Organic Animal Waste as a Growth Substrate for *Metarhizium anisopliae*: A Viability Analysis

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ABSTRACT

Entomopathogenic fungi are important biological control agents and also have a good impact on the environment. One of the many entomopathogenic fungi that is safe for use by humans is Metarhizium anisopliae. Besides being parasitic, this type of fungus is also saprophytic in that this fungus utilizes organic matter as food. The organic material used will later become the media or substrate for the propagation of Metarhizium anisopliae. The substrate used is usually PDA (Potatoe Dextrose Agar), but due to the high price, farmers usually try to use other organic materials which are more affordable, and what is often used is corn rice. However, until now it has not been confirmed which substrate is the best for Metarhizium anisopliae. Therefore this study aims to determine the viability of entomopathogenic fungi on organic fertilizer substrates. Analysis of the research parameters was carried out at the Agrotechnology Laboratory, Faculty of Agriculture, University of Jember. The study design used a completely randomized design (CRD) with 5 treatments and 5 repetitions, so there were 25 sample units, with observations for 8 weeks so that the total number of samples was 200 samples. The treatments used included P0: using corn-rice media, P1: using chicken manure media, P2: using cow manure media, P3: using goat manure media, P4: using bat manure media. The research variables used were incubation period, final sample weight, and conidia density. The aim of this study was to determine the most suitable substrate for the growth and effectiveness of Metarhizium anisopliae as a biological control agent. The best-performing media were found to be chicken manure and cow manure. The authors suggest further emphasis on the scientific contribution of this research and recommend these substrates for practical field application.

Keywords: *Entomopathogenic Fungus, Incubation, Metarhizium anisopliae, Viability*

INTRODUCTION

Entomopathogenic fungi are important biological control agents and also have a positive impact on the environment (Mora et al. 2017). Entomopathogenic fungi have their natural habitat in the soil (Lozano-Tovar, et al. 2013). There are various types of entomopathogenic fungi, one of which is commonly used and safe for human use is Metarhizium anisopliae (Nourrisson et al. 2017). Jeong et al. (2022) stated in their research that *M. anisopliae* is a promising alternative to replace chemical pesticides. Novianti (2017), in her research, stated that *M. anisopliae* is used as a biological agent capable of controlling several types of pests from the orders Coleoptera, Lepidoptera, Homoptera, Hemiptera, and Isoptera, with attack characteristics including the insects dying, shrinking, and hardening like mummies, as well as turning pale in color.

M. anisopliae can also be referred to as a soil-borne microbe (Fernández-Bravo, et al. 2021). Ilmiyah and Rahma (2021), in their research, argue that *M. anisopliae* is not only parasitic on some insects, but this fungus is also saprophytic in the soil, using organic matter as its food source. Organic matter contains nutrients that can be utilized by *M. anisopliae*. Permadi et al. (2020) in their research suggest that the medium used for propagating entomopathogenic fungi in the laboratory is PDA (Potato Dextrose Agar). Entomopathogenic fungi can be mass-produced using various media, provided the substrate is suitable, supporting rapid fungal growth without losing virulence over several generations (Keppanan et al. 2018). The selection of substrate for the propagation of entomopathogenic fungi should be easily obtainable and have a long shelf life to ensure optimal growth of entomopathogenic fungi (Afifah et al. 2020).

Rohman et al. (2017) stated in their research that the growth of spores and hyphae in entomopathogenic fungi is influenced by the nutrient content in the propagation medium. The optimal growth medium for entomopathogenic fungi is one that contains a high amount of organic nutrients as a source of energy and food for microorganisms. Yusdian et al. (2018) suggest that solid organic fertilizers can function as a source of energy and food for microorganisms in the soil. Additionally, solid organic fertilizers have a relatively long shelf life. The type of solid organic fertilizer used is solid organic fertilizer from animal manure (cattle, goats, chickens, and guano). Some of the above animal manures are easily found in the community, so these animal manures will be optimized for use as a new innovation, namely as a substrate medium for the propagation of the entomopathogenic fungus *M. anisopliae*.

Zimmermann (1982) argues that several specific characteristics, such as pathogenicity to various insects, simple mass production, storage, preparation, and good viability in soil, have made this entomopathogenic fungus highly promising for development. Conidia viability has become the primary parameter used to determine the effects of various environmental factors on entomopathogenic fungi (Oliveira et al., 2015). Viability is an important factor in determining whether the substrate used is appropriate or not. In this study, viability testing was conducted by examining spore density and measuring the optimal growth time using a storage method over an 8-week period, in line with the research by Daoust et al. (1983). The importance of viability testing is closely linked to the success we aim to achieve; by understanding the viability results of the microbes, we can determine the most effective time to apply organic materials containing these microbes. In practice, the use of organic materials supplemented with microbes can contribute to one component of Integrated Pest Management (IPM) and promote environmentally friendly pest control.

RESEARCH METHOD

Research Location and Time

Research on “Viability Testing of Entomopathogenic Fungi (Metarhizium anisopliae) in Various Organic Animal Waste Media” will be conducted from November to December. The research will be conducted at the Agropharmaceutical Laboratory, Faculty of Agriculture, University of Jember.

Research Tools and Materials

Research preparation activities were carried out by preparing research tools and materials, including the following materials: *M. anisopliae* fungal isolates, chicken manure fertilizer, goat manure fertilizer, cow manure fertilizer, guano fertilizer, corn rice, PDA, water, vegetable oil, distilled water, and Tween. This research used tools such as an autoclave, laminar air flow, Bunsen burner, vortex, haemacytometer, 500 ml Erlenmeyer flask, analytical balance, petri dishes, spatulas, spoons, micropipettes, reaction tubes, 1 ml syringes, heat-resistant plastic (HDPE) containers (0.5 kg), paper, donut boxes, black cloth, matches, office supplies, and a camera.

Research Population and Sample

This study was conducted using an experimental method with a completely randomized design (CRD) with corn rice as the control variable. The factors used were various types of animal manure, consisting of chicken manure, goat manure, cow manure, and bat manure. The study was repeated five times with five treatments as shown in the research design (Table 1). Observations were conducted once a week on 25 sample units, with a total observation period of eight weeks, resulting in a total of 200 sample units studied.

