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Viability of Entomopathogenic Fungi (*Metarhizium anisopliae) in* Residual Media Maggots and Pupa Shell Waste

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Abstract: *Metarhizium anisopliae* is a fungus that acts as an entomopathogen, commonly used to control pest insects (Biological Control Agent). This saprophytic mushroom requires sufficient organic nutrients in the growth medium. Typically, rice or corn is used as the media; however, this poses a challenge as these grains are also consumed by humans as staple foods. Additionally, the price of rice and corn has been increasing steadily. Alternatively, organic materials like maggot residue and pupa shells are byproducts from maggot cultivation that contain high nutritional value but are not utilized optimally. This research aims to identify effective alternative media for the multiplication of *Metarhizium anisopliae*. The research method involved controlled, homogeneous, and sterile experimental conditions, using a completely randomized design with four treatments and six repetitions. The fungus showed a high conidia density in the control media (rice corn), measuring 15.5 x 10^8. In chicken manure, a high density of 14.4 x 10^8 was observed, while the media made from maggot residue and pupa shells showed very low densities of 2.08 x 10^6 and 6.25 x 10^6, respectively. This suggests the presence of antimicrobial peptides (AMP), fatty acids, various bacteria, and lignocellulose compounds in the maggot residue and pupa shells. Overall, the organic materials from maggot residue and pupa shells are not effective as growth mediums for the fungus *Metarhizium anisopliae*.

Keywords: Metarhizium anisopliae; Maggot Residue; Pupa Shell; Viability

Introduction

Metarhizium anisopliae is a fungal entomopathogen commonly used for controlling pest insects (Arsi *et al*., 2020). This fungus exhibits heterotrophic characteristics, allowing it to become pathogenic to insect pests (Tairas and Memah, 2020). The mechanism of attack involves infecting the insect, leading to a white mummification that eventually turns green over several days, resulting in the death of the insect (Permadi *et al*., 2019). Importantly, *Metarhizium anisopliae* does not pose a threat to human health or the surrounding environment, although it can cause mortality in insect populations (Erler and Ates, 2015; Wu *et al*., 2022). Additionally, this fungus is saprophytic, utilizing organic materials for its survival (Abrar and Raharjo, 2017; Ilmiyah and Rahma, 2021).

In laboratory settings, the common medium used for multiplying *Metarhizium anisopliae* is synthetic Potato Dextrose Agar (PDA) (Liu *et al*., 2012). Traditionally, rice corn has also been used as a growth medium for this fungus (Indriyanti *et al*., 2024). However, the availability of rice corn is becoming increasingly competitive, as it is also a staple food for humans (Sanusi, 2023). Additionally, the price of rice corn continues to rise. Therefore, there is a significant need for alternative media that provide adequate nutrition, are easily obtainable, and are economically viable.

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Organic materials that are nutritionally beneficial and affordable for *Metarhizium anisopliae* include maggot residue and pupa shells—both of which are underutilized organic waste products. Maggot residue is the organic waste resulting from the bioconversion process performed by maggots, containing essential nutrients like nitrogen (N), phosphorus (P), and potassium (K) (Purnamasari *et al.,* 2021; Sularno *et al.,* 2023). Pupa shells are the remnants of maggot cultivation, formed from the larval shells after they have transitioned into flies (Rachmawaty *et al.,* 2023).

To evaluate the success of this study, viability tests will be conducted. The study aims to explore the alternative functions of maggot residue and pupa shells as organic waste, as well as to identify effective alternative media for the mass multiplication of *Metarhizium anisopliae* as a bioprotectant for controlling or suppressing the development of plant pest diseases (Figueiredo *et al.,* 2011).

Method

This study was conducted at the Laboratory of Agropharmaceuticals, Faculty of Agriculture, University of Jember, from November 2023 to July 2024. The research employed an experimental method under controlled, homogeneous, and sterile conditions, utilizing a Randomized Complete Design (RCD) to determine the number of treatments and repetitions. The study consisted of six repetitions with four treatments (see Table 1). Observations were carried out weekly over a period of eight weeks, resulting in a total of 192 sample units.

Data collection was conducted once a week through dilution and microscopic examination. A total of six samples were taken from each type of organic material, resulting in 24 sample units collected during each data collection session.

