INTERNATIONAL BIOTECHNOLOGY SEMINAR & 5th KBI Congress 2010

MALANG • 27 - 30 JULY 2010

Biotechnology :
Breakthrough for the Future of Industrial Challenges in Developing Countries

PROCEEDING

ISBN 978-979-796-158-9

Editor in Chief : Suharsono

Editors : Komang G. Wiryawan
Bambang Prasetya
Tri Muji Ermayanti
Puspita Lisdiyanti
Saptowo J. Pardal
Siswa Setyahadi
Abu Amar
Hari Eko Irianto
Wahyu Purbowasito
Hayati Minarsih
Ferry Sandra
Misri Gozan
Setiarti Sukotjo
Saidatul Idiyah
Aris Winaya

PROCEEDING

INTERNATIONAL BIOTECHNOLOGY SEMINAR AND 5th KBI CONGRESS
UNIVERSITY OF MUHAMMADIYAH MALANG, JULY 27-29, 2010

Theme:
"Biotechnology: Breakthrough for the Future of Industrial Challenges in Developing Countries"

Chief Edior:
Suharsono

Editors:
Komang G. Wiryawan
Bambang Prasetya
Tri Muji Ermawati
Puspita Lisdiyanti
Saptowo J. Pardal
Siswa Setyahadi
Abu Amar
Hari Eko Irianto
Wahyu Purbowasito
Hayati Minarsih
Ferry Sandra
Misri Gozan
Setiarti Sukotjo
Saidatul Idiyah
Aris Winaya

Organized by :

Center for Biotechnology Development, University of Muhammadiyah Malang and Konsorsium Bioteknologi Indonesia (KBI)

PROCEEDING OF THE INTERNATIONAL BIOTECHNOLOGY SEMINAR AND 5th KBI CONGRESS
JULY 27-29, 2010

ISBN: 978-979-769-158-9

Published by:
Center for Biotechnology Development, University of Muhammadiyah Malang
Jl Raya Tlogomas 246, Malang, e mail: biotechsem@yahoo.com
and Konsorsium Bioteknologi Indonesia (KBI)
Jl. Raya Bogor Km 46, Cibinong, 16911

Printed by:
Aditya Media
Jl. Bimasakti No. 19 Yogyakarta-55281
Telp. (0274) 520612, 520613

The Effects of Simulated Acid Rain on Total Protein, Total Sugar and Vitamin C of Mulberry Leaves

Jekti Prihatin[1], A. Duran Corebima[2], Ariffin[3], Abdul Gofur[2]

[1]Biology Education Department, Faculty Teacher Training & Education, University of Jember, jekti_pr@yahoo.com

[2] Biology Department, Faculty Mathematic & Science, State University of Malang

[3] Agriculture Faculty, University of Brawijaya

Abstract

An important impact of human activities on plants is acid deposition, commonly called acid rain. Acid rain is widely believed to be responsible for acidifying soil and water. The objectives of this study was to determine the effect of the simulated acid rain (SAR) exposure to total protein, total sugar and vitamin C of mulberry *Morus multicaulis* Perr. leaves. Mulberry plants as many as 180 stems were grown on pots for 8 weeks and exposed to SAR treatments of pHs 5.6, 4.6 and 3.6. As a control were used a well water of pH 7.0 and artificial acid rain without sulphuric acid of pH 6.2. Collected data were analyzed using Multivariate Anava and the significant results were continued by LSD and quadratic regression. The results showed that total protein, total sugar and vitamin C contents were decreased at SAR treatments. Level acidity (pH) 6.2 and duration of watering SAR 4 weeks has highest biochemical leaf as observed, except protein. The highest protein was showed on watering SAR 6 weeks in mature leaf of pH 6.2 (6.32%). The highest total sugar in SAR treatments was 8.28% in young leaf and vitamin C was 167.46 mg/100 g sample in mature leaf for watering 4 weeks. While coefficient regressions of effect acidity level on protein, sugar and vitamin C contents were 0.61; 0.64; 0.80 respectively. Plants watered with well water and artificial acid rain of pH 6.2 exhibited higher biochemical content, as evidenced by mean total protein, total sugar and vitamin C plant leaf, than plants watered simulated acid rain.

