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EVALUATION OF DIFFERENT SOLID AND LIQUID MEDIA FOR THE GROWTH AND SCLEROTIAL FORMATION OF *MACROPHOMINA PHASEOLINA* (TASSI) GOID *IN VITRO*

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ABSTRACT
Eight different media including synthetic and semi synthetic in solid and liquid state were tested for their suitability to the growth and sclerotial formation of the fungus, *Macrophomina phaseolina*. The growth of *M. phaseolina* was significantly more on potato dextrose agar (89.67mm) as compared to the rest but was at par with potato carrot sucrose agar (86.67mm). The sclerotial formation of *M. phaseolina* was high on potato dextrose agar, potato carrot sucrose agar and Richard's agar. Among the various liquid media tested, significantly higher dry mycelial weight was yielded in Richards' solution (776.25mg) as compared to the rest of the liquid media but was at par with Czapek's Dox solution (758.68mg). The sclerotial formation was found high level on Richards' solution, Czapek's Dox solution, potato dextrose broth and potato carrot sucrose broth.

INTRODUCTION

Greengram (*Phaseolus aureus* Roxb.) is one of the important pulse crops, primarily grown for food in India. During the survey, occurrence of leaf blight disease in greengram was noticed in serious proportion inflecting heavy losses around Navsari area. Cultivar GM-2K-5 was found more severely affected in the area during kharif and summer seasons. Considering the seriousness of the problem, the present investigation was carried out to pinpoint exact cause and to find out suitable control measures for the disease. With a view to find out the suitable media for the growth and sclerotial formation of the fungus, three semi-synthetic and five synthetic media solid and broth were studied. Several culture media showed differential effects on the growth and cultural characteristics of different isolates of *M. phaseolina* infecting various host plants (Ratnoo and Bhatnagar, 1991; Singh and Kaiser, 1994). Mukhopadhyay and Nandi (1975); Sahi *et al.* (1992) reported maximum growth of the pathogen (*M. phaseolina*) on potato dextrose agar. Jha and Dubey (2000) recorded the best radial growth and excellent sclerotium formation of *M. phaseolina* on potato dextrose agar medium while maximum mycelial and sclerotial production was recorded on Richard's medium (liquid) when these were incubated at 26 ± 4ºC temperature. Vidhyasekharan and Arjunan (1978) reported that no pycnidia were formed in the agar medium. The pycnidial stage was never produced in culture (Grover and Sakhuja, 1981).The paper deals with suitability of media for the growth and sclerotial formation of fungus.

MATERIALS AND METHODS

With a view to find out the suitable media for the growth and sclerotial formation of the fungus, three semi-synthetic and five synthetic media solid and broth were studied are as under.

(A) Semi-synthetic media

Potato Dextrose Agar (PDA); Potato Carrot Sucrose Agar (PCSA); Oat meal agar

(B) Synthetic media

Czapeck's Dox Agar (CzDA); Richard's Agar (RA); Asthana and Hawker's Agar (A and HA); Elliot's Agar (EA); Brown's Agar (BA)

Agar agar based medium (solid media)

These agar agar based sterilized media were poured into 9cm diameter sterilized petriplates @ 20mL/plate and the petriplates were inoculated aseptically after cooling by placing 5mm diameter culture block in the centre, cut aseptically with cork borer from 5 days old pure culture of *Macrophomina phaseolina*. Three replications were kept for recording observations on colony diameter, sclerotial formation and colony character of the fungus. The observations were recorded on 5th day of inoculation. The petriplates were incubated at room temperature. The data were statistically

analysed. The sclerotial count was recorded after 5 days of incubation. Ten culture blocks of 5mm diameter were suspended in 20mL sterile distilled water, homogenized and a drop from such filterate was examined under microscope. The number of sclerotia per microscopic (10X) field was counted. The sclerotial count was grouped as: - = No; + = 10-20; ++ = 21-30 and +++ = above 30 sclerotia per microscopic field in respect of the sclerotial number (Das, 1988).

