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MANAGEMENT OF TOMATO DAMPING-OFF USING NATURAL PLANT EXTRACTS, *Trichoderma harzianum* AND SELECTED FUNGICIDES IN PENJWEEN, SULAIMANI GOVERNORATE, KURDISTAN, IRAQ

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ABSTRACT

Tomato seedlings were found epidemically infected with damping-off disease at four locations (Kanimasyan, Kanishaban, Kanisev and Qzlja), in Penjween, Sulaimani Governorate, Kurdistan, Iraq during May 2011. The highest occurrence of disease incidence was observed in Kanimasyan (56%). *Fusarium oxysporum* (Schlecht), *F. solani* (Mart.) Sacc., *F. acuminatum* (Ell. and Ev.), *F. equiseti* (Corda) Sacc., *F. compactum*, *Rhizoctonia solani* (Kuhn), *Macrophomina phaseolina* (Tassi) Goid., *Phoma lycopersici* (Kleb), and *Cephalosporium* sp. were isolated from the infected plants and proved pathogenicity on tomato seeds (cv. Super Queen). *Fusarium oxysporum* and *R. solani* showed the highest level of seedling damping-off, with largest effect on decreasing the quality of seedling's vigor. Extracts of *H. triquetrifolium*, *E. billardieri* and *C. rotundus* at (20, 30, 40, 45 mg ml^{-1}) showed inhibitory effect on the mycelial growth of all pathogenic fungi. *In vitro* plant extract of *C. rotundus* revealed the highest inhibitory effects on fungal growth at 45 mg ml^{-1}. Fungicide of Keenol at 50 ppm proved to be chemostatic against all pathogens. *T. harzianum* appeared to be an important antagonistic agent against mycelial growth. Management of the disease was carried out in greenhouse using (*H. triquetrifolium,* Vitavax 200, *T. harzianum* and Keenol). It was found that the combination of (*H. triquetrifolium* + keenol) and (*T. harzianum* + *H. triquetrifolium*) showed impressive effects on plant height, dry and fresh weight when compared to the other treatments and control.

Key words: Damping-off, Tomato plant, Management, Plant extracts, Fungicides, Bio-agent

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill), is one of the most important vegetable crops in all over the world, because of its high nutritive value, an excellent source of vitamin A and it plays a significant role in maintaining the human health being a rich source of lycopene. The tomato has been used in the treatment of cancer (Giovannucci, 1999), it is economically attractive, and the area under cultivation is increasing daily. It is an important vegetable crop with an estimated global production of over 120 million metric tons and Iraq production of 913493 tons (F.A.O, 2007). Among the main constraints of tomato production is the damage caused by pathogens, including viruses, bacteria, nematodes and fungi, which result in severe losses in production (Barone & Frusciante, 2007).

Tomato plants are subjected to attack by several soil-borne fungal pathogens of *Fusarium*, *Rhizoctonia*, *Pythium* and *Phytophthora*, causing serious diseases such as root rot and wilt (Montealegre *et al*., 2003 and Srinon *et al*., 2006). *F. oxysporum* is regarded as a widespread soilborne plant pathogen, causing a vascular wilting and crown and root rot (Ozbay & Steven, 2004).

Plant-derived natural substances are non-phytotoxic compounds and potentially effective against plant pathogenic fungi. These plant products have an ability to inhibit soil borne pathogens that are environmentally potential safe alternatives and as components in integrated pest management programs (Bowers & Locke, 2004). Conventional chemical methods of disease management are expensive and unsuitable for the environment, also are not entirely efficient and may lead to

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the appearance of new resistant strains of phytopathogens (Bruin & Edgington, 1981).

Recently, biological control offers suitable alternatives for plant disease management (Wiest *et al*., 2002). Many isolates of *Trichoderma* spp. can control plant pathogens through secretion of antifungal compounds or parasitism (Bae & Knudsen, 2007).

