



## Use of Microbial Insecticides in Control Stem Borer (*Hexamithodera semivelutina* Hell.) on Clove Plant (*Eugenia aromatica* O.K.)

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**Abstract :** The purpose of this study was to determine *Beauveria bassiana* and *Metarhizium anisopliae* to control clove stem borer (*Hexamithodera semivelutina* Hell.). The study used a randomized block design (RBD). Determination of clove gardens and clove trees using the purposive sampling method, namely by selecting clove trees that have clove stem borer holes that are still active. Data analysis of the results of the study used statistical analysis of the SPSS program Ver. 21. Research results show that microbial insecticides can be used to control *H. semivelutina* in clove (*Eugenia aromatica*) plants. *M. anisopliae* was effective in controlling *H. semivelutina*. Concentrations of *M. anisopliae* spores which were effective in controlling *H. semivelutina* were  $10^8$  and  $10^{10}$  / ml.

**Keywords :** *Beauveria bassiana*, *Metarhizium anisopliae*, *Eugenia aromatica*, *Hexamithodera semivelutina*.

### 1. Introduction

In Indonesia, clove plants are more or less 95% cultivated by the people in the form of smallholder plantations spread throughout the province. In 2004, the planted area was 429,935 ha, whereas in 1982 the area had reached 541,830 ha in 26 provinces. The area of the people's clove plantations has fallen around 120,000 ha for 20 years or every year there is damage of around 6000 ha. Very low clove productivity is only 287.42 kg / ha / harvest. The main factors causing the low productivity of clove plants are pests and diseases such as stem borer, *H. semivelutina* Hell. and *Nothopeus fasciatiennis* Wat. In terms of distribution, *H. semivelutina*'s geographical distribution is North, Central and South Sulawesi, while *N. fasciatiennis* is the islands of Sumatra, Java and Kalimantan<sup>1,2</sup>.

The spread of clove plants in the world is thought to be from Maluku then spread to various corners which later became the center of clove plants both in Indonesia and abroad. In 1800 spread to Malaysia and in 1818 to Zanzibar. Between 1798 and 1924 many cloves were brought to Bengkulu, Penang and Singapore,

Lampung, West Sumatra, Tapanuli and Aceh. Cloves spread to Manado and Minahasa in 1870, which were referred to by some Zending teachers as "Gemeente"<sup>3,4</sup>.

*H. semivelutina* in North Sulawesi was first discovered in Minahasa and Bolaang Mongondow Districts in 1924. At that time many clove plantations in North Sulawesi were attacked by stem borer at 43.2% and plant mortality reached 14%. Stem borer attack rates in Sonder District (600 - 800 m a.s.l.) are higher than in Lembean Kora-Kora Subdistrict (0 - 350 m a.s.l.). Generally these pests are found to attack more clove plants in the highlands. The intensity of borer stem borer attack is more severe in the rainy season compared to the dry season. Damage caused by larvae is caused by larvae eating stem tissue by broaching the tissues on the stem, branches, sometimes reaching the roots. The larvae usually bore down towards the lower surface of the bark of 1-3 cm, but there are also those that bore into the rod 3 - 5cm. Unplanned burrows, irregularly down, deep, and most dangerous are burrows that bore the stem<sup>5,6,7,8</sup>.

The hole in the stem looks wet, emitting brown liquid mixed with dirt that looks like grains of sand or flour. Such circumstances indicate that the larvae are in the burrow and are attacking, so the hoist hole is called an active hole. The number of holes in the clove stem ranges from 20 - 40 and an average of 30, sometimes reaching 100. Galleries of these boring insects are irregular in shape. The length of the tunnels from the top hole to the bottom hole is approximately 2 m some are only 1 m, and an average of 1.5 m, depending on the size of the stem being attacked and the stage of the larvae that are attacking. Damage to the stem, branches and roots occur in the cambium, phloem and xylem tissues, causing plant growth to be stunted and even plants can die<sup>9,10,11</sup>.

