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EFFECT OF DIFFERENT pH LEVELS ON THE *IN VITRO* MYCELIA GROWTH OF *ALTERNARIA* SP, THE CAUSE OF LEAF SPOT OF BITTER GOURD (*Momordica charantia* L)

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Abstract

An in vitro study was conducted to check the mycelia growth of different isolates of *Alternaria* sp on different pH levels (Normal, 6.0 and 8.0) in the Department of Plant Pathology, The University of Agriculture, Peshawar during 2010 growing season of the crop. *Alternaria* sp from all the six isolates was cultured aseptically. The experiment was conducted by using three factors Completely Randomized (CR) design. The responses of all the six isolates (Chamkani, Nasirur, Taimalpura, Jabba Daudzai, Zakhi Miana and Garhi Momin) of *Alternaria* sp were significantly different at various pH (normal, 6.0 and 8.0) levels. Data were recorded after five and ten days of incubation at 25^0C. The mycelia growth of all the six isolates were maximum at normal pH of Potato Dextrose Agar (PDA) medium as compared to 6.0 and 8.0. Locations also affected the response of *Alternaria* sp to different pH levels.

Key words: Bitter gourd, leaf spot, *Alternaria* sp, *in vitro* and pH levels

INTRODUCTION

Bitter gourd (*Momordica charantia* L) is one of the most popular cross pollinated crop. It is normally grown as annual crop but can perform as perennial in areas with mild, frost free winters (Singh *et al*, 2006). There are several verities available, having 3-4 inches to even 12 inches' fruits in length (Abascal and Yarnel, 2005). Bitter gourd also has medicinal values. It is antipyretic tonic, appetizing and stomachic. Bitter gourd is considered one of the most nutritious gourds. It is a rich source of vitamin A, B, C, E and K, carbohydrates, dietary fibers, phosphorous, Ca, Mg, Fe, K and Na (Sridhar *et al*, 2008, Nerukar *et al*, 2006, Abascal and Yarnell, 2005 and Koch *et al*, 2006).

Leaf spots (*Alternaria* sp and *Myrothecium* sp), powdery mildew, white rot of fruit and *Rhizoctonia solani* fruit rot have been reported on bitter gourd (Khan and Kamal, 1963 and Maholay, 1989). *Alternaria* sp, *Aspergillus* sp, *Colletotrichum lagenarium*, *Rhizopus* sp had been isolated from seeds of bitter gourd (Manthachitra, 1971, Maholay, 1989, Nair, 1982 and Mathar, 1990). *Alternaria* sp has a wide host range, causing leaf spots and blights on many plant parts. As a result of mass of sporulation by the pathogen, lesions are restricted to surface of leaves which enlarge causing a target spot with concentric rings. Dark color develops due to spore production which can cause new infection sites (Pati *et al*, 2005 and Krick *et al*, 2008). This research included to check the mycelia growth of *Alternaria* sp on different pH levels (normal, 6.0 and 8.0).

MATERIALS AND METHODS

Leaf samples of bitter gourd infected with *Alternaria*

sp were collected from Peshawar (Chamkani, Nasirpur and Taimalpura) and Nowhera (Jabba Daudzai, Zakhi Miana and Garhi Momin) districts of Khyber Pakhtunkhwa during 2010 growing season of the crop. These samples were brought to the Department of Plant Pathology, The University of Agriculture, Peshawar. All these samples were preserved and then packed in paper bags.

Isolation and identification of the pathogen: Pathogen from all the six locations of District Peshawar and Nowshera were isolated on Potato Dextrose Agar (PDA) medium under aseptic conditions. Media was prepared using the standard procedure (for 1 liter of PDA, 250g peeled potato, 20g agar and 20g dextrose) and was sterilized in autoclave for 20 minutes at 121^0C. Streptomycin was added before pouring to inhibit the bacterial growth. Infected bitter gourd leaves having leaf spots were cut into pieces, surface sterilized with mercuric chloride (0.1% solution) for 15-30 seconds and thoroughly rinsed in sterilized distilled water. The specimens were then placed on Petri plates having PDA and kept at 25^0C and incubated for the growth of the pathogen. After five days of incubation, the pathogen was identified by using the key of Barnet and Hunter (1970).

***In vitro* study:** Experiment was conducted in aseptic conditions. The design used was three factors Completely Randomized (CR) and each isolate was replicated three times. The pH of media was adjusted just before sterilization (normal, 6.0 and 8.0). Inoculum plug of equal diameter was maintained for all the isolates. All the Petri plates were kept in incubator at 25^0C for the fungal growth. Data were recorded on colony diameter after five and ten days of incubation. All the recorded data were pooled for statistical analysis using analysis of variance (ANOVA) and least significant difference (LSD) tests (Dana, 2001).