The objectives of this study are the isolation of fungi from infected tomato seedlings from Penjween district, Sulaimani Governorate, Kurdistan, Iraq. Moreover studying of their pathogenicity, effects of plant extracts (*Hypericum triquetrifolium*, *Eryngium billardieri* and *Cyperus rotundus*) on the fungal growth (*in vitro*) and management of tomato damping-off using the integral of plant extracts, biological control using *T. harzianum* and selected fungicides.

MATERIALS AND METHODS

Field survey and sampling

Seedlings of tomato (CV. Super Queen), showing damping-off symptoms were collected during May 2011 from (Kanimasyan, Kanishaban, Kanisev and Qzlja) Penjween in Suliamani. Three samples (100 selected seedlings for each) were randomly taken at each location for assessment of the disease incidence (Riaz *et al*., 2010) depending on the following formula:

% Disease incidence = No. of infected seedlings / Total No. of seedlings*100

Isolation and identification of the causal agents

Fifty seedling roots of tomato (CV. Super Queen) that had the characteristic damping-off symptoms were washed with tap water for 3 hrs. Before cutting into smaller species 0.5-1 cm, the root tissues were surface sterilized by immersion in 1% NaOCl for 2 min, followed by two rinses with sterile water, then blotted on sterile filter paper and transformed to Potato Dextrose Agar (PDA) with 0.5 mg/ ml Streptomycin sulfate. The pure cultures were grown at 25 ± 2ºC for seven days and stored on a PDA slant at 4ºC (Baxter & Vander Linde, 1999). The cultivated fungi on PDA were purified using the hyphal-tip method to obtain pure cultures then kept in the refrigerator at 4ºC for further studies. All genera of fungi were identified depending on (Barnett & Hunter, 1972). For the species identification, the samples prepared and sent to Dr. Khalid Hassan Taha (College of Agriculture and Forestry, Plant Protection Dept., University of Mosul, Mosul, Iraq).

The following formula used for counting the percentage of fungi (Krebs, 1978):

% Fungi frequency = No. of the colonies (for each fungus) / Total No. of all Fungal colonies * 100

Pathogenicity trial

Isolation of *Macrophomina phaseolina, Rhizoctonia* solani, *Fusarium* spp., *Phoma lycopersici* and *Cephalosporium* sp. were propagated on PDA at 25°C, pots (15cm) contained 3 kg sterilized soil, inoculated by 200 ml spore suspension for each fungal isolate. After 48 hrs, ten seeds which were surface- sterilized with 1% NaOCl were grown and maintained in the greenhouse (25-30°C) for 30 days. Pre and Post-emergence damping-off of seedlings were recorded in addition to measurements of plant vigor. Reduction of seedling height was calculated according to the following equation (Heather *et al*., 1977):

% Inhibition = Plant height in control – Plant height in treatment / Plant height in control* 100

Preparation of plant extracts

Mature plants of *Hypericum triquetrifolium*, *Eryngium billardieri* and *Cyperus, rotundus* collected in July 2010 from different areas in Sulaimani governorate including (Baziyan and Ziewi). A selection of these plants that were chosen based on their importance in folk medicine and anti-microbial activity (Ahmed, 2007; Boskani, 2008). Plant samples formerly identified in Herbarium Section (College of Agriculture, University of Baghdad, Bagdad, Iraq). Plants were washed out of soil particles and spread onto the cardboard carton in the shade to air dry indoors at an ambient temperature (18-25°C) (Subhan *et al.*, 2009). The *Eryngium* roots of the plants were washed out well with tap water before furnishing and drying and then cut into small pieces to facilitate the process of crushing, and later preserved at 4ºC.

