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INTERACTION OF *TRICHODERMA SPP* WITH *RHIZOCTONIA SOLANI* DURING PATHOGENESIS TO FABA BEAN ROOTS AND THE EFFECT ON THE INTRINSIC PLANT RESISTANCE.

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ABSTRACT

Trichoderma harzianum and *T. viride* were used to study their effect against faba bean root rot disease caused by *Rhizoctonia solani.* Application of the antagonists as seed treatment, significantly decreaced pre and post-emergence damping-off, compared with untreated control, especially for *T. harzianum* (isolate no 2). Culture filtrates of the three *Trichoderma* isolates showed considerable reduction in polygalacturonase activity (PG) of *R. solani* during examination periods. Seed treatment with the three tested isolates of *Trichoderma spp* also resulted in appreciable reduction in (PG) activity in infected faba bean roots compared with untreated infected control, that showed a gradual increase in (PG) activity during examination period.

The decrease in polygalacturonase activity was found to be associated with noticeable increase in total terpenes content (Phytoalexine) and greater increase in peroxidase activity in faba bean roots of pre-treated seed with *Trichoderma spp*. Furthermore these increases were much higher and recorded many folds in the pretreated infected plants compared with untreated infected control. Additional to the improved resistance in plant application the treatment was associated with significant increase in fresh and dry weight of plants as well as the number of nodules per plant, in both healthy and infected plants compared with untreated ones

Key words: Faba bean, *Rhizoctonia solani, Trichoderma spp,* polygalacturonase, terpenes, Peroxidase, plant growth and nodulation.

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important legume crops which is used as human food or animal feed. Root rot disease caused by different soil borne fungi are considered the limiting factors affecting growth and yield. Damping off and root rot disease caused mainly by *R. solani* is subject to many studies. (Omar, 1986; and Sepulveda, 1991). Biological control is an environmental friendly measure and efficient alternative to chemical fungicide management of this pathogen.

Trichoderma spp. have Long been known for their potential in plant pathogenic fungi control. The primary mechanisms were considered to include direct effects on plant pathogenic fungi such as mycoparasitism, antibiosis and competition, More recently, many more mechanisms, including suppression of pathogenicity enzymes. (Kapat *et al* 1998; Elad 2000; and Levy *et al* 2004). The suppression of pathogenicity enzymes might lead to accumulation of oligogalacturonoide which acts as elicitors of defense mechanisms of host plants and thereby reduced the disease severity. (Cervone *et al* 1989 and Zimand *et al* 1996), and /or phytoalexins as terpenoid. (Hammerschmidt *et al* 2001; Howell *et al* 2000; Hanson and Howell 2004). While defense related enzymes as peroxidases, which play a key role in the synthesis of phenolic compounds and the formation of structural barriers were also considered (Dalisay and kuc 1995; Shao *et al.* 2005; and Karthikeyan *et al*. 2006). The application of *Trichoderma spp*. was reported also to increase plant growth by solubilization of nutrients in soil or by directly enhancing plant uptake of nutrients. (Yedidia *et al.* 2000; and Gravel *et al* 2007). These diversity of effects indicate that these beneficial fungi have multiple modes of action. (Elad 2000).

The objective of this investigation is to study the effect of *Trichoderma spp* against faba bean root rot disease caused by *R. solani* under greenhouse condition, and on the activity of the major pathogenicity enzyme (polygalacturonase) produced by the pathogen *in vitro* and *in vivo* as well as the role of *Trichoderma spp* in induced resistance as shown by terpenes content; peroxidase, nodulations; fresh and dry weight of plants.

MATERIALS AND METHODS

Source of *Trichoderma spp* and rhizobium strain:

Trichoderma harzianum (1), *T. harzianum* (2) and *T. viride* were isolated from rhizosphere of faba bean plants collected from El-Sharkia, Nubaria and Dakkhlia governorates.

Rhizobium strain (*Rhizobium Leguminosarum*) was kindly provided from Dept of Microbiology, Soil, Water and Environment Res. Inst ARC. Giza.

Preparation of *Trichoderma* and rhizobium strain:

The fungi in concern were grown on PDA medium for 10 days at 25^{o}C. Spore suspension of each isolate was prepared and adjusted to about 2.0 x 10^{7} spore/ml using sterilized water.

Rhizobium strain (*Rhizobium Leguminosarum*) was cultured as recommended in yeast extract mannitol broth medium 250 ml flasks, and incubated at 25^{o}C for 7 days.

Seed treatment:

Surface sterilized faba bean Giza -3 seeds were treated with 2% solution of carboxymethyl cellulase (CMC) as sticker and air dried. Seeds were soaked for 1h. in the spore suspension of *T. harzianum* , isolate (1), *T. harzianum*, isolate (2) and *T. viride* and were allowed to air dry.

Preparation of fungal inoculum:

Glass bottles (500 ml in volume) containing corn meal sand medium (3.1 w/w) were autoclaved at 121^{o}C for 30 min. The sterilized bottles were then inoculated with discs (5mm) of 7 days old culture of *R. solani* and incubated at 25^{o}C for 15 days.

Soil infestation was carried out by mixing fungal inoculum with autoclaved potted soil at the rate of 3% (w/w). and watered for 7 days to enhance growth.

