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Influence of *Glomus intraradices* and *Trichoderma harzianum* on Pepper Fusarium Wilt Control

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ABSTRACT

Two beneficial microorganisms *Glomus intraradices* and *Trichoderma harzianum* were tested for their ability to suppress Fusarium wilt of Pepper caused by *Fusarium oxysporum* f.sp. *capsici*. The results showed that *G. intraradices* and *T. harzianum* controlled the wilt disease reducing disease incidence to (28 ,19.6% and 23 ,14,6%) compared with control (38.3 ,25%) in the two verities of pepper plant California Wonder and Local verity respectively . This pathogen significantly reduced plant growth as measured by dry root weight and dry shoot weight . Overall, *T.harzianum* and *G. intraradices* caused significant increasing in phenol amount (91.3,118.7 and 80.3,104.4 µg g^{-1} FW) , chlorophyll contents (28.3,31.7 and 23.7, 26.4 mg/g FW), % Carbohydrate (1.63,1.82% and 1.39,1.53%) compared with control (63 ,81.9 µg g^{-1} FW ,18.4,20.55 mg/g FW and 1.08, 1.19%) in the two verities of pepper plant California Wonder and Local verity respectively .

تأثير معاملة نباتات الفلفلِ بالفطر *Glomus intraradices* والفطر *Trichoderma harzianum* في مكافحة الذبول الفيوزارمي

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الخلاصة

تم اختبار قدرة نوعين من الأحياء الدقيقة كعوامل للمقاومة الإحيائية هما فطر المايكورايزا *Glomu intraradices* والفطر *Trichoderma harzianum* للسيطرة على الفطر *F. oxysporum* f. sp. *capsici*.. وبينت النتائج قدرة كل من *G. intraradices T. harzianu* على خفض نسبة الإصابة بالذبول الى (28 ،19.6 % و 23 ، 14.6 %) مقارنة بمعاملة المقارنة (38.3 ، 25 %) للصنفين اعجوبة كاليفورنيا والصنف المحلي على التوالي . وكذلك بينت النتائج تسبب المرض في خفض معنوي في نمو النبات متمثلا بالوزن الجاف للمجموعين الخضري والجذري وكان لعاملي المكافحة الإحيائية *T. harzianum* و *G. intraradices* دور في إحداث زيادة معنوية في كمية الفينولات (91.3 ، 118.7 و80.3، 104.4 مايكروغرام /غرام وزن رطب والكلوروفيل (28.3 ، 31.7 و 23.7 ، 26.4 ملغم/غرام وزن رطب) و% للكاربوهيدرات (1.63 ،1.82% و 1.39، 1.53 %) مقارنة بمعاملة المقارنة (63 ، 81.9 مايكروغرام /غرام وزن رطب) و(18.4، 20.55 ملغم/غرام وزن رطب) و(1.08، 1.19 %) للصنفين اعجوبة كاليفورنيا والصنف المحلي على التوالي.

الكلمات المفتاحية:
F. oxysporum
T. harzianum
G. intraradices
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Introduction:

Vascular wilt diseases caused by pathogenic genus *Fusarium* constitute some of the most important and extensive commercial losses in many crops throughout the world that reduce both quality and yield. Of these, *Fusarium oxysporum* f.sp. *capsici* is one of the most destructive pathogens of pepper (Moreno, 2002).Arbuscular mycorrhizal fungi (AMF) have been frequently shown to reduce damages and the growth of soil borne pathogens in a wide range of mycorrhizal plant

