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Effect of *Trichoderma Harzianum* against *Thielaviopsis Paradoxa* and Their Pathological Potential on Date Palm Seedlings

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Abstract Eight isolates of *Trichoderma harzianum* showed antagonistic properities toward *Thielaviopsis paradoxa* on bi-culture test. *Trichoderma* isolates were varied of antagonistic properities toward *T. paradoxa*. *Trichoderma harzianum* (No. 1) was highly suprssed and showed over growth on *T. paradoxa* , meanwhile *T. harzianum* (No. 8) reduced linear growth of *T. paradoxa* by 44 % and recorded high inhibition zone than the all *Tricoderma* isolates tested. Germinated seeds of date palm were soaking for 1 hour on *T. harzianum* isolate (No. 8) before, after and during inoculation by *T. paradoxa*. Different treatments of soaking date palm seeds on *T.harazianum* isolate (No.8)showed significantly reduced rot root of date palm seedlings than control. Germinated seeds of date palm were soaked with *T. harzianum* before inoculation of *T. paradoxa* gave more effective to reduce root rot of date palm seedlings than treatment after inoculation with pathogen. Germinated seeds of date palm were soaked with *T. harzianum* alone and followed by *T. harzianum* before inoculation with pathogen stimulated growth of date palm seedlings *i.e.*, length and fresh weight of shoot and root systems. It was preliminary investigation on screening antagonistic biocontrol agents against fungal pathogen causing root rot disease of date palm.

Keyword: Date palm, *Trichoderma harzianum*, *Thielaviopsis paradoxa*, root rot, biological control

Introduction

Date palm (*Phoenix dactylfera* L.) is one of the most important fruit crops in tropical and subtropical region in the world. In Egypt, the cultivated area is about 14 million trees (FAO, 2009). Date palm trees are infected by *Thielaviopsis paradoxa* causing several diseases *i.e.*, root rot (Baraka *et al*., 2011 and Arafat, *et al*.,2012), black scorch (Farrag Eman) and Abo-Eyousr, 2011) and inflorescence brown rot disease of male and female plants which seriously losses of kerena production (Ziedan *et al.*, 2013). Biological control of plant pathogens by microorganisms has been considered more natural and environmentally acceptable alternative to chemical (Eziashi *et al.*, 2007). *Trichoderma* spp. are common saprophytic

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fungi which were found in almost any soil and rhizosphere microflora. They have been investigated as potential biocontrol agents because of their ability to reduce the incidence of disease caused by plant pathogenic fungi, particularly many common soil borne pathogens. Ziedan and Elewa (2000) were used *Trichoderma* spp. for control wilt disease of sesame caused by *Fusarium oxysporum* f.sp. *sesami*, wilt of sunflower (Nawar and Lobna *et al*., 2001). Root-rot of grapevine caused by *Fusarium oxysporum* and *Rhizoctonia solani* (Ziedan *et al*., 2005) and *Fusarium* wilt of banana caused by *Fusarium oxysporum* f.sp.*cubense* (Ziedan *et al.,* 2007). Several reports using nonpathogenic fungi used as biocontrol agents against *Thielaviopsis* diseases *i.e*., *Chaetomium* species for control *Thielaviopsis* bud rot of *Hyophorbe lagenicaulis* (Soytong *et al.,* 2005), *Trichoderma logibrachiatum* against *T. paradoxa* (Sanchez *et al.,* 2007) and *Trichoderma viride* recorded as a mycoparasite against *Ceratocystis paradoxa* as well as *T. polysporum*, *T. hamatum* and *T. aureoviride* were significantly reduced growth of *Ceratocystis paradoxa* (Eziashi *et al*., 2007). Furthermore recently in Egypt, *Mucor hiemalis* showed antagonistic properties against *T. paradoxa* (Ziedan *et al*., 2013). Also, three isolates of *Streptomyces bobilii*, *Streptomyces albolongus* and *Streptomyces grisiobrunne* reported to control root rot of date palm seedlings (Arafat *et al*., 2012). The objective of this study was to screen effective biocontrol agents, *Trichoderma* spp. to control root- rot of date palm caused by *T. harazianum* fungi.

Materials and methods

Pathogen and antagonistic fungi

Thielaviopsis paradoxa isolates was obtained from Plant Pathology, Department, National Research Centre, Dokki, Cairo, Egypt.

Isolates of *Trichoderma harzianum* were obtained from isolation of the samples of healthy root of date palm which collected from Gharbeia and Sohage Governorate. Small pieces (2 cm) of root samples were surface sterilized by sodium hypochlorite 2% for 2 minutes then washing several times by sterilized water then dried between two layers of sterilized filter papers. Sterilized tissue pieces were plated on potato dextrose agar (PDA) medium (4 pieces per plate), supplemented with streptomycin sulfate (50 µg/ml). Plates were incubated at 28℃ for 3-5 days. Fungal growth were transferred to PDA until get pure cultures which also get it by single spore isolation. Fungal isolates were identification according to Barnett and Hunter (1998) and other literatures.