Pots (35cm – diam) containing infested soil were sown with faba bean seeds previously infested with *T. harzianum* (1), *T. harzianum* (2) and *T. viride.* Seeds treated with the fungicide (Rhizolex-T) 3g/kg seed were used as cheek and untreated seeds were used as control. Four replicates were used for each treatment and 10 seeds were sown in each pot. Another group of pots containing un-infested soil were sown with the seeds of the same treatments. Cell suspension of

rhizobum strain (10^7 cfu/ml) was added to the soil at the rate of 10 ml/pot.

Pre and post-emergence damping-off was recorded 15 and 30 days after sowing respectively. Number of rhizobia per plant was recorded 50 days after sowing. Fresh and dry weight of plants was recorded 75 days after sowing.

A. *In vitro* determination of PG:

- Preparation of culture filtrate of Trichoderma:

Erlenmyer flasks (100 ml) containing (50 ml) Gliotoxin medium (Brain and Hemming, 1945) were inoculated with discs (5 mm diam) of 7 days old culture of *T. harzianum* isolate (1), *T. harzianum* isolate (2) and *T. viride,* separately; and inocubated at 25°C for 15 days in dark. Then, the cultures were centrifuged, and The supernatants were collected and sterilized by filteration using centered glass (G5).

- Polygalacturonase (PG) activity:

Flasks (250 ml) containing 100 ml of modified Czapek's liquid medium (the carbon source was replaced by 1.2% pectin) were used for determination of PG activity of *R. solani* (Talboys and Busch, 1970). Five ml of the sterilized filtrate of each isolate of *Trichoderma* was poured separately in each of the aforementioned flasks. Blank Czapek's liquid medium were used as a control. All flasks were inoculated with discs (5mm diam) of *R. solani* and incubated at 25°C . Cultures were filtrated 5, 10 and 15 days after inoculation. The filtrates were centrifuged 3000 rpm for 20 min. the clear supernatants were utilized as crude enzyme preparation. The relative activity of polygalacturonase (PG) was determined by measuring the reduction in viscosity by Ostwaled viscometer. The substrate used for measuring activity was 1.2% pection in phosphate buffer solution at PH (5.6). Five ml of crude enzyme was added to 10 ml of buffered substrate, then incubated at 30°c.

The enzyme activity was determined intervally after incubation by measuring the loss in viscosity of reaction mixture. Control with distilled water was also run. The reduction in viscosity was expressed as percentage loss in viscosity over control calculated by the following formula:

Percentage loss in viscosity over control = $((T_0 - T_1)/ (T_0 - T_w)) \times 100$

Where: T_0 = flow time of reaction mixture at zero hour,

T_1= flow time of reaction mixture at a given time interval.

T_w = flow time of distilled water.

B. *In vivo* determination of PG:

For *in vivo* studies of PG activity, faba bean samples (roots), untreated or pretreated with Trichoderma isolates (Healthy or infected with *R. solani*) were collected 10, 15 and 20 days after sowing and the extracts were prepared according to the method described by Chan and Sockston (1972). Subsequent determination of PG activity in extracts was made as mentioned before.

Total terpenes:

The experiment was carried out in Central Laboratory of Faculty of Agriculture- Cairo University.

Faba bean root samples from each treatment (Healthy or infected with *R. solani*) were collected 10. 15 and 20 days after sowing to estimate total terpenes.

Extraction and estimation:

The dried roots sample (0.1g) was boiled with 15 ml of 40% ethyl alcohol for 4 hr, then a small amount of activated charcoal was added and the extract was filtered through whatman filter paper No. 41. The extract was completed to 50 ml (in a measuring flask) with distilled water, and subjected to total terpene estimation, spectorphotometrically at 473 mm. (Ebrahimzadeh and Niknam, 1998).

Peroxidase activity:

Faba bean samples (roots) from each treatment (Healthy or infected with *R. solani*) were collected 10, 15 and 20 days after sowing. Enzyme extract was obtained by grinding root tissues in 0.1 M sodium phosphate buffer (PH 7.1, 2ml/g tissues) in a porcelain mortar. The extracted tissues were strained through four layer of cheesecloth. Filtrates were centrifuged at 3000 rpm for 20 min at 6^{o}C. Peroxidase activity was expressed as changes in absorbance/min at 425 nm according to the method of Allam and Hollis (1972).

RESULTS AND DISCUSSION

Three different isolates of *Trichoderma spp* were used as seed treatment to study their efficiency against root rot disease caused by *R. solani* compared with seed treatment with fungicide (Rhizolex-T.)

Data presented in Table (1) showed that all treatments significantly reduced per-and post emergence damping-off and

increased healthy survival plants compared with untreated infected control.

Table (1): Effect of seed treatment with *Trichoderma spp* on pre and post-emergence damping-off caused by *R. solani* under greenhouse conditions.

Treatment	Damping-off %		Total	Efficiency (%)	Survival (%)
	Pre-emergence (%)	Post-emergence (%)			
T. viride	17.5	5.0	22.5	(57.14)	77.5
T. harzianum (1),	15.0	2.5	17.5	(66.67)	82.5
T. harzianum (2)	7.5	0.0	7.5	(85.71)	92.5
Fungicide (Rhizolex-T)	5.0	0.0	5.0	(90.47)	95.0
Control infested	35.0	17.5	52.5	-	47.5
L.S.D. 5%	9.00	6.74			8.20

It could be concluded that application of *Trichoderma spp* as seed treatment provided a good protection against damping-off caused by *R. solani*, with minor variation among the tested isolates. *T. harzianum* (2), however was the most effective treatment, as shown by the lowest percentage of pre-emergence damping-off and complete inhibition of post-emergence damping-off (85.71% efficiency). Meanwhile, *T. viride.* was the least effective treatment (57.14%). Seed treatment with Rhizolex-t fungicide was the most effective in this regard and gave approximately similar efficiency as application with *T. harzianum* (2).