species and pathogenic organisms (Smith, *et al* 1997). These fungi constitute Arbuscular mycorrhizal fungi (AMF) are key components of soil microbiota and form symbiotic relationships with the roots of most terrestrial plants, improving the nutritional status of their host and protecting it against several soil-borne plant pathogens (Harrison, 1999; Bi *et al*., 2007). The incidence and the effect of root colonization vary depending on the plant species and the AMF they are influenced by soil microorganisms and environmental factor. (Pozo, *et al*, 2002 and VanLoon *etal*,2006) *Trichoderma* spp. is a common microorganism of rhizosphere soil and has been reported to suppress a great number of plant diseases, some strains, also, have been reported to colonize the root surface, enhancing root growth and development, crop productivity, resistance to abiotic stresses, and the uptake of nutrients (Elad, 2000 and Howell, 2003). Several reports have demonstrated that the interaction of these two groups of microorganisms may be beneficial for both plant growth and plant disease control, the possibility of combining AMF and *Trichoderma* spp. also have been advantageously explored in the past and synergistic interactions with regard to plant growth especially occurred in dually inoculated plants (Saldajeno *et al*., 2008; Martnez-Medina et al., 2009, Rashmi Srivastava, *et al*,2010 Tchameni *et al*,2012). Reports in a number of studies illustrate changes of the root exudation pattern through mycorrhization expressed as a different effect of root exudates on soil microorganisms and have been suggested to be at least partially involved in the altered susceptibility of mycorrhizal plants towards soil-borne microorganisms (Jones *et al*. 2004;Vierheilig and Piche 2002; Vierheilig 2004).Roots exude a wide range of compounds into the rhizosphere soil. such as sugars, amino acids, organic anions , phenolics ,and high molecular weight organic exudates such as proteins, pigments, mucilage .Secreted roots exude from plants have an important function in determining the positive or negative outcome of interactions in the rhizosphere.(Shi, *et al*,2009) Recently, it has been shown that root exudates of tomato stimulate the microconidia germination of the tomato pathogen *Fusarium oxysporum* (Steinkellner *et al*., 2005), similar pattern of microconidia germination was found in the presence of root exudates from non-host plants, such as sweet pepper, bean, barley, tobacco and cucumber (Steinkellner *et al*., 2005 Scheffknecht *et al*., 2009). Kamilova *et al*. (2006) reported on changes of the composition of organic acids and sugars in tomato root exudates due to the presence of *F. oxysporum* f. sp.*radicis-lycopersici*. Other contrast reports on the susceptibility of mycorrhizal tomato plants to *F*. oxysporum. In several studies demonstrated bioprotectional effect of *G. intraradices* a gainst *F. oxysporum* f. sp.*capsici* (Akkopru and Demir, 2005).

The goal of this study was to evaluate the effect of *T. harzianum* and *G. intraradices* to promote pepper growth and induce resistance against Fusarium wilt,and initiated the plants a series of morphological as well as biochemical changes which are considered to be part of the plant defense response.

Materials and methods:

Arbuscular mycorrhizal fungi:

Glomus intraradices (Manufactured by reforestation Technology International reforest .com .1341 Byton street Suite G .Salius .Ca.93901 USA.80 spores per CC) inoculums was multiplied as pot cultures with barley and consisted of mixtures of soil and root fragments.

Isolation of pathogen and pathogen inoculum preparation:

Infected pepper plants collected from pepper fields in Mosul. To isolate *Fusarium* spp. plants were washed thoroughly in running water, cut into small pieces, surface-disinfected with 0.6% sodium hypochlorite (v/v) for 5 min, and placed on potato-dextrose agar acidified with lactic acid to pH 5.0. Species identification was according to Nelson *et al.* (1983). The pathogen inoculum was prepared as spore suspension obtained from 7 day-old cultures. The appropriate concentration was $2x10^6$ measured by using heamocytometer.

Host Plant:

Pepper seeds (*Capsicum annuum*) (California Wonder, Local verity Hot pepper**)** were surface-sterilized with 0.6% sodium hypochlorite (v/v) for 3 min Seedlings of the plant were raised in trays (35x15x10 cm) filled with soil sterilized with formalin. Seedlings were transplanted after 4 weeks into plastic pots (20 cm diameter and 30 cm deep) filled with sterilized soil. Plants were inoculated with *T. harzianum* and *G. intraradices* in a greenhouse Here, the AM inocula were mixed at a rate of 20 g kg−1 of soil, while *T. harzianum* was added to reach population density of 4×10^6 conidia ml^{-1},

Five treatments were used in this study.

(i) Uninoculated control,

(ii) Inoculated with *Fusarium oxysporum* f.sp. *capsici.*(FOC)

(iii) Inoculated with *G. intraradices*

(iv) Inoculated with FOC and *G. intraradices* simultaneously

(v) Inoculated with FOC and *T.harzianum* simultaneously

(vi) Inoculated with *T.harzianum*

(vii) Inoculated with *G. intraradices* ,*T.harzianum* simultaneously and FOC .

