EFFICACY AND RESIDUAL ACTIVITY OF *Lecanicillium kalimantanense* and *Helicoverpa armigera* Nucleopolyhedrovirus (*Hear*NPV) AGAINST CORN EARWORM *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) UNDER FIELD CONDITIONS

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ABSTRACT

Helicoverpa armigera is one of two most important pests attacking corn in Indonesia, Virus *Hear*NPV and *Lecanicillium kalimantanense* are insect pathogens that can be used for pest management. This research sought to test the efficacy of *Hear*NPV and *L. kalimantanense* applied singly or mixed against *H. armigera* under field conditions. This study was conducted from January to April 2017, with the field phase using randomized block design consisting of three treatments and control, with four replicates, in the IPB Cikabayan experimental garden**.** Laboratory studies were conducted in the Insect Pathology laboratory, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural Institute (IPB). The treatments consisted of viral suspension of *Hear*NPV, conidial suspension of *L. kalimantanense,* mixture of viral and conidial suspension. The corn silk were sprayed using a hand sprayer with entomopathogen suspension after artificial infestation of young larvae at 45 days after planting. Cob damage and larval population were observed and the pathogen was reisolated from corn and soil. All treatments effectively lowered the larval population. Cob damage were reduced by 50% following treatment by fungal suspension whereas cob damage after treatment with viral and mixture of (viral + conidial suspension) were 17.5 % and 22.5 % respectively. No viable conidia could be isolated from corn silk, whereas 13 CFU (colony forming units) was obtained from soil. Polyhedra of *Hear*NPV could be detected from both corn silk and soil 15 days post treatment. Additive effects on mixed applications did not occur in viruses, when compared to the *Hear*NPV application, mixed application was not recommended because it decreased HearNPV virus activity.

Key words: cob damage, entomopathogens, number of larvae, residue, sweet corn.

INTRODUCTION

Corn earworm or *Helicoverpa armigera* (Hubner) is one of two most important pest of sweet corn in West Java Province, along Asiatic corn borer, *Ostrinia furnacalis* Guen. (Lepidoptera: Crambidae). Losses due to the corn earworm is often significant. This polyphagous insect attacks cotton, beans, sorghum, sunflower, soybeans, peanuts, and tomato (Tay et al. 2013). Decrease in yield due to borer attack in the island of Sulawesi reached 51.9 - 53.4% (Karim et al. 2013). The average cob damage in East Java province reached 21.5% (Sarwono et al. 2003). Chemical control is preferably avoided on food crops to minimize the risk of poisoning. In Indonesia, sweet corn is consumed soon after harvest, either as plain food or mixed with vegetable. Application of chemical pesticides might entail negative

impacts to beneficial organisms such as honey bees, parasitoid *Trichogramma* sp. and earwigs. Insecticides like pr ofenofos, endosulfan, and cyfluthrin have negative effect on the natural enemies of *H. armigera* such as *Paederus* sp. (Coleoptera: Staphylinidae), *Camphyloma* sp. (Hemiptera: Miridae), *Chrysopa* sp. (Neuroptera: Chrysopidae), and spider (Araneae: Araneidae) (Nurindah and Subitakto *in* Laba (2010). The use of entomopathogenic microorganisms that are very specific are expected to control the pest population while being environmentally safe for beneficial arthropods.

Entomopathogens evaluated against *H. armigera* include *Beauveria bassiana, Metarhizium anisopliae, Nomuraea rileyi* and *Hear*NPV (Qayyum et al. 2015). *Hear*NPV was more effective against *H. armigera* than mixed application (*Hear*NPV and *Lecanicillium* sp.) in the laboratory, the value of LT_{50} in *Hear*NPV application against *H. armigera* larvae was 2.03 days, while mixed applications (*Lecanicillium* sp. and *Hear*NPV) took 3.23 days (Ginting et al. 2018). *Lecanicillium* sp. PTN01 was able to inhibit *H. armigera* egg hatching by 13.75%, and the first instar larval survival 98.75% in the laboratory. Virulence test showed the highest *H. armigera* larval mortality of 41.25%, at 10^7 conidia/mL density. The DNA sequence analysis of ITS 1 and ITS 4 primers showed that *Lecanicillium* sp. PTN01 was similar to fungus species *L. kalimantanense* strain BTCC F23 with 94% homology (Ginting et al. 2019). *Lecanicillium kalimantanense* strain BTCC F23 was isolated from exoskeleton of (Staphylinoidea: Coleteopera) in *Asplenium nidus* which grows in tropical rainforests, in East Kalimantan (Sukarno et al. 2009).

