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Research Article

Control of Rice Powder Pest *Sitophilus oryzae* L. with Entomopathogenic Fungus *Metarhizium anisopliae* (Metch.)

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Abstract

Background and Objective: Rice is one of the most important staple foods in the world, especially on the Asian Continent. Postharvest pests can reduce the quality and quantity of rice by up to 25%. Control using pathogenic microbes is the best choice in controlling postharvest pests in general and *Sitophilus oryzae* rice powder pests in particular. Research has been carried out to know the effect of dosage concentrations of pathogenic cultures and LC_{50} values of pathogenic *Metarhizium anisopliae* cultures on the control of *S. oryzae* rice powder pest. **Materials and Methods:** The concentration dose of the pathogenic culture of *M. anisopliae* acted as a treatment. The test insect population for each treatment was 25 imagos. The study of the effect of dose concentration of *M. anisopliae* culture on the mortality of *S. oryzae* imago used analysis of variance. Determination of the effective concentration of LC_{50} using probit analysis. **Results:** The higher the concentration of the pathogenic culture of *M. anisopliae*, the higher the mortality of the adult rice powder pest *S. oryzae*. The highest mortality of rice powder pest *S. oryzae* occurred at a dose of 50% concentration of the pathogen *M. anisopliae* culture, namely 89.33%, followed by a concentration of 40% with a mortality of 72.0%, a concentration of 30% with a mortality of 57.33%, a concentration of 20% with a mortality of 13.33% and the lowest dose concentration of 10% with a mortality of 1.33%. **Conclusion:** The effective concentration dose (LC_{50}) of the pathogenic culture of *M. anisopliae* in controlling the rice powder pest *S. oryzae* was 31.88%.

Key words: *Sitophilus oryzae* L., entomopathogen, rice, fungus, *Metarhizium anisopliae*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice is one of the most important staple foods in the world, especially in Asia. Based on the report of the Organization for Economic Cooperation and Development (OECD), the rice consumption of Asian residents was the highest in the world, at 77.2 kg per person per year during 2018-2020[1]. This figure is projected to be 77.5 kg per person annually by 2030, Asia. The Asian continent is also the home of farmers who produce about 90% of the world's total rice production[2]. In Indonesia, about 97% of the population consumes beras. This keadaan means that Indonesia must be able to provide mem production of beras to memenuhi keb's food needs[3,4].

Postharvest rice management techniques, namely in the form of rice, significantly affect the storage, quality and nutritional duration[5]. Rice is particularly susceptible to pest disturbances that can degrade its quality and nutritional content[6]. The quality and quantity of beras can be used as several pests, rats, birds and micro-organismes[7]. Postharvest losses of rice in developing countries caused by mishandling, decay and pest attacks can reach 25%[8]. The presence of insect pest attacks causes heavy exposure to postharvest materials, including rice stored in the warehouse[9].

The use of post-deposit materials or stash materials is very important. It has an important value in economics because: (1) The material is ready for consumption, (2) It consumes quite a lot of costs, namely starting from the cultivation, the soil processing, planting, maintenance and harvesting[10]. So these that little in postharvest materials has become a different loss compared to the attacks of pest organisms in plants in plants[11,12].

Until now, no effective control method has been found to control the pest of *S. oryzae* rice powder. Chemical control, especially spraying, cannot be done because it can poison rice so that it can poison consumers[13]. The use of pathogenic microbes is the best option for pest control of *S. oryzae*. The control method by utilizing insect pathogens is environmentally friendly and does not cause poisoning of postharvest materials or stash materials[14,15].

The microscopic fungi *Metarhizium anisopliae* are widely studied as entomopathogens, including in mosquitoes[16] and warehouse pests[17]. However, application as a pest control of *Sitophilus oryzae* L., rice powder is still very little reported. Research has been conducted on using the pathogenic fungus *Metarhizium anisopliae* (Metch.) as a microbial insecticide in the pest control of *Sitophilus* rice powder, *Sitophilus oryzae* L.