Research Design

The experimental design in this study employed a completely randomized factorial design, consisting of two factors: the type of organic media and the storage duration. The first factor, which is the type of organic media (P), included five levels: P0 representing corn rice, P1 chicken manure, P2 cow manure, P3 goat manure, and P4 guano manure.

Table 1. Layout of the experimental unit for week 1

Replication 1	Replication 2	Replication 3	Replication 4	Replication 5
P0	P0	P0	P0	P0
P1	P1	P1	P1	P1
P2	P2	P2	P2	P2
P3	P3	P3	P3	P3
P4	P4	P4	P4	P4

Research Procedures

The research procedure was carried out by implementing the predetermined stages. The stages to be carried out include:

1. Prepare tools and materials

Preparation of tools and materials not only ensures that the tools and materials used are available, but also involves weighing the required materials, with each sample weighing 50 grams and requiring 5 replicates. Each week requires 250 grams, with a total observation period of 8 weeks, resulting in a total requirement of 2000 grams or 2 kilograms per sample type. In addition to weighing, other important preparations include preparing the medium and isolating *M. anisopliae*. The preparation of the medium and isolate is carried out using the following method. Add water to the medium until the moisture content reaches 30%.

a. Media preparation involves several stages, as follows:

Preparing tools and materials
↓
Put 50 grams of media into heat-resistant HDPE plastic
↓
Add water to the medium until the water content reaches 30%.
↓
Add 0.5 ml of vegetable oil.
↓
Wrapping plastic fertilizer using paper
↓
Sterilize the media using an autoclave.

Figure 2. Media Preparation

b. Preparation of *M. anisopliae* isolates

Rejuvenate the *M. anisopliae* isolate until the conidia fill the Petri dish.
↓
Pour 10 ml of aquades containing 0.1% tween onto the rejuvenated *M. anisopliae* isolate.
↓
Scraping *M. anisopliae* isolates that have been treated with distilled water using a spatula
↓
Pour all the isolate solution into the Erlenmeyer flask.
↓
Count the density of conidia using a Haemocytometer, then ensure that the density reaches 10^6.

Figure 3. Isolate preparation *M. anisopliae*

1. Inoculation of *M. anisopliae* isolates on corn rice and organic fertilizer media

Inoculation is carried out in a pre-sterilized laminar air flow. This inoculation begins with preparing the media as described above, then taking 1 ml of the previously prepared *M. anisopliae* isolate using a 1 ml syringe, and then inoculating it into the prepared media.

2. Variable observation

a. Incubation Period

Observations during the incubation period were conducted during the first and second weeks, where visual observations were made to determine when condia first appeared in the organic medium used.

b. Conidia Density

Subsequent observations were conducted once a week to observe whether there were any *M. anisopliae* conidia. During these observations, dilution was performed. Dilution was carried out by diluting 50 grams of each sample with 450 ml of sterile water. This was followed by manual stirring using a spoon to ensure the solution was homogeneous. Dilution is performed as needed and can be done up to a maximum of 105. If *M. anisopliae* conidia are present, the conidia density must be calculated using a haemocytometer.

The following is the formula for calculating conidia density using a haemocytometer based on the Indonesian National Standard (SNI):

$$S = \frac{\chi}{l \; x \; d \; x \; Fp} \; x \; 10^3$$

Description ;	S	= Conidia Density
	χ	= Number of Conidia in The Upper of Lower Box
	l	= Area of the counting box (0,04 mm^2)
	d	= Deepth of the counting box (0,1 mm)
	Fp	= Dilution Factor

Data Analysis

The data analysis used in this study was Analysis of Variance (ANOVA) processed using the SPSS program, in accordance with the design used, namely the Completely Randomized Design (CRD). This analysis is also presented in the form of a diagram to show the developmental phases of the M. anisopliae fungus..

RESULT AND DISCUSSION

Morphology of Entomopathogenic Fungus *M. anisopliae*

The entomopathogenic fungus isolate *M. anisopliae* originated from the Laboratory for Observation of Plant Pests and Diseases in Food Crops and Horticulture, located in the Tanggul area, Jember, East Java. The strain of *M. anisopliae* used in this study is Metarhizium anisopliae var majus (Figure 4b). This strain of *M. anisopliae* var majus was first discovered in 2000 by a researcher named Driver (Driver, et al. 2000).

A	*B*	*C*
Source jurnal Driver, et al. (2000)	*Source observation documentation*	*Source observation documentation*

Figure 4. a. *M. anisopliae var. majus*, b. *M. anisopliae var. majus*, c. Colony color of *M. anisopliae var. majus*

M. anisopliae var. majus is similar to *M. anisopliae* in general, but differs in size (Pupin, et al., 2000). *M. anisopliae* var. majus reaches a length of 10 micrometers (Driver, et al., 2000). *M. anisopliae* var. majus also has larger, longer, and more elongated conidia, but with a less regular shape. Rapidly growing colonies of *M. anisopliae* var. majus produce dark green colonies on Potato Dextrose Agar medium (Figure 4c).

Growth of Entomopathogenic Fungus Metarhizium anisopliae on Growth Media

1. Incubation Period

The incubation period is the time required for fungi to infect a host or medium until the first visible symptoms appear (Silvani et al., 2022). *M. anisopliae* var. majus showed the fastest growth on chicken organic material medium, followed by cow organic material, goat organic material, and finally guano organic material. The research results indicate that this incubation period can serve as an indicator of the growth rate of entomopathogenic fungi on the media used. The first inoculation was performed on October 10, 2023, and by October 12, 2023, growth of *M. anisopliae* was already visible on some of the media used. The average incubation period for *M. anisopliae* on the organic materials used was 2 days. The incubation period for Trichoderma on organic materials was also an average of 2 days (Gusnawati et al., 2017). The incubation period was marked by a change in color on the media used, making it visually observable.

