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DIVERSITY AND ABUNDANCE INDEX OF ENTOMOPATHOGENIC FUNGI AND THEIR HOSTS IN RICE PLANTATIONS BOLAANG MONGONDOW REGENCY, INDONESIA

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Abstract

Research on the diversity and abundance of entomopathogenic fungi and their hosts in rice plantations in Bolaang Mongondow Regency was an attempt to find potential local biological control agents. Integrated pest management as often employed in agricultural pest control, may be a way to address these issues. The purpose of this study was to determine the diversity and abundance of entomopathogenic fungi and their hosts, to find pest insect species that attack rice plants, and to look for potential host species and entomopathogenic fungi in Bolaang Mongondow. The results showed that entomopathogenic fungi that have been identified through the research are Beauveria bassiana, Hirsutela sp., and Metarhizium anisopliae. Hosts that have been found due to fungal entomopathogenic infections are Nilaparvata lugens, Scotinophara coarctata, Leptocorisa oratorius, and Paraeucosmetus pallicornis. The highest diversity index in Bolaang Mongondow was found in Nilaparvata lugens (1.07) and for entomopathogenic fungi was found in Beauveria bassiana (1.34). The highest abundance index levels in Bolaang Mongondow were found in the same host and fungi.

***Key words*:** *Nilaparvata lugens, Beauveria bassiana,* diversity index, abundance index, biological control agents

INTRODUCTION

As a staple food and main source of calories in Indonesia, rice plants (*Oryza sativa* L.) have experienced pest attacks by at least 21 insect species which are categorized as major pests [3; 21]. For these reasons, and many others, we all share the responsibility to ensure that new agricultural technologies will be available continuously. Generally, pest control uses synthetic insecticides because they are easy to use and have a quick killing effect; however, those synthetic insecticides can lead to long term negative effects, such as environmental pollution, the emergence of resistant pest insects, the killing of natural enemies, increased residual yields, and health problems for users [17; 4; 1].

Biological control is expected to inhibit the growth of pest insects by using biological control agents which are natural enemies of target insects, such as parasitoids, nematodes, viruses, fungi, bacteria and other predatory insects [1; 10]. The biological control commonly used is entomopathogenic fungi because it is an eco-friendly alternative; such as soil fungus (rhizosphere) which is able to attack insects by producing extracellular enzymes in the form of chitinase, lipase, and protease, which are important components of fungal infection process into insect bodies [24; 26].

Entomopathogenic fungi have great potential as biological pest control agents, and have been developed throughout the world for controlling a variety of important agricultural insect pests [12; 17]. The diversity of entomopathogenic fungi in a particular land may provide information about the potential of local pest control from entomopathogenic fungi that are naturally present at the site. However, *B. bassiana* isolates have different level of virulence at each host and location [17]; so that the local potentials of entomopathogenic fungi are needed to be known scientifically as a consideration for the supply of biological control agents.

In recent years, Bolaang Mongondow Regency has become the center of rice production and granary in North Sulawesi. This region has experienced pest attacks [18; 15; 13] recently, and to overcome this problem, it is necessary to explore local potential entomopathogenic fungi isolates to control agricultural pests. Based on the description above, the problem of this research is how the diversity and abundance levels optimize the effectiveness of the entomopathogenic fungi and what insect species of pests and host species are potential to be controlled with entomopathogenic fungi, to obtain species of entomopathogenic fungi which are potential to be used as biological control agents in Bolaang Mongondow.

MATERIALS AND METHODS

This research took place from April 2019 to February 2020; including exploratory research using 1 m x 1 m plot size method, which was conducted in three districts of North Dumoga, Central Dumoga and East Dumoga as location samples. Furthermore, as the sampling location of each sub-district sample, three stations in the rice field area were determined with a purposive random sampling method adjusted to the age of rice plants. Sampling areas were chosen by making 10 plots of 1 m x 1 m size. Insects that have been infected with fungi were taken as samples; after being identified at the Laboratory of Ecology and Conservation FMIPA Sam Ratulangi University Manado. The number of entomopathogenic fungi was calculated based on the host species infected by the entomopathogenic fungi species. Diversity of infected host species and fungal species was calculated using the Shannon-Wiener Diversity Index [25].