Table 1. Floor Plan Sample

A1	B1	C1	D1
A2	B2	C2	D2
A3	B3	C3	D3
A4	B4	C4	D4
A5	B5	C5	D5
A6	B6	C6	D6

Information:
A: Maggot Residue
B: Pupa Shell
C: Rice Corn
D: Chicken Manure

The study began with the preparation of tools and materials, which included an autoclave, laminar airflow cabinet, vortex mixer, hemocytometer, Bunsen burner, analytical scales, petri dishes, spatulas, spoons, pipettes, reaction tubes, glass slides, sprayers, hot plastic stands (HDPE) of size 1 kg, thin-walled containers, Erlenmeyer flasks, buckets, microscopes, orbital shakers, inoculation needles, fungus isolate *Metarhizium anisopliae*, corn rice, maggot residue, maggot pupa shells, chicken feces, synthetic PDA media, distilled water, vegetable oil, Tween, and alcohol.

The study procedure included the following stages:

1) Media preparation

Preparation involved the following steps: Gather tools and materials; Place each type of media into its respective receptacle in the required amounts; Soak the corn rice media for one hour; For the media composed of maggot residue, pupa shells, and chicken feces, slowly add water to each receptacle while stirring until reaching 30% field capacity; Drain the media, weigh each sample to 50 grams using analytical scales, and add 0.5 ml of vegetable oil; Shake the mixture in plastic containers until it is evenly distributed, then flatten the plastic; Wrap the plastic in HVS paper and sterilize the media in an autoclave at 121°C for one hour; Allow the media to cool.

2) *Preparation* of *Metarhizium anisopliae* Isolate

The preparation of the *Metarhizium anisopliae* isolate included: Reviving the *Metarhizium anisopliae* isolate until it fills the petri dish; Pouring 10 ml of distilled/stirred sterile water containing 0.1% Tween into the *Metarhizium anisopliae* isolate; Using a spatula to extract the *Metarhizium anisopliae* with the Tween solution; Pouring the isolate into a 500 ml Erlenmeyer flask and stirring it with an orbital shaker for one hour; Taking 1 ml of the mixture and transferring it to a reaction tube containing 0.1% Tween; then homogenizing it with a vortex mixer for one minute; Using a micropipette to take 0.2 ml and checking the conidia density of *Metarhizium anisopliae* using a hemocytometer, ensuring the density reaches 10^6.

3) *Inoculation* of Isolates on Organic Media

Inoculation required the following steps: Take 1 ml of the prepared *Metarhizium anisopliae* isolate using a micropipette and add it to the plastic container containing the media (1 ml capacity); Shake the media to ensure even distribution of the isolate; Place the inoculated media into thin-walled containers for storage; Store the thin-walled containers containing samples in a cupboard.

By completing these procedures, the study aimed to investigate the effects of various treatments on the growth and development of the selected fungus isolate.

Results and Discussion

Viability Metarhizium anisopliae

Viability refers to an organism's ability to grow and sustain its life by utilizing nutrients from the growth medium. Several factors influence the viability of fungi, including pH, temperature, and the nutrient content of the growth medium (Barnett and Hunter, 1960; Namasivayam *et al.*, 2015). For mushrooms to grow successfully, they require essential nutrients, including carbohydrates, proteins, and phosphorus.

According to the results of media content tests that have been conducted, as stated in (Table 2). The fungus *Metarhizium anisopliae* utilizes organic material as a resource and recycles nutrients such as carbon and nitrogen (Sahid and Kusumaningtyas, 2023). Common organic materials used as growth media include rice, bran, and corn (Indrayani and Prabowo, 2016; Riani and Futeri, 2023). All of these growth media contain organic nutrients that can be used by fungus to grow.

The nutritional content of the media used in the treatments is as follows:

Table 2. Contents Nutrition in Media Materials Organic

Media	C- Organic	N	P	K
Chicken Manure	15.17%	2.94%	0.75%	0.56%
Maggot Residue	23.76%	3.36%	2.59%	2.32%
Pupa Shell	30.08%	7.48%	2.7%	1.13%

Source: Jember State Polytechnic Lab Test Results, 2023; Jember University Soil Science Lab Test Results, 2024.

