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Adaptability of *Macrophomina phaseolina* isolates of pigeonpea (*Cajanus cajan* L.) to different temperature and pH

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ABSTRACT

Sixty-one isolates of *Macrophomina phaseolina* were isolated and studied for their adaptability to different temperature and pH. Higher relative growth rate was observed at 30 °C followed by 35 °C. Similarly, Higher mean dry mycelial weight at pH 6 and 7, clearly indicated the preference of isolates to particular range of pH. Different nitrogen sources were found to have a significant effect ($p \leq 0.01$) on the physiology of M^+ and M^- isolates of *M. phaseolina*.

KEYWORDS

Global Warming; Growth Rate; *Macrophomina Phaseolina*; Temperature.

INTRODUCTION

Global warming and its associated affect on plant disease induced by changing climate has become a serious issue with uncertain predictions. The increase in global temeperature might affect the plants or the pathogen or it may also affect both eventually affecting their interaction (Garrett *et al*., 2006). The issue also becomes an important one because usually short-term weather conditions are used for disease modelling and management. Therefore, it is important to study the pathogen biology in its ecological niche in order to understand its possible interaction with the host under certain environmental conditions.

Macrophomina phaseolina has been recently reported as an emerging phytopathogen (Kaur *et al*., 2012c). *M. phaseolina* is an anamorphic fungus in the ascomycete family Botryosphaeriaceae (Crous *et al*., 2006). Macrophomina is primarily soil and seed-borne fungal pathogen that incites disease by producing microsclerotia/pycnidia (Pun *et al*., 1998). With a wide host range of approximately 500 species in 75 plant families, the pathogen exhibits heterogeneous host specificity i.e., the ability to infect monocots as well as dicots and exhibits non-uniform distribution in the soil (Mayek-Perez *et al*., 2001). *M. phaseolina* consists of only one species. In spite of being a mono-specific genus, *M. phaseolina* exhibits a high degree of morphological (Mayek-Perez *et al*., 1997), pathogenic (Su *et al*., 2001), physiological (Mihail and Taylor, 1995) and genetic (Babu *et al*., 2007) variability probably due to the presence of heterokaryosis (Beas-Fernandez *et al*., 2006). It cause stem canker, seedling blight, charcoal rot, stem rot and root rot diseases in various crops. Different abiotic stress has been reported as disease conducive in different crops. The pathogen has a wide host range and survives in the form of microsclerotia in soil and plant debris. *M. phaseolina* becomes aggressive during summer and at a soil temperature of 80-95 °F (27-35 °C) (Yang and Navi, 2003).

These challenges have added to the difficulty of designing effective and sustainable disease management strategies. Recent reports of Macrophomina induced disease incidence and the adjunct global warming highlights the significance

of studying the effect of different factors on the pathogen biology. In this context, the study was conducted to evaluate the effect of different temperature and pH on the growth rate and biomass of *M. phaseolina*, respectively.

MATERIALS AND METHODS

Collection of diseased plants

A roving survey of pigeonpea grown at various places in Varanasi and Mirzapur districts was conducted at the pre-maturity stage of the crop. Three fields were randomly selected in each village. The diseased and healthy plant counts were made from 4m × 4m area of four randomly selected spots in each selected field. Plants showing typical symptoms of stem canker from each place were collected for further studies.

Isolation, purification and maintenance of the isolates

The symptomatic parts of diseased plants collected from different places during survey were thoroughly washed under running tap water and blotted to dry on previously sterilized blotting paper. Upper surface of infected stem was peeled off and cut into pieces of five to six mm size and surface sterilized by dipping in 1.0% (w/v) sodium hypochlorite in 10% ethanol for 30 sec. After thorough washing in sterile distilled water, the pieces were then transferred aseptically on to sterilized 1% (w/v) agar and incubated for 72 hrs at 25±1^{o}C. The hyphal tips of the growing mycelium were marked on the underside of the Petri dish with glass marker under the light microscope. The hyphal tips from margins of resulting colonies were cut with sterilized cork borer and transferred to Petri dish containing fresh potato dextrose agar (PDA). The isolates were transferred to PDA slants and stored at 4±1 ^{o}C for further studies. The culture of each isolate was regularly sub-cultured at three months interval.

