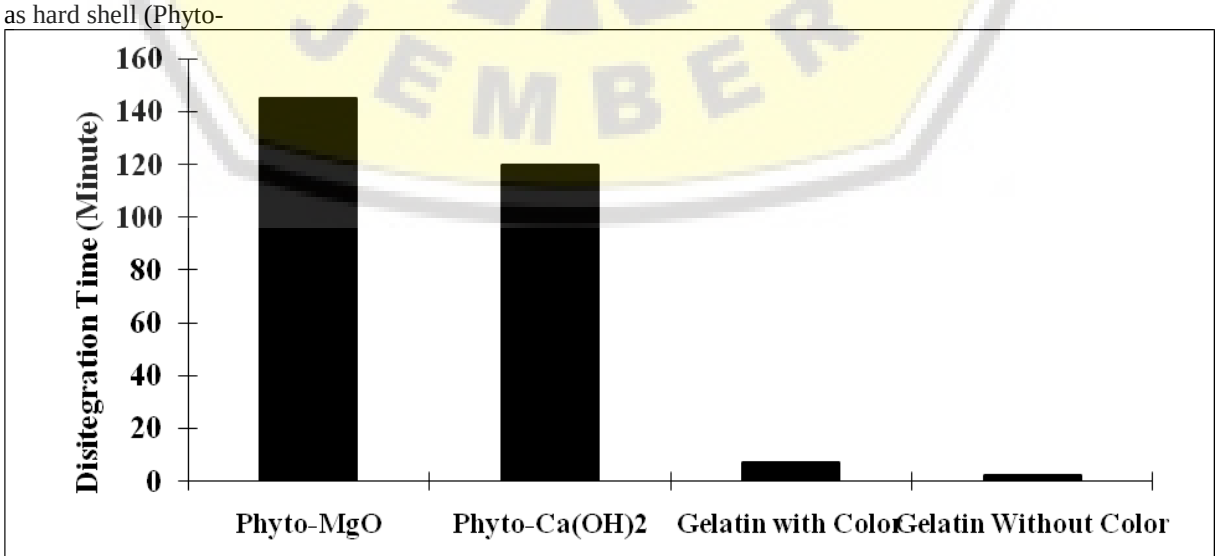

Figure 1. Disintegration time of Capsule

Results and Discussion

The morphology of the Phyto-capsule had smooth surface. Disintegration time is one of the quality to determine of the solid dosage form [1]. The results of disintegration capsule showed in Figure 1. In Figure 1 showed that Phyto-capsules that made from polysaccharides, sorbitol and 0.05 g of bases, MgO or 0.05 g $Ca(OH)_2$ as crosslinker had higher disintegration time compared with gelatin capsule both color and colorless. The longer time of disintegration of Phyto–capsule caused the interaction of polysaccharide with crosslinker (MgO or Ca $(OH)_2$), but it had ability to develop as a hard capsule.

Conclusion

The disintegration time of phyto-capsules both with MgO and Ca(OH)2 were considerably higher compared to the gelatin capsules both color and colorless.

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DBLβC2 DOMAIN of VAR GENE of INDONESIAN *Plasmodium falciparum* HAD AN ASSOCIATION with SEVERITY of MALARIA

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Abstract

Malaria in Indonesia is still a major health problem. Most of severe cases of malaria is due to *P. falciparum* infection. In malaria falciparum, PfEMP1 is supposed to play an important role as cytoadherence ligand on the surface of infected erythrocytes and thereby contributing to the distinct pathogenesis of malaria. It is a large and polymorphic protein and is encoded by *var* gene family. The extra-cellular highly polymorphic portion contains of three distinct binding domains: Duffy binding-like (DBL), Cysteine-rich interdomain regions (CIDR) and C2. PfEMP1 varies in domain composition and binding specificity. The study explored the characteristic of Indonesian DBLβC2-*var* genes and investigated the role of the sequence to the severity of malaria. Seventeen blood samples from clinically mild to severe malaria patients in Jember-East Java, a hypo-endemic malaria area in Indonesia were collected for DNA extraction. Diagnosis confirmation by Giemsa-stained thick blood smear showed four of them were pure infection of falciparum malaria infection, four samples were pure infection of *P. vivax* and the rest 9 samples were mixed infection of *P. falciparum and P. vivax*. PCR using specific primer designed based on reference sequences targeting on full-length DBLβC2 resulted approximately 1,7 kb band in a sample. In conclusion, the DBLβC2-var gene of Indonesian isolates was 1,7 kb in length and there was a possible association of the existence of DBLβC2 domain with the severity malaria outcome.
[Times New Roman 10, justified]

Keywords: DBLβC2, var gene, *Plasmodium falciparum*, Indonesia. *[Times New Roman 10, justified]*

Introduction

Malaria is a global health problem with hundreds of million people around the world suffering from this disease each year. In Indonesia, it is an important health problem with the Annual Parasite Index (API) 1.96 per 1000 people and causing 432 deaths in 2010 [1]. There are four established Plasmodium parasites causing human malaria and *Plasmodium falciparum* is the deadly malaria parasite because of the capability to evoke broad spectrum of clinical symptoms from asymptomatic to severe malaria event leading to death. SEARO-WHO reported 47.9 % of death in 2013 due to malaria is caused by *P. falciparum* [1].

There two characteristics relevant to the severity of malaria falciparum: the capability to invade all stages of erythrocytes leading to very high parasitaemia and the ability of infected erythrocytes to adhere to vascular endothelium and other host cells, called cytodherence. Cytodherence might result in obstruction of the microcircualtion leading to poor perfusion of host tissues, hypoxia, dysfunctin of affected organs resulting in multiple organ failure [2,3]. One of the proteins responsible for cytoadherence is *P. falciparum* erythrocyte membrane protein 1 (PfEMP1). PfEMP1 is secreted during the erythrocytic cycle, it is exported from the parasite to the surface of infected erythrocyte approximately 18 hours post invasion onwards. In addition to cytodherence mechanism, PfEMP1 has an immunoregulatory effects on host immune cells through binding to other host receptor and antigenic variation with consequent immune evasion [4,5].

PfEMP1 is a large and highly polymorphic protein that varies in domain composition and binding specificity. It possesses an extra-cellular and intra-cellular part. The extra-cellular part is highly polymorphic and contains N-terminal segment (NTS) followed by three distinct binding domains: Duffy binding-like (DBL), Cysteine-rich interdomain regions (CIDR) and C2 [6].

PfEMP1 is encoded by the highly divers *var* gene family consisting of approximately 60 variable genes per haploid genome of the parasite [7]. The *var* genes have two exons, the first exon is highly polymorphic, encodes the extracellular part of protein and trans-membrane (TM) domain and the second exon, in contrast, is more conserved and encodes the intracellular region. Although the *var* gene family is highly diverse in terms of overall organization and sequence, the majority of *var* genes contain a number of conserved motifs including several homologous cysteine-rich domains known as Duffy binding-like (DBL) domains. Based on the consensus motifs, DBL domains have been classified into six types; α, β, γ, δ, ε, and x. Each *var* gene potentially encodes between two and seven DBL domains [8].

DBL2β domain in tandem with C2 domain mediates binding to ICAM-1 in several *P. falciparum* isolates. The ICAM-1 binding required both DBLβ and C2 domain including 16 conserved cysteine residues for ICAM-1 binding isolates [9-11]. In this study, we reported the characteristic of DBLβC2-*var* genes from field isolates collected in a hypo-endemic area of malaria in Indonesia and investigated the role of the sequence to the severity of malaria.

Methods

Subjects

Malaria patients were enrolled from the Ledokombo and Sumberjambe Primary Health Care in Jember district, East Java, Indonesia. All patients were informed of the study and written informed consent was obtained. Inclusion criteria were infection with *P. falciparum* either pure or mixed infection confirmed with microscopic examination of thin and thick blood smears stained with Giemsa. The ethical approval was obtained from the Ethical Committee of Research of Faculty of Medicine, University of Jember, Indonesia.

Isolation of Genomic DNA

Genomic DNA (gDNA) was directly isolated from blood samples obtained from malaria patients by TIANamp Blood DNA kit (Tiangen Biotech) according to the manufacturer's instructions.

Identification of DBLβ-C2 domain of each field isolates

Specific primer for the DBLβ-C2 sequence were designed according to several referred sequences of the DBLβ-C2 domain: JDP8 (AY028643), isolate IT4/24/25 (IT-ICAM var) (AY578326), clone A4 strain IT4/25/5 (L42244.1), clone A4tres isolate IT (AF193424) and FCR3var CSA (AJ133811). The primers were: DBL2F1 (5'-AGT GTG TTG AAG GAC GTA TGT-3') dan DBL2R3 (5'-CCA AAC ATA TAT CTC TAT AAT CTC C-3'). The cycle conditions for the PCR were as follows: initial denaturation at 95ºC for 4 minutes, followed by denaturation at 95ºC for 60 seconds, annealing at 65ºC for 60 seconds and extension at 65ºC for 3 minutes, for 30 cycles. The amplified fragments from PCR were visualized using UV light transilluminator.

Results and Discussion

Characteristics of Subjects

Seventeen blood samples of malaria patients from Sumberjambe and Ledokombo Primary Health Care were enrolled in the study after written informed concern. Characteristics of patients were figured out in Table 1. Sixteen out of 17 patients (94.1%) were male and the rest was female. This is in accordance with the fact that most patients were migrant working in Kalimantan and Nusa Tenggara. They were infected and returned as a malaria infected person.

Tabel 1. Characteristics and clinical manifestation of subjects

No	Sex	Age - years	Microscopical Diagnosis	Clinical manifestation
1.	L	35	*P. falciparum*	Moderate malaria
2.	L	28	*P. falciparum*	Severe malaria
3.	L	25	*P. falciparum*	Mild malaria
4.	L	43	*P. vivax*	Mild malaria
5.	L	32	*P. falciparum*	Mild malaria
6.	L	15	*P. vivax*	Mild malaria
7.	L	30	*P. vivax*	Mild malaria
8.	L	46	*P. falc+P.viv*	Moderate malaria
9.	P	38	*P. vivax*	Mild malaria
10.	L	51	*P. falc+P.viv*	Mild malaria
11.	L	39	*P. falc+P.viv*	Mild malaria
12.	L	27	*P. falc+P.viv*	Moderate malaria
13.	L	28	*P. falc+P.viv*	Mild malaria
14.	L	30	*P. falc+P.viv*	Mild malaria
15.	L	28	*P. falc+P.viv*	Moderate malaria
16.	L	35	*P. falc+P.viv*	Moderate malaria
17.	L	39	*P. falc+P.viv*	Moderate malaria

Microscopical examination using Giemsa stained confirmed 4 sampels (23.5%) pure *P. falciparum* infection, another 4 samples (23.5%) *P. vivax* infection and the rest 9 samples (53%) mixed infection of *P. falciparum* and *P. vivax*. There were neither *P. malaria* nor *P. ovale* infection. Clinical manifestation showed a wide range of symptom, where only a patient with *P. falciparum* infection showed a severe malaria with an anaemia as a prominent symptom, six patients revealed moderate malaria and ten patients had a mild malaria.

According to WHO (2000), criteria for severe malaria is one of the following symptoms: severe anaemia, prostration, convulsion and respiratory distress, metabolic acidosis or cerebral malaria with

impaired consciousness and coma [12]. In our study the severe malaria patient had a severe anaemia with Hb 5 g/dl.

3.2 DNA isolation

Blood DNA extraction were conducted in all samples with *P. falciparum* infection either pure or mixed infection. As many as 12 blood samples were isolated and the results showed in Fig. 1.

Figure 1. Electrophoresis of gDNA in 1.5% agarose. The results of DNA isolation from sample 1, 2, 3, 10, 12, 13, 14.

3.3 Amplification of DBLβC2-*var* genes

Amplification of the DBLβC2-*var* genes were conducted in all samples using specific primer designed based on reference sequences. Primer pair DBL2F1 and DBL2R3 amplified the whole sequence of DBLβC2 domain as long as ± 1.7 kb in sample 2, as shown in Fig.2.

Figure 2. Amplification result using specific primer DBL2F1 and DBL2R3. There was a ± 1.7 kb in sample 2. Sampel 1, 3, 5, 8 did not show any band but sample 15, 16, 17 and 15 yielded multiple bands.

Fig. 2. perfomed the PCR results using specific primer DBL2F1 and DBL2R3. Only sample 2 yielded a single band of ± 1.7 kb, other samples showed either no band or multiple bands with different size. Based on reference sequences, the sequence of DBLβC2-var genes is approximately 1.7 kb. The sequences encode for DBLβC2 domain of PfEMP1. Some studies reported that the domain consist of 522 amino acid [13], 70 amino acid longer than previous study [6].

Multiple bands showed in three samples (15, 16, 17). We assumed that bands were part of DBL domain of PfEMP1, as previous mentioned that there are several subclass of DBL domain i.e. DBL α, β, γ, δ, ε, ζ and χ [6].

The result of 1,7 kb band in sample 2 indicating the presence of DBLβC2 domain in the sample. The DBLβC2 domain is one of the PfEMP1 domain responsible for binding with host receptor Intercellular Adhesion Molecule 1 (ICAM-1) locating at the surface of endothelial cells and leucocytes. The binding of PfEMP1 through DBLβC2 domain with ICAM-1 is the basic pathology of severe malaria or malaria with complications, due to some findings: (1) the adhesion to ICAM-1 tended to be higher in patients with cerebral malaria; (2) a co-localization of ICAM-1 with parasite sequestration in brain vessels in autopsy samples from cerebral malaria patients; (3) up-regulation of ICAM-1 expression on endothelium during malaria infection [14,15]. As previous data reported that sample 2 had a clinicaly severe malaria with an anaemia as a major complication.

The ICAM-1 binding required both DBLβ and C2 domain for ICAM-1 binding isolates [9, 10, 11]. Both two binding domains contain contact residues required for ICAM-1 binding and there is a functional interdependence between two binding domains from the same protein, where recombination construct of DBLβ and C2 domain of ICAM-1 binding and non-binding isolates as well as chimera containing the DBLβ and C2 domain from two different ICAM-1 binding isolates did not bind ICAM-1 ([9,16].

Alignment of ICAM-1 binding DBLβ-C2 domains from three isolates (A4, A4tres and JDP8) revealed significant homology, sharing 16 conserved cysteine residues and a number of conserved hydrophobic amino acid residues, indicating conservation of the structure as well as three-dimensional structure. However, multiple alignments of ICAM-1 binding and non-binding DBL2β-C2 domains suggested that the binding region for ICAM-1 is unlikely to lie in a linear sequence stretch within DBL2β-C2 [10].

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ODONATA of ISLAND GARDEN CITY of SAMAL and ITS RELATION to OTHER SMALL ISLANDS in THE PHILIPPINES

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Abstract

The first record of odonata fauna in the Island Garden City of Samal is presented with comparison to other small islands in the Philippines. Opportunistic and photo documentation were employed in all fluvial systems surveyed between April 2014 to April 2015. Thirty one (31) species belonging to 7 families and 22 genera was recorded. Twelve species (12) or 39% are zygopterans and 19 species or 61% are anisopterans. A relatively low level of endemism (35%) is recorded which is attributed to the different habitat modifications of its fluvial systems. Kroeber's percentage of similarity revealed Island Garden City of Samal shares similar odonata species with Siargao and Saranggani Islands characterized by karst ecosystem with low lying topography. Creation of local policy to protect the head waters where most of the endemic species found is urgently needed.

Keywords: odonata, survey, karst, conservation, Samal.

Introduction

Odonata are not only aesthetically pleasing insects inhabiting our rivers and lakes but also serve as bio-indicators of water and habitat quality and control insect-borne diseases [3, 7]. Hämäläinen [1] reported the Philippines as one of the 17 mega diverse countries in the world and a home to 300 odonata species of which 90% are mostly Zygopterans and 40% Anisopterans. Mindanao the second largest island in the country holds 130+ species [1]. Hämäläinen & Muller [5] provided the most comprehensive account of the odonata species in various islands of the country however many islands remain unexplored including the Island Garden City of Samal (IGACOS) [6].

The Island Garden City of Samal (IGACOS) is one of the small islands belonging to the Mindanao faunal region. It is a 300 km^2 with unique geologic position bordered by Davao del Sur and Davao City on the west, Compostela Valley Province on the north, and Davao Oriental on the east where Mount Hamiguitan Range Wildlife Santuary a UNESCO World Heritage is located. It is relatively flat with no defined mountains and its highest elevation is only 410 masl. It has only two major river systems, Tagbaobo River and Binoling River. The island receives fair rainfall all throughout the year with no pronounced dry or wet season [8].

Recently the island is gaining popularity as one of the major tourist destinations in Region XI (Davao Region). With the recent transformation of the island into a tourist destination, much of the island's forested land is transformed into commercial and agricultural purposes. More importantly, its fluvial systems are transformed into commercial tourist destination. Cottages and other amenities were built inside Tagbaobo and Binoling Rivers which may cause disturbance to its Odonata inhabitants which is virtually unknown until the 2014-2015 Odonata expedition in the island. This paper provided the first list of Odonata in Samal Island and comparison to other small islands in the Philippines is also presented.

Materials and Methods

Opportunistic and photo documentation were employed in the following fluvial systems, Sampao Falls, Tagbaobo River in Barangay Tagbaobo (7°0'25"N 125°46'43"E), Hagimit Falls, Binoling River in Barangay Cawag (7°3'43.2"N 125°43'44.4"E), Bagsak Lake in Barangay Aumbay (7°3'0"N 125°45'52.2"E) and Bangkalan Lake in Barangay San Agustin in the months of April 2014 and April 2015. Species were collected by hand-picking and hand netting for elusive species. Vouchers were soaked for 24 hours in acetone, air dried, and placed in individual paper triangles. Endemicity and conservation status were obtained using the IUCN Red List of Threatened Species [13].

Figure 1. Map of the A. Philippines, B. Map of IGACOS

Results and Discussion

Thirty one (31) species belonging to seven (7) families and twenty two (22) genera were documented. Twelve (12) or thirty nine percent (39%) are zygopterans and nineteen (19) or sixty one percent (61%) are anisopterans. Family Libillulidae comprised majority of the species (17 spp.) followed by Coenagrionidae (7 spp.), and Platycnemididae and Gomphidae with 2 species each and the rest of the families Platystictidae, Protoneuridae and Chlorocyphidae represented by only 1 species (Table 1).

High representation of the Oriental species from family Libellulidae like *Orthetrum pruinosum clelia, Diplacodes trivialis, Neurothemis ramburii, N. terminata, Trithemis aurora, Trithemis festiva, Brachydiplax chalybea chalybea* and *Acisoma panorpoides* was observed in its fluvial systems which experience various modifications such as establishment of resorts, human habitation, and agricultural use in all sampling sites. Family Libillulidae is known as one of the largest families that dominate standing water and have high tolerance to anthropogenic disturbances. They dominate lentic ecosystems of Bangkalan and Bagsak Lakes which are gradually converted into agro-ecosystems.

A few endemic species like *Drepanosticta flavomaculata, Risiocnemis tendipes, Risiocnemis atripes, Gomphidia kirchii, Diplacina bolivari,* and *Diplacina braueri* were recorded near the water sources with remaining well vegetated parts of Sampao Falls, Tagbaobo and Binoling River. The presence of endemic species at the water sources in the island calls for an alarming imbalance ecosystems. These water sources served as the remaining haven of these endemic species [9] that urgently needs protection. Continuous modification up to the water sources of each fluvial system would lead to site extinction of endemic species which is crucial to the future of IGACOS ecosystem.

Among of the fluvial systems surveyed Sampao Falls and Tagbaobo River host most number of endemic species. This can be attributed to the remaining thick vegetation fragments to some parts of Sampao Falls and Tagbaobo River. The high endemism level in Sampao Falls and Tagbabo River showed that zygopteran species mostly endemic prefer shaded fluvial systems [4]. The low number of endemic species found in Binoling River implies the detrimental effect of anthropogenic disturbances like the commercialization of the falls into resort and human habitation. Likewise with Bagsak and Bangkalan lakes which are now converted into agricultural uses.

Table 1.List of Odonata Species in IGACOS with their Geographical Distribution

Family	Species	Geographic Distribution
Chlorocyphidae	*Rhinocypha colorata* (Hagen *in* Selys, 1869)	PE
Coenagrionidae	*Agriocnemis f. femina* (Braeur, 1868)	O
	Argiocnemis rubescens intermedia	O
	Coeriagrion lieftincki Asahina 1967	O
	Ishnura senegalensis Rambur 1842	O
	Pseudagrion pilidorsum (Brauer, 1868)	O
	Pseudagrion microcephalum Rambur 1842	O
	Teinobasis samaritis Ris 1915	PE
Platycnemididae	*Risiocnemis appendiculata* (Brauer, 1868)	ME
	Risiocnemis atripes Needham &Gyger, 1941	ME
	Risiocnemis atripes Needham &Gyger, 1941	ME
Platystictidae	*Drepanosticta flavomaculata*	ME
Protoneuridae	*Prodasineura integra* (Selys, 1882)	PE
Gomphidae	*Gomphidia kirschii* (Selys, 1878)	PE
	Heliogomphus bakeri Laidlaw, 1925	PE
Libellulidae	*Acisoma p. panorpoides* (Rambur, 1842)	O

	Agrionoptera insignis (Rambur, 1842)	O
	Brachydiplax chalydea chalydea	O
	Crocothemis s. servilia (Dury, 1770)	O
	Diplacina bolivari Selys, 1882	PE
	Diplacina braueri Selys, 1882	PE
	Diplacodes trivialis Rambur, 1842	O
	Neurothemis ramburii (Brauer, 1866)	O
	Neurothemis terminate Ris, 1911	O
	Orthethrum pruinosum clelia (Selys, 1878)	O
	Orthethrum sabina sabina(Drury, 1770)	O
	Pantala flavescens Fabricius, 1798	O
	Potamarcha congener (Rambur, 1842)	O
	Ryothemis phyllis subphylis Sulzer, 1776	O
	Tetrathemis irregularis irregularis Brauer 1868	O
	Trithemis aurora (Burmeister, 1839)	O
	Trithemis festiva Rambur, 1842	O
TOTAL	31	

Legend: O (Oriental), PE (Philippine endemic), ME (Mindanao endemic)

Eleven out of 31 species or thirty five percent (35%) are endemic of which 8 are found distributed in the Philippines and 3 species are confined to Mindanao.

This is relatively lower compared to other islands in the Philippines which have a comparable size to IGACOS like Babuyan Group and Siargao Islands as recorded by Villanueva [2, 10] with endemism levels of 45% and 38% respectively.

However, its species richness does not lag behind other islands except for Siargao Island which has 47 recorded species. Among of the small islands with relatively low level of endemism compared to IGACOS are Batanes Group of islands and Saranggani with 13% and 18% endemism [10,11]. Villanueva [2] attributed the low endemism of Saranggani to the low topography of the island which is not favorable for surface flowing water and various anthropogenic disturbances the island experiences which is very similar with Island Garden City of Samal.

Kroeber's Percentage of similarity showed odonata of IGACOS is most similar to Siargao and Saranggani Islands which are also karstic in geology and have almost the same topographic description mainly low lying and no considerable high elevation. Among of the taxa shared by the different islands compared are the Oriental species from the family Libellulidae indicating high distribution of this family to the different parts of the archipelago (Table 3). For Zygoptera *Rhinocypha colorata* and *Agriocnemis femina femina* are abundantly distributed in all islands compared (Table 3). Most of the taxa not shared are the endemic zygopterans like *Euphaea refulgens, Amphicnemis dentifier, Neurobasis sp. (luzonensis)* and possible island endemic *Drepanostica sp., Risiocnemis atripes* which are confined to specific islands. Siargao hosts the most unique species not found in other islands like *Pseudagrion schieli, Teinobasis filamentum, Lestes p. praemorsus and Gynacantha bayadera*.

The unique species found in IGACOS not shared with other islands include the endemic species *Risiocnemis atripes*, and *Gomphidia kirchii* of which the latter is rare in most islands in the Philippines except Polillo[12]. No island endemic species is recorded in IGACOS

Table 2.Odonata fauna of IGACOS and other small islands in the Philippines

	IGACOS 300 km^2	Babuyan Group 50-196 km^2	Balut Island 80 km^2	Batanes Group	Bucas Grande 130 km^2	Siargao Isl. 436 km^2	Saranggani Isl. 70 km^2
Total Species Recorded	31	29	23	30	24	47	17
% of Endemism	35%	45%	26%	13%	38%	38%	18%
# of Species Shared	-	17	15	10	14	23	13
# of Species Not Shared	-	12	8	20	10	24	4
Kroeber's % of Similarity	-	56.73%	56.8%	32.79%	51.75%	61.65%	59.2%

Conclusion and Recommendation

The endemic species observed in the semi-forested area of Sampao Falls and Tagbaobo River is a good indication that the island still hosts to a few endemic species. However, the dominance of Oriental species mainly from Libillulidae in almost all fluvials systems surveyed indicates detrimental effect of various habitat modifications like resorts, human habitation, and clearing of forest for agricultural use. An immediate conservation effort such as the creation of a policy to protect the head water sources where most of the endemic species were found is urgent.

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ORCHID MYCORRHIZAE FUNGUS: IDENTIFICATION of *RHIZOCTONIA* in WEST BORNEO

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Abstract

Orchid is one of the ornamental plants that have high economic value. This effected an excessive exploitation of orchids. As a result some orchid species become threatened or even endangered for its existence in the population especially epiphytic orchid (*Appendiculla* sp., *Calanthe vestita* and *Bulbophyllum beccarii*) in West Kalimantan. The discovery of the interaction between orchids and mycorrhizal fungi raises the possibility of conservation of orchids *ex situ*. Generally, mycorrhizal fungi which frequently interact with the orchid is from the group of *Rhizoctonia* spp. Interaction between epiphytic orchid with *Rhizoctonia* is specific and effect the role of *Rhizoctonia* as mycorrhiza, saprophytic or parasitic. Therefore, this study aimed to identify the orchid mycorrhizal fungus on orchid plants in West Kalimantan. The methode of research were done exploration in forest of Raya Pasi and Bawang mountains, West Kalimantan, and isolated the healthy roots orchid. Fungi of isolation was identified using characteristic of morphology and isolates grouping. The results of research showed that fungi that isolated from the healty roots orchids (*Appendiculla* sp., *Calanthe vestita* and *Bulbophyllum beccarii*) were identified as *Rhizoctonia* spp., namely *Ceratoriza*, *Ephuloriza* and *Rhizoctonia* like. This results were the early information to study deeply about the role of orchid mycorrhiza fungi (*Rhizoctonia* spp.) to increase growth and as biocontrol of orchids plant.

Keywords: Orchid mycorrhizae, *Rhizoctonia*, identification, morphology characteristics

Introduction

Epiphytic orchid plant is one of many types of plants that grow in wet areas such as tropical rain forests of West Kalimantan. Some types of orchids have high economic value, such as *Appendiculla* sp, *C. vestita* and *B. beccarii*. For *ex-situ* conservation, the cultivation of orchids have many constraints, especially for mature growth, in addition, propagation by tissue culture is also not easy. In nature, Cultivation of orchids require the presence of mycorrhizae to initiate germination and seedling development. Orchid mycorrhiza is formed on the family Orchidaceae, which helps the absorption of ions essential for orchid growth (Peterson & Farguhan, 1994).

Orchid mycorrhizae-forming fungi is *Rhizoctonia*-like fungi. *Rhizoctonia* spp. will penetrate and invade the plant embryo when the seed begun to germinate [20]. Futhermore, fungal hyphae coiled around the plant root cells forming the peloton. It is one of the morphological characteristics of the orchid mycorrhizae.

Rhizoctonia grouped based on morphological

Material And Method

characteristics, the number of cell nuclei per cell hyphae and anastomosis (fusion of hyphae). Characteristics of *Rhizoctonia* morphological include colony color, concentric circles, sclerotia and hyphae size. Based on the number of the cell nuclei, *Rhizoctonia* is divided into 3 groups, namely uninucleate (anamorphs: *Ceratobasidium*), binucleate (anamorps: *Ceratobasidium*), and multinucleate (*Rhizoctonia* sp.) [20]. Based on the ability of the anastomosis, *Rhizoctonia* multinucleate consists of 14 AG (AG 1-13 and AG-BI) [1], AG1-1D (subgroup of group AG 1) [18], *Waitea circinata, R. globulis* and *Tulasnella* sp. (orchid mycorrhizal fungi)[2]. Binucleate *Rhizoctonia* group consisting of AG-A - AG-U [10], *Ceratorhiza cerealis, C. ramicola, Epulorhiza repens* and *E. calendulina,* (orchid mycorrhizal fungi) [2].

Information of *Rhizoctonia* as orchid mycorrhizal fungi is important to study because in the nature, this fungi can be as saprophyte, mycorrhizal fungi and pathogen [20]. Specifity of the association between *Rhizoctonia* species with orchid is effected by the vegetation diversity as the major forest component. Therefore the orchid culture in conservation ex-situ is influented by the success of *Rhizoctonia* colonization (as mycorrhiza association) in root.

Exploration and Isolation of Orchid Mycorrhizal Fungi (*Rhizoctonia*)

Exploration of orchids are taken from the orchids in West Kalimantan, the location shooting done in some natural forests of West Kalimantan. They are forest of Raya Pasi and Bawang mountains. Healty roots of orchids were cutted (1 cm), their surface-sterilized with consecutive washes of 70% ethanol (1 min), 2.5% sodium hypochlorite (30 s), and a final rinse of 70% ethanol (1 min), for isolation of orchids mychorrizae. *Rhizoctonia* spp. were isolated using method of [19]. The roots pieces were cultured in 10 ml potato dextrose agar (PDA) with 50 ppm each streptomycin, tetracycline, and penicillin, incubated at 25 ^{o}C. Each 10 ml PDA in *petri* dish contained 5-6 roots pieces. The growth of fungi (2-3 days after isolation) was observated under microscopy to determine *Rhizoctonia* isolates. The hyphal tip of *Rhizoctonia* was cutted and cultured in 10 ml soil extract [8]. This experiment was repeated 5-8 times to obtain pure isolates, and incubated at a temperature of 25-28 ^{o}C for morphological characteristics.

Morphological characteristics

Rhizoctonia sp. isolates were observed based on colony colour, sclerotia and fungal dimensions (hypha cell diameter and the number of nuclei per cell hypha) [22;6]. Mycelia plug was cultured in water agar (WA) on glass slide, incubated for 2-3 days. The number of nuclei were determined with the fluorescence mycroscopy after stained with safranine O dye. This observation was done as many as 30 fields of view for each isolate.

Isolates Grouping of *Rhizoctonia*

Isolates grouping of *Rhizoctonia* spp. were observed following the procedure Rauf *et al.* (2007) modified by [20], based on the criteria [14]. Anastomosis was tested by opposing isolates on PDA medium in Petri dishes (diameter 9 cm). Single *Rhizoctonia* sp. isolate was tested against one isolate else. Mycelial plug (diameter 6 mm) of *Rhizoctonia* sp. (4 days old) was plated 2-3 cm away from each other on WA in Petri dish. The dishes were incubated at 25 ^{o}C (2-3 days) by the time the growing hyphae of the isolates get in touch and overlapped with the hyphae of the other strain. Each coupling was done at least two times. A 0.5 cm^2 section detached from the area of contact was placed on a microscopic slide in a solution containing one drop of alkaline safranin 0 solution and one drop of 3.0% w/v potassium hydroxide (KOH).

Result

Observations pelotons done in root cells of terrestrial and epiphytic orchids (*B. becarii*, *Appendicula* sp. and *C. vestita*). Peloton is one of characteristics of the formation of orchid mycorrhizae. Thus, it was used as the basis of presence orchid mycorrhizal fungal detection (Figure 1). Orchid mycorrhizal fungi detected as the group of *Rhizoctonia*. Spesific characteristic of *Rhizoctonia* both macro and micro were shown in Table1,2and 3.

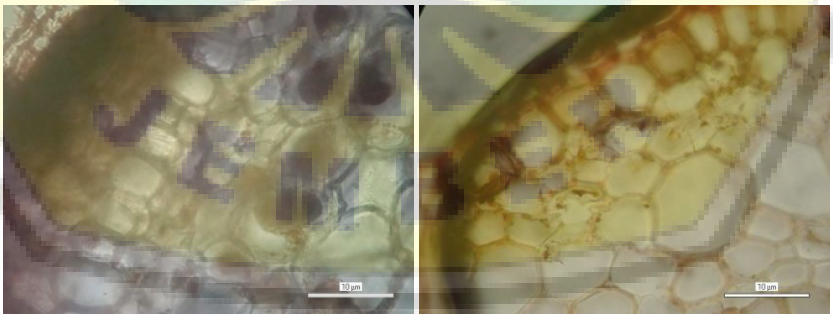

Figure 1. Orchid root cells (a and b); the peloton (red arrow)

Table 1. Morphological of orchid mycorrhizal fungi (*Rhizoctonia* spp.)

Note : RBBE (*Rhizoctonia* sp. isolate from *B. Beccarii* roots), RAPP (*Rhizoctoia* sp. isolate from *Appendicula* sp. roots), RCVE (*Rhizoctonia* sp. isolate from *C. Vestita* roots)

Isolates	Colony colour	Hyphal size		Sclerotia colour	Concentric circles
		Length	Width		
RBBE	White to light beige	68,19 ± 36,78a*	4,79 ± 0,66 a*	-	Apperent
RAPP	Light brown	49,93 ± 38,20a	4,61 ± 1,20a	Brown	Apperent
RCVE	White	54,88 ± 30,70a	5,02 ± 1,09a	White	Look less obvious

Description of the colony character, hypha, the number of cell nuclei and monilioid cells represent to the introduction of the types of orchid mycorrhizal fungi.

The mycellium colour of *Rhizoctonia* were observed on 7th days, RBBE isolate (*Rhizoctonia* from *B. becarii* roots) was white to light beige, RAPP (*Rhizoctonia* from *Apendiculata* sp. roots) was light brown and RCVE (*Rhizoctonia* from *C. vestita* roots) was white. The length and diameter of hyphae in each isolates were 16,19 µm and 4,79 µm (RBBE); 49,93 µm and 4,16 µm (RAPP); 54,88 µm and 5,02 µm (RCVE). Production of sclerotia is one of *Rhizoctonia* characteristic but it is not all *Rhizoctonia* produce sclerotia. The observation of research obtained two isolates of three isolates that produced sclerotia. Sclerotia from RBBE was produced on day 14 while RAPP on day 10 th (Figure 2 and Table 1). In addition of the hyphae, observation of monilioid cell was done. Moniloid cells of RBBE isolate are brown, elipsoidal shape, irreguler monilioid cells of RAPP isolate are hyalin to brown, formed at the tip cells of the main hypha and RCVE isolate are hyalin, elongate shape (Figure 2a, c, e and Table 2).

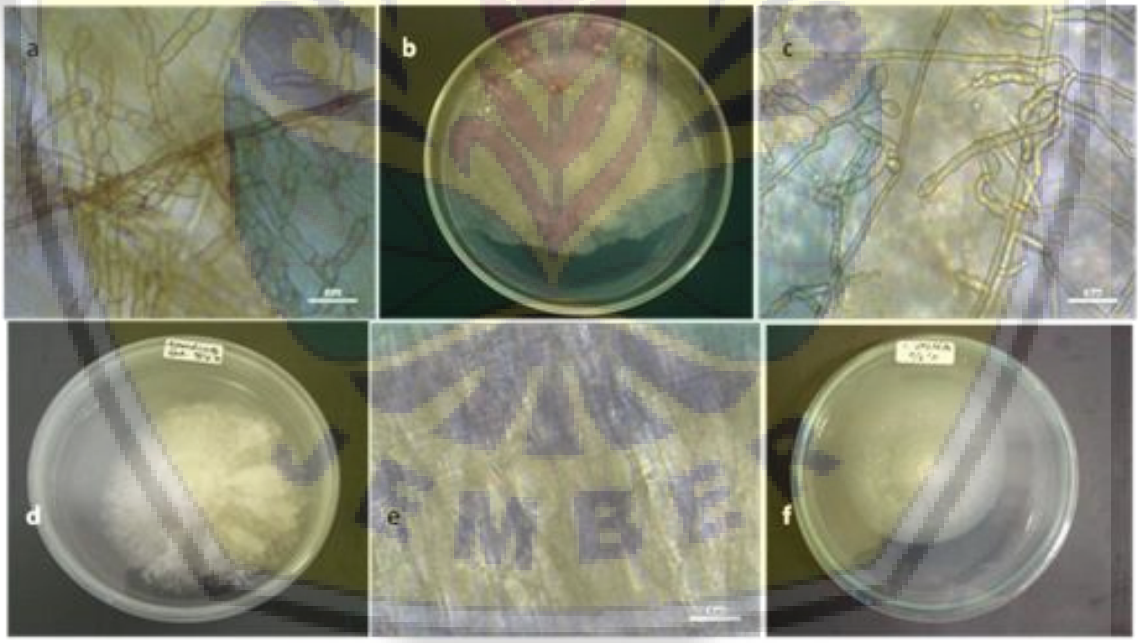

Figure 2. Monilioid cells (a, c, e) and isolate of *Rhizoctonia* (b, d, f). RBBE (a, b), RAPP (c, d), RCVE (e, f)

Table 2. Monilioid cells of *Rhizoctonia*.

Isolates	Moniloid cells		Shape
	Length	Length	
RBBE	19,00 ± 5,88b*	8,94 ± 2,70b*	Elipsoidal
RAPP	15,83 ± 3,82b	4,61 ± 1,20b	Irreguler, formed at the tip cells of main
RCVE	05,64 ± 1,82a	4,06 ± 0,44a	Irreguler

* sign followed by the same number indicated no difference significantly at significant level 95%.

Table 3. Average cell nuclei of *Rhizoctonia* spp.

Isolate	A number of cell based on anumber of hyphal cell nuclei				Average nuclei / hyphal cell	Note
	1 nuclei	2 nuclei	3 nuclei	4 nuclei		
RBBE	7	14	1	8	2,33	Binucleate
RAPP	8	13	7	2	2,10	Binucleate
RCVE	7	9	4	8	2,30	Binucleate

Note : RBBE (*Rhizoctonia* sp. isolate from *B. beccarii* roots), RAPP (*Rhizoctonia* sp. isolate from *Appendicula* sp. roots), RCVE (*Rhizoctonia* sp. isolate from *C. vestita* roots).

Table 4. Isolate grouping based o n the ability of the anastomosis (McNish et al., 1994).

Isolate	RBBE	RAPP	RCVE
RBBE	C3	C0	C0
RAPP		C3	C0
RCVE			C3

Note : RBBE (*Rhizoctonia* sp. isolate from *B. beccarii* roots), RAPP (*Rhizoctoia* sp. isolate from *Appendicula* sp. roots), RCVE (*Rhizoctonia* sp. isolate from *C. vestita* roots).

The number of nuclei/hyphal cells determined the classification of *Rhizoctonia* into groups binukleat, multinucleate or uninucleate. Table 3 showed that the average nuclei/cells of hyphae in each isolate were 2,33 nuclei (RBBE), 2,10 nuclei (RAPP) and 2,30 nuclei (RCVE). Some hyphal cells had a nuclei more than two and less than two, but its intensity were smaller than a cell with two nuclei. RBBE had 7 mononucleate cells (contain one nuclei), 14 binucleate cells (contain two nuclei), 9 multinucleate cells (contain several nuclei). RAPP had 8 mononucleate cells, 13 binucleate cells and 9 multinucleate cells. RCVE had 7 sel mononucleate, 9 sel binucleate and 12 sel multinucleate (Table 3).
Grouping *Rhizoctonia* can be done based on the ability to anastomose with tester isolate (isolate have known their AG). In this research there was no tester isolate that could only be done grouping among isolates were tested (Tabel 4). The results showed that there were no hyphal fusion between isolates (C0) so that it could be said that the three isolates of different species.

Discussion

C. vestita, *Appendiculata*, dan *Bulbophyllum* were epiphytic orchids in West Kalimantan. Viability of this orchids is very depend on mycorrhizal orchids (*Rhizoctonia*). As a cosmopolitan fungi, *Rhizoctonia* can be isolated in various methods [20; 22; 13; 16] but *Rhizoctonia* which can be mycorrhizal fungi, can only be isolated by using healty orchid roots, it was like the method of endophytic fungi isolation. This is because the ability of *Rhizoctonia* can form mycorrhizae in orchid roots while in the root of other plants also act as a saprophytic or parasitic well [20]. But [5] explained that they are fundamentally different from the highly specialised fungi forming other types of mycorrhizas.
Spesific characteristic of *Rhizoctonia*-like fungi is the hyphal branch at right angle and a septum at right angles branches, formed monilioid cells and sclerotia. *The* results of identification showed that isolates from orchids were *Ceratorhiza* (RBBE), *Epulorhiza* (RAPP) and *Rhizoctonia*-like (RCVE) (Identifitacation of *Rhizoctonia* based on [2]. The early detection of orchid mycorrhizae
Spesific characteristic of *Rhizoctonia*-like fungi is the hyphal branch at right angle and a septum at right angles branches, formed monilioid cells and sclerotia. The results of identification showed that isolates from orchids were *Ceratorhiza* (RBBE), *Epulorhiza* (RAPP) and *Rhizoctonia*-like (RCVE) (Identifitacation of *Rhizoctonia* based on [2]. The early detection of orchid mycorrhizae could be recognized from the pelotons that were formed in the host plant root cells. The peloton is hyphae that form masses of tightly-interwoven coils. The pelotons formation is influenced by the orchids type (epiphytic and terrestrial) and their habitats. [17] provided that 75% - 80% of the pelotons were found in the roots of some orchids cortex cells, whereas others only 5% - 10%. The presence of

hyphae penetrate the root cell wall and the presence of the peloton in the cortical region showed tolypophagy infection. This infection explained that the fungal hyphae penetrated the cell wall, forming a peloton (young and old) subsequently seen the digestion of hyphae [7]. It indicated that there is the mycorrhizal symbiosis between orchid plant roots with certain fungi. The presence of mycorrhizal orchid is based on the formation of the peloton while identification orchid mycorrhizal fungi are based on morphological characteristics and the number of cell nuclei/cell fungal hyphae.

Morphological properties that can characterize inter-genus, is isolate colour. The isolate colour of *Rhizoctonia* spp. varied (Table 1). The difference of isolate colour was influenced by age and species of *Rhizoctonia*. Some of research showed that *Rhizoctonia* spp. were white to dark brown [20;12; 18]. [19] explained that brown colour indicated the decomposition of melanin in the cell wall of hyphae. Therefore, it would be difficult to distinguish the role of *Rhizoctonia* (whether as a saprophyte, pathogen / parasite or mycorrhizal fungi) based on the colour of isolate. Beside that, the size of hyphal cell could not distinguish between *Rhizoctonia* as mycorrhizal fungi or not. The result showed there was no difference in cell size in each isolate (Table 2). The range of hyphal cell size varied, they were 1,60 - 2,23 µm (diameter) with a length 18,30 - 24,29 µm [20], 3 – 7 µm (diameter) [19, 20], 2,50–17,50 µm (diameter) and 15,00 – 382,50 µm (length) of hypha cells size of orchids mycorrhizal fungus [11]. Therefore, differences in the size of *Rhizoctonia* hyphae could not be used as a special characters for grouping *Rhizoctonia* [19]. The difference was caused by genetic and environment factors such as media and the growth temperature [1]. Unlike the case with moniliod cell, monilioid cell shape and size were different for each species of *Rhizoctonia*. The difference of monilioid cell shape and size could be used to distinguish species of *Rhizoctonia*, although it would be complete if equipped with molecular identification.

Rhizoctonia can distinguished by the number of nuclei in each cell of hyphae, which uninucleate (one nuclei), binucleate (two nuclei) and multinucleate (more than three nuclei). The number of nuclei in cell hyphae of *Rhizoctonia* range from 1 – 3 nuclei thus classified as binucleate. Isolat RBBE had 2,33 binucleate hyphal cells, the most widely among the three other isolates, while the smallest was RAPP (2,10 cells) (Table 3). There were some cells that had 1 nuclei, at a tip of hyphal cells or cells of young hyphae [15].

Rhizoctonia spp. from terrestrial orchid roots were binucleate. On the other side, binucleate and multinucleate *Rhizoctonia* are not much symbiosis with epiphytic orchid except uninucleate [4]. From this research could be proved that binucleate *Rhizoctonia* was associated with epiphytic orchid (in West Kalimantan). Therefore it was important to study specificity between uni-, bi- and multinucleate *Rhizoctobnia* with habitat type of orchid (terrestrial and epiphytic).

Identification of isolate based on morphology (*Ceratorhiza* sp., *Epulorhiza* sp. and *Rhizoctonia* like), could be proved with isolate grouping. [19] explained that the presence of tester AG for *Rhizoctonia* could be substituted by grouping with other contact at all three isolates (C0). Two of *Rhizoctonia* isolates are grouped in the same of AG if occurred the perfect of hyphal fusion, (there is a genetic suitability of vegetative structures *Rhizoctonia* / a same clone such as RBBE with RBBE, RAPP with RAPP and RCVE with RCVE, and if happened wall and cell membrane fusion followed by lysis of cells (somatic incompatibility response / a different clone) [15]. Associated between *Rhizoctonia* mycorrhizae with orchid was is specific especially epiphytic orchid.

Conclusion

Fungi isolated from the healty roots of *B. becarii*, *Appendiculata* sp. And *C. vestita* were identified as binucleat *Rhizoctonia* that associated with orchid roots. The third of *Rhizoctonia* isolates were not the same clones. They were *Ceratoriza* sp., *Epulorhiza* sp. And *Rhizoctonia* like. The success of orchid mycorrhizal fungi colonization determines the success of orchid cultures.

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Sauropus androgynus (L.) MERR. LEAF VARIATION THAT GROWS in THE AREA of SOME TRADITIONAL SOCIETIES in EAST JAVA .

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Abstract

Community life will continue to change along with the development of modern technology. This can effect change in culture and preservation of biological resources. Katuk is Indonesian native plants whose existence is increasingly rare . The preservation of plants katuk, if its use as a facilitator milk that is passed from generation to generation on the wane, it is feared the wild population will decline, due to a lack of knowledge in society. The introduction of the character of the plant is part of the knowledge society in an effort to optimize their utilization. The purpose of this study was to determine variations katuk leaves in various regions in East Java, especially in the area of traditional society. The results showed a variation of leaves katuk in all regions studied. Variations are found on the upper surface of the leaf (adaxial), while on the lower surface of leaves (abaxial) was not found. Overall katuk leaves on all the communities were studied showed four variations of the leaves that are light green with white patches of irregular, color dark green leaves with white patches regularly centered around the midrib, green color with patches regularly at the edge of the leaf, and color dark green with a smooth surface without spotting. The amount of variation katuk leaves showed differences in all areas studied.

Keywords: leaf variaty, *Sauropus androgynus,* traditional society, East Java.

Introduction

Katuk (*Sauropus androgynus* (L.) Merr.), including members of familia Euphorbiaceae [1; 2; 3; 4] is a native plant in Indonesia. Plants are scattered in Asian countries temperate (China) and tropical Asia such as India, Sri Lanka, Vietnam, Indonesia, Malaysia, Papua New Guinea, and the Philippines [5]. Generally, plants katuk used as a vegetable, especially for mothers who are breastfeeding because of its potential launch breast milk (ASI). According [6] and [7] katuk as a traditional medicine in Indonesia because of its potential as a facilitator of breast milk. Results of the research today shows that katuk as traditional medicinal plants, not only as a facilitator of breast milk, but it is useful also as an antioxidant [8; 9; 10]. Katuk leaves as an antioxidant because it contains vitamin C [10;8]. In addition to vitamin C, a natural antioxidant also found that flavonoids. Results of research in West Java, from 11 vegetables native (indigenous) consumed showed the highest flavonoid content (831.7 mg / 100 g) is in katuk [11]. The results of other research showing the benefits katuk as antimicrobial or antifungal [12; 13]. Generally Sauropus androgynus (L.) Merr. known as Katuk in Indonesia Pak-Wanban in Thailand. The term in Indonesia [14], among others: babing (Java), simani (Minangkabau). According [15] this plant is also known by the name of Katu or katukan (Java), katuk (Sunda), and kerakur (Madura). Other designations are katuk shoots in Riau [16] and kayu manis in Bali [17].

Consideration of a species is said to be native to a particular area or not, according to[18], can be observed among other aspects of habitat, geographic distribution, and reproduction patterns. Initial survey results on the field against katuk plant in 2011 to 2012 in the city of Malang shows the morphology of structural variation in leaf organs. Likewise, the results of the initial survey in some areas traditional societies in East Java (2013), found a variation on the leaf surface katuk found.

According [19] morphological variation (phenotypic) is a response to environmental conditions such as climate, soil, altitude, and temperature. Morphological variation is more common in the genus category, for example, morphological variation in the genus Kyllinga [20]. In Law No. 12 of 1992, section 1, point 2, germplasm is defined as a substance contained in a group of living things and is a source of offspring traits that can be exploited and developed to create the kind of superior or new cultivars. Examples germplasm for katuk named Bastar, Paris, Kebo and Zanzibar [21]. Is the leaf morphological variation found on katuk (category species) is the character of the individuals in a population that is effected by environmental factors, it is still necessary to study further.

According to the viewpoint of ethnobotany, a branch of biology that studies the interaction of plants with the community, that traditional communities have local knowledge (traditional wisdom) to the existing plants in the vicinity. Local wisdom is the wisdom that is understood at a certain cultural communities [22]. Community life will continue to change along with the development of modern technology. This can effect change in culture and preservation of biological resources. For example in the preservation of plants katuk, if its use as a facilitator milk that is passed from generation to generation on the wane, it is feared the wild population will decline, due to a lack of knowledge in the community about katuk plants.

Euphorbiaceae has approximately 7200 species include 300 clans spread across the tropics [4]. All members of this family have the character of a single flower in the armpit leaves lying alternate on the stem branch, has hermaphrodite flowers or female flowers and male flowers within an individual plant. One member of this family is a plant katuk which has the scientific name of the genus Sauropus BL, with floral characters in a single leaf armpit, initially forming one or several female flowers, then formed several male flowers, not found crown (corolla). There are three known species, one of which is cultivated for vegetables (cultivated vegetable) is Sauropus androgynus species (L.) Merr., While the other two species is S. macranthus Hassk. and S. rhamnoides BI. Morphological characters to the three species, according to [2] can be based on the color of the female flower calyx, calyx shape of the male flowers, fruit color, and habitat. Characters on Sauropus androgynus (L.) Merr. ie female flowers patterned calyx color red (dark red or yellowish mottled dark red), the male flowers form repand calyx with six lobes, fruit white to pink, and habitat includes curbside, living fences, teak rain at an altitude of between 5- 1300 meters above sea level (asl). What about the character of the leaves still need further study.

According to [1] and [4] katuk plants are classified into divisio Spermatophyta, Angiospermae subdivisio, classis Dicotyledoneae, order geraniales, familia Euphorbiaceae, Sauropus genus, and species Sauropus androgynus (L.) Merr. However [3] classify plants into divisio katuk, subdivisio, classis, and different orders, namely divisio Magnoliophyta, Magnoliopsida subdivisio, classis Rosidae, and order euphorbiales. As a medicinal plant germplasm, karaktersisasi katuk has not been done to the level of species or varieties. The introduction katuk in the field is still limited to katuk green, marked in green leaves and red katuk marked with green leaf color red [23]. However, the National Commission Plasma nuftah has spread germplasm katuk terms clones or varieties Paris, Kebo, Bastar and Zanzibar [21]. According [24] characterize the upper surface of the leaves is used to characterize clones katuk ie plain green leaves (Bastar), leaves with white patches spread (Paris), and the white spot centered in the middle (Zanzibar).

The purpose of this study was to determine variations katuk leaves in various regions in East Java, especially in the area of traditional society. In particular the research is expected to provide information on the characterization of variations in leaf katuk. For further be used as a baseline (data base) in terms of distribution katuk through mapping, as well as plants katuk ethnobotany in the use and management aspects of cultivation in the society, particularly traditional communities in East Java. Generally exploring herbs local (indigenous plants) with potential (bioprospect), increasing the quantity of mapping the biodiversity of plants in nature, and promote empowerment of local knowledge as a strategic effort conservation katuk-based society, particularly traditional communities in East Java, as well as in general in the country Indonesia.

Materials and Methods

This research was done during October to December 2014 in areas that represent traditional communities in East Java with a majority population of ethnic and traditional behavior which is still attached to people [22] . Area community that have been chosen the appropriate altitude habitat katuk, between 5-1300 m asl [2] .

Samples of katuk leave were performed on five areas of traditional communities in East Java , ie. the Java Community Mataraman (Pacitan at an altitude of between 29-824 m asl), Community of Pandalungan (Lumajang at an altitude of between 25-210 m asl) , Society Madura Island (Sumenep at an altitude of between 16-218 m above sea level) , the Society of Osing (Banyuwangi at a height of between 171- 453 m asl), and the society of Tengger (Malang at height about 868 to 1119 m asl). Data variaty of katuk leave in traditional communities were analyzed based tabulation descriptively.

Maps of the area were sampled as shown in Figure 1.

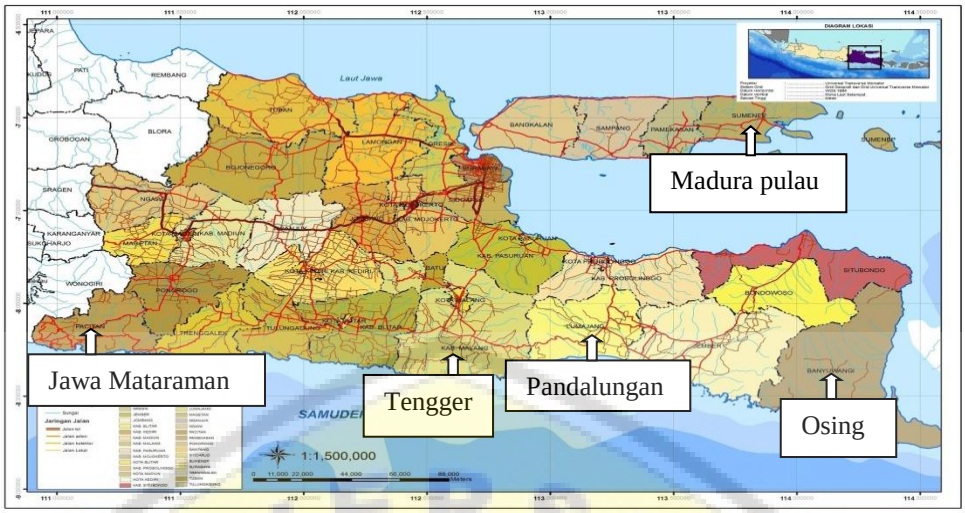

Figure 1. Map of East Java with traditional public area were marked arrows (Source : www.google.com)

Results and Disscusions

Katuk exploration results in the area of plant communities studied showed variations on the upper surface of the leaf (adaxial), while on the lower surface of leaves (abaxial) was not found.

The lower surface of the leaves on all the plants of katuk were studied has green rather young colour (Scale number 3, according [25]. Description of the lower surface of leaves katuk shown in Figure 2.

Variations on katuk leaf surface in area of all traditional societies studied are described in Table 1.

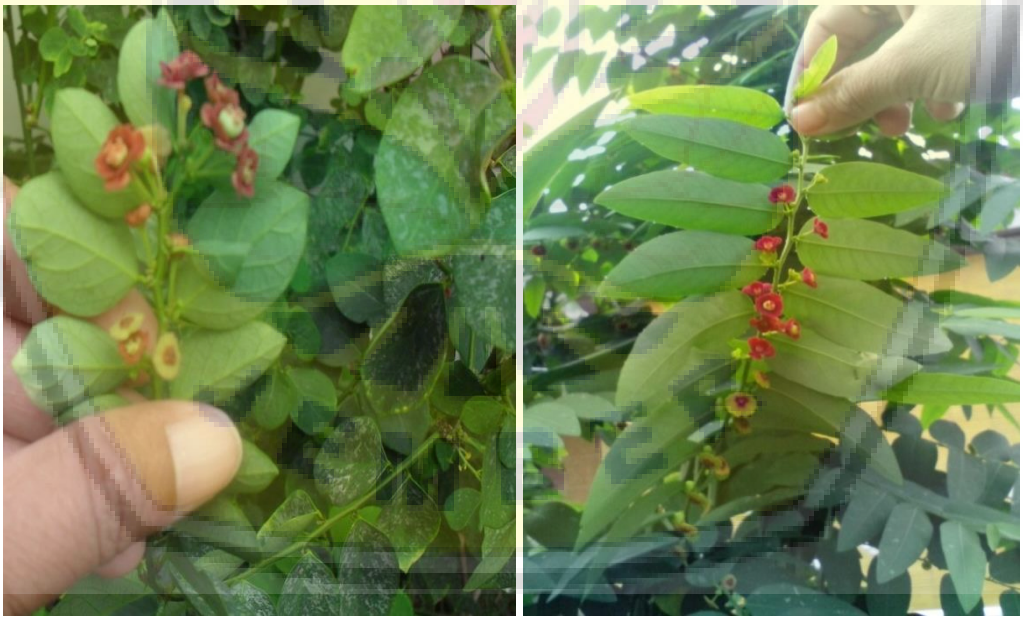

Figure 2. The lower surface of the leaves of katuk have a light green color plain without spotting (Source: Personal Document, 2014)

Table 1. Description of the variation on the leaf surface of katuk

No	Location	Variation amount	Variation amount
1	Osing	plain; white spot in the center; white patches spread	3
2	Jawa Mataraman	White spot in the center; white patches spread	2
3	Pandalungan	plain; white spot in the center; regularly white patches on the edge; white patches spread	4
4	Madura pulau	plain; white spot in the center; white patches spread	3
5	Tengger	plain; white patches spread	2

In Table 1 is found variations in color upper surface of katuk leaves and its amount. All of the area were studied is found four variations i.e. plain (color dark green with a smooth surface without spotting); white spot in the center (color dark green leaves with white patches regularly centered around the midrib); regularly white patches on the edge (green color with patches regularly at the edge of the leaf); and white patches spread (light green with white patches of irregular). Description of these variations is shown in Figure 3.

Figure 3. Variations of upper surface of katuk leaves. A. White spot in the center. B. White patches spread C. Regularly white patches on the edge. D. Plain (Source: : Personal Document, 2014)

Based on the amount of variation that was found highest in public areas Pandalungan (4 Variations), followed by Madura island and Osing (3 Variations), and the fewest are found in areas of Java Mataraman and Tengger (2 variations).

Based on the location of traditional communities studied demonstrable differences in the percentage of variation, ie variation of scattered white spots exist in all areas of traditional communities studied (100%). Further variations on the central white spot is found in four areas, namely area Osing, Java Mataraman, pandalungan, and Madura island (80%). While in public areas Tengger not found such variations. Likewise, the smooth variation (80%) was found in four areas, namely community Osing, Pandalungan, Madura Island, and Tengger. While in the area of Java Mataraman not found a plain variety. Only one variation, regularly white patches on the edge is found in Pandalungan (20%). Differences katuk leaf surface variations was found in the communities studied possible areas related to its potential value. Other possibilities may be due external factors such as the environment in which the katuk plants grow or internal factors.This matter still needs further study.

Knowledge of the many potential katuk in the community is expected to increase the population katuk in nature, so that maintained continuity. For example in Probolinggo, katuk plants that are known to have nutritional value and high in vitamin C used as one solution to overcoming malnutrition. Once the importance of tackling malnutrition, so Probolinggo Regent Tree Planting Katuk launched on November 15, 2013 in the District Maron. The activity was followed by other activities, namely the creation of copyright festival menu of processed food made from the leaves katuk by Probolinggo District Health Office [26] .

The intervention of Local Government and related agencies be great opportunities in katuk conservation in the community. Katuk farming activities in the community also opened up opportunities for more specialized studies on the characteristics of plants katuk as a justification of the importance of plant communities katuk.

Conclusions

The results showed a variation of leaves katuk in all regions studied. Variations are found on the upper surface of the leaf (adaxial), while on the lower surface of leaves (abaxial) was not found. Overall katuk leaves on all the communities were studied showed four variations of the leaves that are light green with white patches of irregular (white patches spread) , color dark green leaves with white patches regularly centered around the midrib (white spot in the center), green color with patches regularly at the edge of the leaf (regularly white patches on the edge), and color dark green with a smooth surface without spotting (plain). The amount of variation katuk leaves showed differences in all areas studied. The most variations in public areas Pandalungan (4), followed by Madura island and Osing (3), and least in Java Mataraman (2).

Acknowledgment

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CHARACTERISATION of SYMBIOTIC BACTERIA ISOLATED from SPONGE *Haliclona* sp.

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Abstract

Marine sponges are invertebrates in marine ecosystem which have the essential value such as pharmaceutical resources and ecological aspect in marine ecosystems. Exploitation on this sponge recently improved and several aspect of it also more have attention. Based on sponge characteristics, many microorganisms have the relationship with this creature. The microorganisms can be isolated to the numerous aspect of human life. This study purposes are isolate and determine the type and the characteristics of symbiotic bacteria on a sponge *Haliclona* sp. from Situbondo coastal water, East Java. The focus of characterizations is extracellular enzymes including amylolytic, proteolytic, and cellulolytic. This research was conducted qualitatively by using descriptive methods, which is data analysis was depicted in graphics and figures. The results showed that the overall bacteria symbiotic with sponges *Haliclona* sp. had activity in producing the enzyme amylase, cellulose, and protease and bacteria that have the best activity in producing an enzyme identified as *Enterobacter* sp.

Keywords: *Enterobacter* sp., symbiont bacterial sponges, enzyme activity, pathogen bacteria

Introduction

Sponges are invertebrates included in the phylum Porifera. Sponges are the oldest class of metazoan animals were able to survive until today, despite sponge still has the function of organs and tissues that are very simple [1]. Sponges are animals including a porous filter feeder [2]. The way of sponge take a feeding is filtering water through the pores, making the sponge as a habitat or a host for microorganisms, such as algae, bacteria, phytoplankton and fungi [3]. Microorganisms have two important roles in biological systems sponge, which is as a food source [4] and living in symbiosis [5]. The bacterial symbiosis with sponges *Haliclona simmulans* is quite diverse [6]. Five phyla of bacteria found in symbiosis in sponges, including the phylum Actinobacteria, Bacteriodetes, Firmicutes, Proteobacteria α-and γ-Proteobacteria [7].

Sponges produce chemical compounds as part of a defense system against predators and competitors [7]. The chemical compounds will also induce symbiotic microorganisms with a sponge to produce specific secondary metabolites [8]. Specific secondary metabolites produced by microorganisms that are symbiotic with sponges is strongly associated with chemical compounds generated by its host sponge [9]. Specific secondary metabolites has produced by symbiont bacteria bacteria with the sponge have variety of biological activities. *Alteromonas* sp., *Bacillus* sp. and *Halomonas* sp. symbiotic with sponges sp. reportedly can produce β-lactamase inhibitor [11]. The symbionts bacteria with sponges *Dendrilla nigra* identified as *Pseudomonas* sp. (MSI057) has a lipase-producing activity known as psychrophilic alkaline lipase characteristics that can be applied in industrial activities [11].

Material and Method

This research was conducted qualitatively by using descriptive method, which aims to illustrate the nature of a condition existing at the time of the study and explore the cause of the symptoms that occur [12]. This study will describe the activity of extracellular enzymes (amylolytic, cellulolytic and proteolytic) and characterization of bacterial isolates symbiosis with marine sponges.

Bacteria Isolation

Isolation from Spons *Haliclona* sp.

Sponge cleaned first with seawater flowing prior to the isolation of bacteria. Spongy tissue sections were taken by cutting the sponge as much as one gram then crushed using a mortar that had previously been sterilized 13(Kim *et al*., 2006). Results grinding as much as one gram is inserted into a test tube and diluted with nine mL of sterile seawater (salinity 33ppt). The solution was further diluted to 10^{-3}. Samples were inoculated with the surface plate method, by taking a sample of one ml of medium inoculated in TSA aseptically, then incubated in an incubator at 30°C for 24-48 hours [14]. Colonies of bacteria that grow on the media

observed. Each bacterial colonies growing separated by color and form colonies, and purified using the same media [4].

Isolation from Seawater

Samples obtained from marine waters Situbondo. Each one ml of the sample is then inoculated with a surface plate method at 10% SWC media and incubated for 48-72 hours at a temperature of 30ºC [15]. Colonies of bacteria that have grown on the medium were then observed. Each bacterial colonies growing separated by color and form colonies, and purified using the same media [4].

Extracelulair Enzyme Activity test

Amilolitic activity test

Extracellular enzymes amylase activity test using disc diffusion method based on research [16]. Pure isolates obtained from the purification grown in TSB media in mikrotub diluted with sterile sea water (salinity 33ppt). Isolates in mikrotub then incubated in the incubator for 24 hours at 30°C. Isolates were grown visible color change from cloudy to mikrotub. Isolates were then inoculated on paper disc, by dipping a paper disc on bacteria in TSB media. Inokulat then planted in the media with the addition of 0.2% NA starch diluted with sterile sea water (salinity 33ppt). Isolates were then incubated at 30ºC for 24-48 hours.

Proteolitic activity test

Production of protease enzyme activity test is based on research [16]. Pure isolates obtained from the purification grown in TSB media in microtubes diluted with sterile seawater (salinity 33ppt). Isolates in microtubes then incubated in the incubator for 24 hours at 30°C. Isolates were grown visible color change from cloudy to microtube. Isolates were then inoculated on a paper disc, by dipping a paper disc on bacteria in TSB media. Inoculate then planted on NA medium with the addition of 5% skim milk diluted with sterile seawater (salinity 33ppt). Isolates were then incubated at 30ºC for 24-48 hours.

Selulolitic Activity Test

Cellulolytic enzyme production activity test is based on Shanmugapriya's research [17]. Pure isolates obtained from the purification grown in TSB media in microtubes diluted with sterile seawater (salinity 33ppt). Isolates in microtubes then incubated in the incubator for 24 hours at 30°C. Isolates were grown visible color change from cloudy to microtube. Isolates were then inoculated on a paper disc, by dipping a paper disc in TSB media containing bacterial isolates. Inoculate then planted on NA medium with the addition of 0.5% carboxyl methyl cellulose diluted with sterile seawater (salinity 33ppt). Isolates were then incubated at 30ºC for 24-48 hours.

Isolates Identification of Bakteria

Isolates identification of bacteria were made by some of the observations that morphological observation colony, gram stain, catalase test, test oxidase, test the motility and the production of compound indole, test O/F (Oxidative/ Fermentative), test TSIA (Triple Sugar Iron Agar), and the sugar test.

Colony morphology observation was conducted to determine the color of the colony, the colony edge, elevation or surface colonies and form colonies. Catalase test aims to determine the activity of bacteria to produce the enzyme catalase. Oxidase test is done to determine whether their oxidase enzyme produced by bacteria. Motility test is performed to determine whether the bacteria are motile or non-motile. Test O/F medium (Oxidative/Fermentative) aims to determine the nature of oxidation or fermentation of bacteria to glucose. Sugar test aims to determine the ability of bacteria to degrade the sugar and produce organic acids [18].

TSI slant performed referring to Lehman [19] with the aim to differentiate types of bacteria based on their ability to break down lactose, glucose and sucrose and to determine the ability of bacteria to produce H2S.

Discussion

Isolation of Symbiotic Bacteria from Spons *Haliclona* sp.

Based on the research conducted, from a sponge colony Haliclona sp. weighing one gram has been obtained bacterial colonies growing on media Tryptic Soy Agar (TSA), having incubated for 24-48 hours. From the results obtained 74 separate colony isolation.

A total of 74 pure isolates were tested for activity to produce extracellular enzymes (amylolytic, proteolytic and cellulolytic), the media SA, SMA and CMCA (Feby and Nair, 2010; Shanmughapriya et al., 2010). Producing enzyme activity characterized by a clear zone around the media indicating that bacteria can produce extracellular enzymes [20]. After testing the activity of the enzyme producer, will be chosen isolates with the best activity to be characterized by testing colony morphology, gram staining, and biochemical tests to identify bacterial symbiosis with sponges *Haliclona* sp. (Figure 1. A-B)

Isolation of Bacteria on Seawater

Isolation of bacteria that have been made of sponge living aquatic environments where Haliclona sp. obtained seven separate bacterial colonies that grows on TSA media. Bacteria that grow on TSA media is dominated by colonies of bacteria with

cream-colored colony morphology, elevation convex, rounded shape colony diameter ranging from 0.1 mm sd 1mm with a flat edge of the colony. Bacterial colonies have growing on the medium and then purified TSA to form a single colony. Seventh isolates will then be characterized by testing colony morphology, gram staining, and biochemical tests to identify the bacteria present in the aquatic environment sponge *Haliclona* sp. (Figure 1. C-D)

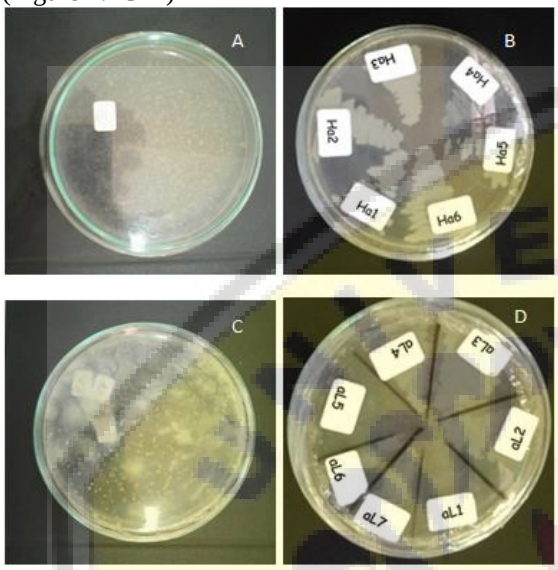

Figure 1. Isolation and purification of bacteria in the sponge symbiont *Haliclona* sp. (A-B) and isolate and purification of acterial seawater (C-D).

Extracellular Enzyme Activity Test

Amylolytic Activity Test

Amylolytic activity test performed on media Nutrient Agar (NA) by the addition of 0.2% starch diluted with sterile seawater (salinity 33 ppt) [16]. The test results from amylolytic activity characterized by a clear zone around the paper disc that has been inoculated bacteria. Based on the test results amylolytic activity against 74 bacterial isolates, all isolates were capable of hydrolyzing enzymes amylolytic to wide diameter clear zone produced ranges from 10mm up to 19 mm.

Proteolytic Activity Test

Proteolytic activity test performed on media Nutrient Agar (NA) by the addition of 0.1% skim milk diluted with sterile seawater (salinity 33ppt) [16]. The test results from proteolytic activity characterized by a clear zone around the paper disc that has been inoculated bacteria. A total of 74 bacterial isolates capable of hydrolyzing enzymes amylolytic to wide diameter clear zone produced ranges from 12 mm up to 20 mm.

Activity Test Cellulolytic

Cellulolytic activity test performed on media Nutrient Agar (NA) by the addition of 0.5% carboxyl methyl cellulose diluted with sterile seawater (salinity 33ppt) [17]. The test results from cellulolytic activity characterized by a clear zone around the paper disc that has been inoculated bacteria. The test results from cellulolytic activity against 74 bacterial isolates obtained to isolate amylolytic enzyme capable of hydrolyzing to a wide diameter clear zone produced around 8-14 mm.

Identification of Symbiotic Bacteria Isolates from Marine Sponge *Haliclona* sp.

Based on the test results of the activity of producing extracellular enzymes (amylolytic, proteolytic and cellulolytic) each earned eight isolates with the best in the hydrolyzing activity of extracellular enzymes indicated by the size of the diameter of clear zones that are relatively higher when compared with the others. Diameter clear zone formed qualitatively show will be the high capability of extracellular enzymes produced or can also indicate a high number of enzymes will be produced and released by bacteria (and, consisted Subagiyo, 2012). The eighth isolates each with a code Ha2, Hb31, Hb42, Hc49, Hc57, HC62, Hd67 and Hd72. Eight isolates were further identified by test colony morphology, gram staining and biochemical tests subsequent test results will be compared with Bergey's Manual of Determinative Bacteriology for analysis determine the species of bacteria.

Results of the test bacteria morphology based on macroscopic observations on TSA media, as well as microscopic observations by staining gram with the aim to see the shape and nature of the cells can be found in the annex grams 9.Hasil macroscopic observations showed that the eight isolates have colored colony morphology beige, elevation convex shape and forms spherical colonies with a flat edge. Based on Gram staining of eight isolates, whole isolates are Gram-negative rod-shaped. Based TSIA test in eighth biochemical test isolates could break down lactose and sucrose are marked in yellow on the media slant (slant) and upright (butt). In the test oxidase, indole production, H2S production, gas production and the ability to ferment inositol showed negative results. Being the catalase test, test motility and ability to ferment glucose, sucrose, maltose, arabinose, mannitol and lactose showed positive results. The characterization of the results of the eight isolates showed the character that corresponds to a group of Enterobacter sp. identified based on the characters in Bergey's Manual of Determinative Bacteriology [21].

Identification of Bacterial Isolates from Seawater

Testing of bacterial isolates identified by colony morphology, gram staining, and biochemical tests subsequent test results will be

compared with Bergey's Manual of Determinative Bacteriology. The test results will be analyzed to determine the genus of bacteria. Results of the test bacteria morphology based on macroscopic observations on TSA media, as well as microscopic observations by staining gram with the aim to see the shape of the cell and the nature of gram can be seen in Table 1 and Tabel 2. Based on observations of colony morphology, gram staining, and biochemical test of the seventh isolates can be seen that there are some isolates that have similar characteristics. Detail information of biochemical test in Table 3.

Tabel 1. The morphological characteristics of symbiotic bacterial colonies isolated from sponge *Haliclona* sp.

No.	Isolate	Colony Morphology			
		color	edge	Elevation	configuration
1	Ha2	cream	flat	Convex	rounded
2	Hb31	cream	flat	Convex	rounded
3	Hb42	cream	flat	Convex	rounded
4	Hc49	cream	flat	Convex	rounded
5	Hc57	cream	flat	Convex	rounded
6	Hd62	cream	flat	Convex	rounded
7	Hd67	cream	flat	Convex	rounded
8	Hd72	cream	flat	Convex	rounded

Isolates with code AL1, AL3 and al5 identified as a group of Gram-negative bacteria are rod-shaped, can produce H2S and Gas, oxidase and catalase positive, non-motile, can ferment glucose, sucrose, and maltose. The third negative isolates the indole production test, and showed no reaction in the test O / F. Based on the characteristics of the three bacterial isolates can be identified into the genus *Pseudomonas* [21]. Isolates AL2 and al7 including a group of Gram-positive, rod shape, non-motile, catalase positive, capable of fermenting glucose, sucrose, arabinose, mannitol, and maltose. Both of these isolates also known oxidase negative and can not ferment lactose and inositol. Characteristics of the isolates AL2 and al7 can be identified as the genus *Bacillus* [21]. Further Isolates aL4 and aL6 known as a group of Gram-negative bacteria, the form of rods, motile, are fermentative, showed positive results in catalase test, oxidase test, and indole test. Both isolates can ferment glucose, mannitol, and maltose. But can not ferment lactose, sucrose, arabinose and inositol. With such characteristics, the isolates aL4 and aL6 belonging to the genus Vibrio [21].

Discussion

Isolation of bacterial symbiosis with sponges *Haliclona* sp. TSA may use the media that is reconstituted with sterile sea water [14]. Bacterial colonies that grow after incubated for 24 hours was dominated by spherical colonies with colonies spread evenly in all parts of the TSA media. A total of 74 separate colonies result from isolation, have extracellular enzyme-producing activity. Whole bacterial isolates obtained from sponge Haliclona sp. capable of producing the enzyme amylase, protease and cellulase. Almost 12% bacterial symbiosis with marine sponges can produce two to three different types of enzymes [16]. Production of extracellular enzyme activity characterized by a clear zone around the bacterial inoculant, a clear zone can be formed because the bacteria can hydrolyze starch, protein and cellulose in each test medium-producing activity of the enzyme.

Tabel 2. The morphological characteristics of symbiotic bacterial colonies isolated from seawater.

No.	Isolate	Colony Morphology			
		color	Edge	elevation	Configuration
1	aL1	cream	Flat	convex	Rounded
2	aL2	white	Flat	convex	Rounded
3	aL3	cream	Flat	convex	Rounded
4	aL4	cream	Flat	convex	Rounded
5	aL5	cream	Flat	convex	Rounded
6	aL6	cream	Flat	convex	Rounded
7	aL7	white	Flat	convex	Rounded

Bacteria have amylolytic activity, proteolytic and cellulolytic by producing the enzyme amylase, protease and cellulase is secreted into the environment [20]. The third function of these enzymes to break down the composition of chemical compounds contained in food remains a sponge. Extracellular amylase enzyme used to hydrolyze the compounds are starch, extracellular protease enzyme used to hydrolyze the compounds are proteins into simpler compounds such as oligopeptides, short-chain peptides and amino acids. The function of extracellular also as cellulase enzymes is hydrolyzing cellulose.

Enzymes are produced by bacteria to break down the rest of the food contained in the body of the sponge, then the rest of the feed that has been reformed into simpler compounds can be directly used as the compliance component of bacterial nutrient for growth [20]. Enzymes that have been produced by the bacteria can also help the process of digestion of food by sponge *Haliclona* sp., simple compounds on food remains after

overhauled by the enzyme can be digested again by sponge [9].

Isolates bacteria symbiotic with sponges Haliclona sp. produce extracellular enzymes as a form of symbiotic mutualism [4], symbiotic mutualism is a form of association between two organisms of each benefit [22]. Simibiosis form of mutualism between sponges *Haliclona* sp. with bacteria, can be seen from the sponge that provides shelter and protection from competitors for the bacteria. As well as bacteria that produce extracellular enzymes to aid digestion of food by sponge.

The activity of bacteria in produce extracellular enzymes indicated by the formation of a clear zone, the diameter of clear zone formed indicating that there is a revenue-producing activities of enzymes, as well as qualitatively show will be the high ability of enzymes produced is also high number of enzymes that are produced and released by bacteria [20]., Under these conditions, found eight isolates with a clear zone diameter larger than the clear zones produced by other bacteria. Eighth these isolates was isolate the code Ha2, Hb31, Hb42, Hc49, Hc57, HC62, Hd67 and Hd72, to test the results of the activity of the extracellular enzyme-producing bacteria are symbiotic with sponges Haliclona sp. can be found in annex 3, 5 and 7. Based on the test characterization of eight isolates of bacteria by using morphological observation test, Gram staining and biochemical tests. Eighth isolates have the same characteristics, the eight isolates were identified as groups of bacteria are Gram-negative, rod-shaped, can not produce H2S and Gas, oxidase negative, catalase positive, motile, can ferment glucose, lactose, sucrose, arabinose, mannitol and maltose , Eighth isolates were also negative in indole production test, and the test is fermentative O/F. Based on these characteristics eighth symbiotic bacteria with a sponge *Haliclona* sp. showed identical characters with character bacteria in Bergey's Manual of Determinative Bacteriology identified as *Enterobacter* sp. [21].

The physiological and biological character of *Enterobacter* sp. is Gram-negative, rod shape, motile and can ferment sucrose, raffinose, sorbitol and glycerol [23]. *Enterobacter* sp. is also oxidase, indole and H2S negative. With a character which is owned in common between bacteria symbiotic with sponges *Haliclona* sp. with characters from *Enterobacter* sp., it can be concluded that the eight isolates of bacteria symbiotic with sponges *Haliclona* sp. the best extracellular enzyme-producing activity is *Enterobacter* sp.

According to Munn [24], *Enterobacter* sp. is a bacterium which belongs to the family γ-Proteobacteria. The γ-Proteobacteria families of bacteria is known to form a symbiosis with the sponge *Haliclona simulans* origin Irish waters, Ireland [6]. The γ-Proteobacteria families, including *Enterobacter* sp., also can form a symbiosis with the sponge *Dysidea granulosa* origin Kavaratti islands, the islands of Lakshadweep, India is endosymbiosis extracellular namely symbiosis that occurs inside the sponge body [23]. It is known that the bacterial symbiosis with the sponge in the extracellular contained in mesohyl layer that is outside the cell sponge [25].

Isolation of bacteria that have been made from sponge aquatic environment Haliclona sp. obtained seven separate bacterial colonies that grow on TSA media. Bacteria that grow on TSA media is dominated by colonies of bacteria with cream-colored colony morphology, convex elevation, a circular shape colony diameter ranging from 0.1 mm sd 1mm with a flat edge of the colony. Based on the observation of colony morphology, gram staining, and biochemical tests of seven bacterial isolates can be seen that there are some isolates that have similar characteristics.

Isolates aL4 and aL6 known as a group of Gram-negative bacteria, the form of rods, motile, are fermentative, showed positive results in catalase test, oxidase test and indole test. Both isolates can ferment glucose, mannitol and maltose. But can not ferment lactose, sucrose, arabinose and inositol. With specific characteristics, the isolates aL4 and aL6 included in the genus Vibrio, according to Garrity [21] genus *Vibrio* has the characteristics of Gram-negative, have a variety of forms ranging from stem slightly curved, arched resembles a comma, facultative anaerobic, are fermentative, can ferment glucose, mannitol and maltose. Positive results are show in catalase test, oxidase test and indole test. *Vibrio* can grow well at a temperature of 30-370C.

Isolates AL2 and al7 including a group of Gram positive, rod shape, non-motile, catalase positive, capable of fermenting glucose, sucrose, arabinose, mannitol and maltose. Both of these isolates also known oxidase negative and can not ferment lactose and inositol. With specific characteristics, the isolates AL2 and al7 can be identified as the genus *Bacillus*, according to Garrity [21] Bacillus has the characteristics of Gram-positive, rod-shaped with a slightly curved part resembles a comma, a non-motile, aerobic facultative and non-aerobic facultative, showing results positive in catalase test, can ferment glucose, sucrose, arabinose, mannitol and maltose.

Isolates with code AL1, AL3 and al5 identified as a group of Gram-negative bacteria are rod-shaped, can produce H2S and Gas, oxidase and catalase positive, non-motile, can ferment glucose, sucrose and maltose. The third negative isolates the indole production test, and showed no reaction in the test O / F. Based on the characteristics of the three bacterial isolates can be identified into genus Pseudomonas, according to Garrity [21] genus

Pseudomonas has the characteristics of Gram-negative, rod-shaped, aerobic, oxidase and catalase positive, non-motile, can ferment glucose, sucrose and maltose. Can grow in the temperature range 04-450C, but grows optimally at a temperature of 280C.

Seven isolates were obtained in aquatic environments sponge *Haliclona* sp. divided into three different genera, the genus *Bacillus* are, *Pesudomonas* and *Vibrio*. The third genus is known to have broad pattern of spread in the environment, especially in aquatic environments [24]. Several many factors governing the distribution spread of *Bacillus*, *Pesudomonas* and *Vibrio* but there are some of the most important factors in influencing patterns of deployment include inorganic nutrients, carbon source available, temperature, salinity, dissolved oxygen, and the depth below sea level [21].

The research that has been done, it can be seen that in general isloat symbiotic bacteria with a sponge *Haliclona* sp. the coastal waters of northern origin Situbondo, East Java, forming a symbiotic manner extracellular endosymbiosis. This form of interaction occurs through horizontal transmission occurring as a result of eating habits sponge as a filter feeder make symbiotic bacteria inside the sponge body, and also known as endosymbiosis extracellular [5]. Bacterial symbiosis with the sponge in the extracellular contained in mesohyl layer that is outside the cell sponge [25]. This statement is supported from the isolated bacterial symbiosis with sponges Haliclona sp. and isolated the bacteria in aquatic environments Area Haliclona sp. Results of macroscopic observation of colony morphology was found that some bacteria found in aquatic environments area *Haliclona* sp. also found in bacteria symbiotic with sponges *Haliclona* sp.

The test results further identification by gram staining and biochemical tests it was found that of the symbiotic bacteria with a sponge *Haliclona* sp. and have the best production of an enzyme activity is *Enterobacter* sp., but did not rule out the possibility that the genus Bacillus, *Pseudomonas* and *Vibrio* are found in aquatic environments area sponge *Haliclona* sp. also there and form a symbiosis with the sponge *Haliclona* sp. because of the macroscopic observations also found that the same colony morphology between bacteria from aquatic environments with symbiotic bacteria with a sponge *Haliclona* sp.

Enterobacter sp. symbiotic with sponges *Haliclona* sp. has the best activity to produce extracellular enzymes can indicate that the *Enterobacter* sp. is a species of bacteria associated with sponge *Haliclona* sp. through vertical transmission which is a form of interaction between the bacteria with a sponge since the sponge in the larval stage [7]. One quirk of this transmission is secondary metabolites form produced by bacteria have a better activity when compared to bacterial symbiosis with the sponge of the other part [4].

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ANTIBACTERIAL ACTIVITY of INFUSION and ETHANOL EXTRACT of SOME MEDICINAL PLANTS as ANTIDIARRHEA BASED on BANGKA SOCIETY'S KNOWLEDGE

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Abstract

Since ancient time, plants have been used as a source of medicine. One of common disease which happen frequently in tropical country is diarrhea. Based on Bangka society's knowledge, some plants which common used as medicine to cure diarrhea are *Adinandra sorasanthera, Baeckea frustescens, Rhodomyrtus tomentosa*, and *Trema orientalis*. *A.sorasanthera, R.tomonetosa, T.orientalis* were used by Lom community (indigenous community of Bangka) and *B.frustescens* used by other local society. Both the water infusion and the ethanol extract of those plants leaves were tested their effectiveness against four enteropathogenic bacterias (Enterophatogenic *Escherichia coli, Pseudomonas aeruginosa, Shigella dysentriae*, and *Staphylococcus aureus*) by disc diffusion method. The results showed that both water infusion and ethanol crude extract showed different antibacterial activity against tested bacteria. The ethanol extract of *B. frustescens* was the best extract to inhibit Enterophatogenic *Escherichia coli* even compared with kanamysin and chloramphenicol at the same concentration. Phytochemical tested showed that this extract contained alkaloids, flavonoids, phenols, saponins, steroids, tannins, and triterpenoids.

Keywords: antibacterial, *Adinandra sorasanthera, Baeckea frustescen, Rhodomyrtus tomentosa, Trema orientalis*

Introduction

As developing country, Indonesia faces the infectious diseases such as diarrhea. Some enteropathogenic bacteria cause diarrhea are resist to many antibiotic and this has created immense clinical problem in treatment of infectious disease. The resistance of the organism increase due to the indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious disease [1]. [2,3] stated some enteropathogenic bacteria such as EPEC (Enteropathogenic to antibiotic such as kanamysin, sefotaksim, amoxylin, chloramphenicol and tetrasiklin. Their research showed that kanamysin at 30 mg/mL was the best antibiotic to inhibit EPEC and *P.aeruginosa* while kanamysin at 40 mg/mL and chloramphenicol 50 mg/mL were the best antibiotic to inhibit *Shigella dysentriae E.coli*), *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella dysentriae* were resist and *S.aureus* respectively. Resistance case of microorganism to many antibiotic force the scientist to search new antimicrobial compound from various sources including medicinal plants. Drugs from the plants are easily available, inexpensive, safe, efficient, and rarely accompanied by side effects [4,5]. Antibiotics may also cause adverse effects on the host including allergies, hypersensitivity and immune-suppression [1]. Due to these reasons, medicinal plants which perform the same antibacterial effect with chlorampenicol and kanamysin against enteropathogenic bacteria need to be explored.

Indonesia possesses diversity of medicinal plants. Bangka Belitung Islands is a province in Indonesia which also rich of folk medicine. The used of plants traditionally by indigenous society can provide the foundation to modern pharmaceutical [6]. The use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community [7]. One of indigenous society in Bangka Belitung province is Lom. This society use some medicinal plants to cure diarrhea. Some plants are used by Lom society to cure diarrheae such as *Adinandra sorasanthera* (Micq) (Lom ethnic mention as "pelempang itam"), *Rhodomyrtus tomentosa* (Ait) Hassk (Lom ethnic mention as "karamunting"), *Trema orientalis* (L.) Bl. (Lom ethnic mention as "mengkirai") [8]) and *Baeckea frustescens* (L.) (Bangka society mention as sapu-sapu).

A. sarosanthera is one genus with *A.nitida*. *A.nitida* is known as traditional tea rich in phenols and flavonoids [9] known as "Shiyacha [10,11]. *A.nitida* known have curative effect such as antibacterial, antitumor, analgesic, and antifungi [12,13]. *B. frustescens* of the family Myrtaceae is a medicinal plant that has an essential oil which has been used as a traditional drug in South East Asia. In China, this plant is used to cure fever, in Malaysia is used as anticancer and antimicrobe [14]. [15] stated that leaves and bark of

B.frustescens is used as anti-diarrhea, analgesic, anti-acne. *R. tomentosa* is a herb which has many medicinal properties such as antidiabetic, diarrhea, burn [16] treat oral, gastrointestinal, urinary tract infections, and used as an antiseptic wash for wounds [17]. *T. orientalis* plants has many medicinal properties, such as hypoglycemic activity, analgesic, anti-inflammatory activities, anti-plasmodial activity, diuretic activity, anti-convulsant activity, anti-helmintic activity, anti-sickling effect, anti-oxidant, and anti-bacterial activity [18]. In Congo, this plant is used to treat several ailment such as diabetes mellitus, respiratory diseases, and malaria [19].

Those medicinal plants has a high value to treat the disease. Therefore, it is important to do this research. The aim of this research are to know the antibacterial activity of these plant and compared to commercial antibiotic (chloramphenicol and kanamysin). This research can open new opportunity in treatment of diarrhea

Materials and Methods

Plant material.

Four medicinal plants used in this study were *Adinandra sarosanthera, B.frustescens, Rhodomyrtus tomentosa* and *T. orientalis*. Fresh leaves of the plants were collected from Merawang district, Bangka, Indonesia in August 2014. The collection were identified in Bangka Belitungense Herbarium, Bangka Belitung University.

Extraction of plant material.

The fresh samples were washed, dried and ground to be fine powders using a blender. The fine powder samples were then soaked in ethanol (1:3) for 3 days at room temperature. The solvent-containing extract were then filtered. The filtrate from extractions were evaporated using a rotary evaporator to give crude ethanol extract. Extract were stored at 4°C for determination of antibacterial activity.

Infusion.

The fresh samples were washed, dried and ground to be fine powders using a blender. The fine powder samples were then put into boiled water at 90°C and soaked for 15 minutes in water bath. Temperature was constant at 90°C. The solvent-containing extract were then filtered and the solvent tested against enteropathogenic bacteria.

Phytochemical tested.

Phytochemical analysis were analyzed by using qualitative method (colorimetric). This analysis was use to know the active compound of plants tested. There are some active compound that analyzed such as alkaloids, flavonoids, phenols, saponins, steroid, tannins, and triterpenoids. Alkaloid were detected by Robinson's method [20] while flavonoids, phenols, saponins, steroids, tannins, and triterpenoids were detected by Harborne's method [21].

Test microorganism.

Four enteropathogenic bacterial strains were used in this study: EPEC, *P.aerugionosa, S.aureus*, and *Shigella dysentriae*. The bacteria obtained from the Microbiology Laboratory, Bangka Belitung University, Indonesia. Bacterial strains were cultivated at 37°C and maintened on NA (Oxoid, USA) slant at 4° C.

Antimicrobial activity assay.

Antimicrobial activity was determined against four bacterial pathogens by papper disc diffusion method. The crude ethanol extract and commercial antibiotic (chloramphenicol and kanamysin) were dissolved in aquades. Extract and antibiotic were tested using three concentration (30mg/mL, 40 mg/mL, and 50 mg/mL). 20 mL of Nutrient Agar and 100µL of bacterias at log phase (10^6-10^8 cell/mL) were poured into petridishes (90mm each side), mixed and made homogent. After the agarplate solid, 6 mm diameter of blank disc which had been soaked in extract and antibiotic with different concentration in 5 minutes, were placed on the surface of the inoculated agar plates. The plates were incubated at 37° C for 24 hour. The antibacterial activity test were done three replicate. The antibacterial activity was evaluated by measuring the zone of growth inhibition sorrounding the disc. Negative control were using papper disc soaked with aquades. The diameter of inhibition zone was measured by digital Vernier Caliper. Inhibition zone was calculated with formula: Diameter of total inhibition zone- diameter of papper disc (6mm).

Results and Discussion

Phytochemical test.

Phyochemical tested showed that both infusion and ethanol extract of *A.sarosanthera*'s leaves had the same active compound while there were differences in other plants (Table 1).

Antibacterial Test.

Natural product perform various function, and many of them have biological activities. Both antibiotic and tested plants showed antibacterial activity against enteropathogenic bacteria (Table 2).

The inhibition zone formed by infusion of tested plant leaves were smaller than ethanol extract (Table 2). Infusion of *B.frustescens'*s leaves made the best inhibition zone against EPEC (2.14±2.87mm) at concentration 50 mg/mL, and infusion of *T.orientalis'*s leaves made the best inhibition zone against *P.aeruginosa* (2.2±0.31mm) at the same concentration. Extract ethanol of *B.*

*frustescens'*s leaves showed the best antibacterial activity among tested plant.

	A.sarosanthera		*B.frustescens*		*R.tomentosa*		*T.orientalis*	
Compound	eth	Inf	Eth	Inf	eth	inf	eth	inf
Alkaloids	+	+	+	+	-	-	-	-
Flavonoids	-	-	+	+	-	-	-	-
Phenols	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+
Steroids	+	+	+	-	+	-	+	-
Tannins	+	+	+	+	+	+	+	+
Triterpenoids	+	+	+	-	+	-	-	-

Table1. Result of phytochemical tested of plant leaves infusion and extract
Note: += detected, -= not detected, eth= ethanol extract, inf=infusion

Extract ethanol of *B. frustescens'*s leaves could inhibit all of tested bacterias especially the best inhibition against EPEC (12.15±2mm) even compared to antibiotic chloramphenicol (2.53±0.89mm) and kanamysin (2.99±0.62mm) at concentration 40mg/mL. It showed that *B. frustescens* effective against enteropathogenic bacteria both Gram positive bacteria (*S.aures*) or Gram negative (EPEC, *P.aeruginosa*, and *Shigella dysentriae*). [22] stated that *B.frustescens* have the most important applications against human pathogens, including those that cause enteric infections. In their research, appreciable antibacterial activity against all gram positive cell wall (MRSA, *S.aureus*, and *Bacilus*) and moderately active against gram negative bacteria *E.coli*.

Extract ethanol of *B. frustescens'*s leaves contained flavonoids while other plants both infusion or extract ethanol did not contained this compound. It can be assumed that flavonoids which contained in *B. frustescens*'s leaves as main antibacterial compound. [23] stated that flavonoids is a main compound that believed had a role as antimicrobial activity. The mechanisms of flavonoids as antimicrobial can be classified as the inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism [24]. Flavonoids are found to be effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to make complex with extracellular and soluble proteins and to complex with bacterial cell wall [25].

Ethanol extract of *B.frustescens'*s leaves contained alkaloids, flavonoids, phenols, saponins, steroids, tannins, triterpenoids. The optimal effectiveness of a medicinal plant may not be due to the one main active constituent, but may be due to the combined action of different compounds originally in the plant which are classified as bioactive antimicrobial compounds [26]. It can be assumed that both ethanol extract and infusion of medicinal plants showed difference against bacteria tested, depend on active compound contained in each extract.

Most alkaloids act as antimicrobial through efflux pump inhibition [27]. [28] stated that the antimicrobial effects of the alkaloid may be through another mechanism, since the compound is known to be a DNA intercalator and an inhibitor of DNA synthesis through topoisomerase inhibition. Phenol compound is mayor antimicrobe compound in plant. It was reported that an antimicrobial action of phenolic compounds was related to inactivation of cellular enzymes, which depended on the rate of penetration of the substance into the cell or caused by membrane permeability changes [29]. Phenol compounds have capacity to link with proteins and bacterial membrane to form complexes [30].

Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes [31]. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell [32]. [33] stated that antibacterial of tannin act by interfere cell membrane of bacteria. Tannins act by iron deprivation, hydrogen bounding or non specific interactions with vital proteins such as enzymes [34]. Triterpenoid act by interfere the permeability of the membrane of the pathogenic bacteria and increase of non-specific cell membrane permeability for the antibacterial molecule [35].

Although all of tested plant didn't have antibacterial activity as good as *B.frustescens* but *T.orientalis, R. tomentosa*, and *A.sarosanthera* still have antidiarrhea potency due to all of tested plants

contain antibacterial compound. Differences in the antimicrobial effects of plant species, due to the phytochemical properties among species of plants. [36] stated that some of the plants that less effective may have contained antibacterial constituents, just not in sufficient concentrations. It is also possible that the active chemical constituents were not soluble in ethanol or water. It assumed that by increasing the concentration level or other type of extraction and other solvent, leaves of tested plants may be able to inhibit the tested bacterias.

Type and kinds of Sample	Concentration mg/Ml	Inhibition zone (mm) EPEC	P.a	S.a	Sh
Infusion water of *A.sarosanthera*	30	0.09±0.08	0.47±0.22	0.59±0.51	1.05±0.42
	40	0.04±0.02	0.34±0.12	0.49±0.06	0.77±0.11
	50	0.36±0.34	0.55±0.36	0.75±0.08	0.99±0.55
B.frustescens	30	0.14±0.06	0.8±0.43	0.92±0.46	1.5±0.64
	40	0.12±0.05	1.01±0.24	0.82±0.48	1.07±0.62
	50	2.14±2.87***	1.24±0.26	1.35±0.10***	1.45±0.41***
R.tomentosa	30	0.04±0.00	0.63±0.49	1.27±0.24	0.8±0.66
	40	0.19±0.05	1.27±0.91	0.94±0.61	0.79±0.16
	50	0.76±0.96	0.85±0.45	1.17±0.50	0.74±0.18
T.orientalis	30	0.06±0.06	0.77±0.31	0.88±0.73	1.44±0.94
	40	0.21±0.13	1.71±0.80	0.96±0.17	1.46±0.52***
	50	1.25±1.57	2.2±0.31***	1.29±0.15	1.22±0.63
Maceration ethanol of *A.sarosanthera*	30	0	0.95±0.88	1.77±1.21	0.91±0.29
	40	0.31±0.53	1.54±1.34	2.60±0.86	1.28±0.48
	50	0	2.66±0.21	2.52±0.53	1.89±0.15
B.frustescens	30	9.99±1.64	4.18±0.48	9.11±2.52	7.28±0.99
	40	12.15±2*	4.41±0.72	10.29±1.01	11.45±1.52**
	50	12.07±3.47	6.02±0.50**	12.10±1.21**	10.5±0.69
R.tomentosa	30	0	0.62±0.76	0.61±0.58	0.31±0.54
	40	0	0.58±0.50	1.58±0.58	0
	50	0	1.69±0.59	1.40±0.39	0.49±0.85
T.orientalis	30	0	0.61±0.46	0.52±0.12	0.34±0.58
	40	0.31±0.53	0.82±0.23	0.64±0.34	0.04±0.06
	50	0	1.17±0.37	0.70±0.21	0.61±1.06
Antibiotics Chloramphenicol	30	2.93±0.64	11.14±2.77*	18.94±1.34	17.81±3.10
	40	2.53±0.89	8.83±0.68	17.08±0.86	20.12±2.41
	50	4.34±1.04	10.02±1.30	22.35±1.03*	22.98±2.23
Kanamysin	30	3.08±0.19	8.03±0.97	18.82±1.81	23.16±1.34
	40	2.99±0.62	7.91±0.80	19.42±1.39	23.36±1.17
	50	2.92±1.09	10.31±2.06	22.29±1.07	28.63±1.91*

Table 2. Results of antimicrobial tested of the investigated plants in papper disc diffution assay
Note: *= the best inhibition zone of extract against each bacteria compared to antibiotic, **=the best inhibition zone of extract against bacteria, ***=the best inhibition zone of infusion against bacteria.
EPEC= Enteropathogenic *E.coli*, P.a= *Pseudomonas aeruginosa*, S.a= *Staphylococcus aureus*, Sh= *Shigella dysentriae*

According to [37], both crude methanol and aqueous root extracts of *T. orientalis* showed activity against Gram-negative bacteria and aqueous extract showed higher anti-microbial activities than methanol extract. In this research also showed that water infusion of *T.orientalis's* leaves had better antibacterial activity than ethanol extract. The leaves of *T.orientalis* contain tannins, saponins, flavonoids, triterpenoid (simiarenol, simiarenone, trematol) [18]. [38] isolated rhodomyrtone from *R. Tomentosa*'s leaves. Their study showed that rhodomyrtone had antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Compound present in *R. tomentosa* such as glycoside, antraquinon. [39] reported that *R.tomentosa* contained limonene, β-pinena, and rhodomyrtone.[16] stated that compound present were aleuron, starch, tannin, cathekol, carbohydrat, alkaloid, 1,8 dioksiantrakinon, and saponin

A. sarosanthera contained alkaloids, phenols, saponins, steroids, tannins, and triterpenoids. There is no supported research about this plant but [13] stated that extract flavonoids from *A.nitida* which one genus of this plant significantly inhibit the growth of *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium* and *Listeria.*

In this research, chloramphenicol and kanamysin was used as compared commercial antibiotic to inhibit enteropathogenic bacteria. Chloramphenicol has been shown to exert a strong inhibitory action on microbial protein synthesis [40] by inhibit peptide bond formation by binding to a peptidyltransferase enzyme on the 50S ribosome . The aminoglycoside kanamycin acts primarily on is the 30S ribosomal subunit resulting in prevention of protein elongation. In addition to targeting the protein synthesis machinery, kanamycin also inhibited the synthesis of DNA and targeted the cellular membrane composition [41].

Conclusions

All of tested medicinal plant in this research are potentially anti-diarrhea agent especially *B. frustescens*. *B. frustescens's* leaves exhibited the best antibacterial activity against EPEC even compared with kanamysin and chloramphenicol. The extract of *B.frustescens* may investigate further (such as fractination) to give better antibacterial activity and to know antibacterial compound of this plant. The resulting information will contribute better understanding mechanism of the antibacterial activity of this plant and open new opportunity in diarrhea treatment.

Acknowledgement

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DIVERSITY and DISTRIBUTION of DRAGONFLIES (ODONATA) in BROMO FOREST AREA (BKPH LAWU UTARA : KPH SURAKARTA) CENTRAL JAVA

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Abstract

Abstract. Odonates diversity in Bromo forest area (KPH Surakarta) was observed, where we recorded 21 species. Libellulidae was the richest family with 12 species and *Orthretum* was the most common genera. The sub-order Zygoptera was represented by 7 species and 14 species represents sub-order Anisoptera. River stream along the forest area with multiple vegetation structure may provides good habitat to Odonata lives. Mostly odonates were aggregated due to habitat specific nature, the presence of family Gomphidae, *P.reinwardtii* in this study showed there is a good condition of water. A detailed list of odonates recorded from Bromo forest area is presented.

Keywords: Damselfly, Dragonfly, Diversity, KPH Surakarta

Introduction

The order Odonata which consist of two suborders, Anisoptera and Zygoptera belong to amphibiotic insects. A major part of their life cycle is spent in freshwater ecosystem as larvae. The adults are generally predacious insects. All of them have been described and they are distributed in the tropics, where the greatest numbers and diversity occur. Based on the latest finding by [8, 9], the Malaysian odonate fauna comprises about 342 species. About 239 species are reported from Sabah, Sarawak and Brunei and 226 species are listed from Peninsular Malaysia including Singapore [7]. In java there is 88 species of Odonata, consist of 23 species from sub-order Zygoptera and 65 species belong sub-order Anisoptera [11]. Libellulidae is the most dominant family found in various ecosystems in Peninsular Malaysia.

Many species of Odonata are restricted to specific habitats both during larval and adult life stages especially the stenotopic species (limited to a single habitat) [8]. These species are highly sensitive to factors such as the amount of sunlight and water movement. Generally, the stenotopic species (Calopterygidae, Platystictidae, Platycnemididae and Protuneuridae) are very abundant in primary forest especially in mixed dipterocarp and freshwater swamp.

Bromo forest area is the Perum Perhutani field area located in Central Java, belong to BKPH Lawu Utara under KPH Surakarta, the forest area has multiple vegetation structure (Pine, Mahogany, Rosewood and Mixed). Besides its main fuction to provide wood and resin, the forest area also used to recreational field. This study is aim to investigate the diversity and distribution of Odonata in Bromo forest area, the early study to provide ecosystem services by serving the composition of Odonata there, in order to attract people caring the greater nature.

Materials and Methods

Study area

Collections of Odonata were conducted in Bromo Forest Area, Western of Mount Lawu (BKPH Lawu Utara : KPH Surakarta) Central Java, the PT. Perhutani Indonesia areas. Located between 300-400 m above sea level, this area has some kinds of vegetation structure, there are pine forest, mixed forest, mahogany forest, and rosewood forest. We choose sampling sites along river stream and a reservoir beside the forest area, named Tirtomarto reservoir.

Sampling Methods

Odonata sampling was carried out from June to August, 2014. We used direct searching observation method with opportunistic sample collection of Odonates from different habitats. In this method target species or group of species observed visually and it is a very effective method for day flying butterflies, moths, dragonflies and damselflies. Mostly species were photographed, but some were also collected with insect catching nets for the proper identification. The collection preference is for adults species, collected with entomological nets and packed live into triangular

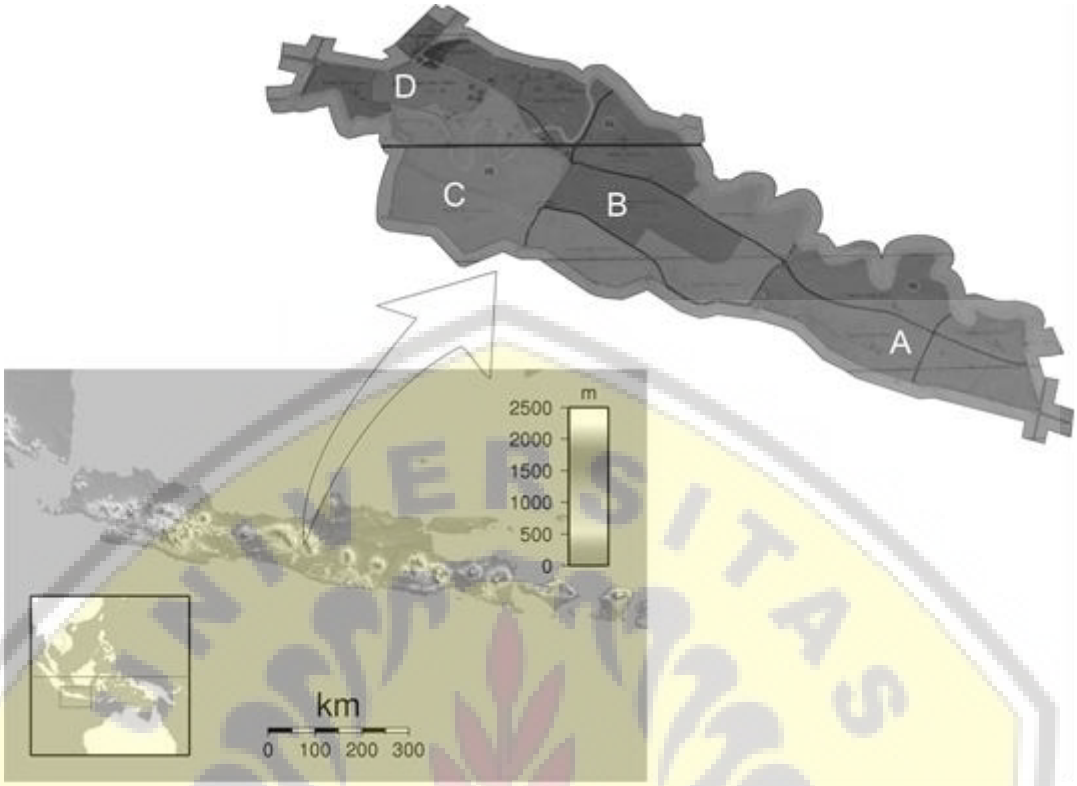


Figure 1. Sampling sites along river stream in Bromo forest area and Tirtomarto reservoir, Karanganyar, Central Java. (A) pine forest, (B) rosewood forest, (C) mixed forest, (D) mahogany forest.

envelopes (12 x 8 cm) of thin paper. The sampling effort consisted of an active search, usually lasting between 9 and 15 hours of the day, always by the same person. The specimens were nominally recognized to the level of genus and species when possible. Collected and photographed species were identified with the help of standard identification guide [12, 13].

Analysis of data

The degree of species diversity (H') for each site was determined by using the Shannon Wiener Index. This index indicates the degree of species composition per unit area. The analysis of data use descriptive method by comparing all data measurement and the habitat differences. The relative abundance and distribution of Odonata measured by following formulas :

$$KR = \frac{ni}{N} \; x \; 100\%$$

KR: relative abundance
ni : number species of Odonata
N : total number of Odonata

$$H = -\sum_{i=1}^{s} pi \ln pi$$

H : Shanon Wiener species diversity indices
pi : proportional abundance

$$S^2 = \frac{\sum (x - \bar{x})^2}{n-1} \text{ dimana, } \bar{x} = \frac{\sum x}{n}$$

x : number of individuals each species
n : number of sampling sites
ẍ : average number of species each sampling sites

Results

Faunal composition. A total 21 species representing 16 genera from 7 family were recorded from multiple vegetation structure in Bromo forest area (Table 1). Libellulidae was the dominant family with 12 species, followed by Coenagrionidae (4) and Aeshnidae (1), Gomphidae (1), Chlorocyphidae (1), Platicnemididae (1), Protoneuridae (1). Orthetrum was found to be the most species rich genera with 4 species. Species from family Aeshnidae are typically difficult to collect, thus collecting their diversity in an area is normally needs longer sampling periods.

Species abundance. The relative abundance of Odonates show that O. sabina was the most abundance species in all sampling sites (28.2 %), followed by *A. femina* (13.3 %), *P. flavescens* (9.3%), *B. contaminata* (6.8 %), *C. marginipes* (6.8 %), *R. fenestrata* (5.9 %), *N. ramburii* (5.4 %), *C. servillia* (4.8 %), *P. pruinosum* (4.8 %), *D. trivialis* (3.4 %), *I. senegalensis* (1.7 %), *O. glaucum* (1.4 %), *P. autumnalis* (1.4 %), *N. terminata* (1.2 %), *O. testaceum* (1.1 %), *P. congener* (1.1 %), *O. chrysis* (0.8 %), *A. pygmaea* (0.8 %), and the least

abundance followed by *A. guttatus* (0.5 %), *P. O. sabina* has high tolerance to environmental changes, so does in polluted water system [12]. *O. sabina* not only be the most abundance species but also be the most widespread species in Indonesia [14].

Species Diversity and Distribution. During the study Shanon-wiener indices were calculated as measure of in different habitat of multiple vegetation structure in Bromo forest area. The Shanon-wiener diversity index indicated that mixed forest is relatively diverse (2.37) followed by Tirtomarto reservoir (1.84), Pine and Mahogany forest (1.66), the least Rosewood forest (1.60) (Figure 3). The distribution of Odonates in different habitat were assessed and result revealed that 23.8 % were randomly distributed and 76.2 % were aggregated. The aggregated distribution indicated the habitat preference where random distribution indicates the available resource use and suitability to survival (Table 2).

Discussion

The diversity of Odonata species is explained by the high diversity of aquatic environments in tropical forests [9]. Information of Odonata from Java Indonesia presented 88 species [11], but it still need another scientific information to confirm the total species of Odonata in java, another information of Odonata in java there was 142 species [3]. Java is the most densely populated region in Indonesia, some study of environment showed that forest area are decreasing by the time.

In the present study we mainly focused on the diversity of Odonata in the forest area including multiple vegetation structure and we recorded 21 species. The river streams with riparian zones along the forest area provide good opportunity to the Odonata to flourish. The presence some species of Odonata can indicate the condition of water there, *reinwardtii* (0.5 %), *C. lineata* (0.5 %) (Figure 2). like the presence of *B. contaminata* in high number of population indicates that the area has poor water quality [2, 6]. In this study, *B. contaminata* only found in Tirtomarto reservoir but not in high number of population. In mixed forest found a species of Gomphidae belonging to endemic java species, it was *P. reindwartii.* The presence of this family can indicates there is clear and undisturbed water area [2].

Almost Zygoptera found along border side of Tirtomarto reservoir, being perched on a riparian plant like *Imperata cylindrica*, *Ipomoea aquatica*, and the other from family Cyperaceae and Dipterocarpaceae, it was the suitable habitat for them [5]. Dragonflies are ectotherm insect, they regulate their body temperature by behavior and physiological response to solar input [1]. Therefore, shade or the solar intensity affects the dragonflies thermoregulation, so does their abundance and distribution. Shade affected the Anisoptera habitat selection in small reservoir in South Africa become decreasing [10]. The maximum species abundance and diversity was recorded at Tirtomarto reservoir, there are low cover percentage shade, floating and submerged macrophytes and higher cover percentage reeds, seem to following research [4].

Data sharing among researchers are useful to complete the information of Odonata in Indonesia, especially java. In India has a lot information of Odonata, in Maharashtra, the largest state in India there is 134 species of Odonata [15]. Nowadays, through social networks researchers could freely communicate about information species and site studies. However, checklists of regions should be made by experts to avoid false presence data, they may result into usable species data. It was necessary information to understand changing species distributions and the causes of this change.

Table 1. List of Odonates with the number of collected individuals recorded in Bromo forest area (KPH Surakarta) Central Java, Indonesia.

