Virulence And LC50% Levels Of Beauveria Bassiana Entomopathogenic Fungi In Croccidolomia Binotalis Zell.

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Abstract : This study aims to look at the level of virulent entomopathogenic fungi against larvae Croccidolomia binotalis and LC 50%, after obtaining entomopathogenic fungi isolates through exploration, isolation and identification. Entomopathogenic fungi are obtained from the Sibolangit conservation forest and from the Berastagi vegetable plantation land. The long-term goal is to reduce the use of chemical pesticides through the development of fungi bioinsecticides obtained through exploration from different isolate sources that are around the Brastagi vegetable fields and Sibolangit conservation forest., Virulence test was carried out in vitro. Fungi that have high virulence will be applied in planta in Brastagi vegetable land. The fungus produced from this research is recommended as a local alternative bioinsecticide to reduce or replace the use of synthetic pesticides which have been threatening the environment and human health. From the results of the study note that the death of larvae in vitro with the treatment of 2 types of entomopathogenic fungi is relatively low. The lowest yield was 0 deaths from FHHKS18 fungi while the highest yield from FPKSB8 fungi was 4 larvae at a concentration of 2000 ppm. The highest percentage of mortality of C.binotalis larvae occurred at the concentration of B. bassiana 4000 ppm, the fastest death on day 4 or 79 hours after treatment. Whereas lethal time 50 (time to kill 50% of C.binotalis larvae) occurs on the 9th day or about 210 hours after treatment.

Index Terms : Virulance, LC50%, Entomopathogenic Fungi, Beauveria bassiana, Croccidolomia binotalis Zell., Bioinsecticide, Berastagi

1. INTRODUCTION

Nearly one million species of insects are known, around 15,000 species are known as pests and around 300 species require control. Fortunately, most insect pests have pathogenic microorganisms associated with them. Among the natural enemies of pest control are pathogenic fungi in insects. In general, pathogenic fungi in insects include Metarhizium anisopliae, Beauveria bassiana, Nomuraea rileyi, Paecilomyces farinosus and Paecilomyces fumosoroseus (Takhur and Sandhu, 2010), Lagenidium, Coelomomyces, Conidiobolus, Entomophaga, Entomophthora, Erynia, Neozygites, Pandora, Zoophthora, Cordyceps, Hypocrella, Torrubiella, Aschersonia, Hirsutella, Tolypocladium, and Verticillium. Pesticides are widely used in agricultural production to prevent or control pests, diseases, weeds, and other plant pathogens in an effort to reduce or prevent yield losses and maintain high product quality.. Although pesticides are developed through a very strict regulatory process functioning with adequate certainty and minimal impact on human health and the environment, serious concerns have been raised regarding health risks due to occupational exposure and from residues in food and drinking water. Control of pest insects with pesticides is a serious problem for human and animal health, therefore the use of biological control is a modern era for pest control. Biological control is a key component of the 'system approach' for integrated pest management, for controlling pests that are resistant to pesticides, withdrawing chemicals and minimizing the use of pesticides (Bale et al, 2008). Cultivated land in Simpang

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Empat District, Tanah Karo Regency, North Sumatra, is a horticultural land that is dominated by cabbage, mustard greens, broccoli, beans, tomatoes and several types of fruits. Exploration of entomopathogenic fungi on this land is to determine the diversity of fungi and see the impact of the use of massive pesticides on the presence of entomopathogenic fungi. Sibolangit Conservation Forest is a forest with a tropical rain forest ecosystem that is still relatively intact. The ecological process runs naturally and not much pressure from the surrounding community. Podsolic soil type and crystal texture so it is easy to absorb water and washed away. It has a wavy topography with a slope factor of 5-10% while the altitude is 558 m above sea level. including in climate type B with rainfall 2,500-30,000 mm / year with humidity between 60-80% maximum average temperature of 35.6ºC and minimum of 25.3ºC. Natural Conditions The forest is thought to have the potential for entomopathogenic fungi that live in association with the roots of forest plants with moisture that is conducive to fungal life. There are no reports of exploration of entomopathogenic fungi in the Sibolangit conservation forest, so the potential of existing fungi is unknown and has not yet been utilized. The urgency of this research is to obtain data on the potential of local entomopathogenic fungi that can be used as bio-insecticides to replace synthetic pesticides. To determine the level of virulence of entomopathogenic fungi and LC 50% and LT 50%.