Key words: Simulated Acid Rain, total protein, total sugar, vitamin C, Mulberry leaf.

Introduction

Acidic deposition results from the combustion of fossil fuels, which provides heat and/or energy. When coal, oil, or other fossil fuels are burned, acid-rain precursors are emitted into the atmosphere. These include nitrogen oxides (NO_x) and sulfur dioxide (SO_2). Once in the atmosphere, NO_x and SO_2 are transformed, depending upon atmospheric conditions, into acid nitrate and acid sulfate, otherwise known as nitric acid and sulfuric acid, and fall back to earth in rain, snow, fog, cloud water, particles, and gases (NYSERDA, 2005).

Acid rain can adversely affect plants in many ways including: 1) damaging leaves directly by damaging the protective waxy coating which allows acids to diffuse into them, interrupting the evaporation of water and gas exchange; 2) litter and soil leaching and element uptake by root (Haines & Swank, 1988); 3) releasing aluminum ions attached to insoluble soil particles which then hinder the uptake and use of nutrients and water by the plant; 4) leaching essential metal nutrients (such as potassium, calcium, and magnesium) from topsoil (Cronan, 1991; Schaberg *et al.*, 2000); (5) generally weakening plants such that they become more susceptible to damage from insects, disease, drought, or environmental extremes (Haines & Swank, 1988); and 6) caused considerable changes both in photosystem 2 functional activity (Velokova and Yordanov, 1996).

Less people aware that pollutants as sulfur dioxide and nitrogen oxides contain essential macronutrients, nitrogen and sulfur respectively, thus having some beneficial nutritional effects on crops growing in nutrient-poor soils. This area of 'pollution-science' has not been adequately studied and the literature is scattered (Gupta, 2005).

Mulberry (*Morus sp.*) leaves are the only source of nutrition for silkworm. The growth and development of the larva and subsequent cocoon production are very influenced by its nutritive value (Gosh *et al.*, 2003). Effects of acid rain to mulberry plant is very important to study. The objectives of this study was to determine the effect of the simulated acid rain (SAR) exposure to total protein, total sugar and vitamin C of mulberry *Morus multicaulis* Perr. leaves.

Methods

The research was conducted at Field Laboratory of Agricultural Department of East Java Province, at Mulyoagung Dau Malang, Indonesia on October until December 2009. The experimental design in the research was factorial randomized complete block design with two factors. The first variable of acidity level of simulated acid rain treatments were pHs of 5.6, 4.6, 3.6 and control of artificial rain water (pH 6.2), and control of well water (pH 7.0). Meanwhile, the second variables were artificial acid rain watering for two, four, six, and eight weeks. As a whole there were twenty combinations, each with three replications. Each replication consisted of three plants. Therefore, mulberry plants are required to study as many as 180 plants. Total protein, total sugar, and vitamin C in young leaves and mature leaves were observed and analyzed. Four month-old vegetative twigs of selected Mulberry plant of *Morus multicaulis* Perr. was used in the research.

Artificial acid rain formulation using observation results of polluted rain at Sidoarjo, Jawa Timur on March until April 2009 as follow:

Table 1. Formulation of Artificial Acid Rain

Chemicals	Contents (mg/L demineralization water)
Ammonium sulphate - $(NH_4)_2SO_4$	7.2538
Natrium sulphate - Na_2SO_4	9.8040
Magnesium sulphate - $MgSO_4$	8.3009
Calcium Chloride - $CaCl_2$	13.4125
Kalium Nitrate - KNO_3	4.9897
Natrium Nitrate - $NaNO_3$	3.2459
Natrium Fluoride – NaF	0.3525
Magnesium Chloride - $MgCl_2$	2.1493
Demineralization water (TDS < 1.7)	

An amount of H_2SO_4 was added to bring the initial solution to pH 5.6, 4.6, or 3.6. Artificial rain with pH 6.2 (without H_2SO_4) and well water with pH 7.0 were used as a control. The pH value of all solutions were checked with a digital pHmeter. Mulberry was watered every two days for two, four, six and eight weeks with approximately 500 ml per polybag. Biochemical content of young and mature leaves were measured, according to AOAC (1988).

