

Effect of Botanicals and *Trichoderma harzianum* on Fusarium Wilt of Tomato (*Lycopersicon esculentum* Mill.)

¹ Habib Rahman Shirzad Ghorbandi, ² Abhilasha A Lal, ³ Sobita Simon

¹ Department of Plant Protection and Quarantine, Ministry of Agriculture, Irrigation and Livestock, Kabul, Afghanistan

^{2,3} Department of Plant Pathology, SHIATS, Allahabad, Uttar Pradesh, India

Abstract

Tomato (*Lycopersicon esculentum* Mill.) is economically the most important and popular fresh market vegetables throughout the world. It occupies number one position in its nutrient contribution to human diet. Fusarium wilt is known as one of the most devastating diseases of tomato worldwide. It is an important soil inhabiting fungi, and is known to be phylogenetically diverse. Most strains assigned to this species are saprophytic or non-pathogenic. Pathogenic fungi of the genus fusarium that are the causal agents of tomato wilt cause root and basal stem deterioration and result in the wilting of vegetable plants. Browning of the vascular tissue is strong evidence of fusarium wilt. The experiment was conducted under *in-vitro* and field culture conditions to observe the effect of bio-agents, botanicals and fungicide against *Fusarium oxysporum*. For the *in-vitro* studies five treatments were studied. Therefore, the Maximum percentage of inhibition was recorded in *Trichoderma harzianum* 74.53%, Ashoka 28.82%, *Psidium guajava* 23.61%, *Aloe-vera* 20.48%, *Lantana camera* 3.47%, followed by carbendazim 100%. The inhibition and effect of bio agent (*Trichoderma harzianum*) and leaf extracts against tested pathogen was due to competition and preventing the mycelia growth of the pathogen, which is very valuable and riskless in eco-friend, animals and human health.

Keywords: Botanicals, Fusarium wilt, *Fusarium oxysporum*, Tomato, *Trichoderma harzianum*

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is economically the most important and popular vegetables throughout the world. Successful cultivation of tomato is hindered by various diseases (Neela *et al.*, 2014) [7]. Tomato is one of the most popular fresh market vegetables grown commercially (Kaiser and Ernst, 2011) [5]. Tomato is a major contributor to the fruit vegetable diet of humans. It is cultivated in essentially all countries either in fields or in protected culture. Its many varieties are now widely grown, sometimes in greenhouses in cooler climates (Akrami and Yousefi, 2015) [2]. Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* is a disease that causes serious economic loss. Fusarium species causes a huge range of diseases on an extraordinary range of host plants. The fungus can be soil borne, airborne or carried in plant residue and can be recovered from any part of the plant from the deepest root to the highest flower (Singha *et al.*, 2011) [10]. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and root rot minimal or absent crop yield. The disease causes great losses, especially on the susceptible varieties of tomato especially when soil and air temperature are rather high during the warm season (Akrami and Yousefi, 2015) [2]. In view of the high cost of chemical pesticides and their hazardous consequence, use of biodegradable and different material like fresh plant extracts from different parts of the plants gained importance during the last three decades for plant disease control. Unfortunately, the chemical fungicides are not readily biodegradable, tend to persist for years in the environment and few fungi have developed resistance to them. Use of natural products like botanical amendments or botanical extracts for the management of fungal diseases (Ramaiah and Garampalli, 2015) [9]. The present studies entitled, Effect of botanicals and

bio-agent on fusarium wilt of tomato (*Lycopersicon esculentum* Mill.).

