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PREVALENCE OF EARLY BLIGHT OF TOMATO AND DIFFERENCES AMONG ISOLATES OF *ALTERNARIA SOLANI* IN PESHAWAR DIVISION

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ABSTRACT

A survey was conducted in Peshawar division to determine the incidence of early blight of tomato caused by *Alternari asolani*. High disease incidence (100%) was recorded in Pathwarbala, Sufaid sang, kanderysadin and Shahibala. Regiaftezai and Malakandhir had the lowest (50%) incidence of the disease. *Alternaria solani* was successfully isolated on PDA medium from the infected tomato leaves of three locations. Comparison of the isolates showed Regilalma isolate to be the most vigorous. The conidia of this isolate were found to be wider than those of the other isolates. All the three isolates grew better at pH 6.0 and 5.5 as compared to pH4.5. Regilalma and Nasir bagh isolates registered more abundant growth than the Malakandhir isolate. These result shows that isolates of *Alternaria solani* may differ in their growth and virulence over short distances.
Keywords: *Alternaria solani,* disease incidence, PDA medium, pH, isolates.

INTRODUCTION

Tomato (*Lycopersicum esculentum*) which belongs to the family *Solanaceae* is believed to be first domesticated in Mexico (anonymous 1983). It is the second most important vegetable crop after potato. Present world production is about 126 million tons (FAO statistics database, 2009). Tomato fruit is rich in vitamins A and C and contains an antioxidant, lycopene (Jones 1999). Statistical data for the year 2008-2009 shows that total area under tomato cultivation in Pakistan is 53400 hectares, with a total production of 561900 tonnes and 10.5 tonnes/hectare, whereas tomato crop in KPK occupied 16500 hectares with a total production of 161800 tonnes and 9.8 tonnes/hectares (MINFAL,2008-2009).

Tomato plants are subject to a large number of pests and diseases from the time of emergence to harvest. Among these, early blight disease induced by *Alternariasolani* is one of the most important limiting factors in tomato production (Rich, 1983). It is widespread in the tropics, subtropics and temperate zones and can attack the plants at any stage of development causing a significant risk to crop productivity in the field and quality in the market (Anonymous 1983). Early blight, caused by *A.solani* is a common and serious fungal pathogen of tomato as it results in yield loss and reduced fruit quality (Jones et al. 1991).

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Early blight inflicts a wide range of symptoms at all stage of plant growth. It can cause damping-off, collar rot, stem cankers, leaf blight and fruit rot. The classic symptoms occur on the leaves where circular lesions up to 1/2" in diameter are produced. Within these lesions, dark concentric circles can be seen. The leaf blight phase usually begins on the lower, older leaves and progresses upwards. Infected leaves eventually wither, die, and fall from the plant (Sherf and Macnab, 1986).

All *Alternaria* species are highly resistant to adverse weather. They develop in a wide range of temperatures and use the locally available source of moisture. They sporulate best on necrotic and dead tissues and produce a relatively small number of spores, mainly at the end of the season, and they are decisively affected by the age condition and susceptibility of host plants (Rotem, 1998). The best way to manage the disease is to use preventive measures by combining many control measures together. Once early blight is established in the crop, it is very difficult to be controlled (Smith and kotcon, 2002). Management of early blight disease in tomato currently relies on fungicides such as Ridomil Gold, Sulphur and copper compounds (Markham 1990). The widespread use of these fungicides have significant drawbacks including increased cost, handling hazards, pesticide residues in agricultural commodities, and threat to human health and environment (Paranagamaetal., 2003).

Alternaria genus is cosmopolitan in occurrence. The members of this species like

*Alternaria alternata, A. solani, A. porri, A. dauci, A. helianthi, A. carthami*and *A. macrospore* causing different diseases in their respective host (Rotem, 1998). Among them,*A.solani causing* early blight of potato (*Solanum tuberosum*) and tomato (*L.esculentum*) is the most destructive (Rotem, 1998) disease of field crops. It causes diseases on foliage (blight), basal stems if seedlings (collar rot and damping off), stems of adult plants (stem lesions), fruits (fruit rot) of tomato and may also infect egg plant and pepper. This disease can be very destructive if left uncontrolled, often resulting in complete defoliation of plants (Dater and Mayee, 1985).
Therefore, there is dire need for advance planning and research to increase food production and improve quality in order to meet the needs of ever increasing population.
Considering the importance of tomato, the costs of diseases in terms of yield reduction, expenditure on their control, the present study is designed to investigate the prevalence of early blight in tomato in Peshawar division, variation among different isolates and their responses to different pH levels.