12 October 2023	12 October 2023	12 October 2023	13 October 2023	05 November 2023
				
A	B	C	D	E

Figure 5. a. Corn rice media, b. Organic chicken manure media. c. Organic cow manure media, d. Organic goat manure media, e. Organic guano media

Based on the results of the study in terms of color change, the growth media that showed positive changes were corn rice media as the control media. Chicken organic material and cattle organic material, these three media showed growth on October 12, 2023 (Figures 5a, 5b, and 5c), so it can be concluded that *M. anisopliae* var. majus requires 2 days to infect the medium. Goat organic material showed color changes on October 13, 2023 (Figure 5d), while guano organic material did not show any color changes until the eighth week of storage (Figure 5e). The visualization of color changes caused by *M. anisopliae* var. majus on the organic material media used, in order of the growth media showing the most positive color changes, are corn rice media, chicken organic material, cow organic material, goat organic material, and finally guano organic material.

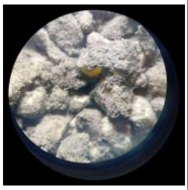	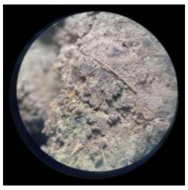	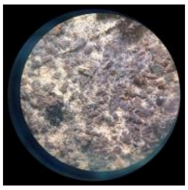	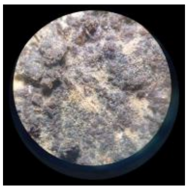	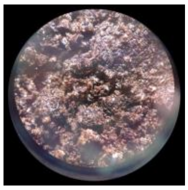
A	*B*	*C*	*D*	*E*

Figure 6. Macroscopic appearance of mycelium on organic material; a. Corn rice medium, b. Chicken manure organic medium, c. Cow manure organic medium, d. Goat manure organic medium, e. Guano organic medium

The color characteristics of fungal colonies cannot be used as a specific feature for grouping, because color differences are caused by different nutrient contents in each medium (Rosmini, et al., 2021). The use of media affects the visual color of the resulting colonies (Figure 6). Corn rice medium as a control medium produces a dark green color similar to that on Potato Dextrose Agar (PDA) medium. Chicken organic material medium produces a dark green colony color. However, cow organic material and goat organic material media produce yellowish-brown colony colors. Trizelia et al. (2015) reported that the colony color of *M. anisopliae* on tomato plant organic material is yellowish-green. Colony color was not observed on guano organic material, as the growth of entomopathogenic fungi was very low on guano fertilizer, making the color difference in colonies not clearly visible. Day et al. (2022) reported that changes in the visualization of Trichoderma fungal colony color on organic media resulted in a dark green color on media with the most positive growth and a white color on media with less positive growth.

2. Fungal Viability

Viability is the ability of an organism to survive in a specific environment (Nona et al., 2021), and it is used as a reference to determine the quality of an organism (Hasri, 2021). In fungi, viability refers to the ability to survive and grow in a specific medium (Utami et al., 2023). Viability is typically measured to determine which medium can optimally support fungal growth (Mackay, 1972). Fungal viability can be measured by calculating conidia density, with the number of conidia being directly proportional to the viability of the fungus (Ozkaya et al., 2022). Fungal viability can be measured by calculating conidia density (Barnett and Hunter, 1972), but conidia density in fungi is also influenced by several factors, including temperature, humidity, light, and nutrient content of the growth medium (Fatu et al., 2020). These factors must align with the fungus's natural habitat.

Table 4. 1. Results of Media Content Testing Laboratory of Soil Science, Faculty of Agriculture, University of Jember, 2024

No.	Media Perlakuan	C-Organik	N	P	K	Kadar Air	C/N
1.	Bahan Organik Ayam	15,17%	2,94%	0,75%	0,56%	18,63%	5,16 %
2.	Bahan Organik Sapi	7,06%	0,98%	0,05%	0,48%	20,57%	7,20%
3.	Bahan Organik Kambing	21,33%	1,40%	0,07%	0,56%	26,82%	15,24%
4.	Bahan Organik Guano	21,93%	5,18%	0,48%	0,45%	16,67%	4,23%

Decomposition is the process of breakdown by decomposers to obtain energy for their reproduction, which is influenced by environmental factors such as humidity and temperature (Thalib, et al. 2021). The general requirement for the C/N ratio needed in the decomposition process of organic matter is 20-30. A C/N ratio of 30 results in the highest decomposition rate (Marlina et al., 2020). Microbial population dynamics are influenced by nutrient availability in the substrate, as explained by the C/N ratio (Marlina et al., 2020). Based on Table 4.1, all nutrient contents of the growth medium are required by *M. anisopliae* for conidia growth. Nitrogen is one of the components required by *M. anisopliae* to trigger hyphal growth and germination (Anisa'a et al., 2020).

a. Corn Rice

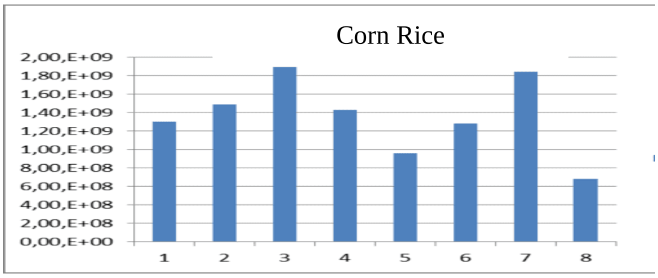

Figure 7. growth of *M. anisopliae* fungus on corn rice medium

Based on Figure 7, it shows that the growth of *M. anisopliae* observed by counting the density of conidia shows the highest density in the third week. The calculation of conidia density shows the highest viability in the third week with the number of conidia after inoculation of 1.9 x 109 and a decrease until the eighth week. In the first and sixth weeks, growth was nearly uniform. Similarly, in the second and fourth weeks, growth was also nearly identical. One of the key factors supporting the successful growth of *M. anisopliae* is the growth medium.

