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Exploring and Identifying the Effects of Entomopathogenic Fungi to Rice black bugs, *Scotinophara* sp in Rice paddy in Bolaang Monondow

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Abstract: *Scotinophara* sp is a major pest that damages rice fields in Bolaang Mongondow. The use of insecticides failed to stop the rice black bugs attacks. Entomopathogenic fungi can be used to reduce rice black bugs population. However, the function of the type of fungus that can eradicate rice black bugs was not yet identified. This experimental research was conducted to identify the types of fungus that attack rice black bugs by taking samples of nymphs and imago attacked by entomopathogenic fungi in Bolaang Mongondow Regency. Sampling of rice black bugs was done 3 times. Koch's postulates test was employed to see the fungus that infect the rice black bugs. Samples were isolated on PDA media (Patato dextrose agar). Entomopathogenic fungi were purified, then inoculated on healthy rice black bugs. Rice black bugs infected with fungi were isolated again on PDA media. In a mass, of fungus colonies showed reddish white field and after being isolated on PDA media of the colony agregate, entomopathogenic *Paecilomyces* sp. fungus and *Fusarium* sp were found. Fungi as pathogenic agent was only found in *Paecilomyces* sp. The rice black bugs (*Scotinophara* sp.) were isolated on culture media and inoculation in healthy *Scotinophara* sp showed the same color of the colony during sampling of *Scotinophara* sp. infected with fungus. Both fungi were scattered at the location of *Scotinophara* sp infected with fungi.

Keywords: Type of fungus, rice black bugs, rice paddy.

Introduction

Rice paddy is a strategic agricultural commodity in improving the economy in Indonesia, so the lack of rice supply can have a significant impact on its people's lives. In North Sulawesi, the area of rice paddy can be found in Bolaang Mongondow within the area of 22.4 399 ha¹. Most of the population in Bolaang Mongondow depends their income on rice paddy. Therefore pests are still a threat to maintain rice paddy production. In North Sulawesi, there are 22 types of insects of rice plants. Starting from the pests that attack the young plants

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to the pests that attack the-ready-to-harvest paddy, but only a few species that are classified as important including *Nephotettix virescens*, *Scirpophaga innotata*, *Pareucosmetus* sp., *Leptocorixa acuta*, *Scotinophara* sp. and *Spodoptera* sp.^{2,3,4}.

Scotinophara sp. are the scientific name of rice black bugs attacking rice paddy by sucking its liquid stems which cause the attacked plants' color turns into reddish brown or yellow. Rice black bugs attack in the vegetative phase which cause the number of tillers to decrease and its growth to stunt. If an attack occurs after the reproductive phase, the rice paddy produces a dwarf panicles, incomplete panicle filling thus this make the grain become empty. High population causes plants to turn yellow or reddish brown eventually wilt and die^{5,6}. In addition to rice paddy as its host, rice black bugs also attack corn, and grass^{6,7}.

Efforts to control rice black bugs by using synthetic insecticides and resistant varieties have not been able to solve the problem of the damage that rice black bugs make to rice paddy plants. In North Sulawesi, farmers mixed several types of insecticides to control the growth of rice black bugs. By mixing some types of insecticide, the farmers hope to minimize the population rice black bugs. Since when the population of rice black bugs is still high, the production of rice paddy will be low^{2,3}. The use of insecticides is actually not a wise solution to control the growth of pests. Since they will become resistance by insecticides, resurgence will arise and environmental pollution will occur.

A good prospective control to pests which can be categorized as environmentally sound is biological control. Biological control by utilizing pathogenic fungi in pests (insects) is a bioinsecticide that has not been studied and tried to rice black bugs in North Sulawesi. In the Philippines, a pathogenic fungus that is reported to be able to infect the rice black bugs are *Beauveria bassiana*, *Metarhizium anisopliae* and *Paccilomyces lilacinus*⁸.⁹ reported that the *Verticillium lecanii* could infect *Scotinophara* sp. and could colonize the ladybug eggs, *Riptortus linearis*, so that the eggs did not hatch. Rice black bugs can be attacked by pathogenic fungi that is potential as plant pest control agents in Bolaang Mongondow^{2,3}, however the type of fungi that infects rice black bugs is not yet known. Each fungus within the same species can be different in its virulence in each region. Before knowing the virulence of entomopathogenic fungi, the type of fungus that infects the rice black bugs should first be examined.

