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Effectiveness of Various Media in Propagating Local Isolates of Metarhizium anisopliae

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ABSTRACT: One of the most important commodities in the South Central Timor Regency is sweet potatoes. However, one significant factor limiting production is the attack of the Cylas formicarius insect. Biological pest management is the ideal method to address this issue due to its lack of adverse effects. Entomopathogenic fungi are a type of biological agent used for this purpose. Previous research by the author demonstrated that local isolates of the entomopathogenic fungus Metarhizium anisopliae, when supplemented with insect flour, could enhance conidia production, viability, and virulence. This makes the isolate suitable for development as a bioinsecticide. For the growth of entomopathogenic fungi as biological agents, a sufficient supply of high-quality inoculum and efficient production methods are essential. Currently, solid media such as rice and maize are used for propagation, which is costly. Therefore, there is a need for new alternative media that are economically viable, nutrient-rich, effective, easy to obtain, and abundant in raw materials. Additionally, the media must support the growth and proliferation of entomopathogenic fungi. The objective of this study is to identify the most effective media for the propagation of M. anisopliae. The study employed a completely randomized design (CRD) with 8 treatments and 4 replications, resulting in 32 experimental units. The treatments included various media: rice bran, tofu leftovers, broken maize, sweet potatoes, cassava, husks, and green beans. Data were processed using variance testing, followed by the BNT test at a 5% significance level. The study's findings indicate that the rice bran medium had the highest growth percentage of M. anisopliae, reaching 100%. It also proved to be the best medium in terms of incubation time, fungal colony diameter, and conidia/mg.

KEYWORDS: Biological pest management, Cylas formicarius, Entomopathogenic fungi, Metarhizium anisopliae, Propagation media, Sweet potatoes.

INTRODUCTION

Sweet potato cultivation in South Central Timor Regency faces challenges due to the pest attack by Cylas formicarius, leading to significant yield losses. Various studies have explored effective pest control methods. Research has shown that the use of Tagetes erecta (Tagetes) plants can reduce the intensity of weevil attacks on sweet potatoes, potentially enhancing crop growth and minimizing pest damage [1]. Additionally, the successful eradication of C. formicarius over a wide area has been achieved through a combination of male annihilation technique (MAT) and sterile insect technique (SIT) applications, showcasing the feasibility of biocontrol methods in managing this destructive pest [2]. Furthermore, studies on odorant-binding proteins (OBPs) in C. formicarius have highlighted their role in the detection of sex pheromones and host plant volatiles, indicating the potential for RNA interference as a means of disrupting pest behavior and reducing crop damage [3], [4]. These findings collectively support the use of biotherapy as a promising and environmentally friendly approach for controlling C. formicarius and mitigating yield losses in sweet potato production. Pest attacks by Cylas formicarius, such as the sweet potato weevil, can indeed lead to a loss of yield in crops like sweet potatoes. Research has shown that C. formicarius is a significant pest that attacks sweet potato plants, causing crop failure due to weevil infestations [5]. Additionally, the presence of C. formicarius can lead to a decrease in tuber dry weight, affecting the overall productivity of sweet potato plants. Studies have highlighted the impact of C. formicarius on sweet potato cultivation, with larvae feeding and tunneling within storage roots, resulting in root malformation and a bitter taste, ultimately reducing the quality and quantity of the crop yield [6]. Implementing strategies such as using barrier plants or beneficial nematodes like Heterorhabditis indica have shown promise in managing C. formicarius populations and reducing the damage caused by these pests, potentially improving crop yield [7]. Pest control Biotherapy is the best way because it has no negative impacts. One of the biological agents is entomopathogenic fungi. The local isolates of the entomopathogenic fungus *Metarhizium anisopliae* enriched with insect flour were able to increase conidia production, viability and virulence, making it suitable for development as a bioinsecticide [8].

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Production in adequate quantities and good quality inoculum are important components to support the development of entomopathogenic fungi as biological agents. Propagation can be done using liquid and solid media. Since liquid media cannot produce conidia as well as solid media can, it is not a suitable choice for field application. Solid media also tends to be more stable [9].

