Biological Forum – An International Journal **15(10): 679-683(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Impact of Culture Media and Temperature on Growth and Sclerotial Formation of *Macrophomina phaseolina* (Tassi) Goid. causing dry Root Rot of Mothbean

Megha Jaimini[1*], J.R. Verma[2], Dama Ram[3], Surjeet[1] and Govind Junjadia[1]
[1]Ph.D. Scholar, Department of Plant Pathology,
College of Agriculture, Jodhpur, Agriculture University, Jodhpur (Rajasthan), India.
[2]Professor, Department of Plant Pathology,
College of Agriculture, Jodhpur, Agriculture University, Jodhpur (Rajasthan), India.
[3]Assistant Professor, Department of Plant Pathology,
College of Agriculture, Jodhpur, Agriculture University, Jodhpur (Rajasthan), India.

(Corresponding author: Megha Jaimini)*
(Received: 07 August 2023; Revised: 01 September 2023; Accepted: 24 September 2023; Published: 15 October 2023)
(Published by Research Trend)

ABSTRACT: *Macrophomina phaseolina* causal agent of dry root rot and charcoal rot in various pulses crops. Due to fluctuation of temperature and climatic disturbance in arid region is quite difficult to manage the dry root rot. To reduce the number of sclerotia in soil or to minimize the contact of the inoculums and the host, six different culture media and temperature were tested for their suitability on mycelial growth and sclerotial formation of the *M. phaseolina in vitro* conditions. Among the six media PDA recorded highest (90.00 mm) followed by Richard's agar (86.75 mm) media best for mycelial growth as well as for sclerotial formation. While, minimum growth (43.00 mm) was recorded in Sabouraud's agar medium. Six different temperature levels *viz.*, 15, 20, 25, 30, 35 and 40ºC were used for the study. The results indicated that the mycelial growth of *M. Phaseolina* was significantly grew best at 30ºC temperature (90.00 mm) followed by 35ºC temperature (84.50 mm), which was reduced significantly below 15°C and above 40°C.

Keywords: Mothbean, media, temperature, sclerotia, PDA, *Macrophomina phaseolina*.

INTRODUCTION

Mothbean [*Vigna aconitifolia* (Jacq.) Marechal], 2n = 22 a drought resistant legume belongs to family *Fabaceae*. It is commonly grown in arid and semi arid region of India and locally known as matbean, mothbean, matki, Turkish gram or Dew bean. It has an exceptionally hardy and thermo tolerance legume qualities, due to this it is very commonly survive in arid region and helps prevent soil erosion by conserving soil moisture (Sharma *et al*., 2014). Mothbean is having areas under cultivation in India, Pakistan and Myanmar (Bisht and Singh 2013) globally, India is the key grower and producer with an area more than1.5 million hectares, mostly confined to Rajasthan, Gujarat, Uttar Pradesh, Haryana, Punjab, Madhya Pradesh, Maharashtra and Karnataka states with the production of 0.4 million tons with the productivity of 133 kg-^{1} (Anonymous, 2022). In India, Rajasthan and Gujarat is the foremost mothbean producing states (Sharma *et al*., 2016). In the state of Rajasthan, mothbean cultivation is prevalent in Bikaner, Barmer, Jodhpur, Jaisalmer and Churu districts.

Dry root rot or Charcoal rot is an emerging mothbean soil- borne disease incited by *Macrophomina phaseolina* (Tassi) Goid. has been identified as most destructive and economically important disease of western part of Rajasthan which cause 15-26% considerable yield losses in mothbean growing areas. (Anonymous, 2020). Maximum damage reported to be causes at seedling and seed maturity stage of the crop (Chaturvedi, 2017). The degree of infection upsurges with higher soil temperatures and low soil moisture. Germination of the microsclerotia occurs when the temperature is between 28-35 °C.

Macrophomina phaseolina is a soil borne fungus, under *in vitro* conditions, the fungus grows rapidly on potato dextrose agar medium. The mycelium of the fungus is dull white in colour that becomes brown to grey with age. The fungus may produce abundant aerial mycelium with branched septate hyphae bearing a number of sclerotia. The microsclerotia serve as the primary source of inoculums and have been found to persist within the soil up to three years (Sharma *et al.*, 2021). Root rot disease has not been controlled consistently and economically due to prolonged viability, unpredictable nature of fungal propagules (Khan, 2007; Ndiaye *et al*., 2010) and lack of knowledge about the components that influence the growth. Therefore, development of dry root rot due to fluctuation of temperature and climatic disturbance is prevalent in arid region and it is the need of the hour to strengthen the management tactics for management of root rot in mothbean. Keeping this in view, overcome the disease

prior to find out suitability of media and temperature for the growth and sclerotial formation of fungus through which easily carried out suitable control measures for the disease.

