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ABUNDANCE AND DIVERSITY OF ENTOMOPATHOGENIC FUNGI IN SIBOLANGIT CONSERVATION FOREST AND BERASTAGI VEGETABLE LAND

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Abstract. *This study aims to determine the diversity and abundance of entomopatogenic fungi that exist around the Brastagi vegetable field and Sibolangit conservation forest. From the results of the study it was found that the Sibolangit Conservation Forest found 2 types of entomopatogenic fungi Beauveria bassiana and Metarhizium anisopliae. The level of abundance is quite low, namely the highest average larvae infected by B.bassiana 3 larvae per sample site and 2 larvae infected with Metarhizium per sample soil. While infection in larvae is higher in the Berastagi community vegetable garden. From the vegetable garden, the Berastagi community found 2 types of entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae. The level of abundance is higher than that of fungi originating from sibolangit conservation forests, namely the highest average larvae infected by B.bassiana 5 larvae per sample soil location and 3 larvae infected with Metarhizium per sample soil.*

Keywords: Entomopatogenic fungi, exploration, isolation, diversity, abundance

INTRODUCTION

Nearly one million known insect species, around 15,000 species are known as pests and about 300 species need control. Fortunately, most pest insects have pathogenic

microorganisms associated with them. There are two economic aspects caused by insects. The first problem is the loss of production due to crop damage by insects and health problems for humans and livestock due to the use of pesticides. The second problem is the cost to prevent or control loss of production. At the same time as increasing agricultural activity the insect population also increases and becomes an important competitor of human food damage or even the cause of food destruction[1] (Bale et al, 2008).

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 loss and maintain high product quality. Although pesticides are developed through very strict regulatory processes 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.[2] (Damalas and Eleftherohorinos, 2011).

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 a 'system approach' for integrated pest management, to control pests that are resistant to pesticides, withdrawing chemicals and minimizing pesticide use.

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[3] (Takhur and Sandhu, 2010), Lagenidium, Coelomomyces, Conidiobolus, Entomophaga, Entomophthora, Erynia, Neozygites, Pandora, Zoophthora, Cordyceps, Hypocrella,

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[1] Bale, J. S. Lenteren, F. Bigler. (2008). Biological Control and sustainable food Production. *Philos. Trans.R.Soc.Lond. B. Biol.Sci.* pp. 54 - 67

[2] Damalas, Christos, A. Eleftherohorins**, J.** (2011). Pesticide Exposure, Safety Issues, and Risk Assessment Indicators. *Int J Environ Res Public Health*, 8(5), pp. 1402–1419

[3] Takhur, Rupesh, Sandhu,Sardul, S. (2009). Distribution, occurrence and natural intervertebrate host of indigenous entomopathogenic fungi of Central India. *Indian J Microbiol,* 50(1), pp. 89 96

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Torrubiella, Aschersonia, Hirsutella, Tolypocladium, and Verticillium[4] (Pell and Shah, 2003).

Cultivated land in Gajah village, Simpang Empat Subdistrict, Tanah Karo District, North Sumatra, is a horticultural land that is dominated by cabbage, mustard, broccoli, beans, tomatoes and several fruits. Exploration of entomopatogenic fungi in this field is to determine the diversity of fungal species and to see the impact of massive pesticide use on the presence of entomopatogenic fungi.

Sibolangit Conservation Forest is a forest with a tropical rain forest ecosystem that is still relatively intact. The ecological process goes naturally and does not get much pressure from the surrounding community. Podsolic soil type and crystal texture so that it easily permeates water and is washed away by water. Has a wavy topography with a slope factor of 5 - 10% while the height is 558 m above sea level. including in type B climate with rainfall of 2,500-30,000 mm / year with humidity between 60-80% maximum average temperature of 35.6º C and minimum of 25.3ºC. Natural Conditions The forest is thought to have the potential of entomopathogenic fungi living in association with the roots of forest plants with moisture conducive to fungal life. There has been no report on entomopathogenic fungi exploration in the Sibolangit conservation forest, so that the existing fungus potential is unknown and untapped.

This study aims to determine the potential of insect pathogenic fungi found in vegetable and fruit fields in Tanah Karo Regency and Sibolangit Conservation Forest in North Sumatra, its diversity and abundance.

METHODOLOGY

This research was conducted using Tenebrio molitor larvae as a method of exploration to obtain entomopatogenic fungi in the vegetable garden in Brastagi. And sibolangit conservation forest

[4] Pell, J.K. Shah, P.A. (2003). Entomopathogenic fungi as biological control agents. *Appl Microbial Biotechnol, 8(3), p. 76-78*

Stage of exploration

The step of the research is to do mushroom exploration in the soil around the roots of plants with a depth of 5 to 15 cm. Soil samples are taken according to sampling determination. The soil is then put into plastic and mixed until homogeneous. The soil sample obtained is placed in a plastic container, filled about half of the volume of the container. 3. Before the Tenebrio molitor caterpillar is inserted, the soil in the container is moistened by adding enough water. 4. Putting the caterpillar Tenebrio molitor on the ground surface in a container, the caterpillar that is inserted is the new molting caterpillar (replace the skin) which is white 5. Then the container is closed using gauze so that the caterpillar does not come out of the container, then incubated for 1 to 2 weeks in a dark place so that the trap caterpillar is active, so it is easy to contact the entomopathogenic fungi that are in the soil sample.

