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Efication of Some Entomopatogen Fungus on Green Ladybug Imago (Nezara Viridula Linnaeus) (Hemiptera: Pentatomidae)

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Abstract *: Green ladybug (Nezara viridula L.) is an important pest in some plants, including soybeans. N. viridula attacks cause a decrease in yield of up to 80%. Environmental friendly pest control studies are needed to overcome the problem of resurgence caused by N. viridula, among others by utilizing entomopathogenic fungus as their natural enemies. This study discusses about the efficacy of entomopathogenic fungi against N. viridula imago. The research is conducted at the Agricultural Agrotechnology Laboratory, Muhammadiyah University of South Tapanuli. The study uses a completely randomized design, nine treatments (control and eight isolates of entomopathogenic fungus) with three replications. The conidia density used is 108 conidia / ml. The entomopathogenic fungus application was performed on N. viridula imago. The results shows that MetKP fungus isolates are able to kill N. viridula imago with the highest mortality rate of 78.33%. The isolates of MetKM fungi and M. anisopliae caused the lowest imago mortality of 61.67%. Isolate of B. bassiana fungus had the lowest LT_{50} with a time of 5.66 HSA and the isolate of the MetTmM fungus had the highest LT_{50} with a time of 7.27 HSA. In the immature body part of N. viridula infected with fungus is enveloped by entomopathogenic fungal mycelium.*

Keywords *: Metarhizium anisopliae; Beauveria bassiana; mortality; pests; isolates*

I. Introduction

Green ladybug (*Nezara viridula L.)* is an important pest in some plants, including soybeans. The development of green ladybugs was first reported in Ethiopia, then spread to tropical and subtropical countries in continental Europe, Asia, Africa and America (Squitier 2017). *N. viridula* comes to the soybean crop before the flowering phase to lay the eggs. Signs of *N. viridula* attack can be seen from mouth puncture marks on the skin of the pods and seeds. *N. viridula* will damage the pods and seeds until near harvest. The attack directly decreases the quality and yield of seeds (Asadi 2009). *N. viridula* attacks can cause a decrease in yield of up to 80% (Correra and Azevedo 2002).

N. viridula resurgence problems due to chemical insecticides that are used continuously can be overcome by using biological control, among others, by utilizing natural enemies, such as entomopathogenic fungus, predatory insects and parasitoid (Norris et al. 2003). Entomopathogenic fungi are one of the natural enemies that can be used as pest control agents. Entomopathogenic fungi reported to be able to infect pest insects are *Lecanicillium lecanii, Metarhizium anisopliae, and Beauveria bassiana.* Entomopathogenic fungi are very suitable to be chosen as bioinsecticides to control *N. viridula* because entomopathogenic fungus can infect *N. viridula* through the cuticle, in contrast to viruses and bacteria.

Biological control and utilization of entomopathogenic fungi (Prayogo et al. 2012) have various advantages, which have high reproduction, short life cycle, can form conidia that can last long in nature even in conditions that are unfavorable, relatively safe, selective, compatible with various insecticides, it is relatively easy to produce, and the possibility of

causing resistance is very small. Therefore, it is necessary to test the efficacy of several entomopathogenic fungi against *N. viridula* as an environmentally friendly pest control.

II. Research Method

This research was conducted at the Agrotechnology Laboratory, Faculty of Agriculture, Muhammadiyah University of South Tapanuli. *N. viridula* for propagation was taken from paddy fields, long beans and green beans in the city of Padangsidimpuan. This study used a completely randomized design (CRD) method, with 9 treatments (control and 8 isolates of entomopathogenic fungi) and 3 replications.

2.1 Propagation of Green Ladybugs (*N. viridula*)

The obtained imago group was put in a gauze cage. The group of nymphs obtained was put into a plastic box and the group of eggs obtained was inserted into the petri dish. In gauze cages and plastic boxes filled with long beans that have been washed with water to be free of synthetic insecticide residues. Furthermore, the eggs, nymphs and imago groups were maintained in the laboratory. Every day the feed is replaced with fresh beans that are still fresh.

Each nymph stage with the same age is put in a plastic box to avoid competition between the ages of the insect stage. The imago group is also put in the same screen to get the eggs produced by the imago. Imago used in the study was imago aged 1 day after changing the skin.