L. lecanii is able to infect eggs of soybean bug *Riptortus linearis* (F.) (Hemiptera: Alydidae) (Prayogo 2004). Corn cob stalk borers (*Helicoverpa armigera*) can be controlled by using the fungus *Beauveria bassiana* (Ritu *et al*., 2012), and *L. lecanii* for corn stalk borers *(Ostrinia furnacalis)* (Agustin 2014). NPV isolated from a cotton field was effective against *H. armigera* (Diyasti 2016). The fungus *Lecanicillium* and NPV are hypothesized to be effective against corn earworm larvae. Because the mode of actions exhibited by the two entomopathogen are not similar, *contact poison* for fungus and *per oral* for NPV, it is not intended to study deeply the effect of joint action between two entomopathogen. This research sought to measure the efficacy of *Hear*NPV and *L. kalimantanense* applied separately and as a mixture against *H. armigera* under field conditions.

MATERIALS AND METHOD

Corn cultivation. Sweet corn variety Talenta was planted in polybags (25 cm diameter and 50 cm height) in order to minimize contamination by other microorganisms from the environment to the soil, on January 7, 2017. Soil and cow manure mixture (2 part of soil and 1 part of manure) was used as growing media. One seed was placed at a depth 3 cm in the soil and fertilizer was applied according to the manual of corn cultivation (System Information Management Development Rural, 2000). All polybags were laid out in the experimental field of the University Farm at Cikabayan (Bogor), Faculty of Agriculture, Bogor Agricultural University. The distance between polybag in the row was 40 cm, and the distance between rows was 70 cm. The plants were watered twice a day at the vegetative stage and reduced to twice a week, in the morning. How many weeks old when wataering was done 2x a week?

Introduction of *H. armigera* eggs. *H. armigera* larvae were obtained from Situ Gede (Bogor, Indonesia), and maintained in a laboratory using artificial diet (Grzywacz et al. 2011). Infestation of plant with *H. armigera* eggs was carried out after the silk (female flower) were completely formed or 45 days after corn planting. In the laboratory, the egg were laid by moth on gauze cloth. The cloth with eggs then cut and sticked to the corn silk. To each cob, 20 eggs have been introduced. After the plants were infested with eggs, corn cob were encased by fine gauze cloth to prevent predators and parasitoids, because *H.armigera* larvae are cannibal so the number of larvae was left on each cob each one larvae.

Preparation and application of biopesticides. The treatment consisted of *Hear*NPV polyhedral suspension, *L. kalimantanense* conidial suspension and mixture of polyhedra and conidia suspensions and control (water sterile). *L. kalimantanense* were cultured using parboiled rice media and incubated in the incubator at 24 C. After 21 days, conidia were harvested, filtered and counted by using haemocytometer (Goettel and Inglis 1997). Final density of conidia obtained was 2.8 x 10^8 conidia/mL. The conidia were suspended in a solution of Triton X-100 in sterile water (0.1 %). One cob received 11.2 mL suspension using the recommended rate of 400 L spray volume for 1 ha of corn. Hand sprayer was used to deliver 11.2 mL suspension to each cob. Suspension of *Hear*NPV polyhedra was prepared from cadavers of *H. armigera* infected by NPV homogenized in mortar and filtered with fine nylon cloth. The sediment was centrifuged at 2975 g to separate the polyhedra from the larval debris that passed the cloth. Polyhedral suspension mixed with sterile water containing Triton X-100 (0.1% concentration) and the density of polyhedra inclusion body (PIB) were counted using a haemocytometer. *Hear*NPV with final concentration of 2.8 x 10^6 PIB/mL was used as treatment. The choice of the concentration of conidia and polyhedra is based on preliminary study in the laboratory which caused about 70% mortality of second instar larvae. The mixture of two biopesticdes was made by mixing equal volumes of the two suspensions. All preparations were done on the same day as the application.

The application of biopesticides to the cob was realized by spraying with a hand sprayer, 3 days after the eggs hatched. Each cob received 11.2 mL suspension of corresponding treatment. Borders and rows between plots were left untreated (240 plants). Spraying was done twice with three days interval. Three weeks after application of fungal and viral biopesticides, the residual effects of the biopesticides on corn silk and in the soil were assessed according the method described below. The experimental treatments followed group randomized design.