Polygalacturonase (PG) activity of *R. solani* was estimated in the presence or absence of culture filtrate of *Trichoderma spp* to study its effect on PG activity of *R. solani*.

Data presented in Table (2) showed a pronounced increase in PG activity of *R. solani* during the examination period, in absence of culture filtrate of *Trichoderma spp* (control). Maximum increase in PG activity was recorded 15 days after inoculation.

Table (2): Polygalacturonase (PG) activity of *R. solani* as affected periodically by culture filtrate of *Trichoderma spp.*

Treatment	% Polygalacturonase activity after days					
	5 days	Reduction (%)	10 days	Reduction (%)	15 days	Reduction (%)
T. viride	30.79	(23.24)	40.00	(31.88)	41.20	(44.32)
T. harzianum (1)	23.12	(42.36)	24.00	(59.12)	29.24	(60.48)
T. harzianum (2)	20.53	(48.81)	27.79	(52.67)	32.07	(56.66)
Control	40.11	-	58.72	-	74.00	-

In the presence of culture filtrate of *Trichoderma spp*, however the PG acivity showed pronounced decrease during the examination period. Culture filtrates of *T. harzianum* (1) and *T. harzianum* (2) coused greater decrease than culture filtrate of *T. viride.* After 5 days of inoculation *T. harzianum* (1) and *T. harzianum* (2) recorded 42.36 and 48.81% reduction in PG activity respectively. Meanwhile, *T. viride* recorded 23.24% reduction. Then, the reduction in PG activity increased periodically till it reached 60.48, 56.66 and 44.32% reduction 15, days after inoculation, in the presence of culture filtrate of *T. harzianum* (1), *T. harzianum* (2) and *T. viride* respectively.

Data in Table (3) showed that application of *Trichoderma spp* as seed treatment resulted in a pronounced decrese in Polygalacturonase (PG) activity in infected treated plants compared with untreated infected control, which recorded progressive increase in PG activity with higher amounts during examination period.

T. harzianum (2) was the most effective treatment indicating by the lowest activity of PG during examination periods (6.43, 7.91 and 12.56% activity respectively. Followed by *T. harzianum* (1). While *T. viride* was the least effective treatment. It recorded 17.13, 19.94 and 23.30% PG activity during examination period.

Table (3): Effect of seed treatment with *Trichoderma spp* on Polygalacturonase (PG) activity in faba bean roots.

Treatment	% Polygalacturonase (PG) activity after days					
	Infected with *R. solani*			Healthy		
	10 days	15 days	20 days	10 days	15 days	20 days
T. viride	17.13	19.94	23.30	1.11	1.50	3.00
T. harzianum (1),	11.00	7.48	14.33	2.15	1.90	3.71
T. harzianum (2)	6.43	7.91	12.56	2.93	2.16	1.56
Control	28.47	39.14	45.25	1.80	2.33	1.07

Healthy plants either untreated or treated with *Trichoderma spp* recorded the lowest activity of PG during examination period. NO correlation could be noticed between seed treatment with *Trichoderma spp* and PG activity in healthy treated plants

Table (4) showed that only slight increase in total terpenes content was present in roots of untreated faba bean plants infected with *R. solani* compared with untreated healthy control

Table (4): Effect of seed treatment with *Trichoderma spp* on total terpenes in faba bean roots.

Treatment	Total terpenes mg/g root after		
	10 days	15 days	20 days
T. viride	70.0	97.5	95.0
T. viride + *R. solani*	90.0	127.5	100.0
T. harzianum (1),	65.0	115.0	85.0
T. harzianum (1) + *R. solani*	112.5	140.0	130.0
T. harzianum (2)	107.5	112.5	82.5
T. harzianum (2)+ *R. solani*	180.0	185.0	145.0
Control (1) untreated healthy	50.0	72.0	80.0
Control (2) untreated infected	60.0	95.0	80.0

Seed treatment with *Trichoderma spp* resulted in concedrable increase in total terpenes content in roots of healthy or infected plants

compared with untreated infected control. Furthermore, the increase was much higher in infected treated plants compared with healthy treated ones. Pronounced increase was recorded during early stage of planting (10 and 15 days after sowing).

As for pre-treated infected plants, *T. harzianum* (2) was the most effective treatment judged by the highest increase in total terpenes content during the examination period than untreated infected control, similar trend could be noticed with, *T. harzianum* (1). The lowest increase in total terpenes, however was recorded for *T. viride,* during the examination period.

Data presented in Table (5) showed that All treatments of *Trichoderma spp* increased peroxidase activity in roots of pre-treated plants (healthy or infected with *R. solani*) compared with untreated ones.

In healthy treated plants slight increase in proxidase activity was found as a result of application *Trichoderma spp* as seed treatment. Meanwhile, the increase in peroxidases activity was more noticeable in infected pretreated plants. Isolates of *T. harzianum* (1) and (2) recorded maximum increase in proxidase activity in infected pre-treated plants. This increase was more than 2.3, 1.5 and 1.5 fold over infected control, during 10, 15, 20 days respectively after soweing. At the same time, *T. viride* increased peroxidase activity in infected treated plants by 1.5, 1.2 and 1.1 time over infected control during the same periods, respectively.

