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Morphological variability of *Macrophomina phaseolina* (Tassi) Goid, causal agent of dry root rot disease of chickpea (*Cicer arietinum* L.)

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ABSTRACT

To find out the best sources of nutrients for the fungal growth, different solid media were tested. Maximum radial growth was recorded on PDA and Czapek's agar with mean colony diameter of 90mm and on par with each other compared to other media tested. Carrot agar recorded maximum number of sclerotial production (48 per microscopic field) followed by PDA (46 per microscopic field) and the minimum sclerotial production was observed in Czapek's agar (39.33 per microscopic field). PDA and carrot media supported good mycelial growth and sclerotia production. The maximum sclerotial size was observed in PDA (0.5×0.4 µm) whereas Czapek's agar recorded minimum size of sclerotia (0.3×0.2 µm). Ellipsoidal shape of sclerotia was observed in Czapek's agar whereas, irregular shaped sclerotia were observed in other media. Maximum dry mycelial weight was recorded eighth day of incubation (150mg).

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the major legume crop widely grown in the Indian sub continent. India is the largest producer of chickpea accounting for about 75 per cent of world production that grown in an area of about 8.21 million ha producing 7.48 million tonnes with the productivity of 911 kg/ha. Karnataka is one of the major chickpea producing states in the country and it is grown over an area of 0.97 m /ha with a production of 0.57 million tonnes having an average productivity of 622 kg/ha during *Rabi* 2009-10 (Viswanatha *et al*., 2011). The production of chickpea in the Indian sub-continent and in other Asian countries is severely affected by many plant pathogenic fungi, bacteria, viruses, and nematodes which cause the diseases such as Fusarium wilt, dry root rot, *Ascochyta* blight, collar rot, bacterial blight, filiform virus and root nematode (Nene and Sheila, 1996). Now a day's dry root rot caused by *Macrophomina phaseolina* is the most important soil borne disease is becoming severe in most of the chickpea growing regions of India. Hence, an attempt was made to find out the best sources of nutrients for the fungal growth by using different media. The solid media was used to determine the radial growth of the fungus whereas; liquid media was used to determine the dry mycelial weight of the fungus.

MATERIAL AND METHODS

For identifying the best medium for the dry root rot pathogen the following eight different media were evaluated *viz.*, Potato dextrose agar, Malt extract agar, Czapek's agar, Oat meal agar, Sabouraud's agar, Host seed extract agar, Carrot agar and Richard's agar media.

Solid media :

Twenty ml of each solid media was poured into 90 mm diameter Petriplates. Inoculation was made by transferring half a cm disc of mycelium taken from periphery of seven days old culture of *M. phaseolina*. The plates were incubated at 27 ± 1°C for five days. For each different media, differences in topography, rate of growth, colour, number of sclerotial bodies formed per microscopic field (10x) at three spots in a Petriplates were noted. The sizes of the sclerotial bodies were recorded by using standard stage and ocular micrometer. The results were analyzed statistically.

Liquid media :

Twenty ml of PDB was poured into each of the 150 ml conical flasks and the flasks were sterilized at 1.1 kg/cm^2 for 20 min. Inoculum disk of five mm diameter from periphery of seven day old culture were transferred aseptically into the PDB flasks separately. Each treatment was replicated twice. The flasks were incubated at 27 ± 1°C. Twice flasks were harvested separately, starting from second day onwards upto 15th day by leaving a gap of 24 hr between the two successive harvests. The cultures were filtered through previously weighed Whatmann number 42 filter paper discs of 90 mm diameter which were dried to constant weight at 60°C in an electric oven prior to filtration. The mycelial mat on the filter paper was thoroughly washed with sterile distilled water to get rid of the salts likely to be associated with the mycelium. The filter paper along with the mycelial mat were dried to a constant weight at 60°C and weighed immediately on an analytical balance. The differences between final and initial weight of filter paper discs was taken as weight of the mycelia. The data were analyzed statistically.

RESULTS AND DISCUSSION

The mycelium was pale white in colour in the initial stages of the growth but later turned to dark brown to black as and when sclerotial formation started. Mycelia showed right angled branching. The sclerotia varied in shape and size with growth pattern of scattered to clustered (Plate 1). These observations are in line with the description provided by Butler for Pycnidial stage *M. phaseolina* and other characters described by Ashby (1927) and Manjunatha (2009).

There was a significant variation in radial growth of *M. phaseolina* recorded among eight media. Significantly maximum radial growth was recorded on PDA and Czapek's agar with mean colony diameter of 90 mm and at par with each other compared to other media tested. On six days of incubation followed by host extract agar with mean colony diameter of 88.83 mm and least mean colony diameter of 84.33 mm was observed in Richard's agar (Table 1 and Plate 2).

There were slight variations in sclerotia production on all the eight media. Among the eight media, carrot agar recorded maximum number of sclerotial production (48 per microscopic field) followed by PDA with sclerotial production (46 per microscopic field) and the minimum sclerotial production was observed in Czapek's agar (39.33 per microscopic field).