MATERIALS AND METHODS

Diseased survey and collection of specimen:
The survey was conducted at different locations of Peshawar division. Early blight infected leaves were collected in polythene bags and brought to the laboratory of department of plant pathology, The University of Agriculture, Peshawar-Pakistan, where they were stored at 4^0c until isolation of *Alternaria solani*was made.

Disease Incident (%):
Diseases incidence in tomato fields was recorded by randomly throwing a 1m diameter ring. The total and infected plants inside the ring were than counted and % disease incidence was calculated by the formula:

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$$\frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

Isolation of *Alternariasolani*:
Alternaria solani was isolated from diseased leaves on PDA medium. PDA medium was prepared using standard procedure (for one liter medium, 250g potato, 20g dextrose and 20g agar). The medium was sterilized at 121^0c for 20 minutes. After sterilization streptomycin was added for the inhibition of bacteria growth while pouring media into Petri dishes.
The infected leaves were cut into small pieces, surface sterilized with mercuric chloride (0.1%) for 15-30 seconds and then rinsed with sterilized distilled water thrice. The small pieces were placed on PDA medium (4-5 pieces/petri dish). The plates were then incubated at $25C^0$ for growth of *Alternaria solani*. Pure cultures of *A. solani* were prepared on fresh PDA medium by sub culturing.

Comparison of*Alternariasolani* isolates:
Alternaria solani were inoculated at the centre if fresh PDA medium in petridishes under aseptic conditions; for each isolates four petridishes were used in completely randomized (CR) design. The plates were incubated at 25^0c for the growth of the isolates. Colony diameters of the isolates were measured periodically along two perpendicular lines. Slides were prepared for determining the size of conidia through micrometry. Average conidium size was calculated from 20 individual measurements.

Response of *Alternariasolani* isolates to changes in pH of PDA medium:
Alternaria solani isolates were culture on PDA medium adjusted to pH levels of 4.5,5.5 and 6.0 pH was lowered or raised by adding hydrochloric acid (HCL) or Sodium Hydroxide (NaOH) to PDA medium, respectively, prior to sterilization. The factorial experiment (isolates and pH levels) was replicated four times. After incubation at 25^0Cdata on colony diameters were recorded periodically as described above. At the end of the experiment, the petridishes were flooded with sterile distilled water (10 ml each) conidia were detached from the cultures by rubbing gently with a glass rod. The spore concentration was determined with the help of Haemocytometer. The data were analyzed statistically by ANOVA and LSD test at 5% probability level.

RESULTS

Incidence (%) of early blight diseases of tomato

Survey conducted on diseases incidence at different locations of Peshawar division (Table 1). The highest diseases incidence (100%) was recorded in areas of Pathwarbala, Sufaid sang, kandarysadin and Shahibala. Areas of Malkandhir and RegiAftezai had the lowest diseases incidences (50%).

Table-1: Disease incident of early blight disease in Peshawar Division

S. No.	Location	Incidence (%)
1	PathwarBala	100
2	RegiAftezai	50
3	Malkandhir	50
4	Sufaid Sang	100
5	Kandarysadin	100
6	Regilalma	80
7	Nasir bagh	70
8	Amangharh	60
9	Jamma khan kalay	60
10	Shahibala	100
11	Palosi	60
12	Safdarabad	70
13	Azakhailbala	75
14	AzakhailPayan	80
15	Peer Bhai	60

Comparison among *Alternaria solani* isolates in terms of colony diameter and conidia size

Data presented in table-2 shows comparison of three isolates in terms of colony diameter and conidia size. After 10 days of incubation, the highest colony diameter (55.53) was recorded for isolate Regilalma. Isolate Malakandhere and Nasir Bagh were statistically at par with each other. However, the three isolates did not differ significantly in conidium length and width (Table-2).

Table-2: Comparison among *Alternariasolani*isolates in terms of colony diameter and conidia size

Location	Colony diameter (mm) after 3 days	Colony diameter (mm) after 10 days	Spore width (µm)	Spore length (µm)
Malakandhir	17.00 B	43.66 B	0.453	1.17
RegiLalma	20.33 A	55.33 A	0.463	1.14
Nasir Bagh	18.44 AB	45.11 B	0.46	1.13

Response of *Alternaria solani* isolates to changes in pH:

After 3 days of incubation, colony diameters recorded for the three isolates (Table-3) revealed that isolates of Regilalma and Nasir Bagh had grown significantly more than Malakandhir isolate. On the other hand, maximum growth (19.83mm) was recorded at pH 6.0, which reduced significantly as the pH level was decreased to 5.5 and 4.5. However, isolates did not interact significantly with the pH levels.