Clove stem borer attack in North Sulawesi, was first discovered in Minahasa and Bolaang Mongondow Districts in 1924. At that time many clove plantations in North Sulawesi were attacked by stem borer which was 43.2% and plant mortality reached 14%. Observations at two different locations above sea level (a.s.l.) show different levels of attack. Stem borer attack rates in Sonder Subdistrict (600 - 800 m a.s.l.) were higher than in Lembean Kora-Kora Subdistrict (0 - 350 m a.s.l.). Generally these pests are found to be more attacking clove plants in the highlands<sup>12,13,1,14</sup>.

The intensity of clove stem borer attack is more severe in the rainy season compared to the dry season. Damage caused by larvae is caused by larvae eating stem tissue by broaching the tissues on the stem, branches, sometimes reaching the roots. The larvae usually bore towards the bottom surface of the bark 1 - 3 cm, but there are also larvae that bore into the stem 3 - 5 cm. Borer tunnels that were made irregularly, some downward, inward, and most dangerous were tunnels that encircle the trunk<sup>15,16,14,17</sup>.

The stems that are being attacked emit a brown liquid mixed with fine impurities such as grains of sand or flour. This sign indicates that the larvae are in the tunnel and are attacking, so the tunnel is called an active hole. The number of holes in the clove stem ranges from 20 - 40 and an average of 30, sometimes reaching 100. Borer tunnels are irregular in shape. The length of tunnels can reach approximately 2 m some are only 1 m and an average of 1.5 m, depending on the size of the stem being attacked and the stage of the larvae that are invading<sup>5,9,18,19</sup>.

The main problems in clove farming in Minahasa District are: (1) clove crop productivity is still low due to *H. semivelutina* attacks, (2) control is carried out in a conventional manner, namely physical and / or mechanical and chemical control. The physical and / or mechanical method is to search for *H. semivelutina* larvae by injuring the stem so that the stem becomes damaged interfering with the growth of the clove plant, and (3) chemical methods use synthetic insecticides which pollute the environment. The purpose of this study was to determine the ability of *B. bassiana* and *M. anisopliae* to control clove stem borer (*H. semivelutina*).

The objectives of the study were to: (1) determine whether microbial insecticides can control *H. semivelutina* larvae, (2) test whether *M. anisopliae* or *B. bassiana* are effective in controlling *H. semivelutina* larvae, and (3) determine the concentration of spores of *M. anisopliae* and *B. bassiana* which effectively controls *H. semivelutina* larvae.

## 2. Material and Methods

The research was carried out on clove plantations in Kombi Subdistrict, Minahasa District, North Sulawesi, from March - August 2019. The study used a Randomized Block Design (RBD). Determination of sample clove trees using a purposive sampling method was done by selecting clove trees with holes. clove stem borer still active. Active hole is known with symptoms of discharge and the rest of the movement in the hole.

Microbial insecticides research using the fungus *M. anisopliae* and *B. bassiana* local strain in North Sulawesi. The purposes of this study were to know: (1) the ability of *B. bassiana* and *M. anisopliae* to control clove stem borer, and (2) effective spore concentrations for *B. bassiana* and *M. anisopliae* in controlling clove stem borer.

Application of *B. bassiana* and *M. anisopliae* were carried out in active holes. The treatment consisted of four levels of spore concentration that was  $10^4$  / ml;  $10^6$  / ml,  $10^8$  / ml,  $10^{10}$  / ml, and  $10^0$  / ml as a control. Each treatment was repeated four times, and each test consisted of five holes. The research layout can be seen in Figure 1.

