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IN VITRO GROWTH COMPARISON OF VARIOUS ISOLATES OF LEAF SPOT OF BITTER GOURD PATHOGEN (*ALTERNARIA* SP)

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Abstract

An *in vitro* study was conducted to characterize ten (10) isolates of *Alternaria* sp, the cause of leaf spot of bitter gourd. Isolates were collected from Peshawar (Chamkani, Nasirpur, Taimalpura, Yaseen Abad and Budhni) and Nowshera (Jabba Daudzai, Zakhi Miana, Ghari Momin, Tarujabba and Qasim Ali Baig) districts during 2010 growing season of the crop. *Alternaria* sp from all the ten locations were isolated in aseptic conditions. Then, an experiment was framed using Completely Randomized (CR) design. Each isolate was replicated five times. Data were collected after five and ten days of incubation at 25°C of *Alternaria* sp. Significant differences were observed in colony growth of *Alternaria* sp after five and ten days of incubation. The colony diameter of *Alternaria* sp isolated from Taimalpura was the highest (7.9 cm), whose growth was the fastest and lowest (4.5 cm) of Nasirpur and Jabba Daudzai having slowest mycelia growth.

Key words: Leaf spot, Bitter gourd, *Alternaria* sp, Characterization, Isolates and Comparison.

INTRODUCTION

Momordica charantia L is a tropical and sub-tropical vegetable belongs to the family cucurbitaceae. It is widely grown in India, Nepal and other parts of the Indian sub-continent, South Asia, China, Africa and the Caribbean (Abascal and Yarnell, 2005). Bitter gourd is cooked and eaten mainly as a vegetable. It is an excellent source of vitamin A, B, C, E and K, carbohydrates, dietary fiber, phosphorous, Ca, Fe, Mg, K and Na (Abascal and Yarnell, 2005). It has been used in various traditional medicines for cancer, diabetes, viral and bacterial infections (Josef and Jini., 2013). It is useful for diabetic patients by increasing insulin sensitivity (Sridhar et al, 2008 & Josef and Jini. 2013). *Alternaria* leaf spot has become a major threat to bitter gourd in Khyber Pakhtunkhwa. The pathogen (*Alternaria* sp) caused great losses to the crop ranging

from 80-88% on pumpkins and water melons in India (Bhargava and Singh, 1985). The responsible fungus is *Alternaria cucumerina* (Ellis and Everth) Elliot (Ibrahim et al, 1975 and Jakson, 1959). The other crops infected by the pathogens are water melons, musk melons, pumpkins and cantaloupes (Bhargava and Singh, 1985, Chandler and Thomas, 1991, Ibrahim et al, 1975, Latin, 1992, Rotem, 1994, Schenk, 1968, Thomas, 1983 and Thomas et al, 1990). Environmental conditions especially temperatures (20-32°C) prevailing during the growing season and high relative humidity or dew presence favor the disease development. The disease attack the leaves especially lower leaves of cucurbit crop causing large necrotic areas at favorable conditions and sporulation of the fungus in the form of dark brown growth could be observed on the upper surface of necrotic areas (Batta, 2003). Because of the importance of the crop

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and the disease, this research study was initiated to isolate and identify the causal organism of leaf spot of bitter gourd and assess the colony growth of the ten different isolates of *Alternaria* sp.

MATERIALS AND METHODS

Isolation and identification of the pathogen:

Isolates (10) of leaf spot of bitter gourd (*Alternaria* sp) were collected from Peshawar (Chamkani, Nasirpur, Taimalpura, Yaseen Abad and Budhni) and Nowshera (Jabba Daudzai, Zakhi Miana, Ghari Momin, Tarujabba and Qasim Ali Baig) districts of Khyber Pakhtunkhwa, Pakistan during 2010 growing season of the crop. These were brought to the laboratory of the Department of Plant Pathology, The University of Agriculture, Peshawar and preserved. Pathogen from all the ten isolates were isolated on general medium, potato dextrose agar (PDA) medium under aseptic condition. PDA was prepared by using the standard procedure (for 1 liter medium, 250gm potato, 20gm agar and 20gm dextrose). Medium was sterilized at 121°C for 15 minutes. Streptomycin was added for the inhibition of bacterial growth and then poured it into the Petri plates.

For isolation, the infected parts of the bitter gourd leaves were cut into small pieces, then surface sterilized by dipping in 0.1% solution of HgCl₂ for 15-30 seconds. After three dips in sterilized distilled water, the specimens were placed on Petri plates having PDA and kept at 25°C in incubator for the growth of the pathogen.