¹⁰ reported that high virulence only happened in the host species from which the fungus was first isolated. ¹¹ differences in entomopathogenic fungus types are related to differences in the characteristics possessed by each of these fungi. Characteristics of entomopathogenic fungi include shape, colony's color, conidiophore's shape, hyphae's color, and spore's shape, sporangiospore's shape, fungus texture, phialide's composition and mortality. This parameter is part to find out the type of fungus that infects the rice black bugs. The purpose of this study was to examine the type of fungi that are pathogenic against rice black bugs, *Scotinophara* sp. and the distribution of entomopathogenic fungi in rice paddy in Bolaang Mongondow region.

Research Method

A study on taking the sample of infected rice black bugs actually has been carried out at the production centers of rice paddy in West Dumoga Districts, East Dumoga and Lolayan Bolaang Mongondow District. This area was chosen as an endemic area of rice black bugs population. The rice black bugs and soil nymphs infected with entomopathogenic fungi sample were taken in the vegetative and reproductive phases of the plants. The infected insects sampling was taken 3 times, with intervals of 3 weeks. In addition, healthy rice black bugs were taken to be cultured in the laboratory in the process of testing fungi as pathogens. The rice black bugs infected with fungi sample was taken by doing a census on rice paddy, specifically by observing all sample of rice paddy clumps measuring ± 0.5 ha per location.

The fungi were isolated on PDA media but macroscopic observations were previously made to obtain preliminary data about the character of the fungus or mycelium as a supporting data in identification. Before being isolated the surface of the body of the rice black bugs were disinfected with 70% alcohol, and NaOCl for 3 minutes then rinsed with sterile water for 3 times and dried with sterile absorbent paper, then cultured on PDA media. After culturing, PDA was incubated for 7 days at a temperature of 24-25°C. The fungus that grows on PDA medium was then purified again on PDA medium. Purified fungi were used in inoculation of rice black bugs immagos to prove that the fungus infects the rice black bugs and is then identified.

The fungus growing on PDA media was taken sufficiently by spores using an ose needle and then dissolved in sterile water in a test tube and rubbed evenly for 10 -15 minutes, so that a suspension is formed. A total of 10 ml of suspension was poured into petri dish and 20 of rice black bugs imago were added and it was repeated for 3 times. Insects that are in petri dish were left for 5-10 minutes, then released in gauze sacks containing rice paddy. Observation was done to see whether there is a symptom of an attack, and to know when the time of imago's death is. Mortality data were observed every day for 10 days after inoculation. The rice black bugs imago that die and appearance of fungi on the surface of the body, was then re-isolated to the PDA media and incubated for 7 days at a temperature of 24-25⁰C. Fungi that grow on PDA media were identified, in order to know the type of pathogenic fungi that infect the rice black bugs. The rice black bugs infected by entomopathogenic fungi from the field, were then taken its spores and mycelium. Spores and mycelium are mixed with sterile water to form a suspension. The suspension is placed in a petri dish and inserted into the rice black bugs for 5-10 minutes, then the imago is released on the rice paddy plants in the laboratory.

The breeding of rice black bugs was done in a 1m x 1m x 1 m gauze cage and a plastic bucket filled with rice paddy plants that have produced panicles were put. Adult ice black bugs taken from the field were put in 10-15 pairs of gauze cages in a field laboratory, these rice black bugs were let to populate and lay eggs in gauze cages. The rice paddy plants that have eggs were separated and then put into other gauze cages. After the eggs hatched, the nymphs were kept until the imago was ready to be used in the Koch's Postulation

Identification of the entomopathogenic fungi characteristics was based on the color of the colony, hyphae, conidiophore, fungus texture, structure of the fungus, spore shape, and so on. Pathogens identification to the level of genus or species was based on its morphology.

Results and Discussion

Rice black bugs infected with entomopathogenic fungi are commonly found at the base of rice paddy stems. In addition to the base of the stems, infected rice black bugs were found in the fronds, but the population was very low compared to the base of the stem of rice paddy. The fungus colony mass was macroscopically reddish white in all sampling locations. The rice black bugs that have grown entomopathogenic fungi on the surface of their body were then isolated on PDA media to find out and obtain what type of fungus causing infection and mortality of the bugs⁴. The state of rice black bugs infected with entomopathogenic fungi with a colony mass and healthy is shown in Figure 1.