$$H' = -\sum_{n=1}^{s} pi\ lnpi$$

H' = Shannon-wiener Diversity Index
pi = comparison of the number of i-th individuals (ni) with the total number of individuals (n)
s = Number of i species

The abundance of infected host species and fungal species was calculated using the Abundance Index [7].

$$A = \frac{\text{counts of individuals of i type}}{\text{counts of individuals of all types}} \text{ x } 100\%$$

A = Abundance Index

Identification of entomopathogenic fungi was done by using a combined method of [19, 27, 9, 16, 28, 20, 30, 23].

RESULTS AND DISCUSSIONS

Results

Exploration of Host Insects and Entomopathogenic Fungus

The results of the exploration of host species and entomopathogenic fungi in Bolaang Mongondow District obtained four host insect species, namely *Nilaparvata lugens*, *Scotinophara coarctata*, *Leptocorisa oratorius*, and *Paraeucosmetus pallicornis*; and three entomopathogenic fungi species found, namely *Beauveria bassiana*, *Hirsutela sp.*, and *Metarhizium anisopliae*. The exploration data can be seen in Table 1.

Table 1. Average Number of Entomopathogens in Bolaang Mongondouw Regency

Type of Host (Individual)		**Type of Fungi (Individual)**		**Total**
	B. bassiana	*Hirsutela sp.*	*M. anisopliae*	
N. lugens	1.47	1.31	0.78	3.56
S. coarctata	0.93	0.98	0.49	2.40
L. oratorius	0.81	0.60	0.18	1.59
P. pallicornis	0.81	0.36	0.36	1.43
Total	4.02	3.25	1.72	

Source: Own calculation.

The highest total number of entomopathogenic fungi was found in N. lugens host (3.56) and the least in *P. pallicornis* host (1.43). The *B. bassiana* fungus is the most common host of *N. lugens* (1.47), *L. oratorius* (0.81), and *P. pallicornis* (0.81). Hirsutela mushroom sp. is the fungus that most attacks the host *S. coarctata* (0.98). Based on these data, the type of host that is potential to be controlled with entomopathogenic fungi is the *N. lugens* host because it has the highest total number of attacks on this pest insect compared to the other hosts. The type of entomopathogenic fungus that is potential to control pest insects in Bolaang Mongondow is *B. bassiana* fungus because this fungus has the highest number of attacks against three of the four pest insects found.

Diversity Index (H')

The fungi diversity index was analyzed using the Shannon-wiener diversity index formula [25]. In this study, there are two forms of diversity index calculation. The first form is based on the type of host with a defining value (ni) which is the number of entomopathogenic fungi that attack one type of host and the value (N) is the total number of entomopathogenic fungi that attack one type of host. The second form is based on the type of entomopathogenic fungus with the defining value (ni), namely the number of similar insects infected by one type of entomopathogenic fungus and value (N), namely the total number of individual insects infected by one type of entomopathogenic fungus found.

There are three categories of diversity indexes, namely Low (H' <1), Medium (1 ≤ H' < 3), and High (H' ≥ 3) [2].

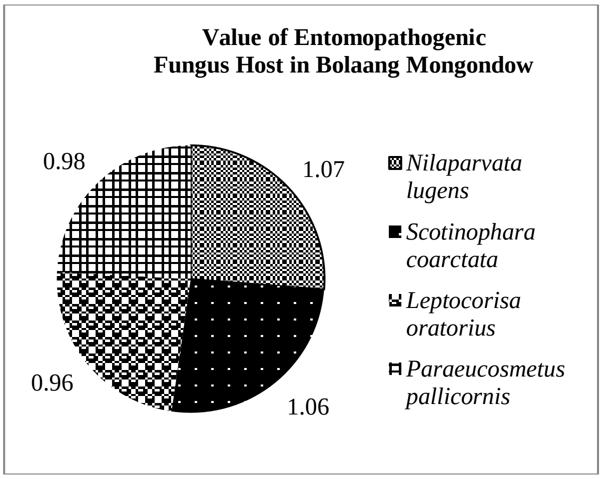

Fig. 1. Entomopatogenic Fungus Host Value in Bolaang Mongondow
Source: Own calculation.