Liquid media

All the solid media used in earlier section were used as broth media with the same ingredients only omitting agar agar. These broths were poured into 100mL cap conical flasks containing 50mL medium per flask. The flasks were plugged with non-absorbent cotton and autoclaved at 1.2kg cm^{-2} pressure for 20 minutes for sterilization. The flasks were inoculated aseptically by placing 5 mm diameter culture block, cut aseptically with a cork borer from 5 days old pure culture. Four replications were maintained for recording mycelial growth and sclerotial formation. The flasks were incubated at room temperature. After 10 days of inoculations mycelial mats were harvested on previously weighted, oven dried Whatman's filter paper no. 42 giving sufficient washing with warm (80ºC) distilled water. The filter papers with mycelial mats were dried in an oven at 60ºC till constant weight was obtained. The observations were recorded to compare the dry mycelial weight. The data were statistically analysed. The sclerotial count was recorded from fourth replication. At the end of incubation period, the whole mycelial substrate was homogenized in 50mL sterile distilled water with the help of homogenizer. A drop of suspension was examined under microscope (10X). The number of sclerotia per microscopic field was counted. The sclerotial formation was grouped as: - = No; + = 10-20; ++ = 21-30 and +++ = above 30 sclerotia per microscopic field in respect of the sclerotial number (Das, 1988).

RESULTS AND DISCUSSION

Eight different media including synthetic and semi synthetic in solid and liquid state were tested for their suitability to the growth and sclerotial formation of the fungus, *M. phaseolina*. The results on the growth and sclerotial formation and the colony/cultural characters of the fungus recorded in different solid media are presented in Tables.

Table 1: Effect of different solid media on growth and sclerotial formation of *M. phaseolina in vitro*

S. No.	Name of the media	Average diameter of pathogen(mm)	Sclerotial formation
1	Potato dextrose agar	89.67	+ + +
2	Potato carrot sucrose agar	86.67	+ + +
3	Richards' agar	79.67	+ + +
4	Oat meal agar	74.33	+ +
5	Asthana and Hawker's agar	68.33	+ +
6	Elliot's agar	61.33	+
7	Brown's agar	58.33	+
8	Czapek's Dox agar	55.00	+
S.Em. ±		1.84	
C.D. at 5 %		5.58	
C.V. %		4.44	

Sclerotial formation (no. of sclerotia per microscopic field); No sclerotial formation; + low level (10-20); + + Medium level (21-30); + + + High level (more than 30)

Table 2: Effect of different liquid media on growth and sclerotial formation of *M. phaseolina in vitro*

S. No.	Name of the media	Dry mycelial weight (mg)	Sclerotial formation
1	Richards' solution	2.89* (776.25)**	+ + +
2	Czapek's Dox solution	2.88 (758.68)	+ + +
3	Potato dextrose broth	2.78 (602.56)	+ + +
4	Potato carrot sucrose broth	2.65 (446.68)	+ + +
5	Oat meal broth	2.58 (380.19)	+
6	Asthana and Hawker's solution	2.42 (263.03)	+ +
7	Elliot's solution	2.13 (134.89)	+
8	Brown's solution	2.01 (102.33)	+ +
	S.Em. ±	0.013	
	C.D. at 5 %	0.039	
	C.V. %	0.89	

* Figures indicate logarithmic transformed values; ** Figures in parenthesis indicate retransformed values; Sclerotial formation (no. of sclerotia per microscopic field); No sclerotial formation; + low level (10-20); + + Medium level (21-30)

Solid media

Among the various solid media tested, the growth of *M. phaseolina* was significantly more on potato dextrose agar (89.67mm) as compared to the rest but was at par with potato carrot sucrose agar (86.67mm). Next best in order of merit was Richards' agar (79.67mm) followed by oat meal agar (74.33mm). The rest of the media viz., Asthana and Hawker's agar (68.33mm), Elliot's agar (61.33mm), Brown's agar (58.33mm) and Czapek's Dox agar (55.00mm) also supported good growth. Thus, among the semi-synthetic media, potato dextrose agar, potato carrot sucrose agar and among the synthetic media, Richard's agar medium proved better for the growth of the pathogen followed by oat meal agar. The sclerotial formation of *M. phaseolina* was high on potato dextrose agar, potato carrot sucrose agar and Richard's agar. It was found medium level on oat meal and Asthana and Hawker's while low in Brown's, Elliot's and Czapek's Dox agar media. The pycnidial stage was not produced in any of the media tested.