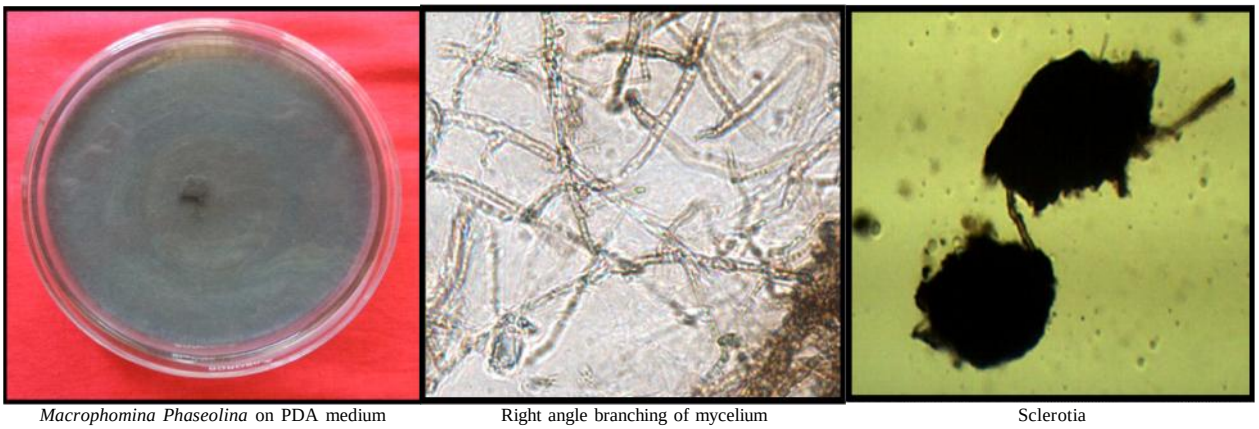
Slight variations were also observed with respect to colony colour, type of margin, topography, size and shape of the sclerotia on different solid media on the end of sixth day of incubation. The fungus showed mycelium having blackish colour with flat growth on all the media tested except the colony colour which was light blackish colour in oat meal agar. All the media recorded sclerotial production of more than 40 sclerotia per microscopic field except Czapek's agar which recorded less than 40 sclerotia per microscopic field and type of margin in all eight media was uniform. Sclerotial size ranged from 0.3 × 0.2 µm to 0.5× 0.4 µm. The maximum sclerotial size

Table 1 : Effect of solid media on radial growth and sclerotia production of *Macrophomina phaseolina* from chickpea

Sr. No.	Media	Radial growth in mm	No. of Sclerotia per microscopic field at 10x
1.	Potato dextrose agar	90.00	46.00
2.	Malt extract agar	86.33	42.66
3.	Czapek's agar	90.00	39.33
4.	Oat meal agar	86.66	42.00
5.	Sabouraud's agar	85.33	45.66
6.	Host seed extract agar	88.33	45.33
7.	Carrot agar	87.67	48.00
8.	Richards agar	84.33	44.00
	Mean	87.33	44.12
	S.E. ±	1.11	1.006
	C.D. (P = 0.01)	3.35	3.018

was observed in PDA (0.5× 0.4 µm) whereas Czapek's agar recorded minimum size of sclerotia (0.3 × 0.2 µm). Shape of sclerotia varied from irregular to ellipsoid, ellipsoidal shape of sclerotia was observed in Czapek's agar whereas, irregular shaped sclerotia was observed in other media (Table 2).

The dry mycelial weight was recorded at different day intervals. As the incubation periods increased from second to seventh day, there was significant increase in growth and also mycelial weight from 18 mg to 141 mg. On eighth day after incubation period, maximum dry mycelial weight of 150 mg



Macrophomina Phaseolina on PDA medium | Right angle branching of mycelium | Sclerotia

Plate 1 : *Macrophomina phaseolina* on PDA medium and its morphological characters

Table 2 : Growth characteristics of *Macrophomina phaseolina* on different solid media

Media	PDA	MEA	CZA	OMA	SA	HEA	CA	RA
Colony colour	Blackish	Blackish	Blackish	Light blackish	Blackish	Blackish	Blackish	Blackish
Type of growth	Flat growth	Flat growth	Flat growth	Flat growth	Flat growth	Flat growth	Flat growth	Flat growth
Type of margin	Uniform	Uniform	Uniform	Uniform	Uniform	Uniform	Uniform	Uniform
Sclerotial production	46.00	42.66	39.33	42.00	45.66	45.33	48.00	44.00
Size of sclerotia (µm)	0.5×0.4**	0.3×0.3	0.4×0.3	0.3×0.3	0.4×0.3	0.3×0.3	0.3×0.2	0.4×0.3
Shape of sclerotia	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Ellipsoid	Irregular

PDA- Potato dextrose agar, MEA- Malt extract agar, CZA- Czapek's agar, OMA – Oat meal agar, HEA- Host extract agar, CA- carrot agar, RA- Richards agar; ** Size of sclerotia taken length × breadth

Table 3 : Growth phase of *Macrophomina phaseolina* in potato dextrose broth

Days after incubation	Mean dry mycelial weight (mg)
2	18
3	33
4	36
5	74
6	124
7	141
8	150
9	141
10	139
11	128
12	123
13	88
14	40
15	31
Mean	985
S.E.±	1.10
C.D. (P = 0.01)	3.35

Front view Dorsal view

Plate 2 : *Macrophomina phaseolina* on different solid media

was recorded, whereas from ninth day to fifteenth day the dry mycelial weight decreased from 141 mg to 31 mg, due to autolysis (Table 3).

Present findings are in conformity with earlier studies conducted by several workers (Anamika and Khare, 2002; Ramaprasad Shresti, 2005 and Sachidananda, 2005). However, these findings are not in agreement with that of Satishchandra (1977) who noticed maximum growth was observed at 11th day incubation period. In the present investigation, maximum dry mycelial weight was observed at eighth day after incubation, Bengaluru isolate may be virulent or adaptation to environmental conditions hence it contributed towards maximum growth on eighth day after incubation itself.

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