Metarhizium anisopliae, an entomopathogenic fungus widely utilized in pest control, can be cultivated on solid media like rice, corn, and other substrates. Research has shown that the Mycoharvester® equipment effectively harvests and separates M. anisopliae conidia from rice, providing a high yield of pure conidia suitable for biopesticide formulation [10]. Additionally, studies have highlighted the potential benefits of amino acid supplementation in improving spore production of M. anisopliae, indicating a possible strategy to enhance cultivation efficiency and reduce costs associated with traditional media like rice and corn [11]. These findings suggest that exploring alternative substrates and optimizing cultivation techniques, along with leveraging advancements in harvesting technologies, could offer more cost-effective and sustainable options for M. anisopliae production in the face of rising prices of conventional media. For this reason, new alternative media are needed that possess low economic value, sufficient nutritional content, effectiveness, ease of acquisition, abundant availability of raw materials, and suitability for the growth and development of entomopathogenic fungi.

Sago dregs, cashew pulp, sawdust, bran, rice, rice husks, and corn were used as media for propagating Trichoderma Gusnawaty et al. The most effective media was bran because the growth ability *of Trichoderma* four days after incubation reached 100% with the highest number of conidia, namely 104.125x10 ^{3}/gram of media. Meanwhile, Kansrini [12] used rice bran, sweet potato, cassava, potato and corn media with research results showing that jicama is the best media for propagating Beauveria bassiana because it produces the highest spore density, namely 1,546,933,333 spores/gram and the highest spore viability, namely 97.42%. The research on utilizing rice, corn cobs, sugar cane bagasse, and coffee husk as a medium for propagating the entomopathogenic fungus Aspergillus flavus revealed that A. flavus cultivated on bagasse media exhibited the highest efficacy, inducing larval mortality of 62.5%, producing an average of 7x10^9 conidia/gram, and achieving a conidia germination rate of 71.75% [13]. Additionally, studies on the environmentally sustainable production of lovastatin by Aspergillus terreus AUMC 15760 under solid-state conditions demonstrated the potential of utilizing sugarcane bagasse as a substrate for valuable chemical production, with lovastatin yields reaching up to 156.43 mg/L through optimized fermentation parameters [14]. These findings underscore the dual benefits of utilizing agricultural waste materials for both biological control purposes and the sustainable production of valuable compounds like lovastatin. When comparing different media for propagating Penicillium sp., tofu dregs demonstrated superior performance, yielding a higher number of conidia and viability than rice, corn, bran, pollar, and sago dregs. Specifically, tofu dregs produced approximately 207.27 x 10^6 spores/g with a viability of 68.15%. This finding aligns with research on the growth dynamics of entomopathogenic fungi, highlighting the importance of understanding fungal growth for assessing virulence and pathogenic potential [15]. Additionally, studies on maize leaf blight caused by Penicillium species in China emphasized the significance of identifying pathogens affecting crop production, with P. oxalicum and P. citrinum being reported as causal agents of maize leaf blight for the first time worldwide [16]

Referring to the results of this research, it is necessary to conduct research regarding the use of the most effective propagation media to increase the virulence of local M. anisopliae isolates enriched with insect flour for the needs of local farmers. The problem that can be formulated in this research is whether there is an appropriate local isolate of M. anisopliae propagation media for mass production. This research aims to find the best media for the propagation of M. anisopliae with a high level of virulence against the pest Cylas formicarius.

The results of this research are a product, namely a local isolate of Metarhizium anisopliae fungus propagation media enriched with insect flour with a high level of virulence. This research is important to carry out because sweet potatoes are an important commodity for the TTS community. The C. formicarius pest is one of the production-limiting factors that must be overcome.

MATERIAL AND METHODS

The research was carried out at the Plant Protection Laboratory, Department of Food Crops and Horticulture, Kupang State Polytani. The research was carried out using a Completely Randomized Design (CRD) with 8 treatments and 4 replications to obtain 32 experimental units. The data obtained was processed using variance testing and continued with the BNT test at a real level of 5%.

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The treatment given is the following propagation media: rice bran, tofu dregs, broken corn, green beans, sweet potatoes, cassava, husks, and rice.