Material Organic Rice Corn

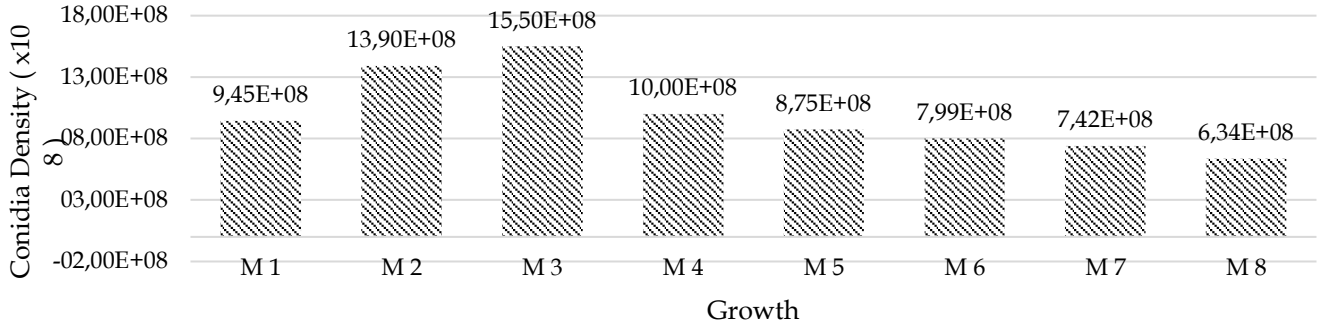

Figure 1. Chart Growth *Metarhizium anisoplia* on Rice media Corn

Based on the growth chart (Figure 1), the density of *Metarhizium anisopliae* in the control media with rice corn was highest in the third week, reaching a density of 15.5 x 10^8. Novianti (2017) noted that using rice corn as a growth medium for *Metarhizium anisopliae* resulted in a good conidia yield of 39.8 x 10^8. The high conidia growth of *Metarhizium anisopliae* during weeks 2 and 3, measuring 13.9 x 10^8 and 15.5 x 10^8 respectively (Figure 1), indicates that the fungus was in the exponential growth phase. The exponential phase is characterized by optimal growth conditions, where the number of cells increases significantly and the activity is at its peak (Soviani *et al*., 2024). During this phase, the fungus effectively utilizes the nutrients in the medium to support hyphal growth and spore germination (Triasih *et al*., 2019).

However, a decline in viability is observed from the fourth to the eighth week, which marks the final observation period, with viability recorded at 6.34 x 10^8. According to Triasih *et al.* (2019), the decline in fungal viability is attributed to the reduction in the organic nutrient content of the growth medium.

Material Organic Chicken manure

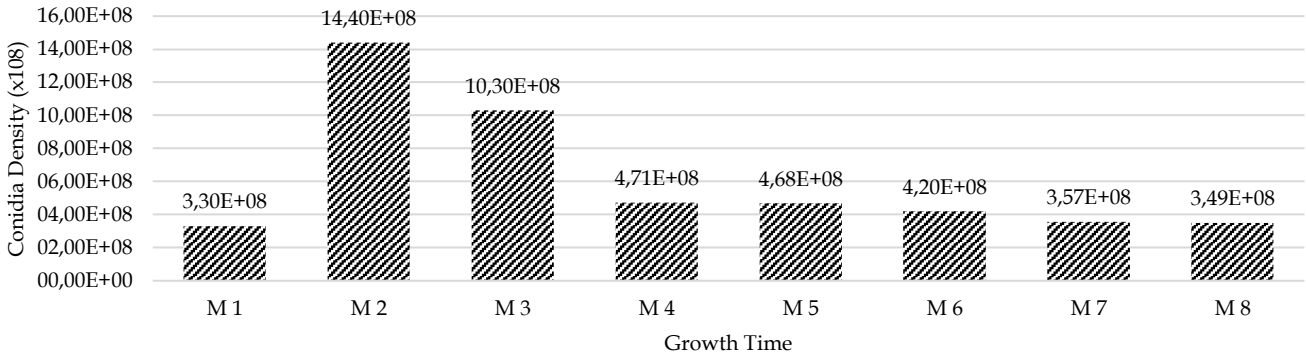

Figure 2. Growth *Metarhizium anisoplia* on the media Chicken manure

Based on the growth chart (Figure 2), the highest density of *Metarhizium anisopliae* observed in chicken dung media during the second week after inoculation was 14.4 x 10^8. During weeks 2 to 3, the fungus experienced an exponential growth phase. The conidial density of *Metarhizium anisopliae* in chicken dung media, at 14.4 x 10^8, was slightly lower compared to the density on rice corn, which reached 15.5 x 10^8. The type of growth media significantly influences the life cycle of the fungus *Metarhizium anisopliae.*

