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The Effectiveness of Trichoderma Harzianum and Trichoderma Viride to Inhibit Pathogenesis Of Rhizoctonia Solani agianst Tomato Plant

Mezeal Ali Intesar, Ibraheem Jameel Liqaa and Mizil Nghamish Shaimaa.

Department of Biology, College of Science, University of AL- Mustansarya, Baghdad, Iraq

Abstract

In vitro testing, antagonistic capability of Trichoderma spp. obtained from the rhizosphere of tomato showed inhibitory effect against growth of R. solani , few biological control agents have achieved success under field conditions. Among the hundreds of organisms identified as potential biological disease control agent, only few have resulted in proving commercially acceptable control of these diseases. Rhizoctonia solani were isolate from a naturally occurring epidemic of damping-off tomato plants grown in Kut governorate, Iraq,In vitro, of 9 isolates belonging to the fungus Trichoderma spp. 5 isolates belonging to the fungus T. viride while diagnosed 4 isolates belong to the fungus T. harzianum isolated from the rhizosphere of tomato plants. Both T. viride and T. harzianum had antagonistic effect on R.solani isolates . Results showed that ability of six isolates of the pathogenic fungus to damping of the tomato plant seedlings under greenhouse conditions , isolates R.solani-M and R.solani-H showed the largest percentage of the disease compared with other isolates(30.2 and 32.1%) respectively, while the effect of isolate R.solani-A was daming of 21.9% from tomato seedlings Pre-emergence.

Keywords*: tomato, biocontrol, damping-off, Trichoderma, Rhizoctobnia .*

*Corresponding E-mail: intesarali664@yahoo.com

INTRODUCTION

Various methods for controlling such diseases have been investigated including the use of resistant varieties (Brisa *et al*., 2007), chemical control, cultural practices (Punja *et al*., 1986), plant volatile compounds (El-Mougy *et al*., 2007), plant extracts(Kumar and Tripathi, 1991) and biological control, particularly with species of Trichoderma and Gliocladium (Ristaino *et al*., 1994). Many researchers have demonstrated the potential of *Trichoderma* spp. in controlling damping-off and wilt diseases of crop plants caused by *Rhizoctonia solani* and *Fusarium* spp. (Dubey *et al*., 2007; Rojo *et al*., 2007). Biological control is an another to the use of chemical pesticides, Biological fungicides may entertainment to suppress the population of the Pathogenic organism complete struggle with pathogenic organisms, stimulate plant growth, which may consent plants to speedily outgrow any pathogen effects, or damage the pathogen by means of toxins produced (Cook, 2000; Gilreath, 2002).

A diversity of soil microorganisms have confirmed action in the control of various soil borne plant pathogens, counting Fusarium wilt pathogens. Of the fungi used for control of soil borne pathogens, various species of *Trichoderma* spp have conventional the most courtesy. *Trichoderma harzianum* is a fungal biocontrol agent that doses a range of phytopathogenic fungi. *T. harzianum* alone or in combination with other Trichoderma species can be used in biological control of numerous plant diseases (Papavizas, 1985; Chet, 1987; Samuels, 1996).

Tomato (*Solanum lycopersicum* L.) is one of the most important expendable and widespread winter as well as summer vegetables through the world , many pathogens occurrence the harvest at different growing periods that eventually experience 30-40% yield loss yearly. One of the imperative soil borne pathogens causing seedling rot is the *R.solani* (Anon, 1992). *R. solani* is one of the phytopathogens that occurrence tomatoes cultured under greenhouse conditions, causing root and crown rot (Latorre, 2004). *R. solani* is controlled using methyl bromide (Apablaza, 2000), recognized for its high poisonousness and its unoriginal effect on the ozone layer (Duniway, 2002) The biocontrol by fungal species of the *Trichoderma* of root and crown rot caused by *R. solani*, are existence used as an substitute to chemical fungicides (Papavizas, 1985; Limon *et al*., 1999; Rey *et al*., 2001). Formulation of the *Trichoderma* spp. to reduce incidence of the diseases caused by soil borne pathogens in the field is of great importance in biocontrol of such diseases. Therefore the present work was aimed to determine the efficacy of application of formulation contained the *Trichoderma* spp. as soil treatment against damping-off and wilt diseases of bean under greenhouse and field conditions.