Figure. 1 Morphology of rice black bugs a) Attacked by the *Paecilomyces* sp. and b) Healthy

Based on the results of observations in the field that entomopathogenic fungi growing on rice black bugs started from the insects' body, especially in the abdomen, then it spreads to all parts of the insect's body. Fungi obtained from rice black bugs in the three locations namely Lolayan District, East Dumoga and West

Dumoga Bolaang Mongondow District were then isolated in PDA media. The results of isolation of fungi that grew on PDA media found different colony mass, can be seen in Figure 2.



A. *Paecilomyces* sp

B *Fusarium* sp

Figure 2. Colonies mass of *Paecilomyces* sp and *Fusarium* sp.

Based on the identification of entomopathogenic fungus colony masses growing on PDA media, two types of fungi have been found, namely *Paecilomyces* sp. and *Fusarium* sp. but from both fungi effects, the cause of mortality of rice black bugs in rice paddy plants were not yet known since inoculation which can cause mortality in the laboratory on healthy rice black bugs is still continued.

Inoculation of entomopathogenic fungi on population of 20 rice black bugs to obtain data that infects rice black bugs has been carried out in two ways namely. The first one was fungi originating from isolation results on PDA media and the second one was the fungi colony mass from the field. From the Koch Postulate test results, a mass of colonies on the fungus *Paecilomyces* sp was obtained from it infected the rice black bugs in the field until inoculation was carried out on healthy bugs, while the fungus *Fusarium* sp. which was applied to healthy rice black bugs had not caused any infections in the bugs' body. This shows that *Fusarium* sp. is a secondary fungus on rice black bugs. *Fusarium* sp. can make a contact on rice black bugs after the *Paecilomyces* sp. appeared. Thus the *Fusarium* sp. is not the main fungus that causes mortality in rice black bugs. The *Fusarium* sp colonies mass is orange brown. *Fusarium* sp. which infects pest is still very inadequate. The result obtained from the colony mass growing on the growing medium, then inoculated on healthy rice black bugs, showed the macroscopic appearance of the same morphology in the colony mass of *Paecilomyces* sp. before and after inoculation on rice black bugs

The colony mass of *Paecilomyces* sp. which is taken from the field separated from the bugs' body and then mixed with sterile water. It then applied to healthy rice black bugs on rice paddy plants in the laboratory. The results showed that it produced a similar morphology of the colony mass to the morphology of *Paecilomyces* sp. originating from the field. Although the colony mass of *Paecilomyces* sp. has already been contaminated with a number of fungi, the *Paecilomyces* sp. keep appeared after it was applied to the insects. Unlike other fungi, *Paecilomyces* sp. must be isolated on the growing media to get pure breed, then the it is applied to insect pests in order to prove that the fungus can infect the pests.

During the application of *Paecilomyces* sp. on healthy rice black bugs, there are bugs that are not infected by the fungi, and thus it has not caused mortality in bugs. Concentration of fungi applied to rice black bugs in the laboratory is still relatively low 10^6 . When the fungus suspension is used, *Paecilomyces* sp. 10^8 will cause mortality in all rice black bugs populations used as samples. The contact of the fungus spores on the insect's body need fine moisture to grow and penetrate to the insect's body. The growth of hyphae that meet the insects' organs will cause death. Fine moisture will grow hyphae, to the body surface of an insect, which then

can transmit to other insects. Rice black bugs infected by fungus are often found in the base of rice paddy stems, because the moisture was pretty fine for *Paecilomyces* sp. to make contact with the bugs.

Morphologically *Paecilomyces* sp. has round to oval conidia with varying sizes between (2-4) x (1-2), fusoid, hyphae with septa, and hyaline, branched conidiophores and phialides at the edges, thin phialides with essentially enlarged and long, sized (5-6,5) x (2,4) μm . The results of this identification are in accordance with those stated by ^{12,13,14}. Classification of the *Paecilomyces* sp, including the Eurotiomycetes class, the order of Eurotiales and Family of Trichocomaceae. The characteristics of *Paecilomyces* sp. is that it has a oval conidia, septa and hyaline⁹.