Implementation stages:

- Propagation of test insects. The test insect used in this study was *Cylas formicarius imago* . The imago is taken from the field, then placed in the laboratory.
- Propagation of isolates. The TTS local isolate *Metarrhizium anisopliae* used in this research was a collection from [8] which was refreshed on PDA media.
- Media preparation is done by weighing 150 grams of each ingredient, then washing thoroughly and steaming, then cooling. The cooled media was put into heat-resistant plastic, 100 grams per plastic, then the plastic was rolled up tightly until there was no more air in the plastic, while 50 grams of media was put in a petri dish. The media was then sterilized again in an autoclave for 1 hour at a temperature of 121 ^{o}C, pressure 2 atm. After being sterilized from the autoclave, the media was cooled. Once cool, the media is ready to use.
- Inoculation of local isolates *of M. anisopliae* was carried out aseptically in an isolation room using LAFC. Local isolates of *M. anisopliae* on PDA media were cut into pieces using *a corkborer* , then pieces of the isolate were taken using a loop needle and then put into each propagation medium and stirred. The media is incubated and ready to be observed.
- The observation variables are incubation period and colony diameter
  a. The incubation period is the time needed foranisopliae to reproduce on each medium, namely the time from inoculation on the medium until it starts to reproduce [17].
  b. The measurement of the diameter of the M. anisopliae colony was carried out 21 days after incubation. By measuring the diameter of the M. anisopliae colony on a petri dish using a calliper, measurements were made at 4 points, and the average of these measurements was taken [18].
  c. Conidia density. Suspension preparation was carried out to count conidia. The number of conidia was recorded and calculated using the formula proposed by [19] as follows:

$$j = \frac{txd}{0{,}25xn}$$

Note: j : Number of conidia in 1 g of media t : Number of conidia in all square boxes counted n : Number of square boxes counted d : Dilution factor 10^{7}
0.25 : Constant

RESULTS AND DISCUSSION

Based on the results of variance analysis, it was found that differences in several treatment media had a significant effect on the incubation period of Metarhizium anisopliae. The average incubation period for M. anisopliae is one day after incubation. On the first day of observation, M. anisopliae had grown to a greenish-white colour and finally became dark green.

Table 1. Average Incubation Period for *Metarhizium anisopliae* in Various Media

Treatment Media	Incubation period (days)
Rice bran	1a
Tofu dregs	1a
Cracked corn	1a
Mung beans	1a
Sweet potato	1a
Cassava	1a
Rice	1a
Husk	5b

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The incubation period for M. anisopliae on tofu dregs, broken corn, green beans, sweet potatoes, cassava, rice bran and rice media occurs 1 (one) day after inoculation (day after inoculation), whereas on rice husk media it only occurs at 5 days after inoculation (day after inoculation) in Table 1. This is because the composition of the media is one of the determining factors for fungal growth [20], [21], [22]. It is suspected that the starch content is very low in rice husks. Different results were shown in research by [23], where the incubation period for M. anisopliae occurred at 3 days using rice, corn, rice bran, sawdust, husks and bran media. According to [17], fungi will grow if they come into contact with the culture media provided because there are nutrients that can be utilized by the fungi.

According to [24], the growth of *M. anisopliae* is directly influenced by the nutrients contained in the growth medium. These nutrients can be used after *M. anisopliae* excretes extracellular enzymes which can break down complex compounds from the substrate into simpler compounds (simple molecules in the form of sugar (monosaccharides and disaccharides) and other components that dissolve around the hyphae that can be directly absorbed. Other more complex molecules such as cellulose, starch and protein must be broken down first before being absorbed into cells.

The influence of various media treatment factors on the diversity of data from measuring the diameter of *M. anisopliae colonies* can be seen in Table 2 below.