Antagonistic potential of T. harzianum against T. paradoxa

Antagonistic activity of *T. harzianum* isolates against *T. paradoxa* was assayed by bi-culture test on on potato dextrose agar (PDA) medium. *T. paradoxa* isolate was placed by disk 4-mm toward the disk 4-mm of *Trichoderma* isolate on other side of plate (7 days old). Five Petri dishes (9-cm) were used for each treatment. Plates were incubated at 28 ℃ for 5 days. Linear growth, inhibition zone and over growth were recorded and calculated at 5 and 10 days after incubation.

Evaluation of T. harzianum to control root rot of date palm seedlings

Trichoderma harazianum spore suspensions ($1x10^6$ cfu/ml) were prepared from 15 days old culture of fungal growth on PDA medium grown at 27 $\pm$ 2 ℃. *Trichoderma* treatment was carried out on date palm germinated seeds on Petri dishes for one week which modified from El Hassina *et al.*, 2007). Date palm seedlings were Zaglol cultivar. Seeds were surface sterilized before germination on wetted filter paper in Petri-dishes (20 cm diameter) at 25-30 ^{C}. Ten germinated seeds were used of each treatment on Petri dishes for a week and ten germinated seeds were served non-inoculated control . Spore suspensions of *T. harzianum* and *T. paradoxa* were adjusted to 1 x 10^6 cfu/ml. Treatments were done as follows:- control (non-inoculated treatment), *T. paradoxa* only, *Trichoderma harazianum* only, *Trichoderma* and followed by *T. paradoxa*, *T. paradoxa* and followed by *Trichoderma*, *T. paradoxa* and followed *Trichoderma*. Data were recorded as percentage of root rot on date palm seedlings at 30 days after treatments.

Statistical analysis

The obtained data were statistical analysis computed by analysis of variance and compared treatment means using Duncan's multiple range test according to Snedecor and Cocharn (1980).

Results

Antagonism potential of T. harazianum. on T. paradoxa

Resulted showed that eight *Trichoderma* isolates gave one or more than antaganostic properaties toward *T. paradoxa*. Six isolates were grown faster than *T. paradoxa* on PDA medium and caused reduction on linear growth of *T. paradoxa* . Four isolates of *Trichoderma* showed over growth on *T. paradoxa* ,without inhibition zone was observed. *Trichoderma* (No. 1) showed highly over growth on *T. paradoxa* followed by No. 2, No. 4 and then No. 3. The best isolates of *Trichoderma harzianum* was No .8 which reduced linear growth of *T. paradoxa* by 44 % and recorded high inhibition zone than other *Trichoderma* isolates tested (Table 1).

Table 1. Antagonism of *Tricoderma harazianum* on *T. paradoxa*

Trichoderma isolates	Antagonism of *Trichoderma harazianum*. on *T.paradoxa*		
	linear growth reduction (%)	zone of inhabition (mm)	over growth 8 days (%)
1	0.00 e[1]	0.0 c	100.0 a
2	12.0 c	0.0 c	75.0 b
3	20.0 b	0.0 c	25.0 d
4	0.00 e	0.0 c	50.0 c
5	12.0 c	0.0 c	0.00 e
6	12.0 c	2.0 b	0.00 e
7	08.0 d	2.0 b	0.00 e
8	44.0 a	5.0 a	0.00 e
free	0.00 e	0.00	0.00 e

[1]Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range tests**.**

Effect of T. harzianum treatments on root rot of date plam seedlings

Result showed that different treatments by *T. harzianum* significantly reduced rot root of date palm seedlings than control as shown in Fig.1. Data It indicated that all germinated seeds soaking of date palm on *T. harzianum* were reduced root rot incidence on date palm seedlings. Soaking date palm germinated seeds on *T. harzianum* before inoculation by pathogen gave more effective to reduce root rot of date palm seedlings than treatment after inoculation by pathogen. Meanwhile, treatment of germinated seed by *T. harzianum* and pathogen at the same time, *T harzianum* treatment alone were recorded as the least root rot incidence of date palm seedlings (Table 2).

Figure 1. Root rot symptoms on date palm seedlings showing stunt and brown discoloration of seedling shoot and root (left) compare healthy seedling (right)

Table 2. Effect of *T. harzianum* on rot root of date plam seedlings

Treatment	Root- rot (%)
T. paradoxa	53.3 a[1]
Trichoderma	10.0 f
Trichoderma then *T. paradoxa*	13.0 e
T. paradoxa then *h*	40.0 b
T. paradoxa and *Trichoderma*	20.0 d
Control	26.7 c

1Values followed by the same letter are not significantly different at *P≥ 0.05* according to Duncan's multiple range tests.

Effect of T. harzianum and T. paradoxa on date plam seedlings

Result showed that all germinated seed soaking of date palm on *T. harzianum* promoted to increase all morphological criteria *i.e.,* length and fresh weight of seedling shoot and root systems than inoculated by sowing pathogen suspension which it significantly reduced morphological characters of date palm seedlings. Soaking date palm germinated seeds on *T. harzianum* alone was the best treatment to stimulate the growth of date palm seedlings and followed *T. harzianum* before inoculation by pathogen which also increased in all morphological criteria *i.e.,* length and fresh weight of seedling shoot and root systems than inoculated by pathogen. Meanwhile, treatment of germinated seeds by *T.harzianum* after and at same time with pathogen inoculation (Table 3).