MATERIALS AND METHODS

The infected mothbean plant showing typical symptoms of root rot like yellowing, sudden wilting were collected from College farm Agriculture University, Jodhpur. Collected samples of infected plant were brought to the laboratory for further investigations. Studies of the following physiological aspects of *M. phaseolina* were conducted *in vitro*.

Effect of culture media and temperature on the growth of *Macrophomina phaseolina*

Effect of different culture media on growth of *M. Phaseolina.* With a view to find out the best medium for the growth and sclerotial formation of fungus, six media in solid states were compared. The compositions of various media used are as under.

1. Czapek's Agar Medium (CzAM)

Sodium nitrate ($NaNO_3$)	: 2.0 g
Dipotassium hydrogen orthophosphate (K_2HPO_4	: 1.00 g
Magnesium sulphate ($MgSO_4.7H_2O$)	: 0.50 g
Potassium chloride (KCl)	: 0.50 g
Ferrous sulphate ($FeSO_4.7H_2O$)	: 0.01 g
Sucrose ($C_6H_{22}O_{11}$)	: 3.00 g
Agar agar	: 20.00 g
Distilled water	: 1000 ml

2. Oat Meal Agar (OMA)

Oats	: 100.00 g
Agar agar	: 20.00 g
Distilled water	: 1000 ml

3. Peptone Sucrose Agar Medium (PSAM)

Sodium glutamate	: 1.0 g
Peptone	: 10.0 g
Sucrose	: 10.0 g
Agar agar	: 17.0 g
Water	: 1000 ml

4. Potato Dextrose Agar (PDA)

Peeled potato	: 200.00 g
Dextrose ($C_6H_{12}O_6$)	: 20.00 g
Agar agar	: 20.00 g
Distilled water	: 1000 ml

5. Richard's Agar Medium (RAM)

Potassium nitrate (KNO_3)	: 10.00 g
Potassium dihydrogen orthophosphate (KH_2PO_4)	: 5.00 g
Magnesium sulphate ($MgSO_4.7H_2O$)	: 2.5 g
Ferric chloride ($FeCl_3.6H_2O$)	: 0.02 g
Sucrose ($C_6H_{22}O_{11}$)	: 50.00 g
Agar agar	: 20.00 g
Distilled water	: 1000 ml

6. Sabouraud's Agar Medium (SAM)

Peptone	: 10.0 g
Dextrose	: 40.0 g
Agar agar	: 15.0 g
Water	: 1000 ml

The cultural study was conducted on different media in Petri plates (90 mm). Sterilized media (20 ml) was poured into each sterilized Petri plate. The plates were inoculated with fungal disc of 5 mm size cut from periphery of 7 days old pure culture of *M. phaseolina* and incubated at 27±1°C. Four replications were maintained for each culture medium. Colony diameter was measured diagonally after 3 days of inoculation. Mycelial growth and number of sclerotia were recorded after 7 days of incubation.

Table 1: Criteria used to record the sclerotial formation of *M. phaseolina.*

Sr. No.	Particulars	Sclerotial production/ microscopic field	Sign indication
1.	No sclerotial formation	Nil	–
2.	Poor sclerotial formation	1-20	+
3.	Moderate sclerotial formation	21- 30	++
4.	Good sclerotial formation	31-50	+++
5.	Excellent sclerotial formation	> 50	++++

Effect of different temperature on the growth of *M. phaseolina.* The growth of *M. phaseolina* was tested at six different temperature levels viz., 15, 20, 25, 30, 35 and 40°C was used for these studies. The temperature study was conducted on PDA in Petri plates (90 mm). Twenty ml of PDA was poured in each of sterilized Petri plate. Each Petri plate was inoculated aseptically by placing in the centre a 5 mm disc from actively growing 7 days old culture on PDA. The inoculated Petri plate was incubated at 15, 20, 25, 30, 35 and 40°C temperature for 7 days with four replications. Observation on mycelial growth was recorded after 7 days of incubation.

