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The Effectiveness of the Fungus *Metarhiziumanisopliae* as a Biocontrol Agent against the *Nezaraviridula* Pestin the Province of Aceh.

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Abstract:*Nezaraviridula is an important pest in soybean plants. Stabbing and sucking is part of the mouthpiece of this pest, and stilet is part of the mouth tool used to damage pods from soybean plants which harm up to 80%.Management of pest N. viridulaso far done by using synthetic insecticides that have a negative impact on theenvironment. Resolving the problem need to control other ways environmentally friendly as use M. anisopliae as a biological agent. Plant Pest Laboratory, Plant Protection Study Program, Faculty of Agriculture, Syiah Kuala University. This is where the research was conducted, from April to July 2018 by adopting a completely randomized design. The purpose of this research to study the effectiveness of M. anisopliae fungi as a bioinsecticide against N.viridulapests in soybean plants. The results showed that M. anisopliae has potential as a bioinsecticide in controlling N. viridula. The activity of M. anisopliae against N. viridulahas damaged the body's tissue system, which affects its slow movement, weakness, not eating and death. ycelia from the fungus M. anisopliae almost covers all integument surfaces to the nymphs of N. viriduladries like a mummy. M. anisopliae conidia with a density of 8g / 100 ml are effective for infecting N. viridulanymphs on instar 2, with a mortality of 87.78% at 6 days after application.*

Keyword: ***Nezaraviridula, pests, biological agents, M. anisopliae, soybeans***

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I. Introduction

Nezaraviridula is an important pest in soybean plants in Indonesia. Stylet in this pest is used for Stab and suck soybean pods until production is reduced by 80% (Kalshoven, 1981). *N. viridula*control is currently underway by using synthetic insecticides and negative impact on the environment. To resolve the issue need other controls that are environmentally friendly,as with the use of entomopathogenic fungi (Sayuthi 2011).One of biological agents that has the potential to control pest insects from the order Lepidoptera, Coleoptera, Isoptera and Hemiptera is *Metarhiziumanisopliae* (Prayogo, 2006). According to Sayuthi et al. (2012) that *M. anisopliae* with a conidia density of 109 / ml is capable of producing mortality up to 90% of the isoptera order. According to Permadi (2016) that with a conidia density of 108 / ml is capable of producing mortality of the lepidoptera order up to 28.33%.The basis of the problem becomes a motivation to learn the action of the fungus *M. anisopliae* as a bioinsecticide against *N. viridula*insect pests.

II. Material and Methods

This research was conducted at the Plant Pest Laboratory Study programPlant Protection Faculty of Agriculture, Syiah Kuala University from April to July 2018. The tool used is Laminar Air Flow Cabinet, petridish, spatula, tweezers,scissors, erlenmeyer, knife, autoclave,shaker,analytic scales,incubator, stove,gas cylinders, filters,bucket,digital cameras (Sony α 5000),microscopebinocular (Swift SM-80),andTheingredient is the N. viridulainsect,flourpotato dextrose agar,corn,*M. anisopliae*Tangse isolate.aquadest,gauze,paper straw, alcohol 70%, aluminum foil,long beans,and heatresistant plastic.

Research procedure

1. Insect Culture of *N. viridula*

*N. viridula*was obtained from the Faculty of Agriculture experimental garden, then bred in the Plant Pest Laboratory Plant Protection Study Program. Imago *N. viridula*obtained from the field is put into a jar measuring 8 x 16 cm which has been filled with long beans as feed and covered with gauze and every day given food After imago populated and produce eggs. Nymph which has become an egg moved to in another jar that has been filled with food like long beans. After Nymph becomes second instar, then used as a test insect in this research

2. Increase *M. anisopliae* on PDA media

An amount of 3.9 g of PDA and 100 ml of distilled water was put into the erlemer. Then the solution is stirred until homogeneous and purified using an autoclave for 30 minutes at 121^{o}C. Then the solution is cooled for 10 minutes. Then the PDA is put into the petridish as much as 10 ml. Rejuvenated tangse isolates. By using an ose needle, *M. anisopliae* fungus was grown on PDA media and incubated at ± 25 oC for 7 days (Figure 1.)