2.2 Propagation of fungus on PDA media

The fungus isolates used in this study can be seen in table 1. Fungal isolates were grown on PDA media. The composition of PDA media used is potato 400 g, dextrose 15 g, so that 15 gr and aquades 1 L (Goettel and Inglis, 1997). PDA media are compacted in a petri dish 9 cm in diameter. The fungus was incubated for 21 days at room temperature.

Table 1. Isomate of Entomopathogenic Fungus

Fungus isolate	Source of Isolate
MetTrP	*Metarhizium* sp. from rizosfer Terong Padangsidimpuan
MetKPP	*Metarhizium* sp. from rizosfer Kacang Panjang Padangsidimpuan
MetKP	*Metarhizium* sp. from rizosfer Kedelai Padangsidimpuan
MetTmM	*Metarhizium* sp. from rizosfer Tomat Madina
MetJM	*Metarhizium* sp. from rizosfer Jagung Madina
MetKM	*Metarhizium* sp. from rizosfer Kedelai Madina
M. anisopliae	Center for Plant Crops and Protection (BBPPTP) Surabaya
B. bassiana	Center for Plant Crops and Protection (BBPPTP) Surabaya

2.3 Preparation of Fungus Suspension for Testing

Each fungus culture is made suspension. The culture of the fungus is taken as a container by means of each cup plus 10 ml of sterile water + Tween 20 as much as 0.1 ml then scraped with a soft brush. The conidia is then put into a test tube and shaken using a vortex for approximately 60 seconds. The conidia density of each suspension was calculated by the haemocytometer Neubauer-improved until the conidia density used was 108 conidia /

ml. The necessary conidia density is obtained by making a multilevel dilution with a sterile + tween distilled mixture (Goettel and Inglis 1997).

2.4 Entomopathogenic fungus application in *N. viridula* Imago

The fungus suspension was applied by spraying directly on the *N. viridula* imago as much as 10 times the spray / experimental unit (20 imago), using a 2 ml size sprayer bottle. Each treatment was repeated 3 times. Before the application of fungi, each *N. viridula* imago group was put in a plastic box with a size of P x L x T = 10cm x 10cm x 4cm which had been coated with tissue. After the application of plastic fungus filled with sufficient amounts of washed beans as an imago feed supply. Then the plastic box is dripped with distilled water every day to maintain moisture.

2.5 Observation parameter

The variables observed were the number of *N. viridula* who died from infection with entomopathogenic fungi, which were calculated from the time of application up to 7 days after application (HSA), and the time of death of *N. viridula* imago after sprayed with conidial fungus suspension. The percentage of imago mortality was calculated using the formula:

$$M = \frac{A}{N} \times 100\%$$

Information :
M = percentage of mortality (%)
A = Number of insects that die from fungal infections
N = Number of insects tested

The data obtained was analyzed by analysis of variance and DNMRT follow-up at a real level of 5%.

III. Results and Discussion

The observation of the percentage of *N. viridula* immunity mortality after application of several entomopathogenic fungi isolates can be seen in Figure 1 below:

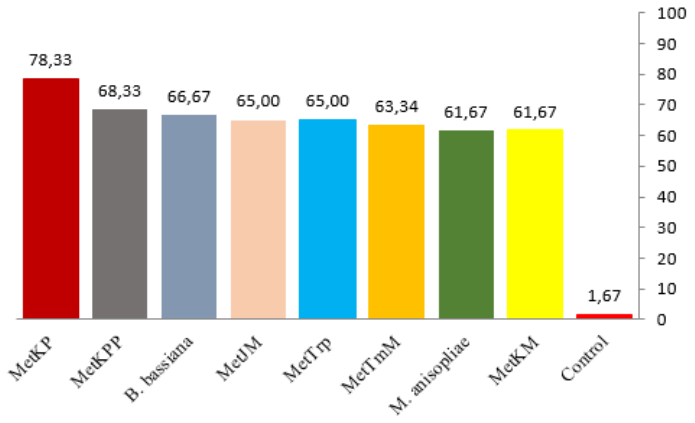

Figure 1. Percentage of mortality of *N. viridula* imago after the aplication of entomopathogenic fungus isolates