MATERIALS AND METHODS

Study area: This research was carried out at the Laboratory of Pests and Plant Penyakits, Department of Pests and Plant Penyakits, Faculty of Pertanian, Sam Ratulangi University, Manado. Penelitian is held for 8 months, from March to October, 2021.

The research used a randomized design of complete and as a treatment, was a dose of the pathogen concentration of *M. anisopliae*. The study was conducted in two stages, namely the study of the effect of *M. anisopliae* concentration on imago mortality of *S. oryzae* and the study of the effective concentration dose (LC_{50}) of the pathogen *M. anisopliae* on imago mortality of *S. oryzae*.

Maintenance of test insects: Rice samples that have been attacked or show symptoms of *S. oryzae* attacks were collected from traditional markets in Manado City and Minahasa Regency and under laboratory to be rearing as the propagation of rice test insects that were scattered g or showed that *S. oryzae* attacks were put in jars of 1 kg each and covered with white azahi cloth. The rice jars were left for 1.5 to 2 months until the pest *S. oryzae* in the rice multiplied into many. The test insect *S. oryzae* is ready to be applied with a microbial insecticide, namely the culture of the pathogen *M. anisopliae* on rice media.

Preparation of *M. anisopliae* pathogen culture on rice media: The rice is steamed until half cooked and then packed in transparent plastic bags per 20 g. Pure culture of *M. anisopliae* was added in each rice pack. After the pathogen *M. anisopliae* has experienced full growth 2-3 weeks after the inoculation culture, the culture is ready to be applied.

Effect of dose concentration of *M. anisopliae* pathogen culture on imago *S. oryzae* mortality: The treatment consisted of 5 levels of dose concentration of *M. anisopliae* pathogen culture with 3 repeats, namely A = 10% concentration (pathogen culture 10 g/100 cc aquades), B = Concentration 20% (pathogen culture 20 g/100 cc aquades, C = Concentration 30% (pathogen culture 30 g/100 cc aquades, D = concentration 40% (pathogen culture 40 g/100 cc aquades, E = Concentration 50% (pathogen culture 50 g/100 cc aquades and K = Control (pathogen concentration 0 g/100 cc aquades.

A total of 25 *Sitophilus oryzae* imago on each petri dish according to the number of treatments and replays. It is also a small jar that has been filled with pest-free rice

according to the number of treatments and tests. Evenly disburse the solution of the pathogen *M. anisopliae* on the test insects according to the level of treatment concentration. Imago *S. oryzae* is applied with a solution of the pathogen *M. anisopliae* in each filled treatment jar of 15 g of pest-free rice each and then a lid with a white azahi cloth. Observations after application with an observation time interval of 2 days and observations were made 5 times. The same procedure was also for the implementation of research on the effective concentration dose (LC_{50}) of the pathogenic culture of *M. anisopliae* in pest control of *S. oryzae* rice powder.

Effective concentration dose research (LC_{50}) of *M. anisopliae* pathogenic culture in pest control of *S. oryzae* rice powder: Three levels of dose of *M. anisopliae* pathogen culture concentration with three repeats, namely A = Concentration of 20% (pathogen culture 20 g/100 cc aquades), B = Concentration 30% pathogen culture 30 g/100 cc aquades), D = Concentration 40% (pathogen culture 40 g/100 cc aquades) and K = Control (pathogen concentration 0 g/100 cc aquades.

Observation: The observed things were 1) growth progression of *M. anisopliae* in *S. oryzae* test insects until the test insect dies and 2) imago *S. oryzae* mortality for each concentration of *M. anisopliae* pathogens due to the application of *M. anisopliae* pathogens. Observations of the growth development of *M. anisopliae* in the test insect were carried out from when the test insect died until the hyfa or fungal mycelia grew on the surface of the test insect's body. The observations made were macro.