\*\*\*\*\*C4      \*\*\*\*\*Bb2      \*\*\*\*\*Ma1      \*\*\*\*\*Bb4  
 \*\*\*\*\*C2      \*\*\*\*\*Ma4      \*\*\*\*\*C1      \*\*\*\*\*Ma2  
 \*\*\*\*\*Ma3      \*\*\*\*\*C3      \*\*\*\*\*Bb3      \*\*\*\*\*Bb1

**Figure 1. Graph development of percentage of holes of larvae *H. semivelutina* inactive (healed) after the application of *M. anisopliae* and *B. bassiana* on Observation Week I-V**

Observations were made on the third day after application, and were made 4 times, with an observation interval of three days. The things that were observed were the active bore-holes and the bore-holes that had healed due to the use of microbial insecticides. The formula used to measure the success of using Microbial insecticides was as follows:

$$P = \frac{x}{y} \times 100 \%$$

where P is the average percentage of the bore-holes that are inactive (healed) after the application of microbial insecticides, x is the number of the bore-holes that are not active (healed), y is the number of the bore-holes observed inactive (cured) after application of antibiotic insecticide.

Research data were analyzed statistically using the SPSS 21, and to determine the significant level between treatments, the least significant difference (LSD) test was used.

### 3. Results and Discussion

The results of experiments using the fungus entomopathogen *B. bassiana* and *M. anisopliae* against *H. semivelutina* in Clove plants can be followed in Table 1.

**Table 1. Average percentage of the bore-holes not active (healed) due to the use of the fungus Entomopathogenic *B. bassiana* and *M. anisopliae***

Treatments	Average the bore-holes healed (%)	Notations
Ctrl	5,00	a
Bb1	10,00	ab
Ma1	10,00	ab
Bb2	15,00	abc
Bb3	25,00	bc
Ma2	30,00	c
Bb4	45,00	cd
Ma3	60,00	d
Ma4	100,00	e

Alpha = 05; \*): Numbers followed by the same letters indicate no significantly different.

Note:

Ma1	=	<i>M. anisopliae</i> spore concentration $10^4$ / ml
Ma2	=	<i>M. anisopliae</i> spore concentration $10^6$ / ml
Ma3	=	<i>M. anisopliae</i> spore concentration $10^8$ / ml
Ma4	=	<i>M. anisopliae</i> spore concentration $10^{10}$ / ml
Bb1	=	<i>B. bassiana</i> spore concentration $10^4$ / ml
Bb2	=	<i>B. bassiana</i> spore concentration $10^6$ / ml
Bb3	=	<i>B. bassiana</i> spore concentration $10^8$ / ml
Bb4	=	<i>B. bassiana</i> spore concentration $10^{10}$ / ml
Ctrl	=	Control (Ma and Bb spore concentration $10^0$ / ml)

The data above shows that *M. anisopliae* was an effective pathogen in controlling stem borer larvae that was able to achieve a 100% healing rate. The high level of healing of the bore-holes in *M. anisopliae* applications because these pathogens are natural hosts. This fungus is the host of *Brontispa longissima* and *Oryctes rhinoceros*. The higher the concentration of these fungal spores, the higher the level of control of *H. semivelutina* larvae in Clove plants, while *B. bassiana* is less effective in controlling *H. semivelutina* larvae.

According to<sup>20</sup> that *Beauveria* sp. and *Metarhizium* sp. known as insect pathogenic fungi, or entomopathogenic fungi, which are fungi that can cause disease in insects. This is due to the fungus can be used to control pests by making the target pests become sick first, then experiencing death. Formulation of *Beauveria* sp. and *Metarhizium* sp. able to control caterpillars, aphids, and leaf beetles in food crops, horticulture, and biopharmacy. In addition, it can be used to control brown plant hopper, green leafhopper, rice stem borer, stinky rice pest, white pest, and soil bed in rice plants. *Beauveria* sp. and *Metarhizium* sp. controlling pests by means of their spores attached to the body of the host insect will germinate and develop to form sprout tubes. After that, the spores enter by penetrating the integument. Spores can enter through the digestive tract, respiratory tract, and integument of pest insects.

The successful use of fungi as a pest controller in the field is greatly influenced by environmental factors (temperature, humidity, and sunlight). *Beauveria* sp. can release beauvericin that will develop in the body of host insects (pests) and attack all body tissues so that the insects die. Insects attacked by *Beauveria* sp. will die with the body hardened like a mummy, while the attacked by *Metarhizium* sp. will die with a fragile body.

