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Effect of temperature on morphology of *Macrophomina phaseolina* causing root rot of cotton

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Abstract : *Macrophomina phaseolina* (Tassi) Goid.ispolyphagous, notorious, soil borne destructive plant pathogen with an almost unlimited host range and is responsible for causing pre and post emergence seedling mortality, rotting of seedlings and mature plants. Sclerotia allow the fungus to survive for prolonged periods of time in the soil. Morphologically, physiologically, genetically, and pathogenically, the fungus varies widely, enabling it to adapt to different environmental conditions and hence has become widely distributed geographically. PDA supported higher mycelial growth of *M. phaseolinathan* CDA medium, whereas, CDA medium was more favourable than PDA for microsclerotia size. Thus, the growth of *M. phaseolinais* strongly influenced by the ingredients of the culture medium in the study conductedat CCS HAU, Cotton Research Station, Sirsa. The mycelia of all the isolates reached the edges of the Petri dishes (90 mm) at 25, 30 and 35ÚC temperature ,whereas, at 15°C and 20°C isolates failed to reach a diameter of 90 mm by 72 hours of inoculation. It was thus concluded thatthe role of temperature on the myclial growth of the pathogen while its impact on microsclerotial size is non significant but size of microsclerotia varied from isolate to isolate. Temperature range of 25-35o C and PDA medium supported maximum growth of this growth and microsclerotial size.

Key words: CDA, cotton, *Macrophomina phaseolina*, PDA, root rot, temperature

The root rot caused by *Macrophomina phaseolina* (Tassi) Goid is one of the most serious diseases of cotton particularly in the northern region of India where cotton is grown on about 19.1 Lakhs ha. area in the states of Punjab, Haryana and Rajasthan (Anonymous, 2021). *Phaseolina* is a soil borne plant pathogenic fungus. The pathogen is seed-borne and seed to-seedling transmission has been documented (Pun *et al.*, 1998). Sclerotia allow the fungus to survive for prolonged periods of time in the soil (Baird *et al.*, 2003).

It is an important phytopathogen distributed worldwide and causes charcoal rot on more than 500 plant species (Das *et al.*, 2008) out of these root rot caused by *M. Phaseolina* is the one of the limiting factor for cotton production. It is a polyphagous, notorious, soil borne destructive plant pathogen with a wide host range (Adams, 1990) and is responsible for causing pre and post emergence seedling mortality, rotting of seedling and mature plants. Seeds undergo complete rotting resulting in poor germination. It also affects the underground part of young seedlings and mature plants. If the affected plants are pulled out and examined, the entire root system may be found rotten. The lateral and thinner roots get completely rotted. Minute fungal sclerotia appear as minute black dots on the surface of woody tissues and the root bark. Moreover, this fungus survives in soil by multicellular jet black microsclerotia produced enormously during parasitic phase or saprophytic phase (Dubey and Upadhyaya, 2001). Sclerotia of *M. phaseolina* are such a potent resting body that each and every cell ispotential to germinate and cause disease.

Cotton root rot exhibits significant morphological, physiological, pathogenic and genetic variability which makes the pathogen more capable of adapting and attacking susceptible hosts in diverse environment. Diversity in *M. phaseolina* occurs due to the heterokaryotic condition of mycelium. (Beas *et al.*, 2006; Reyes *et al.*, 2006). Due to this, the pathogen can adapt to different environmental

conditions and hence become widely distributed geographically (Su *et al.*, 2001). Keeping this in view, the present studies were carried out to see the effect of different medium and temperature on the morphology of *M. phaseolina* causing root rot of cotton at CCS HAU Cotton Research Station, Sirsa

MATERIALS AND METHODS

Six isolates of *M. phaseolina* were collected from major cotton growing areas and their pathogenicity was proved on cotton cultivars *viz.*, HS6 and HD 432 at CCS HAU Cotton Research Station, Sirsa. These following isolates were used for this study.

Sr. No.	Name of the isolates	Source/place
1	M. *phaseolina* MP-1	Hisar
2	M. *phaseolina* MP-II	Sirsa
3	M. *phaseolina* MP-III	Bikaner1
4	M. *phaseolina* MP-IV	Bikaner2
5	M. *phaseolina* MP-V	Nagpur1
6	M. *phaseolina* MP-VI	Nagpur2

Two different media *viz.*, Potato Dextrose Agar (PDA) and Czapek's Dox Agar (CDA) were used to find out the best medium for the growth *M. phaseolina*. 20 ml of each medium was poured in Petri plate and allowed to solidify. Thereafter, 5mm discs of test fungus were cut with the help of sterilized cork borer from seven days old culture grown on PDA medium. One disc of the culture was placed in inverted position in the centre of each Petri plate. The Petri plates were sealed and incubated at 15, 20, 25, 30 and 35 °C. Three replications for each medium and temperature were maintained. The observations on colony characters and diameter were recorded on different culture media by visual observation.