Observations of test insect mortality due to the treatment of *M. anisopliae* began to be carried out the second day after application. Observation of *S. oryzae* mortality was carried out five times and the observation interval was two days. Calculation of *S. oryzae* pest mortality due to the application of the *pathogen M. anisopliae* using the formula:

$$M = \frac{n}{N} \times 100\%$$

Where:

M = Mortality

n = Number of test insects that died as a result of the application of *the pathogen M. anisopliae*

N = Number of test insects per treatment (Manueke *et al.*[15] and Yassin *et al.*[17])

Data analysis: Data on the results of penelitian, namely *S. oryzae* mortality due to the application of the pathogen *M. anisopliae* were analyzed using the statistical analysis program SPSS 21.0. Calculate the effective dose of culture solution of *M. anisopliae* culture (LC_{50}) using probit analysis.

RESULTS

Development of the pathogen *Metarhizium anisopliae* (Metch.) on the test insect *S. oryzae*: Imago *S. oryzae's* behavior after applying the pathogen *M. anisopliae* is that insects stop/do not want to eat, are on the material's surface, are less active/stay silent and die. After the insect dies, two days later, there is a change in color, namely the body's surface becomes pitch black and on the cuticle, a black rickshaw is seen as a trace of mold penetration. Imago *S. oryzae*, who was attacked by *M. anisopliae* in the laboratory, can be followed in Fig. 1 (a and b). Figure 1 showed that after the imago *S. oryzae* dies, the initial symptoms were the appearance of hyfa or white *M. anisopliae* fungal mycelia on the sutura or wing boundaries on the abdomen, the boundary between the abdomen and thoracic and the base of the legs. The subsequent development of mycelia or fungal hypha will cover the entire body surface and change color from white to muscardine green.

Effect of dose concentration of *M. anisopliae* pathogen culture on imago *S. oryzae* mortality: Data from the research on the application of the effect of the pathogen *concentration of M. anisopliae* on the mortality of the rice powder pest *S. oryzae* gave quite satisfactory results. The study's results on the effect of the pathogen concentration of *M. anisopliae* on the mortality of the rice powder pest *S. oryzae* were shown in Table 1.

Table 1 showed that all treatments showed marked differences in control except for treatment A, which was a concentration of 10%. The dose of *M. anisopliae* pathogen culture concentration of 50% gave the highest mortality result of 89.33%, followed by the concentration of *M. anisopliae* pathogen 40% with a mortality of 72.0%, pathogen concentration *M. anisopliae* 30% with mortality 57.33%, pathogen concentration *M. anisopliae* 20% with mortality 13.33% and lowest at the pathogen concentration *M. anisopliae* 10% with a mortality of 1.33%. This study showed that the fungus *M. anisopliae* could be used to control the pest of *S. oryzae* rice powder.

The development of imago *S. oryzae* mortality from the first observation to the fifth observation showed that the increase in the dose of the pathogen concentration of

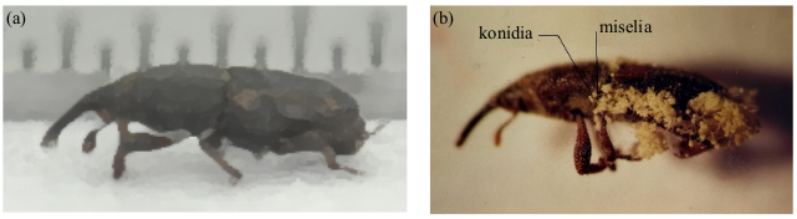

Fig. 1(a-b): Imago *S. oryzae* attacked by *M. anisopliae* pathogen in the laboratory, (a) Imago *S. oryzae* healthy and (b) Imago *S. oryzae* stricken *M. anisopliae*