The success of *M. anisopliae* propagation and pest control is influenced by the selected propagation medium (HS et al., 2017). Corn rice is a commonly used alternative medium for the propagation of entomopathogenic fungi. Corn rice contains 79.08% carbohydrates, 6.80% protein, 6.67% dietary fiber, and 12.78% moisture content (Muhandri and Subarna, 2011). The use of corn rice medium is an appropriate alternative medium, achieving a conidia density of 5.8×10^9 conidia/mg (Novianti., 2018). In addition to corn rice, alternative propagation media for *M. anisopliae* can also utilize organic materials.

Erdiansyah et al. (2024) stated that the use of coarse rice bran waste as organic material in the mass propagation of *M. anisopliae* resulted in a conidia density of 23.69×10^9 conidia/mg. Meanwhile, according to Soviani et al. (2024), in their study using organic materials as microbial growth media, the conidia density was 20×10^8 in corn rice media and 16×10^8 in SDA media. Utami et al. (2023) stated that corn contains complex compounds such as lipids, nucleic acids, carbohydrates, and proteins that promote fungal growth.

b. Organic Chicken Manure

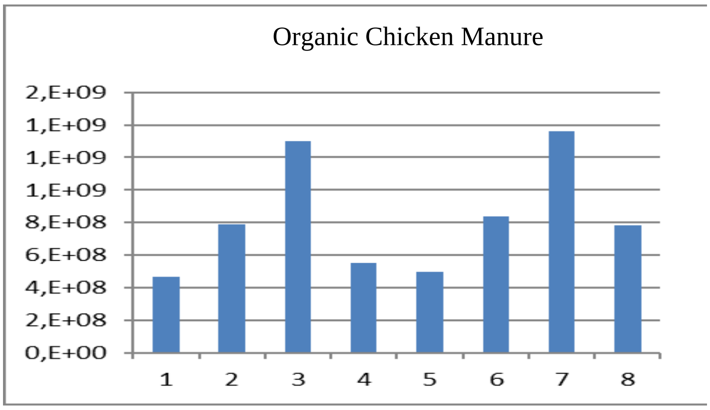

Figure 8. growth of *M. anisopliae* fungus on organic chicken manure medium

Based on Figure 8, it shows that the growth of *M. anisopliae* observed by counting the density of conidia shows the highest density in the seventh week. The calculation of conidia density shows the highest viability in the seventh week, with the number of conidia after inoculation being 1.36×10^9. The treatment using chicken manure as organic material had lower results than the control treatment using corn rice. Additionally, the growth peak lasted longer in the corn rice treatment due to the difference in nutrient content between the two organic materials. In the study by Fauzana and Fadilla (2022), the best composting treatment using a combination of chicken manure and sawdust with *M. anisopliae* produced good fertilizer quality and a conidia density of 57.2×10^6 . The conidia density produced was influenced by the nutrient content of the growth medium.

Table 4.1 shows that the nutrient content of chicken manure is lower than that of the control medium, thereby affecting the growth rate of *M. anisopliae*. Entomopathogenic fungi also exhibit saprophytic behavior on organic media (Wibowo et al., 2022). Ritonga et al. (2022) state that chicken manure is one of the organic wastes produced by chickens that has potential in agriculture, as it contains useful nutrients. The N, P, and K content is derived from the organic matter that is decomposed into nutrients required by the fungus *M. anisopliae* for its growth. Leku et al. (2019) state that the nutrient content of chicken manure organic matter consists of 0.5% Nitrogen (N), 0.25% Diphosphorus Pentoxide (P_2O_5), and 0.5% Potassium Oxide (K_2O), which varies significantly depending on environmental conditions and the feed provided. The nutrient content of chicken manure is as follows: 2.79% N, 0.52% P_2O_5, and 2.29% K_2O (Purba et al., 2019). One of the nutrients that significantly influences conidia growth is nitrogen.

Nitrogen is one of the components required by *M. anisopliae* to stimulate hyphal growth and germination (Anisa'a et al., 2020). In chicken manure organic medium, the nitrogen content is relatively high at 2.94%. The C/N ratio of 5.16 is not too high, allowing microorganisms to grow optimally with the nutrient content of chicken manure organic medium. Akmal et al. (2021) stated that a high C/N ratio can inhibit the decomposition process or microbial activity and nitrogen availability in the growth medium.

c. Organic Cow Manure

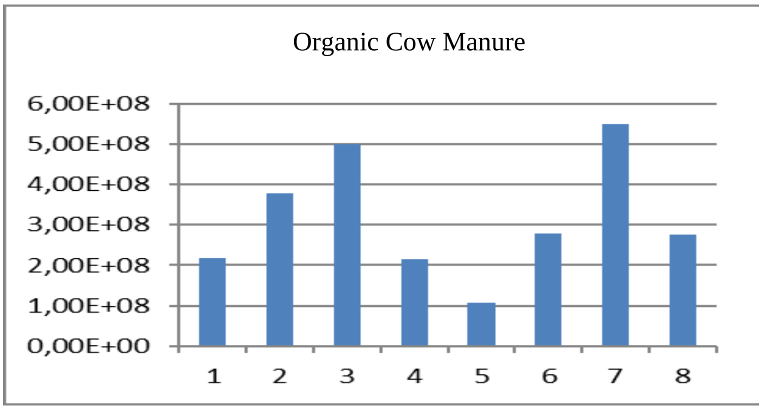

Figure 9. growth of *M. anisopliae* fungus on organic cow manure medium

Based on Figure 9, the growth of *M. anisopliae* fungus showed the highest density in the seventh week. The calculation of conidia density showed the highest viability in the seventh week with the number of conidia after inoculation being 2.78 x 108. In the study by Novianti et al. (2021), the best composting treatment using a combination of cow manure and sawdust with Beauveria bassiana produced good fertilizer quality and a conidia density of 83.2 × 10^8 conidia/ml. The treatment using cow manure as the organic medium had a lower density than the corn rice control treatment. Additionally, the growth peak was longer than the corn rice treatment due to the difference in nutrient content between the two organic materials.