Table (5): Effect of seed treatment with *Trichoderma spp* on Peroxidase activity in faba bean roots (healthy or infected with *R. solani*)

Treatment	Peroxidase activity /minute after					
	Infected with *R. solani*			Healthy		
	10 days	15 days	20 days	10 days	15 days	20 days
T. viride	0.180	0.163	0.157	0.100	0.100	0.105
T. harzianum (1),	0.265	0.201	0.209	0.098	0.112	0.101
T. harzianum (2)	0.268	0.213	0.224	0.095	0.103	0.108
Control	0.115	0.127	0.132	0.090	0.090	0.097

Data presented in Table (6) showed that All treatments significantly increased fresh and dry weight of faba bean plants compared with untreated infected control. Furthermore the increase was much higher in healthy treated plants compared with pre treated infected ones.

Table (6): Effect of seed treatment with *Trichoderma spp* on fresh weight, dry weight and number of nodules of faba bean plants healthy or infected with *R. solani*

Treatment	Fresh weight (g)		Dry weight (g)		Number of nodules/plant (Mean)
	Root	Shoot	Root	Shoot	
T. viride	5.26	18.96	1.71	6.64	62.50
T. viride + *R. solani*	4.73	16.37	1.28	5.12	57.00
T. harzianum (1),	6.79	22.80	1.97	7.91	82.50
T. harzianum (1) + *R. solani*	5.68	19.78	1.56	6.24	78.25
T. harzianum (2)	5.34	22.00	1.58	7.10	118.57
T. harzianum (2) + *R. solani*	4.70	19.10	1.43	6.03	90.50
Fungicide (Rhizolex-T) + *R. solani*	5.05	16.58	1.42	5.18	46.78
Control (1) healthy	5.13	16.46	1.56	5.49	48.75
Control (2) infected	2.39	11.16	0.87	3.12	21.50
L.S.D 5%	0.63	2.65	0.37	0.89	5.14

In infected treated plants, the highest increase in root fresh weight was recorded by *T. harzianum* (1), followed by seed treatment with Rhizolex-T., *T. viride* and *T. harzianum* (2) respectively. Also, in pretreated infected plants, *T. harzianum* (1) and *T. harzianum* (2) recorded the highest increase in root dry weight, followed by fungicide treatment, and *T. viride* respectively.

Shoots were also increased as a result of application of *Trichoderma spp.* In infected treated plants, *T. harzianum* (1) and *T. harzianum* (2) recorded the highest increase in shoot fresh weight followed by fungicide treatment and *T. viride* respectively. The same trend was found in shoot dry weight.

Concerning the number of nodules per plant obtained data in Table (6) showed that application of *Trichoderma spp* as seed

treatment significantly increased number of nodules compared with untreated control (healthy or infected). The increase was higher in healthy treated plants compared with treated infected ones. *T. harzianum* (2) recorded maximum increase in number of nodules in both healthy or infected plants. This increase was more than 4-fold in infected treated plants compared with untreated infected control. Followed by *T. harzianum* (1) and *T. viride* which recorded more than 3 and 2.5 fold increase in infected treated plants respectively compared with untreated infected control while, seed treatment with fungicide (Rhizlex-T) recorded the lowest increase in number of nodules. Although, seed treatment with fungicide increase number of nodules compared with untreated infected control, however *Trichoderma* treatments were superior.

Discussion

Obtained results showed significant reduction in pre and post emergence damping-off caused by *R. solani* in faba bean plants as a result of seed treatment with *Trichoderma spp* or fungicide (Rhizolex – T) compared with untreated infected control.

Fungicide treatment (Rhizolex–T) was superior compared to other treatments however the tested bioagents recorded considerable suppression against damping-off. *T. harzianum* (2) was the most effective treatment as indicated by the highest reduction in damping-off while *T. viride* was least effective one.

Application of *Trichoderma spp* as seed or soil treatment used successfully to control large numbers of soil borne diseases. In this respect, Prasad *et al.* (2002) and Gupta *et al.* (2005) found that application of *T. viride* or *T. harzianum* as seed or soil treatment reduced chickpea wilt complex caused by *Fusarium oxysporum* f. sp *ciceris, Sclerotium rolfesii* and *R. solani*. Jayaraj and Radhakrishnan (2003) demonstrated that cotton seed treatment with *T. harzianum* resulted in better plant stand, plant biomass and less damping-off disease caused by *R. solani* in both greenhouse and field condition. Mahdy *et al.,* (2006) used *T. harzianum* (as seed coating or soil application) for management of root-knod and root disease complex caused by root-knod nematode. *M. javanica* and the fungus *R. solani* on soybean plants. They found that number of galls, root galling, egg masses and disease severity were reduced sharply on plants treated with bioagent, either as seed or soil application, compared with non-

treated plants. Abd-El-Kareem (2007) evaluated *T. harzianum*, as seed coating against root rot of bean caused by *R. solani* and *Fusarium solani*. He found that bean seeds coating with *T. harzianum* decreased disease incidence by more than 85.1 and 81.9% for *F. solani* and *R. solani* respectively under greenhouse condition. Furthermore, under field condition the disease incidence reduced by more than 65.7% during two successive seasons.