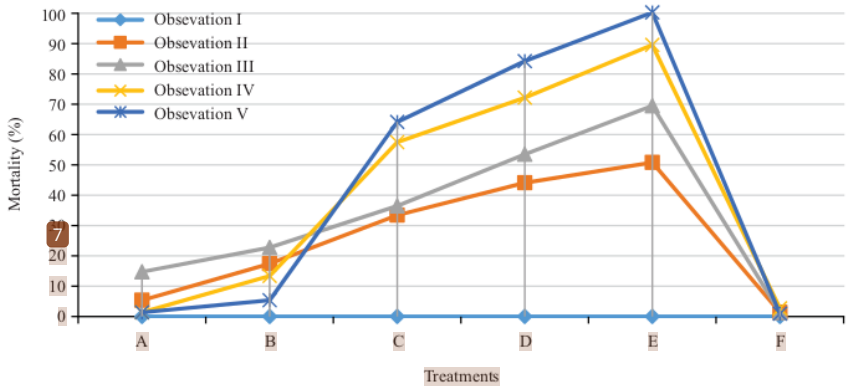

Fig. 2: Graph of the development of mortality of the rice powder pest *S. oryzae* at varying dose levels of the culture concentration of the pathogen *M. anisopliae* from the first observation to the fifth observation, A: Concentration of 10% (pathogenic culture 10 g/100 cc aquades), B: Concentration of 20% (pathogen culture 20 g/100 cc aquades), C: Concentration of 30% (pathogenic culture 30 g/100 cc aquades), D: Concentration of 40% (pathogen culture 40 g/100 cc aquades), E: Concentration 50% (pathogen culture 50 g/100 cc aquades and F: Control (pathogen culture 0 g/100 cc aquades)

Table 1: Table of average mortality of *S. oryzae* rice powder pests due to *M. anisopliae* pathogenic application

Treatment	Mortality (%)
F = Control	1.3333[a]
A = Concentration 10%	1.3333[a]
B = Concentration 20%	13.3333[b]
C = Concentration 30%	57.3333[c]
D = Concentration 40%	72.0000[d]
E = Concentration 50%	89.3333[e]

α = 0.05, A: 10% concentration (pathogen culture 10 g/100 cc aquades, B: Concentration 20% (pathogen culture 20 g/100 cc aquades, C: Concentration 30% (pathogen culture 30 g/100 cc aquades, D: Concentration 40% (pathogen culture 40 g/100 cc aquades, E: Concentration 50% (pathogen culture 50 g/100 cc aquades and F: Control (pathogen concentration 0 g/100 cc aquades)

M. anisopliae was directly proportional to the mortality of imago *S. oryzae* (Fig. 2). Figure 2 showed that the higher the culture concentration of the *pathogen M. anisopliae*, the higher the mortality of *S. oryzae* rice powder. At the first observation, there had been no mortality in the pest *S. oryzae* and mortality began to occur on the second observation (4th day after application) and increased steadily on the third, fourth and fifth observations. Increased mortality of rice powder pest *S. oryzae* occurs due to an increase in the number of spores at each increased dose of concentration of the culture of the pathogen *M. anisopliae*. An increase always follows the increase in spores in the toxins contained in each increased dose of the culture concentration of the *M. anisopliae* pathogen.

Effective concentration dose (LC_{50}) of *M. anisopliae* pathogenic culture in pest control of *S. oryzae* rice powder: The results of the probit test of effective concentration dose (LC_{50}) of the pathogenic culture of *M. anisopliae* in the control of *S. oryzae* rice powder pests were shown in Table 2. The results of the probit analysis showed that the effective concentration dose (LC_{50}) of the *M. anisopliae* pathogenic culture in pest control of *S. oryzae* rice powder was 31.885%. Lethal Concentration 50 (LC_{50}) is a substance that can cause 50% of deaths when exposed to a population of an organism.