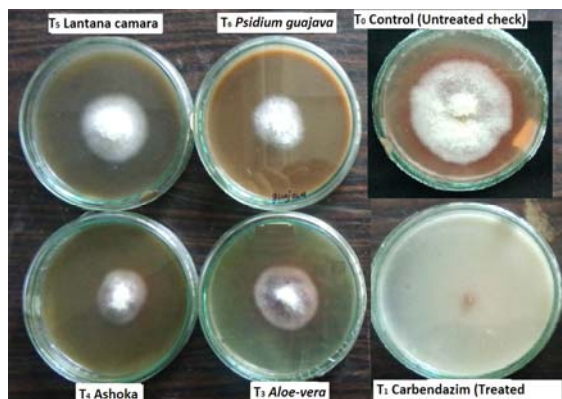
Materials and method

The present study was conducted field condition. Field experiment was laid-out in randomized block design with three replications at research plot of the department of Plant Pathology, Sam Higginbottom Institute of Agriculture, Technology and Science, during the *Rabi* season of 2015-16. The fresh leaves were ground in a mixer of pestle and mortar by using sterile distilled water. The extract filtered through double layered muslin cloth and made to the required concentration by adding distilled water Ramaiah and Garampalli (2015) [9]. The particulars of the botanicals used for the experiment in the field condition as well as *in vitro* condition. In the field 10 ml of extract was mixed with 90 ml of water and applied as soil drench around the tomato plants. In the lab *in-vitro* experiment added 10 ml of leaf extract into 90 ml of PDA, kept flasks in the auto clave for sterilizing. Mass culture of the fungus was prepared by soaking sorghum grains in tap water overnight and then surface dried by spreading on paper towel in laboratory under ceiling fan. Surface dried seeds were put into conical flasks @ 250g flask and the flasks were closed by inserting cotton plugs. These flasks were autoclaved at 15 psi for 20 minutes. The sterilized flasks after cooling were inoculated with ten days old *Fusarium oxysporum* L. actively growing culture, by adding 4 mm agar plugs using sterile cork borer. After 7-10 days the flasks were full of mycelial growth. After plugging these flasks were incubated at $26 \pm 2^{\circ}$ C for 12 days. This culture was mixed thoroughly in 10 g/kg of soil (Singh *et al.*, 2011) [6]. The sorghum grains which contained mycelium growth of the pathogen transferred in the field 3-5 days before the seedling transferring. Five mm diameter of culture disc of fusarium was

kept at the center of each petriplate containing the fungicides of required concentration dissolved in PDA. Three replications will be maintained. The plates will be incubated at 27^o C for ten days and colony diameter was recorded. Per cent inhibition of mycelial growth will be calculated (Vincent, 1947) [12].



placed on PDA medium one cm away from the edge of the plate, separately. *Trichoderma* spp. (9 mm disc) was placed at opposite side of the Petri plate. Three replicate plates for each treatment maintained and incubated at 25±3^o C. Per cent inhibition over control was calculated by the following formul (Sundaramoorthy and Balabaskar, 2013) [11].



In vitro evaluation

The *in-vitro* trial was laid out in completely randomized design (CRD) with four replications and five treatments including check in the experimental laboratory of department of Plant Pathology. The efficacy fungicides were tested for applying poison food and dual culture techniques against. The observation of the mycelial growth inhibition per cent was recorded at 48 - 168 hrs. Observation on disease incidence recorded for a period of 30, 60 and 90 days after transplanting (Wheeler, 1969) [13]. Nine mm disc of fungal cultures were

Result and Discussion

Effect of bio-agent and fungicide on the mycelial growth (mm) of *Fusarium oxysporum* at different time interval after inoculation. The data on the mycelial growth (mm) was influenced by bio-agents and fungicide and are given in the. For eco-friendly system in the nature and sustainable and proper method in management of fusarium wilt disease of tomato, the present study tested for inhibition and antifungal activities of important bio agent (*Trichoderma harzianum*) and some important leaf extracts.

