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Growth, development and viability of *Metarhizium anisopliae* on media with various nutrient sources

■ S.D. PATIL*[1], J.R. KADAM[2], A.G. CHANDELE[2], S.S. WAGH[2] AND R.S. JADHAV[2]

[1]Department of Entomology, Agricultural Research Station, Niphad, NASIK (M.S.) INDIA
[2]Department of Entomology, Mahatma Phule Krishi Vidhyapeeth, Rahuri, AHMEDNAGAR (M.S.) INDIA

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***Corresponding author:**

ABSTRACT

The entomopathogenic fungus, *Metarhizium anisopliae* (Metschinikoff) Sorokin was mass produced in different liquid media. The nine media of various nutrient sources were evaluated to find out the most suitable medium for growth, biomass and viability of *M.anisopliae*. Sabouraud's dextrose broth with yeast extract proved to be the superior which gave significantly highest cfu ($12.33x10^8$/ml) and biomass (7.20g). The next best medium was Sabouraud's maltose broth with yeast extract and potato dextrose broth with yeast extract which registering cfu count of (10.33×10^8 and 10.67×10^8cfu/ml) and biomass (6.27 and 5.73 g), respectively. The lowest (48.33%) medium surface coverage and least biomass (1.57g) and cfu (4.33×10^8/ml) were registered in medium with malt extract. Thus, considering growth, development and viability of *M.anisopliae*, Sabouraud's dextrose broth with yeast extract (SDY) emerged as the most potential medium for biomass production.

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INTRODUCTION

Indiscriminate use of pesticides to control insect pests of crops has disturbed the ecological balance. This leads to use of biological control agent as an important and alternate control practice. Among the various entomopathogenic fungi, *Metarhizium anisopliae* (Metschinikoff) Sorokin has proved best microbial agent against a large number of pests. (Makaka, 2008; Rangel *et al*., 2004 and 2005). It has a wide range of pathogenicity to insects belonging to different orders. Success of any microbial control programme depends on production of sufficient quantity of inoculum for field application. Most of the entomopathogenic fungi are facultative pathogens which can be mass produced in synthetic, semi-synthetic or natural media containing suitable nutrient sources (Niassy *et al.,* 2011). However, selection of suitable media for mass multiplication are important. In the present study, an attempt has been made to develop a mass production medium for the fungus using various nutrient sources media.

MATERIAL AND METHODS

The laboratory evaluations were carried out in Biocontrol Research Laboratory, Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar (M.S.). The present study was conducted for evaluation of nine media of various nutrient sources on the basis of per cent surface coverage and biomass. Sabouraud's dextrose (SD) broth + 1 per cent Yeast extract,

Sabouraud's maltose (SM) broth + 1 per cent Yeast extract, Potato peptone (PP) broth, Yeast extract glucose (YEG) broth, Potato dextrose (PD) broth, Potato maltose (PM) broth, Malt extract (ME) broth, Potato glucose (PG) broth and Potato dextrose (PD) broth + 1 per cent yeast extract were prepared. The empty saline bottles were filled with 40 ml medium. As such different media bottles were sterilized under 15 lbs pressure at 121°C for 15 minutes. Each bottle was inoculated with fungal culture ($2x10^8$cfu/ml) and incubated for 10 days at temperature 25 ± 2°C. The individual fungal mat was separated using pre-eight Whatman no.1 filter paper. The observations on per cent surface coverage on 3,7,10 and 15 days and biomass on 15th day were recorded (Hall and Bell 1961).

The method suggested by Ming-Guang Feng *et al.* (1990) was used for determining the cfu count. The autoclaved Sabouraud's dextrose agar with Yeast extract (SDA) medium in Petridishes (100 mm diameter) was inoculated with the help of micropipette by releasing 1 ml *M.anisopliae* suspension prepared in the distilled water in laminar flow cabinet. Other Petridishes with the medium were prepared in similar manner and inoculated with various dilutions in the series (10^1 to 10^{10} cfu/ ml) at 27 ± 1°C. After 48 hrs from the 10 samples in each Petridish the numbers of colonies/Petri dish were counted and cfu/ml was calculated.

RESULTS AND DISCUSSION

The ultimate suitability of the medium for mass production of the entomopathogenic fungus was determined on the basis of growth, development and viability at 15 DAI, considering surface coverage, biomass development and cfu count, respectively. The surface coverage, biomass development and cfu count in the test media ranged from 48.33 to 100.0 per cent, 1.57 to 7.20 g and 4.33×10^8 to 12.33×10^8cfu/ml, respectively. The nine media of various nutrient sources (Table 1) were evaluated to find out most suitable medium for growth, biomass and viability of *M.anisopliae* (Alves *et al*., 2011).