RESULT AND DISCUSSION

Effect of culture media and temperature on the growth of *Macrophomina phaseolina*

Effect of different culture media on growth of *M. phaseolina.* The results of the experiment revealed that the presented in (Table 2, Fig. 1 and Plate 1) among the six solid media tested, the highest mycelial growth was recorded on PDA (90.00mm) followed by Richard's agar (86.75 mm). The next best media in order of merit were Oat Meal Agar (72.37 mm), Czapek's agar (55.50 mm) and Peptone sucrose agar (46.00 mm) medium while, the lowest growth (43.00 mm) was obtained in Sabouraud's agar medium. The excellent sclerotial formation of *M. phaseolina* was observed on PDA and Richard's agar medium. Moderate sclerotial formation recorded on Oat Meal Agar, while good sclerotial formation was observed on Czapek's Agar and Sabouraud's Agar. Peptone Sucrose Agar revealed very poor sclerotial formation.

The results of present investigation are in agreement to the results obtained by earlier workers. Sobti and Sharma (1992) reported that the fungus was well grown on PDA medium with similar cultural characteristics of mycelium and sclerotial formation after 3rd and 7th day

of incubation, respectively. Suriachandraselvan and Seetharaman (2003) also reported the best radial growth and excellent sclerotial formation of *M. phaseolina* on PDA and Richard's agar medium.

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

Sr. No.	Media	Average colony diameter (mm)	Sclerotial formation*
1.	Czapek's agar	55.50	++
2.	Oat Meal Agar	72.37	+++
3.	Peptone Sucrose Agar	46.00	+
4.	Potato Dextrose Agar	90.00	++++
5.	Richard's Agar	86.75	++++
6.	Sabouraud's Agar	43.00	++

Average of four replications; *+ Poor,++ Moderate, +++ Good,++++Excellent

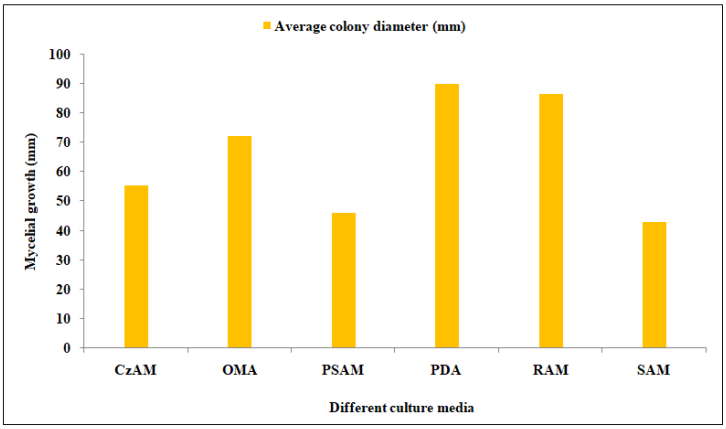

Fig. 1. Effect of different media on mycelial growth of *Macrophomina phaseolina in vitro*.

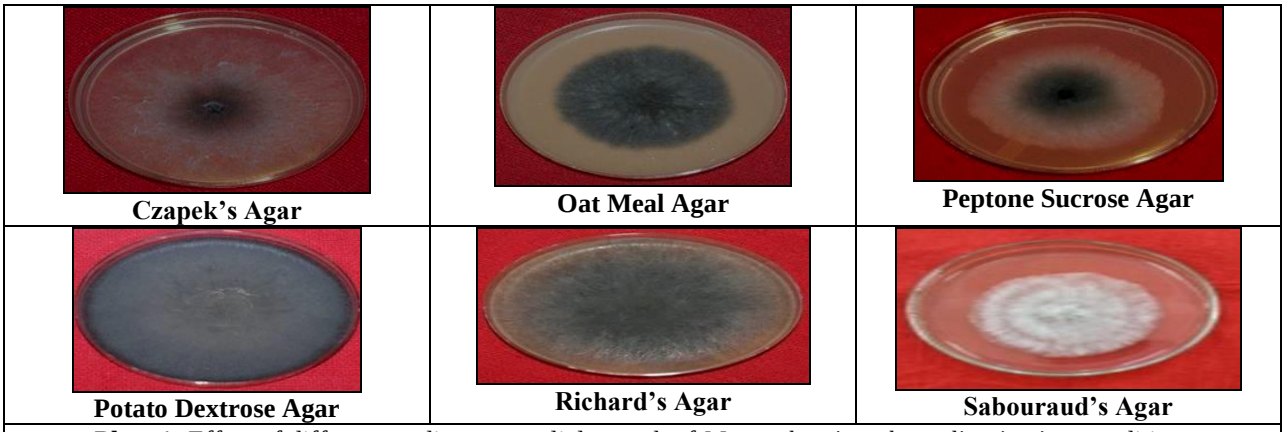

Plate 1: Effect of different media on mycelial growth of *Macrophomina phaseolina in vitro* condition.

