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Original Research Article

Sensitivity of *Macrophomina phaseolina* (Tassi) Goid. isolates of maize (*Zea mays* L.) to different temperature and pH levels

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Abstract

Macrophomina phaseolina is the devastating fungus of many crops. For this study, infected maize samples were collected from four districts (Kasur, Okara, Sahiwal and Pakpatan). Twenty-four isolates of *M. phaseolina* were isolated from infected maize samples and their growth was evaluated at 20, 25, 30, 35 and 40°C as well as at 5.0, 5.5, 6.0, 6.5 and 7.0 pH. The growth of fungal isolates was significantly affected by different levels of pH and temperature. Higher mean dry mycelial weight was observed at 35ºC followed by 40ºC. Higher mean dry mycelial weight at pH 6.5 and 7.0, clearly indicated the preference of isolates to particular range of pH. Mean dry mycelial weight was increased with increase in pH and temperature.

Keywords: Global warming, Mycelial weight, *Macrophomina phaseolina*, Temperature, pH

Introduction

Global warming and its effects on plant disease induced by varying climate have become a serious concern with tentative predictions. The increase in global temperature might affect the plants and the pathogen eventually affecting their interaction (Garrett et al., 2006). The crop yield is badly affected by biotic and abiotic factors especially diseases which depends upon crop germplasm (Bashir and Malik, 1988). The fungus *Macrophomina phaseolina* is the devastating pathogen and distributed in varying climatic conditions (Iqbal and Mukhtar, 2014). Physiological factors like temperature, moisture and heat affect the prevalence of the pathogen (Dhingra and Sinclair, 1978). *M. phaseolina* is a soil and seed-borne fungus that cause disease by forming microsclerotia/pycnidia (Pun et al., 1998). *M. phaseolina* shows very high morphological (Mayek-Perez et al., 1997), pathogenic (Su et al., 2001), physiological (Mihail and Taylor, 1995) and genetic (Babuet al., 2007) variation due to heterokaryosis (Beas-Fernandez et al., 2006). It cause charcoal rot, stem canker, root rot, stem rot and seedling blight in different crops (Sanei and Razavi, 2011). The infection caused by the pathogen is greatly influenced by environmental factors (Maholay, 1992). *M. phaseolina* becomes destructive during summer at

soil temperature of 20-40ºC (Yang and Navi, 2003). Its pathogenicity increases with increase in temperature (Saleh et al., 2010). Low moisture level enhances the growth rate and survival ability of the fungus (Zazarini et al., 1985). Despite extensive studies on the effect of temperature on *M. phaseolina* growth attributes no precise range of epidemiological factors has been described up till now (Csondes et al. 2012). The temperature that affects the growth of the fungus varies with crop species (Das et al., 2008). The optimum temperature for microsclerotial development also varies with region to region (Das, 1988). At lower pH the growth of *M. phaseolina* becomes slower than at higher pH (Kulkarni, 2000). These issues are responsible for planning of efficient and suitable management approaches for the management of disease. Current reports of charcoal rot disease and global warming emphasizes the effect of different factors on pathogen biology. In the above background, the experiment was carried out to find the effect of different temperature and pH ranges on the *M. phaseolina*.

Materials and Methods

Collection of diseased plants

A random survey of major maize growing districts in Punjab (Kasur, Pakpatan, Sahiwal and Okara) was conducted during March 2015 and different villages were chosen for sample collection. Three fields were randomly selected in each village. The selected districts, villages and fields had variations in soil properties, cropping pattern and field history. The charcoal rot infected and healthy plant counts were made from 4m × 4m area of four randomly selected spots in each field. The stem samples with microsclerotia from the symptomatic plants were collected for further studies. The samples were taken in paper bags and then in polythene bags until use (Lotfalinezhad et al., 2013).

Isolation, purification and maintenance of the isolates

The diseased portions of plants were collected from surveyed areas, washed in tap water and dried on sterilized blotting paper. Infected portion was cut into pieces (5 mm) and surface sterilized by dipping in 1% sodium hypochlorite for 30 sec. Sterilized pieces were washed to remove excess sterilant and transferred to 1% (w/v) agar and incubated for 72 hours at 27±1 ºC. Hyphal tips were cut with cork borer and transferred to Petri dishes having potato dextrose agar (PDA) medium and identified by following Barnett and Hunter (1972). The isolates were transferred to PDA slants and stored at 4±1ºC for further studies (Csondes et al. 2012).

