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EXPLORATION OF BEAUVERIA BASSIANA ENTOMOPATHOGEN ON LEPTOCORISA ACUTA IN RICEFIELD

Mohammad Yunus^{1*)}, Salmirna¹⁾, Nur Edy¹⁾

¹⁾Program Study of Agrotecnology, Faculty of Agriculture, Tadulako University.

Correspondence author's: Mohammad Yunus Email : mohyunus125@gmail.com

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ABSTRACT

Beauveria bassiana (Bals.) Vuill is a fungus that infecting the insect *Leptocorisa oratorius* F. naturally in ricefields. This study aimed to obtain isolates of the entomopathogenic fungus *B. bassiana* from the insects body of infected *L. oratorius*, which would later be used as biological agents in controlling rice pests. The exploratory research was carried out at rice production centers in Sidera Village, Sigi Regency and in Dolago Village, Parigi-Moutong Regency, Central Sulawesi. The laboratory tests were carried out at the Plant Disease Laboratory, Faculty of Agriculture, Tadulako University. Research methods include exploring *L. oratorius* insects infected by *B. bassiana* in the field, and then the specimens were brought to the laboratory for isolation and identification, calculation of conidia density, colony relative growth rate, and determination of viability. The results showed that *L. oratorius* was infected with the fungus *B. bassiana* in Sidera and in Dolago. Isolates from both regions had the same macroscopic and microscopic morphological characteristics, but had different characteristics of conidia density, colony relative growth rate, and viability.

Keywords: Rice, L. oratorius, Entomopathogen, B. bassiana.

INTRODUCTION

The fungus *Beauveria bassiana* is one of the entomopathogenic fungus species with a very wide host range, easily found in nature and easily propagated in the laboratory (Trizelia, 2005). The fungus *B. bassiana* can be explored from the soil (Tanada and Kaya, 1993), from plants (Soetopo and Indrayani, 2007), or infected host insects (Hasyim and Azwana, 2003).

Exploring biological agents in the field is an alternative to biological pest control activities. The results of the

exploration of biological agents from the field can be reproduced in the laboratory and then used for insect pest control (Herdatiarni et al., 2014). Entomopathogenic fungi can inhibit growth, inhibit reproduction, or even kill the insect (Dwiastuti and Kurniawati, 2007).

Entomopathogenic fungi's mechanism in infecting host insects starts with fungi that enter the host's body through the mouth or through the integument (Goettel and Inglis, 1997). Infected host insects show symptoms of decreased feeding activity, slowed movement, convulsions, and stiff death. Another symptom appears that the host's body is filled with fungal hyphae (Herlinda et al., 2008). The types of infected host insects are from the orders Lepidoptera, Hemiptera, Homoptera, and Coleoptera (Prayogo et al., 2005). It is vital to investigate B. bassiana in various locations to produce high-quality isolates that may be used again in the field. Further exploration results were characterized morphologically and tested for viability (Utami et al., 2014; Permadi et al., 2018).

This study aims to obtain isolates of the entomopathogenic fungus *B. bassiana* indigenous, which have the potential to become biological agents by exploring L. oratorius insects that were found dead and infected by the fungus in the field and then isolated. Exploration was carried out in two rice production centers in Central Sulawesi, namely in Sidera Village, Sigi Regency, and in Dolago Village, Parigi-Moutong Regency.

MATERIALS AND METHOD

Study sites

The research was carried out by exploring the entomopathogenic fungus *B. bassiana* in the field and continued by observing macroscopic and microscopic morphological characters and viability testing in the laboratory. As a place of exploration, two rice production centers were determined in Central Sulawesi Province, namely Sidera Village, Sigi Regency, and Dolago Village, Parigi-Moutong Regency. As an illustration of the field conditions of the two villages, it is presented in Table 1.

Exploration of the Entomopathogenic Fungus *B. bassiana*.

The method of exploration of entomopathogenic fungi in the field refers to Herdatiarni et al. (2014). Exploration carried out by surveying and was searching for *L. oratorius* insect specimens in the rice *fields*, which were thought to have died from infection by the fungus Beauveria sp. The specimens were collected in a test tube nd then brought to the laboratory. The insect specimen was placed in a nine cm diameter Petri dish, lined with filter paper, then tightly closed to avoid air humidity (Herlinda et al., 2008).

Isolation, purification, identification, and suspension of entomopathogenic fungi.

Isolation was carried out to obtain cultures. Dead specimens of pure Specimens were placed in a 9 cm diameter petri dish containing sterile moist tissue and incubated to stimulate the growth of the fungus. The fungus that came out of the body of *L. oratorius* was taken with an inoculation needle, cultured on potato dextrose agar (PDA) media and incubated for seven days at a temperature of 23-25 °C. On the third day, hyphae began covering the insect body. The hyphal then re cultured to the new PDA in Petridish (Wartono et al., 2016; Rizkie et al., 2017). Purified hyphal on PDA then growth on agar block for three days and observed using a microscope at 400x magnification. Identification was based on morphological characteristics (Barnett and Hunter, 1972) and the key of determination developed by Samson et al. (1988).