The entomopathogenic fungus, *Paecilomyces* sp., which infects rice black bugs is only found one species. The *Paecilomyces* sp. which infects rice black bugs in Bolaang Mongondow may have more than one species if identification is made to species level. Conversely, if the sampling of rice black bugs specimens in several locations in Bolaang Mongondow Regency with an apparently wide spread area, it is possible to find more than one species too, because the location of rice paddy planting in the Bolaang Mongondow regency is restricted to mountains and other agricultural crops than rice paddy are limited to cocoa, corn and horticulture. ¹⁵the appearance, diverse and distribution of entomopathogenic fungi will vary depending on its habitat, location, geography, environmental conditions, plant species and cultivation practices. ¹⁶ argued that the diversity of entomopathogenic fungi in the soil is influenced by several factors, namely soil water content, organic matter content, and temperatur⁹ ¹⁷stated that organic matter will add to the energy needed for the life of ⁷il microorganisms. ¹⁸the presence and distribution of entomopathogenic fungi in the soil are strongly influenced by the pH and soil type, height, habitat, soil temperature and plant species. The dry condition of the will be insufficient to get rice black bugs infected, but rice black bugs infected by entomopathogenic fungi will commonly be found in muddy paddy clumps. The rice black bugs population will still be found in the remaining of the rice paddy clumps have been cut still and it can be infected with entomopathogenic fungi. ¹⁹*Paecilomyces variotii* was the first species to be discovered, and there are approximately 100 recognized species of *Paecilomyces*.

The *Paecilomyces* sp. is very similar to the *Penicillium* sp. but can be easily distinguished from its loose conidiophores branched and cylindrical conidiogen cells with tapered ends. Fungi infected rice black bugs may be able to infect other pests in other rice paddy plants, because *Paecilomyces* sp. has a wide spectrum to ³ards various pests in agricultural crops. Several species of *Paecilomyces* sp. have been isolated in plant pests. Some species of *Paecilomyces* sp. are isolated from several insects, and even are a cause of infection to humans^{20,21}. *Paecilomyces variotii* is a species common³ found after being isolated on various media, including food, soil, indoor air, and wood^{22,23}. *Paecilomyces* sp is a filamentous fungus, commonly found in soil, rotten plants, and food products^{24,25,26}.

After being inoculated by entomopathogenic fungus, *Paecilomyces* sp, there was a change in the rice black bugs' behavior, generally they were not visible on the surface of the rice fronds but settled at the base of the rice stem instead. Rice black bugs become less active until it is dead, after they dead a few days later reddish-white mycelium appears on their body, especially on the abdomen, then it spreads to all parts of their body. Mycelium continues to grow until it covers all parts of the insect's body. *Paecilomyces* sp. need more time to infect the rice black bugs. Healthy rice black bugs inoculated with entomopathogenic fungus, *Paecilomyces* sp., require 8 days to their death, then on the 10th day the mycelium or hyphae began to appear. Six strains of *Paecilomyces* sp. (10^8 spores.ml⁻¹) were inoculated in *Plutella xylostella* larvae for 2.52 days and 6.59 days, whereas for *Spodoptera litura* were inoculated for 2.51 days and 7.09 days. Only two⁵ species can be used as biological control of *P. xylostella* and *S. litura*, namely *P. lilacinus* and *P. javanicus*. For sporulation, red rice or brown rice mixed with rice husk and rice bran are suitable for cultivating *Paecilomyces* sp. isolates²⁷. The lengthy of a rice black bug to die after inoculation to healthy bugs was due to the fact that the application of spores was very low. Although it needed quite long time, the application of the *Paecilomyces* sp. could cause mortality to the bugs. ²⁸the mortality of *Bemisia Tabaci* by *P.fumosoroseus* lasted longer

How the entomopathogenic fungus works is not as fast as synthetic or vegetable insecticides whose results can directly be see through the sudden mortality of the targeted insect. Entomopathogenic fungi take longer time to kill target insects. During the infection process, *Paecilomyces* sp. produce a number of enzymes for their development in the insects' bodies such as prosthesis, chitinase, and lipase which degrade the host cuticle and facilitate the attachment of *Paecilomyces* sp. conidia on the host insects' cuticle²⁹. In addition, ³⁰

stated that entomopathogenic fungi produce several types of toxins which can cause an increase in hemolymph pH, clotting of hemolymph, and cessation of hemolymph circulation. Insects that have been attacked by the *Paecilomyces* sp. become hard and stiff, this may have occurred hemolymph fluid collection.

Conclusion

The fungus associated with the surface of the rice black bugs' body consists of *Paecilomyces* sp and *Fusarium* sp. Both fungi are spread in Lolayan District, East and West Dumoga. The fungus that caused mortality of the rice black bugs investigated using the Koch's Postulate test was only *Paecilomyces* sp, whereas the *Fusarium* sp was a secondary fungus. Mycelium begins to appear on the periphery of the bugs' abdomen, then spreads to all parts of its body. The movements of rice black bugs infected with *Paecilomyces* sp. are rather slow and it eventually die.

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