Stage of isolation

The isolation stages included 1. Tenebrio molitor caterpillars infected with entomopathogenic loss were isolated by implanting infected tissue samples on Potato Dekstrose Agar (PDA) media and incubated for 5 to 7 days 2. Isolation was carried out by dipping infected tissue samples (Tenebrio molitor caterpillars) several when (± 3 minutes) into a solution of chlorox, alcohol, then rinsed with sterile aquadest. 3.fungi that grow on the media are identified.

FINDINGS AND DISCUSSION

The collection of soil samples at this location was taken at 25 locations scattered on the research land which constituted 25 treatments with 4 replications. From the results of exploration, there were 2 types of white fungi thought to be Beauveria bassiana (white muscardine) and the green ones thought to be Metarhizium (muscardine green).

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Table 1. Entomopatogenic fungi T. molitor larvae in the Sibolangit conservation forest with 25 soil samples and 4 replications

No	Source of sample soil	The number of larvae infected with white fungi on repetition				The number of larvae infected with green fungi in replication				Average fungal infection	
		1	2	3	4	1	2	3	4	FP	FH
1	HKS1	2	1	2	3	1	0	2	1	2	1
2	HKS2	3	2	0	0	1	0	0	1	1,25	0,5
3	HKS3	2	1	0	0	1	1	0	0	1	0,5
4	HKS4	0	0	0	0	0	0	0	0	0	0
5	HKS5	2	1	2	2	1	1	2	1	1,75	1,25
6	HKS6	3	0	2	1	1	0	0	1	1,5	0,5
7	HKS7	1	1	0	2	0	1	0	1	1	0,5
8	HKS8	3	2	4	2	1	2	1	1	2,75	1,25
9	HKS9	3	4	3	2	2	2	3	1	3	2
10	HKS10	4	3	3	2	2	2	1	2	3	1,75
11	HKS11	3	2	1	2	2	1	1	1	2	1,25
12	HKS12	1	0	1	1	0	0	0	0	0,25	0
13	HKS13	2	1	3	0	1	1	0	0	1,5	0,5
14	HKS14	3	2	2	1	1	1	1	0	2	0,75
15	HKS15	2	1	3	1	1	2	1	1	1,75	1,25
16	HKS16	2	3	3	1	1	0	1	1	2,25	0,75
17	HKS17	2	3	2	2	2	1	3	1	2,25	1,75
18	HKS18	2	4	1	2	2	3	2	1	2,75	2
19	HKS19	1	2	2	0	1	2	2	0	1,25	1,25
20	HKS20	3	3	2	4	2	3	1	1	3	2
21	HKS21	2	2	1	3	1	1	0	1	1,75	0,75
22	HKS22	4	2	3	1	2	1	2	0	2.5	1.25
23	HKS23	1	1	3	2	0	0	2	2	1,75	1

24	HKS24	3	0	3	2	1	0	3	3	2	1,75
25	HKS25	1	2	1	1	1	0	1	0	1,25	0,5

Information: HKS: Sibolangit Conservation Forest
FP: White fungi
FH: Green fungi

From the above data it can be seen that the presence of entomopathogenic fungi is not much or abundant, the highest average white fungal infection in T. molitor larvae is 3 medium larvae, the highest average green fungi infection in larvae is 2. The abundance of fungi from soil samples in the forest Sibolangit Conservation is a green fungi. White fungi, which is suspected to be Beauveria bassiana, are higher in abundance than the green fungi suspected by Metarhizium anisopliae, according to Anna et.al. (2015) this occurs because B. bassiana forms more colonizing units in the soil than organic land compared to other fungi. Apart from the fact that although organic fields are considered to be more suitable environments for entomopatogenic fungi, abiotic factors and plant practices such as tillage may have a greater impact on their abundance (Cliffton, et al. 2015). In Sibolangit Conservation Forest, fertile organic soil conditions are very suitable for the growth of entomopatogenic fungi, but biotic factors, namely the presence of insects as hosts is very minimal, so the abundance of entomopatogenic fungi tends to be low.

Entomopatogenic exploration of fungi in Berastagi vegetable garden

The collection of soil samples at this location was taken at 25 locations scattered on the research land which constituted 25 treatments with 4 replications. Land is taken on post-harvest land. This land is still overgrown with vegetable remnants, fertile and loose soil but no longer applied with pesticides, so that around many plants there are plutella xylostella and Crocidolomia binotalis in all levels, from eggs, pupae, larvae and imago. From the results of exploration, there were 2 types of white fungi thought to be *Beauveria bassiana* (white muscardine) and the green ones thought to be Metarhizium (muscardine green).