Bioassay and assessment of disease incidence and severity:

Bioassay and assessment of disease incidence and severity was performed 60 days after the experiment was carried out in a greenhouse. Disease incidence was expressed as the percentage of wilting plants in each pot over the total number of plants . Severity symptoms on individual plants were rated on a scale from 0 to 4, according to the percentage of foliage with chlorosis or necrosis in acropetal progression: 0 = 0%, 1 = 1–33%, 2 = 34–66%, 3 = 67–100% and 4 = dead plant (Hervas et al.1997). Biochemical parameters, chlorophylls (a and b) , carbohydrates percentage and total phenols content ,were analyzed at this stage.(Zieslin, and .Ben,1993)

Assessment of *G. intraradices* **root colonization:**

Roots were cut into 1 cm pieces, thoroughly mixed with each other and sets of approximately 1 g were used for assessment G. *intraradices* colonization .The root subsamples were cleared using 10% potassium hydroxide (KOH) and stained with acid fuchsine. They were then mounted on slides and observed using a light microscope. The percentage of root length colonized by *G. intraradices* was calculated by the gridline intersect method (Giovannetti and Mosse, 1980). Positive counts for *G. intraradices* colonization included the presence of vesicles or arbuscules or typical mycelium within the roots.

Collection of root exudates:

After the total growth period of 60 days plants were removed by gently washing the soil off the roots with tap water. The plants were placed in a beaker containing sterile distilled water, such that the roots were completely submerged. The plants were placed under laboratory condition 24h , thereafter removed from the beaker. The volume of exudate obtained was adjusted with sterilized water to 15 ml per g root fresh weight. The exudates were passed through 0.22 µm sterilfilters and stored at -20°C for further investigation.(Steinkellner *et al* ,2009)

Effect of plant root exudates on spore germination:

Root exudates (15 ml) were aseptically mixed with 15 ml of half strength Czapeck Dox liquid (CDL) medium to achieve 50% concentration of the root exudate. Appropriate control treatment consisted of mixing distilled sterile water with half strength CDL medium.Aliquots (1ml)of freshly prewashed microconidial suspensions of *F.oxysporum* f.sp. *capsici* ,was pipette in 100-ml flasks containing the root exudates and media to achieve final spore load of 100 conidia/ml. Inoculated flasks were incubate at 27 C in dark. After 20 h, spores were stained with 0.1% methylene blue and viewed with Microscope at 400x magnification for the percentage of germination of microconidia. The data were expressed as percent spore germination.

Statistical analysis. All calculations were carried out using the Statistic Analysis System, version 9 (SAS Institute, Cary, NC).For all experiments the levels of significance for the experimental repetitions, main treatments, and their interactions were calculated using the General Linear Models

Procedure (PROC GLM). Data were subjected to analyses of variance and treatment means were compared by an approximate Duncan's multiple test (P<0.05).

Results:

Effect of *G.intraradices* and *T.harzianum* on pepper wilt incidence and severity:

The results of one experiment are presented to demonstrate the effect of *G. intraradices* and *T.harzianum*. on control pepper wilt disease caused by *Fusarium oxysporum* f.sp. *capsici* (Table 1). There was no significant difference in disease incidence between *T.harzianum* ,*G. intraradices* and *T.harzianum* (23,21 and 14.6, 12.6%) for California Wonder and Local verity pepper respectively. Disease severity in pepper treated with *T.harzianum* exhibited lower disease severity (0.13 and 0.14) compared to the infected control (0.44 and 0.23) for California Wonder and Local verity pepper respectively. Dual inoculation with *G. intraradices* and *T.harzianum* significantly reduced wilt disease incident and disease severity (21 ,12.6% and 0.12 ,0.11) Table 2 for California Wonder and Local verity pepper respectively compared to al inoculation with *G. intraradices* or *T.harzianum* only.

Table 1: Effect of inoculation with *G. intraradices* and *T.harzianum* on wilt disease incident and severity development in pepper plant .

Treatment	Disease incident %		Disease severity	
	California Wonder	Local verity pepper	California Wonder	Local verity pepper
NC	0 f	0 f	0 f	0 f
FOC (control)	38.3 a	25 c	0.44 a	0.23 b
G. i	0 f	0 f	0 f	0 f
FOC and *G.i*	28 b	19.6 d	0.17 bcd	0.19 bc
FOC and *T.h*	23 c	14.6 e	0.13 cd	0.14 cd
T.h	0 f	0 f	0 e	0 e
T.h, FOC and *G.i*	21 c	12.6 e	0.12 cd	0.11 e

T.h (T.harzianum) G. i(*G. intraradices*)NC (Negative Control)

Means are followed with different letter are significantly different(P<0.05) according to Duncan test.

