

Short Communication

Effectivity of *Spodoptera littoralis* Nucleopolyhedrovirus (*SpliMNPV*) and Natural Additives Mixtures against *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) on Cabbage Plants

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ABSTRACT

Armyworm (*Spodoptera litura* Fab.) is one of the agricultural pests that can cause huge losses especially for Indonesian farmers because it is damaging various crops, especially cabbage (*Brassica olerifera* L.). *Spodoptera littoralis* nucleopolyhedrovirus (*SpliMNPV*) is one of the biological agent which is effective for the management of the *Spodoptera litura*. However, because of UV radiation it easily degraded when applied in the fields. This study was aimed to determine the effectivity of several indigenous plants for UV protectant of *SpliMNPV* for controlling armyworm at greenhouse scale. Extracts of 2% (w/v) of turmeric rhizome, red betel leaf, moringa leaf, and clove flower, were formulated with *SpliMNPV* and sprayed evenly onto two-month-old cabbages. The experiment used five replicates with six periods of sunlight exposures (0, 1, 3, 5, 7, and 15 days). A commercial product of deltamethrin was used as a comparison. The sprayed leaves were then used as a bioassay by using 25 individuals of one day old 1st larval instar by five replicates. The results showed that the turmeric additive was the most effective as a UV protectant and effectively prolonged the half-life of *SpliMNPV* to 4.12 days, while for clove, moringa leaf, and red betel was 2.48, 2.15, and 2.28 days, respectively.

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Pest attacks in agricultural country such as Indonesia can cause huge losses, one of which is the armyworm (*Spodoptera litura* Fab.). Armyworms belong to the Noctuidae family and the order Lepidoptera. The armyworm is a polyphagous insect that eats many plant species. Armyworms chew large areas of the leaf and can defoliate a crop. In such cases, the larvae migrate in large groups to another field in search of food (Reddy 2015). Several plant species in tropical regions that are heavily damaged by armyworm include *Colocasia esculenta*, cotton plants, peanuts, hemp, corn, rice, soybeans, tea, tobacco, vegetables (eggplant, *Brassica*, *Capsicum*, pumpkin, *Phaseolus*, potato, *Vigna*, etc.) (Garad et al. 1984). This pest is a major problem in various areas of agricultural countries.

Pest management mostly relies on using chemical insecticides. However, there are many negative impacts caused by these chemical insecticides, both to organisms and to the environment. The negative effects of pesticides are generally divided into short-term exposure poisoning (eg. rash, headache, blurred vision, cramps, numbness, and death) and long-term exposure (cancer, reproductive disorders, immune system disorders and neurological damage). Potential damage caused by chemical pesticides will be more significant and visible in fetuses, infants, and children whose organs are still in the developing phase, than in adults (Listorti & Doumani 2001).

Due to the many negative impacts caused by chemical insecticides, an effective and environmentally friendly solution is needed to control pests. One of the most widely used bioinsecticides is a virus from the Baculovirus family. Baculovirus is widely used as a bioinsecticide because it is specific for certain pests or organisms, so that it does not have a negative impact on natural enemies or other non-target insect populations (Armenta et al. 2003). The type of baculovirus used in this study belongs to the nucleopolyhedrovirus (NPV) genus, namely *Spodoptera littoralis* nucleopolyhedrovirus (*Sp*lMNPV). Baculovirus that has been swallowed by the larvae will dissolve in the intestine, then will release the virions which will stick and become one with the cells in the midgut. The nucleocapsid goes to the nucleus for replication and transcription. Furthermore, the virus will spread to the ovaries, body fat, and most endothelial cells through the tracheal system (Vialard et al. 1995). Baculovirus is capable of infecting insect cells, causing damage to the peritrophic membrane of mid gut (Lehane 1997; Engelhard & Volkman 1995).