For measuring sclerotial size, slides from 7 day old pure cultures of *M. phaseolina* isolates were prepared and their diameter was recorded. Sizes of twenty randomly selected sclerotia were measured and their means were calculated.

Statistical analysis

Data were analysed statistically after angular transformation using appropriate designs (Panse and Sukhatame, 1978). The treatments were compared at P = 0.05.The significance of treatment was judged with the help of F test. The experimental design employed for analysis of variance in the experiments was completely randomized design. The critical difference was calculated.

RESULTS AND DISCUSSION

Morphology of six isolates of M. *phaseolina* was investigated on PDA and CDA culture media at different temperatures *i.e.* 15, 20, 25, 30 and 35°C. The data (Table 1) revealed that colour of mycelium of all the six isolates of *M. phaseolina* on PDA and CDA ranged from white to black at different temperatures. With the passage of time, the colour of the cultures became darker. On CDA medium, the shiny surface of the cultures was observed at all temperature. While colony colour of the all the six isolates of *M. phaseolina* was variable on the both media *i.e.* PDA and CDA. Colonies were mostly grey, dark grey, or black all the six isolates. The white colony colour however was recorded at lower temperature *i.e.*, 15 and 20°C on both media. On the CDA medium, isolates were of dark grey colour at 25 and 30°C as compared to PDA. While at 35°C, the colour of all isolates was black on both media. Morphological variability has also been reported by many workers in terms of growth and colour, among different isolates of *M. phaseolina* on different hosts (Dhingra and Sinclair, 1973, 1978; Pearson *et al.*, 1986; Atiq *et al.*, 2001; Riaz *et al.*, 2007) which supported the present findings. Aboshosha *et al.*, (2007) also observed white, grey, black, dark green and brown colony colour phenotypes; grey being the most frequent.

The colony growth patterns of the isolates (Table 2 and 3), revealed that maximum growth

Table 1: Effect of different temperature on the colony colour of *M. phaseolina* isolates

Temperature (°C)	Isolates	PDA	C(DOX)
15	MP-I	Dull white	White
	MP-II	Dull white	White
	MP-III	Dull white	White
	MP-IV	Dull white	Dull white
	MP-V	White	White
	MP-VI	White	White
20	MP-I	Dull white	Dull white
	MP-II	Dull white	Dull white
	MP-III	Dull white	White
	MP-IV	Dark grey	Grey
	MP-V	White	White
	MP-VI	White	White
25	MP-I	Grey	Dark grey
	MP-II	Grey	Grey
	MP-III	Grey	Grey
	MP-IV	Dark grey	Dark grey
	MP-V	Grey	Grey
	MP-VI	Grey	Dark grey
30	MP-I	Grey	Dark grey
	MP-II	Grey	Dark grey
	MP-III	Grey	Dark grey
	MP-IV	Dark grey	Dark grey
	MP-V	Grey	Dark grey
	MP-VI	Grey	Dark grey
35	MP-I	Black	Black
	MP-II	Black	Black
	MP-III	Black	Black
	MP-IV	Black	Black
	MP-V	Black	Black
	MP-VI	Black	Black

of the fungus was recorded after 72 hr on PDA medium, as compared to CDA at lower temperatures *i.e.*, 15 and 20°C however, mycelia growth and microsclerotial colonies were similar on both media. It was also observed that PDA supported maximum growth of the pathogen compared CDA medium. On PDA, the mycelial growth of all the isolates reached the edges of the Petri dishes by 72 hrs at 25, 30 and 35ÚC temperatures. However, at 15 and 20°C the mycelial colonies failed to attain a diameter of 90mm up to 72 hrs of incubation. The growth of all the isolates at 15°C was very slow; the maximum growth was observed in MP-II isolate (9.75mm after 24 hours). The MP-I isolate showed the minimum growth at 15, 20 and 25°C. Whereas, at 30°C slowest growth was observed in MP-II while at 35°C it was slowest in MP-IV isolate.