Table 1: Isolates of *Macrophomina phaseolina* collected from maize plants from districts of Punjab

Sr.No.	Isolate code	District	Locations
1	K-1	KASUR	Bheala
2	K-2		Garewala
3	K-3		Talwandi
4	K-4		Atari
5	K-5		Noor Pur
6	K-6		KhudianKhas
7	O-1	OKARA	Aktharaabad
8	O-2		Ahmadabad
9	O-3		BasirPur
10	O-4		HavaliLakha
11	O-5		Hujra Shah Muqeem
12	O-6		Renala Khurd
13	S-1	SAHIWAL	Kassowal
14	S-2		Chak 42/12 L
15	S-3		Chak 21/11 L
16	S-4		Chak 44/12 L
17	S-5		AddePur
18	S-6		Bashera
19	P-1	PAKPATAN	Chak 17 SP
20	P-2		JamanBodla
21	P-3		Bunga Hayat
22	P-4		Malka Hans
23	P-5		Chak 50 SP
24	P-6		Chak 30 SP

Effect of different pH on fungal mycelial weight

Isolates of *M. phaseolina* were grown on the Potato Dextrose Broth in selected pH levels of 5.0, 6.0, 6.5 and 7.0. The pH was maintained by 1N NaOH or HCl. Conical flasks having 30 ml medium at different pH levels were inoculated with 7 days old mycelial discs of the isolates. Three replicates were used and flasks incubated at 27± 1°C (Sukanya and Jayalakshmi, 2017).

Effect of temperature on fungal mycelial weight

Growth of each isolate was tested at 20, 25, 30, 35 and 40°C. Thirty ml of potato dextrose broth was poured into 150 ml conical flasks and sterilized. Ten days old, 5 mm mycelial discs of the isolates were inoculated

separately into conical flasks. Three replications were maintained and incubated at selected temperatures (Bekadda et al. 2008).

Measurement of fungal dry mycelial weight

The broth was centrifuged at 12,000 × g for 15 min at 4±1ºC after 10 days of incubation. Pre-weighed Whatman filter paper No. 1 was used for filtration of broth. The filter paper along with fungal mat was oven dried at 60±1 ºC for 24 hours. The dried fungal mycelium was kept in desiccators and weighed using weighing balance (Iqbal and Mukhtar, 2014).

Statistical Analysis

Collected data was interpreted by statistical analysis. Analysis of variance (ANOVA) technique was selected for data analysis by using MINITAB/STAT statistical analysis software (Minitab, 2010).

Results

Effect of different pH on fungal biomass

Variation in *M. phaseolina* biomass accumulation due to change in hydrogen ion concentration (pH) was recorded. The fungal mass of all the isolates differed with changing pH (Fig. 1). Fungal biomass and pH are directly proportional to each other. Mean dry mycelial weight increased at pH range 6.5 and 7 that more clearly indicated the preference of isolates to high pH. Highest mean (321 mg) dry mycelial weight was observed at pH 7.0 followed by 282 mg at pH 6.5. Least growth (83 mg) was observed at pH 5.0 indicating its inability to support the growth of *M. phaseolina* isolates. Out of 24 isolates, 9 were grouped in the lowest range of mycelial weight (132-170 mg), 4 were ranked in the middle range (171-207 mg) and 11 were in the range of (208-244 mg).

Effect of temperature on fungal biomass

The growth of all fungal isolates was significantly affected by varying temperatures. However, isolates showed different response in terms of dry mycelial weight at all temperature ranges. The individual effects of temperatures on the fungal biomass of different isolates are given in (Fig.1). Among the different temperatures tested at 35ºC was most favorable (331 mg) and it was closely followed by 40ºC (297 mg) for the growth of *M. phaseolina*. At 20ºC, 25ºC and 30ºC poor growth was observed indicating isolates' preference towards higher temperature for the growth. Fungal biomass increased with increasing temperatures up to 35ºC but at 40ºC it suddenly decreased. Among 24 isolates, 15 were in the range of (155.9-198.0mg) and 9 isolates grouped in the range (199.0-241.1mg) (Table. 2).