The nutrient content in the media, including elements such as carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), and potassium (K), supports optimal fungal growth (Sadad *et al.,* 2014). Differences in conidial viability arise from variations in the organic media used (Triasih *et al.,* 2019). The carbohydrate content in chicken dung is lower compared to the control media of rice corn. High levels of carbohydrates are essential for optimizing the vegetative growth of mushrooms (Sadad *et al.*, 2014). Carbohydrates are converted into carbon compounds, which serve as energy sources and resources for carbon (Agustin *et al.,* 2023) and for the formation of new cells (Nurfitriani *et al.*, 2014). A decline in carbon and nitrogen sources negatively affects spore viability (Kansrini, 2015).

According to a study by Sadad *et al.* (2014), the optimal growth of *Metarhizium anisopliae* occurs on paddy bran media, which has an average diameter of 6.8 cm, primarily due to its carbohydrate content reaching 84.36%.

Material Organic Maggot Waste

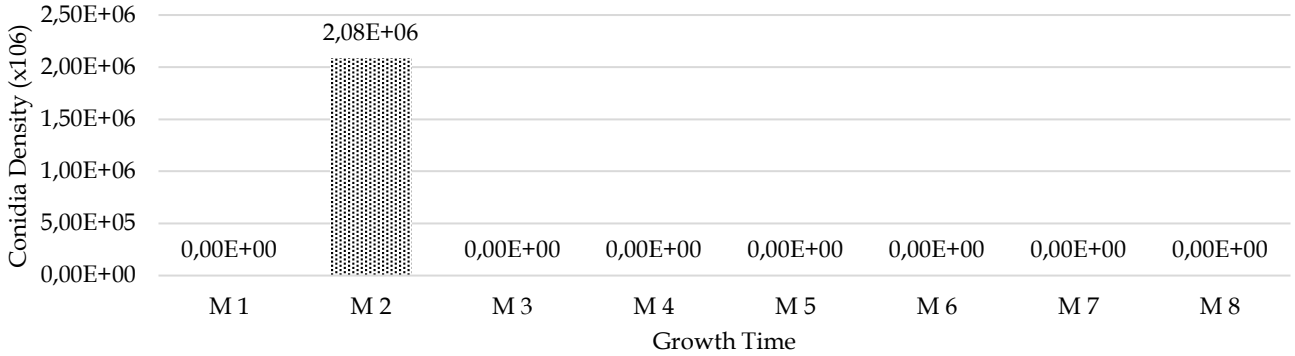

Figure 3. Growth *Metarhizium anisoplia* on Maggot Residue media

Black Soldier Fly (BSF) larvae contain a high level of antimicrobial peptides (AMPs) which function as antimicrobial, antifungal, and antibacterial agents (Parhusip and Gandhy, 2023; Anisa *et al*., 2021). The antimicrobial peptides in BSF larvae are also secreted into the residue known as kasgot, indicating that BSF larvae biomass has the potential to be a superior feed (Gold *et al.,* 2018).

In addition to their high AMP content, BSF larvae are also highly nutritious. They are rich in fatty acids (Anisa *et al.,* 2021), including lauric acid, which has antibacterial properties (Parhusip and Gandhy, 2023), accounting for up to 49.18% of their composition. These fatty acids play a vital role in boosting immunity and promoting wound healing (Sartika, 2008). Saturated fatty acids, such as hexadecanoic acid (also known as palmitic acid), are commonly found in milk, butter, cheese, and other dairy products (Sinaga and Siahaan, 2018). This organic compound can inhibit the growth of microbes (Illing *et al*., 2021) and possesses antifungal properties. It works by disrupting the cell walls and membranes of molds and has an activity similar to that of various active compounds, including terpenoids, which can enhance antifungal activity (Wahyuni *et al.,* 2019).

The residue from BSF larvae also contains a variety of bacteria secreted during the decomposition process. Vandeweyer *et al.* (2023) noted that there is a diverse bacterial population in the residue from BSF larvae, with the composition influenced by the type of feed or substrate used. For instance, chicken feed and food waste from catering services or supermarkets produce distinct bacterial profiles. Some of the bacteria identified in kasgot include *Acetobacter sp., Lactobacillus sp., Limosilactobacillus sp., Latilactobacillus sp., and Pseudomonas sp.* (Vandeweyer *et al.,* 2023).