Subordo / Famili / Jenis	Number of individuals				
	Pine forest	Rosewood forest	Mixed forest	Mahogany forest	Tirtomarto reservoir
I. Anisoptera					
A. Aeshnidae	-	-	1	-	1
1. *Anax guttatus* Burmeister 1839					
B. Gomphidae					
2. *Paragomphus reinwardtii* Selys 1854	-	-	2	-	-
C. Libellulidae					
3. *Brachythemis contaminata* Fabricius 1793	-	-	-	-	24
4. *Cratila lineata* Brauer 1878	-	-	2	-	-
5. *Crocothemis servilia* Drury 1770	2	-	-	-	15
6. *Diplacodes trivialis* Rambur 1842	1	-	3	-	8
7. *Neurothemis ramburii* Brauer 1866	7	6	-	6	-
8. *Neurothemis terminata* Ris 1911	2	-	2	-	-
9. *Orthetrum chrysis* Selys 1891	-	2	1	-	-
10. *Orthetrum glaucum* Brauer 1865	-	-	3	2	-

11.	*Orthetrum sabina* Drury 1770	21	13	8	11	47
12.	*Orthetrum testaceum* Burmeister 1839	1	-	2	-	1
Subordo		**Number of individuals**				
Famili		Pine forest	Rosewood forest	Mixed forest	Mahogany forest	Tirtomarto reservoir
Jenis						
13.	*Pantala flavescens* Fabricius 1798	-	-	-	-	33
14.	*Potamarcha congener* Rambur 1842	2	-	2	-	-
II.	**Zygoptera**					
D.	**Chlorocyphidae**					
15.	*Rhinocypha fenestrata* Rambur 1842	2	7	9	3	-
E.	**Coenagrionidae**					
16.	*Agriocnemis femina* Brauer 1868	-	-	-	-	47
17.	*Agriocnemis pygmaea* Rambur 1842	-	-	-	-	3
18.	*Ischnura senegalensis* Rambur 1842	-	-	-	-	6
19.	*Pseudagrion pruinosum* Rambur 1842	-	2	8	7	-
F.	**Platycnemididae**					
20.	*Copera marginipes* Rambur 1842	8	10	1	5	-
G.	**Protoneuridae**					
21.	*Prodasineura autumnalis* Fraser 1922	-	-	5	-	-
Total		46	40	49	34	185
Total all individuals				354		

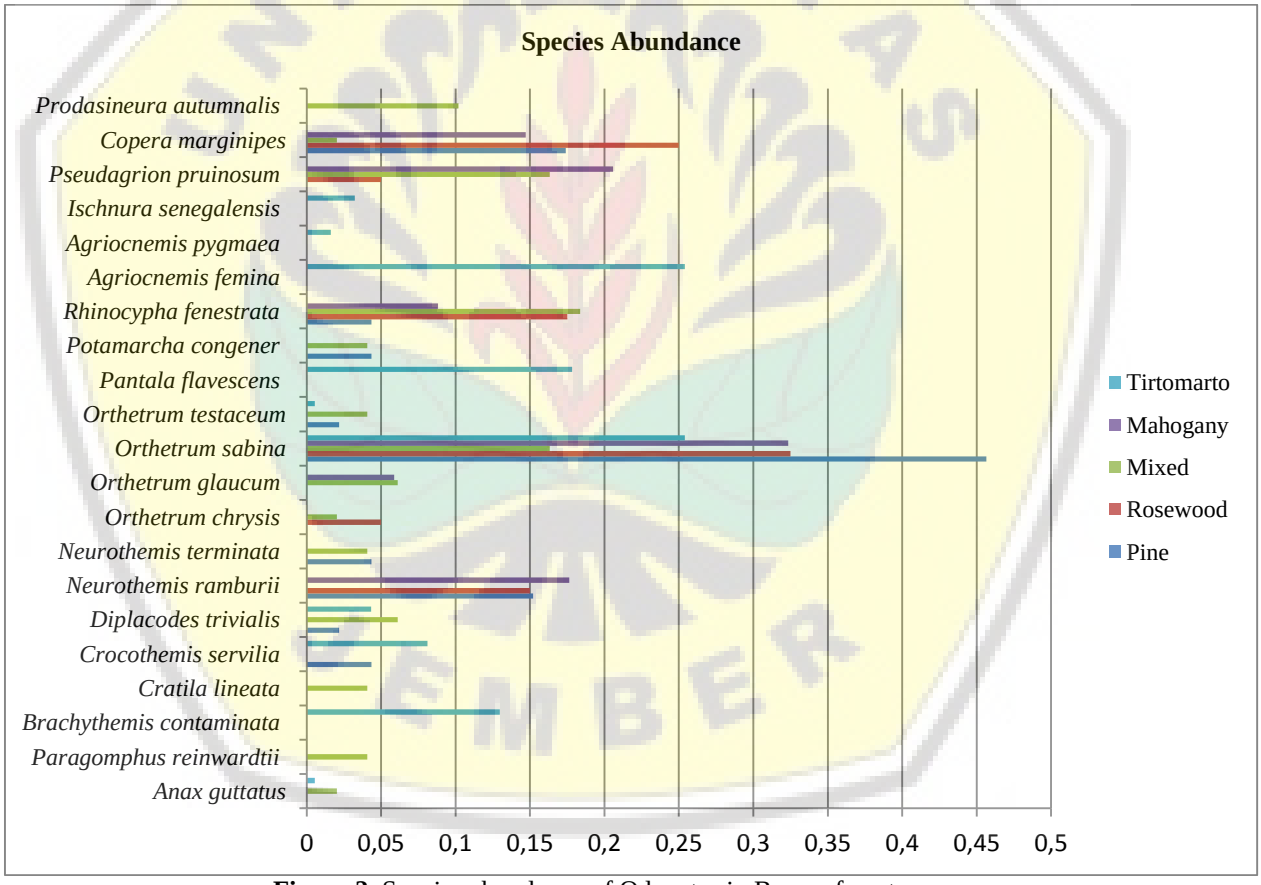
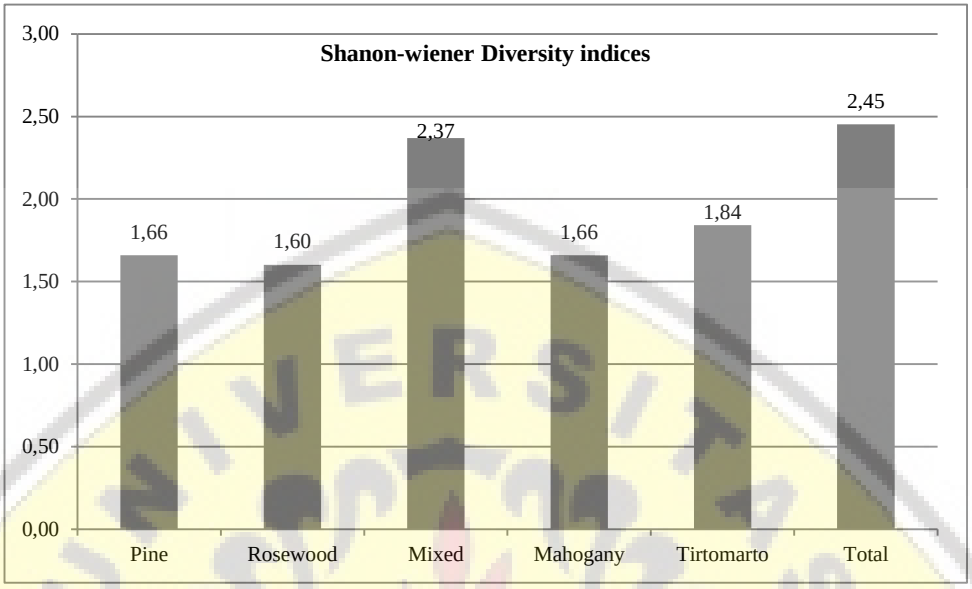


Figure 2. Species abundance of Odonates in Bromo forest area

Figure 3. Species diversity indices (Shanon-wiener) of Odonates in Bromo forest area



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GIVING of PROTEIN DIETARY LEVEL BROODSTOCK of CATFISH (*Clarias sp*) and LASERPUNCTURE INDUCTION to ESTROGEN LEVEL and GSI

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Abstract

Laserpuncture induction at the reproductive acupoint can be increases production of gonadotropin hormone and triggering the oocyte maturation at broodstock catfish. Supporting this action, nutrition factor enriched by protein is also important. However, the effect of laserpuncture induction at post protein levels of dietary on estrogen level and Gonado Somatic Index (GSI) has not been known.. This study aims to determine the effect of laserpuncture induction and protein level of dietary on estrogen level and GSI. Three protein levels (30%, 35% and 40%) of dietary were given to the female catfish broodstock along with and without laserpuncture treatment. Laserpuncture was induction on 2/3 ventral part of body in 15 sec/week during 8 weeks. We used 172 of mature female catfish broodstock around 1-1.5 years old with 900-1500 g of body weight. Blood and gonad were taken from 4 samples per treatment group in each week. Estrogen level was analyzed by ELISA. This study showed that laserpuncture induction along with any protein levels of dietary significantly increase estrogen level and GSI value ($P<0.05$). Giving of 40% protein dietary level and laserpuncture induction reached the highest serum estrogen level and GSI at 3th and 6th week. Meanwhile, giving of 40% protein dietary level without laserpuncture induction reached the highest serum estrogen level and GSI at 6th week. Although 40% and 35% protein dietary level have more essential amino acid excess than 30%, the economical reason suggest that 30% protein dietary level was an efficient level to produce vitellogenin.

Keywords : catfish, dietary protein level, estrogen, GSI, laserpuncture induction

Introduction

Gonadotropin hormone (GtH) release and protein dietary are some methods to accelerate gonad maturation. The female broodstock of *Clarias gariepinus* dietary application with 30-40% protein has proven increased growth performance, gonad development and maturity [1,2].

Giving of 30%-40% level of crude protein dietary able to affect the activity of the hormone gonadotropin. The availability of nutrients to the good quantity-quality protein in particular to support the process of reproduction and the hormones able to enhance normally [3,4,5]

Since level of crude protein dietary increases GtH I production, it is also considered to be able to enhance the production of estrogen hormone in order to stimulate the vitellogenin synthesizing process and gonad maturity [3]. In addition, to accelerate the release of gonadotropin hormone [6] and estrogen [7] and stimulate gonadal maturation using technology of laserpuncture induction [8].

Laser is an electromagnetic wave energy radiation which can be a biostimulation [9, 10]. Laserpunture was induced on acupoint reproductive contained by active cells in reproductive system to stimulate peripheral nervous system and the increase of GtH. GtH regulates gametogenesis through production of gonadal steroid, e.g. estradiol-17β (Estrogen, E2) and 17,20 β-dihydroxy-4-pregnen-3-one (17,20 β-P) [11] also stimulating gonad maturation [6].

Considering that the estrogen release in catfish, highest GSI after laserpuncture induction at reproduction acupoint, and give of different level protein dietary have not been recognized, the paper presented our result of experiment in order to prove that laserpuncture induction at reproduction acupoint and that giving different level protein dietary could stimulate the release of estrogen, then stimulate vitellogenin synthesis with the indicators of gonad maturation with GSI control the advanced in catfish. This study aims to determine the effect of protein level of dietary and laserpuncture induction on estrogen level and GSI. Three protein levels (30%, 35% and 40%) of dietary were given to the female catfish broodstock along with and without laserpuncture treatment

Methode

Acclimatization and treatment conditions

This research was carried out in December 2012 to August 2013 at Unit Pengelola Budidaya Air Tawar (UPBAT) Kepanjen, Malang.

We used mature gonad catfish broodstock (8-9 month). It was resulted by female Sangkuriang and male Paiton catfish broodstock hybridization from

same population in UPBAT Kepanjen Malang and has not spawned before. The research used Completely Randomized Design Nested with three levels of treatment and 4 times replication. The level of treatment that consisted of: 1) protein dietary level (30%, 35%, and 40%); 2) laserpunc ture induction (with and without induction) nested in the protein level; 3) treatment duration (0 – 8 weeks) nested in laserpuncture induction. Laserpuncture was induction in once a week (15 sec) during 8 weeks. Laserpuncture was shot at the reproduction acupoint, exactly 2/3 of the ventral body for 15 seconds, every week for 2 months or up to gonad broodstock maturity according to Kusuma *et al*. [12]. We repeated each treatment in 4 times repetition.

We used 172 female catfish broodstock (900-1500 grams) and 172 male catfish broodstock (1140-1750 grams). The average of catfish age is 1-1.5 years. Male catfish broodstock was used in spawning process only. The female catfish brood stock were separately maintained in tarpaulin fish pond in 2 m x 2 m x 90 cm size. Acclimatization was held during one week. Initially, all experi-mental catfish was given by 6% of dietary twice a day (morning and evening) that contain 30% protein. After acclimatization, the fish were placed in 43 tarpaulin ponds (2 x 2 x 1 m^3) and each group consisted of 4 females. The female catfish brood stock has similar initial condition with without mature eggs after spawned in a week [12]. The catfish were given 30, 35 and 40% protein-dietary [1].

Estrogen Hormone Level Measurement

Blood samples were collected from caudal vena of catfish by without heparinized syringe, then centrifuged at 3000 rpm for 10 minutes. The serum was stored at -20^{0}C up to the analysis [13]. Estrogen level was measured by using ELISA according to Elisa kit manual (Cusabio; catalog number of CSB-E13017Fh).

Gonado Somatic Index Measurement

The female catfish broodstock was scaled and opereted to get the gonad. Gonad was also scaled. The data of bodyweight and gonad weight were used to determine GSI. GSI = Wg/(Wt-Wg) x 100.

The prosedure discribed before was done on each week (from 0 – 8th week). We used 4 samples per treatment for each week.

Data were analyzed by analyze of variance-nested with Duncan's Multiple Range Test.

Results and Discussion

Estrogen level

Profile of serum estrogen levels after giving of protein dietary level variation with and without laserpuncture induction in female broodstock catfish during 8 weeks showed upward and downward trend until certain value. The estrogen level of female broodstock catfish after giving of 40% protein level was higher than 35% and 30% both with and without laserpuncture induction. Giving of 30%, 35%, and 40% protein dietary level with laserpuncture induction successively increase 48.30%, 6.94%, and 5.52% serum estrogen level compare to without laserpuncture induction (Figure 1 and Table 1).

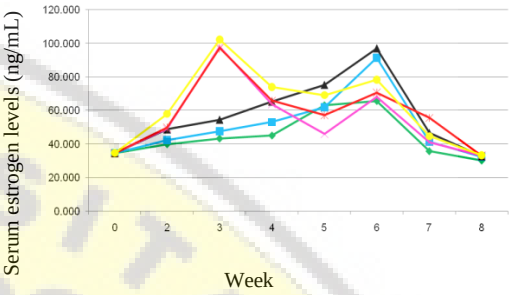

Figure 1. Serum estrogen level profile of female broodstock catfish

Table 1. Trend of serum estrogen levels in female broodstock catfish *(Clarias sp).* The peak of serum estrogen level after giving of 30%, 35%, and 40% were reached at 3th week (with laserpuncture induction) and 6th (without laserpuncture induction)

Protein Deviation Dietary Estrogen Level (ng/mL)	Lasepucture	Mean of Serum Level	Percent of Estrogen Level Enhancemen (%)
30%	Without laser	65,495 ± 3,076^{a}	
30%	Laser	97,128 ± 5,143^{bc}	48.30
35%	Without laser	91,48 ± 3,604^{b}	
35%	Laser	97,833 ± 4,731^{c}	6.94
40%	Without laser	96,968 ± 1,417^{bc}	
40%	Laser	102,32 ± 4,057^{c}	5.52

Values in the column followed by different superscript showed significant difference (P<0.05)

Our study showed that serum estrogen level enhancement of 30% protein dietary level with laserpuncture induction compare to without laserpuncture induction was higher than 35% and 40% (Table 1). It is because 30% protein dietary level with laserpuncture induction on reproductive acupoint in female broodstock catfish stimulated relation among GABA (Gamma Aminobutyric

Acid), hypothalamus, and pituitary neurons in the brain. It stimulated gonadotropin (GtH-I and GtH-II) releasing from pituitary neuron and increased GtH in serum. GtH was carried in blood stream into gonad. In the gonad, GtH was involved in steroidogenesis to produce steroid hormone like 17β-estradiol or estrogen. The normal physiological production of estrogen needs sufficient protein. Giving of 30% protein dietary level had been proven increase estrogen level compare to 35% and 40% (Table 1). Protein is a raw material in hormone production. The sufficient protein can stimulate physiological activity of estrogen production. Meanwhile, laserpuncture induction stimulated granulose cell to produce estrogen. According to Oyelese [14], giving of 35%, 39%, 43% and 48% protein dietary level increased gonad development.

Estrogen level of 40% protein dietary without laserpuncture induction was relatively same at 6th week. Meanwhile, the same estrogen level of 30% protein dietary was reached at 3th week ($P<0.05$). This data showed that protein dietary level combination between 30% and laserpuncture induction in female broodstock catfish had been proven increased serum estrogen level (Table 1). Suitable with Hariani [15], our study showed that 30% protein dietary level and laserpuncture induction in female broodstock catfish increased 49.6 % serum estrogen level compared to without laserpuncture induction.

This research showed that giving of combination treatment between protein dietary level (30% up to 40%) and laserpuncture induction reached two peaks at 3th and 6th week (Figure 1). This combination treatment stimulated follicular cell proliferation in the gonad and activated granulose cell to produce more estrogen level is 3 weeks sooner compare to wihout laserpuncture induction because pre vitellogenic occurred at 2nd week. In the middle of 2nd and 3th week was vitellogenic stage. The first peak was reached in the middle of 3th week. This condition showed that oocyte was in late stage of vitellogenic. On this period, oocyte was responsive to produce highest serum estrogen level that was proven by quick oocyte development. Giving of protein dietary level and laserpuncture induction accelerated pre vitello genic and vitellogenic process also accelerated oocyte maturation in the end of 3th week. The second peak was reached at 6th week, but the serum estrogen level was lower than the first peak. At 7th and 8th week, serum estrogen level decreased and oocyte was in post vitellogenic. On this period, oocyte has matured, ovulated and ovopositioned. In this time, serum estrogen level decreased because vitellogenesis had done and its physiological func tion has been changed by gonadotropin hormone (GtH-II). .

Suitable with Karu [9] and Koutna [10] our result showed than Helium-Neon laserpuncture induction stimulated biological organ such as follicular cell proliferation, enzyme and hormone activity. According to Lefler *et al*. [16], protein dietary level determines oocyte number in follicle to produce estrogen. Yaron *et al*. [4] and Aizen *et al*. [5] showed that protein dietary level determines GtH release and estrogen synthesis. Laserpuncture induction in female broodstock catfish increase GtH-I level up to 48% before spawning [17]. Hariani [15] suggest that laserpuncture induction had been proven increase estrogen level.

The reproductive acupoint is a sensitive point caused by existing of low resistance high potential active cell. These cells are sensitive to laserpuncture induction. Laserpuncture rays penetrate into epidermis and dermis. There are many periphery nerves between epidermis and dermis. Laserpuncture rays on reproductive acupoint release electromagnetic wave. This energy will be changed into electric signal; causes depolarization in neuron membrane and generate action potential [18, 19]. The action potential in neuron membrane stimulates releasing of neurotransmitter. The action potential causes Ca^{2+} channel be opened by voltage gate calcium channel and calcium sensing receptor. Ca^{2+} extracellular penetrates into the cell and causes Ca^{2+} concentration enhancement.

Ca^{2+} intracellular concentration enhancement stimulates synapse cyst release neurotransmitter by exocytosis into synapse. Neurotransmitter will bind to receptor in post synapse. If the effect is excitatory, impuls will carry to the brain. In the brain, complex physiological reaction stimulates Glutamic Acid Dicarcoxylase 65 (GAD65). The active GAD65 stimulates GABAergic neuron to secrete Gamma Aminobutyric Acid (GABA). GABA will stimulate hypothalamic neuron to release Gonadotropin Releasing Hormone (GnRH). GnRH gradually stimulates pituitary neuron to relase GtH-I and II. GtH-I and II. GtH-I is involved in steroid hormone synthesize in the gonad (testosterone and estrogen). Laserpuncture induction increased this hormone production. Testosterone is carried into granulose to stimulate the releasing of cytochrome P450 aromatase and change the testosterone to 17β-estradiol (estrogen). It means that laserpuncture induction increased estrogen level production more than without laserpuncture induction. According to Okuzawa *et al*. [20], Kah and Dufour [21] and Urbatzka *et al*. [22], the main regulator of reproductive cycle in fish is in axis brain - hypothalamus – pituitary to regulate reproductive hormone production.

GSI

Gonado Somatic Indes (GSI) value of female broodstock catfish after giving of protein dietary level both with and without laserpuncture induction

during 8 weeks showed upward trend until certain value and gradually decreased. The GSI value of 35% protein dietary level treatment is higher than 30% and 40% both with and without laserpunture induction. Giving of 30% protein dietary level and laserpuncture induction increased 7.77% GSI value compared to without laserpuncture induction. Giving of 35% protein dietary level and laserpuncture induction increased 9.93% GSI value compared to without laserpuncture induction. While, giving of 40% protein dietary level and laserpuncture induction increased 5.15% GSI value compared to without laserpuncture induction (Figure 2 and Table 2).

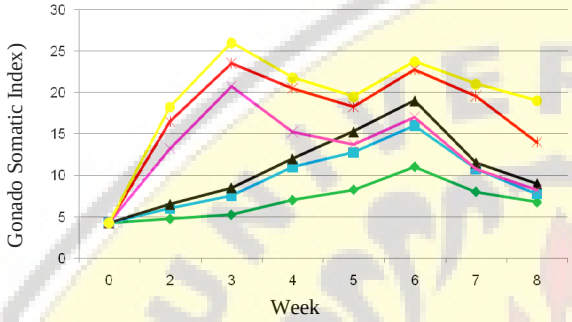

Figure 2. Gonado Somatic Index of female broodstock catfish

Table 2. The peak value of Gonado Somatic Index of catfish (*Clarias sp*) after giving of 30%, 35% and 40% protein dietary level at 3th week (with dietary level at 3th week (with laserpuncture induction) and 6th week (without laserpuncture induction)

Protein Dietary Level	Lasepucture	Mean ± Deviation Gonado Somatic Index	Percent of Gonado Somatic Index Enhancemen (%)
30%	Without laser	19.17 ± 0.87[a]	
30%	Laser	20.66 ± 0.99[b]	7.77
35%	Without laser	19.73 ± 0.82[ab]	
35%	Laser	21.57 ± 0.99[cd]	9.33
40%	Without laser	21.35 ± 0.68[bc]	
40%	Laser	22.45 ± 0.83[d]	5.15

Values in the column followed by different superscript showed significant difference (P<0.05)

The GSI value of 35% protein dietary level treatment was higher than 30% and 40% both with and without laserpunture induction (Table 2). It because the treatment combination between 35% protein dietary level and laserpuncture induction on reproductive acupoint in female broodstock catfish activated impuls in the brain to release Gama Aminobutyric Acid (GABA), Gonadotropin Hormone (GtH), and estrogen. Estrogen was carried in blood stream and entered to hepatic cell to regulate vitellogenic synthesis. This vitellogenin entered to gonad. In the gonad, vitellogenin was absorbed mediated by vitellogenin receptor. Accumulation of vitellogenin increased oocyte size, gonad weight, and GSI value.

Giving of 40% protein dietary level and laserpuncture induction resulted in highest GSI value compare to 30% and 35%, but lowest GSI enhancement in without induction treatment. It might caused by maximum enzyme capacity to digest protein was not more than 40%. If the female broodstock catfish was given more than 40% protein dietary level, the protein decomposition into amino acid decreased. It means that absorbed protein will decrease and be taken out by defecation. We suggested that 30% protein was an optimal level to increase GSI value. This capability was also supported by laserpunture induction. According to Ibim and Sikoki [2], the 40% protein dietary level in female African catfish increased gonad weight and GSI value compare to less than 31% protein dietary level. Kusuma [17] showed that laserpuncture induction in female broodstock catfish accelerated gonad maturation and increased GSI value. Gonad maturation acceleration and GSI value enhancement is determined yolk production enhancement.

Our study showed that protein dietary variation level was determines GSI value. The high protein dietary level significantly (P<0.001) increased the GSI value (Figure 2). Protein dietary level without laserpuncture induction increased GSI value gradually during 2nd week until 6th week. On this period, oocyte is in vitellogenesis. On the vitellogenin stage, estrogen level is high and be used to stimulate hepatic cell to synthesis vitellogenin. This vitellogenin will be stored and accumulated in oocyte (yolk). The yolk (protein vitelin) enhancement was indicated by GSI enhancement value. The same condition was maintained in the middle of 6th week (late vitellogenic stage). The peak of GSI values was reached in the end of 6th week. It indicated that oocyte has matured caused by vitellogenin accumulation. Oocyte gradually hydrated that was indicated by maximum gonad weight and GSI value. Araoye [23], Laleye *et al.* [24] and Shinkafi & Ipinjolu [25] showed that the maximum GSI value was reached before spawning. At 7th week and 8th week, GSI value decreased. It indicated that oocyte was in post vitellogenic stage because vitellogenesis has finished and catfish has spawned. Suitable with Utomo *et al*. [26], reduction of GSI value in female zebra fish indicated that vitellogenesis has finished. GSI value indicated gonad maturation. Gonad maturation after 40% protein dietary level without laserpuncture induction was reached at 6th week. Meanwhile, giving of 30% protein dietary level with laserpunture induction reached the highest GSI value at 3th week (P<0.05) (Tabel 2). It means that

the effective combination treatment to increased GSI value was 30% protein dietary level with laserpuncture induction because it increased GSI value 3 weeks sooner than other. According to Kusuma *et al.* [12], Hariani & Kusuma [27,28], laserpuncture induction on reproductive acupoint in every week during 15 second accelerated gonad maturation and GSI value in female broodstock catfish.

The broodstock catfish need sufficient protein to maintain oocyte growth and development, follicle formation and yolk synthesis. This sufficient protein associated with sufficient amino acid in the dietary. Based on this research, we suggest that the total amino acid necessary for broodstock female was 14.76%. Essential amino acid test result of 30%, 35% and 40% protein successively contained 13.29%, 15.26% and 17.33% essential amino acid. Our study showed that 35% protein dietary level was enough to suffice vitellogenin protein necessary. Although 40% and 35% protein dietary level have more essential amino acid excess than 30%, the economical reason suggest that 30% protein dietary level was an efficient level to produce vitellogenin. Kusuma (2013) showed that laserpuncture induction increased gonad maturation and GSI value. Cerda *et al.* [26] showed that GSI value indicated gonad development and maturation catfish.

Our study showed that GSI value of combination treatment between protein dietary level variation (30-40%) and laserpunture induction reached two peaks (3^{th} and 6^{th} week) (Figure 2). Laserpuncture induction on reproductive acupoint stimulated reproductive activity to increase vitello genin production. This physiological activity is also need good quality of dietary to produce and accumulate the yolk in oocyte. The combination between optimal protein dietary level and laserpunc ture induction accelerated yolk protein accumul ation 3 weeks sooner and maintained. quality of egg. The yolk accumulation in egg increased egg size and gonad weight. It caused GSI value gradually increased. Hariani *et al*. [27] and Hariani (2014) showed that broodstock catfish given by commercial dietary with laserpuncture induction can increase their GSI value 3 weeks sooner. On the maturation time, hydrated oocyte will be bigger followed by maximum gonad weight and GSI value.

Conclusions

Giving of 40% protein dietary level and laserpuncture induction reached the highest serum estrogen level and GSI value at 3^{th} and 6^{th} week. Meanwhile, giving of 40% protein dietary level without laserpuncture induction reached the highest serum estrogen level and GSI value at 6^{th} week. Although 40% and 35% protein dietary level have more essential amino acid excess than 30%, the economical reason suggest that 30% protein dietary level was an efficient level to produce vitellogenin. The combination treatment between protein dietary level and laserpuncture induction had been proven accelerate estrogen release and accelerate gonad maturation 3 weeks sooner compare to without induction.

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FISH DIVERSITY of *CYPRINIDAE* FAMILY BASED on DNA BARCODES in HARAPAN RAINFOREST, JAMBI

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Abstract

A total of 65 individual member of Cyprinids (*Cyprinidae*), representing 17 genera and 26 species had been sequenced, to analyzed phylogenetic relationship of *Cyprinidae* family. Aligned nucleotide sequence produced 500 bp in length consisted of 295 bp conserve and 205 bp variable nucleotide. The composition of nucleotide bases T (*U)* = 28.5%, C = 27.1, A = 27.4%, and G = 17.0%. Partial nucleotide sequence of *Cyprinidae* COI gene has similarity of 89% comparing to Genebank database. Reconstruction of phylogenetic tree showed two different main cluster of *Cyprinidae* family, represented by *Puntius* and *Rasbora*.

Keywords: COI, mtDNA, Sumatra

Introduction

Harapan Rainforest is the first ecosystem restoration in Indonesian. The area was 101,000 ha [15], used to be utilized by logging company. This area is located in Jambi and South Sumatera Province. The remaining forest now is a mixture of secondary forest and degraded forest. There are four primary watersheds in Harapan Rainforest i.e., Meranti, Kapas, Lalan, and Kandang. There are at least 37 aquatic zones in Harapan Rainforest with various types of aquatic ecosystems such as weak-current large river, lake or swamp flood. Fish diversity in Harapan Rainforest consist 123 species, 62 genera, and 23 families. Highest number is Cyprinids (*Cyprinidae*) 59 species (48%) that has distinct character on the body character such as the presence of large transverse band (bar), stripe as well as small spots [16] . Fish with transverse band compose of the Sumatra barb *Puntiugrus tetrazona* and *Puntiugrus anchiporus* . The spanner barb is *Puntius lateristriga*, the hampala barb is *Hampala macrolepidota* (and *Hampala bimaculata* . Group of striped fish are the lined barb *Striuntius lineatus* and *Desmopuntius gemellus*). Fish with spot consist*;* the beardless barb are *Cyclocheilichthys apogon* and *Cyclo-cheilichthys repasson.* Fish within same group tends to have similarity in morphological character, thereby morphological identification mislead to ambiguity taxonomical status. Morphological identification can not reveal the presence of cryptic species, sub-species or sibling species, and the species within the development stage, so therefore more accurate identification is required such as molecular identification. Ref [12] stated that traditional identification was difficult for the assessment of biodiversity conservation and management of fish globally, so the molecular identification using DNA barcode is necessary.

DNA barcode is a mitochondrial DNA (mtDNA) sequences with the size of 650 base pair (bp) in the area of *cytochrome oxidase I* (COI) that can be used to identify animal species [16]. It is designed to identify species accurately, efficiently and quickly through short sequences in the area of genome target species in comparison to a library of known sequences [16]. DNA barcode today is an effective tool to deal with any confusion in the identification and taxonomic conflict because it can reveal the on set of genetic diversity and can distinguish the species by either just using short sequence of mtDNA COI of 650 bp [19]. The objective of this study was to analyzed phylogenetic relationships in the family of *Cyprinidae* in Harapan Rainforest, Jambi based on DNA barcode using COI gene as a genetic marker. This result will be then useful to assist monitoring of biodiversity.

Material and Method

Research was conducted in September 2012 - February 2014. Sampling locations was in the aquatic area of Harapan Rainforest Jambi, such as Kapas River, Lalan River, Kandang River,

Rohani Lake, 41 Lake, Tiung Luput Lake, Base camp Lake and Klompang Swamp. Morphological analysis was carried out in the Laboratory of Fish Resources Research Centre in Bogor Agriculture University and Research centre for Biology, Indonesian Institute of Science. Identification of fish was undertaken on both morphometric and meristic characters, refer to several identification book such as: [1] [11], [18], [14], [10], [2]). Molecular analysis was conducted in Biotechnology Laboratory Primate Study Centre Bogor Agriculture University. Specimen will be stored in Bogor Zoological Museum (MZB) to acquire catalogue number.

DNA Extraction

Prior to be transported to Lab for DNA extraction right pectoral fin was cut and preserved in absolute alcohol solution. DNA extraction was carried out using a DNeasy from Qiagen. DNA extraction performed was in a Bio Safety Cabinet (BSC) based on the Quick-Star Protocol for Qiagen tissues. Intact DNA samples were feasible as DNA template for amplification.

Species Barcode and PCR Condition

COI gene amplification was conducted using Polymerase Chain Reaction (PCR). Primers used were FishF1 primer (forward) 5'TCAACCAACCACAAAGACATTGGCAC3' and FishR1 (Reverse) 5'TAGACTTCTGG GTGGC CAAAGAATCA3' [18]. PCR mixture was performed using Gotag mix. Total volume of 25 µl PCR was needed, consisting of 1 µl of each primer, 12.5 µl Gotaq master mix, 5.5 µl of nuclease free water, and 5 µl of DNA samples. PCR preparation was performed in a laminar cabinet. PCR was performed using 40 cycles with Gene Amp 9700 PCR machine (PE) from Applied Bio systems. PCR conditions was 94ºC in 5 seconds (s) for pre-denaturation, 94ºC in 30 s for denaturation, 52ºC for anneling, 72ºC for 30 s for extensions, and 72ºC in 5 s for post PCR.

Visualization of DNA bands

PCR products were visualized using 1.8% agarose gel. Those were electrophoreses in a horizontal electrophoresis machine. 20µl of each PCR product was run into the chamber with 100 watt voltages for 45 minute. Follow that PCR bands was visualized under UV gel doc. Intact PCR product more sent to be sequenced 1st Malaysia.

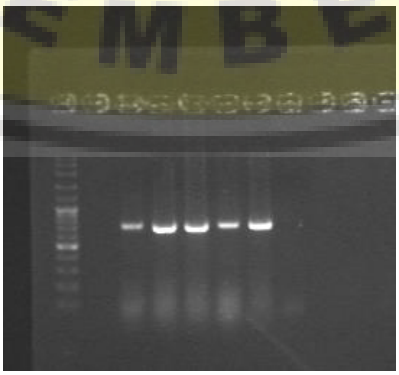
Data Analysis

Nucleotide sequence acquired from sequencing products were analyzed using BIOEDIT. Those were then corrected with primer FishF1 and FishR1 using MEGA 5.1 [17]. Prior to that, nucleotide sequences were matched and analyzed use DNA library in geneBank NCBI using BLAST, under user name tedjo_sukmono.

Reconstruction of phylogenetic was built using maximum likehood method with 1000x bootstrap neighbour joining (NJ). Genetic distance was analyzed using Kimura 2 parameter (K2P). Nucleotide sequence of family *Cyprinidae* were deposit in geneBank, Barcode of Life and FishBold, obtain DNA barcode under username of tedjo_sukmono

Result

A total of 65 *Cyprinidae* consisting 17 genera and 26 species were processed for DNA barcode using COI gene. PCR product showed intact band with 650 bp in length (Figure 1) that we good enough to be processed for sequencing. Specimens of fish have been deposited in the MZB Research centre for Biology, Indonesian Institute of Science with number catalog MZB 22168 until 22243.



M 1 2 3 4 5 -C

M = marker, 1= *Hampala macrolepidota*, 2= *Croossocheilus oblongus*, 3= *Puntius lateristriga*, 4= *Rasbora elegans*, 5= *Cyclocheilictyhs apogon*, -C= negative control

Figure 1.DNA band using electrophoresis 5 sample of Cyprinidae family (650 bp)

Nucleotide variation

Aligned nucleotide sequence family *Cyprinidae* resulted in 500 bp consisting 295 conserve and 205 variable nucleotides. Analysis of the nucleotide position was conducted to find out the location of transition substitutions and transverse. *Puntius lateristriga* used as a comparison in-group and the Indonesian snakehead *Channa micropeltes* family *Channidae* and the slender walking catfish *Clarias nieuhofii* family *Claridae* accession number JF280836 used as out group. Transitions substitution occurs in the purine ↔ purine (A↔G) or pyrimidine ↔ pyrimidine (C↔T), transverse substitution occur in the purine ↔ pyrimidine (C↔A, A↔T, C↔G and T↔G). There are nucleotide substitutions of family *Cyprinidae* compared with out group in 4 sites, at the nucleotide position to 55 (C↔A), 174 (T↔C), 273 (A↔T), 347 (G↔A), and 489 (A↔C).

Phylogenetics of family *Cyprinidae*

Cyprinidae nucleotide sequences were aligned using MEGA 5 (Tamura *et al*. 2011) to determine reconstruction of phylogenetic tree. Reconstruction of phylogenetic was perfomed using maximum likehood method with 1000x bootstrap neighbour joining (NJ). Phylogenetic tree results in two distinct groups of *Cyprinidae* species as genus *Rabora* and P*untius* (Figure 2).

Discussion

FishF1 and FishR1 primers were successfully able to distinguish CO1 gen from 26 Jambi *Cyprinidae* species. Comparison of Jambi *Cyprinidae* CO1 gene and geneBank data using BLASTn confirmed 89 - 99% similarity. It is in accordance with [12]) saying that similarity among *Cyprinidae* family was > 85%. Ref [6] stated that COI gene can produce a high resolution for species identification as a standard barcode. According to Herbert *et al*. 2003 identity of more than 3% showed high similarity species and this suggests that bioinformatics databases of CO1 gene sequences can be used as a global bio-identification of animals. Consolidation of bioinformatics data is needed for implementation through provision of library genes. In this research there are five species of the nucleotide base sequence is not yet available in the NCBI Genebank, i.e. *Hampala ampalong*, the signal barb *Labiobarbus fastivus*), *Luciosoma trinema*), *Puntius* sp "Harapan", and *Rasbora borneensis*. These sequences will be deposited in NCBI Genebank and Barcodelife. Nucleotide sequences of T (U) (*Puntius* and *Hampala*). Cluster B is composed two groups (III and IV), group III consists of three genera, i.e., *Parachella, Malayochella*, and *Luciosoma*, meanwhile group IV consists

= 28.5%, C = 27.1%, 27.4% and A = G = 17.0.%, Closely confirmed with [12] resulting nucleotide composition of the *Cyprinidae* family were T = 29.6%, C = 26.1%, A = 27.4%, and G = 16.6%. Based on the analysis of nucleotide variation length 475 bp, there are 283 conserve and 192 variables. This suggests that these genes are retained from his ancestors as primitive character (symplesiomorphy) of 59.6%, and the character of derivative (synapomorphy) of 40.4%. Analysis of nucleotide variation indicates that there is difference between the nucleotide compositions of the family *Cyprinidae* compared out group both *Channidae* and *Claridae* family. Nucleotide substitutions family *Cyprinidae* with out group occur at 5 sites, that is position nucleotides 166 (A↔G), 185 (G↔A), 286 (A↔T), 347 (G↔A), and 439 (A↔C). This showed that the 5th sites of the nucleotides can act as the primary identifier of the family *Cyprinidae*.

Striuntius lineatus and *Striuntius* sp of Harapan Jambi showed high similarity with *Puntius johorensis* in GeneBank (99%), those were phenotypic similar as having longitudinal striped body. According to Haryono (2005) the striped *Puntius* split into four species of all known the stripped barb *Puntius eugrammus.* Ref [8] grouped striped *Puntius* divided into 4 species, in this case *P. eugrammus* divided into three types (*P. johorensis, P. gemellus,* and *P. trifasciatus,* and *P. lineatus* now call name *Striuntius lineatus*. Colour pattern is the main character to illustrate the differences of striped *Puntius* because other morphometric and meristic characters fail to distinguish the three species. This indicates that the difference in colour pattern is not strong enough to distinguish DNA on striped *Puntius* group. There are a monophyletic group in striped *Puntius*, in other words *P. eugrammus* as ancestor had primitive character (symplesiomorphy) such as striped however, each member also has a unique character of synapomorphy or automorphy characters as the derivative from different numbers of striped and colour pattern. However, in this case automorphy was not able to analyze.

The resulting phylogenetic tree shows that the *Cyprinidae* family is divided into two main cluster consisting of cluster A and cluster B. Cluster A consists of two main groups (group I and II). Group I consist of 8 genera *Osteochilus, Labiobarbus, Barbichtys, Barbonymus, Crossocheilos, Cyclocheilicthys, Puntioplites and Puntius.* Group II comprised two genera

of *Rasbora*. *Genetic* distance between cluster A and B is 0.267 i.e. *Striuntius lineatus* and *Rasbora elegans*. Genetic distance within cluster A between group I and group II was 0.213 i.e. *Labiobarbus occelatus* and *Striupuntius lineatus*, whereas cluster B between group III and group IV was 0.210 i.e. *Parachella oxgastroides* (Bleeker 1852) and *Rasbora borneensis* (Bleeker 1860).

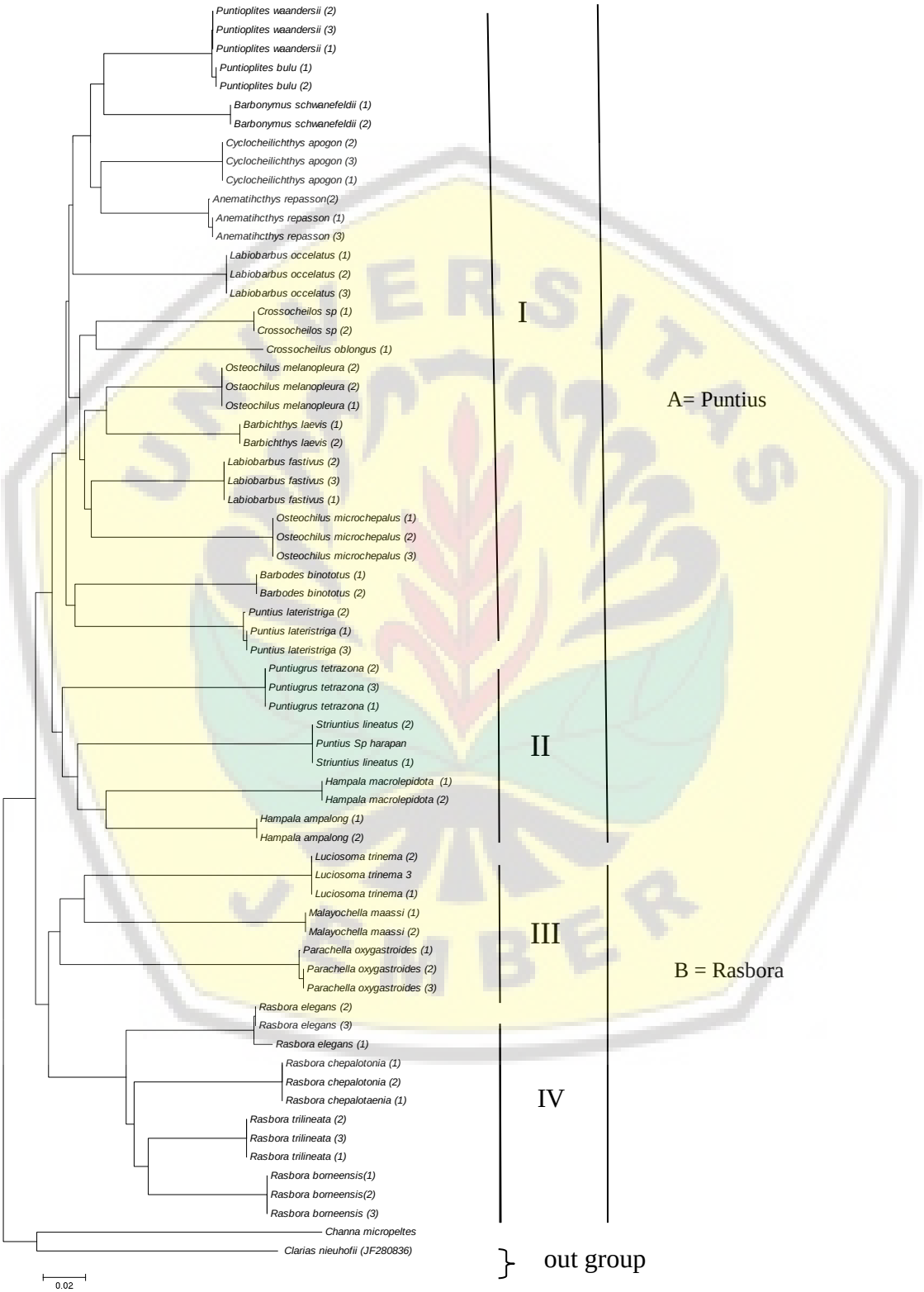


Figure 2. Reconstruction of phylogenetic tree Cyprinidae family using NJ- boostrap 1000x (475 bp)

In *Cyprinidae* phylogenetic, there are several new genus based [9] and [3] i.e. *Puntiugrus*, *Striupuntius*, and *Barbodes* that originally came from the genera *Puntius*. Some of these species are separated into groups but still within a cluster that cluster A which represents the genus *Puntius.* According to Kottelat 1996, the genus *Puntius* initially split into two genera *Puntius* and *Puntioliptes*, based on different finger fins. *Puntius* has weak last fingers of the anal fin, whereas in the *Puntioliptes* the last anal flipper finger was hardened. Furthermore, *Puntius* genera split into 3 genera based on scale structure which are *Puntius*, *Poropuntius*, and *Barbodes*. According to [3]), *Puntius* resplit into 6 genera of *Puntius*, *Desmopuntius*, *Pethya Puntigrus*, *Striuntius*, *Dawkinsia*, *and Hyselobarbus*. Likewise, there has been a name change in the name of *Puntius tetrazona* being *Puntigrus tetrazona* and name of *Puntius binototus* was change to *Barbodes binototus*, and *Puntius lineatus* being *Striuntius linetus*. Phylogenetically, those species are in different groups.

Acknowledgements

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BROWN PLANTHOPPER POPULATIONS on SOME RICE VARIETIES

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Abstract

Outbreak of brown planthopper was happened when The Government introduced rice varieties with high rates production. Previous local farmers grow rice varieties and never outbreak of this pest. To overcome the brown planthopper attack, government released rice resistance variety of brown planthopper (VUTW). Research carried out to find out the brown plant hopper populations in some local rice varieties and VUTW. The study was conducted in Delanggu Klatean starting in January 2015 to July 2015. Rice varieaties were used Rojolele, Pandanwangi, Rojopusur, Ciherang, Situbagendit, Membramo, and IR 64. Twenty five days after planting (DAP) , plants were infested with 5 pairs of adult brown planthoppers. Observations were made every day by noted the age of adults infestation, nymphs age and counting the number of nymphs and adult were formed. The result showed that the fastest adults death were occurred on IR 64 variety ie 4 days after infestation (DAI). On Rojolele, adult death were occurred at 12 DAI. All adults died at 19 DAI. Eggs hatched at 8 DAI and adult emerged 21 DAI. The peak of nymph population was occured at 12 – 18 DAI with Membramo variety (348 nymphs) and Ciherang (256 nymphs) higher than other test varieties that ware relatively similar. Adult arising from Membramo variety was higher than other varieties. The death of aduts were begun at 29 DAI. This research shows that there were differences of planthopper population and the age of this pest between the test rice varieties.

Keywords : Brown planthopper, local rice varieties, population

Introduction

Brown planthopper (BPH), *Nilaparvata lugens* Stall. (Homoptera: Delphacidae) is an important pest of rice crops in rice-producing regions in South Asia and Southeast Asia, including Indonesia [4,2]. In Indonesia the first time of BPH attack has happed in 1968 covering an area of 52,000 Ha. In 1975-76 year, these pests back inflict damage on an area of 242 427 ha [4], and the highest attack in 1998 reached 14 645 ha. In 2010 BPH attack occurred in some rice-producing areas in Central Java with a total area of 3,340 ha [3]. Based on data from the Department of Agriculture Central Java province in 2010, the intensity of the BPH attack has also increased significantly, which covers 32 065 ha and 4,149 ha of which have puso (crop failure). Brown planthopper attack in 2010 was able to break-resistant rice varieties planted by farmers.

Planting rice varieties resistant to brown planthopper (VUTW) is still the main choice of farmers and policy makers to control the brown plant hopper attacks [1,2]. In fact, planting VUTW which has a gene resistant (bph), can be broken resistance in the growing season 3-4 only [4] because new biotypes of BPH appeared to break the resistance of the only regulated by a single gene bph.

Indonesia is one of the world's biodiversity centers. In the case of rice varieties, there are quite a lot of local varieties that are believed to also have a high genetic diversity of nature as well, including the diversity of potential resistance including the diversity of potential resistance to brown plant hopper pest. Local varieties of rice that can survive up to now clearly has specific advantages and stress resistant properties (stress), both abiotic and biotic, because it has adapted to the environment in a very long period of time. Local rice varieties such as Rojolele, Rojopusur and Pandanwangi also has certain superior properties as a characteristic as resistance to pests. Nevertheless, the identification of potential local varieties resistant to brown plant hopper pest planthoppers to overcome the problems that have not been done. Identification of rice resistant to BPH can be done by knowing the BPH population that was reared in some rice varieties.

Material and Method

The research was conducted at field laboratory of Gita Pertiwi NGO in Klaten Central Java, from January 2015 to June 2015. This research used 7 rice varieties such as IR-64, Ciherang, Membramo, Situbagendit, Rojolele,

Rojopusur, and Pandanwangi . Each varieties were replicated by 5 times.

Brown Planthopper Preparation : BPH population was originated from nimfe and adult collected from paddy crops in Klaten, Central Java. BPH were reared in 20x20x10cm3 wooden cage using paddy seedling as died. BPH were reared to obtain of adult enough to infestation.

Plant Test Preparation : Seedling was conducted in plastic trays for each variety. Transplanting was conducted when the seedlings were 20 days old. Rice were planted in a 25cm-diameter plastic pot filled with paddy soil and manure in the ratio 1: 1. At of 25 days after transplanting (DAT), the plants were caged with mica plastic diameter of 25 cm with a height of 1m .

BPH Infestation : BPH infestation was conducted when the plant aged 25 DAT. A total of 5 pairs BPH adults were infested on the test plants. Observation was done every day to knowing adult (Fo) died, time of nymphs emerged, number of nymphs, time of adult emerged (as F1), and number of adult (F1) emerged.

Results and Discussion

Adults (Fo) death : BPH Adult's death began occurred 4 days after infestation (DAI) ie varieties IR 64. In Rojolele as local varieties, adults died occurred 12 DAI. In general, an increase of adult's death occurred at 12 DAI. All of adults died occurred at 19 DAI (Fig 1). The period of BPH adults are about 9-12 days [8] according to the research results. The difference in the life of adult in each test varieties can be used as indicators of the suitability of host plants as BPH feeding. IR 64 is high yielding varieties (VUTW) that was knew resistance to brown planthopper biotype 1, 2, and moderately resistant to brown planthopper biotype 3. Membramo resistance to biotypes 1,2 and moderately resistant to biotype 3. Ciherang resistant to biotype 2 3 . Situbagendit is a group of upland rice can be grown in paddy fields.

Pandanwangi the local varieties are susceptible biotypes 2 and 3. Meanwhile, Rojolele and Rojopusur the local varieties that are susceptible to brown planthopper. BPH adults were able to survive on the variety IR 64, showed that BPH colony Klaten have been able to break the resistance of this varieties. Based on Balitpa survey at 2003, some varieties of rice such IR 64, Ciherang and Membramo were the varieties that were preferred by farmers and were planted extensively. According [7], the reaction varieties of rice in the field against the brown planthopper after several years may change as IR 64 resistant to BPH Klaten colony eventually turned into moderatly resistant.

[1] showed that brown planthopper Klaten colony has been transformed into biotype 4 so as to break the resistance of varieties VUTW that is currently widely grown by farmers.

Based on these observations can be seen, generally BPH adults able to survive at all a of rice test varieties (VUTW and local varieties). This suggests that BPH has been adapted to all varieties of the test so as to break the resistance.

Nymphs Population : The result of BPH adult infestation showed, nymphs begin to appear at 8 DAI according to [8] the periods of BPH eggs are about 8-9 days. This indicates, BPH aduts immediately lay eggs after were infested. At the beginning, the most number of nymphs were showed in Rojolele followed by Membramo varieties. The peak of nymphs occurred at 13-18 DAI. Of all the varieties tested, the highest nymph populations were observed at Ciherang (256 nymphs) and Membramo (348 nymphs). [9], the last stage of a search by the insect to host is the host suitability. Host suitability is characterized by long life cycle, number of offspring, and the survivorship. The results showed that BPH Klaten colony able to live in all varieties tested. The high population in Ciherang and Membramo showed that both varieties capable of supporting the needs of BPH. Situbagendit nymph population was relatively less than the other varieties. Situbagendit is upland rice that can be grown in paddy fields [10]. Nymph population decreased 19 DAI (Fig.2). This happens because most nymphs have become adults and some of them died as a result of the plant dried because it was not capable to supporting BPH life. All of Rojolele plants died at 26 DAI. Generally the death of host plants occurred at 28 DAI. With the death of host plants means adults formed were not optimum.

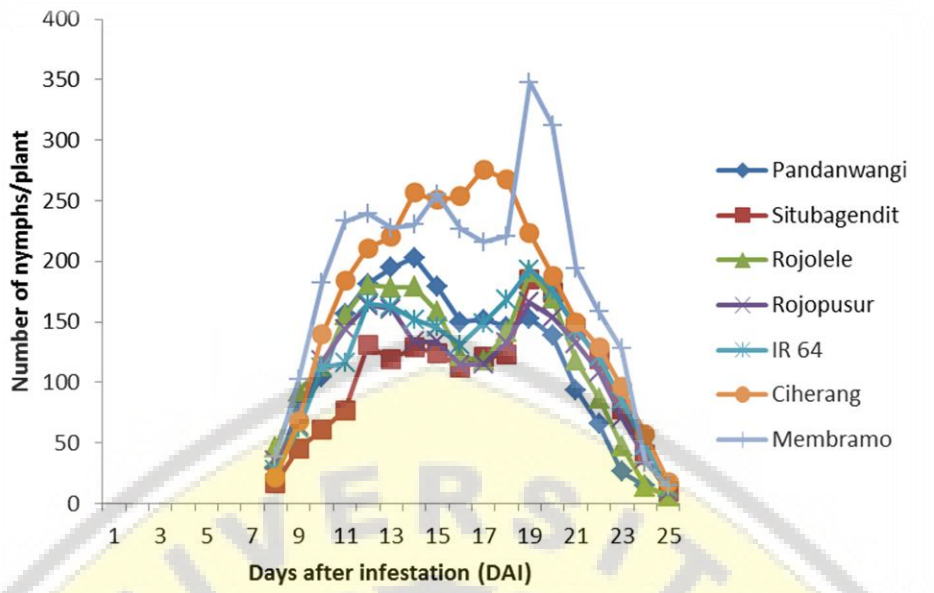

Figure 2. Population of BHP Nymphs on some rice varieties

Adult F1 Population : BPH adults emerged at 21 DAI or 13 days after eggs hatching. [8], the periods of nymphs is 13-15 days. In all rice varieties, nymphs succed be adults. The low adult population in Rojolele accured the plant due to dry before all the nymphs become adult (Fig 3). Among varieties tested, Membramo showed the highest adult population followed by Ciherang . The lowest adult population showed on IR 64. Population nymph and adult on Rojopusur and Pandanwangi were lower than Ciherang and Membramo that knowing resistant to BPH biotype 2. Based on the research, IR 64 and Situbagendit fewest number of nymph and adult generated. Besides that the two varieatas be able to survive longer than most others.

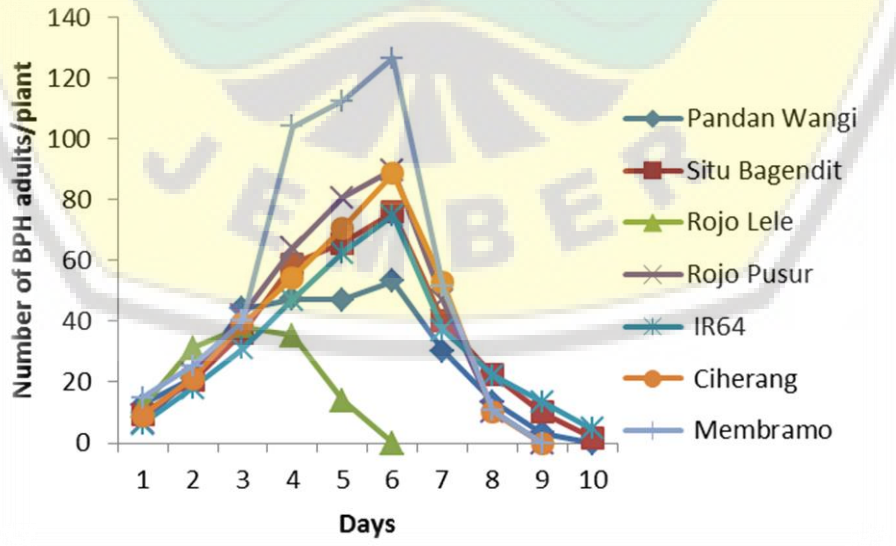

Figure 3. Number of Brown Planthopper adult on Some Rice Varieties

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MORPHOLOGICAL VARIATION of LOCAL DURIAN (*Durio zibethinus* Murr.) on THE TERNATE ISLAND

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Abstract

Local durian of Ternate island is a term variations local name durian (*Durio zibethinus* Murr.) on the Ternate island. Local durian on the Ternate island has a diverse morphological variations. The purpose of this study was to analyze the variation of morphological characters 13 of durian local names collected from the island of Ternate. The research was conducted at three locations: Tobololo, Sasa and Tongole villages from April to June 2013, using descriptive method with purposive sampling. Morphological characters based on the standard IPGRI which were modified to 59 characters were used in this experiment. Analysis phenetic between durian was conducted using UPGMA method. At Ternate island, it was found 13 species of local durian which have various fruit shape, aril color and texture, seed shape, and form of spine. Based on the analysis of 59 morphological characters, a phenetic tree which was constructed showed three main groups of local durian Ternate namely: group I (local durian Udi and Sina); group II (local durian Air tege-tege and small Luri); group III (local durian Mentega and Gajah kuning).

Keywords : local durian, morphology variation, ternate island

Introduction

Durian (*Durio zibethinus* Murr.) is a plant family Bombacaceae from Southeast Asia, especially Indonesia [2,13]. Durian is also called *King of fruits* and have economic prospects are enaugh good value beside other fruits. Today marketing durian has increase from year to year. Durian is increasingly favored by the public so that the price of durian fruit growing [1].

North Maluku has the potential diversity of tropical fruits, one of which is the durian (*Durio zibethinus* Murr). Ternate Island is one of the durian production centers. BPS North Maluku province (2012) reported that the production of durian in northern Maluku fairly increased by an average of 2, 92 tons annually with productive area's are: Jailolo, Tidore and Ternate. At Ternate island it was found 13 species of local durian which have various fruit shape, aril color and texture, seed shape, and form of spine. The variation of durian fruit morphological has to led the phenomenon of namely many different locally at any morphological variation durian.

Many studies have been conducted are identified in several places durian diversity using morphological characters durian, includes: in Langkahan Aceh [5], in Kasembon the district of East Java (Utomo, 2011), and in Tanah Datar [14]. Inventory and morphological characterization of durian (*Durio zibethinus* Murr.) This research was expected to be used as a reference to introduce the types of local durian on the island of Ternate, utilization and conservation. The purpose of this study was to develop a *database* of durian diversity by identifying and characterizing the morphology of local durian on the island of Ternate.

Material And Methods

This research was conducted by using descriptive method of exploration. Sampling methode with purposive sampling. The experiment was conducted in three locations: Tobololo, Sasa and Tongole village from April to June 2013. Observations focused on 13 morphological characters durian which has been outstanding for more than 15 years. Morphological characters based on the standard IPGRI (1987) modified to 59 characters, namely: habitus, stem diameter, tree height, color and size of the leaf, stem color and size, color and size of fruit, colour and shape of seeds, and form of spines. Analysis phenetic between local durian using dendrogram construction with UPGMA method.

Results Inventory local Durian on the island of Ternate

Based on the exploration of the types of local durian from the Ternate island it was found that there were 13 variations of local durian at three observation point (Table 1.)

Table 1. The Local Name of Durian at Ternate island

Code	Local Name	Site
TI	Durian Cinta	Tobololo
T2	Durian Gajah Abu-abu	Tobololo
T3	Durian gajah Kuning	Tobololo
T4	Durian Boso	Tobololo
T5	Durian Pare	Tobololo
T6	Durian Mentega	Sasa
T7	Durian Luri kecil	Sasa
T8	Durian Luri besar	Sasa
T9	Durian Ratem	Sasa
T10	Durian Air tege-tege	Tongole
T11	Durian Biji mati	Tongole
T12	Durian Sina	Tongole
T13	Durian Udi	Tongole

Morphological characters of Local Durian from Ternate Islands

The 13 Ternate local durian can be distinguished from each other based on vegetative and generative character (Fig. 1, 2, 3, 4, and 5).

Relationship of 13 types local durian Ternate

Relationship analysis of 13 variations of local durian Ternate based on 59 morphological characters using UPGMA shown in dendrogram (Figure 1).

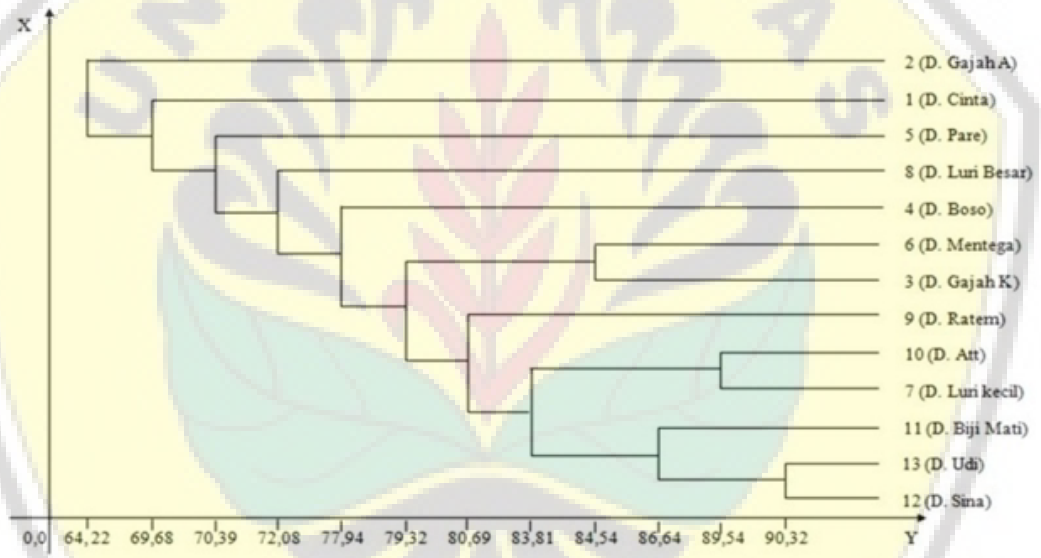

Figure 1. Dendogram Genetic Relationship of 13 Local Durin (*D. Zibethinus*) at Ternate Island base of Morphological Character.

Discussion

The ternate island were divided into 3 research areas : North, South and Central Ternate. From those areas there were found 13 local varieties of Ternate durian. In this research the presence of a durian populations were observed at the altitude of 500-700 meters above sea level (asl). Each area had different varieties based on the naming by the local community. From Subdistrict North Ternate (Tobololo) there were 5 varieties of durian found: Cinta (VL1), Gajah abu-abu (VL2), Gajah kuning (VL3), Boso (VL4) and Pare (VL5). From Sub-district South Ternate there were 4 varieties of durian: Mentega (VL6), Luri small (VL7), Luri Besar (VL8), Ratem (VL9) found. From Subdistrict Central Ternate there were 4 durian varieties: Air tege-tege (VL10), Biji Mati (VL11), Sina (VL12) and durian Udi (VL13) found. Durian trees grow well at altitude of 1-800 meters asl and can grow optimally at a height of 50-600 meters asl [7]. In its natural habitat, durian can reacrh the age up to approximately 200 years. The height of durian tree ranges from 20-50 meters [10]. The highest durian tree at the time of the observations was showed by durian Mentega (V6) 40 meters and the lowest was showed by durian Biji mati (V11) 19 meters. In general, durian tree will continue to grow until the limit of life. Ternate local durian trees in general has monopodial branches (Figure 1)

Figure 2. Monopodial branching tree on Local Durian Ternate

Durian is an annual plant that has the type of growth model of Roux and characterized by the dominance of stem growth continuous monopodial orthotrop (continuous growth) [8]. (The shape of the leaves of the plant durian observed were: elliptic and oval. Most durian showed the form of ellipse (Figure 2)

Figur 3: Type of elliptic and oval leaves on Local durian Ternate island

Durian leaf is incomplete because it only consists of the petiole and leaf blade. Durian leaves has elliptic to oval-shaped with the base of the stalk and leaves are round (Hardiantono (1992). There are variation of the leafe surface of durian. In general, the color of the lamina is light green and dark green colored. The leaf color of a plant species can change according to the circumstances in which it grows and linked to water and food supplies as well as irradiation Tjitrosoepomo (2005). The upper leaf the time of the observations was showed by durian Mentega (V6) 40 meters and the lowest was showed by durian Biji mati (V11) 19 meters. In general, durian tree will continue to grow until the limit of life. Ternate local durian trees in general has monopodial branches (Figure 1)

The shape of the leaves of the plant durian observed were: elliptic and oval. Most durian showed the form of ellipse (Figure 2) surface is generally curved to follow the pattern of the veins, but there is also a flat or smooth. The lower surface of leaves of durian has a different color to the surface upon which predominantly green color.

While the lower surface of the leaf colored greenish white, beige, brown and brown. Stem shape by cross-sectional durian is round (teres). In observation of the color bar, there are four categories of properties acquired, namely: gray, brown, dark brown and green moss, but from the entire sample of dark brown color is more dominant. The diameter of the largest trunk found.

Figure 4: The shape and color steam of local durian Ternate

According to Gardner, Pearce and Mitchell 1991 cit [9] stem diameter will increase in size when the food needed plants are in adequate amounts. Based on qualitative properties (surface stem and stem color) of each accession was found four kinds of stem durian namely: (1) smooth, (2) coarse, (3) very rude and (4) scales, to the surface of the stem. As for the color bars are: (1) gray, (2) Brown, (3) dark brown and (4) Green moss. Flowers durian can not be observed for all local durian, is due at the time of observation, durian were not in the flowering period, most of the plants have started to bear fruit, some of them are waiting for the next flowering season. Observation of the fruit, there are 3 types of fruits are globose without lobes and petals, globose with lobes without stamens and shirts (Figure: 4). The texture of durian flesh meat locally observed that there are two types: soft and rough, while the color of the fruit flesh at the local durian there are 3 colors: white, pale yellow and dark yellow (Figure 5). The texture and colour flesh of local durian Ternate (figure 5).

Figure 6: Color and Texture flesh local durian Ternate

Relationship of analysis is used to determine of relations between plant taxa using morphological caracter of a plant. Morphological caracter can be used for the describe the types of relationship level. The types related have many similarities from one species to another (Davis and Heywood 1973 cit [9].

Based on the analysis of known dendogram there are 3 clusters (groups) on the main local durian Ternate. Cluster I is the cluster with the highest similarity index and the closest cluster first found in varieties of VL 12 and VL 13 (durian Sina and Udi), with the rank of the similarity of 90.32%. Durian is living in the same habitat that is in the village District of Tongole Central Ternate. Cluster II contained of VL7 and VL10 are durian Luri small and durian Air tege-tege, with the index of 89.54%. Durian has a different habitat, durian small Luri at Sasa village located in the South District of Ternate while durian Air tege-tege at Tongole Central sub District of Ternate. Cluster III contained VL3 and VL6 are durian gajah kuning and durian Mentega), with an index of 84.54%. Same of the case with durian small Luri and durian Air tege- tege, durian Mentega and durian Gajah kuning also different habitats. Statement of Sitompul and Guritno (1995) in [14,11], plants need certain environmental conditions are optimum environmental conditions to fully express its genetic program. Properties which appear on every individual is the interaction between genes and the environment. Local durian Udi and Sina has a high degree of similarity as a durian that are in the same habitat, so that the expression of genes and environment interactions the same.

Qualitative characters that cause these differences such as branching models, shapes and colors stem, shapes and colors of leaves, fruits and seeds. Quantitative characters such as plant height, length and width of leaves, flowers, fruits and seeds. Differences between plant morphological characters in the population durian also influenced by genetic and environmental (Indriani et al, 2008). Furthermore, [6] stated that the differences and similarities beyond the morphological appearance of a plant species can be used to find much close relationship. These three main clusters of local durian population Ternate is contained in a low range as the geography of the islands with different environmental factors that are not too extreme. Morphological variation among local durian on the Ternate island is not too much difference.

Conclusion

Based on the research that has been conducted on the local durian Ternate found 13 local durian morphological variation island of Ternate. Durian plant morphological variation observed based on different forms of branching, the shape and color of the stems, leaves, fruits and seeds. kindship analysis based on morphological characters of the 13 local durian Ternate obtained three main clusters with the highest degree of similarity, resemblance rate reached 90.32 % on the local durian Udi and Sina.

Unknowledgment

We would like to thanks to the, A.R Tolangara and team Fundamental Research special to Ismat Ishak and Fadli for that supports the colection local durian at Ternate island.

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MEALYBUGS and THEIR NATURAL ENEMIES DIVERSITY on CASSAVA CROPS (*Manihot esculenta* Crantz)

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Abstract

These recent two years, cassava production has been threatened by mealybugs invasion. Cassava mealybugs are new pest that potentially decrease crop production, especially cassava. One of pest control towards mealybugs on cassava is using their natural enemies like parasitoids, predators, and pathogens. This research conducted to discover mealybugs and their natural enemies diversity on cassava crops. This research using direct observation method with simple random sampling technique applied at four different locations in Banyuwangi. This research was held on November 2014 until December 2014. The result showed that there are two specieses found at four defined locations in Banyuwangi. Those two specieses are *Paracoccus marginatus* and *Ferrisia virgata*. The highest diversity index was found at Glenmore, Sub District Glenmore (H' = 2.47) and the lowest diversity index was found at Sobo, Sub District Banyuwangi (H' = 1.63). The highest average of *P. marginatus* was also found at Glenmore, 28.65; while the lowest average of *P. marginatus* was found at Secang, 6.5. The highest average of *F. virgata* population was also found at Glenmore, 13.6 and the lowest average of *F. virgata* population was found at Secang, 1.3. There was only one mealybugs predator found in this research, *Hemerobius* spp. The conclusion of this research are that mealybugs' diversity index was put on medium category and there were two specieses found, *P. marginatus* and *F. virgata*. Mealybugs natural enemies diversity was on low category, with only one mealybugs predator found from *Hemerobius* spp species.

Keywords: mealybugs. natural enemies, diversity

Introduction

Cassava is one of lots important crops for human being, because of their advantage as food, animal feed, as industrial and bioethanol raw materials[1]. In these recent two years , cassava production sustainability was threatened by mealybugs invasion . *Phenacoccus manihoti*, *Paracoccus marginatus*, and *Ferrisia virgata* are three mealybugs species attacked cassava crops. Mealybugs are kind of pest with wide range host such as physic nut, cashew nut, tomato, avocado, melon and papaya. Pesticides are the common mealybugs pest control taken by lots of farmers. But the use of pesticides at some defined concentrations will raise some side effect to the environment and to the mealybugs natural enemies as well. Considering that pesticides can cause a resistance, an environmental friendly pest control for long term is badly needed. One possible alternative is using their natural enemies, such as predators, parasitoid or pathogens[2]. This research conducted to discover mealybugs diversity on cassava crops (*Manihot esculenta* Crantz) because mealybugs is one of some factors that affected cassava production. This research is also conducted to discover any mealybugs natural enemies as the pest control for long term application.

Methods

This research has been taken for two months, from November to December 2014 at four different locations in Banyuwangi District. Those four locations are Secang, Kemiren, Sobo, and Glenmore. The methods using on this research were direct and sample observations. The procedures taken during this research are sample collection, rearing, and natural enemies' identification. Samples are collected by taking some infected leaves from some different plants. Rearing process was held for 7 to 14 days to trap mealybugs parasitoids.

Shannon-Wienner index[3] was used to discover species' diversity at each locations, with equation below :

$$H' = -\Sigma pi \ln pi \ , \ pi = \frac{ni}{N}$$

with :
H' = Shannon Wiener Index
ni = amount of pest observed
N = total amount of all pest caught

Diversity index categorized into three category. If H' < 1, the species diversity was on low category. If H'= 1 < H' < 3, the species diversity was on medium category. And if H' > 3, it means the species diversity was on high category.

Sorensen index of similarity[4] was used to count the species similarity between two defined locations.

$$IS = \frac{2c}{A+B} \times 100\%$$

with :
IS = Sorensen index of similarity
A = amount of mealybugs species at location1
B = amount of mealybugs species at location 2
C = amount of same mealybugs species at both locations.

If index value > 50%, it means species similarity between two locations compared is on the high category, and so otherwise, if index value < 50% means species similarity between two locations compared is on the low category. 3.

Result and discussion

The result achieved through this research is that only two mealybugs species found through this research. Those two species are *P. marginatus* and *F. virgata*. The amount of *P. marginatus* caught at Secang, Kemiren, Sobo, and Glenmore are 130; 250; 173; and 573. And the amount of *F. virgata* found at Secang, Kemiren, Sobo, and Glenmore are 26; 47; 61; dan 272. The biggest amount of *P. marginatus* and *F. virgata* was found at Glenmore. Diversity index at Secang, Kemiren, Sobo, and Glenmore are 1.80; 1.63; 1.89; 2.47.

Figure 1. *Ferrisia virgata*

Figure 2. *Paracoccus marginatus*

The highest mealybugs index of diversity was found at Glenmore with some reason. Glenmore has the highest population of mealybugs and cassava crops as the main host observed. Mealybugs population defined by some factors and host population is one of them. It explains why the highest mealybugs index of diversity was found at Glenmore, the location with the widest field of cassava crops. Other plants being surround main host also define mealybugs abundance, and affecting the species distribution pattern as well. The distribution pattern of mealybugs was determined by the values of z, $\bar{x}$, dan v. Value for z achieved by using equation z = ($\bar{x}$ - v)/ (std/√n). Z value determining the species distribution pattern at defined locations. If z value is -1.96 > z > 1.96, the distribution pattern is random one. If counted z is beyond that value and average point ($\bar{x}$) is larger than diversity value (v), it means the species distribution pattern is regular pattern. And if the average point is smaller than the diversity value, it means the species distribution pattern is the clustered one[5].

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F. virgata distribution patterns for all locations are clustered, except at Kemiren. Clustered distribution pattern happened when a species is already settled down at the defined location for long enough. *F. virgata* distribution pattern at three locations showing that this species was already invaded the locations for years. While why *F. virgata* distribution pattern at Kemiren is still on random pattern which happened when a species is just fill in the place is that because at Kemiren there are some other plants that possibly become alternative hosts for mealybugs attacked cassava crops. The plants are papaya, tomato, physic nut and chili pepper. These plants are placed close enough to the cassava crops, that some mealybugs migrations are possible to be happened between cassava crops and the surround plants or otherwise. It explains why the distribution pattern of *F. virgata* at Kemiren is random. *P. marginatus* distribution patterns are clustered for all locations except at Secang. The explanation for this case is also about the others plants around cassava crops field. Some plants found surrounding cassava crops field at Secang are silk tree, eggplants, chili pepper and papaya which planted randomly close enough to the cassava crops field as the main observation objects. Some plants surround cassava crops are suspected causing the *P. marginatus* distribution pattern at Secang is random one. Those plants are available to be another hosts for *P. marginatus* especially papaya plants which are the main host of *P. marginatus*. *P. marginatus* distribution pattern at other locations are clustered, which means that this pest is already settled down at those locations eventhough *P. marginatus* is a newly attacking pest. It showed that this pest invasion was quietly fast through these years.

The amount and diversity of mealybugs species was also affected by abiotic factors such as temperature, lights, and rainfall intensity beside biotic factors such host and food abundance, surround plants, and natural enemies being.

Index of similarity of all species found are in high category, with index value 100%. The mealybugs species found at four locations are all the same. The species similarity determined by host similarity and abiotic factors at the location observed.

Hemerobius spp is the only mealybugs predator found through this research. There is no mealybugs parasitoid found at four defined locations in Banyuwangi District.

Conclusion

There are two mealybugs species found at four different locations in Banyuwangi District. Those two species are *P. marginatus* and *F. virgata*. The diversity index of mealybugs are on medium category for all locations. *Hemerobius* spp. is the only mealybug predator found through this research and there is no mealybug parasitoid found.

Unknowledgment

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LOCAL WISDOM in THE MAKING of PLANTS BASED BIOPESTICIDES by ORGANIC RICE FARMERS in EAST JAVA

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Abstract

The use of botanical pesticides derived from plants has been developed as more safe and environmentally friendly pesticides. It is also one of the traditional method of controlling pests and diseases that have long been known by the rice farmers of Indonesia. This research aims to identify the making process of plants based biopesticides by organic rice farmers (composition and procedures) and the application method (method of administration, concentration, and time) against the pests that attack the organic rice. This research was a descriptive research by conducted surveys and interviews with ten of organic rice farmers in four districts of East Java, which covers Malang (Ds. Sumber Ngepoh Lawang and Kepanjen), Mojokerto (Ds. Kesemen Ngoro and Ds. Seloliman-Trawas), Jombang (Ds. Pojok Kulon Kesamben and Ds. Ngagri Megaluh) and Madiun (Ds Kaibon Geger). Data were analyzed by qualitative descriptive analysis. The results showed that each plant pests were controlled by different plants based biopesticides. The common method of making biopesticides was by soaking the plants that have been mashed in water, mixed with certain materials for fermentation process and incubated for a certain period. The method of administration was sprayed the biopesticides at the start of rice planting until the rice grains. The biopesticides were given once in two weeks and the concentration of which varies depending on the type of the pests.

Keywords: Local wisdom; organic rice; plants based biopesticides

Introduction

Agro-ecosystems are deemed productive if there is a balance between the soil, nutrients, humidity and organisms that provide good growing conditions and sustainable results. The balance can be achieved through the implementation of organic farming system. Organic farming is an agricultural activity without the use of synthetic fertilizers and pesticides also genetically modified seeds [1].

Eleven districts in East Java have implemented organic farming system, especially for rice. On organic rice farming, farmers faced with the problem of the availability of fertilizers, seeds and biopesticides. Pests are often attacked organic rice plants are borers, moths, rice bugs, leafhoppers and mice. Farmers have attempted to make botanical pesticide by using plants that exist in the environment such as betel, yam, and Mindi in order to cope with the pests.

The use of botanical pesticides or bioactive compounds derived from plants considered more safe and friendly for the environment and not affect non-target organisms. The nature of plant compounds are generally easy to degrade, therefore the residue does not caused a negative impact on the environment. The plants produce secondary metabolites such as phenolic compounds, alkaloids, terpenoids, and sulphur compounds as a defense mechanism against pests [2].

Various studies have been conducted to utilize secondary metabolites as biopesticides. For example, plants that can be utilized as a pesticide are *Tinospora tuberculata*, *Dioscorea hispida* Dennst, and *Pluchea indica* L. *Tinospora tuberculata* is known to contain a compound pikoretin, berberine, and palmatin, which included alkaloid compounds; pikroretosid and tinokrisposid which are a compound glycosides and triterpenoid compounds [3]. The sap of *Dioscorea hispida* Dennst is used to treat snake bites and the rest of the processing flour is used as an insecticide [4, 5]. Yam tubers toxicity caused by the dioscorin. *Pluchea indica* L. known to contain alkaloids, flavonoids, phenols hydroquinone, tannins, and essential oils that can be used as herbicides or insecticides [6].

The use of plants as materials for plant-based pesticides is the knowledge gained from life experience, indigenous knowledge, and local wisdom. Local wisdom began forgotten since the widespread use of synthetic pesticides. However, the side effects caused by synthetic pesticides, such as pest resistance, pest resurgence, secondary pest explosion and environmental pollution make a natural pesticide developed [7]. Based on this conditions, this research aimed to identify local wisdom in the making process of plants based biopesticides by organic rice farmers (composition

and procedures) and the application method (method of administration, concentration, and time) against the pests that attack the organic rice. The results of this study can be used as a basis to improve plant-based biopesticides for pest control in organic farming administration, concentration, and time. Data were analyzed by qualitative descriptive analysis.

Methods

This study is a descriptive research conducted with interview of organic rice farmers in four districts of East Java to identify the making process of plants based biopesticides and the application method against the pests that attack the organic rice. Data of the making process included included the composition and procedures. Meanwhile the application method included method of administration, concentration, and time. Data were analyzed by qualitative descriptive analysis.

Results and Discussion

The interviews with organic farmers in four districts of East Java, namely Malang, Mojokerto, Jombang and Madiun indicate that organic farmers have used a variety of methods to make plant based biopesticides. The farmers also using varies application method. The data were shown in Table 1. of plants based biopesticides and the application method against the pests that attack the organic rice. Data of the making process included the composition and procedures, meanwhile the

Table 1. Composition, Procedure, and Application of Plant Based Biopesticides

No	Area	Composition	Procedure	Application		
				Administration Methods	Concentration	Time
1	Dsn. Jampang, Ds. Kesemen, Kec. Ngoro, Kab. Mojokerto	<u>Leafhoppers and white moths</u> a. *Pangium edule* (leaves) 1 oz. b. *Azadirachta indica* (leaves) 1 oz. c. *Artocarpus heterophyllus* (rinds)1 kg d. *Derris elliptica* (roots) 1 oz. e. *Durio zibethinus* (rinds) 1 kg f. Chicken gizzards g. Water <u>Rice bugs</u> a. *Capsicum annum* L. (fruits) 1 kg b. *Dioscorea hispida* (tubers) 1 kg c. Water	<u>Leafhoppers and white moths</u> 1. Mashed all of the composition 2. Added 10 l of water 3. Fermented with fluid of chicken gizzards 4. Incubated in a tank at least for 10 days <u>Rice bugs</u> 1. Mashed all of the composition 2. Added 2 l of water, then squeezed 3. Diluted 500 ml of juice with 12 liter of water	Sprayed	50 l/ha <u>Leafhoppers and white moths</u> 500 ml of fermented water/12 l of water <u>Rice bugs</u> 500 ml of fermented water/12 l of water	▪ Given only when pests attack ▪ Once in a week when pest attack
2	Dsn. Pojok Kulon, Kec. Kesamben,	a. *Azadirachta indica* (leaves) ½ kg b. *Dioscorea hispida* (tubers) 1 kg	1. Mashed all of the composition 2. Added leaves of *Piper betle* L. (½ kg/50 l)	Sprayed and flushed	10 l/ha	At the start of rice planting

No	Area	Composition	Procedure	Application		
				Administration Methods	Concentration	Time
	Kab. Jombang	c. *Nicotiana tabacum* 1-2 oz. d. *Piper betle* L. (leaves) ½ kg	3. Soaked with hot water 4. Fermented for two weeks			until the rice grains
3	Dsn. Sono, Ds. Ngogri, Kec. Megaluh, Kab. Jombang	a. *Melia azedarach* (leaves) b. *Dioscorea hispida* (leaves) c. *Annona muricata* L.(leaves) d. *Nicotiana tabacum* (leaves) e. *Aegle marmelos* L. (fruits)	1. Mashed all of the composition 2. Fermented for two weeks	Sprayed	5 l/ha	At the start of rice planting until the rice grains, once in two weeks
4	Ds. Sumber Ngepoh, Kec. Lawang, Kab. Malang	<u>Stem borers</u> a. *Zea mays* (seeds) b. *Allium sativum* L. (tubers) c. *Kaempferia galanga* (tubers) d. *Citrus auranticum* (leaves) e. *Capsicum annum* L. (fruits) f. Shrimp paste <u>Mice</u> a. *Dioscorea hispida* (tubers) 1 kg b. *Aleurites moluccana* 10 grains c. Salted fish 1 oz. <u>Rice bugs</u> a. *Annona muricata* L. (leaves) ½ oz. b. *Acorus calamus* 1 oz.	<u>Stem borers</u> Mixed and mashed all of the composition <u>Mice</u> 1. Mashed all of the composition 2. Mixed with 3-5 kg of bran 3. Formed and dried (prepared as pellets) <u>Rice bugs</u> 1. Mixed all of the composition 2. Added 5 l of water 3. Incubated for two weeks 4. Added 1 sack of *Nicotiana tabacum* dregs if needed	<u>Stem borers</u> Sprinkled to bring the red ants that eat caterpillars <u>Mice</u> Inserted into the mice nest <u>Rice bugs</u> Sprayed	<u>Stem borers</u> 15 kg / ¼ ha	<u>Mice</u> Given at 25-30 days after planting <u>Rice bugs</u> Once in 3-5 days

No	Area	Composition	Procedure	Application		
				Administration Methods	Concentration	Time
		c. *Allium sativum* L. 8 tubers d. Water e. *Nicotiana tabacum* (dregs) 1 sack				
5	Dsn. Biting, Ds. Seloliman, Kec. Trawas, Kab. Mojokerto	Rice bugs a. *Melia azedarach* (leaves) 1 oz. b. *Sapindus rarak* 1 oz. c. *Dioscorea hispida* (tubers) 1oz. d. *Moringa oleifera* (leaves) 1 oz. e. Water *Fusarium* a. *Allium sativum* L. 1 oz. b. *Alpinia galanga* 1 oz. c. *Curcuma aeruginosa* 1 oz. d. Water Caterpillar a. Calcium b. Shrimp paste c. *Tithonia diversifolia* d. *Artocarpus heterophyllus* (rinds)	Rice bugs 1. Mashed all of the composition 2. Diluted with water 3. Incubated in an aerator for one week *Fusarium* 1. Mashed all of the composition 2. Diluted with water, then squeezed Caterpillar Mixed all of the composition and incubated for at least one month	Sprayed	Rice bugs 250 ml of biopesticide to be diluted with 12 l of water (50 l/ha) *Fusarium* 250 ml of biopesticide to be diluted with 12 l of water (50 l/ha)	Once in a week when pest attack
6	Ds. Kaibon, Kec. Geger, Kab. Madiun	Rice bugs a. *Musa paradisiaca* L. (leaves) 2 kg b. *Tinospora crispa* L. (leaves) 0.5 kg c. *Nicotiana tabacum* 5 leaves d. Water	Rice bugs 1. Mashed all of the composition 2. Boiled in 4 l of water 3. Left until the boiling water only 2 l, then filtered	Sprayed	Rice bugs 100 ml of biopesticide to be diluted with 14 l of water (per ha) Stem borers 5 ml / 15 l of water	At the start of rice planting until the rice grains, once in

No	Area	Composition	Procedure	Application		
				Administration Methods	Concentration	Time
		Stem borers a. *Nicotiana tabacum* 1 stem b. Camphor 1 oz. c. Water Armyworms a. *Areca catechu* Linn. (fruits) 2 kg b. *Dioscorea hispida* (tubers) 1 kg c. *Acorus calamus* 0.5 kg d. Water	Stem borers 1. Soaked the stem of *Nicotiana tabacum* in 5 l of warm water 2. Mixed with mashed camphor 3. Homogenized, then filtered Armyworms 1. Mashed the fruits of *Areca catechu* Linn. and *Acorus calamus* 2. Added 1 kg of grated *Dioscorea hispida* tubers and 5 l of water, then filtered		Armyworms 100 ml of biopesticide to be diluted with 15 l of water	3-4 days

Data in Table 1 shows each districts has a different composition of biopesticide for handling pests on organic rice farming. Each pest is also dealt with different materials. The procedures of biopesticides making also vary, but in general, any material mashed then soaked in water and incubated for a certain time. Some area also conducted the fermentation process by adding materials, such as fluid from chicken gizzards.

Most of biopesticides made by organic rice farmers administered through the spraying method. Concentration and time to apply biopesticides were varies.

Dioscorea hispida is a plant species found in each area as the biopesticide composition. This plant has been known for its function as a pesticide. These plants produce secondary metabolites called dioscorin. Dioscorin are toxic and can suppress the growth of pest populations through antifeedant activity [7].

The plants contain secondary metabolites such as tannins, alkaloids, flavonoids, saponins, and phenols which are usually produced by plants for defense against pest. These compounds have a mechanism that can inhibit the metabolism of target organisms [8]. Alkaloid and flavonoids are inhibiting insects eating activity, and also toxic [9]. Saponins can decrease the activity of digestive enzymes and food absorption. The secondary metabolites will caused the death at an early age, the relatively short life span, adulthood body size shrinks, the growth rate declined, the morphology of insects become abnormal and the incidence of anxiety and other abnormal behavior [10].

Conclusion

The common method of making biopesticides was by soaking the plants that have been mashed in water, mixed with certain materials for fermentation process and incubated for a certain period. The method of administration was sprayed the biopesticides at the start of rice planting until the rice grains. The biopesticides were given once in two weeks and the concentration of which varies depending on the type of the pests.

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GC-MS ANALYSIS of PHYTOCOMPONENTS in THE METHANOLIC EXTRACT of *Justicia gendarussa* Burm. f

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Abstract

Justicia gendarussa Burm f. belongs to the family *Acanthaceae*. The objective of the study was to identify the present in the methanolic extract of *Justicia gedarussa* Burm f. by GC-MS analysis. Twenty three compounds were identified. The major constituents are 2- ethylidene-1-methyl-3-phenylimidazolidine, 9,12-Octadecadienoic acid (Z,Z), and 5,6,8,9- tetramethoxy-2-methylpepero (3,4,5-jk)-9,10-dihydrophenanthracene.

Keywords: GC-MS, *Justicia gandarussa Burm. f.*, *Acanthaceae*, Phytocomponents

Introduction

Justicia gadarussa Burm.f. belongs to *Acanthaceae.*and common in forests of West Paapua (Indonesia). Traditionally, the extract of this plant is consumed by the West Papua community a male contrasetive [1]. In Ayurveda, the plant is useful for treatment of inflamation, bronchitis, vaginal discharges, eye diseases, dyspepsia, and fever. The decoction of the leaves and tender shoots are diaphoretic and they are given in chronic rheumatism. Oil prepared from the leaves is useful in eczema and the mixture of leaves is given internally for hemiplegia, cephalalgia, facial paralysis [2]. In Indonesia, the plant is clinically using for male contrasetive drug [3]. Gandarussa is from South Asia and South East Asia. Gandarusa is erect undershrub, 0.6-1.2m in height with subterete branches. This plant grows wildly in the forest, river embankment, curbs and shrubs, ranging from lowland to the altitude of 1,500 m asl. This crop is planted as a living fence and preserved as a medicinal plant. In Java, willow grows at an altitude of 1-500 m asl [4].

Plants of different habitats will produce different profiles of secondary metabolites as well. *Piper crocatum* leaf extract, acollection of nursery Palembang contain essential oils such as sesquisabinene hydrate (22.83%), β-bisabolo (17,24%), γ=curcumene (11.16%), anymol (3.9%), and trans-caryophyllane (2.37%) [5]. While GC-MS analysis of P. crocatum leave extract from Magelang Cenral Java contains 16 component. The main component consist of sabinen (44.91%) and β=mirsen (18.8%) [6].

The present work was carried out to identify some of the phytocomponents present in the methanolic extract of the leave of *J. gadarussa* Burm.f. by GC-MS technique, to ascertain the medicinal properties of the plant

Material and Method

Collection of the plant material

Justicia gendarussa Burm.f. plant was collected from the Taman Husada Graha Family, Surabaya, Indonesia. The leave of Justicia gendarussa Burm.f. was identified and authenticated at the Biology Department, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia.

Preparation of the extract

Justicia gendarussa Burm.f. leaf was shade dried, crushed by hand and ground into coarse powder using a mortar. Twenty grams of the powdered plant material was subjected to three methanol washes followed by filtration of the combined filtrate through the filter paper and evaporated to give a final yield of 7% extract. The extract was subjected to GC-MS analysis.

GC-MS analysis

The GC-MS analysis of the sample was performed using a Agilent 6980N Network GC System with autosampler, Detector Agilent 5973 inert MSD, fitted with J&W Scientific, HP-5 5% fenilmetil-siloksan capillary column (30m X0.32 mm, with 0,2um film thickness). The oven temperature was programmed from 50^0C to 280^0 C at 100^0C/min and a hold for 10 min. Helium was used as carrier gas at flow 1.3 mL/min. The injector temperature was 280 ^{0}C, injection size 1 uL neat, with split ratio 1:10. The interface and MS ion source were maintained at 230^0C and 150^0C, respectively, the mass spectra were taken at 70eV with a mass scan range of 200-700 amu. Data handling was done using GCMS solution software. The identification of compounds was based on comparison of their mass spectra with those of WILEY version 8.0 Libraries

Result and Discussion

The GC-MS chromatogram of *Justicia genda-russa* Burm.f. methanolic extract showed 23 peaks (Figure 1) and have been identified after comparison of the mass spectra with WILEY version 8.0 (Table 1), indicating the presence of 23 phytocomponents. From the results, it was observed that 2-ethylidene-1-methyl-3-phenylimidazolidine (14.351%), 9,12-Octadecadienoic acid (Z,Z) (13.731) and 5,6,8,9-tetramethoxy-2-methylpepero (3,4,5-jk)-9,10-dihydrophenanthracene (11.440) were the major components in the extract. The methanolic extract of *Justicia wynaadensis from* Irpu Hills, Western Ghats, Karnataka by GC-MS analysis to ascertain it's usage by the local community as a plant possessing medicinal properties. Twenty four compounds were identified. The major constituents are Dihydrocoumarin, Phytol and Palmitic acid. Significant quantities of Linoleic acid, Stearic acid, Squalene and phytosterols such as Campesterol and Stigmasterol were also present [7].

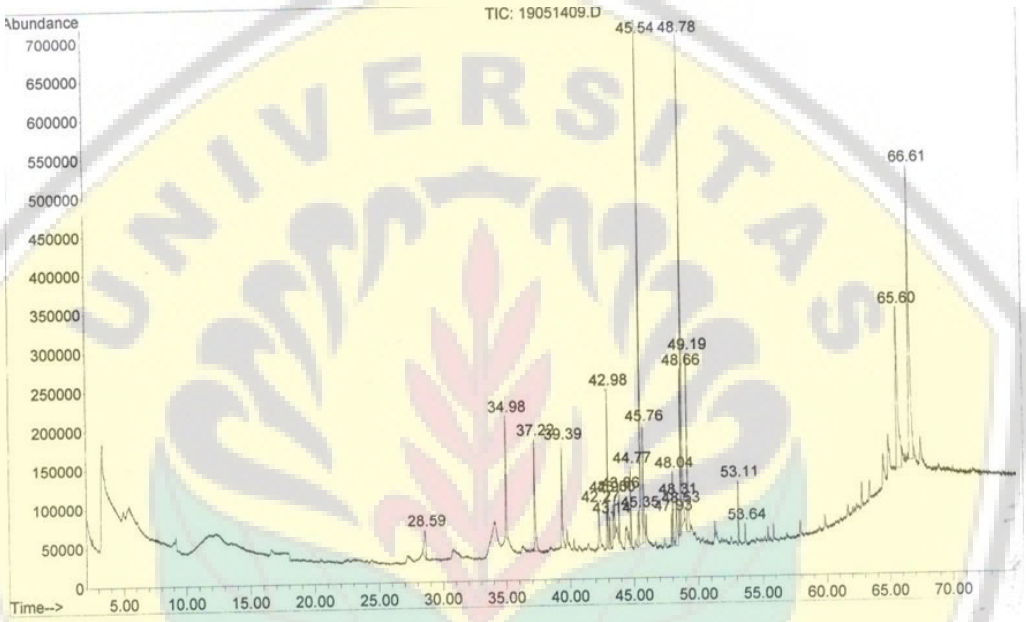

Figure 1 *GC-MS chromatogram of the methanolic extract of the aerial parts of Justicia gendarussa* Burm.f.

From the results, it was observed that 2-ethylidene-1-methyl-3-phenylimidazolidine (14.351%), 9,12-Octadecadienoic acid (Z,Z) (13.731) and 5,6,8,9-tetramethoxy-2-methylpepero (3,4,5-jk)-9,10-dihydrophenanthracene (11.440) were the major components in the extract. The methanolic extract of *Justicia wynaadensis from* Irpu Hills, Western Ghats, Karnataka by GC-MS analysis to ascertain it's usage by the local community as a plant possessing medicinal properties. Twenty four compounds were identified. The major constituents are Dihydrocoumarin, Phytol and Palmitic acid. Significant quantities of Linoleic acid, Stearic acid, Squalene and phytosterols such as Campesterol and Stigmasterol were also present [7]. The quantitative estimation of phyto constituents of leaves of *Justicia gendarussa* were collected from Anand farm and nursery Gandhinagar, Gujarat contain carotenoids (7.88 ±0.394 %), Alkaloids (1.62 ±0.081 3%), Phenolics (2.21 ±0.11 %), Flavonoids (2.03 ±0.105 %), Triterpenic acids (0.199 ±0.009 %), Sugar (8.74 ±0.435%) and Starch (5.85 ±0.292%) [8]. *Justicia gendarussa* was collected from Botanical garden, Forest Research Institute, New Forest, Dehradun, contains β-sitosterol, b-Sitosterol-b-D-glycoside and aromadendrin [9]. Samples of *Justicia gendarussa* were collected from Kishoregonj, Bangladesh contains three compounds, stigmasterol, lupeol, 16-hydroxylupeol [10].

Table 1. Phytocomponents identified in the methanolic extracts of *Justicia gendarussa* Burm.f. leaf by GC-MS

No	Name of Compund	Retention Time (min)	Average %age of Compounds in Leaf
1	Dodecamethylcyclohexasiloxane	28.587	1.672
2	Cycloheptasiloxane Tetradecamethyl-	34.983	3.647
3	4-oxo-4H-pyrido [1,2-a] pyrimidine-3-carbonitrile	37.222	4.008
4	Cycloheptasiloxane, hexadecamethyl-	39.385	2.968
5	5,7-Indolinedicarboxaldehyde, 1-methyl-	42.274	1.717
6	4-(3,4-Dimethoxybenzylidene)-1-(4-nitrophenyl)-3-phenyl-2-pyrazolin-5-one	42.900	1.254
7	Neophytadiene	42.984	3.152
8	2-Hexadecene, 3, 7, 11, 15-tetramethyl-, [R- [R@, R@-(E)]]-	43.137	0.710
9	Ethyl 5,6,7,8-tetrahydroquinoline-3-carboxylate	43.504	0.991
10	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	43.856	1.032
11	Hexadecanoic acid, methyl ester (CAS)	44.765	1.640
12	2-ethylidene-1-methyl-3-phenylimidazolidine	45.537	14.351
13	Hexadecanoic acid (CAS)	45.759	3.245
14	Phosphorochloridothioic acid, O,O-diethyl ester	47.929	0.667
15	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	48.043	1.603
16	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	48.311	2.273
17	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS)	48.533	0.563
18	Heptadecanoic acid, 16-methyl-, methyl ester	48.662	4.283
19	9,12-Octadecadienoic acid (Z,Z)-	48.785	13.731
20	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	49.190	4.759
21	Octadecanoic acid	53.110	1.358
22	Hexanedioic acid, bis(2-ethylhexyl) ester	53.637	0.446
23	5,6,8,9-tetramethoxy-2-methylpepero (3,4,5-jk)-9,10-dihydrophenanthracene	65.605	11.440

Conclusion

The methanol extract of gandarussa leaf contains 23 compounds and the major components in the extract are 2-ethylidene-1-methyl-3-phenylimidazolidine (14.351%), 9,12-Octadecadienoic acid (Z,Z) (13.731) and 5,6,8,9-tetramethoxy-2-methylpepero (3,4,5-jk)-9,10-dihydrophenanthracene (11.440).

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THE USE of THE LOCAL FLORA as BIOPESTICIDES by ORGANIC RICE FARMERS in EAST JAVA

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Abstract

Indonesia has a high diversity of local flora that can be used as environmentally friendly biopesticide. This study aims to identify the local flora used by organic rice farmers as biopesticides, and identify the pests that attack crops of organic rice. This study was a descriptive research by conducted surveys and interviews with ten of organic rice farmers in East Java, which covers four districts of East Java, there were Malang (Ds. Sumber Ngepoh Lawang and Kepanjen), Mojokerto (Ds. Kesemen Ngoro and Ds. Seloliman-Trawas), Jombang (Ds. Pojok Kulon Kesamben and Ds. Ngagri Megaluh) and Madiun (Ds Kaibon Geger). Data were analyzed by qualitative descriptive analysis. The results showed that the most common pests in organic rice farming were borer (sundep), moth, walang sangit, leafhoppers and mice. While the local flora used as biopesticides by organic farmers were the members of Annonaceae, Araceae, Liliaceae, Solanaceae, Dioscoreaceae, Euphorbiaceae, Gramineae, Rutaceae, Myrtaceae, Asteraceae, Palmae, Papillionaceae, Piperaceae, Meliaceae, Achariaceae, Moraceae, Fabaceae, Bombaceae, Sapindaceae, Moringa, Menispermaceae, and Musaceae. The results also showed that each pest was coped with different plant species.

Keywords: Local flora, biopesticides, organic rice

Introduction

Agricultural extensification and intensification have change ecosystems, as indicated by the decrease of the biodiversity of fauna and flora that also simplifies the ecosystems. Agriculture is deemed efficient if it can deliver a highest production with maximum profit level, but it resulted in a decrease in the potential of the land and the environment (abiotic and biotic) exceed the ability of ecosystems to recover [1]. During this time, the use of synthetic pesticides is increasing because in some circumstances it consider as an effective and efficient way to control pest and disease. Nevertheless, synthetic pesticides can cause side effects of environmental pollution by residue / residual pesticide active compounds that are toxic in the soil, and it also affects non-target organisms [2].

In accordance with the strategy of the management of agro-ecosystems and people's desire to back to nature, the organic farming is further developed. Organic farming is universally understood as farming without using synthetic fertilizers, herbicides and pesticides. Instead, farmers rotating the types of crops, using green manure, compost, and biopesticide, which means also that organic farming, avoid the pollution of soil, water and air.

The main purpose of organic farming is to provide agricultural products that is safe for the producers and consumers and does not cause the damage of the environment. Indonesia has a huge potential to compete in the international market. This is because various comparative advantages include: 1) there are a lot of land resources which can be opened to develop organic farming systems, 2) the technology to support organic farming is available such as composting, planting without tillage, biological pesticides and others.

The preliminary studies showed that organic farming has been developed in East Java includes eleven districts. The market demand for organic rice is quite high but like other organic farming, the main obstacle to improving productivity and quality of plants is the presence of pests and diseases. Interviews with organic farmers in the area of Malang, Mojokerto, Jombang and Madiun obtain information that pests that often attack the organic rice plants are borer, moth, rice bug and *Sclerotium*. To overcome the pest, the farmers have attempted to make biological pesticide using plants that exist in the environment, even though it is still not optimal to resist pests so that there is a shortage of production 10-20% per hectare.

The use of botanical pesticides or bioactive compounds derived from plants has been developed as a safer and friendlier method to the environment and not affects non-target organisms. The use of botanical pesticide is one of the traditional method of controlling pests and diseases that have long been known by the people of Indonesia. The method is the heritage that comes from life experience, knowledge of native (indigenous knowledge) and local knowledge (local wisdom).
Besides producing the primary compound in metabolism, plants also produces secondary

metabolites such as phenolic compounds, alkaloids, terpenoids, and sulfur compounds [3]. In the last 30 years, approximately 850-1500 species of plants have been reported active against insects, or adversely affect plant pests. Various parts of the plant can be used as a biopesticide such as leaves, stems, flowers, seeds, and fruit [4]. Secondary metabolites such as tannins, alkaloids, flavonoids, saponins, phenols are produced by plants and used for defense against insects, because these compounds have a mechanism that can inhibit the metabolism of insects [5]. The effects of secondary metabolites that act as insecticides are the death at an early age, the growth rate declined, shrinking body size, the relatively short life span, the abnormal morphology and the incidence of anxiety and other abnormal behavior of the insects.

Based on these conditions, this study aimed to identify target organisms / pests that attack organic rice and identify the various flora in the environment surrounding organic farming that being utilized by organic rice farmers in East Java as biopesticides. By knowing the different types of local flora for biopesticides it is expected to further development by utilizing owned plant secondary metabolites as a biopesticides.

Methods

This study is a descriptive research conducted with surveys of organic rice farmers in East Java to identify species of local flora used as a biopesticide. The procedures were a) define the areas in East Java which will be used as a research location. Four of the 11 district selected includes 7 sub-districts were used as a source of research data. Selection is based on the area that has the potential for organic farming (area and commitment of organic farmers to agricultural development), b) made the instrument to interview the organic farmers, c) conducting interviews with 10 organic rice farmers, d) identify the species of local flora used as biopesticides, e) identify the target pest and the flora that is used to control it. Data were analyzed descriptively qualitative.

Results and Discussion

The survey results and interviews with organic farmers in 7 districts and four regions of East Java, namely Malang (Ds. Sumber Ngepoh Lawang and Kepanjen), Mojokerto (Ds. Kesemen Ngoro and Ds. Seloliman-Trawas), Jombang (Ds. Pojok Kulon Kesamben and Ds. Ngagri Megaluh) and Madiun (Ds. Kaibon Geger) indicate that organic farmers have used a variety of plants in the surrounding environment as biopesticide. The data of the local flora is shown in Table 1 and the data of pests / target organisms in Table 2.

Table 1. Data of local flora used as biopesticide by organic rice farmers in East Java

No	Area	Botanical Biopesticide			Compounds
		Local name	Scientific Name	Family	
1	Dsn. Jampang, Ds. Kesemen, Kec. Ngoro, Kab. Mojokerto	a. Kluwek (leaves) b. Nangka (rinds) c. Mimba (leaves) d. Tuba (roots) e. Durian (rinds) f. Cabe (fruits) g. Gadung (tubers)	a. *Pangium edule* b. *Artocarpus heterophyllus* c. *Azadirachta indica* d. *Derris elliptica* e. *Durio zibethinus* f. *Capsicum annum* L. g. *Dioscorea hispida*	a. Achariaceae b. Moraceae c. Meliaceae d. Fabaceae e. Bombaceae f. Solanaceae g. Dioscoreace	a. Palmitic acids, oleic acids and linoleic acids b. Morin, cyanomaklurn (tanning substances), flavon and tannin c. Azadirachtin, meliantriol, and salannin d. Rotenone e. Essential oil, flavonoid, saponin, cellulose, lignin and starch f. Capsaicin, capsanthin, carotenoid, alkaloid g. Dioscorin (alkaloid)

No	Area	Botanical Biopesticide			Compounds
		Local name	Scientific Name	Family	
	Dsn. Pojok Kulon, Kec. Kesamben, Kab. Jombang	a. Mimba (leaves) b. Gadung (tubers) c. Tembakau (dregs) d. Sirih (leaves)	a. *Azadirachta indica* b. *Dioscorea hispida* c. *Nicotiana tabacum* d. *Piper betle* L.	a. Meliaceae b. Dioscoraceae c. Solanaceae d. Piperraceae	a. Azadirachtin, meliantriol, and salannin b. Dioscorin (alkaloid) c. Nicotine d. Essential oil, sesquiterpen, starch, and phenol
	Dsn. Sono, Ds. Ngogri, Kec. Megaluh, Kab. Jombang	a. Mindi (leaves) b. Gadung (leaves) c. Sirsak (leaves) d. Maja (buah) e. Tembakau (leaves)	a. *Melia azedarach* b. *Dioscorea hispida* c. *Annona mucirata* L. d. *Aegle marmelos* L. e. *Nicotiana tabacum*	a. Meliaceae b. Dioscoraceae c. Annonaceae d. Rutaceae e. Solanaceae	a. Azadirachtin, meliantriol b. Dioscorin (alkaloid) c. Tannin, annonain and alkaloid d. Saponin, tannin, flavonoid and poriphenol e. Nicotine
	Ds. Sumber Ngepoh, Kec. Lawang, Kab. Malang	a. Sirsak (leaves) b. Dringu c. Bawang putih (tubers) d. Tembakau e. Gadung (tubers) f. Kemiri (fruits) g. Jagung (seeds) h. Cabe merah (fruits) i. Kencur (tubers) j. Jeruk purut (leaves) k. Salam (leaves) l. Paitan (leaves) m. Kelapa (water) n. Kacang tanah (oilcake)	a. *Annona mucirata* L. b. *Acorus calamus* c. *Allium sativum* L. d. *Nicotiana tabacum* e. *Dioscorea hispida* f. *Aleurites moluccana* g. *Zea mays* h. *Capsicum annum* i. *Kaempferia 164aradisi* j. *Citrus auranticum* k. *Syzygium polyanthum* l. *Tithonia diversifolia* m. *Cocos nucifera* n. *Arachis hypogea*	a. Annonaceae b. Araceae c. Liliaceae d. Solanaceae e. Dioscoreaceae f. Euphorbiaceae g. Gramineae h. Solanaceae i. Rutaceae j. Rutaceae k. Myrtaceae l. Asreraceae m. Palmae n. Papillionaceae	a. Tanin, annonain and alkaloid b. Asarone, kolamenol, and kolamen c. Saponin, flavonoid, and essential oil, d. Nicotine e. Dioscorin (alkaloid) f. Saponin, flavonoid and poliphenol g. C-glycosylflavones h. Capsaicin, peroksidase i. Essential oil, cinnamal, aldehide, motil p-cumaric acids, annamat acids, ethyl acetate and pentadekan j. Citric acids k. Saponin, triterpenoid, flavonoid, poliphenol, alkaloid, tannin and

No	Area	Botanical Biopesticide			Compounds
		Local name	Scientific Name	Family	
					essential oil (sesquiterpen, lakton and phenol) l. Essential oil, treterpenoid, steroid, phenol, and alkaloid m. Poliphenol, sugar, vitamin, mineral, amino acids, and phytohormon n. Fatty acids, saponin, oleic and linoleic acids
Dsn. Biting, Ds. Seloliman, Kec. Trawas, Kab. Mojokerto	a. Mindi (leaves) b. Klerek (leaves) c. Gadung (tubers) d. Kelor (leaves) e. Tapak liman f. Babadotan	a. *Melia azedarach* b. *Sapindus rarak* c. *Dioscorea hispida* d. *Moringa oliefera* e. *Elephantopus scaber* f. *Ageratum conyzoides*	a. Meliaceae b. Sapindaceae c. Dioscoreaceae d. Moringaceae e. Asteraceae f. Asteraceae	a. Azadirachtin and meliantriol b. Saponin and flavonoid c. Dioscorin (alkaloid) d. Saponin e. Flavanoid, phenol, saponin, steroid, tannin and terpen f. Amino acids, essential oil, kumarin, *Ageratochromene*, friedelin, cytosterol, and stigmasterol
Ds. Kaibon, Kec. Geger, Kab. Madiun	a. Brotowali (leaves) b. Pisang (leaves) c. Tembakau (leaves) d. Pinang (fruits) e. Gadung (tubers) f. Dringu	a. *Tinospora crispa* L. b. *Musa 165aradisiacal* L. c. *Nicotinia tabacum* d. *Areca catechu* Linn. e. *Dioscorea hispida* f. *Acorus calamus*	a. Menispermace b. Musaceae c. Solanaceae d. Palmae e. Dioscoreaceae f. Araceae	a. Pikoretin, berberin, and palmatin (alkaloid) b. Water, nitrogen, and lipid c. Nicotine d. Arecoline (alkaloid, similar to nicotine) e. Dioscorin (alkaloid) f. Saponin, flavonoid, essential oil

Data in Table 2 indicates that the pest in organic rice farming that is often encountered is the stem borer, moth, rice bug, leafhoppers and mice. Data also shown that each type of plant pests coped with different specific biopesticide plants.To overcome the pests, the farmers makes botanical pesticide from plants that exist in the environment such as betel, yam, mindi, brotowali, dringu, nut, lerak, garlic, moringa and so on that are included in the family of Annonaceae, Aracea, Liliaceae, Solanaceae, Dioscoreaceae, Euphorbiaceae, Gramineae, Rutaceae, Myrtaceae, Asteraceae, Palmae, Papillionaceae, Piperaceae, Meliaceae, Achariaceae, Moraceae, Fabaceae, Bombaceae, Sapindaceae, Moringa, Menispermaceae, and Musaceae (Table 1). This result is similar with other studies of secondary metabolites of plants that show a variety of plants that have high potential to be developed as insecticides are a group of Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiate, Aristolochiaceae, Malvaceae, Zingiberaceae, and Solanaceae [6,7]. The plants contain secondary metabolites such as tannins, alkaloids, flavonoids, saponins, and phenols which are usually produced by plants as defense against insects. These compounds able to inhibit the metabolism of insects [5].

Terpenoids compounds can bind to molecules of protein and lipids that can affect the physiological function of cell membrane proteins and enzymes. Alkaloids and flavonoids are plant defense compounds that also an antifeedant for insects, and also toxic [8]. Saponins are glycosides which after hydrolysis will produce sugars (glikon) sapogenin (aglycone). Saponins together with other plant substances act as a defense against insects, because the saponin can decrease the activity of digestive enzymes and absorption of the food. There are some symptoms due to the compounds act as insecticides, such as the death at an early age, the growth rate declined, shrinking body size, the relatively short life span, the insects morphology become abnormal and the incidence of anxiety and other abnormal behavior [9].