Observation variables. One treatment consisted of 10 plants and each plant had one larva. Each treatment was repeated 4 times so that there were 40 plants for one treatment. The total plant for 4 treatments are 160 plants.

The percentage of damage to corn cobs was calculated at harvest time using the formula:

$$P = (n / N) \times 100\%$$

where P = Percentage of cob damage (%), n = Number of damaged corn cobs (fruit), and N = Total number of cobs observed.

Residue of fungal conidia on corn silk and soil. The soil was sampled from each polybag and mixed. From these soil aggregate samples, 10 g were weighed and suspended with 90 mL of sterile distilled water in a 250 mL Erlenmeyer and vigorously shaken. Serial dilution was made from 1 mL soil suspension until 10^{-3} dilution and 1 mL was spread onto sterile PDA in a 9 cm Petri dish. After several days, the number of fungal colonies were counted and when the conidia were fully formed and developed, microscopic observation were performed at 21 days after inoculation. The fungi were identified based on their morphological characters according to the key determinations of Becnel (1997). The same procedures were applied to assess the residue of conidia from the corn silk.

Residue of *Hear*NPV. Extraction of occlusion bodies from corn silk and soil was performed at 15 days after application using the method of Hunter-Fujita and co-workers (1998). The silk of each cob were cut and mixed, from which 12.5 gram silk were randomly sampled. Soil weighing 12.5 gram was collected from each polybag at approximately 5 cm depth. It was then mixed until homogeneous. The corn silk and soil samples were each cleaned for 5 min at 4 ºC using ultrasonic cleaner to release the biopesticide. Then to the sonicated samples, 25 mL SDS desorbent (0.1%) were added and allowed to stand for 90 minute. The supernatant was separated from the sediment. The sediment was taken and

allowed to stand for 20 min in desorbent, before sonication for 5 min at 4°C. The sediments were discard while the combined supernatants were centrifuged for 5 min at 180 g. The sediment were discarded and the supernatant was centrifuged again during 20 min at 2975 g. The sediments were resuspended in 100 mL of sterile water and centrifuged for 20 min at 2975 g to remove impurities. The supernatant was discarded and the samples were again suspended in 3 mL deionized water and observed under a microscope. The polyhedra inclusion body/mL concentration were calculated using a haemocytometer. The suspension that contained polyhedra were used as virus suspension treated to larva *H. armigera* through feed contamination. Subsequently disease symptoms and the presence of polyhedra was observed as a part of biological identification of NPV.

Data analysis. The experimental data were analysed by ANOVA using SPSS program version 16.0 (Sujianto and Agus 2009). In case of any significant difference between treatments, data analysis was continued with Least Significant Difference (LSD) in level α 0.05.

RESULTS AND DISCUSSION

Larval population. The application of all entomopathogen isolates had a significant effect on the population of *H. armigera* larvae (F= 70.154, df =15, P= 0.000). The population of *H. armigera* larvae were significantly different between treatment and control (Table 1). The number of larvae in the control treatment (10 plants) was 9.75, or in average one larva per cob. This was because the control plants were not treated with isolates and so there was no interference with larval feeding activity during which the insect larvae stage had high feeding activity. The number of larvae in the control treatment was higher than the other treatments, this was because the control plants were not applied to insect pathogens.Treatment of *L. kalimantanense* alone caused high mortality of larvae, yet only 1.75 larvae were found on 10 plants. The highest mortality was shown by treatment involving *Hear*NPV. The as low number as 0.75 larva was observed in the treatment of both *Hear*NPV alone and mixture *Hear*NPV and *L. kalimantanense.*

Table 1. Larval survival after application of entomopathogenic isolates of and HearNPV on the *H. armigera* larvae (n = 10)

Treatment	**Number of larvae ± SE (Average)**
Control	9.75 ± 0.25a
HearNPV	0.75 ± 0.47b
Mixture of HearNPV and *L. kalimantanense*	0.75 ± 0.75b
L. kalimantanense	1.75 ± 0.47b

The numbers followed by different letters in the same column are significantly different according to the LSD test 5% level.

The results were consistent with preliminary laboratory experiment data, where HearNPV was found more effective in controlling *H. armigera* larvae and capable of causing 100% mortality within 4 days at concentrations of 10^7 polyhedra inclusion body (PIB)/mL whereas *L. kalimantanense* was able to control only 41.2% within 7 days at a density of 10^7 conidia/mL. NPVs belong to the group of viruses that have rapid infection course to killing insects in 4-7 days (Grzywacz et al. 2011). HearNPV have been shown effectively control *H. armigera* in the field at concentration level of HearNPV 6 g /liter (Ompusunggu et al. 2015).

