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Cultural studies of *Macrophomina phaseolina* causing Root Rot disease of Groundnut (*Arachis hypogaea* L.)

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Abstract
Groundnut is an important oil seed crop. It is attacked by large number of soilborne fungal diseases amongst them the dry root rot is caused by *Macrophomina phaseolina.* It is an important soilborne pathogen. It causes severe damage to crops. The aim of present investigation was to study cultural variations of *Macrophomina phaseolina* isolated from rhizosphere soil of groundnut on different media. The different media influences the radial growth, texture, colony colour, pigmentation, biomass, sclerotia formation and sclerotia diameter. The most suitable medium for the growth and biomass production are Potato Dextrose Agar followed by Czapek's Dox Agar. The minimum growth was observed on nutrient agar and there was no sclerotia formation.

Keywords: cultural variations, *Macrophomina phaseolina*, dry root rot and groundnut

Introduction
Groundnut is one of the most important leguminous oil seed crops of our country (K. S. Jadem *et al.*). India is the second largest producer of groundnut. This crop having a high nutritive value and along with that it increases the soil fertility by fixing the nitrogen (Rajamohan, K and Balabaskar, P.). Groundnut is the primary source of vegetable oil and it occupies 43% of total oil production. Groundnut is known as king of oil seed (Sridevi *et al.* 2011) [13].

Groundnut is attacked by large number of soilborne diseases among them the dry root rot is caused by *Macrophomina phaseolina* (Moradia, 2011) [1]. This pathogen having a wide host range and it infects more than 500 economically important crops such as soybean, corn, sorghum, cotton, cowpea and groundnut (Mame *et al*, 2014). It induce the diseases to host plants like seedling blight, damping off and stem rot which result in decreased stem height, girth as well as weight and also death of affected plant. This pathogen favors high soil temperature range from 30º C to 42º C and low humidity or deficiency of water (Kumar and Thirumalai (2015) [3], Shekhar *et al.* (2015) [5] and Stojsin *et al.*).

Dry root rot is serious problem in Rajasthan, Uttar Pradesh, Tamil Nadu, Andhra Pradesh and Maharashtra. The symptoms of dry root rot occurs on the stem just above the ground level as water soaked necrotic lesions on the young plant and turn dull brown, girdle the stem or hypocotyl and kill the plant. The older plants are infected near the soil surface; the infection spreads within root system as well as in the shoot system which is followed by wilting and also the roots and pods turned black due the infection. It kills the plants.

The *Macrophomina phaseolina* causes severe seedling mortality and it marks into a patch in standing crop and thus it results in the reduction of crop yield (P. subrahnyam *et al.* 2011). The genus *Macrophomina phaseolina* was studied by different workers for their genetic diversity, morphological and pathogenic variability but the cultural and morphological study of fungal variations on different culture media was less. The aim of a present investigation was to study the cultural and morphological variations on different media by observing characters like colony colour, texture, radial growth, pigmentation and microsclerotial production of *Macrophomina phaseolina*.

Material and methods
Isolation of pathogen
The pathogen was isolated from rhizosphere soil of groundnut collected from Marathwada region. It was isolated by soil dilution method. The selected pathogen was aseptically transferred to Potato Dextrose Agar plates for purification and maintained on Potato Dextrose Agar slant for further study. The identification was done through the microscopic observations and confirmed by using standard manual (Nagamani and C.V. Subhramaniyan, 1971) [8].

Culture media
The cultural variations were observed on six solid media namely Corn Meal Agar, Czapek Dox Agar, Glucose Nitrate Agar, Malt extract Agar, Nutrient Agar and Potato Dextrose Agar and five different liquid media such as Czapek Dox broth, Glucose Nitrate broth, Malt extract broth, Nutrient broth and Potato Dextrose broth.