Protein content was analyzed by the Semi-Micro-Kjeldahl. A total of 2 g of pulverized leaf was inserted into a 500 ml Kjeldahl flask and add 5 ml of H_2SO_4 (93-98% free of N). Added 5 g of a mixture of Na_2SO_4-HgO (20:1) and 10 ml of distilled water. The mixture is boiled until clear (approximately four hours) and reboiling for 30 minutes. After added 35 ml cold distilled water and added 8.5 mL 45% NaOH solution were followed by distillation. Distillate is collected in 6.5 ml solution containing boric acid (H_3BO_3 4%) and a few drops of the indicator methyl red / methylene blue. As many as 25 ml distillate was titrated with 0.02 N HCl. Total protein was calculated using the following formula.

$$\text{Protein (\%)} = \frac{\text{ml titration} \times \text{N HCl} \times 14.008 \times 6.25 \times 100\%}{\text{g sample} \times 1000}$$

Total sugar was measured by Anthrone method using spectrophotometry. Leaf samples are crushed and given 10 g of distilled water until it reaches the volume of 100 ml and then filtered. As many as 5 ml of the filtrate were then given 10 ml of anthrone reagent and heated in a waterbath at 100°C for 10 minutes. Appear green color as a sign of total sugar, then cooled quickly in a glass beaker containing cold water. After Cu_2O (red) was formed, then was transferred to cuvette and shakered until homogeneous. Further on the value of absorbance using UV-vis spectrophotometer at a wavelength of 620 nm with a red mark was observed. Standard glucose was used as a standard.

Vitamin C content was estimated by titrametric method (Method-Iodometri Jacŏb). Leaf samples were mashed with a mortal and weighed. As many as 10 g crushed leaves were added with distilled water and stirred until homogeneous. The solution was filtered until reached 100 ml. A total of 25 ml of the filtrate were added with 0.5 ml of 1% starch indicator. Subsequently, the filtrate was titrated with 0.01 N I_2 standard solution until the color change to blue was remain for 10-15 seconds.

$$\text{Vitamin C (mg/100g)} = \frac{\text{ml titration} \times 0.88}{\text{mass of sample}} \times \frac{\text{volume of solution total}}{\text{volume of titrated}} \times 100$$

Data was analyzed using a Multivariate ANOVA by statistical analysis software SPSS 16. If the analysis of the data shows there are significant differences, then continued with the least significant difference test (LSD) 5%. Moreover, to know the effect of treatment at pH 5.6, 4.6, and 3.6 was conducted regression analysis on exposure 2, 4, 6, and 8 weeks.

Results and Discussion

This research has shown a decrease biochemical content of mulberry *Morus multicaulis* Perr. leaves, especially on total protein, total sugar, and vitamin C due to the effect of acid rain. Simulated acid rain on weak acidity (pH 6.2) showed the highest result compared with the biochemical content of pH 5.6, 4.6, and 3.6. In addition, acid rain watering for 4 weeks showed a higher value than watering for 2, 6, and 8 weeks. This was indicated by the highest total protein, total sugar and vitamin C in Mulberry young leaf were 5.5%, 8.28%, and 161.07 mg/100g, respectively. While in mature leaf, the same parameters showed the highest result were 5.36%, 7. 71%, and 167.46 mg/100g.

Anova analysis showed that significant results were appear on all treatments, except interaction between treatments on vitamin C of mature leaf, with F value 1,94; df 12. The complete data was shown on Table 2.