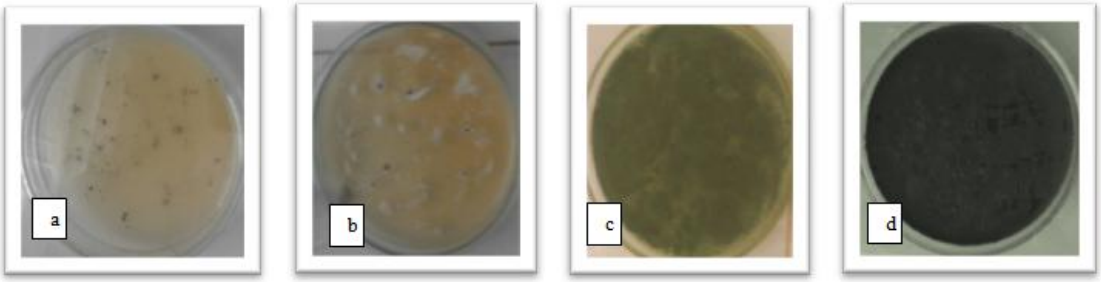

Figure1. Growth and development of *M. anisopliae*Tangse isolates on PDA media: a. 1 Day after inoculation (DAI)., B. 3 DAI., C. 5 DAI., D. 7 DAI

3. Preparation of *M. anisopliae* conidia application

M. anisopliae fungus culture with treatment 4, 6 and 8 g. dissolved into 100 ml of distilled water and stirred using a shaker for ± 30 minutes. Application for each treatment is done by dipping the test insects in each treatment unit (for each test using 10 individual insects in each treatment and for control used aquadest). Then the test insect is put into a jar fed with beans then covered with gauze and observed.

4. Observed variables

Symptoms of *N. viridula*after action by *M. anisopliae*then it appears a form of change in insects due to reactions from pathogens called symptomatology. Observations were made by observing symptoms in *N. viridula*from 1 day after application until behavior changes and morphological changes occur in *N. viridula*, like motion and eating power that is reduced to death.

5. N. viridulamortality (%)

Observations were made by counting the number of *N. viridula*that died from 1 day After Application until the deadline. Calculation of *N. viridula*mortality by using the formula (Abbott, 1925) as follows: Po = r / n x 100%
Information:
Po: Mortality of *N. viridula*
r: Number of *N. viridula*dead
n: Total number of *N. viridula*

6. Time of Death (days)

The time of death is the span of time required by *M. anisopliae* to result in death of the N. viridulainstar 2 nympha. The speed of death is calculated at intervals of 1 day after application (DIA) until death occurs. Calculations using the following formula:
Time of death = (∑ (observation time x number of dead *N.viridula*) / (number of initial *N.viridula*)

III. Result

1. Symptomatology of *M. anisopliae* in *N. viridula*

Action of ***M. anisopliae*s** Bioinsecticide against morphological damage in *N. viridula*pests.The action of *M. anisopliae* as a bioinsecticide against the 2nd instar *N. viridula*nympha was observed on days 1 to 5 (Figure 2: a-e).

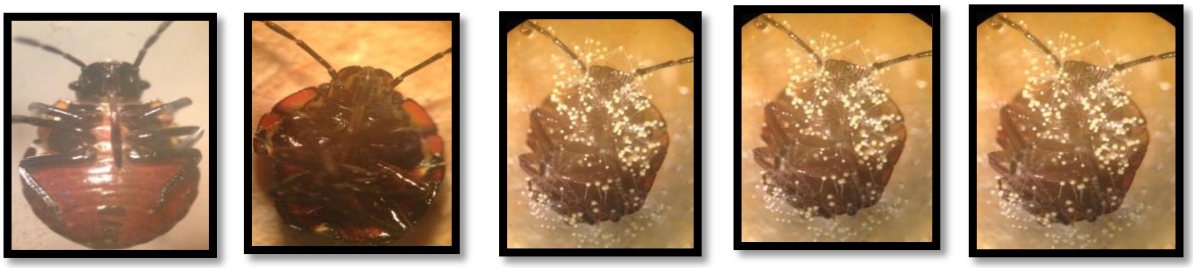

Figure 2. Infection activity of *M. anisopliae* against nympha*N. viridula*instar 2