Alcoholic extraction

One hundred grams of powder samples from *H. triquetrifolium*, roots of *E. billardieri*, and rhizomes of *C. rotundus* put in a thimble inside a soxhlet for continuous extraction using 500 ml ethanol 70-80% in 4-6 hrs at 45-50ºC depending on the species. The extracts were concentrated using Rotary Vacuum Evaporator at 45ºC. The gummy residue of the total plant extracts was weighed and kept in a refrigerator (4ºC) for later use (Boskani, 2008).

The effect of plant extracts on the pathogenic fungal growth and *T. harzianum*

Four concentrations of the whole plant extract (20, 30, 40, and 45 mg ml^{-1}) were prepared by adding 2, 3, 4 and 4.5 g of each plant gummy extract to 100 ml of culture media. The cultures were inoculated in their centers with 5 mm of mycelial plugs of *F. solani*, *F. oxysporum*, *F. acuminatum* and *R. solani* each in addition to *T. harzianum* and incubated at 25±2º C for seven days.

The treatment repeated four times (4 dishes) for each fungus and plant extract and smilar numbers were maintained without plant extracts as control. Mycelial growth Inhibition rate were measured according to (Smith *et al.,* 1991) as follows:

$$\% \text{ MGI} = \text{DC-DT/DC x } 100$$

where: % MGI = percentage of mycelium growth inhibition, DC = diameter of the fungal colony in control and DT = diameter of the fungal colony in treatment.

The antagonistic effect of *T. harzianum* against isolated fungi

In vitro dual culture technique was depended on cultivating *T. harzianum*, *F. oxysporum*, *F. acuminatum*, *F. solani* and *R. salami* separately using PDA medium for seven days at 25±2º C.

Mycelial plugs (5 mm) of *T. harzianum* fungus was inoculated on the right half of the plates contained solidified PDA and 5mm mycelial discs in each of the pathogenic fungus with equal distances of 4 cm between the pathogen and the bio-agent. Numbers of plates was inoculated with each pathogenic fungus only as control. The plates were incubated at 25±2°C for seven days (Dennis & Webester, 1971; Kucuk & Kivance, 2004). Antagonistic effect of *T. harzianum* was recorded using the prementioned formula.

Effect of *T. Harzianum* filtrates on the mycelial growth of the pathogenic fungi

Sterilized liquid media of Potato Dextrose Broth (PDB) with Streptomycin sulfate (100 mg/L) was poured in 250 ml flasks and inoculated with 8 mm Mycelial plugs of 7 days old cultures for each pathogenic fungus. After incubation at 25±2°C for seven days, the flasks were shaked thoroughly and filtered using three layers via Whatman filter paper No. 1. The control (standard treatment) maintained without inoculation. PDA medium was prepared and after cooling (temp. 45°C) two concentrations (5 and 10%) of the filtered bio-agent was added. The flasks were gently shaken and poured into 8.5 cm Petri dishes then inoculated with the 4 mm fungal plugs of isolated fungi before incubating at 25±2°C for eight days to obtain a homogenized solution. The inhibitory effect of the toxicogenic extracted substances of *T. harzianum* fungal growth was calculated by measuring the diameter of colonies of the isolated fungi at each concentration. The measurements were taken in two planes at 90° to each other, and the average was calculated (Ali, 2007).

Effect of two fungicides on the mycelial growth of the pathogenic fungi and *T. harzianum*

Two fungicides of Vitavax200 (Carboxin 37.5% + Thiram37.5%) and Keenol (a.i. Console 50%) at 50, 100, 150 and 200 ppm a. i. of the fungicide 1.33 g of Vitavax200 and 2 ml of Keenol per 1 Litter PDA for each. Four replicates applied in each treatment and control contained distilled water. Plates were inoculated in their centers with 5mm of mycelial plugs and incubated at 25±2ºC for 8 days. The inhibition mycelial growth rate was measured by a formula mentioned by (Sunder *et al*., 1995) as follows:

$$\% \text{ MGI} = \text{X-Y / X* } 100$$

where: X = Growth of the fungal colony in control and the Y = Growth of the fungal colony in treatment.