The value of H' host insect pest species can be seen in Figure 1. The highest value was found on host *N. lugens (1.07),* followed by *S. coarctata* (1.06), *P. pallicornis* (0.98) and *L. oratorius* with the lowest value of 0.96. *N. lugens* and *S. coarctata* hosts are in the moderate category and *L. oratorius* and *P. pallicornis* are in the low category [2]. This shows that *N. lugens* and *S. coarctata* hosts are entomopathogenic fungi that is more diverse than the other two host insect species, so that *N. lugens* is the most potential hosts to be controlled with entomopathogenic fungi.

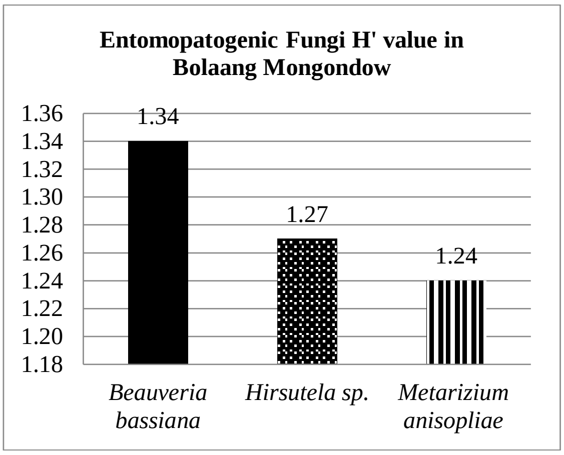

Fig. 2. Entomopatogenic Fungi H' value in Bolaang Mongondow
Source: Own calculation.

The index value of entomopathogenic fungi diversity in Bolaang Mongondow Regency shows that *B. bassiana* (1.34) has the highest index value, followed by *Hirsutela sp.* (1.27), and *M. anisopliae* (1.24) with the lowest index value (Figure 2). Those fungi are classified as the medium category, which means that they have the same diversity of hosts. Based on these data, the type of entomopathogenic fungus that has the potential to control pest insects is *B. bassiana*, based on the highest H' value compared to the other two fungi.

Abundance Index (A)

The entomopathogenic mushroom abundance index was calculated using the formula of abundance index, calculated using two forms of calculation [7]. The first is based on the type of host and the second is based on the type of entomopathogenic fungus. The higher the value of A means the species is more abundant. Based on the host insect species, the results indicated that there were two abundant entomopathogenic fungi in certain species of pest insects. *B. bassiana* fungi were found to be abundant in three host insect species, namely *N. lugens* (41.20%), *L. oratorius* (51.01%), and *P. pallicornis* (56.72%). Meanwhile, *Hirsutella sp.* overflow in *S. coarctata* host (40.74%) (Fig. 3).

The *M. anisopliae* fungus shows the lowest abundance value for all host insects indicated by these values: *N. lugens* (21.85%), *S. coarctata* (20.37%), *L. oratorius* (11.18%), and *P. pallicornis* (18.65%).

Thus, the *B. bassiana* fungus is the most abundant entomopathogenic fungus compared to the other two fungi because it is able to attack three insect pests found; hence, it is the most potential for controlling insect pests.