Village	Distance from Palu	Elevation	Temperature	Humidity	Rainfall
Sidera	15 km	65 m asl	16,4-28,8 °C	76%	761 mm y ⁻¹
Dolago	90 km	10 m asl	22,6-35,9 °C	74%	1,618 mm y ⁻¹

Table 1. Research Site Description.

Source: Profile of Sigi Regency and Profile of Parigi-Moutong Regency in 2019.

Purified fungus on PDA was harvested by adding 10 ml of distilled water. Then the hyphal sterile colony was scraped off using a soft brush sterile, stirring slowly until the mycelium and conidia were released from the PDA. After thoroughly mixed in water, 1 ml of conidia was taken and put into a test tube filled with 9 ml of sterile distilled water. From here, serial solution was made for the next experimental used (Ristiari et al., 2018).

Conidia Density, diameter, relative growth rate, and viability.

Conidia density calculation using Hemocytometer. A total of 0.2 ml of conidia suspension was dripped on the counting plane using a dropper through the upper and lower side canals until the suspension filled the counting plane, then allowed to stand for 1 minute. Conidia density was calculated under a microscope using a magnification of 400x following the following formula (Gabriel and Riyanto, 1989).

$$C = \frac{t}{n \times 0.25} \times 10^6$$

Description:

C = spore density (conidia/ml solution).

- T = Total number of spores in the observed sample box.
- N = Number of observed samples

0.25 = Correction factor

Colony diameter was calculated based on the average value of growth diameter vertically and horizontally on Petridish. Measurements were made every day until the fungus colony filled the Petridish. Colony diameter was calculated using the following formula (Gabriel and Riyanto, 1989).

$$D = \frac{d1 + d2}{2}$$

Description

D = Diameter of colony growth

d1 = Diameter of vertical growth

d2 = Diameter of growth horizontally.

The relative growth rate of *B*. *bassiana* was calculated using a formula

based on colony diameter growth (Lily and Barnett, 1951).

$$LPR = \frac{At - Ao}{t}$$

Description

LPR	= Relative growth rate (cm day ⁻¹)
At	= Colony diameter on the last day
A_0	= Colony diameter on the first day
Т	= Time

PDA media was cut into 0.5 cm diameters using a cork drill. A total of 3 pieces of PDA media as replicates were placed on a glass object using a scalpel. Fungal conidia suspension at a dilution of 10⁻⁶/ml was dropped using a pipette with a volume of 1 ml. Each piece of PDA media was closed using a cover glass and then ready to be observed under a microscope. Conidia viability testing was carried out after 24 hours of incubation and observed using a microscope at 400x magnification. The number of germinating and nongerminating conidia were counted. Conidia viability was calculated using the following formula (DPPKP, 2014).

$$VK = \frac{\sum KB}{\sum KTB + KB} x 100\%$$

Information: VK = viability of conidia KB = germinated conidia KTB = conidia that do not germinate.

RESULTS AND DISCUSSION

Results

During the field exploration, *L. oratorius* insects were found dead infected by entomopathogenic fungi in rice fields in Sidera Village, Sigi Regency, and in Dolago Village, Parigi Moutong Regency, Central Sulawesi Province. Symptoms of infected hosts from both areas have similar morphological characteristics. The host's body is covered with hyphae and a white conidia mass (Figs. 1a and 1b). The host's body appears to be brownish-black in color (Fig. 1c.).



Figure 1. Specimen of L. oratorius died infected by entomopathogenic fungi.

- a). Dead white infected L. oratorius insect, from Sidera,
- b). Dead white infected L. oratorius insect, from Dolago,
- c). L. oratorius body hardened and brownish black.

Table	2.	Macrosco	pic I	Morp	holog	ical	Char	acterist	ics o	f Fungal	l Isolates	on host <i>I</i>	L. oratoriu	ıs
						,								

Sample	Colony	Shape of the colony	Symptoms on infected insect
origin	color		
Sidera	white	Circle on dead insect	Insect body hardened brownish
		body	black
Dolago	white	Circle on dead insect	Insect body hardened brownish
		body	black



Figure 2. Microscopic morphological characteristics of *B. bassiana* on day 20 after incubation

L. oratorius specimens that died infected by the fungus in the field were brought to the laboratory for macroscopic and microscopic morphological identification. The fungus was regrown and isolated to obtain pure B. bassiana isolates, and further identification was carried out.

The results of fungal identification based on macroscopic morphology showed

that the fungal specimens from Sidera had the same characteristics as the specimens from Dolago. The colonies were white and circular (Table 2).