Cultural variability
20ml of each sterilized medium was poured into petriplates and after solidification the mycelial disc from seven day old culture of *Macrophomina phaseolina* was inoculated. Three replications for each medium were incubated at 28^0 C ± 2^0 C. After each 24 hour intervals colony diameter and subsequently three days of incubation the colony characters like colony colour, pigmentation, texture and margin were recorded (Table No. I). for the microscopic observations the plates were incubated up to seven days. To observe the variation in dry

weight of mycelia, 50 ml of each liquid medium was taken into 100 ml conical flask. After inoculation the flask was incubated at 28^0 C ± 2^0 C for seven days and then it was filtered through Whatman No. 1 filter paper. The mycelial mat along with filter paper was dried and weight of dry mycelium on each medium was recorded.

Table 1: Radial growth of *Macrophomina phaseolina* on different growth media

Sr. No.	Media	24 hr.	72 hr.	120 hr.	Mean
1	Corn Meal Agar	3.3	4.9	6.7	4.9
2	Czapek Dox Agar	4.4	7.0	8.5	6.6
3	Glucose Nitrate Agar	2.1	4.8	5.5	4.1
4	Malt Extract Agar	4.9	8.5	9.0	7.4
5	Nutrient Agar	3.5	5.1	6.9	5.1
6	Potato Dextrose Agar	6.4	9.0	9.0	8.1

Table 2: Dry weight of *Macrophomina phaseolina* on different medium

Sr. No.	Media	Dry Weight in mg
1	Czapek Dox broth	965
2	Glucose Nitrate broth	526
3	Malt Extract broth	723
4	Nutrient broth	101
5	Potato Dextrose broth	833

Table 3: Colony characters of *Macrophomina phaseolina* on different medium

Sr. no.	Media	Colony Characters			Sclerotia formation
		Texture	Surface Colour	Reverse colour	
1	Corn Meal Agar	Slightly flat cottony	Neutral Grey	Toner Grey	+++
2	Czapek Dox Agar	Fluffy cottony	Warm Grey	Beet Purple	++++
3	Glucose Nitrate Agar	Flat cottony	Light Grey	Green Grey	++
4	Malt extract Agar	Fluffy cottony	Grey	Walnut	+++
5	Nutrient Agar	Sparse cottony	Ivory	Ivory	- - - -
6	Potato Dextrose Agar	Fluffy cottony	Clay Grey	Slate colour	++++

++++ = Heavy; +++ = Moderate and ++ = poor.