***G. intraradices* root colonization , plant root and shoot dry weight:**

Growth parameters of Pepper plants grown in soils inoculated with G. *intraradices* and *T. harzianum* were variably affected when examined after 8 weeks (Table2) . Plants grown in substrates inoculated with *G. intraradices* were successfully colonized (66 and 68.8%) for California Wonder and Local verity pepper respectively. Dual inoculation of *T.harzianum* and *G. intraradices* under infection condition significantly reduced the level of root colonization by *G. intraradices* for California Wonder and Local verity pepper(61.3 and 58.7%) respectively, treatment involving FOC and *G. intraradices* associated with significant reduced in the level of root colonization by *G. intraradices* for California Wonder and Local verity pepper respectively (52.3 and 46.4 %).The mean dry shoot weight of Pepper plants was in soils inoculated with *G. intraradices* (63.6 and 50.9 g/plant) for California Wonder and Local verity pepper respectively , *T.harzianum* were significantly different to that associated with FOC and the control (Table 2). Dual inoculation, as well as treatment involving *T.harzianum* only, produced significant increasing in root dry weights (42.3,45.6 and 39.3,42.6g/plant) for California Wonder and Local verity pepper respectively, whereas the mean height of plants root dry weights inoculated with *G. intraradices* (56.6 and 47.9g/plant) for California Wonder and Local verity pepperrespectively.

Effect of plant root exudates on spore germination:

The effect of 50% concentration of 20-day-old root exudates of pepper on microconidia germination (%) of FOC was tested in CDL media (Figure 1). the uppermost spore germination was noted with *G. intraradices* (91, 85.5%) for California Wonder and Local verity pepper respectively inoculation with *T.harzianum* only caused lowest significant spore germination

(33,30.1%) for California Wonder and Local verity pepper respectively Overall, *T.harzianum* and *G. intraradices* caused lowest significant spore germination in comparison with the control treatment.

Table 2: Percentage pepper root colonization by *G. intraradices* , Shoot dry weight and root dry weight of pepper plants inoculated with *G. intraradices* and *T.harzianum* .

Treatment	Root colonization (%)		Dry shoot weight (g/plant)		Dry root weight (g/plant)	
	California Wonder	Local verity pepper	California Wonder	Local verity pepper	California Wonder	Local verity pepper
NC	0.0 e	0.0 e	53.0 b	42.4 b	47.0 b	35.4 b
FOC (control)	0.0 e	0.0 e	36.0c	28.8c	30.0c	21.8c
G.i	66.0a	68.8 a	63.6a	50.9a	56.6a	47.9 a
FOC and *G.i*	52.3cd	46.4 d	59.3a	47.4 a	52.3a	45.4 a
FOC and *T.h*	0.0 e	0.0 e	52.4 b	46.9 b	43.3b	40.9 b
T.h	0.0 e	0.0 e	51.3b	41.1b	42.3b	39.3b
T.h, FOC and *G.i*	61.3 b	58.7 c	55.3 b	44.5 b	45.6b	42.6 b

T.h (T.harzianum) G. i(*G. intraradices*)NC (Negative Control)
Means are followed with different letter are significantly different(P<0.05) according to Duncan test.

