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# Toxicology of Nuclear Polyhedrosis Virus, Botanical and Synthetic Pesticides on Mortality Rate of *Crocidolomia binotalis* (Zeller)

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**Abstract:**

*Crocidolomia binotalis* (Cb) (Zeller) is one of the main pests of cabbage which is difficult for farmers to control in the Ijen Crater in Indonesia. The demand for cabbage in Indonesia is increasing every year, so intensification efforts are continuously being made. Farmers intensively use synthetic pesticides as the primary control of Cb. The purpose of this study is to examine several control agents that can be used as an alternative to synthetic pesticides. Then, it provides recommendations to Ijen Crater farmers to adapt in reducing the use of synthetic pesticides. The biopesticide used was based on the Nuclear Polyhedrosis Virus strains of Spodoptera litura (SlNPV) and Helicoverpa armigera (HaNPV), botanical pesticides, and synthetic pesticides as a comparison treatment. SlNPv and HaNPV isolates were obtained from the Indonesian Sweetener and Fiber Crops Research Institute. The botanical pesticide formulas come from a combination of *Azadiracta indica, Aglaia odorata, Ageratum conyzoides* dried for one week and proposed to form flour. Then, it was extracted using 96% ethanol solvent, 0.5% tween 90 using a rotary evaporator at a temperature of 40$^{o}$C. For synthetic pesticide, the treatment used the active ingredient Permethrin 20 mgL$^{-1}$. The cb collected from the field was re-filtered in a Petri dish by providing artificial feed soaked with this material for 5 minutes. The highest mortality test results were found in the botanical pesticide formula with a value of 100% at 72 hours. Mortality in SlNPV and HaNPV tended to be stable with a maximum value of 72.02%. The results of mortality testing in the laboratory can be a recommendation for alternative control of Cb pests in cabbage plants in the field. In this case, it is an effort to reduce farmers' dependence on using synthetic pesticides.

**Keywords**: Ijen Crater, NPV, Preference, Recommendation, Sustainable.

## Introduction:

Asustainable agricultural system is one of the Food and Agriculture Organization (FAO) efforts to alleviate problems of food, production, and malnutrition[1]. One of the implementations of sustainable agriculture is based on ecology, where biological control is an example of environment friendly and conservative control[2,3]. Ijen Crater is an intensive agricultural system and production center for horticultural crops such as cabbage and potatoes in Indonesia. So far, agricultural production has been distributed throughout the islands of Java and Bali. The problem faced by farmers is the attack of *Crocidolomia binotalis* (Cb)(Zeller).

The *Crocidolomia binotalis* (Cb) is the primary pest of cabbage[4-7]. Morphologically, in the imago phase, the Cb has a wingspan of approximately 20 mm, has a filiform antenna shape, and is light brown[8]. Cb attacks during the crop formation phase until harvest. The ability to lay eggs in female Cb reaches 200-300 eggs. Cb is included in complete metamorphosis so that, in the larval phase, there could beseveral changes in size known as instars. Cb has a high level of greed, so it does not take long to finish the cabbage plant. A study reports, Cb has more eating activity along with increasing phases of his life[9].

The challenge in control today must be sustainable. Sustainabilityhas three dimensions, namely social, economic, and environmental. There are several conceptual stages in implementing sustainable agriculture. Before moving to the technical part, the transitional period of the cultivation system transition includes four aspects, including trust, infrastructure, institutional strength, and implementation methods so that these four aspects can accelerate the transition process in shifting from a cultivation system that applies the concept of sustainable agriculture[10].

Typology in the Ijen Crater area based on Rapid Rural Appraisal (RRA) is classified as intensive agriculture. The same thing is shown based on the upstream part's behavior showing similarities in the intensive use of pesticides[11,12]. The impact of the intensive use of synthetic inputs has an impact on environmental quality degradation[13]. Synthetic pesticides will cause resistance to Cb[14-16]. Currently, Indonesia is making an acceleration in producing agricultural products that are safe and free from residues. One of them is through Good Corporate Governance, which has implications for sustainable corporate performance, which has four essential values: economic sustainability performance, environmental sustainability performance, and social sustainability performance[17].