Effect of temperature on growth rate

The mycelial disc (5mm diameter) of different isolates of *M. phaseolina* was transferred aseptically to the centre of a 9 cm Petri dish containing PDA. The petri plates were sealed and incubated at 15 ^{o}C, 20 ^{o}C, 25 ^{o}C, 30 ^{o}C and 35 ^{o}C. Each treatment was replicated four times. Mean diameter of colony (mm) was recorded each 24 hrs interval for up to 120 hrs. The radial growth rate (*Kr*, mm d^{-1}) (average of four perpendicular radial lengths) was measured. RGR was calculated (Eqn.4) as per the method of Trinci *et al*, (1971).

$$Kr = \frac{(R_1 - R_0)}{(t_1 - t_0)}$$

Where R_0 and R_1 are the colony radii at time t_o and t_1, respectively. The *Kr* was calculated by linear regression of colony radius versus time during the phase of linear growth.

Effect of different pH on fungal biomass

Effect of different pH on fungal biomass was studied on the basis of the grouping obtained at 30 ^{o}C. Three isolates from each group was selected for biomass estimation at different pH. The pH of the PDB medium was adjusted to pH 4, 5, 6, 7, 8, 9 and 10. The pH was maintained with 0.5M NaOH and 0.5 M HCl. The mycelial disc (5 mm diameter) was cut from the edge of a 4-day-old *M. phaseolina* culture grown on PDA, and transferred aseptically to 50 ml PDB in a 150 ml conical flask. The flasks were incubated at 30±1^{o}C for seven days. Each treatment was replicated three times.

Fungal biomass measurements

After seven days of incubation, the broth was centrifuged at 12,000 × g for 15 min at 4±1^{o}C. the broth was filtered through pre-weighed Whatman filter paper no. 1 and the filter paper containing fungal mat was oven dried at 45±1 ^{o}C for 24 hrs or until a constant weight was achieved. The dried fungal biomass was kept in desiccator and weighed using weighing balance Sartorius analytic.

Statistical analysis

Hierarchical cluster analysis was done using Ward's squared Euclidean method to group isolates at different temperatures. Analysis of variance was conducted on the group of isolates. A measure of association test was conducted between the group of isolates and different pH range. Data were statistically analyzed by using the multiple analysis of variance (ANOVA) by SPSS Version 16.0 for windows. Treatment means were separated by the Duncan's Multiple Range Test (DMRT), $p \leq 0.05$.

RESULTS

Relative growth rate (RGR) of different isolates of *Macrophomina phaseolina* at different temperatures

Sixty-one isolates of *M. phaseolina* were grouped on the basis of RGR at different temperature (Table 1). At 15 °C, max. frequency (39.34%) of cluster C had cluster mean of 8.36 mm/day while cluster B with mean of 36.61 mm/day had minimum frequency (6.56%). At 20 °C, 32.79% (cluster A) of isolates had min. RGR (15.95 mm/day) while max. RGR (51.62 mm/day) was observed in 14.75% of isolates. 18.03% and 34.43% of isolates had max. RGR (62.04 and 53.71 mm/day), respectively at 25 °C while min. RGR (20.58 mm/day) was observed in only 4.92% of isolates. Similarly, at 30 °C, RGR varied from min. (38.57 mm/day) to max. (67.44 mm/day) with frequency distribution of 11.48% and 19.67%, respectively. Group A and B had equal no. of frequency distribution (24.59%) with RGR 56.50 and 49.51 mm/day, respectively. 22.95% of isolates have max. (67.03 mm/day) RGR while 3.28% of isolates had min. RGR of 29.49 mm/day.