Effect of different temperature on the growth of *M. phaseolina*. The results presented (Table 3, Fig. 2 & Plate 2) indicated that the mycelial growth of *M. phaseolina* was grew best at 30 ºC temperature (90.00 mm) followed by 35 ºC temperature (84.50 mm) and optimum growth (78.00 mm) at 25ºC on 7th day of incubation. There was very poor mycelial growth (37.82 mm) at 15 ºC temperature followed by (48.45) at 20ºC and above 35°C in all the test temperature on 7th day of incubation.

Jha and Sharma (2005) findings supported that the optimum temperature for *Rhizoctonia bataticola* was 30-35°C, both for mycelial growth and sclerotial formation. At 40°C temperature, very poor mycelial growth and no sclerotium was observed. Sandhu and Singh (1999); Sharma *et al*. (2004) also reported higher temperature ranges of 25-30 °C favored the growth of *M. phaseolina*. Naik *et al*. (2010) reported that 30 °C and 35 ºC was congenial for optimum mycelial growth (90 mm and 89.50 mm, respectively) of *Macrophomina phaseolina.*

Table 3: Effect of different temperature on mycelial growth of *M. phaseolina in vitro*.

Sr. No.	Temperature	Average mycelial growth (mm)
1.	15°C	37.82
2.	20°C	48.45
3.	25°C	78.00
4.	30°C	90.00
5.	35°C	84.50
6.	40°C	70.00

Average of four replications

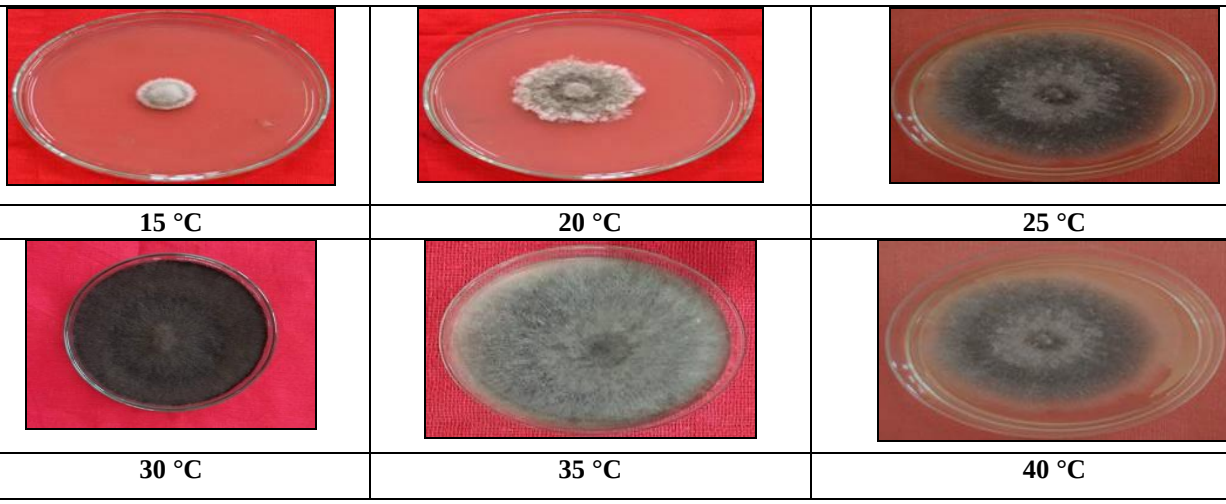

Plate 2. Effect of different temperature on mycelial growth of *Macrophomina phaseolina in vitro* conditions.

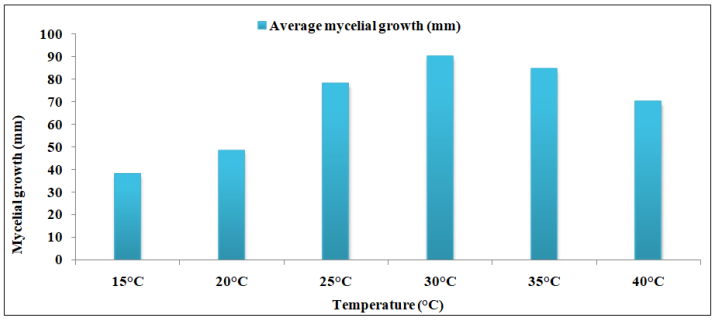

Fig. 2. Effect of different temperature on mycelial growth of *M. phaseolina in vitro.*

CONCLUSIONS

Overall the pathogen associated with dry root rot of mothbean was grow best on Potato dextrose agar media as well as excellent sclerotial formation on PDA at 30ºC temperature which was reduced significantly below 20°C and above 35°C in artificially conditions.