The purpose of this study is to examine the problem of Cb attack that occurred in the Ijen Crater Mountains with the control that implements a sustainable agricultural system, one of which is by using biological control agents using NPV (Nuclear Polyhedrosis Virus), botanical pesticides, and as a comparison using the usual synthetic pesticides used by farmers. NPV effectively kills target pests with specific advantages of targeting[18-20]. Meanwhile, botanical pesticides have properties as biodegradation, have toxic secondary metabolites, and are environmentally friendly[15,21].

## Materials and Methods:

### Location study

Observation and sampling of *Crocidolomia binotalis* were taken from cabbage farms in the Ijen Crater, East Java, Indonesia (Fig 1). This location is one of the centers for producing horticultural crops for distribution around Java and Bali islands. Geographically, the research location is located at an altitude of 1,100 meters above sea level and is at the coordinates Lat -7.996939 and Long 114.150269. The collected Cb wasbrought and refined at the Plant Pest Laboratory, Faculty of Agriculture, University of Jember.

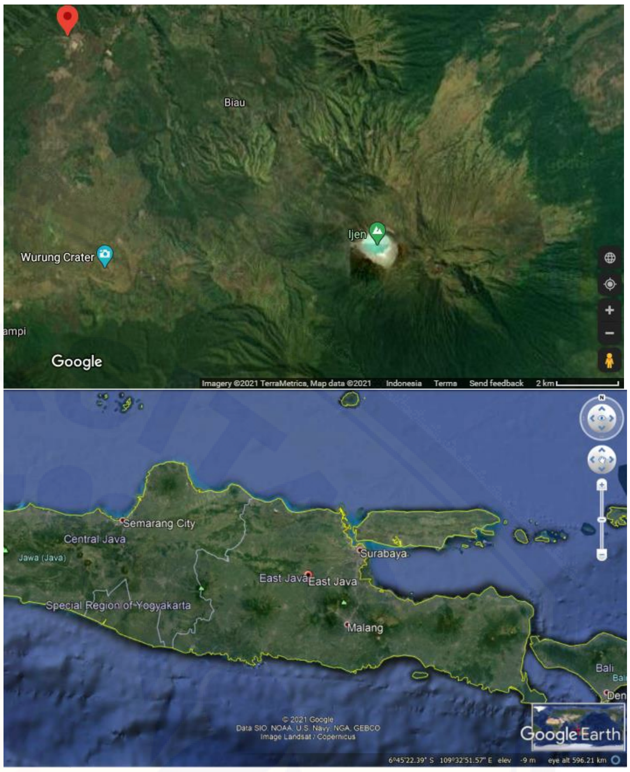


**Figure 1. Research location.**

### Preparation of isolates to control *Crocidolomia binotalis*

**NPV (Nuclear Polyhedrosis Virus).** Two types of viruses used with different strains, namely SlNPV (Spodoptera litura strain nuclear polyhedrosis virus) and HaNPV (Helicoverpa armigera stain nuclear polyhedrosis virus) were obtained from the Indonesian Sweetener and Fiber Crops Research Institute, Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture. The concentrations used were 1.59 x $10^7$ PIBs $(mL)^{-1}$ and 4.24 x $10^9$ PIBs $(mL)^{-1}$ [22].

***Botanical Pesticides.*** The botanical ingredients formula used is neem leaves (*Azadiracta indica*), Chinese henna (*Aglaia odorata*), and babandotan (*Ageratum conyzoides*)[21]. Based on the research, these ingredients are effective in controlling pests of the order Hemiptera and Lepidoptera. The ingredients are air-dried for about one week and then mashed using a blender to obtain a flour formulation. Then the extraction process used a rotary evaporator to obtain secondary metabolites. Each of these materials is weighed as much as 100 g, added 96% ethanol as much as 750 mL, and tween 90 as much as 0.5%[24]. After being homogeneous, it was incubated for 24 hours and extracted using a rotary evaporator at a temperature of 40°C for approximately 2 hours[25]. The extraction results were obtained from botanical pesticides in the form of a paste[26-28]. There are several references in the selection of botanical materials to use. The preferences used can be seen in Table. 1.