MATERIALS AND METHODS

Microorganisms

Pathogenic isolates

Rhizoctonia solani were isolated from naturally infected roots of diseased Tomato plants showing damping-off and wilt symptoms grown in Kut Governorate, Iraq.

Trichoderma spp

For isolation of *Trichoderma* spp., soil samples were collected from the rhizosphere of healthy Tomato plants using dilution plate techniques and purified by the single spore method. The isolated fungi were identified on the basis of their morphological characters (Rifai, 1969).

preparation of Inoculations

R. solani

Inoculate were prepared by growing isolates of the tested fungi in 250 ml conical flasks each containing 100 ml of Czapek's broth medium. They were inoculated separately with 5 mm agar disc obtained from 7-days old cultures of *R. solani*, The flasks were incubated at 28oC for 10 days. Resulting mycelial growth of the tested fungi was decanted, washed with distilled water, suspended in 100 ml of distilled water and blended for 5 minutes using a Warring blender ,the suspensions containing 5x106 cfu/ml .

Trichoderma spp

Mass cultures of *Trichoderma* isolates were made on millet grains, Twelve hour water soaked grains (250 gm) were filled in 1000 ml flask and autoclaved for one hour for three days respectively . After cooling the grains were inoculated with 7 days old culture of *Trichoderma* and incubated at 28oC for 10 days , contents of flasks were transferred to plastic plates under sterile conditions, left to air dry then mixed in a blender to become powder and kept in polyethylene bags at room temperature until used. Colony forming units in all formulae of *Trichoderma* spp. mixtures was adjusted to 3 x 107 cfu/g.

Dual cutler test

Disks from each isolate of Trichoderma spp. (5 mm in diameter) were inoculated on PDA medium in one side in Petri plate and the opposite side was inoculated by R.solani inocula, According to the treatments listed later, three replicates were used for each treatment.After 7 days incubation periods at 28°C, linear growth of R.solani was recorded. Inhibition percent of growth was calculated .

Greenhouse Experiments

The trials were carried out in the glass house of Biology Department, Collage of sciences, AL-Mustansariya University. The formulated antagonists (ThF, Th H and ThH2) .Fungal suspensions containing 5x106 cfu/ml were added to 30 cm diameter pots According to the treatments listed later, at the rate of 1% w/w(Abdel-Kader, 1997). Three replicates were used, Seed infestation was carried out by dipping the seed in 1% sodium hypochlorite solution for 3 minutes, and then rinsing for several times with sterilized water. Pots containing non-infested soil were used as control. Five Tomato seeds were sown in each pot, and three pots were used as replicates for each isolate. Percentages of damping-off incidence of tomato at pre and post-emergence stages were calculated after 10 and 30 days, respectively. Pre-emergence damping-off was based on the number of non-emerged seeds in relation to the number of sown seeds, while post-emergence (%) was based on the number of plants showing disease symptoms in relation to the number of emerged seedlings. Fifty days after sowing, the disease severity (DS) was recorded by using the CIAT scale, 1-9 Van Schoonhoven and Pastor-Corrales, 1987), where 1= no visible symptoms and 9 = plants are dead or severely infected, with 100% foliage showing wilting chlorosis and/or premature defoliation.

Results and discussion

***Trichoderma* isolates and *R.solani* isolates**

Results indicate in Table 1 that he was isolated 9 isolates belonging to the fungus *Trichoderma* showed microscopic examination of isolates to diagnose 5 isolates belonging to the fungus *T. viride* while diagnosed 4 isolates belong to the fungus *T. harzianum* , has been coded as shown in the above table, either in terms of isolates pathogenic fungus *R.solani* has been isolated 6 isolates from different Iraqi provinces as well are encoded as shown in the table above .

Table 1:- isolates of microorganisms

***Trichoderma* isolates**	***R.solani* isolates**

T. viride(Tvf1)	*R.solani-B*
T. viride(Tvf2)	*R.solani-K*
T. viride(Tvf3)	*R.solani-A*
T. viride(Tvf4	*R.solani-M*
T. viride(Tvf5)	*R.solani-D*
T. harzianum(Thf1)	*R.solani-H*
T. harzianum(Thf2)	
T. harzianum(Thf3)	
T. harzianum(Thf4)	