Table 2. Mortality rate of N. viridula imago after application of fungal isolates

Fungus isolate	Instar Nymph mortality II (%)	
MetKP	78.33	A
MetKPP	68.33	A B
B. bassiana	66.67	A B
MetJM	65.00	B
MetTrP	65.00	B
MetTmM	63.33	B
M. anisopliae	61.67	B
MetKM	61.67	B
Control	1.67	C

Description: Numbers followed by the same letters in the same column are not significantly different according to Duncan's test (DNMRT) at the 5% level

In table 2, the MetKP fungus isolates caused the highest mortality of *N. viridula* as large as 78.33% but not significantly different from the isolates of MetKPP (68.33%) and *B. bassiana* (66.67%). Meanwhile the fungus isolates of MetKM and *M. anisopliae* caused the lowest imago mortality of 61.67% respectively, but not significantly different from the mortality mortality due to fungi isolates MetTmM, MetTrP, MetJM, *B. bassiana* and MetKPP. The difference in mortality caused by fungus is closely related to the origin of the fungus isolates. Prayogo et al. (2005) said that growing media, virulence level, viability and pathogenicity of entomopathogenic fungi greatly determine the success of fungi in the process of infecting the host. Several factors that influence the effectiveness of entomopathogenic fungi in infecting insects are fungi, origin of fungus, conidia density, quality of growing media, type of pest controlled, application time, frequency of application, and environmental factors such as temperature, rainfall, ultraviolet light and humidity (Tengkano 2004).

In another study, Sunardi et al. (2013) reported that M. anisopliae fungus could kill *Aphis craccivora* (Hemiptera: Aphididae) by 75% at 108 / ml konidia density. Suryadi and Kadir (2007) obtained the results of the study of M. anisopliae capable of causing mortality of 46% in Nilaparvata lugens (Hemitera: Delphacidae). Prayogo (2004) also reported *M. anisopliae* infect *Riptortus linearis* (Hemiptera: Alydidae) by 40%. Furthermore, Permadi (2017) research obtained the results of *B. bassiana's* research causing mortality of *Diaphorina citri* (Hemiptera: Liviidae) of 53.33%. Ladja et al. (2011) obtained the results of B. bassiana killing 65% *Nephotettix virescens* (Hemiptera: Cicadellidae) at 108 / ml conida densities. Herlinda et al. (2012) also found that *B. bassiana* killed Paracoccus marginatus (Hemiptera: Pseudococcidae) by 75%.

The Agricultural Research and Development Agency (2012) stated that the mechanism of infection of entomopathogenic fungi begins with the attachment of fungal conidia to insect cuticles. Then konida germinate and penetrate into the insect's body. The next stage, the fungus grew and developed in hemolymph. Fungus would accelerate reproduction by separating the body of the hifa to fight the body's defenses. At the same time, antibiotic toxins produced by fungus weaken and kill insects quickly. Furthermore hyphae would grow and fill the entire body of the insect. When fungus begun to develop, insects show symptoms of pain, such as movements that are not coordinated and consequently will cause death in insects.

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In Figure 2, we can see the accumulation of the daily mortality of *N. viridula* imago after application of several fungus isolates. Based on observations, the fungus isolates tested had begun to cause death in *N. virudula* in 1 HSA.