**Table 1. Preferences formula botanical pesticides effectively control pests**

| Material | Formula | Target | Method | References |
|---|---|---|---|---|
| *Azadiracta indica, Aglaia odorata, Ageratum conyzoides* | Paste | *Leptocoriza acuta* | Material is mixed with 96% methanol and extracted using a rotary evaporator | 23 |
| *Azadiracta indica* dan Monocrotophos | Oil | *Spodoptera litura* | Monocrothopos 3% mixed with variant *A. indica* with a concentration of 10%, 20%, 30%, 40%, and 50% and added Tepol 5% | 24 |
| *Ocimum basillicum* dan *Azadiracta indica* (Bioneem) | Oil | *Lymantria dispar* | Leaves were dried to air at a temperature of 22°-25°C. The essential oil composition was detected using GC MS HP 5890 | 25 |
| Muntingia *Calabura*; *Melia azedarach* dan *Azadiracta indica* | Paste; Oil | *Plutella xylustella* | Ingredients are dried in an oven at 40°C for 48 hours. Then it is macerated at a cold temperature for 72 hours using a hexane solvent. Ginseng stems and leaves of A. indica are dried at 50°C for three days and mixed with 70% methyl alcohol. The extraction was then evaporated using a solvent of 500 mL of ultrapure water containing 1 $gL^{-1}$ Triton X-1000 | 24-26 |

***Synthetic pesticides.*** The rapid rural appraisal (RRA) survey results show that the majority of farmers in the Ijen Crater Mountains use synthetic pesticides with the active ingredient Permethrin 20 $mgL^{-1}$. Spraying is done every morning and evening at intervals of 2 days.

### Experimental design and data analysis

The study was designed using a CRD (Completely Randomized Design). The experimental design can be seen in Table. 2. The method of application is by rearing third and fourth instar larvae that have been given artificial feed. Artificial feed using cabbage which is obtained from the origin from which the pest *Crocidolomia binotalis* was collected. Then the leaf pieces were soaked in each of the ingredients starting from NPV, botanical pesticides, synthetic pesticides, and comparative treatments for about 5 minutes. The leaves of the cabbage are cut into 2.5 × 2.5 cm sizes. The artificial feed is then put into a petridish which contains as many as 15 *Crocidolomia binotalis* larvae. Data analysis was done using ANOVA. If the calculation results are significantly different, then a further test is carried out using Tuckey with a confidence level of 95%. The analysis program that was used Minitab version 16.

**Table 2. Design of research experiment**

| Code | Treatment | Times observed |
|---|---|---|
| A | Concentration SlNPV 1.59 x $10^7$ PIBs $mL^{-1}$ | 24 hours; 28 hours; 72 hours; 96 hours |
| B | Concentration HaNPV 4.24 x $10^9$ PIBs $mL^{-1}$ | |
| C | Botanical pesticides with concentration 500 mL $L^{-1}$ | |
| D | Synthetic pesticides with concentration 20 $mgL^{-1}$ (Control +) | |
| E | Mineral water (-) | |

### Pathogenicity Test

Observations are made by calculating the amount of mortality (larval mortality) in Petri dish that occurs after application. A change in skin colour characterizes the dead larvae. The pathogenicity test was calculated by calculating the percentage of dead larvae with the total number of larvae in each treatment during time units ranging from 24 hours, 48 hours, 72 hours, and 96 hours.

## Results and Discussion:

Cb attacked the cabbage crop head by entering the outer leaves (Fig 2). The Cb will gnaw at the base of the cabbage head. During the development phase, Cb will lay eggs between the fruit stalks to hatch and turn into the pupa phase. Pupae have a lifespan of 7.00 ± 0.128 [29]. The pupa will be protected and covered by a white netting membrane (Fig 2a). Cb eats cabbage from the base to the tip of the outer leaves. The largest population of cabbage heads will cause crop failure.