Table 2. Biopesticides and the target organisms

No	Area	Plants	Target Organisms
1.	Malang Ds. Sumber Ngepoh Lawang	a. *Annona mucirata* L. (leaves) b. *Acorus calamus* c. *Allium sativum* L. (tubers) d. *Nicotiana tabacum*	Rice bugs
		a. *Dioscorea hispida* (tubers) b. *Aleurites moluccana* (fruits)	Mice
		a. *Zea mays* (seeds) b. *Capsicum annum* (fruits) c. *Allium sativum* L. (tubers) d. *Kaempferia galanga* (tubers) e. *Citrus auranticum* (leaves)	Stem borers
2.	Malang, Kepanjen	a. *Piper betle* L.(leaves) b. *Tithonia diversifolia* (leaves)	Stem borers
		a. *Dioscorea hispida* (tubers) b. *Melia azedarach* (leaves)	Armyworms
3.	Mojokerto, Kecamatan Ngoro	a. *Pangium edule* (leaves) b. *Artocarpus heterophyllus* (rinds) c. *Azadirachta indica* (leaves) d. *Derris elliptica* (roots) e. *Durio zibethinus* (rinds)	Leafhoppers and moths
		a. *Capsicum annum* L. (fruits) b. *Dioscorea hispida* (tubers)	Rice bugs
4	Mojokerto, Kecamatan Trawas	a. *Melia azedarach* (leaves) b. *Sapindus rarak* (leaves) c. *Dioscorea hispida* (tubers) d. *Moringa oliefera* (leaves)	Rice bugs
5.	Madiun, Ds Kaibon Kecamatan Geger	a. *Tinospora crispa* L. (leaves) b. *Musa paradisiaca* L. (leaves) c. *Nicotinia tabacum* (leaves)	Rice bugs

No	Area	Plants	Target Organisms
		a. *Nicotinia tabacum* (leaves)	Stem borers
		a. *Areca catechu* Linn. (fruits) b. *Dioscorea hispida* (tubers) c. *Acorus calamus* (leaves)	Armyworms
6.	Jombang Ds.Pojok Kulon Kesamben	a. *Azadirachta indica* (leaves) b. *Dioscorea hispida* (tubers) c. *Nicotinia tabacum* (dregs) d. *Piper betle* L.(leaves)	Rice bugs
7	Jombang Ds sono, Ngagri Megaluh	a. *Melia azedarach* (leaves) b. *Dioscorea hispida* (leaves) c. *Annona mucirata* L.(leaves) d. *Aegle marmelos* L. (fruits) e. *Nicotinia tabacum* (leaves)	Leafhoppers

Penetration of chemicals into the body of the insect through epicuticle cause damage to the waxy substances on the cuticle layer, so the insect is losing water and causing death [9]. Phenol compounds can also cause plasmolysis because it can precipitate cell membrane proteins and able to lower the surface tension of the membrane of the cell wall of larvae. The formation of pores in the cell walls facilitates the toxic compounds to entry into cells and interfere the metabolism of the larvae. Contact toxic effects on larvae cause the movement of the larvae became sluggish, shrinking body and eventually die. Another symptom is the decreased of the larvae feeding activity, this condition indicates that the larvae out of energy due to the formation of energy metabolism is inhibited due to the administration of the phenol compound, thereby disrupting the growth and the larvae eventually die.

The use of botanical pesticides on organic farming is expected to conserve natural resources and agricultural productivity in the long term, keeping the environmental impact to a minimum, optimum crop production with minimal chemical inputs, and providing commensurate economic benefits for farmers. By controlling pests permanently expected to help create a balanced agricultural ecosystem and sustainable agriculture.

Conclusion

The results showed that the pests in organic rice farming in the four districts of East Java that is often encountered is the stem borer, moth, rice bug, leafhoppers and mice. While the plants were used as biopesticide comes from the family Annonaceae, Araceae, Liliaceae, Solanaceae, Dioscoreaceae, Euphorbiaceae, Gramineae, Rutaceae, Myrtaceae, Asteraceae, Palmae, Papillionaceae, Piperaceae, Meliaceae, Achariaceae, Moraceae, Fabaceae, Bombaceae, Sapindaceae, Moringa, Menispermaceae and Musaceae.

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INFLUENCE of THE KIND of VERMICOMPOST MATERIAL and EARTHWORM *Pontoscolex corethrurus* POPULATION on THE YIELD and QUALITY of *PHAK-COI* MUSTARD (*Brassica rapa* L.) with ORGANIC POTTING MEDIA

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Abstract

Effects of three kinds of vermicompost materials and *Pontoscolex corethrurus* population on plant yield and quality of Phak-coi mustard were investigated in pot cultures organically. The experiment used a factorial complete randomized design which consisting of two factors. The first factor is the kind of vermicompost material which consists of three levels (the mixture of mushrooms media waste+cow manure+vegetable wastes (V1), mushrooms media waste+cow manure+leaf litter (V2), mushrooms media waste+cow manure+vegetable wastes+leaf litter (V3)). The second factor is the population of *P.corethrurus* consisted of five levels (0, 25, 50, 75, and 100 indiv.m^{-2}) and one control treatment (inorganic treatment). The results of this study showed that the application of various vermicompost had significantly ($p< 0.05$) higher total biomass and marketable weigth of Phak-coi mustard than the control treatment. The highest total biomass (160.7 g plant^{-1}) and marketable weight (155.3 g plant^{-1})were found on the vermicompost made from the V1 and V2 treatment with population of *P.corethrurus* by 75-100 indiv.m^{-2}. Vermicompost application significantly increased the quality of Phak-coi mustard when compared with the control treatment, with increase in the contents of sugar and vitamin C by 75% and 41%, respectively. The treatments using the vermicompost made from the V2 with population of *P.corethrurus* by 50-75 indiv.m^{-2} gave the highest quality. Vermicompost application significantly ($p<0.05$) also increased shelf life of Phak-coi mustard by average weight depreciation of 68,7 % for the treatment of 7 days of storage at room temperature and 17.6% for the treatment of 14 days of storage at cold temperature when compared with the inorganic treatment by 215.9 % and 36.7 %, respectively. The treatments using the vermicompost made from the v3 with population of *P.corethrurus* by 75-100 indiv.m^{-2} gave the best shelf life.

Keywords : Vermicompost, *P.corethrurus*, Phak-coi mustard, vitamin C, sugar content, shelf life.

Introduction

Conventional farm system have been characterized by high inputs of chemical fertilizer and pesticide. In the long term can lead to decreased soil quality and crop yields due to declining soil organic matter content [1; 2; 3]. In recent years, increasing consumer concern about issues such as food quality, environmental safety and soil conservation has lead to a substantial increase is in the use of organic agricultural practices. Organic farming system Involves using techniques to Achieve good crop yields without harming the natural environment or the people who live and work in it. Organic farming systems using organic soil amendment as a source of plant nutrients. It is an alternative agricultural practices for sustaining economically viable production with minimal environmental pollution [4]. Many research results have shown that organic farming practices build soil quality, maintain water quality, biodiversity support, and have potential to mitigate global climate change while supporting an economic bright spot [5].

Long-term beneficial effects of composted materials improves soil physical properties by decreasing bulk density and increasing the soil water holding capacity [6], increases in soil organic carbon and some plant nutrients [7; 8; 9]. In addition to the changes exerted on the chemical and physical properties, composted materials have a clear impact on soil biological properties, such as increases in microbial biomass and activity [10] as well as changes in the activity of soil enzymes [6; 11] and in the structure of the soil microbial community [11].

Among organic amendments, vermicomposts can have potential applications in horticulture. Because vermicomposting is a cheap process, which can process large amounts of organic wastes. Various waste can be used as a medium for the cultivation of earthworms epigeic such as *Lumbricus rubellus*. The different types of waste used will produce different quality vermicompost. During the vermicomposting process, the entire organic matter is decomposed, and its physical and chemical properties are changed with the degradable organic C being oxidized and stabilized

[12]. Many studies report that vermicompos is an excellent soil conditioner and can increase is the growth and yield of vegetables such as tomatoes [13], peppers [14] and Chinese cabbage [15], garlic [16], and strawberry [17]. However, the effect of vermicompost on the growth and yield highly variable. The variability may depend on the cultivation system into the which it is incorporated, as well as on the physical, chemical and biological characteristics of vermicompost, which vary widely depending on the original feedstock, the earthworm species used, the production process, and the age of vermicompost [18; 19; 20]. In order to accelerate the effect of application of vermicompost on the growth of plants, especially plants short-lived needs to be combined with inoculation of endogeic earthworms which plays an important role in the process of decomposition and mineralization of organic materials in addition to the role and function others are not owned by epigeic worm in vermicomposting.

Endogeic earthworms are important soil organisms that can affect important soil properties such as soil aggregate formation [21], water holding capacity, the activity of microflora by mixing plant litter and soil minerals [22]. They are important part in soil functioning by influencing decomposition processes, like fragmentation of organic litter [23] and stimulation of microbial activity [24], and by bioturbation, increasing soil porosity and water infiltration [25; 26]. [27] reported that the presence of worms endogeic *P.corethrurus* can increase the rate of N mineralization of organic materials of low quality and increase of nutrient availability [28]. Because of their effects on these properties, earthworms often affect plant growth [29;30]. Earthworm casts and burrow linings often have a greater microbial biomass and respiration rate compared to bulk soil [31]. Earthworms affect soil microbial activity and community by altering the physical and chemical environment, especially surrounding the channels created by the worm [32; 33; 34]. This study aims to determine the combination effect of the application of various vermicompost and population of endogeic earthworm P.corethrurus on the yield and quality of Phak-coi mustard grown in pots organically.

Materials and Methods

Study Site and Soil Characteristics

This study is a pot experiment conducted in a polyhouse at Tawangargo village, Karangploso district, Malang regency with latitude 07º56 S, longitude 112º36 E, altitude 1060 m above sea level and Inceptisol soil type. in March- June 2015 and the average temperature of 17º-22ºC. Soil samples were air dried and sieved to pass through a 2 mm sieve. The soil is well drained with the following characteristics; pH (H20) 5.3, 2.03 % organic C by Walkley and Black method; 0.47 % total Kjeldahl N; 131.17 mg/kg P (Bray II), 3.26 me/100 g K, cation exchange capacity 42.48 me/100 g soil, and 28 % sand, 56 % silt and 16 % clay.

Preparation of Vermicompost

Vermicompost used in the study is composed of mushrooms culture waste, vegetables residue, leaf litter, and cow manure. The making process of vermicompost is done in a container with the mushrooms culture waste as bedding in the bottom of vermicomposting container. Bedding is functioning to control temperature and humidity vermicomposting, additional food for earthworms and provide a suitable environment for the proliferation of earthworms. In the process of vermicomposting is needed bedding in the bottom of container thickness of 5 cm, and in the upper layer is as thick as 10 cm feed worm. Earthworms feed used there are 3 kinds: (V1) mixture of cow manure + vegetables residue, (V2) cow manure + leaf litter, and (V3) cow manure + leftover vegetables + leaf litter. The amount of feed needed depend on the abilities of earthworm decomposing the organic material. According [35], feed requirements of earthworm by 0.75 kg/kg of earthworms/day with worms to process vermicompsting density is 1.6 kg /m^2. Earthworms *Lumbricus rubellus* (0.32 kg of earthworms for 6.72 kg of organic matter per container with a surface area of 0.2 m2) were added and incubated for 28 days. The moisture of vermicompost was adjusted to 80%. Then, the vermicompost was dismantled and composted by adding egg shells flour and fish bone meal 5g / 100g of material. The composting process lasts for 14 days. Every 2 days, the compost was opened and stirred again so that the temperature remains stable.

Experiment Design

Three kinds of vermicompost has been created, added into the plastic pots as the first factor. Every pot was filled with mixture of soil and cow manure with the ratio of 4: 1. The vermicompost was applied by 200 g per 10 kg media. The second factor is population of earthworm *P.corethrurus* the which consisted of five levels: 0, 25, 50, 75, and 100 indiv.m^{-2}. The earthworms were inoculated into each pot corresponding surface area of the pot. Furthermore, the pots were covered with plastic covers. One plant was grown in each pot three days after inoculation earthworm. Fifteen Phak-coi mustard seedlings were grown in each pot and each treatment was replicated three times with three samples for each replication and one control treatment (inorganic treatment). Total pot used in this experiment was 144 pots. The pots were placed with complete randomized design in the polyhouse. Yield, vitamin C contents, sugar content and shelf

life test were determined 28 days after cultivation. Total nitrogen (N) was measured with the

Kjeldahl method and hydrolyzable N by the alkali distillation method. Available potassium (K) was extracted with 1.0-M NH4OAc (pH=7.0) and then determined by Flame photometer [36]. The available P was extracted with Bray II then the P content was determined by the colorimetric method. The pH values of the mixtures were determined with a soil:water ratio of 1:5(w:v) [37] after shaking for 2 hand filtration through Whatman No.1 Filter Paper. Lignin, cellulose, and ash content by Goering and Van Soest method and polyphenols content by Folin-Denis method [38]. The results of analysis were presented in Table 1.

Table 1. The chemical composition of three kind of vermicompost on dry weight basis

No.	Chemical properties	V1	V2	V3
1	C-organic (%)	17,07	15,44	16,48
2	Polyphenol (%)	0,49	0,47	0,45
3	Celulose (%)	37,21	35,34	34,00
4	Lignin (%)	17,74	17,55	18,66
5	Total N (%)	12,89	12,97	12,62
6	C:N ratio	26,30	24,78	25,87
7	P (%)	0,77	0,87	0,92
8	K (%)	0,59	0,49	0.42
9	pH	6,63	6,97	6,93
10.	Ash (%)	2,72	4,73	1.36

Determination of Plant Yield Quality

Vitamin C content was determined by iodometric titration method. Plant samples crushed with mortar. 30 g slurry was taken and put in a 100 ml volumetric flask. Distilled water is added until the volume reaches 100 ml, then filtered with filter paper. 20 ml filtrate was taken and placed in a 125 ml erlenmeyer flask then added 2 ml of 1% starch solution. The next stage is a standard titration with 0.01 N iodine solution that is made from KI and iodine until the solution blue. [39] states in 1 ml iodine used equivalent to 0.88 mg of vitamin C, so the calculation of the content of vitamin C can be done by multiplying the volume of iodine solution used in the process of titration with 0.88 mg. The content of dissolved solids (sugar) is determined with a hand held refractometer. Mustard crop yields for each treatment were taken a sample of 1 g, and then pulverized using a mortar to remove fluid. The liquid is then used for readings with a refractometer.

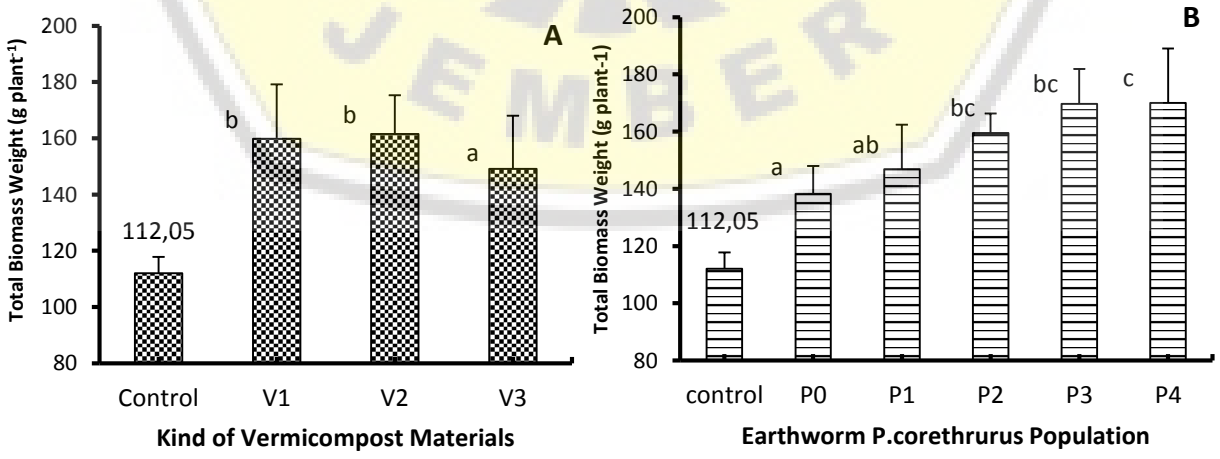
Statistical Analysis

The collected data was statistically analyzed using analysis of variance (F-Test) at level ($P \leq 0.05$) and differences in each treatment were adjudged by Tukey test ($P \leq 0.05$) using Minitab Version 14.12. Dunnett test at 5% level was used to compare all treatments with control. For statistical analysis of data (charts), Microsoft Excel was employed.

Results and Discussion

Effect of Various Vermicompost Material and Earthworm P.corethrurus population on the plant yield

The addition of vermicompost significantly increased the total biomass and marketable weight of Phak-coi mustard when compared with the inorganic treatment (control) and reaching the highest value in the V1 and V2 (Fig 1a and 1c). Inoculation of *P.corethrurus* also increase the total biomass and marketable weight compared to the control treatment (inorganic treatment) or without inoculation of *P.corethrurus* (P0). The population of *P.corethrurus* provided the highest yields are 75-100 indiv.m^{-2} (Fig 1b and 1d).



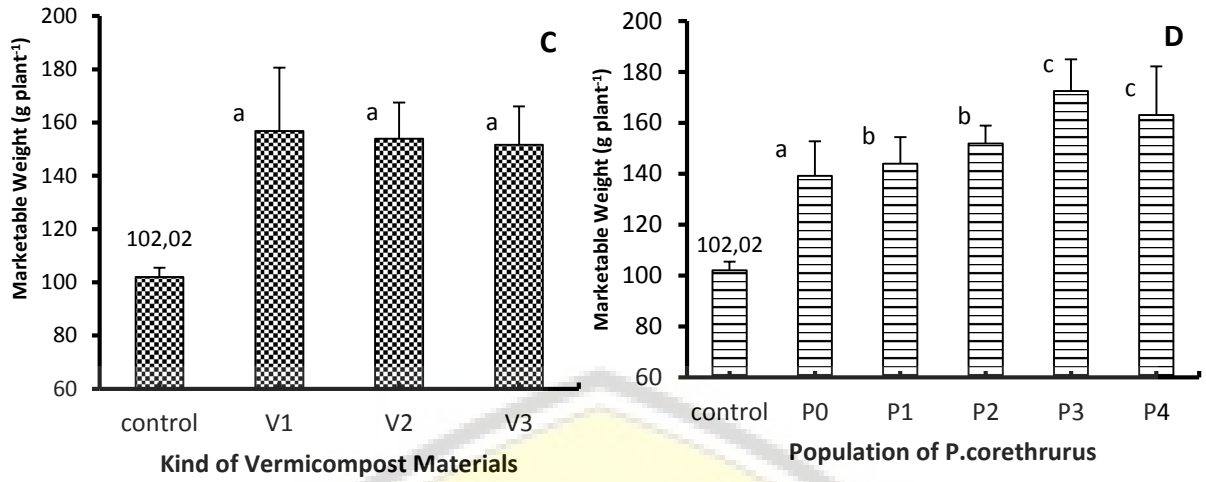


Fig. 1. Total Biomass (A,B) and marketable weight (C,D) of Phak-coi mustard plants harvest after 30 days; plants were subjected to different vermicompost materials and population earthworm *P.corethrurus*. Data are mean value ± SD of three replicates. Different letters showed significant difference at $p<0.05$.

Similar results were observed with earthworm-processed sheep manure on tomato yield [40]. The enhancement of the total biomass and marketable weight is the combined effect of the addition of vermicompost and earthworms *P.corethrurus*. [15] reported that the application of vermicompost had a directly effect on the plant because it contains plant growth regulators and humic acids. [41] indicated resources that humic acids isolated from earthworm compost enhanced root elongation, lateral root emergence, and H^+-ATP ase activity of the plasma membrane of maize roots. The presence of earthworms *P.corethrurus* can increase the nutrient mineralization rate in vermicompost which have an impact on increasing the availability of N, P and K. [27] reported that the presence of endogeic earthworms *P.corethrurus* can increase the rate of N mineralization of low quality organic matter and nutrient availability [28]. Endogeic earthworms *P.corethrurus* play an important role in improving soil structure because of his activities as burrowing [42]. Activities such as these provide a proper environment for root growth [43]. Vermicompost also has a positive effect on vegetative growth, stimulating shoot and root development [44]. This is because vermicompost plant constitutes a source of macro- and micronutrients. Although some of reviews these nutrients are present in inorganic forms and are readily available to plants, most are released gradually through the mineralization of the organic matter, thus constituting a slow-release fertilizer that supplies the plant with a gradual and constant source of nutrients [45]. However, in contrast to chemical fertilizers, the amount of nutrients-provided may vary greatly depending on the original feedstock, processing time and maturity of the vermicompost [46] Fertilization with vermicompost and manure also favored the growth of microorganisms in the soil. The bacterial growth significantly increased after application of the high doses of vermicompost and manure. Organic amendments promoted microbial growth, the which resulted in a higher soil microbial biomass [12]. Results of this study are also consistent with [17] and [47]. Both studies indicated resources a significant increase is in the growth and productivity of cultivated strawberries with 5 and 7.5 t ha^{-1} of vermicompost respectively, in comparison with strawberries cultivated with equivalent doses of mineral fertilizers. Furthermore, many studies have shown that increases in growth and yield often involve changes in plant development and or plant morphology such as increased leaf area, root volume and root branching [47; 48].

Effect of kind of vermicompost material and earthworm P.corethrurus population on the plant yield quality.

The kind of vermicompost material and earthworm P.corethrurus population had significantly effect on the quality of the marketable yield in the Phak-coi mustard plants. The vitamin C and sucrose content were higher in response to vermicompost and earthworm P.corethrurus population than in response inorganic fertilization (control) (Table 2.).The vermicompost treatments increased the contents of Vitamin C and sucrose content in Phak-coi mustard, especially in the V2 treatment combined with inoculation of earthworm P.corethrurus by 50-75 indiv m^{-2} (P1-P3), with increases of 113% and 48%, respectively, compared to the inorganic treatment (Table 2). [15] reported that the applications of vermicompost significantly increased the contents of vitamin C, phenols, and flavonoids.

Table 2. Vitamin C and Sugar contents of Phak-coi mustard plants harvested after 28 days and treated with different vermicompost material (V) and earthworm P.corethrurus population (P).

Treatment	Vitamin C Content (mg/100g)		Sugar Content (Brix)	
Control	19.83		4.33	
V1P0	29.61*	bc	5.00	A
V1P1	34.58*	cd	5.17	A
V1P2	38.21*	de	5.33	A
V1P3	42.01*	ef	6.00	Ab
V1P4	46.63*	fg	6.67	Bc
V2P0	27.94*	B	5.00	A
V2P1	46.22*	fg	5.00	A
V2P2	51.26*	G	7.33	C
V2P3	48.25*	fg	7.33	C
V2P4	38.07*	de	7.33	C
V3P0	20.89^{ns}	A	5.67	ab
V3P1	21.29^{ns}	A	5.67	ab
V3P2	21.24^{ns}	A	6.00	ab
V3P3	25.60*	ab	7.00	bc
V3P4	30.06*	bc	7.33	c
HSD 5 %	6.33		1.31	

*Means followed by different letters in the same column for each treatment combination are statistically significant different at Tukey- test, P=0.05 ; * sign : significant at Dunnet-test vs.control, P= 0.05 ; ns = non-significant*

[49] also observed that the vitamin C content of tomatoes was decreased by high NO_3 - levels, but it was increased in plants grown with chicken manure and grass-clover treatments. [50] found that organic fertilizer increased the levels of vitamin C in marionberry, strawberry, and corn. Phenolic compounds are a large group of plant secondary metabolites with different biological activities; for example, flavonoids show antioxidant activity [51]. [15] reported that the vitamin C of leaves of Chinese cabbage cultivated in plastic pots filled the Vermicompost:soil mixtures with ratios the 4:7 had 5.8-fold higher than that of full soil treatment. [52] reported that increasing the amount of nitrogen fertilizer from 80 to 120 kg ha^{-1} decreased the vitamin C content by 7 % in cauliflower. It means organic treatment can increase the yield quality of plant. [53] also reported that nitrogen fertilizers at high rates tend to decrease the vitamin C content in many fruits and vegetables.

It has been reported that applying N, P, K, and organic fertilizers can increase sugar content of plants [54; 55]. The timing and mode of mineral application, chemical form of the minerals applied, and tomato genotype affect the response to varying mineral concentrations on fruit total soluble solid such sucrose content [56; 57; 58; 59]. Vermicompost that used in this study had a complete nutrient content.

Table 3. Average weight depreciation of marketable yield compared with initial weight (g) at two storage

Cold temperature storage

Treatments	Weight depreciation compared with initial weight (g)													
	2 das		4 das		6 das		8 das		10 das		12 das		14 das	
control	1.96		2.94		5.57		7.53		14.16		20.99		36.73	
V1	0.66	a	1.52	A	2.41	a	3.79	A	5.38	a	8.48	a	13.69	A
V2	0.81	a	1.93	B	3.37	b	4.29	A	5.71	a	7.57	a	16.38	B
V3	1.30	b	2.47	C	4.20	c	5.64	B	8.09	b	10.95	b	22.78	C
HSD 5%	0.19		0.34		0.56		0.65		0.86		1.15		2.39	
P0	1.13	b	2.19	bc	3.57	b	4.91	ab	6.47	ab	9.35	bc	18.29	Abc
P1	1.03	ab	2.22	C	3.86	b	5.68	B	7.75	b	10.71	c	19.99	C
P2	1.03	ab	2.14	B	3.49	ab	4.44	A	6.55	ab	10.00	c	19.43	Bc
P3	0.71	a	1.55	A	2.92	a	3.99	A	6.04	a	7.72	ab	14.70	A
P4	0.71	a	1.77	ab	2.79	a	3.85	A	5.14	a	7.22	a	15.70	Ab
HSD 5%	0.32		0.58		0.96		1.12		1.48		1.98		4.12	

Room temperature storage

Treatments	Weight depreciation compared with initial weight (g)						
	1 das	2 das	3 das	4 das	5 das	6 das	7 das

Control	24.32		34.28		44.25		52.99		145.50		184.80		215.94	
V1	10.74	c	17.93	B	24.75	b	30.01	B	55.68	B	63.27	b	68.94	b
V2	9.10	b	17.86	B	25.21	B	32.06	C	59.44	B	70.36	c	78.29	b
V3	6.42	a	13.17	A	19.33	A	24.89	A	44.93	A	53.13	a	58.74	a
HSD 5%	1.09		1.14		1.42		1.73		6.17		8.08		9.72	
P0	10.52	b	21.23	C	30.29	C	37.78	C	78.93	C	94.45	c	106.70	c
P1	9.24	b	17.56	B	24.82	B	30.66	B	56.28	B	65.74	b	71.80	b
P2	9.79	b	16.93	B	23.69	B	29.80	B	55.65	B	64.57	b	70.72	b
P3	7.21	a	13.07	A	18.51	A	23.59	A	37.85	A	42.84	a	46.05	a
P4	7.02	a	12.81	A	18.19	A	23.11	A	38.05	A	43.66	a	48.03	a
HSD 5%	1.87		1.96		2.43		2.98		10.61		13.90		16.72	

Means followed by different letters in the same column for each treatment of P and V are statistically significant different at Tukey-test, P=0.05; das = day after storage.

The kind of vermicompost material and earthworm P.corethrurus population had also significantly effect on the shelf life of Phak-coi mustard plants that determined with the weight depreciation of marketable yield (Table 3). The treatment using the vermicompost had a smaller weight depreciation of marketable yield than inorganic treatment (control) either cold temperature or room temperature storage. The vermicompost made from the mixture of cow manure+vegetable residue and the mixture of cow manure+vegetable residue+leaf litter with population of P.corethrurus by 75-100 indiv.m-2 gave the smallest weight depreciation (Table 3). The shelf life of food leaves such as mustard greatly depends on storage conditions. The important parameters of microenvironment in the storage conditions are gas composition (oxygen,carbondioxide, inert gases, ethylene, etc), the relative humidity (% RH), pressure or mechanical stresses, light and temperature. Intrinsic factor of the food itself such as moisture and pH also affect the shelf life of a product. It may be possible to manipulate these factors to extend the shelf life of a food [53].

Conclusion

Application of vermicompost yang dikombinasikan dengan inokulasi cacing P.corethrurus dengan system penanaman pot secara organic significantly increased total biomass and marketable yield of Phak-choi mustard, extend the shelf life and increase the content of important nutrient metabolites such as Vitamin C and soluble sugar content compared with the plant grown in inorganic media. The effects of the vermicompost may not only depend on its chemical composition, but also on the population of P.corethrurus inoculated into the media. Vermicompost gave the highest total biomass (160.7 g plant^{-1}) and marketable weight (155.3 g plant^{-1}) were found on the vermicompost made from the mixture of cow manure + vegetables residue and the mixture of cow manure + leaf litter, with population of *P.corethrurus* by 75-100 indiv.m^{-2}. The treatments using the vermicompost made from the mixture of cow manure + leaf litter with population of *P.corethrurus* by 50-75 indiv.m^{-2} gave the highest quality.

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BAT SPECIES RICHNESS of ORDER CHIROPTERA in THE SANCTUARY of DUASUDARA MOUNTAIN, NORTH SULAWESI

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Abstract

This study was carried out to determine the species richness of bat in several vegetation zones of Duasudara Mountain Sanctuary, North Sulawesi. It was done using Mistnet method at 7 vegetations in the Santuary of Duasudara Mountain, moss forest, sub-montana forest, lowland forest, casuarine forest, coastal forest, shrub, and grass. This study was done from November 2013 to August 2014. Bat sampling used 12 x3.6 m bat net set at 1 m and 3 m above the ground. The bats were then identified in Laboratory of Ecology and Conservation, Brawijaya University and Biological Research Center – LIPI Bogor. Results showed that 16 species of 3 families were found. From 16 species identified in the 7 vegetation zones of Duasudara Mountain Sanctuary, there were 13 species previously reported from Sulawesi, *Acerodon celebensis, Boneia bidens, Cynopterus brachyotis, Cynopterus luzoniensis, Cynopterus minutus, Dobsonia viridis, Macroglossus minimus, Nyctimene cephalotes, Rousettus amplexicaudatus, Rousettus celebensis, Thoopterus nigrescens, Megaderma spasma* and *Rhinolopus celebensis.*

Keywords: bat, vegetation zone, Duasudara Mountain Sanctuary.

Introduction

Bats are mammals of order *Chiroptera* with two sub-orders, *Megachiroptera* and *Microchiroptera*, separated from food habits. Order *Chiroptera* possesses 18 clades, 188 genera and 977 species, distributed in the sub-orders. Food feeding-bats (Megachiroptera) have only one clade, *Pteropodidae*, covering 41 genera and 163 species, while insect feeding-bats (Microchiroptera) have 17 clades with 147 genera and 814 species [1]. Indonesian bats are estimated reaching 230 species or 21% of the world bat species, 77 species are grouped into suborderMegachiroptera and the other 153 species belong to suborder Microchiroptera [2]. Sulawesi Island possesses 127 species of mamals, 79 of which are endemic and the most typical in Indonesia [3].

Sulawesi Island has 54 sanctuary areas of 373 sanctuaries in Indonesia [4]. One of the important sanctuaries in Sulawesi is Natural Sanctuary of Duasudara Mountain. This area posesses approximately 8,867 haarea and covers 3 vulcanoes,Tangkoko Mt-1,109 m above sea level, Batuangus Mt.-450 m above sea level, and Duasudara Mt.-1,351 m above sea level.This sanctuary has also 7 vegetation zonations, moss forest, sub-montana forest, lowland forest, casuarine forest, coastal forest, shrub, and grass [5].

Bats are very important for human life because they work as fruit seed dispersers (water rose apple, guava, walnut, sapodila, andsandalwood) and flower pollinators of economic plants (stink bean, durian, mangrove, and kapok), insect controller, guano and phosphate mine producers in the caves, and ecoitourism objects [2]. Moreover, it was reported that the presence of 12 bat species in Sulawesi[2], *Acerodon celebensis, Boneia bidens, Cynopterus brachyotis, Cynopterus luzoniensis, Cynopterus minutus, Dobsonia viridis, Macroglossus minimus, Nyctimene cephalotes, Rousettus amplexicaudatus, Rousettus celebensis, Thoopterus nigrescens, Megaderma spasma*, and *Rhinolopus celebensis.* Nine species were found on 4 vegetations in September – November at 250 m, 500, 750 m, and 1,000 m above sea level, respectively, in the Sanctuary of Duasudara Mountain [6], *Acerodon celebensis, Boneia bidens, Cynopterus brachyotis, Dobsonia exoleta, Macroglossus minimus, Nyctimene cephalotes, Rousettus celebensis, Thoopterus nigrescens,* and *Myotis muricola*. This fact reflects that Duasudara Mountain Sanctuary has very high potential of biodiversity compared to other sanctuaries in Indonesia.

Nevertheless, the occurrence of bats in the sanctuary of Duasudara Mountain is increasingly threatened due to habitat loss and hunting [7], so that their conservational efforts are highly needed to prevent species extinction. This study was aimed to identify the species richness in the natural sanctuary of Duasudara Mountain, North Sulawesi.

Method

This study was conducted in November 2013 to August 2014 in 7 vegetation zones of Duasudara MountainSanctuary, North Sulawesi. Those

vegetations were moss forest, submontana forest, low land forest, casuarine forest, coastal forest, shrub and grass.As preservative, the study used 75% alcohol. Other equipment used were mistnet, calliper, balance (100 g, 600 g, and 1000 g), wingruler, sigmat, nylon line, plastic line, sample bag, head flashlight, camera, pencil, data sheet, and identification book.

Bat sampling was carried out using Mist-net[2,8,6]. Bat collection used 12 x 3.6 m net set at 1 m and 3 m above the ground. Species identification was done in the Laboratory of Ecology and Conservation, Brawijaya University and Biological Research Center – LIPI, Bogor,following Suyanto[2,9].

Results and Discussion

Bat species collected in the sanctuary of Duasudara Mountain, North Sulawesi, during the study belonged to 3 families with 16 species (Table 1).

Table 1. Bat species found in thesanctuary of Duasudara Mountain, North Sulawesi.

No	Suborder	Family		Species
1.	Megachir optera	Pteropod idae	1.	*Acerodon celebensis*
			2.	*Boneia bidens*
			3.	*Cynopterus brachyotis*
			4.	*Cynopterus luzoniensis*
			5.	*Cynopterus minutus*
			6.	*Dobsonia viridis*
			7.	*Macroglossus minimus*
			8.	*Macroglossus*
			9.	*tailiniensis* n. sp.
			10.	*Nyctimene cephalotes*
			11.	*Rousettus*
			12.	*amplexicaudatus*
			13.	*Rousettus celebensis*
			14.	*Rousettus tangkokoensis* n. sp.
				Thoopterus nigrescens
				Thoopterus tailiniensis n. Sp
2.	Microchir optera	Megader matidae	15.	*Megaderma spasma*
		Rhinolo pidae	16.	*Rhinolopus celebensis*

Table 1 shows that suborder Megachiroptera, despite only one family, has more species than suborder Microchiroptera possessing 2 families but less number of species. Bat collection was largely done in open areas, around caves and bat tree holes. Even though many bats were hunted in this study site, particularly fruit feeders (Megachiroptera), their number of species did not decline. However, the insect feeding-bats (Microchiroptera) decreased in numbers resulting from hunting activities in the cave and tree holes through smoking or tree logging.

It was reported that 20% of suborder Megachiroptera and 50% of suborder Microchiroptera denned in the cave [6]. Several bat species [10] chose caves for denning due to humid condition, stable temperature, and remote from noise. In such a condition, the insect feeding-bats (Microchiroptera) could minimize water deficience from evaporation, find the right temperature for their body, and escape from noise that could cause mortality.

Several species found in this study were also previously collected [2,6], such as *Acerodon celebensis, Boneia bidens, Cynopterus brachyotis, Cynopterus luzoniensis, Cynopterus minutus, Dobsonia viridis, Macroglossus minimus, Nyctimene cephalotes, Rousettus amplexicaudatus, Rousettus celebensis, Thoopterus nigrescens, Megaderma spasma*, and *Rhinolopus celebensis.* Similar finding to the previous study could be influenced by several factors, such assuitable environmental condition, food availability, and predator absence, so that there were still many bats staying in the area. Bats had strong instinct home where the selected residence would be maintained through several generations [11]. Nevertheless, if their resindece is disturbed and unfavorable, it will be left.

This study also found 3 different species unreported in previous studies in this area, *Macroglossus tailiniensis* n. sp., *Rousettus tangkokoensis* n. sp., *Thoopterus tailiniensis* n. sp. It could result from observations concentrated or limited to certain locations and extensive study site [12]. This study was conducted in a wide study site covering all vegetation zones in the sanctuary of Duasudara Mountain, so that more different previously unreported species could be encountered. Other causing factors of several unfound bat species in this area are habitat disturbances, such as hunting and logging. Therefore, finding different other bat species from the previous studies and newly reported species could contribute to bat species numbers living in the sanctuary of Duasudara Mountain, North Sulawesi, indicating that this area possesses high species diversity.

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GROWTH RATE of BLACK SOLDIER FLY (*Hermetia illucens*) DURING BIOCONVERSION of RESTAURANT WASTE

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Abstract

Organic wastes are one of major problem in many cities. Common treatment for the wastes usually by sanitary landfill, composting or burning. However, in many cities of developing countries lack of effort to collect, separate, and transform organic waste lead to increasing pile of organic wastes and cost of treatment. Bioconversion of organic wastes by larvae of black soldier fly larvae (BSFL) (Hermetia illucens) into versatile prepupae could be considered as solution for this problem as this processs give economic value to organic wastes. In this study, BSFL was applied as bioconverter of food waste, as the most dominant type of organic wastes of city. Two days old larvae was fed with two types of food wastes, which were dominated with oil and dominated with decaying vegetables, and growth rate of larvae observed until prepupae then compare with larvae fed on rice husk. Result showed growth rate of BSFL fed on organic wastes significanly better. Rate of waste conversion also increased when the size of waste reduced prior applied as BSFL fed and not isolated from other bioconversion agents.

Keywords : bioconserversion, growth rate, *Hermetia illucens*, organic water.

Introduction

Wastes is one of major problems in many cities of Indonesia. Most of the waste, about 60%, consisted of organic waste [1]. Pile of wastes caused problems in health, environment, and aesthetic. Thus, it is necessary to design waste management system that reduce amount of waste while improve environmental quality and provide some benefits for community [2].

Like other developed countries, organic wastes produced by city were food waste originated from spoil food due to unadequate food storage system and over consumption. It was estimated about 40% of the wastes consisted of vegetables and raw material for food [3]. Most of these wastes ended as pile of wastes as collecting and management usually only covered about 50-70% of total population [4][5]. Common management procedure to manage this waste by sanitary landfill. However, lack of fund and infrastructure hindered the benefit of this method. Another method apply to manage organic wastes by composting but limited market and lack of economic value of the product made this method less favor in low income region [4].

Another alternative to manage organic waste by biconversion. Bioconversion, or biotranformation, is a proces to convert organic substance into less or more complex substance using living organisms. There are three types of bioconversion, (1) Enzymatic Hydrolisis, (2) Fermentation that produce gas, and recently developed (3) C.O.R.S. (Conversion of Organic Refuse by Saprophages) [3][6][7][8][9][10][11].

One of the example CORS is composting and decomposting of organic wastes by earthworm (vermicompost). However, uneven quality and contamination by microorganisms of compost produced considered as huge disadvantages of vermicompost [12].

Latest development of CORS was application of insect larvae as bioconversion agent, such as Black Soldier Flies Larvae (BSFL) (*Hermetia illucens*). Studies showed the ability of BSFL to convert various types of organic wastes into biomass which applied as sources of protein for high quality animal feed and lipid for potential source of biodiesel [13][14][15][16][17][18][19].

There are several benefits of application of *H. illucens* as bioconversion agent, especially in tropical region like Indonesia: (1) native in tropic and warm subtropical region [20], (2) they able to suppress population of house flies (*Musca domestica*) through predation and competition for nutrition [14][21], (3) they able to reduce patogen like *Escherichia coli* dan *Salmonella enterica* [22], (4) Adult flies does not eat and female does not oviposit their eggs directly on food source of larvae made them less likely to tranfer disease [23].

Even thoough, this species already applied as bioconversion agen of various types of organic waste, application in Indonesia is rare. However, direct application for Indonesia should considered the difference on types of waste and enviromental condition which might influence the efficiency of bioconversion process. Thus, it is necessary to conduct basic study on the ability of *H. illucens* as

bioconverter of typical food waste in Indonesia which could be represented by type of waste produced by local restaurants.

Materials and Methods

Animal specimen

Larvae of the black soldier fly *Hermetia illucens* L. were obtained from egg purchased from Center of Research and Development of Ornamental Fish Culture. All egg was kept on substance made of cow dung mixed with rotten vegetables (60% moisture) and kept at constant temperature (28ºC, 70%RH) in a container (50 cm x 25 m x 10 cm) in Laboratory of Enviromental Toxicology, School of Life Sciences and Technology, Bandung, Indonesia.

Animal treatment

Growth medium applied in this study were restauran waste dominated with oil and dominated with vegetables. As control, larvae were fed with rice husk. Restaurant waste was divided into 2 types of treatment grinded and not grinded.

Two days old larvae was used in this study. Each treatment (with three replicates for each treatment) contained 20 larvae fed with 100 mg/4 days (wet weight, 60% moisture content). The larvae were initially placed onto the prepared food within plastic cup. Food for larvae were prepared, weighed, and kept frozen 24 hours before treatment.

In order find the possible effect of nutrition competition to waste reduction, combination of organic waste (dominated with oil and dominated with vegetables, 50:50) was inoculated with BSFL then kept on isolated box (screen box with size 25 cm x 25 cm x 50 cm) and not isolated.

All experiments were conducted in room temperature and relative humidity (28-30ºC, RH = 65-25%, 12 hours photoperiod).

Data sampling

Larvae weight was measured every 4 days while level of waste reduction measured every 3 days.

Nutrition content

Protein and lipid were the most important nutrient required and harvested from larvae. Amount of protein, crude protein, crude lipid and ash of the waste and larvae were measured in the BALITSA.

Data analysis

One way ANOVA (P≤0,05) was applied to detect difference on growth rate among all feeding treatment and waste reduction rate between isolated and un-isolated system. Correlation test (P≤0,05) was applied to detect correlation between waste reduction and larvae weight gain. All test was conducted by IBM SPSS ver. 20.

Results and Discussion

Larvae Growth Rate

Highest growth rate was shown on larvae fed on grinded vegetables while larvae fed on rice husk inhibited lowest growth rate. This study also showed significant growth rate of BSFL when fed on food wastes compare with rice husk as feed (One way ANOVA, P<0.0001) (Table 1).

Table 1. Growth rate of BSFL (mg / day)

Growth medium	Grinded	Not grinded (Raw)
Control	11,92 ± 0,007	
Vegetables dominated waste	58,30 ± 0,009a	58,25 ± 0,011a
Oil dominated waste	45,88 ± 0,015a	51,25 ± 0,013a

Difference on growth rate of larvae could be caused by differences in nutrient content of feed material especially protein and lipid [24]. Rice husk had least amount of protein and lipid compared with waste material applied in this study (Fig. 1). Protein and lipid highly needed for larvae development especially when the adult does not eat. Insect development process, especially species with complete metamorphosis, is highly control by hormon and weight both required certain amount of protein and lipid for production [25].

This study showed that not all types of wastes required grinding prior application to BSFL. Growth rate of BSFL consumed grinded and raw vegetables waste were similar while raw oily wastes provided better growth (Table 1).This condition might related with the level of colonization by certain bacteria, namely *Escherichia coli*, which are food substrate for BSFL [26] [27]. Grinding oily waste probably mixed food material into oil which halted growth of bacteria. However, the difference of growth rate were not significant indicated both type of waste whether grinded or raw suitable for BSFL (P>0.08).

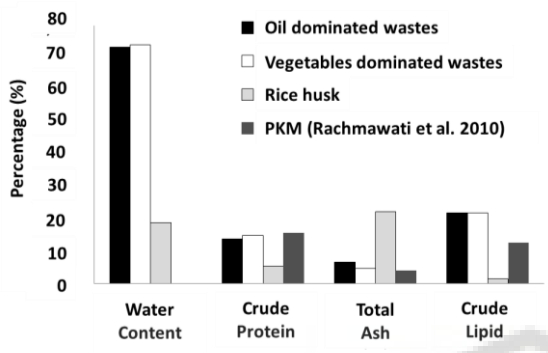

Figure 1. Nutrient content of material applied in the study compared with PKM (Palm Kernel Meal).

Nutrient Content of Larvae

Analysis on nutrient content of larvae showed larvae fed on rice husk had highest water content, crude protein, ash, and lipid (Fig.2). The result contradictive with content of growth medium. There was possibility higher content of all material related with higher metabolism of larvae when fed on low quality diet [3]. In term of harvesting protein and lipid, larvae fed on oil dominated wastes was better as higher weight compensated lower percentage of protein and lipid. Interestingly, even though vegetables dominated wastes had similar amount on lipid, larvae fed on this material reserved less lipid which might related to higher content of fiber and cellulose. Higher fiber and cellulose required more energy to digest thus reduce amount of energy to be reserved as lipid.

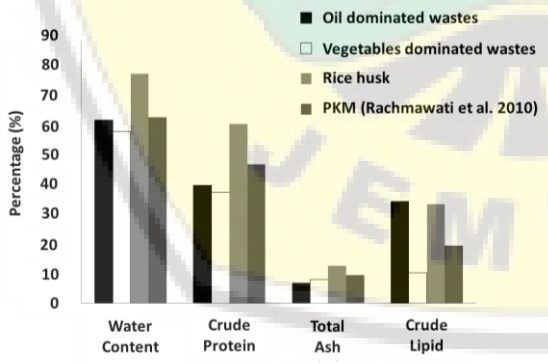

Figure 2. Nutrient content of larvae fed on growth medium applied during study.

Waste Reduction

Waste reduction level effected by growth of larvae (Correlation test, -0.719, P<0.0001) antara penurunan massa sampah dengan kenaikan massa larvae (Fig. 3).

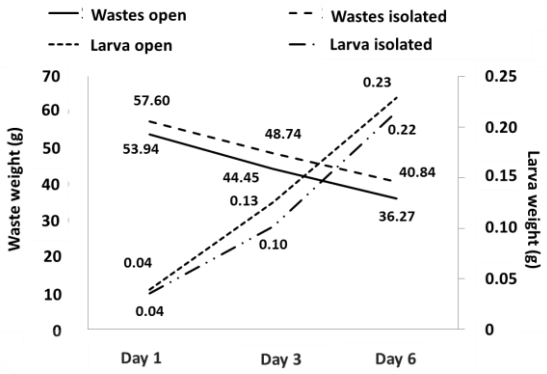

Figure 3. Correlation between waste reduction and larvae growth.

This study showed *H. illucens* would be better to applied as bioconverter in open sytem where decomposition of waste might also involving other organisms (P<0,05) (Fig. 4).

Figure 4. Waste reduction rate between isolated and open system of waste reduction by black soldier fly larvae.

Conclusion

Hermetia illucens could be applied as bioconverter agent of food wastes with without negatively effect the growth rate. This species would act better as bioconversion agent when applied together with other agents.

Future Works

Future work will focused on the design of complete system for food waste management using *H. illucens.* Further study on reproduction success, optimum rate of feed, related organisms, efficiency of digestion are several studies required.

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MYCORRHIZA DIVERSITY from VARIOUS PRIVATE FOREST ECOSYSTEM TYPES in SOUTH SULAWESI

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Abstract

Arbuscular Mycorrhiza are found in various ecosystems. The differences in mycorrhiza species, need to be isolated and identified to recognize of mycorrhiza types and colonization percentage of tree roots in some private forest ecosystems. The methodologies of research included collecting soil samples and tree roots, staining roots and observing arbuscular mycorrhizal fungi infection. Results showed spore types that observed at magnolias', candlenuts' and teaks' roots were *Glomus*, *Gigaspora* and *Acaulospora* spore. Colonization percertage of magnolias' roots was low and whereas candlenuts' and teaks' were intermediate.

Keywords: Mycorrhiza, Vesicular, Arbuscular, Peoples Cultivation Forest

Introduction

Arbuscular mycorrhizal fungi are one of mycorrhiza inducer types that receive attention from environmental specialist and biologist as a future alternative technology for increasing forest trees growth, productivity and quality, particularly for planted trees in nutrient poor-soil. The reports by [1] have proved mycorrhiza fungi significantly increased plant growth and decreased fertilizer needs to 40% that affected the reduction in fertilizer application costs up to 40%.

Arbuscular mycorrhiza are found in various ecosystems. The FMAs' distribution are wide-ranging distribution throughout the world, from arctic to tropical regions. [9] compiled FMA fungis' diversity data from Brazil, Colombia and Zaire and found as many as 16-21 species in natural ecosystems, 10 to 15 spesies in farm ecosystems (low inoculation level) and 6 to 9 species in intensive farm ecosystems, respectively. Study in Jambi and Bengkulu also reported there were 7 to 10 species in forest ecosystems, 8 to 11 species in farm ecosystems and 10 to 11 species in glassland ecosystems [10]. . This diversity difference have showed the existence of different FMA communities types among an environment to others and also FMA composition types in the root zone within a particular ecosystem as affected by soil types.

Due to different mycorrhiza species within some ecosystem types, mycorrhiza fungi need to be isolated and identified from tree rooting zone of some peoples cultivation forest ecosystem types. The findings of this study should become important informations regarding mycorrhiza types and to determine colonization percentage of tree roots from some peoples cultivation forest ecosystem types.

Materials and Methods

Time and Location of Research

This research activities were done at Tree Biotechnology and Breeding Laboratory, Forestry Faculty, Hasanuddin University, Makassar. It was conducted during the period of March up to August 2015. The sample collection locations were done at three peoples cultivation forests: magnolia in North Toraja, candlenut in Maros and teak in Barru.

Research Methodology

Soil Sample and Tree Root Collection

Root samples were taken as many as 18 spots at each following research location: at 0-30 cm soil depth. Soil was chosen from randomly assignedtree by mattock at around root zone in four spots, samples were then composited to one kilogram of soil.

Hairy Root Staining Procedure

FMA observation procedure was root staining technique using root staining method [3], [4] *in* [11]. The procedure steps were : hairy roots were chosen from sample trees and submerged in FAA solution. The roots were then submerged using 10% of KOH for 24 hours at room temperature. KHO solution was removed and root samples were cleaned by water. The clean roots were submerged in hot H_2O_2 solution for 24 hours, then cleanly washed with water. Roots were then submerged in 2% of HCl for 24 hours. The HCl solution was then removed and roots were thoroughly washed with running water. Root samples were then submerged in staining solution for 24 hours, and then submerged once more using staining solution for 24 hours.

Observation of Mycorrhiza Arbuscular Fungi Infection

The measurement of FMA infection was done using infected root length method by Giovannetti and Mosse (1980) in [2]. Five stained roots were cut at approximately 1 cm in length and arranged on a microscopic glass slide. The sliced roots on a microscopic glass slide were observed from every angle. Field of view that showed the colonizations would be sign (+), while if there were no sign of colonization, they would be sign (-).

Variable observation

The observed variable in this study were :

a. Arbuscular Mycorrhiza Fungi (FMA) Spore Characteristic
   The spore that found in this study was observed on its morphological characteristics, such as shape and colour.
b. Arbuscular Mycorrhiza Fungi (FMA) Type
   The observed spore type at root samples was identified up to genus class
c. The Colonized/Infected Root Percentage
   Root colonization was analyzed based on root infection percentage by counting FMA structure using [8] 's formula :

$$\text{Colonized Percentage} = \frac{\text{Number of infected root}}{\text{Total number of observed field of view}} \times 100\%$$

Statistical Analysis

The observation data were analyzed and showed in tabulation and picture/figure forms. Number of FMA infected root criteria would be categorized into 4 classes.

Table 1. The Number of Infection Classification by Connor *et al* (2001) *in* [4]

No	Colonization Percentage	Category
1	0	No colonization
2	≤ 10	Low
3	10-30	Intermediate
4	≥ 30	High

Results and Discussion

Arbuscular Mycorrhiza Fungi Spore Characteristic

Results of identification analysis indicated there were three spore types that found at sample collecting sites: Glomus, Gigaspora and Acaulospora. The observation of three locations and spore types are presented in Table 2.

Table 2. Identification of Spore Types in Each Observed Location

No	Private Forest Location	Spore Type
1	Magnolia in North Toraja	Glomus , Gigaspora
2	Candlenut in Maros	Glomus, Acaulospora
3	Teak in Barru	Glomus, Gigaspora dan Acaulospora

Based on the observation, Glomus spores were found at magnolia, candlenut and teak tree in varied environment conditions. Gigaspora spores were found at magnolia and teak tree, while Acaulospora spores were in candlenut and teak tree. All of three spore types had different characteristics in shapes and colour.

Morphological shape of these three spore types that found at each observed location are presented in Figure 1 (see below).

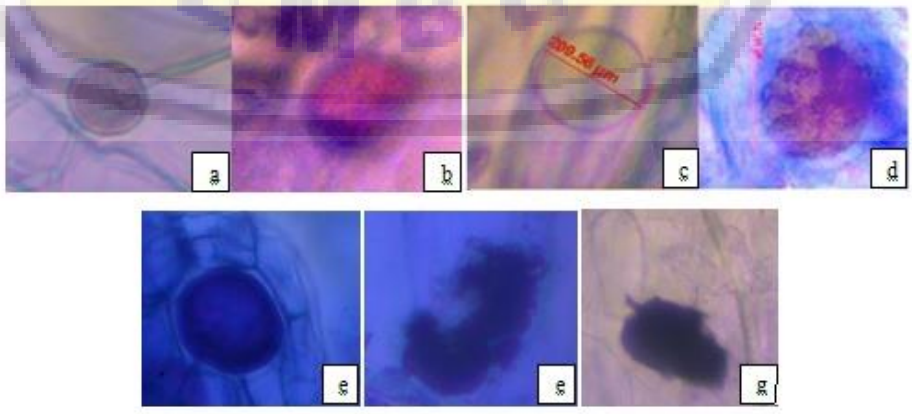

Figure 1. The identified FMA spore types at magnolia tree (a) *Glomus* spore, (b) *Gigaspora* spore*;* at Candlenut tree (c) *Glomus* spore, (d) *Acaulospora* spore*;* and at Teak tree (e) *Glomus spore*, (f) *Gigaspora* spore and (g) *Acaulospora* spore

Results of the study shows *Glomus* sporesthat identified at three observed locations commonly have oval-shaped, ellipse-shaped and round-shaped, as well as coloured in blue, light brown, dark brown, spore wall is soft and transparent/whitist. Findings by [7] stated Glomus spores commonly had round-shaped to ellipse-shaped, whitist/transparent yellow to reddist brown colour, had thin and relatively soft wall spore surface.

Based on morphological characteristics, observed Gigaspora spores at Magnolias' and Teaks' roots had oval-shaped, ellipse-shaped and round-shaped as well as blue, dark brown, brownish yellow and blackish brown in colour. Wall layer was regular and also irregular.

Acaulospora spores which found at candlenuts' and teaks' roots were round-shaped and ellipse-shaped, had thick and irregular wall spore, brownish yellow-coloured and had black spots in them. According to [6] and [5], Acaulospora spores had ellipse-shaped, relatively irregular and thick wall spore. In addition, spores colored in dark brown and browish yellow, and had black spots over their surface.

A. Root Colonization Percentage

Root colonization percentage calculations were varied at all of three observed locations. The histogram of colonization percentage at each location can be seen at Figure 2.

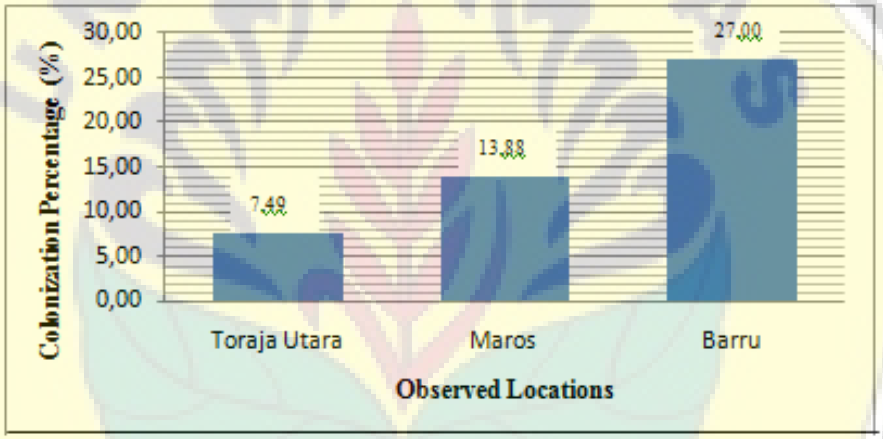

Figure 2. The histogram of FMA Colonization Percentage in Root Sample at Each Location.

Figure 2 describes that Barru is location with highest infection level as compared to other locations, North Toraja and Maros. Moreover, Colonization percentage based on infection level classification showed location with lowest colonization percentage level was North Toraja (7,49%), whereas both other locations, Maros and Barru, were intermediate at 13,88% and 27%, respectively. The presence of colonization level variation depends on environment conditions, such as pH soil, C-organic content as well as water level.

Results of soil analysis indicated the soil condition in North Toraja had a pH ranging between 5,21-5,56; 5,31-5,56 in Maros, while 6,5-6,82 in Barru, respectively. Correlation between pH and mycorrhiza is pH determines whether nutrients is easily absorbed by plants, as phosphorus, if pH is low, the plant growth will be inhibited by low availability of essential nutrients, such as phosphorus and nitrogen [5]. Tuheteru (2003) *in* [8] declared some FMA can be well multiplied, where optimal pH for FMA development are ranged between 5,6-7 for *Glomus* and 4-6 for *Gigaspora*.

C-organic content in each observed location ranged from low to intermediate level; about 1,09%-1,41% in North Toraja (low), 1,66-2,52 % in Maros (intermediate) and 1,95-2,49 % in Barru (intermediate), respectively. [5] reported a linear correlation between C-organic content and mycorrhiza number, where the higher C-organic content in the soil, the higher number of mycorrhiza is obtained since the C-organic can secure mineralization process which may provide nutrients for vesicles, arbusculars and hyphaes development symbiosis.

Conclusion

1. The observed spore types at magnolias', candlenuts' and teaks' roots were *Glomus*, *Gigaspora* dan *Acaulospora*.

2. Colonization percentage at magnolias' roots was low and at candlenuts' and teaks' roots were intermediate.

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THE ENVIRONMENTAL SECURITY PERSPECTIVE on THE GOVERNANCE of HERBAL MEDICINE in INDONESIA

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Abstract

The abundance of Indonesia's biodiversity is a blessing. As second largest biodiversity in the world, Indonesia has a lot of potent in natural resources of herbal medicine. The ability of Indonesia's technology to extract this source is capable. However, there are some political issues that needs to be adressed in using this potent. In environmental security perspective, the potentiality and sources of herbal medicine are one of the way to preserve biodiversity in Indonesia. Moreover, it will be very useful if it is utilized efficiently by Indonesian side. Herbal medicine is national assets, under this consideration, to describe governance of herbal medicine in Indonesia through environmental security perspective, this paper using descriptive case study from interview and literature review to present broader issues in this matter.

Keywords: Herbal medicine, Environmental security, governance, Indonesia.

Introduction

Herbal medicinal practices in Indonesia have been in use long before modern medicine from western world. In addition, its extension, cosmetic and physical maintenance, have also been used long before Dutch colonialization times. By the closing years of the 2000, medical preferences of the Indonesian public had changed. Indonesian society began to demand more choices and increased participation in decisions about their healthcare—and in particular they demanded increased access to herbal medicine. They were not abandoning modern western medicine, but rather requesting additional interventions with herbal medicine. Especially at present, one could buy easily readymade jamu which had produced by industry in the form of powder, pills, capsules, or drinking liquid [1] [2].

Although early attempts to establish a system of state-endorsed medicine was vigorously contested, by the early decades of the 2000, the fortunes of the herbalists were beginning to fade as those of medical practitioners and the herbal medicine corporation rose due to demand from the society[2]. In herbal medicine context, the environment serves various purposes, including the space for the medical landscape. This landscape includes the built environment as the source of medicinal flora, fauna, and minerals, and the places where interactions among practitioner, compound, and patients occur. In addition, the quality of the environment will influence the health conditions of the people and thus help to define the number of people seeking medical care services. However, this environment is threatened by many different conditions.

The opportunities environment provide for future development of herbal medicine, however, are threatened by serious and increasingly environmental problem. In international relation level, transnational environmental crime include illegal logging, trafficking of flora and fauna which has medicine potential, and dumping of toxic waste which destroy herbal medicine environment of live. It is a rapidly rising threat to the environment where sustainable development of herbal medicine becomes more increased in demand [3].

Many regions of herbal medicine environment in the world now have become vulnerable to environmental risks. For instance, most of conservasionist said that South East Asia is the most acute problem in ilegal wildlife[4]. Moreover, it has hub for smugglers to distribute its products to be traveled globally. It has been provoking the debate about risky society, and the need for environmental security. Likewise the transnational environmental crime, the intensification of economic activities, exploitive and extractive industries have become focal points for environmental security of herbal medicine.

Indonesian people exhibit a wide range of health based on herbal medicine that are widely linked to social, economic, cultural, and environmental conditions. The selection of traditional medicine is directly and indirectly linked to economic conditions and that are largely determined by the globalization process [4]. Herbal medicine is not immune from vulnerabilities that caused by political, economic and environmental

changes that are influenced by local and globalization forces. They are also vulnerable to changes happening in the environment and policies aimed at protecting threatened biological resources that are widely used in traditional herbal medicine practices.

Result

Environmental conditions and social processes impact people's health and traditional herbal medicine arrangements in many different ways, as well as their access to medically-important flora and fauna. The combined environmental and health issues in Indonesia are only now beginning to receive adequate security attention, due to the lack of herbal medicine resources. Like other urbanizing cities in the developing world is recording numerous health changes on multiple fronts such as diseases that are directly linked to poverty and also related to living in a contaminated environment. These health challenges have overwhelmed the existing medical facilities, and created opportunities for herbal medicine which can be found in environmental neighborhood to intervene to support health programs.

In implementing the social objective laid down by the State, and the National Health Policy, the Ministry of Health of Indonesia is taking the responsibility of providing promotive, preventive, curative and rehabilitative services to raise the health status of the Indonesian population. Herbal medicine roles are also becoming important as the needs for collaboration in health become more prominent.

Knowledge about the medicinal value of indigenous plants plays a central role in the health and well-being of Indonesian people. If the government can develop effective program and policies to promote sustainable production of these indigenous plants it can lead to maintain societal health [5]. According to Indonesia Health Research in 2010, a half of Indonesian peolpe (49,53%) have been using herbal medicine. From this big number, 4,36 % consume herbal medicine routinly every day, and 45,17 % consume herbal medicine when necesary or they intent to consume it. A half from them 55,16 % consume liquid herbal medicine 43,99 % in powder compound, rest of it consume herbal medicine in many ways, such as, capsule and external usage. [6] The dependence on traditional herbal medicine illustrates how the destruction of the environment not only threatens biodiversity, but also has severe ramifications for local people. This acts as a great incentive for conservation and careful nurturing of the environment. Many herbal medicine practitioner and user are well aware of the value of the environment and how to care for the plants they depends upon. They never cut the whole tree, taking only a few branches or some bark as compound in herbal medicine they usually used. Factors such as over-grazing, deforestation, and extreme weather as a result of climate change, severely threaten the environmental where the herbal medicines can growth.

The Government Indonesia has a commitment for the socioeconomic development of the citizens of the State through herbal medicine which has been being combined with contemporary modern medical science from western world in some hospital in Indonesia. Conservation of the state's rich biodiversity, its sustainable use for expanding livelihood security and ensuring equitable sharing of its benefits have been part of this larger commitment of Indonesia government. Within contemporary herbal medicine, traditional knowledge and modern science are combined to provide a unique meeting of patient, practitioner and plant, the state government have been took early steps to develop a comprehensive view on the sustainable use of state's natural resources and for the conservation of its rich biodiversity.

Evidence based medical science—provides on medicinal plants used by herbal medicine or jamu can be understood as 'folk knowledge'. This knowledge references is to myths, literature and popular culture, information about habitat, cultivation and harvesting [7] are all included in the development of an understanding the herbal medicine. This knowledge had been decrease by time, especially in urban area or big cities in Indonesia. To create resilience in herbal medicine in an urban environment, government can create a citywide cultures by facilitate herbal clinic and clinic garden in neighborhood environment.

Beside those strategic planning, the practical security action for herbal medicine environment can be done through eco-friendly agriculture involving a practices based on locally available biodegradable waste, bio-fertilizers and bio-pesticides. This plan could also to promote the cultivation of medicinal and aromatic plants. Government action to protect herbal medicine plant needs public support and public understanding. For instance, creating medical plant garden within a public parks or botanic gardens can be used by society to treat and learn herbal medicine plant. The purpose is to attract people to see the plants growing in their natural environment and its knowledge of herbal medicine utility in Indonesia.

However, the big issue for the development of herbal medicine in Indonesia is illegal and uncontrolled exploitation of natural resources. It is now widely recognized as a significant threat to both the environment and to sustainable development. Deforestation rate in Indonesia is the biggest rate of clearing forest nation in the world in 2012 [8]. It is also scare biodiversity in Indonesia because Indonesia is the heaven of rainforest in the

world which most of every species in the land very dependent to the existence of rainforest in Indonesia.

In addition, illegal wildlife trafficking is also the obstacle for the biodiversity in Indonesia. The high demand from China for herbal medicine extract from animals in Indonesia, for instance, a lot of Indonesian tigers are caught and being killed by hunters to be sold in black market in China [9]. It will be used as indregients for herbal medicine in China. Tigers is not only one species that to be hunted and killed, but also the others species that very valuable for the herbal medicine utilization in China. It needs awareness from Indonesian government to protect its biodiversity and practically, it is more advantegous for Indonesia to establish cooperation transnationally to combat illicit wildlife trafficking as transnational crime. It is must be securitized and raising its awareness of local people that every species in Indonesia is national asset and must be preserved. Practically, referent object in this case is national biodiversity that must be protected and it can cause threat for the environmental security. Unless, it could be claimed or developed by other countries with little benefit for Indonesia.

Discussion

As the second largest biodiversity in the world, Indonesia has to deal with a lot of things that can preserve its biodiversity. Firstly, the threat of exploitation its biodiversity potential. With the lack of sources of research funding in herbal medicine development, the potential of Indonesia's biodiversity are threatened by the high demand of herbal medicine abroad. Most of Indonesian researcher said that the biggest obstacle to develop herbal potential in Indonesia is funding. Research budget in Indonesia only 0,09 percent of GDP [10]. It is very smaller than China which in 1,98 percent of GDP [10]. It is surpassed European Union. The research funding for phytopharmaca medicine is approximately 1-5 billion rupiah [11] which is very costly for Indonesian researcher. Therefore, it is inevitable for Indonesian researcher to accept funding from abroad to develop its herbal medicine with the possible of patent losses of plants or tablets. It is endanger the ownership of local people in their environment and tend to evoke exploitation of Indonesia natural resources by foreigner without giving a lot of added value to its environment.

Secondly, the gap between the development in Java and the other islands in Indonesia can be the factor of increasing vulnerability to the biodiversity in Indonesia. Moreover, there are only one integrated research centre for herbal medicine in Indonesia and it is located in Java island. Tawangmangu research center for herbal medicine in difficulty now to suplly raw materials of herbal medicine in all over Indonesia. Consequently, the development of herbal medicine has been centralized only in Java Island. It needs more than one research center for huge potential of biodiversity in Indonesia especially for herbal medicine. Every island in Indonesia should has at least one research center for developing herbal medicine and its biodiversity to secure every natural sources that can be useful for herbal medicine.

Thirdly, Indonesia has lack of capable human resources in herbal medicine. There are a lot of expert in developing herbal medicine and also big industry in this field. However, the application of herbal medicine in health system still get big obstacle, especially the lack of middle skill workers to support that. Although there are stated in the regulation of health workers in 2014, traditional health workers yet cannot qualify professional health workers. According to basic health research report from health ministry, the workers in herbal medicine health workers is only graduated from elementary school predominantly. There are 70 percent of it [12]. In addition, only two universities in Indonesia which offer the study that clearly focus on herbal medicine development; University of Indonesia and Airlangga University. Moreover, only one university that really can provide middle skill workers in herbal medicine that provide diploma in herbal medicine specialist.

On the other hand, in China, this such gap is filled with the national policy that Traditional Chinese Medicine (TCM) is the part of national health system and national education for health workers in China. This policy makes China can develop its TCM easier and be supported by its human resources.

Fourthly, the dynamics of politics in herbal medicine has been stalling since 2012 in parliament with no clear vision [13]. There are no good sense of belonging between stakeholders in Indonesia to develop herbal medicine and its natural resources togetherly. Every party seems to work in its criteria and cannot cooperate so well. The problem root is in its political will of leadership. Many leaders only prioritize jamu or herbal medicine as the material of its political campaign, for example Jamu drinking campaign, but cannot integrate every aspect and stakeholders in this field to work together. As a result, business sector tend to save its investment by only reach standarized herbal drugs in its product as a respond to its low integration and low real commitment of the government in this sector. It also has to save its investment by only spending a research and development budget until standarized herbal drugs.

The main problem is in bureaucrat itself as leading sector for developing herbal medicine. Classical issue such coordination intra and inter

ministry such as in health ministry itself to integrate or at least to introduce herbal medicine in health system still get a low respond from its stakeholders. It is more pathetic that to know in the strategic plan services and herbal medicine. Health ministry will emphasize herbal medicine in preventive and promotive sectors in health system. Unless, It will not be the initial step from health ministry to develop herbal medicine in Indonesia.

To preserve its natural resources and biodiversity, there are must political will from every ministry in Indonesia to integrate every aspect in developing herbal medicine in Indonesia. For the security sector in hard approach, police and immigration officer must prosecute every activities that illegally endanger biodiversity in Indonesia nationally and transnationally. Foreign affairs minister also have to establish and strengthen the existence of international cooperation that to establish and combat wildlife trafficking as national interest in Indonesia's foreign policy.

The high demand of Indonesia natural resources for herbal medicine development abroad is the crucial factor that is unavoidable for Indonesia government to protect all aspect of Indonesia biodiversity in herbal medicine development. In soft approach, business sector can cooperate with academician to develop its product and also can empower local farmers to preserve its nature potential in Indonesia. The protection of Indonesia's herbal medicine product abroad also must be prioritized by its trade attache in every embassy of Indonesia.

Conclusion

Herbal medicine is the reliable potential source for Indonesia. With the richness of its biodiversity, Indonesia is heaven for every herbal medicine company to develop its product. However, there are a big challenge to preserve its natural resources with the high demand of herbal medicine raw materials internationally. Moreover, in this end year there will be ASEAN Economic Community which increasing its vulneravility in international politics and economy. Indonesia needs to anticipate it by securitizing its environment and biodiversity richness with the rising political commitment and cooperation between stakeholders nationally. The environmental security matters in this term can be securitized by the strengthening and integrated of Indonesia policy of herbal medicine.

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POTENTIAL of LOCAL FOOD PUMPKIN (*Cucurbita moschata* Duch) as DIVERSIFICATION of RICE to FOOD SECURITY

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Abstract

Demand for food continues to increase in line with population growth and improved quality of life, the need for food such as rice demand is driven up the price is expensive and where rice is shrinking due to environment factors, and improved seed. To anticipate the problem of availability of food needs by making use of local food pumpkin (*Cucurbita moschata* Duch) as the diversification of rice. Pumpkin has a good nutrient content, in addition to high levels of carbohydrates which are also rich in provitamin A, which is a distinctive pumpkin. Pumpkin is not only used the leaves as a vegetable but a fruit and also in the seeds. By utilizing pumpkin as diversification of rice so food security can be resolved.

Keywords: Food Security, Diversification, Pumpkin (*Cucurbita moschata* Duch)

Introduction

Indonesia as a country with a large population facing a very complex challenge in meeting the food needs of its population. Therefore the policy (strengthening) food security is a central issue in development and is a major focus in the development of agriculture. Strengthening food security policies in this regard including the establishment of national food stability [1].

Food security is an important part of the right to food as well as a human right. Food security is also a very important part of national security. In this case the right to food should receive equal attention to the business of enforcing human rights. Hunger and food shortage is the worst form of poverty faced by the people, where the famine itself is a process of cause and consequence of poverty.

Food security includes not only the sense of the availability of adequate food, but also the ability to access (including purchase) of food and non-occurrence of food dependence on any party. In this case, the farmer has a strategic position in food security: farmers are food producers and farmers at the same time also the largest consumer group that most are still poor and needy enough purchasing power to buy food. Farmers should have the ability to produce food at the same time must also have sufficient income to meet their own food needs [2].

The global food crisis impact felt by the entire country through a variety of forms. Although the new food crisis was evident at this time, but the process lasts longer in line with the development of the food supply system oriented capital accumulation globally. Indonesia state has a lot of potential to be able to cope with the food crisis. One way is to develop sources of food that can replace the presence of rice were dwindling. This shrinkage is caused by a growing population. Many sources of food that can be produced to replace the presence of rice. One source of food is pumpkin (*Cucurbita moschata* Duch) [3].

Pumpkin usually cultivated in dry land and only as a secondary crop when approaching dry season. Pumpkin has a very high nutritional value and very possibly used as an alternative to rice food so if optimized to be able to help tackle the food crisis. Pumpkin plant includes in family of *Cucurbitaceae*. The plant are annual crops that are spreading (vine) by means of a flat shaped tool holder. The stems strong enough and a length of 12 m, on the surface of hair ness rods are rather sharp and triangular rod shape, the surface dark green stems [4].

Pumpkin plant leaf is a single leaf that has compound leaf. The leaf are spread along the stem and leaf rounded shape, the color of the leaf surface dark green and dark green no white patches. While the flowers of pumpkin is monoceous uniseksual red yellow. Pumpkin plant can be grown in lowland and highland. While the ideal altitude is between 0 m - 1500 m above sea level [5]. This article will explain about the morphology pumpkin, the content of pumpkin and use as diversification rice.

Morphology of Pumpkin *(Cucurbita moschata* Duch*)*

Pumpkin plant is a vegetable plant species spread from the family *Cucurbitaceae*, which belong to the type of crops that will soon die after fruiting. Pumpkin plant has been cultivated in many countries of Africa, America, India, and China [6]. Pumpkin plant can be grown in lowland and highland. While the ideal altitude is between 0

m - 1500 m above sea level [5,7]. According to Hutapea (1994) [8], pumpkin plant *Cucurbita moschata* Duch can be classified as follows:

Kingdom	: Plantae
Divisi	: Spermatophyta
Sub divisi	: Angiospermae
Kelas	: Dicotyledonae
Ordo	: Cucurbitales
Familia	: Cucurbitaceae
Genus	: *Cucurbita*
Spesies	: *Cucurbita moschata* Duch.

Pumpkin is an annual plant that is spread or propagate by intermediaries who shaped flat tool holder as shown in (Figure 1). Strong enough and long stem and on the surface of the rod are hair ness rather sharp. Leaf width (10-25 cm up to 13-35 cm), and on the surface there are green leaf innocent and there is also green but there are white patches on the surface of the leaf, flower have five petals and five sepals [9].

a b c

Source: Suwanto [3]

Figure 1. Morphology of pumpkin *(Cucurbita moschata* Duch)
Description : (a) stem and petiole; (b) Spots on the green leaf of pumpkin; (c) parts of flower consist of 5 sepals and 5 petals

Pumpkin shaped fruit flat round, oval, round egg upside down, bottles, elongated oval, round, long, and a bokor with lots of grooves (15-30 groove) as shown in (Figure 2). Large fruit and the colors vary (although the fruit is old but the color of the fruit still young green because of fruit pumpkin has not been picked in the stalk so that the chlorophyll in the fruit is still there, while the fruit has been harvested yellow whitish because of fruit pumpkin after being picked from stems and stored so that the chlorophyll in the fruit is not there). Thick thick flesh of about 2 to 3 cm and slightly sweet taste. Weights pumpkin fruit an average of 3-5 kg, for large-size pumpkin can weigh up to 20 kg per fruit. Pumpkin fruit has a very thick skin and hard, so it can act as a barrier between the respiration rate of water out through the evaporation process, and incoming air causes oxidation process. This causes the pumpkin is relatively resilient compared to other fruits. resistance pumpkin fruit can reach 6 months or more, depending on storage, but the pieces already cut must be processed because it would be very easy to be damaged. It is an obstacle in the utilization of a pumpkin on a domestic scale because large pumpkin can not be processed at once [10].

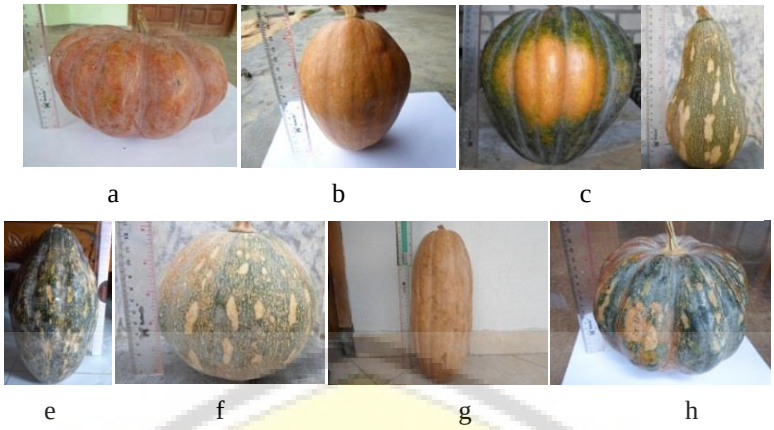

Figure 2. Variations fruit shape pumpkin (*Cucurbita moschata* Duch)
Description: (a) Round flat; (b) Oval; (c) Round egg; (d) Bottle (e) Elongated oval ; (f) Round; (g) long; (h) Bokor.

Nutritional contents of Pumpkin (*Cucurbita moschata* Duch)

Pumpkin has potential nutrition in fruits, seeds, and green leafe. Green leafe can be used as a vegetable and is a source of Ca, P, Fe, vitamin C, vitamin A. Pumpkin contain a lot of calories, in addition to the carotenoid content is quite high, especially pro-vitamin A and carotenoids (eg, β-carotene). Ripe fruit calorie content of approximately 50 kkal per 100 grams. The largest contribution to food calories come from grains, with more than 550 kkal per 100 grams of fresh seed. As for the content of seeds as a source of protein, fat, carbohydrates, and minerals [11,12]. Pumpkin seed oil is generally dominated by oleic (± 50%), linoleic (± 30%) and palmitic acid (± 15%) [7]. Pumpkin seed have pharmacological activity as an antidiabetic, antifungal, antibacterial, and has anti-inflammatory activity, antioxidant effects, and prevent the growth of, and reduce the size of the prostate [13].

Table 1. List of Food Nutritional Composition. Source: [14]

No	Nutrient content	Unit	Value
1	Calory	Kal	29,00
2	Protein	Gram	1,10
3	Fat	Gram	0,30
4	carbohydrate	Gram	6,60
5	Calcium	Mg	45,00
6	Phosphor	M	64,00
7	Iron	Mg	1,40
8	Vitamin A	SI	180,00
9	Vitamin B1	Mg	0,008
10	Vitamin C	Gram	52,00
11	Water	Gram	91,20
12	BDD (part to be eaten)	%	77,00

Quality and durable power pumpkin during storage are known by the level of maturity at the time of picking. The right level of maturity that can reduce the damage and extend the shelf life of pumpkin. Although it has a high water content, pumpkins own a long shelf life. Pumpkin ripe, intact, and without sores can last up to one year in natural storage. Pumpkin should be stored at a temperature 24-29^{o}C during the first two weeks after picking. In this condition, the skin becomes hard pumpkin. Subsequent storage conditions are 10-13^{o}C with 70-75% humidity. Storage areas should be selected that are clean and have good air circulation [15].

Pumpkin not stand stored in cold temperature. If it should be stored at low temperature (0-5^{o}C), required high humidity and not stored too long [15].

Pumpkin potential as a diversification of rice

Increased food production is essentially ensure the survival of the population, improve their living standards, as well as physical and mental endurance. Increased food products in the form of carbohydrates, proteins, and fat in value less than perfect if not supported with food product of higher nutritional value. Pumpkin has a fairly good nutrient content, high levels of carbohydrate are also rich in provitamin A, which is a privilege of a pumpkin that is useful for our health.

Pumpkin has a role in preventing degenerative diseases such as diabetes mellitus, asteroklerosis, coronary heart disease, high blood pressure and can even prevent cancer. During this time we know the extent pumpkin for use as a vegetable. As a variety of other snack that are still not widely known. Through the first steaming can be made of various kinds of snacks such as pudding, layer cake, cake, pie. Through first shredded and then squeezed for

example; yellow rice. Furthermore, it can also preserved with flour made in advance. From the results of testing recipes and flavor assessment of a variety of processed foods is great pumpkin recipes to be developed and manufactured a wide variety of foods such as; cakes, drinks, appetizers, main meals, and desserts.

Results of the research that has been done [16] use pumpkin puree raw material for the manufacture of cookies, [17] pumpkin flour utilization for the manufacture of biscuits. from some of the benefits of pumpkin that has been described above, then the existence of rice can be replaced with a pumpkin as a source of food every day.

Conclusion

From the above explanation that can be used as a diversification yellow rice for food sources, with pumpkin diversification society depends not only on the rice. Pumpkin has a fairly good nutrient content, in addition to high levels of carbohydrates are also rich in provitamin A, which is a feature of a pumpkin. Pumpkin is not only used on the fruit, but the leaf as a vegetable and seed.

With the diversification pumpkin hence food security can be met though use of rice as a source of food, given the availability of rice has been getting down because it is influenced by demand and environmental factors are unstable and pests that attack rice plants so that the productivity of rice getting down rice market also on the wane, because pumpkin can be used as a diversification of rice.

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INCREASING CONCENTRATIONS of THE BIOGAS and STORAGE CONDITIONS

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Abstract

Livestock waste can produce greenhouse gases (GHG) in the form of gas N_2O; NO_x; CH_4; CO_2, but if managed in an aerobic will produce CH_4 (50-70%), CO_2, H_2S and other gases are small. Biogas to be used as a substitute for fossil fuels need to be purified from impurities that methane (CH_4) concentration becomes large. The study says that biogas can be filtered by dry ferrihydrate, ferrihydrate + water (1: 1.25), dry calcium hydroxide, calcium hydroxide + water (1: 1.25), and calcium hydroxide + ferrihydrate with results each called P_1, P_2, P_3, P_4, and P_5 respectively. It turned out very significantly different ($P < 0.01$), which is included in the purified biogas can be compressed into a cylinder tank but the pressure drops about 2 atm than in the compressor. Conclusions: 1). P3 and P5 have a pretty good ability to filter biogas, 2). Purified biogas can be compressed in a cylinder tank, but the pressure is decreased 2 atm compared to biogas in the compressor. Suggestion: to improve the quality of biogas can be carried out by filtering using calcium hydroxide or a combination of ferriydrate and calcium hydroxide.

Keywords: livestock waste, greenhouse gases, ferrihydrate, calcium hydroxide

Introduction

Animal waste is one contributor to greenhouse gas emissions that would cause the temperature of the earth is getting hotter. Livestock waste, if left in place will produce N_2O, NOx, CH_4, CO_2 and others [1]. Such gas hereinafter referred to as greenhouse gases (GHG). Most greenhouse gas emissions come from methane gas emissions from the cow's digestive system.

Farm business accounts for as much as 51% GHG [7]. Lately, many livestock development directed at increasing cattle population. Eventually many of methane gas produced from livestock waste. Availability of livestock waste contained in the public number is not small, in 2014 the number of livestock waste amounted to 494,861.00 tons / day or 180,558,565.00 tons / year. If the input is made of organic sludge biogas units will be 1,577,029.00 tons / day or 575,615,585.00 tons / year [6].

Based on the above, the livestock waste that will be used as input units require biogas digester tank of 1,971,287.00 m3. According to Werner, Stohr and Hees, 1989, states that every 1 m3 tank of 0.18 m3 digester will produce biogas, while according Kadenwal (1990) of 0.34 m3 and depends on the volume of digester tank. So the animal waste is able to produce biogas [2]. Based on the above opinion, the total production of biogas Indonesia amounted to 512,534.5 m^3 / day or 187,075,092.5 m^3/year, equivalent to fossil fuels such as gasoline as much 151,530,824.93 liters / year, it is necessary purification of biogas in order to increase the methane gas content and can ditabungkan in bio pressurized gas cylinders.

Based on the content of the most biogas is methane gas and CO_2 as well as a bit of sulfur gas (H_2S). At least the sulfur gas resulting bio gas burner be easily corroded resulting in rapid deterioration. It is necessary for the capture of sulfur content and that the concentration of methane to CO_2 increases. Materials can be a gas catcher ferry Hydrat and calcium hydroxide. The existence of the two materials is the content of bio-gas will be absorbed and will likely turn into mineral fodder in the form FeS and $CaCO_3$, while wastes are useful for fodder [3]. Both of these minerals are essential to the life of non-ruminant and ruminant livestock. For that we need the handling of CO_2 and H_2S in biogas products in order to clean and improve the fuel and put it into a pressurized gas cylinder.

Research methods

Implementation of this research requires the material in the form of materials and tools. Materials used ferry hydrate ($Fe(OH)_3$). calcium hydroxide ($Ca(OH)_2$) and bio gas. Tools required: 1) plastic bottle of 250 ml, 2) Hose 15 m, 3) Glue plastic, 4) plastic 5) tupperware, 6) scales, 7) masking tape, 8) bucket, 9) funnel and 10) markers.

Sampling, sample source biogas obtained from livestock farmers who: a). Age of biogas units over 5 years. b). The number of cattle more than 5 livestock unit (ST). c). Never experiencing congestion and d). The waste is used as fertilizer and animal feed / fish.

Treat the attempted is 1). manufacture of bio gas filtration materials which include: a). Dry Ferrihydrite b). Ferrihydrate + water (1: 1.25), c). Dry calcium hydroxide. d). Calcium hydroxide + water (1: 1.25), e). Ferrihydrite + Calcium hydroxide (1: 1) and 2). Biogas compressor which is filled with pressure a). 7 atm, b). 8 atm, c). 9 atm, d) .10 atm, e). 11 atm.

The variables measured were: a). Saturated filter time (hours). The time required to bio gas is odorless, namely saturated time minus the start time filtering. b). Pressure compressor is a suction pressure at the end of the bio gas digester tank c). Pressure compressor to the compressed gas cylinders (CNG) compressor pressure is stated with atm. Data analysis. Research done using a completely randomized design random models. The experiments were performed using 5 treatment with 4 replications. Test of mean using Duncan's Multiple Range Test.

Results and Discussion

The observation time saturated filter material used to filter the biogas after statistically analyzed it produces a highly significant difference (P <0.01). The averages are as shown in Table 1.

Table 1. Mean time biogas saturated filter material

Saturated time of filtering (hours)	Average		
P_1	2.53	±	0.479^c
P_2	1.93	±	0.450^a
P_3	130.13	±	7.189^e
P_4	3.08	±	0.309^b
P_5	127.25	±	6.788^d

Based on Table 1 turns P_3 and P_5 have the ability to absorb H_2S is very long. That is material used as a filter that is able to survive in the treatment of H_2S and possibly also absorb CO_2. Because Ca $(OH)_2$ is able to react with CO_2 and become $CaCO_3$. The shape of the increase and decrease in saturated time can be described as Figure 1.

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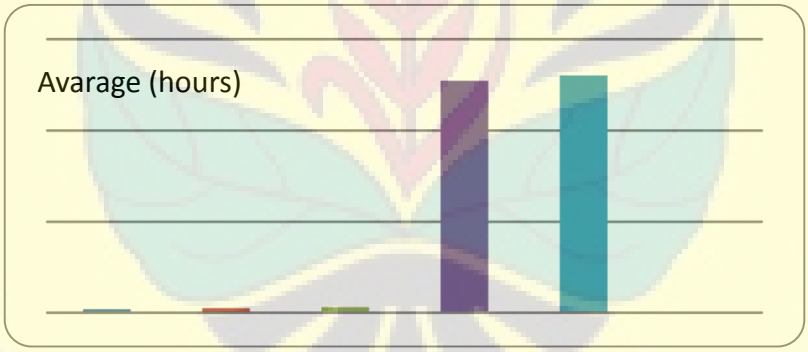

Figure 1. The shape of the increase and decrease in saturated time

Other researchers have stated that a mixture of filter materials can use 50% zeolite, bentonite 10%, 15% lime, sugar drops 5%, calcium 15%, 5% silicone and water. Differences with Werner, Stohr and Hees (1989), which states that the materials to purify the biogas can be done by running the ferrihydrite powder. Biogas without being filtered every 1 m^3 may ignite the stove approximately 4 hours [4]. According Buren (1979) states, $1m^3$ 1 ATM biogas at a pressure equivalent to 60-100 watts of electrical power operation during 6-7 hours and the kinetic energy of 1 m3 of biogas equivalent to 1 hp for 2 hours or comparable to from 0.6 to 0.7 kg kerosene. So that the gas cylinder with a volume of 50 L and 110 L each have a calorific value comparable with 0,3 kg and 0,8 kg kerosene or equal to 0.12 to 0.41 12 kg LPG cylinders. Therefore it is necessary compression biogas or compressed biogas (biogas filling into the tank), that can be taken anywhere or can be traded.

Biogas compression process from initial screening results fed into the compressor. The observation shows that the gas pressure inside the compressor is higher than the inside tube pressurized biogas (TPBG) or compressed natural gas (CNG). Results of the data analysis of the difference in pressure between the compressor and CNG cylinder was not significantly different (P> 0.05), while average looks like Table 2.

Table 2. Difference pressure CNG compressor and cylinder

Pressure of Compressor (atm)	Average		
P_1	1.95	±	0.129
P_2	2.00	±	0.183
P_3	2.05	±	0.238
P_4	2.03	±	0.150
P_5	2.00	±	0.183

Forms of filtering devices, bio-gas compressors, compressed gas cylinders and gas stoves bio can be described as in Figure 2.

Figure 2. Equipment of pressurized biogas, Ngawi Local Government, East Java Indonesia.

Noting Table 2 and Figure 2, it turns out biogas can be purified and incorporated in compressed gas cylinders. According to [6], mentions that the pressurized gas cylinder can be made of iron or steel pipes are modified. However, it should be re-examined about it. To save gas pressure should refer to the draft ISO pressurized biogas in 2014, which states that the purified biogas into biometan can be stored up to 200 bar on CNG tube.

Conclusion

Dry calcium hydroxide and Ferrihydrite + calcium hydroxide (1:1) have the ability as biogas filter material better. Ability press purified bio gas compressor to the compressed gas cylinder bio (Compressed Natural Gas = CNG) is lower than the pressure inside the compressor

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PROXIMATE ANALYSYS of FLOURS DERIVED from PEEL and KERNEL of GEDONG GINCU MANGO

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Abstract

Gedong gincu mangos (GGMs) which has the reddish to yellow skin color are one of the best mangos developed in West Java, especially in Cirebon. At present mango peels and seeds which are the main by-product in mango processing, are simply dumped in landfills. Although considered as a waste, mango peels and seeds are actually still contain a lot of protein, vitamin and carbohydrate that has economic value. The aim of this study is to produce flours from peel and kernel of GGM and to analyze their proximate composition. The result revealed that the content of ash, moisture, and fats in GGM kernel flour is higher than those in GGM peel flour. Moreover the content of carbohydrate and ash in GGM kernel flour is lower than those in GGM peel flour. Thus, vitamin C in GGM peel flour is higher than that in GGM kernel flour with the value of 647.87 mg/100 g and 484.93 mg/100 g respectively.

Keywords: Mango peels, Mango seed kernels, Mango peels flour, Mango seed flour, Gedong Gincu Mango

Introduction

Gedong gincu mangos (GGM) which has the reddish to yellow skin color are considered as one of the best and very special mango in Indonesia. The taste is sweet and has quite good smell. GGM is also called apple mango because the shape, size and color are similar to those of apple. During the kingdom era, the GGM could only be consumed by Cirebon kings and their family members. Ordinary people were prohibited to eat this kind of mangos.

According to Department of Agriculture of Cirebon, Cirebon has mango trees occupying total area of 12,205 hectares. GGM trees occupy about 24% of the total area comprising 2,929 hectares, with GGM total production of 12,460 ton in 2012. In 2013, GGM plantation area increased around region III of Cirebon such as Sedong, Beber, Greged, Astanajapura, Lemah Abang, Susukan, Duku-puntang, palimanan, Sumber, and Talun districs.

GGM has been in demand of domestic and export market. Main export destinations for GGM are Kuwait, Saudi Arabia, Qatar, Dubai, Singapore, Malaysia, HongKong Bahrain. In 2012, Cirebon exported 2,250 ton of GGM or about 18% of total production.

At present, mango are processed to obtain products such as puree, syrup, dodol, nectar, canned slice, juices, fruit bars and pies. During those processing, peels and seeds are the main by-product and are simply dumped in landfills. A previous study in several varieties of mangos showed that the seed represents from 20% to 60% of the whole fruit weight, depending on the mango variety and the kernel inside the seed represents from 45% to 75% of the whole seed [1]. Research conducted by Nzikou et al [2] showed that seed kernel contains carbohydrate, fatty acid and a low amount of crude protein.

On the other hand, peel contributes about 15-20% of the total weight of fruit. Although considered as a waste, mango peels are actually potential sources of phytochemicals like polyphenols, carotenoids, vitamin E, dietary fibres and vitamin C and it also exhibited good antioxidant properties [1,3,4].

If not properly handled, the peel and seed will become a source of pollution to environment and a potential hazard to public health. On the other hand because of their nutrient content, peel and seed of mango can be utilized as flour additives in cookies or cake formulation in addition to wheat flour. The objective of present study was to report on proximate analysis of flours derived from peel and seed of GGM.

Material and Methods

Materials

Gedong gincu mango fruits were collected during peak season of 2015 from the local market in Cirebon. Mango seeds and peels as by-products (waste) were collected from small industry producing mango syrup in Cirebon area.

Methods

Mango peel and kernel were cut into 0.3-0.5 cm in size. Then they were washed with tap water to remove dirt particle. After washing they were immersed in aqueous solution of $NaHSO_3$ and NaCl with the ratio 1:1 for 12 hours. The bleaching solution was removed and replaced with warm water (70-80 ^oC). After 5 minutes of immersion, they were dried and ground into powder. The powder was passed through 70 mesh sieves to obtain finely ground flours.

Proximate analysis was performed in Food and Nutrition Study Center, Gadjah Mada University. Protein content was analyzed by micro-Kjeldahl method, the total of fat content was analyzed by soxhlete extraction method, the ash and moisture content was determined finely and total carbohydrate was determined by difference.

Result and Discussion

The color of flours derived from peel and kernel of GGM is easily changed because of oxidative discoloration. To prevent this oxidation, peel and kernel were immersed in sodium bisulfite and sodium chloride solution. Flours obtained was shown in Figure 1.

Figure 1. Photograph of flours derived from kernel (a) and peel (b) of GGM.

Proximate composition is important in determining quality of raw material. It is often used as a basis for establishing the nutritional value and overall acceptance of developed food products [5]. The proximate composition of gedong gincu mango peel flour (GGMPF) and gedong gincu mango kernel flour (GGMKF) is shown in Table 1.

Table 1 shows that the moisture content of GGMPF samples is on average 8.6% while the moisture content of GGMKF samples is on average 9.1%. Moisture content in GGMKF is higher than that in GGMPF. This result is in agreement with the data obtained by Bandyopadhyay et al [6] and Ashoush et al [7]. They reported that moisture content in MKF is higher than that in MPF by 20%.

Table 1: Proximate composition of MPF and MKF

Characteristics	GGMPF	MPF ref [6]	MPF ref [7]	GGMKF	MKF ref [6]	MKF ref [7]
Moisture (%)	8.6	5.9	4.92	9.1	7.1	6.57
Crude protein (%)	5.9	2.8	3.6	7.9	7.2	7.76
Fats/oils (%)	5.4	1.4	1.23	8.7	9.8	8.15
Ash content (%)	4.1	4.2	3.88	2.2	2.1	1.46
Vitamin C (mg/100 g)	647.87	-	-	484.93	-	-
Total carbohydrate (%)	76.0	75.7	-	72.1	73.1	-

Compared to other mango in those literature, the moisture of gedong gincu peel and kernel flour is higher. Table 1 shows that the moisture content of GGMPF is on average 8.6% while the moisture content of MPF derived from other mango is on average 5.9%. Same phenomenon is also observed in GGMKF and MKF.

Although GGMPF and GGMKF have higher moisture content, their crude protein content is found higher than that of other MPF and MKF in reference. GGMPF has crude protein content two times more than that found in other MPF. However in GGMKF, the crude protein content different is not much different. Table 1 also shows that protein crude protein content in MKF is higher than that in MPF. However this is not general cases. Seleim et al [8] reported that mango seed kernels have a low content of protein compared to mango peel.

Total lipid contents of GGMPF were found to be 5.4% on a dry weight basis. This value is much higher than that of other MPF. However in GGMKF, the total lipid content is almost similar to that of other MKF. Table 1 also shows that generally lipid content in MKF is higher than that in MPF.

The most important difference between GGMPP and GGMKP is the higher in the moisture, protein and fat contents in GGMKP incomparable with GGMPP, while a significant lower in ash and occurring in GGMKP.

Conclusion

Based on above results, it could be concluded that the Gedong Gincu mango peels and seed kernels flours has good nutrition and could be used as a potential source for food additive in cake and cookies formulation. Thus both of the flours contain vitamin C as a good antioxidant.

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EFFECTS of AMINOETHOXYVINYLGLYCINE, PLASTIC WRAPPING, and STORAGE TEMPERATURES on FRUIT SHELF-LIFE and QUALITIES of 'CAVENDISH' BANANA

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Abstract

'Cavendish' banana is a worldwide banana cultivar which has a very short fruit shelf-life and quickly decreased qualities when its ripening is promoted. To find the methods on how to inhibit its fruit deterioration, this research was conducted to study the effects of applications of aminoethoxyvinylglycine (AVG), plastic wrapping, storage temperatures, and their interactions on the shelf-life and quality of 'Cavendish' banana fruits. This research was conducted in the Horticultural Postharvest Laboratory, Faculty of Agriculture, Lampung University from September to October 2014. Treatments were arranged in a completely randomized design with three replications and laid out in a 2 x 2 x 2 factorial design. The first factor was AVG (with and without 1.25 ppm AVG), the second factor was plastic wrapping (with and without one layer of plastic wrapping), and the third factor was storage temperature (a room temperature and 20 °C). The results showed that (1) AVG significantly lengthened fruit shelf-life, but did not affect physical and chemical fruit qualities such as fruit firmness, weight loss, soluble solid and acid contents, and sweetness; (2) plastic wrapping significantly lengthened fruit shelf-life, decreased fruit weight loss, accelerated fruit softening, but did not affect chemical fruit qualities; (3) lower storage temperature significantly lengthened fruit shelf-life, inhibited fruit softening, increased soluble solid content and sweetness, but did not affect fruit weight loss and acid content; (4) a combined application of AVG, plastic wrapping, and low storage temperature was best in lengthening fruit shelf-life, but did not affect physical and chemical fruit qualities.

Keywords: banana, postharvest, aminoethoxyvinylglycine, wrapping, storage temperature, shelf-life, quality, fruit

Introduction

'Cavendish' banana is a climacteric fruit, harvested in a green stage of stage I, and remains green in vacuum polyethylene bags inside cartoon packs during transportation as long as its ripening is not promoted with ethylene gassing. However, once its ripening is promoted and reached stadium III (an early ripening stage of greenish yellow), a stadium when the fruits are started to be distributed locally, in 3-4 days the fruits quickly reach the end of the ripening stage of stage VII (yellow with brown spots), a fruit stage of the end of economical values. Therefore, retarding the stadium development of stages III to VII, not from stadium I (a green stage) as conducted by [1] and [2], is the matter of postharvest handling that has an economical benefit.

In an attempt to retard the quick fruit deterioration of 'Cavendish' banana, [3] applied an anti-ethylene 1-methylcyclopropene (1-MCP) to both stages of stage III and V (perfectly yellow with green tip and greenish-yellow peduncle). Their results showed that the fruits receiving no treatment (control) deteriorated quickly, while those receiving 1-MCP application reached more than 5 days storage. 1-MCP was best if it was applied at fruit yellowing stage (stage V), because at earlier stage (stage III) the application resulted in imperfect fruit color development [3]. The results suggested, therefore, that any attempts to retard banana fruit ripening should be addressed at stage V.

Among anti-ethylene substances, aminoethoxyvinylglycine (AVG) is believed as the most potent chemical because its mode of action is directly as an inhibitory of ACC synthesis activity mechanism [4 and 5]. By applying AVG to inhibit ethylene synthesis, in combination with plastic wrapping to decrease respiration and transpiration rates [6 and 7] and a low storage temperature, lengthening the fruit shelf-life and maintaining qualities of 'Cavendish' banana are expected.

This research was aimed at studying the effects of applications of aminoethoxyvinylglycine (AVG), plastic wrapping, storage temperatures, and

their interactions on the shelf-life and quality of 'Cavendish' banana fruits.

Materials And Methods

This research was conducted on September-October 2014 in the Horticultural Postharvest Laboratory, Department of Agrotechnology, Faculty of Agriculture, University of Lampung, Bandar Lampung, Indonesia. The samples of 'Cavendish' banana fruit of stages V (perfectly yellow with green tip and greenish-yellow peduncle; [3]) were received directly from PT Nusantara Tropical Farm (PT NTF), Way Jepara, East Lampung, Indonesia.

Treatments were arranged in a completely randomized design with three replications and laid out in a 2 x 2 x 2 factorial design. The replications were applied to each experimental unit consisting of one cluster fruit of two fingers each.The first factor was AVG (with and without 1.25 ppm AVG, by dipping for 10 minutes), the second factor was plastic wrapping (without and with one-layer plastic wrapping of Total Plastic Wrap®), and the third factor was storage temperature (a room temperature and 20 °C). Treated fruits were then placed in storage rooms of a room temperature of 28 ± 1 ºC and a low temperature of 20 ± 1 ºC. The storage room temperature of 20 ± 1 ºC was the lowest possible temperature that could be achieved in the storage room of 5.8 x 2.8 x 3.15 m^3 with two ACs, one humidifier, and one thermohygrometer.

Observations were made on fruit shelf-life, weight loss, firmness (with a penetrometer typed FHM-5,with a cylindrical point of 5 mm in diameter of Takemura Electric Work, Ltd., Japan), ºBrix (with an Atago N-1E hand refractometer), titratable acidity (titrated with 0.1 N NaOH and phenolphthalein as an indicator), and sweetness level (as °Brix/titratable acidity ratios). A unit treatment was ended when the banana fruit reached stage VII (yellow with browning spots; [3]). All data were analyzed with ANOVA, and further tested with Least Significantly Difference (LSD) at 5%.

Results And Discussion

'Cavendish' banana is a climacteric fruit. However, different from most other climacteric fruits, its fruit remains green and its qualities are maintained during storage as long as its ripening is not promoted with ethylene gassing and its fruit weight loss and shrunken or wilted rind are avoided. Consequently, its postharvest handling problems are not laid before ethylene gassing [1 and 2], but after it. Once its ripening is promoted and reached stadium III and the fruits are started to be distributed locally, in 3-4 days brown spots are developed on its yellow rind and the fruits are quickly soften or its flesh firmness is quickly decreased.

However, consumers usually judge the quality of 'Cavendish' banana based on its rind color, not on its firmness. On the beginning of this research, its fruit firmness was 2.31 kg/cm^2 (Table 1, footnote) and in 4-8 days storage the fruits soften quickly, reached as low as 0.30 kg/cm^2. The data in Table 1 showed that AVG significantly lengthened its fruit shelf-life by slightly less than one day compared to the control, but its fruit firmness and weight loss were not affected significantly. This indicated that rind color stadium development and fruit flesh softening were two different development processes that did not have to be perfectly correlated each other. A thick rind of banana might play a barrier for ethylene or anti-ethylene to take into effects differently on the ripening rind color stadium development and flesh softening [8].

AVG, however, did not affect other quality parameters, such as soluble solid (°Brix) and free acid contents, and consequently, its sweetness (Table 2). These three fruit quality parameters were not affected because the observation was terminated at the same stage [3, 9, 10, 11, 12, and 13].

Plastic wrapping played a physical barrier for O_2 and CO_2 movements, and developed a modified atmosphere around the fruits with less O_2 and more CO_2. This modified atmosphere decreased respiration rate of the fruit [9], and consequently, lengthened the shelf-life of the 'Cavendish' banana fruits (Table 1). One-layer plastic wrapping also inhibited water loss from the fruits, and consequently, maintained a significantly lesser fruit weight loss than the control (Table 1) as also reported by [13, 14, and 15].

While other fruit qualities, such as soluble solid (°Brix), free acid, and sweetness, were not affected, one-layer plastic wrapping significantly decreased fruit firmness or accelerated fruit softening. Heat deliberated from respiration and trapped inside wrapping might increase temperature inside wrapping, and consequently, accelerated fruit softening [16].

The treatment of low temperature of 20 ± 1 °C applied in this experiment significantly lengthened its ripening rind color stadium development of the fruit which was perceived as fruit shelf-life (Table 1). If a much lower temperature than that of 20 ± 1 °C was applied, we believed that a much longer fruit shelf-life could be achieved because 'Cavendish' banana fruits are usually shipped under a low temperature of 13.33-14.44 °C (56-58 °F; Rachmansyah A. Wardhana, Chairman of R&D Division of PT NTF, personal communication).

Low temperature also significantly delayed fruit softening (Table 1) because it might delay

respiration rate [17]. Consequently, while free acid was not affected, other fruit qualities, such as soluble solid (°Brix) and sweetness were significantly maintained higher than the control (Table 1).

A more significant effect of individually applied wrapping was significantly dominated when it was applied in combination with AVG and low storage temperature (Table 1).

Table 1. Effects of AVG, plastic wrapping, and storage temperature on the fruit shelf-life, firmness, and weight loss of 'Cavendish' banana[1]

Treatments	Shelf-life (days)	Firmness (kg/cm^2)	Weight loss (%)
AVG:			
Control (A0)	5.00 b	0.62 a	7.79 a
AVG (A1)	5.92 a	0.77 a	9.07 a
Wrapping:			
Control (W0)	4.17 b	0.96 a	9.64 a
1-layer (W1)	6.75 a	0.43 b	7.21 b
Temperature:			
Room (T0)	5.00 b	0.54 b	8.05 a
Cool (T1)	5.92 a	0.85 a	8.81 a
AVG*Wrap.:			
A0W0	4.00 c	0.80 ab	9.37 a
A0W1	6.00 b	0.45 b	6.21 b
A1W0	4.33 c	1.12 a	9.92 a
A1W1	7.50 a	0.42 b	8.22 ab
AVG*Temp.:			
A0T0	4.83 a	0.53 a	8.16 a
A0T1	5.17 a	0.72 a	7.42 a
A1T0	5.17 a	0.56 a	7.94 a
A1T1	6.67 a	0.98 a	10.20 a
Wrap.*Temp.:			
W0T0	3.67 b	0.70 b	9.03 ab
W0T1	4.67 b	1.21 a	10.26 a
W1T0	6.33 a	0.39 b	7.07 b
W1T1	7.17 a	0.48 b	7.36 b
AVG*Wrap.* Temp.:			
A0W0T0	4.00 cd	0.58 bc	10.09 ab
A0W0T1	3.33 d	0.82 bc	7.97 bc
A0W1T0	5.67 bc	0.47 bc	6.23 c
A0W1T1	7.00 ab	0.30 c	7.91 bc
A1W0T0	4.00 cd	1.01 ab	8.64 abc
A1W0T1	5.33 bc	1.42 a	11.87 a
A1W1T0	6.33 ab	0.43 c	6.20 c
A1W1T1	8.00 a	0.54 bc	8.52 abc

[1]Values in the same column of each treatment followed with the same letters were not significantly different at LSD 5%. Fruit firmness in the day of treatment (shelf-life 0 day) was 2.31 kg/cm^2.

Table 2. Effects of AVG, plastic wrapping, and storage temperature on the fruit °Brix, acidity, and sweetness of 'Cavendish' banana[1]

Treatments	°Brix (%)	Acidity (mg/100 g)	Sweetness[2]
AVG:			
Control (A0)	6.70 a	0.35 a	19.01 a
AVG (A1)	6.83 a	0.35 a	19.39 a
Wrapping:			
Control (W0)	6.77 a	0.35 a	19.20 a
1-layer (W1)	6.77 a	0.35 a	19.20 a
Temperature:			
Room (T0)	6.50 b	0.35 a	18.45 b
Cool (T1)	7.03 a	0.35 a	19.96 a
AVG*Wrap.:			
A0W0	6.77 a	0.35 a	19.20 a
A0W1	6.63 a	0.35 a	18.82 a
A1W0	6.77 a	0.35 a	19.20 a
A1W1	6.90 a	0.35 a	19.58 a
AVG*Temp.:			
A0T0	6.87 b	0.35 a	19.49 b
A0T1	6.53 bc	0.35 a	18.54 bc
A1T0	6.13 c	0.35 a	17.41 c
A1T1	7.53 a	0.35 a	21.38 a
Wrap.*Temp.:			
W0T0	6.70 ab	0.35 a	19.01 ab
W0T1	6.83 ab	0.35 a	19.39 ab
W1T0	6.30 b	0.35 a	17.88 b
W1T1	7.23 a	0.35 a	20.52 a
AVG*Wrap.* Temp.:			
A0W0T0	7.27 abc	0.35 a	20.62 abc
A0W0T1	6.13 d	0.35 a	17.41 d
A0W1T0	6.47 cd	0.35 a	18.35 cd
A0W1T1	6.13 d	0.35 a	17.41 d
A1W0T0	6.27 d	0.35 a	17.78 d
A1W0T1	7.40 ab	0.35 a	21.00 ab
A1W1T0	6.80 bcd	0.35 a	19.29 bcd
A1W1T1	7.67 a	0.35 a	21.75 a

[1]Values in the same column of each treatment followed with the same letters were not significantly different at LSD 5%. °Brix, acidity, and sweetness in the day of treatment (shelf-life 0 day) were 8.00%, 0.35 g/100 g, and 22.70, consecutively; [2]°Brix/acidity ratios.

In fact, a combined application of AVG, plastic wrapping, and low storage temperature increased fruit shelf-life of more than four days storage compared to the control, but generally they did not affect physical and chemical fruit qualities.

Conclusion

The results showed that (1) AVG significantly lengthened fruit shelf-life, but did not affect physical and chemical fruit qualities such as fruit firmness, weight loss, soluble solid and acid contents, and sweetness; (2) plastic wrapping significantly lengthened fruit shelf-life, decreased fruit weight loss, accelerated fruit softening, but did not affect chemical fruit qualities; (3) lower storage temperature significantly lengthened fruit shelf-life, inhibited fruit softening, increased soluble solid content and sweetness, but did not affect fruit weight loss and acid content; (4) a combined application of AVG, plastic wrapping, and low storage temperature was best in lengthening fruit shelf-life, but did not affect physical and chemical fruit qualities.

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AMYLASE ACTIVITY of FISH VENTRICULI after VARIOUS STORAGE TEMPERATURES and PERIODS

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Abstract

The one of digestive enzymes in animals is amylase. Amylase is used for breaking down the complex carbohydrates to be simpler ones. This concept is important to be studied by undergraduate students of animal physiology class. This experiment aims to identify the activity of ventriculi's amylase of five fish species, *Oreochromis mossambicus*, *Oreochromis niloticus*, *Cyprinus carpio*, *Clarias batrachus* and *Pangasius pangasius*, after various storage temperatures and periods. The results will be useful for determining the fish ventriculi that are probably suitable as sources producing amylase, related to storage temperatures and periods in laboratory work of animal physiology class. The data were gained through examining enzyme activities using benedict reagent. They were analyzed qualitatively and descriptively based on the appearance of colors in the range between dark blue and dark green representing the levels of amylase content on fish ventriculi. Based on the data gained, blue to green appear after fourth day of storage in all various storage temperatures and all species. The green appeared in *Oreochromis mossambicus*, *Cyprinus carpio*, *Clarias batrachus* of ventriculi extracts just after 4 days storage, and the blue appeared until the last day of storage. Overall, amylase activity could be tested on fish ventriculi of 5 fish species and various storage temperatures and periods, and can be used as matterials of laboratory work in animal physiology class.

Keywords: amylase, fish ventriculi, storage temperatures, and storage periods

Introduction

Enzyme is one of basic concepts that relates to a wide range of physiological processes matter that should be understood by students in animal physiology class. Amylase is one of enzyme that examined in laboratory work of animal physiology class.

Previously, the laboratory work used fish intestine of *Cyprinus carpio* as an organ that produces amylase. The organ shows its function on chemical digestion by enzyme activities test. However, after monitoring their activities during 2 semesters, the appearance of enzyme activities changed to be worse. It can be because of the food consummed. Reference [6] said that fishes with relatively broad diets can modulate digestive enzyme activities in response to changes in dietary composition.

Digestion is a process of simplifying complex organic molecules to be some simpler compounds [13]. Carbohydrate is the first source of energy in animals. Reference [3] observed that carbohydrates comprise at least 25-35% of the total diet, and contained in commercial diets in order for reducing costs. Mostly, the animals get this compound in the form of polysaccharide, as starch.

At the first step of starch digestion, amylase plays an important role to breaks starch down into some maltose and glucose molecules. Some studies identified the enzyme in the form of alpha-amylases [1] and it shows that temperature storage influences the activity level of enzymes [17].

The goals of this study is to identify the activity of ventriculus amylase of five fish, *Oreochromis mossambicus*, *Oreochromis niloticus*, *Cyprinus carpio*, *Clarias batrachus* and *Pangasius pangasius*, after various storage temperatures and periods. The results will be useful for determining the fish ventriculi that can be as sources of amylase instead of intestine relating to storage temperatures and periods in laboratory work of animal physiology class.

The data gained were the amylase activities examined from 5 species ventriculi extracts after storing at 15 °C, 25 °C and 35 °C during 4, 7 and 10 days. The ventriculi used were got by cutting the anterior part of digestive tract. After being cut longitudinally and washed by distilled water, they were crushed in 20 ml of 50% of glycerin. Then, 5 drops of toluene were added into the solutions. The extracts resulted were kept in a closed dark bottle and stored at 15 °C, 25 °C and 35 °C for 4, 7, and 10 days in dark chambers. The amylase activities were examined after 0, 4, 7, and 10 days of storage. After each period of storage, the extracts were filtered and the level of amylase activities were examined qualitatively using benedict reagent. The levels were determined based on the color resulted,

i.e. dark blue, greenish blue, light green, and green, representing the level of amylase activities from the lowest until the highest ones. The data gained were analyzed qualitatively and descriptively.