Sabouraud's dextrose broth with yeast extract was significantly superior medium for the growth among all the nine test media. It showed maximum (100 %) surface coverage at 3 DAI. It was followed by Sabouraud's maltose broth with yeast extract (98.33 %) and Potato dextrose broth with yeast extract (93.33 %). At 7 DAI, the both media recorded cent per cent surface coverage. However, at 15 DAI, Sabouraud's dextrose broth with yeast extract was most adequate as judged from significantly highest cfu count (12.33×10^8/ml) and biomass (7.20g) (Fig. 1). The next best media were Sabouraud's maltose broth with yeast extract and Potato dextrose broth with yeast extract registering cfu count of 10.33

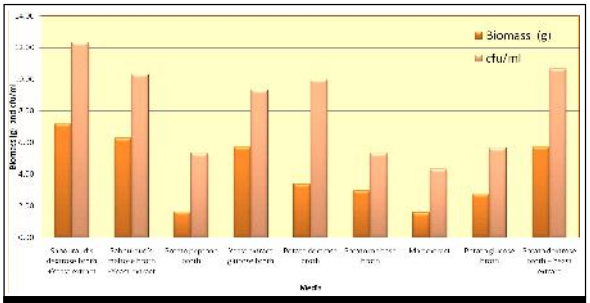

Fig. 1 : Influence of the test media on biomoss and viability of *M.anisopliae*

Table 1 : Influence of test media on growth, development and viability of *M. anisopliae*

T. no.	Treatments	Surface coverage (%) on 3 DAI	7 DAI	10DAI	15 DAI	Biomass g/40ml medium	cfu (× 108/ml)
T_1	Sabourauds dextrose broth + yeast extract	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	7.20	12.33 (3.58)**
T_2	Sabourauds maltose broth + yeast extract	98.33 (82.51)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	6.27	10.33 (3.29)
T_3	Potato peptone broth	23.33 (28.86)	28.33 (32.14)	48.33 (44.03)	51.67 (45.97)	1.63	5.33 (2.42)
T_4	Yeast extract glucose broth	93.33 (75.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	5.73	9.33 (3.13)
T_5	Potato dextrose broth	93.33 (75.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	3.37	10.00 (3.24)
T_6	Potato maltose broth	83.33 (65.88)	96.67 (79.53)	100.00 (90.00)	100.00 (90.00)	2.97	5.33 (2.42)
T_7	Malt extract	23.33 (28.86)	26.67 (31.11)	40.00 (39.23)	48.33 (44.03)	1.57	4.33 (2.20)
T_8	Potato glucose broth	85.00 (67.21)	95.00 (77.08)	100.00 (90.00)	100.00 (90.00)	2.77	5.67 (2.48)
T_9	Potato dextrose broth + yeast extract	98.33 (82.51)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	5.73	10.67 (3.34)
	S.E ±	1.94	1.28	0.64	0.45	0.07	0.09
	C.D. (P=0.05)	5.77	3.80	1.92	1.33	0.21	0.26

* and ** indicates of significance of values at P=0.01, 0.05, respectively DAI = Days after inoculation CFU = Colony forming unit

$\times 10^8$/ml and 10.67 $\times 10^8$/ml with the biomass of 6.27 and 5.73 g, respectively.

The lowest per cent surface coverage at 3 (23.33%), 7 (26.67%), 10 (40.00%) and 15 (48.33%) DAI with least biomass (1.57g) and viability (4.33 $\times 10^8$cfu/ml) at 15 DAI was recorded in Malt extract. Thus, considering growth, development and viability of *M. anisopliae*, Sabouraud's dextrose broth with yeast extract (SDY) emerged as the most potential medium for the biomass production. Akbar *et al.* (2012) evaluated the toxicity of insecticides and fungicides on spore production and mycelial growth of *M. anisopliae*, showing that the chemicals composed by chlorpyrifos, phosphorus, metalaxyl + mancozeb and pro-fenofos were the most toxic to mycelial growth and conidial germination. Meanwhile, the chemicals that contain aceta-meprid, cypermethrin, emamectin, imidacloprid and sinophos were less toxic to mycelial growth and spore production. Spinosad and indoxacarb were considered safe and compatible with *M. anisopliae*. The same methodology employed here was used by Tonussi *et al*. (2012) to evaluate the toxicity of deltamethrin on *M. anisopliae*. The authors observed that the concentration of 50 µg/ml reduced and delayed conidia germination and it was 100 per cent inhibited by high concentrations. Ultra diluted treatments were not inhibitory with concentration of 31.25 ρg/ml and also with the concentration of 31.25 ηg/ml increased the germination of conidia, indicating a possibly occurrence of hormesis effect.

A recent study conducted by Fabrice *et al*. (2013) showed the efficiency of germination speed parameter to evaluate the compatibility of *M. anisopliae* Sorokin with fungicide thiophanate-methyl.

During the present investigation considering Sabouraud's dextrose broth with yeast extract emerged as the most potential medium for biomass production for *M. anisopliae.* It was in conformity with Manjula and Krishnamurthy (2005) who obtained excellent growth of *Nomuraea rileyi* in medium with similar ingredients. Im *et al.* (1988) found that yeast extract was necessary for mycelial growth while dextrose was required for sporulation. Sabouraud's dextrose broth with yeast extract showed best performance for growth, development and viability of *M. anisopliae* in present studies which is in corroboration with the findings reported by Bulla *et al.* (2013); Kulat *et al.* (2002); Sharma *et al*. (2002) and Pandey and Kanujia (2008). All the workers got highest sporulation and viability of *M.anisopliae* in the medium.

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