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Nutritional and Physiological Requirement of *Macrophomina phaseolina* (Tassi) Goid. Causing Dry root rot of Chickpea

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A B S T R A C T

An experiment was conducted to study the nutritional requirement and effect of physiological parameters like temperature and pH on mycelial growth of different isolates of *Macrophomina phaseolina* causing dry root rot of chickpea. Five different solid media, five temperature range and five pH levels were tested for their effect on mycelial growth. All tested culture medium, temperatures and pH levels supported the mycelial growth of different *M. phaseolina* isolates. The potato dextrose agar was found better for mycelial growth of all the isolates followed by Czapek's dox agar. *M. phaseolina* isolates could grow over a wide range of temperature i.e. 20^0C to 40^0C but optimum growth was observed at 30^0C temperaturefollowed by 35^0C, 25^0C, 20^0C and minimum at 40^0C. The pH requirement of the *M. phaseolina* were showed that all the isolates could grow over a wide pH range of 5.0 to 9.0 but the optimum pH for its growth was found to be pH 7.0 followed by pH 6.0. The growth of the fungus was reduced both below and above the optimum pH value.

Introduction

Chickpea (*Cicer arietinum* L.) also known as Bengal gram is one of the most important winter season food legume crops grown in India. Chickpea is a member of the family *Fabaceae* and believed to be originated in South-Eastern Turkey. Chickpea is valued for its nutritive seeds; it is an essential source of cheap protein in many parts of the world for those who follow to a vegetarian diet. Chickpea is also rich in mineral content like phosphorus, calcium, magnesium, iron and zinc. Therefore, chickpea is an excellent heart healthy food that may be beneficial to coronary and cardiovascular disease. The importance of chickpea to include in crop rotation lies due to its ability to bind free

atmospheric nitrogen in soil, which make it's a valuable crop for maintaining soil fertility (Ferguson *et al*. 2010).

Worldwide, the major chickpea producing countries are India, Australia, Myanmar, Ethiopia, Turkey, Russian Federation and Pakistan. India is the world leader in chickpea, share 67 % production followed by Australia. In India, it is grown about an area of 10.56mha with production of 11.23 m tonnes and productivity of 1063 kg/ha. India is the largest chickpea producing country, highest production received from Madhya Pradesh (41%) followed by Maharashtra (16%) and Rajasthan (15%) (Anon., 2017-18a). The total area and production of chickpea in Rajasthan are 1.57 m ha and 1.67 m tonnes, respectively having productivity of 1062 kg/ha (Anon., 2017-18b).

Dry root rot (DRR) of chickpea caused by nectrotropic fungus *Rhizoctonia bataticola* (Taub.) Butler [Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid.] is emerging as a serious threat to the chickpea production worldwide (Pande and Sharma, 2010). Dry root rot in chickpea was first reported from India by Mitra (1931). The first report of root rot occurrence in chickpea along with wilt was made by Padwick (1948). Dry root rot (DRR) is a potentially emerging disease of chickpea in rainfed ecologies worldwide. The disease is gaining importance under the changing scenario of climate particularly in the semi-arid tropics of Ethiopia and in central and southern India (Pande *et al.,* 2012). It causes enough yield losses that vary from 5% to 50% and may cause 100% losses in susceptible cultivars under favorable conditions. Higher temperature coupled with soil moisture deficit during crop growth stage especially at post-harvesting is predisposing chickpea to dry root rot infection (Sharma and Pande, 2013). In order to culture the fungi artificially, it is necessary to supply all essential nutrients needed for their growth and development. Similarly, suitable temperature is also important for growth and metabolic processes of fungi. Therefore, the present investigation was carried out to study the nutritional requirement and effect of physiological parameters like temperature and pH on mycelial growth of different isolates of *M.phaseolina in vitro.*