Besides the many advantages of baculovirus as a bioinsecticide, it has one disadvantage which is easily degraded in nature due to exposure to UV light (McPartland et al. 2000). Exposure to UV-B rays with wavelengths above 280 nm can cause inactivation of baculovirus bioinsecticides. Therefore, inhibition on the inactivation of baculovirus by UV-B rays can be done by providing additives as UV protectants. Additives that have the potential as UV protectants during in vitro tests (Sukirno et al. 2019), namely moringa leaf (*Moringa oleifera* Lam.), turmeric rhizome (*Curcuma longa* L.), cloves (*Syzygium aromaticum* L.) and red betel leaf (*Piper crocatum* Ruiz & Pav.), were tested under sunlight exposures. All additives used in this study generally contain flavonoids. Flavonoids can absorb UV radiation and act as a sunscreen. In addition, there is a study that shows exposure to UV radiation can induce higher levels of flavonoids in plants (Sisa et al. 2010). After absorbing photons from UV irradiation at a certain wavelength, flavonoid molecules have different energies from the ground state through excitation. Flavonoids also have potential as sunscreens because they have chromophore groups (conjugated single double bonds) that can absorb UV-A and UV-B rays. Flavonoids are strong antioxidants as well as metal ion binders which are thought to be able to prevent the harmful effects of UV rays (Svobodová et al. 2003).

This research began with armyworm collection from cabbage plantations in Kopeng, Magelang, Central Java. The collection was done by direct collection of infested cabbage leaves, then kept in plastic jars. Armyworms were reared in the Entomology Laboratory of Faculty of Biology UGM using white bean-artificial diet (Sutanto et al. 2017; Sukirno et al. 2018). The rearing procedure was following Sukirno et al. (2018). One day old 1st larval instar of F4 were used for bioassay.

Each additive that has been mashed and frozen, weighed 100g and then made an extract at a concentration of 10% (w/v) as much as 100 mL. Furthermore, 2% extract of each additive was used to make a solution of bioinsecticide at a concentration of LC95 (8 x 10⁶ PIB/ml) (Sukirno et al. 2019). A total 5 mL of Littovir® was suspended in 495 mL of 2% extract to make 500 ml suspension for each treatment. Deltamethrin at recommended concentration (1ml/l) was used as a positive control.

This research was conducted through 2 stages; the first stage is spraying the liquid formulation of the *Spodoptera littoralis nucleopolyhedrovirus* (Littovir®), natural additives and also adhesive liquid (sticker) on cabbage leaves (*Brassica oleraceae*), while the second stage is testing on armyworm larvae by giving the collected leaves which are then placed in agar medium. The first stage was carried out with 5 treatments for the 1st instar larva of *S. litura*, namely the formulation of a bioinsecticide with four additives and Deltamethrin (Decis®) (Bayer, Indonesia) as a positive control. The formulation of bioinsecticides and additives, as well as positive controls were then sprayed onto the leaves of the cabbage plant (*Brassica oleraceae*) and the leaves would be picked according to a predetermined period. The time in the first stage is called the exposure period, namely 0, 1, 3, 5, 7, 10, and 15 days after treatment (bioinsecticide spraying). Then in the second stage, the collected cabbage leaves along with caterpillar larvae will be placed on the agar medium which will then be observed for 7 days (duration of treatment).

Table 1 showed that there were significant differences both with the addition of additives to Littovir® and positive control treatments. The period of exposure to bioinsecticides and additives at 0-5 days is effective for causing death (mortality) in caterpillars with rates ranging from 90-40%.

Table 1. Mortality of *Spodoptera litura* larvae to the *Spodoptera littoralis nucleopolyhedrovirus* (Littovir®) after addition of natural additives for a fifteen day exposure periods.

