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Effect of Culture Media on Growth, Sporulation, Viability and Biomass of *Lecanicillium psalliotae* Zare & Gams (Hypocreales: Cordycipitaceae)

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Abstract

Lecanicillium psalliotae (MB-APKU-01) isolate was grown in different media to assess the effect of media on growth, sporulation, viability and biomass. Among the six media evaluated, the maximum radial growth (58.5 mm), highest sporulation (7.87×10^8 spores/ml), higher cfu (3.50×10^8 cfu/ml) and maximum biomass (1.93g) was recorded in Sabouraud Maltose Agar with Yeast Extract (SMAY). The lowest growth (49.5 mm), sporulation (5.37×10^8 spores/ml), cfu (1.50×10^8 cfu/ml) and biomass (0.99g) was recorded in Czapek Dox Agar (CDA). Therefore, the medium identified as most suitable for the mass production of the *Lecanicillium psalliotae* (MB-APKU-01) isolate was SMAY.

***Key words*:** *Lecanicillium psalliotae,* Entomopathogen, Culture media, Radial growth, Sporulation, Viability, Biomass

With the discovery of synthetic insecticides, pest management strategies began to depend heavily on chemical solutions, marking a significant shift in agricultural practices. Unlike pesticides, which cause secondary outbreaks, resurgence, resistance development in pests, and health hazards to humans, biocontrol agents stand as an eco-friendly alternative. *Lecanicillium psalliotae* Zare & Gams (Hypocreales: Cordycipitaceae) is known to infest insects, nematodes, and fungi [1-3]. A successful biocontrol agent should be able to mass produce with high number of spores for application in field [4]. Lecanicillium psalliotae Zare & Gams (Hypocreales: Cordycipitaceae) is a widely recognized entomopathogenic fungus known for its ability to parasitize a range of hosts, including insects, plant-parasitic nematodes, and phytopathogenic fungi. Due to its broad-spectrum activity, this fungus holds significant potential as a biocontrol agent in integrated pest management (IPM) strategies.

A critical attribute of any effective biocontrol agent is its ability to be mass-produced efficiently, ensuring a high yield of viable propagules, such as conidia or blastospores, that can be easily formulated and applied in agricultural settings. High spore production is essential for enhancing field efficacy, as a greater number of spores increases the probability of successful host infection and colonization, ultimately leading to effective pest suppression. Moreover, large-scale production methods must be cost-effective, ensuring that the biocontrol agent remains economically viable for commercial use.

For *Lecanicillium psalliotae*, optimizing growth conditions, nutrient formulations, and fermentation techniques is crucial to achieving high spore yields. Solid-state and submerged fermentation techniques are commonly explored to enhance sporulation, with factors such as temperature, humidity, carbon and nitrogen sources, and pH playing a significant role in maximizing spore production. Additionally, developing formulations that improve spore stability and viability under field conditions is essential for maintaining its effectiveness as a biocontrol agent.

Thus, the ability of *Lecanicillium psalliotae* to be mass-produced with a high spore count directly influences its practicality and success as a sustainable alternative to chemical pesticides in pest and disease management programs.

As part of survey of All India Coordinated Research Project (AICRP) on Biocontrol and Conservation Research Project (BCCP), Thrissur, Kerala Agricultural University, one isolate of entomopathogenic fungus, *Lecanicillium psalliotae* (MB-APKU-01) was obtained from the mycosed cadaver of mealybug complex in cassava. The pure culture of this isolate was maintained in the laboratory of AICRP on BCCP, Thrissur. Entomopathogenic fungi can be grown in natural media, semi-synthetic or synthetic media. However, standardization of suitable media for the mass production of entomopathogenic fungal isolates is a critical step in developing efficient and cost-effective biological control strategies. The choice of culture media directly influences the growth, sporulation, and infectivity of fungal isolates [5]. In this context, the present study was carried out to identify the suitable medium for the mass production of the isolate of *Lecanicillium psalliotae* (MB-APKU-01).

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MATERIALS AND METHODS

Lecanicillium psalliotae (MB-APKU-01) was grown in six different media viz., Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA), Sabouraud Maltose Agar (SMA), Sabouraud Maltose Agar with Yeast Extract (SMAY), Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Agar with Yeast Extract (SDAY) to evaluate the effect of media on radial growth. Broths of different media were prepared for studying the effect of media on sporulation, viability of spores and fungal biomass. The laboratory study was carried out in a Completely Randomized Design with 4 replications.

Radial growth

A 9 mm disc of the actively growing culture of fungal isolate was placed at the centre of the medium plate. The sides of the Petri plates were covered using parafilm. The inoculated plates were incubated at 28°C in BOD incubator under dark condition. The radial growth was recorded at three days interval [6].

Sporulation

The discs of 9 mm size from actively growing cultures of fungus were inoculated into sterile broths. Inoculated broths were incubated at 28°C for 14 days. The spore count was enumerated and calculated using improved Neubauer haemocytometer [7].

Viability of fungal spores

Dilution plate method was used to study the viability of spores in 14-day old broths. One mL of spore suspension from 10^{-8} dilution was poured into the Petri plate and rotated clockwise and anticlockwise for equal distribution of the suspension. Then respective molten agar medium at bearable temperature was poured over the suspension and rotated clockwise and anticlockwise. After solidification of the medium, the sides of the Petri plate were sealed with parafilm. Then the plates were incubated at 28°C in a BOD incubator for five days under dark condition. The plates were monitored daily and the count of colonies was recorded on fifth day. The colony count was estimated based on the formula given by Dale and Shinde [8].

$$\text{Number of cfu/ml} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of the sample plated (ml)}}$$

Production of biomass

Actively growing fungal discs of 9 mm were inoculated into 250 ml sterile broths. They were incubated at 28°C for 14 days. The fungal culture in different broths was filtered through previously dried and weighed Whatman No.1 filter paper under aseptic condition. The mycelial mat collected was dried in hot air oven at 70°C until a constant weight was attained. The biomass produced by the fungal isolate was calculated by subtracting the weight of the filter paper from the weight of the filter paper along with the fungal mat after drying [9].