Table 1: Mycelial radial growth (mm) of *Fusarium oxysporum* affected by different treatments

S. No.	Treatments	Radial growth (mm) of <i>Fusarium oxysporum</i>					
		48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
T ₁	Carbendazim (Treated check)	0.00	0.00	0.00	0.00	0.00	0.00
T ₂	<i>Trichoderma harzianum</i>	13.67	23.50	28.17	36.50	38.00	39.17
T ₃	<i>Aloe vera</i>	14.00	20.50	27.50	29.67	37.00	38.17
T ₄	Ashoka	10.67	17.17	21.83	25.83	30.67	34.17
T ₅	<i>Lantna camara</i>	10.83	20.33	27.00	34.33	40.67	46.33
T ₆	<i>Psidium guajava</i>	12.83	18.83	23.83	26.33	31.67	36.67
T ₀	Control (untreated check)	9.83	15.17	24.00	34.33	43.00	21.00
	F-test	S	S	S	S	S	S
	S E m=	3.59	4.00	3.51	5.18	3.74	5.35
	CD (5%)	4.98	5.55	4.88	7.20	5.19	7.43

After 48 hours minimum average radial mycelial growth of *Fusarium oxysporum* wilt pathogen of tomato was observed in T₀ (9.83 mm) after inoculation followed by T₄, T₅, T₆, T₂ and

T₃, as compared to T₁ (0). After 72 h observed in T₀, followed by T₄, T₆, T₅, T₃, T₂ and as compared to T₁ (0). After 96 h observed in T₄, T₆, T₀, T₅, T₃, T₂, compared to the T₁. After 120

h observed in T₄, T₆, T₃, T₀, T₅, T₂, compared to T₁. After 144 h observed in T₄, T₆, T₃, T₂, T₅, T₀, compared to T₁. After 168 h observed in T₀, T₄, T₆, T₃, T₂, T₅, compared T₁.

Using leaf extracts and essential oils against *Fusarium oxysporum* f. sp. *pisi*. Extract of *Aloe-vera* was most effective in inhibiting mycelial growth (69.25%) followed by *Lantana camara* (50.09%) (Ali *et al.*, 2013) [1]. The efficacy of the native isolates of *Trichoderma* species to promote the growth and yield parameters of tomato and to manage *Fusarium* wilt disease under *in vitro* and *in vivo* conditions. Fifteen native *Trichoderma* antagonists were isolated from healthy tomato rhizosphere soil in different geographical regions. Under *in vitro* conditions, the results revealed that *Trichoderma harzianum* isolate was found to effectively inhibit the radial mycelial growth of the pathogen (by 53%) when compared to all other isolates (Sundaramoorthy and Balabaskar, 2013) [11]. Biological control of *Fusarium oxysporum* f. sp. *L.* was studied *in vitro* conditions, dual culture technique showed that *Trichoderma harzianum* inhibited the radial colony growth of the test pathogen (Alwathnani and Perveen, 2012) [3]. Carbendazim and leaf extracts were evaluated for their effect on the inhibition of mycelial growth and spore germination of *Fusarium oxysporum* (Nisa *et al.*, 2011) [8].

Table 2: *In-vitro* growth inhibition of *Fusarium oxysporum* affected by different treatments

Treatments	% inhibition
T ₁ Carbendazim (treated control)	100.00
T ₂ <i>Trichoderma harzianum</i>	74.3
T ₃ <i>Aloe-vera</i>	20.48
T ₄ Ashoka	28.82
T ₅ <i>Lantana camara</i>	3.47
T ₆ <i>Psidium guajava</i>	23.61
T ₀ Treated Control	0
F- test	S
S. Ed. (±)	0.286
C. D. (P = 0.05)	0.606

However, the treatments were significant and statistically at par with each other.

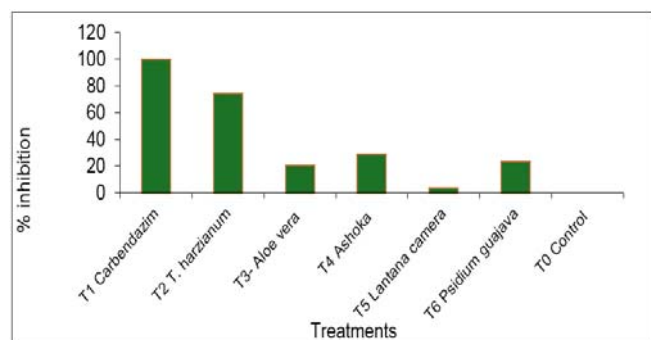


Fig 1: *In-vitro* mycelial growth inhibition of *Fusarium oxysporum* affected by different treatments.