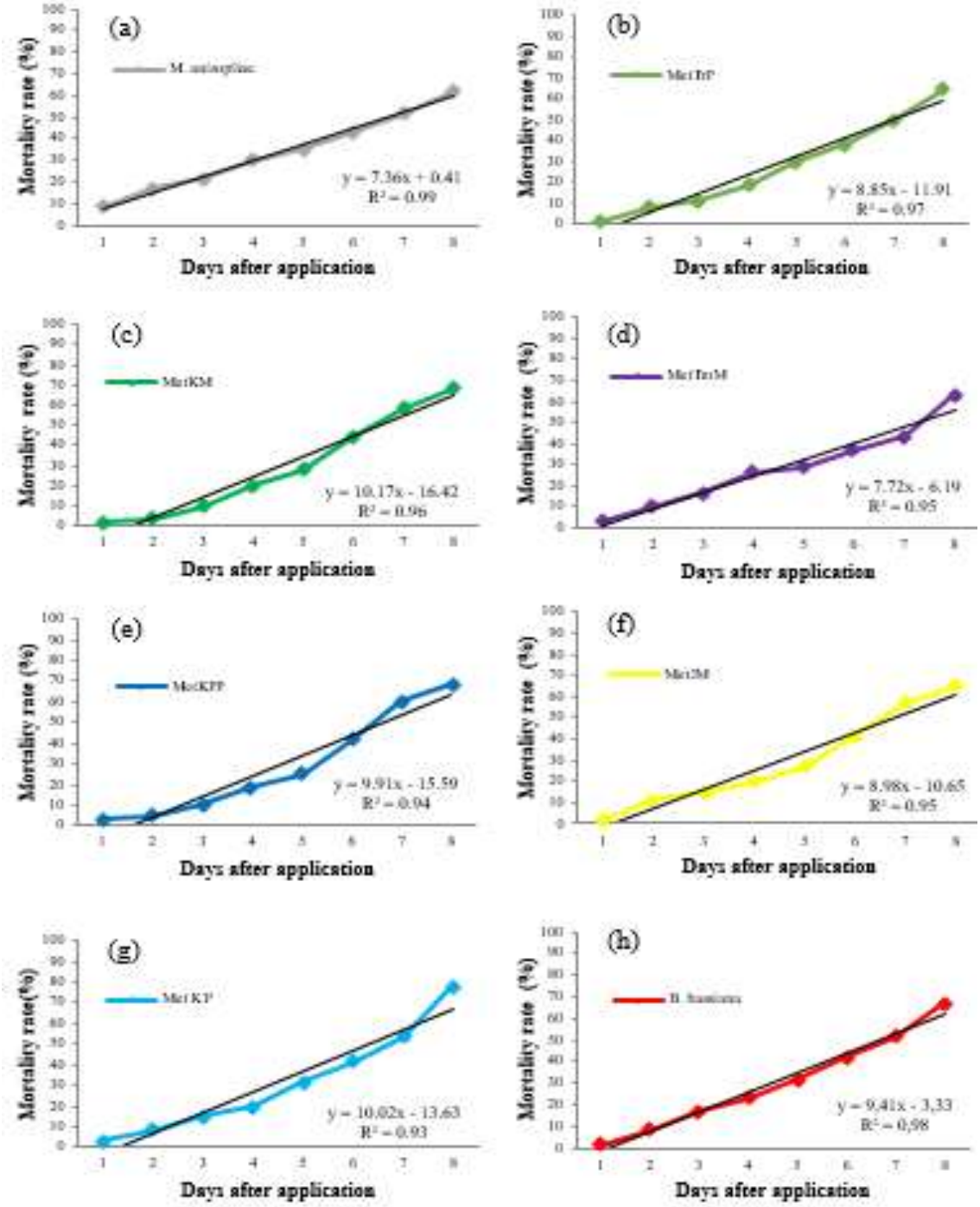

Figure 2. Graph of accumulated daily mortality of *N.viridula* imago due to fungal infection (a) M. anisopliae, (b) MetTrP, (c) MetKM, (d) MetTmM, (e) MetKPP, (f) MetJM, (g) MetKP, (h) *B. bassiana*

The fastest fungus isolate causing mortality in *N. viridula* imago was *M. anisopliae* fungus isolates (5 individuals), MetTmM (2 individuals), MetKP (2 individuals), MetTrP (1 tail), MetJM (1 tail), and B. bassiana (1 tail) occurred in 1 HSA (Figure 2). Furthermore, isolates of the MetKM fungus and the new MetKPP can cause death in 2 HSAs, each with 1 tail and 2 tails. The advantages of MetKPM in the speed of shutting down host insects were influenced by the ability of these fungus isolates to produce enzymes that play a role in penetration and invasion in the insect's body. The body defenses possessed by the *N. viridula* imago also affect the time of *N. viridula* imago death. Weak body defenses from *N. viridula* imago will cause death at the beginning. Then the ability of the fungus to adjust to the body of the *N. viridula* imago to develop and obtain nutrients so as to be able to kill the *N.viridula* imago on the first day.