Table 4.1 shows that the nutrient content of cow manure organic material was lower than that of the control medium, thereby affecting the growth rate of *M. anisopliae*. The N, P, and K content is derived from the decomposition of organic matter into nutrients required by the fungus *M. anisopliae* for its growth. The nutrient composition of solid cow manure organic material consists of a mixture of 0.40% nitrogen (N), 0.20% diphosphorus pentaoxide (P_2O_5), and 0.10% potassium oxide (K_2O) (Gole et al., 2019). Cattle manure contains 0.5% N, 0.25% P, and 0.5% K (Hafizah and Mukarramah., 2017). The nutrient content in the organic cattle manure medium that significantly influences conidia growth is nitrogen and carbon.

Nitrogen and carbon are components required by microorganisms for their metabolic activities (Purnomo et al., 2017). Carbon is used for microbial growth, while nitrogen is used to synthesize proteins as building blocks for microbial cells (Kurniawan et al., 2023). In organic cow manure media, the C/N ratio is not too high at 7.20, allowing microorganisms to develop optimally with the nutrient content of the organic cow manure media. Akmal et al. (2021) stated that a high C/N ratio can inhibit the decomposition process or microbial activity and nitrogen availability in the growth medium.

d. Organic Goat Manure

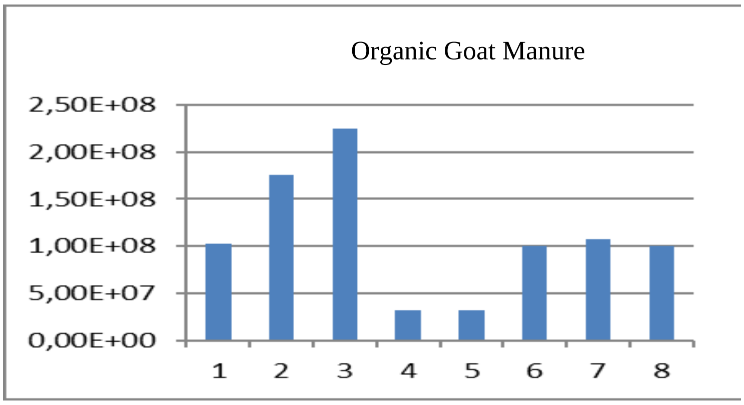

Figure 10. growth of *M. anisopliae* fungus on organic goat manure medium

Based on Figure 10, the growth of *M. anisopliae* showed the highest density in the third week. The calculation of conidia density shows the highest viability in the third week, with the number of conidia after inoculation being 2.25 x 10^8. The treatment using goat manure organic medium was lower than the control treatment using corn rice. Additionally, the growth peak was longer in the corn rice treatment due to the difference in nutrient content between the two organic materials.

Table 4.1 shows that the nutrient content of goat manure organic material was lower than that of the control medium, thereby affecting the growth rate of *M. anisopliae*. The N, P, and K content was obtained from the decomposition of organic material into nutrients required by the *M. anisopliae* fungus for its growth. Pancapalaga et al. (2021) stated that the nutrient content of goat manure is as follows: Nitrogen (1.49%), Phosphorus (0.13%), Potassium (0.62%), Organic Carbon (31.65%), C/N ratio (21.2%), and Moisture content (47.7445%). The nutrients that significantly influence conidia growth are nitrogen and carbon.

Microbes break down carbon as an energy source and use nitrogen for protein synthesis (Ismayana et al., 2012). In goat manure organic material media, the C/N ratio is quite high at 15.23, so microorganisms do not develop well with the nutrient content of goat manure organic material media. This indicates that the nitrogen content is quite low. Meanwhile, nitrogen is very much needed by microorganisms during the composting process because it breaks down organic material (Suparto et al., 2025).

e. Organic Guano Manure

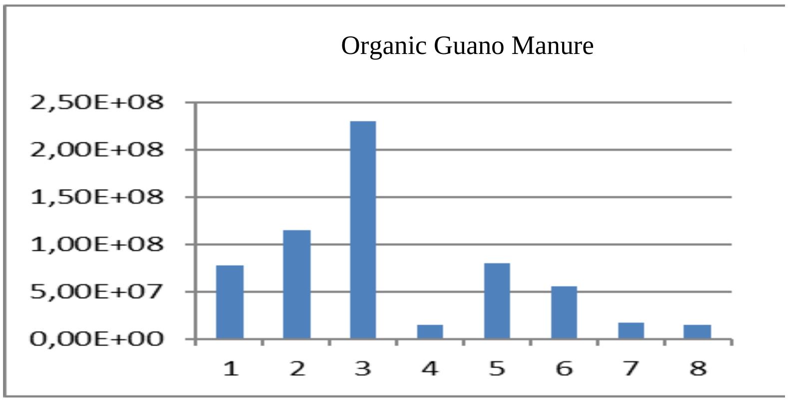

Figure 11. growth of *M. anisopliae* fungus on organik guano manure medium

Based on Figure 11, the growth of *M. anisopliae* showed the highest density in the third week. The calculation of conidia density shows the highest viability in the third week, with the number of conidia after inoculation being 2.30 x 10^8. The treatment using bat guano organic material had lower density than the corn rice control treatment. Additionally, the growth peak lasted longer in the bat guano treatment compared to the corn rice treatment due to the difference in nutrient content between the two organic materials.

Table 4.1 shows that the nutrient content of guano organic matter is lower than that of the control medium, thereby affecting the growth rate of Metarhizium anisopliae. The N, P, and K content is derived from the decomposition of organic matter into nutrients required by the fungus *M. anisopliae* for its growth. The nutrient content of guano fertilizer is 7–17% nitrogen (N), 8–15% phosphorus (P), and 1.5–2.5% potassium (K) (Syofiani and Oktabriana, 2018).

The nutrients that significantly influence conidia growth are nitrogen and carbon (Kusherawati et al., 2024). In bat guano organic material medium, the C/N ratio is not too high at 4.23, so microorganisms should be able to grow optimally. A low C/N ratio indicates that carbon is effectively utilized by microorganisms as an energy source (Achmad, 2021). However, the moisture content in bat guano organic medium is insufficient, preventing microorganisms from growing properly.