The results of microscopic morphological identification showed that the fungal isolate from Sidera had the same characteristics as the isolate from Dolago. The hyphae were insulated, conidiophores growing in clusters, branching and zigzag in shape. The conidia were round and colorless, and grew at the conidiophores' tip (Figure 2).

Conidia density calculation is used to determine the nature of the fungal isolates. The results of observations of isolates from Sidera in various dilution levels always had higher conidia density than isolates from Dolago. The higher the dilution level of the conidia suspension, the lower the density level.

The fungus B. bassiana from Sidera showed that in a 10-5 dilution it had a conidia density of 48.880x106/ml, when the dilution level was increased to 10-8, the conidia density decreased to 14,880x106/ml. The fungus B. bassiana from Dolago showed that in the 10-5 dilution, the conidia density was 34.960x106/ml, and when the dilution level was increased to 10-8, the conidia density decreased to 13.546x106/ml. The results of the calculation of the conidia density of the two isolates of B. bassiana can be seen in Table 3.

Observation of daily colony diameter is helpful in determining the increase in the size of the fungus colony. The growth of colonies of B. bassiana isolates Sidera took nine days to fill a petri dish with a diameter of 9 cm, while a colony of B. bassiana from Dolago took 25 days to fill a petri dish of the same size

Table 3. Density of Conidia B. bassiana from Sidera and Dolago.

No	Dillution/	Conidia density from Sidera	Conidia density from Dolago
1	10-5	48,880 x 10 ⁶ /ml	$34.960 \ x \ 10^{6}$ /ml
2	10-6	33,946 $x \ 10^{6}$ /ml	29.706 $x \ 10^6$ /ml
3	10-7	$20,720 \ x \ 10^{6}/\text{ml}$	21.626 $x 10^6$ /ml
4	10-8	14,880 $x \ 10^{6}$ /ml	$13.546 \ x \ 10^{6}$ /ml



Figure 3. Colony Growth Diameter of *Beuveria bassiana*, from Sidera and Dolago on PDA media at room temperature 24°C.

- a) isolate Sidera in one day after inoculation (DAI)
- b) isolate Sidera in five days after inoculation (DAI)
- c) isolate Dolago in one day after inoculation (DAI)
- d) isolate Dolago in eleven days after inoculation (DAI)

No	Sample origin	Relative growth rate of fungal colonies (cm/day)
1	Sidera	0.91 ± 0.02
2	Dolago	$0.33 \pm 0,004$

Table 4. Relative Growth Rate of Fungus Colonies B. bassiana. Data is average from $n=10\pm SD$.

The relative growth rate of B. bassiana fungus colonies was calculated based on data on the increase in colony diameter measured from one day after inoculation (1 DAI) until the colony filled the surface of a 9 cm diameter petri dish. The relative growth rate refers to the formula (Lily and Barnett, 1951). The observation showed that the isolates from Sidera had a colony growth rate of 0.91 ± 0.02 cm/day, higher than the isolates from Dolago with a growth rate of 0.33 ± 0.004 cm/day (Table 5).

The difference in growth rate between the two isolates was massive and may relate to the isolates' effectiveness in killing insect pests.

The growth rate of fungal colonies describes the effectiveness of the fungus in infecting its host. The faster the growth, the more effective it is in suppressing the growth of the host population. It is indicated that the Sidera isolate grew faster, so it was more effective in suppressing the population of L. oratorius.

Conidia germination percentage was used to determine viability. The viability of the conidia of the fungus describes the ability to grow. The observations of isolates from Sidera showed that conidia viability was higher than that of Dolago isolates. The viability of conidia B. bassiana from Sidera and Dolago were different at 10⁻⁵, 87.81%, and 69.39% respectively. The results of the calculation of the viability of the two B. bassiana isolates can be seen in Table 4.

Discussion

In agricultural ecosystems, tritrophic interactions occur between plants, pests, and natural enemies. The results showed that tritrophic interactions occurred in the ecosystem at the observation site, between rice (trophic-1), L. oratorius insects (trophic-2), and the fungus B. bassiana (trophic-3). Insect L. oratorius is a rice's main pest, and in high population, it is always followed by the fungus B. bassiana as its natural enemy. The presence of Beauveria sp. as a natural enemy will reduce the population of L. oratorius so that the damage to rice will also decrease.

According to Arsi et al. (2020), Besides being found to infect L. oratorius, Beauveria bassiana was also found to infect Nilaparvata lugens Stall. The fungus B. bassiana was very effective in killing N. lugens, according to Minarti (2018) B. bassiana isolates BPcMs shaker was more effective in killing N. lugens nymphs (100%), while Minarni et al. (2020) found that B. bassiana had a lower ability to kill N. lugens, which was 70-80%.