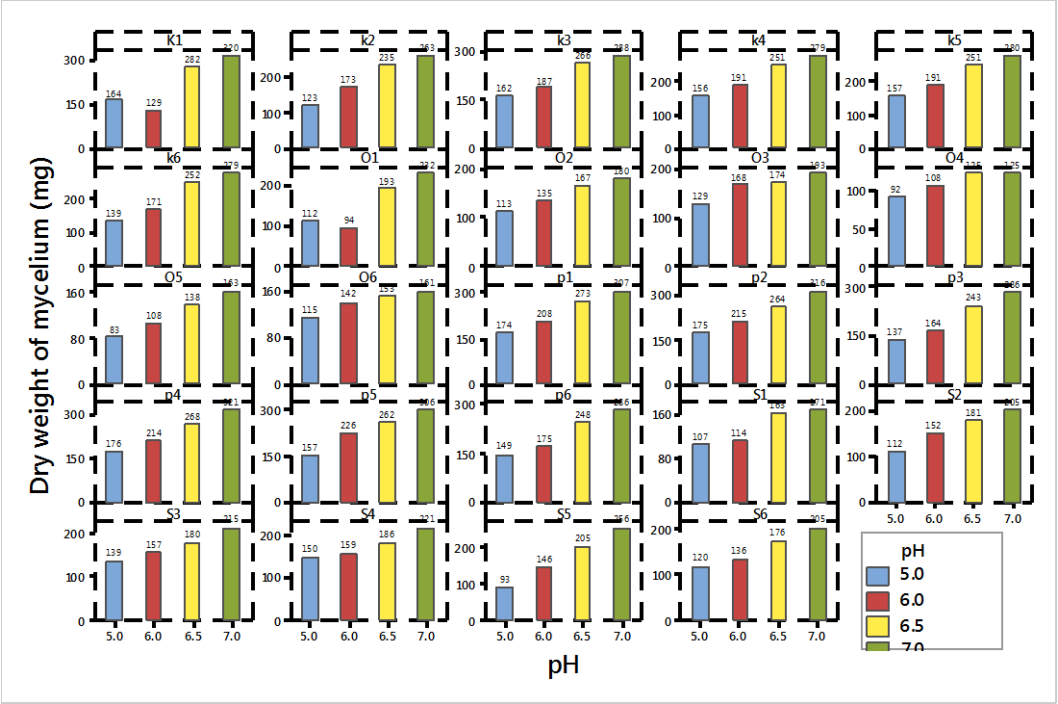

Fig 1: Effect of pH on dry weight of *M. phaseolina* isolates

Table 2: Grouping of isolates of *Macrophomina phaseolina* based on dry mycelial weight at different pH levels

Group	Range (mg)	Number of isolates	Location of Isolates
1	207.49-244.76	11	Bheala, Talwandi, Atari, Noor Pur, Khudian Khas, Chak 17 SP, Jaman Bodla, Bunga Hayat, Malka Hans, Chak 50 SP, Chak 30 SP
2	170.21-207.48	4	Garewala, Chak 21/11 L, Chak 44/12 L, AddePur
3	132.93-170.20	9	Aktharaabad, Ahmadabad, Basir Pur, Havali Lakha, Hujra Shah Muqeem, Renala Khurd, Kassowal, Chak 42/12 L, Bashera