Table 2. Biochemical Content (M±SD) of Mulberry Leaves on Simulated Acid Rain

Treatments		Total Protein (%)		Total Sugar (%)		Vitamin C (mg/100 g)	
		Young Leaf	Mature Leaf	Young Leaf	Mature Leaf	Young Leaf	Mature Leaf
Watering for 2 weeks	Well water, pH 7.0	4.84 ± 0.05hi	4.99 ± 0.02klm	7.84 ± 0.17hi	7.39 ± 0.12j	148.47 ± 1.06ij	161.49 ± 1.91
	Artificial rain, pH 6.2	5.01 ± 0.06 k	5.17 ± 0.03n	8.04 ± 0.02j	7.66 ± 0.07kl	141.65 ± 2.87fg	164.67 ± 0.99
	pH 5.6	4.76 ± 0.08gh	4.92 ± 0.03k	7.80 ± 0.18gh	7.44 ± 0.14jk	145.63 ± 3.59hi	154.64 ± 1.83
	pH 4.6	4.44 ± 0.04e	4.59 ± 0.08ij	7.02 ± 0.19e	6.66 ± 0.17gh	133.52 ± 1.83d	147.28 ± 2.03

	pH 3.6	4.12 ± 0.13c	4.38 ± 0.03h	6.25 ± 0.09c	5.92 ± 0.18e	109.52 ± 1.12b	123.94 ± 3.80
Watering for 4 weeks	Well water, pH 7.0	4.94 ± 0.03ijk	5.01 ± 0.01lm	7.94 ± 0.12ij	7.36 ± 0.10j	153.91 ± 0.91k	151.65 ± 2.11
	Artificial rain, pH 6.2	5.40 ± 0.02m	5.36 ± 0.06o	8.28 ± 0.06k	7.71 ± 0.14l	161.07 ± 1.92l	167.46 ± 1.11
	pH 5.6	5.00 ± 0.08jk	4.97 ± 0.01kl	8.09 ± 0.07jk	7.55 ± 0.21jkl	156.90 ± 2.19k	157.63 ± 2.66
	pH 4.6	4.67 ± 0.11g	4.63 ± 0.02j	7.37 ± 0.05f	6.79 ± 0.12hi	144.55 ± 2.07hi	149.22 ± 0.12
	pH 3.6	4.36 ± 0.07de	4.25 ± 0.04g	6.51 ± 0.07d	5.76 ± 0.02e	121.15 ± 3.66c	127.57 ± 0.88
Watering for 6 weeks	Well water, pH 7.0	4.9 ± 0.00ij	4.66 ± 0.03j	7.74 ± 0.05gh	7.02 ± 0.11i	149.33 ± 0.21j	130.14 ± 1.66
	Artificial rain, pH 6.2	5.19 ± 0.07l	5.05 ± 0.04m	8.05 ± 0.04j	7.41 ± 0.15j	156.32 ± 1.80k	138.78 ± 3.34
	pH 5.6	4.72 ± 0.03g	4.35 ± 0.08h	7.60 ± 0.09g	6.53 ± 0.12fg	148.27 ± 2.84ij	108.28 ± 5.43
	pH 4.6	4.29 ± 0.04d	3.88 ± 0.04e	6.97 ± 0.09e	6.31 ± 0.08f	139.29 ± 2.59ef	87.74 ± 1.66
	pH 3.6	4.06 ± 0.06c	3.45 ± 0.04c	6.07 ± 0.10c	6.00 ± 0.13e	108.23 ± 2.03b	64.98 ± 1.75
Watering for 8 weeks	Well water, pH 7.0	4.55 ± 0.03f	4.15 ± 0.07f	7.06 ± 0.14e	5.36 ± 0.22d	140.02 ± 2.14ef	110.75 ± 3.03
	Artificial rain, pH 6.2	4.84 ± 0.06hi	4.53 ± 0.06i	7.34 ± 0.18f	5.91 ± 0.15e	147.59 ± 1.80hij	119.51 ± 1.80
	pH 5.6	4.16 ± 0.05c	3.64 ± 0.08d	6.67 ± 0.13d	4.77 ± 0.16c	137.67 ± 1.96e	86.66 ± 2.68
	pH 4.6	3.92 ± 0.05b	3.04 ± 0.04b	5.69 ± 0.11b	3.87 ± 0.11b	124.10 ± 3.63c	63.23 ± 1.78
	pH 3.6	3.52 ± 0.06a	2.83 ± 0.02a	4.74 ± 0.17a	3.23 ± 0.08a	94.23 ± 1.96a	45.12 ± 2.00

Means within a column followed by the same letter are not significantly different ($P > 0.05$; GLM) in ANOVA (LSD).