The highest larval mortality attained was from HearNPV and the mixture at 92.5% while the lowest mortality was from *L. kalimantanense* at 82.5%. The results are consistent with 70-100% mortality of *H. armigera* from HearNPV treatment (Miranti 2001; Ompusunggu et al.2015)

Percent corn damage. The application of all entomopathogen isolates had a significant effect on percent corn damage (F= 78.152, df =15, P= 0.000). Treatment with *Hear*NPV protected the cob effectively as only 17.5 % cob were damaged. When the fungal suspension were added to the viral suspension, the damage increase until 22.5 %. Treatment by fungal suspension alone, only protected the cob by 50%. The highest damage was noted in the control treatment (97.5%) (Table 2). All treatment of biopesticide either applied singly or in combination significantly lowered cob damage as compared with control. The low percentage of corn cob damage with *Hear*NPV treatment was due to larval mortality. It has been demonstrated that NPV can also effectively infect the corn ear worm (Tenrirawe 2011)

Table 2. Percent corn cob damage after entomopathogen applications

Treatment	Corn cob damage (%) ± SE	Reduction in corn cob damage (%)
Control	97.5 ± 0.25a	-
*Hear*NPV	17.5 ± 0.47b	17.94
HearNPV and *L. kalimantanense* mixture	22.5 ± 0.40b	23.07%
L. kalimantanense	50.0 ± 0.47c	51.28%

The numbers followed by different letters in the same column are significantly different according to the LSD test at 5% significance level.

Larvae treated with *Hear*NPV die because polyhedra inclusion body (PIBs) enter the mesenteron, the main digestive organ which functions as an absorber of nutrients and secretion of digestive enzymes. When the virus infects the insect mesenteron, the histological structure of the peritrophic membrane which is very important in the digestive process is damaged and the digestive process is disrupted and ultimately the larvae die (Granados and Orsaro 1990; Grzywacz et al. 2011).

The amount of cob damage was closely related to the number of *H. armigera* larvae (Table 2). Larvae that bore into the cob will leave dirt on the cob and creates a climate suitable for the growth of fungi that produce mycotoxins that damage the cob (Zaidun (2004). Additive effects on mixed applications did not occur in viruses, when compared to the *Hear*NPV application. The same thing was reported by Gundannavar et al. (2004), in the combined pathogen of *N. rileyi, B. bassiana* and *Hear*NPV, the application of a single virus isolate caused mortality of *H. armigera* larvae to be higher than the combined isolates, but in this study mixed applications (*L. kalimantanense* and *Hear*NPV) more effective than application *L. kalimantanense*, cob damage in mixed applications was 22.5%, and 50% in *L. kalimantanense*. The results are consistent with laboratory results that demonstrated the disruption of larval feeding activity in a mixture of HearNPV and *L. kalimantanense* thus the amount of virus consumed was reduced. Therefore, when compared to viral treatment, lower mortality of *H. armigera* was observed when *L. kalimantanense* was used.

Fungal conidia on corn silk and soil. The result of isolation from corn silk of the plant treated with fungi *L. kalimantanense* showed that no conidia residue have been successfully isolated after 15 days of conidia application; similar result was obtained from soil of control plant. However, from the soil on which the plants have been sprayed previously by conidial suspension, 13 cfu (colony forming units) grew in the PDA media, but none were found on corn silks or in control soil. It is assumed that those fungal conidia grew in the media, originated from the spray droplets that felt to the soil. In the soil, the conidia remained protected against degradation by UV sunlight.

The isolation results on corn did not show conidial residue at 15 days after application, as well as control soil. The results of this study are consistent with Lerche et al. (2009) who reported that *Lecanicillium* spp. exposed to UV-B rays for three hours were still able to grow, but exposure for four

hours caused the fungus to die. UV-A and UV-B originating from sunlight directly cause cell death and mutation (Valero et al.2007; Begum et al. 2009). UV-C caused delays and decreased germination of fungal conidia caused by increased respiration and metabolic activity, thereby reducing food reserves in the conidia (Rahmatzadeh and Khara 2007).