Amylase activities of five species for four storage periods (0, 4, 7, and 10 days), and at four temperatures (control, 15, 25 and 35 °C) can be seen in Table 1. Based on the data, the amylase activities appear on the ventriculi of five fish species (*Oreochromis mossambicus, Oreochromis niloticus, Cyprinus carpio, Clarias batrachus* and *Pangasius pangasius*) extracts by dark blue, blue, greenish blue, light green and green. The greenish blue appears in patches. However, it could be seen that there are some tendencies of the spreading colors, so that it seem varies among species, and temperatures and period of storages. The appearances of the greenish blue mean that amylase could be got from the ventriculi of the five species, furthermore, they are active. Some authors also identified amylase in some fish and aquatic animals [15, 6, 8, 7, 17, 18].

Table 1 shows data of amylase activities that are different (indicated by different colors) among five fish species. In general, amylase activities shown by greenish blue appear after 4 days of storage and for all storage temperatures. However, there was no amylase activity on fish ventriculi execution.

Samples after 4 days storages were appeared greenish blue to green. At 15°C, the amylase activities in all samples are around greenish blue to blue. On the other hand, amylase activities at 25 °C quite different with 15 °C temperature among five fish species. Surprisingly, at 35 °C, three of five fish species, *Oreochromis mossambicus, Cyprinus carpio* and *Clarias batrachus*, had higher amylase activities shown by green. The enzyme of *Oreochromis niloticus* and *Pangasius pangasius* had the same activities shown by the same as greenish blue.

The table shows that after 7 days of storage, the activities of amylase of 5 fish ventriculi are similar at all storage of temperatures, being around blue and light green, although *Pangasius pangasius* at 25°C storage still shows high amylase activity (light green). Moreover, after 4 to 7 days storages, most of the activities decreased from green and light green to be blue.

In last group of storage period, 10 days, compared with the shorter periods, it had various results due to different storage temperatures. All of amylase activities of five fish species tend to unchanged in blue at 35°C. On the other groups, at 15 and 25 °C, amylase activities of *Oreochromis mossambicus, Oreochromis niloticus* and *Clarias batrachus* slightly increase become greenish blue, but decreased steadily on *Cyprinus carpio'* and *Pangasius pangasius'*. However, at 35 °C, the amylase activities tend to be unchanged at blue between seven and ten days of storage although the activity of *Cyprinus carpio*'s was lower than group at 25 °C.

Table 1. Amylase activity of fish ventriculi

Temp (°C)	Species of Fish	Storage Period (day) 0	4	7	10
Control	*O. mossambicus*	0	0	0	0
	O. niloticus	0	0	0	0
	C. carpio	0	0	0	0
	C. batrachus	0	0	0	0
	P. pangasius	0	0	0	0
15	*O. mossambicus*	-	2	1	2
	O. niloticus	-	2	1	2
	C. carpio	-	2	1	1
	C. batrachus	-	2	1	2
	P. pangasius	-	2	1	2
25	*O. mossambicus*	0	3	1	2
	O. niloticus	0	3	1	2
	C. carpio	0	2	2	1
	C. batrachus	0	2	1	2
	P. pangasius	0	2	3	2
35	*O. mossambicus*	-	4	1	1
	O. niloticus	-	2	1	1
	C. carpio	-	4	1	1
	C. batrachus	-	4	1	1
	P. pangasius	-	2	1	1

Notes:							
Scale	0	1	2	3	4	5	6
Red	0	0	0	40	11	44	0
Green	0	153	255	252	199	136	102
Blue	255	255	255	141	33	44	0
Color Level	0: Dark blue 1: Blue 2: Greenish blue 3: Light green			4: Green 5: Very green 6: Dark green			

Reference [4] suggests that the production of amylase is family-specific. It may relate to anatomical and functional differences of the gastrointestinal tract and associated organs [10]. Reference [14] identified groups of fish based on types of food, carnivorous and omnivorous. The fish have different structure of histology and histochemistry.

Reference [12] identified differences of the amylase activity in *L.vannamei* fed with different protein sources. The authors explained that a decrease in amylase activity and disappearance of one amylase isoform appeared when the amount of dietary casein was increased. It may because of the

changes in expression of several genes that relates to metabolic and physiological adjustments to assimilate the type of food provided [5]. Reference [3] adds that the ability to digest different food items relate to enzyme profile of a given species. The presence of numerous amylase isoforms maybe an ecological advantage and may indicate that species are able to benefit from carbohydrates in diet.

Storage of ventriculus extracts during periods of time was done for extracting enzyme from ventriculus glands cells. Besides relating to species differences, the amylase activities differ among periods of storage. There was no amylase activity without storage period or just after ventriculus extraction shown by dark blue resulted. The activities, shown by blue, greenish blue, light green, and green, started from day 4 until the end of experiment (day 10). Overall, the highest activities were after 4 days of storage. These phenomena may due to the work of toluene on the cell membrane of ventriculus gland cells that contain amylase produced. The same phenomena occur in the extracts of intestines of the same species [11]

Toluene is an organic solvent that acts on cell membrane by solubilizing phospholipids and denaturing proteins. Reference [16] added that lipophilic material could be dissolved in toluene. Just after extracting ventriculus, this toluene had not worked on the cell membrane. In general, in relation to periods of storage, mostly the optimum activities occur among species. It happens on 4 days storage period in *Oreochromis mossambicus*, *Oreochromis niloticus*, *Cyprinus carpio*, *Clarias batrachus and Pangasius pangasius*. These phenomena may due to different structure of cell membrane of amylase glands, so that it influences the works of toluene. Reference [9] reviewed that structure of gastrointestinal tract differ among animal species, especially membrane composition of enterocytes, and different quantity and location of proteins. Reference [14] supported that different animals have different structure of histology and histochemistry.

The amylases activities vary among temperature storages of ventriculus extracts seem different among species. It may due to various factors. Reference [11] said that many samples of *Oreochromis mossambicus* and *Oreochromis niloticus* extract, followed by *Cyprinus carpio*'s, *Clarias batracus*'s, then *Pangasius pangasius's*. It means that the amylase activities tend to dominate in the intestine of the first two species. Reference [2] added that the temperature profile study revealed that the neutral amylase isoform with optimal pH 7 showed optimal temperature characteristics at 40 °C and the alkaline amylase isoform with optimal pH 9 had optimal temperatures of 30 and 50 °C.

Conclusion

In conclusion, based on the results, amylase activities could appear during examination of fish ventriculi extracts of five fish species. However, they are influenced by and various storage temperatures and periods. Thus, the ventriculi can be used as matterials of laboratory work in animal physiology class.

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EFFECT of PH AND FERMENTATION TIME on PATCHOULI LEAF FEREMTATION in A STIRRED FERMENTOR

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Abstract

Patchouli oil produced from patchouli leaves is one of the essential oils that are important in the perfume industry. Several chemical extraction and fermentation process has been used to obtain the patchouli oil is of good quality and high yield. However, until now the fermentation process of patchouli leaf by using fungi still not getting the optimum operating conditions. The purpose of research was to determine the effect of pH on patchouli leaf fermentation in stirred fermentor by using *Phanerochaete chrysosporium* as degrading agents. The research was conducted with the following stages: pre-treatment (preparation) patchouli leaves, regeneration of Phanerochaete chrysosporium fungi, fermentation patchouli leaf, patchouli oil refining and analysis test on patchouli oil. Variables used include independent variables were fermentation time (15, 16, 17, 18, 19, 20 and 22 days) and without supplies of air (only stirred). While the dependent variable was pH (4, 5, and 6). The parameters measured were yield, patchouli alcohol (PA) concentration, and refractive index. The results of research showed that the longer the fermentation time and greater pH, the concentration of PA and the refractive index increases. While smaller the pH, the greater the patchouli oil yield. Optimum operating conditions in the fermentation of patchouli leaves is reached at the time of fermentation for 22 days and pH 6 for PA (37.69%), and refractive index (1.4922). While the largest yield was obtained at the time of fermentation for 22 days, and pH 4.

Keywords*:* Fermentation, *Phanerochaete chrysosporium*, Patchouli oil, Patchouli alcohol, Yied.

Introduction

Patchouli oil is one of the essential oils that are important in the perfume industry, where patchouli oil export to various countries of not less than 1,200 tons / year [2, 27]. However, there are still many problems to maintaining the quality of patchouli oil which patchouli oil export quality is still often mixed with other vegetable oils.

Some patchouli oil processing technology has been used, either using chemical extraction or fermentation process of patchouli leaves [3, 9, 19]. One method of fermentation developed to improve the quality of patchouli oil is the use of fungi *Phanerochaete chrysosporium* [5]. However, to find the best conditions of the fermentation process is still being conducted through various research [16, 23, 24, 25]. The purpose of the research was to determine the effect of pH and fermentation time on the fermentation process of patchouli leaves in a stirred fermentor by using *Phanerochaete chrysosporium* as an agent that degrade cellulose and lignin.

Patchouli oil is the essential oil that can be obtained from the leaves, rods and flowers patchouli with its main component is patchoulol, where the levels of patchoulol in patchouli oil may reach 50-60% [17, 20].

Patchouli leaf composed of multiple tissues are upper epidermis, palisade, spongy parenchyma and below epidermal tissue. Cell or oil glands on the patcouli leaf are found in vacuoles on palisade and spongy parenchyma tissue.

The palisade and parenchymal tissues is protected with cell walls that composed of cellulose, hemicellulose and pectin, where hemicellulose are bound by lignin and other phenolic by covalent bonds while the bonds between cellulose and lignin unknown [17].
Thus, the presence of lignin and hemicellulose surrounds the cellulose is a major obstacle in isolating vacuoles (patchouli oil bags). It is necessary for the fungus *Phanerochaete chrysosporium* has the ability to break down lignin and oil glands in the vacuole so as to isolate the patchouli oil contained in the vacuole [11].

Lignoselulotik *Phanerochaete chrysosporium* is a fungus that produces an enzyme protease, quinone reductase, and cellulases that are able to degrade cellulose. *Phanerochaete chrysosporium* have optimum growth temperature of 40 ° C, pH 4-7, and aerobic [5, 21].

Patchouli oil isolated from the fermentation process usually followed by a purification process of patchouli oil are the extraction process In the extraction process, there are several factors that affect the quality of the extraction, among other things: the quality of raw materials, the type of solvent, contact time and the extraction procedure [12,16]. Type of solvent which has a low boiling point have a greater risk of loss due to the

evaporation process. While the solvent has a high boiling point should be separated at a higher temperature. Thus, the extraction product should be free of residual solvent in order not to affect the aroma [8]. Type of solvent that is often used is n-hexan. Meanwhile, to improve the quality of patchouli oil especially to provide a clearer color needed adsorption process using activated carbon or bentonite.

Method

The research was conducted in laboratory scale using closed fermentor with a capacity of 12 lt made of glass. Fermentor filled with substrate patchouli leaves in media as 60% of fermentor volume so that there is air space by 40%. Fermentor having stirrer rotating at a speed of 60 rpm. While microorganisms are used to degrade patchouli leaves is *Phanerochaete chrysosporium.* Experiments conducted in several stages, among others: pre-treatment leaves (preparation), regenaration of fungi *Phanerochaete chrysosporium*, patchouli leaf fermentation, purification patchouli oil and analysis of patchouli oil. While the variables used are pH (4, 5 and 6); fermentation time (15, 16, 17, 18, 19, 20, 21 and 22 hours).

The research was initiated with the pretreatment of patchouli leaves, which patchouli leaves are dried, reduced in size (patchouli leaf powder) then analyzed the water content, lignin and cellulose using Cheson methode [13]. At the stage regeneration, *Phanerochaete chrysosporium* isolates were grown on PDA (Potatoes Dextrose Agar) to achieve productive phase. Then make a growth curve with media NLM (Nitrogen Limited Media) to determine time the growth of *Phanerochaete chrysosporium* and measuring the amount of *Phanerochaete chrysosporium* using dry cell mass. In the fermentation stage, patchouli leaf powder put in the fermentor is then added isolates.

Phanerochaete chrysosporium and using solvent buffer pH 4, 5 and 6. Comparison of solvent with patchouli leaves is 1 liter NLM: 180 grams of powder patchouli leaves, then add 20% of *Phanerochaete chrysosporium* and 1.6% molasses as nutrients for fermentation [6]. Fermentation running for 22 day, in which the sampling begins at the 15th day.

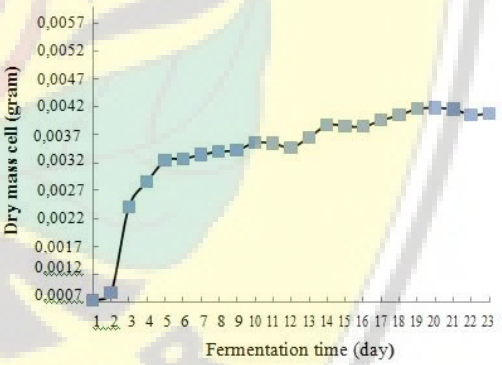
Futhermore, the results of the fermentation was macerated with solvent (n-hexane) for 5 days solids. Liquid (filtrate) is composed of two layers are the top layer (patchouli - n-hexane) and the lower layer (water). The second layer was separated using a separating funnel. Furthermore, patchouli oil and n-hexane was separated again using a distillation so that the n-hexane obtained as a distillate.

Patchouli oil from the distillation then adsorption using bentonite at a temperature of 55C (for 1 hour) and then allowed to stand for 24 hours. Then filtering to separate the oil from the bentonite. Patchouli oil obtained analyzed for concentration of Patchouli alcohol (PA), the yield and the refractive index.

Result and Discussion

Results of analysis of water content inpatchouli leaves before fermentation by 4.63%. Meanwhile, reference [20] mentions that the water levels are good by 12-15%. The low water levels caused by storage factors patchouli leaves too long before it is used for research.

Results of measurement of the number of isolates of *Phanerochaete chrysosporium* using dry mass cell showed that on 5th day occurred stationary early phase of growth of *Phanerochaete chrysosporium* (Figure 1), Where the number of *Phanerochaete chrysosporium* maximum growth. Thus, on 5th day start LiP and MnP enzyme produced by *Phanerochaete chrysosporium*, where these enzymes a very important role in the isolation of patchouli oil in patchouli leaves.so that



all the oil can be removed. Results maseration is filtered to separate the liquid from

Figure 1. The growth curve of *Phanerochaete chrysosporium*

Results of analysis of the content of lignin and cellulose patchouli leaves before fermentation as well as lignin and cellulose33.83% and 34.22% respectively Lignin and cellulose content after fermentation are 6.56% and 10.22% respectively. Thus occurred the reduction of 80.61% lignin and 70.13% cellulose

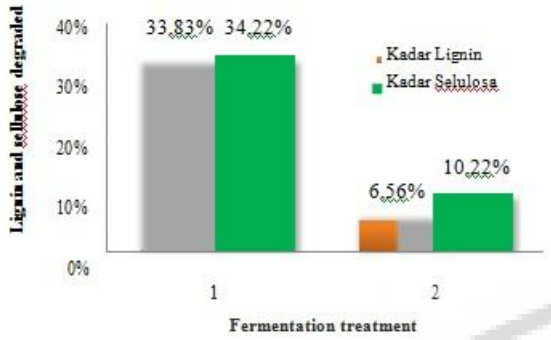


Figure 2. The lignin and cellulose level for before and after fermentation
Remark: 1 :Before fermentation
2 : After fermentation

Reference [14] mentions that the use of *Phanerochaete chrysosporium* of fermentation process can reduce the lignin content of 96.88%, whereas on 30th day, the lignin which decreased by 96.88% while cellulose decreased by 17.70%. while Reference [26] mentions that the white rot fungus effective in degrading cellulose compared lignin.

Effect of pH on patchouli oil yield

The analysis result of patchouli oil fermentation showed that the pH constant, the value of yield increases with fermentation time. Meanwhile, the lower the pH value, the greater the yield obtained (Figure 3).

Increased yield are caused the longer fermentation time which the ability of *Phanerochaete chrysosporium* fungus to produce enzymes to degrade lignin the higher so that the cell wall constituent of lignocellulose as patchouli leaves much degraded tissues [15]. The amount of lignocellulosic much degraded indicated by decreased levels of lignin and cellulose are high on patchouli leaves after fermentation treatment [26].

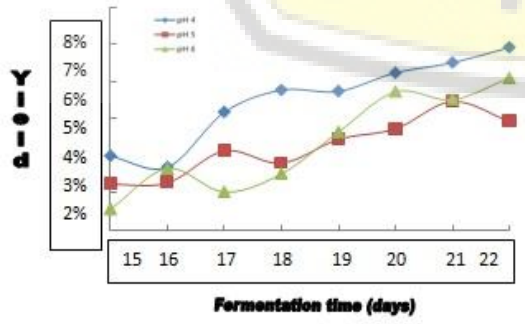


Figure 3. Patchouli oil yield during fermentation at different pH

Content after fermentation shows that there were a decrease in the concentration of both the material. This shows that both the material (lignin and cellulose) has undergone a process of degradation by enzymes produced by *Phanerochaete chrysosporium*.

The highest yield was achieved in 22 days of fermentation time and pH 4 that is equal to 6.920%. While the lowest yield achieved on fermentation time of 15 days and a pH of 6 is equal to 2.565%. Reference [7, 22] mentioned that fermentation using *Phanerochaete chrysosporium* on patchouli leaves can produce the highest yield of 10%.

The magnitude of the yield is influenced by several factors, among others, the factors of physical properties of patchouli leaves which have a water content of 4.63% (less than that required for fermentation). Reference [1], mentions differences in the composition and amount of oil constituent are caused by the variability of subspecies of plants.

pH is one of the factors that influence the fermentation process. Figure 3 shows that the lower the pH the greater the yield generated, where the highest yield of 6.92% achieved at pH 4 with 22-day fermentation time.

Reference [13] states that the optimum pH for fermentation using *Phanerochaete chrysosporium* at pH 5, whereas at pH <3 the fermentation reduce its performance. This is due *Phanerochaete chrysosporium* in the activity produces an enzyme protease and sellulose which can lower the pH value.

Effect of pH on Patchouli Alcohol (PA)

The quality of patchouli oil can be seen from the amount of Patchouli alcohol levels. In the figure 4 shows that the longer the fermentation time, the greater the level of PA in patchouli oil. Furthermore, the greater the pH, the greater the level of PA in patchouli oil. The PA level highest is 37.69% achieved during the 22-day fermentation and pH 6. The PA levels lowest was 29.63% achieved in the fermentation time of 15 days and a pH of 4.

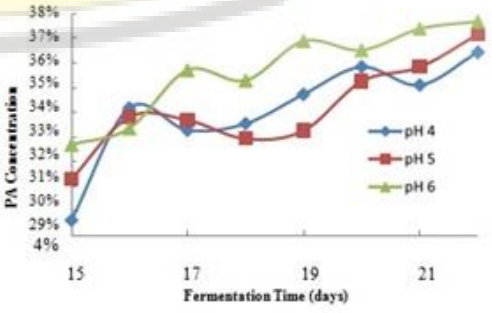


Figure 4. Levels of Patchouli alcohol (PA) during fermentation at different pH

The PA is also influenced by the process of separation (extraction) and purification (adsorption). Reference [10] mentions that solvent separation techniques to determine the amount and quality of residual solvent extraction.

Effect of pH on refractive index

Figure 5 shows that the highest refractive index of 1.4922 is achieved during the 22-day fermentation and pH 6. The low refractive index is 1.4724 at 16-day fermentation and pH 4.

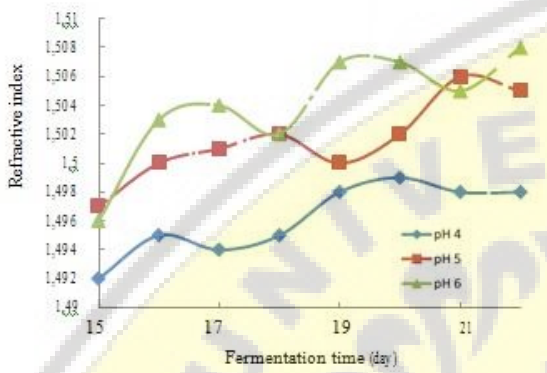


Figure 5. Refractive Index patchouli oil during fermentation at different pH

The refractive index of patchouli oil according to (SNI 06-2385-2006) is 1.507 - 1.515. Figure 5 shows that the refractive index of patchouli oil is best of 1.4922 at pH 6 and 22-day fermentation period that does not meets the standard SNI. The high refractive index at 22 days fermentation caused by the separation of oil fractions having a long chain of carbon atoms in the distillation process. So it is with length of maceration process will increase the value of the refractive index. This is because the longer the time the extraction process, the more weight fraction extracted components that increase the refractive index [9]. The refractive index increases in patchouli oil which has a constituent component of the carbon chain length and also the presence of a number of double bonds [12]. The presence of a long chain of carbon atoms may inhibit and deflect (refract) the rate of the light.

Reference [26] mentions that ligninolitik enzyme activity increased in the presence of oxygen and pH optimum conditions, where the rate of remove of lignin increased with increasing content of oxygen (aerobic conditions) and the optimum growth of fungi *Phanerochaete chrysosporium* at pH 5. However, the amount of transmitted through the medium of patchouli oil.

Conclusion

It can be concluded that: The best operating conditions in the fermentation of patchouli leaves is reached at the time of fermentation for 22 days and pH 6 for PA (37.69%), and refractive index (1.508). While the largest yield obtained at the time of fermentation for 22 days, and pH 4 was 6.92%.

Acknowledgments

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FRAGMENT DNA 387BP GENE LECTIN of SOYBEAN (*Glycine max* (L.) Meriil) VARIETIES DETAM 2

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Abstract

Lectin gene is a housekeeping gene that can be used as a molecular marker soybean (*Glycine max* (L.) *meriil*.). This study aimed to obtain the identity of the lectin gene molecular markers for breeding purposes. This descriptive study was performed using PCR amplification and identification of sequences using a lectin gene fragment sequencing techniques and phylogenetic search using Mega Tree programme. Lectin gene identification has been conducted on variety Detam 2. The results obtained are lectin gene fragment along 387bp used primer Leic Foward GCGGAAACTGTTTCTTTCAGCTGG and primer Leic Reverse CGGAAAGTGTAAACTCAACAGCG.

Keywords: soybean variety detam 2, lectin gene, housekeeping gene, molecular marker

Introduction

Protein, fats, and carbohydrates in soybeans has been studied several decades. Especially since the discovery of compounds isoflavones in soy could be expected to reduce the risk of cancer, heart disease, and osteoporosis, and also reduce the symptoms of menopause. Soybeans in Indonesia has a high protein content such as varieties detam 2. Soybean varieties is a kind of black soya. The highest protein content mainly conceived in black soya beans (Glycine max (L.) Merrill) variestas Detam2 with protein content reached 45.58% dry weight [3].

This study investigates soy DNA fragments (387bp) fragment of the lectin gene of soybean (*Glycine max*) varieties detam 2. Soybean lectin (SBL) specifically binds to terminal N-acetyl-D-galactosamine with greatest affinity and to a lesser extent with D-galactose. Lectins are carbohydrate-binding proteins or glycoproteins that occur widely in plants, animals and microorganisms (Vural, *et al.*, 2010). Protein lectins have been found mostly in seeds of Legumes [4]. Lectin gene is a housekeeping gene that can be used as a molecular marker soybean *(Glycine max* (L.) Meriil.). Lectin protein in soybean plants can be found in vegetative organs such as leaves, stems and roots. lectin protein content in vegetative organs is less than the levels in soybean seeds [6]. Differences in levels of protein lectins not only the location but also can be different for each variety of soybean [2]

PCR has already proved its worth as an analytical method for the detection of genetic organism material in seed or leaf, through its simplicity, specificity, and sensitivity in this study we isolated genomic DNA from soybean seed coat varieties detam-2 with DNA techniques. We use RIDE DNA technique modification that combines conventional DNA isolation techniques on animal tissues and DNA isolation technique with TriPure solution. We assume the isolation of DNA in the seed coat requires special techniques and require a long time to lyse cell walls and cell membranes.

Materials and Methods

DNA Isolation

Soya bean seeds soaked in water for 10-15 minutes, then take the skin of the seeds and weigh 0.3 grams. Next, prepare a mortar and pestle to soften the seed coat by means pounded, after fine inserted into microtube 1,5µL. Insert a 1 ml cell lysis DNA, Proteinase K 70µL, 80µL 20% SDS solution into the microtube. Then incubated for 2 hours. Insert a 7.4 ph STE 600µL into microtube, then the formation of pellet and supernatant portion. A total of 500µL supernatant was transferred to a new microtube, then put 1 ml Tripure. Samples were incubated for 5 min at room temperature (while inverted until homogeneous), then centrifuged at 10,000 rpm for 12µL. Formed supernatant and pellet, then take a supernatant. Add Tripure into supernatant, then added 100µL klroform cold, then incubated for 8 minutes, centrifuged 10,000 rpm for 13 minutes. Take the supernatant, and then is moved to mikrotube. Then added 500µL isopropanol. The next step, the sample divortex, then incubated at room temperature for 5-10 minutes. Then centrifuged 10,000 rpm for 10 minutes Discard the supernatant formed after the vortex, then add 1000µLdan cold 75% ethanol, and do Centrifuge 7500 rpm for 5 minutes. Discard the supernatant, then vacuum until

dry and added as much as 50µL buffer Rehydration Solution.

PCR Primers

Design primers Leic for amplification of regions of the soybean lectin gene. Leic Primer foward dan reverse use sequence nucleotide : Leic Foward 5'GCGGAAACTGTTTCTTTCAGCTGG'3 (24bp), %GC : 50%. and primer Leic Reverse 5'CCGGAAAGTGTCAAACTCAACAGCG'3(25 bp), %GC : 52% with size fragment PCR product 387bp.

Standard PCR Assays

Each amplification reaction contained 1× reaction 5.5ul nuclease free water, 2ul DNA genom, 1ul Leic Primer foward, 1 ul Leic primer reverse and 12.5ul Kappa Taq Polymerase,were as follows: denaturation for 3 min at 94ºC; 40 cycles of 30 s at 94ºC, 30 s at 60ºC, and 45s at 72ºC; and a final extension o10 min at 72ºC.

PCR Fragment Analysis

Analysis of amplified DNA fragments were electrophoresed on 2% agarose gels in 1× TAE buffer, and bands were visualized by ethidium bromide staining and UV transillumination.

Result and Discussion

Research produces DNA that can be used as the identity of molecular lectin gene in the soybean Glycine max (L) Merr, and produce primers products foward and reverse for lectin gene of soybean Glycine max (L) Merr which can be used as reference gene identification lectins in beans soy soybean varieties other. This research proved that the seed coat can be made of soybean genomic DNA isolation. The mature seed contains about 3% of the weight of it (Laija et al.,2010). The biological activities like anti-tumor, anti-proliferative, immune potentiating, antibacterial, antifungal, anti-insect, and antiviral activities have been found in lectins. Lectin compounds in black soya beans have been known to have hemagglutination activity, the enzyme reverse transcriptase inhibitor of HIV-1,

The concentration of soybean genomic DNA obtained: 68.08 ug /ul and purity of the DNA of 1,619. purity DNA results are still contaminated by protein and phenol as Tripure solution in addition to isolating the DNA also can isolate RNA and proteins in a single reaction. But that does not mean there are no soybean genomic DNA. No soybean genomic DNA and results in the isolation of proteins and there is still a phenol solution covering absorbance values. Results of DNA purity value which little can be enhanced with PCR amplification methods. PCR amplification methods require 100ng / ul of genomic DNA. The concentration of soybean genomic DNA obtained 68.08 ug / ul, the volume of soya bean DNA used for PCR amplification techniques as much as 2 ul or 136.16 ug / ul.

Conclution of soya bean lectin gene amplification fragment 387 bp (Figure.1).

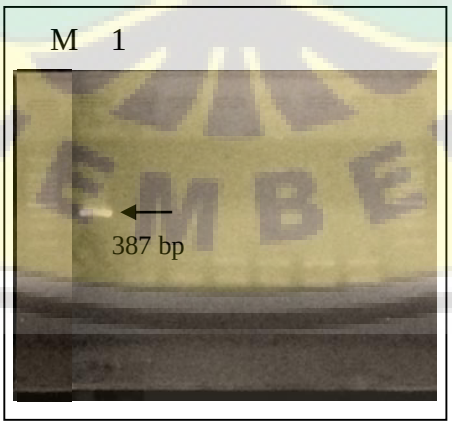

Figure 1: Fragments lectin gene of soybean Glycine max (L) Merr 387bp length using gel electrophoresis agarose 2%

antitumor and can bind to specific carbohydrate compounds that make up the cell membrane of bacteria and viruses [2]. Consumption of lectins derived from soy canincrease the activity of the pancreas to produce insulin in diabetics [4]. Lectin proteins also recognize receptors on the tumor cells so that the lectin protein can also act as an agent of the target molecule tumor cell death [1]. Lectin compounds have useful functions in the field of health.

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RESPONSE of SIX GENOTYPES SOYBEAN on THE DOSE of NPK FERTILIZER

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Abstract

National soybean production can only meet 30% of needs. In connection with the production of soybeans are not enough national needs it is necessary to increase the production. Effort to increase soybean production can be done through the use of improved seed and fertilizer. The purpose of this study are: 1) Examine the response of six genotype soybeans on the dose of NPK fertilizer, 2) Determine the protein content in the seeds of six genotype soybeans, 3) Knowing the interaction between soybean genotypes with dose of NPK fertilizer on the growth and yield. The experiment was conducted in plastic house experiment Faculty of Agriculture, University of General Soedirman. Research carried out since July 2011-September 2011. The experimental design used was Randomized Block Design with 3 replications. The treated factors were the soybean strains or soybean varieties and four levels NPK fertilizer. Soybean strains or soybean varieties consisted of: L/S:B6-G1 (V1), L/S:B6-G3 (V2), L/S:B6-G4 (V3), Grobogan (V4), Burangrang (V5), and Argomulyo (V6). Four levels NPK fertilizer were: 0 kg/ha (N0), 100 kg/ha (N1), 200 kg/ha (N2), and 300 kg/ha (N3). Data was analyzed using F test, if there was varied among treatments followed by Duncan's Multiple Range Test (DMRT) at 5% error level of significance. Regression test is then performed to obtain the response pattern of soybean varieties or soybean starains of the levels NPK fertilizer. The results showed that the genotypes that had been give different response to the increase dose of NPK fertilizer. The best response obtained in strains of soybean L/S: B6-G1 at dose of NPK 300 kg / ha with the highest seed number per plant 184 seeds (32,5 g). The results of this strain was higher than comparable varieties Agromulyo to increase results by 46%. The increased of protein content caused by addition of NPK fertilizer. The highest protein content found in Argomulyo that is 34,5% at application of NPK fertilization 300 kg/ha. In addition there is a positive interaction between the six genotypes tested at a dose of NPK fertilizer in a variable number of flowers per plant, number of seeds per plant, grain weight per plant and protein levels.

Keywords: dose, genotypes, soybean, NPK fertilizer

Introduction

Soybean has long time cultivated in Indonesia, but the development of planting area is very slow in the pattern of agricultural cultivation, soybean never became a major crop. Soybean production in Indonesia in 2013 only 851.647 tons of dry beans and productivity 1.48 tons ha^{-1} while the national soybean needs as much as 2.3 million tons. National shortage of soybean demand is supplied through imports as much as 1.7 million tons in 2010 and increased to 1.9 million in 2012 [3]. According [9] as a source of vegetable protein, soybean contains a variety of nutrients, ie every 100 g of material contained 330 calories, 35 g protein, 18 g fat, 35 g carbohydrates, 227 mg calcium, 585 mg of phosphorus, 8 mg iron, 110 SI vitamin A and 1 mg of vitamin B.

One effort to improve soybean productivity is the use of high yielding varieties. Attempts to obtain improved varieties can be done through breeding programs [12]. Multi-location test is an effort that aims to identify promising lines that show high and consistent results. If the test results in some places getting strains consistent high yield, then these strains can

be recommended to be used as new varieties. The strain used for this test is strain L/S: B6-G1, L/S: B6-G3 and L/S: B6-G4 originating from Faculty of Agriculture Jenderal Soedirman University. These strains are descended from crosses between Lokon Sindoro males and females. The new strains are expected to have a high yield, aged early maturing and high protein content as suitable for the manufacture of tempeh [13].

So, soybeans can produce well require macro nutrients N, P, and K and micro nutrients. The nutrients can be taken up by plants in the form, if available, while the nutrients in the soil are not always in the form available to plants. If in such circumstances, the plant growth will languish and results unsatisfactory. Fertilization in soybean can increase growth and yield if sufficient nutrients are available. That the results appear faster then use mainly inorganic fertilizer NPK compound fertilizer because it contains three main elements as

well and is available in the form of organic ions that plants need in infancy [2].

This study aims to: 1) Examine the response of six genotype soybeans on the dose of NPK fertilizer. 2) Determine the protein content in the seeds of six genotype soybeans. 3) Knowing the interaction between soybean genotypes with dose of NPK fertilizer on the growth and yield.

Research and Methods

The experiment was conducted in a plastic house the Faculty of Agriculture, Jenderal Soedirman University, Purwokerto District of North Village Karangwangkal, Banyumas regency, Central Java. Planting is done in a polybag containing homogeneous soil. The type of soil used was Inceptisol. Altitude of 110 meters above sea level (asl), the average temperature of 29^{o}C, the average rainfall of 284 mm/month, and an average humidity of 93%. Materials used in this study includes three lines of soybean, L/S: B6-G1 (V1), L/S: B6-G3 (V2), L/S: B6-G4 (V3), and 3 varieties of soybean seed Burangrang (V4), Grobogan (V5) and Argomulyo (V6). NPK compound fertilizer, some pesticides, fungicides, and insecticides adapted to the symptoms of an attack. The equipment used in this study include hoes, sprinklers, oven, rope, measuring cups, stationery, long ruler, label paper, envelope paper, plastic, electric scales, stationer. This study is a pot experiment, treatment forms factorial design with randomized treatment groups (RAK). Factors to be tested in this research that soybean genotypes consisting of 3 lines and 3 varieties, and 4 levels of NPK fertilization. The first factor is 3 lines of origin UNSOED L/S: B6-G1 (V1), G3 (V2), G4 (V3) and three varieties of soybean is Grobogan (V4), Burangrang (V5), and Argomulyo (V6). The second factor is the level of NPK fertilizer with four levels ie NPK 0 g/polybag (N0), 0.6 g/polybag (N1), 1.2 g/polybag (N2), 1.8 g/polybag (N3). Combination treatment there were 24 with three replications and each combination treatment consisting of three polybags, so that all are 216 polybag. Variables observed included plant height, number of leaves per plant, number of flowers per plant, days to flowering, harvesting age, the number of productive branches per plant, number of branches per plant, number of pods per plant, number of seeds per plant, seed weight per plant, weight of one hundred seeds, and protein content. Data were analyzed by F test to determine the effect of each treatment were tested. If there is a significant DMRT followed by 5%. Regression test is then performed to obtain the pattern of response of soybean varieties or strains of the level of NPK fertilizer.

Result and Discussion

Table 2. Matrix analysis variance of the experiment

No.	Variable	V	N	VxN
1.	Height of plant (cm)	**	**	ns
2.	Number of leaves (leaf)	**	**	ns
3.	Number of flowers per plant (flower)	**	**	*
4.	Days to flowering	**	ns	ns
5.	Harvesting (days after planting)	**	ns	ns
6.	Number of productive branches per plant (fruit)	**	**	ns
7.	Number of branches per plant (fruit)	*	**	ns
8.	Number of pods per plant (fruit)	**	**	ns
9.	Number of seeds per plant (fruit)	**	**	*
10.	Seed weight per plant (g)	**	**	**
11.	One hundred seed weight (g)	**	ns	ns
12.	Protein content (%)	**	**	**

Note: V = genotypes (varieties or lines); N = Dose of NPK fertilizer; VxN = interaction of genotype with NPK fertilizer; ** = very significant; *= significant; ns = not significant.

Table 2. Average variable observation of the response of six genotypes soybean on the dose NPK fertilizer

Treatment	HoP (cm)	NoL (leaf)	NoFPP (fruit)
Genotypes (V)			
V1 (Lines L/S: B6-G1)	69.1b	21.0b	34.0d
V2 (Lines L/S: B6-G3)	58.9bc	15.6c	45.2bc
V3 (Lines L/S: B6-G4)	101.7a	24.8a	39.9cd
V4 (Grobogan)	57.3c	16.7c	47.6b
V5 (Burangrang)	67.3bc	16.8c	59.4a
V6 (Agromulyo)	59.6bc	19.0b	44.4bc
Dose of NPK (N)			
N0 (Control)	59.7b	16.5c	33.8c
N1 (NPK 0.6	71.1a	18.5b	45.3b

g/polybag)			
N2 (NPK 1.2 g/polybag)	71.7a	19.4b	47.0b
N3 (NPK 1.8 g/polybag)	73.4a	21.6a	54.2a
Treatment	DF (dap)	H (dap)	NoPBPP (fruit)
Genotypes (V)			
V1 (Lines L/S: B6-G1)	32.3b	82.2a	2.4b
V2 (Lines L/S: B6-G3)	29.5d	79.3c	2.4b
V3 (Lines L/S: B6-G4)	33.1b	81.3ab	3.3a
V4 (Grobogan)	30.1cd	78.9c	2.3b
V5 (Burangrang)	34.3a	80.0bc	2.5b
V6 (Agromulyo)	30.8c	79.3c	2.6b
Dose of NPK (N)			
N0 (Control)	32.1a	80.1a	2.0b
N1 (NPK 0.6 g/polybag)	31.4a	79.6a	2.7a
N2 (NPK 1.2 g/polybag)	31.4a	80.2a	2.7a
N3 (NPK 1.8 g/polybag)	31.9a	80.7a	3.0a
Treatment	NoBPP (fruit)	NoPPP (fruit)	NoSPP (fruit)
Genotypes (V)			
V1 (Lines L/S: B6-G1)	69.1b	21.0b	34.0d
V2 (Lines L/S: B6-G3)	58.9bc	15.6c	45.2bc
V3 (Lines L/S: B6-G4)	101.7a	24.8a	39.9cd
V4 (Grobogan)	57.3c	16.7c	47.6b
V5 (Burangrang)	67.3bc	16.8c	59.4a
V6 (Agromulyo)	59.6bc	19.0b	44.4bc
Dose of NPK (N)			
N0 (Control)	59.7b	16.5c	33.8c
N1 (NPK 0.6 g/polybag)	71.1a	18.5b	45.3b
N2 (NPK 1.2 g/polybag)	71.7a	19.4b	47.0b
N3 (NPK 1.8 g/polybag)	73.4a	21.6a	54.2a
Treatment	SWPP (g)	OHSW (g)	PC (%)
Genotypes (V)			
V1 (Lines L/S: B6-G1)	19.3a	16.4b	33.3d
V2 (Lines L/S: B6-G3)	12.4c	18.6a	33.5c
V3 (Lines L/S: B6-G4)	16.3b	15.6bc	33.3e
V4 (Grobogan)	12.2c	15.5bc	34.2b
V5 (Burangrang)	12.6c	14.5cd	34.2b
V6 (Agromulyo)	13.1c	13.7d	34.4a
Dose of NPK (N)			
N0 (Control)	10.7c	15.6a	33.8c
N1 (NPK 0.6 g/polybag)	13.0b	15.5a	33.8b
N2 (NPK 1.2 g/polybag)	14.3b	15.8a	33.9a
N3 (NPK 1.8 g/polybag)	19.11a	16.0a	33.9a

Remarks: Figures followed by the same letter are not significantly different at the 5% level DMRT. Height of plant (HoP), NoL (number of leaves), NoFPP (number of flowers per plant), DF (days to flowering), H (harvesting), NoPBPP (number of productive branches per plant), NoBPP (number of branches per plant), NoPPP (number of pods per plant), NoSPP (number of seeds per plant), SWPP (seed weight per plant), OHSW (one hundred seed weight), PC (protein content).

Differences Between Soybean Genotypes In The Growth and Yield

The results showed that the growth variables and the results of that plant height, number of leaves per plant, number of flowers per plant, days to flowering, harvesting age, the number of branches per plant, number of productive branches per plant, number of pods per plant, number of seeds per plant, weight of seeds per plant, weight of one hundred seeds, and protein content give a significant response. High crop strains L/S: B6-G4 is a genotype which has a plant height highest of 101.7 cm whereas the varieties Grobogan is a genotype which has a plant height of most low at 57.3 cm and not significantly different from the strain L/S: B6-G3 (58.996 cm), L/S: B6-G1 (69.106 cm), varieties Burangrang (67.307 cm), and the varieties Argomulyo (59.643 cm). The difference in mean value between the genotypes of high plant caused by the genetic characteristics of each genotype soybeans. The ability of plants in response to environmental conditions to grow indirectly illustrates the power of adaptability to its environment [4].

Genotype had the highest number of piece of leaves is strain L/S: B6-G4 (24.8 leaf), whereas genotype with the lowest number of leaves is strain L/S: B6-G3 (15.6 leaf). Strain L/S: B6-G1 (21.0 leaf) has a piece of trifoliate leave number per plant more varieties of Grobogan (16.7 leaf), varieties Burangrang (16.8 leaf), and varieties Argomulyo (19.0 leaf). Leaves necessary for the absorption and

conversion of solar light energy into growth and yield crops through photosynthesis [5].

The number of flowers per plant that formed most varieties Burangrang (59.4 flower) while the number of flowers per plant that is at least possessed strain L/S: B6-G1 (34.0 flower). Grobogan varieties (47.6 flower) did not differ with strain L/S: B6-G3 (45.2 flower), and varieties Argomulyo (44.4 flower) but different from the strain L/S: B6-G4 (39.9 flower). This interest is due to differences in the number of plant responses to day length and temperature range as well as an assortment of other environmental factors [13]

Age flowering of genotypes tested ranged from 29.5 to 34.3 days after planting. Strain L/S: B6-G3 is the fastest flowering genotype compared to another genotype is 29.5 days after planting but not significantly different plant varieties Grobogan (30.1 dap), and the varieties Argomulyo (30.8 dap). Varieties Burangrang has a lifespan of flowering longer is 34.3 days after planting it in accordance with the variety description Burangrang showing the old days to flowering 35 days and significantly different from the strain L/S: B6-G4 (33.1 dap) and L/S: B6-G1 (32.3 dap). The duration of flowering at the age of genotypes tested is determined by the length of the vegetative phase to determine the extent of the resulting pile photosintate at flowering, because most of the dry weight of the plant comes from assimilates produced after flowering (Goldsworthy, 1992).

In Table 2, looks varieties Grobogan (78.9 dap) has the fastest harvest age but not significantly different from the strain L/S: B6-G3 (79.3 dap) and varieties Argomulyo (79.3 dap). Harvesting the longest owned by strain L/S: B6-G1 (82.2 dap) and significantly different from the strain L/S: B6-G4 (81.3 dap) and varieties Burangrang (80.0 dap). Krisnawati and Adie (2007), states that soybean short life measured from harvesting plants that are less than 80 days. While the strain L/S: B6-G1 (V1), L/S: B6-G4 (V3) and varieties Burangrang (V5) ranges of more than 80 days that includes genotyping age being.

Establishment of productive branches soybean crop is affected by the absorption of nutrients and light reception. The number of productive branches at most is strain L/S: B6-G4 (V3) as many as 3.3 fruit and number of branches earning the least are varieties (V4) is 2.3 fruit but not significantly different from the strain (V1) 2.4 fruit, strain (V2) 2.4 fruit, varieties (V5) 2,5 fruit and varieties (V6) 2.6 fruit. Strain L/S: B6-G4 (V3) has a number of pods plant Most ie 63.4 fruit, not significantly different from the strain L/S: B6-G1 (V1) and significantly different from the strain L/S: B6-G3 (V2), varieties Grobogan (V4), varieties Burangrang (V5) and varieties Argomulyo (V6). The difference between the number of pods per plant strains and varieties allegedly caused by plant morphology among strains with different varieties, visible from the plant height, the number of piece of trifoliate leave per plant and the number of different flowers.

Number of seeds per plant on the lines and varieties tested ranged from 72.3 to 122.0 fruit. Strain L/S: B6-G1 has a number of seeds per plant at most that 122.0 fruit significantly different from the strain L/S: B6-G4 is 103.4 fruit, varieties Grobogan 84.6 fruit, varieties Burangrang 92.4 fruit, and varieties Argomulyo 94, 6 fruit , while the strain L/S: B6-G3 has the lowest number of seeds of plants which 72.3 fruit. The number of seeds per plant associated with the large number of productive branches and number of pods per plant. In this study, the genotype that has a number of productive branches and number of pods per plant that is strain L/S: B6-G4 (V3). According [11], the yield per plant related to the number of pods, number of seeds and seed weight per plant.

Seed weight per plant indicates the magnitude of each plant's ability to produce seeds. Research data shows that there are very real differences between the strains and varieties tested. Strain L/S: B6-G1 (19.3 g) and L/S: B6-G4 (16.3 g) has a weight of seeds per plant were higher than the strain L/S: B6-G3 (12.4 g), varieties Grobogan (12.2 g), varieties Burangrang (12.4 g), and the varieties Argomulyo (13.1 g). According [7], seed weight per plant has close links with the yield components include the number of pods per plant and seed size.

The results showed the weight of one hundred seeds were tested turned out to have a weight of 100 g seeds ranged between 13.7-18.6 g. Strain L/S: B6-G3 (18.6 g) having the highest weight of one hundred seeds among genotypes tested. This is supported by a number of factors that determine the size of soybean seeds, including genetic factors, according to [1] the weight and size of the seeds is the quantitative nature of the genetic factors that in certain circumstances may change according to the environment to grow plants.

Differences in levels of soybean protein closely related to genotype tested and micro-climatic conditions at the time of the study. Highest protein content owned by varieties Argomulyo (34.4%), while the levels of the protein was low at strain L/S: B6-G4 (33.3%) and a significantly different genotype other is strain (V1) 33.37%, strain (V2) 33.5% varieties (V4) 34.2%, and varieties (V5) 34.2%. It is influenced by differences in genetic characteristics of each plant in addition to the respiration and photorespiration soybean crop increases with increasing ambient temperatures also increase the ratio of O_2/CO_2 available [8]

Effect of Dose NPK Fertilizer on Growth and Yield

The results showed that a dose of NPK fertilizer significantly in almost all the variables observed except at the age of flowering and harvesting. Soybean plants treated with doses of N0, N1, N2 and N3 show growth and results vary, it shows the response of varieties or strains of the dose of NPK fertilizer. Results of the analysis of plant height highest to lowest in Table 2 show the dosing N3, N2, N1 and N0 is 73.4 cm; 71.7 cm; 71.1 cm; and 59.7 cm. Number of leaves per plant at most until a little fertilizer dosage indicated by N3, N2, N1 and N0 is 21.6; 19.4; 18.5; 16.5 in units of strands per plant. Increased plant height as a factor of NPK nutrients that function in the formation of protoplasm, cells multiply and vegetative growth and improve yield and grain protein content [5].

Apparently, the amount of interest, the number of productive branches and the number of branches per plant affected by the provision of different fertilizers Table 2. The highest amount of interest at a dose of N3 (54.2 fruit) and lowest in N0 dose is 33.8 fruit. The highest number of productive branches also on the dose of N3 (3.0 fruit) but the lowest on N1 dose (2.0 fruit). Total number of branches per plant also showed the same thing, at doses of N3 resulted in a number of branches per plant at most that 3.1 fruit and lowest in N0 dose is 2,0 fruit per plant.

Differences in the use of NPK fertilizers also cause differences in the results obtained from soybeans. N3 dose weight of seeds per plant showed the highest of 19.1 g dose followed N2, N1 and N0. In the soybean crop production component elements are the elements most responsible NPK nutrients in addition to the other, because the role in the formation of pods, charging pods, seed formation, protein formation, root growth, and strengthen the growth of plants [10].

Table 2 shows the number of pods per plant was the most widely produced by the treatment of N3 is 60.9 fruit and most low at N0 40.7 fruit per plant. The number of seeds per plant at most also generated by N3 is 120.0 fruit per plant followed, namely 98.2 fruit N2, N1 87.6 fruit lowest and N0 as much as 73.7 fruit per plant. Results of the analysis showed significant different influenced NPK elements that play a role in increasing the number of cells and chlorophyll is able to increase the yield assimilate. Cell formation is very important to keep the plant cells where photosynthesis and assimilate the process can be maintained well, the results are quite assimilates will increase the number of pods and seeds of soybean.

NPK fertilizers are also significantly different from the results of the analysis of one hundred seeds weight and protein content. Data Table 2 results show the highest weight of one hundred seeds at a dose of N3 (16.0 g) and the highest protein content is also on the dose of N3 (33.9%). This is presumably because phosphorus is absorbed when the plant experienced periods of vegetative partially contained in the meristem tissue for protein synthesis, whereas in plants that experienced generative period, the element phosphorus will accumulate in the fruit and seeds [6].

Interaction Between Genotypes with a dose of NPK

The results showed that the differences between varieties or strains with a dose of NPK fertilizer caused the number of flowers per plant, number of seeds per plant, seed weight per plant, significantly different protein content, while at the other observation variables showed no significant

differences. Variable observations did not show any significant interaction between varieties or strains with a dose of NPK fertilizer showed that the difference in uniform on each variable due to the influence of each factor independently treatment.

Table 3. Interaction between soybean genotypes with NPK fertilizer dose on a variable number of flowers per plant

	NPK			
	N0	N1	N2	N3
V1	28.3aA	31.6 aC	36.4 aB	39.8 aC
V2	34.0bA	44.5 abBC	49.7 aAB	52.5 aBC
V3	33.5 aA	39.2 aBC	43.3 aAB	43.6 aBC
V4	39.1 bA	47.1 abB	49.6 abAB	54.8 aB
V5	34.4 dA	67.5 bA	53.1 cA	82.5 aA
V6	33.4bA	42.2 abBC	50.2 aAB	51.7 aBC

Note:
1. The numbers followed by lower-case letters (a, b and c) of the same on each genotype (V) in a row showed no significant difference in DMRT with error level of 5%.
2. The numbers followed by a capital letter (A, B and C) are equal to each dose of NPK fertilizer (N) in the column showed no significant difference in DMRT with error level of 5%.

Based on the Table 3, there is an interaction between the genotype with NPK fertilizer dose on a variable number of flowers per plant, apparently increasing doses of NPK fertilizer can increase the number of flower formation, it is because the plants are able to absorb nutrients given subsequently used for the formation of flowers.

Table 4. Interaction between soybean genotypes with NPK fertilizer dose on a variable number of seeds per plant

G	NPK			
	N0	N1	N2	N3
V1	13.5bA	14.5AB	16.7bA	32.4aA
V2	10.1aA	10.7aB	13.2aA	15.5aB
V3	12.3bA	17.3abA	15.4abA	20.0aB
V4	9.4Ba	12.7abAB	11.2abA	15.2aB
V5	9.9bA	12.5abAB	12.8abA	15.3aB
V6	9.2bA	10.4bB	16.7aA	16.1aB

Note:
1. The numbers followed by lower-case letters (a, b and c) of the same on each genotype (V) in a row showed no significant difference in DMRT with error level of 5%.
2. The numbers followed by a capital letter (A, B and C) are equal to each dose of NPK fertilizer (N) in the column showed no significant difference in DMRT with error level of 5%.

Basing Table 4 shows the results increase the dose of fertilizer increases the number of seeds per plant. There is an interaction between genotypes at a dose of NPK fertilizer on the variable of seeds per plant and the greater the dose of NPK fertilizer given the higher seed yield per plant. This is consistent with the statement Hardjowigeno (1995), NPK play an important role in cell division, energy transformation, assimilation of fat, improve work efficiency chloroplasts, stimulate flowering and accelerate the ripening seeds.

Table 5. Interaction between soybean genotypes with variable dose of NPK fertilizer on grain weight per plant

G	NPK			
	N0	N1	N2	N3
V1	33.2cE	33.3bD	33.4aE	33.4aE
V2	33.5aC	33.5ac	33.5aD	33.5aD
V3	33.3aD	33.3aD	33.3aF	33.3aF
V4	34.1bB	34.2bB	34.2aC	34.3aB
V5	34.1bB	34.2aB	34.2aC	34.2aC
V6	34.4bA	34.4abA	34.4abA	34.5aA

Note:
1. The numbers followed by lower-case letters (a, b and c) of the same on each genotype (V) in a row showed no significant difference in DMRT with error level of 5%.
2. The numbers followed by a capital letter (A, B and C) are equal to each dose of NPK fertilizer (N) in the column showed no significant difference in DMRT with error level of 5%.

The addition of seed weight per plant in response to increasing doses of fertilizer depends on the varieties or strains (genetic trait) plants. Each varieties or strains have different genetic characteristics from each other. Seeds per plant weight difference between varieties or strains and doses of fertilizer due to differences in genetic characteristics and its response to increasing doses of fertilizer in question.

Table 6. Interaction between soybean genotypes with a dose of NPK fertilizer at variable levels of protein

G	NPK			
	N0	N1	N2	N3
V1	33.2cE	33.3bD	33.4aE	33.4aE
V2	33.5aC	33.5ac	33.5aD	33.5aD
V3	33.3aD	33.3aD	33.3aF	33.3aF
V4	34.1bB	34.2bB	34.2aC	34.3aB
V5	34.1bB	34.2aB	34.2aC	34.2aC
V6	34.4bA	34.4abA	34.4abA	34.5aA

Based on the analysis of data shows the interaction of varieties or strains with a dose of NPK fertilizer. NPK fertilizer effect on protein content and the effect depends on the varieties or strains tested. Effect of NPK fertilizer doses cause increased protein content.

The protein content is highest in the V6 is Argomulyo varieties (34.5%) at doses of N3 and significantly different from the other genotypes at the same dose of fertilizer. Strain V1 (L S: B6-G1) is a genotype with the lowest protein content is 33.2% and was significantly different from the other genotypes fertilization with the same level. Contributions phosphate are quite high which is used as a constituent of ATP, ADP, NAD, NADPH which is the energy required transferor molecules in protein assimilation. According to [4], nitrate reduction process that occurs before the production of amino acids and other chemical compounds N combinations, requires electron; The main donor of electrons nicotinamide adenine dinucleotide is (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH), which is a result of photosynthesis.

Optimal dose of NPK Fertilization on Soybean Genotypes

G	NPK			
	N0	N1	N2	N3
V1	13.5bA	14.5bAB	16.7bA	32.4
V2	10.1aA	10.7aB	13.2aA	15.5aB
V3	12.3bA	17.3abA	15.4abA	20.0aB
V4	9.4bA	12.7abAB	11.2abA	15.2aB
V5	9.9bA	12.5abAB	12.8abA	15.3aB
V6	9.2bA	10.4bB	16.7aA	16.1aB

Optimal fertilization on each of the different varieties or strains according deangan ability of each variety or strain (Sunarto, 2008). The results showed significantly different responses from fertilizer NPK fertilization with four doses of the strains or varieties tested. NPK fertilizer application on the strains or varieties were tested with the highest level of high yields as well. The best fertilizer to obtain maximum results that the fertilization of 300 kg/ha. This situation is due to NPK nutrients that can increase the number of cells, chlorophyll, assimilate, formation and increase the number and seed weight.

Conclusions

1. Genotype who tried to have a different response to the increase of NPK fertilizer. The best response obtained in strain L/S: B6-G1 in the dose of 300 kg/ha with the result the number of seeds per plant and seed weight per plant highest of 184 fruit of seeds per plant and 32.5 g of seeds per plant. Results of this strain is higher than the varieties Argomulyo and increase in the result by 46%.
2. Provision of NPK fertilizer can increase the protein content of soybean seeds. The highest protein content contained in Argomulyo varieties (34.5%) with a dose of NPK fertilization of 300 kg/ha.
3. There is a positive interaction between the six soybean genotypes were tested with a dose of NPK fertilizer on a variable number of flowers per plant, number of seeds per plant, seed weight per plant and protein content.

Suggestions

Need to do advanced research in the field for the strain L / S: B6-G1 and L / S: B6-G4 in order to determine the production on a large scale and can be applied directly in the field.

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DEVELOPMENT and VALIDATION ANALYSIS METHOD of SODIUM CYCLAMATE and ACECULFAM-K in SUPPLEMENTS DRINK USING HIGH PERFORMANCE LIQUID CHROMATOGHRAPHY METHOD (HPLC)-UV

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Abstract

Na-cyclamate and acesulfame-K has been widely used in various types of food products included in the supplement drink. Methods High Performance Liquid Chromatography (HPLC) recommended by the *European Committee for Standardization* (CEN) was developed to separate and analyze Na-cyclamate and acesulfame-K on a supplement drink and determine parameters of the validity of the analytical methods are developed meet the criteria of AOAC as well as determine levels of inherent meet BPOM requirements. This study was conducted with a simple method without derivatization using a C_{18} column (10 µm; 3.9 x 300 mm); phosphate buffer mobile phase: acetonitrile (85:15); a flow rate of 1 ml / min; UV-200 nm detector. Linearity standard curve is generated each sweetener showed a good value (r> 0.99). Accuracy and precision shown good results, for Na cyclamate and acesulfame-K with an average of respectively 99.11% and 99.50%, while the value of the resulting relative standard deviation of each sweetener is 0.82% and 0 , 19%. Limits of detection for the Na-cyclamate and acesulfame-K was 6.25 ppm and 18.95 ppm, while the limit of quantitation of each sweetener 1.40 ppm and 4.23 ppm. Sweetener levels in samples supplement drink of 679.89 mg / kg product for Na-cyclamate and 209.22 mg / kg product for acesulfame-K. levels of Na-cyclamate and acesulfame-K obtained does not exceed the maximum limit set by the POM is 1000 mg / kg and 600 mg / kg of product.

Keywords: Na-cyclamate, acesulfame-K, supplements drink, validation, HPLC-UV.

Introduction

Na-cyclamate and acesulfame-K is a type of artificial sweetener that is widely used in food products or beverages in containers that are currently circulating in the market, especially in the beverage supplements. Na-Cyclamate is the sodium salt of the acid cyclamate which cause a sweet taste without the sense of follow-up (no after-taste it), has a sweetness 30 times the sugar and the price is relatively cheap, but Na-Cyclamates can bring many health problems, including tremor, migraine, memory loss, confusion, insomnia, irritation, asthma, hypertension, diarrhea, abdominal pain, allergies, impotence and sexual dysfunction, baldness, cancer of the brain and the bladder [1]. Acesulfame-K is a compound that does not smell, in the form of white crystalline powder, readily soluble in water and sweet taste with a relative sweetness level of 200 times the sweetness of sugar but not calories. Some studies show that acesulfame-K can not be digested, is nonglikemik and non carcinogenic. However, experiments in mice using high doses can cause genetic damage in mice [2].

To avoid the effects mentioned above, the predetermined maximum limit use of Na-Cyclamate and Acesulfame-K in beverages in Indonesia. POM (Food and Drug Administration) in Indonesia has set limits ADI for Na-cyclamate and acesulfame-K is 11 mg/kg and 15 mg/kg. POM also set maximum levels of use of Na-cyclamate and acesulfame-K in the product supplement drink each 1000 mg/kg and 600 mg/kg product [3].

To ensure that the levels of Na-cyclamate and acesulfame-K were added qualify are allowed BPOM, it is necessary to analyze the levels of artificial sweeteners that do not endanger the health if consumed by the public. The method recommended by the European Committee for Standardization (CEN) to analyze the Na-cyclamate and Acesulfame-K is the HPLC (High Performance Liquid Chromatography). This method has a good separation, sensitive, rapid, selective and sensitive, easy sample preparation and can be connected with an appropriate detector that is suitable for analyzing the content of the compound Na-cyclamate and acesulfame-K on a beverage supplement [4,5]. In the method of reference published by CEN EN 1999 with the number 12856, a method of analysis by HPLC-UV is specifically only intended to analyze the artificial sweetener acesulfame-K, aspartame and saccharin [6]. In the same year, with the number CEN EN 12857 issued methods for analyzing Na-cyclamate, through some special

stages that do trinitrobenzensulfonat derivatization by addition of acid, then analyzed by HPLC-UV method. Although both methods of analysis of artificial sweeteners are equally using HPLC-UV method, but there are some differences in the preparation of the artificial sweetener. Therefore, the researchers intend to develop a method of analysis with reference to the European Committee for Standardization (CEN) in order to obtain a new valid method for analyzing Na-cyclamate and acesulfame-K in the drink supplement with the HPLC method. The development is done in this method is that sample preparation is done without derivatization, using a mobile phase of phosphate buffer: acetonitrile (85:15) and using a single wavelength for the two compounds (200 nm). System suitability test and validation of the method refers to the Association of Official Analytical Chemist (AOAC) include: accuracy, precision, linearity, limit of detection (LOD) and Limit of Quantitation (LOQ) [7]. The purpose of this study is to determine the analytical methods developed can be used to separate and analyze Na-cyclamate and acesulfame-K in the product supplement drinks in containers and meet the validity criteria of AOAC as well as determine levels of Na-cyclamate and acesulfame-K contained in the product beverage supplement in the packaging meets the requirements of BPOM.

Research Methods

Material

Materials used in this study is a standard Na-cyclamate (Supelco), standard acesulfame-k (Supelco), acetonitrile HPLC grade (JT Baker), potassium dihydrogen orthophosphate pa (KH2PO4) (Merck), orthophosphoric acid 5% pa (Merck), akuabides (Ikapharmindo), supplement beverage samples containing Na-cyclamate and acesulfame-k, potassium hexacyanoferrate (II) trihydrate pa (K4 [Fe (CN) 6] .3H2O) (Sigma Adrich), zinc sulfate heptahydrate pa (ZnSO4. 7H2O) (Merck).

The equipment used is a suite of tools HPLC (Waters e2695), UV-Vis detector (Waters 2489), C18 column (300 mm x 3.9 mm x 10μm) (Waters μ Bondapak), centrifuge (Hanil), analytical balance semi-microbalance (Mettler Toledo SX 205 series), ultrasonic bath (Branson), a set of glasses (flask, pipette measuring, measuring cups, glass beaker) (Pyrex), solvent filtration membrane (MS®) and 0.45 μm syringe filter (Agela).

Optimization methods of analysis

Development of the method in this study begins with making optimization of analytical methods. This process is intended to get a good method or system that is capable of detecting and quantifying a mixture of Na-cyclamate and acesulfame-k in the sample supplement drink. Modifications made to the development of this method over the adjustment / possible design a mobile phase composition. The next best will be selected and the system can be validated.optimization of the mobile phase. The designs are designed based on the 2^2 factorial design, with four.

Table 1. Reference design methods of Na-cyclamate and acesulfame-K in accordance with CEN [8, 9]

	Mobile phase	The combined polarity index
Acesulfame-K reference method [18]	Acetonitrile: phosphate buffer (10 : 90)	8,68
Na-cyclamate reference method [19]	Methanol: Water (80 : 20)	5,88

Table 2. Design development of analytical methods Na-Cyclamate and Acesulfame-K

	Mobile phase	The combined polarity index
factorial design A	Acetonitrile: phosphate buffer (45 : 55)	7,56
factorial design B	Acetonitrile: phosphate buffer (35 : 65)	7,88
factorial design C	Acetonitrile: phosphate buffer (25 : 75)	8,20
factorial design D	Acetonitrile: phosphate buffer (15 : 85)	8,52

HPLC system set in the same conditions, the column C18, a flow rate of 1 mL/min and injection volume of 20 uL.

The extraction solution [8, 9]

Carrez solution 1

Dissolved 7.5 g of potassium hexacyanoferrate (II) trihydrate (K4 [Fe (CN) 6] .3H2O) into 50 mL aquabidest.

Carrez solution 2

Dissolved 15 g of zinc sulfate heptahydrate (ZnSO4.7H2O) into 50 mL aquabidest.

Making the mobile phase [8, 9]

a. Phosphate buffer solution (KH2PO4) = 0.0125 mol / L pH 4.3
   Dissolved 0.85 g of potassium dihydrogen orthophosphate with akuabides 400 mL in 500 mL glass beaker, pH adjusted to 4.3 using 5% orthophosphoric acid. Transfer to a 500 mL volumetric flask and add akuabides to mark boundaries.
b. Acetonitrile mobile phase and phosphate buffer solution (15:85)
   Put as much phosphate buffer solution into a 425 mL volumetric flask of 500 mL, 75 mL acetonitrile are added and mixed until homogeneous. Mobile phase made solution is filtered using 0.45 µm membrane filter and do degas for 5 minutes using ultrasonic bath.

Making stock solution (1500.4 ppm and 503 ppm)

Carefully weighed Na-cyclamate and acesulfame-k each as much as 75.02 mg and 25.15 mg. Na-cyclamate and Acesulfame-K are weighed and then dissolved in 50 mL volumetric flask with akuabides up to the mark (this solution contains

Making a series of levels

Made solution of Na-cyclamate/acesulfame-k level series with a concentration of 30.01/10.06 ppm, 60.02/20.12 ppm, 120.03/40.24 ppm, 240.06/80.48 ppm, 300.08/100.6 ppm, 600.16 /201.2 ppm using 10 mL volumetric flask. The concentration of each obtained with a pipette 0.2 mL, 0.4 mL, 0.8 mL, 1.6 mL, 2 mL and 4 mL of stock solution and placed in a 10 mL volumetric flask, added akuabides to mark boundaries. Level series solution made up to 20 mL was injected on HPLC. Each area values obtained for Na-cyclamate and acesulfame-k is used to create a line equation y = bx + a.

HPLC conditions

Column : C18 [8, 9]
UV detector : 200 nm.
Vol. Injection : 20 mL [8, 9]
Flow rate : 1 mL / min [9, 10]
Mobile phase : phosphate buffer: acetonitrile (85:15).

Conformance test system [10, 11, 12]

Calculation of the test the suitability of the system include: the capacity factor (k '), resolution (Rs), factor tailings (T), and the number plate (N), the calculation results compared to the value on its recommendation by the AOAC and the Food and Drug Administration (FDA) , The value of k '> 2, Rs> 1,5, T≤ 2, N> 2000.

Determination of retention time (TR)

Taken mix standard solution were injected as much as 20 mL in HPLC. Then determined the retention time of the Na-cyclamate and acesulfame-k. Na-cyclamate 1500.4 ppm and 503 ppm acesulfame-k).

Method Validation [7, 10, 11]

a. Linearity
   Linearity test performed on the raw equation y = bx + a is obtained. Correlation coefficient (r) the good is 0.99.
b. Determination of the limit of detection (LOD) and the limit of quantitative (LOQ)
   LOD and LOQ determined through a standard curve obtained. LOD value equivalent to 3 x (Sy / x) / b while the LOQ value equivalent to 10 x (Sy / x) / b.
c. Precision
   Tests conducted by the method of repeatability (repeatability). Used concentration of 300 ppm and 100 ppm of standard Na-cyclamate and acesulfame-k, 20 mL injected in HPLC for 6 times, then the value of the area acquired, determine the average value of the area, SD and RSD.
d. Accuracy
   The accuracy of testing performed by standard addition method (standard addition). It is estimated that each of the concentrations of Na-cyclamate and acesulfame-k in packaging supplement beverage samples is 120 ppm and 40 ppm.

Recovery of 80%

Pipette 10 mL of sample and 3.2 mL of Na-cyclamate and acesulfame-k than 1500/500 ppm stock solution, put in a 50 mL volumetric flask akuabides add as much as 5 mL, was added 0.5 mL of extracting 1, mixed and added 0.5 mL of extracting 2, mixed until the solution is homogeneous and then let stand for 10 minutes at room temperature (25oC), then added akuabides up to the mark (the concentration of standard solution of Na-cyclamate and acesulfame-k becomes 96/32 ppm). Then, do centrifugation for 10 min, the supernatant was taken and filtered using 0.45 µm membrane filter and injected into the HPLC.

Recovery of 100%

Pipette 10 mL sample solution and 4 mL of Na-cyclamate and acesulfame-k than 1500/500 ppm stock solution, put in a 50 mL volumetric flask akuabides add as much as 5 mL, was added 0.5 mL of extracting 1, mixed and added 0.5 2 mL extraction solution, mixed until the solution is homogeneous and then let stand for 10 minutes at room temperature (25oC), then added akuabides up to the mark (the concentration of standard solution of Na-cyclamate and acesulfame-k becomes 120/40

ppm). Then, do centrifugation for 10 min, the supernatant was taken and filtered using 0.45 µm membrane filter and injected into the HPLC.

Recovery of 120%

Pipette 10 mL of sample and 4.8 mL of Na-cyclamate and acesulfame-k than 1500/500 ppm stock solution, put in a 50 mL volumetric flask akuabides add as much as 5 mL, was added 0.5 mL of extracting 1, mixed and added 0.5 mL of extracting 2, mixed until the solution is homogeneous and then let stand for 10 minutes at room temperature (25ºC), then added akuabides up to the mark (the concentration of standard solution of Na-cyclamate and acesulfame-k becomes 144/48 ppm). Then, do centrifugation for 10 min, the supernatant was taken and filtered using 0.45 µm membrane filter and injected into the HPLC.

Preparation and assay of Na-cyclamate and acesulfame-k in the product supplement drink

a. Sampling

Sampling was done by purposive method samplingg, the sampling technique is not random / non-random. Considered not all the population has an equal chance to be selected into the sample and did not involve an element of chance, the selection of the sample taking into account the existing products on the market that meet the criteria of the researchers, in this case containing a combination of sweeteners Na-cyclamate and acesulfame-k. With a level of confidence (CI) of 98%, a standard deviation of 2%, and the percent relative error of 2%, the samples were used as much as 8 cup. Supplement drink products used are Phanter with different batch numbers. Supplement drink contains water, sugar, acidity regulator (Citric Acid and Sodium Citrate), Flavor Flavor Assorted Fruit (Artificial), Sweetener (Sodium Cyclamate) and Acesulfame-K, Taurine, Preservatives Sodium Benzoate, Caffeine, Inositol, Dyes Tetrazine Cl 19140.

b. Sample preparation and assay

First supplement drink homogenized, then pipette 10 mL of sample, put in a 50 mL volumetric flask akuabides add as much as 12 mL, was added 0.5 mL of carrez 1, mixed and added 0.5 mL of carrez 2, mixed until homogeneous then the solution let stand for 10 minutes at room temperature (25oC), then added aquabides up to the mark. Then, a centrifuge for 10 min, the supernatant was taken and filtered using 0.45 µm membrane filter and injected into the HPLC.

Results And Discussion

a. Analytical Method Development

Optimization method development begins with the mobile phase. Optimization of mobile phase aims to determine the best mobile phase for separating compounds tested seyawa. In this study the compound separated is Na-cyclamate and acesulfame-K. If the mobile phase optimization works well, it will be able to separate the compounds to be analyzed perfectly and produce a chromatogram good views of conformance test system that includes the capacity factor, resolution, tailing factor and number of theoretical plates. This is an early stage can determine the qualitative and quantitative analysis carried out subsequently.

b. Conformance Test System

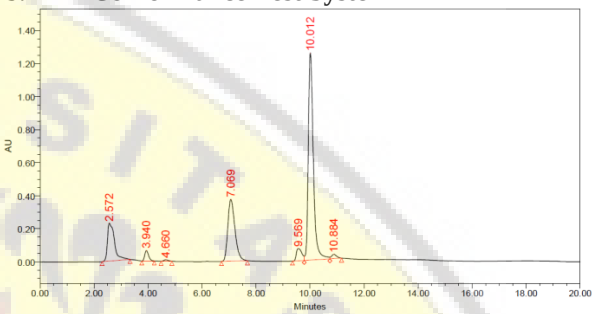


Fig. 1. sample chromatogram

Caption:
UV detector : 200 nm
Column : C18 (30 mm x 3.9 mm x 10 m)
Injection vol : 20µL
Flow rate : 1 mL / min
Mobile phase : phosphate buffer : acetonitrile (85:15)

Components of the system suitability test factors include capacity, resolution, tailing factors, and the number of theoretical plates. System suitability test results can be seen in Table 3.

Table 3. Results of the analysis of system suitability test Na-cyclamate and Acesulfame-K

No	Parameter	Result		Specification requirements
		Na-cyclamate	Acesulfame-K	
1	capacity factor (k ')	1,04	0,73	0,5-20
2	Resolution (Rs)	1,66	1,82	>1,5
3	Tailing factor	0,10	0,97	≤ 2
4	Number of theoretical plates (N)	2171,56	1140,50	>2000

Generally, there are two criteria that must meet the minimum requirements for conformity of the system showed that both [5]. Overall test results

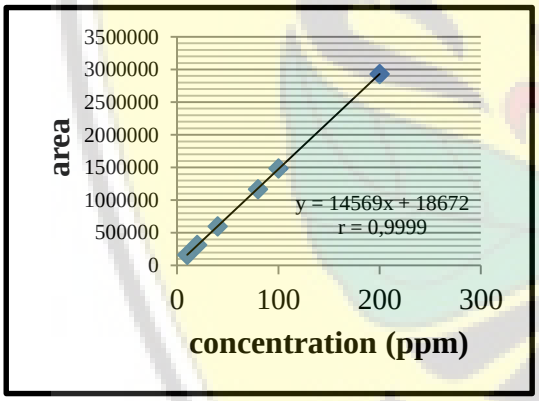
(Table I) show that the capacity factor and tailing factors obtained meets the requirements that are used, so the method has a good system and capable of achieving the accuracy and precision that is acceptable.

c. Validation Methods

This needs to be validated HPLC method because this method has been modified/development, is used in different laboratories, carried out by different analysts, and worked with different tools. To prove that the parameters meet the requirements in use. The parameters that need to be examined include: linearity, Limit of Detection (LOD), Limit of Quantitation (LOQ), precision, and accuracy.

1. Standard curve and linearity

Standard curve is a curve that is made by comparing the series between the concentration levels with a spacious area. Through a standard curve, it can be seen linearity methods used. Linearity is the ability of a method to obtain test results which are directly proportional to the concentration of the analyte in the given range. The correlation coefficient (r) is the most



common parameters used to determine the linearity of a method. R value is an indicator of the quality of liniearitas parameters that describe the analytical response (area) to the measured concentrations [7]. Ryang value recommended by AOAC is> 0.99 [7]. Standard curve Na-cyclamate and acesulfame-K can be seen in Figures 2 and 3.

Figure. 2. Graph linear regression equation Na-cyclamate

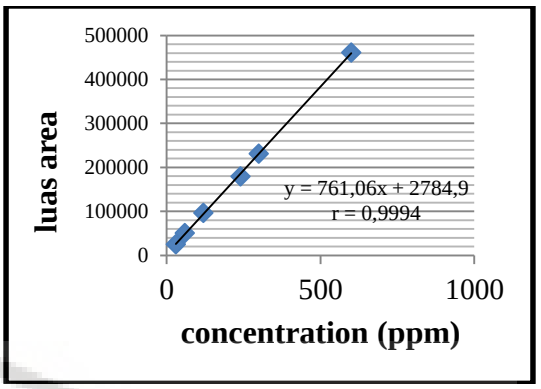


Figure. 3. Graph linear regression equation Acesulfam- K

Based on Figure 3 and 4, the correlation coefficient obtained showed good results as> 0.99. This shows that there is a proportional relationship between analytic response to the measured concentration.

2. Determination of the LoD and LoQ

The detection limit is defined as the smallest concentration that can be detected but not necessarily quantitatively, whereas the definition of limit of quantitation is said to be the smallest concentration of analyte that can be measured quantitatively. After calculation, obtained LoD and LoQ values as shown in Table 4.

Table 4. LoD and LoQ Na-cyclamate and acesulfame-K.

No.	name of compound	LoD (ppm)	LoQ (ppm)
1.	Na-cyclamate	6,25	18,95
2.	Acesulfame-K	1,40	4,23

Based LOD and LOQ values were obtained (Table 4), it is known that the HPLC method can detect the presence of Na-cyclamate in the sample when the levels contained more than equal to 6.25 ppm. Na-cyclamate to its lowest level in a sample can be determined with precision and accuracy that are acceptable to the operational conditions of this method was 18.95 ppm. HPLC method can detect the presence of acesulfame-K in the sample when the levels contained more than or equal to 1.40 ppm. Acesulfame-K to the lowest levels in a sample can be determined with precision and accuracy that are acceptable to the operational conditions of this method was 4.23 ppm.

LOD and LOQ values obtained showed that acesulfame-K has a value of LOD and LOQ smaller than the Na-cyclamate, so that it is more sensitive

method to analyze the content of sweetener acesulfame-K in the sample.

3. Precision

Precision is a measurement repeatability of analytical methods and is usually expressed as a relative standard deviation of a number of different samples was statistically significant [13]. Injection precision expressed as RSD (Relative Standard Deviation) shows the performance of HPLC, including pipe columns and environmental conditions, at the time the sample was analyzed. Precision made is precision in a single day (repeatibility or intraday precision). Preferred levels are 120 ppm for Na-cyclamate and 40 ppm for acesulfame-K. The concentration replicated six times. Levels have been selected to represent the range of samples analyzed.

RSD values obtained at levels of 120 ppm of 0.82% for the Na-cyclamate and 0.19% for acesulfame-K. The results showed a good value, so that the method used is valid. According to the Association of Official Analytical Chemist (AOAC) at levels of 10-100 ppm, qualified RSD is <6% and at levels of 100-1000 ppm <3% [7].

4. Determination Accuracy

Accuracy is the accuracy of the method of analysis or proximity between the measured value with the value received good conversion value, true value or reference value [9]. According to the AOAC value good accuracy to levels of 10-100 ppm is 80-115% and for 1000 to 10,000 ppm level is 90-108% [7]. Research conducted generate value accuracy Na-cyclamate and acesulfame-K as in tables 5 and 6.

Table 5. Na-cyclamate accuracy value

accuracy	replication	% recovery	Average
80%	1	98,59%	99,57%
	2	101,98%	
	3	98,15%	
100%	1	104,09%	102,09%
	2	99,97%	
	3	102,21%	
120%	1	95,98%	95,68%
	2	94,70%	
	3	96,36%	
Average			99,11%

Tabel 6. Asesulfam-K accuracy value

accuracy	Replication	% recovery	Average
80%	1	96,35%	98,44%
	2	95,09%	
	3	103,87%	
100%	1	100,84%	100,72%
	2	101,84%	
	3	99,49%	
120%	1	99,88%	99,35%
	2	100,30%	
	3	97,87%	
Average			99,50%

Based on the tables 5 and 6, the value of Na-cyclamate percent recovery was on average 99.11% and 99.50% acesulfame-K. Percent recovery values obtained can be accepted by AOAC criteria, so that the method used has good accuracy.

d. Determination of Levels of Samples

Measurements are performed in the final stage of the study, after the results showed that the method used has been validated properly. Measurements of samples obtained by mengintrapolasikan value area on the raw curve equation. Measurements were carried out, resulting in the data as shown in table 7.

Table 7. Level calculation results Na-cyclamate and acesulfame-K

name of samples	The average levels of Na-cyclamate (mg / kg product)	The average levels of Acesulfam-K (mg / kg product)
EA 139.2R	665,04	214,63
RK 039.1R	669,28	209,35
RK 237.2R	729,81	217,59
RK 059.2R	655,44	195,30
Average	679,89	209,22
SD	33,78	9,88
RSD	4,97%	4,72%

Description of food = 177,89 g

Based on the results obtained from the research content of Na-cyclamate contained in the sample supplement beverage is at 117.81 mg / serving (177.89 g) and acesulfame-K amounted to 36.24 mg / serving (177.89 g), concentration obtained is different from the content of Na-cyclamate and acesulfame-K stated dikemasan, ie 110 mg/serving of Na-cyclamate and 30 mg/serving

for Acesulfame-K. These results also indicate levels of Na-cyclamate is 679.89 mg / Kg of product and Acesulfame-K is 209.22 mg / kg of product. Levels of Na-cyclamate and acesulfame-K levels were obtained did not exceed the maximum limit set by the POM is 1000 mg/kg and 600 mg/kg of product.

Conclusion

1. The analytical method developed can be used to separate and analyze Na-cyclamate and acesulfame-K to supplement beverage products in the packaging.
2. The development of analytical methods which do meet the criteria for the validity of the Association of Official Analytical Chemist (AOAC) with a standard curve linearity values> 0.99, Na-cyclamate precision levels of 0.82% and 0.19% acesulfame-K with the value% recovery Na -siklamat and acesulfame-K on average each ie 99.11% and 99.50%.
3. Content obtained artificial sweeteners in beverages supplement of, 117.81 mg/serving (177.89 g) of Na-cyclamate and 36.24 mg/serving (177.89 g) for Acesulfame-K. These results also indicate levels of Na-cyclamate 679.89 mg/Kg of product and acesulfame-K 209.22 mg/kg of product. Na-cyclamate levels and levels of acesulfame-K obtained is still included in the maximum limit set by the POM is 1000 mg/kg and 600 mg/kg of product.

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DEVELOPMENT and VALIDATION of ANALYTICAL METHOD of SODIUM CYCLAMATE and ASPARTAME in POWDER BEVERAGE USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY UV-DETECTOR

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Abstract

European Standardization (EN) recommend a method analysing cyclamate and aspartame using High Performance Liquid Chromatography (HPLC) in beverages because it has good selectivity and sensitivity. The aim of this study is to develop and validated the method in order to obtain a simple method without derivatization using a C_{18} column (10 µm; 3.9 x 300 mm); mobile phase acetonitrile:phosphate buffer (15:85); flow rate 1 ml/min; lambda 200 nm. The result of this study showed that HPLC-UV method could be developed and had good validity. The linierity value of cyclamate and aspartame is > 0.99 respectively; recovery of Na-cyclamate is 99.09%, aspartame is 98.12%; precision of cyclamate and aspartame were 1.00% and 0.54% respectively; detection limit and quantitaion limit of aspartame was less than Na-cyclamate. Concentration of cyclamate and aspartame in instant powder drink were 0.16 mg / kg of product and 0.12 mg / kg of the product. Both validation parameters and sample consentration meet the acceptance criteria defined in AOAC and BPOM requirement.

Keywords : aspartame, cyclamate, beverages, method validation, HPLC-UV.

Introduction

Cyclamate and aspartame is a type of artificial sweetener found in many food products and instant drinks on the market. Cyclamate has a level of 30 to 40 times the sweetness of sucrose [1], whereas the aspartame has a level of 160 to 220 times the sweetness of sucrose [2]. In the metabolism of aspartame produces phenylalanine can cause adverse effects. For example, in patients with phenylketonuria cannot metabolize phenylalanine may occur accumulation of phenylalanine in the brain and cause mental disability [3]. It has been reported in a study that the use of artificial sweeteners in large quantities (more than 1680 mg / day) can increase the risk of bladder cancer [4].

The use of food additives such as artificial sweeteners in food products has been regulated in the Decree of the Head of BPOM Republic of Indonesia Number: HK.00.05.5.1.4547 [5]. BPOM set maximum levels of use of aspartame and cyclamate in instant powder drink products is 350 mg / kg and 600 mg / kg of product. To ensure that the levels of cyclamate and aspartame are added in powder beverage products eligible BPOM, it is necessary to analyze the levels of Na-cyclamate and aspartame are added in instant powder drink products that are not harmful to health when consumed by the public.

To ensure that the levels of cyclamate and aspartame are added qualify are allowed BPOM, it is necessary to analyze the levels of artificial sweeteners that do not endanger the health if consumed by the public. The method recommended by the European Standardizatino EN 12 856: 1999 and 12857: 1999) to analyze the Na-cyclamate and aspartame is the HPLC method because it has a separate power selective, and sensitive. but the sample preparation in the Na-cyclamate done derivatization and wavelength for the two compounds is different, the researchers will development of methods that get simpler method.

Development of methods of analysis to be performed is cyclamate sample preparation without derivatization,, using a mobile phase of phosphate buffer: acetonitrile (85:15) and using a single wavelength for the two compounds (200 nm), and meet the criteria of AOAC validation (conformance test system , accuracy, precision, linearity, limit of detection (LOD) and Limit of Quantitation (LOQ)) as well as the levels of Na-cyclamate and aspartame contained in instant powder beverage products in the packaging meets the requirements of BPOM.

Material and methods

Chemicals

Standard cyclamate (Supelco), standard aspartame (sulpelco), acetonitrile HPLC grade (JT Baker), potassium dihydrogen phosphate pa (KH_2PO_4) (Merck), orthophosphoric acid 85% pa (Merck), akuabides (Ikapharmindo), samples of powder drink instant containing Na-cyclamate and aspartame taste soursop, potassium hexacyanoferrate (II) trihydrate pa ($K_4[Fe(CN)_6].3H_2O$) (Sigma Adrich), zinc sulfate heptahydrate pa ($ZnSO_4.7H_2O$) (Merck).

a set of tools HPLC (Waters e2695), UV-Vis detector (Waters 2489), C18 column (300 mm x 3.9 mm x 10µm) (Waters µ Bondapak), centrifuge (Hanil), semimicrobalance analytical balance (Mettler Toledo series SX 205), ultrasonic bath (Branson), a set of glasses (flask, pipette measuring, measuring cups, glass beaker) (Pyrex), solvent filtration membrane (MS®) and 0.45 µm syringe filter (Agela).

Optimazition Methods

Tabel 1. Design development method mobile phase Na-cyclamate and Aspartame

Design	Mobile Phase	The combined polarity index
I	Acetonitril : Buffer phospat (35 : 65)	7,88
II	Acetonitril : Buffer phospat (25 : 75)	8,20
III	Acetonitril : Buffer Phospat (20 : 80)	8,36
IV	Acetonitril : Buffer phospat 15;85)	8,52

The Carrez Solution[8,9]

1. Dissolved 7.5 g of potassium hexacyanoferrate (II) trihydrate (K4 [Fe (CN) 6] .3H2O) into 50 mL aquabidest
2. Dissolved 15 g of zinc sulfate heptahydrate (ZnSO4.7H2O) into 50 mL aquabidest

0.0125 mol / L pH 4.3
Dissolved 0.85 g of potassium dihydrogen orthophosphate with aquabides 400 mL in 500 mL glass beaker, pH adjusted to 4.3 using 5% orthophosphoric acid. Transfer to a 500 mL volumetric flask and add aquabides to mark boundaries.

Carefully weighed cyclamate and aspartame respectively 50 mg and 10 mg. Na-cyclamate and aspartame were weighed and then dissolved in 50 mL volumetric flask with aquabides up to the mark (this solution contains 1000 ppm Na-cyclamate and aspartame 200 ppm)

Sample preparation

One gram sample weighed, and then put in a 50 ml flask. Then add 40 ml aquabides and placed in ultrasonic bath at 40 ° C for 20 minutes. The solution was cooled to room temperature, then 0.6 ml of solution ditimbahkan Carrez 1, mixed and added another 0.6 ml Carrez 2. Strong shaken sample solution and left for 10 minutes then added aquabides room temperature up to the mark. Then centrifuge for 10 minutes (1400 g), the supernatant was taken and filtered using 0.45 µm membrane filter and injected into the HPLC.

Conformance test system[7,8,9]

Calculation of the test the suitability of the system include: the capacity factor (k '), resolution (Rs), factor tailings (T), and the number plate (N), the calculation results compared to the value on its recommendation by the AOAC and the Food and Drug Administration (FDA) , The value of k '> 2, Rs> 2, T <1,5, N> 2000.

Validation method

Linierity

Made solution of cyclamate levels series / aspartame with a concentration of 25/10 ppm, 50/10 ppm ppm 100/20, 200/40 ppm ppm 400/80, 500/100 ppm to use 10 mL volumetric flask. The concentration obtained respectively with 0.25 ml pipette; 0.5 ml; 1 ml; 2 ml; 4ml; 5 ml of stock solution and placed in a 10 mL volumetric flask, added akuabides to mark boundaries.

Level series solution made up to 20 mL was injected on HPLC. Each area values obtained for Na-cyclamate and aspartame is used to create a line equation y = bx + a

Determination of the limit of detection (LOD) and the limit of quantitative (LOQ)

LOD and LOQ determined through a standard curve obtained. LOD value equivalent to 3 x (Sy / x) / b while the LOQ value equivalent to 10 x (Sy / x) / b.

Precision

Tests conducted by the method of repeatability (repeatability). Used concentration of 100 ppm and 20 ppm of standard Cyclamate and aspartame, injected 20 mL of the HPLC 6 times, then the value of the area acquired, determine the average value of the area, SD and RSD.

The accuracy of testing performed by standard addition method (standard addition). It is estimated that each of the concentrations of Cyclamate and aspartame in packs of instant powder drink samples is 80/16ppm, 100/ 20 ppm, 120/24ppm.

Result And Discussion

Development of the method in this study begins with a design of experiments. This process is intended to get a good method or system, so it is capable of detecting and quantifying the mixture of Na-cyclamate and aspartame in the beverage powder samples. Modifications made to the development of this method with the adjustment of mobile phase optimization. Mobile phase of phosphate buffer and acetonitrile selected based on the level of polarity Na-cyclamate and aspartame. Mobile phase optimization results best in show at the design IV in comparison acetonitrile: phosphate buffer (15:85).

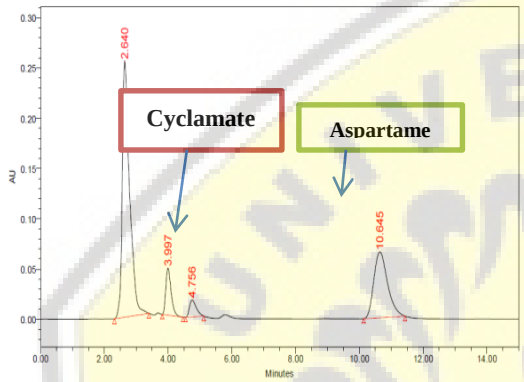

Figure 1. The chromatograms of samples cyclamate and aspartame IV design acetonitrile: phosphate buffer (15:85)

Conformance Test System

System suitability test is done by calculating the capacity factor, tailing factor, resolution and number of theoretical plates.

Table 2. Comformance test system

Parameter	Acceptance criteria[8]	Result	
		cyclamate	Aspartame
Capacity factor (k')	0,5-20	1,10	3,70
Tailing factor (T)	> 1,5	1,20	1,10
Resolution (Rs)	> 1,5	1,50	6,90
Theoritical plats (N)	> 2000	1168,40	1345,10
Mobile phase	Dapar fosfat : Asetonitril (75:25)		
Stationary phase	C_{18} 300 mm x 3,9 mm 10 µm (Phenomenex)		
Flow rate	1 mL/mnt		

Table 2 shows the results based only on the value (N) which does not meet the criteria, but overall had a good value so the method has a good system and capable of achieving the accuracy and precision that is acceptable.

Vadilasi Methods

1. Linearity

Cyclamte's liniear regression is y= 986,14x - 1540 and the value of r is 0,999. Aspartam's liniear regression is y= 56745x – 21282. it can be concluded that there is a proportional relationship between analytic response to the measured concentration. Linearity test results show on Figures 2 and 3.

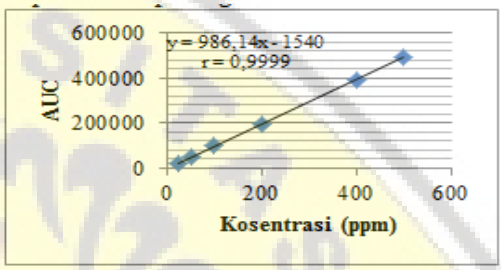

Figure 2. Linear regression and correlation coefficient (r) Cyclamate

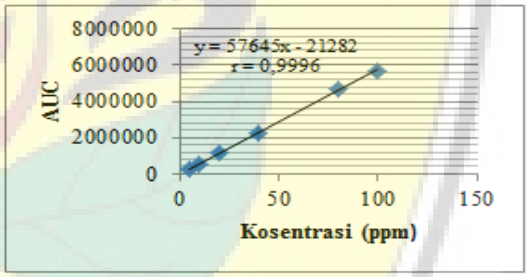

Figure 3. Linear regression and correlation coefficient (r) aspartame

Determination of LoD and L0Q

Limit of Detection (LoD) and Limit of Quantitation (LoQ) shows the sensitivity of the method, the smaller the LOD and LOQ value the more sensitive the method also vice versa. The LoD value of cyclamate and aspartame are 7,67 ppm and 1,64ppm. The LoQ value of cyclamate and aspartame are 23,23ppm and 4,98ppm.

3. Accuracy

Percent recovery value of the average cyclamate and aspartame are 99.09% and 98.12%. Percent recovery obtained were in the range

percent recovery which meet the criteria of AOAC, so that the method used has good accuracy. The result show on table 3 and 4.

Table 3. Accuracy aspartame

Accuracy	Percent recovery	Average
80%	97,291% 97,820% 96,787%	97,30%
100%	98,98% 98,42% 98,38%	98,59%
120%	98,14% 99,24% 97,99%	98,46%
	Average	**98,12%**

Table 4. Accuracy Cyclamate

4. Precision

In this study conducted repeatability precision

Accurasy	Percent recovery	Average
80%	102,50% 102,06% 102,68%	102,41%
100%	97,35% 89,71% 96,45%	94,50%
120%	100,48% 100,08% 100,48%	100,35%
	Average	**99,09%**

testing. According to AOAC precision determination of the levels of 10-100 ppm eligible if the value of RSD <6%, while for the 100-1000 ppm concentration was <3% (39). Based on the table 7 can be inferred value of RSD cyclamate (1.00%) and Aspartame (0.54%) meet satandar range (<6%) were determined by AOAC resulting value good precision.

5. Assay

Based on the results obtained from this study about levels of cyclamate and aspartame in the beverage powder was 0.16 mg / kg of product and 0.12 mg / kg of the product, the samples obtained entered the range specified by
BPOM is 350 mg / Kg of products and 600 mg / kg of product. According to the Word Health Organization (WHO) declared cyclamate is safe for human consumption as an artificial sweetener with the Acceptable Daily Intake (ADI) of 11 mg / kg body weight and safety limit aspartame for consumption is as much as 40 mg / kg body weight so that powder drink if consumed per day, the level of sweetener contained each mg / serving is within the ADI allowed, so that these products are safe to use. The result of cyclamate and aspartame on table 5 and 6.

Table 5. Determination of cyclamte

Sample	Cyclamate	
	Amount found (mg/saji)	Amount found (mg/kg)
MS0216	1,410 1,406	0,163 0,163
MS0516	1,386 1,396	0,160 0,161
MS0816	1,452 1,457	0,168 0,168
MS1116	1,433 1,421	0,164 0,162
Rata-rata	**1,42**	**0,16**
SD	**0,026**	**0,003**
RSD	**1,81%**	**1,82%**

Table 6. Determinatin of aspartame

Aspartam	
Amount found (mg/saji)	Amount found (mg/kg)
0,974	0,113
0,971	0,112
0,953	0,110
0,956	0,110
0,995	0,115
1,010	0,117
1,105	0,126
1,081	0,124
1,01	**0,12**
0,057	**0,006**
5,71%	**5,25%**

Conclusion

High Performance Liquid Chromatography (HPLC) method developed can be used to separate and analyze cyclamate and aspartame in the beverage powder. Validity of analytical methods developed meet the validity criteria AOAC, and also the levels of Na-cyclamate and aspartame contained in instant powder drinks do not pass the safety limit set by BPOM so it is safe for consumed.

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THE USING WOOF IS COMPOSED of FERMENTED-ECENG GONDOK (*Eichchornia crassipes*), TAHU DREGS and DRIED-KANGKUNG (*Ipomoea aquatica*) as THE RUMINANT LIVESTOCK WOOF FORMULATION

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Abstract

There are two major aims of the research. First, aimed is to find the best woof formulation to get the best sheep growth. Second, this research want to create the woof formulation that increase protein percentage in the sheep meat. The type of this research is experimental design with the three treatment. There are three kinds of woof formulation. Woof formulation I is composed (30% tahu dregs, 35% fermented-eceng gondok and 35% dried-kangkung) , woof formulation II is contained (35% tahu dregs, 30% fermented-eceng gondok, and 35% dried-kangkung), woof formulation III is compiled (35% tahu dregs, 35% fermented-eceng gondok, and 30% dried-kangkung). The woofs is given for three group of the sheep, each group have nine members. The sheep is given the woof for ten days. Then, the sheep body weight is measured. In addition, the sheep meat protein percentage is measured too. The conclusion of the research is the woof formulation III is the best media to increase the sheep body weight (0,71Kg/ten days) and the sheep meat protein percentage increase 1%/ten days.

Keywords: Fermented-eceng gondok, tahu dregs, dried-kangkung, woof formulation

Introduction

Eceng Gondok (*Eichchornia crassipes*) can grow so quickly in the water and disturb and damage the water environment [1]. Because of that, some efforts should be made to handle it to protect the negative effect of water environment disturbing. One kind of the solution is utilizing the plant as animal feed so that weeds waters become something of economic value. This is possible item because of high nutrient content in the Eceng Gondok. The plant contain dry matter of about 7%; 11.2% crude protein; 18.3% crude fiber; BETN 57%; crude fat 0.9%; 12.6% ash; Ca 1.4%; and P of 0.3% [46].

Recently a lot of research is done on the using of Eceng Gondok for animal feed. First, the research related to the using Eceng Gondok for the ducks feed and the duck is given the feed produced the high levels protein eggs. Eceng Gondok is also good fish feed especially Nila (*Oreochromis niloticus*), as well as feed ruminant animals such as goats because of high protein and carbohydrate content in thes plant- fermented [12]. According to the [13] biomass of
goat that is given the feed of Eceng Gondok-fermented increased high. The protein content of the goat meat 1% higher than that is given with conventional feed [40].

The fermentation process is very important to be applied to the plant in order to the plant can be used as the feed that have a higher nutritional value and better of level digesting. Some studies fermentation in Eceng Gondok was done. [46] reported that the best long fermentation of the plant with Aspergillus niger is 6 weeks, with PK levels of 18.84% and 15.73% SK levels. In this case the Aspergillus niger is a probiotic. The addition of probiotics increase the acceleration of the fermentation process. Probiotics are living microorganisms that can improve the health and physiological benefits when consumed [49].

Several studies related to the using of probiotics was done. The research conducted by [20] has succeeded in developing a probiotic that can be used to decompose the materials is derived from plants quickly. The other researchers was successfully to conducted the research related to rice straw, corn straw and soybean hay fermentation and implemented in ruminants [20]. The using of mixture of various types probiotics such as of sellulolitik, proteolytic and lipolytic microorganisms produced high quality feed for the cattle from the raw materials of corn straw , from the raw materials of rice straw [17] and from the raw materials of soybean straw [35]. The using of a probiotic mixture of different types of microbes more advantageous than the using of a single microbial as tha fermentation agents. In addition the using of EM (Effective Microorganism) as the probiotics can also speed up the process of organic material decomposition of [11]. Therefore, in this study will be used the ragi tempe (one kind of the yeast) as the fermentation agents of Eceng Gondok

fermentation process. The yeast is a mixture of various microorganisms.

The fermentation process will be improve digestibility, increase nutrient absorption, improve rumen microflora balance, increase endurance, and eliminate or decrease pathogenic microorganisms [7]. In the fermentation process, the protein will turn into peptides, amino acids, ammonia, the fats will turn into volatile fatty acids, and carbon dioxide [48].

There are several important points that must be had the probiotic bacteria that is normally present in the digestive tract. The bacteria must have a shorter regeneration time, produce substances to block the growth of pathogenic microorganisms and strong enough to withstand the packaging process (manufacturing) and distribution so that it can be moved into the intestine in a state live [30].

As a support to obtain these properties, in the fermentation process is added molasses, according opinion of [33] which states that molasses is a major waste of sugar refining industry. Molasses has a crude protein content (PK) 3.1%, crude fiber (SK) 0.6%, extract materials without nitrogen (BETN) 83.5%, crude fat (LK) 0.9%, and ash 11.9 % so as to improve the fermentation nutrients. Based on the nutritional content, there are two kinds of molasses: (1) Cane-molasses, molasses has a sucrose content of 25-40% and 12-25% reducing sugar with a total sugar content 50-60% or more. Levels of crude protein (CP) approximately 3% and ash content of about 8-10%, which is largely made up of potassium, calcium, chloride, and sulfate salts; (2) Beet-molasses¬ a laxative feed which is normally given to cattle in small quantities of about 0.5% [33].

According to [15] more and more types fresh feed of plants is given to the cows will be better, because the element of nutrients (carbohydrates, proteins, fats, vitamins, and minerals) will be more complete. Based on this study it was not good to apply the Eceng Gondok as the singly animal feed. In this research the plant was mixed with the other water plant namely Kangkung (Ipomoea aquatica). The Kale contain some kinds of substances such as vitamin A, vitamin B1, vitamin C, protein, calcium, phosphorus, iron, with a protein content of 3% and energy 29 kcal. Increasing the protein content to the feed is done by adding ampas tahu (solid waste in the tahu production process) to the feed. Ampas tahu has a high nutritional content such as protein and carbohydrates to 17% to 67% in addition to the substance of these nutrients, ampas tahu also have other nutrient content is 3.79% fat, water and ash 51.63% 1 , 21%, it is possible pulp to be added into animal feed.

This study has been carried out by the manipulation of feed formulation levels of Eceng Gondok-fermented, dried kale and ampas tahu. Fermentation techniques were applied following the best results of previous studies [12] so that the research will produce the best formula of ruminant feed that contain three ingredients as mentioned above. Furthermore, the feed formula is implemented to three groups of goat. Each group have nine members of goat. After ten days the biomass of goat and the protein level in it's meat is measured.

Method

This study was an experimental study. Manipulation variables in this study was the percentage component of ruminant feed formula. There are three formulas, namely the formula I that contain (ampas tahu/ pulp 30%, 35% Eceng Gondok-fermented, dried kale 35%), the formula II (ampas tahu/pulp 35%, 30% Eceng Gondok-fermented, water spinach Rendeng 35%) and formula III / III ration (pulp 35%, 35% fermented water hyacinth, dried kale 30%). The response variable in the research is biomass of goats and the level protein in the goat meat. Controle variabel of this research is the age and condition of Eceng Gondok and kale, types of probiotics, , goats gibas weighing 20-25 kg, and the probiotic inoculum as much as 0.15 grams for each treatment and conditions of ampas tahu. Then, the feed is applied to three group of goat (each group have nine members of goat) for 10 days, then the goat's weight and protein content is measured. The data is analyzed with qualitative and quantitative descriptive analysis to obtain the findings or conclusions.

Result

The results that have been achieved in this research include place identification to get the plant, their extraction process, the process of Eceng Gondok fermentation, analysis the nutritional content of fermented feed, kale processing into rending/dried kale, collecting pulp/ampas tahu, formulation of feed, application the feed to goat and measuring the goat weight and meat protein level of goat dafter 10 days application. The detailed description of each product are as follows.

Getting the Eceng Gondok from the river is based on an abundance of the plant. The place is Kali(river) Rungkut. This river is the border between Surabaya and Sidoarjo. The Eceng Gondok in these waters is very abundant, can even cover almost the entire surface of the river and potentially harmful aquatic organisms in that place.

Intake of the Eceng Gondok is done by cutting the stems and leaves. Eceng Gondok is taken a number of ± 1000 Kg. After that, the stems and leaves of Eceng Gondok is chopped in a

composting Nursery, in Bratang. The fermentation process carried out in the Laboratory of Mycology, Department of biology, Faculty MIPA, Unesa.

After the chopping process, Eceng Gondok is boiled until cook. Furthermore is dried up in the sun until rather dry. The plant then put in plastic basket that is lined with banana leaves and fermented with yeast of tempe. To accelerate the fermentation process, the molasses is added on the material. The material is fermented for 10 days, and then is analyzed the nutritional content of feed.

The feed then is analyzed protein content by the proximate analysis. The proximate analyzes performed in the Laboratory of Animal Feed, Faculty of Veterinary Medicine, University of Airlangga.

Table 1. The Proximate Analysis Results of Fermentation Feed during 5 days at various concentrations Ragi of Tempe

NO	KODE SAMPEL	HASIL ANALISIS (%)							
		Bahan Kering	Abu	Protein Kasar	Lemak Kasar	Serat Kasar	Ca	KH	ME (Kcal/kg
1	V0.L5	32.4970	8.1681	4.6958	2.4114	10.9431		17.2217	587.23
	V1.L5	23.8642	6.2166	4.5914	2.2253	6.2500		10.8309	496.66
	V2.L5	25.9936	5.7981	5.1146	2.7824	6.4569		12.2985	601.07
	V3.L5	28.8609	6.4102	4.8301	2.4662	7.3903		15.1544	641.78
	V4.L5	25.6951	5.8889	4.8843	2.2343	6.8651		12.6876	554.22

Table 5. The Proximate Analysis Results of Fermentation Feed during 10 days at various concentrations Ragi of Tempe

NO	KODE SAMPEL	HASIL ANALISIS (%)							
		Bahan Kering	Abu	Protein Kasar	Lemak Kasar	Serat Kasar	BETN	ME (Kcal/kg	KH
1	V0L10	40.0630	9.4630	3.6525	1.7091	16.6299	8.6085	601.23	25.2384
2	V1L5.K	75.6872	17.5757	10.2941	5.8537	15.4093	26.5544	1778.08	41.9637
3	V1L10	42.4818	10.8233	6.9862	2.2918	12.7431	9.6374	782.82	22.3805
4	V2L10	40.4395	10.7599	7.1514	2.6139	10.0352	9.8791	813.92	19.9143
5	V3L10	42.6386	10.3097	6.6413	1.5904	13.0414	11.0558	773.74	24.0972
6	V4L10	44.0817	11.5536	6.9251	1.4271	12.3775	11.7984	797.17	24.1759

Rendeng is ready to be used as a feed component to be made in this study.

The ampas tahu is used as a mixture of feed formula in this study is the solid waste from the tahu factory. The waste collected by buy it. The ampas tahu is dried until the water does not drip. The ampas tahu that had not drip the water is ready to be mixed into the feed formula.

In this study, there are three kinds of feed formula that contain of three materials as mention above as follows.

- Formula I (ampas tahu/pulp 30%, 35% Eceng Gondok-fermented, dried kale 35%),
- Formula II (ampas tahu/pulp 35%, 30% Eceng Gondok-fermented, dried kale 35%)
- Formula III (ampas tahu/pulp 35%, 35% Eceng Gondok-fermented, dried kale 30%)

The feed formulas then is applied as feed for goats for 10 days as an independent feed without any mixture or the addition of another feed. Each feed formula is given for nine goats with 2 times application daily at 09.00 and 17.00. The goat is maintained in Tegalrejo, Bareng, Jombang.

Calculation of weight gain of sheep is done by reducing the weight of sheep after treatment with lamb weight prior to treatment.

Table 3. Weight Gain of the goat which is given feed formula I, II and III for 10 days

Treatment	Re-peat	The prior goat weight (kg)	The last goat weight (kg)	The adding goat weight (kg)	The average adding goat weight (kg)
Feed formula I	1	24,5	25,17	0,67	0,67
	2	22	22,68	0,68	
	3	23,5	24,18	0,68	
	4	22	22,67	0,67	
	5	24	24,68	0,68	
	6	22	22,66	0,66	
	7	24	24,68	0,68	
	8	23	23,67	0,67	
	9	23,5	24,18	0,68	
Feed formula II	1	23	23,67	0,67	0,67
	2	23	23,67	0,67	
	3	22	22,66	0,66	
	4	24,5	25,18	0,68	
	5	22,5	23,16	0,66	
	6	24,5	25,17	0,67	
	7	23	23,67	0,67	
	8	24	24,67	0,67	
	9	23,5	24,16	0,66	
Feed formula III	1	24	24,7	0,7	0,71
	2	22,5	23,19	0,69	
	3	24	24,69	0,69	
	4	22	22,68	0,68	
	5	23,5	24,19	0,69	
	6	22	22,67	0,67	
	7	25	25,9	0,9	
	8	22,5	23,16	0,66	
	9	24,5	25,18	0,68	

After aplication of the feed formulas the goats are slaughtered to get their meat and then is analyzed the nutritional content of the meat. The nutritional value of goat meat are shown in Table 4 below.

Table 4 The nutritional value of goat meat

No.	Sam-pel Code	Nutritional value (%)							
		Dried materials	ash	Crude pro-tein	Crude fat	Crude cellulose	Ca	BETN	ME (Kcal/Kg)

1	Ran-sum 1	22,9249	1,141 5	17,0198	2,2518	1,8735	3,0041	0,6383	757,33
2	Ran-sum 2	30,6002	0,9665	18,2870	2,3878	1,7605	2,6529	7,1984	1050,39
3	Ran-sum 3	28,402	1,0629	18,8037	2,2833	1,2584	2,3251	4,9935	977,78

Discussion

Ruminants abdomen consists of four parts, namely the rumen, reticulum, omasum, and abomasum with the size of which varies according to age and natural food. Feed rumen capacity to accommodate as much as 80%, 5% reticulum, omasum 7-8%, and abomasum 7-8%. This division can be seen from the shape muscle spincter during contraction. When considered from the food storage capacity can be known that the major ingestion of food occurred in the rumen.Rumen media containing a liquid that is a result of changes in foodstuffs that have been mixed with water from food, drinks and water from the water contained in saliva. Saliva contains a large amount of sodium bicarbonate which is very important to maintain the proper pH and serve as a buffer against volatile fatty acids is produced by bacterial fermentation. Saliva is also important to keep the amount of water in rumen fluid [45]. The process of digestion in the stomach depends on rumen temperature 37-39 ^{0}C and pH 6.0 to 6.7 and in the anaerobic state is the best condition for fermentation and the end of the fermentation process will be absorbed continuously by the reticulo rumen [47]. In the rumen there are a large number of microorganisms, which are primarily anaerobic who do a symbiotic mutualism with the host animal. The rumen microorganisms play an important role in digesting food enzymatically to produce simple organic substances are readily absorbed in the digestive system further. Rumen microorganisms secrete extracellular enzymes to enzymatically digest food.

In the digestive system of cattle ruminasia there is a process called cud (rumination). Food that has been chewed by an animal in his mouth, then is swallowed to enter into the rumen. In the rumen occurs destruction of the cell walls of forage, then the nutrients locked inside the walls of cellulose can be overhauled by enzymes produced by microorganisms. The first reform process is certainly not perfect, it will spew ruminant animals back food contained in the rumen back into the mouth. The animal will repeat to chew food for a few moments. Furthermore swallowed back and get into the rumen to digest enzymatically. This continued three to four times, so when we look at ruminant animals have characteristics always perform continuous mastication.

Goat is one kind of ruminant digestion fermentative do. Fermentative digestion is done with the help of rumen microorganisms. On ingestion fermented foods will be overhauled by rumen microorganisms into other compounds of different chemical properties as an intermediate substance. Microorganisms are involved in the digestive process this has cellulolytic and proteolytic properties. At ruminants, digestion in the rumen fermentation occurs and in the reticulum. Fermentation produces protein peptides, amino acids, ammonia, volatile fatty acids, and carbon dioxide [48].

Based on the food digestion process then will be greatly assisted if the food consumed by animals exist in the form of "partially digested" therefore The Eceng Gondok is used as one component of the feed formula should be fermented in advance to assist in the digestion of food is to mechanical or chemical, kale should be made rending to be more subtle (mechanical aids digestion of food) and ampas tahu as a third component, add the protein content in feed formula and bring a distinctive aroma that stimulates appetite goats.

Provision of various types of feed formulas were created role in this study have an impact on weight gain or animal biomass trials. Based on Table 3 on weight gain of goats, feed formula III gives the best average weight gain than that for formula I and II. Weight gain of goats with feed formula III is 0.71 kg within 10 days or 2.13 kg per month. While weight gain of goats that is given feed formula I and II 0.67 per 10 days or 2,01 kg per month. When compared to weight gain with conventional feed goats (1.5 Kg per month) are actually three types of rations were formulated in this study is better than conventional feed, but the feed ration III is the highest weight gain results.

Feed formula III consists of components ampas tahu/pulp 35%, 35% Eceng Gondok-fermented, dried kale 30% while the feed formula I contain ampas tahu/ pulp 30%, Eceng Gondok-fermented 35%, 35% dried kale. Feed formula II consists of ampas tahu/pulp 35%, 30% Eceng Gondok-fermented, dried kale 35%. When considered in the feed formula III the pulp out high and high Eceng Gondok-fermented strongly supports the quality of feed. The ampas tahu contains protein, Eceng Gondok-fermented also highly nutritious and livestock are also high appetite with weight gain

goat thus also the highest. The dried kale high percentage less supportive of growth in weight because it has not become fodder "partially digested" so that undernutrition can be taken to the maximum.

Actually, all three types of feed formulas are made in this research have high nutritional value and can trigger weight gain better than conventional feed. High nutritional value of feed can definitely trigger weight gain faster. Mc Donald et al. (2002) states that the growth of livestock is controlled by the consumption of nutrients, especially energy consumption.

Weight gain, due to the dry ingredients in the feed requirements have been met, and also due to the results of protein and carbohydrate fermentation product which is higher than conventional feed so that the resulting growth is also better. This is consistent with the statement of Soepranianondo (2005), that if the process of metabolism in ruminant good, then the fermentation product in the form of amino acids, ammonia-N and volatile fatty acids in the rumen will be high. As we know that for the growth of livestock amino acid required for the formation of the protein network while volatile fatty acids are used as a source of energy that the rest will be used as a fat or energy reserves.

Boediono (1997) said that the increase in the rate of weight gain can be obtained by increasing the amount of feed composition, as is well known that the feed containing nutrients in sufficient quantities allowing livestock to grow. Therefore, the overall goat feed formulas in this study grew faster than those fed conventional goat.

Based on Table 4 above it can be seen that the protein content of goat meat that is given three kinds feed formula is made in this research is higher (feed I = 17.0198%, ration II = 18.2870, ration III = 18.8037) when compared with the levels of protein goat meat with the conventional feed(16.6%) [7]. In addition to increased protein content, the feed formulas is developed in this study also produce goat meat is low fat content. According [7] the fat of meat goat generally contain up to 9.2%, while based on the results of the analysis of goat meat with feed formula I is 2.2518% fat content, the fat content of goat meat with feed formula II amounted to 2.3878% and ration III produce goat meat with fat content 2.2833%. Based on the results of the analysis of the data obtained can be stated that the feed formulas were developed in this study has the potential to produce goat meat with the low fat and high protein.

Composition and nutritional value of feed is very influential on the physical and physiological condition of the goat. According [38], nutrient content of feed affects the quality of goat meat and goat hormonal conditions. Furthermore, the physiological processes that occur will affect the quality of meat especially level of protein and fat.

