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Effect of Different Culture Media on Growth and Biomass of *Macrophomina Phaseolina*

P. Deepthi[*], E. Siva Sankar Reddy[**], C. S. Shukla[***] and K. P. Verma[****]

ABSTRACT: *Experiment was conducted out to see the effect of different media on growth and biomass of Macrophomina phaseolina. Out of seven media tested significantly higher mycelial growth of M. phaseolina was observed in Malt extract medium and Potato dextrose agar medium and are statistically at par with each other followed by Asthana and Hawker's medium, Richard's medium, PDA with 50% dextrose, Potato agar medium and no mycelial growth was noticed on Peptone dextrose rose bengal agar medium. Biomass of fresh mycelium was significantly more on Malt extract medium and Richard's medium and least weight was found in Asthana and Hawker's liquid medium. No fresh mycelial growth was obtained in Potato dextrose broth with 50% dextrose, Potato broth and Peptone dextrose rose bengal medium.*

Key words: *Macrophomina phaseolina , Media, Growth, Biomass*

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the important oilseeds crops in India occupying the third position. Dry root-rot of sesame caused by *Macrophomina phaseolina* is the most serious disease affecting the crop at later stages of growth (Buldeo *et al.*, 1979). Every living being requires food for its growth and reproduction and the fungi are not an exception. Fungi derive the food from the substrate upon which they grow. In order to culture the fungi artificially it is necessary to supplement in the medium, those essential nutrients needed for their growth, development and other metabolic processes. To find out the best sources of nutrients for the fungal growth, different synthetic and non-synthetic growth media were tested.

MATERIALS AND METHODS

Experimental Site

The present investigation was carried out at the Department of Plant Pathology, IGKV, Raipur. All *in vitro* studies on *Macrophomina phaseolina* were conducted in laboratory of the Department of Plant Pathology.

Collection of Diseased Sample

Naturally infected stem of sesame crop with the typical charcoal rot symptoms *i.e.* spindle shaped spots with light grey centers surrounded by brown margins were collected from the oil seed research farm of the university. Collected samples were brought to the laboratory for critical examination of the symptoms, identification and isolation of the pathogen.

Isolation & Purification

The entire work of isolation and purification was done in laminar air flow, which was sterilized by alcohol or formaldehyde and UV tube light prior to use. The fresh infected stem of sesame was cut in to small pieces measuring about 2 mm and surface was sterilized with 0.1% mercuric chloride ($HgCl_2$) solution followed by three subsequent washings with sterile water. Such pieces were aseptically transferred into petridishes containing 20 ml potato dextrose agar

* Ph.d, Dept of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Viswa Vidyalaya, Raipur, Chhattisgarh, *E-mail: deepthireddy03@gmail.com*
** M. Sc, Dept of Genetics & Plant breeding, College of Agriculture, Indira Gandhi Krishi Viswa Vidyalaya, Raipur, Chhattisgarh.
*** Professor, Dept of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Viswa Vidyalaya, Raipur, Chhattisgarh.
*** Senior Scientist, Dept of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Viswa Vidyalaya, Raipur, Chhattisgarh.

(PDA) medium and incubated at 27 ± 2°C for ten days. Pure culture of the fungus was obtained and purified by adopting single hyphal tip method .

Effect of Media on Radial growth and Biomass

In the present investigation seven different media *viz.* potato dextrose agar, PDA with 50% dextrose, potato agar, peptone dextrose rose bengal agar media, malt extract, richard's and asthana and hawker's media were used to find out the best medium for the growth and biomass of *M. phaseolina* and composition of media are given in Table no : 1. 20 ml of each medium was poured in petriplate and allowed for solidification. Thereafter, 5 mm discs of test fungus were cut with the help of sterilized cork borer from seven days old culture grown on PDA medium. One disc of the culture was placed in inverted position in the centre of each petriplate. Three replications for each medium were maintained. The observations for colony characters and diameter were recorded on different culture media by visual observation. For biomass, Erlenmeyer conical flask containing 50 ml sterilized broth of each media was inoculated with 5 mm disc of test pathogen and thereafter incubated at 27 ± 2^0C for 7 days and observations were recorded for fresh and dry mycelial weight. For dry mycelial weight, collected mat was dried in oven at 60^0C till constant weight was achieved.