2. Mortality*N. viridula*(%)

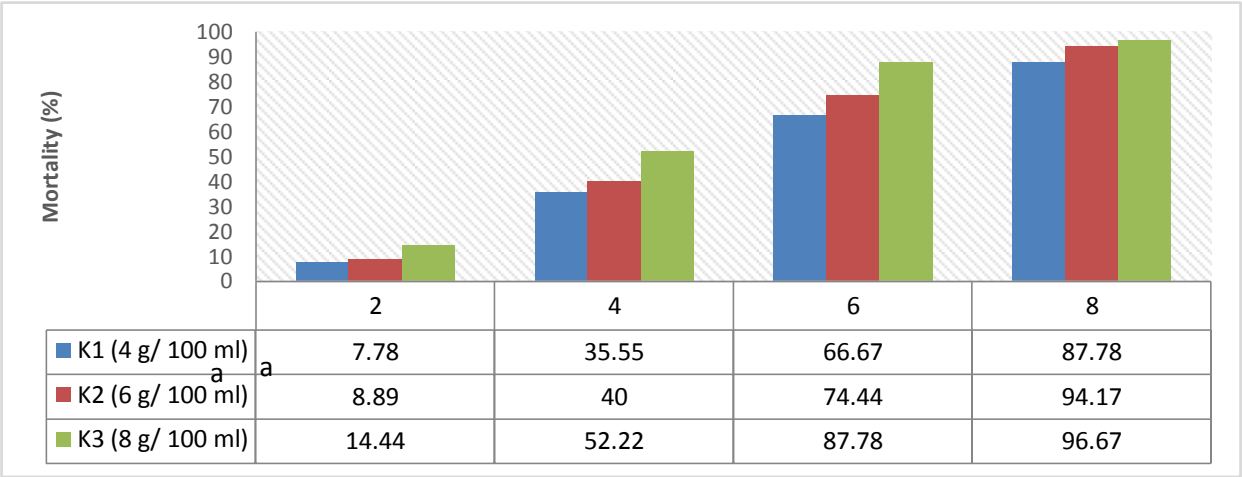

Figure 3. Average Mortality of *N. viridula*after the action of *M. anisopliae* using 4g / 100ml, 6g / 100 ml, 8g / 100 ml treatments which was observed at 2,4,6.8 days

3. Percentage of Food Inhibition (%)

The average percentage of food inhibitors against nympha*N. viridula*instar 2 (Figure 4).

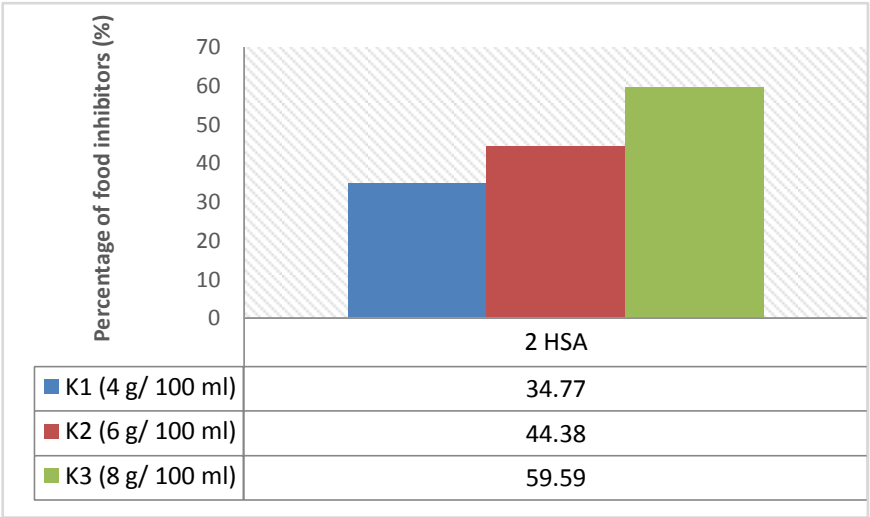

Figure 4. Percentage of inhibitors to eat nympha*N. viridula*instar 2 after several *M. anisopliae* treatments

4.Time of death (days)

Observation of the average death time of instar nympha 2 from *N. viridula*shows that there are significant differences from each treatment of *M. anisopliae* towards the time of death of *N. viridula*instar 2. The time of death was fastest in the K3 treatment (4.49 days), and followed by K2 (5.20 days) and K1 (5.50 day).