Based on the results of probit *B. bassiana* analysis, the fastest kill of 50% of *N. viridula* imago with 5.66 HSA while the fungus isolate MetTmM at the most killed 50% of *N. viridula* imago with a time of 7.27 HSA. There are several factors that cause differences in the speed of lethal *N. viridula* death by several fungi isolates used including the type of isolate, the origin of isolates, the physiology of each isolate, the amount of conidia produced by isolates, conidia sprout power, and virulence (Widayat and Rayati 1993) .

Regression equation for *N. viridula* imago mortality due to *M. anisopliae* infection is y = 7.36x + 0.41 with R2 = 0.99. The formed slope value is 7.36. If the R2 value is getting bigger, then the population response to treatment is increasingly homogeneous. Each individual has a relatively similar sensitivity if the population is homogeneous (Himawati 2003). Immuno *N. viridula* mortality data due to infection of MetKP fungus isolates (y = 10.02x-13.63, R2 = 0.93) were fungal isolates which had the lowest R^2 value. This regression equation is useful for estimating the percentage of mortality (y) obtained on a given day (x).

Table 3. LT50 several isolates of Metarhizium sp. in N. viridula imago

Fungus	LT50	Regression equation	R^2
B. bassiana	5.66	y = 9.41x – 3.33	0.98
MetKP	6.23	y = 10.02x – 13.63	0.93
MetKM	6.53	y = 10.17x – 16.42	0.96
MetKPP	6.61	y = 9.91x – 15.59	0.94
M. anisopliae	6.73	y = 7.36x + 0.41	0.99
MetJM	6.75	y = 8.98x – 10.65	0.95
MetTrP	6.99	y = 8.85x – 11.91	0.97
MetTmM	7.27	y = 7.72x – 6.19	0.95

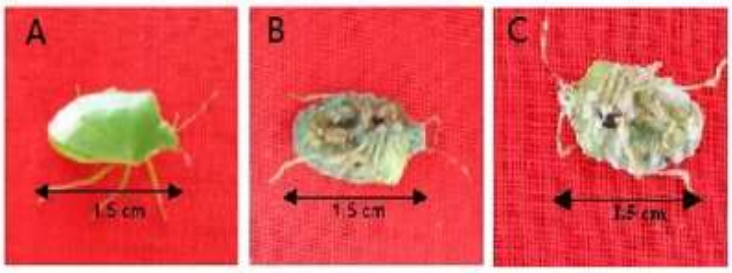

Figure 3. N. virudula (A) Imago healthy, (B) Imago infected wirh fungus Metarhizium sp., (C) Imago infeceted wih B. bassiana fungus

In the picture above the healthy *N. viridula* imago has a bright color. Whereas *N. viridula* imago infected with fungus *Metarhizum* sp. looks covered in green mycelium. As stated by Prayogo (2006) symptoms that arise in insects infected with fungus *Metarhizium* sp. is the presence of mycelia in insects characterized by the growth of entomopathogenic fungal mycelium in parts of the body which are initially white and will then turn dark green. *N. viridula* Imago which has been infected with *B. bassiana* fungus appears to be enveloped in white mycelium.

At the beginning of the death of *N. viridula* imago there are no signs of mycelium growing in the *N. viridula* imago organ. The new fungal mycelium appears after several days of *N. viridula* imago death. Mycelia begins to grow and is found in the articulated organs, especially in the legs, then the mouthpiece, then develops on the thorax and abdominal segments. This is because the articulation organs are areas that are very flexible so that they are more easily penetrated by the conidia of the fungus.

IV. Conclusion

MetKP fungus isolates were able to kill *N. viridula* imago with the highest mortality rate of 78.33%. The isolates of MetKM fungi and *M. anisopliae* caused the lowest imago mortality of 61.67%. Isolate of *B. bassiana* fungus had the lowest LT50 with a time of 5.66 HSA and the isolate of the MetTmM fungus had the highest LT50 with a time of 7.27 HSA. In the immature body part of *N. viridula* infected with fungus is enveloped by entomopathogenic fungal mycelium.

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