*Biopestic. Int.*16(2):141-148 (2020)
DocID: https://connectjournals.com/02196.2020.16.141

ISSN 0973-483X / e-ISSN 0976-9412

The Combination Effect of *Spodoptera litura* Multicaspid Nuclear Polyhedrosis Virus, *Lecanicillium lecanii*, and Neem Seed Extract to Soybean Pests and Predators in East Java Agroecosystem

MAHANANI TRI ASRI*, EVIE RATNASARI AND WINARSIH
Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Surabaya
Surabaya 60213, East Java, Indonesia

KEYWORDS

Biopesticides
Combination effects
Lecanicillium lecanii
Soybean pests
Spodoptera litura
Multicaspid Nuclear
Polyhedrosis Virus
Neem seed extract

ABSTRACT The objective of this study was to elucidate the biopesticidal effect on the relative pest abundance, leaf damage, and soybean yield (on dry weight basis). This study was conducted in 2 areas, Bedali soybean center, Lawang and Sumobito, Jombang, East Java, Indonesia. The study was conducted in 100 m^2 soybean field having 600 plants in 5 treatment groups. *Spodoptera litura* Multicaspid Nuclear Polyhedrosis Virus, an entomopathogenic fungus (*Lecanicillium lecanii*), and *Azadirachta indica* (neem or mimba seed extract) were used in kaolin and ethyl *p*-methoxycinnamate in the ratio of 1:1:1:4:15%. The relative pest and predator populations were recorded and soyabean yield was determined. After treatment, total pests and predators found in the Bedali Lawang area were 269 and 548 compared to 390 and 456 in Sumobito-Jombang area. The damage to the soybean crop in the Bedali Lawang treated area was (42%) but more severe (63.08%) in Sumobito-Jombang area. The soybean yield in Bedali-Lawang and Sumobito-Jombang in treated area was 16.84 and 47.09 g/plant, which was significantly higher than recorded in controls (13.38 and 25.80 g/plant).

INTRODUCTION

Various insect pests recorded in the soybean agroecosystem in Indonesia are soybean leaf beetles, *grayak* caterpillars (*Spodoptera litura*), ssethe sucking pests such as *Aphis glycines* and *Bemisia tabaci* Genn., *Nezara viridula*, *Piezodorus hybneri*, *Riptortus linearis*, *Riptortus* spp., *Melanocanthus* sp., and *Plautia* sp; there are only 2 soybean pod borers, *Etiella zinckenella* and *Etiella hobsoni* recorded along with 2 types of pod-eating pests, *Heliothis* spp. and *Phalandra inclusa* (Arifin, 1991; Tengkano *et al.*, 1992).

Integrated pest management is one method to reduce the pest population, but integrated pest control has not been done properly by farmers. The pest control in soybean agroecosystem in East Java still use synthetic insecticide (Roel *et al.*, 2010). Synthetic insecticides are known to have negative impacts, so pest control systems are now turning to bioinsecticides. The introduced bioinsecticides to farmers are made from active microorganisms, such as viruses *Spodoptera litura* Multiple Nucleopolyhedrosis Virus (*Splt*MNPV), fungi (*Lecanicillium lecanii*), and extracts form *Azadirachta indica* (neem or mimba) (Roel *et al.*, 2010).

*Corresponding author: E-Mail: mahananiasri@unesa.ac.id

Journal Homepage : www.connectjournals.com/bi

Published & Hosted by : Connect Journals
www.connectjournals.com

According to Arifin *et al.* (2010), bioinsecticides are effective against grayak caterpillar. Similar results have also been found by Ratnasari *et al.* (2016), who tested the three active ingredients in a greenhouse experiment; the active ingredients could kill 93.5% of the 2nd instar grayak worm. The three bioinsecticides have a complementary mechanism of action; The *Splt*MNPV acts as a stomach poison, *L. lecanii* is the contact poison, and mimba seed extract as the hemolymph toxin that causes repellence, insects' development inhibition, fertility and fecundity reduction, and also the behavioral and physiological changes that lead to death (Roel *et al.*, 2010). Besides, these bioinsecticides can disrupt the pest's hormonal system so that its growth and development will be disrupted and cause deformation, disability, and even infertility. The difference in dosage and exposure time also lead to various reactions in each pest (Martinez, 2002). Several studies on the bioinsecticidal virus (*Splt*MNPV) showed that the virus had a specific host, *S. litura,* which had a special receptor formed by chlatrin compounds that were compatible with the GP 64 glycoproteins, the viral fusion proteins that are inserted into the cell membranes (Rohrmann, 2008; Passarelli, 2011). *L. lecanii* has hydrolytic enzymes, such as chitinase, protease, lipase, and amylase (Hasan *et al.*, 2013). The fungi are capable to release toxins that can kill insects, such as dipicolinic acid and cyclodepsipeptide (Cloyd, 2003). Neem seed extract contains insecticidal toxic compounds such as azadirachtin, salanin, meliantriol, nimbin, and nimbidin (Koul and Wahab, 2004). Those properties make the bioinsecticide to have a broad spectrum so that some pests in an agroecosystem can be controlled.