Exposure Time (d)	Mortality (%)				
	Turmeric	Clove	Moringa	Red Betel	Deltamethrin
0	76.27 ± 4.26c	90.14 ± 5.67c	86.10 ± 4.55c	69.70 ± 8.50b	78.33 ± 4.64c
1	86.26 ± 6.02c	27.71 ± 8.92a	34.72 ± 3.14b	51.59 ± 4.76b	25.96 ± 6.33ab
3	35.42 ± 5.58b	61.77 ± 4.05b	35.97 ± 9.60b	47.42 ± 4.14b	48.08 ± 9.32b
5	45.06 ± 9.01b	35.28 ± 8.35ab	34.91 ± 8.70b	46.07 ± 10.16b	98.89 ± 1.11c
7	23.87 ± 3.66ab	13.85 ± 7.07a	32.90 ± 4.39ab	8.39 ± 3.24a	47.61 ± 7.65b
10	23.18 ± 1.87ab	11.14 ± 5.40a	15.90 ± 3.55ab	11.82 ± 4.33a	34.40 ± 2.11ab
15	7.64 ± 1.64a	14.57 ± 4.77a	7.69 ± 1.62a	8.95 ± 0.63a	9.76 ± 3.58a

*Note: Numbers followed by the same letter in the same column showed significant difference at α : 0.05 of Tukey' HSD test.

After passing the 5th day of exposure period, mortality began to experience a drastic decline, ranging from <30%. The decrease in the effectiveness of this bioinsecticide is because Littovir® has an effective time range of about 2-4 days after treatment.

This is in accordance with research conducted by by Jha et al. (2015) in which they succeed to lyophilize adenovirus and retain its infectivity over a period of 6 months when stored at room temperature and 4°C, also there is no significant difference in the infectivity or TCID₅₀ titer was observed in the lyophilized virus as compared to the stock virus. Occlusion body (OB) is the infective part of Baculovirus and is quite stable under various conditions. Generally BV (Budded Virus) is less stable than OB, but can be stored for years in tissue culture media at a standard temperature of 4°C and will be even more stable at -85°C. The most detrimental factor in BV storage is light. Therefore, care needs to be taken when using light-tight boxes or when wrapping storage containers with aluminum foil (Lynn & Harrison 2016).

In addition to Littovir® treatment with four natural additives, the treatment carried out in this study was a positive control. Positive control using the chemical insecticide Decis®. The control treatment was applied to cabbage (*Brassica oleracea* L.) in the same way as the bioinsecticide treatment with additives. In the positive control, larval mortality showed a higher value than the negative control because the positive control treatment contained the chemical insecticide Decis®. The chemical insecticide Decis® or also known as Deltamethrin is included in the pyrethroid insecticide and is an insecticide with a broad scope. Pyrethroid insecticides are synthetic compounds created to mimic pyrethrins isolated from chrysanthemums. The mechanism of the chemical insecticide Decis® in infecting insects is to interfere with the function of nerve cells in sending signals by interfering with the opening and closing of small gates in the cell (NPIC 2012).

Although chemical insecticides, especially Decis®, are considered effective in insect control, Decis® has a negative impact on the environment. The use of Decis® for a long time can cause several natural insect biotypes to become resistant, not only to the chemical insecticide Decis® but also to other group 3A insecticides. Resistance can occur because of the variability in the insect's genes and result in dominance in an insect population. Furthermore, the effectiveness of the chemical insecticide Decis against resistant insects can be significantly reduced (Bayer Crop Science 2021). In addition, the negative impact is that this chemical insecticide has a half-life in the soil of 5.7-209 days, which means it takes a long time to decompose because it has a strong binding capacity to soil particles (NPIC 2012). So it is likely to disrupt the balance of the ecosystem in the soil in the long term.