Materials and Methods

The experiment was conducted at Department of Plant Pathology, College of Agriculture, SKRAU, Bikaner (Rajasthan). Dry root rot infested chickpea plant samples were collected from different chickpea growing areas of Rajasthan *viz.,* Alwar, Bikaner, Churu, Hanumangarh, Jaipur, Jaisalmer, Jhunjhunu, Jodhpur, Sikar, Sri Ganganagarand Udaipur. Isolation of the pathogen was made from infested root portions of chickpea and purified by hypal tip method. The isolated fungus was identified on the basis of morphological characters. Confirmed pure cultures were observed under microscope and the stock cultures maintained on PDA slants and stored in refrigerator at 4^0C for further studies. A total of ten isolates of *M. phaseolina* were isolated and subjected to nutritional requirement and physiological variability studies. These isolates were differentiated and codes were given as per Indian Railway abbreviations. The list of *Macrophomina phaseolina* isolates used in present study as under:

Effect of different solid media

The study of different solid media was undertaken to find out the superior media for the mycelial growth of *M. phaseolina.* Five different solid media, two semi-synthetic i.e. Potato dextrose agar (PDA) and Czapek's (Dox) medium and three synthetic media *viz.,*

Richard's agar, malt extract agar and Sabouraud dextrose agar were used and compared for this purpose. These agars based sterilized media were poured aseptically in to 90 mm diameter previously sterilized Petri plates @ 20 ml plate^{-1} which were sterilized in an electric oven at 180^{0}C for 2 hours. The Petri plates were then inoculated aseptically by placing 5 mm disc taken out from the periphery of 7 days old culture and incubated for 7 days for media at 28±2^{0}C. The growth of pathogen was recorded on different media.

Effect of temperature

It is a well-known phenomenon that the temperature yields considerable influence on the biochemical activity of pathogens. Twenty ml of PDA was poured in each of sterilized Petri plates. Each Petri plate was inoculated aseptically by placing a 5 mm disc in the centre from actively growing 7 days old culture on PDA in Petri dishes. The inoculated Petri dishes were incubated at20, 25, 30, 35 and 40±1^{0}C temperature for 7 days.

Effect of hydrogen ion concentration (pH)

The study of different pH levels was undertaken with a view to ascertain the effect of different hydrogen ion concentration of the medium on growth of the fungus. The initial pH of the basal medium before autoclaving was adjusted from 5.0 to 9.0 with a difference of 01 using N/10 NaOH or N/10 HCl. After autoclaving the pH was again tested. The inoculated Petri plates were incubated at 28±2^{0}C for 7 days.

Results and Discussion

Effect of culture media

The mycelial growth of different *M. phaseolina* isolates on different culture media was presented in Table 2. The significant difference in mycelial growth was found between the isolates on different culture media. Maximum mycelial growth of isolates AWR (82.06 mm), BKN (90.0 mm), CUR (90.0 mm), DPA (86.67 mm), HMH (86.18 mm), JSM (84.46 mm), JU (76.91 mm), JJN (84.12 mm), SGNR (82.40 mm) and UDZ (72.10 mm) was observed on potato dextrose agar (PDA) as compared to Czapek's (dox), malt extract agar, Richard's agar and Sabouraud dextrose agar.

On the basis of mean, the most appropriate culture media for mycelial growth among the isolates of *M. phaseolina* was found in order to potato dextrose agar (83.49 mm), Czapek's dox (75.61 mm), Richard's agar (74.60 mm), malt extract agar (68.97 mm) and Sabouraud dextrose agar (65.64 mm). All the tested culture medium supported the mycelial growth of different *M. phaseolina* isolates. The potato dextrose agar was found best for mycelial growth of all the isolates of *M. phaseolina*. Mycelial growth was also found better on Czapek's dox for AWR, BKN, JSM and SGNR; Richard's agar for CUR, HMH, JU, JJN and UDZ; malt extract agar for DPA isolates.

Among the different media (non synthetic and synthetic), potato dextrose agar supported the *M. phaseolina* with highest mycelial growth followed by Czapek's dox, Richard's agar, malt extract agar, while, minimum growth was recorded on Sabouraud dextrose agar. This finding coincides with EI-Wakil *et al.* (1985) reported Potato dextrose agar, Czapek's dox agar and Richard's medium supported the good growth of *M. phaseolina*. Several earlier workers tested and evaluated different culture media and they suggested that Potato dextrose agar was the best culture medium for mycelial growth and sclerotial formation of *M. phaseolina* (Ratnoo and Bhatnagar, 1991; Sharma *et al.*, 2004; Salunkhe*et al.*, 2009; Khan *et al.*, 2012 and Parmar *et al.*, 2018).