Pectin-degrading enzymes are the first class of enzymes produced during plant infection, accounting for the rapid and extensive degradation of cell wall and cell death, and reproduce the major symptoms of diseases caused by many necrotrophic pathogens, particularly those which produce soft-rot diseases. Alghisi and Favaron (1995). Whereas. Bateman (1964) stated that Polygalacturonase produced by *R. solani* was primarily responsible for tissue maceration.

In the present investigation, *Trichoderma spp* showed profound decrease in polygalacturonase (PG) activity of *R. solani* .

In vitro studies, gradual increase in PG activity of *R. solani* was found during examination period in the absence of culture filtrates of *Trichoderma spp.* Meanwhile, considerable reduction in PG activity was found in the presence of culture filtrates of *Trichoderma spp. T. harzianum* (1) and (2) recorded maximum reduction in PG activity.

In vivo studies, application of *Trichoderma spp* as seed treatment resulted in noticeable reduction in (PG) activity in pre-treated infected plants compared with untreated infected control, which showed a progressive increase during examination periods. *T. harzianum* (2) was the most effective treatment whereas *T. viride* was the least effective one. Also, obtained results showed correlation between the capacity of enzyme inhibition by the tested isolates of *Trichoderma* and disease suppression. Isolate of *T. harzianum* (2) , a better biocontrol agent is a better inhibitor of PG activity of *R. solani*. The reduction in PG activity of *R. solani* must be due to the presence of protease or other toxic compounds in culture filtrate of *Trichoderma* isolates as shown by Elad and Kapat (1999), Mandavia *et al* (1999) and Elad (2000) along with the possible suppression of pathogenicity enzymes might lead to an accumulation of oligogalacturonoide elicitors that in turn activate plant defence, Zimand *et al.* (1996). Since these fragment are released earlier in the incompatible interaction, a possible involvement as signaling molecules that activate defence

responses. Alghisi and Favaron (1995). Meanwhile, the obtained results were agreement with those reported by Elad (2000), Roco and Perez (2001), Murugesan *et al.*(2002), Dutta and Chatterjee (2005) and Haggag *et al.* (2006). They indicated that the biocontrol agent *Trichoderma* caused appreciable reduction in pectolytic enzymes activity of the plant pathogenic fungi *In vivo* and *In vitro.*

Recently, research enphasis has been expanded to include induction of defense responses in the host plant by *Trichoderma spp* . The obtained data indicated that treatment faba bean seeds with biocontrol isolates of *Trichoderma* triggers plant defense responses in the developing seedling radicles. Apart of this response apears to be stimulation of terpenes in the root system. The increase in total terpenes in treated infected plants was much higher compared with untreated infected control. A comparison of biocontrol efficacy and induction of terpenes in faba bean roots by *Trichoderma spp* showed that, the most effective isolate *T. harzianum* (2) stimulated maximum production of terpenes (3. 1.9 and 1.7 fold increase than untreated infected control during 10. 15 and 20 days after sowing respectively) whereas, the least effective isolate *T. viride* stimulated the minimum production of terpenes (1.5, 1.3 and 1.1 fold increase than untreated infected control respectively). Hanson and Howell (2004) indicated that effective biocontrol strains of *Trichoderma* were found to simulate greater terpenoid levels in cotton root than noneffective strains.

Terpenoids are known to be toxic to certain pathogens Hunter *et al.* (1978), Mace *et al.*(1990), Zhang *et al.* (1993) and Howell *et al*. (2000). So the presence of terpenoid in induced seedling roots might well prevent subsequent development of pathogen hypha attacking the root, this may explain the lower levels of disease incidence. Hanson and Howell (2004) and Howell (2006).

Also, Hanson and Howell (2004) isolated some elicitors from culture filtrate of *Trichoderma virens.* They found that , 18 KD_a protein gave significant stimulation of cotton terpenoid production in cotton roots.

Obtained results were coinside with those reported by Howell *et al.*(2000), Puckhaber *et al.*(2002) and Howell (2006) they indicated that seed treatment with *Trichoderma* increased terpenoied in cotton root in soil infested with *R. solani* . Bioassay of these compound against *R. solani* showed strong inhibition to the pathogen .

The reduction in disease incidence was also associated with a remarked increase in peroxidase activity in faba bean plants pretreated with *Trichoderma spp* and infected with *R. solani* compared with untreated infected control. Furthermore, these increases reached many fold than the untreated infected control and found to be continued till the last incubation period.

Proxidase play a pivotal role in giving resistance to the plants by involved in the oxidative polymerization of hydroxycinnamyl alcohols to yield lignin (Vance *et al.*,1980) and crosslinking isodityrosine bridges in cell walls (Fry, 1982). These compound act as barriers against pathogen invasion and hence constitute part of host resistance mechanism. Peroxidase also produces free radicals and hydrogen peroxide, which are toxic to several pathogens (Peng and kuc, 1992).

Many investigations reported that application of *Trichoderma spp* as seed or soil treatment to control soil borne diseases was accompanied with great increases in peroxidase activity after challenge inoculation with the pathogen (Yedidia *et al*, 2000; Hanson and Howell 2004; Shao *et al* 2005 and Karthikeyan *et al.,* 2006).

Also, the results showed that, application of *Trichoderma spp* as seed treatment was accompanied with significant increase in fresh and dry weight of faba bean treated plants compared with untreated ones. The increase was higher in healthy treated plants compared with infected treated ones. Isolates of *T. harzianum* were more effective compared with isolate of *T. viride*. They recorded maximum increase in fresh and dry weight of plants.