Integrated management of tomato damping-off

Isolated *F. oxysporum*, *F. solani*, *F. acuminatum and R. solani* were propagated on PDA at 25±2°C. Pots (15cm) contained 3kg sterilized soil were inoculated with 200 ml spore suspension for each suspected fungus. The pots were watered, and after two days of resting and bio-activating of the fungi, four tomato seedlings/ pot – at 3-4 true leaves age – were planted. The treatments were as follows:

1. *H. triquetrifolium,* ethanol extract 30 g/pot
2. Vitavax 200, 2g/pot.
3. Keenol, 1ml/pot.
4. Bio-agent *T. harzianum* 100 ml "pot calculated spore suspension by haemocytometer $5x10^{-5}$ spores/ml with previously thoroughly mixed with the soil.
5. *T. harzianum*, 100 ml "pot + *H. triquetrifolium* extract 15g/pot.
6. *H. triquetrifolium* extract 15g + Vitavax 200 with 2g/pot.
7. Vitavax 200 2g + Keenol 2ml/pot.
8. Control: Included soil in a pot contaminated with each pathogenic fungus only). Each treatment repeated 4 times. The following parameters were measured in the experiment:
   a) Pre and Post emergence damping-off.
   b) Plant vigor's of seedling height, fresh and dry weight (Ismael and Salih, 2015).

Statistical analysis

For all lab. experiments CRD design and for green house experiment factorial CRD design has been applied with four replicates. Means compared according to the Duncan's multiple range test ($p \leq 0.05$). XLSTAT computer programming has been used for data analysis.

RESULTS AND DISCUSSION

Field survey

Tomato plants showed the distinctive symptoms of damping-off, the highest infection was prevailed in Kanimasyan compared to Kanishaban and Kanisev, respectively (Fig. 1). This may be due to conducive weather conditions in these areas and hence caused severe losses every year. Jiskani *et al*., (2007) explained the variation of associated soil borne diseases at different places. Reasons of the disease is due to an increase accumulation of Fungal inoculums fungi in the field because of repeated crops and the reduction of efficiency of pesticides used or neglected disease control in a timely manner, in addition to the ability of most types of *Fusarium* to recolonization in the soil of the field to be a source of infection in subsequent re-culture seasons (Haleem, 2001).

Frequency of isolated damping-off fungi

Damping-off fungi including *Fusarium oxysporum*, *F. solani*, *F. acuminatum*, *F. equiseti*, *F. compactum*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Phoma lycopersici and Cephalosporium* sp. Isolated from the four locations. The highest frequency of *R. solani* and *F. oxysporum* were isolated in Kanisev 36.66% and 28.33% respectively; these two pathogens were the most common fungi that were found in Kanimasyan 31.66% and 26.66%. *R. solani* known to attack seedling of several crops (Khan *et al*., 1973 and Abawi & Martin, 1985). The highest incidence of *R. solani* and *F. oxysporum* f. sp. *lycopersici* were showed damping-off symptoms (Moustafa & Khafagi, 1992; Jiskani *et al*., 2007 & Zaghloul *et al*., 2008). *Fusarium* wilt also considered one of the most significant and common disease of tomato in both fields and greenhouse (Amini & Sidovich, 2010). *R. solani* is a very common soil-borne pathogen, with global distribution and with a great diversity of host plants (Agrios, 1997). The highest frequency of *F. solani* was recorded in Kanisev (16.66%), while the lowest rate was recorded in Kanishaban (3.33%). Studies have pointed to the importance of fungus *F. solani* which cause damping-off, root rot and crown rot of many crops (Ikediugwu & Ejale, 1980). The highest frequency of *M. phaseolina* was appeared in Kanishaban (11.66%), *Fusarium* spp. adapt to live within range of a broad environmental and types can maintain to a large extent on crop residues as saprophytes and causing damping-off, to the next season plants (Gergis *et al*., 1992). The frequency of *Phoma lycopersici* was recorded in Kanimasyan and Kanishaban. It was found that *P. lycopersici* cause stem rot of tomato (Phillips, 1959). The highest frequency of *F. equiseti* was appeared in Qzlja (10%) (Table 1). The isolated fungi were *Fusarium* spp., *M. phaseolina* and *R. solani* recorded as the primary causes of the root diseases of vegetables (EL-Mougy *et al.*, 2011).