RESULTS AND DISCUSSION

A significant difference in radial growth was observed for *Lecanicillium psalliotae* (MB-APKU-01) in different media (Table 1). SMAY recorded the highest radial growth of 15 mm and 49 mm 3 and 12 DAI, respectively, and was on par with SMA (14.5 mm and 48.5 mm). The highest radial growth of 58.5 mm was recorded in SMAY, and the lowest growth in CDA (49.5 mm), 15 DAI. Similar findings were recorded by Banu and Rajalakshmi [10], where they registered the highest radial growth of *Lecanicillium lecanii* (16.90 mm) in SMA and the least growth in CDA (13.10 mm), seven days after incubation. Isolates of *Lecanicillium* spp. from various places showed different growth in Malt Extract Agar and Potato Dextrose Agar (PDA) [11].

Table 1 Effect of different media on radial growth of *Lecanicillium psalliotae* (MB-APKU-01)

Treatment	*Colony diameter (mm) Days of incubation					Days for full growth
	3	6	9	12	15	
Potato Dextrose Agar (PDA)	13.75^{b}	22.75^{c}	32.75^{d}	42.50^{c}	52.25^{e}	30
Czapek Dox Agar (CDA)	12.50^{c}	20.50^{d}	29.25^{e}	39.25^{d}	49.50^{f}	31
Sabouraud Maltose Agar (SMA)	14.50^{a}	24.50^{b}	36.25^{b}	48.50^{a}	55.75^{b}	29
Sabouraud Maltose Agar with Yeast Extract (SMAY)	15.00^{a}	26.50^{a}	37.25^{a}	49.00^{a}	58.50^{a}	28
Sabouraud Dextrose Agar (SDA)	12.75^{c}	23.75^{b}	34.25^{c}	44.75^{b}	53.75^{d}	30
Sabouraud Dextrose Agar with Yeast Extract (SDAY)	13.00^{c}	24.50^{b}	35.50^{b}	45.00^{b}	55.00^{c}	30
CD (P=0.05)	0.65	0.82	0.76	0.65	0.72	

*Mean of four observations
Within column means followed by the same letter do not differ significantly

Table 2 Effect of different media on sporulation, viability and biomass of *Lecanicillium psalliotae* (MB-APKU-01)

Treatment	*Sporulation (Spores/ml)	*Colony forming units (cfu/ml)	*Biomass (g)
PD broth	$6.38 \times 10^{8\,b}$	$2.0 \times 10^{8\,bc}$	1.61^{b}
CD broth	$5.37 \times 10^{8\,c}$	$1.50 \times 10^{8\,c}$	0.99^{c}
SM broth	$6.5 \times 10^{8\,b}$	$2.25 \times 10^{8\,bc}$	1.78^{ab}
SMY broth	$7.87 \times 10^{8\,a}$	$3.50 \times 10^{8\,a}$	1.93^{a}
SD broth	$6.12 \times 10^{8\,b}$	$1.75 \times 10^{8\,bc}$	1.69^{b}
SDY broth	$6.62 \times 10^{8\,b}$	$2.50 \times 10^{8\,b}$	1.83^{ab}
CD (P=0.05)	0.69	0.89	0.21

*Mean of four observations
Within column means followed by the same letter do not differ significantly

The result of the sporulation, viability and biomass of *Lecanicillium psalliotae* (MB-APKU-01) in different media are depicted in Table 2. SMY broth supported the maximum sporulation (7.87×10^8 spores mL^{-1}) while the lowest sporulation was observed in CD broth (5.37×10^8 spores mL^{-1}). SMY broth produced a higher number of cfu (3.50×10^8 cfu mL^{-1}), while CD broth had a lower cfu count (1.50×10^8 cfu mL^{-1}). The highest mycelial biomass of 1.93g was produced by *Lecanicillium psalliotae* (EPF 5) in SMY broth. The lowest mycelial biomass was yielded in CD broth (0.99g). In a study conducted by Senthamizhlselvan *et al.* [12], *Verticillium psalliotae* isolates (VpPmKKL2120 and VpMLKKL2121) recorded the highest sporulation of 5.92×10^8 and 3.31×10^8 spores mL^{-1} in SD broth and 5.33×10^8 and 3.08×10^8 spores mL^{-1} in PD broth, respectively. Previous studies had reported that peptone as nitrogen source might be the reason for high sporulation, biomass and growth of fungus in Sabouraud's medium [13].

Dale and Shinde [8], who recorded that *B. bassiana* isolates in CDA gave low viable conidia (9.50×10^9 cfu g^{-1}) while PDA recorded more colony forming units (30.50×10^9 cfu g^{-1}). The medium rich in nutrient composition produced high sporulation and colony count [14]. CDA contains binding materials such as 30.0 - 45.0% cellulose, and 20.0 - 47.0% lignin, which imparts hardness to medium. In addition, high content of silica (94.0%) and low nitrogen content (3.27%), suppress the growth of fungus might be the reason for less growth in CDA [15].

CONCLUSION

The present study investigated the effect of different media on growth, sporulation, viability and biomass production of *Lecanicillium psalliotae* (MB-APKU-01). SMAY was found to be the most suitable medium for the mass production of *Lecanicillium psalliotae* (MB-APKU-01).

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