Table 1: Grouping of the *Macrophomina phaseolina* isolates at different temperature

Temperature (°C)	Group	Isolate no.	Cluster Mean	Frequency (%)
15	A	8, 14, 7, 11, 22, 49, 36, 48, 42	27.58	14.75
	B	24, 38, 20, 26	36.61	6.56
	C	1, 5, 2, 4, 9, 37, 16, 25, 57, 23, 45, 12, 60, 43, 41, 56, 33, 55, 58, 10, 13, 3, 59, 6	8.36	39.34
	D	15, 31, 14, 54, 34, 53, 47, 35	21.88	13.11
	E	21, 46, 39, 29, 52, 19, 30, 32, 27, 40, 18, 61, 28, 51, 17, 50	15.16	26.23
20	A	40, 61, 6, 12, 29, 2, 16, 25, 3, 13, 19, 32, 18, 36, 58, 17, 8, 26, 33, 56	15.95	32.79
	B	21, 28, 44, 53, 11, 45, 46, 4, 10, 60, 7, 59, 41, 54, 34, 39, 23, 55, 1, 22, 38, 9, 57, 30	24.34	39.34
	C	5, 43, 47, 31, 35, 42, 15, 52	35.01	13.11
	D	48, 49, 27, 24, 50, 14, 20, 37, 51	51.62	14.75
25	A	59, 61, 27, 60, 12, 13, 28, 58, 3, 6, 37, 51	34.35	19.67
	B	15, 19, 17	20.58	4.92
	C	23, 26, 34, 47, 50, 41, 33, 53, 22, 30, 48,	62.04	18.03
	D	36, 49, 38, 5, 29, 9, 35, 43, 52, 57, 2, 24, 7, 1, 39, 16, 46, 20, 54, 4, 10	53.71	34.43
	E	18, 25, 11, 31, 56, 42, 45, 8, 40, 21, 44, 14, 32, 55	46.41	22.95
30	A	59, 61, 23, 25, 15, 33, 38, 60, 4, 6, 22, 7, 30, 5, 46	56.50	24.59
	B	40, 51, 21, 28, 31, 9, 48, 41, 35, 56, 58, 10, 18, 32, 44	49.51	24.59
	C	1, 19, 11, 12, 14, 13, 20	38.57	11.48
	D	39, 47, 43, 50, 27, 36, 37, 54, 2, 34, 45, 42	61.91	19.67
	E	52, 53, 29, 26, 57, 3, 16, 49, 55, 8, 17, 24	67.44	19.67
35	A	11, 61, 4, 33, 59, 30, 39, 53, 50, 60, 42, 5, 37, 45, 54, 34, 57, 36, 6	60.89	31.15
	B	26, 46, 24, 48, 29, 47, 55, 52, 8, 22, 17, 38, 49, 3	67.03	22.95
	C	14, 15	29.49	3.28
	D	28, 32, 20, 58, 41	47.03	8.2
	E	12, 23, 13, 18, 51, 2, 9, 10, 43, 25, 35, 40, 19, 21, 27, 56, 7, 16, 31, 1, 44	53.46	34.43

Table 2 Effect of temperature on the relative growth rate of *Macrophomina phaseolina*

Temperature (°C)	Groups*					Mean**
	A	B	C	D	E	
15	27.58[a]	36.61[b]	8.36[c]	21.88[d]	15.16[e]	21.92[e]
20	15.95[a]	24.34[b]	35.01[c]	51.62[d]		31.73[d]
25	34.35[a]	20.58[b]	62.04[c]	53.71[d]	46.41[e]	43.42[c]
30	56.50[a]	49.51[b]	38.57[c]	61.91[d]	67.44[e]	54.79[a]
35	60.89[a]	67.03[b]	29.49[c]	47.03[d]	53.46[e]	51.58[b]

*Means for groups in homogeneous subsets displayed (horizontally) by same letters do not differ significantly ($p \leq 0.05$)

**Means for relative growth rate at different temperatures in homogeneous subsets displayed (vertically) by same letters do not differ significantly ($p \leq 0.05$).