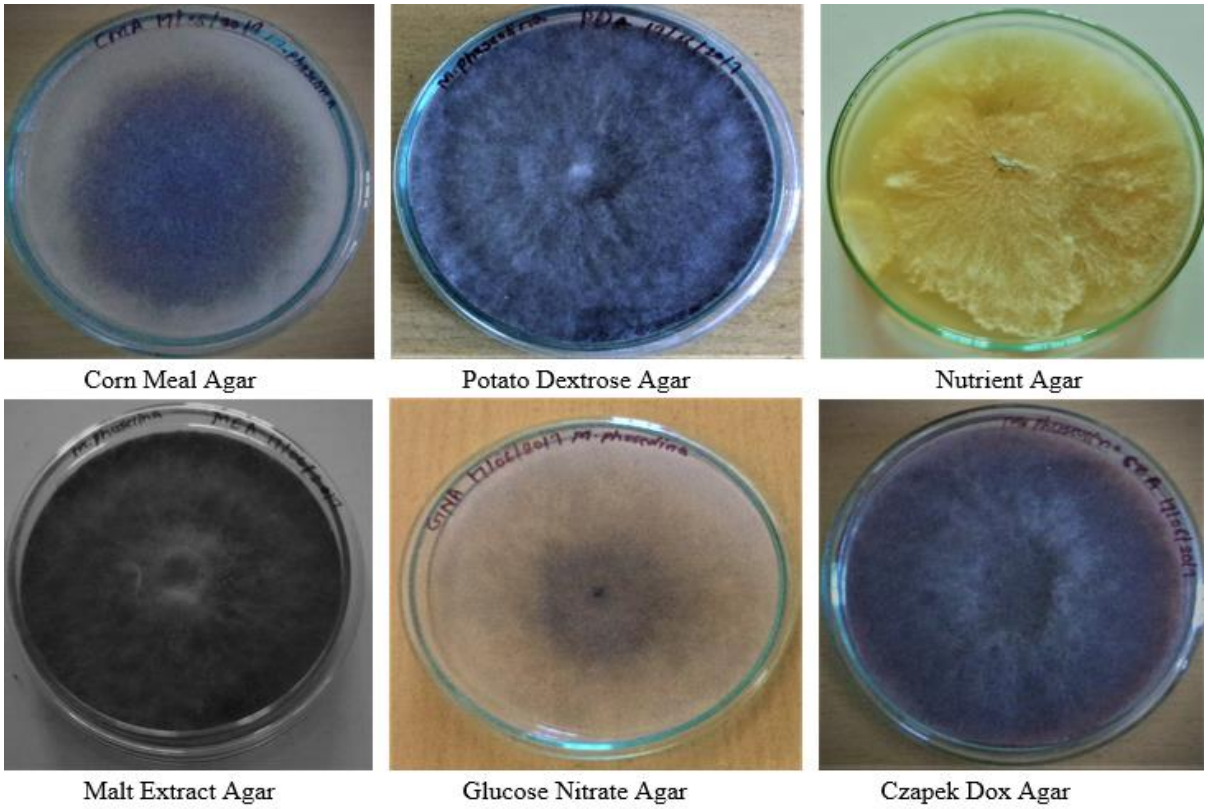

Fig 1: Cultural Variations on Different culture media

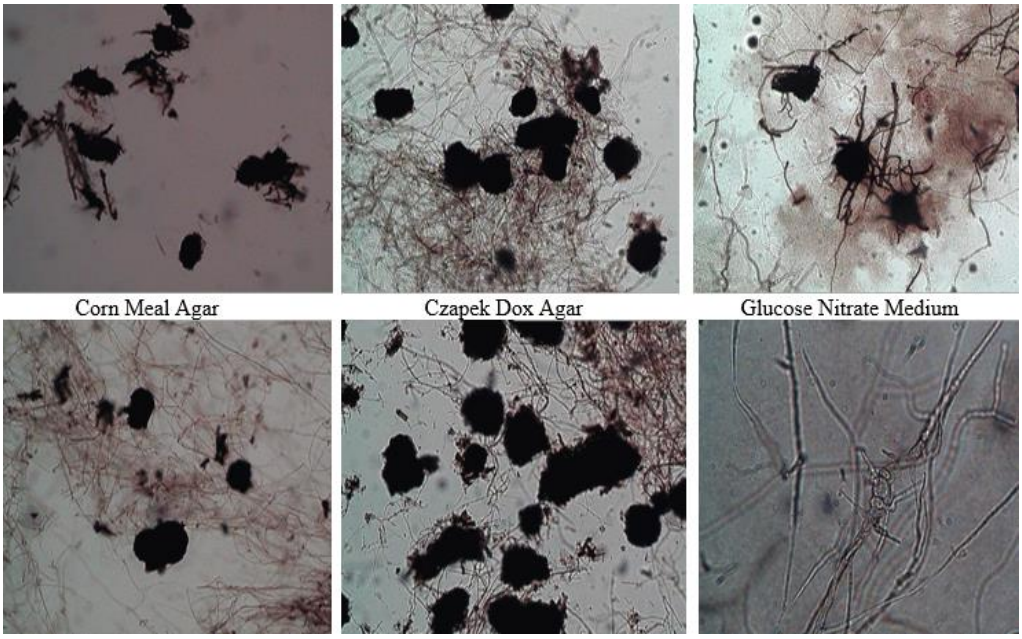

Fig 2: Sclerotial Production on Different culture media

Result and Discussion

The results revealed that there was difference in radial growth, colony texture, colony colour, sclerotia formation and mycelial weight of *Macrophomina phaseolina*. These cultural variations were studied by using different six solid media and five liquid media. There was a significant difference in radial growth on different culture media. The maximum radial growth was observed on Potato Dextrose Agar (8.1 cm) followed by Malt Extract Agar (7.4 cm), Czapek Dox Agar (6.6 cm), Nutrient Agar (5.1 cm), Corn Meal Agar (4.9 cm) and least growth was observed on Glucose Nitrate Agar (4.1 cm). The maximum radial growth was 9 cm. It was firstly recorded on Potato Dextrose Agar medium and secondly on Malt Extract Agar. It was followed by Czapek Dox Agar (8.5 cm) and Nutrient Agar (6.9 cm). Amongst all culture media the Potato dextrose Agar was significant medium followed by Malt Extract Agar. The minimum radial growth (2.1cm) was recorded on Glucose Nitrate Medium and it was followed by Nutrient Agar (3.5cm). These two media are less efficient for the radial growth. The radial growth was influenced by different culture media with respect to incubation period. The Malt Extract Agar shows maximum growth 9 cm after 72 hours but in case of Potato Dextrose Agar the same growth was reported after 48 hours. The effect of incubation period was observed on radial growth of fungi on all medium, this was due to the difference in media composition (Table No. I).

There were three different types of colony texture of *Macrophomina phaseolina* such as fluffy, flat and sparse cottony. The fluffy cottony growth was seen on Czapek Dox Agar, Potato Dextrose Agar and Malt Extract Agar media. On Glucose Nitrate Agar a slightly flat cottony growth was recorded and a flat cottony growth on Corn Meal Agar, however sparse cottony growth was obtained on Nutrient Agar.

There was a quite variations in the production of surface and reverse colony colour on different media such as a grey coloured mycelium on the Malt Mxtract Agar, clay grey coloured mycelium on the Potato Dextrose Agar, light grey coloured mycelium on Glucose Nitrate Agar, warm grey coloured mycelium on Czapek Dox Agar and neutral grey coloured mycelium on Corn Meal Agar. From all the medium Nutrient Agar was revealed the different mycelial colour i.e. ivory. Likewise there were variations observed in the production of reverse colour on different media (Table No. – III).