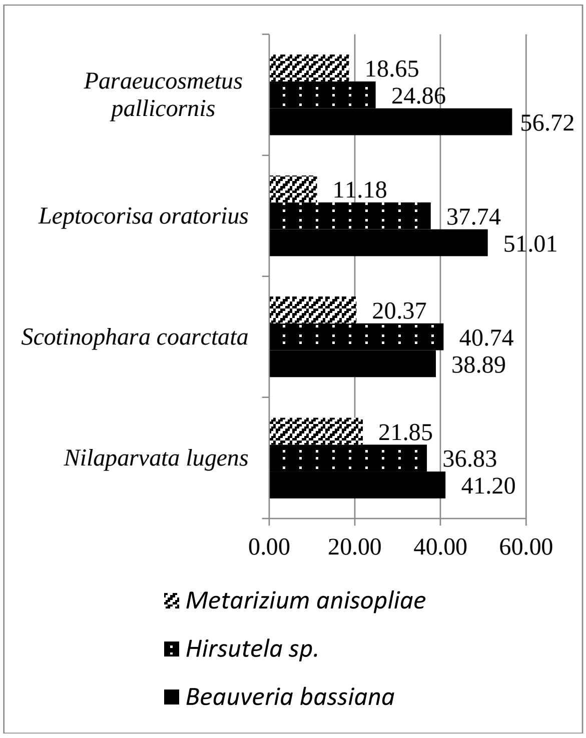

Fig. 3. Abundance index of host infected by entomopathogenic fungi in Bolaang Mongondow
Source: Own calculation.

Based on entomopathogenic fungi species, the results showed that *N. lugens* was the host with the highest index of abundance of entomopathogenic fungi, namely *B. bassiana* (36%), *Hirsutela Sp.* (40%), and *M. anisopliae* (45%) (Fig. 4).

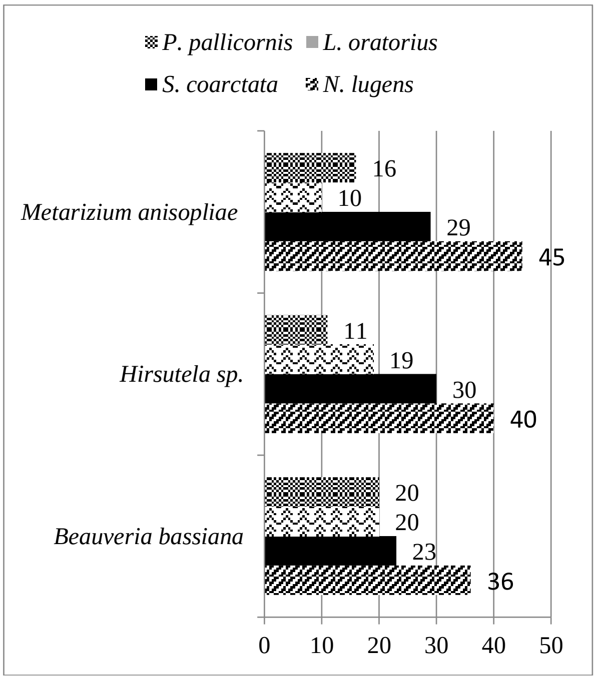

Fig. 4. Entomopathogenic Fungi Abundance index based on Host Type in Bolaang Mongondow (%)
Source: Own calculation.

P. pallicornis hosts showed the lowest index of fungi abundance in the species of *Hirsutela sp.* (19%) and *L. oratorius* host in *M. anisopliae* fungal species (10%); both of them have the lowest abundance index value in *B. bassiana* fungus (20%). This shows that the *N. lugens* pest is an insect that is potential to be controlled with entomopathogenic fungi because of the high abundance index of each entomopathogenic fungus found in this pest.

Discussions

Exploration of insects infected with entomopathogenic fungi in Bolaang Mongondow shows that the insect pests that has the tendency to become host is *N. lugens* insects with entomopathogenic fungus *B. bassiana*. This was influenced by several factors including the use of pesticides [22; 28] and the virulence ability of entomopathogenic fungi [17].

The use of pesticides can affect the ability of entomopathogenic fungi to infect *T. molitor* larvae [28]. It is noted that on certain field area where pesticides are not applied, high percentage of *T. molitor* larvae infection were found. This means that the use of pesticides can affect the ability of entomopathogenic fungal infections, but the level of influence of the use of pesticides on the ability of entomopathogenic fungal infections in Bolaang Mongondow has not been scientifically tested. The use of pesticides that are still massive is suspected to be the cause of the reduced number of infected insect pests because their pathogenicity is disrupted due to pesticide residues. This applies specifically to the *M. anisopliae* fungus because the results show that this fungus has the lowest number of infections of all hosts. The use of imidacloprid-based pesticides can inhibit sporulation of this fungus; but in different strains the opposite effect may occur, so it still needs in-depth investigated [8].