Conclusion

Feed formulas were developed in this study resulted in weight gain of goats is larger (2:07 kg per month) than that of goats with a conventional feed (1.5 kg per month). Feed formula III is the best formula to trigger weight gain goat (2.13 kg per month) is compared with feed formulas I and II (2:01 kg per month). While the protein content of goat meat with feed formulas are developed in this study increase of about 1% when is compared to the goat meat with the feed convensional feed.The level of fat in the goat meat with the feed formula are developed in this study experienced a decline of about 7%.

Suggestion

Feed formulas that have been developed in this study need to be implemented on other ruminants other than goats. Research on the manufacture of feed formulas based on the waste to produce meat low in fat and high protein remains open to do.

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THE EFFECT of LIME, PHOSPHORUS and POTASSIUM FERTILIZER on THE GROWTH and PRODUCTIVITY of BLACK SOYBEAN UNDER SATURATED SOIL CULTURE on TIDAL SWAMP

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Abstract

Saturated soil culture (SSC) is a cultivation technology that gives continuous irrigation and maintains water depth constantly and makes soil layer in saturated condition. By keeping the water-table constantly, soybean will be avoided from negative effect of inundation on soybean growth because soybean will be acclimatized and improve its growth. This technology is appropriate to prevent pyrite oxidation on tidal swamp and has been proved to increase the productivity of yellow soybean on tidal swamp. The objective of the research was to study the effect of lime, P and K fertilizer on the growth and productivity of black soybean. The experiment was conducted at Banyuurip Village, Tanjung Lago Sub District, Banyuasin District, South Sumatera Province (28 m above sea level, $2^0$39'32" South Latitude, and $104^0$43'618" East Longitude), from May to September 2015. The experiment used a randomize complete block design with three replications. . The first factor was two levels of P fertilizer consisted of : 36 and 72 kg P_2O_5 ha^{-1}. The second factor was two levels of K fertilizer cobsisted of: 30 and 60 kg K_2O ha^{-1}. The third factor was four levels dolomite consisted of ; 0, 1000, 2000, and 3000 kg of Ca Mg $(CO3)_2$ ha^{-1}. The results showed that there was an interaction between P and K on the plant height, leave number, branch number, root nodule dry weight, and productivity, interaction P fertilizer and dolomite on the leave number at 8 WAP (Week After Planting); and interaction K fertilizer and dolomite on the branch number. Soybean productivity on 72 kg P_2O_5 ha^{-1} was 3.93 tons ha^{-1} higher than the half dose. Soybean productivity on 30 kg K_2O ha^{-1} was 3.77 tons ha^{-1}. The highest soybean productivity was produced on dolomite treatment 3 tons ha^{-1} as much as 4.06 tons ha^{-1}.

Keywords: black soybean, dolomite ameliorant, fertilizer, marginal land

Introduction

The national productivity of soybean in Indonesia is still low, it was only 1.4 tone/ha in 2012. The national production only fulfill by 30 % of national demand [1]. Therefore, a special effort is needed to fulfill the national demand on soybean, i.e. either to increase crop productivity or to expand the production sites.

One of the alternatives to develop soybean cultivation in Indonesia is to optimize the use of marginal land, and tidal swamp is one of the potential ecosystems for future soybean production. Indonesia has ± 20 millions ha tidal swamps, and 9 millions ha are appropriate for agriculture, and 2 millions is suitable for soybean [2].

The major constrain of soybean production in tidal swamp is high pyrite content, when pyrite is oxidized, soil pH will decrease. "Reference [3]" reported that high pyrite content suppressed the productivity of soybean on tidal swamps, approximately 800 kg/ha.

Saturated soil culture is a technology in cultivation that gives water permanently, maintains and keeps its depth constantly (± 5 cm USS). This makes soil layer in saturated condition. In SSC, watering is started from the beginning of growth to maturity stage. By keeping the water-table constantly, soybean will be avoided from negative effect of inundation on soybean growth, because soybean will acclimatize and improve its growth [4, 5].

Soil water management can be applied to reduce pyrite content where the soil is in reductive condition and able to support soybean growth. SSC technology is one of soil water managements that has been studied in highland and succeed to increase soybean production [6, 7]. This offers the chance to reduce the pyrite, hence increase soybean production on tidal swamps.

Response of soybean to saturated condition varied between varieties and the later maturing soybean was better than the earlier one [4, 6, 8, 9]. "Reference [10]" found on black soybean that

Cikuray, Ceneng, and Lokal Malang as adaptive varieties under saturated condition on tidal swamps.

The soil fertility condition in tidal swamp were indicated by high pyrite, low pH, medium P availability, low K availability, and high Al [11].Therefore the objective of the research was to study the effect of lime, P and K fertilizer on the growth and productivity of black soybean.

Methods

This experiment was conducted on tidal swamps land in Banyuurip Village of Tanjung Lago Sub District, Banyuasin District, South Sumatera Province, Indonesia (28 m above sea level, 2039'32" South Latitude, and 104043'618" East Longitude) from May to September. The experiment used a randomize complete block design with three replications. The first factor was two levels of P fertilizer consisted of: 36 and 72 kg P_2O_5 ha^{-1}. The second factor was two levels of K fertilizer consisted of: 30 and 60 kg K_2O ha^{-1}. The third factor was four levels dolomite consisted of; 0, 1000, 2000, and 3000 kg of Ca $Mg(CO3)_2$ ha^{-1}.The each plot with size 2 m x 3 m was surrounded by furrow irrigation. Water was given at planting time and kept until the maturity stage and made plots in wet condition. The bed width and trench size can be seen in the figure 1.

Two weeks before planting, plots were applied with dolomite, SP36, and KCl. Black soybeans were sprayed with 10 g Urea/l water at 2, 4, 6 weeks after planting to support acclimatization.

The experiment used Cikuray Variety. At planting date, seeds were inoculated with Rhizobium sp and treated with insecticide with active agent Carbosulphan 25.53 %. Seeds were planted in planting distance 40 cm x 12.5 cm, 2 seeds/ hole. The observed variables were: plant height, leave number, and branch number at 2-12 week after planting (WAP); nodule, root, stalk, leave, and pod dry weight at 10 WAP; fill pod per plant, seed dry weight per plot, and 100-seed dry weight at harvest time.

Figure 1. The bed width and trench size on saturated soil culture

Result And Discussion

The Effect of Single factor of Lime, P, and K Fertilizer

The single factor of P only affected on the plant height at 2 WAP, leave and branch number at 6 WAP, and seed dry weight per plot. The plant height at 2 WAP, leave and branch number at 6 WAP, and seed dry weight per plot at 72 kg P_2O ha^{-1} were higher than 36 kg P_2O_5 ha^{-1}. The effect of P fertilizer on the fill pod, 100 seed dry weight, and soybean productivity can be seen on the Table 1.

Table 1.The effect of P fertilizer on the fill pod, 100 seed dry weight, and soybean productivity.

	P_2O_5 (kg ha^{-1})	
Variable	36	72
Fill pod number/plant	92.3	97.7
100-seed-dry weight	14.0	13.9
Seed dry weight/2.4 m^2 (g)	1007b	1110a
Productivity (ton ha^{-1})	4.19b	4.62a
Productivity-15 % area of trench (ton ha^{-1})	3.56b	3.91a

Note: numbers followed by the same letter at the same row are not significantly different with Duncan multiple range test 5%.

The single factor of K affected on the plant height at 2 WAP, pod dry weight, and it did not affect on the seed dry weight and the other variable. The plant height at 2 WAP and pod dry weight at 60 kg K_2O ha^{-1} were higher than 30 kg K_2O ha^{-1}. The effect of K fertilizer on the fill pod, 100 seed dry weight, and soybean productivity can be seen on the Table 2.

Table 2.The effect of K fertilizer on the fill pod, 100 seed dry weight, and soybean productivity.

	K_2O (kg ha^{-1})	
Variable	30	60
Fill pod number/plant	94.3	95.7
100-seed-dry weight	13.9	13.9
Seed dry weight/2.4 m^2	1063	1054

(g)		
Productivity (ton ha^{-1})	4.43	4.39
Productivity-15 % area of trench (ton ha^{-1})	3.76	3.73

Note: numbers followed by the same letter at the same row are not significantly different with Duncan multiple range test 5% .

The single factor of lime affected on the plant height at 2 WAP, branch number at 4-8 WAP, and seed dry weight per plot. The lime increased plant height at 2 WAP, but it was not different of plant height at 1, 2, and 3 ton ha^{-1}.

Table 3. The effect of K fertilizer on the fill pod, 100 seed dry weight, soybean productivity.

Variable	Lime (ton ha^{-1})			
	0	1	2	3
Fill pod number/plant	91.3	95.6	96.0	97.0
100-seed dry weght (g)	14.1	14.0	13.8	13.9
Seed dry weight/2.4 m^2 (g)	970b	1024ab	1090ab	1148a
Productivity (ton ha^{-1})	4.04b	4.27ab	4.54ab	4.78a
Productivity-15 % area of trench (ton ha^{-1})	3.43b	3.63ab	3.86ab	4.06a

Note: numbers followed by the same letter at the same row are not significantly different with Duncan multiple range test 5%.

The lime increased branch number at 4-6 WAP. The branch number at 3 ton ha^{-1} was higher than the other treatment, and it was not different between 1 and 2 ton ha^{-1}. The branch number at without lime was lower than the other treatment. At 8 WAP, the branch number at 3 ton ha^{-1} was higher than the other treatment, and it was not different between 1-3 ton ha^{-1} (Table 3)

The lime increased soybean productivity. The seed dry weight per plot at 3 ton ha^{-1} was higher than the other treatment, but it was not different between 1-3 ton ha^{-1}. The effect of lime on the fill pod, 100 seed dry weight, and soybean productivity can be seen on the Table 3.

"Reference [12]" reported that lime has increased soil pH higher than straw ash, because the lime has Content higher than straw ash. "Reference [13]" reported that optimum dose of lime at 72 kg P_2O_5 ha^{-1} under saturated soil culture as much as 1 ton ha^{-1}.

The Effect of Interaction Between Lime, P, and K

The interaction between P and K fertilizer affected on the plant height at 6 WAP, leave number at 8 WAP, branch number at 4 and 6 WAP, nodule dry weight at 8 WAP, and seed dry weight per plot (Table 4). At the fertilizer 30 kg K_2O ha^{-1}, the observed variables at 36 kg P_2O_5 ha^{-1} were lower than 72 kg P_2O_5 ha^{-1}, but the opposite result showed that at the fertilizer 60 kg K_2O ha^{-1} , the observed variables at 36 kg P_2O_5 ha^{-1} were higher than 72 kg P_2O_5 ha^{-1}.

Table 4. The effect of interaction between P and K fertilizer on the plant height, leave number, branch number, and seed dry weight per plot

Variable	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	
		30	60
Plant height 6 WAP (cm)	36	80.11b	83.78a
	72	83.28a	81.65b
Leave number 8 WAP	36	20.93b	22.13a
	72	22.13a	20.30b
Branch number 4 WAP	36	1.32b	1.60a
	72	1.70a	1.43b
Branch number 6 WAP	36	2.71b	3.08a
	72	3.35a	3.03b
Nodule dry weight 8 WAP (g/plant)	36	0.66b	1.07a
	72	0.89a	0.69b
Seed dry weight/2.4 m^2 (g)	36	952b	1062a
	72	1174a	1046b
Productivity (ton ha^{-1})	36	3.97b	4.42a
	72	4.89a	4.36b
Productivity-15 % area of trench (ton ha^{-1})	36	3.37b	3.76a
	72	4.16a	3.71b

Note: numbers followed by the same letter are not significantly different with Duncan multiple range test 5%.

The interaction between lime and phosphorus fertilizer affected on the leave number at 8 WAP. The highest leave number was obtained at the lime 3 ton ha^{-1} and P fertilizer 36 kg P_2O_5 ha^{-1} (24.43), and the lowest leave number was obtained at the lime 1 ton ha^{-1} and P fertilizer 36 kg P_2O_5 ha^{-1} (19.87) (Table 5).

Table 5. The effect of interaction between lime and phosphorus on the leave number at 8 WAP

Lime (ton ha^{-1})	P_2O_5 (kg ha^{-1})	
	36	72
0	20.93bc	20.27bc
1	19.87c	20.60bc

2	20.90bc	21.10bc
3	24.43a	20.90bc

Note: numbers followed by the same letter are not significantly different with Duncan multiple range test 5% .

The interaction between lime and potassium fertilizer affected on the branch number at 12 WAP. The highest branch number was obtained at the lime 2 ton ha^{-1} and K fertilizer 30 kg K_2O ha^{-1} (4.70), and the lowest branch number was obtained at the lime 0 ton ha^{-1} and K fertilizer 30 kg K_2O ha^{-1} (3.50) (Table 6).

Table 6. The effect of interaction between lime and potassium on the branch number at 12 WAP

Lime (ton ha^{-1})	K_2O (kg ha^{-1})	
	30	60
0	3.50c	4.30ab
1	4.33ab	4.13ab
2	4.70a	4.17ab
3	4.17ab	3.70bc

Note: numbers followed by the same letter are not significantly different with Duncan multiple range test 5% .

Conclusion

The single factor of P affected on the plant height at 2 WAP, leave and branch number at 6 WAP, and seed dry weight per plot. The productivity at 72 kg P_2O_5 ha^{-1} (3.91 ton ha^{-1}) were higher than 36 kg P_2O_5 ha^{-1} (3.56 ton ha^{-1}).

The single factor of K affected on the plant height at 2 WAP, pod dry weight, and it did not affect on the seed dry weight and the other variable. The single factor of lime affected on the plant height at 2 WAP, branch number at 4-8 WAP, and seed dry weight per plot. The lime increased soybean productivity. The seed dry weight per plot at 3 ton/ha was higher than the other treatment, but it was not different between1-3 ton/ha. The highest soybean productivity was produced on dolomite treatment 3 tons ha^{-1} as much as 4.06 tons ha^{-1}.

The interaction between P and K fertilizer affected on the plant height at 6 WAP, leave number at 8 WAP, branch number at 4 and 6 WAP, nodule dry weight at 8 WAP, and seed dry weight per plot . At the fertilizer 30 kg K_2O ha^{-1}, the observed variables at 36 kg P_2O_5 ha^{-1} were lower than 72 kg P_2O_5 ha^{-1}, but the opposite result showed that at the fertilizer 60 kg K_2O ha^{-1}, the observed variables at 36 kg P_2O_5 ha^{-1} were higher than 72 kg P_2O_5 ha^{-1}.

The interaction between lime and phosphorus fertilizer affected on the leave number at 8 WAP, and the interaction between lime and potassium fertilizer affected on the branch number at 12 WAP.

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EFFECTS of AMINOETHOXYVINYLGLYCINE, PLASTIC WRAPPING, and STORAGE TEMPERATURES on FRUIT SHELF-LIFE and QUALITIES of 'CAVENDISH' BANANA

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Abstract

'Cavendish' banana is a worldwide banana cultivar which has a very short fruit shelf-life and quickly decreased qualities when its ripening is promoted. To find the methods on how to inhibit its fruit deterioration, this research was conducted to study the effects of applications of aminoethoxyvinylglycine (AVG), plastic wrapping, storage temperatures, and their interactions on the shelf-life and quality of 'Cavendish' banana fruits. This research was conducted in the Horticultural Postharvest Laboratory, Faculty of Agriculture, Lampung University from September to October 2014. Treatments were arranged in a completely randomized design with three replications and laid out in a 2 x 2 x 2 factorial design. The first factor was AVG (with and without 1.25 ppm AVG), the second factor was plastic wrapping (with and without one layer of plastic wrapping), and the third factor was storage temperature (a room temperature and 20 °C). The results showed that (1) AVG significantly lengthened fruit shelf-life, but did not affect physical and chemical fruit qualities such as fruit firmness, weight loss, soluble solid and acid contents, and sweetness; (2) plastic wrapping significantly lengthened fruit shelf-life, decreased fruit weight loss, accelerated fruit softening, but did not affect chemical fruit qualities; (3) lower storage temperature significantly lengthened fruit shelf-life, inhibited fruit softening, increased soluble solid content and sweetness, but did not affect fruit weight loss and acid content; (4) a combined application of AVG, plastic wrapping, and low storage temperature was best in lengthening fruit shelf-life, but did not affect physical and chemical fruit qualities.

Keywords: banana, postharvest, aminoethoxyvinylglycine, wrapping, storage temperature, shelf-life, quality, fruit

Introduction

'Cavendish' banana is a climacteric fruit, harvested in a green stage of stage I, and remains green in vacuum polyethylene bags inside cartoon packs during transportation as long as its ripening is not promoted with ethylene gassing. However, once its ripening is promoted and reached stadium III (an early ripening stage of greenish yellow), a stadium when the fruits are started to be distributed locally, in 3-4 days the fruits quickly reach the end of the ripening stage of stage VII (yellow with brown spots), a fruit stage of the end of economical values. Therefore, retarding the stadium development of stages III to VII, not from stadium I (a green stage) as conducted by [1] and [2], is the matter of postharvest handling that has an economical benefit.

In an attempt to retard the quick fruit deterioration of 'Cavendish' banana, [3] applied an anti-ethylene 1-methylcyclopropene (1-MCP) to both stages of stage III and V (perfectly yellow with green tip and greenish-yellow peduncle). Their results showed that the fruits receiving no treatment (control) deteriorated quickly, while those receiving 1-MCP application reached more than 5 days storage. 1-MCP was best if it was applied at fruit yellowing stage (stage V), because at earlier stage (stage III) the application resulted in imperfect fruit color development [3]. The results suggested, therefore, that any attempts to retard banana fruit ripening should be addressed at stage V.

Among anti-ethylene substances, aminoethoxyvinylglycine (AVG) is believed as the most potent chemical because its mode of action is directly as an inhibitory of ACC synthesis activity mechanism [4 and 5]. By applying AVG to inhibit ethylene synthesis, in combination with plastic wrapping to decrease respiration and transpiration rates [6 and 7] and a low storage temperature, lengthening the fruit shelf-life and maintaining qualities of 'Cavendish' banana are expected.

This research was aimed at studying the effects of applications of aminoethoxyvinylglycine (AVG), plastic wrapping, storage temperatures, and their interactions on the shelf-life and quality of 'Cavendish' banana fruits.

Materials And Methods

This research was conducted on September-October 2014 in the Horticultural Postharvest Laboratory, Department of Agrotechnology, Faculty of Agriculture, University of Lampung, Bandar Lampung, Indonesia. The samples of 'Cavendish' banana fruit of stages V (perfectly yellow with green tip and greenish-yellow peduncle; [3]) were received directly from PT Nusantara Tropical Farm (PT NTF), Way Jepara, East Lampung, Indonesia.

Treatments were arranged in a completely randomized design with three replications and laid out in a 2 x 2 x 2 factorial design. The replications were applied to each experimental unit consisting of one cluster fruit of two fingers each.The first factor was AVG (with and without 1.25 ppm AVG, by dipping for 10 minutes), the second factor was plastic wrapping (without and with one-layer plastic wrapping of Total Plastic Wrap®), and the third factor was storage temperature (a room temperature and 20 °C). Treated fruits were then placed in storage rooms of a room temperature of 28 ± 1 °C and a low temperature of 20 ± 1 °C. The storage room temperature of 20 ± 1 °C was the lowest possible temperature that could be achieved in the storage room of 5.8 x 2.8 x 3.15 m^3 with two ACs, one humidifier, and one thermohygrometer.

Observations were made on fruit shelf-life, weight loss, firmness (with a penetrometer typed FHM-5,with a cylindrical point of 5 mm in diameter of Takemura Electric Work, Ltd., Japan), °Brix (with an Atago N-1E hand refractometer), titratable acidity (titrated with 0.1 N NaOH and phenolphthalein as an indicator), and sweetness level (as °Brix/titratable acidity ratios). A unit treatment was ended when the banana fruit reached stage VII (yellow with browning spots; [3]). All data were analyzed with ANOVA, and further tested with Least Significantly Difference (LSD) at 5%.

Results and Discussion

'Cavendish' banana is a climacteric fruit. However, different from most other climacteric fruits, its fruit remains green and its qualities are maintained during storage as long as its ripening is not promoted with ethylene gassing and its fruit weight loss and shrunken or wilted rind are avoided. Consequently, its postharvest handling problems are not laid before ethylene gassing [1 and 2], but after it. Once its ripening is promoted and reached stadium III and the fruits are started to be distributed locally, in 3-4 days brown spots are developed on its yellow rind and the fruits are quickly soften or its flesh firmness is quickly decreased.

However, consumers usually judge the quality of 'Cavendish' banana based on its rind color, not on its firmness. On the beginning of this research, its fruit firmness was 2.31 kg/cm^2 (Table 1, footnote) and in 4-8 days storage the fruits soften quickly, reached as low as 0.30 kg/cm^2. The data in Table 1 showed that AVG significantly lengthened its fruit shelf-life by slightly less than one day compared to the control, but its fruit firmness and weight loss were not affected significantly. This indicated that rind color stadium development and fruit flesh softening were two different development processes that did not have to be perfectly correlated each other. A thick rind of banana might play a barrier for ethylene or anti-ethylene to take into effects differently on the ripening rind color stadium development and flesh softening [8].

AVG, however, did not affect other quality parameters, such as soluble solid (°Brix) and free acid contents, and consequently, its sweetness (Table 2). These three fruit quality parameters were not affected because the observation was terminated at the same stage [3, 9, 10, 11, 12, and 13].

Plastic wrapping played a physical barrier for O_2 and CO_2 movements, and developed a modified atmosphere around the fruits with less O_2 and more CO_2. This modified atmosphere decreased respiration rate of the fruit [9], and consequently, lengthened the shelf-life of the 'Cavendish' banana fruits (Table 1). One-layer plastic wrapping also inhibited water loss from the fruits, and consequently, maintained a significantly lesser fruit weight loss than the control (Table 1) as also reported by [13, 14, and 15].

While other fruit qualities, such as soluble solid (°Brix), free acid, and sweetness, were not affected, one-layer plastic wrapping significantly decreased fruit firmness or accelerated fruit softening. Heat deliberated from respiration and trapped inside wrapping might increase temperature inside wrapping, and consequently, accelerated fruit softening [16].

The treatment of low temperature of 20 ± 1 °C applied in this experiment significantly lengthened its ripening rind color stadium development of the fruit which was perceived as fruit shelf-life (Table 1). If a much lower temperature than that of 20 ± 1 °C was applied, we believed that a much longer fruit shelf-life could be achieved because 'Cavendish' banana fruits are usually shipped under a low temperature of 13.33-14.44 °C (56-58 °F; Rachmansyah A. Wardhana, Chairman of R&D Division of PT NTF, personal communication).

Low temperature also significantly delayed fruit softening (Table 1) because it might delay respiration rate [17]. Consequently, while free acid

was not affected, other fruit qualities, such as soluble solid (°Brix) and sweetness were significantly maintained higher than the control (Table 1).

A more significant effect of individually applied wrapping was significantly dominated when it was applied in combination with AVG and low storage temperature (Table 1).

Table 1. Effects of AVG, plastic wrapping, and storage temperature on the fruit shelf-life, firmness, and weight loss of 'Cavendish' banana[1]

Treatments	Shelf-life (days)	Firmness (kg/cm^2)	Weight loss (%)
AVG:			
Control (A0)	5.00 b	0.62 a	7.79 a
AVG (A1)	5.92 a	0.77 a	9.07 a
Wrapping:			
Control (W0)	4.17 b	0.96 a	9.64 a
1-layer (W1)	6.75 a	0.43 b	7.21 b
Temperature:			
Room (T0)	5.00 b	0.54 b	8.05 a
Cool (T1)	5.92 a	0.85 a	8.81 a
AVG*Wrap.:			
A0W0	4.00 c	0.80 ab	9.37 a
A0W1	6.00 b	0.45 b	6.21 b
A1W0	4.33 c	1.12 a	9.92 a
A1W1	7.50 a	0.42 b	8.22 ab
AVG*Temp.:			
A0T0	4.83 a	0.53 a	8.16 a
A0T1	5.17 a	0.72 a	7.42 a
A1T0	5.17 a	0.56 a	7.94 a
A1T1	6.67 a	0.98 a	10.20 a
Wrap.*Temp.:			
W0T0	3.67 b	0.70 b	9.03 ab
W0T1	4.67 b	1.21 a	10.26 a
W1T0	6.33 a	0.39 b	7.07 b
W1T1	7.17 a	0.48 b	7.36 b
AVG*Wrap.* Temp.:			
A0W0T0	4.00 cd	0.58 bc	10.09 ab
A0W0T1	3.33 d	0.82 bc	7.97 bc
A0W1T0	5.67 bc	0.47 bc	6.23 c
A0W1T1	7.00 ab	0.30 c	7.91 bc
A1W0T0	4.00 cd	1.01 ab	8.64 abc
A1W0T1	5.33 bc	1.42 a	11.87 a
A1W1T0	6.33 ab	0.43 c	6.20 c
A1W1T1	8.00 a	0.54 bc	8.52 abc

[1]Values in the same column of each treatment followed with the same letters were not significantly different at LSD 5%. Fruit firmness in the day of treatment (shelf-life 0 day) was 2.31 kg/cm².

Table 2. Effects of AVG, plastic wrapping, and storage temperature on the fruit °Brix, acidity, and sweetness of 'Cavendish' banana[1]

Treatments	°Brix (%)	Acidity (mg/100 g)	Sweetness[2]
AVG:			
Control (A0)	6.70 a	0.35 a	19.01 a
AVG (A1)	6.83 a	0.35 a	19.39 a
Wrapping:			
Control (W0)	6.77 a	0.35 a	19.20 a
1-layer (W1)	6.77 a	0.35 a	19.20 a
Temperature:			
Room (T0)	6.50 b	0.35 a	18.45 b
Cool (T1)	7.03 a	0.35 a	19.96 a
AVG* Wrap.:			
A0W0	6.77 a	0.35 a	19.20 a
A0W1	6.63 a	0.35 a	18.82 a
A1W0	6.77 a	0.35 a	19.20 a
A1W1	6.90 a	0.35 a	19.58 a
AVG*Temp.:			
A0T0	6.87 b	0.35 a	19.49 b
A0T1	6.53 bc	0.35 a	18.54 bc
A1T0	6.13 c	0.35 a	17.41 c
A1T1	7.53 a	0.35 a	21.38 a
Wrap.*Temp.:			
W0T0	6.70 ab	0.35 a	19.01 ab
W0T1	6.83 ab	0.35 a	19.39 ab
W1T0	6.30 b	0.35 a	17.88 b
W1T1	7.23 a	0.35 a	20.52 a
AVG*Wrap.* Temp.:			
A0W0T0	7.27 abc	0.35 a	20.62 abc
A0W0T1	6.13 d	0.35 a	17.41 d
A0W1T0	6.47 cd	0.35 a	18.35 cd
A0W1T1	6.13 d	0.35 a	17.41 d
A1W0T0	6.27 d	0.35 a	17.78 d
A1W0T1	7.40 ab	0.35 a	21.00 ab
A1W1T0	6.80 bcd	0.35 a	19.29 bcd
A1W1T1	7.67 a	0.35 a	21.75 a

[1]Values in the same column of each treatment followed with the same letters were not significantly different at LSD 5%. °Brix, acidity, and sweetness in the day of treatment (shelf-life 0 day) were 8.00%, 0.35 g/100 g, and 22.70, consecutively; [2]°Brix/acidity ratios.

In fact, a combined application of AVG, plastic wrapping, and low storage temperature increased fruit shelf-life of more than four days storage compared to the control, but generally they did not affect physical and chemical fruit qualities.

Conclusion

The results showed that (1) AVG significantly lengthened fruit shelf-life, but did not affect physical and chemical fruit qualities such as fruit firmness, weight loss, soluble solid and acid contents, and sweetness; (2) plastic wrapping significantly lengthened fruit shelf-life, decreased fruit weight loss, accelerated fruit softening, but did not affect chemical fruit qualities; (3) lower storage temperature significantly lengthened fruit shelf-life, inhibited fruit softening, increased soluble solid content and sweetness, but did not affect fruit weight loss and acid content; (4) a combined application of AVG, plastic wrapping, and low storage temperature was best in lengthening fruit shelf-life, but did not affect physical and chemical fruit qualities.

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CHARACTERIZATION and MOLECULAR IDENTIFICATION of *Lactobacillus* spp. ISOLATED from FECES of HEALTHY INFANTS for LOCAL PROBIOTIC DEVELOPMENT in BALI

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Abstract

The main objectives of this research were to characterize and identify isolates obtained from feces of healthy infants by applying molecular techniques. Some 21 isolates of lactic acid bacteria (LAB) considered to be potential for local probiotic development were selected among 103 isolates in this investigation. Of these 21 isolates, 13 and 3 isolates were found to be resistance to extremely low pH (pH 2) and extremely high concentration of NaDC (0.6mM), respectively following exposure for 3 hours to those extreme conditions. All isolates were found to survive at pH levels higher than 2 as well as at NaDC concentrations lower than 0.6 mM, indicating that they were potential for local probiotic development, although further safety tests need to be conducted. In the molecular identification, all 21 isolates were found to be closely related to *Lactobacillus rhamnosus* GG strain GG (ATCC 53103) with percentage of similarity of 99%. Some isolates were also found to have 99% similarity with *Lactobacillus rhamnosus* strain JCM 1136 and *Lactobacillus zeae* strain RIA 482, but their total score (based on GeneBank data) were lower than that of *Lactobacillus rhamnosus* GG strain GG (ATCC 53103)..

Keywords: *Lactobacillus* spp., Probiotic, Bali, Infant feces.

Introduction

The most well-known probiotics commercialized and intensively developed recently belong to genera of *Lactobacillus* and *Bifidobacterium* [1]. These two genera are part of normal flora of healthy intestinal tract of human. In the human intestinal tract, they provide some beneficial effects, such as preventing or curing diarrhea, stimulating immune system, reducing alergic effect of foods, preventing people from having colon cancer, producing vitamins and enzymes for food digestion, as well as reducing the blood cholesterol content [2, 3, 4]. Besides, probiotics have also been used to heal infections of urinary tract [5].

According to [3] and [6] each strain of probiotic is host-specific which means that it works best in specific hosts. Therefore, in the development of human probiotics, the strain candidates should be isolated from the intestine of healthy digestive tract of human, because they have been well adapted to the human intestine [7]. This implies that if the probiotics are to be developed in Indonesia, the agents must be isolated from the intestinal tract of Indonesian people,
because the probiotics claimed to be effective in other countries are not necesarily effective when appied to Indonesian people or vise versa [4].

For safety reasons, before being developed as potential probiotics, the candidates have to pass several sequential *in vitro* and *in vivo* examinations,
including examination on metabolic and ezymatic activities that may have potential to produce bad side

effects to human health, toxicity test, adhesion test on the lining of intestinal tract, survival or competitive
test in the intestinal tract, and application test of the candidate in various food products [8]. Besides these tests, the candidates must also be clearly identified at least up to species level.

Based on the above background, lactic acid bacteria (LAB) were isolated from feces of healthy infants and followed by molecular identification of those isolates with a view for the development of local probiotic candidates. The main objectives of this research were to screen potential isolates and to identify the molecular identity of those potential isolates before being developed as local probiotics.

Materials and Method

Isolation of the LAB candidates

Probiotic candidates were isolated from feces of three healthy infants aged between 1 – 6 months

(normal birth) using a serial dilution plating method as specified in [4]. All colonies suspected to be LAB were preserved at -40 ºC (in 30% glycerol in MRS broth medium) and tested for gram stain reaction, catalase, and gas production from glucose metabolism in the preliminary identification by applying the methods specified by [9;4;10), respectively.

Preliminary characterization of LAB candidates

All LAB isolates were preliminarily characterized by applying several tests, such as tests for resistance to acidic condition (low pH test) and resistance to high concentration of NaDC in order to investigate the possibility of their survival when exposed to conditions of the upper part of human intestinal tract.

Resistance test of LAB isolates to acidic condition

The test followed the method of [4] with small modification. Some 50 µL of isolate in the glycerol stock culture was re-suspended in 5 mL MRS broth, incubated at 37 ºC for 24 h, vortexed, and transferred to 3 new Eppendorf tubes (100 µL each) containing 900 µL MRS broth medium with various pH levels (2, 3, or 4), incubated at 37 ºC for 3 h, centrifuged at 7000 rpm followed by removal of supernatant. The pellet obtained was then washed twice by suspending the pellet in 300 µL saline solution followed by centrifugation at 7000 rpm for 5 minutes and removal of supernatant. The pellet was next re-suspended in 300 µL, inoculated (50 µL) to 5 mL MRS broth medium pH 7, incubated at 37 ºC overnight under anaerobic condition, and measured for turbidity at 660 nm (OD 660nm). An isolate was considered to be resistant to acidic condition if its absorbance reading was ≥ 0,1 [11; 12]. To obtained representative data, the experiment was in triplicates.

Resistance test of LAB isolates to NaDC

Similar procedures as in the resistance test to acidic condition were applied in this test, but the pellets of the isolates were exposed to medium of MRS broth containing NaDC at various concentrations (0.2, 0.4, or 0.6 mM NaDC). MRS broth medium without NaDC served as control.

Isolation of genomic DNA of the isolates

The genomic DNA of the isolates were isolated using Isoplant II Kit (isoplant code No. 310-04151, Nippon Gene, Toyama, Japan). Some 1 mL isolate suspension previously grown in 5 mL MRS broth was centriuged at 5000 rpm for 7 minutes to obtain cell mass following removal of the supernatant. The cell mass was then washed twice with sterile distiled water, vortexed, added with 300 µL solution I, vortexed again for 1 – 3 seconds, added with 150 µL solution II, and vortexed again for 5 seconds. The mix was next added with solition IIIA and IIIB (75 µL each), vortexed for 2 seconds, put on ice for 15 minutes, and centrifuge at 15,000 rpm and 4 ºC for 30 minutes. Some 200 µL of the DNA solution was subsequently taken, added with 400 µL of 99% etanol, centrifuged at 15,000 rpm and 4 ºC for 30 minutes to obtain DNA pellete, added with 100µl 70% etanol, centrifuged as above, evaporated, added with 20-25µl TE pH 7.5, and vortexed. Electrophoresis was run to check whether or not the isolation of DNA succeeded.

Amplification and sequencing of 16s rDNA

Identification of probiotic candidates was conducted by sequencing the composition of rDNA partially at the variable areas of V1, V2, and V3 using primers of 27F (3'-AGA GTT TGA TCC TGG CTC AG-5') and 520R (3'-ACC GCG GC(G/T) GCT GGC-5') (Tannock, 1999). The total volume of the PCR mix reaction was 12.5 µL and consisted of dNTPs (10 mM each), primers of 27F and 520R (25 pmol), 1X PCR buffer II, 75 mM MgCl2, 0.45 U ApliTaq, and 1 µL DNA. The PCR was run at the following conditions; one cycle was at 94 ºC for 5 minutes followed by 35 cycles at 94 ºC for 20 seconds, 55 ºC for 2 minutes, and 72 ºC for 30 seconds. The process was ended with a cycle at 75 ºC for 5 minutes. The PCR product was electrophoresis in 1% agarose in 1X TAE buffer solution, stained with EtBr (50ng/mL), visualized under UV illuminator, and photographed.

The PCR product was next purified using PCR SUPREC™ PCR (Takara Biomedicals, Otsu, Japan) and sequenced with Big Dye Primer Cycle Sequencing FS Ready Reaction Kit (Applied Biosystem) using automated sequencing 3100 Genetic Analyzer (PE Applied Biosystems). The nucleotide sequence of the 16s rDNA obtained in this process was then compared with that available in the NCBI or GenBank in order to find out the homology of the isolates with that available in the GenBank.

Data Análisis

The data obtained in this research was analysed descriptively.

Results and Discussion

Isolation was focused on rod and homofermentative lactic acid bacteria. It was found in this research that 21 isolates showed those criteria and selected as candidates for future probiotic development, among 106 isolates. In acidic conditions, all selected isolates showed positive growth response in MRS broth medium

pH 3 or 4, although their growth was not as good as in the control medium pH 6.5, following 3 hour incubation (Table 1). Three hour incubation was suited with the time needed by foods to travel along the stomach where the pH could be extreemly low [13].

As shown in Table 1, 13 isolates were found to be resistant to pH 2, although their growth was found to be slower when compared to pH 3, 4, or control. Under certain condition (when it is empty) the pH of the stomach may reach 2 or less [14]. The mechanism by which these 13 isolate tolerated the pH condition was not clear. However some scientists, such as [15], reported that the ability of an organism to maintain its internal pH higher than that of its surrounding determines its survival in an environment with extremely low pH level. To do so, this organism will need to actively pump out H^+ ions from its cytoplasm, and this mechanism needs a certain amount of ATP [16]. Although it was not elucidated in our research, the survival of the 13 isolates at pH 2 may be due to the above mechanism.

Table 1: Survival of LAB isolates following exposure to low pH conditions

Isolate codes	Growth indication and OD reading at 660nm*			
	Control (pH 6,5) (OD ± SD)	pH 2 (OD ± SD)	pH 3 (OD ± SD)	pH 4 (OD ± SD)
FBB 4	+ (2.205 ± 0.009)	+ (0.183 ± 0.061)	+ (1.993 ± 0.041)	+ (2.034 ± 0.034)
FBB 5	+ (2.192 ± 0.043)	+ (0.156 ± 0.007)	+ (1.787 ± 0.018)	+ (2.022 ± 0.011)
FBB 9	+ (2.166 ± 0.043)	+ (0.104 ± 0.007)	+ (1.942 ± 0.114)	+ (2.032 ± 0.041)
FBB 10	+ (2.104 ± 0.026)	+ (0.203 ± 0.044)	+ (1.458 ± 0.285)	+ (1.997 ± 0.030)
FBB 13	+ (2.079 ± 0.051)	- (0.024 ± 0.005)	+ (1.862 ± 0.013)	+ (1.994 ± 0.040)
FBB 18	+ (1.979 ± 0.087)	- (0.013 ± 0.007)	+ (1.689 ± 0.028)	+ (1.950 ± 0.100)
FBB 21	+ (2.084 ± 0.024)	- (0.029 ± 0.020)	+ (1.866 ± 0.034)	+ (2.029 ± 0.041)
FBB 22	+ (1.981 ± 0.046)	- (0.024 ± 0.007)	+ (1.476 ± 0.360)	+ (1.996 ± 0.088)
FBB 26	+ (2.108 ± 0.008)	- (0.018 ± 0.016)	+ (1.502 ± 0.034)	+ (2.208 ± 0.110)
FBB 38	+ (2.025 ± 0.023)	- (0.013 ± 0.002)	+ (1.921 ± 0.046)	+ (2.008 ± 0.030)
FBB 40	+ (2.054 ± 0.075)	- (0.024 ± 0.014)	+ (1.873 ± 0.042)	+ (2.020 ± 0.103)
FBB 41	+ (2.016 ± 0.061)	- (0.021 ± 0.007)	+ (1.656 ± 0.026)	+ (2.007 ± 0.046)
FBB 42	+ (2.045 ± 0.054)	+ (0.553 ± 0.246)	+ (1.894 ± 0.061)	+ (1.983 ± 0.040)
FBB 52	+ (2.082 ± 0.061)	+ (0.953 ± 0.085)	+ (1.740 ± 0.067)	+ (2.014 ± 0.013)
FBB 57	+ (2.020 ± 0.016)	+ (0.128 ± 0.041)	+ (1.930 ± 0.013)	+ (2.059 ± 0.068)
FBB 59	+ (2.048 ± 0.025)	+ (1.017 ± 0.102)	+ (1.902 ± 0.039)	+ (1.965 ± 0.024)
FBB 60	+ (2.020 ± 0.046)	+ (1.191 ± 0.155)	+ (1.748 ± 0.153)	+ (1.981 ± 0.026)
FBB 72	+ (2.038 ± 0.063)	+ (1.155 ± 0.095)	+ (1.895 ± 0.023)	+ (2.000 ± 0.052)
FBB 74	+ (2.062 ± 0.053)	+ (0.761 ± 0.201)	+ (1.330 ± 0.017)	+ (1.991 ± 0.029)
FBB 75	+ (1.974 ± 0.019)	+ (0.793 ± 0.024)	+ (1.690 ± 0.377)	+ (1.825 ± 0.058)
FBB 81	+ (2.062 ± 0.016)	+ (0.290 ± 0.041)	+ (1.305 ± 0.006)	+ (1.818 ± 0.035)

Note: + = A ≥ 0.1 (resistant to acidic condition) ; - = A < 0.1 (not resistant to acidic condition)
***Values ± Std in Table 1 are OD reading of each isolate following exposure to low pH. Each value is an average of triplicates.**

Resistance properties the 21 isolates on high concentration of NaDC were also studied in the preliminary characterization of those isolates. This characteristic is also important to study before they are developed as potential probiotics, because the probiotic candidates will be exposed to such bile salt in the small intestine before they reach colon where they are supposed to produce functional effect to their hosts [4].

In this test, some 21 isolates were grown in MRS broth medium (pH 7.2) containing NaDC with various concentrations (0.2, 0.4, or 0.6 mM), and the results are shown in Table 2.

All isolates grew well in the MRS medium containing 0.2 mM NaDC. As the concentration of the NaDC increased, less isolates were found to grow (Table 2). Only 3 isolates were found to be resistant to 0.6 mM NaDC. These results were in line with that reported by [17; 18; 19].

According to [20] tolerance characteristic of an isolate to bile salt is related to the protein and fatty acid composition of bacterial cell membrane. In addition, [21] reported that some LAB produce bile salt hydrolase that cleave bile salt so that it becomes non toxic to the these group of bacteria. The resistance properties shown by our isolates

(Table 2) were probably due to one or more mechanisms above, although further examinations need to be conducted. The results shown in Table 2 provided initial indication that some of those isolates have potential to be developed as local Indonesian probiotics.

In the molecular identification, which is based on 16s RNA sequence similarity analysis, showed compared to the previous strain (*Lactobacillus rhamnosus* strain GG strain GG (ATCC 53103). This provides us with information that isolates showing different morphological and enzymatic characteristics in the preliminary characterizations do not necessarily belong to different species, or vise versa. In other words, identification of bacterial isolates that relay on morphological and that all of our isolates were closely related to *Lactobacillus rhamnosus* strain GG strain GG (ATCC 53103) with 99% similarity. Some of our LAB isolates were also fond to have 99% similarity with strains *Lactobacillus rhamnosus* strain JCM 1136, and *Lactobacillus zeae* strain RIA 482, but their total score, in term relatedness based on the GeneBank data, was lower when enzymatic characteristics can only be used up to genus level and the results tend to be inaccurate (less accurate when compared to molecular method). Similar results were also reported by [22] who compared morphology and morphometric characteristics of grouper fishes traded in Bali with that of their nucleotide sequence analysis.

Table 2: Resistance of LAB isolates following exposure to various levels of NaDC.

Isolate codes	Control (OD ± SD)	Growth indication and OD reading at 660nm*		
		NaDC 0.2 Mm (OD ± SD)	NaDC 0.4 mM (OD ± SD)	NaDC 0.6 mM (OD ± SD)
FBB 4	+ (1.926 ± 0.011)	+ (1.400 ± 0.231)	+ (0.130 ± 0.085)	- (0.036 ± 0.010)
FBB 5	+ (1.937 ± 0.012)	+ (0.495 ± 0.069)	- (0.025 ± 0.013)	- (0.019 ± 0.010)
FBB 9	+ (1.964 ± 0.014)	+ (0.622 ± 0.181)	- (0.069 ± 0.006)	- (0.027 ± 0.025)
FBB 10	+ (1.971 ± 0.039)	+ (0.911 ± 0.144)	- (0.094 ± 0.051)	- (0.061 ± 0.047)
FBB 13	+ (1.973 ± 0.011)	+ (0.500 ± 0.064)	- (0.053 ± 0.035)	- (0.016 ± 0.010)
FBB 18	+ (1.985 ± 0.099)	+ (0.342 ± 0.009)	- (0.046 ± 0.037)	- (0.032 ± 0.009)
FBB 21	+ (1.910 ± 0.021)	+ (0.376 ± 0.064)	- (0.018 ± 0.010)	- (0.012 ± 0.003)
FBB 22	+ (1.940 ± 0.038)	+ (0.502 ± 0.014)	+ (0.129 ± 0.053)	- (0.045 ± 0.008)
FBB 26	+ (2.002 ± 0.032)	+ (0.488 ± 0.085)	+ (0.198 ± 0.036)	- (0.047 ± 0.010)
FBB 38	+ (1.925 ± 0.003)	+ (1.598 ± 0.017)	+ (0.139 ± 0.013)	- (0.046 ± 0.013)
FBB 40	+ (1.918 ± 0.022)	+ (1.838 ± 0.067)	+ (0.843 ± 0.065)	+ (0.221 ± 0.030)
FBB 41	+ (1.960 ± 0.023)	+ (0.785 ± 0.063)	+ (0.121 ± 0.031)	+ (0.124 ± 0.008)
FBB 42	+ (1.945 ± 0.013)	+ (1.813 ± 0.111)	+ (1.066 ± 0.027)	+ (0.219 ± 0.022)
FBB 52	+ (1.955 ± 0.028)	+ (0.861 ± 0.014)	- (0.022 ± 0.012)	- (0.010 ± 0.009)
FBB 57	+ (1.964 ± 0.014)	+ (1.074 ± 0.049)	- (0.094 ± 0.005)	- (0.013 ± 0.001)
FBB 59	+ (1.989 ± 0.064)	+ (1.082 ± 0.072)	- (0.033 ± 0.036)	- (0.025 ± 0.024)
FBB 60	+ (1.934 ± 0.032)	+ (0.877 ± 0.078)	+ (0.141 ± 0.083)	- (0.057 ± 0.016)
FBB 72	+ (1.930 ± 0.024)	+ (1.222 ± 0.062)	+ (0.125 ± 0.081)	- (0.049 ± 0.007)
FBB 74	+ (1.925 ± 0.028)	+ (1.678 ± 0.101)	- (0.068 ± 0.095)	- (0.034 ± 0.016)
FBB 75	+ (1.955 ± 0.005)	+ (1.835 ± 0.045)	+ (0.177 ± 0.020)	- (0.054 ± 0.002)
FBB 81	+ (2.028 ± 0.005)	+ (1.065 ± 0.092)	+ (0.309 ± 0.010)	- (0.043 ± 0.012)

Note: + = A ≥ 0.1 (resistant to NaDC) ; - = A < 0.1 (not resistant to NaDC)
*Values ± Std in Table 1 are OD reading of each isolate following exposure to various levels of NaDC. Each value is an average of triplicates.

Lactobacillus rhamonus is one of the most common isolates found in the feces of post natal healthy infants exclusively breastfed for several months. The reults obtained in our research are in line with those reported by [23; 24; 25] who found *Lactobacillus rhamonus* as the predominant species in the feces of healthy infants. Other species, such as *L. paracasei* ssp. *Paracasei* [24], *Lactobacillus salivarius* CECT 5713 [26], and *Lactobacillus salivarius* [27] are often found in the feces of infants exclusively breastfed.

Due to its adaptation ability and become predominant in human intestinal tract, *L. rhamnosus* has been intensively studied for probiotic development [8; 28]. Based on the preliminary characterization results in this research, all isolates appeared to be potential for local probiotic development, although further test,

especially safely aspects of the isolates for human probiotics, need to be conducted.

Conclusion

All isolates showed potential to be developed as local probiotics, although further tests, especially safety aspects of the isolates for human probiotics need to be conducted. All of those isolates were found to be closely related to *Lactobacillus rhamnosus* GG strain GG (ATCC 53103) with 99% similarity in term of 16s RNA nucleotide sequence. Some of those isolates also had 99% similarity with strains *Lactobacillus rhamnosus* JCM 1136 and *Lactobacillus zeae* RIA 482, but their relatedness with these two strains is lower than that with *Lactobacillus rhamnosus* GG strain GG (ATCC 53103), based on total score specified by GeneBank data.

Acknowledgment

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POTENTIAL of BIOLOGICAL CONTROL for OIL PALM BEETLE (*Oryctes rhinoceros*) with *Metarhizium anisopliae* and *Beauveria bassiana*

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Abstract

Oryctes rhinoceros has now been readily developed to a notorious pest of young oil palm plantations. Biological agents as controlling oil palm beetle have specific properties such as host specific, located spesific and narrow spectrum that have potential synergies with environment. Therefore, the necessary exploration of oil palm beetle control technology with biological control to increase productivity while maintaining a safe environment quality. The purpose of this study was to exploration of biological control for oil palm beetle with *Metarhizium anisopliae* and *Beauveria bassiana* from many localities.This research is compiled in a Completely Randomized Design non factorial with some kinds of biological control isolate : 1. *M. anisopliae* isolate Kedu; 2. *M. anisopliae* isolate Jombang; 3. *M. anisopliae* isolate Jember; 4. *B. bassiana* isolate Kedu; 5. *B. bassiana* isolate Jombang; 6. *B. bassiana* isolate Jember with spore density applications equally 10^7/ml. The result showed that : 1) Biological agents *Metarhizium anisopliae* and *Beauveria bassiana* all isolates from Kedu, Jombang, Jember had potential as a biological control of *O. rhinoceros*; 2) *Metarhizium anisopliae* isolate Jombang has the highest effectiveness in influencing mortality (80% in 144 h-after death) at the grubs of *O. rhinoceros*. ; 3) .*B. bassiana* tends to be slower in reducing mortality of *O. rhinoceros* because more spesific host

Keywords : biological control, oil palm beetle

Introduction

Indonesian palm oil sector experienced a significant development , it is seen from the total area of oil palm plantations are growing to 7.3 million hectares in 2009 from 7.0 million hectares in 2008. While the production of palm oil (crude palm oil / CPO) continued to increase from year to year from 19.2 million tons in 2008 increased to 19.4 million tons in 2009. Meanwhile, total exports also increased , in 2008 stood at 18.1 million tonnes and then to 14.9 million tons in 2009. palm oil still has a fairly bright prospects for development . Oil palm , either raw or processed products , ranked as the third largest non-oil contributors of foreign exchange for Indonesia after the rubber and coffee (Pahan , 2010) .

Problems that are often faced by farmers in the development of oil palm in Indonesia is a drag on the cultivation technologies, such as seed selection, planting, fertilization, pest and disease control, as well as post-harvest handling. Yields of quality palm oil, other than specified by the maintenance and fertilization also depends on how to deal with pests and disease. One of the major pests of oil palm trees are weevils shoots (*Oryctes rhinoceros*). Damage by Oryctes beetles is characteristized by the often triangular gaps in the leaves of oil palm crowns, while fan palms show a row of large holes in the leaflets. The adults bore into the growing spear lef cluster, causing emerging leaves to be shortened, broken and distorted even kill young palms (Kalshoven, 1981).

Chemical control is one way that is often done by farmers due to chemical insecticides have killing power rapid, broad-spectrum so immediately visible results. Pest control with chemical insecticides will have a positive impact with the death of the pest. Control weevils shoots in the field conducted if the beetle population or new damage > 5 per ha with chemical control using insecticides . If the beetle population and new damage is < 5 per ha of the insecticide used is an insecticide with the active ingredient carbosulfan dose of 5 g of product per tree per 2 weeks sown diketiak leaves or sipermetrin concentration of 2 % as much as 100 cc of solution per tree was sprayed with a knapsack sprayer from shoots up to 2 midrib underneath (IMC Plantation , 2011) .But chemical insecticides also will have a negative impacts such as resistance, resurgence, and the eruption of the second pest. It also impair human health and environmental balance, due to high residue on the component production and ecosystems (Erawati, 2009a). One alternative pest control that is safe for the environment and can reduce chemical residues in agricultural products is the biological control.

Erawati (2012) reported that the pest and disease control tobacco plants using biological control has no effect on crop productivity quantitatively . The development of biological agents such as *B. bassiana* to control pests and Trichoderma sp . to control the disease have the potential and good prospects because of the specific nature of the host that is not harmful to humans , natural enemies and the environment . Controlling costs can be reduced as a biological control can be reproduced itself . Other advantages are the residue and the accumulation of toxic compounds that have the potential to pollute the environment is very low because biological agents are more easily biodegradable .

Biological control has great potential as a natural control of pests of oil palm shoot borers are entomopathogenic fungi *Metarrhizium anisopliae* and *Beauveria bassiana*. Erawati (2006) reported that the pathogenicity test results entomopathogenic fungus *Beauveria bassiana* showed that the percentage of dead larvae of *S. litura* infected with *B. bassiana* strain 725 with a density of 10^7 spores / ml was 32% at 48 hours after application and reached 60% at 72 hours after application. Marheni, et al (2011) reported that the application of *M. anisopliae* with 20 grams of corn media showed the highest mortality of larvae of *O. rhinoceros* to 100%. Entomopathogenic fungi's ability to kill insects and pests vary greatly influenced by the character of physiology and genetics of fungi (Trizelia 2005 *in* Hamdani, dkk., 2011).

Biological control of *M. anisopliae* and *B. bassiana* entomopathogenic fungi known as having a broad host range , but still has a host of specific characteristics and specific locations as a typical characteristic of a biological control (Gabarty , et.al , 2011). Development of a biological control technology *M. anisopliae* and *B. bassiana* effective and efficient as *O. rhinoceros* pest control is very important to be able to increase productivity of oil palm plantations with regard to the quality of the environment . Therefore, it is necessary to exploration isolates from several areas to develop technologies such as biological control entomopathogenic fungi *M. anisopliae* and *B. bassiana* as a pest control *O. rhinoceros*.

Materials and methods

The research was conducted at the Laboratory of Plant Protection and Laboratory of Biosain Polytechnic of Jember , from March to September 2015. The study was prepared based on Completely Randomized Design (CRD) single factor with five (5) replicates. Treatment : 1) *M. anisopliae* isolates Jember ; 2) *M. anisopliae* isolates Jombang ; 3) *M. anisopliae* isolates Kedu ; 4) *B. bassiana* isolates Jember ; 5) *B. bassiana* isolates Jombang ; 6) *B. bassiana* isolates Kedu with each concentration of spore density 10^7spores / ml . As a comparison treatment with the application of synthetic chemical insecticide concentration of 2 mg / liter and without treatment .

Preparation of Biological Control

Entomopathogenic fungi in the form of isolates of *M. anisopliae* and *B. bassiana* is obtained from Pusat Penelitian Kopi dan Kakao Indonesia in Jember, Balai Besar Perbenihan dan Proteksi Tanaman Perkebunan Surabaya in Jombang and Laboratorium Pengamat Hama dan Penyakit Kedu. Isolates obtained on the media multiply Potatto Dextrose Agar (PDA). Isolates collected from areas with altitude ranges from 0-90 m asl and isolated from the order Coleoptera to maintain biological control properties that tend to specific locations and specific host

Preparation *O. rhinoceros* Larvae

Test larvae of *O. rhinoceros* be collected from the field in Dusun Kepel Rekesan Desa Lojejer Kecamatan Ambulu Kabupaten Jember. Insects reared test that will be used to achieve uniformity 3 instar larvae and selected body size , normal and healthy to maintain the homogenecity of the material .

Treatment Application (*Screening Test*)

1. Prepare test larval, third instar larvaae that had previously been starvation for 24 - 48 hours. Test larvae placed in vials of insects which have filled the media feed each one larvae and 5 replications.
2. Prepare each isolate to be tested by setting the appropriate concentration treatment. Spore density of *M. anisopliae* and *B. bassiana* calculated by haemocytometer equally 10^7 spores/ml.
3. The application of treatment by using three methods:

a. Treatment application series 1

1. Determination of application methods for the application of the treatment series 1 with immersion method in a solution isolates of biological control for 10 minutes
2. Preparation of test insects by starvation for 24 hours before treatment application.

b. Treatment appliacation series 2

1. Determination of application methods for the application of the treatment series 2 with feeding superbly isolates infected biological control in each test insects
2. Preparation of test insects by starvation for 48 hours before treatment application series 2

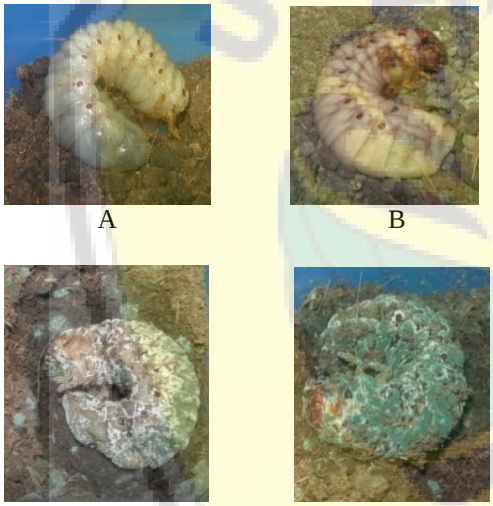
c. Treatment application series 3

1. Determination of application methods for the application of the treatment series 3 with the cadaveric trapping method 2. Preparation of the test insects without starvation period before the application of the treatment series 3.
2. The test larvae were observed and maintained with regular feeding up to one replay achieve 100% mortality.

Results and discussion

Symptoms of Death

Observations of insect mortality symptom test is done through observation of the activity and color change cuticle until the mummification process on the test insects as one of the signs of death caused by infectious biological control of *M. anisopliae* and *B. bassiana*. Third instar larvae of *O. rhinoceros* are healthy and have normal body size 10 -12 cm , have three pairs of legs thoracal and strong mandible. Larval cuticle has a white body with the head blackish brown



A B C D

Figure 1. Stages symptoms of death *O. rhinoceros* larva by *M. anisopliaee* infection (A – D)

Test insects were infected with *M. anisopliae* at first they were active (Figure 1 A) . When the infection rates began to rise , pale colored cuticle evenly on the entire body and test larvae will die with smaller posterior section (Figure 1 B) . Larval body becomes rigid and will appear white hyphae at 2 days dafter death, especially in the anterior and posterior . On 3 days after death , the fungus will sporulate green color (Figure 1 C) . Cadaveric body green fungus on 4-10 days after death (Figure 1 D) .

C D

Figure 2. Stages symptoms of death *O. rhinoceros* larvae by *B. bassiana* infection (A – D)

Test insects were infected with *B. bassiana* will decrease its activity . The cuticle is still bright and shiny body size is normal (Figure 2 A) . After the test larvae die , the body secretes the fluid, looks wet and smelled like ethanol . Shortly after the test larvae die , the body is still weak and not stiff . Cuticle pale brownish color and curvy posterior part and shrink. Furthermore cadaveric body will look dull , dry and stiff and begin to grow hyphae , especially in the thoracic and abdominal (Figure 2 B and 2 C) . White miselum will grow cadaveric body wrap at 5-12 days after death (Figure 2 D)

Mortality

Power infection is the ability of a deadly biological agent in the test insects . Each treatment gives a different effect on the death rate test insects listed in figure 3.

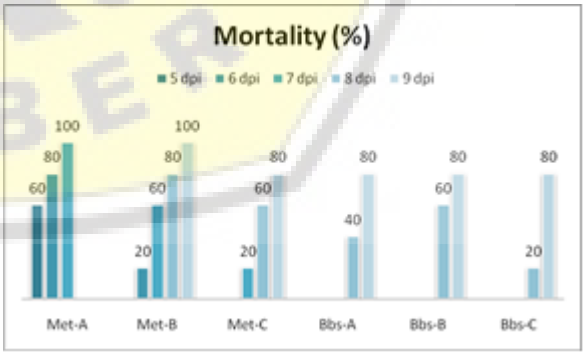


Figure 3. Mortality of test insect

Figure 3 shows that the treatment of all isolates are capable of causing death in test insects . M. anisopliae is able to kill test insects on average by 80 % - 100 % in 7 day to 9 day post infection . Treatment Met- A (isolates Jombang) has the highest mortality rate of 60% in 5 day post

infection , 80 % in 6 d-post infection and 100 % at 7 day post infection . B. bassiana treatment showed an average mortality of 80 % in 9 day post infection.

Treatment Met- A (isolates Jombang) provide the highest emphasis on mortality of larvae test O. rhinoceros . M. anisopliae isolates Jombang effective cause of death because the isolate are host -specific and site-specific . Jombang *M. anisopliae* isolates isolated from larva *Oryctes rhinoceros* at altitude 0-70 m. While M. anisopliae Kedu isolated from larvae *Lepidiota stigma* and *M. anisopliae* Jember isolated from larvae *Stephanoderes hampei* . Sambiran and Hosang (2003 *in* Marheni , 2011) showed that the best host for the developing of *M. anisopliae* is *O. rhinoceros* .

Biological control of *M. anisopliae* and *B. bassiana* entomopathogenic fungi known as having a broad host range , but still has a host of specific characteristics and specific locations as a typical characteristic of a biological control (Gabarty , et.all , 2011)

M. anisopliae infection mechanism is classified into four stages of insect disease etiology . The first stage is the inoculation , the contact between fungal inoculum with the body of the insect . The second stage is the process of attachment and the germination of fungal spores in the insect integument . The third stage is the penetration and invasion , which formed tube sprouts and entered emnembus insects integument . The fourth stage is the destruction at the point of penetration and the formation of blastospora which then spread into hemolimfa and form a secondary hyphae to invade other tissues . After the dead insects , fungi continue the life cycle in a saprophytic phase to colonize the host body and the production of infective spores (Freimoser , et . all, 2003 *in* Marheni , 2011) .

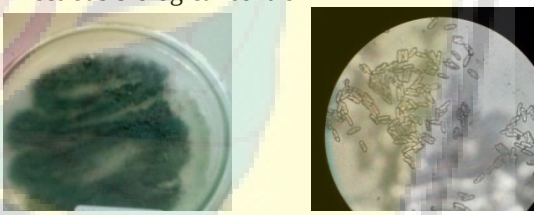
M. anisopliae is generally entered through spiracles and holes in body organs of insects . At the time of first entry in the body of the insect , fungal hyphae producing lateral used as a tool or channel to siphon off part of the body of the insect . Hyphae grow steadily until the insect's body is filled with mycelia . When the inside of the body was eaten by insects , fungus damage the cuticle and do sporulation. *M. anisopliae* can release spores (conidia) in conditions of low humidity (< 50 %) . The fungus can also produce secondary metabolites that are toxic.

B. bassiana infection is slow due to infection mechanism starts with the attachment of conidia on the test larval cuticle then germinate and grow inside the host body . Infected insect show early symptoms such as insects become weak , the sensitivity and the feeding activity is reduced gradually insects will die . Insect death marked the end of the parasitic phase of development of the fungus . Furthermore, mycelia will grow saprofit meet all the body tissues of insects (Ferron , 1981 *in* Erawati, 2009b)

Beauveria bassiana holding insect penetration into the body through the skin between the body segments . Mechanism begins with the penetration of the cuticle spore growth which in turn will issue a fungal enzyme chitinase , lipase and proteinase at 12-24 hours before the penetration process which is able to decipher the composition of insect cuticle . In the body of the insect , the fungus is growing and enter the bloodstream . B. bassiana also produce toxins such as beauverisin , beauverolit , bassianolit , isorolit and oxalic acid which in its mechanism of action leads to increase in blood pH , blood clotting and cessation of blood circulation . This fungus also causes damage to tissue or organ haemosel mechanically such as the gastrointestinal tract , muscles , nervous system and respiratory system . As a result of the whole process will result in the death of insects (Robert , 1981) .

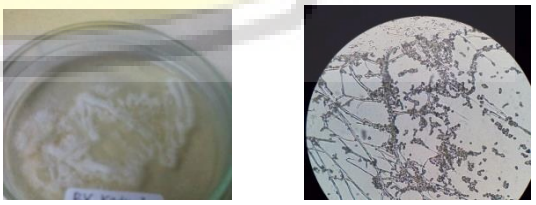
Surgery Test Insects

Surgery test insects (cadaver) is dissected larval body test to ensure that the death was caused by infectious biological control



A B

Figure 4. The results of the purification of isolates of *M. anisopliae* from insect cadavers test
A Colony of fungi on PDA
B. *M. anisopliae* spores (microscopic)



A **B**

Figure 4. The results of the purification of isolates of *B. bassiana* from insect cadavers test
A Colony of fungi on PDA
B *B. bassiana* spores (microscopic)

Figure 4 and Figure 5 are the results of the purification of isolates of *M. anisopliae* and *B.*

bassiana on the insect cadaver body part in the test . A source of inoculum for refining taken from cadavers hemolimfa test insects to maintain the purity of each isolate *M. anisopliae* colonies on PDA showed a dark green color that is characteristic of sporulation of *M. anisopliae* (Figure 4 A) . *M. anisopliae* spores rod-shaped or capsule (Figure 4 B) .

B. basiiana colony on PDA showed white color that is characteristic of sporulation of B. bassiana (Figure 5 A) . Spores *B. bassiana* round or ovoid (Figure 5 B) . *B. bassiana* has been known to attack several species of insects from different orders with the characteristic mushroom -shaped conidiophores single / group and bulging at the bottom. Conidia form one cell , hyaline , ovoid shape , rounded , formed akropetal . Infection of *Beauveria bassiana* on the insect pests causing decreased feeding activity , the weak and the cuticle is white because of the closed mold spores (Erawati , 2009b).

Conclusions

1. *Metarhizium anisopliae* and *Beauveria bassiana* all isolates from Kedu, Jombang, Jember had potential as a biological control of *O. rhinoceros*;
2. *Metarhizium anisopliae* isolate Jombang has the highest effectiveness in influencing mortality (80% in 144 h-after death) at the grubs of *O. rhinoceros*.
3. *B. bassiana* tends to be slower in reducing mortality of *O. rhinoceros* because more spesific host

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EFFECT of SOIL MICROBES EFFECT on PRODUCTIVITY CAYENNE PEPPER (*Capsicum frutencens* L).

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Abstract

Soil microbes are microorganisms that can improve soil fertility and help provide nutrients plants, it can apply on horticulture plants e.g cayenne pepper plant (*Capsicum frutencens* L.). The purpose of this study was determining the soil microbes effect on the productivity of cayenne pepper (*Capsicum frutencens* L.). This research used RAK (*Randomized block design*) experimental methods which were applied at 4 treatment; P1 = without fertilizers, P2 = NPK, P3 = NPK + microbes, P4 = microbes and repeated triplicates. The parameters included of amount of flower, amount of fruit. Data were analyzed using *one way* ANOVA test at level $\alpha = 0.05$ and *post-hoc* of Tukey. The test results showed that there were significant differences in the amount of flower and amount of fruit cayenne pepper plant (*Capsicum frutencens* L.). Results of research on the treatment P4 showed the highest results for the parameter amount of flower (24,78) and the amount of fruits (24,11) cayenne pepper plant *(Capsicum frutencens L)*.

Keywords : *Capsicum frutencens* L, Soil Microbe, Productivity

Introduction

Cayenne pepper (*Capsicum frutescens* L) is considered as one of holticultural plant which is so important for Indonesian, especially the society of east java in the daily consumtion. The contents of cayenne pepper, among others: fat, protein, carbohydrates, calcium, phosphorus, iron, vitamin A, B1, B2, C and alkaloid compounds such as capsaicin, oleoresin, flavonoids and essential oils used as flavoring food, traditional medicine (industry pharmaceutical), food industry, and poultry feed [1].

According to Central Bureau of statistics of Indonesia, the productivity of cayenne pepper in east Java continue to increased, in 2010 production 142.109 ton, in 2011 production 181.806 ton, in 2012 production 244.040 ton, in 2013 productivity of cayenne pepper decreased becomes 227.486 and 4.49 ton / ha [2]. In addition to declining production in recent years, the cost of cayenne pepper fluctuates from normal cost of about 20,000.00 reached 100,000.00 on the market, it because the production of cayenne pepper do not meet demands of the market and most farmers failed to harvest.

Application of chemical fertilizers is the way which done by the farmers to increase the productivity of cayenne pepper. However, the application of chemical fertilizers is continuously making the soil more acidic conditions [3].

Alternative use of fertilizers that are environmentally friendly and have a positive impact on the ground is indispensable. One of them is the use of microorganisms, microorganisms can serve as fastening a particular nutrient or facilitate the availability of nutrients in the soil for plants [4].

Some soil microbes used are non-symbiotic nitrogen fixation microbes (*Azotobacter* sp and *Azospirillum* sp), microbial solvent phosphate (*Bacillus* sp and *Pseudomonas* sp) and *Cytophaga* sp. *Azotobacter* sp and *Azospirillum* sp is a bacteria vital to the nitrogen cycle [5]. *Bacillus* sp and Pseudomonas sp is phosphate solvent, this bacteria can increase element P in soil into available forms [5] and *Cytophaga* sp as decomposers of organic material. Therefore, this study was designed to determine the effect of soil microbes on produktivtas cayenne pepper plants.

Materials and Methods

Seed of cayenne pepper

Cayenne pepper seeds obtained from farm shops in Jember. Cayenne pepper seed was initially soaked in water for 6 hours at room temperature. Then the seeds were selected for seed sinking.

Preparation of soil microbes and NPK fertilizer

Microbes fertilizer : 0,3ml × 36 plants = 10,8 ml. diluted in 1 liters of water.
NPK fertilizer : 15 grams dissolved in 1 liter of water, 200 ml dose of each plant.
NPK+Microbes fertilizer : ½ from dose of NPK + ½ from dose of Microbes fertilizer.

Planting process

The soil is destroyed so homogeneous, then put in a polybag nursery and planting. Cayenne pepper seeds have been soaked, it planted in a polybag nursery, age 25 days the cayenne pepper seed is moved in polybag planted.

Fertilizer application

Microbes and NPK fertilizer is applied when the plants have aged 1 week and 1 month in polybag planting. Treatment P1 = without fertilizers, P2 = NPK, P3 = NPK + microbes, P4 = Microbes**.**

Statistical Analysis

All treatments repeated triplicates, and the observed parameters are the amount of flower and the amount of fruit were analyzed using *one-way* ANOVA test at 5% level and Tukey *post-hoc* test.

Results and Discussion

The amount of flower and the amount of fruits used several treatments showed significant differences (Table 1). Treatment using soil microbes showed the highest results for parameters the amount of interest (24.78 ± 4.44 b) and the amount of fruit (24.11 ± 4.57 b). The results showed treatment with the use of soil microbes showed higher results than without fertilizer.

Table 1. Means amount of flower and amount of fruit of cayenne pepper *(Capsicum frutencens* L*)* using *post-hoc tukey test.*

Treatment	Amount of flower	Amount of fruit
P1 (Without fertilizer)	18,33 ±4,44 [a]	17,67 ±4,57 [a]
P2 (NPK)	20,11 ±4,44 [ab]	19,33 ±4,57 [ab]
P3 (NPK+Microbes)	21,78 ±4,44 [ab]	21,56 ±4,57 [ab]
P4 (Microbes)	24,78 ±4,44 [b]	24,11 ±4,57 [b]

Information: Notation with the same show the difference is not real test of Tukey level 5%.

Table 2. Statistical analysis with *One-way Anova* amount of flower cayenne pepper *(Capsicum frutencens* L*)*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	202.750	3	67.583	4.424	.010
Within Groups	490.000	32	15.313		
Total	24.240	35			

Table 3. Statistical analysis with *One-way Anova* amount of fruit of cayenne pepper *(Capsicum frutencens* L*)*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	210.889	3	70.296	4.300	.012
Within Groups	523.111	32	16.347		
Total	734.000	35			

Parametric analysis using one-way ANOVA test showed significant value on the amount of flower is (0.010 <0.05) and amount of fruit is (0.012 <0.05) there were significant different on the amount of flower and the amount of fruit cayenne pepper (*Capsicum frutencens* L).

Results showed the highest in parameters the amount of flower and the amount of fruit is the use of soil microbes (table 1). This is because the nutrients produced by soil microbes are able to meet demands of cayenne pepper (Capsicum frutencens L.).

Azotobacter sp and *Azospirillum* sp is bacteria fixation of nitrogene. These microbes have the enzyme nitrogenase. This enzyme serves to catalyze the free nitrogen that can not be reached by the plant. *Azotobacter* sp able to change the nitrogen (N_2) in the atmosphere into ammonia (NH_4^+) through nitrogen fixation in which the ammonia produced is converted into proteins needed by plants [5].

Nitrogene has the function to stimulate the growth of plants and all plant tissue formation [7]. If all during the vegetative growth of the plants grow well then it will have effect on the plant generative period.

Bacillus sp and *Pseudomonas* sp capable of anchoring phosphate become available to plants cayenne pepper as much as 50% by removing the organic acids such as citrate, glutamate, succinate, and glioksalat that can break metal bond Fe, Al, Ca, and Mg so phosphate is bound to be dissolved and the sufficiency of P [8]. Phosphate serves for the stimulation of flowering plants cayenne pepper, fulfillment of the element P causes the maximum flowering process [7].

Conclusion

Soil microbial influencial on productivity of plant cayenne pepper (Capsicum frutencens L). The use of soil microbes showed the highest in parameters the amount of flowers (24.78 ± 4.44 b) and the amount of fruit (24.11 ± 4.57 b) the cayenne pepper plant *(Capsicum frutencens L).*

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POTENTIAL TEST TRICHODERMA INDEGENUS SOUTHEAST SULAWESI as to *Fusarium oxysporum* BIOFUNGISIDA THE IN-VITRO

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Abstract

This study aimed to test potential of several isolates of *Trichoderma* spp indigenus Southeast Sulawesi as biofungisida in inhibiting the growth of *F. oxysporum* in-vitro. Inhibitory potency test isolates of *Trichoderma* spp method, the double test on PDA. The experiment was conducted using a completely randomized design (CRD) consisting of 11 treatments, 11 isolates of *Trichoderma* spp indegenus Southeast Sulawesi with three replications. The parameters measured were trichoderma indigenus inhibition on the growth of *F. oxysporum*. The results showed that the 11 isolates tested trichoderma indegenus potential as biofungisida against *F. oxysporum* because it can inhibit the growth of pathogens in vitro. DKT isolates showed inhibition of the highest percentage of 60.12% with the mechanism of inhibition of rapid form of competition space, mikoparasit and allegedly produce antibiosis form of chitinase enzyme that can lyse cell wall of *F. oxysporum.*
Keywords : Indigenus, Isolates, *F. oxysporum*, Southeast Sulawesi, *Trichoder*

Introduction

Fusarium oxysporum is an important pathogen that frequently infects pepper plants in the Southeast. *F.oxysporum* causes of jaundice in pepper plants. The pathogen is soil borne pathogens that spread very easily in the garden farmer, can associate and lead to serious illness and even be lethal pepper quickly.

Reports loss due to jaundice so far no, but the symptoms of jaundice have been found in pepper crops in Konawe, South Konawe and Kolaka (data not yet published) Efforts to control the disease is not yet fully done so it can be a potential source of inoculum in the orchard farmers always available.

Control methods are often done by farmers that use chemical pesticides that exceed recommended doses and used continuously, resulting in the accumulation of high pesticide causing negative impacts on the environment and health. So it takes control alternatives such as the use of biological agents in the form of local Southeast Sulawesi trichoderma indigenus that have adapted to the environment of origin which is expected to be an effective biological control in Southeast Sulawesi.

That the specific biological control is local, that antagonistic microorganisms contained in an area will only give good results in the area of origin [1]. Results of the study that *Trichoderma* sp. isolate South Kalimantan has a better ability to control rice leaf sheath blight disease compared with *Trichoderma* sp. Yogyakarta origin of tidal land areas in South Kalimantan[2]. It is proved that isolates trichoderma indigenus have a better potential in suppressing pathogenic than using isolates from other areas.

That the mechanism of biological agents *Trichoderma* sp. against the pathogen is competition, mikoparasit and antibiosis [3]. Besides the fungus *Trichoderma* sp. also has some advantages such as easily isolated, wide adaptability, can grow quickly on a variety of substrates, have a wide range mikroparasitisme and not pathogenic to plants.

Currently there are 11 isolates trichoderma indegenus Southeast Sulawesi which has been explored from the rhizosphere of some plants cultivated in Southeast Sulawesi and have been characterized based on morphology [4] and have tested its potential as biofungisida against *Phytopthora capsici* [5], against *Colletotrichum* sp [6] .

Based on the above, it is necessary to research on potential test isolates trichoderma indigenus Southeast Sulawesi as biofungisida against *F. oxysporum* origin pepper plants in vitro.

Methods Research

This study uses a completely randomized design (CRD) of which 11 isolates trichoderma indigenus Sulawesi Landmarks disimbol with (T) consisting of isolates DKT (FT1), isolates BPS (FT2), isolates LKA (FT3), isoat ASL (FT4), isolates LTB (FT5), isolates APS (FT6), isolates LPS (FT7), isolates LKP (FT8), isolates DPA (FT9), isolates LKO (FT10) and isolates DKP (FT11) will be tested power potential inhibitory

against *F. oxysporum* with the symbol F. Thus, there are 11 combinations of treatments were repeated 3 times so that there are 33 experimental units.

Prosedur Research

Sampling Plant Pathogen Infected

Samples of infected plant pathogen *F. oxysporum* taken the form of leaves**,** stems and roots are still symptomatic further between the plants that have been infected and any part of the plant that is still fresh then put in a plastic bag in order to retain moisture until use. Samples were infected with the pathogen must be isolated to avoid microbial contamination other than pathogens desired**.**

Isolation *F.oxysporum*

Isolation of *F. oxysporum* can be done by isolating the infected parts of the plant pathogen isolates .Apabila have found what we want and then purified to get really isolate expected in accordance with the identification by [7]

Isolate Rejuvenation *Trichoderma* spp.

Rejuvenation isolates of Trichoderma spp. done in a way that isolates mediated regrow new PDA then diingkubasi for seven days until it is ready to be tested.

Inhibition Test *Trichoderma* spp. against *F. oxysporum*

Testing inhibition of the fungus *Trichoderma* spp. against *F. oxysporum* carried out using test methods double on PDA. One trichoderma isolates and pathogenic aged 7 days were planted in sterile PDA medium with a distance of 3 cm in the opposite place them in a petri dish measuring 9 cm. Each isolate the fungus *Trichoderma* spp. testing with repetition three times on each pathogen test.

Variable observation

Inhibition *Trichoderma* spp. against the pathogen test

Observations were made every day (for seven days) on the growth of pathogens by measuring the radius of the growth of pathogens towards the edge of the Petri dish (R1) and the radius in the direction of growth of pathogenic fungi *Trichoderma* spp. (R2). The acquired data is used to calculate the power resistor (DH) isolates of the fungus *Trichoderma* spp. against fungal pathogens, which are determined by the formula that is used [8]

$$DH = \frac{R_1 - R_2}{\text{———}} \times 100\%$$

Description:

R1 = radius of the growth of fungal pathogens towards the edge of the petri

R2 = radius of the growth of pathogenic fungi isolates toward trichoderma

The mechanism of inhibition of *Trichoderma* spp. against the pathogen descriptive test.

The mechanism of inhibition of *Trichoderma* spp. against the pathogen descriptive test was observed by looking inhibitory activity that occurs dimedium PDA.

Data analysis

Data were analyzed using analysis of variance. If the analysis of variance there is a real effect, then continued with Duncan Multiple Range Test (UJBD) at the level of 95% and for the mechanism of inhibition of *Trichoderma* spp. against *F. oxysporum* were analyzed descriptively.

Results and Discussion

Inhibition of *Trichoderma* spp. against Fusarium oxysporum

Inhibition of *Trichoderma* spp. At any time observations are presented in Table 1.

Mechanism of Inhibition of *Trichoderma* spp. against *Fusarium oxysporum* in- vitro

Based on descriptive observations, there are several mechanisms of inhibition of *Trichoderma* spp. against *Fusarium oxysporum*. The mechanism of inhibition of each isolate are presented in Table 2.

Fusarium oxysporum is a soil borne pathogen that always infects pepper plantations in Southeast Sulawesi. Control solution more effective and environmentally friendly in controlling both the pathogen, one of which is the use of biological agents such as trichoderma indigenus. Trichoderma is a soil fungus that is saprophyte and antagonistic to pathogenic fungi, for example, *P. infestan* causes late blight and potato tubers [9]), *Pythium* sp plant pests that cause disease on seeds of durian [10] and *F. oxysporum* causes wilt disease in tomato plants [11].

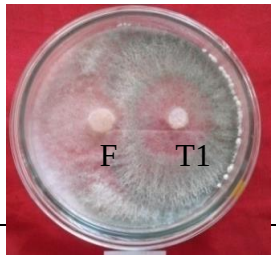
Based on the results of test observations antagonist *Trichodrma* spp. against *Fusarium oxysporum* showed that the growth of colonies of pathogens fingers towards the midpoint of the PDA medium slower than the growth of colonies trichoderma fingers [9] states that *Trichoderma* sp. is a type of microorganism potential for biological control of disease. Results of the research that has been done to support the opinion that the 11 isolates of *Trichoderma* spp. tested could inhibit the growth of *F. oxysporum* in PDA medium in vitro. This means that Trichoderma indigenus Southeast Sulawesi able to utilize nutrients, space,

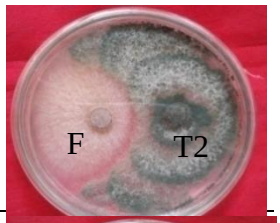
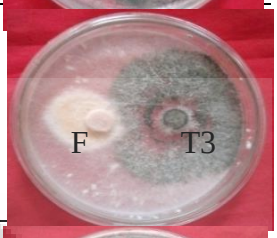
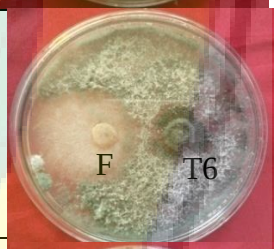
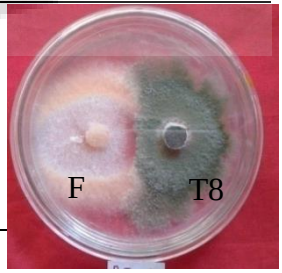
and capable of producing a compound suspected of antibiosis and parasitism of pathogens.

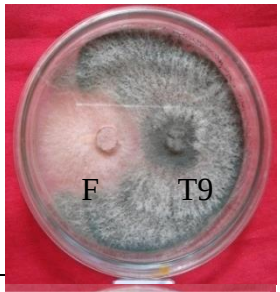
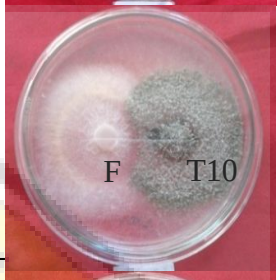
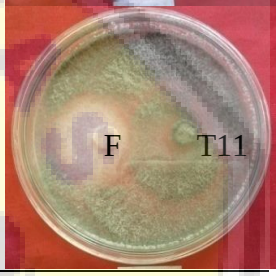
Trichoderma spp. tested differences in the ability to perform inhibitory activity against *F. oxysporum.* Such differences allegedly due to different characteristics of each isolate *Trichoderma* spp. related to the speed of growth in the medium as well as the mechanisms in the inhibitory activity against *Fusarium oxysporum* power. That the critical factor determining antagonist activity, to control pathogenic microorganisms is to have a higher growth rate so as to compete with the pathogens in terms of mastery of space and food, which in turn can suppress the growth of pathogenic fungi [12].

The observation of the 11 isolates of Trichoderma spp. tested against *Fusarium oxysporum*, on average, can inhibit the growth of observations 2 observations 1 HSI HSI whereas inhibition has not occurred in which each of pathogenic fungi and *Trichoderma* spp. grow without affecting each other as the distance grows both cultures are still quite wide on PDA medium. In observation of 2 HSI has taken place in which both pathogen inhibition mechanisms closer together and formed inhibition zones. The inhibition zones are not fixed during the observation this is because the 11 isolates of *Trichoderma* spp. still active in inhibitory activity. According [9] that the fast-growing fungus is able to outperform in the control room and can eventually suppress the growth of fungi opponent. In addition to the competition mechanism of the 11 isolates chamber is also expected to inhibit pathogens through antibiosis mechanism characterized by the depletion of colonies of pathogens due to enzyme produced, [13] suggests that antibiosis is the antagonism mediated by specific or non-specific metabolites, enzymes, volatile compounds, or toxic substances (toxins) produced by other microbes. Furthermore [14] states that when it reaches its host mikoparasit, hifanya then twist or choke the host hyphae to form structures such as hooks (hook-like structure) and then absorb the nutrients host., and according to [15] that *Trichoderma* sp. able to produce cellulase enzymes to degrade cellulose, which cellulose is the main component of fungal cell wall constituent. Mechanism of inhibition to -11 isolates of *Trichoderma* spp. against *F. oxysporum* is generally in the form of competition space, mikoparasit and antibiosis. Besides allegedly because the enzyme chitinase which is owned by the fungus *Trichoderma* sp. can cause lysis of the cell wall chitin of fungi *F. oxysporum*. According to [16] The fungus *T. harzianum* and *T. hamate* act as mikoparasit against the fungus *Rhizoctonia solani* and *Sclerotium rolfsii*, produce enzymes ß (1,3) glucanase and chitinase which causes eksolisis the host hyphae. Further *T. hamate* also produce cellulase enzymes, thus adding capabilities as mikoparasit against pathogens that constituent cell wall is cellulose. According [17], the combination of the two enzymes cellulase and chitinase increase the synergistic fungus *Trichoderma* spp. as an antifungal. Based on the observations of inhibition of *Trichoderma* spp., Isolates DKT an isolate that has the best ability to inhibit *Fusarium oxysporum* that is equal to 60.12%. This is because trichoderma isolates DKT has a mechanism of competition space and nutrients that are very fast compared to other isolates, then after these isolates colonized the space to grow and come into contact with colonies of pathogens suspected mechanisms of antagonist else is happening in the form of antibiosis characterized by the ability of causing the cell wall of the pathogen lysis so that colonies of pathogens and narrow, besides other mechanisms such as mikopasit which isolates DKT able to grow on colonies pathogens. Isolat which has the lowest potential in suppressing the growth of *Fusarium oxysporum* was isolated LKP is equal to 43.89%. This is because the LKP isolates had growth tends to be slower than other isolates. Nevertheless isolates pertubuhan LKP still can inhibit pathogens. This is because the LKP isolates had growth tends to be slower than other isolates. However isolates LKP still can inhibit the growth of pathogens.

Table 2. Mechanism of Inhibition of *Trichoderma* spp. against *F.oxysporum*

Isolates Code	Inhibition Mechanism	Description	Picture
DKT (FT_1)	Competitions space and nutrients, Mikoparasit, antibiosis	*Trichoderma* colonies meet the surface of the medium, colonies of pathogens lysis characterized by the depletion of colonies of pathogens and parasitism	

BPS (FT_2)	Competition nutrition space, Mikoparasit, antibiosis	*Trichoderma* colonies meet the surface of the medium, colonies of pathogens lysis characterized by the depletion of colonies of pathogens and parasitism	
LKA (FT_3)	Mikoparasit, space and nutrients Competition	*Trichoderma* colony grows fills the entire surface of the media and parasitism	
ASL (FT_4)	Competition nutrition space, Mikoparasit, antibiosis	*Trichoderma* colonies meet the surface of the medium, colonies of pathogens lysis characterized by the depletion of pathogen colonies	
LTB (FT_5)	Competitions space and nutrients, mikoparasit	*Trichoderma* grows meet the entire surface of the media and trichoderma riding on the surface of the pathogen colony	
APS (FT_6)	Competition nutrition space, mikoparasit, antibiosis	*Trichoderma* colonies meet the surface of the medium, colonies of pathogens lysis characterized by the depletion of colonies of pathogens and parasitism .	
LPS (FT_7)	Mikoparasit, competition space and nutrients and antibiosis	*Trichoderma* colonies meet the surface of the medium, colonies of pathogens lysis characterized by the depletion of colonies of pathogens and parasitism	
LKP (FT_8)	Competitions space and nutrients, antibiosis	*Trichoderma* colonies meet the surface of the medium, colonies of pathogens lysis characterized by the depletion of colonies of pathogens and parasitism .	

DPA (FT_9)	Competitions space and nutrients, antibiosis, mikoprasit	*Trichoderma* colonies meet the surface of the medium, colonies of pathogens lysis characterized by the depletion of colonies of pathogens and parasitisme .	
LKO (FT_{10})	Competitions space and nutrients, antibiosis	*Trichoderma* colonies meet the surface of the medium, colonies of pathogens lysis characterized by the depletion of colonies of pathogens and parasitisme	
DKP (FT_{11})	Competitions space and nutrients, antibiosis, mikoparasit	*Trichoderma* colonies meet the surface of the medium, colonies of pathogens lysis characterized by the depletion of colonies of pathogens and parasitisme	

Description : F= *Fusarium oxysporum*, T = isolate *Trichoderma* sp, 1,2.3 dst = code isolate

Conclusions

The eleven isolates trichoderma indigenus Southeast Sulawesi potential as biofungisida against *F. oxysporum* in vitro and DKT isolates that have the best potential of 60.12% with a mechanism in the form of competition space quickly, mikoparsit and allegedly produce antibiosis form of chitinase enzyme that can lyse cells dinding *F. oxysporum*. The eleven isolates trichoderma indigenus Southeast Sulawesi potential as biofungisida against *F. oxysporum* in vitro and DKT isolates that have the best potential of 60.12% with a mechanism in the form of competition space quickly, mikoparsit and allegedly produce antibiosis form of chitinase enzyme that can lyse cells wall *F. oxysporum*

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LIPID ANALYSIS of SOME POTENTIAL MICROALGAE for FOOD SUPLEMENT CANDIDATE

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Abstract

Microalgae are microscopic algae commonly found in freshwater and seawater. There are four components of the main substances contained in microalgae, namely carbohydrates, proteins, nucleic acids, and total lipids. All of these components are components of food that is needed by the human body. Microalgae culture can address the needs of the food available in nature. This study aims to determine the composition of lipids at several microalgae. Working method used is descriptive method that can be interpreted by the fact-finding proper interpretation. Methods of data collection include primary data and secondary data. Activity analysis process lipids are carried on numerous microalgae culture reached logarithmic and stationary phases. The lipid content is always decreasing in every phase of growth so that the largest lipid content in logarithmic phase 1. Lipids extraction of microalgae carried out by the Bligh and Dryer method. The principle of this method is based on the gravimetric of two layers separation based on their molecular weight. The solvent used was methanol (polar) to bind water and chloroform (nonpolar) for lipid binding. Microalgae *Tetraselmis* sp. has the larger lipid weight on day 8 than *Cosmarium* sp. and *Scenedesmus* sp. The lipid weight respectively is 540 mg/l, 481.25 mg/l, and 8831.25 mg/l

Keywords: microalgae, lipids, analysis, food supplement

Introduction

Food supply from the agricultural sector has a number of challenges in the future is the increasing number of the world's population is expected to reach 2.3 billion people over the next 40 years, which occur especially in developing countries. The other reason for the limited supply of fresh water is causing crop failures that led to the decrease in productivity generated. In addition to these two reasons, the issue of global warming is also quitecrucial reason to the availability of food in the world. [1]

One alternative food sources are the type of microalgae that grow in fresh and marine waters. Marine microalgae are photosynthetic microorganisms producing numerous bioactive molecules of interest for health and disease care such as lipids rich in omega-3 fatty acids -as eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3)- and carotenoids (*e*.g., β-carotene, fucoxanthin, astaxanthin) [2]. Other than as feed in aquaculture activities biota hatcheries, microalgae also have more value as an ingredient for therapy. Therapeutic supplements from micro-algae comprise an important market in the which compounds such as β-carotene, astaxanthin, polyunsaturated fatty acids (PUFAs) such as DHA and EPA and polysaccharides such as β-glucan dominate [3].

The percentage of the four components is varying depending on the type of microalgae. Microalgae lipid content depending on the type of microalgae, the average growth and culture conditions microalgae [4]. Lipid is soluble in non-polar organic solvents such as benzene and n-hexane. Therefore, the extraction process is required to obtain the content of lipids in microalgae. With the resulting lipid extract, then the potential of microalgae as a source of nutrition. Under these conditions, the activity of the Field Work Practice in microalgae lipid extraction techniques was performed in the Department of Biotechnology LIPI Cibinong is very essential.

These study purposes are to determine the content of fat in several microalgae Chlorophyceae; *Scenedesmus* sp., *Cosmarium* sp., and *Tetraselmis* sp. The nutritional value produced can be arranged of those microalgae as a food supplement or other purposes in the future.

Material and Method

Microalgae Culture Preparation

Procurement of stock culture performed on an agar medium is then performed in a liquid medium. The manufacture of culture media stocks performed on an agar medium slant. Stock culture media that

carried out on 5 g Bacto order that dissolved the commercial media then carried by surface streak to use OSE. Once the medium is ready and then added 200 ml inoculants microalgae.

Stock culture liquid medium is done for preparation before making large-scale culture. A step of making the stock liquid culture medium that adds a number of chemicals and culture media until the volume of distilled water is 5 ml. Test tubes are closed and sterilized at autoclave at a temperature of 121oC for 15 minutes, then allowed to stand at room temperature. The addition of 3 ml of inoculants microalgae into test tubes was done in laminar air flow. Then the stock cultures put on the shelf lighting culture with 2000 flux. A stock culture of microalgae further cultured on media larger than 100 ml, 500 ml, one-liter and two liters after entering the logarithmic phase [5].

Microalgae Culture

Culture of *Scenedesmus* sp. and *Cosmarium* sp. was performed by a commercial media, while *Tetraselmis* sp. was cultured in Johnson media (Table 1). Each of these media constituent chemicals dissolved in 500 ml of distilled water then put the inoculants and water until the volume to one liter. This microalgae culture performed on the medium which has a salinity of 27 ppt and a pH value of 7. The culture microalgae culture is done in glass bottles with a volume of 1000 ml were placed on a shelf fitted aerator and culture with 40-watt lamp lighting with the light intensity of 2000 lux [5].

Table 1. Chemical composition of Commercial Media and Johnson Media

Media	Chemical Composition	Number (/500 ml fertilizer)
Commercial Media	Urea	1 g
	ZA	0.8 g
	TSP	0.3 g
	Gandasil	1 g
Johnson Media	$MgSO_4$,	0.5 g
	$MgCl_2$,	1.5 g
	$CaCl_2$,	0.2 g
	KNO_3 (PA),	0.5 g
	KH_2PO_4 (PA),	0.035 g
	Soda Kue,	0.045 g
	Fe EDTA,	1 ml
	NaCl,	27 g
	Mikronutrien mix.	1 ml

2.3 Culture Density Analysis

Observations on microalgae growth of *Scenedesmus* sp., *Cosmrium* sp., and *Tetraselmis* sp. was performed by measuring the daily Optical Density (OD). The third growth of microalgae is experiencing growth phases. This is consistent with the pattern of growth based on the number of cells that are grouped into five phases: lag phase, logarithmic phase, a phase of decline in the growth rate, stationary phase, and the phase of death. The number of sampling microalgae for lipid analyzes is 20 ml and conducted duplicated. This decision begins when microalgae culture reached doubling in optical density than the first day culture. It means that microalgae in the growth phase and entering the logarithmic phase. Logarithmic phase began with rapid cell division which growth rate increased intensively [6].

Analysis of Fat Content of microalgae

Lipid analysis performed refers to the method of Bligh and Dryer. Samples were taken in duplicate with a volume of 20 ml, at 3500 rpm centrifugation for 10 min. The biomass is separated from the supernatant and was given 5 ml Bligh and dryer for extracting lipids. The sonication performed with a frequency of 40 Hz then re-centrifuged. Supernatant was collected until the biomass into a pale, pale yet when biomass extraction is carried back. The supernatant was collected given 3 ml of chloroform and 2 ml of distilled water. The solution was vortexes to separate the lipid contain. The bottom layer is a lipid and transferred to a porcelain cup that had been weighed previously. After that, the samples has oven for 24 hours and weighed.

Result and Discussion

Microalgae growth experienced some growth phase. This growth phase will affect the content of the microalgae biochemical owned. In a study conducted sampling at several stages of growth to determine the difference in every phase. In addition, the density of the optical density analysis was conducted to determine growth patterns mikrolagae. Population density measurement results are presented in Figure 1 below.

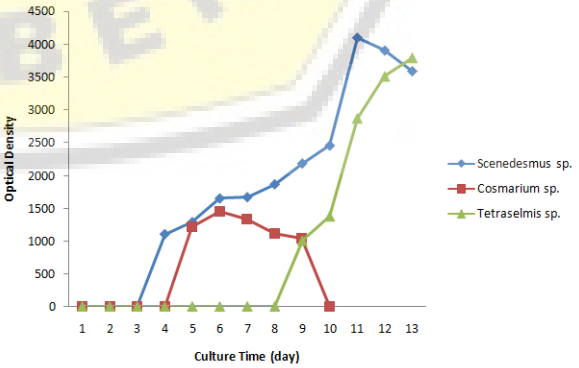

Figure 1.Growth charts of three types of microalgae with Optical density measurement method.

Based on the chart above, it is known that the species experience density *Cosmarium* sp. highest

peak on day 6 of culture. This differs from the species *Scenedesmus* sp. which has the highest density on the 11th day of culture. Although *Scenedesmus* sp. and *Cosmarium* sp. using the same type of fertilizer, but the resulting growth response was very different. *Scenedesmus* sp. with commercial nutrient density can be obtained even though the highest density achievable on the 11th day of culture.

On growth charts *Tetraselmis* sp. showed a different response. On the first day until the eighth day of a period of adaptation for this species. Long enough period of adaptation which is possible because Johnson's use of the media has an indirect nutrient easily absorbed by the microalgae. On day 13, the peak density of the optical density values obtained and nearly equaled the highest density of species *Scenedesmus* sp. using commercial fertilizers media.

Observations microalgae lipid weight has having an average weight that can be seen in Table 2. Based on observations during the one-month weight maintenance lipid was produced by each different microalga. Microalgae *Scenedesmus* sp. has reach the largest lipid weight on eighth day of culture is 540 mg/l. Than *Cosmarium* sp. Has highest lipid content at ten day of culture is 481.25 mg/l. Another microalga, *Tetraselmis* sp., has the largest lipid weight on eighth day of culture 8831.25 mg/l (Figure 2). Microalgae *Tetraselmis* sp. has the highest weight among the others. The weight of microalgae lipid contained in the lipid phase of logarithmic means the average weight increased during the growth of microalgae. The higher fatty acid production during the growth of microalgae [7]. The formation of lipids started at the beginning of the logarithmic growth phase and stop when entering late stationary phase with the formation of lipids at most during the logarithmic phase [8].

Although the content of *Scenedesmus* sp. have a low lipid content when compared *Tetraselmis* sp. However, the species has a fatty acid composition that is quite good and has potential as a biofuel. In addition to the potential as a fuel, *Scenedesmus* sp. as well as species in wastewater treatment that is as absorbent elements nitrogen and phospos [9].

The use of extraction methods with the Bligh-Dyer method generates sufficient lipid content when compared to other methods that use ethanol as a solvent. Lipid content and fatty acid composition produced is not significantly different. It can be concluded that the use of ethanol can be used in the extraction of lipids from wet biomass more efficiently and have a huge potential for the extraction of lipids in large scale [10].
Nannochloropsis sp., *Dunaliella* sp., *Scenedesmus* sp., Contains biochemical rich protein content while in tow, nutritious polyunsaturated fatty acids (such as ALA, ARA, and EPA), and antioxidant pigments. These contents can be compared also with other microalgae that have been popular as *Chlorella* and *Spirulina* **[**11].

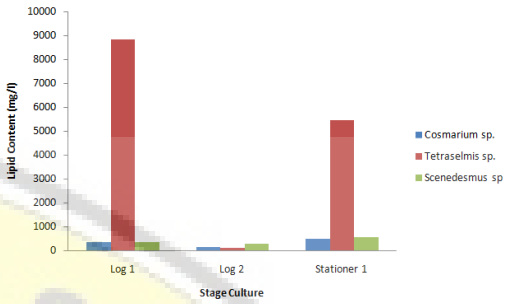

Figure 2. Lipid weight in 20 ml 3 Microalgae species in several Stadia Growth

Based on the weight charts on each microalgae lipid that has been in the culture can be seen that the lipid content in each phase is decreased even though there are some that just the opposite. The formation of lipids started in the early exponential phase of growth and stop type entered late stationary phase with the formation of lipids at most during the exponential phase.

Conclution

Microalgae lipid extraction techniques on using the method of Bligh and Dyer with methanol: chloroform: distilled water at a ratio of 2: 1: 0.8 can be used as a method of isolation of lipids in microalgae. The methods now can isolate the lipids in several species of microalgae that Scenedesmus sp. with lipid produced ranged from 261.25-540 mg/l, Cosmarium sp. the lipid content ranged 124.50-481.25 mg/l, and Tetraselmis sp. containing lipid ranged from 105.00-8831.25 mg/l.

Acknowledgment

We would like thank to all reseachers at LIPI Cibinong Bogor, especially in Fresh Water Microalgae Laboratorium and under supervision of Mr. Sapto Andriyono for their excellent collaboration and cooperation.

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ENCAPSULATION of EMBRYOGENIC CALLUS and SHOOT TIPS for STORAGE of SUGARCANE (*Saccharum officinarum* L.)

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Abstract

In the future, propagation via somatic embryogenesis potential is applied to the plants that will be widely exploited because the seeds can be derived from the somatic cells and can be applied to the seed storage in the form of synthetic seeds with minimal growth technique so that the culture may be stored in a relatively long time. The resulting synthetic seeds are highly similar to zygotic seed so it can be applied as a means of vegetative propagation as well as a storage device. Therefore, this study aimed to get synthetic techniques of seed formation and storage of sugar cane explants with minimal growth method. Encapsulation one month after culture from callus of mannitol 0% that was no callus capable of forming buds, instead of explans buds capable of forming buds to 20% of cultures formed the highest shoots resulting from the treatment of mannitol 1 and 3% of explants shoots. At paclobutrazol 0 mg/l no callus and shoots are capable to regeneration. The highest percentage of culture formed buds resulting from the treatment of paclobutrazol 1 and 3% better than the bud explants or callus, while the concentration of 3% paclobutrazol culture's ability to grow to form buds around 40%. These results contrast with the results of other studies that stated that the addition of mannitol and paclobutrazol treatment in the media was able to inhibit the growth of shoots.

Keywords: encapsulation, embryogenic callus, sugarcane

Introduction

Applications somatic embryos can be used in the formation of synthetic seeds and can be stored in a relatively long time with minimal growth method. Synthetic seeds are highly similar to zygotic seed because of somatic embryos wrapped in the mantle (capsule) that serves as the endosperm that contains a source of carbon, nutrients, plant growth regulators, and anti-microbial agent. Today, the definition of synthetic seeds developed further because of explants used are not limited to somatic embryos but also the terminal bud, axillary buds, nodes, and other meristematic tissue. In addition to the purpose of propagation of seedlings, seed technology synthetic can also be used for conservation purposes through the application of minimal growth technique culture can be stored in the medium term ie in a few months to years [1, 2, 3, 4, 5, 6, 7].

In Indonesia, the techniques of synthetic seed formation and storage through minimal growth in sugarcane has not been reported. [8] successfully *Withania somnifera* storage at the plant in the form of synthetic seeds on MS medium with the addition of 0.5 mg/l IBA. *Rauvolfia serpentina* with storage plants encapsulation technique shoots *in vitro* at a temperature of 20^0C successfully performed by [2]. [9,10] have successfully established a synthetic seed plants *Psidium guajava* L. with a combination of
sucrose and ABA using explants of somatic embryos with a high degree of regeneration. [5] managed to make synthetic seed storage at the plant *Plumbago indica* for 6 months has high regeneration ability. [7] successfully storage of *Sequoia sempervirens* plant in the medium term through the creation of synthetic seed.

A procedure of forming the seed synthetic and storage techniques minimal growth that had been successfully applied to the species or varieties of certain plants, could not always be directly applied to the species or varieties of other crops because of the response of each species or varieties may be different so we need a modification of the technique already there to get synthetic techniques formation and storage of somatic embryo effective and efficient so that the seeds can be stored in long time. Therefore it is very important searched protocols synthetic seed formation and minimal storage methods sugarcane effectively and efficiently.

Storage techniques with minimal growth in sugarcane in Indonesia has not been reported. Conservation techniques *in vitro* through the

method of minimal growth in the form of seed synthetic also has many successfully applied to various types of plants, such as *Acca sellowiana* (Berg) Burret [1], *Psidium guajava* L. [10], *Solanum nigrum* L. [11], *Zingiber officinale* Rosc. [4], *Sequoia sempervirens* (D. Don.) Endl. [7], and *Billbergia zebrine* [6].

Methods

The plant material used in this study was *in vitro* shoots and somatic embryos from sugarcane cv PS 864 where this variety is widely used varieties that farmers and governments to be developed. Retardants or osmotic regulator used was mannitol and paclobutrazol. The experiment was arranged in a completely randomized design environment. Taraf paclobutrazol used is 0, 1, 2, and 3 mg/ ml. 1, 3, and 5%. Each treatment was repeated 10 capsules. Explants encapsulated with a 3% sodium alginate containing MS medium without growth regulation. Encapsulation process performed by the method $CaCl_2.2H_2O$ drops to a solution of 100 mM and soaked for 15 minutes with shaking to form a capsule. Incubation at a temperature of 25^0C in the culture room with 16-hour fotoperiodisitas 800-1000 lux light intensity. whereas for mannitol concentrations used were 0,

The observed response was the percentage of vitality, the percentage of power regeneration, and the percentage of culture that permeates the capsule. Cultures that still survive are then transferred to MS medium that best shoot regeneration for recovery and regeneration of somatic embryos cane.

Results and discussion

Encapsulation age of one month of treatment mannitol showed that at concentrations of 0% no embryogenic callus capable of forming buds opposite of ekplan buds capable of forming buds by 20% (Figure 1). The highest percentage of culture formed buds resulting from the treatment of mannitol 1 and 3 mg/l of explants shoots. These results contrast with the results of other studies that stated that the addition of mannitol treatment in the media is able to inhibit the growth of shoots. Instead of these findings with the addition of mannitol can stimulate bud formation in sugarcane. This suggests that while not exactly given mannitol treatment to inhibit the growth of sugarcane so that mannitol is not recommended for storage or conservation of sugar cane.

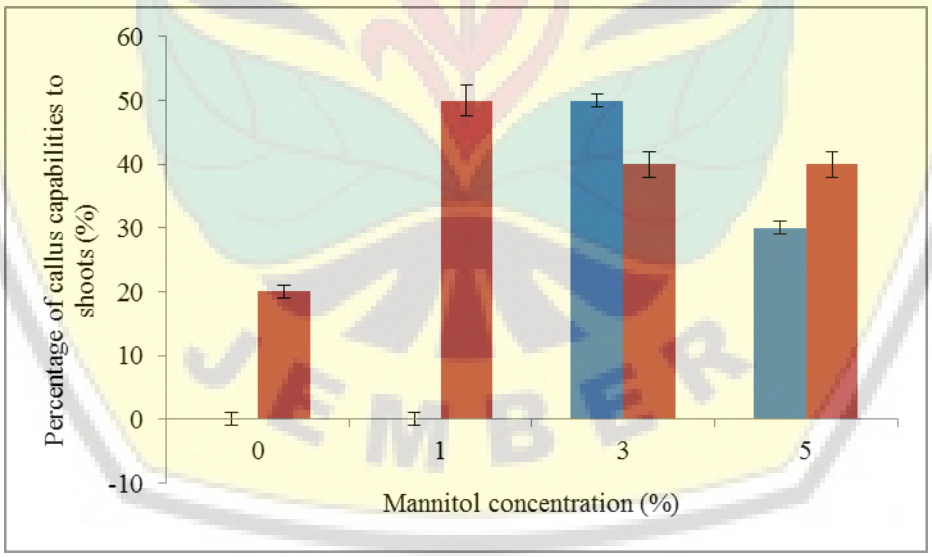

Figure 1. Effect of the addition of mannitol (%) in the percentage encapsulation media capabilities shoots explant and callus of sugarcane PS 864 forming buds (%) aged one month after planting. SE from 10 replications. Notes: blue for callus and red for shoots.

At paclobutrazol showed that without paclobutrazol there were no callus and shoots capable of forming buds (Figure 2). The same results in the treatment of mannitol. The highest percentage of culture formed buds resulting from the treatment of paclobutrazol 1 and 3 mg/l kind of explants shoots embryogenic or callus, while the concentration of paclobutrazol ability to grow to form buds around 40%. The addition of paclobutrazol in the culture medium was able to stimulate the formation of buds, it was contrary to the results of other studies. This suggests that while the treatment was not appropriate given paclobutrazol to inhibit the growth of sugarcane so that paclobutrazol was not recommended for

storage or conservation of sugarcane by using embryogenic callus explants.

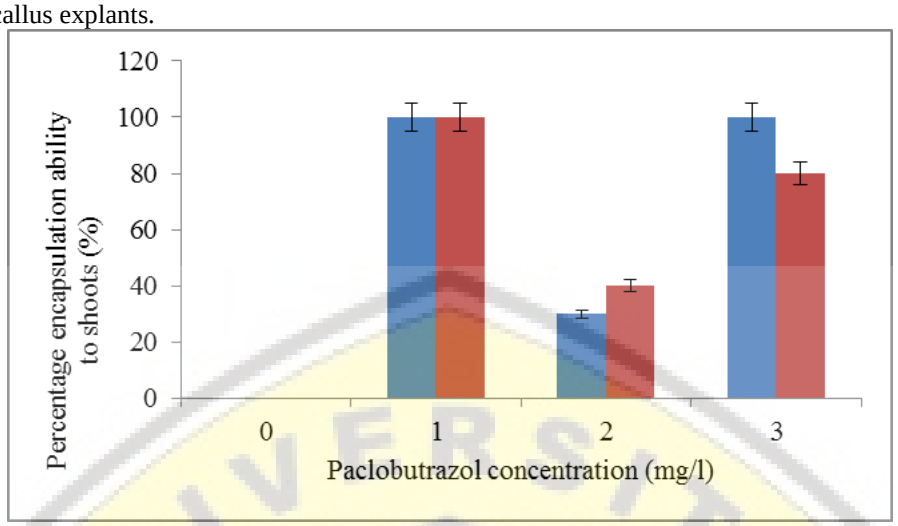

Figure 2. Effect of the addition of mannitol (mg / l) in the media encapsulation of the percentage of explants ability buds and callus of sugarcane PS 864 forming buds (%) aged one month after planting. SE from 10 replications. Notes: blue for callus and red for shoots

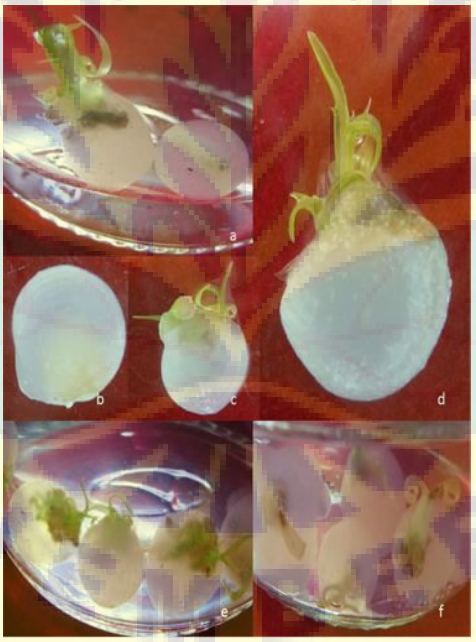

Figure 3. Seed Synthetic sugarcane PS 864 on storage media mannitol treatment and paclobutrazol at one monthafter encapsulation by using embryogenic callus explants. (a, c, d) shows the shoot were able to penetrate the capsule; (b) capsules with explans embryogenic callus were not able to grow; (e) capsule with explans embryogenic callus capable of forming buds and pierce the capsule; (f) browning shoots.

Conclusion

At one month encapsulation from the explant callus of mannitol treatment 0% no callus capable of forming buds but instead of ekplan buds capable of forming buds by 20% of cultures formed the highest shoots resulting from the treatment of mannitol 1 and 3% of explants shoots. At paclobutrazol 0 mg/l no callus and shoots were capable of forming buds. The

highest percentage of culture formed buds resulting from the treatment of paclobutrazol 1 and 3% better than the bud explants or callus, while the concentration of 3% paclobutrazol culture's ability to grow to form buds around 40%. These results contrast with the results of other studies that stated that the addition of mannitol and paclobutrazol treatment in the media was able to inhibit the growth of shoots.

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MORPHOLOGICAL CHARACTER of MOSSES from FAMILY *BRYACEAE* from MOUNT ARGOPURO

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Abstract

Total three species mosses from family of Bryaceae had been collected from Mount Argopura during field research at April 2015. Identification based on its morphological character was done by using microscope observation in Herbarium Jemberiense. *Bryum mamillatum* Lindb., *Rhodobryum giganteum* (Schwägr.) Paris, and *Bryum billarderii* Schwägr are three species that successfully identified. Here we provided the detail description based on sporophytes and gametophytes character with their taxonomical references.

Keywords: Mosses, Bryaceae, Mount Argouro

Introduction

Mosses (Byophyta) with 13,000 species (Shaw & Goffinet, 2009) is the second largest group of land plants after the angiosperms. Mosses have two distinct alternating generations. The leafy gametophyte is the dominant generation, responsible for photosynthesis and sexual reproduction, while the sporophyte generation, which consists of an unbranched axis with a spore-producing capsule at its apex (Vanderpoorten & Goffinet, 2009). Bryaceae is a large cosmopolitan acrocarpous moss family with a diplolepidous-alternate peristome. subfamily Bryaceae, based on capsule orientation and peristome architecture (Pedersen *et al.*, 2007).

Mount Argopuro is located in East Java, 3088 mdpl. It has an unique Tropical montane forest, "Hutan lumut" 1975-2000 mdpl that almost of the tree surfaces is covered by mosses (Bksdajatim, 2012). At the moment, limited research had been reported about the flora diversity especially bryophes in this area. The aims of this research is to observe the presence of Bryophytes from Bryaceae family in Mount Argopuro with taxonomy references.

Methods

1.1 Field Collection

Field research for specimen collection on Hutan lumut was carried out during April 2015 in 20 plots. The mosses were taken on the tree surfaces (>20cm dbh). Sporophytic was preferred. Specimens was packed by using plastic bag to keep the moisture. Global Positioning System (GPS) devices were used to document the general locations of the sites. Thermometer, Luxmeter, Higrometer were used to record temperature, light intensity, and humidity respectively.