Antagonistic effect of *Trichoderma* isolates against *R. solani* isolates

Table 2:- Inhibition of radial growth in *R.solani* isolates

***Trichoderma* isolates**	**%Inhibition of radial growth in *R.solani* isolates**					
	R.solani-B	***R.solani-K***	***R.solani-A***	***R.solani-M***	***R.solani-D***	***R.solani-H***
T. viride(Tvf1)	**92.5**	**85.4**	**89.1**	**74.7**	**91.3**	**73.5**
T. viride(Tvf2)	**97.3**	**92.5**	**98.1**	**86.2**	**95.6**	**73.3**
T. viride(Tvf3)	**34.7**	**49.1**	**32.3**	**21.5**	**52.9**	**12.5**
T. viride(Tvf4	**28.5**	**33.4**	**22.9**	**12.1**	**11.4**	**11.2**
T. viride(Tvf5)	**98.1**	**96.5**	**92.5**	**73.3**	**96.8**	**88.9**
T. harzianum(Thf1)	**11.3**	**24.5**	**12.1**	**35.2**	**9.3**	**7.5**
T. harzianum(Thf2)	**82.9**	**95.1**	**77.5**	**58.3**	**92.1**	**17.7**
T. harzianum(Thf3)	**9.1**	**14.3**	**12.5**	**18.3**	**12.5**	**2.9**
T. harzianum(Thf4)	**99.5**	**92.9**	**97.4**	**88.1**	**91.6**	**82.1**

The results of dual culture of antagonists and pathogen demonstrated that both *T. viride* and *T. harzianum* had antagonistic effect on *R.solani* isolates The highest inhibitory effect on growth of pathogenic isolates of *R.solani* was achieved by *T. viride*(Tvf1) against *R.solani*-B (92.5%) while the lowest inhibitory effect was against *R.solani*-H (73.5%) . Concerning the isolate *T. viride*(Tvf2) t was clearly frustrated radial growth in *R.solani*-A and *R.solani*-B (98.1and97.3%) respectively, while the lowest inhibitory effect was against *R.solani*-H (73.3%) . On the other hand, the highest inhibitory effect obtained by *T. viride*(Tvf5) was against *R.solani*-B (98.1%) , and lowest inhibitory effect was against *R.solani*-M (83.3%) , *T. harzianum*(Thf2) inhabited radial growth in the isolate *R.solani*-K clearly was(95.1%) , while not inhabited just (17.7%) of radial growth in *R.solani*-H, the isolate *T. harzianum*(Thf4) was The highest inhibitory effect against radial growth of all isolates of *R.solani* (Table 2). the isolates *T. viride*(Tvf3), *T. viride*(Tvf4) , *T. harzianum*(Thf1) and *T. harzianum*(Thf3) not showed high inhibitory effectiveness in radial growth against *R.solani* isolates therefore were not used in the further experiments .

In vitro testing, antagonistic capability of *Trichoderma* spp. obtained from the rhizosphere of tomato showed inhibitory effect against growth of *R. solani* , There are many mechanisms suggested to clarify the role of antagonistic organisms in suppression of growth pathogens and thus to control diseases. Their action could be through antibiosis (Ghisalberti and mycoparasitism . Rowl and, 1993 ;Haran *et al*., 1996). The competition for nutrients and/or space (Inbar *et al*., 1994)this fact was already observed in the interaction among *Trichoderma* and other pathogens (Melo, 1991) the other mechanisms involved in *Trichoderma* are induction of resistance in plants (Yedidia *et al*., 1999).

Trichoderma viride and *T. harzianum* were reported by several workers as the best antagonists against several soil and seed borne plant pathogens (Poddar *et al*., 2004). The potentiality of *Trichoderma* spp. as biocontrol agents of phytopathogenic fungi in several

crops is well known especially to *Fusarium* spp. and *Rhizoctonia* spp. (Poddar *et al*., 2004; Rojo *et al*., 2007).

Effect of *Trichoderma* isolates and *R.solani* isolates on incidence of damping-off disease in tomato

Results showed a table 3 refer to the ability of six isolates of the pathogenic fungus to damping of the tomato plant seedlings under greenhouse conditions , isolates *R.solani*-M and *R.solani*-H showed the largest percentage of the disease compared with other isolates(30.2 and 32.1%) respectively, while the effect of isolate *R.solani*-A was daming off 21.9% from tomato seedlings Pre-emergence (table 3).