Based on the rearing of Cb in Petri dishes, various treatments gave different visual symptoms (Fig 3). Observations were made using a Leica microscope with a magnification of 100 times. In Fig. 3, the codes (a, b, c) are visual symptoms that have been applied to botanical pesticides. The formula with a combination of *Azadiracta indica*, *Aglaia odorata*, *Ageratum conyzoides* based on test results on stink bug populations in the field is very effective in reducing population levels[23]. Therefore, the application ability of Cb Petri dish shows a brownish-yellow discoloration on the cuticle part. The position of insect mortality tends to be elongated. There are black dots along his body that are still visible. The mood of entry mechanism of botanical pesticides based on research results varies depending on the secondary metabolite compounds produced [30]. Azadirachtin compounds produced by *A. indica* can inhibit the activity of acetylcholinesterase in insects. This case is confirmed in the histopathological analysis and enzyme activity of *Nilaparvata lugens*[31].

In NPV treatment by referring to Fig. 3 with codes (d, e, f), the symptoms of morphological changes in Cb pests applied by NPV (Nuclear Polyhedrosis Virus) curved and hung. This symptom is typical in insects infected by the virus. The NPV used comes from the Indonesian Sweetener and Fiber Crops Research Institute with strains from *Spodoptera litura* NPV (SlNPV) and *Helicoverpa armigera*(HaNPV). The advantage of NPV is that it has a specific killing power of targeting[32,18,19].Viral protein will enter through insect digestion, causing insects to become paralyzed, the digestive system will be damaged, and the body size becomes smaller. Therefore, insect pests from the order Lepidoptera infected with the virus will be characterized by hanging and the body is wet and slimy. Whereas in the treatment of synthetic pesticide applications, the Cb experienced a greenish discoloration of almost the entire body surface. The active ingredient contained is Permethrin. The mechanism of action of the active ingredient is contact and stomach poison. The poison will spread starting from the digestion of the insects and spread throughout the body. The body changes color from green to dark black.


**Figure 2. Cb behavior and symptoms caused by pests in attacking cabbage crop heads. Note: A) pupa phase is found in cabbage leaf bones, B) larval phase attacks the cabbage plant flesh, C) Larvae continues to gnaw at the cabbage fruit, D) larvae will rotate around the cabbage head, and E) the cracker is marked with a hollow leaf, the emergence necrotic patches.**

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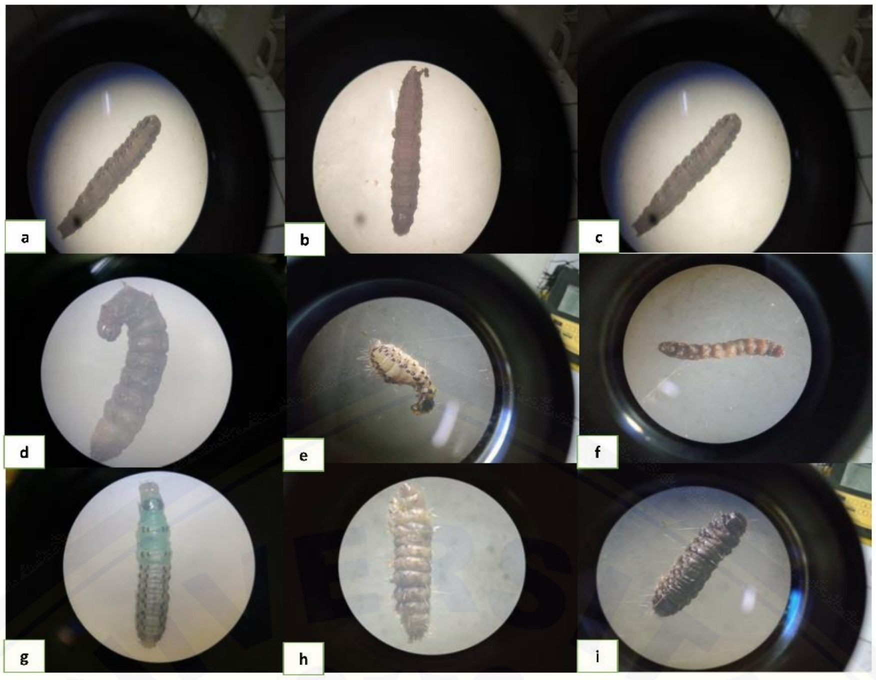


**Figure 3. Visual symptoms in Cb that have died due to several applications. Observations were made under Leica microscope with a magnification of 100 times.**