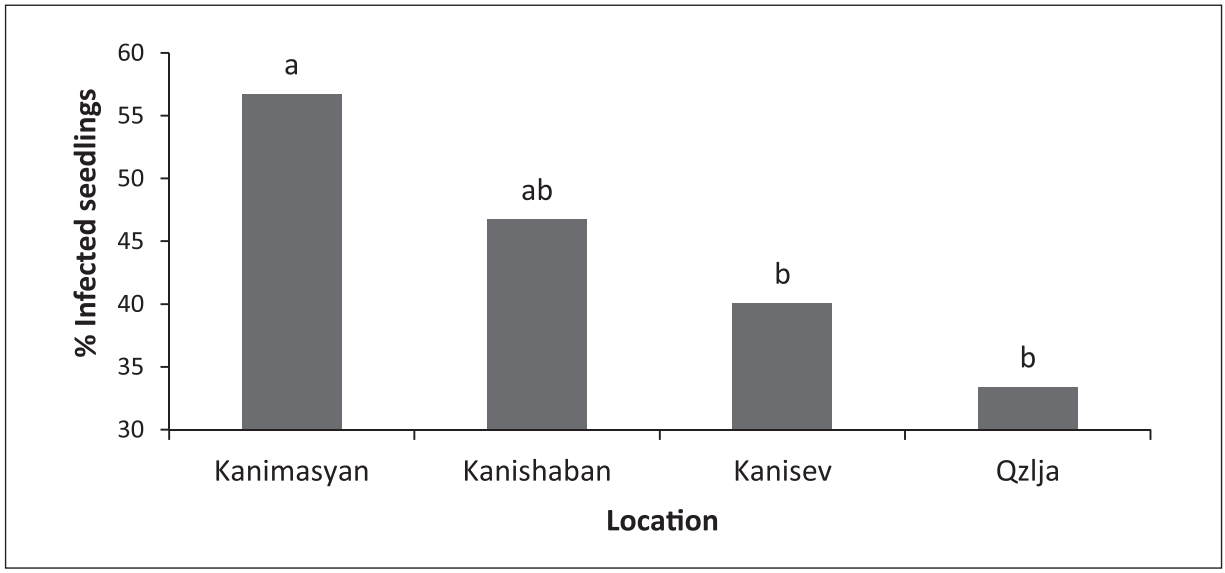

Fig. 1. Occurrence of a tomato seedling damping-off in Penjween district, Sulaimani governorate.

Table 1. Isolation and frequency of damping-off fungi from infected tomato seedlings (CV. Super Queen) from different locations in Penjween district, Sulaimani governorate

Fungi	Locations			
	Kanimasyan	Kanishaban	Kanisev	Qzlja
Rhizoctonia solani	31.66 a*	26.66 ab	36.66 a	30.00 a
Fusarium oxysporum	26.66 a	28.33 a	28.33 ab	25.00 ab
F. solani	3.33 b	13.33 bc	16.66 bc	6.66 bc
Macrophomina phaseolina	3.33 b	11.66 c	3.33 c	6.66 bc
F. compactum	5.00 b	3.33 c	5.00 c	6.66 bc
F. acuminatum	3.33 b	8.33 c	10.00 c	16.66 abc
Phoma lycopersici	3.33 b	3.33 c	0.00 c	0.00 c
F. equiseti	8.33 b	6.66 c	5.00 c	10.00 c
Cephalosporium sp.	1.66 b	1.66 c	0.00 c	0.00 c

*Data are means of four replicates, means followed by same letter within columns are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test.