Table.1 List of *Macrophomina phaseolina* isolates

S. No.	Code	Place of collection of isolates
1.	Mp-AWR	Agricultural Research Station, Navgaon, Alwar
2.	Mp-BKN	Agricultural Research Station,Beechwal, Bikaner
3.	Mp-CUR	Farmer's field- Ratangarh, Churu
4.	Mp-DPA	Rajasthan Agricultural Research Institute,Durgapura, Jaipur
5.	Mp-HMH	Agricultural Research Sub-Station, Hanumangarh
6.	Mp-JJN	Farmer's field-Chidawa, Jhunjhunu
7.	Mp-JSM	Farmer's field- Suthar mandi, Jaisalmer
8.	Mp-JU	Agricultural Research Station, Mandor, Jodhpur
9.	Mp-SGNR	Agricultural Research Station, Sri Ganganagar
10.	Mp-UDZ	Rajasthan College of Agriculture, Udaipur

Table.2 Effect of different culture media on mycelial growth of *Macrophomina phaseolina* isolates

Isolates	Mycelial growth (mm) on different culture media					Mean
	Czapek's (Dox)	Malt Extract Agar	Potato Dextrose Agar	Richard's Agar	Sabouraud Dextrose Agar	
AWR	81.93	65.00	82.06	70.33	64.80	72.82
BKN	84.50	82.00	90.00	82.33	71.33	82.03
CUR	84.84	85.67	90.00	87.67	73.67	84.37
DPA	78.18	81.33	86.67	54.67	58.00	71.77
HMH	76.04	60.67	86.18	83.00	70.00	75.18
JSM	83.54	77.67	84.46	80.00	65.33	78.20
JU	71.04	65.00	76.91	79.33	62.50	70.96
JJN	59.98	60.00	84.12	72.33	62.00	67.69
SGNR	75.68	60.00	82.40	73.00	69.00	72.02
UDZ	60.33	52.33	72.10	63.33	59.80	61.58
Mean	75.61	68.97	83.49	74.60	65.64	73.66
		S.Em (±)		CD (P=0.05)		CV (%)
Isolates		0.59		1.60		3.09
Media		0.42		1.13		
Isolates X Media		1.31		3.58		

Table.3 Effect of different temperature on mycelial growth of *Macrophomina phaseolina* isolates

Isolates	Mycelial growth (mm)on different temperature					Mean
	20^{0}C	25^{0}C	30^{0}C	35^{0}C	40^{0}C	
AWR	52.67	67.83	76.33	70.33	17.67	56.97
BKN	69.33	84.67	90.00	88.00	31.00	72.60
CUR	55.00	69.00	78.00	72.67	18.33	58.60
DPA	67.67	80.67	84.00	82.33	23.67	67.67
HMH	66.67	77.33	80.17	72.33	16.33	62.57
JSM	52.00	65.00	79.07	80.33	19.33	59.15
JU	57.67	73.33	70.67	69.67	20.00	58.27
JJN	48.33	60.33	74.67	67.33	13.00	52.73
SGNR	42.00	57.00	73.50	64.67	10.67	49.57
UDZ	48.33	60.33	58.67	56.67	9.00	46.60
Mean	55.97	69.55	76.51	72.43	17.90	58.47
		S.Em (±)		CD (P=0.05)		CV (%)
Isolates		0.50		1.37		3.32
Temperature		0.35		0.97		
Isolates X Temperatue		1.12		3.06		

Table.4 Effect of different pH levels on mycelial growth of *Macrophomina phaseolina* isolates

Isolates	Mycelial growth (mm) on different pH levels					Mean
	5.0	6.0	7.0	8.0	9.0	
AWR	64.83	63.70	56.33	59.33	49.58	58.76
BKN	77.70	82.20	83.10	72.00	64.00	75.80
CUR	70.63	75.25	80.00	64.33	60.07	70.06
DPA	82.90	90.00	90.00	76.00	64.32	80.64
HMH	73.37	77.70	82.20	67.00	62.67	72.59
JSM	76.57	79.10	82.67	71.00	63.47	74.56
JU	64.47	73.50	70.00	59.00	61.77	65.75
JJN	70.30	63.00	76.33	64.33	55.82	65.96
SGNR	70.93	75.60	77.33	64.67	63.75	70.46
UDZ	56.50	52.50	60.67	51.33	45.62	53.32
Mean	70.82	73.26	75.86	64.90	59.11	68.79
		S.Em (±)		CD (P=0.05)		CV (%)
Isolates		0.70		1.90		3.92
pH		0.49		1.34		
Isolates X pH		1.56		4.25		