Treatment of acid rain showed that at pH 5.6, 4.6 and 3.6 had lower biochemical content than controls. Increased acidity level was followed by biochemicals decrease. The lowest result was at pH 3.6 on the acid rain watering for 8 weeks. In young leaves, the lowest total protein, total sugar and vitamin C in young leaves were 3.52%, 4.74%, and 94.23 mg/100g. Whereas in mature leaves on the same parameters were 2.83%, 3.23%, and 45.12 mg/100g.

Due to the data showed curve line on scatter plots, quadratic regression was used on the analysis. The regression coefficient was written on Table 3.

Table 3. Quadratic Regression Coefficient (R^2) of Acidity Level Effect to Biochemical Contents of Mulberry Leaves

Parameters	Young Leaf	Mature Leaf
Protein	0.612	0.054
Total Sugar	0.646	0.295
Vitamin C	0.806	0.303

Effect of acidity level to protein, sugar and vitamin C contents of mulberry leaves in young leaf was greater than in mature leaf. The greatest effect was 0.806 in vitamin C parameter. It means that 80.6% the vitamin C was affected by acidity level, and 19.4% was affected by the other factors.

Increasing of total protein content at pH 6.2 can be understood. Typically, rainwater is naturally slightly acidic (pH slightly under 6), because carbon dioxide (CO_2) in air is dissolved by rain water will form carbonic acid, a weak acid (Re Velle & Re Velle, 1992). Weak acid is beneficial because it helps dissolve the minerals in the soil which are needed by plants. Potassium and Magnesium, which is an coenzyme, was tied to the soil grains are released and absorbed by plant roots when exposed to weak acid. Mg^{2+} is a coenzyme that can improve efficiency in the metabolism of nitrogen into proteins. Along with protein increasing, Wang *et al.* (2006) also stated that longterm exposure of simulated acid rain affected to changing of soluble sugar phosphate and soluble protein in eggplant that caused the growth of red spider mite *Tetranychus cinnabarinus* (Acarina: Tetranychidae) was promoted. However, strong acid rain (pH<3.0) inhibited the growth of both host plant and the mite. Acid rain also caused releasing of Ca in the soil. Halman *et al.* (2008), suggested that the Ca-addition treatment significantly increased fructose and glucose concentrations foliar red spruce (*Picea rubens*).

Decreasing of vitamin A, E, and C content in Mulberry leaf at SAR exposure of pH below 5.6 was occured. It was more their role as a nonenzymatic antioxidant system. According to Munzuroglu *et al.* (2005), vitamins A, E, and C contents in strawberry fruit was decreased with declining of rain water pH after 24-hour treatment. There are two basic reason for the vitamin reduction in the fruit. The first, the increasing the free oxygen radical formation in the plant and the fruit due to acid rain stress, and plants use of the antioxidant system in order to resist the stress which results in a loss in vitamin levels. The second, in autotrophy crops, vitamin synthesis are inhibit by acidic condition. So, that acid stress changed their metabolism.