The viral bioinsecticides (*Splt*MNPV) and fungi (*L. lecanii*) are known to be susceptible to sunlight radiation so photo-protectant compounds could protect these bioinsecticides. According to Asri (2013), Kaolin and Ethyl P-methoxycinnamate (EPMS) can protect *Splt*MNPV from solar radiation for 12 h. The photo-protectant compounds can protect *Splt*MNPV and *L. lecanii* fungus by reflecting sunlight through the small kaolin particles. These particles also help the absorption of oxygen free radicals, which could be formed from the absorbed energy. When the kaolin is sprayed into the plant, it will become a film layer that protects the plants from pests and even kill the insects (Aquino *et al.*, 2011). The second photo-protectant compound is EPMS with a long chain and a conjugated double bond system that will undergo a resonance during ultraviolet exposure (Taufikurohmah, 2003).

The objective of the present study was to elucidate the effect of *Splt*MNPV, *L. lecanii,* and neem seed oil extract in photo-protectant formulas on the pests abundance, soybean plant damage, and soybean yield. Overall objective was to prevent crop damage and subsequent increase in soybean production.

MATERIALS AND METHODS

Bioinsecticide Propagation

*Sl*MNPV was replicated *in vitro* epithelial cell culture of *S. litura* larvae. The midgut epithelial cells of 5th instars were separated from their tissues aseptically and grown on EX-CELL® 405 Serum-Free Medium for insect cells with fetal bovine serum, antibacterial drugs (penicillin, streptomycin, and gentamicin), and amphotericin-B (antifungal compound). The cell culture was incubated at room temperature for 5 days and subsequently infected with *Splt*MNPV broken down polyhedra and incubated for 10 days. The virus was harvested by centrifugation and used for the experiments. The used dose was 1.2×10^{12} polyhedra inclusion body/mL (Arifin, 1991).

The neem seeds (50–75 g) ground and then macerated with ethanol in the ratio of 1:3 (w/v) for 72 h. The extract was sterilized for 24 h and then filtered. The extract was washed with ethanol twice, allowed to stand further for 24 h and filtrate then combined with earlier obtained filtrate and concentrated using a rotary evaporator. The final dried extract was used for the study at the rate of 20 g/L (Sikka, 2009).

The propagation of *L. lecanii* was done in Potato Dextrose Agar (PDA) and incubated at room temperature for 18 days. The spores and mycelium were harvested using spatulas. Spores and mycelium fragment concentration were calculated using hemocytometer and the used dose was 108 spores/mL as described earlier (Afandhi and Syamsidi, 2010).

Bioinsecticide Formulation

The fungus, spores, virus, and the neem extracts were formulated with the kaolin and EPMS photoprotectant compounds in a 1: 1: 1: 4: 15% ratio (Ratnasari *et al.*, 2016).

The field trials were conducted in 2 areas, Bedawi Soybean Center in Lawang, East Java and in the pests' endemic areas at Jombang, East Java. The test area was treated by a local farmer with a demo plot of 50 m² each; 600 plants were used for the study. The spraying was done 3 times at the vegetative period of soybean plants (1–4 weeks after plantation or until the first flower appears), the flowering period (5–7 weeks after plantation with a flowering period of 3–5 weeks), and the pods' formation (7–10 days after the first flower appeared). The spraying was done in the afternoon around 16.00-17.00 local time.