Pathogenicity trial

All isolated fungi except *Cephalosporium* sp. were caused pre- and post- emergence damping-off. In Mortality rate of the tomato seedlings by *F. oxysporum* and *R. solani* were 50-66.67% (Table 2). Pre-emerging and post-emergence damping off symptoms were appeared on young plants seeded directly and attacked the stem below and above the soil surface (Nawar, 2008). Seedling height was inhibited by 79% and 76.40% respectively. Inhibition of shoot weight was more than 80%. These results were in agreement with those obtained by Zaghloul *et al.*, 2008 who was found that *F. oxysporum* and *R. solani* caused the highest percentage of pre-emergence damping-off in tomato. However, biotic or abiotic factors in the soil cause excess of injuries that lead to increased plant respiration and consumption of carbohydrates of diseased plants and eventually to weakness and stunting symptoms, which reducing both of the fresh and dry weight (Garrett, 1970).

The total extract

The results of total extract in (Table 3) show the average weight of total gummy alcoholic extracts, which prepared from 100 g dry powders of each plant. The highest concentration was found in *E. billardieri* with 24.47 g while the lowest gummy extract found in *C. rotendus* (7.35 g); similar observations reported by (Ahmed, 2007).

Effect of two fungicides on mycelial inhibition of isolated fungi

As shown in (Table 4), five concentrations of two fungicides were tested in an experiment on the growth of *F. solani*, *F. oxysporum*, *F. acuminatum*,

Table 2. Pathogenicity test of fungi isolated from the roots and stems of tomato seedlings (CV. Super Queen) collected from Penjween district, Sulaimani governorate

Fungi	Diseases incidence		% of Inhibition of plant vigor				
	% of Pre-emergence	% of Post-emergence	Plant height	Fresh weight**		Dry weight	
				Shoot	Root	Shoot	Root
Fusarium oxysporum	66.66 a*	53.33 a	79.90 a	86.10 a	85.33 a	84.33 a	80.33 a
Rhizoctonia solani	50.00 ab	26.11 b	76.40 a	84.20 ab	79.20 c	80.50 b	77.00 b
F. acuminatum	40.00 b	19.04 b	73.00 a	81.20 abc	78.40 d	78.24 c	75.00 c
F. solani	33.33 b	24.99 b	71.66 a	80.00 abc	80.22 b	77.20 d	76.50 b
Macrophomina phaseolina	33.33 b	19.72 b	48.10 b	78.10 bc	77.32 e	74.80 e	73.86 cd
F. equiseti	33.33 b	15.86 b	40.31 b	75.31 c	76.00 f	73.26 f	73.00 d
F. compactum	30.00 b	13.64 b	30.00 c	65.40 d	63.20 g	60.50 g	58.30 e
Phoma lycopersci	6.66 c	12.72 b	15.00 d	63.25 d	60.70 h	60.20 g	57.00 e
Cephalosporium sp.	0.00 c	0.00 b	3.33 e	31.20 e	29.30 i	27.50 h	27.00 f
Control	0.00 c	0.00 b	0.00 e	0.00 f	0.00 j	0.00 i	0.00 g

*Data are means of four replicates; means followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test.
** fresh and dry weights = g/plant

Table 3. Weights of the gummy concentrated extracts from different plant species

Plant species	Organs*	Weight of gummy (g)
Hypericum triquterifolium	F+L+S+R	22.30
Eryngium billardieri	Root	24.47
Cyperus rotundus	Rhizomes	7.35

*F=Flower, L=Leaf, R=Root and S=Stem.