Differences in sampling locations also affect the pathogenicity of entomopathogenic fungi in terms of the mortality rate of host insects. Mortality rate of *L. oratorius* (*walang sangit*) due to infection with *B. bassiana* and *Metarhizium sp.* was different; depending on the location where the entomopathogenic fungi are isolated [6]. This relates to the location of sampling.

There are three sample locations, which are the three districts in Bolaang Mongondow, namely North Dumoga District, Central Dumoga District, and East Dumoga District. The characteristics of these three regions need to be investigated further, whether it affects the virulence differences of the entomopathogenic fungi that exist in each place, where the *B. bassiana* fungus was also reported to have a different virulence due to differences in location and host where the fungus was isolated [17].

The index of abundance of pest insect species shows that all types of hosts in all locations have the highest abundance index due to fungal infection of *B. bassiana,* because *B. bassiana* has a high conidia germination (> 70%) when compared to *Metarhizium sp.* with conidia germination <60% [19], so the ability to infect host insects is increased. *B. bassiana* is known to have a high pathogenicity compared to *Metarhizium sp* [6]. In addition, *B. bassiana* fungi showed the highest mortality rate of *L. oratorius* (walang sangit) (73.3%) compared to *Metarhizium sp.* (70%). In addition, *B. bassiana* is an entomopathogenic with a broad spectrum of attack, so its abundance against various host insects is higher.

Based on the calculation of diversity and abundance index values, the potential host insect to be controlled with local isolate entomopathogenic fungi is brown plant hopper (*N. lugens*). These insects are pests that damage plants by sucking cell fluids and are able to migrate for a long distance [14]. These insects can become more virulent by increasing the expression of genes associated with the digestive system, salivary secretion, detoxification, metabolism of carbohydrates, lipids, and amino acids [5].

Control using synthetic pesticides may give resistant effect on these insects. Extreme resistance occurs with imidacloprid and moderate to high resistance occurs with thiamethoxam [31]. Controlling *N. lugens* with pesticides is feared to have a pesticide-resistant effect on Bolaang Mongondow farm. Another concern is shown by the extraordinary migration tray of *N. lugens.* There was a spike in the population of *N. lugens* in the vicinity of the Yangtze River due to N. lugens immigrants reproducing there [11]. Migration that occurs there comes from *N. lugens* that are around the area, meaning that migration of *N. lugens* can occur between agricultural land. If there is a migration of *N. lugens* that has become pesticide resistant to other agricultural land, then the use of pesticides will not have a good impact to control the pest population and actually harm the soil ecosystem where entomopathogenic fungi are located.

Instead of using synthetic pesticides, the selection of local entomopathogenic fungi is highly recommended. Based on the calculation of diversity and abundance index values, locally isolated entomopathogenic fungi that have the potential to control pests are *B. bassiana. B. bassiana* fungi will be hampered by the process of spore formation if there is a fungicide present around it, but imidacloprid-based insecticides can stimulate the growth and sporulation of this fungus [8], so that the use of *B. bassiana* fungi can be applied in fields that have used insecticides. This is also the reason the diversity and abundance index value of *B. bassiana* fungi in Bolaang Mongondow is higher than the other two species of entomopathogenic fungi, if indeed there is a high enough pesticide residue in the area. In addition, the use of *B. bassiana* to overcome insect pests that are resistant to imidacloprid-based insecticides may become a new alternative. Besides being able to play a direct role as a biological control agent, secondary metabolites of the fungus *B. bassiana* can be used as insecticides; because the fungus *B. bassiana* produces toxins from the mycelium which can be used as an insecticide in the process of destroying the stomach tissue of the insect's body. This toxin can be extracted, so that *B. bassiana* in the future can potentially become a biological agent providing N-hexadecanoic acid-based insecticide [29].

CONCLUSIONS

The highest diversity index in Bolaang Mongondow was found in the host *Nilaparvata lugens* (1.07) and for entomopathogenic fungi was found in *Beauveria bassiana* (1.34), while the highest abundance index was also found in the host *Nilaparvata lugens* and for fungi was found in *Beauveria bassiana* fungi. With data examined above, it may be concluded that the most potential host insects to be controlled with entomopathogenic fungi is *Nilaparvata lugens*, with the most potential entomopathogenic fungi to control pests is *Beauveria bassiana*.

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