The main observed parameters were pest and predator abundance, leaf damage, and soybean plant productivity. The data collection was done once a week for about 3–4 months. The pest and predator abundance observation was conducted in the soybean planting period, from the first leaf appearance until harvest time (75–100 days). The pests and predators were captured using a net and calculated using the formula of relative abundance:

$$N1/NTotal \times 100\% \quad (1)$$

The identification of pests and predators was done by morphological observation, guided by insect identification books. The soybean leaf damage level by pest was observed in both control and treatment plots continuously per week. The damage was measured by calculating the percentage of damaged leaves compared to healthy leaves with the following leaf damage category: 0–30% = +1 (low), 31–60% = +2 (moderate), 61–100% = +3 (severe). The sampling was done randomly from 60 plants out of a total of 600 plants (10%). The sampling plants were taken by the diagonal method at 5 points per plot. The soybean plant productivity was measured by the total soybeans yield divided by the number of sample plants at each observed point/plot (60 plants).

Data Analysis

All of the pests and predators' abundance data were analyzed with descriptive quantitative analysis. The significance level between control and treatment plot in each location for the leaf damage and soybean productivity were analyzed by Student's *t*-test ($\alpha = 0.05$).

RESULTS

Pest and Predator Abundance in Soy Agroecosystem

The relative pest abundance was calculated based on the number of pests found in the treatment plot and the control plots as listed in Table 1.

Previously, the main pest in the Bedali Lawang treatment area was *S. litura*, which was not found in this observation due to its immunity against the insecticides and these pests were the target of *Splt*MNPV. Based on Table 1 data, it can be seen that the number of pests in the control group was higher (869 individual) than the treatment (548 individual) in the Bedali, Lawang area. There were 6 types of pests with decreased abundance (from 37.9 to 23.7%) and 8 types of pests that increased (from 50.7 to 64.7%). Meanwhile, the predators were relatively more abundant in the control (4.8%) than the treatment (3.8%). The unidentified pests ranged from 7.5 to 7.8%. The most abundant pests were *Amrasca*

Table 1. Pest and predator relative abundance in soybean agroecosystems after combined treatment of *SpltMNPV*, *Lecanicillium lecanii*, and neem seed extract at Bedali, Lawang

No	Types of pest / predator		Relative abundance (%) in control	Relative abundance (%) in treatment
	Pest	Predator		
1	*Spodoptera litura*		2.0	0
2	*Coccinella* sp. (larva)		1.5	0.5
3	*Bemisia tabaci*		10.0	6.4
4	*Trips parvipinus*		8.5	3.8
5	*Aphis gossypii*		14.5	12.6
6	*Chrysolina coerulans*		1.4	0.4
7	*Amrasca* sp.		49.3	54.4
8	*Lamprosema* sp.		0	0.4
9	*Plutella* sp.		0.2	0.3
10	*Oxya chinensis*		0	1.3
11	*Phaedonia* sp.		0.5	2.2
12	*Longitorsus* sp.		0	2.2
13	*Nezara viridula*		0.5	2.9
14	*Riptortus linearis*		0.2	1.0
15		*Paederus* sp.	3.5	2.2
16		*Menochilus sexmaculatus*	1.0	1.6
17		*Atractomorpha crenulata*	0.3	0
18	Unidentified		7.5	7.8
	Total		100	100
	Total of pests and natural enemies		869	548

sp., both on control (49.3%) and treatment (54.4%) followed by *Aphis gossypii* (14.5% in control and 12.6% in treatment) and *B. tabaci* (10% in control and 6.4% in treatment) (Fig. 1).

The percentage of pest and predator relative abundance in Sumobito-Jombang is presented at Table 2. The number of pests was higher in control (456 individuals) than treatment (390 individuals). There were 5 pests showed decreasing trend (from 36.0 to 23.07%) compared to other 5 pests having increased numbers (from 21.1 to 25.13%) (Table 2). The relative abundance of predators was higher in the treatment area (12.7%) than in control (8.3%). The unidentified pests ranged from 7.5 to 7.8%.