2. MATERIALS AND METHODS

This research was carried out, after the exploration of entomopathogenic fungi with larvae bait Tenebrio molitor aims to obtain pathogenic fungi in vegetable growing land in Brastagi and in the Sibolangit conservation forest. From the Research Stage of the exploration, isolation and identification of entomopathogenic fungi, two species of fungi were found in the two research sites, Beauveria and Metarhizium. Tenebrio molitor larvae infected with both Beauveria bassiana and Metarhizium anisopliae were isolated by implanting infected

tissue samples in Potato dextrose agar (PDA) media and incubated for 5 to 7 days. Isolation is done by dipping a sample of infected tissue (Tenebrio molitor larvae) for a few moments (± 3 minutes) in a chlorine solution, alcohol, then rinsed with sterile aquadest. fungi growing on media are identified and transmitted (reinoculation) to test insects (Khan, et.al. 2012) Then the level of entomopathogenic fungi virulence test is carried out to develop virulent fungi into bioinsecticides. . Virulence test was performed by calculating a 50% lethal concentration (LC50) and a 50% lethal time (LT50). This test was conducted in vitro at the Biology Laboratory of FKIP UISU. Entomopathogenic fungi Beauveria bassiana or Metarhizium anisopliae which have high virulence levels are then propagated on rice or corn propagation media and applied to prepared vegetable farms. Vegetable fields are planted and maintained without using synthetic pesticides.

3. RESULTS

3.1. Beauveria bassiana Virulence Test

The results of the study obtained white and green entomopathogenic fungi from the Berastagi vegetable growing land and from the Sibolangit Conservation Forest. A total of 25 soil samples taken from the Sibolangit Conservation Forest were found to be the most larvae infection in HKS 18 soil samples, on average white fungal infections 2.75 larvae and 2 larvae infected with green fungi and HKS 8 soil samples with an average of 2.75 larvae infected with white fungi and 1,25 larvae infected with color fungi. Whereas 25 soil samples from the Berastagi vegetable garden obtained the three highest samples namely KSB 8 with an average of 5 larvae infected by white fungi and 2.75 larvae infected by green fungi, KSB 9 with an average of 5 larvae infected by white fungi and 2.5 larvae infected with green fungi and 17 KSB on average 4.75 infected with white fungi and 2.25 larvae infected with green fungi. Data can be seen in Table 1. From the 5 samples with the highest infection, in vitro virulence testing was carried out on the larval insects of Crossidolomia binotalis with a conidia concentration of 20000 ppm. The treatment for 10 days was then observed how many larvae deaths occurred through the virulence test.

Table 1. *Highest Fungi infection data for Tenebrio molitor larvae from Forest soil samples Sibolangit conservation and from the Berastagi vegetable land. White fungi are Beauveria bassiana and green fungi are Metarhizium anisopliae*

No.	Sample soil source	Number of white fungi infected larvae on the test				Number of larvae infected with green fungi on the test				Average fungal infection	
		1	2	3	4	1	2	3	4	FP	FH
1	HKS18	2	4	1	2	2	3	2	1	2,75	2
2	HKS8	3	2	4	2	1	2	1	1	2,75	1,25
3	KSB8	5	6	4	5	3	2	3	3	5	2,75
4	KSB9	4	5	5	6	2	3	2	3	5	2,5
5	KSB17	4	5	7	3	2	4	1	2	4,75	2,25

Note: HKS: Sibolangit Conservation Forest
LSB: Berastagi Vegetable land
FP: white fungi
FH: Green fungi

From the selected fungi then identified both macro and microscopic to determine the type of fungi. So from microscopic observation with the help of a microscope and compared with reference sources, that the white fungus is Beauveria bassiana while the green fungus is Metarhizium anisopliae, as in the following figure.