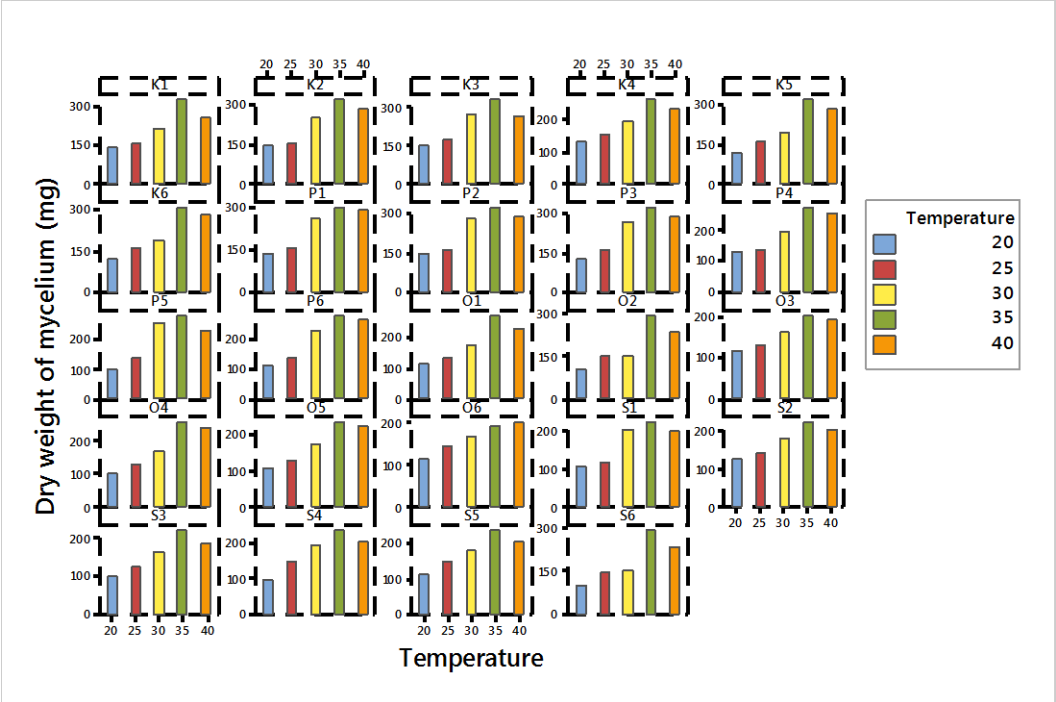

Fig 2: Effect of temperature on dry weight of *M. phaseolina* isolates

Table 3: Grouping of isolates of *Macrophomina phaseolina* based on dry mycelial weight at different temperatures

Group	Range (mg)	Number of isolates	Location of Isolates
1	199.0-241.1	9	Bheala, Noor Pur, Khudian Khas, Aktharaabad, Ahmadabad, Basir Pur,Havali Lakha, Hujra Shah Muqeem, Renala Khurd
2	155.9-198.00	15	Garewala,Talwandi, Atari, Chak 17 SP, Jaman Bodla, Bunga Hayat, Malka Hans, Chak 50 SP, Chak 30 SP, Kassowal, Chak 42/12 L,Chak 21/11 L,Chak 44/12 L, AddePur,Bashera

Discussion

Temperature and pH ranges varied greatly for *M. phaseolina* in different experiments, which may be the effect of crop specie, growing media and growing conditions (Ratnoo and Bhatnagar, 1991). The most favorable temperature for *M. phaseolina* growth was 35ºC followed by 40ºC while reduced growth rate was recorded at 20 ºC and 25 ºC. Csondes et al. (2012) stated that infection caused by *M. phaseolina* in sunflower growing areas is greatly affected by the environmental variable such as pH and temperature. These results are supported by the findings of (Vianaet al., 2002) who recorded 35ºC as the optimum temperature for *M. phaseolina* isolates obtained from common bean (*Phaseolus vulgaris* L.) fields. According to Maholay (1992) mycelia growth and microsclerotial development was best at 30ºC. High temperature (25 to 35°C) enhanced *M. phaseolina* dry weight (Sharma et al., 2004). In Hungary, *M. phaseolina* isolates were subjected to different temperature ranges and best growth was recorded at 25-35 ºC (Csondes et al., 2007). Manici et al. (1995) obtained fungal isolates from different ecological zones of Italy and found that 30-35ºC was temperature for growth. High temperature may exert selection pressure on the pathogen which adapt to survive at higher or lower temperatures.

A survey was conducted in different climatic zones of Varanasi and described that due to increased activities of fungus at high temperature and in drought conditions high charcoal rot disease incidence was recorded (Kaur et al., 2012a). At high temperature, the concentration of hydrolytic enzymes were increased in microsclerotia of *Macrophomina* (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. Jha and Dubey (2000) isolated *M. phaseolina* from okra to check the effect of pH and maximum fungal dry weight was recorded at pH 7. The mycelium of *M. phaseolina* isolated from muskmelon was developed best at pH 5.0 and pH 6.0 (Singh and Chohan, 1982). Nischwitz et al. (2004) used different irrigation types in melon fields and found pH 4.0 and 6.0 as the best for *M. phaseolina* growth. These results contradict with present study which may be due to soil conditions, crop specie and cultural practices.

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 implicated that predicted global warming is likely to increase the range and severity of *Macrophomina* charcoal rot disease. Edaphic factors, such as temperature and pH critically affect the survival of *M. phaseolina*as well as influence the disease incidence in various crops.

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