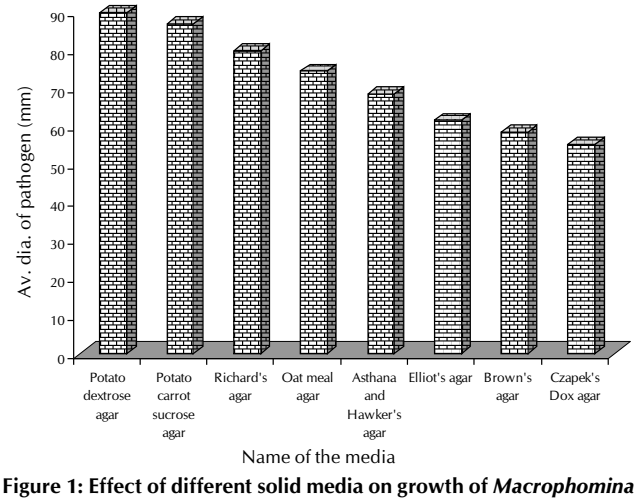

Figure 1: Effect of different solid media on growth of *Macrophomina phaseolina in vitro*

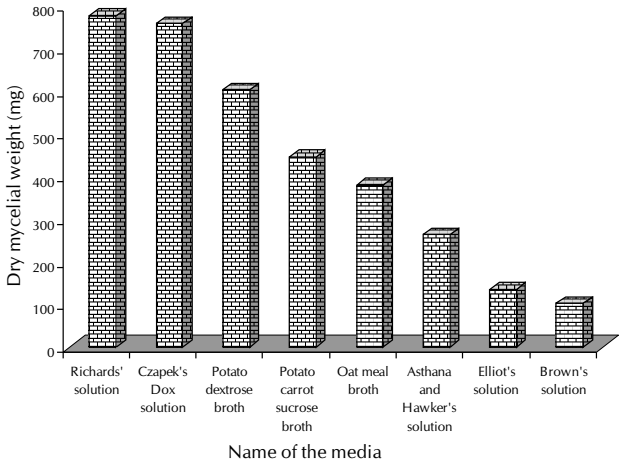

Figure 2: Effect of different liquid media on growth of *Macrophomina phaseolina in vitro*

Table 3: Colony/Cultural characteristics of *M. phaseolina* in different solid media

Name of media	Colony characters
Potato dextrose agar	Fluffy growth, mycelium dirty white and carbon blackish substratum
Potato carrot sucrose agar	Fluffy growth, mycelium dirty white, blackish substratum
Richards' agar	Fluffy growth, mycelium dirty white, black substratum
Oat meal agar	Slightly flat and fluffy growth, mycelium dirty white, dark grayish substratum
Asthana and Hawker's agar	Fluffy growth, mycelium dirty white and blackish substratum
Elliot's agar	Sparse growth, slightly aerial mycelium, substratum blackish
Brown's agar	Flat, sparse growth, mycelium dirty white later turn black
Czapek's Dox agar	Flat slightly dirty white aerial mycelium

The results presented in Table indicated that the fungus showed fluffy growth with dirty white mycelium and blackish substratum in potato dextrose, potato carrot sucrose, Richards' and oat meal media.

This result supported the findings of Sahi *et al*. (1992) who reported potato dextrose agar as the best medium for the growth of *M. phaseolina*. Jha and Dubey (2000) also reported that the best radial growth and excellent sclerotial formation of *M. phaseolina* occurred on potato dextrose agar medium. Suriachandraselvan and Seetharaman (2003) reported that the mycelial growth of *M. phaseolina* was highest in potato dextrose agar medium followed by Richards' agar and oat meal while the lowest growth was recorded in Czapek's Dox agar medium. The pycnidial stage was never produced in culture (Grover and Sakhuja, 1981).

Liquid media

Among the various liquid media tested, significantly higher dry mycelial weight was yielded in Richards' solution (776.25mg) as compared to the rest of the liquid media but was at par with Czapek's Dox solution (758.68mg). The next best medium in order of merit was potato dextrose broth (602.56mg). The dry weight of mycelium was moderately good in potato carrot sucrose broth (446.68mg) followed by oat meal (380.19mg) and Asthana and Hawker's solution (263.03mg) while in Elliot's solution (134.89mg) and Brown's solution (102.33mg), it was very poor.

Jha and Dubey (2000) and Suriachandraselveran and Seetharaman (2000) also found maximum mycelial and sclerotial production of *M. phaseolina* on Richard's medium followed by Czapek's Dox broth. It was minimum in oat meal broth. The present finding tallies with these.

Thus, among the various solid media, potato dextrose agar and potato carrot sucrose agar were the best solid media whereas Richards' solution and Czapek's Dox solution were the best liquid media for the mycelial growth and sclerotial formation of *M. phaseolina*.

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