3. Relationship between Incubation Period and Fungal Viability

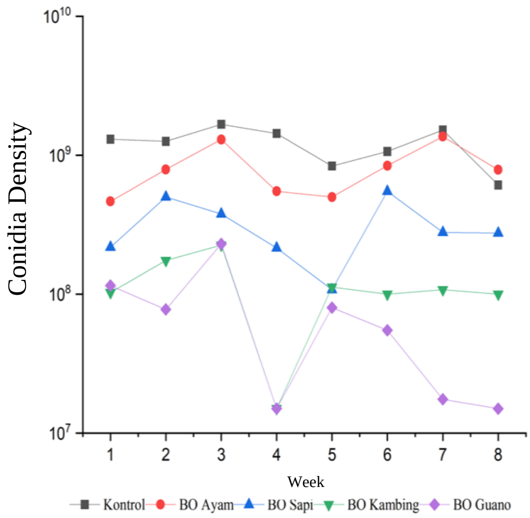

Figure 12. Conidia density on each organic material

The minimum time required to achieve 100% growth is 3 weeks (Aw and Hue, 2017). Based on Figure 12, it can be seen that the type of medium affects the growth rate of the entomopathogenic fungus *M. anisopliae*. The highest conidia density was observed in P0, where the medium was the control medium, namely corn rice, with an average conidia density of 10^9. This is because corn rice, as the control medium, contains the complete nutrients required by Metarhizium anisopliae, specifically carbohydrates, which are complex sugars used as food by *M. anisopliae*. Metarhizium anisopliae requires nutrients that can be utilized, and nitrogen is one of the components needed by *M. anisopliae* to stimulate hyphal growth and germination (Anisa'a et al. 2020).

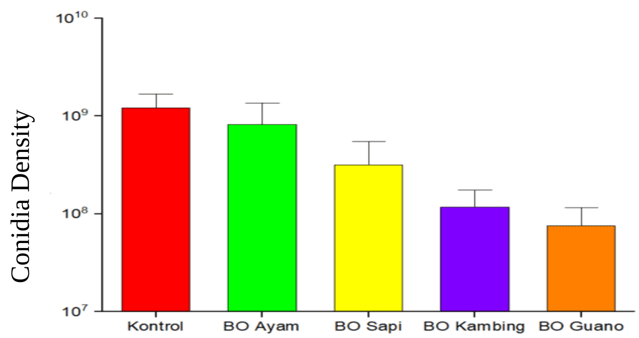

Figure 13. Average conidia density in each organic material

Based on Figure 13, it can be seen that the medium showing the most positive growth is chicken manure. The average density reached 10^9. This is because chicken manure has a relatively high nitrogen content. Nitrogen is required by microorganisms for protein synthesis (Fauza, 2025). Novitasari and Caroline (2021) noted that chicken manure has a lower C/N ratio compared to organic materials from cow and goat manure. An appropriate C/N ratio maintains the population of active microorganisms in the composting process of organic materials (Fauza, 2025). Additionally, chicken manure has a higher moisture content than cow and goat manure. This factor causes the chicken manure medium to have high conidia growth.

In cow manure organic material, the average conidia density reached 10^8. Cow manure organic material has a sufficient C/N ratio, but it is higher than that of chicken manure organic material. This is why the density of cow manure organic material is lower than that of chicken manure organic material. In goat manure organic material media, the average conidia count reached 10^8 but remained lower than that of cow manure organic material. This is because goat manure organic material has a relatively high C/N ratio, making it difficult for microorganisms to grow optimally. The lowest conidia density is found in bat manure organic material, with an average conidia density of 10^7. Bat manure organic material has a low C/N ratio but also has a low moisture content. (Fauza. 2025), the appropriate C/N ratio will maintain the active microbial population during the composting process of organic materials. This factor is what causes microorganisms to struggle to develop optimally..

4. Final Weight of Fungi

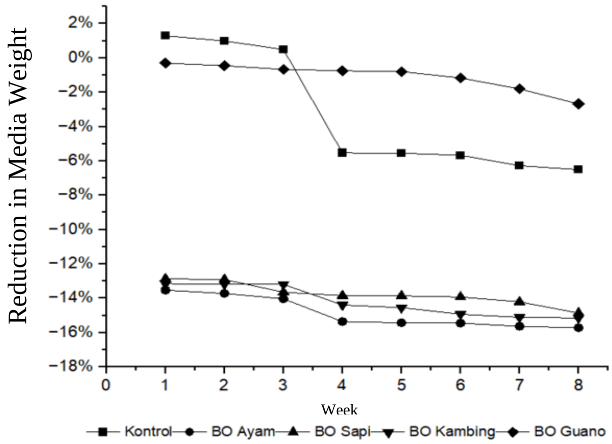

Figure 14. Final weight of each organic material

The influence of fungi in utilizing organic media does not significantly reduce the weight of the media, but fungi utilize water content to support their activity, thereby affecting the decreasing water content in the media (Adiwena, et al. 2022). Differences in media types and water content affect the weight of the treated media. Widarti et al. (2015) found that water content also influences the duration of organic material decomposition. Water content must align with fungal requirements; excessively low water content can inhibit fungal growth, while excessively high water content leads to decay (Risdiyanti et al., 2022). Moisture content is related to oxygen availability to support microbial activity (Yulina et al., 2023). This results in suboptimal microbial activity and prevents the medium weight from decreasing drastically.

The treated media had an average wet weight of 70 grams/sample at the start of inoculation for corn rice media, chicken, cow, and goat organic materials, while guano organic material had a weight of 80 grams/sample. Viareco et al. (2024) stated that the decrease in medium weight is caused by nutrient absorption and water content utilization in the medium by *M. anisopliae*. Water content is crucial in organic material decomposition due to water availability (Ramadhany et al., 2023). The greater the reduction in medium weight, the higher the nutrients utilized by the fungus (Rahmiyah et al., 2023). Microbial activity causes a decrease in medium weight, indicating that microorganisms can grow and utilize medium nutrients effectively (Anthonio et al., 2023).