Table 2. Colony diameter of *M. aniso pliae*

Treatment Media	Average Colony Diameter (cm)
Rice bran	9c
Tofu dregs	9c
Cracked corn	9c
Mung beans	7.5b
Sweet potato	9c
Cassava	9c
Rice	9c
Husk	3a
	BNT $_{0.05}$ = 0.47

The results of analysis of variance (Table 2) show that the multiplication of *M. anisopliae* in several treatment media had a very significant effect on the diameter of colony growth. The diameter of *M. anisopliae* colonies on several treatment media was found to be the same diameter, namely 9 cm, which was significantly different on the green bean (7.5 cm) and rice husk (3 cm) media. The lowest diameter of *M. anisopliae* in rice husk media was 3 cm, this is because rice husks contain less carbohydrates and water content compared to other treatment media. Visually it can be seen that the sawdust media is too dry compared to other media. It is suspected that rice husk media is not good for growing fungi because the C content is not sufficient for fungal growth. Based on observations that the treatment media experienced color changes at 4 days and 7 days. The color of the media changed from cloudy white to dark greenish because the fungus *M. anisopliae was growing* . At 1 day the fungus grows on the surface of the media, so that the media appears to change color and at 5 days the fungus has started to spread downwards. At 7 days the media looked dark green because *M. anisopliae* had grown evenly.

The total density of spores was obtained from observations with a Haemocytometer under a microscope, calculated based on the average number of spores observed multiplied by a constant and a dilution factor.

Table 3. Total Spore Density

Treatment Media	Average conidia density (conidia/mg)
Rice bran	71.5c x 10 ^{10}
Tofu dregs	5.3b x 10 ^{10}
Cracked corn	70.7c x 10 ^{10}
Mung beans	5.5b x 10 ^{10}
Sweet potato	72c x 10 ^{10}

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Cassava	71.2c x 10 ^{10}
Rice	71c x 10 ^{10}
Husk	0.05a x 10 ^{10}
	BNT $_{0.05}$ = 1.22

Table 3 shows that *Metarrhizium anisopliae* can be grown on all treatment media but with different numbers of conidia. The highest conidia density was on rice bran media, namely 71.5 x 10 ^{10} conidia/mg, which was not significantly different from the density of conidia on cassava, rice, sweet potato and broken corn media, but was significantly different from green bean media, namely 5. 5 x 10 ^{10} conidia/mg and tofu dregs 5.3 x 10 ^{10} conidia/mg. The lowest conidia density was found in rice husk media, namely 0.05 x 10 ^{10} conidia/mg.

The results of research by [23] show that bran media is the most effective medium to use as a medium for propagating the *M. anisopliae fungus* because each observation variable shows the ability of *M. anisopliae* to grow and develop better than other growing media. This is thought to be due to the relatively high content of carbohydrates, starch and other elements in rice bran. Apart from that, rice bran also contains sugar which fungi really like for growth. The growth of *M. anisopliae* is highly dependent on the availability of carbohydrates and is used as an energy source for its growth. Ingredients that contain high concentrations of carbohydrates will encourage fungal growth. High growth will produce a greater number of conidia, while low growth will produce a lower number of conidia.

In line with the opinion of [25] that nutritional sources can influence the growth of entomopathogenic fungi. Moore and Landecker (1972) stated that mushroom media must contain organic substances as a source of C, a source of N, inorganic ions in sufficient quantities as a supplier of growth and a source of vitamins. Numerous studies have shown that high carbohydrate consumption promotes vegetative growth of fungi. Apart from that, mushrooms also need micronutrients (calcium, iron, copper and manganese) which are usually found in raw materials.

CONCLUSION

1. The average incubation period *for M. anisopliae* is 1 (one) day after incubation on tofu dregs, broken corn, green beans, sweet potatoes, cassava, rice bran and rice. Meanwhile, on rice husk media this only occurred 5 days after incubation.
2. M. anisopliae colonies on several treatment media was found to be the same diameter, namely 9 cm, which was significantly different on the green bean (7.5 cm) and rice husk (3 cm) media. The lowest diameter of M. anisopliae in rice husk media is 3 cm.
3. The highest conidia density was on rice bran media, namely 71.5 x 10 10 conidia/mg, which was not significantly different from the density of conidia on cassava, rice, sweet potato and broken corn media, but was significantly different from green bean media, namely 5. 5 x 10 ^{10} conidia/mg and tofu dregs 5.3 x 10 ^{10} conidia/mg. The lowest conidia density was found in rice husk media, namely 0.05 x 10 ^{10} conidia/mg.

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