Table 1
Composition of Media used during the Course of Investigation

Media	*Ingredient*	*Quantities (g)*
PDA	Peeled potato	200.00
	Dextrose	20.00
	Agar agar	20.00
	Distilled water	1000 ml
PDA with 50% dextrose	Peeled potato	200.00
	Dextrose	10.00
	Agar agar	20.00
	Distilled water	1000 ml
Potato agar	Peeled potato	200.00
	Agar agar	20.00
	Distilled water	1000 ml
Malt extract	Malt extract	20.00
	Agar agar	20.00
	Distilled water	1000 ml
Peptone dextrose Rose Bengal agar	Agar agar	20.00
	Potassium dihydrogen phosphate	1.00
	Magnesium sulphate	0.50
	Peptone	5.00
	Dextrose	10.00
	Rose Bengal	3.30
	Distilled water	1000 ml
Asthana & Hawker's	Potassium dihydrogen phosphate	1.75
	Magnesium sulphate	0.75
	Potassium nitrate	3.50
	Sucrose	5.00
	Agar agar	20.00
	Distilled water	1000 ml
Richard's media	Potassium nitrate	10.00
	Potassium dihydrogen phosphate	5.00
	Magnesium sulphate	2.50
	Ferric chloride	0.02
	Sucrose	50.00
	Agar agar	20.00
	Distilled water	1000 ml

RESULTS AND DISCUSSION

Isolation, Purification and Identification

The disease samples of sesame were collected from the IGKV research farm and brought to the laboratory. From the infected stem, pathogen was isolated, purified by single hyphal techniques and multiplied on potato dextrose agar media and stored at 4°C. The isolated fungus was matched with the available literature and references for identification **(Plate 1).**

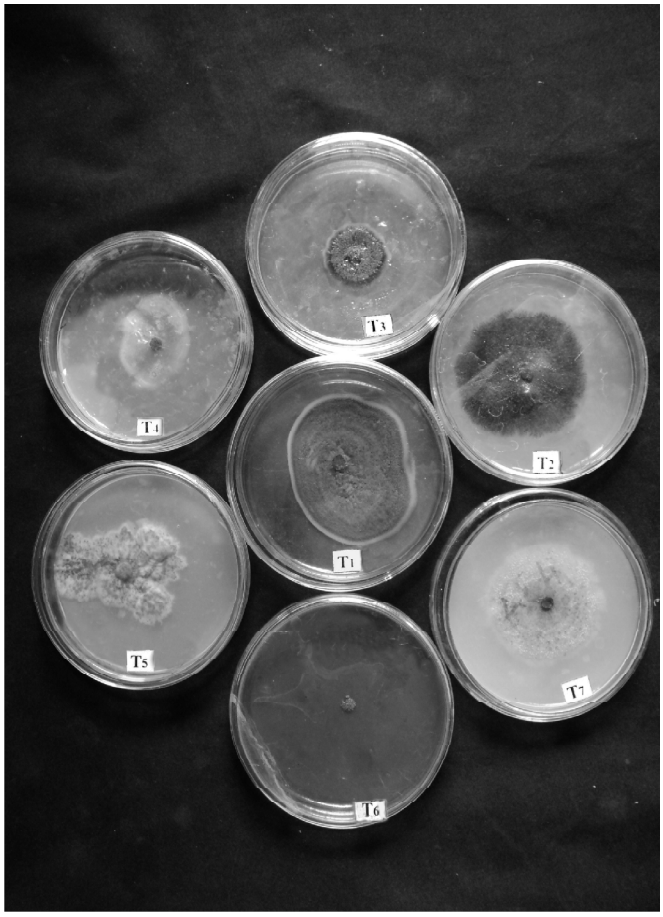
The pathogen was identified on the basis of character of the mycelium and sclerotia. The mycelium was septate, light brown and sclerotia were brown to black colour, rounded or oblong in shape **(Plate 2).** The characters were compared with the standard description of *M. phaseolina* from literature (Singh, 1998).

Effect of Media on Radial Growth, Growth Characteristics and Biomass

Seven media with three replicated plates for each were tested to understand the cultural behaviours such as growth, colony characters and biomass to identify the best growth supporting medium of *M. phaseolina.*

The results obtained are presented in Table 2. malt extract medium was found as the best medium for growth of *M. phaseolina* over other media after seven days, followed by potato dextrose agar, asthana and hawker's, richard's, PDA with 50% dextrose and potato agar medium. Growth of fungus in all the media differ significantly with each other while growth on malt extract medium and potato dextrose agar medium was at par. No growth was recorded on peptone dextrose rose bengal agar medium clearly indicating their unsuitability.