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Table 2. Entomopatogenic fungi T. molitor larvae in the Berastagi vegetable garden with 25 soil samples and 4 replications

No	Source of sample soil	The number of larvae infected with white fungi on repetition				The number of larvae infected with green fungi in replication				Average fungal infection	
		1	2	3	4	1	2	3	4	FP	FH
1	KSB1	5	3	4	3	3	2	2	2	3,75	2,25
2	KSB2	3	4	5	4	2	3	3	2	4	2,5
3	KSB3	2	1	0	0	1	1	0	0	1	0,5
4	KSB4	1	2	2	2	1	2	2	0	1,75	1,25
5	KSB5	2	1	2	2	1	1	2	1	1,75	1,25
6	KSB6	3	0	2	1	1	0	0	1	1,5	0,5
7	KSB7	6	4	3	2	3	3	2	0	3,73	2
8	KSB8	5	6	4	5	3	2	3	3	5	2,75
9	KSB9	4	5	5	6	2	3	2	3	5	2,5
10	KSB10	2	6	4	3	2	3	4	3	3,5	3
11	KSB11	6	6	5	3	3	2	3	3	5	2,75
12	KSB12	4	5	3	6	3	3	4	2	4,5	3
13	KSB13	5	6	4	3	3	3	3	2	4,5	2,75
14	KSB14	3	2	3	3	2	1	1	3	2,75	1,75
15	KSB15	3	5	4	2	3	3	2	4	3,5	3
16	KSB16	5	4	3	3	2	2	1	4	3,75	2
17	KSB17	4	5	7	3	2	4	1	2	4,75	2,25
18	KSB18	5	2	2	3	3	2	2	3	3	2,5
19	KSB19	2	3	4	5	2	1	4	1	3	2
20	KSB20	5	5	4	6	2	3	3	2	5	2,5
21	KSB21	4	2	2	1	1	1	0	0	2,25	0,5
22	KSB22	1	2	2	0	1	1	1	0	1,75	0,75
23	KSB23	3	2	1	4	1	1	1	0	2,5	0,75

24	KSB24	4	5	3	3	2	2	1	2	3.75	1,75
25	KSB25	4	6	3	2	2	4	3	1	3,75	2,5

Information: HKS: Sibolangit Conservation Forest
FP: White fungi
FH: Green fungi

From the above data it can be seen that the presence of entomopathogenic fungi is more abundant than the location of Sibolangit conservation forest, the highest average white fungal infection in T. molitor larvae is 5 medium larvae, the highest average green fungal infection in larvae is 3. The abundance of fungi from soil samples in the vegetable garden Berastagi is a white fungus that is more dominant than green. White fungi, which is suspected to be Beauveria bassiana, are higher in abundance than the green fungi suspected by Metarhizium anisopliae, according to Anna et.al. (2015) this occurs because B. bassiana forms more colonizing units in the soil than organic land compared to other fungi. Apart from the fact that although organic fields are considered to be more suitable environments for entomopatogenic fungi, abiotic factors and plant practices such as tillage may have a greater impact on their abundance (Cliffton, et al. 2015).

In Sibolangit Conservation Forest, fertile organic soil conditions are very suitable for the growth of entomopatogenic fungi, but biotic factors, namely the presence of insects as hosts is very minimal, so the abundance of entomopatogenic fungi tends to be low. Host abundance factors for entomopatogenic fungi appear to play a role in the abundance of fungi on vegetable plantations, even in cabbage and leaf mustard greens there are many insects with crossidolomia binotalis larvae infected with Beauveria bassiana, the body covered with whitened conidia and some larvae are infected with Metarhizium and appear green. The use of synthetic pesticides, synthetic fertilizers and other toxic substances can indeed suppress the growth of entomopatogenic fungi, but the influence of biotic factors (Cliffton, et. Al. 2015), namely the presence of host insects is one of the causes of the abundance of entomopatogenic fungi in the post-harvest Berastagi vegetable land.

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CONCLUSION

Tenebrio molitor larvae infected with fungi were isolated by implanting infected tissue samples on Potato Dekstrose Agar (PDA) media and incubated for 5 to 7 days. Isolation is done by dipping infected tissue samples (Tenebrio molitor caterpillar) for a few moments (± 3 minutes) into Clorox or alcohol solution, then rinsing with sterile aquadest. Dried with sterile tissue and placed on sterile moist filter paper. Conidial entomopatogenic fungi that emerge from the body of the larvae are cultured on PDA medium using an ose needle at room temperature. This isolation often fails because the media is not sterile, contaminated with other types of fungi or bacteria. So the isolation process must be repeated frequently until the fungi that infect T. Molitor larvae are obtained.

The fungi that were successfully grown in the media were then morphologically identified with the help of relevant literature. From isolation obtained Beuveriana and Metarhizium fungi isolates from the Sibolangit Conservation Forest and Beuveriana and Metarhizium fungi isolates from the Berastagi community vegetable garden. The fungi are then propagated on rice or corn media to further test the virulence of entomopatogenic fungi in insect larvae.

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