Soil Science Laboratory tests at the University of Jember (Table 2) indicate that the nutrients found in the organic residue from BSF larvae can support mushroom growth. However, as shown in the growth chart (Figure 3), the growth of *Metarhizium anisopliae* conidia is very low during the exponential phase (weeks 2-3), measuring only 2.08 x 10^6. This low fungal growth is attributed to several factors, including the presence of AMPs, fatty acids, and various bacteria in the residue maggot substrate, which collectively disrupt the growth of entomopathogenic fungi in this environment.

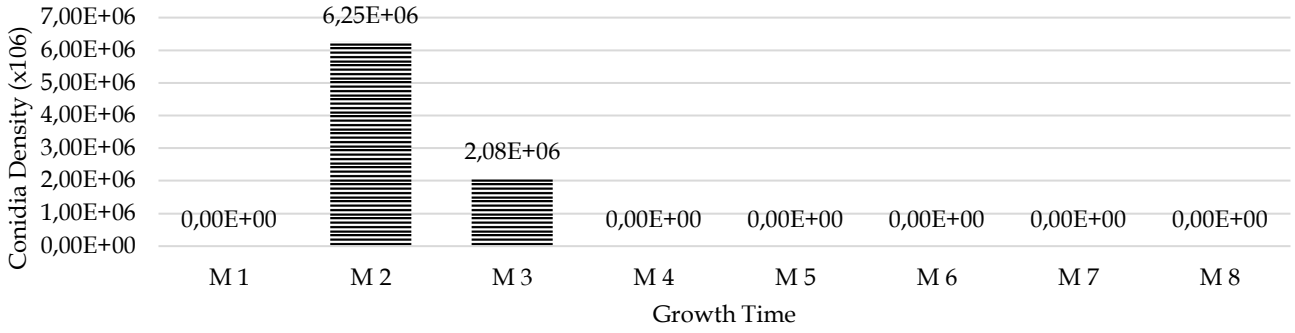

Figure 4. Chart Growth *Metarhizium anisoplia* on Pupa Shell media

The second organic material is the pupa shell. The results of the Soil Science Laboratory Test at the University of Jember (see Table 2) show that the nutrient content in the pupa shell can support fungal growth. Based on the growth graph (Figure 4), the growth of Metarhizium anisopliae conidia was very low in the exponential phase (weeks 2-3), which was only 6.25 x 106. This low conidia growth was caused by the high lignocellulose content in the growth medium. Lignocellulose is a compound that is widely found in plant cell walls, especially woody plants (Chen, 2014), as well as in agricultural and industrial waste (Ciptadi et al., 2022). Lignocellulose consists of cellulose, hemicellulose, and lignin (Riyanti, 2009). One of its components, namely lignin, functions to inhibit the growth of Metarhizium anisopliae in organic media. Lignin is considered a secondary metabolite that helps organisms fight diseases and pathogens (Dalimunthe and Rachmawan, 2017), as well as an antimicrobial or antifungal agent against various fungi, such as Candida albicans (Wu et al., 2008), Cryptococcus neoformans (Kumari et al., 2019), and Aspergillus parasiticus (Pizzolitto et al., 2015). Phenolic compounds disrupt the cell cycle in fungi, especially during replication, thereby inhibiting fungal growth and causing mitochondrial damage (Dewi et al., 2019).

Based on Jember State Polytechnic Lab Test Results (2023), pupal shells have a lignocellulose content of 56.98%. This compound is also found in maggot organic residues, but in slightly lower amounts, namely 47.35% (Jember Polytechnic Practical Results, 2023). High cellulose and lignin content and complex structure (Hartati et al., 2023) make it difficult for Metarhizium anisopliae to decompose it efficiently, so it requires a long degradation process (Sadad et al., 2014; Wahyuni and Nst, 2019). Lignin degradation requires the assistance of ligninolytic microorganisms, such as fungi that can produce lignocellulolytic enzymes, namely from the white rot fungi group such as Trichoderma viride (Madadi and Abbas, 2017), Volvariella volvacea, Marasmius sp., and Trametes hirsuta. These fungi have varying degradation rates. Risdianto et al. (2007) noted that Marasmius sp. degrade lignin faster than Trametes hirsute, by utilizing biochemical methods, namely releasing amylase and protease enzymes to break down nutrients in the media, including carbohydrates and proteins, while also producing lignin-degrading enzymes, such as lignin peroxidase, which functions as the main catalyst in the lignolysis process by breaking down non-phenolic components of the lignin structure (Yenie and Utami, 2017).

