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Control of wilt disease (Sudden Decline Syndrome) on date palms in Iraq

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Abstract

A pots and laboratory experiments were conducted in Plant Protection Department of the Directorate of Diyala Agriculture during season 2010 to evaluate the efficiency of *Trichoderma harzianum* and Beltanol fungicide to prevent the infection by *Fusarium solani* on date palms. The results of this study showed that Beltanol fungicide concentration of $1cm^3$/ liter and *Trichoderma harzianum* were reduced the rate of radial growth of *Fusarium solani* on culture media PSA which was 0.5, 3.25 cm respectively and the inhibiting percentage of *Fusarium solani* in the previous treatments was 89.55, 31.81 % respectively which were significant of control 0.0%. not significant differences in germination percentage among all treatments, significantly increased the disease severity in *Fusarium solani* which was 50% ,significantly increased the shoot length in Beltanol fungicide which was 52.97 cm while treatment of control recorded maximum of root length which was 42.1 cm , not significant differences in fresh shoot weight and fresh root weight, treatments of *Fusarium solani* with *T.harzianum* and *T.harzianum* were recorded maximum of dry shoot weight which was 1.93 ,1.87 g and treatments of control , *Fusarium solani* with *T.harzianum* and *T.harzianum* were recorded maximum of dry root weight which was 0.68 ,0.61 ,0.50 g respectively.

Keywords: *Fusarium solani* ,*Trichoderma harzianum*, Beltanol and Date Palms

Introduction

Date palm (Phoenix dactylifera L.) is one of the most important fruit crops of the tropical and subtropical regions of the world and grown in large area in Iraq. Roots of Date palm are liable to attack by many pathogenic soil borne fungi, that causing serious diseases. Several fungi were recorded as causal pathogens on date palm **(Mansoori and Kord, 2006; El Deeb *et al*., 2007; Samir *et al*., 2009; Arafat, 2011).**

Date palm trees in Diyala province in Iraq particularly Kazania and Baldroz are suffering from a serious disease which is the sudden decline syndrome. This disease destroyed many orchards and dispersed trees at this area. The number of infected trees is increasing day after day. Nowadays sudden decline became a real threat for current date palm cultivation in Diyala rather than entire Iraq. In addition it restricts the extension of new cultivations. The symptoms are starting with orange yellowish coloring for the fronds' then the leaflets. Such symptom starts from outer-lower frond toward central younger fronds. Within a single frond drying begins from the terminal part to cover the whole frond. Eventually, the entire frond turns to pale brown color and the tree dies within few months.

In date palm world, several reports on the isolation of Fusarium species from roots, fronds and trunks of date palm trees showed wilt and decline. *Fusarium solani* and *F. oxysporum* were the most frequent and most abundant in the roots of date palm trees showing decline in middle of Iraq (**Sarhan, 2001**). In Iraq, a similar disease symptoms caused by *F. solani* have been reported (**Al Yaseri *et al*., 2006**). *Fusarium solani* and *F. monliforme* were found associated with declined date palm trees in Egypt (**Rashed and Abdel Hafeez, 2001**) .Recently; a serious disease of date palm was reported caused by *F.*

solani associated with yellowing and death of the fronds. The disease occurred in date palm groves in Kazeron district, west of Fars province in Iran (**Mansoori and Kord, 2006**). An investigation was reported on the incidence of date palm disease in Saudi Arabia and in particular in Al Qassim and Al-Medina Al-Monawara regions, several trees showed symptoms of wilt and dieback very similar to those caused by *Fusarium oxysporum* albedinis. Three Fusarium species were isolated from the infected fronds and roots of the date palm trees. These identified as *F. proliferatum*, *F. solani* and *F. oxysporum* (**Abdalla *et al*., 2000**).

This study aimed to isolate and identification of *Fusarium solani* and evaluates the efficacy of *Trichoderma harzianum* and beltanol fungicide to protection seedling of date palms from pathogen fungi in pots experiment.