Effect of temperature

The effect of temperature on mycelial growth of various isolates of *M. phaseolina* was studied by incubating Petri dishes at different temperatures ranging from 20 to 40^{0}C. Maximum mycelial growth of isolate BKN (90.0 mm), DPA (84.00 mm), HMH (80.17 mm), CUR (78.0 mm), AWR (76.33mm), JJN (74.67mm) and SGNR (73.50mm) were observed on 30^{0}C. Isolate JU (73.33mm) and UDZ (60.33mm) were showed maximum mycelial growth on 25^{0}C, while isolate JSM (80.33 mm) was on 35^{0}C temperature.

Isolate HMH, JU and UDZ showed comparatively increased mycelial growth on 25^{0}C as compared to 35^{0}C. Minimum mycelial growth was observed in all the isolates on 40^{0}C temperature (Table 3).

The significant effect of temperature range was found on mycelial growth of different isolates of *M. phaseolina*. The overall mycelial growth among the isolates of *M. phaseolina* were recorded maximum at 30^{0}C than 35^{0}C, 25^{0}C, 20^{0}C and minimum at 40^{0}C.

The mycelial growth of *M. phaseolina* isolates were increased when increase in temperature up to 30^{0}C but there after increase in temperature decreased the growth. A significant difference was found among the isolates, different temperatures and their interactions. This result coincides with the finding of Dhingra and Sinclair (1973), Patel and Patel (1990), Ratnoo and Bhatnagar (1991), Khan *et al.* (2012), Parmar *et al.* (2018) and Thombre and Kohire (2018), they reported that the optimum temperature range of 25 to 35°C found optimum for growth and sclerotial formation by *M. phaseolina* whereas, < 15^{0}C and >40^{0}C temperature did not favour the mycelial growth as well as sclerotia production.

Effect of pH

Hydrogen ion concentration also affected the growth of *M. phaseolina* tested over a wide range of pH 5.0 to 9.0. The pH 7.0 was found optimum for maximum growth of isolate BKN, CUR, DPA, HMH, JSM, JJN, SGNR and UDZ, while AWR and JU isolate was at pH 6.0.

The mean optimum pH for all the isolates were 7.0 followed by 6.0, 5.0 and 8.0 and least was pH 9.0. The data revealed that all the isolates showed good growth over a wide range of pH 5.0 to 9.0. There was a significant difference between among the isolates, different pH levels and their interactions (Table 4).

In present investigations the maximum growth was observed at 6.0 to 7.0 pH as concluded by Singh and Chauhan (1982) reported the growth of *M. phaseolina* between pH 2.0-9.0, where pH 5.0-6.0 were found optimum. Jha and Dubey (2000) also recorded that the pathogen *M. phaseolina* causing charcoal rot of okra grew on wide range of pH i.e. between 4.0 and 8.0, where pH 6.0 to 7.0 being optimum. Khan *et al.* (2012) investigations correlate with the present findings, it could grow over a wide pH range of 3.0 to 9.0 but the optimum pH for its growth was found to be 5.5. Thombre and Kohire (2018) reported that a wide range of pH supported the growth of *M. phaseolina*. The best pH level for mycelial growth was found to be 7.0.

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References

Anonymous. 2017-18a. Directorate of Economics and Statistics, Department of Agriculture and Cooperation, Ministry of Agriculture & Farmers Welfare, Govt. of India, New Delhi. 2018. www.agricoop.nic.in.

Anonymous. 2017-18b. DOA, Department of Agriculture, Govt. of Rajasthan, Jaipur, www.krishi.rajasthan.gov.in

Dhingra, C. D. and Sinclair, J. B. 1973. Variation among the isolates of *Macrophomina phaseolina* (*Rhizoctonia bataticola*) from different regions. *Phytopathology*. 76: 200-204.