Table 3. Analysis of variance for the effect of temperature on relative growth rate on different isolates of *Macrophomina phaseolina*

Temperature (°C)	df*	MS	F value
15	4	358.05	358.05**
20	3	709.99	709.99**
25	4	799.09	799.09**
30	4	378.46	378.46**
35	4	628.19	628.19**

* Degree of freedom for groups; **Highly significant at $p \leq 0.01$

Table 4. Effect of temperature and group of isolates on the relative growth rate of *Macrophomina phaseolina*

Source of Variation	Df	Mean Square	F value
Temp	4	2729.97	2729.97**
Group of isolates	4	332.36	332.36**
Temp × Group of isolates	15	630.38	630.38**
Error	48	1.00	

**Highly significant at $p \leq 0.01$

Table 5 Effect of pH on the biomass accumulation in *Macrophomina phaseolina*

pH	Biomass (mg)*					Mean
	1**	2**	3**	4**	5**	
4	0.6261[a]	0.9458[b]	0.8721[b]	0.6243[a]	0.8895[b]	0.79[e]
5	0.6006[a]	0.8351[b]	0.9869[c]	0.8095[b]	0.8099[b]	0.81[d]
6	0.8069[a]	0.8613[abc]	0.8795[bc]	0.8420[ab]	0.9154[c]	0.86[a]
7	0.3848[a]	0.8924[b]	0.9362[b]	0.9174[b]	1.1122[c]	0.85[b]
8	0.7492[a]	0.8050[b]	0.7726[a]	0.7929[b]	0.7788[a]	0.78[f]
9	0.7554[a]	0.5833[b]	0.8950[c]	0.9676[d]	0.8945[c]	0.82[c]
10	0.5463[a]	0.7774[b]	0.7633[b]	0.8758[c]	0.8979[c]	0.77[f]

*Means for groups in homogeneous subsets displayed (horizontally) by same letters do not differ significantly ($p \leq 0.05$)

**Representative isolates from the population of *M. phaseolina* selected from the grouping at 30°C.

Table 6 Effect of pH on population variance in *Macrophomina phaseolina*

Source of Variation (pH)	Eta	Eta Squared
pH4	0.84	0.71
pH5	0.82	0.67
pH6	0.57	0.32
pH7	0.96	0.93
pH8	0.53	0.28
pH9	0.95	0.90
pH10	0.93	0.86

Table 7. Analysis of variance for the effect of pH on dry weight accumulation in *Macrophomina phaseolina*

Source of Variation	df*	Mean Square	F value
pH4	4	0.214	24.455**
pH5	4	0.170	20.869**
pH6	4	0.015	4.740**
pH7	4	0.673	131.333**
pH8	4	0.004	3.868
pH9	4	0.210	91.020
pH10	4	0.175	63.589**

*Degree of freedom for isolates;
**Highly significant at $p \leq 0.01$

Effect of temperature on relative growth rate of *Macrophomina phaseolina*

Groups varied significantly ($p \leq 0.05$) within each temperature. Maximum mean RGR was observed at 30 °C followed by 35°C while minimum mean RGR was observed at 15 °C. Means for homogenous subsets are displayed (Table 2; Fig 1). Analysis of variance described a significant effect of temperature within each group (Table 3). Temperature (Fss=2729.97), Group (Fss=332.36) and Temperature × Group (Fss=630.37) had a significant ($p \leq 0.01$) effect on RGR (Table 4). Estimated marginal means were plotted to study the interaction of different temperatures within each group. Non parallel lines suggest the possibility of an interaction taking place among different tempera tures (Fig. 2).

