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In vitro efficacy of *Trichoderma harzianum* Rifai against various isolates of *Alternaria* sp

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Abstract
An *in vitro* study was conducted to determine the efficacy of *Trichoderma harzianum* against ten isolates of *Alternaria sp*, the cause of leaf spot of bitter gourd. Isolates were collected from Peshawar (Chamkani, Nasirpur, Taimulpora, Yaseen Abad and Budhni) and Nowshera (Jabba Daudzai, Zakhi Miana, Ghari Momin, Tarujaba and Qasim Ali Baig) districts during 2011 growing season of the crop. All the ten isolates were cultured in aseptic conditions. Then, an experiment was conducted using Completely Randomized (CR) design. Each isolate was replicated three times. Significant differences (P<0.05) were observed in colony diameter of *Alternaria* isolates after four and eight days of incubation against *T. harzianum*. *Trichoderma harzianum* reduced the mycelia growth of *Alternaria* isolates by 70.4 to 88.1%, with lowest (70.4%) of Jabba Daudzai and highest (88.1%) of Chamkani as compared with their respective control, when *T. harzianum* was applied from two sides. It was 76.0 to 92.3% with lowest (76.0%) in Jabba Daudzai and highest (92.3%) of Chamkani, when *T. harzianum* was applied from four sides to the pathogens as compared to respective control.

Keywords: *In vitro*, biological control, *Trichoderma harzianum*, *Alternaria* sp, bitter gourd

1. Introduction
Bitter gourd (*Momordica charantia* L) belongs to family Cucurbitaceae. It is widely grown in India, Nepal and parts of the Indian sub-continent. It is an excellent source of vitamin A, B, C, E, and K, carbohydrates, phosphorus, Ca, Fe, Mg and Na. [1]. It has some medicinal values i.e useful for diabetic patients, stimulate digestion, have anti-malarial activity and effective for HIV infection [1, 9, 17]. *Momordica charantia L* is attacked by a number of diseases e.g leaf spots (*Alternaria* spp, *Cercospora* spp *Myrothecium roridum*) powdery mildew, white rot of fruit and *Rhizoctonia solani* fruit rot [13, 15].
Alternaria leaf spot has become a major threat to bitter ground in Khyber Pakhtunkhwa. The pathogen *Alternaria sp* cause great losses to the crop ranging from 80-88% on pumpkins and water melons in India [4]. The responsible fungus is *Alternaria cucumerina* (Elis and Everth) Elliot [6, 11]. The other crops infected by the pathogen are water melons, musk melons, pumpkins and cantaloupes [4, 5, 12, 14, 19-22].
Under local conditions, introduction of a large number of hybrid, bitter gourd in to the Pakistani cultivars, demonstrated the presence of high incidence of the disease. The environmental conditions especially temperatures (20-32) prevailing during the growing season and high relative humidity or dew presence favors the disease development [3].
Because of the importance of the crop and the disease (leaf spot of bitter gourd), this research study was proposed having the objectives as, (i) Isolation and identification of *Alternaria sp* from infected bitter gourd leaves collected from ten different locations of Peshawar and Nowshera and (ii) To test the efficacy of *Trichoderma harzianum* against ten different isolates of *Alternaria sp*.

2. Materials and Methods
2.1 Isolation 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, Garhi Momin, Tarujabba and Qasim Ali Baig) districts during 2011 growing season of the crop. Pathogen from all the ten isolates was isolated on general medium, potato dextrose agar (PDA) medium under aseptic conditions. PDA was prepared by using the

standard procedure (for 1 liter medium, 250 gm potato, 20 gm agar and 20 gm dextrose). Medium was sterilized at 121 ^{0}C for 15 minutes. Streptomycin was added for the inhibition of bacterial growth and then poured it into the petri plates.

For isolation, infected parts of the bitter gourd leaves were cut into small pieces, then surface sterilized by the dipping in 0.1% solution of Mercuric Chloride ($HgCl_2$) for 15-30 seconds. After three dips in sterilized distilled water, the specimens were plated on Petri plates, having PDA, and kept at 25 ^{o}C in incubator for the growth of the pathogen.

2.2 Identification of pathogen

Temporary slides were made from specimen and pure culture of the pathogen and were identified by using the key of Barnet and Hunter [2].

