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Research Note

Effect of different media on growth and sporulation of *Metarhizium anisopliae*, a fungal bioagent

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ABSTRACT: An Influence of different culture media containing host extract as substrate on hyphal growth and sporulation of *Metarhizium* was evaluated in laboratory studied. The minimum average of 5.6 days was required for the sporulation of the fungus in Sabouraud's dextrose agar (SDA) medium supplemented with host extract (*Pyrilla* nymph / adult), where as in Emerson YPSS medium it was 7.3 days. The SDA medium in combination of host extract of the insect was found significantly superior over the other entire medium tested. Radial growth of 4.03 cm at days after inoculation (DAI) was observed in SDA medium supplemented with insect extract followed by SDA alone and Emerson YPSS medium with radial growth of 3.5 and 3.01 cm, respectively.

KEY WORDS: Culture media, host extract, hyphal growth, *Metarhizium anisopliae*, sporulation

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Entomopathogenic fungi as biological control agents show promise in reducing insect pest populations and damage in different agro-ecosystems (Inglis *et al*., 2001). *Metarhizium anisopliae* (Metschnikoff) Sorokin has shown broad spectrum pathogenicity (Martin *et al.,* 2000) with varying degree of virulence and host specificity. The virulence of fungus is influenced by the strain and the culture media in which fungus is grown. Several methods and techniques are available for mass production, but mostly designed to yield infective conidia. However meagre information is available on various media for spore and mycelial production. The present studies were undertaken to evaluate different culture media for the growth and sporulation (conidial production) of an isolate of *Metarhizium anisopliae* obtained from NBAII, Bangalore.

A laboratory study with different treatments (culture media) having three replications was carried out. Eight different culture media with their respective compositions / 1000ml sterlized distilled water were used, *viz*., Emerson YPSS medium (yeast extract 4.09 g + starch 15.0 g + dipotassium phosphate 1.0 g + magnesium sulphate 0.5 g + agar-agar 20 g), Sabouraud's dextrose agar (SDA) medium (dextrose 40 g + peptone 10 g + agar-agar 20 g), Sabouraud's dextrose agar supplemented with host extract (nymph/ adult of pyrilla) medium in ratio of 1:10 (extract and medium), Asthana and Hawker's medium (glucose 5 g + potassium nitrate 3.5 g + potassium hydrogen phosphate 1.75 g + magnesium sulphate 0.75 g + agar-agar 20.0 g), Czapek's medium (sucrose 30 g + sodium nitrate 2 g + potassium dihydrogen phosphate 1.0 g + magnesium sulphate 0.5 g + potassium chloride 0.5 g + ferrous sulphate 0.01 g + agar-agar 20 g), corn-meal agar (corn-meal 20 g + glucose 20 g + agar-agar 20 g), potato-dextrose agar (PDA) medium (peeled potato 200 g + dextrose 20 g + agar-agar 20 g) and only agar-agar medium (agar-agar 20 g) were prepared as per standard procedure. The entire medium was autoclaved for 30 minutes at 15 lbs pressure and poured in Petri-plates separately and a disc of 5mm of *M. anisopliae* was aseptically transferred into the different media and incubated at 25 ± 2°C.

Observations were recorded on the number of days required for the sporulation of the fungus on different media. The radial growth in cm was recorded at 3rd, 5th, 7th, and 10th days after inoculation (DAI). The observations at 10 DAI were considered for the evaluation.

The spore count ml^{-1} of fungal suspension was recorded after 10, 15 and 20 days after inoculation. Conidia were counted with a haemocytometer and expressed as mean count of conidia ml^{-1} of *M. anisopliae*.

The highest radial growth (4.03 cm) at 10 DAI was observed in SDA + host extract medium followed by SDA

Table 1. Influence of different culture media on the growth and sporulation of *Metarhizium anisopliae*

Culture medium	Average no. of days for sporulation	Average radial growth (cm) at 10 DAI ml^{-1}	Average spore count of fungal suspension (ml^{-1})
Emerson YPSS	7.33	3.01	6.60×10^6
Asthana Hawker's medium	11.66	1.65	1.12×10^6
Czapek's medium	11.33	2.03	4.26×10^6
Corn Meal	12.33	3.0	1.40×10^6
PDA	11.66	3.0	5.26×10^6
Plain Agar	–	1.30	–
SDA	11.66	3.50	6.16×10^6
SDA + extract	5.60	4.03	8.24×10^6
CD (P = 0.05)	0.94	0.60	0.24×10^6

DAI = days after inoculation

and Emerson YPSS medium (3.5 cm and 3.01 cm, respectively (Table 1). On average, 5.6 days were required for the sporulation of the fungus in SDA + host extract medium followed by Emerson YPSS (7.33 days). The SDA + host extract medium was found superior to other media. Highest spore count of 8.24×10^6 spore ml^{-1} was obtained in SDA + host extract medium (nymph / adult of *Pyrilla*) followed by Emerson YPSS medium 6.60×10^6 spore ml^{-1} .

Spore production of entomopathogenic fungi is affected by the content of the growing substrate (Hajek *et al*., 1990; Ignoffo, 1992; Magathaes, 2000). According to Smith and Grula (1981), various carbon sources, chitin and low level of fatty acids were found to be efficient for better sporulation. Similarly, Schaerffenberg (1964) reported increase in sporulation and virulence of the entmopathogens grown on animal fat incorporated culture medium. Samuels and Coracini (2004) reported that conidial production of *B. bassiana* was higher when it was grown on insect cadaver of *Blissus antillus*. It is concluded that hyphal growth and conidial production of *M. anisopliae* can be increased by culturing them on medium supplemented with host extract (nymph / adult of pyrilla).

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