According to<sup>21</sup> that entomopathogenic fungi enter the body of host insects through the skin, digestive tract, spiracles and other holes. The fungus inoculum attached to the body of the host insect will germinate and develop to form a germination tube, and then enter through the body's skin. Penetration is done mechanically and / or chemically by releasing enzymes or toxin. The fungus will develop in the body and attack all body tissues, so the ladder dies. Fungi mycelium comes out of the insect body, grows covering the host body and produces conidia. However, if the conditions are less favorable the development of the fungus only takes place in the host body.

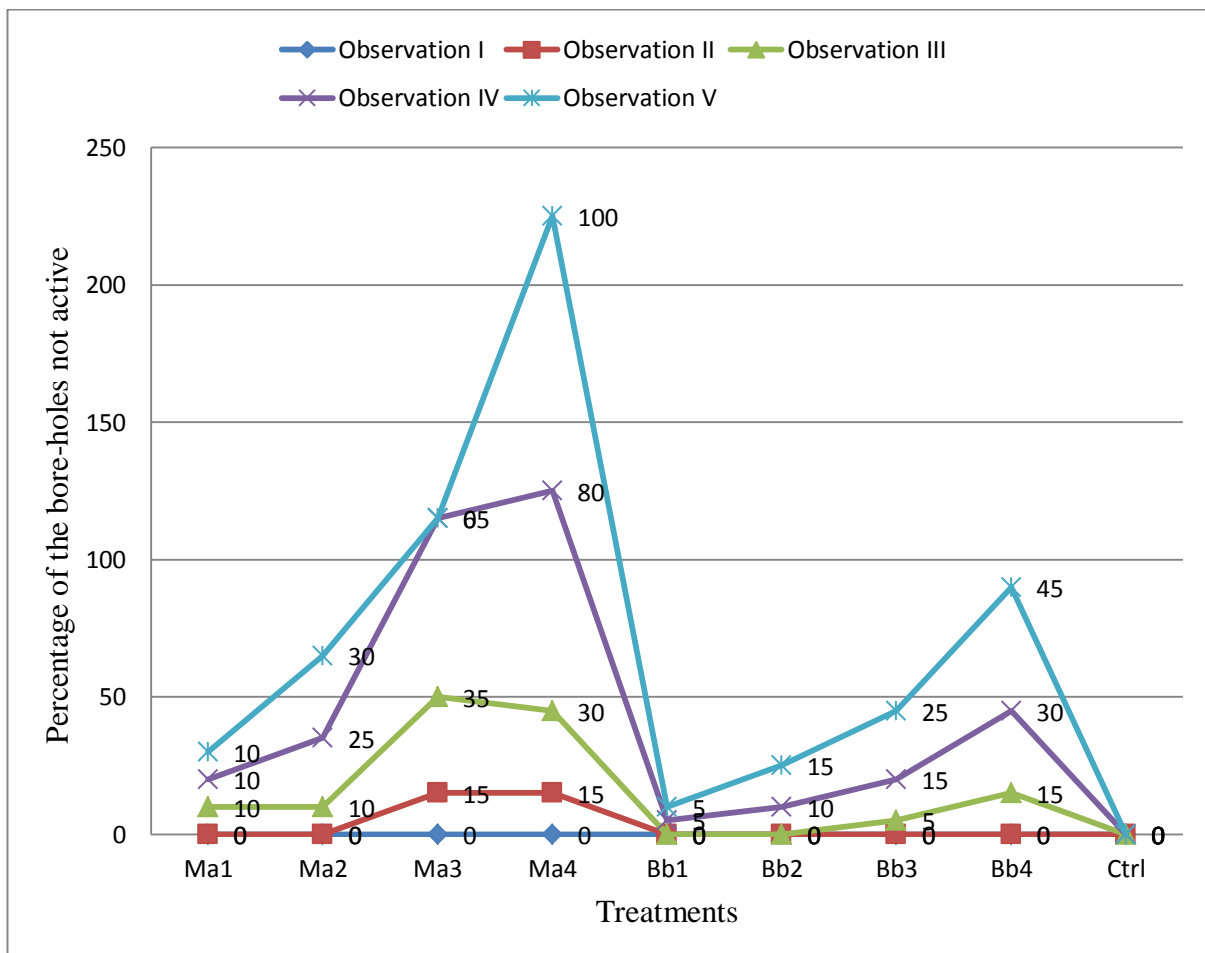
According to Steinhaus (1967) in<sup>22</sup> that *M. anisopliae* fungus has larvicidal activity because it produces cyclopeptide, destruxin A, B, C, D, E and desmethyl destruxin. Destruxin has been considered as a new generation of insecticide. The testosterone effect affects target organelles (mitochondria, endoplasmic reticulum and membranous nucleus), causing cell paralysis and abnormal function of the middle stomach, tubulus malphighi, hemocyte and muscle tissue. The fungus *M. anisopliae* has long been recognized as a pathogen that attacks many pests of the Coleoptera order, especially in the *O. rhinoceros* pest as a pest in the palm plants. Insects infected with *M. anisopliae* will first turn pale yellowish, the movement becomes slow and eating activity decreases. Insects start from the soft parts of the body. Conidia enter the body and spread throughout the body cavity (haemocoel) and penetrate the integument. The typical symptom of the *M. anisopliae* fungus is that the infected larvae will die hard and stiff, but not smell.