2.3 *In vitro* study

The experiment was conducted in aseptic conditions, using three replicate Completely Randomized (CR) design. *Tricherma harzianum* culture was obtained from the Department of Plant Pathology, The University of Agriculture, Peshawar. Inoculum plug of uniform diameter was used of all the isolates of *Alternaria* sp and of *T. harzianum*. *Alternaria* sp was kept in the center and *T. harzianum* was applied from two sides (T_1) and from four sides (T_2). One Petri plate was kept as control (T_0). This practice was conducted for all the isolates. Then, all the Petri plates were kept in incubator at 25 ^{0}C for fungal growth. Data were recorded on colony diameter of *Alternaria sp* after four and eight days of incubation.

2.4 Statistical analysis

All the recorded data were pooled for statistically analysis using analysis of variance (ANOVA) and Least Significant Difference (LSD) test [7].

3. Results and Discussion

3.1 Identification of pathogen

Alternaria sp was identified by using the key of Barnet and Hunter [2].

3.2 Control of Different isolates of *Alternaria sp* against *Trichoderma harzianum*

Data present in Table 1 indicated that there were significant differences among the colony diameter of *Alternaria sp* against *T. harzianum* in districts Peshawar and Nowshera with the highest (21.4 mm) colony diameter in Peshawar. The response of the isolates to *T. harzianum* were significantly different (P<0.05). The colony diameter of these isolates varied from 13.31 to 25.2 mm with the higest (25.2 mm) in Budhni and lowest (13.1 mm) in Jabba Daudzai after four days of incubation at 25 ^{0}C. *Trichoderma harzianum* restricated the growth of all the ten isolates of *Alternaria sp*. It was 29.6 to 81.6% with the lowest (29.6%) of Zakhi Miana and highest (81.6%) of Chamkani isolates, when *T. harzianum* was applied from two sides in the Petri dishes, as compared with their respective control. Also in T_2 (when *T. harzianum* was applied from four sides), the response of isolates to *T. harzianum* were significantly (P<0.05) different. Here, *T. harzianum* reduced the mycelial growth of different isolates by 39.7 to 85.5%, as compared with their respective control. *T. harzianum* restricated the mycelial growth (Table 2) of different isolates significantly (P<0.05). The Tarujabba isolate produced the lowest (18.0 mm) while the highest (30.9 mm) by Budhni. *T. harzianum* reduced the mycelial growth of *Alternaria sp* by 70.4 to 88.1% with the lowest (70.4%) in Jabba Daudzai and highest (88.1%) in Chamkani, when *T. harzianum* was applied from two sides to *Alternaria sp*, as compared with their respective control. When *T. harzianum* was applied from four sides, it was 76.0 (Jabba Daudzai) to 92.3% (Chamkani) as compared with their respective control, after 8 days of incubation (Table 2).

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 rates, timing of sporulation, number of spores produced and optimal conditions 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 distinctive 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. Certain authors reported the antagonistic effect of *T. harzianum* to many fungi causing soil borne diseases [10, 18]. *T. harzianum* exercises its antagonistic effect to phytopathogenic fungi through secretion of antifungal antibiotics called viridin or through production of antifungal substance called gliotoxin where both of them inhibit the germination of phytopathogenic fungal conidia and growth of mycellia. Recently, the antagonistic effect of *T. harzianum* to *Alternaria* sp was also reported on leaves and fruits [3, 8]. The probable mode of action of *T. harzianum* against these fungi might be through the production of viridian and gliotoxin [3].

Table 1: *In vitro* efficacy of *Trichoderma harzianum* against ten different isolates of *Alternaria sp* after four (4) days of incubation at 25^{0}C.

Districts (D)	Locations (L)	Treatments (T)	Colony Diameter (mm)	Colony Diameter (mm) (DxL)	Colony Diameter (D)
Peshawar	Chamkani	T_0	51.7 A* (- -)	22.9 AB*	21.4 A*
		T_1	9.5 H-K (81.6)[1]		
		T_2	7.5 B (85.5)		
	Nasirpur	T_0	41.0 B (- -)	21.4 BC	
		T_1	12.7 F-J (69.0)		
		T_2	10.7 G-K (73.9)		
	Taimalpura	T_0	37.7 B (- -)	18.1 DE	
		T_1	9.3 H-K (75.2)		
		T_2	7.33 JK (80.5)		
	Yaseen abad	T_0	39.5 B (- -)	19.5 CD	
		T_1	10.5 G-K (73.4)		
		T_2	8.5 IJK (78.5)		
	Budhni	T_0	47.2 A (- -)	25.2 A	
		T_1	15.2 EFG (68.0)		
		T_2	13.1 F-I (72.2)		