Significant difference in inhibition percent of mycelial growth was observed among the treatments. Maximum percentage of inhibition was recorded in T₂ *Trichoderma harzianum* 74.53%

followed by T₄ Ashoka 28.82%, T₆ *Psidium guajava* 23.61%, T₃ *Aloe-vera* 20.48%, T₅ *Lantana camara* 3.47%, followed by T₁ carbendazim 100% and.

The percentage of plant height growth of tomato after 30, 60 and 90 days.

Table 3: The percentage of plant height growth of tomato after 90 days.

Treatments	1st	2nd	3rd
T ₁	25.58	31.13	38.28
T ₂	23.99	28.24	34.81
T ₃	23.16	30.22	35.53
T ₄	26.37	29.77	40.55
T ₅	22.42	30.39	38.05
T ₆	24.56	30.87	36.33
T ₀	25.45	28.17	37.73
F- test	S	S	S
S. Ed. (±)	0.975	0.691	1.082
C. D. (P = 0.05)	2.067	1.465	2.294

So, the T₃ (*Aloe-vera*) had the maximum height growth around 40% and T₁ (carbendazim) minimum height growth around 35%. But all are between 35-40%.

Plant height after 90 days 3rd

However, the treatments (T₂, T₃, T₆, T₀), (T₆, T₀, T₅ T₁), (T₀, T₅, T₁, T₄) were non-significant and statistically at par with each other.

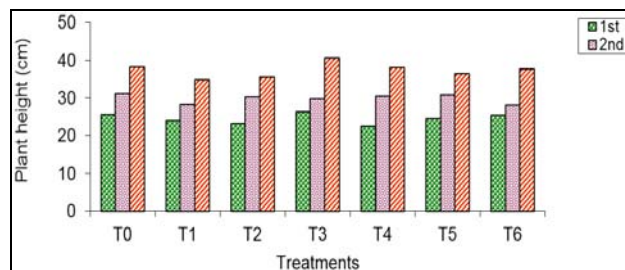


Fig 2: The percentage of plant height growth of tomato after 90 days.

Table 4: Percent disease incidence recorded observation in field condition.

Treatments	Disease incidence
T ₀	11.00
T ₁	4.00
T ₂	7.67
T ₃	9.33
T ₄	9.33
T ₅	9.33
T ₆	11.33
F- test	S
S. Ed. (±)	1.500
C. D. (P = 0.05)	3.179

Disease incidence

However, the treatments (T₂, T₃, T₄, T₅, T₀, T₆) were statistically non-significant at par with each other. And (T₁) was significant,

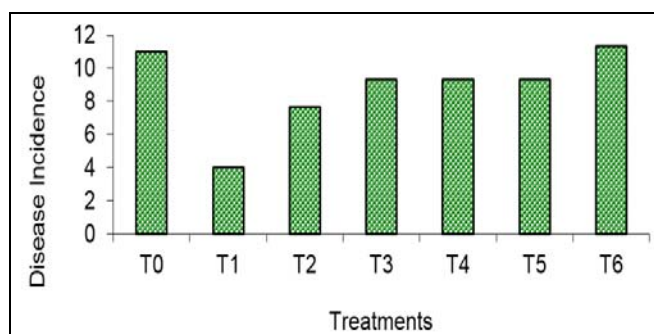


Fig 3: Percent disease incidence recorded observation in field condition:

In the all 21 plots in the field condition disease incidence was observed and analyzed in the above table and diagram. Therefore, the maximum disease incidence observed in the T6 (11.33) and T0 (11.00) and the minimum incidence T1.