Table 3:- Percentage of damping off disease in tomato

Treatments	%Pre-emergence damping off	%Post-emergence damping off
control	9.1	8.4
R.solani-B only	27.5	25.3
R.solani-K only	29.4	26.7
R.solani-A only	21.9	20.2
R.solani-M only	30.2	27.4
R.solani-D only	22.7	19.3
R.solani-H only	32.1	28.3
T. viride(Tvf1) only	3.4	0.0
T. viride(Tvf1)+ R.solani-B	11.5	9.3
T. viride(Tvf1)+ R.solani-K	12.9	11
T. viride(Tvf1)+ R.solani-A	15.6	11.3
T. viride(Tvf1)+ R.solani-M	17.9	16.8
T. viride(Tvf1)+ R.solani-D	11.1	9
T. viride(Tvf1)+ R.solani-H	18.8	15.8
T. viride(Tvf2) only	5.3	3.7
T. viride(Tvf2)+ R.solani-B	12.1	10.1
T. viride(Tvf2)+ R.solani-K	9.5	7.8
T. viride(Tvf2)+ R.solani-A	13.6	10.4
T. viride(Tvf2)+ R.solani-M	19.3	16.3
T. viride(Tvf2)+ R.solani-D	10.1	6.3
T. viride(Tvf2)+ R.solani-H	16.5	13.5
T. viride(Tvf5) only	8.3	6.9
T. viride(Tvf5)+ R.solani-B	14.4	13.1
T. viride(Tvf5)+ R.solani-K	11.8	10.5
T. viride(Tvf5)+ R.solani-A	15.7	11.7
T. viride(Tvf5)+ R.solani-M	19.1	15.4
T. viride(Tvf5)+ R.solani-D	10.6	10.1
T. viride(Tvf51)+ R.solani-H	17.4	13.4
T. harzianum(Thf2) only	5.2	4.1
T. harzianum(Thf2)+ R.solani-B	13.4	9.5
T. harzianum(Thf2)+ R.solani-K	15.2	11.6
T. harzianum(Thf2)+ R.solani-A	11.8	10.5
T. harzianum(Thf2)+ R.solani -M	17.2	12.2
T. harzianum(Thf2)+ R.solani -D	12.5	8.9
T. harzianum(Thf2)+ R.solani -H	18.3	17.3
T. harzianum(Thf4) only	3.2	0.0

T. harzianum(Thf4)+ R.solani-B	**10.3**	**8.5**
T. harzianum(Thf4)+ R.solani-K	**12.6**	**11.4**
T. harzianum(Thf4)+ R.solani-A	**11.2**	**8.1**
T. harzianum(Thf4)+ R.solani -M	**14.6**	**12.3**
T. harzianum(Thf4)+ R.solani -D	**11.5**	**10.8**
T. harzianum(Thf4)+ R.solani -H	**15.4**	**11.3**

The results also showed that addition of biological control agent *T. viride*(Tvf1) only led to reduce the percentage of disease to 3.4 % per emergence and completely been eliminated proportion of damping off post emergence, As well that addition *T. viride*(Tvf1) with the pathogenic fungus into the soil led clearly to reduce the percentage of the damping off of tomato seedlings and were more reduce the percentage of disease in treatment (*T. viride*(Tvf1)+ *R.solani*-D), It was (11.1 ,9%). Pre and post emergence respectively, As for the fungus isolates used in the experiment, they were not significantly different for isolation, as all isolates led an active role in the inhibition of pathological Activity and reducing the rate of the disease clearly, it is worth mentioning that isolates pathological isolates *R.solani* –M and *R.solani* -H were the most effective in the pathogenicity of the tomato plant.

T. harzianum(Thf4)Were more isolates biological control factors influence the disease when added alone to the soil led to the reduction of the disease . It was (3.2 ,0.0%). Pre and post emergence respectively, as well as when added to the isolates different Pathogenic isolates were the results of Table 3 indicated clearly to the effective role of the transactions this isolation with six isolates of *R.solani* in reducing the proportion of damping off of the tomato plant under the conditions of the experiment. The result are in agreement with Roy *et al*.,(1998) and Faruk *et al*., (2002) and Islam and Faruq., (2008) who employed biocontrol agents for the disease control and revealed the inhibitory effect was probably due to hyerparasitism mycoparasitism, competition for space and nutritional source and antagonistic chemicals produced and released into the environment *Trichoderma* sp., have been reported to produce antibiotic compounds (Trichodermin) extracellular enzymes (chitinase, cellulose) unsaturated monobasic acids (Dermadine) and peptides that either damage plant pathogen or enhance their population in biota.

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