In this research, total protein, total sugar and vitamin C due to SAR exposure for 4 weeks was increased. It was because effect of sulfur in SAR was more accumulated. Sulfur is one of the macro elements to promote plant growth (Ashenden, 2002). The high sulfur content will increase the number of amino acids, especially cysteine and methionine. Besides as building block of proteins, amino acids also play as a precursor for a number of biomolecules, including porphyrin. Porphyrin complex is a molecular compound containing four pyrrole compounds covalently bounded to be a ring, where in the chlorophyll, Mg is located at center of porphyrin. Mg in the chlorophyll promotes formation of chlorophyll, which facilitates light capture. Thus, glucose produced in photosynthesis reactions become more numerous. However, when SAR exposure more than 6 weeks, all biochemical contents in Mulberry will be decreased. On our observation, the mulberry leaves were yelowish due to vegetative twigs age more than 2.5 month after pruning.

The amino acid cysteine and methionine contain approximately 90 percent of total sulfur in most crop plants with almost all of these amino acid found in protein (Rowland *et al*., 1988 *cit.* Gupta, 2005). Damage to membrane protein, perhaps by oxidation of susceptible amino and sulfhydryl groups on the surface of the membrane protein, may be the cause of alterations in permeability of the plasmalemma (Dominy & Heath, 1985 *cit.* Gupta, 2005). Interaction of SO_2 with amino acid residues in proteins of membranes and of enzymes would amplify any deleterious effects. Decrease in total protein content due to exposure to SO_2, could be the result of a decrease in the rate of protein synthesis and/or an increase in the rate of protein degradation.

In soil system, acidic deposition accelerates Ca, Mg, and K losses. Increased acidity solubilizes Al, and then displaces Ca, Mg, and K from soil exchange sites, which then leach in association with SO_4 (Elliot *et al*., 2008). In recent study, as watershed budget studies at the Hubbard Brook Experimental Forest (HBEF), New Hampshire, USA, have demonstrated high calcium depletion of soil during the 20th century due, in part, to acid deposition. Over the past 25 years, tree growth (especially for sugar maple) has declined on the experimental watersheds at the HBEF (Juice *et al*., 2006). Aluminum toxicity is considerd as one of the major soil factors limiting root growth in acidic soils. Because of the increase in organic matter content in the upper few centimeters of soils under no-till systems (NTS), most Al in soil solution may be complexed to dissolved organic C (DOC), thus decreasing its bioavailability (Alleony *et al*., 2010).

Conclusions

Plants watered with simulated acid rain (below pH 5.6) exhibited lower biochemical content, as evidenced by mean total protein, total sugar and vitamin C Mulberry leaf, than plants watered with well water of pH 7.0 and artificial acid rain of pH 6.2. Simulated acid rain on weak acidity (pH 6.2) and SAR for 4 weeks showed a highest biochemicals value.

References

Alleoni LRF, Cambri MA, Caires EF, and Ĝarbuio FJ. 2010. Acidity and Aluminum Speciation as Affected by Surface Liming in Tropical No-Till Soils. *Soil Science Society America Journal*. 74:1010-1017.

AOAC, 1988. Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists. Washington, DC.

Ashenden TW. 2002. Effects of Wet Deposited Acidity. *In Air Pollution and Plant Life*. 2nd Ed. Bell, J.N.B. & Treshow, M. (Eds). New York: John Wiley & Sons.

Cronan CS. 1991. Differential adsorption of Al, Ca, and Mg by roots of red spruce (*Picea rubens* Sarg.). *Tree Physiol.* 8(3):227-37.

Elliot KJ, Vose JM, Knoepp JD, Johnson DW, Swank WT and Jackson W. 2008. Simulated Effects of Sulfur Deposition on Nutrient Cycling in Class I Wilderness Areas. *J. Environ Qual* 37:1419-1431

Haines BL and Swank WT. 1988. Forest Hydrology and Ecology at Coweeta: Acid Precipitation Effects on Forest Processes. *Ecological Studie,* Vol. 66.

Halman JM, Schaberg PG, Hawley GJ, Eagar C. 2008. Calcium addition at the Hubbard Brook Experimental Forest increases sugar storage, antioxidant activity and cold tolerance in native red spruce (*Picea rubens*). *Tree Physiol*. 28(6):855-62.

Juice SM, Fahey TJ, Siccama TG, Driscoll CT, Denny EG, Eagar C, Cleavit NL, Minocha R, Richardson AD. 2006. Response of sugar maple to calcium addition to northern hardwood forest. *Ecology* 87(8):2131.