More than one mechanism (e.i suppression of mainor plant pathogen, production of phytohormones and nutrient availability) participate in *Trichoderma* plant interactions leading to growth promotion effect. Shaban (2004), Khatun *et al* (2005) and Gravel *et al* (2007).

Significant increase in number of nodules per plant was found as a result of application of *Trichoderma spp* as seed treatment compared with untreated control. This increase was much higher in healthy treated plants compared with treated infected ones *T. harzianum* (2) was the most effective one followed by *T. harzianum* (1) and *T. viride*. They recorded more than 4, 3 and 2.5 fold increase in number of nodules respectively in treated infected plants compared with untreated infected control. Nodulation might be inhibited by the toxic substances secreted by the pathogens (Jain and Gupta 2002) in

untreated infected plants. Whereas the increase in number of nodules in treated plants must be due to production of phytohormones by *Trichoderma spp*. (Khatun *et al* (2005). All phytohormons are implicated in nodule formation in one way or another (Hirsch *et al* (1997). The effects of some phytohormons are indirect, as they stimulate root growth, providing further sites for infection and nodulation (Zhang *et al.,* (2004).

Application of *Trichoderma spp* as seed or soil treatments usually associated with increase in plant growth, fresh and dry weight as well as number of nodules. Kadam *et al.* (2004) study the effect of sulfer oxidizing microorganisms (*Thiobcillus thiooxidans* and *Trichoderma harzianum*) on soybean nodules formation. They found that the number of nodules on soybean roots significantly increased due to inoculation of the sulfer oxidizing microorganisms. Gupta *et al.* (2005) indicated that the reduction in wilt disease complex of chickpea as a result of seed treatment with *T. viride* was associated with a great increase in dry weight of plants, grain yield and enhancing chickpea-Rhizobium symbiosis. Also Rudresh *et al*. (2005) found pronounced increase in plant growth of chickpea, nodulation and nutrient uptake when *T. harzianum* was inoculated along with phosphate solubilizing bacterium and rhizobium. Mahdy *et al* (2006) found that fresh shoot and root weight, dry weight, number of bods and bacterial nodules of soybean were markedly increase in soybean plants treated with *T. harzianum* either as seed treatment or soil application compared to plants grown in infested soil with the pathogen alone.

REFERENCES

Abd-El-Kareem, F. (2007). Induced resistance in bean plants against root rot and Alternaria leaf spot diseases using biotic and abiotic inducers under field conditions. Research Journal of Agriculture and Biological Sciences, 3(6): 767 – 774.

Alghisi, P. and Favaron, F. (1995). Pectin-degrading enzymes and plant-parasite interactions. European Journal of Plant Pathology, 101: 365 – 375.

Allam, A.I. and Hollis, S.P. (1972). Sulfide inhibition of oxidase in rice root. Phytopathology, 62: 634 – 639.

Bateman, D.F. (1964). The macerating enzyme of *Rhizoctonia solani.* Phytopathology, 54: 1178 – 1186.

Brain, P.W. and Hemming, H.G. (1945). Gliotoxin a fungistatic metabolic product of *Trichoderma viride.* Ann . Appl. Boil., 32: 214 – 220.

Cervone, F.; Hahn, M.G.; Lorenzo, G.D.; Darvill, A. and Albersheim, P. (1989). Host pathogen interaction. XXXIII. A plant protein converts a fungal pathogenesis factor into an elicitor of plant defense responses. Plant Physiology. 90, 542 – 548.

Chan, Y.H. and Sockston, W.F. (1972). Production of pectolytic and celluloltic enzymes by virulent and avirulent isolates of *Sclerotium bataticola* during disease development in sunflower. Can. J. Bot., 50: 2449 – 2453.

Dalisay, R.F. and Kuc, J.A. (1995). Persistence of induced resistance and enhanced peroxidase and chitinase activities in cucumber plants. Physiol Mol. Plant Pathol. 47: 315 -327.

Dutta, S. and Chatterjee, N.C. (2005). Interaction of *Trichoderma viride* with *Rhizopus stolonifer* during its pathogenesis and the associated resistance of the host. Acta phytopatholagica et Entomologica Hungarica, 40 (3/4): 315 – 322.

Ebrahimzadeh, H. and Nikman, V. (1998). A revised spectrophotometric method for determination of triterpenoid saponin. Indian Drugs. 35: 379 – 381.

Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Protection, 19 (8/10): 709 – 714.

Elad, Y. and Kapat, A. (1999). The role of *Trichoderma harzianum* protease in the biocontrol of *Botrytis cinerea*. European Journal of Plant Pathology, 105 (2): 177 – 189.

Fry, S. C. (1982). Isodityrosine, a new amino acid from plant cell wall glycoprotein. Biochem. J., 204,449- 455.

Gravel, V.; Antoun, H.; and Tweddell, R.J. (2007). Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil Biology and Biochemistry, 39 (8): 1968 – 1977.

Gupta, S.B.; Thakur, K.S.; Singh, A.; Tamrakar, D.K. and Tedia, K. (2005). Exploiting *Trichoderma viride* for improving performance

of chickpea in wilt complex affected area of Chhattisgarh. Advances in Plant Sciences, 18 (2): 609 – 614.