Table 2: Effective concentration dose (LC_{50}) of *M. anisopliae* pathogenic culture in pest control of *S. oryzae* rice powder

LC_{50} value calculation results for observation IV Confidence limits		
	95% Confidence limits for doses	95% Confidence limits for log (doses)[a]
Probability	Estimate	Estimate
0.010	06.363	0.804
0.020	07.512	0.876
0.030	08.346	0.921
0.040	09.034	0.956
0.050	09.635	0.984
0.060	10.179	1.008
0.070	10.680	1.029
0.080	11.150	1.047
0.090	11.595	1.064
0.100	12.021	1.080
0.150	13.956	1.145
0.200	15.713	1.196
0.250	17.396	1.240
0.300	19.061	1.280
0.350	20.745	1.317
0.400	22.481	1.352
0.450	24.299	1.386
0.500	26.231	1.419
0.550	28.316	1.452
0.600	30.606	1.486
0.650	33.166	1.521
0.700	36.097	1.557
0.750	39.551	1.597
0.800	43.788	1.641
0.850	49.302	1.693
0.900	57.238	1.758
0.910	59.339	1.773
0.920	61.709	1.790
0.930	64.424	1.809
0.940	67.598	1.830
0.950	71.409	1.854
0.960	76.161	1.882
0.970	82.440	1.916
0.980	91.595	1.962
0.990	108.132	2.034

[a]Logarithm base = 10 and LC_{50} = 26.23 mL

Typically, this parameter is important regarding the toxicity of a substance or chemical substance related to water or contained in 100 cc or 1000 cc of water. The LC_{50} refers to the lethal concentration of a substance that can cause 50% death when exposed to a population of test organisms. The unit of measurement for LC_{50} is in milligrams per cubic meter or ppm.

DISCUSSION

The initial symptoms of *S. oryzae* pests after being applied with the pathogen *M. anisopliae* are insects that stop eating, are on the surface of the material, less active/more silent. After the insect dies, two days later, there is a change in color, namely the body's surface becomes pitch black and on the cuticle, a black rickshaw is seen as a trace of mold penetration. The appearance of hyphae or mycelia of the fungus *M. anisopliae* is white on the sutura or the boundaries of the wings on the abdomen, the boundary between the abdomen and thoracic and the base of the legs. The subsequent development of mycelia or fungal hypha will cover the entire body surface and change color from white to mustard green.

The initial symptoms of insects attacked by the fungus *M. anisopliae* do not want to eat, so the body becomes weak[18]. It lacks orientation, over time, it is silent and dies. The insect changes color and on the cuticle, a black rickshaw is visible as a trace of penetration of the fungus. If environmental conditions are favorable, white mycelia will appear on the surface of his body. The affected larva usually secretes a reddish discharge from its mouth continuously. After death, the body begins to become soft and, within 5 hrs, becomes stiff (mummy). A day later, his body was covered with mycelia. Dead larvae attacked by the fungus *M. anisopliae* will later harden and stiffen. The skin of the larvae will be covered with white flour that will change color to dark green[19].

The color of all isolates of *M. anisopliae* fungi macroscopically at the beginning of white growth, then turns into a dark green color[20]. Microscopically hyaline spores, cylindrical in shape and form chains. Spores of *M. anisopliae* enter the body of an insect through the skin. Spores that have entered the insect body begin to form hyphae ranging from the tissues of the epidermis until the entire tissues of the insect's body are filled with hyphae. Once the host is killed the group of hyphae will form primary and secondary spores, depending on weather conditions, as the weather favors the spores appearing on the insect's cuticle[20,21]. According to Athifa *et al.*[22] that infection and the spread of spores are influenced by several factors, namely wind, humidity and host solids. Strong winds and high humidity can help spread spores and the even distribution of infection throughout individuals in the host population[22]. Entomopathogenic microorganisms widely used to control pest attacks are microorganisms from the entomopathogenic fungi group[18]. Fungi widely used as bioinsecticides are from the genus *Metarhizium* and *Beauveria*, namely *M. anisopliae* and *B. bassiana*. The *M. anisopliae* and *B. bassiana* were found to have attacked many invading pests of the orders Hemiptera and Coleoptera. Some entomopathogenic fungus species that can be considered as biological insecticides are *B. bassiana, M. anisopliae, Verticillium lecanii and Hirsutella thompsonii*[23]. The fungus *M. anisopliae* can infect insects of the order groups of Orthoptera, Coleoptera, Hemiptera, Lepidoptera and

Hymenoptera. *M. anisopliae* could be used to control the pest *Oryctes rhinoceros*. The *M. anisopliae* carried by imago *O. rhinoceros* can infect and kill the larvae of *O. rhinoceros*[23]. The research results of Kastilong *et al.*[23] showed that the application of *M. anisopliae* PH04 fungi could reduce the population of *Lepidiota stigma* larvae in sugarcane plantations and increase crop yields by more than 60%. The fungus can survive on the farm for more than six months, potentially controlling *L. stigma* in the long term.