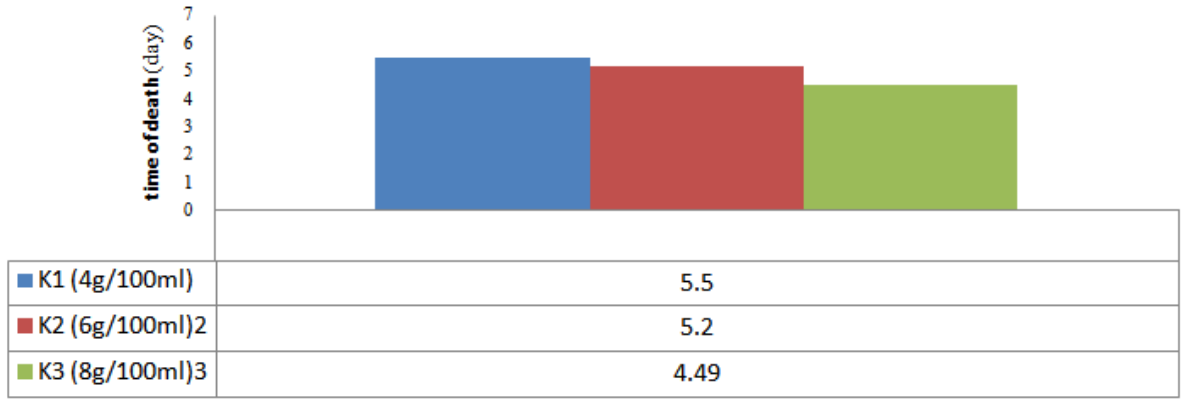

Figure 5. Time of death from nympha*N. viridula*instar 2, after application of each treatment from *M.anisopliae* (4g / 100ml, 6g / 100ml, 8g / 100 ml).

IV. Discussion

1. Symptomatology of *M. anisopliae* in *N. viridula*

After application of *M. anisopliae* to nympha*N. viridula*instar 2 with some predetermined treatments. has caused *N. viridula*to move slowly and not eat. It is suspected that *M. anisopliae* began to react to damage the body's tissue system *N. viridula*but micelia has not been seen (1 HSA) (figure 1).The body of instar nympha 2 is stiff and doesn't move and doesn't eat. His body had begun to shrink but the growth of fungus mycelia on the surface of the insect's body had not been detected visually (2 HSA) (figure 1).N. viridulainstar 2 turns black and the white mycelium has appeared on the ventral surface of the host body in white on day 3 (Figure 3).*N. viridula*instar 2 is black and greenish-white fungus spores appear on the ventral surface of the host body (4 HSA) (Figure 4).

According to Tanada and Rich (1993) insects infected by fungi have become sick and move slowly.According to Wang et al. (2009) secretion of active cuticle degeneration enzymes from 24-72 hours after application.Adult insects have a stronger immune system than young insects,where the cuticle layer of young insects is thinner than adult insects.This condition makes conidia of *M. anisopliae* easier to penetrate the cuticle layer of young insects than old insects (Prayogo, 2010).According to Lawrence (2011) changes in the color of the insect's body due to the response of the pathogen as the host. Generally mycelium growth from fungus occurs on the cuticle surface of insects such as the abdomen, mouth instrument, antennae, and legs (Schapovaloff et al., 201). The sporulation process causes hyphae to infect the host cuticle and form a more dense host tissue until green spores are formed until the body of the insect looks like a mummy or dies (Sayuthi et al., 2011)

2. PestMortality*Nezaraviridula*(%)

Figure 3. Observation results in 2 to 8 days after the application of *M. anisopliae*there are significant differences between each treatment. The highest mortality was found in K3 namely 96.67%, and followed by K2 (94.17%) and K1 (87.78%) at the observation 8 Days After Application. High and low mortality is thought to be due to differences in conidia concentrations and the content of enzymes and toxins produced. The higher the conidia concentration applied to the *N. viridula*pest, the higher the mortality.

The *M. anisopliae* secretes the enzymes chitinase and protease in degrading insect cuticles. After the fungus conidia enters the body cavity of the insect, the resulting destruxin toxin can dehydrate utrients to damage body tissue until the insect dies. According to Moraes et al. (2003) *M. anisopliae* produces chitinase and protease enzymes to degrade the cuticle of pest insects. According to Schrank&Vainstein (2010) that M. anisopliae secretes Destruxin A, E and B toxins (DA, DE, DB).According to Han et al. (2014) after the conidia of the fungus attaches to the cuticle of the insect, the fungus enters the insect's body until the mycelium grows and spreads throughout the body and forms hyphae that produce blastospores.