Table 4. Effect of two fungicides on mycelial inhibition of virulent isolated fungi

Fungicides	Fungi	Inhibition Fungicide	Percentage Concentration	Of Mycelial (ppm)	Growth
		Control	50	100	150
Vitavax 200	*F. solani*	0.00 j	76.47 h	89.11 f	100.00 a
	F. oxysporum	0.00 j	75.29 i	91.17 d	100.00 a
	F. acuminatum	0.00 j	90.58 e	93.81 b	100.00 a
	R. solani	0.00 j	80.14 g	92.90 c	100.00 a
Keenol	*F. solani*	0.00 j	100.00 a	100.00 a	100.00 a
	F. oxysporum	0.00 j	100.00 a	100.00 a	100.00 a
	F. acuminatum	0.00 j	100.00 a	100.00 a	100.00 a
	R. solani	0.00 j	100.00 a	100.00 a	100.00 a

*Data are means of four replicates; means followed by the same letter within the treatments are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test.

R. solani. Fungicide Keenol (a.i. Chinosol 50%) showed inhibitory effects on all tested fungi at 50 ppm dosage) compared to Vitavax200 (a.i. Carboxin +Thiram) when got the same results at 150 ppm. The efficiency of Keenol contributes to the active integration of Chinosol with heavy elements and then configures the complexes which are difficult to be absorbed by the pathogen (Al-Jburi, 2002). Shekhani (2008) & Ali (2007) reported the positive effects of Chinosol on inhibition of *R. solani*, *F. oxysporum*, *F. solani* and *P. lingam* at 50ppm. Al-Adil & Abid, 1979 illustrated the mechanism of Carboxin action on such sensitive fungi and absorbing a large amount of the fungicide quickly by the fungi, but non-sensitive fungi as *F. oxysporum* consumed tiny quantities of the fungicide at a slow rate. (Sultana & Ghaffer, 2010) using concentrations (10, 20, 50,100, 500, 1000) ppm Vitavax 200 gave an inhibition rate (36.4, 68.7, 89.4 and 94.4% respectively) of *F. solani* in cucumber.

Effect of different plant extracts on the inhibition of isolated fungi

The higher mycelial growth inhibition achieved for *F. solani*, *F. oxysporum*, *F. acuminatum* and *R. Salami* when *Cyperus rotundus* extract used at the concentration 45 mg ml^{-1} while the lowest growth inhibition showed at 20 mg ml^{-1} of *C. rotundus* (Table 5). The phytochemical investigation of *C. Rotundas* rhizome has revealed the presence of polyphenols, flavonols, glycosides, alkaloids, saponins, sesquiterpenoids and essential oils (Sharma and Singh, 2011a). The mode of action of essential oils is to disrupt the cell membranes of the plant pathogens (Cowan, 1999). This result was in agreement with (Al-Ani *et al.*, 2003) which proved that ethanolic extract of the tubers of *C. rotundus* gave significant mycelial inhibition against *Fusarium* spp. at 5% and 10% concentrations. There was a significant effect of the *E. billardieri* extract on the growth *F. solani*, *F. oxysporum*, *F. acuminatum* and *R. solani*, the maximum mycelial growth inhibition percentage of the *E. billardieri* at 45 mg ml^{-1}, while the lowest mycelial growth inhibition of *E. billardieri* at 20 mg ml^{-1}. *E. billardieri* contains many secondary metabolites, the a.i. are saponins. Its roots are very rich in these substances, alkaloids, phenols, glycosides and coumarin (Hiller & Friedrich, 1974; Abou-Judah *et al.,* 2002; Sefidkon *et al.,* 2011 & Cardozo, 2004). The mechanism of action of saponin is reducing the surface tension, and this leads to tearing of the thin fungus cell wall, and then complete the process of inhibition (Karumi *et al*., 2004 & Chapagain *et al*., 2006). These results are agreed with observations

Table 5. Percentage of mycelial growth inhibition of some fungi influenced by different concentrations of plant extracts