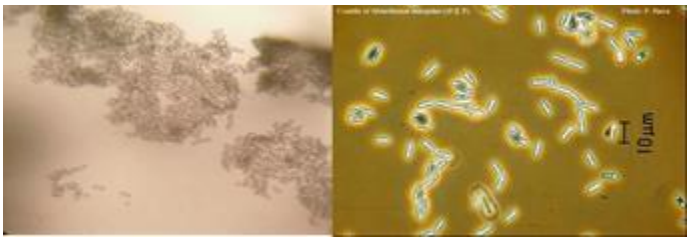

Figure 1. *Appearance of the conidia of Metarhizium anisopliae by microscopy.*

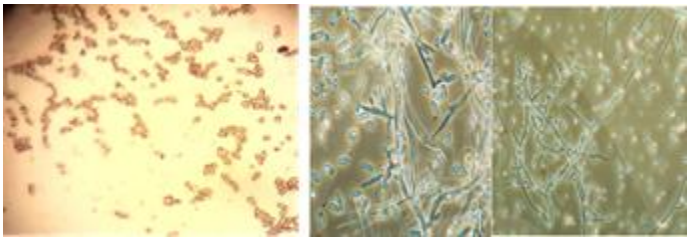

Figure 2. *Appearance of Beauveria bassiana conidia with microscope observation*

The entomopathogenic fungi samples selected from the two land sources were then carried out in vitro virulence tests at a concentration of 2000 ppm in the Biology laboratory FKIP UISU. Virulence test results can be seen in the following table 2.

Table 2*. Results of Beauveria bassiana and Metarhizium anisopliae virulence tests in vitro with a concentration of 2000 ppm in 10 larval Crossidolomia binotalis*

No	Jenis dan Sumber Fungi	Hari setelah aplikasi										Jumlah kematian larva
		1	2	3	4	5	6	7	8	9	10	
1	FPHKS18	0	0	0	0	0	0	0	1	1	2	2
2	FHHKS18	0	0	0	0	0	0	0	0	0	0	0
3	FPHKS8	0	0	0	0	1	1	1	2	2	2	2
4	FHHKS8	0	0	0	0	0	0	0	0	1	1	1
5	FPKSB8	0	0	0	0	1	1	2	2	3	4	4
6	FHKSB8	0	0	0	0	0	1	1	2	2	2	2
7	FPKSB9	0	0	0	0	0	1	1	2	2	3	3
8	FHKSB9	0	0	0	0	0	1	1	1	2	2	2
9	FPKSB17	0	0	0	0	1	1	2	2	3	3	3
10	FHKSB17	0	0	0	0	0	0	1	1	1	1	1

Note:
FPHKS: White fungi from Sibolangit Conservation Forest soil samples
FHHKS: Green color fungi from Sibolangit Conservation Forest soil samples
FPKSB: White fungi from the Berastagi Vegetable Garden soil sample
FHKSB:: Green color fungi from the Berastag Vegetable Garden soil sample

Figure 3. *Insect larvae infected with Beauveria bassiana*

From the results of Table 2 it can be seen that the mortality of larvae in vitro by treatment of 2 types of entomopathogenic fungi is relatively low. The lowest yield was 0 deaths from FHHKS18 fungi while the highest yield from FPKSB8 fungi was 4 larvae. Data shows that entomopathogenic fungi with a concentration of 2000 ppm cannot reach LC 50 or LT 50. The highest mortality is only 40% of the total larvae. These results indicate that the concentration of 2000 ppm is still too low for the application of fungi in C. Binotalis larvae in vitro. Another factor could be because the fungi originated from the Sibolangit and Berastagi areas in the highlands with low temperatures while the virulence test was carried out in Medan as a terrain. low with a relatively higher temperature than Berastagi and sibolangit. An important factor for B. bassiana virulence is the presence of protease and chitinase enzymes in the cuticle penetration of insect larvae (Fan, et al. 2007). Increased production of B. bassiana fungal enzymes can be done by fungal genetic manipulation (Jiménez, et al. 2016). The virulence of B. bassiana is also influenced by the type of nutrition, the ability of the fungus to produce conidia, environmental factors such as temperature, tolerance to ultraviolet radiation and tolerance to heat (Santoro, et al. 2014).