Figure 1 shows that the Littovir® formulation with turmeric additive has a higher mean percentage mortality value than other natural additives. All additives showed similar effectiveness in the exposure period of day 0 to day 5. Entering the exposure period of day 7 and so on, all additives showed a significant decrease in value. The R² value of the four additives did not show

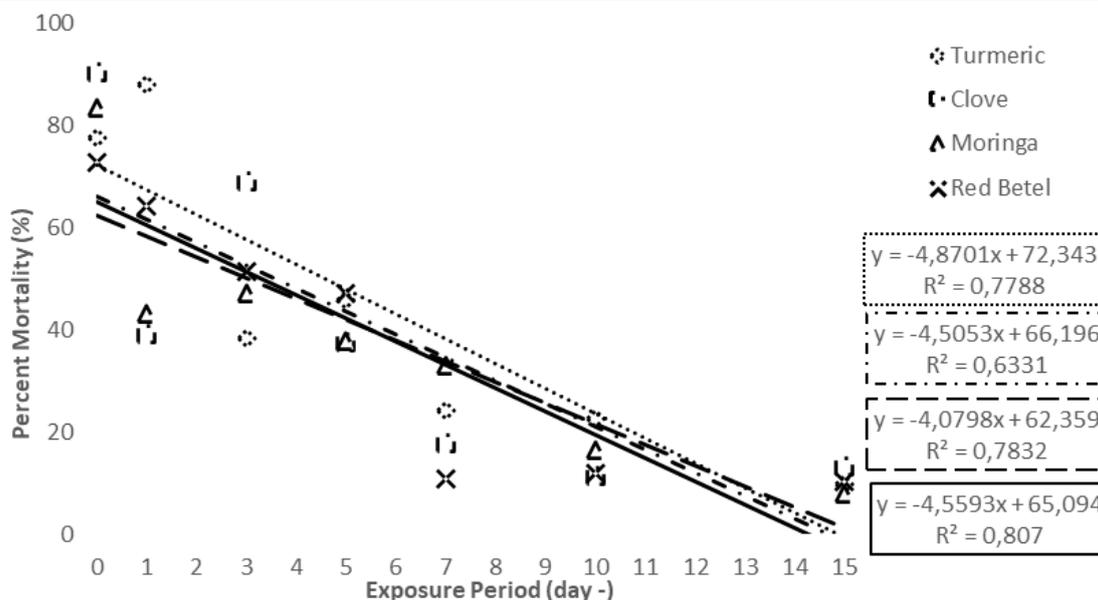


Figure 1. Regression correlation of all natural additives treatment on mortality of *Spodoptera litura* larvae during the fifteen day exposure period.

negative results, so it can be interpreted that all variables x (exposure period) have an effect on variable y (percent mortality).

Based on the results obtained, as described by research conducted by [Bambal & Mishra \(2014\)](#), which compared the formulation of turmeric rhizome extract with *Butea monosperma* flower extract, both of which were mixed with ethanol extract to determine the SPF (Sun Protection Factor) value contained. in it after being exposed to UV light with a wavelength ranging from 290-320nm (UV-B). The results of the study showed that turmeric rhizome extract got a higher SPF value than *B. monosperm* flower extract. It can be concluded that turmeric (*Curcuma longa* L.) rhizome extract showed significant activity as a sunscreen (UV-protectant).

[Khan et al. \(2010\)](#) revealed that the phytochemicals in *Curcuma longa* L. contain alkaloids, glycosides, flavonoids, tannins, phenolics, phytosterols, essential oils, and others. The content of flavonoids in turmeric can act as a scavenger of superoxide anion, singlet oxygen, hydroxyl radicals and lipid peroxy radicals. Many types of flavonoids such as quercetin, luteolin which is a better antioxidant than vitamin C, vitamin E and β -carotene. Therefore, flavonoids belonging to phenolic compounds may be beneficial in preventing UV-induced formation of oxygen free radicals and lipid peroxidation ([Svobodová et al. 2003](#)).

Figure 2 shows the half-life data of Littovir® with the four additives (turmeric, clove, moringa, and red betel). The half-life is the amount of time it takes half of a compound to decay. Half-lives are generally determined in a laboratory where temperature, humidity, light, and pH, which are the determining factors, are tightly controlled. Whereas in actual use (in the field), the half-life is only useful as a reference point, which means the half-life is a variable that depends on interacting field factors such as temperature, humidity, microorganisms, light, soil pH and others.