RESULTS AND DISCUSSION

Identification of the pathogen: Temporary slides were prepared from the culture as well as from the affected leaves. Those were observed under the microscope on different magnifications. The observations were matched with the text and photographs present in the Barnet and Hunter (1970). Thus the pathogen was identified as *Alternaria* sp.

Effect of pH on the mycelia growth of *Alternaria* sp: Response of six different isolates of *Alternaria* sp to different pH levels were significantly different (Table 1). The colony diameter of six different isolates of *Alternaria* sp were non-significant at normal pH of Potato Dextrose Agar medium. Maximum (5.0, 5.1, 4.8, 5.1, 4.4 and 4.3cm) colony diameter were of isolates Chamkani, Nasirpur, Taimalpura, Jaba Daudzai, Zakhi Miana and Garhi Momin at normal pH as compared to pH 6.0 and 8.0 of PDA. Overall effect of pH on isolates of District Peshawar were 3.5-3.8cm while 4.1-4.9cm of District Nowshera.

Data presented in Table 2 indicated significant ($p<0.05$) effect of pH on isolates of *Alternaria* sp after ten days of incubation at 25^0C. Colony diameter of District Peshawar isolates were affected more (6.5cm) as compared to District Nowshera (7.7cm). The colony diameter of District Peshawar isolates were 5.7-6.9cm, with maximum of location Chamkani (6.9cm). Maximum (8.4cm) colony diameter was of location Jaba Daudzai among the isolates of District Nowshera while minimum (7.0cm) of location Garhi Momin. The mycelia growth of *Alternaria* isolates were more (8.3-8.9cm) at normal pH of PDA as compared to 6.0 and 8.0 of all the locations, with maximum (8.9cm) of Jaba Daudzai.

The pH levels affect the *in vitro* cultural characteristics viz. colony color, margin, topography, zonation, pigmentation, colony diameter (mm) and sporolation (Hubbali *et al*, 2010 and Farooqi *et al*. 1985). Anderson and Frisvad (2002) examined fifty eight isolates of *Alternaria* sp. He concluded that pH levels strongly affect the growth rate. The requirement for optimum mycelia growth was different for different species. *Alternaria citri* and *Alternaria tenuis* were studied by Hasija (1970) for their growth on media having different pH levels. He concluded that both fungi were able to grow at a pH range of 2.7-8.0, with optimum being at 5.4. In this study too, different pH levels had a significant effect on the mycelia growth of different isolates of *Alternaria* sp.

Table 1. Effect of different pH levels on the mycelia growth of different isolates of *Alternaria* sp after 5 days of incubation.

Districts (D)	Locatios (L)	pH levels	Colony diameter (cm)	Colony diameter (DxL)	Colony diameter (D)
Peshawar (D_1)	Chamkani (L_1)	Normal	5.0 A (--)	3.8 BC	3.6 B
		6.0	3.6 CDE (28.0)*		
		8.0	2.8 DE (44.0)		
	Nasirpur (L_2)	Normal	5.1 A (--)	3.5 C	
		6.0	2.7 DE (47.0)		
		8.0	2.6 DE (49.0)		
	Taimalpura (L_3)	Normal	4.8 AB (--)	3.5 C	
		6.0	3.3 CDE (31.2)		
		8.0	2.4 B (50.0)		
Nowshera (D_2)	Jaba Daudzai (L_1)	Normal	5.1 A (--)	4.9 C	4.4 A
		6.0	4.8 AB (5.9)		
		8.0	4.9 A (3.9)		
	Zakhi Miana (L_2)	Normal	4.4 ABC (--)	4.1 BC	
		6.0	3.4 BCD (22.7)		
		8.0	4.1 ABC (6.8)		
	Garhi Momin (L_3)	Normal	4.3 ABC (--)	4.2 B	
		6.0	4.0 ABC (6.8)		
		8.0	4.1 ABC (6.4)		
LSD value	--	--	1.2	0.69	0.4
CV (%)	--	--	18.2	18.2	18.2

*Decrease than normal pH level.

Values followed by different letters are significantly different from one another at 5% level of significance.

Table 2. Effect of different pH levels on the mycelia growth of different isolates of *Alternaria* sp after 10 days of incubation.

Districts (D)	Locatios (L)	pH levels	Colony diameter (cm)	Colony diameter (DxL)	Colony diameter (D)
Peshawar (D_1)	Chamkani (L_1)	Normal	8.8 A (--)	6.9 BC	6.5 B
		6.0	5.5 CD (37.5)*		
		8.0	6.5 CD (26.1)		
	Nasirpur (L_2)	Normal	8.7 A (--)	6.7 C	
		6.0	6.3 CD (27.6)		
		8.0	5.2 D (40.2)		
	Taimalpura (L_3)	Normal	8.6 A (--)	5.7 D	
		6.0	5.8 CD (32.6)		
		8.0	2.7 E (68.6)		

Table 2 Continued….					
Nowshera (D_2)	Jaba Daudzai (L_1)	Normal	8.1 A (--)	8.4 A	7.7 A
		6.0	8.0 A (10.1)		
		8.0	8.3 A (6.7)		
	Zakhi Miana (L_2)	Normal	8.7 A (--)	7.5 B	
		6.0	7.9 AB (9.2)		
		8.0	6.0 CD (31.0)		
	Garhi Momin (L_3)	Normal	8.3 A (--)	7.0 BC	
		6.0	6.2 CD (25.3)		
		8.0	6.6 BC (20.5)		
LSD value	--	--	1.3	0.78	0.45
CV (%)	--	--	11.6	11.6	11.6

[*]Decrease than normal pH level.