3.2. LC50 and LT 50 Test

From the results of the virulence test, LC 50 and LT 50 tests were carried out on the FPKSB8 fungi, which caused the highest mortality of 4 larvae (40%) C. binotalis in vitro. This test treats concentrations ranging from 2000 ppm, 3000 ppm and 4000 ppm. The LC50 and LT 50 test sites are in the Biology Laboratory of FKIP-UISU with a room temperature of around 30 ^{0} C. . LC50 and LT 50 Test Results can be seen in the following table 3.

Table 3*. Test Results of LT50 and LC 50 fungi of Beauveria bassiana against Crossidolomia binotalis larvae (10 larvae per petri dish) in vitro for 10 days of observation*

No	Konsentrasi Beauveria bassiana isolat FPKSB8	Hari kematian larva setelah aplikasi										Jumlah Kematian
		1	2	3	4	5	6	7	8	9	10	
1	2000 ppm	0	0	0	0	1	2	2	3	3	4	4
2	3000 ppm	0	0	0	1	1	2	3	4	4	5	5
3	4000 ppm	0	0	0	1	2	3	4	4	5	7	7

Figure 4. *Beauvria bassiana LC 50 and LT 50 test against C.binotalis larvae from fungi isolates FPKSB8*

From Table 3 it is known that the highest percentage of mortality of C.botalis larvae occurs at a concentration of B. bassiana 4000 ppm, the fastest death on day 4 or 79 hours after treatment. While lethal time 50 (time to kill 50% of C.binotalis larvae) occurs on the 9th day or about 210 hours after treatment. LC 50 of the test results is a concentration of 4000 ppm. Concentration of 4000 ppm resulted in the number of C.botalis larvae deaths being relatively higher at 70% compared to B. passiana conidia concentrations of 3000 ppm and 2000 ppm which resulted in 50% and 40% larval death respectively. The greater the concentration of B. bassiana conidia the higher the mortality rate of C.binotalis larvae. In vitro treatment is relatively slightly influenced by environmental factors, all factors other than treatment can be controlled properly. These results can certainly differ from applications in the field, because environmental factors are difficult to control such as rain, wind, solar heat, humidity, the use of pesticides in the environment around the study and other environmental factors. LC 50 is determined by the level of viability and pathogenicity of Beauveria bassiana and conidial concentrations, the higher the conidial concentration of LT50 is also faster (Santoro, et.al. 2010).

4. DISCUSSION

From the results of the study note that the death of larvae in vitro by treatment of 2 types of entomopathogenic fungi is classified as low. The lowest yield is 0 deaths from FHHKS18 fungi while the highest yield from FPKSB8 fungi is 4 larvae. Data shows that entomopathogenic fungi with a concentration of 2000 ppm cannot reach LC 50 or LT 50. The highest mortality is only 40% of the total larvae. Meanwhile, according to Mora (2016), Bb79MI strain shows 78% mortality in T. molitor, the level of virulence of fungi is influenced by sunlight radiation (Bugeme et.al. 2008). Chemicals and the presence of host insects. The highest percentage of mortality of C.binotalis larvae occurred at the concentration of B. bassiana 4000 ppm, the fastest death on day 4 or 79 hours after treatment. While lethal time 50 (time to kill C.binotalis 50% larvae) occurs on the 9th day or as much as 210 hours after treatment. LC 50 of the test results is a concentration of 4000 ppm. According to Bugeme et.al. (2008) LT50 values ranged from 4.2 to 8.1 days and LT90 values from 5.6 to 15.1 days, with B. bassiana isolate ICIPE279 having the shortest lethal time value of 4.2 (LT50) and 5.6 days (LT90). The percentage of larval mortality is influenced by the high concentration of fungi applied to insect pests.

5. CONCLUSION

From this research it is known that the virulence of Beauvriana bassiana is different between sampling. The highest virulence in FPKSB8 can kill up to 40% of C.binotalis larvae with a concentration of 2000 ppm, while a lethal time 50 occurs on the 9th day or around 210 hours with a concentration of 400 ppm.

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