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Efficacy of Native *Trichoderma* spp. in Controlling *Fusarium* wilt of Tomato Plants in Green House, Yemen

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Abstract

This study investigated the ability of *Trichoderma* spp. to control the Fusarium wilt of tomato, as well as the effect of these isolates on tomato plant growth in green house. Two native *Trichoderma* isolates [*T. citrinoviride*, (T33) and Trichoderma sp. anamorph of *H. semiorbis*, (T15)] significantly reduced tomato Fusarium wilt severity (19.77%) and increased all plant growth parameters (root length, 6.65 cm; shoot length,18.29; root fresh weight, 2.94g; shoot fresh weight, 28.66g; root dry weight, 0.46g; shoot dry weight,4.03g and no. of leaves, 24.33. The combination of both species of Trichoderma were more effective in controlling the *Fusarium* wilt disease compared to their application as alone.

Key Words: *Tomato; Fusarium wilt; Trichoderma; biocontrol; green house*

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important edible and nutritious vegetable crop grown all over the world [1]. Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *Lycopersici* that is one of the economically most important disease in major tomato growing regions worldwide [2]. It is a highly destructive pathogen, causing 10 to 50% yield loss in many tomato production areas [3]. However, the widespread use of chemicals fungicides has been a subject of public concern and security due to their potentially harmful effects on environment and human health and their undesirable effect on non-target organisms [4,5].

The control of *Fusarium* wilt of tomato is very difficult because pathogen progress within the vascular tissues which limits the effectiveness of fungicides. Biological control of plant pathogens through antagonistic offer environmentally safe and cost effective alternative to chemicals [2]. *Trichoderma* has gained immense importance since last few decades due to its biological control ability against several deadly plant pathogens [6] for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses, uptake and use of nutrients [7]. Several studies have been published on ability of Trichoderma in biological control of *Fusarium* wilt of tomato [8-10]. Therefore, the objective of the present study was to assess the ability of two *Trichoderma* spp. in decreasing Fusarium wilt of tomato under *in vivo* conditions in Yemen.

Materials and Methods

Origin of *Trichoderma* isolates

Native isolates *T. citrinoviride* (T33) and *Trichoderma* sp. anamorph of *Hypocrea semiorbis* (T15) were isolated from rhizosphere soil of corn in Yemen and selected for their high biocontrol activity against different soil-borne plant pathogens.

Origin of *Fusarium oxysporum*

F. oxysporum (AUMC 208) was collected from Mycological Center, Faculty of Science, Assiut University, Assiut, Egypt.

Sterilization of soil and seeds

Soil used through this experiment was clay soil, sand and peat moss at a ratio of 3:1:1 (w/w) then autoclaved for three successive days at 121°C for one hour/day. Pots of 15 cm ×10 cm diameter were surface sterilized with 1% sodium hypochlorite and were filled with autoclaved soil [11, 12]. Seeds of tomato were surface sterilized with 1% sodium hypochlorite for 2 min, rinsed three times in sterile distilled water and air dried [13].

Preparation of *F. oxysporum inocula*

Fusarium oxysporum inocula was prepared for incula by growing on PDA and incubated for 7 days at 28°C. The suspension for inoculation was prepared by pouring 30 ml of sterile distilled water into each of petri dishes containing *F. oxysporum* culture and the conidia were dislodged with a cell spreader, filtered through cheesecloth and counted with a haemacytometer. It was set to $1x10^6$ conidia/ml [14].