According to<sup>23</sup> that there are several advantages of the insect pathogenic fungus *M. anisopliae* as a natural pesticide, namely: (1). Selective against target insects so as not to harm other non-target insects, such as predators, parasitoids, pollinating insects, and useful bees of honey bees, (2). Does not leave toxic residues on agricultural products, in the soil or in natural water courses, (3). Does not cause phytotoxins (poisoning) in plants, and (4). This fungus is easily produced with a simple technique. The results of<sup>21</sup> regarding the efficacy

of several *M. anisopliae* formulations on the percentage of mortality of *O. rhinoceros* gave very good results. The treatment of 30 g of formulation in corn flour caused the percentage of *O. rhinoceros* larvae mortality to reach 100%.

As stated by<sup>24</sup> that there were four stages in the etiology of the disease caused by the fungus *M. anisopliae* in insects. (1). Contact between fungus propagules and insects. Mucopolysaccharide compounds play an important role in the process of contact between fungi and insects. (2). Attaching and germination of fungus propagules on the insect integument. High humidity, sometimes even water is needed for germination of fungus propagules. The fungus can utilize the compounds contained in the integument. Insect integument contains compounds that can function as stimulants for fungal growth, while compounds in insect cuticles are inhibitors for fungus development, (3). Penetration and invasion stages. The fungus when penetrating the integument, forming the germ tube and the point of penetration is strongly influenced by the integument morphological configuration. The fungus also forms the appressorium to penetrate the integument. Penetration is done mechanically and / or chemically by removing enzymes or toxins. (4) Destruction stage. Near the penetration point blastospores form which then circulate in the hemolymph by forming hyphae secondary to attacking other tissues. In general, insects are dead before the proliferation of blastospores. The development of fungi can be slow or very extensive. After the insect dies the development phase of saprophyte begins with attacking the tissue and ends with the formation of the reproductive organs. In general all insect tissue can be attacked. The fungus colonization in the body of the insect and insect body fluids is immediately used up by the fungus, so the insect dies with the body hardened like a mummy.

The bore-holes by *H. semivelutina* larvae which healed after application of microbial insecticides were directly proportional to the increase in the concentration of pathogenic spores. The development of a cavity healed due to microbial insecticide treatment from the first observation to the fifth observation can be followed in Figure 2.