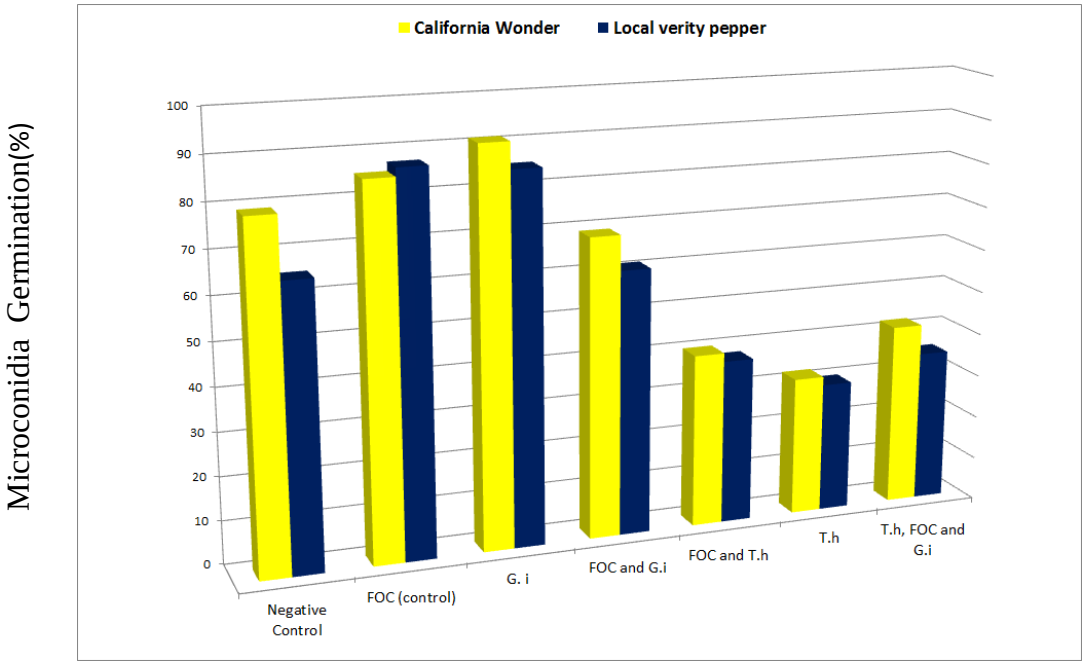

Fig. 1 Effect of root exudates from pepper plants on microconidia germination of *Fusarium oxysporum* f. sp. *capsici.*

Effect of *Trichoderma* and *G. intraradices* strain on total phenols, chlorophyll and Carbohydrate %:

The amount of total phenolic compounds was higher in *T.harzianum &G. intraradices* inoculated plants (114.3, 156.2 µg g^{-1}) for California Wonder and Local verity pepper respectively *G. intraradices* inoculations provided the highest increase in the amount of total phenolic compounds when compared with negative control ,significant differences were observed for dual inoculation with *G. intraradices* and *T.harzianum* when compared with negative control. *T.harzianum* and *G. intraradices* not only suppressed Fusarium wilt but also significantly

promoted chlorophyll contents and % Carbohydrate in Pepper plants . Dual inoculation with *G. intraradices* and *T.harzianum* caused the highest amount of chlorophyll contents (32.3 ,31.5 mg/g fresh weight) for California Wonder and Local verity pepper respectively. While overall , *T.harzianum* and *G. intraradices* has recorded significant increasing in % Carbohydrate in comparison with the control treatment (Table3).

Table 3: Total phenol , Chlorophyll a & b and %Carbohydrate in pepper plants inoculated with *G. intraradices* and *T.harzianum* .

Treatment	Total phenols µg g^{-1} of fresh weight		Chlorophyll a & b (mg/g fresh eight)		%Carbohydrate	
	California Wonder	Local verity pepper	Califor nia Wonder	Local verity pepper	Califor nia Wonder	Local verity pepper
NC	53.0 i	68.90 gh	29.9ab	32.2a	1.7ab	1.67a
FOC	63.0h	81.900 f	18.4e	20.55e	1.08e	1.19de
G.i	76.0 fg	98.8 de	27.7cd	25.3db	1.33cd	1.42bd
FOC and G.i	80.3 f	104.4 cd	23.7bc	26.4ac	1.39bd	1.53ac
FOC and *T.h*	91.3 e	118.7 b	28.3ac	31.7a	1.63ac	1.82a
T.h	112.3 bc	148.8 a	25.0bd	27.8ac	1.46bd	1.53ac
T.h, FOC and G.i	114.3 bc	156.2 a	32.3a	31.5a	1.66ac	1.78 a

T.h (T.harzianum) NC (Negative Control)

Means are followed with different letter are significantly different(p=0.05)according to Duncan test.