The pest with the largest abundance in this area was *Longitorsus* sp., both in control (12.1%) and treatment

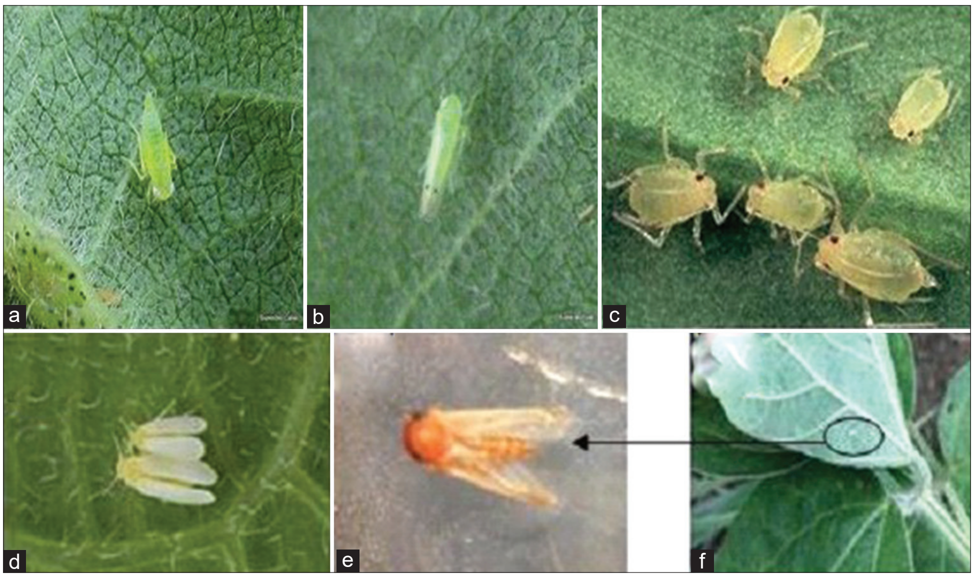

Fig. 1. Three pests with the highest abundance in soybean agroecosystem of Bedali-Lawang area. (a) *Amrasca sp.* (nymph). (b) *Amrasca sp.* (imago). (c) *Aphis gossypii*. (d-f) *Bemisia tabaci*.

Table 2. The percentage of pest's relative abundance in soybean agroecosystem with microbial and vegetable bioinsecticide treatment in photo-protectant formula in Sumobito area, Jombang, East Java

No	Types of pest/predator		Relative abundance (%) in control	Relative abundance (%) in treatment
	Pest	Predators		
1	*Coccinella* sp (larva)		4.6	1.30
2	*Amrasca* sp.		5.3	3.11
3	*Bemisia tabaci*		10.1	5.96
4	*Lamprosema* sp.		6.1	4.15
5	*Oxya chinensis*		9.9	8.55
6	*Trips parvipinus*		0	0.26
7	*Aphis gossypii*		4.6	4.66
8	*Phaedonia* sp.		3.7	5.96
9	*Longitorsus* sp.		12.1	13.21
10	*Riptortus linearis*		0.7	1.04
11		*Menochilus sexmaculatus*	0.4	0.26
12		*Paederus* sp.	7.9	12.44
13	Unidentified		34.6	39.10
	Total		100	100
	Total of pests and natural enemies		456	390

(13.21%), followed by the *Oxya chinensis* (9.9% in control and 8.55% in treatment) and then *B. tabaci* (10.1% in control and 5.96% in treatment) (Fig. 2).

Leaf Damage Level

The treatment plot showed lower leaf damage (24%). Comparatively, the control plots showed moderate damage (42%). To know the difference of leaf damage in both plots, a T-test was conducted after analyzing the data's normality (Table 3).

The result of the Kolmogorov–Smirnov normality test was 0.274 >0.05, which means that the data are distributed normally. The *t*-test showed the t count >t table (11.611 >1.68488) so there was a significant difference between the treatment and control plot on soybean leaf damage. This was at par with the pest observation results; the control had more pests (869 individuals) than treatment (548 individuals) thus causing more leaf damage in control plants compared to treated ones.

From Table 3, the damage in the Sumobito-Jombang treatment plot showed moderate damage (57.08%) while the damage in the control plot was severe (63.08%). Kolmogorov–Smirnov normality test showed 0.408 >0.05 value so the data were distributed normally. The *t*-test showed a higher t count (22.096 >1.66724), indicating a significant difference in leaf damage between treatment and control. This is consistent with the pest abundance results, which was higher in the control (456 individuals) than the treatment (390 individuals).

Soybeans Production

The soybean plants' productivity was based on the total average weight of soybeans after harvest. The observation result obtained after 12 observations in 5 plots has been recorded in Table 4.

