

# Physiology Characterization of Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* on Different Carbohydrate Sources

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**Abstract.** Insect pathogens that are often used to control insect pests are fungi *Beauveria bassiana* and *Metarhizium anisopliae*. One of the agar media, which is often used in the entomopathogenic function of propagation in the laboratory, is PDA (potato dextrose agar). The price of instant PDA is quite high, starting from Rp. 750,000 per 250 g doing the research costs quite large. Cheap and easily obtained abundant natural resources can be used as an alternative medium for microorganism growth to reduce the overall costs to be incurred in research. Carbohydrate sources used in this study were potatoes, cassava, sweet potatoes, corn, and rice, which were made into the agar media. Observation parameters were viability rates, colony growth rates, and sporulation of *B. bassiana* and *M. anisopliae* fungi isolates. The experiments are used in a completely randomized design (CRD) with two treatment factors. The data obtained were processed with variance and continued with the DNMRT test at 5% significance level. The results showed that cassava agar caused the highest germination at 24 hours. The highest diameter was fungi grown on potato dextrose agar. The highest sporulation was fungi grown on corn agar.

## 1. Introduction

Insect pathogens which are often used to control insect pests are fungi *Beauveria bassiana* and *Metarhizium anisopliae*. The fungus *B. bassiana* has explored its ability to control pests. Several researchers have tested the effectiveness of this fungus against several types of pests including *Frankliniella occidentalis* [1], *Spodoptera litura* [2];[3], *Riptortus linearis* [4], and *Diaphorina citri* [5]. Utilization of *M. anisopliae* for pest control has also been widely reported. The effectiveness of this fungus has been tested against several types of pests including larvae of *Oryctes rhinoceros* [6], *Crociodomia pavonana* [7], larvae *Spodoptera exigua* [8], *Chilo sacchariphagus* [9], and the nymph *Nezara viridula* [10].

Propagation of fungi in the laboratory requires a medium that contains all the essential nutrients fungi need [11]. [12] say that the source of nutrition is a determinant of the growth and virulence of entomopathogenic fungi because the rate of germination, growth, and sporulation are indicators of the level of virulence. Before propagating the fungus it is necessary to know the growth requirements of the entomopathogenic liquid media will determine the growth rate and virulence of fungi [13].



One of the agar media, which is often used in the entomopathogenic fungus propagation in the laboratory is PDA (potato dextrose agar). PDA has a low pH (pH 4.5 to 5.6) that inhibits the growth of bacteria that require a neutral environment with a pH of 7. The price of an instant PDA is quite high starting from Rp. 750,000 per 250 g doing research costs quite large. Some researchers have succeeded in finding alternative media for fungal growth from protein sources, namely cowpea, green beans, and black soybeans [14]. In addition to research with protein sources, various carbohydrate sources have also been used successfully as alternative media such as cassava starch [15], palmyrah tuber flour and tubers of sweet potato [16]. Abundant natural resources can be used as alternative media for microorganism growth. Alternative media from materials that are easily available and inexpensive can reduce the overall costs that must be incurred in research. Based on the description above, it is necessary to test various agar media from different carbohydrate sources to compare the growth of *B. bassiana* and *M. anisopliae*.

## 2. Materials and methods

This research was conducted at the Laboratory of Agrotechnology, Faculty of Agriculture,. The experiments were arranged in a completely randomized design (CRD) with two treatment factors. Factor 1 is the type of fungi (*B. bassiana* and *M. anisopliae*), factor 2 is the type of propagation agar media (cassava agar, potato dextrose agar, corn agar, rice agar, and sweet potato agar). Each treatment was repeated three times. The data obtained were processed with variance and continued with DMRT test at 5% significance level.

### 2.1. Making agar media

Cassava, sweet potatoes, corn, rice and potatoes which will be used as media to be peeled and washed using clean water, then are weighed as much as 400 grams, then cut into small pieces of size + 1 cm. Next step, boiled with distilled water as much as 1 litre until boiling, then filtered, put each solution into a glass breaker. Subsequently added agar and dextrose of 15 grams each were stirred evenly while heated, distilled water was added to reach a volume of 1 litre in the event of evaporation. Then the solution is put into a Schott bottle for sterilisation using an autoclave.

### 2.2. Conidia germination

Media in the form of slabs with a size of about 1 cm<sup>2</sup> and 1-2 mm thick is placed on a sterile object-glass. 10 µl conidia suspension containing 10<sup>6</sup> conidia/ml from each entomopathogenic fungus was dripped on top of the media and then inserted into a sterile petri dish filled with moist filter paper and incubated at 25°C for 18 hours. Each treatment was repeated 3 times. Observation using a light microscope with a magnification of 400 times. The percentage of germination calculated from 100 conidia. A conidium is germinated if the length of the sprout tube exceeds the conidia diameter.