Gupta, US. 2005. *Physiology of Stressed Crops*,Volume II: Nutrient Relations. New Hampshire: Science Publishers, Inc.

Gosh L, Alam MS, Ali MR, Shohael AM, Alam F and Islam R. 2003. Changes in Biochemical Parameters of Mulberry (*Morus sp.*) Leaves after Infected with Leaf Spot Disease. *Online Journal Biological Sciences* 3 (5): 508-514.

Munzuroglu O, Obek E, Karatas F and Tatar SY. 2005. Effects of Simulated Acid Rain on Vitamins A, E, and C in Strawberry (*Fragaria vesca*). *Pakistan Journal of Nutrition*, 4 (6): 402-406.

NYSERDA (New York State Energy Research and Development Authority). 2005. Acid Rain – Learning from The Past and Looking to The Future: A Primer. http://www.nyserda.org/programs/environment/emep/acidRainPrimer.pdf, (accessed: May 28, 2009).

Schaberg PG, Dehayes DH, Hawley GJ, StrimbeckGR, Cumming JR, Mukarami PF, Borer CH. 2000. Acid mist and soil Ca and Al alter the mineral nutrition and physiology of red spruce. *Tree Physiol.* 20(2):73-85.

Seip and Munzen. 2002. Air Pollution as a Climate Forcing: Workshop Proceeding. Honolulu, Hawaii, April 29-May 3, 2002.

Velokova V and Yordanov I. 1996. Changes In Prompt Chlorophyll Fluorescene and Oxygen Evolution After Bean Plant Treatment with Artificial Acid Rain. *Bulg. J. Plant Physiol.* 22 (3-4), 14-24.

Wang, J.J., Zhang, J.P, He, L. and Zhao, Z.M. 2006. Influence of Long-Term Exposure to Simulated Acid Rain on Development, Reproduction and Acaricide Susceptibility of the Carmine Spider Mite, *Tetranychus cinnabarinus*. *Journal of Insect Science*. Vol.6. Number 19.

Iskandar
Biofarma

Ismu Prastya wati Sukanto
PT Biofarma (Persero) Jl. Pasteur No.28 Bandung. Phone 08122036084
Email : waty@biofarma.co.id

Jamaluddin
STISIP Muhammadiyah Sidrap Jl Ap Rani No 25 Rappang. Phone 081241560777

Jeffry L. Lazaroni
Under Graduate Student of Department of Fisheries Science, Faculty of Agriculture and Animal Husbandry Muhammadiyah University of Malang
Email : lazaronivirgo@yahoo.com

Jekti Prihatin
Department of Biology, Faculty of Teacher Training and Education
Jember University, Jember-East Java. Phone : 08123485445
E-mail: jekti_pr@yahoo.com

Joko Waluyo
Department of Biology, Faculty of Teacher Training and Education
Jember University, Jember-East Java. Phone: 08123485445
E-mail: jokowaluyo.fkip@unej.ac.id