Discussion:

The results of this study Showed that *Fusarium oxysporum f.sp. capsici* are responsible for wilt disease on Pepper. *Fusarium oxysporum f.sp. capsici* are very common in field soil and all have been reported as causal agents wilt (Black and Rivelli 1990 , Moreno ,2002). *Trichoderma* have attracted much academic and commercial interest as bioprotective agents against fungal pathogens. The mode of action appears to be very complex indicating a possible mechanism such suppresses disease incidence and severity ,antibiotic production, mycoparasitism, the production of cell wall-degrading enzymes and competition for nutrients or space are considered as the actions involved in biocontrol of pathogen (Harman,2000, Harman *etal*,2004a and b).*Trichoderma* also prevention of fusaric acid (an important virulence factor) production. Pathogenicity factors are required by plant pathogens to cause wilt disease(Nosir et al ,2011). During direct contact, lectins in the host's cell wall can induce coiling of the *Trichoderma* around the host hyphae and mycoparasite can produce appressorium-like structures to destroy the pathogen (Zeilinger and Omann, 2007; Vinale *et al*., 2008)..Inoculation of *T. harzianum* either prior to pathogen infection or simultaneously with the pathogen was effective in controlling wilt disease of Pepper Many studies have reported that only a well-established AMF symbiosis could reduce damage caused by root pathogens suggesting that not only direct (fungus mediated) but also indirect (symbiosis-mediated) effects are responsible for the results. a few researchers have reported that the simultaneous addition of AMF with the pathogen could also reduce severity of some root diseases (*Martinez et al*.,2009 and *Martinez et al*.,2011).Observation has been illustrated the effectiveness of *G.mosseae* in controlling *Phytophthora parasitica* infection in tomato roots suggested that the activation of local as well as systemic mechanisms were responsible for the bioprotective effect (Pozo *et al*, 2002).AMF interactions are known to differ even with the strains of the same species of saprophytic fungi (Green *et al*., 1999; *Martinez et al*.,2004).In addition interestingly, combined applications of each PGPF plant growth promoting fungi with *G. mosseae* were more effective in controlling *R.solani* than the inoculation of each species alone. The enhanced capacity in protecting plants achieved by co-inoculation could be due to combination of various mechanisms such as competition for host photosynthates and / or colonization sites, altered root exudations, anatomical and morphological changes in the root system, antibiosis and induced plant defense

systems activated by both the endophyte and saprophyte. Previous study, revealed that PGPF used in this study do not produce antibiotics .The level of colonization of PGPF plant growth promoting fungi isolates as shown not always to link the levels of disease protection (Hyakumachi and Kubota, 2004). Thus interaction of AMF and PGPF on activation of defense systems could be essential for the enhanced protection levels. (Chandanie *et al*,2009).The altered effect of root exudates from mycorrhizal plants on Fol microconidia germination which have been observed could be due to: 1 the presence of new compounds in root exudates stimulatory for microconidia germination, 2 enhanced levels of compounds stimulatory for microconidia germination, or 3 reduced levels of inhibitory compounds in addition to an overall stimulatory effect of the root exudates.(Scheffknecht *et al*,2009) .Chlorosis of plant tissue is a common visible symptoms following infection with phytopathogenic fungi. It may result as a consequence of either a (photo oxidative destruction of existing pigments, or b) inhibition of pigment synthesis. It is possible that the effect of the phytopathogenic fungi on chlorophyll and carotenoids is an attenuation of the biosynthetic rate rather than a breakdown of pigments already formed. Adverse effect of fungal pathogen on chlorophyll pigments may be due to the fact that the fungal toxins form iron-chelate, transforming iron to become unavailable to participate in chlorophyll synthesis. Treatment with the biocontrol agents *Trichoderma* appeared to stimulate chlorophyll synthesis or at least eliminate the adverse effect of the phytopathogens on pigment formation (Ibraheem,2009) .Toxic metabolites of the pathogen may activate phenol - oxidizing enzymes causing high accumulation of phenol .The phenol-oxidizing enzyme plays a vital role in tissue browning by way of its capacity to oxidize phenols to quinines, which increase host resistance against the invading pathogen (Brunner ,et al 2005).

References:

Akkopru A and Demir S (2005) Biological control of Fusarium wilt in tomato caused by Fusarium oxysporum f. sp. lycopersici by AMF Glomus intraradices and some rhizobacteria. J. Phytopathol 153:544–550

Bi, H.H., Song, Y.Y., Zeng, R.S., 2007. Biochemical and molecular responses of host plants to mycorrhizal infection and their roles in plant defence. Allelopathy J.20, 15–27.

Black, L.L and V. Rivelli (1990). Fusarium wilt of pepper in lousiana. Pageuuin: proc. 101 t1 Natl. Pepper conference, 25-27 June.

Brunner K; S. Zeilinger; R. Ciliento; L.S. Woo; M. Lorito; C.R. Kuicek and R.L. Mack (2005): Improvement of fungal iocontrol agent *Trichoderma atroviride* to enhance oth antagonism and induction of plant systemic disease resistance. Appl. Environ. Microiol. 7: 3959-3969.