Materials and Methods

Isolation and identification of *Fusarium solani*

Samples were taken from root of date palms at least 15-20 cm apart then were washed carefully and surface sterilized with 1% sodium hypochlorite solution for 2 minutes followed by three times rising with sterilized distilled water. The pieces were then placed on pre-sterilized blotting paper to remove excess moisture. The pieces of sterilized root were transferred on potato sucrose agar (PSA) plates and incubated at 25±2°C for seven days. After incubation, colonies were observed and identified on the basis of morphological and reproductive characters. Emerged fungi were isolated and purified using the single spore technique and/or the hyphal tip method according to **Wang and Wen (1997).** Stock cultures were maintained on PSA slants and kept in a refrigerator at 5°C for further studies. The fungal colonies growing in the culture plates were identified according to their morphological characteristics according to **Barnett and Hunter (1999)** and **John and summerell (2006)** in college of sciences, department of biology, university of Baghdad**.**

Mass multiplication of *Trichoderma harzianum* on Millet (*Pennisetum glaucum*)

Trichoderma harzianum acquired from ministry of sciences and technology in Iraq.

The Millet grains were soaked partially for one hour in warm water (40 to 45 C) and then spread on the clean blotting paper for air drying. About 150 g moistened grains were filled in each 250 ml flask with 10 ml water and autoclaved for 30 minute at 15 lbs psi pressure. The mycelium bit of pure culture of *Trichoderma harzianum* were inoculated under aseptic condition in those flask containing grains and incubated at 28± 20 C for 10 days. Meanwhile flasks were shaken to avoid clumping of grains and to facilitate early growth of the fungus. The grains turn greenish due to mycelial growth of the test fungus. For soil application in pots experiments, the grains colonized by *Trichoderma harzianum* were mixed in soil 3 g/ pot **(Kamdi *et al.* 2012)**.

In vitro assay of *T. harzianum* against *F. solani*

Trichoderma harzianum was evaluated for this activity against soil-borne pathogenic fungi, Discs of *Trichoderma harzianum* (5 mm) were remove from the edge colonies of active cultures and placed on one side of a Petri dishes containing Potato Sucrose Agar (PSA) medium. Similar dishes of each pathogenic fungus isolates grown in the same manner were placed on the opposite side of Petri plates and made three replicates. The three Petri plates were inoculated only with *Fusarium solani* were kept as control. Cultures were observed daily and recorded for antagonism of *Trichoderma harzianum* against *Fusarium solani* after 8 days.

The percent inhibition of mycelia growth of the pathogens was calculated using following formula **(Singh *et al*., 2002):**

$I = (C-T/C) \times 100$

Where, I = inhibition (%), C = colony diameter in control plate and T = colony diameter in treated plate.

Efficacy of fungicide assay

In vitro evaluation of beltanol fungicide with dose 1 cm^3/ L to check the colony growth of the fungus *F. solani* was done through poisoned food technique described by **Borum & Sinclair (1968)** on potato sucrose agar (PSA) medium. After autoclaving, 25 ml of PDA medium amended with beltanol fungicide in 100 ml flasks were poured in sterilized 90 mm Pyrex Petri plates. The PSA medium without fungicide was kept as control. The fungus *F. solani* was picked from purified culture in the form of a 5 mm agar disc and inoculated in the center of each Petri plate with three replicates. The dishes were incubated at 25°C. The percent inhibition in growth was computed after 8 days of inoculation as follows:

Mycelial growth inhibition (%) = [(dc-dt) / dc] × 100 (%)

Where dc = average diameter of fungal colony in control, and dt= average diameter of fungal colony in treatment group.

Experiment of pots

Experiment carried out using the complete Randomized design (CRD) with six treatments and five replicates in soil brought from the nursery Diyala agriculture directorate after sterilized in autoclave at a temperature of (121) C and pressure (1.5 kg). Cm^{-2}, and placed in the pots by (1.5) kg / Pot, planted the seeds of date palm by 1 seed / pot, add *T. harzianum* to soil of pots by 3 g / pot from culture of millet seeds with irrigation water. *F. solani* was added by two Petri dish / pot with irrigation water and treatments of experiment was as follows:

T1: Control

T2: *F. solani*

T3: *T. harzianum*

T4: Beltanol

T5: *T. harzianum*+ *F. solani*

T6: Beltanol+ *F. solani*

Observation were recorded

- Seed germination percent at 30 days after planting
- Shoot length (cm)
- Fresh and dry shoot weight (gm)
- Fresh and dry root weight (gm)
- Disease intensity (%) were calculated at 252 days after planting by using the following formula:

$$\text{Disease severity (\%)} = \frac{\sum \text{Scale} \times \text{number of plants infected}}{\text{Highest scale} \times \text{total number of plants}} \times 100$$

Chastanger and Ogawa (1979)

Disease severity rate (DSR) was determined by using scale of the disease severity was classified as follows:

0= No of apparent symptoms.