CONCLUSION

Based on morphology, the type of metarhizium produced in this study was *M. anisopliae* var majus. The growth medium used in this study was found to influence the color of *M. anisopliae* colonies. On corn rice medium, the colonies exhibited a pale green color, while on chicken organic material, they also exhibited a pale green color. However, on goat organic material and cattle organic material, the colonies exhibited a brownish-yellow color. On guano organic material, no color change was observed due to the low number of conidia produced. The moisture content and nutrient levels in each medium influenced the density of conidia produced. Corn rice medium as the control medium produced the highest average total conidia count, with an average conidia density of 10^9. Conidia density increases with incubation time. Potato Dextrose Agar as the primary isolate produced an average total conidia density of 10^7. The highest conidia growth occurred at 3 weeks, but thereafter it entered a logarithmic phase (growth slowdown), resulting in a decrease in growth. Nevertheless, the corn rice medium was only able to maintain conidial density at 10^9 during the initial week. In contrast, organic media were able to sustain conidial density for several weeks, although the levels varied. This is due to the slow-release mechanism of organic materials compared to the faster nutrient release in corn rice. Moreover, based on the research findings, the organic media that showed the highest viability were chicken manure, followed by cow manure.It is recommended to always conduct virulence testing of the entomopathogenic fungus *Metarhizium anisopliae* on Hong Kong caterpillars as test insects.

REFERENCES

Afifah, L., Desriana, R., Kurniati, A., & Maryana, R. (2021). Viability of entomopathogenic fungi Metarhizium anisopliae (Metsch) Sorokin in some alternative media and different shelf-life. *International Journal of Agriculture System 8*(2), 108-118.

Aw, K. M. S., & Hue, S. M. (2017). Mode of infection of Metarhizium spp. fungus and their potential as biological control agents. *Journal of fungi*, *3*(2), 30.

Bamisile, B. S., Akutse, K. S., Siddiqui, J. A., & Xu, Y. (2021). Model application of entomopathogenic fungi as alternatives to chemical pesticides: Prospects, challenges, and insights for next-generation sustainable agriculture. *Frontiers in Plant Science 12*, 741804.

Barnett, H. L., & Hunter, B. B. (1972). Illustrated genera of imperfect fungi. *Illustrated genera of imperfect fungi.*, (3rd ed).

Brunner-Mendoza, C., Reyes-Montes, M. d. R., Moonjely, S., Bidochka, M. J., Toriello, C., & Technology. (2019). A review on the genus Metarhizium as an entomopathogenic microbial biocontrol agent with emphasis on its use and utility in Mexico. *Biocontrol Science*, *29*(1), 83-102.

Daoust, R. A., Ward, M. G., & Roberts, D. W. (1983). Effect of formulation on the viability of Metarhizium anisopliae conidia. *Journal of Invertebrate Pathology*, *41*(2), 151-160.

De-Lira, A. C., Mascarin, G. M., & Júnior, Í. D. (2020). Microsclerotia production of Metarhizium spp. for dual role as plant biostimulant and control of Spodoptera frugiperda through corn seed coating. *Fungal biology*, *124*(8), 689-699.

Debrah, P. Y., Afriyie-Debrah, C., Nuhu, F., Ribeiro, P., & Obeng, E. A. (2021). Response of Cabbage (Brassica oleracea var capitata L.) to Organic and Inorganic Fertilizer. *Journal of Experimental Agriculture International*, *43*(2), 105-114.

De Sá Santos, P., Abati, K., Mendoza, N. V. R., Mascarin, G. M., & Júnior, I. D. (2021). Nutritional impact of low-cost substrates on biphasic fermentation for conidia production of the fungal biopesticide Metarhizium anisopliae. *Bioresource Technology Reports*, *13*, 100619.

Fathin, S. L., Purbajanti, E. D., & Fuskhah, E. (2019). Pertumbuhan dan hasil Kailan (Brassica oleracea var. Alboglabra) pada berbagai dosis pupuk kambing dan frekuensi pemupukan Nitrogen. *Jurnal Pertanian Tropik*, *6*(3), 438-447

Fernández-Bravo, M., Gschwend, F., Mayerhofer, J., Hug, A., Widmer, F., & Enkerli, J. (2021). Land-use type drives soil population structures of the entomopathogenic fungal genus Metarhizium. *Microorganisms*, *9*(7), 1380.

Gebremariam, A., Chekol, Y., & Assefa, F. (2021). Phenotypic, molecular, and virulence characterization of entomopathogenic fungi, Beauveria bassiana (Balsam) Vuillemin, and Metarhizium anisopliae (Metschn.) Sorokin from soil samples of Ethiopia for the development of mycoinsecticide. Heliyon, 7(5).

Gole, I. D., Sukerta, I. M., & Udiyana, B. P. (2019). Pengaruh dosis pupuk kandang sapi terhadap pertumbuhan tanaman sawi (Brassica juncea L.). *Agrimeta: Jurnal Pertanian Berbasis Keseimbangan Ekosistem*, *9*(18).

Hawksworth, D. L., Ainsworth, G. C., & Sutton, B. C. (1983). *Dictionary of fungi [including the lichens]*. Commonwealth Mycological Institute.

Ilmiyah, N., & Rahma, Y. A. (2021). EKSPLORASI DAN IDENTIFIKASI CENDAWAN ENTOMOPATOGEN Metarhizium sp. DENGAN METODE BAITING INSECT. *Jurnal Matematika dan Sains*, *1*(2), 87-92.

Irshad, M., Eneji, A. E., Hussain, Z., & Ashraf, M. (2013). Chemical characterization of fresh and composted livestock manures. *Journal of soil science and plant nutrition*, *13*(1), 115-121.

Jeong, S.-G., Kim, H. M., Kim, J., Kim, J. S., & Park, H. W. (2022). Effect of storage conditions on the shelf-life extension of fungus-colonized substrates based on Metarhizium anisopliae using modified atmosphere packaging. *Scientific Reports*, *12*(1), 1-8.

Keppanan, R., Sivaperumal, S., Aguila, L. C. R., Hussain, M., Bamisile, B. S., Dash, C. K., & Wang, L. (2018). Isolation and characterization of Metarhizium anisopliae TK29 and its mycoinsecticide effects against subterranean termite Coptotermes formosanus. *Microbial pathogenesis*, *123*, 52-59.