2.2 Identification

Description and identification was carried out by morphological observation with microscope and optilab. As references Bryophyte nomenclature generally follows Missouri Botanical Garden's TROPICOS nomenclature database (http://www.tropicos.org) or Malesian Mosses book (Eddy, 1998).

Results and Discussions

Field work collection had been conducted in hutal lumut Mount Argoporo in 20 plot (Fig. 1). Environmental condition about the research site indicated that hutan lumut is situated between 2023-2179 mdpl. It had cool temperature (16°-25° C), moist air with humidity: 34-71, and low light intensity: 425-736 cd. This habitat condition is supporting that Bryophytes are shaded plants (Marschall & Proctor, 2004).

Fig. 1. Research Site and Plots Position

Total 3 Species from Family Bryaceae had been identified, i.e. *Bryum mamillatum* Lindb., *Rhodobryum giganteum* (Schwägr.) Paris, and *Bryum billarderii* Schwägr. *Bryum mamillatum* Lindb found in a plot 3, 6, 18, 19, *Rhodobryum giganteum* (Schwägr.) Paris found in a plot 6, 15, 19, 20, and *Bryum billarderii* Schwägr. found in a plot 16 and 19.

The family Bryaceae is characterized by a large cosmopolitan acrocarpous moss family with a diplolepidous-alternate peristome (Pedersen *et al.*, 2007). Compared to investigation of Bryaceae falimy in other mountain e.g. Mount Halimun, there were two species were recorded, *Bryum apiculatum* Schwaegr and *Brachymenium nepalense* Hook. (Tan *et al.*, 2006). On the other hand, bryophyte diversity of the mountain is well represented from of the Malesian flora, no local endemics has been observed but several uncommon Malesian bryophyte species were recorded (Gradstein, 2011).

Description of each species is represented below:

Bryum mamillatum Lindb
Division : Bryophyta
Class : Bryopsida
Family : Bryaceae
Genus : Bryum
Species : *Bryum mamillatum*

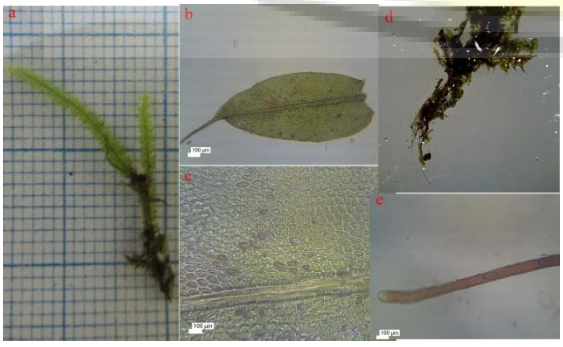

Fig. 2. *Bryum mamillatum* Lindb. (a) Habit; (b) leaf; (c) leaf cells; (d) rhizoid; (e) rhizoid tip

Epiphytic; erect stems, brown stalk, fork branch, 20 mm long, 0,0737 mm diameter; stems surface covered by leaves, rhizoid on the base of the stem, brown, unbranched, long 1 mm , the diameter of 0,0765 mm; rhizoid non-septate; spiral fronds formation; leaves green, oval , emarginated tips, 0,688 mm long, 0,289 mm wide; cell leaves elongated.

Rhodobryum giganteum (Schwägr.) Paris

Division : Bryophyta
Class : Bryopsida
Family : Bryaceae
Genus : Rhodobryum
Species : *Rhodobryum giganteum* (Schwägr.) Paris

Fig. 3. *Rhodobryum giganteum* (Schwägr.) Paris. (a) Habit; (b) leaf; (c) leaf margin; (d) rhizoid; (e) rhizoid (f) leaf arrangement (g) sporangium

Lithophytic; erect stems; brown seta, stem produce stolon, 50 mm long, 2 mm diameter; rhizoid on the base of the stem, brown, branched, 1 mm long, 0,1437 mm diameter, spectate; leaves green , lanceolate, one costa, 10 mm long, 3 mm wide, cell leaves hexagonal; acrocarpus sporophytes, 50 mm long, 0,0602 mm diameter, capsule globular elongated, ends flattened and no hair, long capsule 7 mm, 2 mm wide, calyptra rounded, green, 1 mm long, peristome 0.75 mm long.

Bryum billarderii Schwägr.

Division : Bryophyta
Class : Bryopsida
Family : Bryaceae
Genus : Bryum
Species : *Bryum billarderii* Schwägr.

Fig. 4. *Bryum billarderii* Schwägr. (a) Habit; (b) leaf; (c) leaf cells; (d) rhizoid; (e) rhizoid branches

Lithophytic; erect stems; stalk brown; stem form stolons, 8 mm long, 1 mm diameter; rhizoid on the base of the stem, brown, branched, 1 mm long, 0,0522 mm diameter, rhizoid with septate; spira fronds arrangement, leaves green, lanceolate, one costa to the leaf apex, leaf flattened, 3 mm long, 1 mm wide, cell leaves hexagonal.

Conclusions

Three species of Bryaceae from Mount Argopuro had successfully identified, i.e. *Bryum mamillatum* Lindb., *Rhodobryum giganteum* (Schwägr.) Paris, and *Bryum billarderii* Schwägr. All speciments were shaded mosess based on their habitat condition.

Acknowledgments

This research was one of my project which was supported by CIM. Returning Experts. Many contribution from students and colleague from Lab. Botany in Biology Department, Jember University.

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A COMPARISON of EFFECTIVENESS of *ACORUS CALAMUS* L. EXTRACT and NEEM-BASED INSECTICIDE in THE FIELD AGAINST COFFEE BERRY BORER, *HYPOTHENEMUS HAMPEI* FERRARI (COLEOPTERA: CURCULIONIDAE)

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Abstract

Coffee berry borer (*Hypotenemus hampei* Ferrai) is a major threat for coffee plantation and industry. Several insecticide derived from plant are now available. This study evaluated the effectiveness of neem-based commercial insecticide with hexan fraction of *A. calamus* rhizome which is being developed for the control of the coffee berry borer in the field. Organeem at 0.3% and hexane fraction of *A. calamus* at 1.2% dosages were tested in the small plot in the field. The study concluded that the field efficacy of neem-based insecticides reduced the borer infestation higher than hexane fraction of Acorus at 7 and 15 days after application. However, Acorus extracts showed pronounced action to retard the borer infestation at 21 days after application as compare to neem-based insecticides. This preliminary field data resulted a good signal to develop *A. calamus* as a friendly environment insecticides.

Keywords: *Acorus calamus*, Neem-based insecticides, *Hypotenemus hampei*, Field, Botanical Insecticides

Introduction

Throughout history, plants that produce secondary metabolites have been widely used as insect repellents and insect antifeedants. This is apparent from a number of studies on the effects of chemicals produced by plants which disturbed insect growth and development [6,8]. One of the plants which has insecticidal compounds is *Acorus calamus* L. The active ingredients in rhizome of *A. calamus* widely reported to have β-asarone, saponin and flavonoid [3,4,7].

There are some botanical insecticides available in the market that are friendly to the environment, safe to the human healths and cheap. Therefore, evaluation of the commercial botanical insecticides had been compared to the *A.calamus* hexane fraction. This potential extracts still have been evaluated their efficacy againts the coffee berry insect (*Hypotenemus hampei*).

H. hampei caused severe damage on the coffee berry. Their infestation on the coffee berry had been identified start at eight weeks after flowering until the time of harvest. This beetle caused damage on the storage as well [1].

Methods

Plant material

Rhizomes of *A.calamus* were collected from the Jember district, East Java in Indonesia in April 2013. The rhizome were cut thinly and dried up at room temperature for one week. Dried rhizome (3 kg) was powdered mechanically using a commercial electrical stainless steel blender for getting the powder.

Extraction and fractionation of extracts from this plant were conducted in the Chemical Organic Laboratory UNEJ. The powder of *A.calamus* (each of the 500 grams) was extracted using 2l of ethanol for 24 hours at room temperature. The mixtures were filtered using cloth and vacuum on a Buchner funnel. The combined filtrates was concentrated under reduced pressure at 40-50˚C in a flash evaporator at around speed 5 to yield about 30 ml of extract.

The extracts was then fractionated by using fraction tunnel. 500ml of methanol were added on the extract and then followed by adding 500ml of hexane. Each of layers were separated by using fraction tunnel. The hexane filtrate were then evaporated in a flash evaporator until getting the extract and the residue thus obtained was stored at 4˚C.

Field Trial

The field trial was conducted to assess the repellent action of hexane fraction of *A.calamus* at 1.2% against the pest. An 800 m coffee plantation with 36-40 plants of robusta coffee were divided into 3 groups. First groups were sprayed with hexane fraction of *A.calamus* and second groups were sprayed with organeem at 0.3%, another groups of the same area with water as control. Each plants from these groups were marked randomly and the number of infested fruits and total fruits on four branches selected in each plant were counted before and after spray applications. Data were recorded on 0 day (before spraying), the 7^{th} and 21^{th} day after treatment.

Data Analysis

The data were analyzed bu using ANAVA factorial (α=5%). When the results meaningful (p<0,05) then followed by test Duncan 5%.

Research Results

Table 1 provides the data obtained from the field trial with hexane fraction of *A. calamus* and organeem. Organeem application brought down the pest infestation considerably to low level as compared to the control and Acorus. This can be identified until 7 days after spraying. However, this result cannot be found at 21 days after spraying. At this time the percentage of infected coffee berry number were significantly increased.

Table 1. Interaction among botanical insecticides againts pest coffee berry

Perlakuan	H0 (mean±SE)	H7 (mean±SE)	H21 (mean±SE)
Control (water)	0.00 ± 0.000[a]	3.41± 2.448[ad]	2.12 ± 0.730[g]
Acorus (1.2 %)	7.29 ± 2.597[b]	9.39 ± 2.896[be]	2.10 ± 0.734[h]
OrgaNeem (0.3%)	2.12 ± 0.730[c]	2.10 ± 0.734[f]	6.35 ± 1.491[i]
Ftest insektisida	F(2,423=30.91; Pr>F=0.0001)		
Ftest time	F(2,423=78.02; Pr>F=0.0001)		
Ftest interaction of insektisida and time	F(4,423=38.09; Pr>F=0.0001)		

Note. H0: observation on day 0
H7: observation on day 7
H21: observation on day 21

The contradicted results were found at the application by using hexane fraction of *A. calamus.* at this time, the fraction reduced the percentage of infested coffee berry significantly at 21th after spraying. The number was lower than the control. It indicated that Acorus might work slower than Organeem.

Discussion

This study shows very promising results which might be contributed to the management of controlling the *H.hampei.* The hexane fraction of *A.calamus* showed repellent activity which was slower than Organeem. The efficacy of neem which reduced significantly after one week application, could be explained by their instability characteristic in the open field. Azadirachtin which is the main compound of Organeem, is unstable under hot and humid conditions, light and sensitive to strong acid and alkali [2,5].

The interesting results had been found on the application of hexane fraction of *A. calamus*. Their activity increased significantly at 21 days after application. Based on the observation on the field, the scent of the Acorus still detected among the fruits in each branch. This might explain the repellent activity which is still available. The odour, had strong activity to repel the beetle out from the coffee berry.

Conclusions

It is concluded from the study that efficacy of hexane fraction of Acorus in the field more active than Organeem. Therefore, in the future research, the appropriate formulation could obtained for getting good results.

Acknowledgments

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SYNTHESIS and CHARACTERIZATION of 1-(4-TRIFLUORO METHYL BENZOYL OXIMETHYL)-5-FLUOROURACIL

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Abstract

5-Fluorouracil (5-Fu) is a potential antimetabolite for cancer treatment. Many research have develop many routes to synthesize derivative compounds from 5-Fu. In this research, we suggest a visible synthesize route to produce an ester-based derivative compound from 5-Fu. The compound is synthesized based on benzoylation reaction between 5-fluoro-1-(hydroxymethyl)-uracil, a hydroxylated 5-Fu on it N-1 atom, and 4-trifluoromethyl benzoyl chloride. The optimum condition for synthesis was carried out in ice bath for 11 hours. The product is a white crystal needle with melting point range 172-174°C. The structure of synthesis product was also confirmed with FTIR and ^{1}HNMR characterization.

Keywords: 5-fluorouracil, benzoylation, 1-(4-trifluorometilbenzoil oksimetil)-5-fluorourasil.

Introduction

Cancer is a leading health problem since it causes more than 15% of death rate in the world. It is about 1.45 million new cancer cases present every year (Avendano and Menendez, 2008). Chemotherapy has been one alternative way to cure cancer. Therefore, drug development on cancer research to find better activity drug has attracted many researchers .

5-fluorouracil (5-Fu) has widely used as anticancer agent to inhibit the biosynthesis of timidilic acid (Mycek et al, 2001). Research on 5-Fu has worked on modifying 5-Fu by combining it with amino acids, peptides, phospholipids and polymers (Ozaki et al, 1984; Zhang et al, 1998; Zhang et al, 2006; Jung et al, 2000; Lee et al, 2001). Tian et al (2007) has modified 5-Fu at position N-1, then showed their pharmacology and pharmacokinetics as better prodrugs than the leading compound, 5-Fu. Other researchers also have modified 5-Fu at position N-1, N-3, or both using nucleoside analogues like FdUMP, or conjugation with peptides, amino acids and glucose (Sun et al, 2006; Yin et al, 2008; Xiong et al, 2009; Daumar et al, 2011).

Esterification is a favorite method in modifying 5-Fu since, theoretically, it is easy to do, cheap, and shows great bioactivities for 5-Fu ester based products. However, those ester products synthesized by Tian et al (2007) were produced in small yield percentage and required further purification since they were mixed with the catalysts.

5-Fu has been modified via alkylation and esterification process. First step reaction alkylates 5-Fu at position N-1 with formaldehyde to form 1-hydroxymethil-5-Fu (Tian et al, 2007). This reaction product is then esterified using another acyl source such as benzoyl chloride. Since benzoyl chloride and its derivatives are reactive chemicals, so it does not require a catalyst to undergo a reaction. Hence, separation of reagents does not need to do in this case.

In this research, 4-trifluoromethyl benzoyl chloride was used to produce 1-(4-trifluoromethyl benzoyloxymethyl)-5-Fu. Reaction conditions on this organic synthesis also have been optimized for its temperature and time of reaction at second stage. Pure product was then characterized using FTIR and ^{1}H NMR to confirm the real structure of synthetic product.

Materials and Methods

Chemicals used in this research are included of 5-fluorourasil pro synthesis (Merck), 4-trifluorometilbenzoilklorida *p.a* (Sigma), formaldehyde (Merck), aseton *p.a* (Merck), aquades, triethylamine *p.a* (Merck), HCl (Merck), $NaHCO_3$ (Merck), sodium sulfat anhydrous (Merck), n-hexane *p.a* (Merck), ethyl acetic *p.a* (Merck), and TLC plates (Silica gel 60 F_{254}) (Merck).

Some equipment that have been used in this research are Electrothermal Melting Point apparatus, ^{1}HNMR Jeol 500 MHz, FTIR KBr Perkin Elmer-Spectrum One KBr *pa* e-Merck, kamera digital UV, and ChemBioOffice 2008.

Methods

General Procedure for Synthesis of 5-fluorouracil derivatives, 4a – 4g

The first stage was carried out by refluxing 5-Fu in the excess of formaldehyde and water at 60°C for 6 hours. Solvent in the mixture was evaporated and then was dissolved in acetone. Triethylamine and benzoyl chloride was reacted in separating funnel and then applied drop wise into the flask containing synthesis product at first stage while stirring at optimum condition, ice bath for 11 hour reaction.

Product was dried using rotary evaporator under reduced pressure below 40°C.

The mixture product was extracted with ethyl acetate and washed with hydrochloric acid pH 3-4 for three times. The phase of ethyl acetate was washed with $NaHCO_3$ pH 7-8 for three times. Then the product was dried over anhydrous sodium sulphate followed by evaporating the solvent under reduced pressure. The remaining residue, as crude product, was purified by silica gel column chromatography with eluent hexane: acetone (6:4).

Characterization of the Synthesis Products

Synthesis products was characterized using TLC on Kieselgel 60 F_{254}. The compound spots on chromatograms were tested for their purity using TLC scanner (densitometer) recorded on Shimadzu densitometer. ^{1}HNMR (399.84 MHz) spectra were recorded on an Agilent400-vnmrs400 spectrometer. Chemical shifts (δ) are given in parts per million (ppm) up field from tetramethylsilane (TMS) as an internal standard. IR spectra (KBr pellets) were recorded on a Shimadzu spectrometer. Melting points (*mp*) were determined on an electrothermal melting point apparatus Stuart and are uncorrected.

consisted of reaction in icebath (0-5°C), room temperature, and heating of reflux at 40°C.

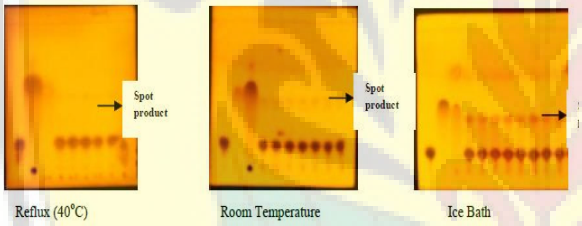

Figure 1. TLC chromatogram from optimization of reaction condition (temperature) at second step reaction: reflux (40°C), room temperature (25-27°C), ice bath (0-5°C).

The chromatograms (Figure 1) show that reflux does not help the reaction to work better than other conditions. The expected spot, as the product, still does not appeared though it has been taking seven hour time of reaction on reflux at 60°C, while the spot of starting materials still thin.

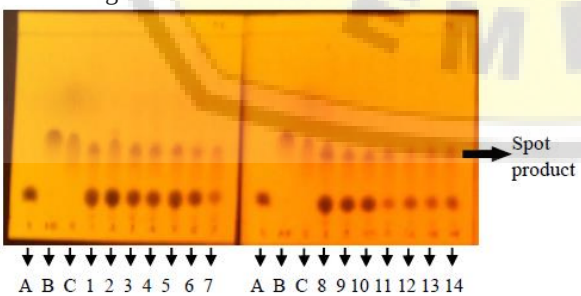

Figure 2. TLC chromatogram for optimization of time of reaction. Lane A: 1-hydroxymethyl-5-fluorouracil. Lane B: benzoic acid. Lane C: 4-trifluoromethylbenzoylchloride. Lane 1-14: time of reaction (step two) at certain hours.

Result

Synthesis was carried out in two step reactions, alkylation of 5-Fu and esterification process through benzoylation of alkylated 5-Fu. The crude synthetic product was then identified based on its separation on thin layer chromatographic using eluen of hexane:aceton (6:4). This mixture composition of eluen was applied since it gave better separation on silica plates with Rs 1,57 compared to hexane:acetone (7:3).

First step reaction was established to run at 60°C for 6 hours (Tian et al., 2007). While second step reaction, as esterification process, was investigated to work at three possible temperatures, and to be affected by time of reaction itself. These conditions were optimized to obtain the best contact time and temperature among the reagents in producing a synthetic product. Three conditions in this research are

Then, the best reaction condition is obtained from the reaction on ice bath. During 14 hour reaction, it has been selected that time of reaction for 11 hours results in the smallest comparison of spot area starting material (product of first time reaction) and the synthetic product (Figure 2).

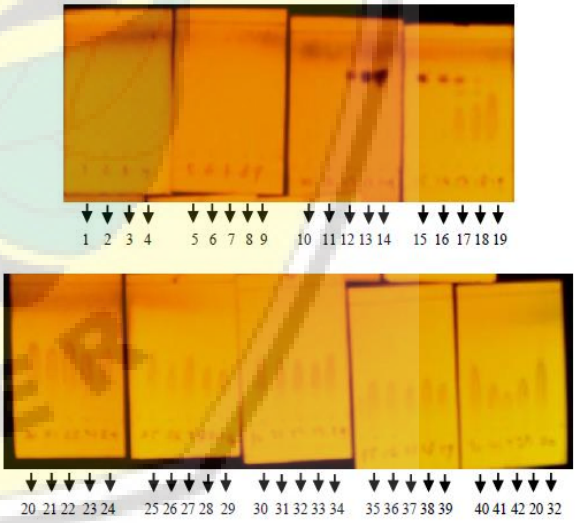

Figure 3. TLC chromatogram of fractions from separation using silica column chromatography. Number 1-42 shows the number of fractions.

The crude synthetic product was then purified using column chromatographic of silica-gel with eluen hexane:aceton to result in four main fractions. The second main fraction contains only one spot, Rf 0,7, and the fourth one cannot be determined since the spot was tailing (Figure 3).

Table 1. Interpretation of ^{1}HNMR spectrum

Proton	Chemical Shift (ppm)			Multiplicity
	Exp Result	Prediction *ChemBioOffice*	Literature	
H1 (NH)	10,6676	10,0	10,0	Singlet
H2 (CH)	7,8957	7,73	7,0-8,0	Doublet
H3 (CH)	8,2420	7,98	7,0-8,0	Doublet
H4 (CH)	7,9112	7,73	7,0-8,0	Doublet
H5 (CH)	8,2576	7,98	7,0-8,0	Doublet
H6 & H7 (CH_2)	6,0110	6,12	4,93	Singlet
H8 (H)	8,1097-8,1227	8,04	6,5 – 8,0	Doublet

The second fraction was subjected to analysis using ^{1}H NMR and FTIR. Based on the data on Table 1, it can be suggested that the structure of the compound on reaction product is shown on Figure 4.

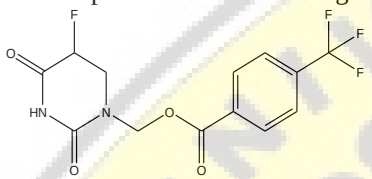

Figure 5. Chemical structure of purified product synthesis: 1-(4-trifluoromethyl benzoyloxymethyl)-5-Fu.

This statement has been supported by FTIR data (Table 3). In addition, the experiment results were in line with literature. Figure 5 illustrates the UV spectrum of the synthetic product.

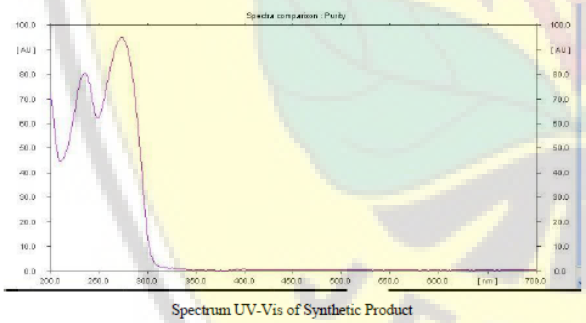

Figure 5. Spectrum UV-Vis of synthetic product.

Conclusion

Derivate of 5-Fu has been synthesized through two step reactions, alkylation and esterification. The synthetic product has also been confirmed its structure using FTIR and ^{1}HNMR.

Acknowledgement

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FUNGAL ISOLATION and ARTIFICIAL INOCULATION INDUCED AGARWOOD in GAHARU TREE (*Aquilaria malaccensis*)

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Abstract

Fungus are often used to artificial induced make "gubal gaharu" (agarwood) in *Aquilaria malaccensis* (Lam.) trees. This study was conducted to isolation, making growth filtrate fungus, artificial inoculated and evaluate the effect fungus on formation agarwood. Fungus isolated from ectoderm, periderm till living phloem region of tree and growth in pottato dextrose agar (PDA) with Streptomycin 100 ppm. For this purpose. 11 fungal isolates were colected from the ectoderm 10 isolates AW1 – AW10, and AW11 isolates from periderm or living phloem which are potential grow on wood extract. AW11 isolate growth in potatto dextrose broth medium for produce secundary metabolities. The crude extract of these fungus were tested, inoculated and against to phloem of *Aquilaria malaccensis* trees to artificial induced gubal gaharu. Typical changes in the length and light intensity of resulting discoloritation were observed After 7 months periods following inoculation.

Keywords: Isolation, fungus, artificial inoculated, *Aquilaria malaccensis* agarwood

Introduction

Gaharu tree or *Aquilaria malace*nsis (Family Thymelacaceae) is a major agarwood tree in Indonesia and Asia. Agarwood tree is to product a secunder metabolith like resins group. The economical market and high market demand for agarwood tree has very seriously affected natural sources this species of agarwood.

The agarwood is used in many fungtions like religious ceremonies, raw material of perfumes, medicine and also as ornamental material (Kakino *et.al*., 2010). Prices of agarwood a many variation from cheaper US$100 until very-very expensive to US$100,000 per kilograms for superior pure agarwood (Naef, 2011). In this same article, agarwood is a resinons wood substance that is produced by the tree as natural inoculated, infected by microbial, or inoculated and infested microbial by vector of insects (Naef, 2011). In natural can be produced agarwood in the long time, may be until 50 years ago. The resinous wood substance to wounding insects ar other anainals ar microbial invation or infection. The resin contain are tree extraxtive with aromatic chemist like terpenes. The main active compound in agarwood derivates of sesquiterpenes an 2-(2- phenyleththyl cromon (Naef, 2011).

In traditional methods for agarwood induction used in many countries include deliberate wounding on epidermic or wood with knives or with hammering nails from woods or nails metal. The reaction of induction need on long time because by spontaneous inoculation. Over the years, the practice of induction by toxic chemical (Sodium Chlorida, NaCl) or invation animals or microorganisms. The toxic chemical or microorganism to accelerate the resin production of agarwood in trees. In this study, we examined the effect of isolate fungi and time on wood induction in *Aquilaria malacensis* in the fields.

Method

Fungus isolated from ectoderm, periderm till living phloem region of tree of *Aquilaria malacensis.* Eleventh isolate of fungi (mold) were tested, but only one isolated (Figure 1a) effective for introduce a gubal gaharu (agarwood). Fungi cultures were maintained in slant *Potato Dextrose Agar* (PDA, Oxoid Ltd., England) (Figure 1b) and were used for preparing bimassa and spore suspention at concentration of 10^3-10^7 spore/ml in the steril distilled water. Spore inoculated on Potato Dextrose Broth (PDB) with streptomycin 100 ppm and shaker till seven days 50 rpm. Filtrate inoculant gaharu ready for used (Figure 1c). Six years old of *Aquilaria malaccensis* trees were grown at field in Banyuwangi East Java, the tree were about 5 m high and a diameter between 12 to 15 cm. Tree were inoculated by making wounds using an electrical drill with a bit size 6 mm in diameter. The first wound was 40 cm above the ground and the next wounds were made above the precident wound at 10 cm interval until we reached 20 wounds. The wounds were drilled in to tree 4 to 7 cm depths. The filtrate were injected into the drilled wounds using a syringe, and the wound were isolated by wood bamboo with same diameter. Evalation in 3 weeks, 6-7 months after inoculation.

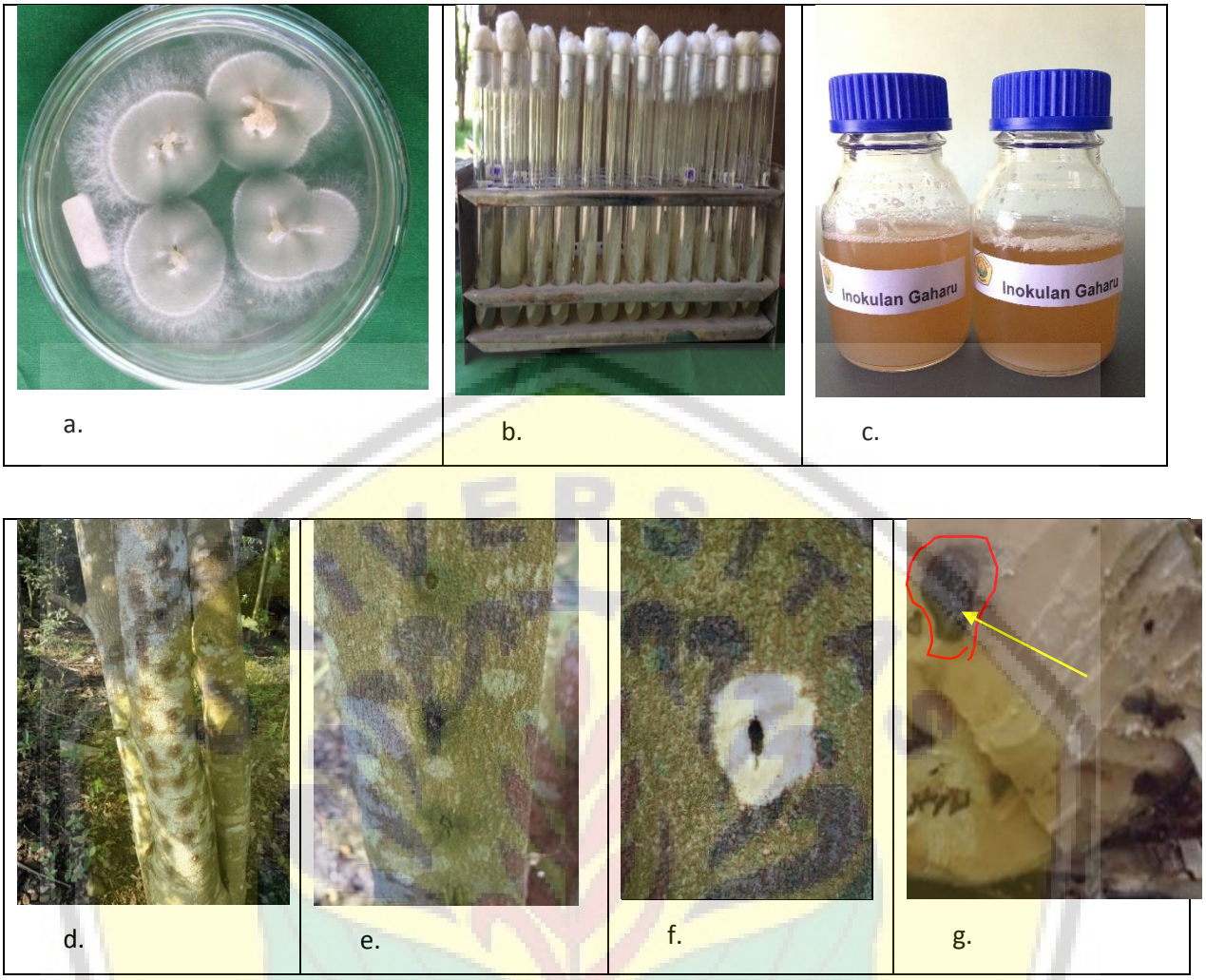

Figure 1. a, Isolate AW11 on PDA; b, Isolate AW11 on PDA slant; c, Filtrate of isolate AW11 on PDB; d, *Aquilaria malacensis* inoculated 3 weeks by filtrate; e, *Aquilaria malacensis* inoculated 7 months by filtrate; f, *Aquilaria malacensis* inoculated 7 months by filtrate; g, gubal *Aquilaria malacensis* inoculated 7 months by filtrate

Result

The results that have been achieved in this Wood sample were evaluation after 3 weeks after inoculation (Figure 1d) and after 6 to 7 month (Figure 1e,f and g). Discoloration zone agarwood were formed the effect of artificial fungal inoculation in 6 to 7 months. The darkened area surrounding the wounding site contains agarwood substance in the core of wood tree. This has been observed using visual method and measured wide of zone, where dark brownish to black zone. The area of resinous part was measured in the horizontal section. The cross sections of the trees treated by the inducers are shown in Figure 1g. Resinous wood formed slowly after treatment isolate filtrate of fungi. Resin accumulated in the wood over time, a brown area and a thin resinous layer appeared inside the trunk in the 3 weeks (Figure 1e). Puce wood and a thick resinous layer were observed all over the trunk after 6 to 7 months (Figure 1f). The whole tree was harvested after 6 to 7 months. All of the samples treated with agarwood inducers developed resinous wood and dark areas, agarwood inducers play an important role in formation of resinous wood.

Figure 1g., discolorization zone observe on wood samplaes harvested 3 weeks and 6 to 7 months after fungi inoculation. The darker substance (arrow) souronding the drilled wound appeared broaser and darker in minimum 6 to 7 months. The darkened area souronding in the woundeing site contain agarwood substances. Many-many enzymes was work like cellulase, pectinase, peroxidase and polyphenol oxidase activities were determined in healthy, naturally infected and inoculated agarwood (*Aquilaria malaccensis*) plant parts at various time intervals to study the changes in activities of these enzymes during pathogenesis. This has been observes using visual method only. Sensory evaluation not detected.

The crude extract of these fungus were tested, inoculated and against to phloem of *Aquilaria malaccensis* trees to artificial induced gubal gaharu (agarwood). Typical changes in the length and light intensity of resulting discoloritation were observed after 7 months periods following inoculation.

Acknowledgement

This work was supported by Lembaga Penelitian The Jember University and field of *Aquilaria malacensis* ora gaharu tree in Banyuwangi East Java.

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KINSHIP of BANTENG (*Bos bibos*, d'Alton) and BALI CATTLE (*Bos sondaicus*, Muller).

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Abstract

Bali cattle probably derived from domestication of the Banteng, to clarify the kinship between the two, measurements of 15 characters at 6 cranium of Bali cattle and 6 cranium of the Banteng. Six Bali cattle cranium collected from Den Pasar Bali in 2013, three Banteng cranium comes from Baluran National Park (TNB) collection 2012-2013, two cranium comes from Zoology Laboratory FMIPA UNEJ collection 1992, and one cranium comes from Merubetiri National Park (TNAB) collection 2013. The results of a cluster analysis of the 15 characters (variables) of the 12 cranial showed that six of the cranium of Bali cattle are in one group, six cranium separated into three subgroups. The first sub group consists of three cranium Banteng TNB collection 2012-2013, second sub group consisting of the two cranium Banteng TNB collection 1992, and the third sub group of the cranium Banteng TNAB collection 2013. Based on the coefficient of the closeness of the relationship, Bali cattle closer to with Banteng TNB collection 2012-2013 and Banteng TNAB collection 2013. The conclusion of this study was Bali cattle different groups with the Banteng. Banteng TNB collection 1992 different from collection 2012-2013. Banteng TNAB closer to the Banteng TNB collection 2012-2013 than with a collection of 1992.

Keywords:

Introduction

Bali cattle (*Bos sondaicus* Muller) is a type of Indonesian native cattle who allegedly centuries ago as a result of the domestication of the Banteng (Wibisono, 2010; Mason *et al.* 1982). In accordance with the Banteng, Bali cattle adaptable in a bad neighborhood, feed not selective food, fast breed, docile, easily controlled, and have a good digestibility of the fibrous food (Batan, 2006). Bali cattle bred as a superior local cattle for meat producer.

The kinship between Bali cattle with Banteng can be seen from the morphological similarities between both male and female, but the posture of Bali cattle is smaller than the Banteng. Posture differences between the two maybe occur because of Bali cattle have genetically decreased during centuries domestication.

To determine the kinship between Bali cattle with Banteng can be done with DNA analysis techniques (molecular analysis) and taxonomically (fenetic method). Fenetic method system easier to implement because the system fenetic learn and observed directly the animal character or characteristics (Ardian, 2012).

According to Frandson RD (1992) the cranium is part of the real body of elders passed down genetically to the next generation. The result of crosses between different parents, will find a different size and shape. Bone characteristics, including the shape of the cranium and mandible, is phenotype of the genes inherited from the parents (Goto *et al*., 1991).

Cranium characteristics between one individual to another can be connected using statistics (Hayashi et al, 1981, 1982[a], 1988[a], 1988[b]). Variable selection must be in accordance with the theories and concepts that are commonly used and have to rational. This rationally is based on the theory that explicitly or from previous studies. The selected variables is a variable that can characterize the object to be grouped and specifically must correspond with the purpose of cluster analysis (Santoso, 2004).

Materials and Methods

Chemicals used in this research are included of 5- This study was conducted on August to October, 2013, in the Laboratory of Zoology, FMIPA UNEJ. Number and composition of the spesimens used in the present study are shown in Table 1.

Table 1. Skull samples of Banteng and Bali Cows

Skull	Origin	Samples Collection	Total
Banteng 1, 2, 3	TN Baluran	2012 - 2013	3

Bantenng 4, 5	TN Baluran	1992	2
Bantenng 6	TN Merubetiri	2013	1
Balicows 1-6	Den Pasar-Bali	2013	6
Total			12

TN = National Park

The ages of specimens were determined by the status of eruption and wear of the teeth. Young adults, whose skull sutura were not well connected and might not have reached final proportions, were excluded from the present data so that the analysis would not be complicated by variations due to growth. All skull observed from male.

The 12 cranial measurements used in this study were those defined by Hayashi (1981, 1982[a], 1988) (Figure 1A-B and Table 2)

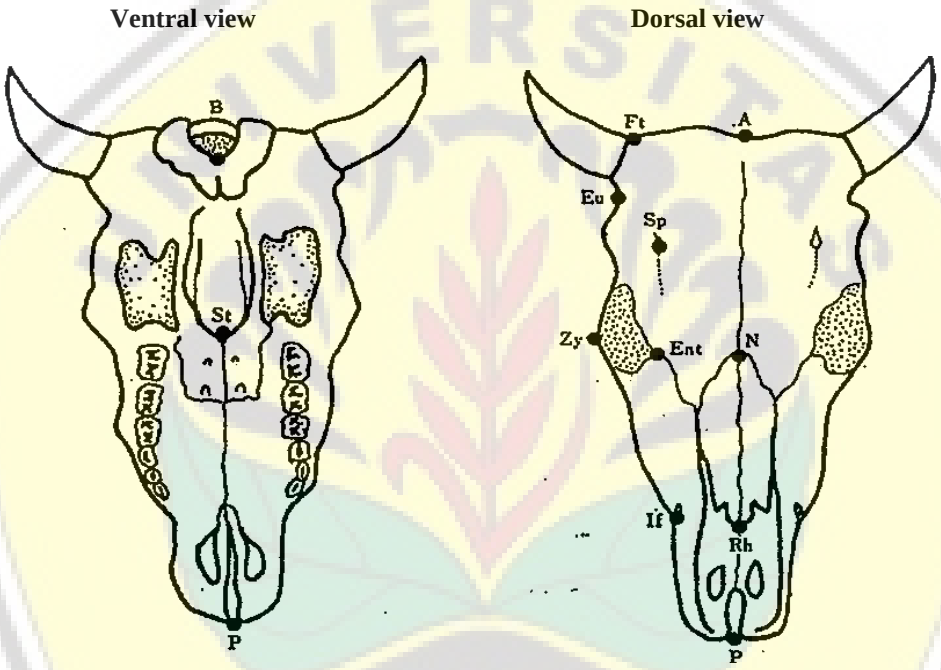


Figure 1-A The name of points measurements of dorsal dan ventral view (Hayashi *et al.*, 1981)

Caption:

A	: Akrokranion	Eu	: Euryon
N	: Nasion	Sp	: Supraorbitale
B	: Basion	Ft	: Fossotemporale
P	: Prosthion	St	: Staphylion
Ent	: Entorbitale	If	: Infraorbitale
Rh	: Rhinion	Zy	: Zygion

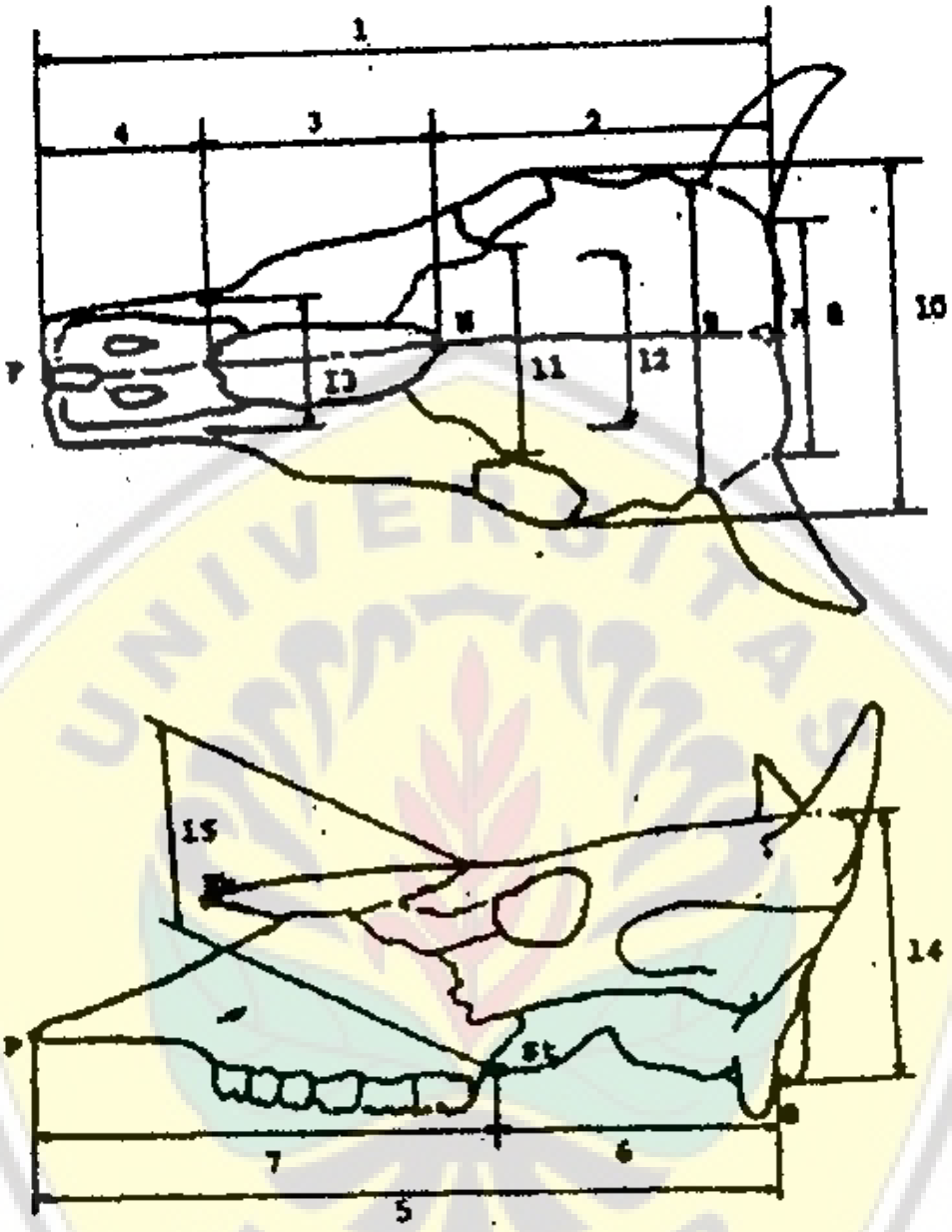


Figure 1-B Measurements of the skull of Banteng and Bali Cows
A: Akrokranium, B: Basion, N: Nasion, P: Prostion, St: Staphylion (Hayashi *et al*., 1982[a])

Table 2. Measurements of the skull of Banteng and Bali Cows

No	Cranium	
1	Profile length	Akrokranium (A) – Prostion (P)
2	Median frontal length	A – Nasion (N)
3	Length of the nasals	N – Rhinion (Rh)
4	Height of the naris	Rh – P
5	Basal length	Basion (B) – P

6	Basion – Staphylion (St)	
7	Median palatal length	St – P
8	Least breadth between the bases of the horncores	
9	Breadth of neurocranium	Euryon (Eu) – Eu
10	Greatest breadth of skull	Zygion (Zy) – Zy
11	Least breadth between the orbits	Entorbitale (Ent) – Ent
12	Breadth between the supraorbital foramina: Supraorbitale (Sp) - Sp	
13	Breadth between the supraorbital foramina: Infraorbitale (If) - If	
14	Height of the occipital region	A - B
15	Height of the nasal region	N - St

Source : Hayashi *et al*., 1988)

All measurements were taken with sliding or spreading calipers (Prohex) and were recorded to the nearist 0.01 milimeter. The analysis of data 15 cranial measurements using SPSS Statistics 17.0 (cluster analysis).

.

Result

Table 3 shows the means of 15 cranial measurements for 3 kinds of male Banteng and Bali cattle. In general, the measurements of Banteng significantly larger than those of Bali cattle.

Tabel 3 Mean of 12 cranial measurements for male Banteng 1-3, Banteng 4-5, Banteng 6 and Bali Cattles

Variable	Bali Cows Mean (cm)	Banteng 1-3 Mean (cm)	Banteng 4-5 Mean (cm)	Banteng 6 (cm)
1	39.902	49.32	49.08	49.66
2	18.813	23.44	21.66	22.24
3	13.097	16.35	17.34	16.98
4	7.220	8.44	9.07	8.34
5	38.030	45.38	45.04	45.22
6	12.391	16.44	15.15	15.18
7	24.996	30.67	29.24	30.10
8	10.261	14.83	11.34	11.40
9	16.543	20.40	19.54	19.75
10	18.001	22.40	22.04	22.67
11	11.370	16.20	15.76	17.38
12	8.193	10.38	9.00	10.16
13	6.249	9.19	9.02	8.91
14	15.931	20.45	16.47	19.58
15	12.691	13.33	16.60	13.53

Based on the results of measurements on all the variables, Bali cattle skull is smaller than in the three groups of Banteng. This results indicates that the Bali cattle smaller than Banteng. If it is mentioned before that Bali cattle originate from domesticated Banteng, within a very long time, then there has been a decline in the quality of genetic because the influence of domestication. For Bali cattle genetics improvement to its future, need to be back cross with Banteng.

Among three groups Banteng, number 4-5 have a smaller size for some character such variable 2 (median frontal length), 7 (median palatal length), 11 (least breadth between the orbits), 12 (breadth between the supra-orbital foramina), and 14 (height of the occipital region) compared to the Banteng number 1-3 and Banteng number 6. But for the

character variable 3 (Length of the nasals), 4 (Height of the naris), and 15 (Height of the nasal region), Banteng number 4-5 greater. Between Banteng number 1-3 and 6 there is a considerable difference in the character variable 2 (Median frontal length), 6 (Basion), and 9 (Breadth of neurocranium). The tentative conclusion shows that there are variations between the Banteng.

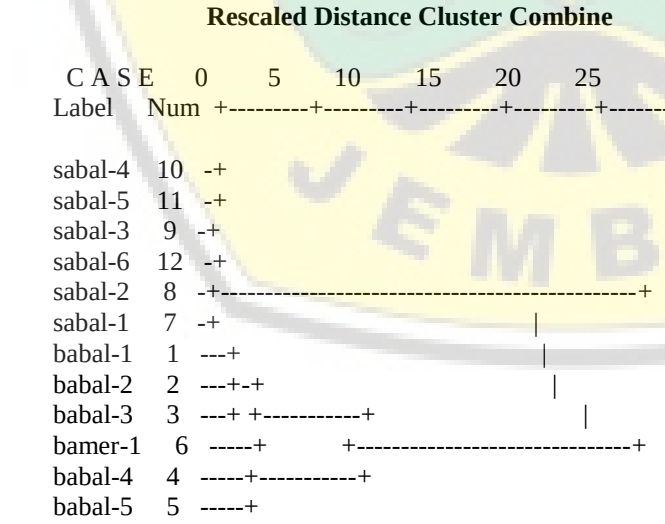
Table 4 shows the kindship of Bali cattle with Banteng based on measurements of the cranium.

Table 4: **Agglomeration Schedule**

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	10	11	.598	0	0	2
2	9	10	.683	0	1	3
3	9	12	.885	2	0	4
4	8	9	1.023	0	3	5
5	7	8	1.464	0	4	11
6	1	2	2.824	0	0	7
7	1	3	4.315	6	0	8
8	1	6	5.574	7	0	10
9	4	5	6.275	0	0	10
10	1	4	16.582	8	9	11
11	1	7	49.913	10	5	0

Caption : Number 1 – 6 Banteng and number 7 – 12 Bali cattle

Figure 2 shows the dendogram results of grouping between Bali cattle with Banteng, 15 based on the characteristics of the cranium.



Caption: sabal 1-6 = Bali cattle; babal 1-5 Banteng Baluran; bamer 1 = Banteng Merubetiri

Figure 2: Grouping of Bali Cattle with Banteng based on cranium

Table 4 and Figure 2 shows the grouping based on the kinship between Bali cattle with the Banteng. Number 1-6 is Banteng and number 7-12 is Bali cattle. Kinship of Bali cattle are very close or homogeneous with coefficient 0.598 – 1.464. This number can be assumed as the homogeneity of a species. While the Banteng glaze occurred is considerable variation between Banteng Baluran National Park the collection in 1992 with a collection of 2012-2013, as well as Banteng Merubetiri National Park collection of 2013. The variation may be caused by sample size, number of the sample cranium can not much as collecting in the national park. Difficulties due to find the cranium of Banteng old dead in the very wide area on national park.

Bali cattle number 1 with Banteng Baluran number 1 represents two distinct kinship between the group of Bali cattle and group of Banteng. Conclusions of this study are: 1) Bali cattle are not closely related to the Banteng and 2) between Banteng there were variations based on the collection of samples.

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THE DIVERSIFICATION of FOOD CONSUMPTION and THE IMPROVEMENT of DESIRABLE DIETARY PATTERN (DDP) with THE APPLICATION of SUSTAINABLE RESERVED FOOD MODEL (SRFM) in MADIUN REGENCY

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Abstract

Food diversification involves diversifying and improving food nutrient quality. It is also an effort to reduce community dependence on rice and to support the sustainability of food self-sufficiency. One alternative for food diversification is the exploration of local food from natural resource. Lack of optimization of local food to support food consumption diversification has caused a lower score of Desirable Dietary Pattern (DDP) in Madiun Regency. To obtain the expected condition, the Initiative to Accelerate Food Consumption Diversification Based on Local Resource is then conducted by targeting at PPH score of 95 in 2015. A part of this initiative is a of Sustainable Reserved Food Model (SRFM) which aims to meet the daily food demand of the household and to produce the household with food self-sufficiency. Research begins from January to December 2013 in Madiun Regency, East Java. The objective of this research is to identify the type of food consumption diversification and to evaluate PPH. Research involves several methods to collect the data. Survey is used to obtain baseline data. Structured interview is performed with questionnaire given to the cooperator households which are randomly selected from each stratum of targeted households. The questionnaire helps the author to secure the data of household profile, rural DDP score, house yard utilization, and actual/existing cultivation technology. Result of research indicates that vegetables are widely cultivated in two villages, Wonorejo and Blabakan, followed by crops, fruits, and herbs. The cultivated vegetable species include rice, corn and peanut. Herbs are red ginger and turmeric. The early DDP Score for herbs at Wonorejo Village is 65.90 and after SRFM, the DDP Score reaches 77.80. At Blabakan Village, the early DDP Score is 69.80 but after SRFM, the vDDP Score increase to 88.70. Indeed, the program of SRFM can increase DDP Score of all food groups at Wonorejo Village and Blabakan Village, Mejayan District, Madiun Regency, each for 18.1 % and 45.9%.

Keywords: Food Diversification, Desirable Dietary Pattern (DDP), SRFM, Madiun Regency

Introduction

The application of Sustainable Reserved Food Model (SRFM) Program in rural or urban area can help to satisfy the demand of daily food in the households and also to produce the households with food self-sufficiency. The President of Indonesia Republic in the address before the Conference of Food Self-Sufficiency Board in Jakarta Convention Center on October 2010 has said that food self-sufficiency must start from household level. One way to achieve food self-sufficiency is by food diversification with numerous food providers (the Ministry of Agriculture, 2010). Food diversification is a process of food product development that avoids the dependence on one food species but utilizes various food materials to improve community nutrient quality. Household food diversification can be measured by the score of Desirable Dietary Pattern (DDP). Therefore, DDP Score represents the indicator of nutrient fulfillment and household food diversification. The ideal DDP Score is 100. The measurement of DDP Score is a parameter of successful implementation of SRFM activities.

The activities in SRFM are aimed (1) to fulfill the demand of food and nutrient at household and community levels by optimizing the utilization of house yard, (2) to improve the capacity of household and community in utilizing house yard for the cultivation of crops, fruits, vegetables and herbs, in rearing livestock and fishes, and in performing food diversification, (3) to develop the sources of seeds in order to preserve the sustainable utilization of house yard and to conserve local crops in the future, and (4) to develop productive economic activities such that the households can

increase their welfare and create clean, healthy and self-sufficient green environment [5,29].

Pursuant to President Decree No.22 of 2009, the score of Desirable Dietary Pattern (DDP), characterizes food consumption diversification of a community group. The less optimum of local food role to support food consumption diversification has reduced DDP Score. In general, Indonesian mostly consumes carbohydrate (rice and equivalents) than other food materials [13,31]. To obtain the expected condition, the Initiative to Accelerate Food Consumption Diversification Based on Local Resource is then conducted by targeting at DDP score of 95 in 2015. Desirable Dietary Pattern (DDP) is the arrangement of food group based on its energy contribution to meet the demand of nutrient in terms of quantity, quality, diversity, and various aspects such as society, culture, economic, religion and taste. All of these terms are represented by DDP Score. A governmental program to encourage the community to consume various local foods and to persuade them toward nutrient improvement is Sustainable Reserved Food Model (SRFM) in 2012. This activity is organized by the Ministry of Agriculture through the principle of utilizing house yard in manner of environmental friendly to meet the demand of food and nutrient of the household.

In Indonesia, house yard has great potentials such as to provide food materials for household, to reduce household expense for food, and also to increase the income for household, especially farmers [4]. House yard also becomes the important place for the conservation of genetic resources such as crops or other creatures [15, 30, 25]. According to [17] and [24], house yard can be defined as the agriculture system which combines social, spiritual and economical functions. In relative with social function, house yard can be the spot used as gathering, child playground and garden. Economical function of house yard is shown by the presence of agricultural ecosystem which produces various food sources, energy materials and fibers [14]. House yard is also useful to increase the income through planting crops, herbs, and trees, and also by rearing livestock and fishes.

In 2013, the successful land utilization program has been appreciated by FAO through "Award Outstanding Progress in Fighting Hunger and Undernourishment" because Indonesia is the first target of global development agenda (MDGs/Millenium Development Goals) where the first goal of MDGs is that in 2015, every nation must have capacity to alleviate poverty and hunger for a half of earlier proportion in 1990. Land utilization program is also expected to meet beyond Energy Adequacy Level (EAL), from earlier of 2,200 ccal/cal/day to 2,400 2,200 ccal/cal/day [2].

East Java has the width of 47,157.72 km^2. The potential house yard is quite large with more or less 626,740 Ha and 10,385,281 households. However, households who cultivate house yard for farming work are still very few [9]. Quite big potential will improve the production and consumption of vegetables and fruits such that DDP Score can also be increased [3]. The objective of this research is to understand the impact of SRFM program on the availability of crops, vegetables and fruits in households and the effect on the increment of DDP Score.

Methods

Research begins from January to December 2013 at Santan Hamlet of Blabakan Village, and Wonorejo Hamlet of Wonorejo Village, which all of them remain in Mejayan District, Madiun Regency, East Java. The activities of research include:

1. The implementation of SRFM at Blabakan Village is carried out by Farmer Women Group (FWG) "Anggrek" at Santan Hamlet of Blabakan Village, Mejayan District at coordinate $7^0$35'1, 60"S, $111^0$40"8, 58"E, altitude of 111 m a.s.l, with 45 members. The implementation at Wonorejo Hamlet of Wonorejo Village, Mejayan District is at coordinate $7^0$35'1, 60"S, $111^0$40"8, 58"E, altitude of 111 m a.s.l, also with 45 members.
2. Food consumption survey is conducted to measure PPH Score in the early and end of the activity.

The Implementation of SRFM

Field activity starts by constructing Village Seed Garden (VSG). Various vegetable seeds are spread onto VSG. Vegetable and fruit seeds are sorted. Other preparation includes polybag, pot, organic fertilizer made of livestock waste and household trash, inorganic fertilizer, and other planting materials. To increase the knowledge and awareness about the importance of nutrient food consumption and also land utilization, therefore, four schedules of socialization are managed for Women Farmer Group (VSG) during the activity.

The Measurement of DDP Score

1. Food consumption survey is performed to identify the quantity and species of food consumed by households as the implementer of SRFM in a day before the survey. Participants fill into questionnaire list comprising of personal data, household member characteristic, and the yesterday food consumption expense. To

facilitate the Arrangement of National Expectancy Food Pattern, data of food consumption are needed which include seven groups such as Rice, Tuber, Animal-Based Food, Oil and Fat, Fruit/Oily Seed, Pea, and Vegetable and Fruit.

2. PPH Score reflects the consumption level of households which is known by the proximate of yesterday food expense. Data of food quantity per food group are then converted into Actual Energy Quantity (ccal/cal/day) and then, % Actual, % Energy Adequacy Level (EAL), EAL Score, and DDP Score are counted.

Table 1. The Example of DDP Calculation

No.	Food Group	Energy Consumption		% Actual	% EAL	Weight	Actual Score	EAL Score	Maximum Score	DDP Score
		Expectancy	Actual							
1	2	3	4	5	6	7	8	9	10	11
1	Rice	1000	1125	66,9	56,3	**0,5**	33,5	28,2	**25,0**	25
2	Tuber	120	62	3,7	3,1	**0,5**	1,9	1,6	**2,5**	1,6
3	Animal-Based Food	240	82	4,9	4,1	**2,0**	9,8	8,2	**24,0**	8,2
4	Oil and Fat	200	156	9,3	7,8	**0,5**	4,7	3,9	**5,0**	3,9
5	Fruit/Oily Seed	60	36	2,1	1,8	**0,5**	1,2	0,9	**1,0**	0,9
6	Peanut	100	48	2,9	2,4	**2,0**	5,8	4,8	**10,0**	4,8
7	Sugar	100	84	5,0	4,2	**0,5**	2,5	2,1	**2,5**	2,1
8	Vegetable and Fruit	120	64	3,8	3,2	**5,0**	1,9	16,0	**30,0**	16,0
9	Others	60	24	1,4	1,2	**0,0**	0,0	0,0	**0,0**	0,0
	Total		1681	100	84,1	11,5	61,3	65,7	100,0	62,5

Source : Anonimous, 2009

Note:

AKE = 1681/2000 x 100 = 84.1 %

Level 2000 = national average of energy adequacy level (based on National Workshop of Food and Nutrient, conducted once in 5 years)

Column 5 → the contribution of actual energy consumption (column (4) / total in column 4)

Column 6 → % against actual energy total (column (4) / 2000)

Column 8 → % actual multiplied by weight (column (4) / column (7))

Column 9 → % AKE multiplied by column weight (column (6) x column (7))

Observation

In PPH measurement, the compiled data are the width of house yard in each household and also the composition, species and quantity of plants cultivated in house yard. In food consumption survey, the compiled data include the species and quantity of food consumed per capita in one day before survey. Data analysis is subjected to 30 household samples of each village.

Data Analysis

Primary data are obtained using questionnaire which is arranged based on research goals. The collected data are tabulated and analyzed using software to calculate DDP Score.

Results

A strategic framework is used by Assessment Institute for Agricultural Technology (AIAT) of East Java to apply SRFM. It concentrates on the fostering of citizens, households or household heads, and targeted households who are assigned into few strata based on the width of house yard owned or available to every household, rather than on economic capacity level of household. Four strata are considered, respectively

Stratum 1 (household with very narrow house yard or without house yard), Stratum 2 (household with house yard width of < 120 m^2/ narrow category), Stratum 3 (household with house yard width of 120-400 m^2/ moderate category), and Stratum 4 (household with house yard width of > 400 m^2/ wide category).

The development of SRFM begins with the counting of number of household in all strata. The result is 70 households covering 2 villages, precisely Blabakan Village (Santan Hamlet) and Wonorejo Village (Wonorejo Hamlet). During the implementation of SRFM program, some persons who do not belong to the member of SRFM and also persons who come from other hamlets are taking the seeds from Village Seed Garden (VSG). They are not the citizens of Blabakan Village and Wonorejo Village but they plant the seeds into polybag and their house yard. On July 2013, the number of household planter increases to 80, or there is increment by 14.29 % compared to early number. On December 2013, there are 97 household planters, or there is increment by 38.57 % compared to early number. This increment is shown in Table 2 and 3. The number of households as the implementer of Sustainable Reserved Food (SRF) at both villages until December 2013 has increased by 38.57 % (compared to the beginning of SRFM on May 2013).

Table 2. The Early Number of SRFM Implementer (May 2013) on July and December 2013 based on Number of Stratum at Wonorejo Village

No.	Strata	Early on May 2013	July 2013	December 2013
1	Stratum 1	0	0	0
2	Stratum 2	32 households (91.43%)	39 households (96.67%)	46 households (93.88%)
3	Stratum 2	3 households (91.43%)	6 households (96.67%)	3 households (93.88%)
4	Stratum 2	0	0	0
	Total	35 households	45 households	49 households

Table 3. The Early Number of SRFM Implementer (May 2013) on July and December 2013 based on Number of Stratum at Blabakan Village

No.	Strata	Early on May 2013	July 2013	December 2013
1	Stratum 1	0	0	0
2	Stratum 2	33 households (94.29%)	38 households (95.0%)	44 households (91.67%)
3	Stratum 2	1 households (2.86%)	1 households (2,5%)	2 households (6.25%)
4	Stratum 2	1 households (2.86%)	1 households (2,5%)	3 households (2.08%)
	Total	35 households	40 households	48 households

Mostly, SRFM implementers at both villages who have house yard width Stratum 2 are 92.78 % with house yard width of < 120 m^2. This stratum remains within narrow category (the Ministry of Agriculture, 2012). SRFM implementers with house yard width Stratum 3 or moderate category with house yard width of 120-400 m^2 are 6.19 %. Those with Stratum 4 or wide category of house yard width above 400 m^2 are 3.06 %. Water availability is relatively adequate throughout years and has been used by SRFM implementers to cultivate fish pools at width of 10-300 m^2 to rear various species, although it is dominated by catfish and indigo fish.

The Construction of Village Seed Garden (VSG) and The Implementation of SRFM at Wonorejo Village and Blabakan Village

The attractive moment of implementation is during the visualization of M-KRPL at research location. Visualization at both locations of SRFM is begun with the construction of Village Seed Garden (VSG). It aims to internalize a mindset into SRF citizens to be self-sufficiency in meeting the demand of seeds, especially those of vegetables, tubers, herbs and fruits.

Main activities in SRFM are to construct Village Seed Garden (VSG); to provide the grant of vegetables, fruits, fish juveniles and others; and to organize trainings and technological counseling. The preparation of nursery spot and Village Seed Garden (VSG) has been agreed in a house of SRFM implementer either at Wonorejo Village and Blabakan Village to make easier the rearing and maintenance. The result of nursery cultivation on VSG will be supplied to SRFM implementer to be planted into polybag/pot or house yard. The excess of seeds can be sold at price Rp. 150,- or Rp. 250,- depending on whether it is vegetable or fruit.

The construction of Village Seed Garden (VSG) involves 2 units each for Wonorejo Village and Blabakan Village in Mejayan District. VSG construction starts from designing, assembling, rising VSG office and arranging the shelves of seedlings into Village Seed Garden (VSG). The size of Village Seed Garden (VSG) is 6 m x 3 m (18 m^2) to facilitate the placement of seedlings shelves into VSG. The design and assembly of VSG start from 25 April to 5 May 2013, while the construction of VSG and seedlings shelves begins from 7 May to 15 May 2013 (Figure 1 – 4).

The facility in Village Seed Garden is functioned as the sustainable provider of seeds to the community. The existence of VSG may be beneficial because it not only acts as seed supplier but also becomes sustainable finance source to VSG. The user of production seeds from VSG is not only the members, but also other buyers from immediate community, including schools or the outsiders who pass by or are attracted to VSG. According to Harmaini et.al. (2012), Village Seed Garden is a garden to produce and to distribute seeds to citizens/communities as SRF implementers. The management is performed by the organization as SRF implementer. VSG in SRFM has strategic value because it not only provides seedlings for each SRF but also becomes useful place for learning for immediate communities. The user of production seeds from VSG is not only limited to the members, but it has been known to immediate community or government institution, such as schools, or the outsiders who pass by or then being attracted to buy (Susanti and Subagiyo, 2013).

The demand of seeds for food cultivation and for household herb is also supplied by Village Seed Garden (VSG). A SRFM must be minimally 1 (one) unit. The operational cost includes the seed production cost incurred by SRF unit and the cost to sell the seed out of the region. VSG can be developed further into the nursery pool of freshwater fish. The demand for livestock descendants (poultry, ruminant, or fish) and structure/infrastructure of production/plant may be fulfilled from outside (private or government) by applying business partnership with farm organization in the village.

Figure 1-4. The Activity and Arrangement of VSG

The functions of VSG include (1) the function of production and distribution, meaning that commodities at VSG can be produced in sustainable manner; (2) the function of diversity, meaning that commodities have horizontal diversity such that it can fulfill the demand of seeds of SRF members; (3) the function of esthetic, meaning that the planting considers vertical diversity aspect such that it will give beautiful regular scenery; (4) the function of environment, meaning that VSG can give pleasant, friendly, creative and health nuances; (5) the function of service, meaning

that VSG can serve the demand of seeds to the members of SRF in every moment of necessity; and (6) the function of sustainability, meaning that VSG is managed professionally by business model.

Village Seed Garden is constructed to provide seeds to SRFM implementer households. It is expected that VSG will facilitate the supply of seeds and there will be good cooperation across SRFM implementer households. The activities in VSG involve: the land management around VSG, the filling of planting media into polybag by FWG, the planting of vegetables into polybag, the maintenance of plants (watering, fertilizing, controlling pest organism in vegetables, composting, planting cassava around VSG, preparing the seed of vegetables and the withdrawal of seeds by SRFM implementer households to be planted into polybag or their house yard). The activity and arrangement of Village Seed Garden at Wonorejo Village and Blabakan Village are presented in Figure 1-4. The active role of citizens is quite apparent during the planting of vegetables into polybag.

The seedlings in Village Seed Garden (VSG) are mostly vegetables such as red pepper, chili, celery, lettuce, cucumber, leek, eggplant and tomato. Vegetable seeds are also planted on seedbed in house yard. The collection of plants in Village Seed Garden , therefore, comprises of leek, kangkong, mustard, red pepper, chili, tomato, lettuce, eggplant, lime, broccoli, cassava, sweet potato and others. The collection of herbs includes turmeric and red gingers. The seeds distributed already to SRFM implementers are spinach, kangkong, mustard, tomato, red pepper, squash, pare, long bean and others.

Species and Number of Plants Favored by SRF Implementers

Until the end of December 2013, the majority of implementers of Conserved Food Housing at 2 villages have planted vegetables around their house yard. The number of vegetable species is 18 at Wonorejo Village and 24 at Blabakan Village. Other dominant plants include fruits (passion, papaya and banana), herbs, crops and plantations.

Table 4. Plant Species Cultivated in House Yard of SRF Implementers at Wonorejo Village and Blabakan Village, Madiun Regency, in 2013

Villages	Variety of Plants					
	Vegetables	Fruits	Spices	Crops	Herbs	Plantations
Wonorejo	18	7	4	2	4	3
Blabakan	24	10	8	4	2	5

In general, vegetables and fruits are widely cultivated in house yard because both are relatively easier to be planted in limited land. These commodities can be planted in shelves, polybags and the used containers. Of many researches on the function of house yard, [12] conclude that the utilization of house yard and farming environments can give high contribution to long-term food security during the period of energy scarcity.

The purchasing power of some communities to allocate food necessity is limited. It is main cause of inadequate or less balanced nutrient. In other case, households can be self-sufficient in producing food source. The utilization of house yard combined with farming cultivation is an alternative useful for households to give food adequacy, to improve nutrient, to improve productive workforces, and to improve housing environment and health, which all of them are driven toward ethe improvement of income and living standard (community welfare). The fulfilled demand for vegetables at household, however, may increase the purchasing power of community to other commodities. Therefore, the negative impact of this increased purchasing power on the less purchasing for animal-based foods and fruits is rejected and also resolved [13].

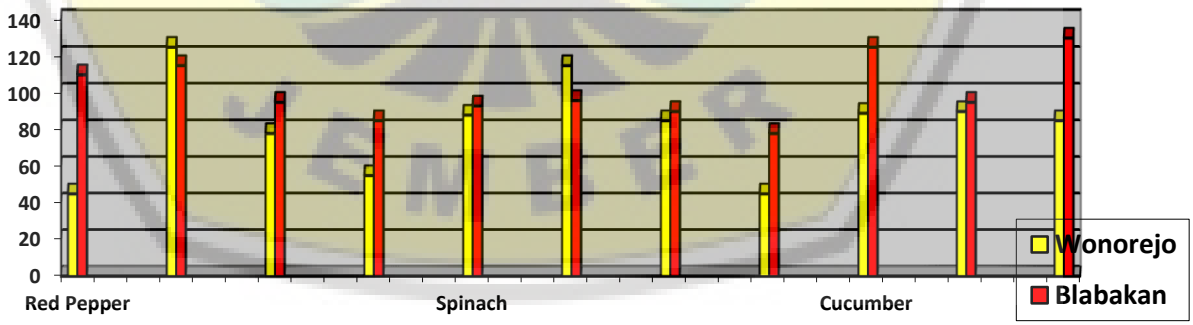
Figure 5 – 7. Structuring yard strata 1, 2 and 3

Reliable food stock at macro levels (national, provincial and regency/city) represents an important factor but it is not quite reliable to secure the availability of food consumption at household and individual levels. The self-sufficiency in fulfilling food necessity will restrain over low food consumption at household level because the problems of distribution and economic access of household to the food source can be resolved [16].

Cultivated Vegetable Commodities

There are 900 – 1,112 plants of vegetables cultivated in house yard with 18 and 24 species planted each at Wonorejo Village and Blabakan Village. There are 15 vegetable species that remain similar at both villages. Moreover, the dominant plant at Wonorejo Village is chili (125 plants), whereas at Blabakan Village, the dominant plants include squash (130 plants), cucumber (125 plants), and chili (115 plants) (Table 5). Vegetables favored by SRF implementers are chili (17.50%), mustard (16.34 %), cucumber (15.25%), and squash (13.62%). It is made by considerations of (1) easily to cultivate, (2) higher economic price, and (3) its great substation value to certain commodity (leek can substitute shallot) (Figure 8).

Figure 8. Vegetable Species Cultivated in House Yard of SRF Implementers at Wonorejo Village and Blabakan Village, Madiun Regency, in 2013



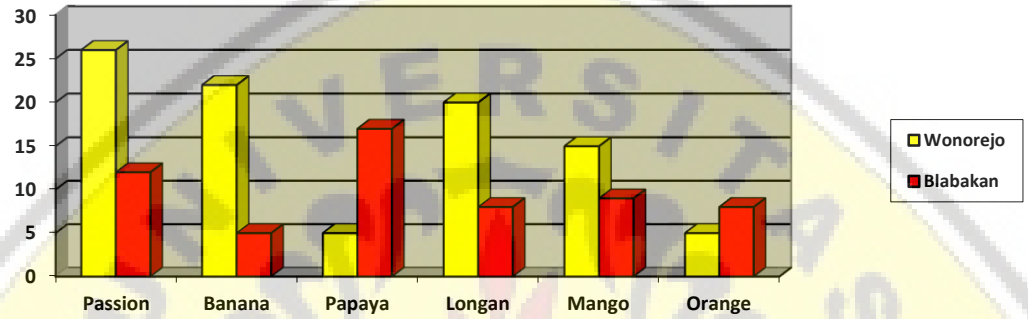
The planting of these vegetables are scheduled in planting pattern of a year (5 – 6 times planting in a year). Commodities are selected based on the tenacity or adaptability of plants to the weather or environmental condition. Horticulture production is deteriorating during water scarcity period (dry season from June to August 2013). After

examining the aspect/component of vegetable horticulture cultivation, either in verticulture system or in seedbed, the number of households that cultivate vegetable horticulture [19] is reduced. The activities to utilize the lands including installing house fence, cultivating non-irrigated plot, below-tree plot, and/or planting commodities such as tubers, fruits and herbs, have encouraged housewives to apply SRF in sustainable manner.

Cultivated Fruit Commodities

Wonorejo Village has 97 plants of 15 fruit species, whereas Blabakan Village has 56 plants of 10 fruit species. Based on species, only 6 species are similar. Passion fruit is dominant in both villages, Wonorejo Village cultivates fruits in more quantity than Blabakan Village, or at percentage point of 55 % (Figure 9).

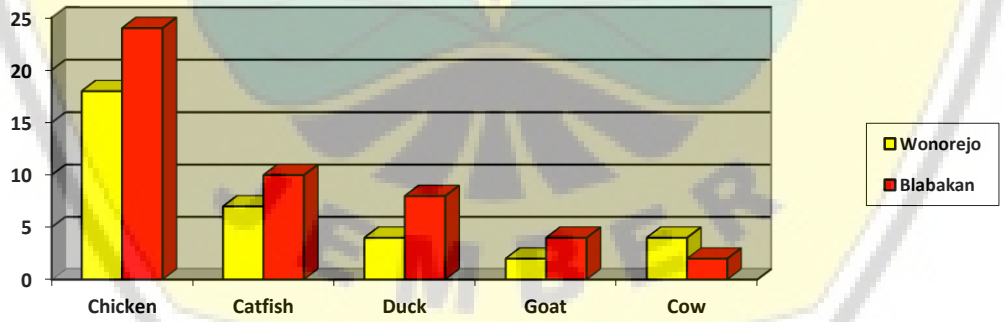
Figure 9. Fruit Species Cultivated in House Yard of SRF Implementers at Wonorejo Village and Blabakan Village, Madiun Regency, in 2013



Reared Livestock Commodities

The variety of reared livestock includes catfish, chicken, duck, goat and cow. The dominant livestock is catfish (70.24%) and chicken (12.46%). Both stocks are easier to rear, not requiring wide place, not involving huge capital, and are easier to sell. The species of livestock reared by SRF implementer households is shown in Figure 10.

Figure 10. Livestock Species Reared in House Yard of SRF Implementers at Wonorejo Village and Blabakan Village, Madiun Regency, in 2013



The above table indicates that the most reared livestock is catfish and chicken. It is because both livestock are easy to rear, not requiring wide place, not involving huge capital and easy to sell. Livestock reared by RPL Implementers can fulfill nutrient demand of the household and also increase household income. The contribution of livestock from SRF can also reduce the gap of real welfare level among Indonesians which is less than 5.3 % of ideal necessity of animal-based protein.

The Analysis over DDP Before and After SRFM Implementation

Desirable Dietary Pattern (DDP) is the arrangement of food group based on its energy contribution in fulfilling the demand of nutrient in terms of quantity, quality, diversity, and various aspects such as society, culture, economic, religion and taste. The higher DDP Score means the higher diversity and balance of nutrients (maximum DDP Score is 100). DDP Score is counted using household proximity or household limit. It means that a household is defined as a group

of people who occupy part or whole physical building and have a meal together from one kitchen. DDP Score is counted as the consumption level of household compared to yesterday consumption expense. Consumption questionnaire is based on National Economic Social Survey (SUSENAS) organized by BPS. Nutrient conversion rate is also based on that used by SUSENAS.

According to Rahayu and Soeharsono (2013), the important aspects of food self-sufficiency involve adequate quantity, good quality and diversity. Food is not only concerning with main material, such as rice, but also related to other materials that are processed to produce new food. Food diversification is one of four successful steps made by the Ministry of Agriculture to reduce community dependence on one of main food materials. Therefore, local food shall be explored to meet the demand of food quantity and nutrient level based on Desirable Dietary Pattern (DDP)

DDP Score, as reported by FAO (1989), is the ideal composition of the consumed main food group which can fulfill the demand of energy and other nutrient substance. The expected DDP Score in national level is 93 in 2014. The Head of Division of Food Consumption and Diversification in Food Self-Sufficiency Agency in East Java Province has informed that in 2010, PPH Score in East Java has reached 86.40 and in 2014, it attains to 93.30 with Energy Adequacy Level (EAL) per capita pointing minimally to 2200 ccal/cal.day and also with protein consumption level that meets to 52 grams per day.

Table 5. The Score of Desirable Dietary Pattern (DDP) based on Food Consumption Survey at Wonorejo Village and Blabakan Village, Mejayan District, Madiun Regency, in 2013

No.	Food Group	Maximum DDP Score	DDP Score at Wonorejo Village			DDP Score at Blabakan Village		
			Before	After	Increment/ Decrement (%)	Before	After	Increment/ Decrement (%)
1	Rice	25,0	20,2	22,9	13,4	15,6	23,7	51,9
2	Tuber	2,5	2,3	1,6	-30,4	1,0	0,8	-20,0
3	Animal-Based Food	24,0	10,4	16,0	53,8	11,8	20,3	72,0
4	Oil and Fat	5,0	4,6	4,5	-2,2	4,4	5,3	20,5
5	Fruit/Oily Seed	1,0	0,9	0,9	0,0	0,5	0,3	-40,0
6	Peanut	10,0	5,4	4,0	-25,9	8,4	7,9	-5,9
7	Sugar	2,5	1,8	2,1		2,0	1,8	-10,0
8	Vegetable and Fruit	30,0	20,3	25,8	16,7	17,1	28,6	67,3
9	Others	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	Total	100	65,9	77,8	18,1	60,8	88,7	45,9

DDP Score reflects the arrangement of food group based on its energy contribution in fulfilling the demand of nutrient in terms of quantity, quality, diversity, and various aspects such as society, culture, economic, religion and taste. The higher PPH Score means the higher diversity and balance of nutrients (Ariani, 2010). Based on the result of analysis over DDP before and after SRFM implementation at two villages (Wonorejo Village and Blabakan Village), it is shown that the average DDP Score increases by 32 %. The increment at Blabakan Village is higher than Wonorejo Village (Table 5).

In Table 4, after one year of SRFM implementation at Wonorejo Village and Blabakan Village, the increment of DDP Score of each village is 18.1 % and 45.9%. Table 4 also shows the result of DDP Score before and after SRFM activity. There is a quite good progress through the existence of SRFM. The increase of DDP Score is also developed from the presence of Sustainable Reserved Food (SRF) in all strata. SRF has been more established and therefore, been also capable of periodically and continuously

supplying food materials such as vegetables, egg, fishes and chicken meats for household consumption. Pursuant to Table 4, the application of Sustainable Reserved Food Model (SRFM) at both villages has increased DDP Score. By the increasing DDP Score, it is expected that RFM program may increase the fulfillment of food necessity and nutrient level of household and community through house yard utilization, and also can support the establishment of food consumption diversification. The goal of DDP Score calculation is to produce a normal (standard) composition of foods in fulfilling the necessity of nutrient in the community by considering nutritional balance supported by taste, quality and affordability [8].

Food diversification is very important to establish food self-sufficiency because the quality of food consumption has been lower as shown by the indicator of Desirable Dietary Pattern (DDP) at national level. In 2009, the score is only 75.7, and it must be increased to achieve the target in 2014 of 95. To keep the sustainability of achievement, the plan of house yard utilization must be updated by taking a lesson from various related programs such as Food consumption Diversification Acceleration and The Women Movement of House Yard Optimization (GPOP) [18].

Desirable Dietary Pattern (DDP) is food diversity which is arranged based on the proportion of food balance. This food balance is established based on the proportion of energy balance from main food group. The ideal food score is 100 and it represents maximum score. DDP Score below ideal score can mean that the quality of communal food stocks is not fulfilling the ideal diversity of food group in every food group [23].

Conclusions

The application of SRFM program at Wonorejo Village and Blabakan Village, Mejayan District, Madiun Regency, can be concluded as follows:

1. The implementation of SRFM at Wonorejo Village and Blabakan Village on early May 2013 is conducted by 70 households. In December 2013, number of implementer increases to 97 households. The increase is counted as increment 28.57 %.
2. The impact of SRFM implementation at Wonorejo Village is influential to the increase of DDP Score from 65.90 before SRFM to 77.80. Therefore, there is increment of 18.06 %. The impact of SRFM implementation at Blabakan Village is influencing the increase of PPH Score from 18.1 before SRFM to 88.70. The increment is by 45.9 %.
3. Village Seed Garden (at Wonorejo Village and Blabakan Village) is functional as the sustainable provider of seeds for the community. The existence of KBD can give huge benefits such as being as the provider of seeds and also the source of sustainable finance to VSG..
4. SRF implementers have favored some commodities. The favored vegetables are red pepper, chili, tomato, mustard, squash, eggplant, kangkung, spinach, cucumber. Herbs species include turmeric and red ginger. Fruits species involve papaya and markisa. Cultivated tubers are cassava and purple sweet potato. The reared livestock is chicken and goat. Fish species include catfish and indigo fish.

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EFFECT of PROPOLIS COATING on ALBUMIN and YOLK INDEX of LOCAL INDONESIA CHICKEN'S EGG

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Abstract

Egg had been considered as one of the best nutrition sources. However, egg consumption in Indonesia still low due to high price and inconsistent supply. Another problem related with this is high rate of egg quality degradation during transportation and storage. One of the solution to reduce the degradation by application of coating. In this study, propolis of local stingless bee was applied as coating for egg as potential organic coating for local egg. Raw propolis was extracted by ethanolic extraction procedure. Propolis extract with concentration 2.5, 5, 10, and 15% was sprayed to surface of egg as treatment and ethanol as positive control. Quality of egg was determined albumin and yolk index which measured weekly for five weeks. Result showed that application of propolis coating by spraying able to maintain the quality of eggs but not increased storage time of egg. This study also showed the quality of egg produced by local farm were not uniform.
Keywords: albumin index, egg quality, organic coating, propolis, yolk index.

Introduction

Eggs are one of the best nutrtion sources for human [1]. However, egg consumption in Indonesia still low due to high price, inconsisten supply, and loss during transportation and storage.
Quality of eggs starts to deteriorate immediately after laid and continue increasing during storage. Factors influencing egg quality are strain and age of hen also storage time and conditons [2][3][4][5]. Egg quality could be measured based on degradation of albumen index (AI) and yolk index (YI) which are when eggs are laid [6][7].

Eggshells are porous and breathable material; therefore they allow movement of moisture and carbon dioxide through the shell [8]. This movement may cause physical and chemical changes in albumen and yolk and also weight loss [9]. Studies showed that preventing this movement minimize detoriation in interior egg [8][10].
Various methods applied for this purpose include mineral and vegetable oil coating [11][12][13][14] [15], coating of egg shell with chitosan, whey protein, and shellac [16][17][18][19], and edible films [20][21].

Another challenge in egg preservation is the increasing consumer's demand for fresh and hygienic. In order to prevent microbial contamination washing technology was introduced in many countries and applied by many egg producers. However, studies showed washing process resulting in deterioting egg quality [16][22][23]. During washing process, usually chemical applied to sanitized or improve preservation of the eggs. Common chemical applied is chlorine. However, chlorine could react with specific amino acids existing in cuticle layer, caused physical change to egg shell [24]. Study by Park et al. [5] showed by combining washing, sanitizing, and coating could significantly increase the shelf-life of the eggs. Thus, application of coating that sanitize egg while reduce the effect of shell degradation would increase the effectiveness of egg preservation procedure. One of the potential coating is propolis.
Propolis is a sticky gummy resinous substance collected by worker honeybees (*Apis melifera*), at temperate regions, and *Trigona* sp., in tropical regions, from the young shoots and buds of certain trees and shrubs [25][26]. This substance known for having strong anti-bacterial, anti-fungal and anti-viral properties i.e. *Bacillus sublitis*, *Bacillis* alvei, *Proteus vulgaris*, *Proteus galangin*, *Salmonella*, *Staphyloccus aureus*, and *Escherichia coli* [27][28][29]. Due to its anti-bacterial effect, propolis has been used on various agricultural product for protection during storage [30][31][32][33].
Since it also posses sticky characteristic, propolis should be test as edible coating for egg during storage which assess by albumen and yolk index of the eggs.

Materials and Methods

This study was conducted at room conditions at the Laboratory of Animal Physiology, Faculty of Science and Technology, Universitas Islam Negeri

Sunan Gunung Djati. Brown-shelled fresh hen eggs were used in this study. Eggs were obtained from a local eggs producer. After oviposition eggs were collected and brought to laboratory for coating treatment with various concentration of propolis extract. The following treatments were included: (1) control without any treatment grouped as control (negative), (2) coating with ethyl alcohol grouped as control (positive), coating with (3) 2.5%, (4) 5%, (5) 10%, and (6) 15% propolis extract in ethyl alcohol.
Treated eggs were stored for five weeks at room condition (26-32 °C, 60-72% RH) and interior egg quality was determined every week. Weekly six eggs were used for determination of albumen and yolk height, length, and width in each group.

Propolis Extraction
Propolis was collected from *Trigona laeviceps* nests in Maribaya, West Java and extracted according to the method suggested by Krell [28]. A 5% propolis solution was prepared by mixing 1900 ml 70% ethanol and 100 g of propolis, a 10% propolis were prepared by mixing 1800 ml 70% ethanol and 200 g of propolis while 2,5% and 15% solution were prepared based on those solution. Solutions were kept in a clean and dark bottle, sealed, and shaken twice daily for one week. Each solution was filtered separately and was kept at 4°C until use.

Egg coating with propolis solution and data sampling
Solution of 2.5%, 5%, 10%, and 15% propolis kept in individual hand sprayer. The distance between head of sprayer was between 10-15 cm. Eggs then dried at room temperature and kept at room condition (26-32 °C, 60-72% RH).
Data of albumin and yolk index were collected weekly for 5 weeks.

Albumen Index
Eggs were broken on a flat surface, using transparant glass plate, where the height of the albumen was measured, half way between yolk and edge of the inner thick albumen, by calliper in mm. Albumen length and width was measured to nearest 0.1 mm.
Albumen index (AI) was calculated by formulae

$$AI = \frac{H}{0{,}5\ (D1 + D2)}$$

where
H = Albumen height (mm)
D1 = outer diameter of thick albumin (mm)
D2 = shorthest diameter of thick albumin (mm)

Yolk Index
Eggs were broken on a flat surface, using transparant glass plate, where the height and widht of yolk was measured by calliper in mm. Yolk length and width was measured to nearest 0.1 mm.
Yolk index (YI) was calculated by formulae

$$YI = \frac{h}{0{,}5\ (d1 + d2)}$$

where
h = Yolk height (mm)
d1 = outer diameter of yolk (mm)
d2 = shorthest diameter of yolk (mm)

Data analysis
One way ANOVA ($P \leq 0{,}05$) with subsequent Tukey HSD tests was applied to detect difference on AI and YI among all treatment.

Results and Discussion

Albumen Index
Highest average of albumen index, during 35 days of observation, was recorded on uncoated egg while the lowest recorded on eggs coated with 2.5%, 5%, and 15% propolis. However, the differences on albumen index among all treatment were not significant (Table 1). This study showed that quality of the eggs produced by local egg farm had AI index less than Indonesia National Standard (SNI 3926:2008) which is 0.090 to 0.120.

Table 1. Average value of albumen index during course of research (different letter indicat

Treatment	Albumen Index			
Control (negative)	0.05	±	0.02	a
Control (positive)	0.03	±	0.01	a
Propolis 2.5%	0.02	±	0.00	a
Propolis 5%	0.02	±	0.01	a
Propolis 10%	0.04	±	0.01	a
Propolis 15%	0.02	±	0.00	a

Different letter indicated significant data (P<0.05)

Weekly observation recorded similar pattern albumen index changes for all treatments (Fig. 1). During first week of storage, all eggs experience decreased in AI with highest drop recorded for eggs coated with alcohol. In general value of AI decreased with increasing storage time agreed with result of Tilki and Saatci [34] and Copur et al. [9].

Interestingly, AI increased 3 weeks after first observation. The reason for this increased is uncleared although high variation of initial quality of egg and uneven application of propolis could explained the results.

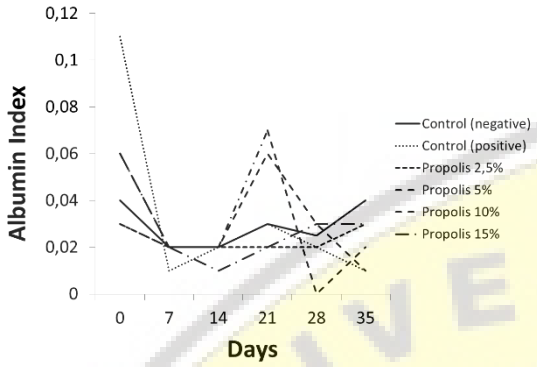

Figure 1. Weekly changes of albumin index

After 35 days, albumin index of both uncoated egg and egg coated with 2.5% propolis remain unchanged while egg coated with ethanol and 10% propolis experienced great reduction of AI (Fig. 2). Great reduction could be caused by uneven application. High storage temperature and excessively coating, that highly prevented loss of water and CO_2, also could produced rapid reduction of AI [5].

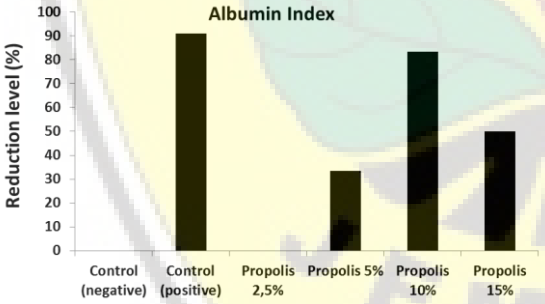

Figure 2. Level of Albumin Index reduction 35 days after treatment.

Yolk Index

Decreasing YI during storage is related with change in albumen condition. As storage time increase, more water diffuse from albumen to yolk, resulting changes in condition of vitellin membrane and liqufaction of the yolk [6][35]. Indonesia National Standard for YI between 0.33 to 0.52 (SNI 3926:2008).

Highest average of albumen index, during 35 days of observation, was recorded on uncoated egg. On the other hand, egg coated with 15% propolis produced significantly lowest YI (Table 2). This study showed, average YI of eggs for all treatment were lower than national standard.

Table 2. Average value of yolk index during course of research

Treatment	Yolk Index			
Control (negative)	0,23	±	0,02	a
Control (positive)	0,20	±	0,02	ab
Propolis 2,5%	0,18	±	0,02	ab
Propolis 5%	0,21	±	0,02	ab
Propolis 10%	0,22	±	0,02	a
Propolis 15%	0,16	±	0,02	b

Different letter indicated significant data (P<0.05)

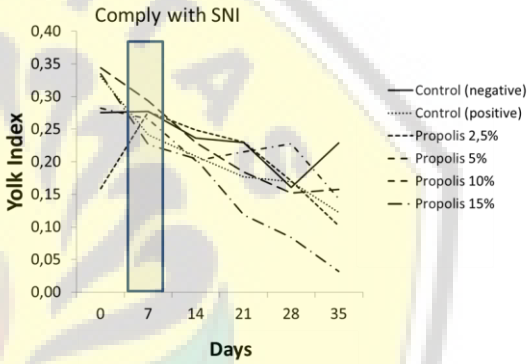

Figure 3. Weekly changes of Yolk Index.

Unlike AI, weekly reduction of YI index much lower (Fig. 3). After 1 week, YI of eggs coated with 10% propolis were still comply national standard. However, at second application of propolis did not able to maintain YI index longer than uncoated egg. This result is contradictive with Copur et al. [9].

After 35 days of observation, this study showed YI of uncoated egg and egg coated with 2.5% propolis achieved similar reduction level (Fig. 4).

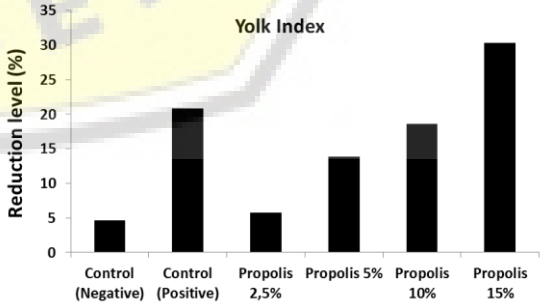

Figure 4. Level of Albumin Index reduction 35 days after treatment.

Conclusion

Egg distributed in local market which originated from local farming mostly below SNI and has various quality.

Propolis coating only able to maintain quality of the eggs but not increasing storage time of eggs. Application of 2.5% propolis is the optimum value to achieved best preservative effect. Application of higher concentration of propolis increase deteriorate rate of eggs.
Coating application by hand spraying may unsuitable for egg shell coating.

Future Works

Studies on others component of internal and external egg quality should be measured. The effects of type of hen, age of hen, and food of hen on the initial egg quality will provide valuable data to determine limiting factors of propolis application for egg preservative. Other studies should conducted with different types of chicken eggs produce in Indonesia.

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MODIFICATION SHREDDED as AN EFFORT to FOOD DIVERSIFIED to REACH FOOD SECURITY

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Abstract

Food as a basic need for human life, affect the quality of individuals. Food consumption patterns in Indonesia is not yet diversified perfect, for food consumption as a whole and also for the foodstuffs. Fish is source of high protein. Catfish processing and banana onthong to be shredded is one of alternatives in diversified processing. Food insecurity is greatly affected by purchasing power of the community being determined level of income. Low income aggravate energy and protein consumption. Shredded made from fish having the high price so to depress the price of shredded products made with animal that combination with plants. Modification shredded as one of many efforts to solving nutrition problem namely protein energy malnutrition (PEM). Modification shredded (100 grams) having energy 338.2 kcal and protein 16.5 gram can donate 15% energy requirement/day and 26,4% protein requirement/day. Nutritional problems have to solving as soon. Food security must bring into reality, because national food security as a basis for the establishment of human resources and good generation, who necessary to build this nation. Food diversified is one of the main pillars as an effort to handle food and nutrition problems which in turn can realize national food security. Efforts to diversify food must keep continue and necessary the consistency of many parties (central and regional governments, food industry, community) to achieve implement in sustained in better coordination; need of further research to know effectiveness of modification shredded on increased community nutritional status.

Keywords: modification sheredded, food diversified, food scurity

1. Food

Food is a human right. Food was the most basic for human[1]. Food also determines quality of human resources a people and food a basis the national security[2]. Food is everything derived from sources biological agricultural products, plantation, forestry, fisheries, livestock, and water, good process or processed provided as of food or drink for human consumption, including an additional material for food, raw materials food, and other ingredients used in the process preparation, processing, and/or manufacture of food or drink [3].

2. Food Security

Food security is a condition fulfill food for individual countries which is seen from the availability of food enough, terms of quantity and quality, safe, various, nutritious, evenly, and affordable and not contrary to religion, confidence, and culture of society , in order to live healthy, active, and productive sustainably. Definition of *food security* in *Rome Declaration and World Food Summit Plan of Action*: *"food security exists when all people, at all times, have access to sufficient, safe and nutritious food to meet their dietary needs for an active and healthy life".* Food security is the pillars of the construction of other sectors. [2] Food security are meant to be summons, because national food security is a pillar for the establishment of human resources and the generation that quality, necessary to build this nation. In realizing national food security, could be pursued in many ways, one of them is by diversified food.

3. Food diversified

Food diversified consumption basically expanding choice of community in activities of consumption in accordance with tastes desired and avoid boredom to get food and nutrition in order to live healthy and active. It is very influenced by purchasing power of the community, knowledge, availability, support policy, and social and cultural factors. In an implicit manner efforts to diversify food can identify with the effort to improve nutrition to get quality of human resources Indonesia capable of defenseless competitiveness with other countries. [4] Diversification food is one of the main pillars as an effort to handle food problems and nutrition which in turn can realize national food security. [5] Food Indonesia consumption patterns is not yet diversified with perfect, good for food consumption as a whole and to foodstuffs. [4] Food diversified is an effort to improve the availability and food consumption diverse, nutritious balanced, and based on the potential of power localized. [3] Three sorts of diversified the

business that one must applied simultaneously to increase the income is diversified production, diversification processing and diversified marketing. Food security national realized when held food supply a stable and enough for the entire population, and each households able to obtain the food in accordance needs. With food security good, there is a a guarantee to all people to get food and enough nutrients.

Food security is a system consisting of subsystem food availability, food distribution, and subsystem consumption. The availability and distribution facilitate food supply a stable and equally in all areas, while subsystem consumption allow any households food have sufficient and use it in a responsible manner to fulfill the nutritional needs for all its members. Thus, food security is issue at the regional level to the household level, with two important element that is food availability and access each individual to food enough.In this regard, food not only rice or food crops (rice, corn, soybean but includes food and beverage was from plants and animals, including the products primary and its derivatives [6]. The outcome of several study showed food availability enough national has proven to be not ensure embodiment food security in regional , households and individual. Data showed that the number of households proportion his undernourished in each province is still high. In this regard, diversified food be one of the main pillar in providing food security to independence food. Food shortages is strongly influenced by purchasing power of the community specified level of income. Low level of income aggravate energy consumption and protein [4]

Vulnerability happened when households, the community or to a definite region experienced insufficiency food to meet standard of a physiological need for growth and the health of individual members. Food shortages comprises of chronic shortages, that is happening by continuous because of incompetence buy or producing food own; and food scarcity while which occurs because the condition unexpected such as a natural disaster or other disaster. Food shortages if there is continuous will impacted on the decline in status nutrition and health [6].

Nutritional status of communities is further results (outcome of food security households, which is a combination of food enough, ability to manage consumption each its members have nutritional intake meet their needs , and the ability tending health and set sanitation environment [6]. The results of Riskesdas 2013, prevalence of malnutrition in children under five of 19.6 % it means problems malnutrition in Indonesia is still a problem.[7] The situation nutritional status of also shows the weak nutrition security occurred in households in Indonesia. When associated with the efforts to build the quality of human, so access food, health services, and education is determine than availability food.

Consumption of protein the inhabitants of east java up to 62,30 gr/hood/day in 2005 or increased by 2,20 gr/hood/day or 3,66 % of consumption of protein of the previous year (2006) as much as 60,10 gr/hood/day. Consumption of protein in fact go beyond 10,30 gr/hood/day (19.61%) of a digit sufficiency a protein that is advisable 52 gr/hood/day. Consumption of protein is supported by an increase in consumption of protein the rural population large enough protein from consumption of rural population the previous year. Consumption of protein an urban population and rural up to 60.70 gr/hood/day and 64,5 gr/hood/day [8]. Consumption of protein an urban population of 60,7 gr/hood/day down by 6.7 gr/hood/day or 9,95 % from previous year which 67.4 gr/hood/day. While, Consumption of protein rural population of 64,5 gr/hood/day increased 6.3 gr/hood/day or 10,82 % from the previous year which 58,20 gr/hood/day. Increase of protein consumption in rural population because the food the rise in public consumption animal in the form of: fish, meat, eggs and milk. Hence, acceleration movement bear directed in an urban focused on diversity food consumption vegetable non rice/wheat flour of tubers, vegetable and fruit, nuts, and food consumption animal nutritious and balance [8].

4. Modification Shredded

Fish is one of food a source of high protein that can be used as a source of food alternatives problem solving of Protein Energi Malnutrition (PEM), especially lacking protein [9]. Fish and onthong bananas is one of foodstuffs are no stranger for Indonesian people. Food security development based on resources and local wisdom through the effort to diversify pagan have to keep excavated and increased.[4] Three kinds of diversification that must be applied simultaneously to increase revenue society is diversified production, diversified processing, and diversified marketing [6]. Catfish dumbo and banana onthong processing to be the product abon is one of alternatives in diversified processing.

Shredded made from fish having high price so as to depress price of abon, products made with animal can combine with vegetable. Modification shredded as an effort to overcome nutrition problem namely Protein Energi Malnutrition (PEM). Shredded modification (100 grams) having energy 338,2 kkal and protein 16.5 grams can donate 15 % sufficiency energy per day and 26,4 % sufficiency protein per day. With reference to the basis of determined in a list Daftar Kecukupan Gizi yang Dianjurkan (DKGA) in 2012 for early adolescence (on average sufficiency energy 2400 kkal per day and protein 62.5 grams per day). Most proteins consumed by community is still derived from food vegetable its quality lower than animal food.

Table 1. Nutrients of Modification Shredded

Nutrients	Result
Energy	290,3 kcal

Protein	18,5 g
Fat	14,3 g
Carbohydrate	23,9 g
Dietary fiber	2,8 g
Vit. A	78,7 µg
Vit. C	3,8 mg
Sodium	1318,5 mg
Potassium	565,2 mg
Calcium	336,2 mg
Magnesium	51,4 mg
Phosphorus	311,8 mg
Iron	4,9 mg
Zinc	0,9 mg

Modification shreedded contain high nutrient, energy 290.3 kcal, protein 18.5 g, fat 14.3 g, carbohydrate 23.9 g, dietary fiber 2.8 g, vitamine A 78.7 µg, vitamine C 3.8 mg, etc.

Reference and appetites someone would has changed from a preferred food simple with a low price to fulfilling their basic needs as only focus on food sources of carbohydrate changed to food a source of protein, vitamin, and minerals, to meet nutritional requirements more complete type and number.[4] Efforts to diversify food must keep continue and necessary the consistency of various parties (central and regional governments, food industry, community) to achieve implement in sustained in better coordination; need of further research to know the effectiveness of products modification shredded on increased community nutritional status

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THE EFFECTIVENESS of STORED *Splt*MNPV on MORTALITY and NORMALITY of *Spodoptera litura*

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Abstract

The storage of *Spodoptera litura* multiple nucleopolyhedrosis virus biopesticide is an important stage and often performed before the virus was applied, to maintain the purity of the virus. This study aims to determine *Splt*MNPV in vitro effectiveness which have been stored for 20 months at -80 ° C on mortality and normality *S. litura*. *Splt*MNPV in vitro at doses of A = 5.95 x 10^8 PIBs / ml, B = 5.95 x 10^7 PIBs / ml. C = 5.95 x 10^6 PIBs / ml and D = Control was infected to third instar Larvae of *S. litura*. This research was conducted in the laboratory with repeatedly for fifth times and each replication used 10 animals caterpillar. Infected Larvae has been incubated for 17 days and had been given an artificial feed.The parameters observed in the form of mortality and normality *S. litura*. Research result shows that in *S. litura* infected with *Splt*MNPV in vitro dose of 5.95 x 108 PIBs / ml caused high mortality of 44%, *S. litura* abnormal approximatly 14% and the rest managed to become a moth normal life. Lowest mortality at doses C = 5.95 x 10^6 PIBs / ml; the mortality of *S. litura* is 38% and abnormalities *S. litura* is about 12%. *S.litura* abnormal morphology was observed in the Larvael stage, PrePupae and Pupae.

Keywords: Effectiveness, *Spodoptera litura* Multiple Nucleopolyhedrosis virus (*Splt*MNPV), mortality, abnormalities, *Spodoptera litura*

Introduction

S*podoptera litura* multiple nucleopolyhedrosis virus (SpltMNPV) is biopesticide made from the virus. The effort hasbeen made to propagate the virus in vitro by culturing cells midgut epithelial cells or ovarian cells of various insect Larvae such as *S. litura*, *S. frugiperda*, *Pseudoletia unipuncta*, *M. sexta* and *Heliothis virescens*. According to a preliminary test by [1], cultured midgut epithelial cells of of S. Litura Larvae grow very fast growing / divide rapidly, reaching 975 times / 24 hours.The culture can be used to produce a wide range of bioproducts such as biopesticide virus production virus (baculovirus) and certain protein product that is typically use for genetic engineering.

The Pathogenicity of SpltMNPV biopesticide a result of in vitro propagation in midgut epithelial cell culture that was recultured second time and formulated with photo-protectant and infected to *S. litura* second instar Larvae, resulting 100 % of S. litura Larvae mortality at the fifth day [1] According to [2], latent virus in the shape of polyhedral or prism in epithelial cells cultured *S. litura* Larvae that have been saved (in the freezer in Grace's medium temperature ± 20^{o}C) for at least 2 years then pathogenecities were 10 percent. However when the virus were infected repeadly to the original host Larvae of *S. litura* their pathogenecitas increased to 14% after the first infection, and 63% in the second infection, and 90% in the third infection.

The factors that determine the level of effectiveness of the virus, among others, are the nature, concentration, application mode, storage, and persistence level of the virus. NPV inoculum are resistant when they are stored in a refrigerator in the form of dry polyhedra.

Mixture polyhedrosis usually last for four to five years, granulosis last for two to three years, and cytoplasmic polyhedrosis last for one year [3] Once applied on the field, the virus will survive for along time if it is not exposed to direct sunlight. If it is directly exposed constantly to the sunlight, then its virulence will reduce by 50 percent each day. According to Pramono (2000), the NPV pathogenicity remains unchanged after 1 year of storage. This is supported by Balitsa Lembang's study. He has packed NPV mixed with talc and then placed it in a plastic bottle. The result showed that the NPV can be stored for more than one year. In addition, based on a study conducted by Klaten research center, NPV was also quite effective in maintaining the stability and effectiveness up to 6 months when placed in a dry room temperature.

Based on the background this study will examine the effectiveness of in vitro propagated SpltMNPV on epithelial cells of *S. litura* Larvae that had been stored for 20 months in Ex-cell medium at -80 ° C, on the mortality and normality of *Spodoptera litura*.

Materials And Methods

A.Equipment and Materials Research

The tools used in this study include the main equipment and ancillary equipment. The main equipment consists of incubator, light microscope, Laminar Air Flow (LAF), incubator, centrifuge, magnetic stirrer, and blender. The support equipment consists of capsule bottles, brushes, gauze box, centrifuge tubes, syringe, aluminum foil, knife, and label.

The materials needed in the study are SpltMNPV Wonosobo, Central Java isolates that are propagated in vitro, third instar Larvae of *Spodoptera litura*, artificial feed for Larvae of *S. litura*, Bayclin, Alcohol 70% as a sterilizing agent, and distilled water.

A. Research Design

This research is an experimental study using a complete randomized design, one factor is the dose SpltMNPV propagated in vitro in the midgut epithelial cells of Larvae of *S. litura* which has been stored at a temperature of - 80 ° C for 20 months, infected Larvae fed by using artificial feed (Balitas , Malang).

This study aimed to test the pathogenicity of in vitro SpltMNPV that has been stored for 20 months on the mortality and normality of *S. Litura*. The study was repeated 5 times and used 10 Larvae in each repetition. Larvae of S. litura were given SpltMNPV in vitro treatment with a dose: A = 5.95 x 108 PIBs / ml; B = 5.95 x 107 PIBs / ml; C = 5.95 x 106 PIBs / ml; D = Control (akuadest). The results of research in the form of mortality and infected normality S. litura were analyzed descriptively.

B. Research Procedure

1. Preparation: The calculation of the concentration of SpltMNPV

SpltMNPV which are newly stored in the refrigerator in the temperature of 80oC adapted beforehand to the same temperature as the room.Virus concentration is calculated using haemocytometer. SpltMNPV concentration used is A. 5.95 x 108PIBs / ml; B. 5.95 x 107PIBs / ml and C 5.95 x 106 PIBs / ml.

2.Propagation test insect (*Spodoptera litura*)

F0 S. litura Larvae captured in the area around the jatropha and tobacco plantation in Malang, and subsequently maintained in the Laboratory of Microbiology UNESA up to the stage of imago and feeded using jatropha leaves as natural food.Imago were moved into screen box with the male and female ratio of 1: 3. Imago were allowed to marry and fed using liquid honey 10%. Eggs produced in this step is an F1 generation. Eggs obtained were subsequently maintained until they hatch into Larvae F1.The Larvae then immediately transferred into bottle maintenance/ bottle capsules with a volume of 50 ml.Feed given to the 1st and 2nd instar caterpillar is the natural feed in the form of jatropha leaves, and artificial feed used to feed instar 3 to 5 use. The composition of 1 liter of artificial feed includes: soybean flour (42 g), agar-agar powder (14 g), yeast (42 g), Wheat germ 36 grams), salt Wesson (10 g), vitamin mix (9.6 gr), parabernsoat (1 g), sorbic acid (1 g), aureomycin (1 g), streptomycin (0.2 g), Benlate (1 g) and formaldehyde (2 ml) that were cooked and printed as gelatin to size 1 x 1 cm square. S. litura Larvae used in this study was the third instar Larvae of *S. litura*.

C. Test of SpltMNPV pathogenicity in vitro which have been kept for 20 months on the Larvae of S. litura

Larvae of *S. litura* were infected orally by dipping Larvae feed on virus suspension. The infected Larvae were incubated in the capsule bottles individually for 17 days (Larvae become moth). Furthermore, the infected Larvae were observed in terms of their mortalities and morphological changes to see normality. Morphological changes of the Larvae were observed every day starting from initial infection until caterpillars' death.

Results And Discussion

A. The mortality and normality of *S. litura* infected with SpltMNPV

The result of this study is the effectiveness of SpltMNPV which were reproduced on midgut epithelial cells of *S. litura* Larvae on the mortality and normality ofS. *litura*, with different SpltMNPV oncentrations and were infected with food dipping method. The treatment was repeated three times and there were 10 Larvae in each repetition. The percentage of mortalities, abnormalities and normalities of *S. litura* infected by SpltMNPV presented in Table 1, as follows:

Table 1. The percentage of mortalities, abnormalities and normalities of *Spodoptera litura* by SpltMNPV in vitro after 20 months of storage (observed at day 17)

Treatment	Imago	Replay					Average
		1	2	3	4	5	
A	Dead	40	50	50	40	40	44
	Abnormal	0	10	20	20	20	14
	Normal	60	40	30	40	40	42
B	Dead	50	20	40	30	60	40
	Abnormal	0	10	10	20	20	12
	Normal	50	70	50	50	20	48
C	Dead	10	50	50	50	30	38
	Abnormal	20	10	20	10	0	12
	Normal	70	40	30	40	70	50
D	Dead	20	10	20	30	10	18
	Abnormal	10	10	20	0	0	8
	Normal	70	80	60	70	90	74

Descriptions :

A = 5.95 x 108 PIBs / ml; B = 5.95 x 107 PIBs / ml; C = 5.95 x 106 PIBs / ml; D = Control

Data desribed as the percentage of mortalities, abnormalities and normalities of *S. litura* that died because of being infected by SpltMNPV (Table 1) can be seen at the seventeenth post infection**.** Based on the data in Table 1 above, it appears that the highest mortality achieved in SpltMNPV in vitro with a dose of 5, 95 x 108 PIBs / ml were 44%, the next row at a dose of 5.95 x 107 PIBs / ml and 5.95 x 106 PIBs / ml with a mortality of 40% and 38%. Compare to the research conducted by [1] the same, but still new cultured, virus which were infected on second instar Larvae of *S. Litura* achieved 100% mortality on the fifth day.The mortality obtained by this study is lower than that was resulted by the new cultured virus. This is caused by the fact that the virus in this study has undergone sufficiently long time storage, that was 20 months.

According to Mangundihardjo et al (1992), the weakness of NPV stored for a long time is the virus does not get live cells so that its activity stops and crystallizes and it needs some time to be active when it find the host. Evidence that the virus which has been stored for a longer period shapes crystal/like a prism has been successfully photographed by [2] using scanning electron microscope, while the NPV which is still isolated from the new host shapes polyhedra (hexagon like dice).In addition, strains of SpltMPNV used were less virulent because the virus has undergone propagation in different environments i.e. in vitro cell culture conditions are not the same when SpltMNPV attack in vivo. In the in vitro conditions, the host's immune system does not exist so that the virus is not adaptive when inserted into the original host body.

According To Pramono, at the virus storage period for 1 year, the pathogenicity of the virus applied in the field reaches the mortality of *S. litura* as much as 49.33% on the tenth day. Thus, the virus storage for 20 months caused mortality of *S. litura* was slightly lower (44%) than that of 1 year virus storage. %. According to Suwandi (2007), NPV stored in a dark room for 24 hours and infected on caterpillar H. Talaka (inch caterpillar on tea leaves), its pathogenicity decreased by 26.66%.In the in vitro conditions, the host's immune system does not exist so that the virus is not adaptive when inserted into the original host body.

Besides, the relatively low mortality was also obtained at post infection on the seventeenth day. In the long period of death, according to Granados and Federici (1986, in Suwandi, 2007), NPV is said to be virulent if it can kill S. litura within 2-5 days, and is said to be less virulent if the NPV can only kill it within 2 to 3 weeks. So, based on the opinion, SpltMNPV in vitro which have been stored at -80 ° C for 20 months will be classified as less virulent.The use of SpltMNPV resulted from in vitro propagation causes the growth of *S. litura* at various abnormal stages (Figure 1,2 and 3).

It can be seen from the evidence that *S. litura* virus were dying in the stage of PrePupae, Pupae and imago. In the Larvael stage, virus rarely died. This shows that in the Larvael stage, the Larvae are able to make high defense against infection of SpltMNPV. Only in the intermediate stage of the Larvae become PrePupae, PrePupae into a Pupae and imago stages.

At this stag**e,** *S. litura* are less able to form a high defense against viral infection. PrePupae is a critical stage because the Larvae feeding activity has stopped. The energy stored when the Larvae are widely used to convert the organs in the body of the Larvae into other organs whose functions change.

For example, the digestive organs was in the form of mouth /chewing food in the form of solids (leaf) will turn into fluid/ honey-sucking mouth. This applies to other organs such as the formation of the wings to fly which did not occur in the Larvae l stage.

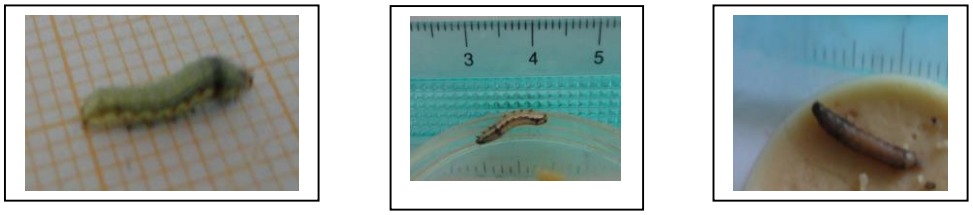
B. The Morphology of SpltMPV *S. litura* infected in vitro

The morphology of *Spodoptera litura* infected with SpltMNPV at various stages was observed to see the abnormalities produced.

Observations on abnormalities done at the Larvae l, PrePupae, Pupae and imago stages.

Table 2. Characteristics of Larvae, PrePupae, Pupae and imago of *S. litura* infected with SpltMNPV in vitro.