Pathogenicity test

Pathogenicity test of *F. oxysporum* was carried out on tomato variety (*Solanum lycopersicum* L., cvs. 'Rio grande, Faten) according to Jasnic., *et al.* [15]. Pathogenicity test was tested by sowing of tomato seeds in artificially infected soil by *F. oxysporum.* Control plants were sown in soil with sterile distilled water. The severity of the Fusarium wilt was assessed from 2 weeks of inoculation up to 45 days. Disease incidence was determined as reported by Khanna., *et al*. [16]:

$$\text{Percentage diseases incidence (DI \%)} = \frac{n}{N} \times 100$$

Where:

n= number of plants showing wilts symptoms

N= Total number of plant sampled

Biological control of *Fusarium* wilt of tomato in greenhouse

The selected antagonists (T15 and T33) were tested for their ability to control the Fusarium wilt of tomato as well as their effect on tomato plant growth under greenhouse conditions. Each pot was taken with sterilized soil. Soil was infested with conidial suspension (30 ml) of *F. oxysporum* ($1x10^6$ conidia/ml). Simultaneously after 2 days of pathogen inoculation, soils were inoculated with (30 ml) of Trichoderma isolates ($1x10^7$ conidia/ml) and then pots were watered for 7 days before sowing. Ten tomato seeds were sown in each pot, three pot replicate for each treatment arranged in completed randomized design. Pots were kept under greenhouse condition till the end of the experiment [17]. The details of total 5 treatments as follow:

T (1): Control (without Fusarium and Trichoderma)

T (2): *F. oxysporum*

T (3): *Trichoderma* sp. anamorph of *H. semiorbis* + *F. oxysporum*

T (4): T. citrinoviride + F. oxysporum

T (5): *Trichoderma* sp. anamorph of *H. semiorbis* + *T. citrinoviride*+ *F. oxysporum*

At the end of experiment, the plants removed from the pots, shoot and root length, fresh weight (FW), dry weight (DW) and number of leaves per plant were determined [18]. The percentage of disease severity was determined using the formula given by Song *et al*., [19]:

$$\text{Disease severity (\%)} = \left[\frac{(\Sigma \text{ scale} \times \text{number of plants infected}) \times 100}{\text{Highest scale} \times \text{total number of plant}} \right]$$

Severity ratings for *Fusarium oxysporum* inoculated plants were done using a scale of 1-6 as described by Marley and Hillocks [20]. Where:

1. no symptoms
2. chlorosis and wilting of the first branches
3. chlorosis and wilting of second and third branches
4. chlorosis above third, second and third branches may be lost
5. chlorosis and partial desiccation
6. complete death of plant

Statistical analysis

Statistical analysis was performed in order to determine the effect of treatments on observed parameters. Significance of treatments was tested by One-way Analysis of Variance (ANOVA) and Tukey's test (at $P< 0.05$) was applied for the differences in mean values. All the statistical analyses were completed using Graphpad 6.01 Statistics.

Results and Discussion

The tomato plants were inoculated with *F. oxysporum* to test the capability of the fungus producing disease symptoms in tomato in vivo. In the present study the inoculated plant showed wilting symptoms and percentage of diseases incidence was (62.64%). The result is supported by the findings of Begum [21] who observed that *F. oxysporum* f. sp. lycopersici was able to produce wilting symptoms in tomato plants.

The results of severity of *F. oxysporum* on tomato gave the highest percentage of disease severity (67.85%) (Figure, 1) and reduced all plant growth parameters (Table, 1). The negative effects of *F. oxysporum* result from disruption to plant growth by the blocking of xylem vessels, causing leaf senescence and reduced photosynthesis [22].

It was revealed from the results, Trichoderma isolates varied in their effect on tomato plants and ability to reduce the effect of *F. oxysporum* when subsequently applied in the pot experiment (Table, 2). Reduction of Fusarium wilt disease was observed by *T. citrinoviride* and *Trichoderma* sp. anamorph of *H. semiorbis* in comparison to control. Best disease control was achieved in treatment (5) demonstrating only 19.77% of disease severity followed by treatment (4) and treatment (3). These results are in agreement with previous studies of several workers [3,8, 23,24] they reported that isolates of Trichoderma showed promise for controlling *Fusarium* wilt and improving the growth and yield of tomato.