After seven days of incubation, significantly higher mycelial growth (66.66 mm) of *M. phaseolina* was observed in Malt extract medium and Potato dextrose agar medium (65.5 mm) and statistically at par with each other. However, growth was



1. Malt extract
2. Potato dextrose agar
3. Richard's
4. Potato agar
5. PDA with 50% dextrose
6. Peptone dextrose rose bengal agar
7. Asthana and Hawker's

Plate 1: Intial symptoms on stem under field conditions
(a) Severe infection under field conditions
(b) Infected and pre-mature opening of pods (c)

Plate 2: Isolation and purification of *M. phaseolina* on PDA

significantly less (21.5 mm) noticed in Potato agar medium followed by PDA with 50% dextrose (31.0 mm), Richard's medium (40.5 mm) and Asthana and Hawker's medium (47.16 mm). No mycelial growth was noticed on Peptone dextrose rose bengal agar medium. Fig. 2

Besides variation in growth rate in different media, the data presented in Table 2 and Plate 1 revealed that *M. phaseolina* exhibited variation in respect of colony colour, margin of colony, zonation and topography of mycelium also. malt extract, potato dextrose agar, PDA with 50 % dextrose and potato agar medium showed dull whitish mycelium, whereas it was dirty white and whitish grey noticed in asthana and hawker's medium and richard's medium respectively. More pycnidial production was found in centre as compared to periphery in malt extract, asthana and hawker's, potato dextrose agar and PDA with 50% dextrose agar medium where as, in richard's medium gave on entire growth. In respect to mycelial growth, irregular growth was recorded in potato dextrose agar, asthana and hawker's, richard's, PDA with 50% dextrose, potato agar medium, while regular growth was noticed in malt extract medium. Plate: 1

In liquid medium, significantly more (1.80 g) fresh mycelial weight of *M. phaseolina* was obtained in Malt extract medium and Richard's medium (1.70 g) and both were at par with each other. However, significantly least fresh mycelial (0.58 g) weight was found in Asthana and Hawker's liquid medium. No fresh mycelial growth was obtained in Potato dextrose broth with 50% dextrose, Potato broth and Peptone dextrose rose bengal medium. Fig. 3

The dry mycelial weight of *M. phaseolina* in different liquid medium was in accordance with that of fresh mycelial weight except Potato dextrose liquid

Table 2
Effect of Different Media on Radial Growth, Growth Characteristics and Biomass of *M.phaseolina*

S.no	*Medium*	*Radial growth (mm)*	*Growth characteristics*	*Fresh mycelial weight (g)*	*Dry mycelial weight (g)*
1	Malt extract	66.66	Regular, flat, smooth without zonation and dichotomous branching growth. More pycnidial production was oticed in centre as compared to periphery	1.80	0.15
2	Potato dextrose agar	65.50	Irregular, dull white, smooth growth at periphery but dark black in centre with zonation and pycnidial production was observed in entire growth.	1.46	0.02
3	Richard's	40.50	Irregular, grey whitish growth at centre but dull white at periphery and raised growth. Pycnidial production observed in entire plate.	1.70	0.05
4	Potato agar	21.50	Irregular dull whitish flat growth and less production of pycnidia.	0.00	0.00
5	PDA with 50% dextrose	31.00	Irregular, smooth and dull whitish growth. More pycnidial production in centre as compared to periphery.	0.00	0.00
6	Peptone dextrose rose bengal agar	0.00	No growth observed.	0.00	0.00
7	Asthana and Hawker's	47.16	Irregular, dirty white, flat growth and more pycnidial production noticed in centre where as at periphery they just initiated and their colour was whitish.	0.58	0.05
	SE(m)	**3.10**		**0.09**	**0.02**
	CD(P=0.05)	**9.40**		**0.29**	**0.07**

medium. Significantly higher (0.15 g) dry mycelial weight of *M. phaseolina* was observed in malt extract medium. However, it was significantly lower (0.02 g) recorded in potato dextrose medium followed by asthana and hawker's (0.05 g) and richard's medium (0.05 g).

The results obtained in the present investigation are confirmed with the studies of CsÖndes *et al.* (2006). They reported malt extract medium and potato dextrose agar medium as the best media for culturing of *M. phaseolina*. Potato dextrose agar medium as good medium for growth and sclerotial formation of *M. phaseolina* was also reported Salunkhe *et al.* (2009), Sharma *et al.* (2004) and Surichandraselvan and Seetharaman (2003).

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