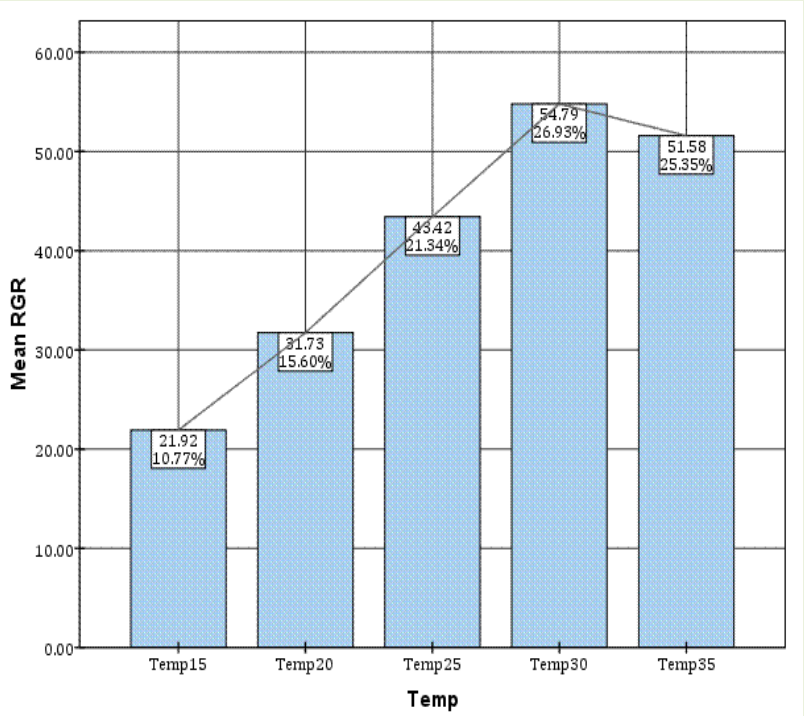

Figure 1 Effect of temperature on relative growth rate of *Macrophomina phaseolina*

Effect of different pH on fungal biomass

Variation in *M. phaseolina* biomass accumulation due to change in hydrogen ion concentration (pH) was recorded. Mean dry mycelial weight at pH 6 and 7 was more clearly indicating the preference of isolates to particular range of pH. Highest mean (0.861 mg/50ml) was observed at pH 6 (Table 5). Maximum variance (92.9%) was observed at pH 7.0 followed by pH 9.0 (90.1%) and pH 10.0 (86.4%) (Table 6). pH had a highly significant ($p \leq 0.01$) effect on fungal biomass (Table 7).