The results of isolation from the soil of plants that have been previously sprayed with conidia suspension treatment and grown on PDA media obtained 13 cfu/mL, but none were found on corn silks or in control soil. Earlier studies demonstrated that *L. lecanii* density in soil decreased rapidly on the first month after inoculation, then stabilized after 6 to 10 months. *L. lecanii* was able to survive on agricultural land for 14 months and at 10^2 conidia/g of soil, growth, sporulation, germination and virulence in aphids did not change (Xie et al. 2015). The presence of conidial residues in treated soil occurs because the soil is an environment suitable for entomopathogenic fungi (Krueger and Roberts, 1997). Conidia in soil can last longer because it is protected from extreme environmental factors such as UV radiation, temperature and drought, but its infectivity was strongly influenced by the physical and chemical properties of the soil (Ekesi et al. 2003). Conidial viability of *Lecanicillium* depended on the duration of sun exposure, 4 h exposure resulted in 19.2% decrease in conidial viability which increased to 78% when exposed for 12 h under sunlight (Prayogo 2004).

Residue of *Hear*NPV. The viral occlusion body (OB) of *Hear*NPV can be detected from corn hair and plant soil treated with *Hear*NPV after 15 days after treatment. The presence of OB on the ground comes from the sprayed material on corn hair which falls to the ground, while the OB on corn hair is ascribed to the insect cadaver. Insect cadaver plays an important role in protecting viral OB from UV radiation. In this study the amount of OB virus residue was not estimated, but the concentration was sufficient to kill the larvae. Qualitative examination of dead larvae after being fed with food contaminated with the OB virus shows that the OB virus has the same properties as OB that were applied, both morphologically (OB form) and biological (virulence OB) (Fig. 1).

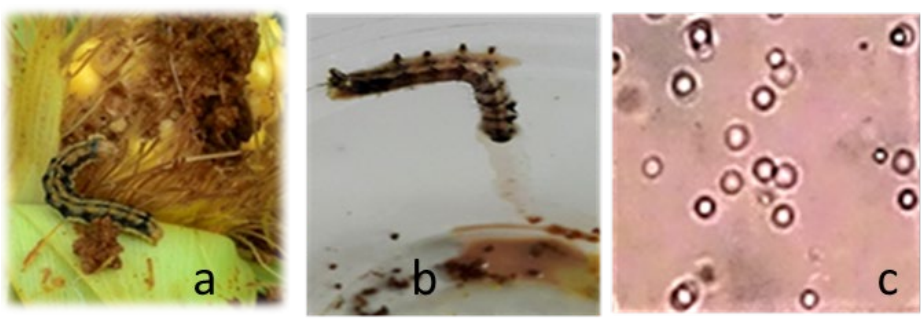

Fig. 1. *Hear*NPV infection in *H. armigera* larvae, a. Healthy larvae, b. Larvae infected by *Hear*NPV, c. PIBs in larvae infected by *Hear*NPV.

*Hear*NPVinfected larvae experience integument color changes, becoming darker, moving more slowly and moving toward the sun. In the final stage of viral infection, the larval body cells become lysis. The infected larva dies in a dependent state forming an inverted V letter. When the integument larvae are torn, the haemolymph fluid contains a lot of polyhedra which spreads.

The recombinant virus expressing neurotoxin (HzSNPV.LqhIT2) and wild type *H. zea* NPV (HzSNPV.WT), sprayed on cotton plants five times during the growing season, were still detected in the soil at 26-35 cm depth and remained distributed between 0-2 cm throughout the plot. The HzSNPV.WT virus was detected as 11-13 OB/g at a depth of 0 -14 cm; while the wild type virus was undetectable below 14 cm. The survival of the virus in the soil was also monitored on three wild-type viruses. *Autographa californica* NPV (AcNPV.WT), *A. californica* NPV expressed scorpion toxin

(AcNPV.AaIT), and *A. californica* NPV expressed juvenile hormone esterase (AcNPV. JHE-S201G). The amount of OB available in the recombinant NPV was greater than that of the wild type virus after 17 months (Fuxa et al. 2001).

CONCLUSION

L. kalimantanense and *Hear*NPV have the potential to control *H. armigera*. The application of HearNPV was more effective than *L. kalimantanense* in the field. Additive effects on mixed applications (*Hear*NPV and *L. kalimantanense)* did not occur, when compared to the *Hear*NPV application. Mixed application was not recommended because it decreases *Hear*NPV virus activity. *Hear*NPV has a higher pathogenicity against *H. arimigera* larvae, compared to *L. kalimantanense*, because *Hear*NPV is a specific host so virulence is higher, while *L. kalimantanense* has a wider range of hosts *L. kalimantanense* and *Hear*NPV are able to develop in nature and cause epizootics, so these are considered important in pest management.

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