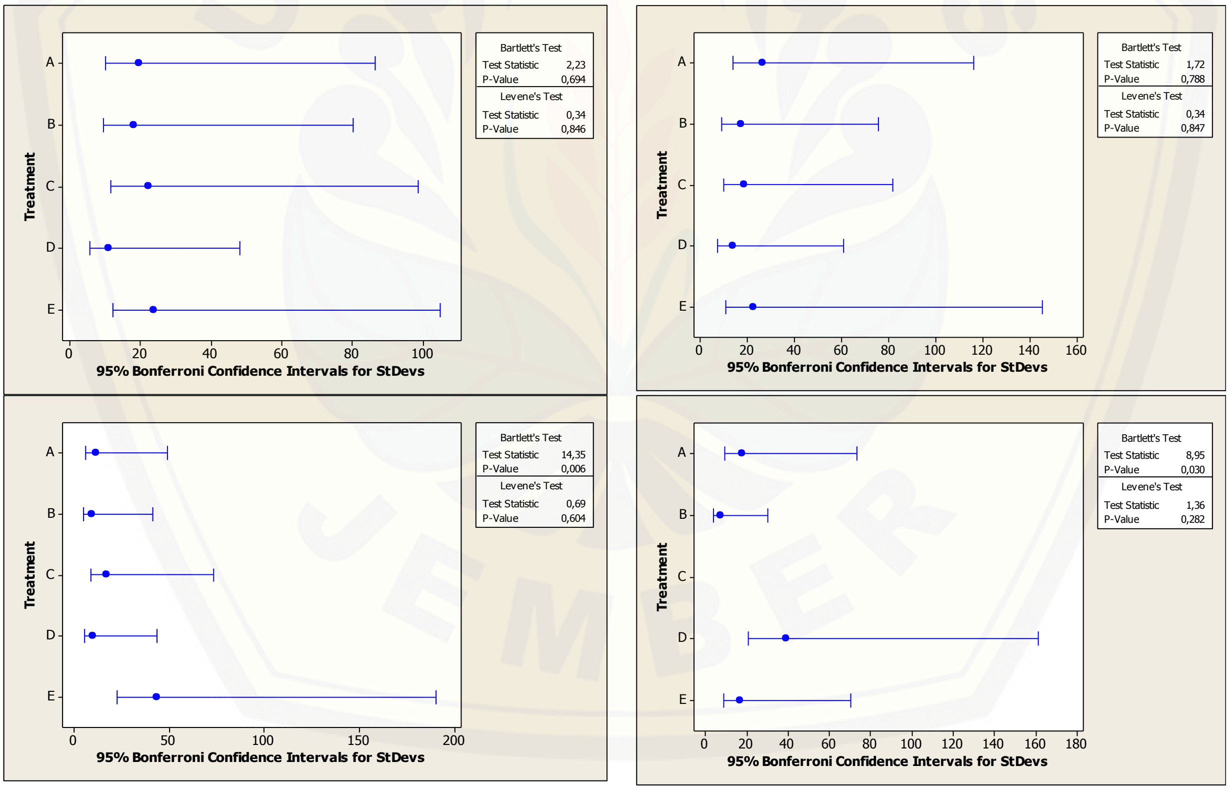


**Figure 4. Bonferanni's analysis results is based on time of 24 h, 48 h, 72 h, and 96 h.**

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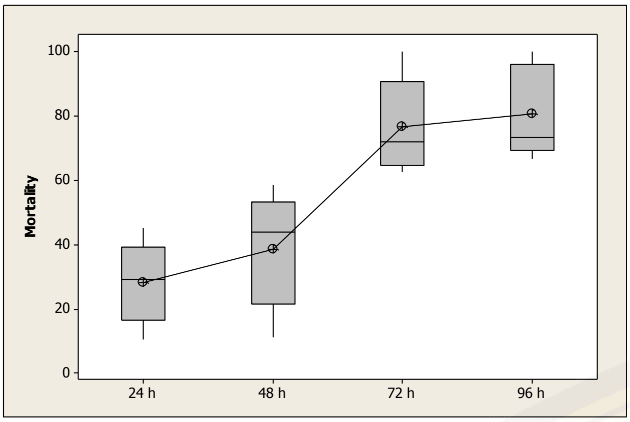