In CDA medium, no mycelial growth was observed upto 24 hrs of incubation at 15°C in any of the isolates under study while it was low at 15 and 20°C in all the isolates and at 20°C, only MP-II isolate showed the largest diameter of 5.5 mm at 24 hrs. Mycelial growth of MP-II and MP-V were 52 mm and 51.25 mm, respectively, at 25°C up to 48 hrs. MP-IV was fastest growing isolate among all the six isolates and achieved 37 mm and 42.75 mm diameter at 15 and 20°C, respectively after 72 hours of inoculation. However, at 30 °C the colony size reached maximum in MP-III and in MP-VI at 35°C after 48 hrs of incubation. Similarly, in CDA medium all the six isolates showed a full (90 mm diameter) mycelium growth at 72 hrs of inoculation at 25, 30 and 35°C. The most

Table 2: Effect of different temperatures on colony size (mm) of *Macrophomina phaseolina* isolates on Potato Dextrose Agar medium

Temperature (°C)	Hours	MP-I	MP-II	MP-III	MP-IV	MP-V	MP-VI
15	24	0.00	9.75	0.00	0.00	0.00	0.00
	48	14.50	27.75	22.75	22.50	28.75	23.25
	72	36.75	52.00	49.25	47.25	52.75	51.00
20	24	0.00	16.25	0.00	0.00	0.00	0.00
	48	18.25	31.00	20.25	23.75	31.50	29.00
	72	46.00	57.00	53.00	50.50	54.50	56.00
25	24	19.50	29.25	27.50	29.25	33.75	33.75
	48	67.50	73.00	71.00	80.00	75.25	79.75
	72	90.00	90.00	90.00	90.00	90.00	90.00
30	24	47.25	31.50	50.00	45.00	38.00	44.50
	48	75.25	63.50	76.25	72.50	71.25	72.00
	72	90.00	90.00	90.00	90.00	90.00	90.00
35	24	39.25	43.75	38.75	29.50	30.00	35.50
	48	69.25	71.00	73.75	57.50	59.00	65.00
	72	90.00	90.00	90.00	90.00	90.00	90.00

C.D. (p=0.05) for temperature (T) = 0.98
C.D. (p=0.05) for hours (H) = 0.76
C.D. (p=0.05) for (TXH) = 1.70
C.D. (p=0.05) for isolates (I) = 1.07
C.D. (p=0.05) for (TXI) = 2.40
C.D. (p=0.05) for (HXI) = 1.86
C.D. (p=0.05) for (TXHXI) = 4.16

favourable temperature regimes for growth of all the six isolates were 25 to 35°C.

These results are in consonance with earlier workers who reported that the growth of M. *phaseolinais* strongly influenced by the ingredients of the culture medium. Singh and Chohan (1982) found that the mycelial growth and microsclerotia development of *M. phaseolina* were most intensive when grown on Czapek's-Dox Agar (CDA) medium, which contained sucrose, while Singh and Kaiser (1994) reported that not only CDA but also potato dextrose agar (PDA), which contained glucose, promoted the rapid growth of the pathogen. On CDA medium containing sucrose, also promotes fungal growth but at slower rate, as observed by Simay *et al.*, (1987). According to Csondes *et al.*, (2012), the infectivity of *M. phaseolina* is highly influenced by the environmental variables. Optimum temperature for the growth of the mycelium and microsclerotia was 30°C (Maholay, 1992) while Viana *et al.*, (2002) recorded optimum growth of M. *phaseolina* at 35°C. It was observed that a higher temperature range of 25 to 35°C favoured the growth of *M. phaseolina* (Sharma *et al.*, (2004) also, Csondes *et al.*, (2007). The isolates collected from various climatic regions of Italy also exhibited 30-35°C as optimum temperature irrespective of the soils from which they were isolated (Manici *et al.*, 1995). Kaur *et al.*, (2012), also observed higher disease incidence in Varanasi region under higher temperature and drought stress conditions.

The sizes(s) of microsclerotia of all the six isolates were examined from one week old cultures and mean diameters of the microsclerotia are presented in Table 4 and 5. Substantial differences in diameter between microsclerotia originating from the same isolate on different media were observed. Considering all the isolates on both media, the diameter ranged *i.e.* in between 74 and 120 µm. CDA medium supported more microsclerotial size of this pathogen, Compared to PDA medium. Nevertheless the shape of the microsclerotia was generally spherical on both media. The microsclerotia were

Table 3: Effect of different temperatures on colony size (mm) of *Macrophomina phaseolina* isolates on Czapek's (Dox) medium