The sclerotia formation was checked after seventh day. Among all media maximum sclerotia formation was observed on Potato Dextrose Agar medium. It was followed by Czapek Dox Agar and Malt Extract Agar. The minimum production of sclerotia was observed on Glucose Nitrate Agar Medium and Corn Meal Agar medium there was no sclerotia formation on Nutrient Agar medium (Photoplate – B).

The dry weight of mycelia was studied on five different liquid media. From all the media maximum mycelial dry weight was obtained on Czapek Dox broth (965 mg) followed by Potato Dextrose broth (833mg), Malt Extract broth (723 mg), Glucose Nitrate broth (526mg) and least dry weight of fungal mycelium was recorded on Nutrient broth (101 mg). For the dry weight of mycelium, Czapek Dox broth was the suitable medium followed by Potato Dextrose broth and Malt Extract broth but for the radial growth of mycelium, Potato Dextrose Agar was the best medium followed by Malt Extract Agar and Czapek Dox Agar. From all the media, these three media with slight fluctuations were beneficial for the radial growth as well as dry weight of mycelium of the selected fungi.

From all tested culture media Potato Dextrose Agar was the best medium for the mycelial growth. It was commonly used culture media and it has balanced nutrient for the mycelial growth and also for the sclerotia production similar findings were reported by Meera Gupta *et al.* (2012) [6] and N. M. Lokesh and V. I. Benagi (2003) [13]. The mycelial growth, colony characters like texture, surface and reverse colour and sclerotia formations were influenced by different culture media. The G. shrama and R. panday (2010) [2] observed the

similar effect of different culture media on fungal colonies isolated from decaying vegetable wastes like *Penicillium*, *Fusarium* and *Chaetomium*. The least mycelial growth and dry weight was recorded on Nutrient media. Similar results were found by Sahana Banakar (2017) [12] and Sahera Nasreen and Vijata Hase (2017) [15]. From obtained results we conclude that the different media influences the growth and dry weight of selected fungi.

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