Host deaths occur because the toxin produced by the fungus causes blood clots and reduced nutrition. Samuels et al. (1988) added that the toxin released by the fungus was able to reduce the host immunity to cause physiological disorders, tissue damage to accelerate the process of host death. Wright & Cornelius (2012) reported that the application of *M. anisopliae*with a concentration of 106 / ml was able to produce mortality up to 7.5% of Coptotermesformosanus and 77.5% with a concentration of 108 / ml after 14 days after application.

The nymphaof Instar 2 has a thin cuticle on the integument, resulting in the enzymes produced by the fungus easily degrade the integument. According to James (2001) nympha instar 2 has a thin waxy coating, which has not been formed optimally, so that *M. anisopliae* is easy to infect the host quickly. The enzymes

produced by *M. anisopliae* are chitinases, proteases and lipases which function to degrade the constituents of cuticles in insects (Bai et. al. 2012 According to Wang et al. (2009) nympha insects die in harsh and rigid conditions because the fluids in the host body have been absorbed by the fungus mycelium *M. anisopliae.*

3. Percentage Inhibition Eat (%)

Figure (4) shows that the average percentage of food inhibitors after the application of *M. anisopliae*with several conidia treatments there is a significant difference for each treatment. The highest percentage of food inhibitors was found in K3 (59.59%), followed by K2 (44.38%) and lowest in K1 (34.77%). High and low percentage of food inhibitors are thought to influence the toxin content in each treatment. The higher the concentration level of *M. anisopliae*given, the level of food inhibitors increases. Conidia of the fungus after entering the body of the insect, the fungus will release Destruxin A, B, and E toxins which can affect the central nervous system which affects the digestive system of insects so that appetite decreases, so that insects are weak and die.

According to Hussain et al. (2009) toxins from the fungus will negatively impact the digestive system, nervous system, and respiratory system. Mycelium is spread in the host insect haemocoel to disrupt host tissue, digestive tract, and muscle tissue (Toledo et al. 2010). According to Schneider et al. (2013) if a pest insect has been infected by a fungus can cause pest insects to be slow to move due to lack of energy.

4. Time of Death (days)

Figure (5)The observations show thatthe higher application concentration of *M. anisopliae*then the death rate from nympha*N. viridula*instar 2 is increasing.This is suspected Bauveriabassiana fungus toxin activity in damaging the body's tissue system *N. viridula*is increasingly active. Until the time of his death more quickly.According to Melanie et al. (2016) application of M. anisopliae with conidia density of 1.5x105 conidia / mlable to produce death of Nympha N. virudula instar 2 in a number of 1.96 days and concentration of 1.5x102 / ml M. anisoplie can produce N. viriduladeath at 3.08 days.

According to Han et al. (2014) application of M. anisopliae at a concentration of 105 / ml resulted in mortality at 4.7 days, compared to a concentration of 108 / ml which resulted in mortality at 2.1 days for larvae of Spodopteraexigua instar 2.Figure 5 deaths from instar nympha 2 from *N. viridula*were significantly different from each treatment from M. anisopliae.The fastest time of death was 4.31 days (N1) and N2 (4.88 days) and N3 (6.00 days).The younger the stage of insect development, the faster the time needed for M. anisopliae to penetrate *N. viridula*and die.

V. Conclusion

M. anisopliae is effective as a bioinsecticide against *N. viridula*pests.infection of *M. anisopliae* to *N. viridula*is in the early stages of the movement of insects to be slow, weak, not eating and insects attached to the culture media walls. Then it doesn't move and is hard or stiff. The next day of observation on the surface of the insect integument grew white mycelium on the limbs, stylet and thorax, the ventral part. Then the mycelium undergoes a sporulation process and a spore colony is formed. Spores cover almost all parts of the surface of the pest until it dries like a mummy. Application of *M. anisopliae* with a concentration of 8g / 100 ml in instar 2 nymphs is effective against *N. viridula*up to 87.78% at 6 days after application 2. High mortality can affect the incubation period, the percentage of food inhibitors and the time of death of *N.viridula.*

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