Temperature (°C)	Hours	MP-I	MP-II	MP-III	MP-IV	MP-V	MP-VI
15	24	0.00	0.00	0.00	0.00	0.00	0.00
	48	7.25	14.50	8.25	12.50	14.50	9.75
	72	24.25	29.25	21.75	37.00	31.00	25.75
20	24	0.00	5.50	0.00	0.00	0.00	0.00
	48	13.25	22.00	15.25	18.25	20.75	16.25
	72	29.25	34.00	31.75	42.75	33.00	31.50
25	24	14.75	23.75	21.75	24.75	26.50	30.25
	48	43.00	52.00	45.50	48.75	51.25	49.75
	72	90.00	90.00	90.00	90.00	90.00	90.00
30	24	26.75	22.00	37.25	20.50	22.50	21.75
	48	49.00	52.00	66.25	42.50	52.50	54.25
	72	90.00	90.00	90.00	90.00	90.00	90.00
35	24	31.75	37.50	31.50	29.00	30.00	35.25
	48	58.25	65.25	61.00	58.00	59.50	67.25
	72	90.00	90.00	90.00	90.00	90.00	90.00

C.D. (p=0.05) for temperature (T) = 0.68
C.D. (p=0.05) for hours (H) = 0.53
C.D. (p=0.05) for (TXH) = 1.18
C.D. (p=0.05) for isolates (I) = 0.75
C.D. (p=0.05) for (TXI) = 1.67
C.D. (p=0.05) for (HXI) = 1.29
C.D. (p=0.05) for (TXHXI) = 2.89

Table 4: Effect of different temperatures on size of sclerotia (µm) of *Macrophomina phaseolina* isolates on Potato Dextrose Agar medium

Temperature (°C)	MP-I	MP-II	MP-III	MP-IV	MP-V	MP-VI	Mean
15	97.05	92.53	79.03	100.05	75.08	106.2	91.65
20	96.55	92.05	78.93	99.98	74.93	105.45	91.31
25	97.60	91.78	78.83	100.15	74.88	106.28	91.58
30	96.83	92.83	80.03	99.50	74.53	105.85	91.59
35	98.00	91.95	79.00	99.88	73.93	106.18	91.49
Mean	97.21	92.23	79.16	99.91	74.67	105.99	

C.D. (p=0.05) for temperature (T) = NS
C.D. (p=0.05) for isolates (I) = 0.46
C.D. (p=0.05) for interaction (TXI) = NS

brownish black in colour on CDA while black on the PDA media. However, there was no effect of different temperature regimes on the size of microsclerotiai.e. the size remained same for a particular isolate on varying temperatures.

On PDA medium the largest size of microsclerotia was recorded in MP-VI isolate (106.28 µ) at 25°C followed by MP-IV (100.15 µm). The smallest size of microsclerotia (73.93 µm) was observed in MP-V at 35 °C. At 25°C, smallest size of microsclerotia (78.83 µm) was noticed in MP-III. The size of microsclerotia was observed larger in CDA as compared to PDA. In CDA, largest size of microsclerotia (120.28 µm) was observed in MP-VI isolate followed by MP-IV (117.15 µm) isolate at 25°C. The smallest size of sclerotia (92.83 µm) was of MP III on CDA medium at 25 °C followed by 92.9 µm size in MP-V isolate at 35°C. Similarly, in CDA medium there was no effect of temperature on the size of sclerotia and there was no variation. It is clear from the literature and present study that variability exists in the microsclerotia formation of *M. phaseolina*, confirming that the size and number of

Table 5: Effect of different temperatures on size of sclerotia (µm) of *Macrophomina phaseolina* isolates on Czapek's (Dox) medium

Temperature (°C)	MP-I	MP-II	MP-III	MP-IV	MP-V	MP-VI	Mean
15	114.05	103.53	93.03	117.05	94.08	120.2	106.99
20	113.68	103.05	92.93	116.98	93.93	119.45	106.67
25	114.75	102.78	92.83	117.15	93.88	120.28	106.94
30	113.83	103.83	94.03	116.5	93.53	119.85	106.93
35	115.00	102.95	93.00	116.88	92.9	120.18	106.82
Mean	114.26	103.23	93.16	116.91	93.66	119.99	

C.D. (p=0.05) for temperature (T) = NS
C.D. (p=0.05) for isolates (I) = 0.47
C.D. (p=0.05) for interaction (TXI) = NS

microsclerotia depends on the nutrients available in the substrate (Monga and Raj, 1994, Sundravadana *et al.*, 2011 and Short *et al.*, 1978).

CONCLUSIONS

Present investigations clearly indicated the role of temperature on the myclial growth of the pathogen while its impact on microsclerotial size is non-significant but size of microsclerotia varied from isolate to isolate. Temperature range of 25-35o C temperature and PDA medium supported maximum mycelia growth and microsclerotial size.

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