The Kolmogorov–Smirnov normality test result on an average soybean dry weight of Bedali area was 0.230 >0.05 so the data were distributed normally. From the *t*-test, it was found that t count > t table (11.61005 >1.8331), so it can be seen that the treatment gave a significant effect on soybean production.

On the other hand, the dry weight of soybean in Sumobito was distributed normally based on the Kolmogorov–Smirnov

Fig. 2. Pests in Jombang. (a) *Longitarsus sp*. (B) *Longitorsus sp* in soybean flower. (c) Green grasshopper/*Oxya chinensis.*

Table 3.The average damage after microbial and neem biopesticide application in photo-protectant formulas in control and treatment plots in Bedali, Lawang and Sumobito-Jombang areas

Plot	Bedali, Lawang				Sumobito-Jombang			
	Control		Treatment		Control		Treatment	
	Average of leaf damage (%)	Level of leaf damage	Average of leaf damage (%)	Level of leaf damage	Average of leaf damage (%)	Level of leaf damage	Average of leaf damage (%)	Level of leaf damage
1	15.97	+	40.99	+ +	47.63	++	63.68	+++
2	29.89	+	50.04	+ +	57.68	++	65.69	+++
3	27.77	+	35.60	++	59.75	++	64.74	+++
4	27.17	+	42.01	+ +	65.95	+++	58.19	++
5	19.08	+	42.38	+ +	54.40	++	61.69	+++
Average	24[a]	+	42[b]	++	57.08[a]	++	63.08[b]	+++

Letters in each land area indicate significant differences based on Student's *t*-test (α0.05)

Table 4. Soybeans dry weight in Bedali, Lawang and Sumobito-Jombang East Java

Land area		Average dry weight (g/plant) of soybean in plot areas					
		I	II	III	IV	V	Average
Bedali Lawang	Control	14.22	11.16	12.35	9.91	19.25	13.38[a]
	Treatment	15.44	16.72	15.51	13.53	22.98	16.84[b]
Sumobito-Jombang	Control	21.15	23.94	26.73	27.55	29.62	25.80[a]
	Treatment	46.11	44.16	44.80	49.51	50.88	47.09[b]

Letters in each land area indicate significant differences based on Student's *t*-test (α=0.05)

normality test (0.128 > 0.05). The *t*-test showed t count >t table (28.488 >1.65776), so the treatment gave a significant effect on soybean production. The highest soybean production was 50.88 g/plant in treatment plot V while the lowest was 21.15 g/plant in the control plot I.

DISCUSSION

Some soybean pests were observed in our study, such as *Amrasca* sp., *A. gossypii*, *B. tabaci*, *Longitarsus* sp., and green grasshoppers (*O. chinensis*). The major pests in the soybean agroecosystem at Bedali, Lawang were categorized as destructive pests with leaf sucker types, such as *Amrasca* sp., *A. gossypii*, and *B. tabaci*. All of these caused damages in soybean with a low to moderate level due to their tiny size. *Amrasca* sp. is a leaf sucker that turned leaves into yellowish to brownish-red and subsequently they wilted giving burnt appearance (Winarno, 2005). *A. gossypii* is known as an aphid and a transmitting vector for various viruses. As the second pest in our present study, *A. gossypii* caused leaf edges to shrink and curl. According to Riyanto *et al.* (2011), the losses due to *A. gossypii* attack can be enormous, especially at the early growth stage. Another insect pest identified was silver leaf whitefly (*B. tabaci*) that sucks leaf's fluid and caused damage to leaf tissues seen as necrotic spots. The reported yield loss after this pest attack was about 20–100%. According to Byamukama *et al.* (2004), *B. tabaci* is also capable of transmitting the pathogenic virus, such as Geminivirus, Closterovirus, Nepovirus, Carlavirus, Potyvirus, and Rod-shape DNA Virus.

In contrast with the Bedali, Lawang soybean agroecosystem, the soybean agroecosystem at Sumobito, Jombang was categorized to have a moderate level of damage. This suggests that the pests in both agroecosystems were different. The major pest in Sumobito, Jombang was a leaf-eating type, such as green grasshoppers and *Longitarsus* sp. Green grasshoppers and *Longitarsus* sp. are known to eat a large number of leaves. Based on the observation, the green grasshoppers attacked the young soybean (shoots). The adult female grasshoppers were large (58–71 mm) than the adult males (49–63 mm), with 2–3 g body weight. Second major pest in Sumobito, Jombang was *Longitarsus* sp. (Chrysomelidae: Coleoptera) that attacked the leaves. The leaves would turn hollow, withered, and dry and eventually fall off. This inhibited photosynthesis system and decreased soybean growth and productivity as a consequence of this damage.