Chandanie , W.A; , M. Kubota and M. Hyakumachi (2009). Interactions between the arbuscular mycorrhizal fungus Glomus mosseae and plant growth-promoting fungi and their significance for enhancing plant growth and suppressing damping-off of cucumber (*Cucumis sativus* L.). Applied Soil Ecology ,41 : 336–341.

Elad, Y., 2000. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Prot. 19, 709–714.

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots.New Phytol84:489–500.

Green, H., Larsen, J., Olsson, P.A., Jensen, D.F., Jakobsen, I., 1999. Suppression of the biocontrol agent Trichoderma harzianum by mycelium of the arbuscular mycorrhizal fungus Glomus intraradices in root-free soil. Appl. Environ. Microb. 65,1428–1434.

Harman , G . E .(2000) . Myths and dogmas of biocontrol change in perceptions derived from research on *Trichoderma harzianum* T22 . Plant Dis. 84: 377 -393 .

Harman , G . E.; C.R. Howell ; A.Viterbo ; I. Chet and M. Lorito (2004a). *Trichoderma* species-opportunistic virulent plant symbionts. Nature Reviews 2:43-56.

Harman, G. E.; R. Petzoldt ; A. Comis and J.Chen(2004 b). Interactions between *Trichoderma harzianum* strain T22 and maize inbred line Mo17and effects of these interactions on diseases caused by *Pythium ultimum* and *Colletotrichum graminicola.* Phytopathology 94:147-153.

Harrison, M.J., 1999. Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. Annu. Rev. Plant Phys. 50, 361–389.

Hervas, A; Linda and B,Jimenez-Diaz RM(1997):Influence of chickpea genotype and *Bacillus* sp on protection from Fusarium wilt by seed treatment with nonpathogenic *Fusarium oxysporum*. Eur J Plant Pathol 103:631–642.

Howell, C.R., (2003). Mechanisms employed by Trichoderma sp. in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis.87, 4–10.

Hyakumachi, M.; M.Kubota(2004). Fungi as plant growth promoter and disease suppressor. In: Arpra (Eds.), Fungal Biotechnology in Agricultural ,Food and Environmental Applications. Marcel Dekker, New York, pp. 101–110.

Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480.

Ibraheem, B.Y. (2009): Induced biotypes from the Fungus *Trichoderma* Types to Improve biocontrol and Enhancement Plant Growth Parameters. Ph.D. Thesis. College of Agriculture and Forestry, Mosul Univ., Iraq (In Arabic with English abstract).

Kamilova, F.;L.V. Kravchenko ; A.I. Shaposhnikov ;N. Makarova and B.Lugtenberg(2006). Effects of the tomato pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* and the biocontrol bacterium *Pseudomonas fluorescens* WCS365 on the composition of organic acids and sugars in tomato root exudate. – Mol. Plant-Microbe Interact. 19: 1121-1126.

Martinez,A.;M. bertello;A.Pardo;j.A. Ocampo and A., Godeas (2004). Interactions between Trichoderma pseudokoningii strains and the arbuscular mycorrhizal fungi Glomus mosseae and Gigaspora rosea. Mycorrhiza 14, 79–84.

Martinez-Medina, A., Pascual, J.A., Lloret, E., Roldن, A., (2009). Interactions between arbuscular mycorrhizal fungi and Trichoderma harzianum and their effects on Fusarium wilt in melon plants grown in seedlings nurseries. J. Sci. Food Agr. 89, 1843–1850

Martinez-Medina,A.; Antonio Roldan and Jose A. Pascual (2011).Interaction between arbuscular mycorrhizal fungi and *Trichoderma harzianum* under conventional and low input fertilization field condition in melon crops:Growth response and Fusarium wilt biocontrol. Applied Soil Ecology, 47: 98–105 .

Moreno, C.P. (2002). Identification of fuugi that cause ((pepper wilt)) in the Bagio Region. Proceedings of the 16th International pepper conference Tampico, Tamaulifas. Mexico, November 10-12-2002.

Nelson ,P.E.;T.A. Toussoun and W.f.O.Marassas (1983). Fusarium species an illustrated for identifcation Pennsylvania Satate University ,University Park and London .193 pp.