No	Stages *S. litura*	The characteristics of *S. litura* abnormal	The characteristics of *S. litura* normal
1.	Larvae	The infected Larvae tend to has bright and shiny colors, especially in the lower abdomen, the mandible when it was touched its response is slow, the Larvae tend to rise to the top (closed bottle), his movements is slow, lazy to feed, time needed by instar to turnover tends to be longer, her body swelled and when died the virus body are easily broken when we touch, it discharged white milk containing viral polyhedra (Figure 1 A).	Color matches the color of the Larvae feed, on the day of the beginning of each instar Larvae tend to be bright colors (beige / brown) but on days 2-3 ahead instar change color becomes darker. The texture /line on the Larvae is clear. When being touched Mandible Larvae will respond quickly. Larvae at Instar 5 tend to be darker. The Larvae eat actively (Figure 1.B, C)
2.	PrePupae	PrePupae color is darker / blackish brown, sometimes the color is uneven (half brown and half dark brown), its body segments are irregularly, if we touch its response was slow, the formation of candidates for the head imago is not perfect, some PrePupae failed to shrink his body, even enlarged/swelling, diabdomen leg is still visible, diabdomen segment of the middle part shrink, soft body and when we touched it discharge milk chocolate which contains many polyhedra (Figure 2A)	PrePupae color is still like instar 5 Larvae that is dark brown, the length and width of the Larvae shrink nearly half the normal size, PrePupae eat inactively, make a nest by collecting leftover food and feces are made such that the whole body covered. Food remains and excrement held together with a kind of yarn produced by saliva. When we touch, PrePupae response is slow, and the body tends to curved (Figure 2B)
3.	Pupae	The size of the Pupae is smaller than the normal Pupae (<2 cm), Pupae color is uneven (there are several bulkhead colored younger), head of the Pupae enlarged, abdominal shrinking, the legs are still seen, bulkhead abdomen retracts but not hardened, its color is dark brown tends to black, head arched, some abdomen / head is soft and easily broken when we touch and issuing white liquid filled with polyhedra. (Fig 3 A)	Pupae normal size (± 1.5 to 2 cm), the color of the Pupae from young to old Pupae terraced ranging from light brown to dark brown. When touched, its response is fast (Figure 3B).
4.	Imago	Imago wing curling, sometimes broken and small, when we touch the response is slow, passive and not actively foraging.	The shape and size of a normal imago (± of 1 - 1.5 cm), grayish-brown color with distinctive spots. When attached to the substrate it resembles an isosceles triangle. Fast response when touched. Tend to be nocturnal, actively approaching the feed in the form of liquid honey.



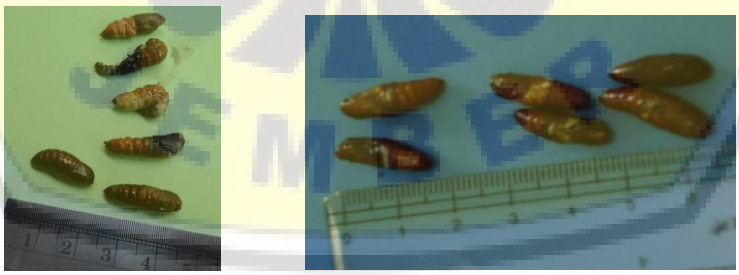
A B C

Figure 1. A third instar Larvae of *S. litura* normal, and B, C third instar Larvae of *S. litura* infected SpltMNPV (abnormal)

Based on Table 2, the changes in morphology and behavior of (healthy) normal *S. litura* Larvae and S. litura Larvae infected with SpltMNPV in vitro (abnormal) can be observed. Healthy *S. litura* Larvae movement is still active, feeds actively, the color of caterpillar is normal, that is cream- brown, dark brown (accordance with the color of the feed)., the color is clear, when it is touched mandible is still moving. Colors on the body of S. litura Larvae is in accordance with the color of the food he ate.

A B

Figure 2. A. PrePupae abnormal because infected SpltMNPV and B. The development of normal uninfected PrePupae SpltMNPV



A B

Figure 3. A. B. Pupae normal abnormal

In this study, the feed used is in the form of artificial feeding (formula of Balitas Malang), cream / yellow colored, so that most of the Larvae color is also cream. The older the instar Larvae, the darker the color of their bodies. Their skin color then fade when the change their skin as a marker of instar stages of change. The color change is due at the time of the skin change, cuticle that has melanin pigment was released. The cuticle layer will be replaced with a new cuticle that has little melanin.

The *S. litura* larvae infected with SpltMNPV in vitro showed changes in morphology and behavior.The changes can be seen in the Larvael body at the beginning of the infection caused by SpltMNPV begin damaging cells it attacked. The initial infection usually begins in the digestive tract. This causes the larvae will not eat, because many cells in the gastrointestinal tract begins to rupture / lysis. This resulted in the production of digestive enzymes are inhibited (eg proteases), metabolism becomes impaired, the energy derived from the metabolism decreases as a result of passive larvae (slow motion).Larvae look began to stop eating, and tends to rise to the end of the bottle as in their natural habitat, that is in the plant, which tend to rise to the top of the plant.The infected Larvae look swollen, especially on the back / dorsal, while it looks redness at the abdominal / ventral.The swelling is caused by the many larvae were infected by lysis so that the cells secrete fluid mixes with hemolimfa. In the abnormal Larvae that died, the color of reddish brown caterpillar can bee seen in the abdomen, and at the back has white colored cream milk or destroyed at the end of the bottle with the liquid that comes out milky white. Soft and when it was touched, it broke and secrete a milky white liquid. Mandible did not move. Caterpillar size is smaller.One characteristic of *S. litura* larvae infected with the virus is dead in a dependent position as the letter "V" inverted with abdominal leg as a footstool. The fluids which broke from the body of dead caterpillars infected SpltMNPV SpltMNPV containing polyhedra which, if left in a few moments, will cause a bad smell. This odor is due to the fermentation of the remnants of larvae metabolites that were out of the lysis cell or because of the degradation of the remnants of lysis cells / tissues / organs.

At normal PrePupae, PrePupae color is still like the 5 instar Larvae, that is dark brown, the length and width of the Larvae shrink nearly half the normal size, PrePupae eat inactively, make a nest by collecting leftover food and feces are made such that the whole body covered.Food and excrement remnants held together with a kind of yarn produced by saliva. When PrePupae is touched, its response is slow, the body tends to curve.

Meanwhile, abnormal PrePupae because of being infected by darker / blackish brown virus, sometimes the color is uneven (half brown and half dark brown), its body segments are irregularly, if it was touched its response was slow, the formation of candidates for the head of imago is not perfect. Some PrePupae failed to shrink his body even enlarged / swollen, diabdomen legs are still visible, diabdomen segments of the middle part shrink, the body is soft and when it was touched it discharged milk chocolate contains a lot of polyhedral.

The normal growth Pupae showed the characteristics of normal size (± 1.5 to 2 cm), the color of the Pupae from young to old Pupae terraced ranging from light brown to dark brown. When it was touched, the response was fast (Figure 3B). Meanwhile, abnormal Pupae has specific characteristics, namely size of the Pupae is smaller than the normal Pupae (<2 cm), Pupae color is uneven (there are several bulkhead colored younger), head of the Pupae enlarged, abdominal shrinking, legs are still visible, abdomen bulkhead retracts but not hardened, dark brown color tends to black, curved head, some of its abdomen / head was soft and easily broken when it was touched and issued white liquid filled with polyhedra. (Figure 3 A).

In the imago stage formed after 8 days old Pupae, morphology imago. The shape and size of imago is normal (± of 1 - 1.5 cm), its color is grayish-brown with distinctive spots. When attached to the substrate**,** it resembles an isosceles triangle. It has fast response when was touched. Tend to be nocturnal, actively approaching the feed in the form of liquid honey. Conversely, abnormal imago has characteristics of curly wings, sometimes broken and small, when it is touch the response is slow, passive and not actively foraging.

Conclusion

Based on the data above it can be seen that SpltMNPV propagated on epithelial cells of *S. litura* Larvae, and had been maintaining in the storage at -80 ° C for 20 months caused the mortality of third instar S. litura Larvae highest achieved at doses SpltMNPV 5.95 x 108 PIBs / ml by 44 %, the next row at a dose of 5.95 x 107 PIBs / ml and 5.95 x 106 PIBs / ml with a mortality of 40% and 38%. The mortality was observed for 17 days after treatment Mortalitas tersebut diamati 17 hari setelah perlakuan. Stadia *S. litura* in the forms of Larvae, PrePupae, and Pupae or imago which were infected with SpltMNPV showed the characteristics of specific abnormalities that are

different from normal.The percentage of abnormal imago decreased is equivalent with the decrease of SpltMNPV concentrations given.

Acknoledgment
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GROWTH PERFORMANCE of SOME VARIETIES CHRYSANTHEMUM as A MOTHER PLANT on *IN-VITRO* PROPAGATION

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Abstract

Chrysanthemum (*Chrysanthemum morifolium* Ramat) is one of the cut flowers are very interested in the community, among others. The diversity of varieties of chrysanthemum flower color and the look of flower shapes. Problems at this stage requires a chrysanthemum seed supply healthy mother's plant through in vitro propagation. The research objective was to determine the performance of some varieties of chrysanthemum growth in vitro at the stage of sub-cultures. Research carried out in the Tissue Culture Laboratory AIAT East Java in June - October 2013. The research using completely randomized design with five varieties of chrysanthemum were cultured on Murashige and Skoog medium that is repeated 6 times with 10 units each replication. Three varieties of chrysanthemums as treatments include: Puspita Nusantara, CFA-03C and Yoko Ono sub-cultured and observed for growth. Observations included: the percentage of contamination, number of leaves, plant height, root length and plant morphology and tissue anatomy. The results showed that there were differences in the results of the subculture of the three varieties of chrysanthemum. Performance differences between the varieties of leaves visible on the shape of the leaf while the rod shape and color of the stem and leaf color did not show differences. Varieties which seem to have high growth in the most prominent is the variety Puspita Nusantara. Yoko ono varieties have a growing number of leaves more than other varieties. Varieties showed the longest root growth is Yoko ono varieties. From the results of cross-sectional anatomy of leaves between chrysanthemum varieties showed different results. Propagation chrysanthemum in vitro can maintain chrysanthemum vigor's plantlet that will be used as the mother plant.

Keywords: *chrysanthemum, varieties, growth, in vitro*

Introduction

In Indonesia chrysanthemum is a commodity in the floriculture industry . Chrysanthemum is one of cut flowers with high economic value . In 2010 the productivity of these plants has reached 185.232.970 stalk , with the market demand, both locally and internationally continues to increase. Productivity and chrysanthemums demand continues to increase from year to year, clearly requires the availability of improved varieties of new and sustainable quality seed. Chrysanthemum varieties are grown in Indonesia comes from the variety introduction of other countries such as the Netherlands as well as the results of research in Indonesia (Dirjen Hortikultura, 2013)

Methods of in vitro tissue culture is an engineering plant propagation by taking parts of the plant such as buds , stems , flowers , leaves, roots , and other parts to be grown in planting medium in the bottle for a certain period of time , until the plants can be planted in the field . Excellence seed chrysanthemum with tissue culture is to get quality seeds (free of pests and diseases) , Rooting stronger so the growth of seedlings better , does not require a large place and treatment continuously , propagation in large amounts with a relatively short time [10]. Results of in vitro propagation of chrysanthemum used as the mother plant shoot tips as explants.

Subculture is the process of cutting and removal of plantlets from old media to new media [10]. Some reasons do subculture according [7] are : the phenomenon due to nutrient deficiency in the media, nutrients in the media dries so that the concentration of salt and sugar is too high, growth has filled space test tubes or bottles, materials needed for further propagation. Media used during subculture is solid MS medium with the addition of coconut water , CaP and GA3 . The specialty solid MS medium is nitrate , potassium and ammonium is high (Lestari , 2008) . The research objective was to determine the performance of some varieties of Chrysanthemum growth in vitro at the stage of sub-cultures

Materials and Methode

Research carried out in the Tissue Culture Laboratory BPTP East Java starting in June-October

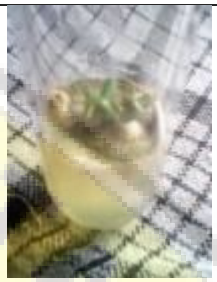
2013. The research using completely randomized design with three varieties of chrysanthemum were cultured on Murashige and Skoog medium that is repeated 6 times with 10 units each replication. three varieties of chrysanthemums as treatments include: Puspita Nusantara, CFA-03C and Yoko Ono sub-cultured and observed for growth. The explants were used for propagation in vitro is a chrysanthemum bud shoots, after a 1.5-month-old plantlets after sub-culture inoculation is done using 2 segment explants. Growth of chrysanthemum culture using modified Murashige and Skoog medium, medium sterilization using autoclave with a pressure of 20 psi for 20 minutes. Cultures are grown at room temperature of 20°C. Observations included: the percentage of contamination, number of leaves, plant height, root length and plant tissue anatomy. Morphology and Tissue anatomy observed when explants grown into plantlets at the age of 1.5 months after culture. Data analysis used F test and continued with LSD at the level of $\alpha = 0.05$ and descriptive analysis of morphology and tissue anatomy.

Results and Discussion

Percentage Contamination

From the results of in vitro culture showed contamination resulting explants do not grow well . The percentage of contamination of each varieties showed significant differences between the varieties of each other in a row Variety Puspita Nusantara = 25 % , CFA varieties 03C = 0 % and Yoko ono varieties = 70 %. Contamination causes impaired growth culture . Organisms source of contamination in all three of these varieties there are several types and have different characteristics , as presented in Table 1

Table 1. Observations organism a source of contamination subcultural

No	the type of organism	Picture	characteristic
1.	Mushroom 1		hyphae white Similar Colonies cotton Basic black colonies
2.	Mushroom 2		Color black hyphae Basic black colonies Similar Colonies velvet
3.	Mushroom 3		Similar Colonies powder The color green hyphae Spores orange Basic orange colonies
4.	bacterium		Similar colonies red mucus

Some types of microorganisms either mushroom or bacterium release toxic compounds into the culture medium can cause death explants [1].

Growth Performance of plantlets

Observations Morphology and Anatomy

The results showed that the chrysanthemum varieties Puspita Nusantara , CFA 03C and Yoko Ono demonstrate the performance is almost the same or different but less real morphology. Performance of light green stem with surfaces that are smooth hair , a round -shaped cross-section rod , a single leaf , the location of the alternate leaves, leaf margin serrated, upper and lower surfaces of leaves hair smooth and there are many shaped root fibers. The performance difference of the three varieties of chrysanthemum visible morphology of stem diameter and the length and width of leaves (Table 3 and Table 4) . Table 3 shows the diameter of the stem , leaf length and width as well as length of segments of plantlets.

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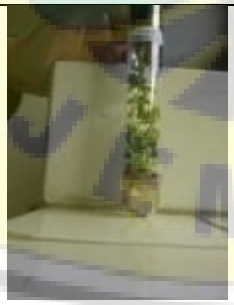
Table 3. The diameter of the stem and leaf size chrysanthemum

Varieties	stem diameter (cm)	Leaf		length of segment (cm)
		Length (cm)	Width (cm)	
PN	0,16	1,9	1,7	0,86
CFA 03C	0,15	2,2	1,5	0,58
Yoko ono	0,12	1,4	1,1	0,50

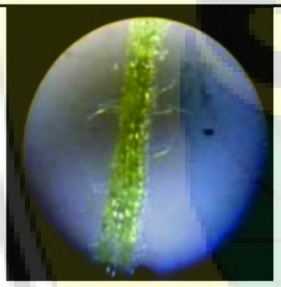
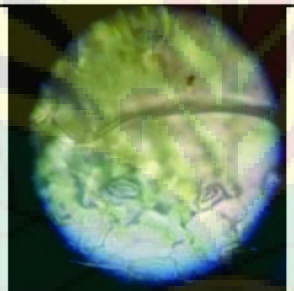
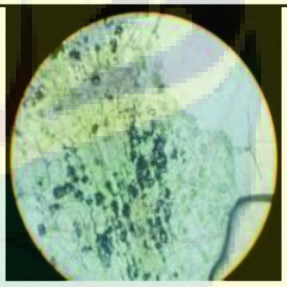
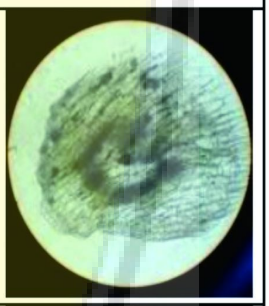
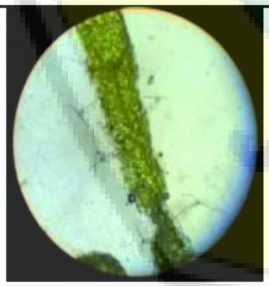
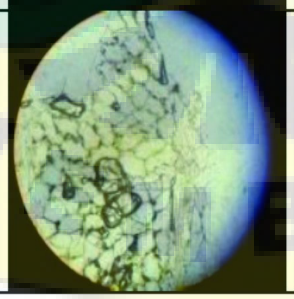
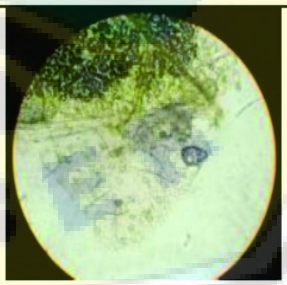
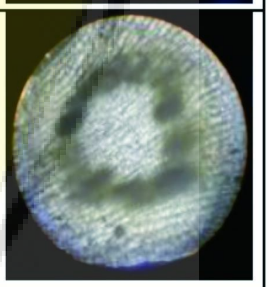
Table 4. Performance of each variety of leaf morphology

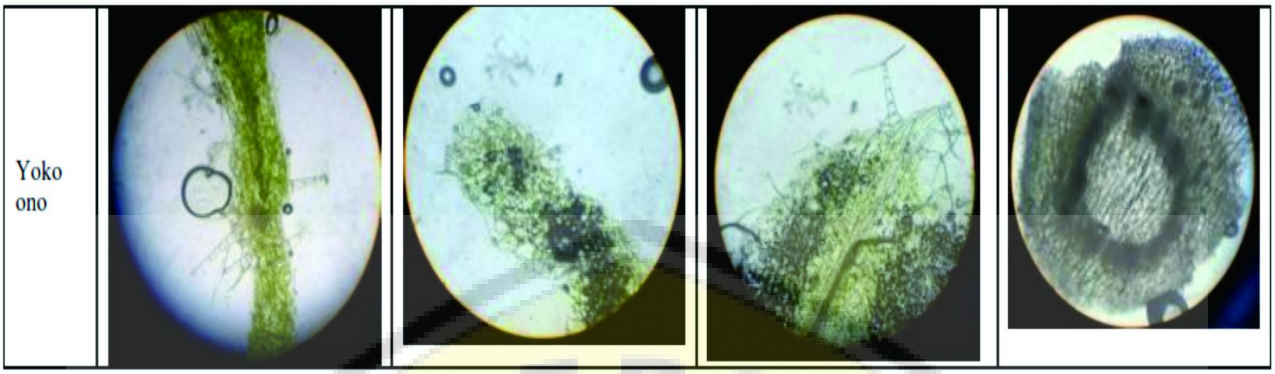
Varieties	Plantlet	Leaf morphology
Yoko Ono		
CFA 03 C		

Puspita Nusantara		

The observation of the cross-sectional anatomy includes stem , upper epidermis , lower epidermis (Table 5) , the cross-sectional leaf , form stomata and trichomes . Kidney-shaped stomata . Number of stomata on the lower epidermis more than the upper epidermis . Trichomes consist of multiple cells and the glandular trichomes , epidermal trichomes are at the top and bottom .

Table 5. Performance of each variety of leaf and Stem Anatomy

Varietas	Leaf			Stem
	cross-section	the upper epidermis	the lower epidermis	cross-section
PN				
CFA 03C				



b.2. The performance of high , number of leaves and root length plantlets

The results showed that there are differences in the performance culture growth of three varieties of chrysanthemum are seen in the growth of plant height , leaf number and root length . As presented in Figure 1 shows a diagram of the high growth of chrysanthemum plants produced subculture

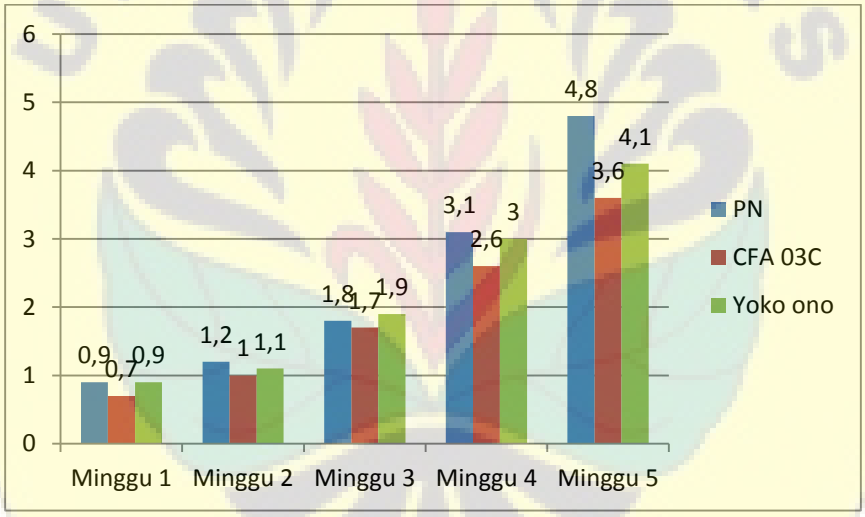


Figure 1. Diagram of plant height subculture results

Based on the diagram can be seen high growth of chrysanthemum plants subculture or hikes every week . High growth of each of the different varieties . Varieties which seem to have high growth is the most prominent varieties of PN (Puspita Nusantara) . Yoko ono varieties have good growth as well , but the high is still less than the PN varieties . CFA varieties 03C has the lowest high when compared with PN and Yoko ono varieties . This suggests that the high growth of chrysanthemum different subcultures each variety .

The performance culture in vitro growth of chrysanthemum seen from the increasing number of leaves as presented in Figure 2 .

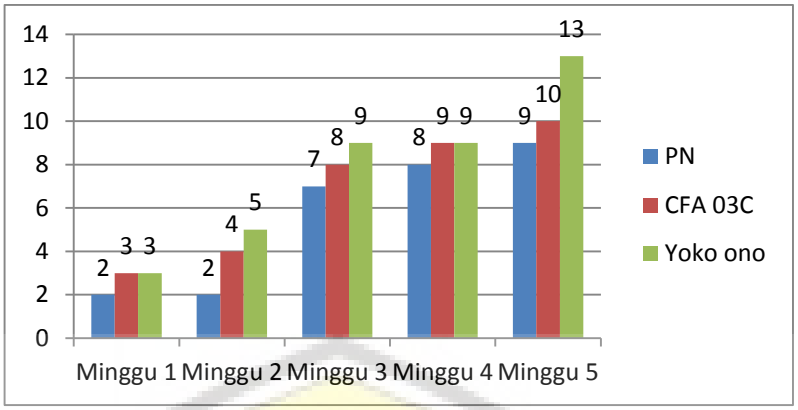


Figure 2. Diagram of the amount of leaf plantlets subculture

Based on the diagram can be seen that the number of plant leaves subculture has increased every week, The most notable growth is from week 2 to week 3, Yoko ono subculture chrysanthemum varieties have a growing number of leaves that are more than Puspita Nusantara and CFA 03C varieties. While the performance of root growth of plantlets are presented in Figure 3

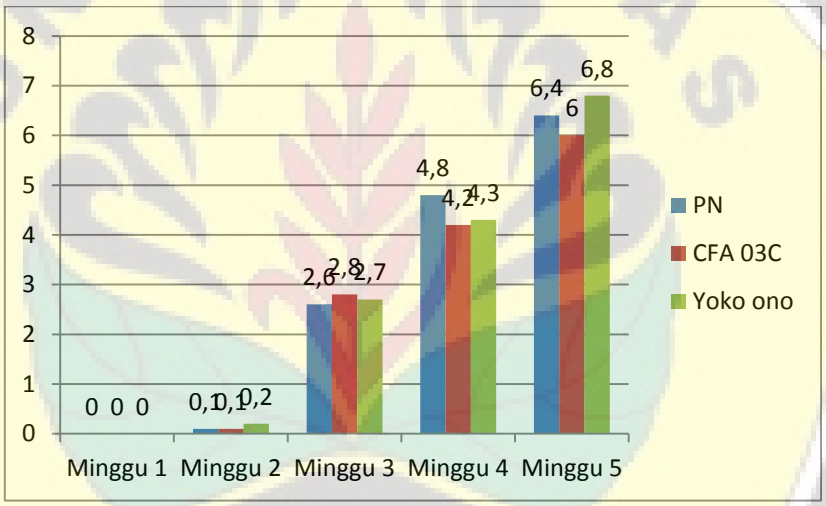


Figure 3 Diagram length of the root plantlets subculture

The results showed that root formation begins at week 2 for all varieties . Length of root growth every week . Varieties showed the longest root growth is Yoko ono varieties . Root length growth of all three varieties showed no significant differences.

The success of plant tissue culture is influenced by several factors , namely explants, growing media , plant growth regulators [6]. Based on the results obtained during subculture and observation , chrysanthemum varieties that can be developed are varieties Puspita Nusantara and CFA 03, but Yoko Ono can be used as a mother plant needs more careful sterilization and modification of the appropriate media. These varieties grow well and also a high percentage of success subculture .

Conclusion

The success of the growth of chrysanthemum plantlets in vitro such as mother plant determined the existence of contaminants in the media. Performance differences between the varieties of morphology leaves visible on the shape of the leaf while the rod shape and color of the stem and leaf color did not show differences. Each varieties of Chrysanthemum in vitro differently of the performance in morphology and anatomy . Plantlet of Puspita Nusantara varieties are more prominent in the performance of high while Yoko Ono varieties stand on the formation of leaves and roots.

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PREFERENCE TEST of SUSTAINABLE FOOD HOUSEHOLD AREA (KRPL)'S PRODUCTS in SUKOREJO VILLAGE, PONOROGO

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Abstract

KRPL is the area/village level region/RW/RT which formed from several Sustainable Food Household (RPL), by applying the principle of optimal utilization of the yard that is based on local resources to meet the food and nutrition needs of the family. Optimal utilization of the yard begins with the cultivate of various crops (mustard greens, tomatoes, corn, cassava and sweet potato). Excess crops in addition to fulfilling the daily needs of the family are also sold in the market. Problems will arise if the crop is not be full absorbed by the market. Hence it is needed diversification processed in order to increase the added value and family income. This assessment aims to determine the KRPL farmer's response and also determine the most preferred KRPL's food products that have been introduced based on the parameters of taste, aroma, texture, color and the general preference. This assessment was carried out from August to September 2014 at groups of women farmers (KWT) MELATI in Sukorejo village, Ponorogo. To determine the panelists preference, the organoleptic test was conducted by the method of "hedonic test" base on parameter of color, aroma, texture, taste and preference level in general. Organoleptic test was conducted on 27 panelists. This research was using the Randomized Block Design statistic method. Processing technology were tested :1) brownies from purple sweet potato, 2) cassava roll cake, 3) mustard greens marble cake, 4) tomatoes cookies and 5) corn crackers. Various products which introduced give significant effect on the preference level base on parameter of color, aroma, texture, taste and general preference. According to the panelist response, preference value of brownies was on the range 3.741-4.000 (which means like). Preference value of roll cake was on the range 2.741-3.333 (which means enough). Marble cake mustard greens have a preference value was on the range 3.370-3.926 (which means enough to like). Tomatoes cookies have a preference value 3.333-3.852 (which means enough to like). Preference value of corn crackers was on the range 3.667-4.148 (which means like). A high preference value base on parameter of color, flavor, aroma was on corn crackers products. Based on the general level of preference, corn crackers occupy the highest value of 3.963, which means like.

Keyword : sustainable food houses area, organoleptic, processing

Introduction

The aim of KRPL program is developing the ability of families and communities in self sufficient the food and nutritions in a sustainable manner [21]. One effort to improve food security and family nutrition can be done through the utilization of the resources that available in the environment by exploiting yard area that managed by the household. Yard area was used to cultivate food crops, horticulture and livestock to meet the daily food needs of the family [12]. Based on this the Ministry of Agriculture through the Agricultural Research Agency develops Sustainable Food Household Area (KRPL) program for optimizing the utilization of yard which managed by the family [13]. This program is collaborating with various government and private institutions such as the Food Security Agency (Badan Ketahanan Pangan), the Department of Agriculture (Dinas Pertanian), local traders and Research Center for Agricultural Research and Development Agency (Balit, Puslit and all of Badan Litbang Pertanian's Scope) (BBP_2TP, 2012). Yard area has a multipurpose function because in narrow land, can produce crops, horticulture and livestock [10].

Yards can be a potential resource to provide nutritious food. Ponorogo (Sukorejo village) is one of KRPL's programs target. The majority of the resident in Sukorejo village work as farmers that still rely on paddy fields to meet the food needs of the family. However, there are still many people who have not been able to meet its food needs despite utilizing the rice field, it is due to its narrow rice field. Even many people who do not have a rice field. With the use of yard area will facilitate the villagers to meet the daily food needs of the family.

In Sukorejo already introduced the cultivation of mustard greens, tomatoes, corn, cassava and sweet potato. Farmers have already started planting these commodities in their yard. Harvest products partially consumed to meet the daily needs, the

others was sold in the market. Farmers started getting problems when the harvest began to excess, because it is not all of the product can absorp by consumer, while commodities are easily damaged. According [4] the problems that faced in the development of KRPL's program was the lack of post-harvest technologies for food commodities locally in yard, the harvest product just to meet the needs of the household. Hence it is need diversification of KRPL commodities in to food product that can be sold to increase the added value and extend the shelf life of commodities. Moreover can increase family income. Processing technology was introduced to farmers, such as 1) brownies from purple sweet potato, 2) cassava roll cake, 3) mustard greens marble cake, 4) tomatoes cookies and 5) corn crackers.

Technology processing which introduced was very easy, it is use the materials and equipment that commonly used by mothers daily. It is expected that farmers can adopt this technology to improve food security and family income. This assessment aims to determine the KRPL farmer's response and also determine the most preferred KRPL's food products that have been introduced based on the parameters of taste, aroma, texture, color and the general preference.

Materials and Methods

This assessment was carried out from August to September 2014 at groups of women farmers (KWT) MELATI in Sukorejo village, Ponorogo. The raw materials was used comes from the KRPL's product in the farmers yards.

To determine the panelists preference, the organoleptic test was conducted by the method of "hedonic test" base on parameter of color, aroma, texture, taste and preference level in general. Organoleptic test was conducted on 27 panelists, come from KRPL's farmers. This research was using the Randomized Block Design statistic method. Processing technology were tested, such as 1) brownies from purple sweet potato, 2) cassava roll cake, 3) mustard greens marble cake, 4) tomatoes cookies and 5) corn crackers. Formulation of the products can be seen in table 1.

Tabel 1. Formulation of Food Product that have been tested

Brownies from Purple Sweet Potato	Cassava Roll Cake	Mustard Greens Marble Cake	Tomatoes Cookies	Corn Crackers
Puree of Purple Sweet Potato 200 g	Cassava flour 5 tablespoon	Margarine 160 g	Tomato puree 100 g	Corn flour 500 g
Wheat 250 g	Egg 5	Sugar 150 g	Cornstarch 150 g	Tapioca 500 g
Egg 10	Sweetened condensed milk 5 tablespoon	Mustard Greens puree 100 g	Wheat 500 g	Salt15g
Cooking oil 350 ml	Sugar 5 tablespoon	Egg yolk 6	Sugar 250 g	Garlic 40 g
Chocolate block 150 g	Jam for filling	Egg whites 4	Margarine 250 g	Water 1,2 l
Chocolate powder 100 g	-	Wheat 200 g	Milk powder 150 g	-
Sugar 450 g	-	-	Egg yolk 4	-

Results and Discussion

Organoleptic Test of KRPL's Product

Organoleptic test is also called the sensory test because his judgment is based on sensory stimuli in sensory organs [18]. Organoleptic test was performed to assess how much consumers preference against products base on the parameters of color, flavor, aroma, texture and general preference. If one of these parameters was rejected, then the consumer will not accept the food product [15]. Hedonic test is one method of organoleptic tests to determine the levels of consumers preference [14]. To determine the panelists preference of KRPL's food product, the organoleptic test was conducted by the method of "hedonic test" base on parameter of color, aroma, texture, taste and preference level in general.

Tabel 2. Organoleptic Test of KRPL's Product

Treatments	Color	Taste	Texture	Aroma	General Preference
Brownies from Purple Sweet Potato	3.889^{a}	3.741^{a}	4.000^{a}	3.778^{a}	3.778^{ab}
Cassava Roll Cake	3.333^{b}	3.148^{b}	3.000^{c}	3.000^{c}	2.741^{c}
Mustard Greens Marble Cake	3.926a	3.852^{a}	3.407^{b}	3.370^{bc}	3.444^{b}
Tomatoes Cookies	3.852^{a}	3.333^{b}	3.407^{b}	3.593^{ab}	3.593^{b}
Corn Crackers	4.148^{a}	3.852^{a}	3.667^{ab}	3.815^{a}	3.963^{a}

Note : Assessment Criteria
1) very dislike;
2) dislike;
3) enough;
4) like;
5) very like;

Based on organoleptic tests, a variety of products that were introduced give significant effect on the preference of color, taste, aroma, texture and general level of preference.

1. Color

The appeal of a particular food is influenced by color, as the integral part of product quality [11]. Colour is the most determining factor of a food product appealing [22]. Color is a property of materials that ascribed from the spread of ray spectrum, besides the color is not a substance or object but rather a sensation of a person due to the stimulation of a radiant energy that falls to the senses of the eye or retina [8].

Various types of products which introduced to farmers give significant effect on the color preferences. A value of the color preference was on the range from 3.333-4.148, which means "like" (Table 2). In general, all panelists liked the color of products were tested. A high value of color preference was on corn cracker, while the lowest value of color preferences contained in Cassava Roll Cake. Corn crackers produced on a golden yellow color that is most preferred by panelists than tawny color in Cassava Roll Cake. This is due to Cassava Roll Cake use 100% of tawny cassava flour. The tawny Cassava Roll Cake occurs because of browning reaction (nonenzimatis reaction) when cassava is processed into flour [19] thus affecting the color of the Cassava Roll Cake.

2. Taste

Taste parameter is different with the aroma and involve the senses of taste. Taste is influenced by several factors, including the chemical compound, temperature, concentration and interaction with other flavor components [23]. Taste arising from the chemical stimulation of the senses that can be accepted by the taster or tongue [15].

Various types of products that introduced give significant effect on the taste preferences. Taste preference value was on the range 3.148-3.852, which means "enough to like". A lowest value of the taste preference was on Cassava Roll Cake (Table 2), a highest value of the taste preference was on Mustard Greens Marble Cake and Corn Crackers. Cassava Roll Cake has the lowest value of taste preference because it is using 100% cassava flour as raw materials. According by panelist Cassava Roll Cake has a strongly cassava felt on the tongue.

Texture

Texture is a group of physical properties caused by the structural elements of food that can be perceived by the senses of touch, is associated with deformation, disintegration and flow of foodstuffs under pressure as measured objectively by a function of massa, time and distance. Texture of the food can be defined as how the various components and structural elements arranged and combined into micro and macrostructur and structure statements out of this in terms of flow and deformation [7]. Texture is also a sensation of pressure that can be observed with the mouth (when bitten, chewed and swallowed) or touching with a finger [8].

Various types of products that introduced give significant effect on texture preferences. A texture preference value was on the range 3.000 – 4.000 (which means "enough to like"). Brownies have a highest value of texture preference, which means that most prefered by panelists (Table 2).

The raw material of brownies using a purple sweet potatoes pasta to substitute wheat (Table 1). Brownies have soft texture and prefered by panelist. [2] states that sponge cake can be substituted with non wheat flour (purple sweet potato) up 100% or less, because it is not require development volume as in donut. In this term still accepted by panelist base on color, taste, aroma and texture parameters.

A lowest value of texture preference was on Cassava Roll Cake. Panelist do not like Cassava Roll Cake' texture because the texture is very hard. The main ingredient of Cassava Roll Cake using 100% cassava flour so that its have hard texture. According with statement [20], cassava flour 's characteristics does not contain gluten so it is not easy to inflate, thus it has a hard texture than the characteristics of product that use a wheat and containing a gluten so it is more easy to inflate and make crumbs on products.

In addition a hard texture is also due to the fiber content that contained in the raw material. The higher of fiber content can increase the hardness of the product [5] and decreased elasticity [5]. Cassava flour which used as a raw material in Cassava Roll Cake contains higher fiber than wheat flour so that Cassava Roll Cake texture is harder. The fiber content of cassava flour at 3.34% [23] whereas the fiber content of wheat at 0.4-0.5% [3 in 17].

Brownies have a highest texture preference value (4.000) that do not significantly effect with the corn crackers texture preference (3.667) (Table 2). According to the panelists, corn crackers has very crispy texture so that preferred by panelists. The more volume crackers development, the more crispy crackers texture. Volume development of corn crackers is due to the content of amylopectin derived from tapioca starch. According Zulviani 1992 [1] crackers with the high content of amylopectin will have a highest crackers development volume because during the heating process it will occurs gelatinization and form an elastic structure which can be inflated at frying step. Crackers with a high development volume, the more crispy crackers texture.

Aroma

Aroma is the flavor caused by chemical stimulation which is wafted by the olfactory nerves are located in the nasal cavity when the food into the mouth [23]. Aroma is one important factor in by consumers. Aroma can be recognized when the form of steam, the smell is generally accepted by the nose and the brain is a lot more variety or a mixture of the four main ingredients are fragrant, sour, rancid and charred [23].

Different types of products that introduced to farmers give significant effect on the value of aroma preference. A value of aroma preference was on the range between 3.000-3.815 which means all of panelist "like" an aroma of product that introduced. A lowest aroma value contained in Cassava Roll Cake at 3.000 which means "enough" determining the quality of foodstuffs. In the food industry, aroma analysis is considered important because it can quickly give an assessment of products, whether or not the product is preferred (Table 2). A highest value of aroma preference found in corn crackers because of the distinctive aroma of corns are very strong and most prefered by panelists. According by panelist, Cassava Roll Cake have a typical cassava flavored. This is due to Cassava Roll Cake use a 100% cassava flour as raw materials. A typical cassava flavored give an effect to Cassava Roll Cake. The distinctive aroma in cassava flour is derived from degraded starch content. According [16] aroma and flavor formation caused by degraded carbohydrate content in tuber.

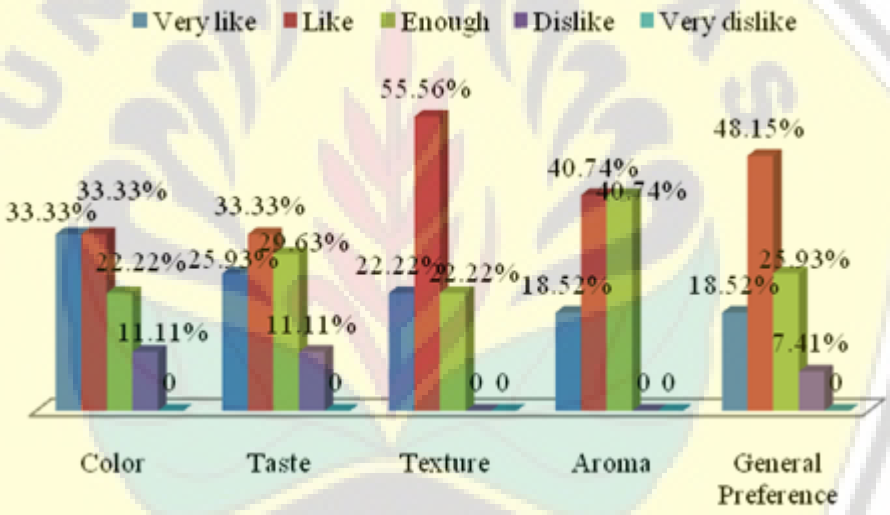

Figure 1. Percentage of Panelists Response on the Brownies

3. General Preference

At general preference, panelists analyze the Figure 1 shows the percentage of panelists response level of the purple sweet potato brownies. In color parameters, a number of panelist (66%) who expressed "like to very like", while only 11.11% panelists who expressed "dislike" and 0% panelist who expressed "very dislike" to brownies color. A number of panelists (59.26%) who expressed "like to very like" to brownies taste. While only 11.11% panelists who expressed "dislike" and 0% panelists who expressed "very dislike" for the taste of brownies. On texture parameters, (77.78%) of panelists expressed "like to very like" and 0% product based on the overall parameter, such as color, texture, taste and aroma. Different types of products that introduced to farmers give significant effect on the general preference. A value of general preference was on the range between 2.741 – 3.963, which means "enough to like" (Table 2). A lowest value of general preference (2.741) found on Cassava Roll Cake, because in terms of color, taste, texture, aroma and general preference, occupies the lowest value preference (Table 2).

The most preferred product which the highest value of a general preference was on corn crackers. Corn crackers have a high value of color, taste and aroma. On texture parameters, the highest value of texture preference was on brownies. However, this value did not give significantly effect to corn crackers texture value. So in general, the most preferred product is corn crackers.

Acceptance panelists response

1. Brownies from Purple Sweet Potato

Panelist response to the purple sweet potato brownies based on the parameters of color, taste, aroma, texture and general preference shown in the following picture of panelists who expressed "dislike" towards brownies texture.

In the aroma parameters, for 59.26% panelists expressed "like to very like" and 0% of panelists expressed "dislike" towards the aroma of brownies. Based on the general preferences, some of 66.67% panelists expressed "like to very like" and 7.41% panelists expressed "dislike".

2. Cassava Roll Cake

Panelist response to the Cassava Roll Cake based on the parameters of color, taste, aroma, texture and general preference shown in the following picture**.**

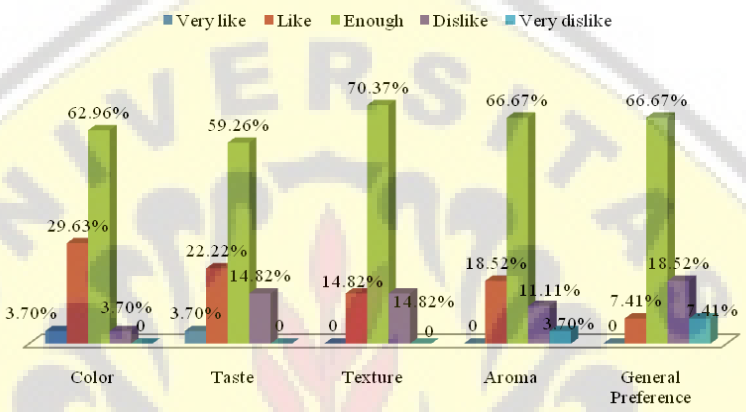

Figure 2. Percentage of Panelists Response on the Cassava Roll Cake

The panelists response to color of Cassava Roll Cake was shown by the number of panelists who expressed "like to very like" at 33.33% panelists, while a number of panelists (3.704%) who expressed "dislike" to the color of Cassava Roll Cake.

In the parameters of taste, some 25.92% of panelists expressed "like to very like" toward taste of Cassava Roll Cake; and amounted to 14.82% of panelists expressed "dislike" to the taste of cassava roll cake.

A number of panelists (14.82%) who expressed "like" to the texture of cassava roll cake. In aroma parameters, (18.52%) of panelists expressed "like" to roll cake aroma and a number of panelist (14.81%) expressed "dislike to very dislike" towards aroma cassava roll cake. Based on the general preference, a number of panelist (7.41%) expressed "like" to cassava roll cake and some 25.93% of panelists expressed "dislike to very dislike" to cassava roll cake.

2. Mustard Greens Marble Cake

Panelist response to the Mustard Greens Marble Cake based on the parameters of color, taste, aroma, texture and general preference shown in the following picture.

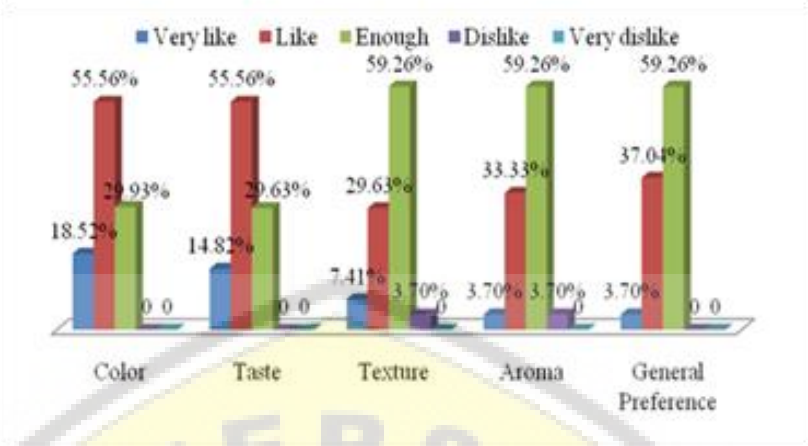

Figure 3. . Percentage of Panelists Response on the Mustard Greens Marble Cake

Panelist response to color of Mustard Greens Marble Cake was shown by the number of panelists who expressed "like to very like" for 74.08% of panelists, and no panelists expressed "dislike" for the color of Mustard Greens Marble Cake.

In the parameters of taste, some 70.38% of panelists expressed "like to very like" and no panelists who expressed "dislike" to the taste of mustard green marble cake.

Panelists who expressed "like to very like" to the texture of mustard greens marble cake at 37.04% panelists. A number of panelists (3.704%) expressed "dislike" to the texture of mustard green Panelists response to color tomatoes cookies was shown by the number of panelists who expressed "like to very like" for 70.38% of panelists, and no panelists who expressed "dislike" for the color of tomatoes cookies. In the parameters of taste, some 33.33% of panelists expressed "like to very like" and a 3.70% of panelists expressed "dislike" towards tomatoes cookies taste. Panelist response to the texture of tomatoes cookies, showed that some 37.04% of panelists expressed "like to very like" and a 3.70% of panelists marble cake.

In aroma parameters, a number of panelists (37.03%) expressed "like to very like" to aroma of mustard green marble cake; while a number of panelists (3.704%) expressed "dislike" towards aroma of mustard green marble cake. Based on the general preferences, some 40.74% of panelists expressed "like to very like" toward mustard green marble cake and no panelists expressed "dislike" towards mustard green marble cake.

3. Tomatoes Cookies

Panelist response to the tomatoes cookies based on the parameters of color, taste, aroma, texture and general preference shown in the following picture.

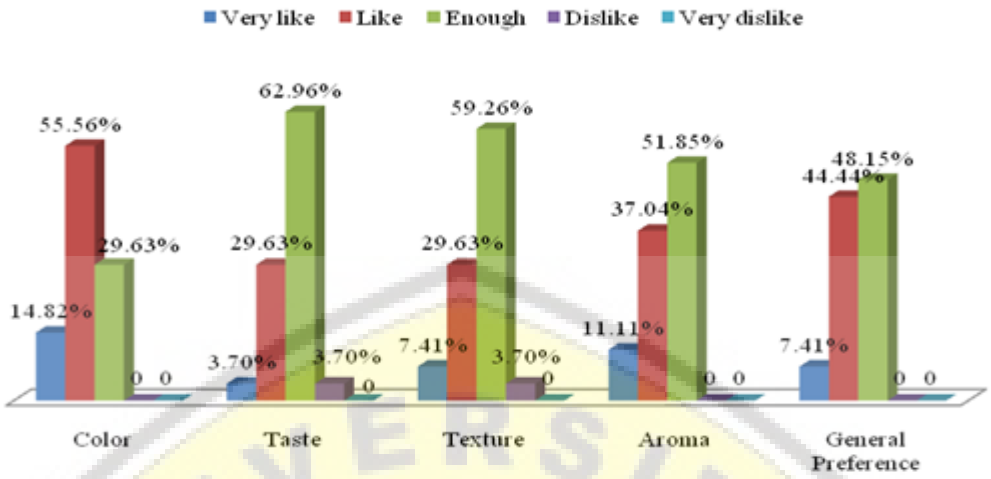

Figure 4. Percentage of Panelists Response on the Tomatoes Cookies

expressed "dislike" for the texture of tomatoes cookies.

At parameter aroma, some 48.15% of panelists expressed "like to very like" for the aroma of tomatoes cookies and no panelists expressed "dislike" to the aroma of tomatoes cookies. Based on the general preferences, some 51.85% of panelists expressed "like to very like" for the tomatoes cookies and no panelists who expressed "dislike" for tomatoes cookies

4. Corn Crackers

Panelist response to the corn crackers based on the parameters of color, taste, aroma, texture and general preference shown in the following picture.

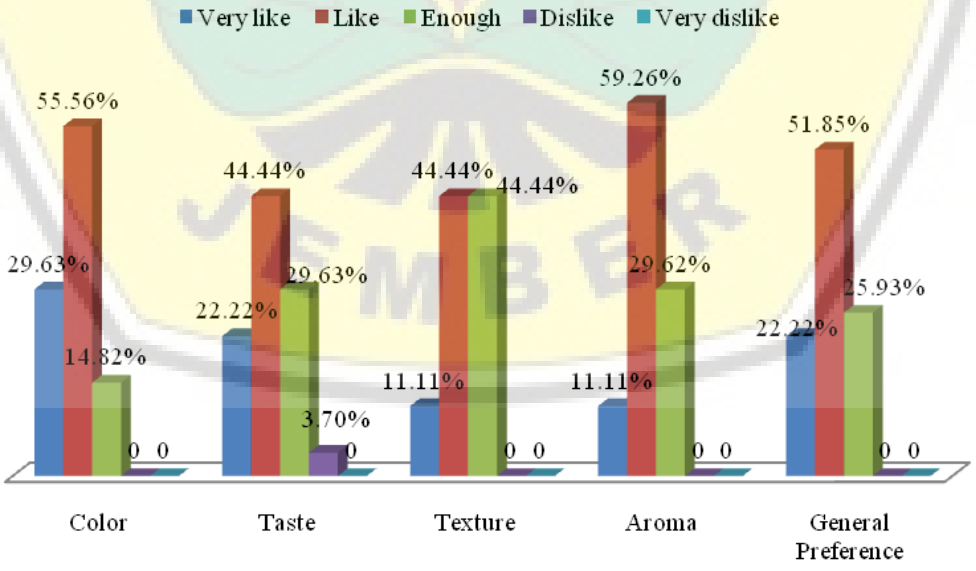

Figure 5. Percentage of Panelists Response on the Corn Crackers

The panelists response to color of Corn Crackers was shown by the number of panelists who expressed "like to very like" at 85.19% and no panelists who expressed "dislike" for the color of corn crackers. In the parameters of taste, some 66.66% of panelists expressed "like to very like" toward taste of corn crackers; and amounted to 3.70% of panelists expressed "dislike" to the taste of corn crackers. The panelists response to texture of Corn Crackers was shown by the number of panelists who expressed "like to very like" at 55.55% and no panelists who expressed "dislike" for the texture of corn crackers. The panelists response to aroma of Corn Crackers was shown by the number of panelists who expressed "like to very like" at 70.37% and no panelists who expressed "dislike" for the aroma of corn crackers. Base on General Preference, some 74.07% of panelists expressed "like to very like" for the Corn Crackers and no panelists who expressed "dislike" for corn crackers.

Conclusion

Commodities that potential to be developed in the yards with the concept of utilization of the yard (KRPL) and other programs toward agro-scale household is cassava, sweet potato, mustard greens, tomatoes and corn. While food processing technology that potential to be developed; 1) brownies from purple sweet potato, 2) cassava roll cake, 3) mustard greens marble cake, 4) tomatoes cookies and 5) corn crackers. The availability of agro-technology, easily carried by the user to increase the added value and supporting agro-industry as well as to support household food security program.

Various products which introduced to farmers give significant effect on the preference of color, aroma, texture, taste and general preference. According to the panelist response, preference value of brownies was on the range 3.741-4.000 (which means like). Preference value of roll cake was on the range 2.741-3.333 (which means enough). Marble cake mustard greens have a preference value was on the range between 3.370-3.926 (which means enough to like). Tomatoes cookies have a preference value between 3.333-3.852 (which means enough to like). Preference value of corn crackers was on the range between 3.667-4.148 (which means like). A high preference value base on parameter of color, flavor, aroma was on corn crackers products. Based on the general level of preference, corn crackers occupy the highest value of 3.963, which means like.

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EFFECT of LIQUID FERTILIZER SUPPLEMENT (PPC) and AN-ORGANIC FERTILIZER DOSAGE on GROWTH and YIELDS of CABBAGE (*Brassica oleraceae* L).

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Abstract

The research objective was to obtain the best dose of supplementary of liquid fertilizer and in-organic fertilizer in supporting the growth and yield of cabbage. Research was conducted in Junrejo village in Batu East Java, September till December 2014. The method used was a Randomized Block Design consisted of seven treatment doses of fertilizer and repeated four times. The treatment were P_0C_0 (30,000 compost+100 kg Urea+ 250 kg ZA + 350 kg SP-36 +200 kg KCl and 0 cc PPC), P_1C_1 (30,000 compost + 100 kg Urea + 250 kg ZA + 350 kg SP-36 + 200 kg KCl and 2 cc PPC), P_1C_2 (30,000 compost of + 100 kg Urea + 250 kg ZA + 350 kg SP-36 + 200 kg KCl and 4 cc PPC), P_1C_3 (30,000 compost + 100 kg Urea + 250 kg ZA + 350 kg SP -36 + 200 kg KCl and 6 cc PPC), P_2C_1 (30,000 compost + 50 kg Urea + 125 kg ZA + 175 kg SP-36 + 100 kg KCl and 2 cc PPC), P_2C_2 (30,000 compost + 50 kg Urea + 125 kg ZA + SP-36 175 kg + 100 kg KCl and 4 cc PPC), P_2C_3 (30,000 compost + 50 kg Urea + 125 kg + 175 kg ZA SP-36+ 100 kg KCl and 6 cc PPC). Variable observed were plant height, number of leaves, canopy diameter, the diameter of the heads and head weight per plant. The data were analyzed by F test and BNT ($\alpha = 5\%$). The results showed that the treatment of P_1C_2 (2 cc/liter PPC at 100 kg Urea + 250 kg + 350 kg ZA Sp-36 + 200 kg KCl / ha) and P_1C_3 (2 cc/liter PPC at 100 kg Urea + 250 kg + 350 kg ZA Sp-36 + 200 kg KCl / ha) have significant effect on head diameter and weight of the head. PPC additions on 2 cc/lt, 4 cc/lt and 6 cc / liter has not been able to reduce the use of in-organic fertilizer till half dose recommendation.

***Keywords**: PPC, inorganic fertilizers, doses o f fertilizer recommendation, cabbage*

Introduction

Plant growth is strongly influenced by the availability of nutrients, both micro and macro. Fertilization help provide nutrients needed by plants to obtain high yields. Fertilization can be done through the roots and the soil in the form of foliar fertilizer, fertilizer that applied through the leaves. The application provision of certain nutrients through the leaves more efficient than the through of land. According [4], there are some plant nutrients are absorbed perfectly by plants when sprayed through the leaves.

The application of complementary liquid fertilizer through the leaves is more effective, because it contains micro elements that quickly absorbed, so as to spur growth and improve the efficiency of metabolism in the leaves. Besides containing micro elements, complementary liquid fertilizer also contains microscopic substances (bio-activator) which supports biosynthesis in plant tissue and as biokatalisator which form the various compounds in the plant cells [1].

Some of the advantages of fertilization through the leaves of were the absorption of fertilizer nutrients given run faster than the fertilizer is given through the roots, avoid the occurrence of volatilization, and keeping the soil structure remains crumb / loose. Plants can experience micro-nutrient deficiencies if only rely on of the NPK fertilizer containing macro nutrient only. With fertilizer leaves the shortfall is expected to be resolved [3].

Kubis (*Brassica oleraceae* L.) merupakan salah satu sayuran yang mendapat prioritas utama dalam pengembangan agrobisnisnya. Pada umumnya, tanaman kubis diusahakan oleh petani di dataran tinggi (pegunungan) dengan ketinggian 1.000– 2.000 meter di atas permukaan laut. Pertumbuhan tanaman kubis memerlukan pupuk cukup banyak dengan unsur hara makro antara lain Nitrogen, Fosfor dan Kalium yang sangat berperan dalam peningkatan hasil dan kualitas kubis. Selain unsur hara makro, tanaman kubis juga memerlukan unsur hara mikro seperti Cu, Mo, Zn, B, Fe dan Mn yang pada umumnya berasal dari pupuk pelengkap cair [2].

Rekomendasi dosis pupuk untuk tanaman kubis dari Balai Penelitian Tanaman Sayur (Balitsa) Lembang adalah 30 ton pupuk kandang + 100 Kg Urea + 250 Kg ZA + 350 Kg SP-36 + 200 Kg KCl per hektar. Namun, petani cenderung memberikan pupuk tidak sesuai dengan anjuran yaitu hanya berdasarkan kemampuan petani. Sehingga perlu dilakukan upaya untuk lebih meningkatkan efisiensi dan efektivitas pemupukan yang umum

dilaksanakan dengan mencari input agroteknologi. Penelitian ini bertujuan untuk memperoleh dosis pupuk pelengkap cair dan dosis pupuk an-organik yang paling baik dalam mendukung pertumbuhan dan hasil tanaman kubis (*Brassica oleraceae* L).

Methode And Materials

The study was conducted in the village of Junrejo, Malang in the rainy season of 2014. The method used was Randomized Block Design consisted of seven treatments (Table 1), each treatment is a combination of doses of PPC and inorganic fertilizers, repeated four times. Data were analyzed by F test and LSD at 5%.

Treatments	Dose of inorganic fertilizer (kg/ha)				Dose PPC (cc /lt)
	Urea	ZA	SP-36	KCl	
P_0C_0 *) (Fertilizer recommendation)	100	250	350	200	0
P_1C_1 (Fertilizer recommendation + PPC 2 cc/lt)	100	250	350	200	2
P_1C_2 (Fertilizer recommendation + PPC 4 cc/lt)	100	250	350	200	4
P_1C_3 (Fertilizer recommendation + PPC 6 cc/lt)	100	250	350	200	6
P_2C_1 (½ Fertilizer recommendation + PPC 2 cc/lt)	50	125	175	100	2
P_2C_2 (½ Fertilizer recommendation + PPC 4 cc/lt	50	125	175	100	4
P_2C_3 (½ Fertilizer recommendation + 6 cc/lt)	50	125	175	100	6

Tabel 1. Perlakuan PPC pada kubis, 2014.

Description: *) dose of recommendation fertilizer the Vegetable Crops Research Institute in Lembang as control

PPC that used on this research was Sidagreen addition to containing some macro nutrients Nitrogen 18.34%; 4.22% P2O5; 3.04% K2O also contains micro elements B, Mn, and Zn (Table 2).

Data in each treatment were taken from an average of five plant samples. Variables measured observation of plant height, leaf number, and the width of the canopy made before the head was formed at the age of 30, 45 and 60 DAP. At harvest carried out observations of heavy head, head diameter. Cabbage is harvested after the age of 81-105 days with the characteristics of the harvest is when the outer edges of the leaves of the crop has been curved out, somewhat colored purple and head parts of the already congested.

Table 2. Results of the analysis of the content of the element in PPC Sidagreen.

Parameter	**Satuan**	**Result**	**SNI 2803: 2010**
Nitrogen (N)	%	18,34	SNI 2803: 2010
Total $P_2 0_5$	%	4,22	ICP-OES
K_2O	%	3,04	ICP
Boron (B)	ppm	30,20	ICP
Mangan (Mn)	ppm	67,50	ICP
Zink (Zn)	ppm	21,70	ICP
Cobalt (Co)	ppm	<0,10	ICP
Coper (Cu)	ppm	3,60	ICP
Molibdenum (Mo)	%	0,02	ICP
Plumbum (Pb)	ppm	21,70	ICP
Cadmium (Cd)	ppm	0,10	AAS Method 957.02 and 986.15*)
Arsen (As)	ppm	0,49	AAS Method 957.02 and 971.21*)
Mercuri (Hg)	ppm	0,01	

Source: Sucofindo, 2013; *) AOAC 18nEd 2005.

Implementation of the research:

1. Land plowed as deep as 20-30 cm and shaped beds with widths of 120 cm. Organic fertilizer is given before planting by broadcasting and mixed evenly over the beds. Grooves made between plots treated as a delimiter.

2. The spacing used was 60 x 50 cm. Seeds that have been aged 3-4 weeks and has 4-5 leaves grown by making the appropriate hole spacing has been determined. Planting is done by removing the seeds of polybags first.
3. Fertilizer given in the planting hole that has been given manure, then covered again with soil. Supplementary fertilization at the age of 25 DAP.
4. Watering is done every day in the morning and the afternoon or in accordance with the conditions of the plant. Weeding, tilling and soil Pembumbunan done at ages 2 and 4 Weeks After Planting (WAP).
5. Application of PPC at the time the plant was 20.30, and 40 days after planting with a dose according to the treatments.

Results and Discussion

This research showed that the given fertilizer treatment did not significantly affect plant height at 30 DAP (Days After Planting), 45 DAP and 60 DAP. At the age of 30 DAP plant height range between 16.60 to 19.67 cm and an average of 18 , 08 cm. At the age of 45 DAP plant height range between 24.47 to 18.87 cm with an average of 22.93 cm. Plant height at 60 DAP the range of 21.20 to 25.33 cm with an average of 24.02 cm (Table 3).

Table 3.Plant height of cabbage at 30 DAP, 45 DAP and 60 DAP by fertilizer recommendation and PPC treatments, 2014.

Treat-ments	Plant height (cm)		
	30 DAP	45 DAP	60 DAP
P_0C_0	18,13 ab	22,00 ab	24,00 ab
P_1C_1	19,67 a	24,47 a	25,33 a
P_1C_2	18,80 ab	23,07 a	24,73 a
P_1C_3	17,53 ab	22,33 a	24,40 a
P_2C_1	16,60 b	18,87 b	21,20 b
P_2C_2	17,93 ab	22,80 a	24.33 a
P_2C_3	17,87 ab	22,93 a	24,13 a
Average	*18,08*	*22,35*	*24,02*
F-Test	*ns*	*ns*	*Ns*
LSD 5%	*2,370*	*3,222*	*2,865*

Descriptions : - ns = non significant at F-test
- the numbers followed by a different letter on the same line are not significant at LSD (α = 5%).

The addition of liquid fertilizer Sidagreen on cabbage was not significant in increased the number of leaves at 30, 45, and 60 days after planting. Each of the average number of leaves strands were 12.11, 15.80 and 16.49 (Table 4). Wide canopy of cabbage was also not affected by fertilizer treatment at the 30 DAP, 45 DAP and 60 DAP. Wide canopy at of 30 DAP was 28.00 to 33.73 cm with an average of 30.56 cm. At 45 DAP, canopy width range is 30.20 to 36.87 with an average of 34.29 cm. Whereas at 60 dap, the width of the canopy has an average of 37.51 cm with a range between 33.80 to 39.33 cm (Table 5).

Table 4. The Number of leaves of cabbage at 30 DAP, 45 DAP and 60 DAP by fertilizer recommendation and PPC treatments, 2014.

Treat-ments	Number of leaves		
	30 DAP	45 DAP	60 DAP
P_0C_0	12,53 a	15,33 a	15,73 a
P_1C_1	12,93 a	15,80 a	16,60 a
P_1C_2	12,53 a	16,40 a	16,93 a
P_1C_3	11,60 a	15,93 a	16,73 a
P_2C_1	11,20 a	15,87 a	16,60 a
P_2C_2	12,33 a	15,40 a	16,40 a
P_2C_3	11,67 a	15,87 a	16,40 a
Average	*12,11*	*15,80*	*16,49*
F-Test	*ns*	*ns*	*ns*
LSD 5%	*1,990*	*1,580*	*1,752*

Descriptions : - ns = non significant at F-test
- the numbers followed by a different letter on the same line are not significant at LSD (α = 5%).

Tabel 5. The Wide of canopy of cabbage at 30 DAP, 45 DAP and 60 DAP by fertilizer recommendation and PPC treatments, 2014.

Treat-ments	Wide of canopy (cm)		
	30 DAP	45 DAP	60 DAP
P_0C_0	30,53 abc	34,47 ab	36,80 ab
P_1C_1	33,73 a	36,47 a	39,33 a
P_1C_2	32,80 ab	36,87 a	39,00 a
P_1C_3	30,00 abc	33,93 ab	38,13 ab
P_2C_1	28,00 c	30,20 b	33,80 b
P_2C_2	28 ,73 bc	33,73 ab	38,07 ab
P_2C_3	30,13 abc	34,33 ab	37,40 ab
Average	*30,56*	*34,29*	*37,51*
F-Test	*ns*	*ns*	*ns*
LSD 5%	*4,450*	*4,444*	*4,826*

Descriptions : - ns = non significant at F-test
- the numbers followed by a different letter on the same line are not significant at LSD (α = 5%).

Liquid fertilizer Sidagreen application was significant effect on the weight and diameter of the head of cabbage (Table 6). The highest weight of cabbage was in the fertilizer dose recommendation

+ Sidagreen 4 cc/lt and were not different on LSD 5% from the weight of cabbage in dose recommendation + sidagreen 6 cc/lt, reached 1.44 kg of weight. The lowest weights of cabbage at ½ Dose recommendations + sidagreen 2 cc/lt (reached 0.92 kg) and it was not different with ½ dose recommendations + sidagreen 4 cc/slt (reached 0.93 kg).

Treat-ments	Weight of head (kg/plant)	Diameter of head (cm)
P_0C_0	1,07 bc	15,40 abc
P_1C_1	1,31 ab	17,96 a
P_1C_2	1,44 a	17,63 ab
P_1C_3	1,44 a	15,01 abc
P_2C_1	0,92 c	13,44 c
P_2C_2	0,93 c	13,95 c
P_2C_3	1,02 bc	14,73 bc
Average	*1,16*	*15,45*
F-Test	*s*	*s*
LSD 5%	*0,344*	*2,965*

Table 6. Weight of head and Diameter of head of cabbage by fertilizer recommendation and PPC treatments, 2014.Descriptions : - s = significant at F-test

- the numbers followed by a different letter on the same line are not significant at LSD (α = 5%).

The highest diameter head of cabbage was at the dose treatment recommendation + Sidagreen 2 cc/lt is 17.96 cm. The lowest diameter of the head of cabbage was at dose of recommendation + ½ fertilizer treatment sidagreen 2 cc/lt (13.44 cm) and not different with ½ dose of ferilizer recommendations + sidagreen 4 cc/lt (13.95 cm).

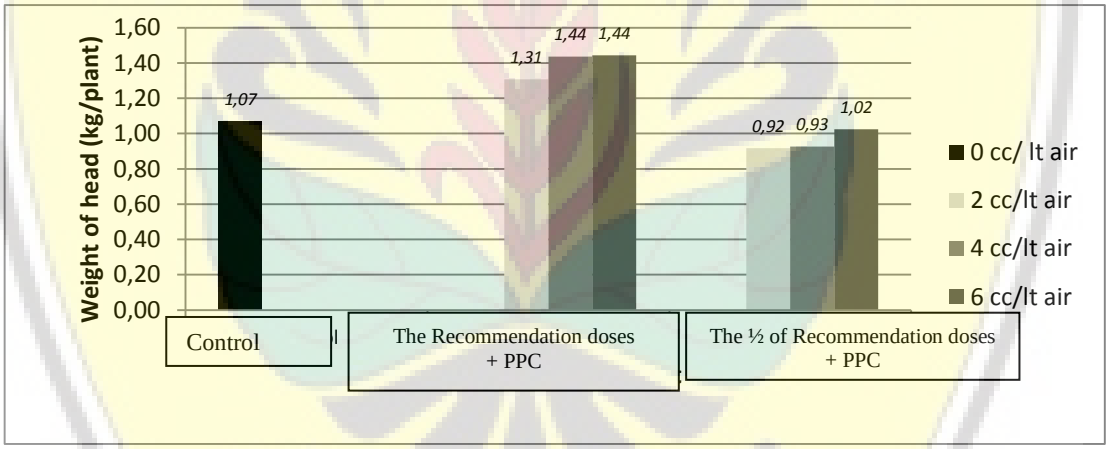

Figure 1. The weight of cabbage on control, on fertilizer dose recommendations and on ½ fertilizer dose recommendations, 2014.

Figure 1 confirms that the addition of PPC Sidagreen 2-4 cc / liter were able to increase the weight of the head from 0.24 to 0.37 kg that means it increase of 22.4 to 34.6% compared to only use doses fertilizer recommendation, without PPC. Giving a 1/2 dose of fertilizer recommendations plus PPC Sidagreen till 6 cc per liter, has not been able to offset the weight of head on the fertilizer recommendation (without the addition of PPC).

Results of correlation analysis showed that there were several variables that have a real correlation, very real and unreal (Table 7). Results of correlation analysis showed that the weight of the head of cabbage was affected by the diameter of the head (DMTR). Its mean that the increasing the diameter of the head will increase the weight of the head of cabbage. The diameter of head were affected by the width of the head. Further, canopy width were affected by the number of leaves, especially at the 45 to 60 days after planting.

Figure 2. The correlation analysis variable of the head weight (bobot), head diameter (DMTR), plant height

	bobot	DMTR	tgg1	tgg2	tgg3	kanopi1	kanopi2	kanopi3	daun1	daun2	daun3
bobot	1	0.6045**	0.13	0.19	0.31	0.21	0.20	0.16	0.03	0.42	0.24
DMTR			0.23	0.40	0.49	0.48108*	0.47613*	0.36	0.05	0.25	0.03
tgg1			1	0.67608**	0.50522*	0.51388*	.64699**	0.653**	0.53325*	0.23	0.34
tgg2				1	0.924**	.66807**	.79156**	.75985**	0.46694*	0.29	0.28
tgg3					1	0.54756*	.66345**	.63872**	0.34	0.31	0.22
kanopi1						1	.88774**	.69954**	0.41	0.32	0.27
kanopi2							1	.90811**	0.49759*	0.38	0.42
kanopi3								1	0.49387*	0.4586*	.57252**
daun1									1	0.09	0.38
daun2										1	.83147**
daun3											1

Descriptions : * = significant on correlation analysis
** = very significant on correlation analysis

(tgg), the width of the canopy (kanopi) and the number of leaves (daun), 2014.

Conclusion

Application of Supplementary Liquid Fertilizer Sidagreen was significantl increasing the diameter and weight of cabbage head compared to the dosage of fertilizer recommendation application only. However, the application of complementary liquid fertilizer Sidagreen have not been able to reduce the use of inorganic fertilizers. The application of ½ dose inorganic fertilizer recommendations plus Sidagreen 2 cc / lt and 4 cc / lt producing head and crop diameter lower than head and crop diameter in dosage recommendations + Sidagreen 2 cc / lt, 4 cc / lt, and 6 cc / lt. For more deep information, this research can tested in different seasons so the response to the PPC Sidagreen cabbage plants can be seen more clearly

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HYDROLYSIS PROFILE of OIL PALM EMPTY FRUIT BUNCH by AN EXTRACELLULAR ENZYME from *Aspergillus niger*

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Abstract

Large quantities of oil palm empty fruit bunch (OPEFB) biomass with low economic value were released during oil palm production. This biomass components are cellulose (41-46%), hemicellulose (25-34%), lignin (27–32%), and C/N ratio 70-100 so that in nature OPEFB decomposition takes much time. To overcome this problem microbial utilization is needed. In this research, an extracellular enzyme from *Aspergillus niger* was used to hydrolyze OPEFB and gave the rate hydrolysis at 1.5% (756ug/ml) for 6 hours respectively. It was estimated by Gas Chromatograph (GC) analysis as alditol acetate proved that the main sugar in hydrolysate as monosaccharide was glucose with the concentration 74.2%. Suggested, much cellulase was released by *A. niger* during solid stated fermentation.

Keywords: *Aspergillus niger*, extracellular enzymes, hydrolysis and OPEFB.

Introduction

Large quantities cellulose and lignocellulose which occurs as micro fibrils embedded in cell wall are most important component of plant biomass. However, during processing, generally all of these materials cannot be processed, and a significant amount remains as organic wastes (1,8). For example, the processing of OPEFB one of the important agriculture industry in Indonesia, which discard a huge amount of secondary organic products of material as OPEFB.

The degradation of lignocellulose to other forms of derivate polysaccharides can be efficiently done with appropriate hydrolyzing enzymes from fungi or bacteria (2,3), which is considered effective methods to manage the organic waste and other forms of pollution (1,9). By degradation followed by biosynthesis, the organic waste material can be transformed to other useful products which may decreased the process cost and solved some of in the environment problems (4,5). In nature, the genus of *Aspergillus* secreted wide range of enzymes and readily degraded a wide variety of polymers, such as cellulose (5), hemicellulose, pectin and lignin (4,6,7,8), and also storage compounds like starch (XX), sucrose and inulin (6,7,8). This capability is due to secrete on of a broad range of enzymes by different member of this genus that can degrade these complex polymers (7,9).

In this research, microbial utilization of OPEFB by introducing *Aspergillus niger* in solid state fermentation, which may degrade cellulose and lignocellulose was reported.

Methode And Materials

a. Crude enzyme production

Aspergillus niger was used for producing crude extracellular enzyme by inoculating to 100 g of sterilized OPEFB in a 5 liter Erlenmeyer flask and at 30^{o}C incubation. After 6 days, the culture was stopped by adding 200 ml water containing 1% NaCl and 0.1% toluene (v/v), followed by shaking at room temperature for 10 hours. The suspension were filtrated using filter paper and centrifuged to recover the supernatant as a crude extracellular enzyme. From this step, 180ml of filtrate as crude enzyme was collected. Then the crude enzyme were re-filtrated at 20 micron and concentrated to about one-tenth of the initial volume by dialysis using hollow fiber 10kD, eluted with acetate buffer 20mM at pH 5. The precipitate was dissolved and dialyzed against water for 3 days. This solution was stored at 4^{o}C till used for OPEFB hydrolysis.

b. Degree of hydrolysis and total sugar content analysis

The degree of hydrolysis was examined by incubating the reaction mixture of concentrated crude enzyme and OPEFB substrate at 37^{o}C. The OPEFB-hydrolyzate was obtained by centrifugation and the release of reducing sugars measured by the method of Nelson (10) as modified by Somogyi (11) using glucose as a standard sugar for calibration. The degree of hydrolysis of okara was calculated as follow:

where:

DH : Degree of hydrolysis (%)

TRSH : Total reducing sugar in hydrolyzate (w/v)

$$DH(\%) = \frac{TRSH}{TS} * 100\%$$

TS : Total substrate (w/v)

The total sugar of OPEFB-hydrolyzate was also measured by the phenol-sulphuric acid method (12).

c. Analysis of sugar composition of OPEFB

Gas chromatograph (G-3000, Hitachi, Tokyo, Japan) was used to analysis sugar composition of OPEFB-hydrolyzate as alditol acetates (13,14) with a few modifications. A sample of 20 mg of OPEFB was fully hydrolyzed with 2 ml of 2 N HCl for 6 hours at 100ºC. The hydrolyzate was filtered, evaporated to dryness. One mg of 2-deoxy-D-glucose was added as an internal standard. The mixture was then reduced with 2 ml of 0.2 M $NaBH_4$ at room temperature, overnight. Five to six drops slurry of dowex resin H type 100-200 mesh (Bio-Rad Laboratories, CA) were then added to the mixture and incubated at room temperature for 1 h, followed by filtration. The filtrate was evaporated to dryness and remaining boric acid residue was removed by repeated evaporation using methanol. The sugar alcohols obtained were acetylated in 2 ml of acetic anhydride:pyridine (1:1) at 100ºC for 10 min. The mixture was then diluted with chloroform:water (1:4), shaken and the upper layer removed by centrifugation at 2000 rpm for 10 minutes. Remaining pyridine was removed from the chloroform extract by washing with water, followed by centrifugation. The resulting alditol acetates were dried and dissolved using chloroform. Gas chromatograph (GC) analysis was performed on a stainless steel column, 2 mm I.D. x 1.83 m, packed with 3% (w/w) ECNSS-M on Gas Chrom Q 100-120 Mesh (GL Sciences, Japan). Nitrogen gas flowing at 30 ml min^{-1} was used as carrier gas with the initial column temperature of 190ºC for 5 minutes and rapidly increased to 210ºC at a rate of 1ºC/min.

Results and Discussion

The hydrolyzing experiment using crude enzyme with 10% substrate of OPEFB unbuffered condition resulted in 1.5% degree of hydrolysis after 6 hours incubation at 37°C. The degree of hydrolysis was increased significantly, and nearly 45% degree of hydrolysis was after 36 hours if the mixture condition was added with 50 mM acetate buffer pH 5 and incubated at the same temperature.

It was reported in previous research (15) that the time delay in degree of hydrolysis was due to the enzyme tend to function at low pH condition with the optimum pH around 4, while the stability in a pH range of 2.5–7.5 (6,8). Thereafter 72 hours, the hydrolysis is still in progress and could presumably attained the same hydrolysis at very much later time. Whereas in control the hydrolysis is almost complete by 72 hours with the same substrate. Furthermore, in previous result (17), we found that the □-L-AFS and E-□GAL purified enzymes also tend to function at low pH condition and exhibited maximum activity at pH 4.5 and 3.6 and retained nearly 100% activity in a pH range of 2-7 and 3-6 respectively.

Analysis of reducing-sugars and total-sugar showed that OPEFB-hydrolyzate was rich in monosaccharides but poor in oligosaccharides, which constituted less than 0.5% of the total sugar content. By the TLC analysis, it was found that the major hydrolysis products of dried-OPEFB at the spots, are monosaccharides, while oligosaccharides were not detected (Fig.1). However, except for glucose spot, the presence of other monosaccharides could not be clearly identified.

A B C

Figure 1. Result of thin layer chromatography (TLC) analysis of OPEFB-hydrolyzates. Ten percent dried-OPEFB with no buffer condition was digested by crude-enzyme 42 hours at 37ºC. OPEFB-hydrolyzate with no enzyme as control (A), product (B) and glucose standards (C) was analyzed by TLC.

To quantification of sugars component of OPEFB-hydrolyzate, GC analysis was used. GC analysis revealed that OPEFB-hydrolyzate consists of four sugars component (Fig. 2) with the sugar constituents are glucose (74.2%), xylose (21.1%), arabinose (3.6%) and mannose (1.1%).

From the hydrolysis process results show that during solid state fermentation process, *A. niger* produced more than one enzyme. Furthermore, hydrolysis showed that the cellulase enzyme is very active as indicated by the high concentration of glucose produced as monosaccharides. Some researchs reported that *A. niger* can produced wide range of enzymes including cellulase (4,7).

Enzyme production by utilizing of OPEFB through solid-state fermentation was also investigated and found to be promising in bioconversion of biomass. Also reported that highest production of cellulase was noted at acid condition (pH 4-6) and 35 - 37 degrees C, under submerged conditions (1). Growth and enzyme production was affected by variations in temperature and pH (7,8)

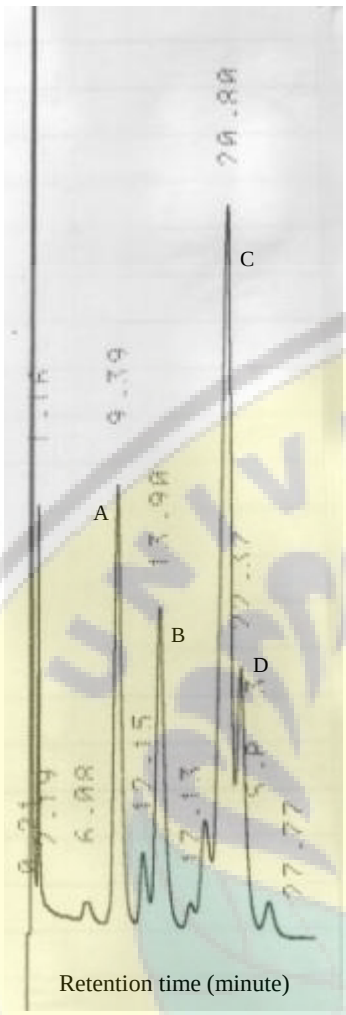

Figure 2. Chromatogram of sugar components in of OPEFB hydrolyzates which detected by Gas Chromatograph (GC). Four sugars were detected as xylose (A), arabinose (B), glucose (C) and mannose (D).

Conclusion

An extracellular enzyme from *A. niger* was used to hydrolyze OPEFB and gave the rate hydrolysis at 1.5% (756ug/ml) for 6 hours respectively. The main sugar in hydrolysate as monosaccharide was glucose with the concentration 74.2%, respectively. Suggested, much cellulase was released by *A. niger* during solid stated fermentation.

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INVESTIGATING CRYOTHERAPY TECHNIQUES to ELIMINATE VIRUS on POTATO SHOOT TIPS

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Abstract

Virus-free, early generation seed is the key in the production of high quality potatoes. Recent methods of virus elimination involve cryotherapy. This study investigated the effectiveness of different cryotherapy procedures evaluated by explant survival rate. Five genotypes were exposed to three cryotherapy methods: 1) encapsulation-dehydration, 2) encapsulation-vitrification, and 3) droplet-vitrification. The first method included pre-culturing shoot tips (1-2 mm) in sucrose media and formation of beads, followed by dehydration for 5 hours in a laminar hood and freezing in liquid nitrogen for 60 minutes. The second method differed by immersion of the beads in a vitrification solution prior to freezing in liquid nitrogen. In the third method, shoot tips were pre-cultured for three days and placed in 5°C to harden the shoot tips, followed by incubation in vitrification solution, then frozen in liquid nitrogen. Following treatment, shoot tips were cultured in standard MS media for recovery, supplemented with 0.5 mg/L IAA, 0.5 mg/L Zeatin riboside, and 0.2 mg/L Giberellic acid and incubated in the dark at 22°C for three days. Shoot tip survival was assessed at four and eight weeks using the following scoring criteria: 1- tissue bleaching and no growth response, 2- brown callus, 3- green callus, 4- shoot growth, and 5- plantlet establishment. Untreated control shoots displayed normal growth (score ≥4). Low survival rate was found on all procedures.

Keywords: cryopreservation, Solanum tuberosum, encapsulation, vitrification, liquid nitrogen

Introduction

Potato (*Solanum tuberosum* L.) is the fourth largest food crop around the world after rice, wheat and corn. Since potatoes are propagated vegetatively, it become easily infected with viral disease and passed from generation to generation. Most common viruses found in seed potato are Potato Virus X (PVX), Potato Virus Y (PVY), and Potato Virus S (PVS). This impacted on reduced potato production from 10% – 80%; and virus elimination process, maintenance and renewal of virus-free stock plants are costly (Zapata *et al.*, 1995; Jianming *et al.*, 2012).

Potato production in Indonesia also experiences a severe decrease due two viral diseases, causing high import both on seed and consumed potatoes. Total potato import during January – June 2013 was 22 thousand ton, value of about $ 14.3 million (BPS, 2013).

Producing and maintaining a virus free plant material *in vitro* is the key for viral control in seed potato production system. A number of techniques have been reported including meristem culture, thermotherapy, thermotherapy follows by meristem culture, and chemotherapy (Zapata *et al*., 1995; Wang *et al*., 2014).

Application of meristem culture for virus eradication resulted in low percentage of virus free plants (Meylbodi *et al*. 2011). On heat therapy method, *in vitro* potato shoot tips were incubated for 6 weeks followed by ELISA test to confirm virus free plants after heat therapy treatment. If virus was still detected, shoot tips will be incubated another 6 weeks and this procedure will be repeated until virus free plants were obtained. This method is time consuming and slow down the process to provide good quality seed potatoes. Virus free plants obtained with this technique were 10%, as reported by Zapata *et al*. (1995).

Cryotherapy is a recent method in virus elimination. This technique involve dipping plant materials, particularly shoot tips, into ultra-low temperature (-196°C) on liquid nitrogen for about one hour. This technique is believed to be able to shorten the time in providing virus free plants (Feng *et al*., 2013, Wang *et al*., 2012).

The main bottleneck in cryotherapy technique is in obtaining survival shoot tips after treatment. According to Wang *et al*. (2006), successful rate is depended upon a number of factors such as genotype and pre-treatment of *in vitro* shoot tips before immersion in liquid nitrogen.

Application of cryotherapy technique has been reported on a number of horticultural crop with varying technique and survival rate of the shoot tips after immersion in liquid nitrogen. Survival rate was vary from 42.5% - 87.5% in Lilium sp. (Yin *et*

al., 2014), raspberry (Wang and Valkonen, 2009), garlic (Kim *et al*., 2007), sweet potato (Wang and Valkonen, 2008) and potato (Wang *et al*., 2014).

Jianming *et al*. (2012) reported survival rate and regeneration rate of potato shoot tips treated with cryopreservation technique were 83.64% and 72.04%, respectively, higher than apical meristem culture technique (46.67% and 31.49%), thermotherapy (72.22% and 44.44%) and thermotherapy combined with apical meristem culture (50.54% and 30.38%).

A number of cryotherapy was described by Wang *et al*. (2014), including encapsulation-dehydration, encapsulation-vitrification and droplet vitrification. This study investigated the three different method in cryotherapy to obtain surviving shoot tips on 4 genotypes of potato shoot tips *in vitro*.

Materials and Methods

2.1. Plant materials

This research was conducted at Horticulture Department, Texas A & M University, College Station, USA, from July to November 2014. Four virus positive potato genotypes from Texas A & M University and University of Idaho, USA, were used in this experiment, namely COTX08121-1Ru, ATX 07305s-1Y/Y, ATTX984 66-5R/WR, and POR0 PG56-1. These selections were chosen for different tuber classes, different type of virus infection and possible differences in response to treatments. Plantlets were maintained *in vitro*, at temperature 22^{o}C ± 1^{o}C under a 16-h photoperiod with light provided by cool-white fluorescent tubes.

2.2. Media preparation

A number of solutions were prepared: 1) one liter liquid MS medium containing MS salts (Murashige and Skoog, 1962) and 30g/L sucrose, pH 5.8; 2) 100 ml calcium chloride solution (contain 0.1 M calcium chloride, 2 M glycerol and 0.4 M sucrose in liquid MS, pH 5.8); 3) 100 ml sodium alginate solution (contains 0.4 M sucrose, 2 M glycerol and 2.5% (w/v) sodium alginate made up in liquid MS medium, pH 5.8); 4) 200 ml of 0.25 M sucrose pre-culture medium containing MS medium with 85.5 g/L sucrose, 2 g/L agar, 5) Recovery medium (contains MS + 30 g/L sucrose + 8 g/L agar + 0.4 mg/L BAP and 1 mg/L GA 3 , pH 5.8); 6) PVS3 (contains 50% (w/v) Glycerol and 50% (w/v) sucrose in MS), Recovery medium Z (contains MS media, with 0.5 mg/L IAA, 0.5 mg/L Zeatin riboside, and 0.2 mg/L Giberelic acid, pH 5.8).

2.3. Methods

Three cryotherapy protocols were investigated in this study: 1) Encapsulation-dehydration, 2) Encapsulation-vitrification, 3) droplet-vitrification. Encapsulation defined as artificial seed that acts as a barrier and protectant for the plant material. Dehydration means decreases water content within the cell to minimize cell damage. Vitrification defined as uses of sucrose (and other sugar based chemicals) to replace water within the cells upon freezing, where the cells enter a glass-likestate.

This experiment employed Randomized Completely Design with 2 factors, i.e. plant genotypes and cryotherapy treatment. Each treatment combination consisted of 8 shoot tips as replicates.

Cryotherapy method generally consists of 6 steps: Pre-culture, pre-treatment, cryoprotectant, dip in liquid nitrogen, warming and culture in recovery media. The three treatments employed in this experiment were differed in those steps, as shown on Table 1.

For treatment 1 and 2, steps in making bead (bead formation) are as follows: shoot tips of about 2 mm were placed on Sodium alginate solution. Pipette tips were cut 1.5 cm from the tips. Then use pipette to draw the Sodium alginate solution and shoot tips into the pipette tip. Drop the Sodium alginate that contained shoot tips into 15 – 20 ml $CaCl_2$ solution. Gently shake the $CaCl_2$ solution and let set for 20 minutes for beads to become firm. Place beads on sterile filter paper in petri dishes to blot dry before proceeding with next steps in protocols.

Treatment 3 did not need encapsulation for the shoot tips, but shoot tips were loaded in loading solution, followed by immersion in vitrification solution before being dipped in liquid nitrogen. On control treatment, liquid nitrogen and warming steps were omitted on the shoot tips.

Variable observed was survival of shoot tips, evaluated using performance of shoot tips 4 and 8 weeks after treatment. Observation was on the following scoring criteria: 1- tissue bleaching and no growth response, 2- brown callus, 3- green callus, 4- shoot growth, and 5- plantlet establishment.

Results and Discussion

Results shows that all treatments exhibit low survival rate regardless of genotypes, in which only swelling and green callus were observed on shoot tips (average score $\leq$ 3) (Figure 1a, b and c). Average score for control plants was also below 5 on all treatments, showing that although shoot tips was not dipped into liquid nitrogen, a number of steps before dipping into liquid nitrogen affecting regeneration ability of the control plants.

Some nodes in treatment three, i.e. droplet-vitrification, survived the liquid nitrogen treatment, COTX08121-1Ru and ATX07305s-iY/Y where 1 shoot each shows normal growth (score = 4, Figure 2). Survival from treatment three indicates that it can be altered to optimize plant recovery.

Droplet-vitrification technique was also been employed on cryopreservation of 12 potato genotypes. The survival rate was reported ranging from 64.0 to 94.4% (Yoon *et al*., 2007). High survival rate on reported study maybe due to difference in vitrification solution employed. Yoon *et al*. (2007) uses PVS2 while this experiment uses PVS3. PVS2 contains DMSO (Dymethyl Sulfoxide), which is known as a cryoprotectant agent, while on PVS3, DMSO was omitted from the vitrification solution. It is suggested that DMSO is the key chemical in the successful of vitrification, resulted in a relatively high rate of plantlet survival.

Encapsulation and vitrification are technique to protect *in vitro* shoot tips from damage due to ultra-low temperature, in which, with encapsulation or exposing to vitrification solution, crystalization in shoot tips will not formed (Kacmarcyzyk *et al*., 2011).

Low survival rate on all attempted treatments may also due to a number factor such as condition of donor plants and position of shoot tips on the explants. Schäfer-Menuhr *et al*. (1996) reported that aeration of culture vessels is necessary to achieve high survival of cryopreserved potato apical shoot tips. Yoon *et al*. (2007) also described that high light intensity, ventilation of culture vessel and low planting density were necessary conditions to obtain healthy *in vitro* plantlets and high recovery after cryopreservation.

Conclusion

It can be concluded that all genotype response differently on each cryotherapy treatment. Droplet–vitrification treatment tended to produce better survival on the shoot tips, and need to be investigated further to obtain higher survival rate.

Tabel 1. Different steps on each cryotherapy treatment.

Steps	**Treatment 1: Encapsulation-dehydration**	**Treatment 2: Encapsulation-vitrification**	**Treatment 3:droplet-vitrification**
Preculture	One day each on 0.25 M, 0.5 M and 0.75 M sucrose in MS and 8 g/L agar media, 23°C	One day in 0.3 M sucrose in MS + 8 g/L agar media, 23°C.	Shoot tips were precultured on MS + 8 g/L agar + 0.3 M sucrose, keep in the dark at 5◦C for 3 days.
Pretreatment	Bead formation using Sodium alginate and Calcium Chloride solution. Dehydrate beads for 5 hours	Bead formation using Sodium alginate and Calcium Chloride solution. Followed by immersion in loading solution containing 2 M glycerol and 0.6 M sucrose in MS for 90 min	Pre-cultured shoot tips were loaded for 30 min with a loading solution containing 2 M glycerol and 0.4 M sucrose in MS.
Cryoprotectant	None	Beads were dehydrated in PVS3: 50% (w/v) Glycerol and 50% (w/v) Sucrose in MS for 4 hours at 4°C.	Dehydrated with PVS3: 50% (w/v) Glycerol and 50% (w/v) Sucrose in MS, for 40 min at 4°C.
Liquid Nitrogen	Tubes set in Liquid Nitrogen for 60 minutes	Tubes set in Liquid Nitrogen for 60 minutes	Dehydrated shoot tips were transferred onto aluminum foils followed by a direct immersion in liquid nitrogen (LN) for 60 minutes.

Warming	Incubated in 38ºC water bath for 3 minutes	Incubated in 38ºC water bath for 3 minutes, followed by immersion in 1.2 M sucrose at 25◦C for 20 min.	Aluminium foil directly plunged in 38ºC water bath for 3 minutes, followed by immersion in 1.2 M sucrose at 25◦C for 20 min.
Recovery	Recovery medium: MS + 30 g/L sucrose + 8 g/L agar + 0.4 mg/L BAP and 1 mg/L GA 3 , pH 5.8.	MS media, with 0.5 mg/L IAA, 0.5 mg/L Zeatin riboside, and 0.2 mg/L Giberelic acid, pH 5.8	MS media, with 0.5 mg/L IAA, 0.5 mg/L Zeatin riboside, and 0.2 mg/L Giberrelic acid. pH 5.8

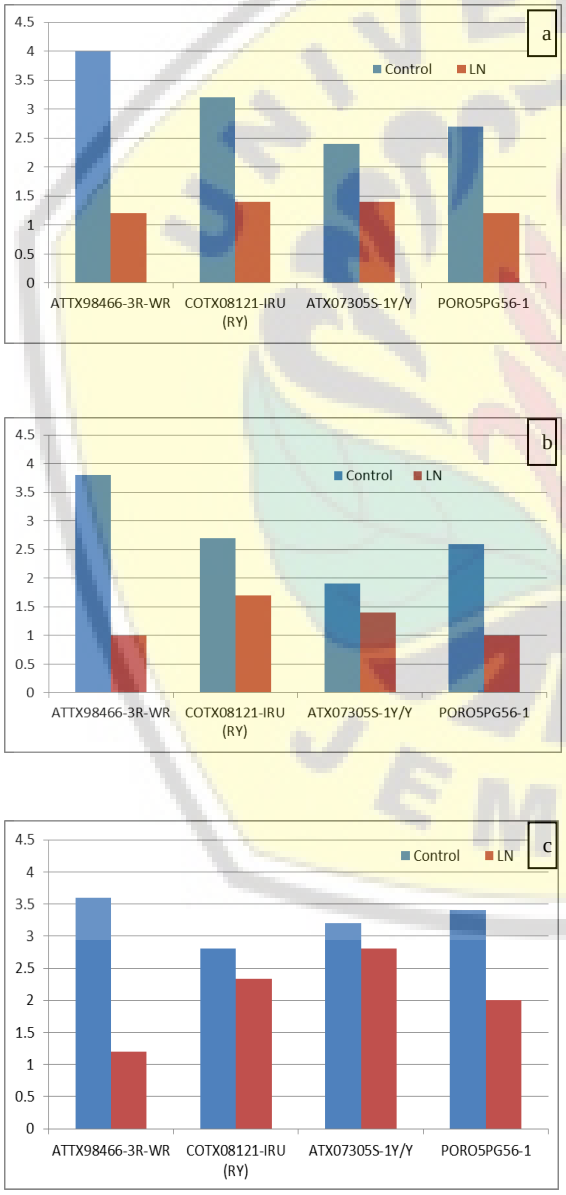

Figure 1. Survival rate of each genotype, 8 weeks after treatment. a. Encapsulation-dehydration, b. Encapsulation-vitrification, c. Droplet-vitrification

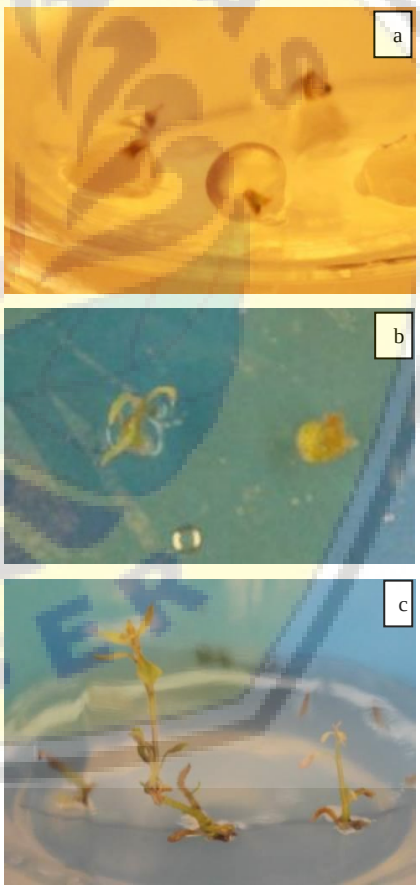

Figure 2. Survival performance of shoot tips after treatment. a. shoot emerge from encapsulation bead. b. Shoot growth after encapsulation – vitrification treatment. c. Shoot growth after droplet –vitrification treatment. d. Control plants.

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Poster Session

AGROFORESTRY of *Apis arana* HONEY BEES' FEED PLANTS in RIGHT FOREST AREA in PANEKAN SUB-DISTRICT, MAGETAN REGENCY

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Abstract

Apiculture that based on beekeeping by utilizing right forest and all agricultural crops around forest stand are policy of society around right forest in Panekansub-district Magetan regency. Society's local wisdom in managing and developing bee as insect that useful and helpful in life is an effort of increasing income through right forest with non-wood result; this situation has already grown for one decade especially in Panekan sub-district and generally in Indonesian society. The apiculture success is an important key in agroforestry management based on honey bee with weft availability by continuous system. Purpose of the research is to know region potential and vegetation variety for fulfillment of honey bee weft also chance to develop continuous apiculture in Panekansub- district by using belt transect method to know variety vegetation for honey bee weft's source. Inventory is done by random method in 20 m x 20 m plots for tree stand and 10 m x 10 m for shrub, measurement is done in area with agroforestry system treatment that can be found in right forest. Honey bees weft availability is calculated based on variety density; inventory is done in area of 10 villages in right forest area, in calliandra area as stand and coffee as shrub plant. Shrub plants' status in plot stand is counted and identified as the source of bees' feed or not. Gotten- Information is in the form of data and then analyzed quantitatively and descriptively. Having observed the Right Forest, the researchers get the result which shows that honey bees' feed resource is from 9 forest trees, 3 plants of Non-timber Forest (HBK), while there are known- versatile plants: 5 types of plants, 7 types of fruit trees and 2 types of shrub plants. Honey bees' feed in the area has different potency based on the density (individual/ha) as many as 258.70 individual/ha caliandra type as pollen producer and 1125.10 individual/ha coffee type as nectar producer. It is found that type of plants for honey bees' feed in Panekan sub-district is abundant and it is the area's potency to develop the cultivation of honey bees.
Keywords : society, honey bees, revenue, policy, wisdom.

Introduction

Forest is an area which has chances to exploit its potencies in beekeeping business. Forest and bees have important role in economics building strategies of rural society and sustainable agriculture sector until now [3]. The activities of right forest and state forest management by using agroforestry system for bees forage provision in order to produce high-quality food products so that it can help increasing the nutrient and rural society's income are very important to pay attention. Through pollination, honeybees also have important role in increasing fruit and seed production and keep the life sustainability and plant diversity [1].

Agroforestry system development by apiculture technique, the combination between forestry plants and beekeeping, is one of business activities which does not harm the land so that it cannot be a rival for agriculture business in general. Until now, beekeepers have a role in optimizing natural resource through nectar and pollen exploitation, two plant products which will be useless if we do not use it for honeybees forage. Doing so, beekeeping is one of activities which can give additional value to plants cultivation.

Bees forage provision from agriculture plants and trees are important in developing beekeeping system. It is important considering Indonesia has big potency in this field [5]. Agroforestry existence is the answer in beekeeping management in Indonesia, for example, the basis of bees forage resource availability throughout the year and honeybees diversity: besides, the society, traditionally, has known the beekeeping.

Magetan regency makes Penekan sub-district as one of honey producer base. That regency has potency, right forest and state forest, where there are a lot of interesting flora and fauna which live between large and various tropical trees. The exist forest potency is grown various types of woods which are very important for commerce such as calliandra,sengon, teak, durian, jackfruit, rambutan and etc. If this honeybees cultivation can be socialized, the government will indirectly be able to realize rural society's welfare especially society around the forest.

Right forest and state forest in Panekan sub-district is a mixture between forest and composing

vegetation diversity plants which can only be exploited by selective logging with low-intensity wood as the main result and non-timber wood result including honey, medicinal plants, grass, flowers, fruits, seed and insects. Vegetation diversity which is getting more various supports honeybees breeding development because biodiversity has important role as food supplier, bees. The influencing factors to support the developing forest honeybees breeding are climate which sustain bees forage throughout the year and condition of the forest/ ecosystem of an area. It is because the honeybees can only breed in the ecosystem which is not relegated. The needs of bees forage must be fulfilled all the time in order to preserve bees' life in which forest honeybees get the forage from nectar of various types of forest trees or the plants around the area. Nectar in all plants' flowers can be used as the main ingredient of honey maker while bees forage can be got from pollen and propolis. The effort to maintain the balance of the ecosystem is very important and need to be done in order to important to do research about categorizing and identifying of honeybees forage plants in Panekan sub-district, Magetan regency.

Material And Methods

The research is done in Panekan sub-district, Magetan regency which consists of 10 villages and data retrieval based on random sampling in retrieving data using plots in the area which has bees forage plants. This research is done in 2013 for 3 months, May- July.

This research is done by recording all potential plants as forest bees' forage. This research method is survey research with quantitative approach. Survey research which is quantitative approach is a research done by taking samples from one population, as a tool to collect main data. The information is qualitatively done in order to get clearer description from the research.

The potency of bees forage plants in the forest can be seen by vegetation analysis counting. Data counting with vegetation analysis, not only the type and the number of plants is counted but also density type value (K). Density type is the ratio between the number of individual in an area and the width of examplesswath.

$$K = \frac{\text{jumlah seluruh tumbuhan}}{\text{jumah seluruh sampling unit}}$$

$$KR = \frac{\text{k suatu jenis}}{\text{k total seluruh jenis}} x\ 100\%$$

$$F = \frac{\text{Jumlah petak contoh ditemukan spesies}}{\text{Jumlah seluruh petak contoh}}$$

$$FR = \frac{\text{frekuensi suatu spesies}}{\text{f seluruh spesies}} x\ 100\%$$

$$\text{Indeks Nilai Penting (INP)} = KR + FR$$

Result And Discussion

The development of beekeeping by providing bees forage is something that must be done if the honeybees farmers want to get benefit and continuous success. The colony of honeybees in its growth is very determined by the availability of nectar and pollen producer plants[2]. The plants as an ideal bees forage is just not enough to support the growing of the colony but abundant so that the cultivation is able to produce good crop. maintain forage resource for forest honeybees.
Considering the condition of that area, it is The main factor that must be done in starting honeybees breeding is collecting the availability of information and the abundance of forage plants. Inventory is very important to do because it is to know the various potential forage and the location of forage resource plants so that it can be determined if an area is supporting or not for breeding honeybees.

Plants flowering time is generally seasonal. It causes the bee's colony, in a certain time, potentially experience food scarcity. When there is no flower, it is the time that beekeepers need to pay attention because it is a critical period, the small colony population. This kind of condition is really affecting the next harvest season because sharp decreasing population will cause slow colony growing while approaching nectar season. This condition causes bees farmers cannot utilize harvest season maximally or even experience crop failure.

Bees Forage Composition and Vegetation

The type of forest around Paean sub-district is important to know for honeybees breeding business. From the identification, we can get data about the types of vegetation which have been found. In Right forest and State forest area in Panekan sub-district, Magetan regency, it is known the types of vegetation as many as 943 individual from 74 types and 52 families. The number of types which is found in the researchlocation referring to the types of vegetation composing forest is quite various.

Various types of plants with mixed composition are found in the plot made with line. There are 63

types of plants which become honeybees forage from 52 families (picture 1).

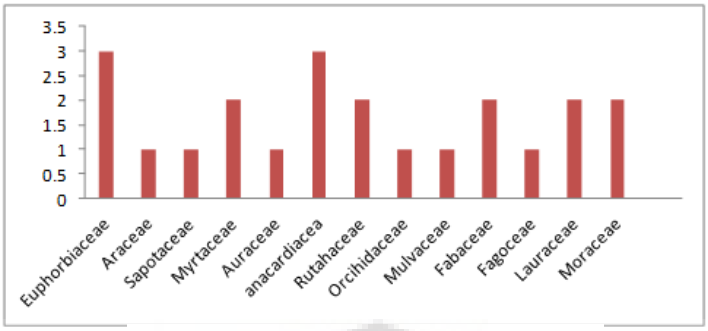

Figure 1. Vegetasi bee food

Honeybees, searching food in plants, will look for substance in the form of sweet liquid called nectar. In the plants and flowers, they have pollen other than nectar. In looking for some food, honeybees have certain time and the climax is around 08:30 up to 10:30.

Plants as Honeybees Forage Potency

Honeybees really like pollen and nectar as the staple food. Bees colonies try to increase protein by utilizing pollen as food main source while nectar as carbohydrate source. All of the elements of this bees forage are taken from plants, especially from flowers. However, for special plants like rubber plant (*Ficus Elastica*) and Acacia (*Acacia spp*), [5]. Nectar is not taken out from the gland in the base part of flowers but from the young leaf buds and base leaf. Honeybees can also get carbohydrate from honeydew, the result of ticks' excretion forming liquid sugar from the plants.

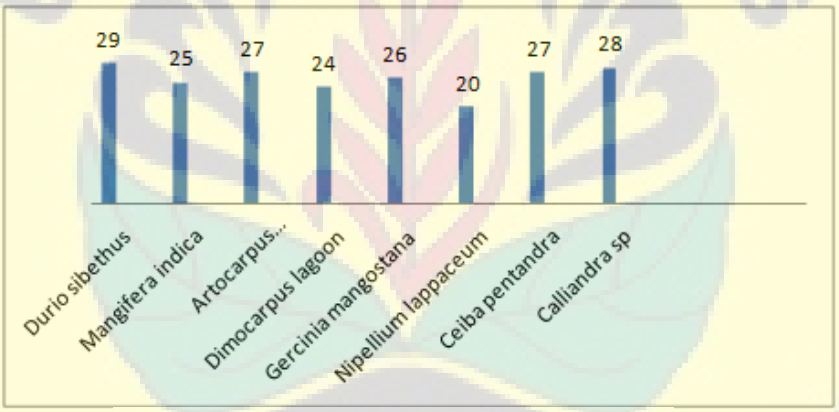

Figure 2. Trees bee food

The done-inventory gets 8 plants which can be used by bees as bees forage resource in Panekan sub-district, for both bred bees and wild bees (not yet bred) by society around the area. The various types of plants as forest honeybees forage resources are jackfruit, longan, calliandra, cotton, mango, durian, and rambutan. Bees' nest is usually kept around durian, calliandra and cotton trees.

Stand Density of Honeybees Forage

The done-Identification and observation get types of forest honeybees stand. In Panekan sub-district, 8 types are found by making plot 48 for forest honeybees stands trees level with total density 112 trunks/ ha (picture 3).

In picture 3, it is seen the types of honeybees forage stand which has density value relatively around 12 at the tree level. The type which has high-density value is the type that is cared with economics calculation so that it is rarely logged by farmers to fulfil their economics need. It is because the society has bred it around their house. At the stand with low density, it is known that there are some factors, natural factor and human intervention factor like logging and the death of the stand.

Density value describes the plant or the stand which is able to adapt well with the pattern of the area will have high-density value. In the stand life rivalry and plants to get sunlight as one of elements is getting higher too. The estimation of total individual of plants type can be done or counted. If the number of individual plants is stated in per width, the value will be called as density.

The complex ecosystem is the series of life which has high diversity or does not experience physical pressure. Populations tend to be controlled

biologically. This condition is used as honeybees forage plants in Panekan sub district and also becomes forest honeybees' nest.

Undergrowth Plants as honeybees Forage

Based on the done- inventory, undergrowth plant in front area is used as forest honeybees forage at undergrowth plants level on 48 plots in Panekan sub-district is found type of undergrowth plant category coffee. It is because there are a lot of coffee plants around the plots managed and cultivated by society and it has potency as commercial plants.

Stand Density of Honeybees Forage

The done-Identification and observation get types of forest honeybees stand. In Panekan sub-district, 8 types are found by making plot 48 for forest honeybees stands trees level with total density 112 trunks/ ha (picture 3).

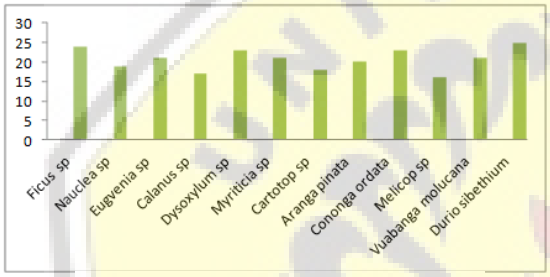

Figure 3. Seedling bee food

Conclusion

1. The done- inventory meets 8 types of plants as honeybees forage resource in right forest, both the bred or not bred by the society around the area.
2. The types of plants as forest honeybees forage for stands are longan, cotton, rambutan, calliandra, durian, mangosteen, jackfruit, while plants as the nest is durian type.

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PREFERENCE TEST of SUSTAINABLE FOOD HOUSEHOLD AREA (KRPL)'S PRODUCTS in SUKOREJO VILLAGE, PONOROGO

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Abstract

KRPL is the area/village level region/RW/RT which formed from several Sustainable Food Household (RPL), by applying the principle of optimal utilization of the yard that is based on local resources to meet the food and nutrition needs of the family. Optimal utilization of the yard begins with the cultivate of various crops (mustard greens, tomatoes, corn, cassava and sweet potato). Excess crops in addition to fulfilling the daily needs of the family are also sold in the market. Problems will arise if the crop is not be full absorbed by the market. Hence it is needed diversification processed in order to increase the added value and family income. This assessment aims to determine the KRPL farmer's response and also determine the most preferred KRPL's food products that have been introduced based on the parameters of taste, aroma, texture, color and the general preference. This assessment was carried out from August to September 2014 at groups of women farmers (KWT) MELATI in Sukorejo village, Ponorogo. To determine the panelists preference, the organoleptic test was conducted by the method of "hedonic test" base on parameter of color, aroma, texture, taste and preference level in general. Organoleptic test was conducted on 27 panelists. This research was using the Randomized Block Design statistic method. Processing technology were tested :1) brownies from purple sweet potato, 2) cassava roll cake, 3) mustard greens marble cake, 4) tomatoes cookies and 5) corn crackers. Various products which introduced give significant effect on the preference level base on parameter of color, aroma, texture, taste and general preference. According to the panelist response, preference value of brownies was on the range 3.741-4.000 (which means like). Preference value of roll cake was on the range 2.741-3.333 (which means enough). Marble cake mustard greens have a preference value was on the range 3.370-3.926 (which means enough to like). Tomatoes cookies have a preference value 3.333-3.852 (which means enough to like). Preference value of corn crackers was on the range 3.667-4.148 (which means like). A high preference value base on parameter of color, flavor, aroma was on corn crackers products. Based on the general level of preference, corn crackers occupy the highest value of 3.963, which means like.

Keyword : sustainable food houses area, organoleptic, processing

Introduction

The aim of KRPL program is developing the ability of families and communities in self sufficient the food and nutritions in a sustainable manner. One effort to improve food security and family nutrition can be done through the utilization of the resources that available in the environment by exploiting yard area that managed by the household. Yard area was used to cultivate food crops, horticulture and livestock to meet the daily food needs of the family. Based on this the Ministry of Agriculture through the Agricultural Research Agency develops Sustainable Food Household Area (KRPL) program for optimizing the utilization of yard which managed by the family. This program is collaborating with various government and private institutions such as the Food Security Agency (Badan Ketahanan Pangan), the Department of Agriculture (Dinas Pertanian), local traders and Research Center for Agricultural Research and Development Agency (Balit, Puslit and all of Badan Litbang Pertanian's Scope). Yard area has a multipurpose function because in narrow land, can produce crops, horticulture and livestock. Yards can be a potential resource to provide nutritious food. Ponorogo (Sukorejo village) is one of KRPL's programs target. The majority of the resident in Sukorejo village work as farmers that still rely on paddy fields to meet the food needs of the family. However, there are still many people who have not been able to meet its food needs despite utilizing the rice field, it is due to its narrow rice field. Even many people who do not have a rice field. With the use of yard area will facilitate the villagers to meet the daily food needs of the family.

In Sukorejo already introduced the cultivation of mustard greens, tomatoes, corn, cassava and sweet potato. Farmers have already started planting these commodities in their yard. Harvest products partially consumed to meet the daily needs, the others was sold in the market. Farmers started getting problems when the harvest began to excess, because it is not all of the product can absorp by consumer, while commodities are easily damaged. According [4] the problems that faced in the

development of KRPL's program was the lack of post-harvest technologies for food commodities locally in yard, the harvest product just to meet the needs of the household. Hence it is need diversification of KRPL commodities in to food product that can be sold to increase the added value and extend the shelf life of commodities. Moreover can increase family income. Processing technology was introduced to farmers, such as 1) brownies from purple sweet potato, 2) cassava roll cake, 3) mustard greens marble cake, 4) tomatoes cookies and 5) corn crackers.

Technology processing which introduced was very easy, it is use the materials and equipment that commonly used by mothers daily. It is expected that farmers can adopt this technology to improve food security and family income. This assessment aims to determine the KRPL farmer's response and also determine the most preferred KRPL's food products that have been introduced based on the parameters of taste, aroma, texture, color and the general preference.

Materials and Methods

This assessment was carried out from August to September 2014 at groups of women farmers (KWT) MELATI in Sukorejo village, Ponorogo. The raw materials was used comes from the KRPL's product in the farmers yards.

To determine the panelists preference, the organoleptic test was conducted by the method of "hedonic test" base on parameter of color, aroma, texture, taste and preference level in general. Organoleptic test was conducted on 27 panelists, come from KRPL's farmers. This research was, using the Randomized Block Design statistic method. Processing technology were tested, such as 1) brownies from purple sweet potato, 2) cassava roll cake, 3) mustard greens marble cake, 4) tomatoes cookies and 5) corn crackers. Formulation of the products can be seen in table 1.