### 2.3. Colony Growth rate

Each entomopathogenic fungus aged 7 days with a diameter of 10 mm was inoculated on the propagation agar medium tested in a Petri dish and incubated at 25°C. Observations of the colony diameter of each fungus were measured every day from 3 days to 14 days after inoculation.

### 2.4. Sporulation

Sporulation of each type of entomopathogenic fungus was calculated by preparing conidia suspension with a density of 10<sup>6</sup> conidia/ml. For each isolate, 0.1 ml of conidia suspension was inserted in a petri dish (diameter 7 cm) which contained each of the various propagation agar media. The culture was incubated for 14 days at 25°C. After 14 days, culture on petri dishes was put into an erlemeyer flask, and 50 ml of sterile aquadest was added. Cultivated be shaken for 5 minutes, filtered and diluted up to 4 times. The conidia density of the suspension is calculated by a haemocytometer.

### 3. Result and discussion

#### 3.1. *Kondia Germination*

The results showed that the isolate *M. anisopliae* tested had the highest germination at 6 HAI (hours after inoculation) compared to isolate *B. bassiana*. However, isolate *B. bassiana* had the highest germination rate at 24 HAI compared to isolate *M. anisopliae*.

**Table 1** Percentage germination of fungus isolates in various agar media

Media Types	6 HAI		24 HAI	
	<i>B. bassiana</i>	<i>M. anisopliae</i>	<i>B. bassiana</i>	<i>M. anisopliae</i>
Sweet potato Agar	0.28 d	19.11 ab	50.04 a	33.84 bc
Cassava Agar	4.13 cd	23.54 a	53.80 a	30.78 cd
Rice Agar	3.80 cd	13.13 abc	41.74 abc	21.45 d
Potatoes Dextrose Agar	1.26 cd	8.37 bcd	52.50 a	19.12 d
Corn Agar	5.73 cd	7.70 bcd	43.92 ab	18.53 d

Note: HAI = hours after inoculation, Numbers followed by the same letters in the same column shows no significant difference based on Duncan (5%)

Cassava agar is more supportive of fungus germination. Cassava agar contains more calcium than other agar media. Conidia fungi isolates need time to germinate. This is due to the appressorium requiring a process to absorb the nutrient in each media. Therefore, before propagation, it is necessary to know the growth requirements of an entomopathogenic fungus. Protein is one of the nutrients for the germination process. But a high amount of protein does not guarantee the conidia's ability to germinate. The suitability of the composition between protein, carbohydrate, starch, glucose also determines the conidia to grow. Incubation time affects the conidial ability of the fungus. This study shows that the longer the incubation time, the higher the conidial germination ability. The longer the conidia are in the incubation media, the more nutrients are absorbed for conidia germination so that the germination continues.

Fungi will grow well if they meet the requirements such as the media must have an appropriate pH, the agar media does not contain any inhibiting substances, the media must be sterile, and the agar media must contain all nutrients that are easy to use fungi. Fungi need nutrients for growth. These nutrients include carbon, protein, nitrogen, fat and Fe, vitamins A and C, water, and energy [17]. Also, the fungus requires additional nutrients that contain essential substances such as sulfur and phosphate [18]. [13] said that nutrition is a determining factor for the growth of entomopathogenic fungi because germination, growth, and conidia density require these nutrients. According to [19], nutrition is needed for fungus for biosynthesis and energy release as the main factors supporting germination, ability to live, and the sustainability of the colony. Germination is a very important parameter to know because germination is very determining the success of fungi in subsequent growth.

#### 3.2. *Colony growth rate*

Results showed that each fungus isolate had a significant difference in colony diameter area. The fungus isolate *B. bassiana* has the highest diameter area. Furthermore, corn agar, rice agar and potato dextrose agar support the growth of fungus colonies compared to other agar media.

**Table 2** Size of the daily diameter of fungi (cm)

Media type	3 DAI		9 DAI		14 DAI	
	<i>B.</i>	<i>M.</i>	<i>B.</i>	<i>M.</i>	<i>B.</i>	<i>M.</i>
	<i>bassiana</i>	<i>anisopliae</i>	<i>bassiana</i>	<i>anisopliae</i>	<i>bassiana</i>	<i>anisopliae</i>
Sweet potato Agar	1.84 bc	1.32 d	4.78 c	1.98 de	7.14 b	2.56 cd
Cassava Agar	1.94 ab	1.34 d	4.90 bc	1.94 e	7.16 b	2.50 cd
Rice Agar	1.92 ab	1.36 d	5.04 b	2.18 d	7.44 a	2.70 c
Potatoes Dextrose Agar	1.72 cc	1.36 d	5.02 b	2.12 de	7.54 a	2.66 cd
Corn Agar	2.04 a	1.40 d	5.32 a	1.92 e	7.58 a	2.42 e

Note: DAI = days after inoculation, the numbers followed by the same letters in the same column shows no significant difference based on Duncan (5%).