EI-Wakil, S. A., Haggag, M. H., Machali, H. H. and Saasd, E. A. 1985. Radiation transfer in dispersive media. *J. Phys. D: Appl. Phys.*, 18: 1469.

Ferguson, B. J., Indrasumunar, A., Hayashi, S., Lin, M. H., Lin, Y. H., Reid, D.E. and Gresshoff, P. M. 2010. Molecular analysis of legume nodule development and autoregulation. *J. Integr. Plant Biol*., 52: 61–76.

Jha, A. K. and Dubey, S. C. 2000. Occurrence of collar rot of okra (*Abelmoschus esculantus*) in the plateau region of Bihar. *J. Res. Birsa Agril. Uni.,* 12(1): 67-72.

Khan, R. A., Bhat, T. A. and Kumar Krishan. 2012. Management of Chickpea (*Cicerarietinum* L.) Dry root Rot Caused by *Rhizoctonia bataticola* (Taub.) Butler. *Intrn. J. Res. Pharma. and Biom. Sci.*, 3(4): 1539-1548.

Mitra, M. 1931. Report of the imperial mycologist. *Sci. Rep. of the Agric. Res. Inst*., 1929- 1930: 58-71.

Padwick, G. W. (1948). Plant protection and food crops of India, plant pest and disease at rice, wheat, sorghum and gram. *Imperial J. Exp. Agri.*, 16: 55-64.

Pande, S. and Sharma, M. 2010. Climate change: Potential impact on chickpea and pigeonpea diseases in rainfed semi-arid tropics (SAT). Presented in 5th International Food Legumes Research Conference (IFLRCV) and European conference on Grain Legumes (AEP VII), April 26-30, Antalya, Turkey.

Pande, S., Sharma, M., Nagavardhini, A. and Rameshwar, T. 2012. High Throughput Phenotyping of Chickpea Diseases: Stepwise identification of host plant resistance. Information Bulletin No. 92 Patancheru 502 324, Andhra Pradesh, India: *International Crops Research Institute for the Semi Arid Tropics.* pp. 56.

Parmar, H., Kapadiya, H. J., Bhaliya, C. M. and Patel, R. C. 2018. Effect of Media and Temperature on the Growth and Sclerotial Formation of *Macrophomina phaseolina* (Tassi) Goid causing Root Rot of Castor. *Int. J. Curr. Microbiol. App. Sci*., 7(02): 671-675.

Patel, K. K. and Patel, A. J.1990. Meteorological correlation of charcoal rot of sesamum. *J. Mycol. Pl. Pathol*., 20(1): 64-65.

Ratnoo, R. S. and Bhatnagar, M. K. 1991. Effect of temperature and pH on growth and sclerotia formation of *M. phaseolina. J. Mycol. Pl. Pathol.*, 21(3): 279-280.

SalunkheVanita, Sarika Armarkar and Ingle, R. W. 2009. Effect of nutritional and physiological factors on growth and sclerotial formation of *R. bataticola* (Taub.) Butler isolates. *J. Pl. Dis. Sci.*,4(1): 44-48.

Sharma, M, and Pande, S. 2013. Unravelling Effects of Temperature and Soil Moisture Stress Response on Development of Dry Root Rot [*Rhizoctonia bataticola* (Taub.)] Butler in Chickpea. *American J. Pl. Sci.*,4: 584-589.

Sharma, V. K., Gaur, R. B. and Bisnoi, H. R. 2004. Cultural, morphological and

physiological variability in *Macrophomina phaseolina*. *J. Mycol. Pl. Pathol*., 34: 532- 534.

Singh, R. S. and Chauhan, J. S. 1982. Physio-pathological studies of *Macrophomina phaseolina* causing charcoal rot in muskmelon. *Indian J. Mycol. Pl. Pathol.*, 12: 81-82.

Thombre, B. B. and Kohire, O. D. 2018. Nutritional and physiological studies of *Macrophomina phaseolina* (Tassi) Goid causing mungbean blight. *International Journal of Chemical Studies*. 6(2): 3058-3062.

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