1= Length of lesions ranged from 1-5 cm.

2= Lesion area was 5mm to 5cm and some of small scattering lesion

3= Infection was randomly distributed and infected tissues began to Collapse.

4= Half or more of the rachis (leaflet) was still alive.

5= Most of the rachis collapsed and dried.

Results and Discussion

Antagonistic effect of *Trichoderma harzianum* and Beltanol on *Fusarium solani*

The results of Table.1 indicated that significantly increased in reduction of diameter growth rate of Fusarium in the treatment of Beltanol 0.5 cm followed by *T. harzianum* 3.25 cm as compared with Control 4.83 cm, the growth inhibition (%) of *Fusarium solani* is significantly increased in the treatment of Beltanol 89.55% followed by *T. harzianum* 31.81% as compared with Control 0.0 %.

The effects of Beltanol was reported to combined with copper mineral which is transfer in plant tissues and kill the pathogens **(Meister, 2000).** The inhibitory effect of *T. harzianum* against tested pathogen was probably due to competition and/or antibiosis.

Table 1: Antagonistic effect of *Trichoderma harzianum* and Beltanol on the growth inhibition (%) of *Fusarium solani*

Treatment	**mean of diameter growth of *Fusarium solani***	**inhibition(%)of *Fusarium solani***
Beltanol	0.5	89.55
T. harzianum	3 .25	31.81
Control	4.83	0.0
LSD 0.05	0.72	14.05

The results of Table 2 revealed that non-significant among all treatments in Seed germination%, significantly reduced the disease severity (%) in all treatments which were found non-significant among themselves as compared with T2 *Fusarium sp*, significantly increased the shoot length in beltanol 52.97 cm as compared with control 39.94 cm, significantly increased the root length in control 42.1cm as compared with beltanol19.25 cm, non-significant among all treatments in fresh shoot and root weight, treatment *Fusarium sp* +*T.harzianum* is recorded maximum increase in dry shoot weight 1.93 g from other treatment and treatment control is recorded maximum increase in dry root weight 0.68 g from other treatment.

Different mechanisms have been suggested as being responsible for *T.harzianum* biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compounds **(Haram and al. 1996; Zimand and al. 1996).** Also, previous studies have demonstrated that before mycelia of fungi interacted, *T.harzianum* produced low quantities of extracellular exochitinases **(Kullnig & al., 2000; Brunner & al., 2003).** The diffusion of these enzymes dissolved cell fragments of host cells. These cell fragments in turn induced the production of additional enzymes and triggered a cascade of physiological changes, stimulating a direct and rapid growth of *T.harzianum* **(Zeilinger & al., 1999).**

Table 2: Effect of *Trichoderma harzianum* and beltanol on the Seed germination%, Disease severity %, Plant length cm, fresh and dry shoots weight (g), fresh and dry root weight (g) of date palm seedling.

treatment		Seed germination%	Disease severity %	Plant length cm		Weight of shoot g		Weight of root g	
				Shoot	root	fresh	dry	fresh	Dry
T1	control	100	12	39.94	42.1	4.57	1.64	2.40	0.68
T2	*Fusarium sp*	80	50	42.85	26.5	2.84	0.93	0.82	0.17
T3	*T.harzianum*	100	12	45.42	30.7	5.25	1.87	3.05	0.61
T4	Beltanol	80	15	52.97	19.25	3.17	0.98	0.91	0.19
T5	*Fusarium sp* +*T.harzianum*	100	16	45.46	38.9	5.83	1.93	2.17	0.50
T6	*Fusarium sp* +Beltanol	100	4	48.22	34.12	3.55	1.11	1.23	0.27
LSD 0.05		NS	46.02	11.56	18.27	NS	0.68	NS	0.19

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