Juwartina Ida Royani
Email: idhajr@yahoo.com

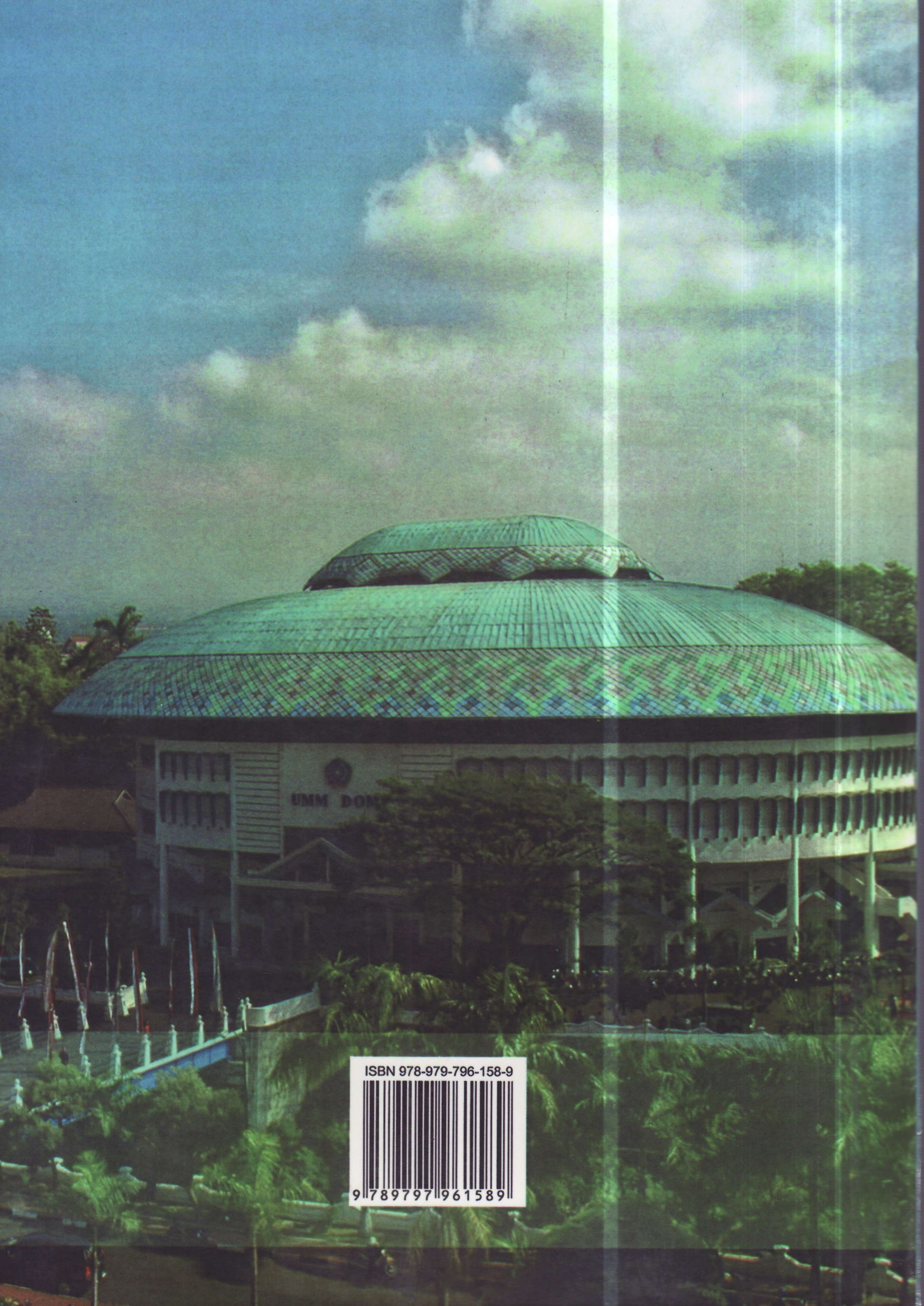
Kartini Zaelanie
Faculty of Fishery and Marine Science Brawijaya University.
Jl Intan 3 Tlogomas Malang, Phone: Telp : 08179637873
Email : kartinizailaniek@yahoo.com

Komang G. Wiryawan
Biological Resources Research and Biotechnology,
Institute Agriculture of Bogor

Kennetth Monjero Igadwa
Kenya Agricultural Research Institute, Biotechnology Center, Plant Biotechnology
PO BOX 5711-00200
Nairobi, Kenya

Khoirin Maghfiroh
Department of Biology, Faculty of Teacher Training and Education
Muhammadiyah Universiyt of Malang Phone: 081335030983
Email : rizinputri@yahoo.com

Khoirul Umam
Department of Biology, Faculty of Teacher Training and Education
Muhammadiyah Universiyt of Malang Phone: 08970236125
E-mail: umm_kkr@ymail.com



ISBN 978-979-796-158-9