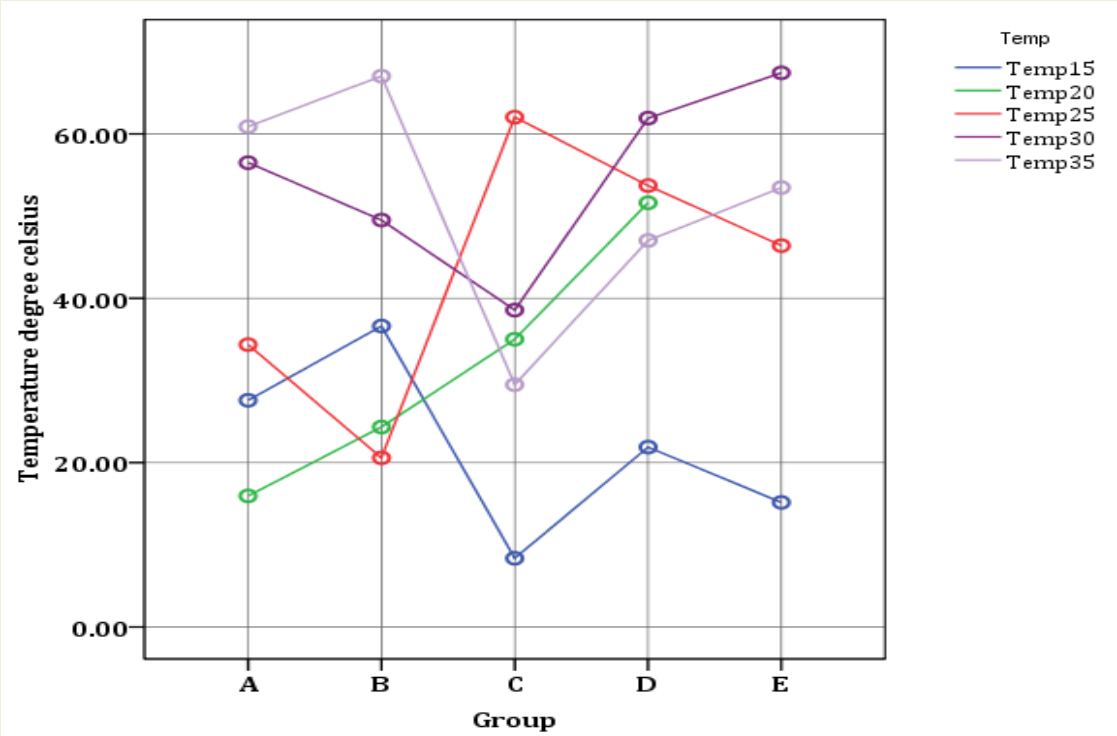

Figure 2 Interaction of different temperatures among different groups.

DISCUSSION

Fungal growth can be quantified easily in linear dimensions on an agar medium. An optimum temperature recorded for the growth of *M. phaseolina* was 30 °C followed by 35 °C while growth rate was reduced at lower temperatures i.e., 15 °C and 20 °C. The interaction between temperature and group of isolates had a significant effect on relative growth rate of *M. phaseolina*. According to Csondes *et al*, (2012), the infectivity of *M. phaseolina* is highly influenced by the environmental variables. The optimum temperature for the growth of the mycelium and development of the microsclerotia was 30 °C (Maholay, 1992). Optimum temperature for *M. phaseolina* was 35 °C (Viana *et al*., 2002). Sharma *et al*, (2004) also observed that a higher temperature range of 25 to 35 °C favored the growth of *M. phaseolina* isolated from pearl millet, sesame, horsegram and mothbean. Csondes *et al*, (2007) also reported 25-35 °C as the optimum temperature for the growth of *M. phaseolina* isolates collected from Hungary. The isolates of various climatic regions in Italy exhibited optimum temperature of 30-35 °C as that of the soils from which they were isolated (Manici *et al*., 1995). This suggests variable soil temperature might exerts selection pressure on the pathogen that results in better adaption to survive at higher or lower temperatures (Manici *et al*., 1995).

Increasing global warming and the adaptability of the fungus at varying range of temperature suggests the presence of adaptive mechanisms for life under variable environmental and host conditions. It might also be envisaged as an approach to exist as saprophyte or latent pathogen in host tissues. It also ensures the continuous vegetative development even under the conditions of unfavorable temperature. Kaur *et al*, (2012a), conducted a survey in different regions of Varanasi and concluded higher disease incidence under the conditions of higher temperature and drought stress. The adaptability towards increasing temperature conditions might play an important role in pathogenesis. High activity of different hydrolytic enzymes were observed in microsclerotial isolate of Macrophomina on apple pomace supplemented with 1% (w/w) rice husk through koji fermentation (Kaur *et al*., 2012b).

The metabolic activities, such as transformation of substrate into products, are carried out with the help of biological catalysts (enzymes) that requires

particular range of pH. Different pH range has been suggested as optimum for the growth of *M. phaseolina*. In the present investigation, variations in fungal biomass accumulation have been observed. Maximum biomass accumulation was observed between pH 6.0 to 7.0, which was in accordance with results obtained by Jha and Dubey (2000) while studying effect of pH on the growth of *M. phaseolina* isolated from okra. Singh and Chohan (1982) also reported pH 5.0 and pH 6.0 as the optimum for the mycelium development of *M. phaseolina*. Nischwitz *et al*., (2004) reported pH 4.0 and 6.0 as the optimum for growth.

CONCLUSION

The present investigation suggests adaptability of the pathogen to wide range of temperature and pH that increases fitness in particular niche. It might also be implicatd that predicted global warming is likely to increase the range and severity of Macrophomina stem canker disease. Devastating epidemics might result in case such effects are ignored, with far reaching socio-economic consequences and threatening wildlife as well (Anderson *et al*., 2004). Edaphic factors, such as soil moisture, temperature, salinity and pH critically affect the survival of *M. phaseolina* as well as influence the disease incidence in various crops. Therefore, it is plausible to interpret on the basis of the above findings that changes in the agro-ecosystem in recent years have encouraged *M. phaseolina* to adapt higher temperatures and pH for growth and reproduction.

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