Haggag, W.M.; Kansoh, A.L. and Aly, A.M. (2006). Proteases from *Talaromyces flavus* and *Trichoderma harzianum*: Purification, characterization and antifungal activity against brown spot disease on faba bean. Plant Pathology Bulletin, 15(4): 231-239.

Hammerschmidt, R.; Metraux, J. P. and Van Loon, L.C. (2001). Inducing resistance: a summary of papers presented at first international symposium on induced resistance to plant diseases. Corfu, May 2000. European Journal of Plant Pathology 107: 1 - 6

Hanson, L.E. and Howell, C.R. (2004). Elicitors of plant defense responses from biocontrol strains of *Trichoderma virens.* Phytopathology, 94 (2):171 – 176.

Hirsch, A.M.; Fang, S.; Asad, S. and kapulnik, Y. (1997). The role of phytohormones in plant-microbe symbioses. Plant and Soil. 194: 171 – 184.

Howell, C.R. (2006). Understanding the mechanisms employed by *Trichoderma virens* to effect biological control of cotton diseases. Phytopathology, 96 (2): 178- 180.

Howell, C.R.; Hanson, L.E.; Stipanovic, R.D. and Puckhaber, L. S. (2000). Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens.* Phytopathology, 90: 248 – 252.

Hunter, R.E.; Halloin, J.M.; Veech, J.A. and Carter, W.W. (1978). Terpenoid accumulation in hypocotyls of cotton seedling during aging and after infection by *Rhizoctonia solani.* Phytopathology, 68: 347 – 350.

Jain, V. and Gupta, V.K. (2002). Effect of rhizosphere fungi on nodule number, shoot and root length of *Vigna mungo*. Indian Phytopathology, 55 (3): 323 – 324.

Jayaraj, J. and Radhakrishnan, N.V. (2003). Development of UV-induced carbendazim-resistant mutants of *Trichoderma harzianum* for integrated control of damping-off disease of cotton caused by *Rhizoctonia solani.* zeitschrift fur pflanzenkrankheiten und pflanzenschutz, 110 (5): 449 -460. (cited from CAB Abstract).

Kadam, R.M.; Shinde, D.B. and Jadhav, A.C. (2004). Effect of sulphur oxidizing micro-organisms on nodulation pattern of soybean. Journal of Maharashtra Agricultural Universities, 29 (3): 300- 302.

Kapat, A.; Zimand, G. and Elad, Y. (1998). Effect of two isolates of *Trichoderma harzianum* on the activity of hydrolytic enzymes produced by *Botrytis cinerea.* Physiological and Molecular Plant Pathology. 52 (2): 127 – 137.

Karthikeyan, M.; Radhika, K.; Mathiyazhagan, S.; Bhaskaran, R.; Samiyappan, R. and velazhahan, R. (2006). Induction of phenolics and defens related enzymes in coconut (*Cocos nucifera* L.) roots treated with biocontrol agents. Brazilian Journal of Plant Physiology. 18 (3): 367 – 377.

Khatun, M.M.; Ali, M.H. and Paderes, E.P. (2005). Effect of Trichoderma on growth response of rice. International Journal of Sustainable Agricultural Technology, 1 (3): 48 – 49.

Levy, N. O.; Elad, Y.; Korolev, N.; and Katan, J. (2004). Resistance induced by soil biocontrol application and soil solarization for control of foliar pathogens. Bulletin – OILB/SROP, 27 (1): 171 – 176.

Mace, M.E., Stipanovic, R.D. and Bell, A.A. (1990). Relation between sensitivity of terpenoid phytoalexins and virulence to cotton of *Verticillum dahliae* strains. Pestic. Biochem. Physiol., 36: 79 – 82.

Mahdy, M.E.; El-Shennawy, R.Z. and Khalifa, E.Z. (2006). Biological control of *Meloidogyne javanica* and *Rhizoctonia solani* on soybean by formulation of *Bacillus thuringiensis* and *Trichoderma harzianum*. Arab Universities Journal of Agriculture Sciences, 14 (1): 411- 423.

Mandavia, M.K.; Gajera, H.P.; Andharia, J.H.; Khandar, R.R. and parameswaran, M. (1999). Cell wall degrading enzymes in host-pathogen interaction of Fusarium wilt of chickpea: Inhibitory effects of phenolic compounds. Indian Phytopath, 52 (3): 285 – 288.

Murugesan, K.; Perumal, K. and Kalaichelvan, P. T. (2002). *Trichoderma viride* paralyses lignolytic ability of a white rot fungus *Ganoderma lucidum.* Journal of Mycology and plant pathology, 32 (2): 225-230.

Omar, S.A. (1986). Pathological studies on root rot disease of faba bean (*vicia fabae* L.) 11th con. Sci, Soc. and Research Cairo. Egypt, pp: 33 – 48.

Peng, M. and Kuc, J.A. (1992). Peroxidase-generated hydrogen peroxide as a sourse of antifungal activity *in vitro* on tobacco leaf disks. Phytopathology, 82: 696- 699.

Prasad, R.D.; Rangeshwaran, R.; Anuroop, C.R. and Rashmi, H.J. (2002). Biological control of wilt and root rot of chickpea under field conditions. Annals of Plant Protection Science, 10 (1): 72 – 75.

Puckhaber, L.S.; Doud, M.K.; Stipanovic, R.D. and Howell C.R. (2002). Toxicity of (+) – and (-) – gossypol to the plant pathogen, *Rhizoctonia solani.* Journal of Agric and food chemistry, 50 (24): 7017 – 7021.