Leku, P. M. N., Duaja, W., & Bako, P. O. (2019). Pengaruh Dosis Kombinasi Pupuk Kandang Kotoran Ayam dan Pupuk Majemuk NPK Phonska terhadap Beberapa Sifat Kimia Tanah dan Hasil Cabai Rawit (Capsicum frutescens L.) pada Alfisol. *Pada Alfisol Effect of Combination Dosage Of Chiken Fertilizer and NPK Phonska Fertilizer On Some Of The Soil Chemical Properties And Results Of Cayenne Pepper*.

Li, Y., Zhao, J., Krooneman, J., & Euverink, G. J. W. (2021). Strategies to boost anaerobic digestion performance of cow manure: Laboratory achievements and their full-scale application potential. *Science of The Total Environment*, *755*

Lozano-Tovar, M. D., Ortiz-Urquiza, A., Garrido-Jurado, I., Trapero-Casas, A., & Quesada-Moraga, E. (2013). Assessment of entomopathogenic fungi and their extracts against a soil-dwelling pest and soil-borne pathogens of olive. Biological Control, 67(3), 409-420.

Maina, U., Galadima, I., Gambo, F., & Zakaria, D. (2018). A review on the use of entomopathogenic fungi in the management of insect pests of field crops. *J. Entomol. Zool. Stud*, *6*(1), 27-32.

Masaji, I. P. A., Suarta, M., & Sudewa, K. A. (2021). The Effect of the Length of Cuttings and the Dose of Rabbit Manure on the Growth and Yield of Purple Sweet Potato (Ipomoea Batatas L. Poiret). *Agriwar Journal*, *1*(2), 44-50.

Misra, P. K., Gautam, N. K., & Elangovan, V. (2019). Bat guano: a rich source of macro and microelements essential for plant growth. *Annals of Plant and Soil Research*, *21*(1), 82-86.

Mora, M. A. E., Castilho, A. M. C., & Fraga, M. E. (2018). Classification and infection mechanism of entomopathogenic fungi. *Arquivos do Instituto Biológico*, *84*.

Nourrisson, C., Dupont, D., Lavergne, R. A., Dorin, J., Forouzanfar, F., Denis, J., ... & Sabou, M. (2017). Species of Metarhizium anisopliae complex implicated in human infections: retrospective sequencing study. Clinical Microbiology and Infection, 23(12), 994-999.

Novianti, D. (2017). Efektivitas Beberapa Media untuk Perbanyakan Jamur Metarhizium anisopliae. *Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam*, *14*(2), 81-88.

Oliveira, D. G. P., Pauli, G., Mascarin, G. M., & Delalibera, I. (2015). A protocol for determination of conidial viability of the fungal entomopathogens Beauveria bassiana and Metarhizium anisopliae from commercial products. *Journal of microbiological methods*, *119*, 44-52.

Pancapalaga, W., Suyatno, S., & Sedlacek, D. (2021). The Use of Rumen Contents as Bio–Activators for Fermentation in Goat Manure Fertilizer Production. In *E3S Web of Conferences* (Vol. 226, p. 00048). EDP Sciences.

Permadi, M., Samosir, B., Siregar, D., & Wayni, M. (2020). *Physiology Characterization of Entomopathogenic Fungi Beauveria bassiana and Metarhizium anisopliae on Different Carbohydrate Sources.* Paper presented at the Journal of Physics: Conference Series.

Pupin, A. M., Messias, C. L., Piedrabuena, A. E., & Roberts, D. W. (2000). Total lipids and fatty acids of strains of Metarhizium anisopliae. *Brazilian Journal of Microbiology*, *31*, 121-128.

Rahman, N. A., Ab Majidb, N. M., & Sallehc, M. S. (2022). EFFECTS OF GUANO MANURE ON HEIGHT GROWTH AND FOLIAR NUTRIENT CONCENTRATIONS OF SELASIH (OCIMUM TENUIFLORUM L.). *Science*, *6*(1), 27-30.

Sasan, R. K., & Bidochka, M. J. (2012). The insect-pathogenic fungus Metarhizium robertsii (Clavicipitaceae) is also an endophyte that stimulates plant root development. American journal of botany, 99(1), 101-107.

Schrank, A., & Vainstein, M. H. (2010). Metarhizium anisopliae enzymes and toxins. *Toxicon, 56*(7), 1267-1274.

Singh, J., Ganbari, B., Kumar, D., & Baleanu, D. (2021). Analysis of fractional model of guava for biological pest control with memory effect. *Journal of Advanced Research32*, 99-108.

Stenberg, J. A., Sundh, I., Becher, P. G., Björkman, C., Dubey, M., Egan, P. A., . . . Jonsson, M. (2021). When is it biological control? A framework of definitions, mechanisms, and classifications. *Journal of Pest Science, 94*(3), 665-676.

Surya, M. S., Santosa, Y. S. J., & Bahri, S. (2022). Kajian Pupuk Organik Cair Dan Pupuk Guano Terhadap Hasil Jagung Manis (Zea mays saccharata). *Prosiding HUBISINTEK, 2*(1), 403-403.

Syofiani, R., & Oktabriana, G. (2018). Aplikasi pupuk guano dalam meningkatkan unsur hara N, P, K, dan pertumbuhan tanaman kedelai pada media tanam tailing tambang emas. *Prosiding SEMNASTAN*, 98-103.

Tallou, A., Haouas, A., Jamali, M. Y., Atif, K., Amir, S., & Aziz, F. (2020). Review on cow manure as renewable energy. *Smart Village Technology: Concepts and Developments*, 341-352.

Tanada Y, Kaya HK. 1993. Insect Pathology. San Diego (US): Academic Press, INC.

Yusdian, Y., Karya, K., & Vaisal, R. (2018). Pengaruh Dosis Pupuk Kandang Ayam terhadap Pertumbuhan dan Hasil Tanaman Kentang (Solanum

Zimmermann, G. (1982). Effect of high temperatures and artificial sunlight on the viability of conidia of Metarhizium anisopliae. *Journal of Invertebrate Pathology 40*(1), 36-40.