After 10 days of incubation, Regilalma isolate maintained its superiority in growth recorded (Table-3), followed by Nasir Bagh isolate. Malkandhir isolate registered the lowest growth. Growth on pH 6.0 and 5.5 was observed to be similar but higher than at pH 4.5. The isolates interacted significantly with pH levels. While Regi Lalma isolate had more

abundant growth at pH 6.0 And 5.5 than 4.5, Nasir Bagh isolate grew as much as at ph 4.5 as at either 6.0 or 5.5. On the other hand,Malakandhir isolate had similar growth at all pH levels.

After 10 days of incubation, colony diameters of RegiLalma and Nasir Bagh Isolates of *Alternaria solani* were statistically at par (Table.3) and significantly higher than that of Malakandhir isolate, similarly, growth at pH 6.0 and 5.5 was similar but higher than that of pH 4.5. The interaction between isolates and pH levels was also significant. Isolates of Regi Lalma and Malakandhir had statistically similar colony diameters at pH 6.0 and 5.5 but Nasir Bagh isolate had significantly more growth at pH 6.0 than at pH 5.5.

Table-3: Colony diameters (mm) after three, seven and ten days of incubation

Location	Colony diameter	pH=4.5	pH=5.5	pH=6.0	Mean
Malakandhir	Colony diameter after 3 days of incubation	15.25	17	19	17.08 B
RegiLalma		17.37	19.62	20.5	19.17 A
Nasir Bagh		17.62	18.25	20	18.63 A
Mean		16.75	18.29 B	19.83 A	
Malakandhir	Colony diameter after 7 days of incubation	27.62 C	45.00 C	45.00 C	38.83 C
RegiLalma		44.00 C	56.62 A	56.00 A	52.21 A
Nasir Bagh		46.12 BC	43.87 C	50.00 B	46.83 B
Mean		39.25 B	48.13 A	50.50 A	
Malakandhir	Colony diameter after 10 days of incubation	33.75 D	66.12 BC	65.25 ABC	55.04 B
RegiLalma		56.00 C	70.12 AB	67.62 AB	64.58 A
Nasir Bagh		61.37 BC	56.62 C	75.12 A	64.37 A
Mean		50.38 B	64.29 A	69.33 A	

Spores concentration of *Alternaria solani* isolates:

Regarding spore concentration, neither the isolates of *A.solani*nor the pH levels differed significantly from one another. The interaction was also non-significant.

Spore concentration of *A. solani* isolates

Table-4 Spore Concentration of *A. solani*isolates

Location	pH=4.5	pH=5.5	pH=6.0	Mean
Malakandhir	12.99	10.66	10.32	11.32
RegiLalma	10.33	10.66	8.32	9.77
Nasir Bagh	10.33	9.82	5.99	8.71
Means	11.21	10.38	8.21	

DISCUSSION

This study found variation in disease incidence of early blight of tomato among different locations of Peshawar division. The variation in disease incidence may indicate the existence of isolates differing in virulence. *In vitro* differences in growth among selected isolates in this study confirmed this assumption. Isolates growing more vigorously on PDA medium caused higher disease incidence in the field. Another reason for variation in disease incidence may be the prevalence of different environmental conditions, such as temperature and humidity, at different locations which influence the final disease outcome. The use of different cultural practices by farmers may also result in different levels of the disease such practices may include the use of resistant varieties, proper time of sowing, sanitations and the use of appropriate fungicides in the proper dose, frequency and time. The choice and availability of different cultural practices may in turn be an indication of the socioeconomic and educational levels of the farmer.

The isolates of *Alternariasolani* grew best at slightly acidic medium. Growth decrease as pH of the medium was reduced. Again, isolates showed differences in their response to pH levels. This finding is similar with the results of Chaerani*et al.* (2007)

The results of the study show the need for further research to elaborate mechanisms of variation among isolates of *Alternaria solani* so that effective strategies could be devised for the management of this economically important disease of tomato in Peshawar and other areas of the province.

CONCLUSION

The prevalence of early blight disease of tomato varied from location to location in Peshawar division indicating pathogenic variation among isolates of *A. solani.*Isolates of *A. solani* showed variation in *In vitro* growth reflecting their filed variation. It is concluded that isolates of *A. solani* grow best at slightly acidic PDA medium and they respond differently to changes in pH levels.

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