Table 3. Effect of *T. harzianum* and *T. paradoxa* on date plam seedlings

Treatment	Shoot		Root	
	length (cm)	fresh weight (g)	length (cm)	fresh weight (g)
T. paradoxa	22.4 c[1]	1.33	21.0	0.32
Trichoderma harazianum	28.3 a	1.64	31.4	0.46
Trichoderma then *T. paradoxa*	28.5 a	1.60	30.7	0.39
T. paradoxa then *Trichoderma*	23.4 c	1.44	27.0	0.37
T. paradoxa and *Trichoderma*	27.6 b	1.52	27.0	0.36
Control	26.0 b	1.58	31.4	0.43

[1]Values followed by the same letter are not significantly different at $P \geq 0.05$ according to Duncan's multiple range tests.

Discussion

Date palms under the Egyptian conditions are subjected to infection with different diseases caused by many soil-borne pathogenic fungi which may cause considerable losses of shoots and trees. The most frequent fungi were *Fusarium* spp. and *Thielaviopsis paradoxa* (Baraka *et al*., 2011 and Arafat, *et al.*, 2012). Also, *T. paradoxa*, caused black scorch (Farrag Eman and Abo-Eyousr (2011) and Ziedan, *et al.* (2013). The use of biocontrol agent for control soil diseases of plant is of because there are no chemical means effectively control to pathogenic fungi of plant and resistant cultivars are not available. *Trichoderma* spp. are common saprophytic fungi which were found in almost any soil and rhizosphere microflora. They have been investigated as potential biocontrol agents because of their ability to reduce

the incidence of disease caused by plant pathogenic fungi, particularly many common soil borne pathogens. In this study, eight isolates of *T. harzianum* showed antagonistic properties toward *T. paradoxa* on PDA medium including over growth, reduction of mycelium growth and inhibition zone of fungal growth. Four isolates of *T. harzianum* showed over growth on *T. paradoxa* without inhibition zone. *Trichoderma* No. 1showed highly effective meanwhile *T. harzianum* No. 8 reduced linear growth of *T. paradoxa* by 44 % and high inhibition zone than other *Trichoderma* isolates. These results are agreed with Sanchez *et al.* (2007) found that *T. longibrachiatum* completely inhibited the growth of *T. paradoxa* on PDA plates by produced non-volatile metabolites. Also, the antagonistic mechanism of *T. viride* that conidia can be penetrated through cell wall of *Ceratocystis paradoxa* and some report indicated that *T. polysporum* and *T. hamatum* produced toxic volatile compounds (Eziashi *et al.* 2007). On this study, different soaking date palm germinated seeds of Zaglol cultivar for one hour of *T. harzianum* No.8 before, during and after inoculation by *T. paradoxa* were significantly reduced rot root of date palm seedlings. *Trichoderma harazianum* treated before inoculation of *T. paradoxa* gave more effective to reduce root rot of date palm seedlings than other treatments after and during inoculation by pathogen. These results were similar proved that *Trichoderma* spp. could control wilt disease of sesame caused by *Fusarium oxysporum* f.sp.*sesami* (Ziedan, and Elewa, 2000), wilt disease of sunflower (Nawar Lobna, *et al.*2001), root-rot of grapevine caused by *F. oxysporum* and *Rhizoctonia solani* (Ziedan *et al*, 2005) and *Fusarium* wilt of banana caused by *F. oxysporum* f.sp.*cubense* (Ziedan *et al*,. (2007) and root rot of date palm was control by *Streptomyces* isolates (Arafat *et al.,* 2012) as results. Moreover, *Trichoderma* spp. were expressed for controlling other soil borne fungi by different mechanisms inhibited growth of *T. paradoxa* by produced toxic volatile and non-volatile

metabolites and produced proteases, beta 1,3-glucanases and chitinases that would be responsible for the degradation of *Thielaviopsis* hyphae (Sanchez *et al*., 2007, Eziashi *et al*., 2007 and Druzhinina *et al*., 2011).

Soaking date palm germinated seeds on *T. harzianum* alone and followed by *T. harzianum* before inoculation by pathogen were stimulated growth of date palm seedlings of all morphological criteria.The enhancement of plant growth characters of date palm seedlings may be due to ability of *T. harzianum* to provide plant by nutritional requirements and plant growth regulators as expectation. In this respect, Vinale (2009) reported that harzianic acid could express an antifungal and plant growth promoting metabolite from *T. harzianum* at low concentration. *Trichoderma* could promote the growth on plant by enhanced biomass production and lateral root growth promotion which reported to be an auxin-dependent mechanism in Arabidopsis (Contreras-Cornejo *et al*., 2009 and Mastouri *et al*., 2012). It can be stated that *Trichoderma harzianum* is one of the best nonpathogenic microorganisms as a promising as alternative control of various fungal plant diseases.

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