Nowshera	Jabba Daudzai	T_0	20.4 DE (- -)	13.1 F	21.4 A*
		T_1	10.4 G-K (49.1)		
		T_2	8.4 IJK (58.8)		
	Zakhi Miana	T_0	19.9 DE (- -)	15.3 EF	
		T_1	14.0 FGH (29.6)		
		T_2	12.0 F-J (39.7)		
	Garhi Momin	T_0	23.8 CD (- -)	18.2 DE	
		T_1	16.3 EF (31.5)		
		T_2	14.3 FGH (39.9)		
	Tarujabba	T_0	27.0 C (- -)	13.2 F	
		T_1	7.3 JK (72.9)		
		T_2	5.3 K (80.4)		
	Qasim Ali Baig	T_0	40.5 B (- -)	18.5 CDE	
		T_1	8.5 IJK (79.00)		
		T_2	6.5 K (84.2)		
LSD Value	..	..	5.5	3.2	1.4
CV (%)	..	..	18.1	18.1	18.1

T = Treatments (T_0 =Control, T_1 =Bio-agent applied at two sides of pathogen and T_2=Bio- agent applied at four sides of pathogen).
*Figures followed by different letters in the same column are significantly different from one another at 5% level of significance.
[1] Percent decrease than their respective control.

Table 2: *In vitro* efficacy of *Trichoderma harzianum* against ten different isolates of *Alternaria sp* after four (8) days of incubation at 25^0C.

Districts (D)	Locations (L)	Treatments (T)	Colony Diameter (mm)	Colony Diameter (mm) (DxL)	Colony Diameter (D)
Peshawar	Chamkani	T_0	71.7 A* (- -)	28.6 A*	26.6 A*
		T_1	8.5 E (88.1) 1		
		T_2	5.5 E (92.3)		
	Nasirpur	T_0	49.7 CD (- -)	23.8 AB	
		T_1	12.3 E (75.2)		
		T_2	9.3 E (81.3)		
	Taimalpura	T_0	54.7 BCD (- -)	24.7 AB	
		T_1	11.5 E (79.0)		
		T_2	7.8 E (85.7)		
	Yaseen abad	T_0	59.5 ABC (- -)	24.9 AB	
		T_1	9.3 E (84.5)		
		T_2	6.0 E (89.9)		
	Budhni	T_0	68.7 AB (- -)	30.9 A	
		T_1	13.5 E (80.3)		
		T_2	10.5 E (84.7)		
Nowshera	Jabba Daudzai	T_0	53.0 CD (- -)	13.1 F	21.4 A*
		T_1	15.7 E (70.4)		
		T_2	12.7 E (76.0)		
	Zakhi Miana	T_0	56.8 BC (- -)	15.3 EF	
		T_1	13.2 E (77.0)		
		T_2	10.2 E (82.0)		
	Garhi Momin	T_0	56.2 C (- -)	18.2 DE	
		T_1	13.0 E (77.0)		
		T_2	9.0 E (83.9)		
	Tarujabba	T_0	41.3 D (- -)	13.2 F	
		T_1	7.5 E (81.8)		
		T_2	5.2 E (87.4)		
	Qasim Ali Baig	T_0	63.2 ABC (- -)	18.5 CDE	
		T_1	12.2 E (80.7)		
		T_2	9.2 E (85.4)		
LSD Value	..	..	14.7	8.5	3.8
CV (%)	..	..	34.8	34.8	34.8

T = Treatments (T_0= Control, T_1= Bio-agent applied at two sides of pathogen and T_2 = Bio- agent applied at four sides of pathogen).
*Figures followed by different letters in the same column are significantly different from one another at 5% level of significance.
[1] Percent decrease than their respective control.

4. Conclusion and Recommendation

Response of *Alternaria sp* isolates to *T. harzianum* was significantly different either applied from two sides or four sides to the pathogens. *T. harzianum* have the ability in arresting the mycelia growth of *Alternaria sp* significantly.
Further exploration for the management of species of *Alternaria* through *T. harzianum* is needed.

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