The reduced *Fusarium* wilt on tomato may have been due to reduced population density of the pathogen [25], parasitism, lysis of pathogenic fungi, competition for limiting factors in the rhizosphere mainly iron and carbon [26,27].

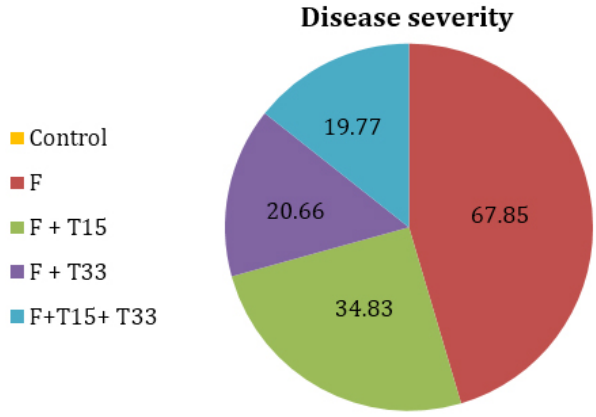

Figure 1: *Disease severity of F. oxysporum on tomato plant.*

The results of *Trichoderma* treatments showed statistically significant effects of *Trichoderma* treatments on plant growth parameters (Table, 1). Root and shoot length, fresh weight dry weight and number of leaves per plant found that the maximum was recorded in the treatment [5]. These results agree with Ozbay and Newman [28] who reported that seedling treated with *T. harzianum* increases seedling emergence, number of true leaves, fresh and dry weights of shoot and root of tomato plants.

Treatments		Plant length (cm)		Fresh weight (g)		Dry weight (g)		No. of leaves
		root	shoot	root	shoot	root	shoot	
T_1	Control	5.53	15.03	1.67	17.5	0.34	2.34	19.55
T_2	F	4.28	13.45	0.9	8.50	0.20	1.31	18.58
T_3	F + T15	5.60	15.76	2.85	18.66	0.38	2.62	20.68
T_4	F + T33	5.75	16.64	2.90	21.12	0.44	3.01	22.62
T_5	F+T15+ T33	6.65	18.29	2.94	28.66	0.46	4.03	24.33
P value		0.0906	0.0548	0.0001	0.0001	0.0021	0.0001	0.0870

Table 1: *Effects of Trichoderma treatments on tomato growth and disease incidence of Fusarium wilt of tomato in green house.*

Sundaramoorthy and Balaskar [29] also observed that tomato plants treated with *T. harzianum* (ANR-1) stimulate plant height by 73.62 cm and increased the dry weight by 288.38 g in comparison to untreated control.

Furthermore, Harman [30] established that *Trichoderma* spp., are opportunistic plant colonizers that affect plant growth by promoting abundant and healthy plant roots, possibly via the production or control of plant hormones. The increased growth response caused by *Trichoderma* isolates may be through modification of the rooting system as Yedida., *et al*. [31] reported for *T. harzianum* inoculation which improved uptake of nutrients by the plants at a very early growth stage.

Conclusion

Richoderma viride and *Trichoderma* sp. Decreased disease incidence of *Fusarium* sp., and increased plant yield in tomato. Moreover, the combination of both *Trichoderma* spp. proved better than their application alone. Thus, the finding of present investigation holds a good promise in tomato wilt management. However, further studies on the effect of these treatments in field conditions need to be undertaken so that *Trichoderma* could be recommended as a biocontrol agent richoderma viride and *Trichoderma* sp. Decreased disease incidence of *Fusarium* sp., and increased plant yield in tomato. Moreover, the combination of both *Trichoderma* spp. Proved better than

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T. citrinoviride (T33) and *Trichoderma* sp. anamorph of *H. semiorbis* (T15), decreased disease severity of *F. oxysporum* and increased plant growth parameters in tomato. Moreover, the combination of both Trichoderma spp. proved better than their application alone in vivo in biological control of Fusarium wilt of tomato.

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