J. Biol. Control, 16(2): 177-179, 2002

Research Note

Influence of different culture media on the growth and sporulation of *Metarhizium anisopliae* (Metschnikoff) Sorokin

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ABSTRACT: **Eight different culture media were tested for the growth and sporulation of the fungus. After inoculation, sporulation initiated in 5.66 and 7.66 days on Emerson YPSS and Barner's medium, respectively while Sabouraud's dextrose agar+yeast (SDA+Y) medium was found to be the best with highest radial growth (4.07cm) followed by Emerson YPSS medium (4.01cm) at 10 days after inoculation (DAI). Highest spore count (9.43×10^6 spores ml^{-1}) of fungal suspension was observed in Barner's medium followed by Emerson YPSS medium (8.29×10^6) and SDA+Y medium (7.16×10^6) at 10 DAI.**

KEY WORDS: Culture media, growth and sporulation, *Metarhizium anisopliae*

The use of entomopathogenic fungi, due to their amenability to mass production, has potential in future strategies of insect pest management. *Metarhizium anisopliae* is known to attack over 200 species of insects from 7 orders (Yendol and Roberts, 1971). The present studies were undertaken to evaluate the different culture media for the growth and sporulation of an isolate of *M. anisopliae* obtained from Sugarcane Breeding Institute, Coimbatore.

Laboratory studies with eight treatments (culture media) having three replications in completely randomized design were carried out in the department of Entomology, College of Agriculture, Nagpur during 1999-2000. Eight different culture media with their respective composition/1000 ml sterilized distilled water were used, *viz*., Emerson YPSS medium (Yeast extract 4.09g+starch 15.0g+ dipotassium phosphate 1.0g+Magnesium sulphate 0.5g+agar-agar 20g), Sabouraud's dextrose agar+yeast (SDA+Y) medium (dextrose 40g+bacto-peptone 10g + yeast extract 1g+agar-agar 20g), Asthana and Hawker's medium (Glucose 5g+potassium nitrate 3.5g+potassium hydrogen phosphate 1.75g+magnesium sulphate 0.75g + agar agar 20.0g), Barner's medium (glucose 1.0g + potassium nitrate 1.0g+potassium triphosphate 1.0g+ammonium nitrate 1.0g+agar agar 20g), Czapek's medium (sucrose 30g+sodium nitrate 2g+potassium dihydrogen phosphate 1.0g+magnesium sulphate 0.5g + potassium chloride 0.5g + ferrous sulphate 0.01g + agar agar 20g), Potato dextrose agar (PDA) medium (peeled potato 200g + dextrose 20g+agar agar 20g), Corn-meal medium (corn-meal 20g+glucose 20g+agar agar 20g) and Plain medium (agar agar 20g) were prepared as per the standard procedure and were sterilized in autoclave for 30 minutes at 1.05kg cm^{2-1} pressure and 160°C temperature. A fungal disc of 6mm diameter was transferred aseptically to the sterilized media in a laminar flow. The Petri-plates were kept

Table 1. Influence of different culture media on the growth and sporulation of *M. anispoliae*

Culture medium	Av. No. of days required for sporulation	Av. radial growth in cm at 10 DAI	Av. spore count ml^{-1} of fungal suspension at 20 DAI
Emerson YPSS	5.66	4.01	8.29×10^6
SDA + Y	15.33	4.07	7.16×10^6
Asthana & Hawker's	11.66	1.65	1.12×10^6
Barner's	7.66	2.03	9.43×10^6
Czapek's	16.33	2.17	6.60×10^6
PDA	18.33	2.34	5.26×10^6
Corn meal	12.33	3.09	1.40×10^6
Plain (agar-agar)	–	1.30	–
SEM ±	0.31	0.20	0.08×10^6
CD (P = 0.05)	0.94	0.60	0.24×10^6

DAI = Days after inoculation

for incubation at 25±2°C temperature and relative humidity 90 percent.

Observations were recorded on the number of days required for the sporulation of the fungus on different media and the average number of days required for sporulation was worked out. The radial growth in cm was recorded at 4, 6, 8 and 10days after inoculation (DAI) by inverting the Petri-plates to avoid the error in measurement. The observations at 10 DAI were considered for the evaluation. The observations on spore count ml^{-1} of fungal suspension were recorded at 15 and 20 DAI and observation of 20 DAI was considered for the evaluation. For this 20g of medium with homogenous fungal growth from each medium was suspended thoroughly in 70ml sterilized distilled water using a rotary mixture for 20 minutes. The homogenate was passed through a muslin cloth followed by Whatman No.1 filter paper. Final volume was made up to 100ml by adding sterilized distilled water. After series of dilutions, condia were counted from each filtrate using a Neubaur's haemocytometer under phase contrast microscope and mean counts of conidia ml^{-1} of fungal suspension were worked out. Mean counts of three such samples were estimated for the evaluation (Puzari *et al.*, 1977).

The data (Table1) revealed that the minimum average of 5.66 days were required for the sporulation of the fungus in Emerson YPSS medium followed by Barner's medium (7.66 days) and these two media were found to be significantly superior over the other media. In this investigation 18.33 days were required for the sporulation of the fungus on PDA medium, not relevant as strains differences exist.

The highest radial growth of 4.07cm at 10 DAI was observed in SDA + Y medium which is in corroboration with findings of Liu *et al.* (1989) followed by Emerson YPSS medium and corn-meal medium in which 4.01 and 3.09 cm radial growth was recorded, respectively.

Highest sporulation of 9.43×10^6 spores ml^{-1} was obtained in Barner's medium at 20 DAI followed

by Emerson YPSS medium (8.29×10^5) and SDA+Y medium (7.16×10^6 spores ml^{-1}). Barnes *et al.* (1975) reported better spore production of *M. anisopliae* in the medium containing yeast, which is consistent with the present findings.

ACKNOWLEDGEMENTS

The authors thank the Director and Drs. S. Eswaramoorthy and N. Mukundhan, Senior Scientists (Entomology), Sugarcane Breeding Institute, Coimbatore, for the supply of mother culture of *M. anisopliae* var. *anisopliae* for these studies.

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