Note: Ma1 = *M. anisopliae* spore concentration 10<sup>4</sup> / ml

Ma2	=	<i>M. anisopliae</i> spore concentration $10^6$ / ml
Ma3	=	<i>M. anisopliae</i> spore concentration $10^8$ / ml
Ma4	=	<i>M. anisopliae</i> spore concentration $10^{10}$ / ml
Bb1	=	<i>B. bassiana</i> spore concentration $10^4$ / ml
Bb2	=	<i>B. bassiana</i> spore concentration $10^6$ / ml
Bb3	=	<i>B. bassiana</i> spore concentration $10^8$ / ml
Bb4	=	<i>B. bassiana</i> spore concentration $10^{10}$ / ml
Ctrl	=	Control (Ma and Bb spore concentration $10^0$ / ml)

**Figure 2. Graph development of percentage of holes of larvae *H. semivelutina* inactive (healed) after the application of *M. anisopliae* and *B. bassiana* on Observation Week I-V**

The data in Figure 2 shows that the recovery of the bore-holes due to *H. semivelutina* larvae has begun to occur in observation II for the treatment of *M. anisopliae* spore concentration of  $10^{10}$  / ml. The treatment of *M. anisopliae* spore concentration  $10^8$  / ml,  $10^6$  / ml,  $10^4$  / ml and treatment of *B. bassiana* concentration of spores  $10^{10}$  / ml,  $10^8$  / ml began to occur at observation III. Treatment of *B. bassiana* with  $10^6$  / ml and  $10^4$  / ml spore concentrations occurred at IV observation. The healing rate the bore-holes were directly proportional to the number or number of pathogenic spores of *M. anisopliae* and *B. bassiana* in a microbial insecticide solution. The greater the content of pathogenic spores in a microbial insecticide solution, the greater the level of healing of the bore-holes due to *H. semivelutina*.

Kaya (1993) in<sup>21</sup> that the virulence of entomopathogenic fungal spores were largely determined by the number and age of spores, and the stages of destruction and colonization of the digestive tract and respiratory system. These processes generally take between 1-2 days depending on the type of fungus and environmental conditions.. These processes generally take between 1-2 days depending on the type of fungus and environmental conditions.. These processes generally take between 1-2 days depending on the type of fungus and environmental conditions. Furthermore Novizan (2002) in<sup>22</sup> that after successfully penetrating and entering the insect's body, the fungus will release toxins resulting in damage to insect body tissue and in two days later the insect will experience death accompanied by the growth of fungal spores on surface of the insect's body. In further attacks the body of the insect will be mummified or the body becomes hard and stiff and overgrown with white mold spores. The effectiveness of entomopathogenic fungi depends greatly on the type of isolate, the density of the spore, the quality of the growing media, the type and age of the host insect, the application time and environmental factors including sunlight (ultra violet) rainfall and humidity.

Mc. Inns (1975) in<sup>25</sup> that fungi that infect insects are called entomopathogenic fungi. Today more than 750 species of entomopathogenic fungi are known and about 100 fungal genera. In contrast to viruses, fungal pathogens enter the body of insects not through food channels but directly into the body through the skin or integument. After the fungus conidia enter the insect's body, the fungus reproduces itself by hyphae formation in epicuticular tissue, epidermis, haemocoel and other tissues, and in the end all tissues are filled with fungal mycelia. In addition there are also some fungi that can affect insect pigmentation and produce toxins that greatly affect the physiology of insects. The spread and infection of fungi are greatly influenced by several factors including host density, spore availability, weather especially wind and wetness. High wetness and strong winds help the spread of conidia and even distribution of pathogenic infections to all individual host populations.

#### 4. Conclusions

1. Microbial insecticide can be used in controlling clove stem borer (*H. semivelutina* Hel.)
2. *M. anisopliae* microbial insecticide was effective in controlling *H. semivelutina*.
3. Concentrations of *M. anisopliae* spores that were effective for controlling *H. semivelutina* were  $10^8$  and  $10^{10}$  / ml.

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