Nosir, W.; J. McDonald and S. Woodward (2011).Impact of biological control agents on fusaric acid secreted from *Fusarium oxysporum* f. sp. *gladioli* (Massey) Snyder and Hansen in *Gladiolus grandiflorus* corms.Journal of Industrial Microbiology & Biotechnology. l 38 :21-27.

Nzanzaa, B.; Diana Maraisa and Puffy Soundyb(2012).Yield and nutrient content of tomato (Solanum lycopersicum L.) as influenced by *Trichoderma harzianum* and *Glomus mosseae* inoculation Scientia Horticulturae. 144 : 55–59

Pozo, M.J.;C. Cordier ;E. Dumas-Gaudot; S. Gianinazzi ;J.M. Barea; and C.Azcon-Aguilar(2002).Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to Phytophthora infection in tomato plants. Journal of Experimental Botany 53, 525–534.

Rashmi Srivastava ; Abdul Khalid ;U.S. Singh and A.K.Sharma(2010).Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation

against *Fusarium oxysporum* f. sp. *Lycopersici* for the management of tomato wilt .Biological Control 53:24–31

Saldajeno, M.G.B.;W.A. Chandanie ;M.Kubota and M.Hyakumachi (2008). Effects of interactions of arbuscular mycorrhizal fungi and beneficial saprophytic mycoflora on plant growth and disease protection. In: Siddiqui, Z.A., Akhtar, M.S., Futai, K. (Eds.), Mycorrhizae: Sustainable Agriculture and Forestry. Springer Sciences, Netherlands, pp. 211–226.

Scheffknecht ,S; R. Mammerler ; S. Steinkellner and H. Vierheilig (2009).Root exudates of mycorrhizal tomato plants exhibit a different effect on microconidia germination of *Fusarium oxysporum* f. sp.*lycopersici* than root exudates from non-mycorrhizal tomato plants. Mycorrhiza .16: 365–370.

Shi ,Shengjing(2009).Influence of Root Exudates on Soil Microbial Diversity and Activity .Ph.D. thesis .Lincoln University.New Zealand 252pp

Smith, S.E.;D.J. Read and J.L.Harley(1997). Mycorrhizal Symbiosis, second ed. Academic Press, London.

Steinkellner, S.;R. Mammerler and H.Vierheilig (2005) Microconidia germination of the tomato pathogen Fusarium oxysporum in the presence of root exudates.J. Plant Interact. 1: 23-30.

Steinkellner , S .; R. Mammerler and , H. Vierheilig (2009).Root exudates as important factor in the Fusarium– host plant interaction. Multitrophic Interactions in Soil . 42:165-168.

Tchameni , S.N. ; M.E.L. Ngonkeu ; B.A.D. Begoude ; L. Wakam Nana ; R. Fokoma ; A.D. Owona ;J.B. Mbarga ; T. Tchana ; P.R. Tondje ; F.X. Etoa and J. Kuaté (2011) Effect of *Trichoderma asperellum* and arbuscular mycorrhizal fungi on cacao growth and resistance against black pod disease . Crop Protection. 30 : 1321-1327 .

Van Loon, L.C.;M.Rep and C.M.J. Pieterse(2006). Significance of inducible defense-related proteins in infected plants. Annual Review of Phytopathology 44, 7.1–7.28.

Vierheilig H, and Y. Piche (2002) Signalling in arbuscular mycorrhiza: facts and hypotheses. In: Buslig B., Manthey J. (eds) Flavonoids in cell function. Kluwer Academic /Plenum,New York, pp 23–39.

Vierheilig,H.(2004)Regulatory mechanisms during the plant- arbuscular mycorrhizal fungus interaction. Can J Bot 82:1166–1176

Vinale, F.,Sivasithamparam ;K., Ghisalberti, E.L.; Marra, R., Barbetti; M.J., Li ; H. Woo and S.L. Lorito (2008).A novel role for *Trichoderma* secondary metabolites in the interactions with plants. Physiol. Mol. Plant Pathol. 72:80-86.

Zieslin, N. and R.Ben-Zaken (1993). Peroxidase activity and presence of phenolic substrate in peduncles of rose flower. Plant Physio.Biochem.31:333-339.

Zeilinger, S. and M. Omann (2007). *Trichoderma* biocontrol: signal transduction pathways involved in host sensing and mycoparasitism. Gene Regul. Syst. Biol. 1,227-234.