The soybean productivity after biopesticide treatment was higher than control in both areas (Table 4). According to Tjahyani *et al.* (2015) too, various pesticide spray on soybean plants showed significantly higher yield than control, as represented in pods number, namely 60.03% higher/ha for vegetable pesticides, 62.58% for Mospilan, and 61.27% for the Ingrofol pesticide. Based on our results, the biopesticide treatment application could reduce the pest number and increase soybean productivity. This was consistent with a previous study by Mahendra and Oktarina (2017) that vegetable biopesticide application significantly decreased leaf damage intensity by pests.

Present study showed different soybean productivity in Bedali Lawang and Sumobito-Jombang. The soybean productivity in Sumibito-Jombang was higher than in Bedali, Lawang. The difference in pest composition may have contributed to this difference. Our results also indicated that the combination of microbial and vegetable bioinsecticides could reduce the pest number, so soybean productivity would increase compared to control plots.

Based on the results of this study, it can be concluded that the abundance of pests and predators in Bedali Lawang and Sumobito-Jombang soybean agroecosystems would decrease after the microbial and vegetable bioinsecticides with photo-protectant formula application. The soybean leaf damage level in both areas was categorized as low to moderate in the treatment plots and moderate to high in the control plots. The addition of microbial and vegetable bioinsecticides in photo-protectant formula, however, significantly impacted the soybean dry weight in both the agroecosystems.

Acknowledgment. The authors gratefully thank the State University of Surabaya for their support. Special thanks to Bedali soybean center, Lawang, East Java, for providing the place for experiment and the resident of pests' endemic area in Jombang, East Java who was very nice.

FUNDING

There is no funding source to be declared for this study.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

MTA carried out the bioinsecticides propagation, participated in data analysis, its design and coordination, also drafted the manuscript. ER carried out the bioinsecticides formulation, designed the study, and performed the statistical analysis. W participated in the bioinsecticides propagation and manuscript drafting. All authors read and approved the final manuscript.

REFERENCES

Afandhi, A. and Syamsidi, S.R.C. (2010) *The Protectant Potential of a Gamblong Flour to Enhance Efficacy of Beauveria Bassiana Fungi Used as a Foliar Treatment Against Spodoptera litura.* Paper Presented at 8th International Symposium on Biocontrol and Biotechnology, King Mongkut's Institute of Technology Ladkrabang and Khon Kaen University, Nong Khai Campus, Thailand.

Aquino, S.D., Cocco, A., Ortu, S. and Schirra, M. (2011) Effects of kaolin-based particle film to control *Ceratitis capitata* (Diptera: Tephritidae) infestations and postharvest decay in citrus and stone fruit. *Crop Prot.*, **30**, 1079–1086.

Arifin, M. (1991) *Peranan Musuh Alami ulat Grayak* (*Spodoptera litura* F.) *Pada Berbagai Kondisi Lingkungan Pertanaman Kedelai* (*Role of Natural Enemies of the Common Cutworm* (*Spodoptera litura* f.) *in Different Environmental Conditions of the Soybean Crops*). Proceedings of the National Seminar on Basic Biology II, Basic Biology in Supporting Productivity and Biological Quality, Bogor.

Arifin, M., Prayogo, Y. and Koswanudin, D. (2010) *Insektisida Biorasional Untuk Mengendalikan Hama Kepik Coklat, Riptortus Linearis Pada Kedelai* (Biorational Insecticides for Control of the Pod Sucking Bug, Riptortus Linearis on Soybean). Paper Presented at National Seminar on Soybeans, Research Institute for Nuts and Tubers, Malang.

Asri, M.T. (2013) *Infection and Effectivity of SpltMNPV Mechanism in vitro in Photo-Protectan Formula to Spodoptera Litura Larvae Mortality* (Doctoral Dissertation), Brawijaya University, Malang.

Byamukama, E., Gibson, R.W., Aritua, V. and Adipala, E. (2004) Within crop spread of sweet potato virus deseases and the population dinamic of its whitefly and aphids vectors. *Crop Prot.*, **23**, 109–116.