Temporary slides were prepared from the infected leaves as well as from the isolated pure culture. These were thoroughly observed under the microscope for the identification of the causal organism. Help were taken from the key of Barnet and Hunter (1972).

In vitro study: Experiment was conducted in aseptic conditions, using sterilized glass wares. The design used was Completely Randomized. Each isolate was replicated five times. Inoculum plug of equal size was maintained for all the isolates. Then, all Petri plates were kept in incubator at 25°C for the fungal growth. Data on colony diameter was recorded by measuring the colony diameter with the help of ruler at two places of the fungal growth in each Petri dish perpendicular to each other. Then averages of the two were determined. This was recorded after five and

ten days of incubation. All the recorded data were then pooled for statistical analysis using analysis of variance (ANOVA) and least significant difference test (Dana, 2001) was performed at 5% level of probability.

RESULTS AND DISCUSSION

Identification of the pathogen: Temporary slides from pure culture as well as from affected leaves were made and thoroughly examined under the microscope. The observations were matched with the photographs and text in the key of Barnet and Hunter (1972). Help was also taken from the internet. The causal organism of leaf spot of bitter gourd was identified as *Alternaria* sp.

Growth of different isolates of *Alternaria* sp: Data presented in Table 1 indicated that there were significant differences among the different isolates of *Alternaria* sp after 5 days of incubation. The colony diameter of *Alternaria* sp isolated from Taimalpura was 6.2cm. This was the highest radial colony growth than the other isolates. This was followed by Yaseen Abad isolate, whose colony diameter was 5.7cm. Isolate of *Alternaria* sp isolated from Zakhi Miana had the lowest colony diameter (2.6cm). The colony diameter of Peshawar isolates was 2.9-6.2cm while that of Nowshera were 2.6-5.5cm.

Data recorded after 10 days of incubation (Table 1) indicated significant differences among different isolates of *Alternaria* sp. The highest (7.9cm) colony diameter was of isolate Taimalpura followed by the colony diameter (7.8cm) of isolate Yaseen Abad. The colony diameter of isolates of *Alternaria* sp isolated from Jabba Daudzai and Nasirpur was the lowest (4.5cm). The colony diameter of Peshawar isolates was 4.5-7.9cm while that of Nowshere were 4.5-7.1cm.

Proper identification of pathogenic species had a direct impact on epidemiological studies and disease management. Different isolates and species have different life histories, such as growth rate, timing of sporulation, number of spores produces and optimal condition for spore germination and growth. All of these factors are important in the development of disease forecasting models, which is critical in optimizing effective and economical control programs. In summary, the most successful

management of *Alternaria* leaf spot of bitter gourd will be achieved only after a definitive assessment can be made of the diversity that exist among different isolates and species of *Alternaria* that occur on the bitter gourd and the potential of these distinct isolates and species that cause the disease. This study revealed

distinct variability in the mycelia growth of *Alternaria* isolates. Variability was also reported by other workers (Sofi et al., 2013) in the isolates of *Alternaria mali*. They reported that some of the isolates were the fastest while others were slowest. The isolates showed periodic changes in growth rate.

District	Locations	Colony growth (After 5 days)	Colony growth (After 10 days)
Peshawar	1.Chamkani	3.0 cde*	4.6 b*
	2.Nasirpur	2.9 de	4.5 b
	3.Taimalpura	6.2 a	7.9 a
	4.Yaseen Abad	5.7 ab	7.8 a
	5.Budhni	3.4 cd	5.0 b
Nowshera	1.Jabba Daudzai	2.8 de	4.5 b
	2. Zakhi Miana	2.6 e	4.6 b
	3.Ghari Momin	5.5 ab	7.1 a
	4.Taru Jabba	5.3 b	6.8 a
	5.Qasim Ali Baig	3.7 c	6.9 a
LSD _{0.05}	---	0.74	1.53
CV (%)	---	14.1	20.1

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

CONCLUSION

There were significant differences between the radial colony growth of different isolates of *Alternaria* sp, the cause of leaf spot of bitter gourd, incubated at 25°C. The colony diameter of the isolates, isolated from Taimalpura was the highest (7.9 cm) and lowest (4.5 cm) of Nasirpur and Jabba Daudzai. On the basis of these results and as far as the problem is concerned, it is recommended for further detail systematic continuous research work to solve the problem of the farmers' community.

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