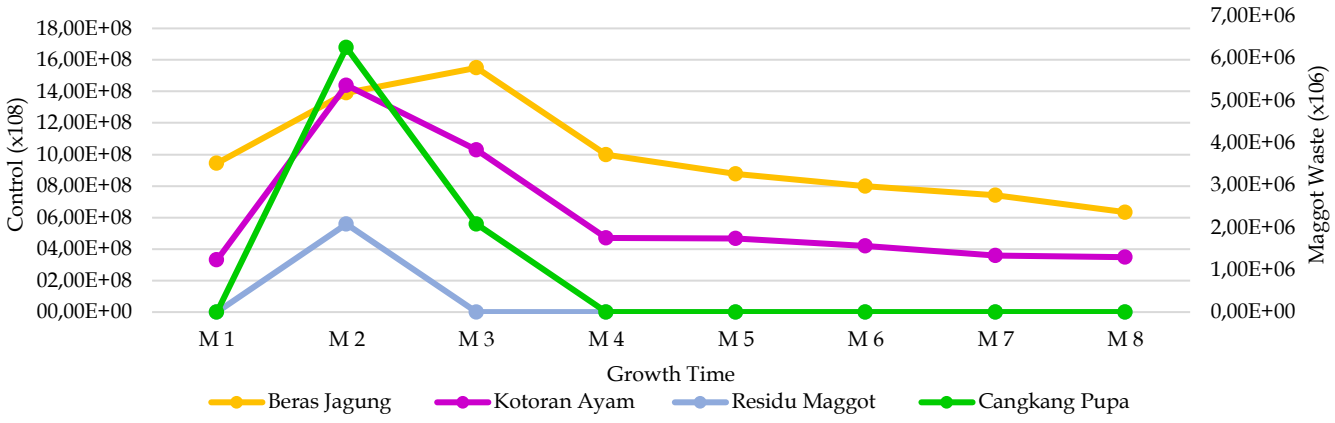

Figure 5. Graph Growth *Metarhizium anisoplia* on Various Media Materials Organic

The viability of fungi is greatly influenced by the nutrient content of the organic media used. According to Kansrini (2015), the nutritional content affects both the growth and viability of entomopathogenic molds. Observations (Figure 5) indicate that the density of conidia for *Metarhizium anisopliae* reached its highest point in the control media of corn rice, measuring 15.5 x 10^8 in the third week. In contrast, the highest density of conidia in organic media was observed at 14.4 x 10^8 during the second week when using chicken feces. The densities of conidia in organic media derived from maggot waste, specifically maggot residue and pupa shells, were significantly lower, at 2.08 x 10^6 and 6.25 x 10^6, respectively.

The difference in conidia density between the corn rice media and chicken feces is not substantial, while a significant difference exists between corn rice media and maggot waste. The growth density of the fungus is supported by the nutrients present in the organic media, including carbon and nitrogen, which promote hyphal growth, while proteins enhance spore germination (Triasih *et al.,* 2019). The nutrient content in corn rice media and chicken feces can be optimally utilized by *Metarhizium anisopliae* due to the presence of essential nutrients such as carbon and nitrogen (Suparti *et al.*, 2016).

Although maggot residue and pupa shells also contain nutrients that can support fungal growth, similar to corn rice and chicken feces, certain inhibitory factors are present in these media. These include AMP, fatty acids, and a high concentration of bacteria in the maggot residue, as well as lignocellulosic compounds in the pupa shells. Therefore, when utilizing organic media for fungal growth, it is crucial to consider the nutrient content thoroughly.

Conclusion

Based on the description above, it can be concluded that the entomopathogenic fungus *Metarhizium anisopliae* shows low viability in media made from maggot residue and pupa shells. This low viability is attributed to the presence of antimicrobial peptides (AMP), fatty acids, various bacterial contents, and lignocellulosic compounds. These elements hinder the utilization of organic nutrients by *Metarhizium anisopliae,* making maggot waste media and pupa shells ineffective as growth mediums for this fungus.

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Conflict of Interest
The content of this article does not create a conflict of interest

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