This study also showed that the increase in dose concentration of the pathogen *M. anisopliae* was directly proportional to the mortality of imago *S. oryzae*. The higher the culture concentration of the pathogen *M. anisopliae*, the higher the mortality of *S. oryzae* rice powder. Increased mortality of rice powder pest *S. oryzae* occurs due to an increase in the number of spores at each increased dose of concentration of the culture of the pathogen *M. anisopliae*. An increase always follows the increase in spores in the toxins contained in each increased dose of the culture concentration of the *M. anisopliae* pathogen.

The increase in the culture concentration of the pathogen *Metarhizium anisopliae* (Metch.) is directly proportional to the mortality of the larvae of *Hexamitodera semivelutina* Hell. on Clove plants. The more high concentrations of *M. anisopliae* pathogenic cultures, the higher the spore content in the pathogen's culture so that the pathogenic culture's killing power also increases and causes the mortality of test insects to increase[24].

The research results by Wamiti *et al.*[25] showed that *Metarhizium* sp. infects the host through four stages: Inoculation, pasting, penetration and digestion. The first stage is the inoculation of contact between the propagules of the fungus and the insect's body. The second stage is the process of pasting and germination of propagules of fungi on the integuments of insects. The third stage is penetration and invasion. Fungi penetrating through the integuments can form sprout tubes (appressorium). The morphological configuration of the integument strongly influences the penetration point. Breakout is carried out mechanically or chemically by secreting enzymes and toxins. The fourth stage is digestion at the end of penetration and the formation of blastospores which then circulate into the hemolymph and form secondary hyphae to attack other tissues. So that in general, all tissues and body fluids are used up by fungi, so insects die with hardened bodies.

The LC_{50} stands for lethal concentration i.e., the parameter for various chemical compounds that describe the amount of substance in a unit volume of water (100 cc or 1000 cc) that can cause death. These two parameters measure the lethal character of a substance when exposed to a population and cause the death of 50% of that population. The LC_{50} is a concentrated dose administered once (single) or several times in 24 hrs of a substance that is statistically expected to kill 50% of test animals[26]. The LC_{50}, commonly abbreviated as LC_{50}, is a calculation to determine an extract's or compound's activeness. The LC_{50} means at what concentration the extract can kill 50% of the test organisms that can be estimated by graphs and calculations or at a specific observation time[27]. The LC_{50} is the concentration at which the extract solution can cause the death of the test organism population up to 50%.

CONCLUSION

The increase in the concentration of the pathogenic culture *M. anisopliae* is directly proportional to the mortality of the rice powder pest *S. oryzae*. The higher the culture concentration of the *pathogen M. anisopliae*, the higher the mortality of *S. oryzae* rice powder. The effective concentration dose (LT_{50}) of the *M. anisopliae* pathogenic culture to control the pest of *S. oryzae* rice powder, which is 31.885%, is a dose of *M. anisopliae* culture concentration which can cause the mortality of *S. oryzae* rice powder pests to be 50%.

SIGNIFICANCE STATEMENT

Rice is the main food ingredient in Asia, including Indonesia. *Sitophilus oryzae* L., becomes a post-harvest pest that reduces the quantity and quality of rice. This research was conducted to provide alternative pest control for *Sitophilus oryzae* L. with biological control using the entomopathogenic fungus *Metarhizium anisopliae* (Metch.). This study found the Entomopathogen *Metarhizium anisopliae* (Metch.) to be effective in controlling *Sitophilus oryzae* L. Further research can be carried out on a field scale.

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