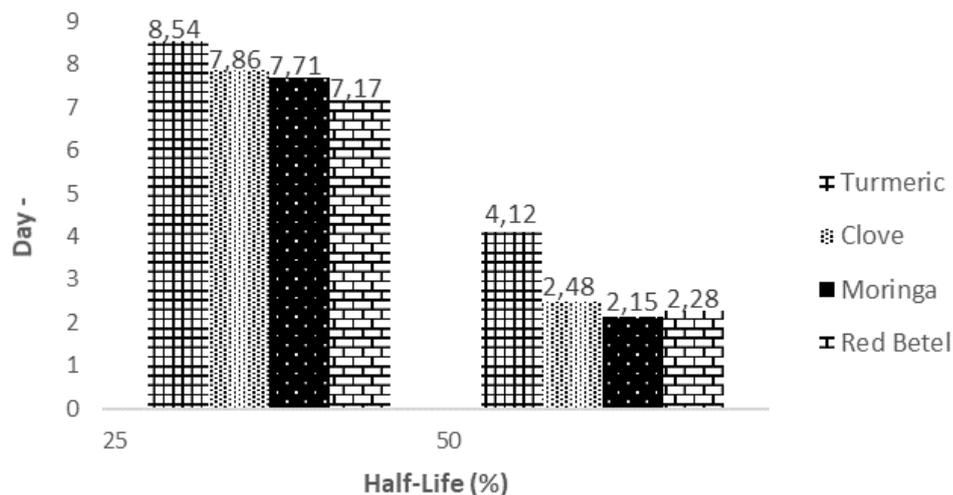


Figure 2. The half-life of *Spodoptera littoralis nucleopolyhedrovirus* (Littovir®) after the addition of natural additives to the leaf surface of *Brassica oleracea* L (The data from Prasetya (2021) was re-analysed).

The presence of UV light is the greatest source of energy that drives the decomposition of most pesticides. However, some chemical pesticide formulations have UV blockers, thereby reducing the amount of photodecomposition of active pesticide ingredients (Cress 1990). Meanwhile, bioinsecticides do not have a protection mechanism like chemical insecticides, so natural additives are needed that function as UV protectors. With the addition of natural additives to bioinsecticides, it is expected to increase the half-life so that it is not easily degraded when applied in the field.

The bioinsecticide formulation that has the longest half-life (50%) is with the natural additive turmeric, which is 4.12 days. Meanwhile, the other three natural additives have no significant difference in half-life. The half-life with clove additives is 2.48 days, the half-life with moringa natural additives is 2.15 days, and the half-life with red betel natural additives is 2.28 days. The bioinsecticide formulation which has the longest half-life (25%) is with natural additives turmeric, which is 8.54 days. Meanwhile, the three formulations of bioinsecticides with other natural additives did not show a significant difference in the second half-life, namely; the half-life with clove additives is 7.86 days, the half-life with moringa natural additives is 7.71 days, and the half-life with red betel natural additives is 7.17 days. Littovir® has effectiveness in controlling armyworm (*Spodoptera litura* Fab.), one of the disadvantages of bioinsecticide is that it is easily degraded when applied in the field. This degradation is caused by UV exposure from sunlight. Natural additives are needed that function as UV protectors for bioinsecticides, so that the effectiveness of bioinsecticides can be increased. Based on the research that has been done, it can be concluded that the most effective natural additive as a UV protectant for *Spodoptera littoralis nucleopolyhedrovirus* (*Spl*MNPV) (Littovir®) is turmeric (*Curcuma longa* L.). The first half-life (50%) indicated by the bioinsecticide formulation with natural additives turmeric is 4.12 days, then the second half-life (25%) will be 8.54 days.

AUTHORS CONTRIBUTION

SS designed the experimentation and managed the research project. BAAP, ASP, and SS run the experimentation, data collection, and perform data analysis. SSu, HP, IS, SuS and RCH supervise and support the experimentation. All the authors read and have the same contributions to the submitted manuscript

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CONFLICT OF INTEREST

All the authors declare that there is no conflict of interest.

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