**Figure 5. The mortality rate of Cb is based on time.**

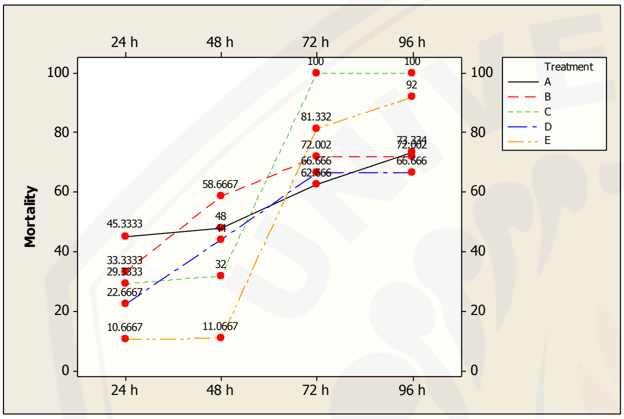


**Figure 6. Development of pest mortality based on time and treatment. Description: A) SlNPV concentration 1.59 × $10^7$ PIBs $mL^{-1}$; B) Concentration of HaNPV 4.24 × $10^9$ PIBs $mL^{-1}$; C) Botanical pesticides with a concentration of 500 mL $L^{-1}$; D) Synthetic pesticides with a concentration of 20 $mgL^{-1}$ (Control +); E) Mineral water (-).**

The ANOVA calculations on Tuckey's analysis show that the P. value is greater than the F. value (Sig) of 0.05%. So the Bonferonni analysis was carried out. The aim is to find out precisely the average between different treatments. Based on Fig. 4, the Bonferonni test results show the average mortality in each treatment against time units. The mean value based on time concluded the mean at 24 h> 48 h> 72 h> 96 h. The closer to 0, the average mortality is significant; conversely, if it is further away from the number 0, H0 is most likely accepted. Based on the calculation of the average mortality in Fig. 5, initially, at 24 hours, it can be seen that the Cb mortality rate is still low. In this case, the treatment reaction will still be accepted by the target. Meanwhile, at the 48 h and 72h, the mortality tended to increase. This is because the toxin is reacting to the insect's body. There has been a work process on insect digestion. Meanwhile, the 96 h is the climax phase of the toxin acting on the body. In the number of insect trials, the mortality rate is higher.

The Cb mortality in various treatments is presented in Fig. 6. The graph of the highest mortality rate lies in treatment C (botanical pesticides with a concentration of 500 $mlL^{-1}$). The formula used in botanical pesticides is *Azadiracta indica, Aglaia odorata, Ageratum conyzoides*[23]. The botanical pesticide formula used contains complex secondary metabolite compounds. Based on other treatments' responses, botanical pesticides have a relatively slower performance compared to NPV and synthetic pesticides. In this case, the performance of secondary metabolites that work requires reaction time in insect digestion. However, it is very effective for the death rate at 96 hours. *Aglaia odorata* contains 8-(70,80,90-propanetriol-40-methoxy-30-O-phenylpropanoid) -7-hydroxy-6-methoxycoumarin, Cyclopentatetrahydrobenzopyran, and triterpenoids[33-35]. While *Ageratum conyzoides* contains phytochemicals AC6: β-sitosterol and stigmasterol, 3-Oβ-D-glucopyranosylβ-sitosterol and precocene I, b. (E) -caryophyllene, precene I and II, 3- (2'-methylpropyl) 2-methyl-6,8-demethoxychrom-4-one, 2- (2'-methylethyl) 5,6-dimethoxybenzofuran, 2,2 -dimethylchromene-7-Oβglucopyranoside, 14-Hydroxy-2H beta3-dihydroeuparine, ageratochromene dimer, and encecanescin[36,37]. The mortality rate was as high as 100% at the time of 72 hours. Meanwhile, in other treatments, Cb still show that some are still alive.