Values followed by different letters are significantly different from one another at 5% level of significance.

CONCLUSION AND RECOMMENDATION

Six isolates (Chamkani, Nasirpur, Taimalpura, Jaba Daudzai, Zakhi Miana and Garhi Momin) of Alternaria sp were tested on different pH levels (normal, 6.0 and 8.0) of Potato Dextrose Agar (PDA) medium for their mycelia growth. The mycelia growth of all the six isolates was maximum at normal pH of PDA as compared to 6.0 and 8.0.

On the basis of this project research, I recommend detail, continuous and systematic research work on the problem concerned.

LITERATURE CITED

Abascal, K.E. and E. Yarnell. 2005. Using bitter gourd to treat diabetes. Alternative and Complementary Therapies. 11(4):179-184.

Anderson, B. and C.J. Frisvad. 2002. Characterization of Alternaria and Penicillium species from similar substrata based on growth at different temperature, pH and water activity. Systematic and Applied Microbiology. 1:162-172.

Barnet, H.L. and B.B. Hunter. 1970. Illustrated Genera of Imperfect Fungi. 3rd Edition. Burgess Publishing Company, Minneopollis, Minnesotar, USA. Pp.203.

Dana, S.D. 2001. Statistical and data analysis for the behavioral sciences. 1st Edition, Von Hofmann Press Incharge, New York, USA. 11:411-454.

Farooqi, W.A., M.A. Malik, G.A. Shaukat and M.S. Ahmad. 1985. Influence of pH on the growth of Alternaria citri on citrus fruit juice. Fla. State Hort. Soc. 98:214-215.

Hasij, S.K. 1970. Physiological studies of *Alternaria citri* and *A. tenuis*. Mycologia. 62(2):289-295.

Hubballi, M., S. Nakkeerram, T. Raguchander, T. Annand and R. Samiyappan. 2010. Effect of Environmental Conditions on Growth of *Alternaria alternata* causing leaf blight. World Journal of Agricultural Sciences. 6(2):171-177.

Khan, S.A. and M. Kamal. 1963. Cercosporae of Sind region including 35 new records. Pakistan Journal of Science and Industrial Research. 6:118-119.

Kirk, P.M., P.F. Cannon, D.W. Minter and J.A. Stalpers. 2008. Dictionary of the Fungi. 10th Edition. Wallingford. CABI. Pp.22.

Maholay, M.N. 1989. Seed borne diseases of cucurbits. III. Bottle gourd (*Lagenaria siceraria* Mol.) Standl. Seed and Farm. 15:30-31.

Manthachitra, P. 1971. Investigations on seed borne fungi of some vegetable crops of Thailand. Summaries of research projects (1967-1988). S.B. Mathur. 1990. Danish Government Institute of Seed Pathology for Developing Countries, Denmark. Pp.18.

Mathur, S.B. 1990. Summaries of Research Projects 1967-1988. Danish Government Institute of Seed Pathology for Developing Countries, Denmark. Pp.111.

Nair, L.N. 1982. Studies on mycoflora of seeds: some cucurbitaceous vegetables. Journal of Indian Botanical Society. 61:342-345.

Neerurkar, P.V., Y.K. Lee and E.H. Linden. 2006. Lipid lowering effects of *Momordica charantia* (bitter gourd) in HIV-1-protease inhibitor treated human hepatoma cells, HepG2. British Journal of Pharmacology. 148(8):1156-1164.

Pati, P.K., Sharma, M. Salar, R.K. Sharma, A.P. Gupta and B. Singh. 2008. Studies on leaf spot disease of Withania somnifera and its impact on secondary metabolites. Indian Journal of Microbiology. 48:432-437.

Singh, N.P., D.K. Singh, Y.K. Singh and V. Kumar. 2006. Vegetable seed production technology. 1st Edition, International Book Distributing Company, Lucknow. 143-145.

Sridhar, M.G., R. Vinayamoorthi, A.V. Suyambunathan, Z. Booby and N. Selvaraj. 2008. Bitter gourd (Momordica charantia L) improves insulin sensitivity by increasing skeletal muscle insulin-stimulated IRS-1 tyrosine phosphorylation in high fat fed rates. British Journal of Nutrition. 99(4):806.