Cloyd, R. (2003) *The Entomopathogen Verticillium lecanii*. Mildwest Biological Control News. University of Illinois. Available from: http://www.ruf.rice.edu/Bioslabs/methods/microscopy/cellcounting.html. [Last accessed on 2017 Feb 02].

Hasan, S., Ahmad, A., Purwar, A., Khan, N., Kundan, R. and Gupta, G. (2013) Production of extracelluler enzymes in the entomophatogenic fungus *Verticillium lecanii*. *Bioinformation*, **9**, 238–242.

Koul, O. and Wahab, S. (2004) *Neem Today and in the Next Millennium*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Mahendra, A.Y. and Oktarina, O. (2017) Respon kedelai edamame (*Glycine max, L Merill*) terhadap waktu aplikasi dan konsentrasi pestisida nabati gadung (Response of edamame soybean (*Glycine max, L Merill*) to the time of application and concentration of yeast vegetable pesticides). *Agritrop J. IlmuIlmu Pertanian*, **15**, 44–54.

Martinez, S.S. (2002) O Neem-azadirachta indica natureza, usos múltiplos, produção. Londrina (Neem-*Azadirachta indica*-a natural insecticide). *Inst. Agronôm. Paraná.*, **142**, 1–7.

Passarelli, A.L. (2011) Barriers to success: How baculoviruses establish efficient systemic infections. *Virology*, **411**, 383–392.

Ratnasari, E., Mahanani, T.A. and Winarsih. (2016) *Pemanfaatan Bioinsektisida Mikroba dan Nabati Dalam Formula Foto-protektan Yang Efektif Untuk Mengendalikan Hama Kedelai dan Aman Bagi Agroekosistem.* (Utilization of Microbial and Vegetable Bioinsecticides in Photo-protection Formula that is Effective for Controlling Soybean Pests and is Safe for Agroecosystems), University Flagship Research Report, State University of Surabaya, Surabaya.

Riyanto, R., Herlinda, S., Irsan, C. and Umayah, A. (2011) The abundance and species diversity of predatory insects and parasitoid of *Aphis gossypii* in South Sumatra. *J. Hama Penyakit Tumbuhan Tropika*, **11**, 57–68.

Roel, A.R., Dourado, D.M., Matias, R., Porto, K.R., Bednask, A.V. and Costa, R.B.D. (2010) The effect of sub-lethal doses of *Azadirachta indica* (*Meliaceae*) oil on the midgut of *Spodoptera frugiperda* (*Lepidoptera*, *Noctuidae*). *Rev. Bras. Entomol.*, **54**, 505–510.

Rohrmann, G. (2008) *Baculovirus Molecular Biology*. 4th ed. Corvallis, Oregon State University.

Sikka, A.A. (2009) *The Effectiveness of Larvaeides of the Ethanol Extract of Neem Leaf (Azadirachta indica) against Aedes aegypti larvae* (Undergraduated Dissertation), Diponegoro University, Semarang.

Taufikurohmah, T. (2003) *Sintesis p-Metoksisinamil, p-Metoksisinamat, dan p-Metoksisinamil Salisilat dari Material awal etil p-Metoksisinamat Hasil Isolasi Rimpang Kencur* (*Kaemferia galanga L*) *Sebagai Kandidat Tabir Surya* (Master Dissertation), Airlangga University, Surabaya.

Tengkano, W., Iman, M. and Tohir, A.M. (1992) Bioecology, attack and control of soybean pod sucking and borer pests. IPM soybean plants. *In* M. Marwoto, N. Saleh, S. Sunardi and A. Winarto (eds.), *Risalah Lokakarya Pengendalian Hama Terpadu Tanaman Kedelai*, (Minutes of Soybean Plant Integrated Pest Management Workshop). Balai Penelitian Tanaman Pangan, Malang.

Tjahyani, T., Wulan, R., Herlina, N. and Suminarti, N.E. (2015) The response kind and time application of pesticides on growth and yield of edamame (*Glycine max (L.) Merr.*). *J. Prod. Tanaman*, **3**, 511–517.

Winarno, D. (2005). *Hama tanaman Rami* (*Boechmeria nivea* (L) *Gaud*) (*Hemp plant pest* (*Boechmeria nivea* (L) *Gaud*)). Balai Penelitian Tanaman Serat Dan Tembakau, Malang.

Received: 26 Aug 2020; Accepted: 15 Dec 2020