Treatments A and B used NPV (Nuclear Polyhedrosis Virus) using SlNPV and HaNPV. In both of these treatments, the mortality rate tends to be stable compared to other treatments. Initial detection is that NPV has a higher and relatively fast killing power compared to other treatments. The process of viral particles enters the digestive tract of insects. The gp64 gene functions as a cell-binding with the endocytosis process [38]. The highest mortality rate was 72.02% at 96 hours. Whereas in treatment D (Synthetic) with the active ingredient Simpermetrin, the symptom of mortality was deficient. Farmers often use these active ingredients. The low mortality is assumed that the Cb has experienced resistance to the active ingredient. Several factors can cause pests to become resistant to an active ingredient due to the large doses and spraying frequency that exceeds the recommendation.

## Conclusion:

Based on the test results in this study, it can be concluded that the response to the use of biological control can provide a high mortality value. This study showed that Cb treated with botanical pesticides had a mortality rate of up to 100%. The test in this study can be a recommendation for Cb pest control for farmers to reduce their dependence on intensive pesticide use. The use of biological agents also minimizes pest resistance that has the potential for an outbreak.

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## Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Authors sign on ethical consideration's approval
- Ethical Clearance: The project was approved by the local ethical committee in University of Jember No.1302/UN25.8/KEPK/DL/2020.

## Authors' contributions statement:

B.H. and F.K.A. conceived of the presented idea. A.P.P. and M.H. carried out the experiment.

S.,F.K.A. and W. conceived and planned the experiments. S.P., F.K.A. and M.W.J. contributed to sample preparation. A.A.H.N. contributed to the final version of the manuscript.

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# سمية فيروس تعدد التعرق النووي والمبيدات النباتية والاصطناعية وتأثيرها على معدل وفيات *Crocidolomia binotalis* (Zeller)

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**الخلاصة:**

(Crocidolomia binotalis (Cb) (Zeller هي واحدة من الآفات الرئيسية للملفوف التي يصعب على المزارعين السيطرة عليها في منطقة بركان ايجن في إندونيسيا. يتزايد الطلب على الملفوف في إندونيسيا كل عام، لذلك يتم بذل جهود مكثفة باستمرار. يستخدم المزارعون بشكل مكثف مبيدات الآفات الاصطناعية كعنصر تحكم أساسي في Cb. ان الغرض من هذه الدراسة هو فحص العديد من عوامل التحكم التي يمكن استخدامها كبديل لمبيدات الآفات الاصطناعية. بعد ذلك، تقديم توصيات إلى مزارعي ايجنCrater للمساهمة في الحد من استخدام المبيدات الحشرية الاصطناعية. اعتمد المبيد الحيوي المستخدم على سلالات فيروس تعدد التعرق النووي من Spodoptera litura ((SlNPV)) و (Helicoverpa armigera (HaNPV)) ، ومبيدات الآفات النباتية ، ومبيدات الآفات الاصطناعية كعلاج مقارن. تم الحصول على عزلات SlNPv و HaNPV من المعهد الإندونيسي لأبحاث التحلية ومحاصيل الألياف. تأتي تركيبات مبيدات الآفات النباتية من مزيج من Azadiracta indica و Aglaia odorata و Ageratum conyzoides والتي تم تجفيفها لمدة أسبوع وتم اقتراحها لتكوين دقيق. بعد ذلك، تم استخلاصه باستخدام 96٪ مذيب إيثانول، 0.5٪ بين 90 باستخدام مبخر دوار عند درجة حرارة 40 درجة مئوية. بالنسبة لمبيدات الآفات الاصطناعية، تستخدم المعالجة باستخدام المكون الفعال بيرميثرين 20 مجم L-1. تمت إعادة ترشيح cb الذي تم جمعه من الحقل في طبق بتري عن طريق توفير علف اصطناعي تم نقعه بهذه المادة لمدة 5 دقائق. تم العثور على أعلى نتائج اختبار الوفيات في صيغة مبيدات الآفات النباتية بقيمة 100٪ في 72 ساعة. تميل الوفيات في SlNPV و HaNPV إلى الاستقرار بحد أقصى 72.02٪. يمكن أن تكون نتائج اختبار الوفيات في المختبر توصية بمكافحة بديلة لآفات Cb في نباتات الملفوف في الحقل. في هذه الحالة، يُعد هذا جهدًا لتقليل اعتماد المزارعين على استخدام مبيدات الآفات الاصطناعية.

الكلمات المفتاحية: Ijen Crater، NPV، الأفضلية، التوصية، والمستدامة.