[20] suggested that the colony diameter, characteristics (texture, surface, and colouring) and fungus sporulation were strongly influenced by the type of propagation agar media used. In general, entomopathogenic fungi need carbohydrates for growth. Carbohydrates are useful as energy for fungi informing cells. This is following the research of [21], who explained that the source of carbohydrates is the most important nutrient for fungal growth and must be available in greater quantities than other nutrients. Carbon sources commonly used by fungi are carbohydrates (polysaccharides, disaccharides, monosaccharides), organic acids, amino acids and natural products.

### 3.3. Sporulation

Factors affecting the production of conidia of the fungus are the type and amount of nutrients contained in the propagation media used. Especially the comparison of the content between carbon and nitrogen. [21] explained that the carbon source (carbohydrate) is the most important nutrient for fungal growth and must be available in greater quantities than other nutrients. The surface area of the growing media also influences the number of conidia produced. The more surface area of the media, the more space the colonies can grow. If the fungus colony is getting wider, the conidia production will be higher. Media that tend to clot will have a narrow surface area so that conidia production is also small. Ideal fungus propagation media are media that not only have a large surface but also which can maintain the integrity of particles during the production process of fungus conidia. High growth will produce a higher number of conidia, whereas a low growth process will result in a smaller number of conidia. The diameter of isolates *B. bassiana* was wider than isolates *M. anisopliae*. This causes isolate *B. bassiana* to produce more conidia than isolate *M. anisopliae*.

**Table 3** Sporulation (conidia/ml) of fungus isolates after 14 DAI in various agar media

Types of Media	<i>B. bassiana</i>	<i>M. anisopliae</i>
Sweet potato Agar	2.88 x 10 <sup>8</sup> b	3.45 x 10 <sup>5</sup> d
Cassava Agar	1.37 x 10 <sup>8</sup> c	4.45 x 10 <sup>5</sup> d
Rice Agar	1.03 x 10 <sup>8</sup> c	1.60 x 10 <sup>5</sup> d
Potatoes Dextrose Agar	1.32 x 10 <sup>8</sup> c	1.97 x 10 <sup>6</sup> d
Corn Agar	3.65 x 10 <sup>8</sup> a	2.30 x 10 <sup>6</sup> d

Note: DAI = days after inoculation, the number followed by the same letter in the same column shows no significant difference based on Duncan (5%).

Entomopathogenic fungi must match the growing media and produce a lot of conidia. Corn media is very supportive of the growth and development of isolates *B. bassiana*. It can be seen from the wide diameter, and conidial production isolates *B. bassiana* were high, respectively by 7.58 cm and 3.65 x

$10^8$  conidia/ml. Whereas the cassava media supported the growth and development of isolates *M. anisopliae* tested. The results showed the diameter and conidia production of isolates *M. anisopliae* grown on cassava media were 2.50 cm and  $4.45 \times 10^5$  conidia/ml, respectively. This proves that each fungus has different nutritional needs for growth and development.

Differences in nutrient content greatly affect conidia production. The selection of entomopathogenic fungus propagation media material must be carried out appropriately, especially choosing materials that can produce conidia consistently. Based on research [22], concentration and vegetable fat/oil affect the growth also affect the development of fungi. A good agar media is one that can increase mycelium growth and conidial density. So that the difference in nutrient content greatly affects the conidia density production. This is confirmed by [20] that the type of media used greatly affects the colony diameter, characteristics and density of the fungus conidia. If the fungus is to be developed as a bioinsecticide, the amount of conidia produced by entomopathogenic fungi is important for mass propagation.

#### 4. Conclusions

Cassava agar caused the highest germination at 24 hours after inoculation. Longer the incubation time, the higher the conidial germination ability. The highest diameter was fungi grown on potato dextrose agar. Entomopathogenic fungi need carbohydrates for growth. Carbohydrates are useful as energy for fungi informing cells growth and must be available in greater quantities than other nutrients. The highest sporulation was fungi grown on corn agar. The surface area of the growing media also influences the number of conidia produced. Wider fungus colony, more conidia will be produced.

#### Acknowledgement

Thank to Universitas Muhammadiyah Tapanuli Selatan for providing research funding through the Hibah Internal APB UM-Tapsel 2018 so that this research can be carried out.

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