Tabel 1. Formulation of Food Product that have been tested

Brownies from Purple Sweet Potato	Cassava Roll Cake	Mustard Greens Marble Cake	Tomatoes Cookies	Corn Crackers
Puree of Purple Sweet Potato 200 g	Cassava flour 5 tablespoon	Margarine 160 g	Tomato puree 100 g	Corn flour 500 g
Wheat 250 g	Egg 5	Sugar 150 g	Cornstarch 150 g	Tapioca 500 g
Egg 10	Sweetened condensed milk 5 tablespoon	Mustard Greens puree 100 g	Wheat 500 g	Salt15g
Cooking oil 350 ml	Sugar 5 tablespoon	Egg yolk 6	Sugar 250 g	Garlic 40 g
Chocolate block 150 g	Jam for filling	Egg whites 4	Margarine 250 g	Water 1,2 l
Chocolate powder 100 g	-	Wheat 200 g	Milk powder 150 g	-
Sugar 450 g	-	-	Egg yolk 4	-

Results and Discussion

Organoleptic Test of KRPL's Product

Organoleptic test is also called the sensory test because his judgment is based on sensory stimuli in sensory organs. Organoleptic test was performed to assess how much consumers preference against products base on the parameters of color, flavor, aroma, texture and general preference. If one of these parameters was rejected, then the consumer will not accept the food product. Hedonic test is one method of organoleptic tests to determine the levels of consumers preference. To determine the panelists preference of KRPL's food product, the organoleptic test was conducted by the method of "hedonic test" base on parameter of color, aroma, texture, taste and preference level in general.

Tabel 2. Organoleptic Test of KRPL's Product

Treatment	Color	Taste	Texture	Aroma	General Preference
Brownies from Purple Sweet Potato	3.889[a]	3.741[a]	4.000[a]	3.778[a]	3.778[ab]
Cassava Roll Cake	3.333[b]	3.148[b]	3.000[c]	3.000[c]	2.741[c]
Mustard Greens Marble Cake	3.926a	3.852[a]	3.407[b]	3.370[bc]	3.444[b]
Tomatoes Cookies	3.852[a]	3.333[b]	3.407[b]	3.593[ab]	3.593[b]
Corn Crackers	4.148[a]	3.852[a]	3.667[ab]	3.815[a]	3.963[a]

Note : Assessment Criteria
1) very dislike;
2) dislike;
3) enough;
4) like;
5) very like;

Based on organoleptic tests, a variety of products that were introduced give significant effect on the preference of color, taste, aroma, texture and general level of preference.

Color

The appeal of a particular food is influenced by color, as the integral part of product quality. Colour is the most determining factor of a food product appealing. Color is a property of materials that ascribed from the spread of ray spectrum, besides the color is not a substance or object but rather a sensation of a person due to the stimulation of a radiant energy that falls to the senses of the eye or retina.

Various types of products which introduced to farmers give significant effect on the color preferences. A value of the color preference was on the range from 3.333-4.148, which means "like" (Table 2). In general, all panelists liked the color of products were tested. A high value of color preference was on corn cracker, while the lowest value of color preferences contained in Cassava Roll Cake. Corn crackers produced on a golden yellow color that is most preferred by panelists than tawny color in Cassava Roll Cake. This is due to Cassava Roll Cake use 100% of tawny cassava flour. The tawny Cassava Roll Cake occurs because of browning reaction (nonenzimatis reaction) when cassava is processed into flour, thus affecting the color of the Cassava Roll Cake.

Taste

Taste parameter is different with the aroma and involve the senses of taste. Taste is influenced by several factors, including the chemical compound, temperature, concentration and interaction with other flavor components. Taste arising from the chemical stimulation of the senses that can be accepted by the taster or tongue.

Various types of products that introduced give significant effect on the taste preferences. Taste preference value was on the range 3.148-3.852, which means "enough to like". A lowest value of the taste preference was on Cassava Roll Cake (Table 2), a highest value of the taste preference was on Mustard Greens Marble Cake and Corn Crackers. Cassava Roll Cake has the lowest value of taste preference because it is using 100% cassava flour as raw materials. According by panelist Cassava Roll Cake has a strongly cassava felt on the tongue.

Texture

Texture is a group of physical properties caused by the structural elements of food that can be perceived by the senses of touch, is associated with deformation, disintegration and flow of foodstuffs under pressure as measured objectively by a function of massa, time and distance. Texture of the food can be defined as how the various components and structural elements arranged and combined into micro and macrostructur and structure statements out of this in terms of flow and deformation. Texture is also a sensation of pressure that can be observed with the mouth (when bitten, chewed and swallowed) or touching with a finger.
Various types of products that introduced give significant effect on texture preferences. A texture preference value was on the range 3.000 – 4.000 (which means "enough to like"). Brownies have a highest value of texture preference, which means that most prefered by panelists (Table 2).

The raw material of brownies using a purple sweet potatoes pasta to substitute wheat (Table 1). Brownies have soft texture and prefered by panelist. [2] states that sponge cake can be

substituted with non wheat flour (purple sweet potato) up 100% or less, because it is not require development volume as in donut. In this term still accepted by panelist base on color, taste, aroma and texture parameters.

A lowest value of texture preference was on Cassava Roll Cake. Panelist do not like Cassava Roll Cake' texture because the texture is very hard. The main ingredient of Cassava Roll Cake using 100% cassava flour so that its have hard texture. Cassava flour 's characteristics does not contain gluten so it is not easy to inflate, thus it has a hard texture than the characteristics of product that use a wheat and containing a gluten so it is more easy to inflate and make crumbs on products.

In addition a hard texture is also due to the fiber content that contained in the raw material. The higher of fiber content can increase the hardness of the product and decreased elasticity. Cassava flour which used as a raw material in Cassava Roll Cake contains higher fiber than wheat flour so that Cassava Roll Cake texture is harder. The fiber content of cassava flour at 3.34% whereas the fiber content of wheat at 0.4-0.5% [3].

Brownies have a highest texture preference value (4.000) that do not significantly effect with the corn crackers texture preference (3.667) (Table 2). According to the panelists, corn crackers has very crispy texture so that preferred by panelists. The more volume crackers development, the more crispy crackers texture. Volume development of corn crackers is due to the content of amylopectin derived from tapioca starch. According Zulviani 1992 in [1] crackers with the high content of amylopectin will have a highest crackers development volume because during the heating process it will occurs gelatinization and form an elastic structure which can be inflated at frying step. Crackers with a high development volume, the more crispy crackers texture.

Aroma

Aroma is the flavor caused by chemical stimulation which is wafted by the olfactory nerves are located in the nasal cavity when the food into the mouth. Aroma is one important factor in determining the quality of foo

dstuffs. In the food industry, aroma analysis is considered important because it can quickly give an assessment of products, whether or not the product is preferred by consumers. Aroma can be recognized when the form of steam, the smell is generally accepted by the nose and the brain is a lot more variety or a mixture of the four main ingredients are fragrant, sour, rancid and charred.

Different types of products that introduced to farmers give significant effect on the value of aroma preference. A value of aroma preference was on the range between 3.000-3.815 which means all of panelist "like" an aroma of product that introduced. A lowest aroma value contained in Cassava Roll Cake at 3.000 which means "enough" (Table 2). A highest value of aroma preference found in corn crackers because of the distinctive aroma of corns are very strong and most prefered by panelists. According by panelist, Cassava Roll Cake have a typical cassava flavored. This is due to Cassava Roll Cake use a 100% cassava flour as raw materials. A typical cassava flavored give an effect to Cassava Roll Cake. The distinctive aroma in cassava flour is derived from degraded starch content. Aroma and flavor formation caused by degraded carbohydrate content in tuber.

General Preference

At general preference, panelists analyze the products that introduced to farmers give significant effect on the general preference. A value of general preference was on the range between 2.741 – 3.963, which means "enough to like" (Table 2). A lowest value of general preference (2.741) found on Cassava Roll Cake, because in terms of color, taste, texture, aroma and general preference, occupies the lowest value preference (Table 2).

The most preferred product which the highest value of a general preference was on corn crackers. Corn crackers have a high value of color, taste and aroma. On texture parameters, the highest value of texture preference was on brownies. However, this value did not give significantly effect to corn crackers texture value. So in general, the most preferred product is corn crackers.

Acceptance panelists response

Brownies from Purple Sweet Potato

Panelist response to the purple sweet potato brownies based on the parameters of color, taste, aroma, texture and general preference shown in the following picture.

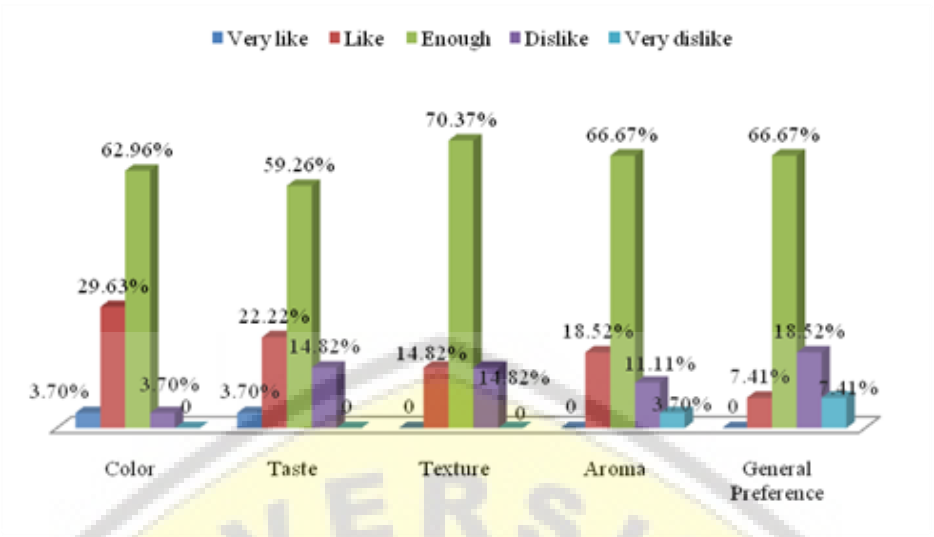

Figure 2. Percentage of Panelists Response on the Cassava Roll Cake

who expressed "like to very like" at 33.33% panelists, while a number of panelists (3.704%) who expressed "dislike" to the color of Cassava Roll Cake. In the parameters of taste, some 25.92% of panelists expressed "like to very like" toward taste of Cassava Roll Cake; and amounted to 14.82% of panelists expressed "dislike" to the taste of cassava roll cake.

A number of panelists (14.82%) who expressed "like" to the texture of cassava roll cake. In aroma parameters, (18.52%) of panelists expressed "like" to roll cake aroma and a number of panelist (14.81%) expressed "dislike to very dislike" towards aroma cassava roll cake. Based on the general preference, a number of panelist (7.41%) expressed "like" to cassava roll cake and some 25.93% of panelists expressed "dislike to very dislike" to cassava roll cake.

Mustard Greens Marble Cake

Panelist response to the Mustard Greens Marble Cake based on the parameters of color, taste, aroma, texture and general preference shown in the following picture.

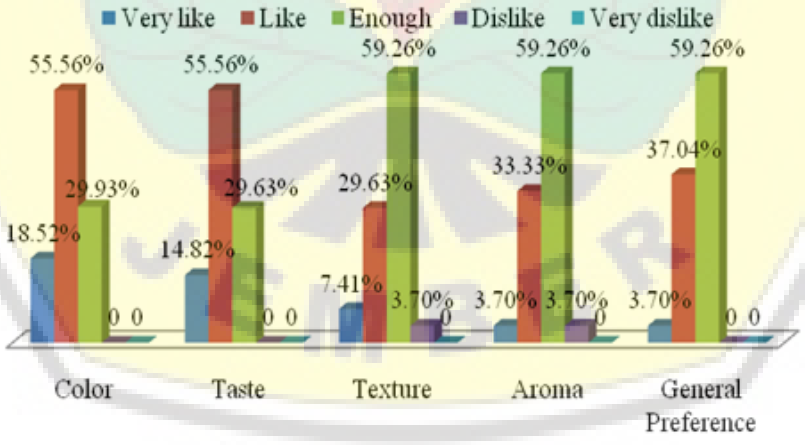

Figure 3. Percentage of Panelists Response on the Mustard Greens Marble Cake

Panelist response to color of Mustard Greens Marble Cake was shown by the number of panelists who expressed "like to very like" for 74.08% of panelists, and no panelists expressed "dislike" for the color of Mustard Greens Marble Cake.

In the parameters of taste, some 70.38% of panelists expressed "like to very like" and no panelists who expressed "dislike" to the taste of mustard green marble cake.

Panelists who expressed "like to very like" to the texture of mustard greens marble cake at 37.04% panelists. A number of panelists (3.704%) expressed "dislike" to the texture of mustard green marble cake.

In aroma parameters, a number of panelists (37.03%) expressed "like to very like" to aroma of mustard green marble cake; while a number of panelists (3.704%) expressed "dislike" towards

aroma of mustard green marble cake. Based on the general preferences, some 40.74% of panelists expressed “like to very like” toward mustard green marble cake and no panelists expressed “dislike” towards mustard green marble cake.

Tomatoes Cookies

Panelist response to the tomatoes cookies based on the parameters of color, taste, aroma, texture and general preference shown in the following picture

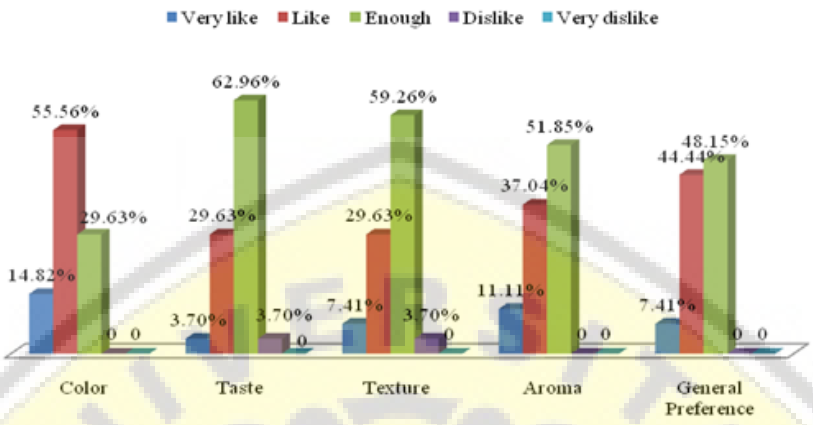

Figure 4. Percentage of Panelists Response on the Tomatoes Cookies

Panelists response to color tomatoes cookies was shown by the number of panelists who expressed “like to very like” for 70.38% of panelists, and no panelists who expressed “dislike” for the color of tomatoes cookies. In the parameters of taste, some 33.33% of panelists expressed “like to very like” and a 3.70% of panelists expressed “dislike” towards tomatoes cookies taste. Panelist response to the texture of tomatoes cookies, showed that some 37.04% of panelists expressed “like to very like” and a 3.70% of panelists marble cake.

In aroma parameters, a number of panelists (37.03%) expressed “like to very like” to aroma of mustard green marble cake; while a number of panelists (3.704%) expressed “dislike” towards aroma of mustard green marble cake. Based on the general preferences, some 40.74% of panelists expressed “like to very like” toward mustard green marble cake and no panelists expressed “dislike” towards mustard green marble cake.

Tomatoes Cookies

Panelist response to the tomatoes cookies based on the parameters of color, taste, aroma, texture and general preference shown in the following picture.

expressed “dislike” for the texture of tomatoes cookies.

At parameter aroma, some 48.15% of panelists expressed “like to very like” for the aroma of tomatoes cookies and no panelists expressed “dislike” to the aroma of tomatoes cookies. Based on the general preferences, some 51.85% of panelists expressed “like to very like” for the tomatoes cookies and no panelists who expressed “dislike” for tomatoes cookies

Corn Crackers

Panelist response to the corn crackers based on the parameters of color, taste, aroma, texture and general preference shown in the following picture

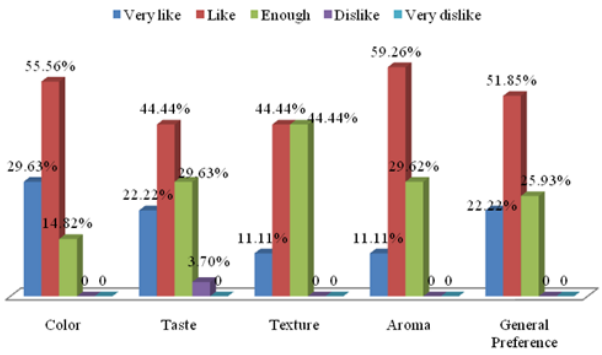

Figure 5. Percentage of Panelists Response on the Corn Crackers

The panelists response to color of Corn Crackers was shown by the number of panelists who expressed "like to very like" at 85.19% and no panelists who expressed "dislike" for the color of corn crackers. In the parameters of taste, some 66.66% of panelists expressed "like to very like" toward taste of corn crackers; and amounted to 3.70% of panelists expressed "dislike" to the taste of corn crackers. The panelists response to texture of Corn Crackers was shown by the number of panelists who expressed "like to very like" at 55.55% and no panelists who expressed "dislike" for the texture of corn crackers. The panelists response to aroma of Corn Crackers was shown by the number of panelists who expressed "like to very like" at 70.37% and no panelists who expressed "dislike" for the aroma of corn crackers. Base on General Preference, some 74.07% of panelists expressed "like to very like" for the Corn Crackers and no panelists who expressed "dislike" for corn crackers.

Conclusion

Commodities that potential to be developed in the yards with the concept of utilization of the yard (KRPL) and other programs toward agro-scale household is cassava, sweet potato, mustard greens, tomatoes and corn. While food processing technology that potential to be developed; 1) brownies from purple sweet potato, 2) cassava roll cake, 3) mustard greens marble cake, 4) tomatoes cookies and 5) corn crackers. The availability of agro-technology, easily carried by the user to increase the added value and supporting agro-industry as well as to support household food security program.

Various products which introduced to farmers give significant effect on the preference of color, aroma, texture, taste and general preference. According to the panelist response, preference value of brownies was on the range 3.741-4.000 (which means like). Preference value of roll cake was on the range 2.741-3.333 (which means enough). Marble cake mustard greens have a preference value was on the range between 3.370-3.926 (which means enough to like). Tomatoes cookies have a preference value between 3.333-3.852 (which means enough to like). Preference value of corn crackers was on the range between 3.667-4.148 (which means like). A high preference value base on parameter of color, flavor, aroma was on corn crackers products. Based on the general level of preference, corn crackers occupy the highest value of 3.963, which means like.

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THE TOXICITY of POLAR and NON-POLAR FRACTION of RHIZOME EXTRACT of *Acorus calamus* L. AGAINST *Hypothenemus hampei* (Ferr.)

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Abstract

Effect of *Acorus calamus* L. rhizome materials, were studied for their control afficacy on the *Hypothenemus hampei* (Ferr.). The powder of *A. calamus* was extracted using ethanol as an organic solvents and then was separated by using methanol (polar solvents) and hexane (nonpolar solvents). This to maximize the withdrawal of active compounds on plants by considering the nature of a compound bioactive, include the nature of that polarity. It was found that hexane fraction of *A. calamus* more toxic than methanol fraction.

Keywords: A*corus calamus*, toxicity, hexane fraction, *Hypothenemus hampei*

Introduction

Dringo (*Acorus calamus* L.) is herbaceous plants compounds containing of saponin active, flavonoid, and a volatile oil called calamus oil [4]. Bioactive compounds is dringo rhizome have diversity in terms of polarity. Thus, to maximize withdrawal compound active in plants have to consider the nature of a compound bioactive these include the polarity properties. Organic compounds in plants is polar and capable of being dissolved in solvent polar, while organic compounds non polar capable of being dissolved in non polar solvent [2].

Based on the description above need to evaluate the effectiveness of extracts dringo rhizomes with polar and non polar solvents. In this research for the assessment of its toxicity extract rhizomes dringo using solvent methanol (polar) and hexsane (non polar) using coffee berry borer (*H. hampei*).

Materials and Methods

Materials

Two fractions of *A. calamus* in polar and non polar solvents in this study had been used.

H. hampei

Population of *H. hampei* were obtained from infected coffee berry borer in the field (Jember). Rearing was done in a secure controlled environment room, 14:10 h (light : dark) at 25 ± 2°C and 54 ± 10% relative humidity. Coffee berry that infested by *H. hampei* were put in a container (π x 6,5 cm x 6,5 cm) with coffee berry without exposure of any insecticides. They were kept caged until adults emerged and mated [5].

Bioassay

A contact methods with filter paper was used to evaluate the toxicity of two fractions (methanol and hexane) of *A. calamus* against adults of *H. hampei*. In brief, filter paper (3 cm in diameter) were dropped with each fraction of *A. calamus*. Seven concentrations of test fractions were dropped by using micropipette. Each concentration was replicated 5 times. The number of insect mortality was observed after 24 hours and 168 hours.

Data Analysis

Data were corrected for control mortality using the formula Abbot, concentration mortality data were subjected to probit analysis to provide the LC_{50} value [3]. The percentage mortality was determined for ANOVA. Treatment means were compare by Duncan test [1].

Discussions

The percentage of *H. hampei* mortality in various concentrations with methanol and hexane fractions of dringo rhizome showed in Table 1.

Table 1. Effect of methanol and hexane fractions of extract dringo rhizome (*A. calamus*) against *H. hampei* at 168 hours after treatment.

Concentration (%)	Mortality (%) (Mean ± SD) *)	
	Methanol fraction	Hexane fraction
0	0 ± 0.55^{b}	0 ± 0.55^{b}
0,1	11.90 ± 2.61^{ab}	88.10 ± 1.73^{a}
0,2	38.10 ± 3.63^{a}	83.33 ± 1.67^{a}
0,4	30.95 ± 2.17^{ab}	95.24 ± 0.55^{a}
0,8	14.29 ± 1.10^{ab}	90.48 ± 0.84^{a}
1	26.19 ± 1.64^{ab}	95.24 ± 0.89^{a}
1,5	19.05 ± 1.10^{ab}	92.86 ± 1.34^{a}
3	30.95 ± 2.49^{ab}	97.62 ± 0.45^{a}

*) means within column followed by the same letter are not significanty different at α=5% (Duncan)

In Table 1 can be seen the percentage of the average death ± SD (standart deviation) *H. hampei* faction on methanol at concentrations 0.1%, 0.2%, 0.4%, 0.8%, 1%, 1.5%, and 3% successive is

11,90% ± 2.61, 38,10% ± 3.63, 30,95% ± 2.17, 14,29% ± 1.10, 26,19% ± 1.64, 19,05% ± 1.10, and 30,95% ± 2.49. While the average death ± SD *H. hampei* on heksan faction with concentration 0.1%, 0.2%, 0.4%, 0.8%, 1%, 1.5%, and 3% respectively is 88,10% ± 1.73, 83,33% ± 1.67, 95,24% ± 0.55, 90,48% ± 0.84, 95,24% ± 0.89, 92,86% ± 1.34, and 97,62% ± 0.45.

Based on the results showed that the concentration of methanol fraction extract dringo rhizome has not significant affect *H. hampei*, while mortality the hexane faction extract dringo rhizome has significant effect.

The effectiveness of fraction extract rhizome dringo using two solvents that have different polarity analyzed, showed (P=0,000)<0,05 significantly differences on methanol fraction and hexane fraction against *H. hampei*. Fraction with hexane solvent compound containing non polar who can easily enter to in the cell membranes through a process diffusion so that led to the cell faster damaged, while polar compound not easily diffuses enter the cell membrane. This resulted polar compound more difficult to get into the in the cell so that their toxicity is lower than hexane fraction [6].

Conclusions

Hexane fraction from extract of dringo rhizome who applied with the residue method showed toxic against *H. hampei* which cause mortality up 88% at the concentrations 0,1%.

Acknowledgments

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DEVELOPMENT of TRANSGENIC SUGARCANE CONTAINING DOUBLE OVEREXPRESSION (STACKED) of THE GENES for SUCROSE-PHOSPHATE SYNTHASE and SUCROSE TRANSPORTER PROTEIN

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Abstract

Sugarcane (*Saccharum officinarum* L.) is a major crop for sucrose production in tropic region. Biosynthesis of sucrose is catalyzed by Sucrose Phosphate Synthase (SPS) in cytosol of photosynthetic leaf cell (sources tissues). The SPS enzyme serves to catalyze the formation of sucrose-6-phosphate from UDP-glucose and fructose-6-phosphate, which is then the sucrose-6-phosphate is converted into sucrose by sucrose phosphate phosphatase (SPP). In sugarcane, sucrose that synthesized in leaf tissue is translocated and accumulated in the stem organ (sink tissues). The sucrose translocation is facilitated by the sucrose transporter protein (SUT). Recently, transgenic sugarcane with overexpression of single gene either gene encoding for SPS enzyme (*SoSPS1*-cDNA) or encoding for sucrose-transporter protein (*SoSUT1*-cDNA) has been previously generated through genetic transformation. However, each of this transgenic sugarcane only increased only in the sucrose biosynthesis or translocation, but did not increase of these two processes both the sucrose synthesis and translocation. To develop transgenic sugarcane with double overexpression (stacked), the transgenic sugarcane with single overexpression of *SoSPS1* gene is transformed with *SoSUT1*-cDNA, and conversely the transgenic sugarcane with overexpression of *SoSUT1* gene is transformed with *SoSPS1*-cDNA. Thus, the transgenic sugarcane containing double genes are expected to have high sugar content in the stems organ due to increasing of sucrose biosynthesis and translocation. The genetic transformation was conducted using *Agrobacterium tumefaciens* vector. Thus, selection for transgenic sugarcane was conducted by incubation of plantlet in a media containing double antibiotic 50 ppm kanamycin and 25 ppm hygromicine. After 5 successive cycles in the selection media, the survived plantlets were acclimated in green house as putative transformant. Genome DNA was isolated from leaves of the putative transformant and subjected for PCR analysis with specific pair of primer for amplification of the DNA markers. The results showed the presence double overexpression (stacked) of the transgenic sugarcane, that were 10 transgenic sugarcane clones of *SoSPS1-SoSUT1* and 11 transgenic sugarcane clone of *SoSPS1-SoSUT1*.

Keywords: Sugarcane transformation, sucrose phosphate synthase, sucrose transporter protein, double overexpression (stacked) gene.

Introduction

Sucrose is the main product of the photosynthesis [1] which is translocated to various tissues for nutritional needs of plant growth [13]. In sugarcane, SUT protein serves as sucrose translokator from photosynthetic tissue (source tissues) to storage tissues (sink tissues) in the form of rods [9].

In phylogenetic analysis, SUT protein-coding genes in plants are divided into three subfamilies, such us SUT1, SUT2, and SUT4. Based on sequence homology and affinity for substrate, protein SUT1 has a high affinity for the substrate but has a lower transportation capacity. SUT2 have an affinity and a low transport capacity. SUT4 has low affinity but has a high transportation capacity. In addition, it was found that only SUT3 gene can be isolated from the tobacco plant and fall within the SUT1 category [5]. Based on these characters, SUT1 more potent in enhancing sucrose accumulation in a plants.

SUT1 gene's function in the accumulation of sucrose has been demonstrated in a study [11] that the over expression of SUT1 genes on potato may increase the rate of sucrose transport, thus increasing its ability to accumulate sucrose to storage organ (sink tissues). Inhibition of expression SUT1 gene on potato plants with antisense techniques also affect biomass reduction during the early stages of tuber development [6]. That technique also affects the decrease in sucrose translocation and tomato fruit development [4]. Sugarcane genetically engineered products 2 and 20 events are *SoSUT1* gene overexpression. Sugarcane

2 and 20 events are transformation results in previous studies. That's sugarcane has a high level of sucrose transport.

SPS enzyme (*Sucrose Phosphate Shynthase*) is a key enzyme in the formation of sucrose in the leaf cell (photosynthetic tissues). Therefore, SPS gene overexpression plants have high levels of sucrose biosynthesis. In the previous study [2], *SoSPS1* gene overexpression can increase sucrose rate in sugarcane. That study explain that sugarcane positive transformants *SoSPS1* gene that has the highest expression levels are 2.2.B event.

This study aims to transform *SoSUT1* genes in sugarcane PRG *SoSPS1* gene overexpression 2.2.B event and to transform *SoSPS1* genes in sugarcane PRG *SoSUT1* gene overexpression 2 and 20 event using *A. tumefaciens* GV 3101 strain. Thus, the transgenic sugarcane containing double genes are expected to have high sugar content in the stems organ due to increasing of sucrose biosynthesis and translocation.

Methods

This research was conducted at the Molecular Biology and Biotechnology Laboratory CDAST (Center for Development of Advanced Sciences and Technology) Jember University.

Explants Preparation For Transformation

Sugarcane in vitro plantlets *SoSPS1* gene overexpression 2.2.B event propagated in selection Mso media (Murashige and Skoog) + kanamycin 50 mgL-1. Sugarcane in vitro plantlets *SoSUT1* gene overexpression 2 and 20 events propagated in selection Mso media + Hygromycine 20 mgL^{-1}.

A. tumefaciens culture and Confirm Existence of Plasmid pAct-*SoSUT1* and pKYS-SoSPS1

Transformation is done by using the A. tumefaciens vector containing PAct-*SoSUT1* and PKYS-*SoSPS1* plasmid. Map pAct-*SoSUT1* construct plasmid can be seen in Figure 1 and PKYS-*SoSPS1* in Figure 2.

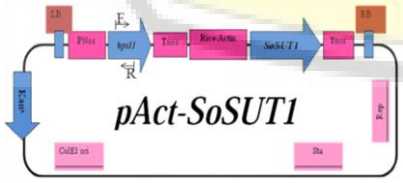

Figure 1 Map construct plasmid pAct-*SoSUT1* composed by boundary T-DNA LB: Left Border and RB: Right Border, P-Nos: Promoter Nopaline synthetase, hptII: Hygromycin Phosphotransferase gene, T-nos: Terminator Nopaline synthetase, Promoter Rice Actin, SoSUT1: Sucrose Transporter in sugarcane [12].

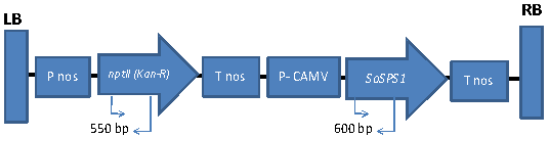

Figure 2. Part of the T-DNA plasmid pKYS-*SoSPS1* construct containing *SoSPS1* genes and antibiotic resistance *nptII* gene.

A. tumefaciens cultured in liquid YEP medium (Yeast Extract Peptone) 2 ml of containing antibiotics selectors Agrobacterium cells. After incubation, carried confirmation stage presence of plasmid on *A. tumefaciens* cell.

Plasmid isolation techniques of bacterial cells is done by Sambrook *et al.* methods in 1989. The resulting DNA plasmid dissolved in TE buffer. DNA was amplified by PCR and confirmed by agarose gel electrophoresis, then visualized by *Gel Imaging System*.

SoSUT1 Gene Transformation

A. tumefaciens starter containing pAct- *SoSUT1* and pKYS-*SoSPS1* plasmid constructs 2 ml subcultured in 50 ml of liquid YEP media containing the selectors antibiotic, such us kanamycin 50 mgL^{-1}, Rifampicin 100 mgL^{-1}, and Gentamycin 12.5 mgL^{-1}, incubated shaker 150 rpm at a temperature of 28°C until the cell density (OD600) reached 0.7.

Plantlets which amounts to ± 50-100 cut the base 0.5 cm. The explants were infected by soaking in a liquid YEP 50 ml media containing culture of *A. tumefaciens* with the addition of 100 mgL^{-1} *acetosyringone* and shaken at 120 rpm, temperature 28°C, in dark conditions, for 15 minutes. Explant incubation results filtered and planted on media kokultivasi for 3 days.

After kokultivasi phase then Explants is washed first with cefotaxime 500 mgl^{-1} and 100 ml of distilled water. Planting explants then performed on the elimination of media consist of MS_O + cefotaxime 500 mgL^{-1} and incubated in bright conditions for 7 days.

Selection process takes 5 cycles, each cycle incubation for 21 days in bright conditions. Sugarcane that pass the five cycle selection referred to as sugarcane putative transformants.

Genomic DNA isolation and analysis of putative plant transformants

Insolation phase genome start the process of confirming the existence of the target gene in the plant genome. The result DNA from purification concentration was measured using nano vue plus and then analyzed PCR (Polymerase Chain Reaction.

PCR analysis performed to detect the presence of target genes that have integrated into the plant genome. PCR was used primer pair *hptII* (Hygromycin phosphotransferase) is *hptII*-F (5'-CCG ATC GGT GGA CAA CAA TA-3 '), *hptII*-R primer (5'-CCC AAG CTG CGA CAT CAT AA-3') were will produce a DNA fragment of 470 bp and *nptII* (neomycin phosphotransferase II) *nptII*-F (5'-GTC ATC TGCC TCC TGC TCA CCT-3 ') and *nptII*-R (5'-GCT GTC GTC TGG ATT TCG TCG-3') which will amplify DNA fragment of 550 bp.

DNA that has been amplified then separated by agarose gel electrophoresis containing 1% 1,5µl EtBr (Ethidium Bromide) with a voltage of 100 volts for 25 minutes. DNA markers that used are 3µl 1 kb marker Ladder. Electrophoresis results can be seen and documented using a *Gel Imaging System.*

Result And Discussion

Base of the buds explants of Sugarcane In Vitro for Transformation.

Shoots multiplication takes ± 4 months to subculture stage in one time for 3 weeks. Shoots multiplication is done for explant transformation, each transformation takes ± 50-100 shoots. The explants used the base of the cane shoots with a length of 0.5 cm, measured from the base of the root towards the tip (Figure 3) . Base cane shoots explants enables the transformation process occurs more effectively. That is because base of the cane shoots consist of meristem tissue that can divide actively and require a relatively short time to regenerate [7].

Sugarcane in vitro *SoSPS1* gene overexpression 2.2.B event planted on MS_O media + kanamycin 50 mgL^{-1} and sugarcane *SoSUT1* gene overexpression 2 and 20 event with the addition of hygromycin 20 mgL^{-1} antibiotics. Kanamycin antibiotics at 50 mgL^{-1} concentration is used for the selection of plants containing a gene *npt* namely kanamycin antibiotic resistance gene. Concentration of 50 mgL-1 kanamycin can already be used to select positive transformants plants because it can inhibit the growth of plants that not transformant [9]. Hygromycin antibiotics are also used for the selection of plants containing hpt gene is an antibiotic resistance gene to hygromycin.

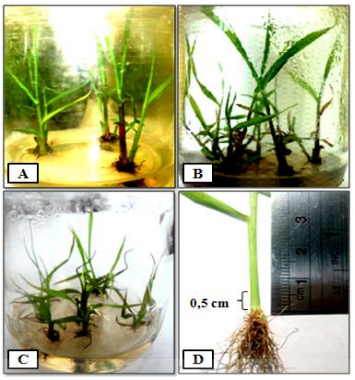

Figure 3.In vitro Sugarcane SoSPS1 gene overexpression 2.2.B event (A), in vitro Sugarcane SoSUT1 gene overexpression 2 event (B), in vitro Sugarcane SoSUT1 gene overexpression 20 event (c), explants were used in transformation is the base of the bud with 0, 5 cm in long (d).

3.2 Confirmation of Existence *hptII* Gen in Agrobacterium cell.

Confirmation of existence *hptII* and *nptII* gene in *A. tumefaciens* is done through PCR analysis using DNA plasmid template and *hptII* and *nptII* primer Forward (F) - Reverse (R). Electrophoresis results showed that there were 470 bp and 550 bp DNA band whose size is determined based Marker 1kb DNA Ladder (Figure 4).

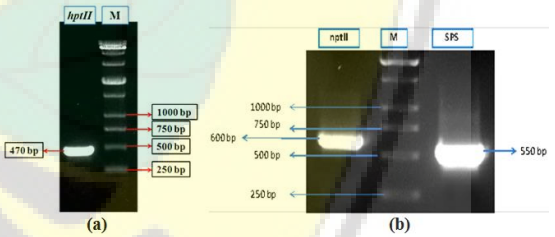

Figure 4. Results PCR using *hptII* (b) and *nptII* (b) primers FR, and also DNA template isolated from the *A. tumefaciens*: M: DNA marker 1 kb Ladder, hptII: *hptII* DNA fragment contained in plasmid construct pAct-*SoSUT1*, nptII*: nptII* DNA fragment contained in plasmid construct pKYS-*SoSPSI.*

The length of DNA bands are visible on electrophoresis results of the DNA fragments amplified successfully with primer pairs FR *hptII* and FR *nptII*, and in accordance with the length of *hptII* (470 bp) and *nptII* (550 bp). The results of the PCR analysis showed that pAct-*SoSUT1* plasmid construct already contained in the *A. tumefaciens* cell, and also that pKYS-*SoSPS1* plasmid construct already contained in the other types of *A. tumefaciens* so that they can be used as a vector transformations in sugarcane.

Gene transformation SoSUT1 on Sugar Cane Plant

After the explants 2.2.B, 2, and 20 event through the process of infection, explant started to show growth of shoots after 3 days at kokultivasi media. Then shoots at the media entrance elimination and shoot regeneration process continues until the end of the selection. At the beginning of the selection period, the buds begin to show a response to the antibiotic kanamycin and hygromycin. The response shown by the events of albino and browning and eventually die. The state of the cane shoots in vitro at every stage of the transformation presented in Figure 5.

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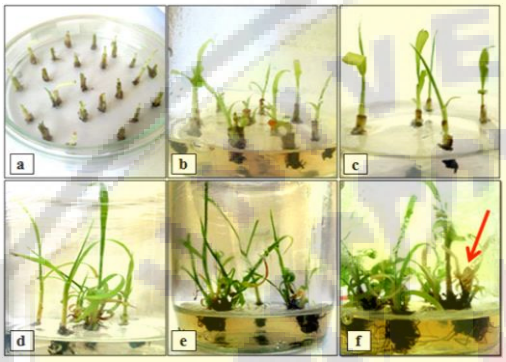


Figure 5. The state of sugarcane buds on the stages of transformation. A: kokultivasi, b: elimination, c: selection I, d: Selection II, e: sleksi III, f: Selection IV, section designated shoots arrows are experiencing albino and browning.

Kokultivasi stage is made by adding the acetosyringone 100 mgL^{-1} that aims to activate Virg proteins and other virulence genes which are responsible for the transfer and integration of target DNA into the genome of plants. The next elimination phase containing cefotaxime 500 mgL^{-1}. Cefotaxime antibiotic is used to eliminate *A. tumefaciens*. Cefotaxime antibiotic is a cephalosporin class of antibiotics which actively inhibit the synthesis of peptidoglycan especially gram-negative bacteria [8]. Optimal concentrations of cefotaxime to eliminate *A. tumefaciens* cells and is not toxic to the explants is 500 mgL^{-1} [7].

Selection phase aims to select plants that already contains a target gene. Selection phase determines the success of a genetic transformation method. Target DNA fragment integrated into the genome of plants and antibiotics in selection media are are used as a selectors for transformant and non-transformant plants.

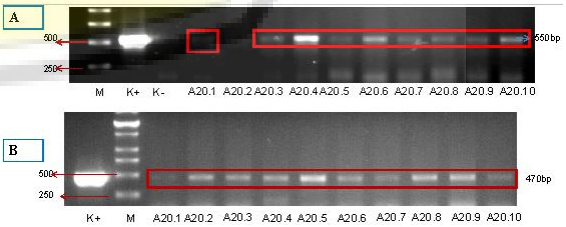
Hygromycin antibiotic can inhibit plant growth through protein synthesis inhibition and cause browning and death [10]. Based on the figure 5 there are explants undergo browning and death , that because resistance *hptII* genes not integrated in plant genomes so they are can not survive when exposure to antibiotics hygromycin. The response to the kanamycin antibiotic is form albinism, the green color of the leaves waning due to disruption of the metabolism of the chloroplast and eventually die. Shoots dead may not have the resistance gene *nptII*, otherwise the shoots that contain the resistance gene *nptII* will keep alive despite being in exposure to the kanamycin antibiotic.

Plants that escaped from the selection media can be referred to as putative plant transformants (Figure 6). Plants pass the selection has a condition that is fresh and good rooting. The total number of putative plant transformants as many as 31 plants from 2.2.B events, 12 plants from 2 events, and 10 from 20 events. Furthermore, the putative transformant plants was done isolation of genomic DNA as a template in PCR analysis.

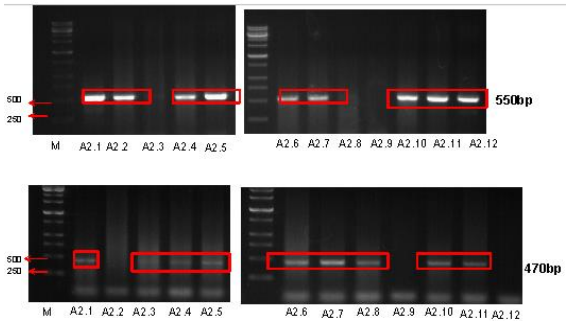
Figure 6. Sugarcane that qualify antibiotic selection media

Sugarcane PCR analysis of *SoSPS1* and *SoSUT1* gene

Confirmation of *SoSUT1* and *SoSPS1* target gene contained in the transformant putative plant is to detect the presence of genes selectors using primers for genes *hptII* and *nptII*. This is in accordance with the map of the construct (Figure 1) that the gene *SoSUT1* are in one cassette T-DNA with *hptII* antibiotic resistance gene, so the presence of the *SoSUT1*gene can be detected by a *hptII* gene. PCR analysis using primers FR *nptII* SoSPS1 based because genes are in one cassette T-DNA with *nptII* antibiotic resistance gene (Figure 7 and 8).

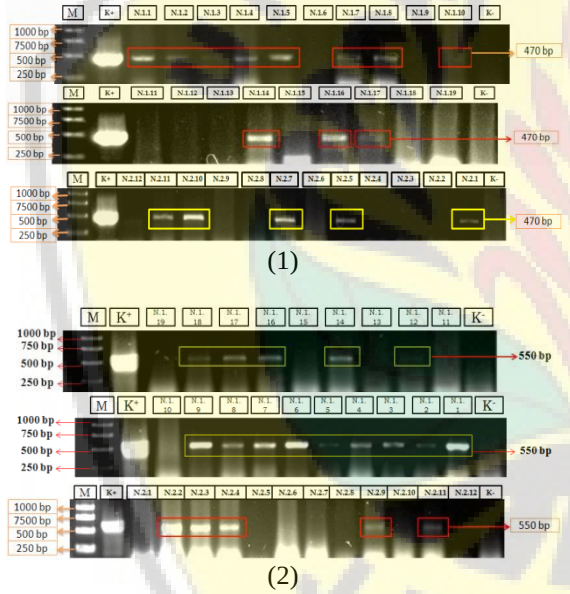


(a)



(b)

Figure 7. Results of electrophoresis selectable marker gene *nptII* and *hptII* to confirm the presence of a target gene, a: 9 planlets event 20 positive transformants *SoSPS1* genes and 10 plants positive transformant *SoSUT1* gene; b: 9 planlets event 2 positive transformant *SoSPS1* genes and 9 plants positive transformants *SoSUT1* genes.



(1)

(2)

FigureTemplate 8. DNA gel electrophoresis results of sugarcane event 2.2.B suspected to contain a target gene, 1: there are 16 plants were positive for the *SoSUT1* target gene; 2: There are 19 plants were positive for the *SoSPS1* target gene.

The success of genetic transformation is determined by the integration of the target gene in the plants genome. Stages to detect the target gene is integrated in the plant genome is the genome isolation and PCR analysis.

Sugarcane who pass the selection of media, but when detected do not contain the *SoSUT1* genes or *SoSPS1* genes, called as sugarcane non transformants protected by the transformant cells. This is because these plants contain the target genes randomly in the tissues, so it is not homogeneous [3].

Existence *hptII* DNA fragment with 470 bp and 550 bp of DNA *nptII* PCR results showed integration pAct-*SoSUT1* plasmid construct on sugarcane genome so that the transformation results can be considered as sugarcane PRG *stacked gene SoSUT1* and *SoSPS1*. *Stacked gene* is a term in the plant assembly by using more than one gene.

Coclusion

There are 31 putative transformants plants 2.2.B events, 10 putative transformants plants 20 events, and 12 putative transformants plants 2 event. Based on PCR analysis of putative transformant plants, plants that are positive for the *SoSUT1* gene are 16 plants (2.2.B), 9 plants (2), and 10 plants (20), and positive for the gene *SoSPS1* are 19 plants (2.2.B), 9 plants (2), and 9 plants (20). Moreover, plants containing both genes SoSUT1 and SoSPS1 are 11 plants (2.2.B), 7 plants (2), and 3 plants (20).

Acknowledgment

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MYCORRHIZA DIVERSITY from VARIOUS PEOPLES CULTIVATION FOREST ECOSYSTEM TYPES in SOUTH SULAWESI

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Abstract

Arbuscular Mycorrhiza are found in various ecosystems. Due to differences in mycorrhiza species, mycorrhiza fungi need to be isolated and identified to elucidate mycorrhiza types and colonization percentage of tree roots in some peoples cultivation forest ecosystems. The methodologies of research included collecting soil samples and tree roots, staining hairy roots and observing arbuscular mycorrhizal fungi infection. Results showed spore types that observed at magnolias', candlenuts' and teaks' roots were *Glomus, Gigaspora* and *Acaulospora* spore. Colonization percertage of magnolias' roots was low at 7.49%; whereas candlenuts' and teaks' were intermediate at 13,88% and 27%, respectively.

Keywords: Mycorrhiza, Vesicular, Arbuscular, Peoples Cultivation Forest

Introduction

Arbuscular mycorrhizal fungi are one of mycorrhiza inducer types that receive attention from environmental specialist and biologist as a future alternative technology for increasing forest trees growth, productivity and quality, particularly for planted trees in nutrient poor-soil. The reports by [1] have proved mycorrhiza fungi significantly increased plant growth and decreased fertilizer needs to 40% that affected the reduction in fertilizer application costs up to 40%.

Arbuscular mycorrhiza are found in various ecosystems. The FMAs' distribution are wide-ranging distribution throughout the world, from arctic to tropical regions. [9] compiled FMA fungis' diversity data from Brazil, Colombia and Zaire and found as many as 16-21 species in natural ecosystems, 10 to 15 spesies in farm ecosystems (low inoculation level) and 6 to 9 species in intensive farm ecosystems, respectively. Study in Jambi and Bengkulu also reported there were 7 to 10 species in forest ecosystems, 8 to 11 species in farm ecosystems and 10 to 11 species in glassland ecosystems [10]. This diversity difference have showed the existence of different FMA communities types among an environment to others and also FMA composition types in the root zone within a particular ecosystem as affected by soil types.

Due to different mycorrhiza species within some ecosystem types, mycorrhiza fungi need to be isolated and identified from tree rooting zone of some peoples cultivation forest ecosystem types. The findings of this study should become important informations regarding mycorrhiza types and to determine colonization percentage of tree roots from some peoples cultivation forest ecosystem types.

Materials And Methods

Time and Location of Research

This research activities were done at Tree Biotechnology and Breeding Laboratory, Forestry Faculty, Hasanuddin University, Makassar. It was conducted during the period of March up to August 2015. The sample collection locations were done at three peoples cultivation forests: magnolia in North Toraja, candlenut in Maros and teak in Barru.

Research Methodology

1. Soil Sample and Tree Root Collection

Root samples were taken as many as 18 spots at each following research location: at 0-30 cm soil depth. Soil was chosen from randomly assignedtree by mattock at around root zone in four spots, samples were then composited to one kilogram of soil.

2. Hairy Root Staining Procedure

FMA observation procedure was root staining technique using root staining method [3, 4, 11]. The procedure steps were : hairy roots were chosen from sample trees and submerged in FAA solution. The roots were then submerged using 10% of KOH for 24 hours at room temperature. KHO solution was removed and root samples were cleaned by water. The clean roots were submerged in hot H_2O_2 solution for 24 hours, then cleanly washed with water. Roots were then submerged in 2% of HCl for 24 hours. The HCl solution was then removed and roots were thoroughly washed with running water. Root samples were then submerged in staining solution for 24 hours, and then submerged once more using staining solution for 24 hours.

3. Observation of Mycorrhiza Arbuscular Fungi Infection

The measurement of FMA infection was done using infected root length method by [2]. Five stained roots were cut at approximately 1 cm in length and arranged on a microscopic glass slide. The sliced roots on a microscopic glass slide were observed from every angle. Field of view that showed the colonizations would be sign (+), while if there were no sign of colonization, they would be sign (-).

Variable observation

The observed variable in this study were :

a. Arbuscular Mycorrhiza Fungi (FMA) Spore Characteristic. The spore that found in this study was observed on its morphological characteristics, such as shape and colour.
b. Arbuscular Mycorrhiza Fungi (FMA) Type The observed spore type at root samples was identified up to genus class
c. The Colonized/Infected Root Percentage Root colonization was analyzed based on root infection percentage by counting FMA structure using [8] formula :

$$\text{Colonized Percentage} = \frac{\text{Number of infected root}}{\text{Total number of observed field of view}} \times 100\%$$

Statistical Analysis

The observation data were analyzed and showed in tabulation and picture/figure forms. Number of FMA infected root criteria would be categorized into 4 classes.

Table 1. The Number of Infection Classification by [4]

Colonization Percentage	Category
0	No colonization
≤ 10	Low
10-30	Intermediate
≥ 30	High

Results And Discussion

Arbuscular Mycorrhiza Fungi Spore Characteristic

Results of identification analysis indicated there were three spore types that found at sample collecting sites : Glomus, Gigaspora and Acaulospora. The observation of three locations and spore types are presented in Table 2.

Table 2. Identification of Spore Types in Each Observed Location

No	Peoples Cultivation Forest Location	Spore Type
1	Magnolia in North Toraja	Glomus , Gigaspora
2	Candlenut in Maros	Glomus, Acaulospora
3	Teak in Barru	Glomus, Gigaspora dan Acaulospora

Based on the observation, Glomus spores were found at magnolia, candlenut and teak tree in varied environment conditions. Gigaspora spores were found at magnolia and teak tree, while Acaulospora spores were in candlenut and teak tree. All of three spore types had different characteristics in shape and colours

Morphological shape of these three spore types that found at each observed location are presented in Figure 1

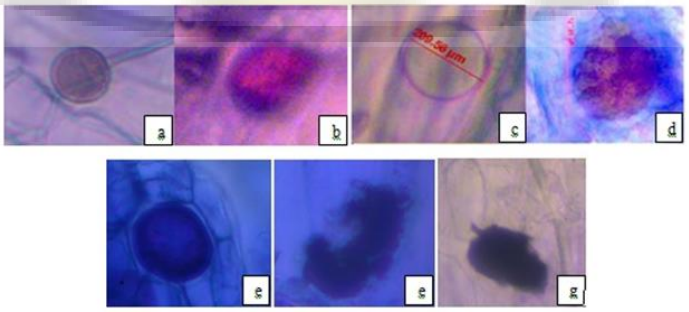

Figure 1. The identified FMA spore types at magnolia tree (a) *Glomus* spore, (b) *Gigaspora* spore*;* at Candlenut tree (c) *Glomus* spore, (d) *Acaulospora* spore*;* and at Teak tree (e) *Glomus spore*, (f) *Gigaspora* spore and (g) *Acaulospora* spore

Results of the study shows *Glomus* sporesthat identified at three observed locations commonly have oval-shaped, ellipse-shaped and round-shaped, as well as coloured in blue, light brown, dark brown, spore wall is soft and transparent/whitist. Findings by [7] stated Glomus spores commonly had round-shaped to ellipse-shaped, whitist/transparent yellow to reddist brown colour, had thin and relatively soft wall spore surface.

Based on morphological characteristics, observed Gigaspora spores at Magnolias' and Teaks' roots had oval-shaped, ellipse-shaped and round-shaped as well as blue, dark brown, brownish yellow and blackish brown in colour. Wall layer was regular and also irregular.

Acaulospora spores which found at candlenuts' and teaks' roots were round-shaped and ellipse-shaped, had thick and irregular wall spore, brownish yellow-coloured and had black spots in them. According to [6,5], Acaulospora spores had ellipse-shaped, relatively irregular and thick wall spore. In addition, spores colored in dark brown and browish yellow, and had black spots over their surface.

Root Colonization Percentage

Root colonization percentage calculations were varied at all of three observed locations. The histogram of colonization percentage at each location can be seen at Figure 2.

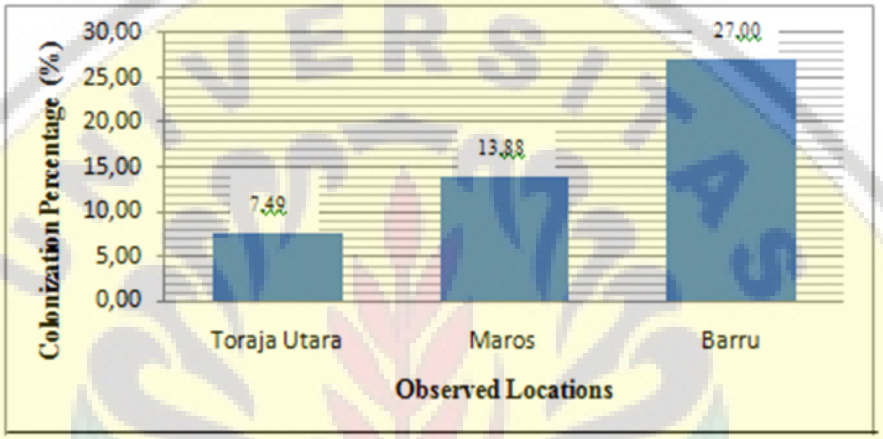

Figure 2. The histogram of FMA Colonization Percentage in Root Sample at Each Location

Figure 2 describes that Barru is location with highest infection level as compared to other locations, North Toraja and Maros. Moreover, Colonization percentage based on infection level classification showed location with lowest colonization percentage level was North Toraja (7,49%), whereas both other locations, Maros and Barru, were intermediate at 13,88% and 27%, respectively. The presence of colonization level variation depends on environment conditions, such as pH soil, C-organic content as well as water level.

Results of soil analysis indicated the soil condition in North Toraja had a pH ranging between 5,21-5,56; 5,31-5,56 in Maros, while 6,5-6,82 in Barru, respectively. Correlation between pH and mycorrhiza is pH determines whether nutrients is easily absorbed by plants, as phosphorus, if pH is low, the plant growth will be inhibited by low availability of essential nutrients, such as phosphorus and nitrogen [5]. [8] declared some FMA can be well multiplied, where optimal pH for FMA development are ranged between 5,6-7 for *Glomus* and 4-6 for *Gigaspora*.

C-organic content in each observed location ranged from low to intermediate level; about 1,09%-1,41% in North Toraja (low), 1,66-2,52 % in Maros (intermediate) and 1,95-2,49 % in Barru (intermediate), respectively. [5] reported a linear correlation between C-organic content and mycorrhiza number, where the higher C-organic content in the soil, the higher number of mycorrhiza is obtained since the C-organic can secure mineralization process which may provide nutrients for vesicles, arbusculars and hyphaes development symbiosis.

Conclusion

1. The observed spore types at magnolias', candlenuts' and teaks' roots were *Glomus, Gigaspora* dan *Acaulospora* spore.
2. Colonization percentage at magnolias' roots was low at 7,49%, whilst at candlenuts' and teaks' roots were intermediate about 13,88% and 27%, respectively.

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RESPONSE of PLANT GROWTH and YIELD of KENCUR (*Kaempferia galanga* L.) by APPLIED DIFFERENT SOURCE of ORGANIC MATTER

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Abstract

One of the important biodiversity in Indonesia is a medicinal plant among others kencur. Until now been released Balitro (The Research Center of Medicinal and Aromatic Plants) varieties of seed kencur National, among others, are Galesia-1, 2 and 3 with the productivity reached 16.2 tons / ha. Demand for medicinal plants is currently experiencing an increase in the cultivation kencur so good and really requires. For healthy seed production and seed production quality assessment done by using various sources of organic fertilizers in order to obtain seed-filled and survive during storage. Purpose of this study is to gain wide media suitable for the production of seed quality seed production. The assessment carried out at the experimental Malang, East Java BPTP in October 2012 to July 2013 in polybags diameter of 30 cm and 40 cm high. Assessment using a randomized block design with 6 treatments planting medium is repeated 5 times, the treatment each is (1) Soil + organic fertilizer (2) Soil + organic fertilizer + NPK (3) Soil + organic fertilizer + NPK + coconut coir (4) Soil + organic fertilizer + NPK + composted municipal waste (5) Soil + organic fertilizer + media sewage fungus (6) Soil + organic fertilizer + NPK + vermicompost. Variables observations include the number of leaves, number of shoots, length and width of the canopy, leaf area, and yields. These data analysis by F test and BNT at α=0.05. The results shows that the highest yield in treatment soil + organic fertilizer (69.44 g / polybag) and the lowest in treatment soil + organic fertilizer + NPK + composted municipal waste (51.79 g / polybag). The main pests are leaf spot disease caused by the fungus *Phyllosticta* sp.

Keywords: kencur, organic matters, seed production, biofarmaka.

Introduction

One of the important biodiversity in Indonesia is a medicinal plant among others kencur. Until now been released Balitro (The Research Center of Medicinal and Aromatic Plants) varieties of seed kencur National, among others, are Galesia-1, 2 and 3 with the productivity reached 16.2 tons / ha. Demand for medicinal plants is currently experiencing an increase in the cultivation kencur so good and really requires. The existence of the productivity gap between the yields of the farmer with the potential productivity of the yields is one indication that the application of the production technology still needs to be improved further.

Productivity medicinal crops in farmers' fields are generally still lower when compared to the potential outcome. This is partly due to the cultivation of land has not been carried out in accordance with the principles of tillage is good and right that a growing medium can not optimally support the growth of plants both in supporting the availability of nutrients and aeration for soil and plants. Besides, the maintenance of plants such as fertilization, and pest control and plant diseases have not been on time, dosage, and type [1].

Other factors that affectof the production was planting material or seed. True seed and the high quality of seed still limited. In fact, the success rate of cultivation of approximately 40% is determined by the quality of the seed. Farmers still do not handle plants for seed production intensively both in the selection of seed sources and in the maintenance of the plants until harvest. Harvesting of medicinal plants for the production of seed rhizomes different from rhizomes of plants for the production of consumption. For the purposes of the provision of seeds, seed rhizomes are harvested older than rhizome production. Using seed from the rhizome of young plants causing low production [4].

According [2] the seed has been produced on farm can not be increased on its quality because storage action seeks only to maintain the quality. That way treatment during on farm was very important in achieving good production to increase the number, quality and sustainability of the production of medical plants in need of good cultivating practices. How to cultivation medical plant including the recommended use of organic fertilizer as an important component and can be added to inorganic fertilizers to increase production. Production or yields kencur on cultivation with organic fertilizer was lower than inorganic fertilizer. However, the yields of kencur that grown with the addition of organic fertilizers

was better in quality than the cultivation with inorganic fertilizer [9]. Fertilization is one of the important technologies component in plant cultivation kencur. Inorganic fertilizer use must be followed by the use of organic fertilizers.

Inorganic fertilizer use is intended to replace the nutrients was transported along with the transport of rhizome yields. As an illustration, to produce fresh rhizomes 82.03 g/plant (equivalent to 16.41 tonnes/ha) of nutrients transported into the rhizome of 415.60 mg N/plant (equivalent to 83.12 kg N / ha), 112.50 mg P/ plant (equivalent to 22.50 kg P/ha) and 571.70 mg K/plant (equivalent to 114.34 kg K/ ha) [7].

The recommendations of inorganic fertilizer for crops kencur is 200-250 kg / ha of urea, 250-300 kg / ha SP-36 and 250-300 kg / ha KCl. Urea is given three times, namely when old plants 1, 2 and 3 months after planting, 1/3 dose respectively. Fertilizer SP-36 and KCl are given once at planting [5]. Organic fertilizer is recommended of 20-40 tonnes / ha [6]. Dose of fertilizer applied is an important factor in fertilization. If the fertilizer is given exceeds the needs of the plant would be economically inefficient and could hamper the availability of other nutrients. The research objective is to obtain information on the type of organic material capable of supporting plant growth so the yields optimally.

Methode And Materials

The research was conducted at KP Karangploso Malang, East Java-AIAT East Java in October 2012 to July 2013 in polybags diameter of 30 cm and 40 cm high. Research using a Randomized Block Design with 6 treatments planting medium was repeated 5 times, the treatment were: (1) Soil + organic fertilizer (2) Soil + organic fertilizer + NPK (3) Soil + organic fertilizer + NPK + coconut coir (4) Soil + organic fertilizer + NPK + composted municipal waste (5) Soil + organic fertilizer + media sewage fungus (6) Soil + organic fertilizer + NPK + vermicompost.

NPK fertilizer used with a dose of 15 g/polybag and given three times during the growing period, at 1 MAP (Month After Planting), 2 MAP, and 3 MAP. Weeding had done from plants 2 week after planting to 3 MAP. Control of plant pests using pesticides in accordance with the recommended dose. Harvesting was done at the plant was 9 MAP indicated with the leaves begin to dry. Observations were conducted on variable number of leaves, number of tillers, length and width of the canopy and yield per polybag. Data collected and analyzed by F test and continued with LSD at the level of $\alpha = 0.05$.

Results and Discussion

The results showed that the treatment significant at the variable of number of leaves and length of the canopy, but not significant affect at canopy width variables (Table 1).

Table 1. Effect of treatment at the number of leaves, length of the canopy and canopy width to the kencur, 2013.

Treatments	Leaves (helai)	Canopy length (cm)	Canopy width (cm)
(1) Soil+ organic fertilizer	20.80 bc	28.25 b	22.23 a
(2) Soil+ organic fertilizer + NPK	19.75 c	28.50 b	24.73 a
(3) Soil+ organic fertilizer + NPK+ coconut coir	22.50 bc	27.65 b	24.80 a
(4) Soil+ organic fertilizer + NPK+ composted municipal waste	25.50 bc	29.45 b	25.05 a
(5) Soil+ organic fertilizer +NPK+ media sewage fungus	46.40 a	33.15 a	26.28 a
(6) Soil+ organic fertilizer +NPK+ vermicompost	26.55 b	34.15 a	24.13 a
CV (%)	*16.03*	*15.87*	*14.00*

Description: The numbers in the same column followed by the same letter are not different shows on BNT ($\alpha = 5\%$).

The variables of highest number of leaves was on the treatment of soil+organic fertilizer+NPK+ media sewage fungus reached 46.40 leaves. The lowest number of leaves was on treatment without the addition of organic matter, soil+NPK+organic fertilizer. Number of leaves were lower in the treatment without the addition of organic matter (Soil+NPK+organic fertilizer) reached 19.75 leaves. The addition of organic matter and organic fertilizer into the soil, make it more friable and allow better root growth. As research results [9], that the used of organic matter in the plant rhizomes give positive effects on growth and yield. Good root growth affecting at performance of root and leaves plant.

Length of canopy on the treatment on soil + organic fertilizer + NPK + media sewage fungus reached (33.15 cm) and on soil + organic fertilizer + NPK + vermicompost (34.15 cm) were higher than other treatments. Furthermore, the treatment had no effect on the variable width of the canopy. Observation of the effect of treatment on the number of tiller and yield were in Table 2.

Table 2. Effect of treatment of the tiller number and yield of kencur, 2013.

Treatments	Number of tiller	Yields (g/ polybag)
(1) Soil+ organic fertilizer	10.10 b	69.44 a
(2) Soil+ organic fertilizer + NPK	11.05 ab	68.39 a
(3) Soil+ organic fertilizer + NPK+ coconut coir	12.85 ab	62.67 b
(4) Soil+ organic fertilizer + NPK+ composted municipal waste	12.15 ab	51.79 c
(5) Soil+ organic fertilizer +NPK+ media sewage fungus	15.35 a	58.41 ab
(6) Soil+ organic fertilizer +NPK+ vermicompost	11.15 ab	57.26 ab
CV (%)	*8.77*	*4.74*

Description: The numbers in the same column followed by the same letter are not different shows on BNT (α = 5%).

The results showed that the highest yield in the treatment of soil+organic fertilizer (69.44 g/polybag) and the lowest was at soil+organic fertilizer+NPK+ composted municipal waste (51.79 gram / polybag). Kencur on the treatment of soil treatment+organic fertilizer showed the highest production so Kusumawati et.al (2010) said that high production because of the balanced of source and sink activity.

The main pests are leaf spot disease caused by the fungus Phyllosticta sp. This pathogen attack symptoms began to appear in 7 MAP. At the beginning of this pathogen attacks cause blotches or spots on leaves. Spotting at first and growing small round or elongated with periphery dark chocolate and surrounded by a yellow circle. These patches usually found in young leaves. Patches will further enlarge and coalesce to form patches larger. If not controlled soon lead to impaired photosynthesis process. Control is done by spraying a fungicide once a week until symptoms was decline occur.

Conclusion

The treatment on this research were significant on variable number of leaves, length of canopy, number of tillers and harvest while it had no significant effect on variable width of canopy. The results showed that the highest yields in the treatment of soil+organic fertilizer (69.44 g/polybag) and the lowest in soil+organis fertilizer+NPK+ composted municipal waste (51.79 g/polybag).

The main pests are leaf spot disease caused by the fungus Phyllosticta sp. This pathogen attack symptoms began to appear in 7 MAP. At the beginning of this pathogen attacks cause blotches or spots on leaves. Spotting at first and growing small round or elongated with periphery dark chocolate and surrounded by a yellow circle.

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DEVELOPMENT of COMMERCIAL NURSERIES on SELLING PLANTS and FLOWERS in DENPASAR, BALI

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Abstract

Bali is one of tourism destination. Tourism activities always related with garden and landscape around hotels or villas. For a decade, customers have changed their mind for plants that will be planted in their place. The study was conducted to investigate what kind of plants that had been used for gardening. Field study was adapted to collect information. Some nurseries were visited and data was analyzed based on plant price, propagation and customer preferency. So far, hotels prefer kind of tree such as palm, Norfolk, grasses, frangipani, bougainvillea, and some bedding plants. For household, customers prefer orchids, rose, euphorbia, frangipani and bedding plants because easier to maintain. The price is increased due to transportation from Java to Bali. Since five years ago, nurseries had more variety for some varieties from Heliconia. At present, orchids still become favourite plants and some bedding plants that has attractive colour.

Keywords: landscape, nursery, plants

Introduction

Nursery is a term that related to plants and seedlings. In addition, nursery also applies for activity on selling plant for gardening. In horticulture, nursery meant a place where plants, young tree and seedlings are grown commercially. In Bali, nursery has an important role to support the view in hotels or villa's landscape.

Ten years ago, nurseries activity only for selling plants and propagate it vegetatively to increase number of seedlings. At this time, using this technique, nursery cannot increase income because budget to rent the land is more expensive. In Florida, nursery industry generated $3 billion in farm gate in 2005 [2]. Therefore, other kinds of work should be done in order to mix between selling plants and offer a work to design in a landscape site as a horizontal garden. Nursery usually offer different kind of plants, such as shrubs, border plants, ground cover, bedding plants, tree, ornamentals and flowering plants as soft material in landscaping. Pots in different size, garden lighting, stones, compost and others as hard material for landscape design.

Vertical gardens (green walls or living walls) can be alternative for landscaper that work in areas with limited space horizontally or want to cover unattractive site as wall in the front building or between two houses. Almost 80% of vertical gardens were constructed by institutions and in public spaces to improve aesthetics [5], such as airport in Changi Singapore, Edmonton Canada or in Mumbai [1]. Green walls is different with green facades. Green walls have growing media supported on the face of the wall. Green facades have pots or containers with media at the base of the wall and support climbing plants.

Horticulture plants were classified into (a) woody plants: tree, shrubs , ground cover; (b) herbaceous plants: flowering and vine; (c) grass or turf [4].

The study was conducted to(1) investigate what kind of plants that had been used for gardening; (2) encourage nurseries to provide various plants and seedlings; (3) give opportunities to consumers for maintaining home garden

Methods

Field study likely survey to nurseries around Denpasar, such as sampling model around Sanur areas such as nurseries on Sedap Malam, HangTuah and Hayam Wuruk street as dominant place for nurseries. Data were collected based on interview about kind of plant, price and how to propagate it that usually offered by nursery.

Results and Discussion

Based on field study, kind of plants that offered by the nurseries are almost similar, that is trees (Table 1) and shrubs (Table 2), even though the price is slightly different. All shrubs including ground covers plants are range from Rp. 2.500 to 3.000 per polybag. The tree's price is depend on type, between Rp.5.000 for Codeaum and roses. The price for bigger tree reach until almost about Rp. 800.000 for hybrid bamboos, red palm and

other exotic plants. Plant price is depend on customer's demand and trend for specific plants. During 2007, *Anthurium* 'wave of love' and adenium became popular and the price increase sharply until hundred thousand rupiahs. However 3 years later, the price dropped. One year ago, *Plumeria* sp become favourite and hybrid frangipani can be sold until almost one million for big and flowering tree

Only 2 nurseries sell orchids in pot. They are Duta Orchids and Flora Bali. The price for orchids is relative stable for Dendrobium (Rp. 15.000-25.000), Cattleya (Rp. 25.000-40.000),Phalaenopsis (Rp. 70.000-125.000), Vanda (Rp. 75.000-150.000). Sometimes, orchid seedlings also available in sterile bottle cultured (Rp. 40.000-45.000) and compots (Rp. 5.000 – Rp. 15.000). Besides selling orchids, they offer rental blooming orchids in pot for office or banks for about 1-2 months until the flowers wilted. Maintenance like watering plants was done by the nursery every 3-4 days.

Almost 50% of the nurseries offer landscaping for hotels, villa and housing. Budget for landscape is depended on wide of the area. Planting grasses is around Rp. 150.000 per m^2. Maintenance should be done by the nurseries until the plants grow well as their guarantee. In addition, transport big tree required special technique to protect plant material from physiological disorders such as water loss and heat stress especially in long journey [7]. Some methods can be applied, such as tree root balls were pressure washed or harvesting tree and then spraying a combination of water and fine granular material designed to hold water at the root zone.

Vertical garden is more famous during 5 years ago. It can be applied for high building or area with limited space. Type of plants that had been used are almost ground cover plants or hanging drop plants. Vertical garden can reduce heat [3] and pollution. Plants are planted in small pot then hanging in a frame that already built over the wall of the building. Vertical gardens can be indoors or outside, freestanding or attached to an existing wall. Green wall may be constructed of modular panels which hold a growing medium with loose media, mat media or structural media.

There are strategies practiced by nursery operators in Florida, included diversifying services and marketing [6]

Table 1. Plant type (shrubs), price and propagation in commercial nurseries around Denpasar.

No	Plant's name	Price in thousand (Rp.)	Propagation
1	Agave	40-45	Shoots division
2	*Aglaonema* sp.	50-75	Sboots division
3	*A.andreanum*	40-50	Shoots division, seed
4	*Dracaena* sp	20-50	Stem cutting
5	Aloe vera	5-10	Shoot division
6	*Sanseviera* sp.	5	Shoot division
7	*Jasminum* sp.	10-15	cutting
8	Bedding plants	2.500-3.000	cutting
9	*Adiantum* sp	10-15	Shoot division
10	*Portulaca*	2-3	Shoot division
11	*Evodia suaveolens*	45-50	Seeds
12			

Table 2. Plant type (trees), price and propagation in commercial nurseries around Denpasar.

No	Plant's name	Price in thousand (Rp.)	Propagation
1	*Bougainvillea* sp.	50-100	cutting
2	Palms	50-500	seeds
3	Frangipani	40-800	cutting, seed
4	Araucaria sp.	100-200	Cutting
5	Bonsai *Ficus* sp	500-1000	cutting
6	Euphorbia	50-100	cutting
7	Fruit trees	100-250	cutting
8	Hibiscus sp.	20-30	cutting
9	*Phaleria macrocarpa*	10-25	seed
10	*Wodyetia bifurcata*	100-250	Shoot division
11	Arecaceae	100-200	Shoot division
12	*Codeaum* sp.	5-7.5	cutting

Conclusion

Nurseries already offered lots of plant's types and varieties to consumers. Besides selling soft and hard material for landscape materials, rental potted exotic plants and blooming orchids also become routine job. Diversifying plants and kind of work can increase income to cover all production process.

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MORPHOLOGICAL CHARACTERISTICS of SOME VARIETIES of CUCUMBER (*Cucumis sativus* L.)

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Abstract

Cucumber (*C. sativus*), which belongs to the cucurbitaceae family, is largely cultivated in Indonesia. They are considered as important vegetables and the most commercially produced among other species in cucurbitaceae. Generally, they are planted conventionally in the field. However, greenhouse-based cucumber production recently becomes popular. In addition, many varieties of cucumber, which have a good performance, have been created to satisfy the customer needs. Each variety possesses particular characteristics that can be distinguished from one another. This present study aimed to characterize the morphological aspects of three commercial cucumber varieties that have been launched in Indonesian market. They are *C. sativus* var Natanz CU 531, var Semi CU 699 and var Image Baby. The morphological characteristics were analyzed descriptively using standard taxonomical key. The results showed that visible differences between three varieties could be easily observed in leaves and fruits. Natanz CU 531 and Image Baby varieties possess a pointed leaves tips. Meanwhile, leaves form of the var. Semi CU 699 performed a blunt tip. The size of the fruits also showed a significant difference in term of their size. *C. sativus* var Natanz CU 531 is longer than var Semi CU699 and ranges from approximately 28 cm and 16 cm, respectively. *C. sativus* var Image Baby possesses the minimum size of fruit with only 10 cm length.
Keywords: *Cucumis sativus*, morphology, var Natanz CU 531, var Semi CU 699 and var Image Baby

Introduction

Cucumber (*Cucumis sativus* L.) is one of popular monoecious plants, which belongs to the cucurbitaceae family. This family comprises of 90 genera and 750 species [1]. They are originally from India and are considered as the oldest vegetables cultivated in the world since 3000 years ago [2]. Recently, they are considered as important vegetables and the most commercially produced among other species in cucurbitaceae. These are mainly due to their high nutritional value. About 100 g of cucumber fruit consist of 3% carbohydrate, 1% protein and about 1.5 total fat [3]. In addition, they possess high water content, making them a good cleansing agent. The fruits are also source of calcium, phosphorus, zinc and many precursors of some vitamins including vitamin A and E. Moreover, they also have a significant amount of antioxidant substances, such as β-carotene and lutein [4].

In Indonesia, cucumber is largely cultivated especially in Java and Sumatera. Due to their economical benefit, they become popular among plant breeders. Many varieties have been created to satisfy the consumer needs. Generally, plant variety can be defined as a taxonomic rank placed below subspecies. They are usually characterized by one morphological character that can be distinguished from one another. Characterization of both morphological and physiological character is extremely important if we are going to evaluate or maintain the homogeneity of individual plant within one variety. This present study aimed to characterize the morphological aspects of three commercial cucumber varieties that have been launched in Indonesian market. They are *C. sativus* var Natanz CU 531, var Semi CU 699 and var Image Baby.

Material And Methods

Plant Material

The cucumber varieties *C. sativus* var Natanz CU 531, var Semi CU 699 and var Image Baby were obtained from the commercial market. The seeds were then soaked in warm water for approximately 2 hours to break seed dormancy. Seeds from each variety were subsequently sown in a growing media (placed in a poly bag) consisting of soil : compost (1 : 1). They were grown in greenhouse until 50-60 days. The plants were then watered regularly [5].

Morphological Analysis

Each variety was observed when they were 60 days old. The observation was conducted by analyzing the physical morphology of the leaves and fruits based on the morphological key determination [6].

Data Analysis

The observation of morphological aspects of each cucumber variety was analyzed descriptively.

Results And Discussion

Leaves Morphology

Leaves are vegetative organs, which play a significant role in plant development. They are food-producing organs and are considered as one of

the key determinant for plant survival and growth [7]. Many reports suggested that variations in leaf treats are mainly due to the process of plant adaptation against environmental condition [8]. The results of the observation displayed descriptively in the table 1.

Table 1. Morphological differences of the leaves of three *Cucumis sativus* L. varieties

Different traits	Natanz	Semi	Image
Heart-like shape (cordated leaves)	√	√	√
Tapered leaf tips	√	-	√
Blunt leaf tips	-	√	-
Serrated edge	√	√	√
Reticulated leaf veins	√	√	√
Coverage of trichome in the surface of the leaf	√	√	√
Dark green leaves	√	-	-
Light green leaves	-	√	√
Notched leaf base	√	√	√

Based on the results, major differences of the three varieties of cucumber are on the leaves shape, especially in the tip region of the leaves and leaf color. Many previous studies showed that difference in leaves shape is regulated genetically. Interestingly, the leaves shapes of certain plants have been also influenced by the environmental conditions [9]. However, many reports have showed that if genetic factor is stronger than environmental factors, the plant will maintain their phenotype according to their place of origin.

Figure 1. Leaves of *C. sativus* var. NATANZ CU 531. White arrow indicates tapered leaf tip

C. sativus var. NATANZ CU 531 has the same leaves shape with other cucumber variety (cordate), which is characterized by the heart-shaped leaves where the petiole is attached to the cleft (figure 1). However, it can be distinguished by the form of their leaf tip and the leaves color, which is *acuminatus* and dark green, respectively. Like the other cucumber variety, the leaves shape of the var. SEMI F1 CU 699 is *cordate* (figure 2). Meanwhile, the leaf tips is included to the *obstusus* or blunt leaf tips. Their color is light green.

The leaves shape of cucumber var. IMAGE BABY is similar to the other cucumber varieties - *cordate* (figure 3). Meanwhile, their leaf tip and color are *accuminatus* and light green, respectively.

Figure 2. Leaves of *C. sativus* var. SEMI F1 CU 699. Black arrow indicates blunt leaf tip

Fruit Morphology

Cucumber belongs to the monoecious plants, which have male and female flowers separately, but still on the same plant. The male flowers will come first and then followed shortly by the presence of female flowers. After pollen has been transferred to the female flowers, the fruit development will takes place. These are the result of the observation regarding to the fruit morphology of the three cucumber varieties (table 2).

Table 2. Morphological difference of the fruit of three cucumber varieties

Parameters	Natanz	Semi	Image Baby
Fruit length ± 28 cm	√	-	-
Fruit length ± 16 cm	-	√	-
Fruit length ± 10 cm	-	-	√
Fruit diameter ± 4 cm	√	√	√
Dark green color	√	-	√
Light green color	-	√	-

Based on the above table, it can be concluded that the major fruit traits that can be used to distinguish easily the character of the three cucumber varieties are the length of the fruit and its color. Previous studies have showed that the traits of the cucumber fruits are genetic-dependent. But in some cases, environmental condition might also play a significant role in determining the fruit morphology [10].

Figure 3. Leaves of *C. sativus* var. IMAGE BABY. White arrow indicates tapered leaf tip

Cucumber var. NATANZ CU 531 is generally consumed for salad. It has approximately 28 cm length and 4 cm diameter (figure 4). The fruit has densed cavity, crunchy and sweet. This variety can be easily propagated, has a dark green color of the fruit and can be harvested within 29-31 days after planting with a potential production of 70 tons per Ha. This type of cucumber is suitable to be planted in low and highland.

Figure 4. Fruit of *C. sativus* var. NATANZ CU 531

Var. SEMI CU 699 is also commobly used as salad. The fruit of this variety is smaller than var. NATANZ, with the average of length about 16 cm and having 4 cm diameter. However, the color of the fruit is lighter than var. NATANZ (figure 5). Interestingly, it has more period of harvesting than the previously described variety, which ranged from 30 to 35 days after planting. This variety is suitable to be planted in the lowland and middle.

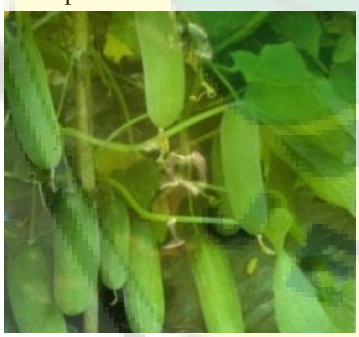

Figure 5. Fruit of *C. sativus* var. SEMI CU 699

Fruit of cucumber var. IMAGE BABY is the smallest compered with other variety described previously. It has only 10 cm length with the same diameter like the other (4 cm). It has similar color with var. NATANZ (dark green) (figure 6). They can be harvested within 28-30 days after planting.

Figure 6. Fruit of *C. sativus* var. Image Baby

Morphological characters that differ between varieties showed genetic diversity among species of *C. sativus* L. Nevertheless, these differences would be strongly influenced also by the environmental condition. Therefore, further research on the morphological and physiological aspects of these three cucumbers varieties would be interesting to be conducted to determine the pattern of response of each variety against environmental changes or stress.

Conclusion

Different varieties of cucumbers can be distinguished by the morphology of the leaves and fruit. major differences of the three varieties of cucumber are on the leaves shape, especially in the tip region of the leaves and leaf color. Meanwhile, the key determinant characters for distinguishing the fruit morphology are the fruit length and color.

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DECOMPOSITION of COFFE PULP POLYSACCHARIDES by *Aspergillus niger* EXTRASELLULER ENZYME

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Abstract

Coffee pulp is agricultural waste which amount of annual production capacity of 336 tons in Indonesia at 2012. The coffee pulp containing a major component in the form of polysaccharides consisting of cellulose, hemicellulose, lignin and C / N ratio is relatively high which causes this agricultural waste takes longer to decompose naturally. *Aspergillus niger* strains B10 MCC-00136 is a fungus that can produce a wide variety of extracellular enzymes that can be utilized for the decomposition process. *A. niger* strain B10 MCC-00136 was grown on medium coffee pulp and crude enzyme to be extracted then carried out using a reducing sugars assay method Somogy Nelson. The optimum activity was obtained on day 5th with reduction sugars produced by 29.9 ug / ml. Testing results crude production of enzymes at pH 3.5 to 6 and a temperature of 30-50 °C showed optimum activity at pH 4, 5 and a temperature of 40 °C. The results of crude enzyme hydrolysis test reaches optimum at the 18th hour with an efficiency of 4,69 %.

Keywords: *Aspergillus niger*, coffe pulp, decomposition, reducing sugar

Introduction

Coffee pulp is agricultural waste which amount of production capacity in Indonesia reached 336 tons in 2012. it contains the main components in the form of polysaccharides consisting of cellulose 18.65 %, hemicellulose 0.98 % and lignin 12 25 % [1] as well as the C / N ratio is relatively high, reaching 57,2 [2] which led to this agricultural waste takes longer to decompose naturally. In addition to the decomposition of a relatively long time, coffe pulp also are compounds such as caffeine, tannins and polyphenols which are compounds that could potentially cause contamination if it accumulates in nature.

Handling of organic wastes can be done by using microorganisms which aim to reduce pollution, reduce the amount of waste or increasing the economic value of waste [3]. Microorganisms such as bacteria and fungi capable of using organic compounds and inorganic compounds as a source of nutrients, carbon source and nitrogen source for the metabolism of primary [4]. Some mikroorganime decomposers have the ability to produce extracellular enzymes capable of hydrolyzing organic polymers. The use of microorganisms as enzyme producers has several advantages such as can be produced in a relatively short time, the production cost is relatively inexpensive, has a high growth rate as well as easy to control [5].

Aspergillus niger strain B10 MCC - 00136 is a filamentous fungus capable of producing a wide variety of extracellular enzymes that can be used for the decomposition process. Produced extracellular enzymes including proteases of *A. niger*, pectinase [6], sellulase, xylanase, invertase, amylase, and inulinase [4]. A variety of extracellular enzymes is what hopefully will be able to decomposing coffee pulp. So the goal was to determine the penilitian extracellular enzyme activity of *A. niger* in soft leather waste mendekompisisi cherries are based on the conditions of pH, temperature, and optimum incubation it..

In the determination of the optimum temperature, 500 mL of coffee fruit soft leather substrate 0.5% pH optimum of 60 mM were incubated at 37 ° C for 20 minutes and were divided into 2 groups: test and control. In the test group added 100 mL of crude enzyme in buffer 50 mM pH optimum and incubated at a temperature variation of 30 ° C - 70 ° C for 2 hours. Then added 0.5 ml of reagent Somogyi. While the control group, after incubated 20 minutes then passed back to the variation of incubation temperature of 30 ° C - 70 ° C for 2 hours. After it was added 0.5 ml of Somogyi reagent and 100 mL of crude enzyme in 50 mM pH optimum. Each then homogenized with vortex engine. Once homogeneous, the solution is heated for 15 minutes. Once cool, Nelson reagent solution was added 0.5 ml and 2.5 ml of distilled water and then dihomegenkan with vortex engine. Each of these is then taken into a 1 ml eppendorf and centrifuged at a speed of 800 rpm for 10 minutes. The supernatant is then taken and the reduction of sugar measured with a spectrophotometer wavelength of 500 nm as much as 2 repetitions. Reduction sugar test results are then compared with standard glucose curve that had been made previously.

Methods

Preparation of materials research

Substrate alkaline extract of coffe pulp

A total of 100 grams of powdered bark of the coffee pulp with water content of less than 1 % were mixed with 80 g NaOH 2 M and dissolved in 1000 ml distilled water. The resulting mixture is then homogenized using a magnetic stirrer for 24 hours. After 24 hours, the pH of the mixture was adjusted to reach 7 by adding a solution of acetic acid (CH_3COOH) little by little. After the pH to 7, followed by filtering the mixture with a filter paper to be taken filtrate. The filtrate obtained is then added 97 % ethanol with ethanol filtrate ratio is 6 : 4. The resulting mixture is then centrifuged and taken pelletnya to then dried at 50 °C.

Standard curves Glucose

Dilution series is made of 500 ug / ml stock glucose to 5 ug / ml , 10 ug / ml , 15 ug / ml , 20 ug / ml , 50 ug / ml and 75 ug / ml . A total of 0.5 ml of Somogyi Nelson reagent and 2.5 ml was added to each dilution series. Reducing sugar content measured by spectrophotometry using a wavelength of 500 nm absorbance value that will be used in the manufacture of a standard curve.

Production of crude extract enzyme

Optimization of the production of enzymes

1 ml suspension of *A. niger* strain B10 MCC - 00136 containing 5.775 x 10^9 spores/ml into 10 grams of coffee pulp substrate saturated with water, and then incubated at 30°C for 7 days and continued harvesting enzymes begin day 1 to day 7. Harvesting is done by adding 20 ml of distilled water containing 1 % NaCl and 0.1 % Sodium Azide and dishaker 10 rpm for 12 hours then filtered using filter paper. The filtrate centrifuged at a speed of 800 rpm for 10 minutes. Centrifugation supernatant was taken and tested the results of its activity using *Somogyi-Nelson* method.

Large -scale production of crude enzyme.

A total of 20 ml suspension of *A. niger* strain B10 MCC - 00136 containing 5.775 x 10^9 spores/ml into 200 grams of coffee pulp substrate saturated with water, and then incubated at 30 °C corresponding optimum incubation time of enzyme production. Harvesting is done by adding 20 ml of distilled water containing 1 % NaCl and 0.1 % Sodium Azide and dishaker 10 rpm for 12 hours then filtered using filter paper. The filtrate centrifuged at a speed of 800 rpm for 10 minutes. Centrifugation supernatant was taken and tested the results of its activity using *Somogyi - Nelson* method.

Characterization of crude enzyme ekstraseuler

Stability and optimum pH

PH stability test is divided into 2 test test test X and Y. A total of 500 mL to 500 mL using a mixed enzyme buffer 50 mM pH varisi (3 ; 3.5 ; 4 ; 4.5 ; 5 ; 5.5 ; 6 ; 6 , 5 ; 7 , 7.5, 8). While the Y test, enzyme treated using sterile distilled water of 500 mL of an enzyme that is mixed using 500 mL sterile distilled water. Each - each mixture is then incubated 4 hours at 37 ° C and reducing sugar testing methods Somogy nelson using soft leather substrate coffee fruit 0.5 % and 500 ul enzyme . After reducing sugar known test through X and Y, then the specified percent of the enzyme activity by comparing the results of reducing sugar X and Y.

In the determination of the optimum pH, 500 mL of coffee pulp substrate 0.5% pH 5 60 mM were incubated at 37 ° C for 20 minutes and were divided into 2 groups: test and control. In the test group added 100 mL of crude enzyme in 50 mM buffer pH variations and incubated at 37 ° C for 2 hours. Then added 0.5 ml of reagent Somogyi. While the control group, after incubated 20 minutes then passed back incubation at 37 ° C for 2 hours. After it was added 0.5 ml of Somogyi reagent and 100 mL of crude enzyme in 50 mM buffer pH variations. Each then homogenized with vortex engine.

Once homogeneous, the solution is heated in a water bath for 15 minutes. Once cool, Nelson reagent solution was added 0.5 ml and 2.5 ml of distilled water and then dihomegenkan with vortex engine. Each of these is then taken into a 1 ml eppendorf and centrifuged at a speed of 800 rpm for 10 minutes. The supernatant is then taken and the reduction of sugar measured with a spectrophotometer wavelength of 500 nm as much as 2 repetitions. Reduction sugar test results are then compared with standard glucose curve that had been made previously.

stability and optimum temperature

Temperature stability test is divided into 2 test, X and Y. 500 mL to 500 mL of enzyme in buffer in 50 mM buffer pH optimum at X incubated test using predetermined temperature variation that is a temperature of 30 °C , 35 °C , 40 °C , 45 °C , 50 °C , 55 °C , 60 °C , 65 °C and 70 °C, while the Y test, 500 mL to 500 mL of enzyme in buffer in 50 mM buffer pH optimum directly tested shortly after being taken from the place storage at -20 °C. Test X performed with reducing sugar analysis using Somogyi - Nelson method using soft leather substrate coffee fruit 0.5 % and 500 ul enzyme. After reducing sugar known test through X and Y, then the specified percent of the enzyme activity by comparing the results of reducing sugar X and Y.

In the determination of the optimum temperature, 500 mL of coffee pulp substrate 0.5% pH optimum of 60 mM were incubated at 37 °C for 20 minutes and were divided into 2 groups: test and control. In the test group added 100 mL of crude

enzyme in buffer 50 mM pH optimum and incubated at a temperature variation of 30 °C - 70 °C for 2 hours. Then added 0.5 ml of reagent Somogyi. While the control group, after incubated 20 minutes then passed back to the variation of incubation temperature of 30 °C - 70 °C for 2 hours. After it was added 0.5 ml of Somogyi reagent and 100 mL of crude enzyme in 50 mM pH optimum. Each then homogenized with vortex engine. Once homogeneous, the solution is heated for 15 minutes. Once cool, Nelson reagent solution was added 0.5 ml and 2.5 ml of distilled water and then homogenized with vortex engine. Each of these is then taken into a 1 ml eppendorf and centrifuged at a speed of 800 rpm for 10 minutes. The supernatant is then taken and the reduction of sugar measured with a spectrophotometer wavelength of 500 nm as much as 2 repetitions. Reduction sugar test results are then compared with standard glucose curve that had been made previously.

Optimization of Coffee Fruit Soft Skin Hydrolysis by Enzyme Ekstraselules A. niger

The determination of the optimum time of hydrolysis was conducted to determine the highest activity of A. niger extracellular enzymes in decomposing waste coffee fruit soft skin over a certain period. A total of 2.5 grams of soft fruit skin smooth substrates coffee in 50 ml crude enzyme and added 500 ul Na Azide 1 %, incubated at 37 ° C for 72 hours. Sampling for the reduction of glucose testing is done by taking 1 ml campuaran substrate and enzyme incubation time stretcher at 0, 6, 12, 18, 24, 30, 36, 48, 60 and 72 hours . Each sample was then tested the reducing sugar by Somogyi - Nelson method. Efisisiensi hydrolysis was calculated using the formula :

$$\text{hydrolisis effiency} = \frac{\sum reduction\ sugars\ produced}{\sum \text{Substrate of polysaccharides}} \text{x100 \%}$$

Result

Optimization of enzyme production

Optimizations have been made known that the day-to - 5 is the optimum incubation time for enzyme production . Total reducing sugar produced on day 5 reached 29.9 ug / ml as seen on the curve 4.2

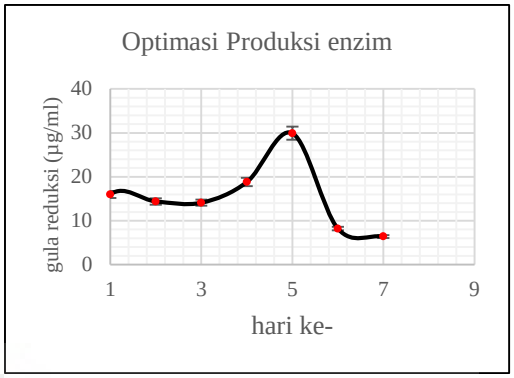

Figure 1. Curve optimization of the production of crude enzyme *A. niger* in media coffee pulp.

The amount of reducing sugar on the first day to the third day tends decreased from 16 pg / ml on day 1 to 14,1 ug/ml on the 3rd day. This happens because the isolates of *A. niger* are still utilizing the reducing sugar is in coffee pulp as a source of carbon. According to [7] on the skin of the cherries are soft sugar reduction of 12.4 % dry weight. A more modest reduction in sugar will tend to be used first by the *A. niger* for its metabolism before eventually break down more complex substrate which in this case is the polysaccharides in the coffee skin, so that the amount of enzyme breaker automatically polysaccharide produced is also relatively less. [8] in his research on the influence of several carbon sources on the growth of fungi, said fungi will tend to utilize a simpler carbon source first.

Stability and Optimum pH

Crude enzyme A. niger has a stable activity at pH has stabilized activity in the range pH 3.5 - 6, while the optimum activity is at pH 4.5 as depicted in the curve below.

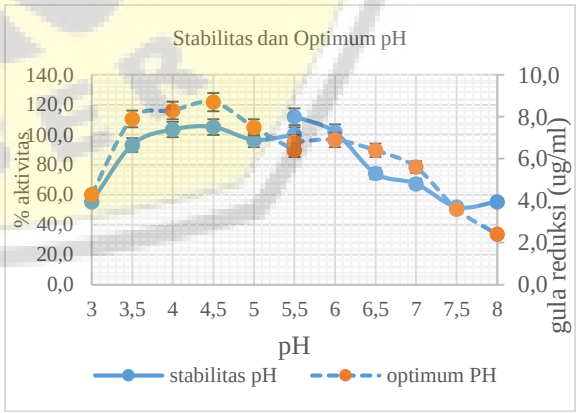

Figure 2. Curve stability and optimum enzyme activity of *A. niger* crude to pH.

On the curve above it can be seen that the enzyme activity is stable in the pH range from 3.5 to 6 and beyond the range of their activities tend to be lower. It can be seen from the % activity of the enzyme. At pH 3, % of enzyme activity reached

103.4 %. Then, at pH 3.5 the enzyme activity increased to 105.2%. Enzyme activity began to show its stability to pH 6 with activity 101.7% which also indicates that the enzyme is in the range of pH. However, At pH 6.5 to 8 relative enzyme activity began to decline with activity only amounted to 74.1 % at pH 6.5 and 55.2 % at pH 8. The enzyme has a structure that is very sensitive to changes in pH. pH that is too acidic or too alkaline will cause ionization of the active site of enzymes that will reduce or even inhibit the enzyme activity. Enzyme activity increases concurrently increase in the pH of the environment until it reaches its maximum activity or the so-called pH optimum, then fell back when the pH the more alkaline environment [9].

Decrease in enzyme activity due to the pH environment according to [10] due to the enzyme is charged molecules, changes in pH that is going to change the charge on the areas that govern the bonding between the substrate and the enzyme that causes the activity will be reduced. PH changes may also cause changes in the charge on the areas far from the active site of enzymes that may play a role in maintaining or tertiary quaternary structures of the enzyme active site. By changing this structure, the structure will change and inhibit enzyme substrate binding [10].

On the curve 4.3 can also be seen that the highest enzyme activity is at pH 4.5 with the amount of reducing sugar output reached 8.7 ug/ml. Its activity has continued to decline relative to pH 8 as seen from the amount of reducing sugar produced only by 2.4 ug/ml. In the study [1] Please also note that the fermentation activity of *A. niger* in coffee pulp also decline little by little towards the end pH of 4.5. [11] reported that the optimum activity sellulase *A. niger* is the range pH 4,5 - high as 7,5. Meanwhile, [12] saying that sellulase activity of *A. niger* showed high activity at pH 4 and then continued to decline until the activity close to 0 ug ml while the pH 9.

Stability and Optimum Temperature

Based on the analysis of the activity of the enzyme is known that the temperature of crude *A. niger* in media activities coffee pulp is stable at a temperature range of 30-50 °C, while the optimum activity is at a temperature of 40 °C, as it is seemingly on the curve below

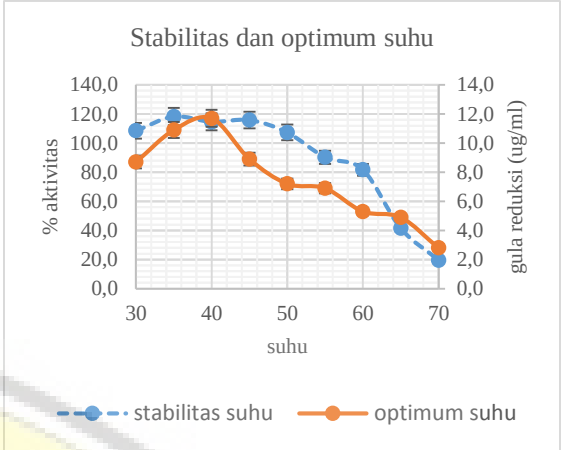

Figure 3. Curve stability and optimum enzyme activity of crude *A. niger* on media suede coffee fruit.

As can be seen on the curve that the activity of the enzyme produced crude stable in the pH range of 30-50 °C. It is seen from the % activity of the enzyme that is relatively stable in the temperature range that the temperature of 30 °C activity reached 108.5%, at a temperature 35 °C reaches 118.3%, amounting to 114.5% of enzyme activity at a temperature of 40 °C, then at a temperature of 45 °C and 50 °C the enzyme showed activity amounted to 115.9 % and 107,3 %. Enzyme activity then decreased gradually as the temperature rises above 50 °C, the enzyme activity by 90,2% at a temperature of 55 °C were reduced to only 19,5% at 70 °C. The enzyme basically can be a pure protein, or is the result of a combination of proteins with other chemical cluster [13]. Such as proteins, enzymes also have a high sensitivity to temperature. The temperature is too low will reduce or even stop their activities, while the temperature is too high will denature or destroy most enzymes [13]. In the structure of enzymes which are part thermosensitive easily undergo denaturation when exposed to temperatures above an area of stability, even if any chemical reaction can be increased concurrently increase in temperature to some extent [9].

[6] said that the *A. niger* has a temperature range of approximately between 6 °C- 47 °C and automatically influence the temperature range in which the enzymes produced work. Point optimum enzyme activity is at a temperature of 40 °C with the amount of reducing sugar produced was 11,7 ug/ml. Then from the optimum point, reducing sugar produced become 8,9 ug/ml at a temperature of 45 °C to be only 2,8 ug/ml of reducing sugar produced at a temperature of 70 °C decrease in this activity. Based on the research [12] activity sellulase of *A. niger* are also reported to decrease after passing a temperature of 50 °C. Based on the above crude enzyme known that *A. niger* is a species that berisifat mesophilic [14].

Coffee pulp hydrolysis by A. niger strain B10 MCC – 00136.

The highest hydrolysis activity is on the clock to- 18 with a reducing sugar produced by 1039 ug/ml with an efficiency of 4.69 % of the total of 2.5 grams substrate. These values are relatively large when compared to the crude enzyme hydrolysis efficiency of *A. niger* on organic wastes such as oil palm empty fruit bunches (EFB) as in [15] which only reached 1,5 % with a reducing sugar of 756 ug/ml.

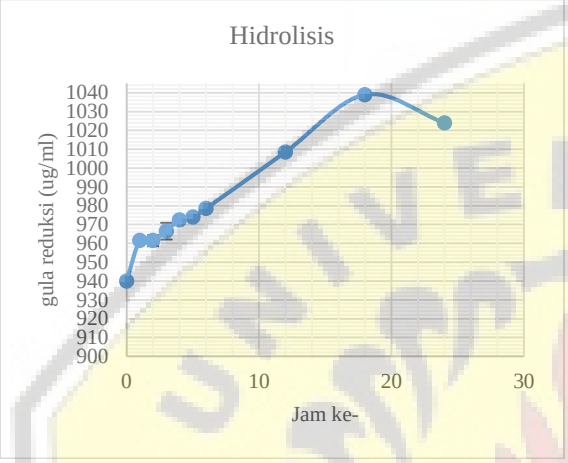

Figure 4. Curve hydrolysis crude *A. niger* extracellular enzymes on the coffee pulp substrate.

On the curve above it can be seen that the crude enzyme hydrolysis activity of extracellular *A. niger* against coffee pulp has increased along with the increase of incubation time to reach the peak at 18 hours incubation time. Then the activity began to decline in the hours to 24, this reduction may occur because due to the feedback mechanism inhibitor, which is the end product of a metabolic path which can stop its own synthesis by inhibiting the activity of one enzyme at the beginning of the track biosyntetic it [13]. According to [16] mentioned that the presence of metabolites such as glucose products will inhibit the enzyme activity lignoselulolitic particularly cellulose degrading manner allosterik attached to the side so that the active enzyme can no longer occupied by the substrate .

Conclusion

Results of the research that has been done can be seen that the *A. niger* strain B10 MCC - 00136 is able to decompose the coffee pulp. Production of enzyme with maximum activity on day 5th with the amount of reducing sugar produced by 29,9 ug/ml. Crude enzyme activity of *A. niger* strain B10 MCC - 00136 is stable at pH 3,5 to 6 with optimum activity at pH 4,5 and the enzyme was also stable at a temperature of 30-50 °C with optimum activity at a temperature of 40 °C. The test results *A. niger* crude enzyme hydrolysis showed that the hydrolysis process reaches optimum at the 18th hour with an efficiency of 4,69 %.

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PRODUCTION SINGLE CELL PROTEINS *Saccharomyces cerevisiae* USING PRODUCT HYDROLYSIS JATROPHA CURCAS CERNEL CAKE FERMENTATION by *Aspergillus niger*

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Abstract

S. cerevisiae can production by using media hydrolysis Jatropha cernel cake yield solid state fermentation by *Aspergillus niger*. Treatment fermentation of jatropha cernel cake with using *Aspergillus niger* can to uptake reduction sugar until 88,87% equal with first concentration before fermentation. In this research succeed growing S. cereviceae in media hydrolysis that. Determination optimum concentration and time for production S. cereviceae in media hydrolysis jatropha cernel cake has been found that in optimum concentration 632.727 µg/ml and time optimum is 60 hour with total cell until 22,756498 x 10^6/ml.

Keywords: *Saccharomyces cerevisiae*, *Jatropha curcas* cernel cake, fermentation, *Aspergillus niger*

Introduction

Jatropha curcas cernel cake (jatropha curcas L) are belongs industrial waste from product manufacture jatropha seed oil. In Indonesia, area crop jatropha curcas around 68.200 hectare in 2007 year with production reach 7.852 ton and advance production in 2009 year reach 8.013 ton (Syakir, 2010). In this production 30% became jatropha seed oil and jatropha cernel cake waste for another. Employing of *Jatropha curcas* cernel cake in Indonesia only became material biobriket not yet optimum to increase addition value jatropha waste (Fahmi, 2013) and employing for animal feed can consequently poisoning because obtain toxin as tannin, alkaloid and saponin (Sanusi *et al*., 2013).

Single cell proteins are manifestation resources high protein production can be used for protein supplement human although for animal. In some single cell proteins, *S. cerevisiae* is contains simple fat, proteins and carbohydrate in absorbed, well, and not toxic (Purwitasari *et al*., 2004). Production *S. cerevisiae* common in general media *Yeast Extract Peptone Dextrose* (YEPD) and *Yeast Extract Peptone Glycerol* (YEPG) (Goeddel, 1990; Purwitasari *et al*., 2004), but also capable growing in easy media as industrial waste. *S. cerevisiae* can growing in fruit shell waste (Wilkins *et al.*, 2007), cassava starch waste capable growing *S. cerevisiae* with high quality (Ejiofor *et al*., 1996), and soybean molasses waste (Siqueira *et al*., 2008).

Jatropha curcas cernel cake are nutrition resources for growing Single cell proteins have less effective because contains compound toxic and high fiber (Tjakradidjaja, 2007; Mahajati, 2008), so needed detoxification and hydrolysis with enzyme microbe. Some microbe have extra-celluler enzyme, but *A. niger* have ability for detoxification compound antinutrition in cake (Belewu *et al*., 2010) and high efectivity for reduction celullosa become glucose because produce high β-glucosidase (Juhasz, 2003; Safaria *et al*., 2013). Some research about solid-state fermentation by *A. niger* can be able to increase release nitrogen into Jatropha cernel cake. So, for increase employing jatropha cernel cake waste be needed hydrolysis by extra-celluler enzyme *A. niger* and then for production single cell proteins *S. cerevisiae.*

Materials and Methods

Collection and processing *Jatropha curcas* cernel cake

Jatropha curcas carnel cake from Indonesian Gresik cement foundation pounding until like pebble and then dry in the sun until 24 hours. After this, jatropha curcas cernel cake can use for material substrate water saturated.

Collection and Pre-culture Isolate Used

Isolate *A. niger* including to o 10 ml media PDA into petridish in streak plate, After this incubation 3 days in temperature 30°C. Futhermore isolate subculture in 5 ml oblique PDA into reaction tube, and incubation until 3 days in temperature 30°C for stoke mold isolate.

S. cerevisiae to get from association knowledge and application technology including to 10 ml media YEPD with streak plate, and then incubation until 24 hours in temperature 30°C for stoke single cell protein isolate.

Fermentation *Jatropha curcas* Cernel Cake Using *A. Niger*

Production hydrolysis filtrate from fermentation in high scale with using 50 gram jatropha cernel cake saturation water included by 5 ml suspension inoculum *A. niger* age 4 days then incubation in temperature 30°C until 4 days. Futhermore, extraction with H_2O equivalent *Jatropha curcas* cernel cake 1:4 and then shaker until 6 hours for mixed soluble. Soluble filtration with paper filter until to get filtrate and then centrifugation in 4000 rpm until 10 minute for separated filtrate with pellet. Hydrolysat filtration with fiber filter 0,2 µm into cool condition and then product filtration incubation in -20°C.

Analysis Concentration First Reduction Sugar With Method Somogyi Nelson

Hydrolysis product fermentation 0,5 ml addition reagen somogyi 0,5 ml for end enzyme reaction and boiling in water steam bath until 15 minute. After not warm, addition reagen nelson for bundle reduction sugar yeald process hydrolysation substrat and then addition aquadest 2,5 ml and measure value absorbantion with spectrophotometer λ 500 nm.

Analysis Optimum Concentration and Time Incubation *S. cerevisiae* in Filtrat Jatropha Cernel Cake

Culture Isolate *S. cerevisiae* incubation 3 days including 100 µl to 20 ml filtrate hydrolysis cernel cake after in variation concentration with 2 refrain. After that, Every suspension measuring with spectrophotometer in 600 nm for first population *S. cerevisiae* and then incubation shaker until 72 hours in temperature 30°C and every 6 hours measuring absorbance with spectrophotometer for final absorbance in every time incubate. Counting total population growing in media filtrate hydrolysis jatropha cernel cake.

Analysis End Concentration Reduction Sugar With Method Somogyi Nelson

End concentration reduction sugar counting with isolate *S. cerevisiae* including to 10 ml media filtrate hydrolysis jatropa cernel cake with conditioning into optimum concentration and time then every day until optimum time production s. cerevisiae carry out shaker 4000 rpm until 20 minute for precipitate *S. cerevisiae*. Residue Filtrate addition 0,5 ml reagent somogyi for finishing enzyme reaction and then boiling into water steam bath until 15 minute. After this, addition reagent nelson 0,5 ml for bundle recidue reduction sugar and then addition aquadesh 2,5 ml and measuring value absorbance with spectrophotometer in 500 nm with 3 refrain.

Results

Concentration filtrate hydrolysis jatropha cernel cake fermentation using A. niger until optimum time

Fermentation 50 gram substrate saturated water jatropha cernel cake by A. niger with total inoculum first spora $83{,}2375x10^6$ in incubation until 4 days production raising concentration reduction sugar 297,727 µg/ml with extraction using aquadest 200 ml.

Tabel 1 yield concentration reduction sugar in optimum time

Treatment	ABS (nm)	Concentration (µg/ml)
Control	0.754	335.000
Fermentation 4 days	1.409	632.727
Fermentation 5 days	1.078	482.272

Aspergillus niger with age 4 days as first inoculum because in time incubation this already give total spora until $83{,}2375x10^6$ according (Mojsov, 2010) total spora until $6x10^6$ are optimum for inoculum process fermentation using *A. niger*. While fermentation until time optimum 4 days for product high concentration reduction sugar 632.727 with raising reduction sugar until 88,87% equal with first concentration before fermentation. According (Sa'adah, *et al* 2010) in incubation 96 hours product high activity celulace enzyme so can production reduction sugar with high concentration.

Production *S. cerevisiae* into media hydrolysis jatropha curcas cernel cake

Population S. cerevisiae in this research measuring with spectrophotometer 600 nm for observe absorbance in every time and concentration filtrate yield hydrolysis jatropha cernel cake by *Aspergillus niger*.

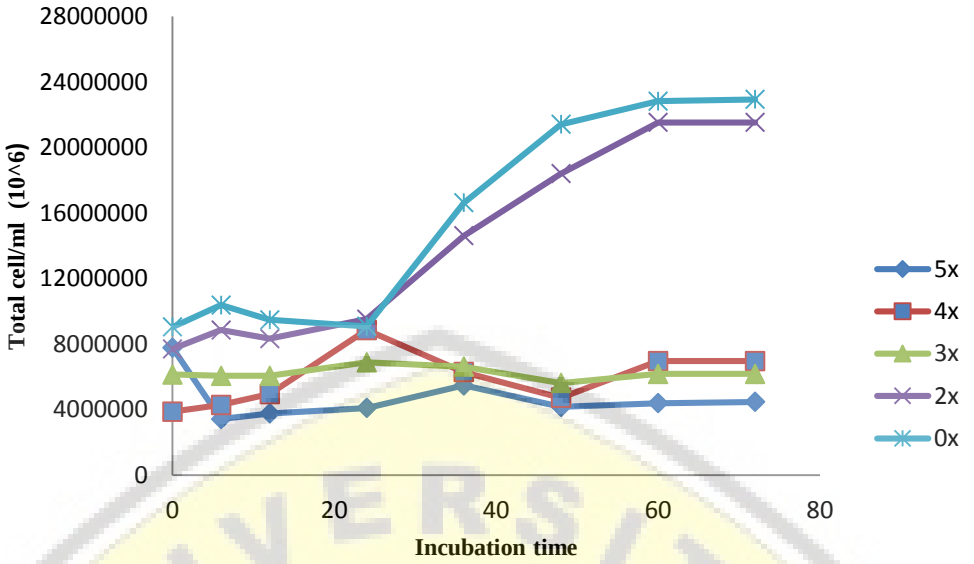


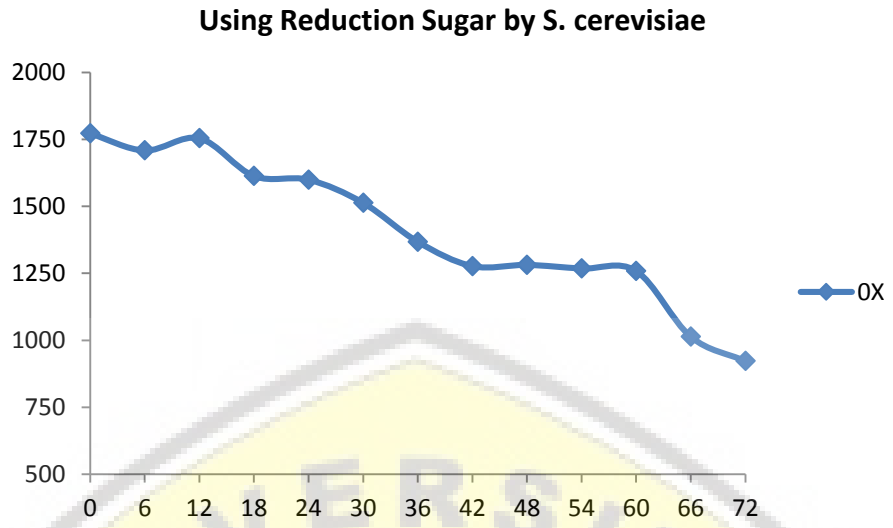
Figure 1. Kurva growth S. cerevisiae in variation Concentration and Time

S. cerevisiae can growing in media contains resource simple carbon and protein. Figure 1 describe growing *S. cerevisiae* in media hydrolysis with variation concentration and time. Every line describe variation concentration hydrolisat, while X axis for describe incubation time and Y axis total cell/ml. In curve can show optimum growing *S. cerevisiae* available in delution filtrate 0x (No delution) from first concentration and time optimum is 60 hour with total cell until 22,756498 x 10^6/ml. Decreasing total cell in lower concentration filtrate Jatroha because in lower concentration have limited nutrition so growing cell will be slow (Button , 1985). *S. cerevisiae* can used nutrition into filtrate yield hydrolysis Jatropha cernel cake for growing. Condition population *S. cerevisiae* in media filtrate can show in figure 2.

Figure 2. *S. cerevisiae* in Media Filtrate yield hydrolysis Jatropha cernel cake using *Aspergillus niger*

End Concentration Reduction Sugar in Time and Concentration Optimum

Analysis end reduction sugar can evidence *S. cerevisisae* using carbon resource in filtrate yield hydrolysis with *A. niger*. Figure 3. can describe ability *S. cerevisiae* using reduction sugar for become energy resource.



Decreasing total reduction sugar because *S. cervisiae* consumption reduction sugar such as glucose yield hydrolysis by enzyme cellulose or glucose and xylose with xylanase enzyme (Lamid, 2011).

Conclution

Fermentation until time optimum 4 days for product high concentration reduction sugar 632.727 with raising reduction sugar until 88,87% And using this hydrolysat for media production *S. cerevisiae* give highest total population until 22,756498 x 10^6/ml in delution filtrate 0x from first concentration and 60 hour.

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