FUTURE SCOPE

Dry root rot of mothbean is a very serious disease in arid western region due to higher soil temperatures and low soil moisture. Integrated approaches for management, molecular techniques have to be employed for identification of pathogen and bio inoculants will be investigated in the lab as well as in the field are the potential way for its management in sustainable manner.

Acknowledgement. I extend my sincere thanks to Prof. (Dr.) J. R. Verma (major advisor) and to my advisory committee members for giving me proper suggestion in preparing the manuscript. I also sincerely thank College of Agriculture, Jodhpur, Agriculture university, Jodhpur for supporting the research financially.

Conflict of Interest. None.

REFERENCES

Anonymous (2022). Crop-wise fourth advance estimates of area, production and yield of various principle crops during 2021-2022. Commisonorate of Agriculture, Krishi Panth Bhawan, Jaipur Rajasthan.

Anonymous, (2020). *Research summary kharif*. ARS, Mandor, Jodhpur.

Bisht, I. S. and Singh, M. (2013). Asian Vigna. In: Singh, M., Upadhyaya, H.D., and Bisht, I.S. (Eds.) Genetic and genomic resources of grain legume improvement. *Elsevier*, pp. 237-267.

Chaturvedi, D. (2017). Incidence and plant stage relationship of root rot incited by *Macrophomina phaseolina* on mothbean using Novel Inoculation Method. *International Journal of Scientific Research and Management*, *5*(6), 5727-5731.

Jha, K. M. and Sharma, N. D. (2005). Influence of temperature and pH affecting *Rhizoctonia bataticola*. *JNKKV Research Journal*, *39*(1), 69-73.

Khan, S. K. (2007). *Macrophomina phaseolina* as causal agent for charcoal rot of sunflower. *Journal of Mycology and Plant Pathology*, *5*(2), 111-118.

Naik, P. J., Gohil, V. P. and Patel, S. N. (2010). Influence of temperature on growth of *Macrophomina phaseolina* (chickpea root rot) *in vitro. Journal of Mycology and Plant Pathology, 40*(4), 613.

Ndiaye, M., Termorshuizen, A. J. and Bruggen, A. H. C. V. (2010). Effects of compost amendment and the biocontrol agent *Clonostachys rosea* on the development of charcoal rot (*Macrophomina phaseolina*) on cowpea. *Journal of Plant Pathology*, *92*(1), 173-180.

Sandhu, A. and Singh, R. D. (1999). Factors influencing susceptibility of cowpea to *M. phaseolina. Journal of Mycology and Plant Pathology*, *29*(3), 421-424.

Sharma, R., Jain, M., Kumar, S. and Kumar, V. (2014). Evaluation of differences among *Vigna aconitifolia* varieties for acquired thermo tolerance. *Agriculture Research*, *3*(2), 104-112.

Sharma, R., Kumar, S., Mahla, H. R., Bhatt R. K. and Kalia, R. K. (2016). Molecular approaches for understanding drought tolerance in mothbean: Our experiences, In:

Bhatt, R.K., Burman, U., Painuli, D.K., Singh, D.V., Sharma, R., Tanwar S.P.S., (Eds.), Climate Change and Agriculture: Adaptation and Mitigation. Satish Serial Publishing House, Delhi, India, pp. 303-317.

Sharma, R., Kumar, S., Mahla, H. R., Khandelwal, V., Roy, P. K. and Sundria, M. M. (2021). Mothbean. The Beans and the Peas from orphan to mainstream crops. *Elsevier*, pp. 67-88.

Sharma, Y. K., Gaur, R. B. and Bisnoi, H. R. (2004). Cultural, morphological and physiological variability in *M. phaseolina*. *Journal Mycology of Plant Pathology, 34* (2), 532-534.

Sobti, A. K. and Sharma, L. C. (1992). Cultural and pathogenic variations in isolates of *Rhizoctonia bataticola* from groundnut in Rajasthan. *Indian Phyto pathology*, *45*, 117-119.

Suriachandraselvan, M. and Seetharaman, K. (2003). Effect of media on growth and sclerotium production of different isolates of *M. phaseolina* infecting sunflower. *Journal Mycology and Plant Pathology*, *33*(2), 226-229.

How to cite this article: Megha Jaimini, J.R. Verma, Dama Ram, Surjeet and Govind Junjadia (2023). Impact of Culture Media and Temperature on Growth and Sclerotial Formation of *Macrophomina phaseolina* (Tassi) Goid. causing dry Root Rot of Mothbean. *Biological Forum – An International Journal, 15*(10): 679-683.