Discussion

Tomato cultivation is affected by a number of diseases. Among them, the wilt disease caused by *Fusarium oxysporum* is a serious disease in major tomato-growing areas. A new approach in crop protection to reduce the disease damage level and microorganisms were reported in many crops for the control of fungal pathogens. The talc-based formulation has been reported for the management of several crop diseases in India (Anitha and Rabeeth, 2009) [4]. In present study, the pathogenic fungus was isolated from infected plant and identified based on morphological and cultural characters as *Fusarium oxysporum* f. sp. L. whose pathogenicity was confirmed by Koch's postulate on tomato seedling (Mishra *et al.*, 2014). Investigated that the *Fusarium* spp. (*F. solani* and *F. oxysporum*) are the important soil-borne pathogens and infects wide variety of hosts. The effects of *Trichoderma harzianum*, *T. asperellum*, and *T. virens* on the wilt disease complex of tomato (*Solanum lycopersicum*) caused by *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani*. Tomato cultivar inoculated with *F. oxysporum* f. sp. *ciceri* and *R. solani*, showed greater wilt incidence, chlorosis of leaves and induced vascular discoloration in roots (Akrami and Yousefi, 2015) [2]. The tomato fusarium wilt is considered as one of the most important diseases of tomato both in field and greenhouse – grown tomatoes worldwide (Amini and Sidovich, 2010).

References

1. Ali M, Lal M, Khan A, Singh V, Singh PS. Evaluation of leaf extracts and essential oils against *Fusarium oxysporum* f. sp. *pisi* the causal agent of pea wilt, Indian Phyto Pathology Journal, 2013; 66(3):316-318.
2. Akrami M, Yousefi Z. Biological control of fusarium wilt of tomato (*Solanum lycopersicum*) by *Trichoderma* spp. As antagonist fungi. Biological Forum Journal, 2015; 7(1):887-892.
3. Alwathnani HA, Perveen K. Biological control of fusarium wilt of tomato by antagonist fungi and cyano bacteria. Biotechnology Journal. 2012; 11(5):1100-1105.
4. Anitha A, Rabeeth M. Control of fusarium Wilt of tomato by bioformulation of streptomyces griseus in green house

- condition. Journal of Basic & Applied Sciences, 2009; 1(1-2):9-14.
5. Kaiser C, Erns M. Organic tomatoes, University of Kentucky College of agriculture, Food and Environment Journal, 2011; (2):132-133.
6. Mishra P, Singh P, Tripathi NN. Evaluation of plant extracts against *Fusarium oxysporum* f. sp. *lycopersici*, wilt pathogen of tomato. Food, Agriculture and Veterinary Sciences Journal, 2011; 4(2):163-167.
7. Neela FA, Sonia IA, Shamsi S. Antifungal activity of a selected medicinal plant extract on *Fusarium oxysporum* the causal agent of fusarium wilt disease in tomato. American Journal of Plant Sciences, 2014; (5):2665-2671.
8. Nisa T, Wani AH, Bhat MY, Pala SA, Mir RA. In vitro inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*, Biopesticides, 2011; 4(1):53.
9. Ramaiah AK, Garampalli RKH. In vitro antifungal activity of some plant extracts against *Fusarium oxysporum* f. sp. *lycopersici*. Plant Science and Research Journal, 2015; 5(1):22-27.
10. Singha IM, Kakoty Y, Unni BG, Kalita MC, Das J, Naglot A, *et al.* Control of *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* using leaf extract of *Piper betle* L. a preliminary study. World Journal of Microbiol Biotechnol, 2011; (10):11274-011.
11. Sundaramoorthy S, Balabaskar P. Biocontrol efficacy of *Trichoderma* spp. against wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*, Applied Biology & Biotechnology Journal, 2013; 1(03):036-040.
12. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature Journal, 1974; 159:239-241.
13. Wheeler BEJ. An introduction to plant disease, John Wiley and Sons, Ltd., London Journal, 1969; (1):302-309.