The mechanism of infection of B. bassiana conidia begins with the attachment of propagules to the cuticle surface of the host insect, the hyphae immediately grow and enter the insect tissue with the help of enzymes or pressure. Fungi produce mechanical enzymes such as lipolytic, proteolytic, and chitinase, which cause hydrolysis of insect integuments. Fungi also produce secondary metabolites in the form of antibiotics, which function to prevent bacterial decay of host insects. The host insect becomes hard and stiff (the mummification process). The growth of external hyphae produces conidia that envelop the host body and spread to the environment that has the potential to infect other healthy insects (McCoy et al., 1988; Tanada and Kaya, 1993; Jauharlina and Hendrival, 2003; Fuxa and Richter, 2004; Arsi et al., 2020).

According to Effendy et al. (2010), L. oratorius nymphs newly infected by B. bassiana showed decreased feeding activity, stayed away from food, and eventually died. The death of L. oratorius by entomopathogenic fungi is not only through biochemical processes but also through physical processes. The growth and development of fungi in the insect's body will inhibit the life process of the host insect.

The isolation and identification results showed similarities in macroscopic and microscopic morphological characters between isolates from Sidera and from Dolago. The macroscopic morphological characters found were the host insect covered in white conidia. While the microscopic characters found are; hyphae insulated, hyaline, straight, and thick; hyaline conidia, round, single-celled, formed solitary at the tip of the conidiophores and attached to short sterigma with alternating growth patterns. Based on the identification results that refer to the identification key Barnett and Hunter (1972) and Samson et al. (1988), entomopathogenic fungus the found belongs to the species B. bassiana.

The macroscopic morphological characteristics of the fungus found follow the findings of Tanada and Kaya (1993); Sari and Rosmeita (2020), that B. bassiana infects the host insect has a macroscopic morphological character of white colonies, circular shape, and covers the body of the host insect. Its microscopic character follows the findings of Boucias and Pendland (1998); Suharto et al. (1998); Sari et al. (2018), that B. bassiana has colorless conidia, round to oval in shape, hyphae insulated, conidiophores branching in a zig-zag shape, the mycelium below swells. Conidiophores are formed singly or in regular groups. Conidia are round, single-celled, hyaline and formed singly on short sterigma.

The density level will reflect the concentration. The higher the density

level, the higher the concentration. The results of the observation showed that isolates from Sidera had a higher conidia density than isolates from Dolago. The higher the dilution level of the conidia suspension, the lower the density level. Higher conidia density is always followed by higher effectiveness. This indicates that Sidera isolate is more effective than Dolago isolate. Several factors that can cause differences in conidia density are the type of culture medium (Herlinda et al., 2006), temperature, and humidity (Suharto et al., 1998; Prayogo et al., 2005). Genetic factors (Nuraida and Hasyim, 2009).

According to Gandjar et al. (1999), several factors that affect the speed of growth and development of fungal the type of substrate, colonies are humidity, temperature, pH, and chemical compounds in the environment. In addition, Herlinda et al. (2005) stated differences in the growth rate of fungal colonies in the types of host insects and in geographical areas. Rohman et al. (2017) added that the growth of B. bassiana colonies was influenced not only by the type of media but also by its nutritional components. The type and concentration of chitin added to the growth medium affected the growth rate of B. bassiana colonies.

Differences influence the viability of fungal conidia in isolates, age of the fungus, germination medium, temperature, pH, and incubation period (Tanada and Kaya, 1993). High conidia germination causes the infection process and the death of the host insect to be faster (Trizelia, 2005). Optimal viability at a certain incubation period, the incubation period of 24 hours of conidia B. bassiana was able to germinate above 80%, and at 48 hours of incubation, the germination of conidia increased up to 88.67% (Jeniesthiana, 2011). The viability of the fungus tends to decrease over time. According to Aregger (1992), the degree of loss of conidia viability varies. Decreasing conidia viability can be a factor that affects the level of poisoning. The magnitude of the decrease in conidia viability correlated with a decrease in the mortality rate of the infected host insect.

CONCLUSION

Beauveria bassiana isolates from and Dolago had the Sidera same macroscopic and microscopic morphological characteristics but had different conidia density, colony relative growth rates, and viability characteristics. Isolates from Sidera had better characteristics than isolates from Dolago. At a dilution of 10⁻⁵, isolates from Sidera had a conidia density of 48.880×10^6 /ml, a colony growth rate of 0.91 ± 0.02 cm/day, and a viability of 87.81%, while isolates from Dolago had a conidia density of 34.960×10^{6} / mL at a dilution of 10-5, the colony growth rate was 0.33 ± 0.004 cm/day, and the viability was 69.39%. Isolates from Sidera can potentially be used as biological agents for insect pest control, especially L. oratorius.

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