Roco, A. and Perez, L.M. (2001). *In vitro* biocontrol activity of *Trichoderma harzianum* on *Alternaria alternata* in the presence of growth regulators. Electronic Journal of Biotechnology, 4 (2): 1 – 6.

Rudresh, D.L.; Shivaprokash, M.K. and Prasad, R.D. (2005). Effect of combined application of Rhizobium, phosphate solubilizing bacterium and *Trichoderma spp*. On growth, nutrient uptake and yield of chickpea. Applied Soil Ecology. 28 (2): 139 – 146.

Sepulveda, R.P. (1991). Identification of *R. Solani* kuhn. Affecting faba bean (*vicia faba L.*) in chile. Agric. Tecnico Santiagoo, 51 (4) : 362 – 363.

Shaban, G.M. (2004). Response of wheat to treatment with Trichoderma isolates having different enzymatic and root colonization activities. African Journal of Mycology and Biotechnology, 12 (1): 35 – 44.

Shao, X., Jinghua, Z.; Yanqing, H., you yoong, Z. and Jie, C. (2005). Induced resistance to *Fusarium oxysporum* in melon treated with combination of *Trichoderma Viride* and entophytic bacteria *Bacillus subtilis*. Chinase Journal of Biological Control, 21 (4) 254- 259. (Cited from CAB Abestract).

Talboys, P.W. and Bush, L.V. (1970). Pectic enzymes produced by *Verticillium spp*. Trans. Br. Mycol. Sco., 55: 367 – 381.

Vance, C.P.; Kirk, T.K.; and Sherwood, R.T. (1980). Lignification as a mechanism of disease resistance. Ann. Rev. Phytopathology, 18: 259 – 288.

Yedidia, I.; Benhamou, N.; Kapulnik, Y. and Chet, I. (2000). Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T. 203. Plant Physiology and Biochemistry 38 (11) 863 – 873.

Zhang, J.; Mace, M.E.; Stipanovic, R.D. and Bell, A.A. (1993). Production and fungitoxicity of the terpenoid phytoalexins in cotton inoculated with *Fusarium oxysporum* F. sp *vasinfectum*. J. Phytopathol, 139: 247 – 252.

Zhang, S.; Reddy, M.S. and Kloepper, S.A. (2004). Tobacco growth enhancement and blue mold disease protection by rhizobacteria: Relationship between plant growth promotion and systemic disease protection by PGPR strain 90 – 166. Plant and Soil, 262: 277 – 288.

Zimand, G.; Elad, Y. and chet, I. (1996) Effect of *Trichoderma harzianum* on *Botrytis cinerea* pathogenicity. Phytopathology, 86: 1255 – 1260.

تفاعل انواع مختلفة من الفطر ترايكوديرما مع الفطر ريزوكتونيا سولانى خلال اصابته لجذور الفول البلدى وتأثيره على المقاومة المصاحبة للنبات

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فى هذا البحث استخدمت 3 عزلات من الفطر ترايكوديرما وهى ترايكوديرما هارزيانم (1) ، ترايكوديرما هارزيانم (2) ترايكوديرما فيردى. وذلك لدراسة تاثير هذه العزلات كمعاملة بذرة على الاصابة بمرض عفن جذور الفول البلدى المتسبب عن الفطر ريزوكتونيا سولانى.

اوضحت النتائج ان معاملة بذور الفول البلدى بعزلات الفطر ترايكوديرما ادى الى حدوث انخفاض معنوى فى نسبة موت البادرات مقارنة بالكنترول الغيرمعامل. وكانت غزلة الفطر ترايكوديرما هارزيانم رقم 2 هى الاكثر فعالية . كما وجد ان استخدام راشح المزارع الخاصة بعزلات الفطر ترابيكوديرما ادى الى انخفاض ملحوظ فى نشاط انزيم البولى جالاكترونيز للفطر رايزوكتونيا سولانى خلال فترات الاختبار تحت ظروف المعمل. وكذلك ادى استخدام عزلات الفطر ترايكوديرما فى صورة معاملة للبذرة قبل الزراعة الى انخفاض شديد فى نشاط انزيم البولى جالاكترونيز فى جذور النباتات المصابة مقارنة بالكنترول المصاب والغير معامل الذى سجل زيادة كبيرة فى نشاط انزيم البولى جالاكترونيز خلال فترات الاختبار.

لوحظ ان الانخفاض فى نشاط انزيم البولى جالاكترونيز فى جذور النباتات المصابة والتى سبق معاملتها بالفطر تريكوديرما مصحوبا بزيادة فى المحتوى الكلى للتربينات فى جذور النبات وكذلك زيادة نشاط انزيم البيروكسيديز. حيث كانت هذه الزيادة كبيرة جدا ووصلت لاكثر من ضعف فى جذور النباتات مقارنة بالكنترول المصاب والغير معامل بالفطر تريكوديرما.

بالاضافة الى استحثاث المقاومة فى النباتات فقد وجدان استخدام عزلات الفطر تريكوديرما فى صورة معاملة بذرة ادى الى زيادة ملحوظة فى الوزن الرطب والوزن الجاف للنباتات وكذلك زيادة عدد العقد الجذرية للنبات فى كل من النبالتات السليمة والمصابة مقارنة بالكنترول الغير معامل.