Plant extracts	Conc. (mg ml^{-1})	Growth Inhibition		Percentage (GI%)	
		F. solani	*F. oxysporum*	*F. acuminatum*	*R. solani*
	0	0.00 L	0.00 l	0.00 j	0.00 h
H. triquetrifolium	20	44.70 k	52.35 k	50.29 i	39.70 g
	30	51.17 j	55.88 j	59.41 h	47.35 f
	40	58.23 i	58.23 i	62.64 g	49.41 f
	45	60.58 h	60.58 h	69.11 ef	53.52 f
	0	0.00 L	0.00 L	0.00 j	0.00 h
E. billarderi	20	61.46 h	62.93 g	67.05 f	68.23 e
	30	64.40 g	65.29 f	69.40 ef	72.05 de
	40	67.64 f	67.93 e	72.05 de	76.17 cd
	45	69.99 e	69.41 d	73.52 d	78.23 bcd
	0	0.00 L	0.00 L	0.00 j	0.00 h
C. rotundus	20	74.40 d	73.82 c	81.46 c	82.05 abc
	30	76.46 c	81.46 b	84.40 c	82.64 abc
	40	80.29 b	84.70 a	92.05 b	84.11 ab
	45	82.34 a	85.28 a	100.00 a	87.34 a

*Data are means of four replicates; means followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test.

reported by Boskani, 2008 who proved that ethanolic extract of the roots of *E. billardieri* gave a good inhibition percentage which was 90.7%.

Biological control using *Trichoderma harzianum* Rifai

Antagonistic tests of *T. harzianum* against damping-off pathogens

The application of *T. Harzianum* (*in vitro*) inhibited all of the pathogenic fungi, particularly when used against *F. solani*, *F. oxysporum*, *F. acuminatum*, *R. solani* (Fig. 2 and 3) since the mycelial growth of these pathogens was reduced by more than 60%. Similar results achieved by Ali, 2007 and El-Haidery, 2007. *Trichoderma* spp. parasite on a broad range of fungi, once the fungi come into contact, whenever to connect to the host can coil around it and form appressoria on the host surface.

Attachment is mediated by the binding of carbohydrates in the *Trichoderma* cell wall to locations on the target fungus. Another mechanism of the mode of action of the genus *Trichoderma* is producing several fungi-toxic cell-wall-degrading enzymes, and probably also a peptaibol antibiotic. These compounds with combined activities can result in parasitism of the target fungus and dissolution of the cell walls, at the sites of the appressoria, holes are produced in the target fungus that causes direct entry of *Trichoderma* hype into the lumen of the target fungus occurs (Harman *et al*., 2004).

Effect of *T. harzianum* filtrates on the mycelial growth of isolated fungi

The maximum mycelial growth inhibition was 71.32% for the fungus *R. solani*, while the minimum rate was 35.75% for *F. acuminatum* using 100 and 50 ppm of *T. harzianum* filtrates respectively (Table 6). The *Trichoderma* spp. excrete one or more of the following antibiotics (metabolites); Viridine, Trichodermine, pachybascine and Gliotoxins, which behave the strong role inhibiting of the pathogenic fungi (Papavizas, 1985). As well as some cell walls degrading enzymes such as chitinases, glucances that break down polysaccharides, chitins and glucanase, thereby destroying the cell wall integrity. These may also play the crucial role integrity before penetration. Possible mechanisms of antagonisms employed by *Trichoderma* spp. include, nutrient and niche competitions, antibiosis by producing volatile components and non-volatile antibiotics that are inhibitory against a broad range of soil borne fungi, as well as parasitism (Akrami *et al.,* 2011).

Inhibitory effects of different plant extract on *T. harzianum*

Different effects of plant extracts on the mycelial growth inhibition of *T. harzianum* showed in *C. rotundus* proved maximum inhibition rate in comparison with other plant extracts and was 80.58% at 45 mg ml^{-1} concentration (Table 7). The phytochemical investigation of *C. rotundus* rhizome has revealed the presence of polyphenol, flavonol

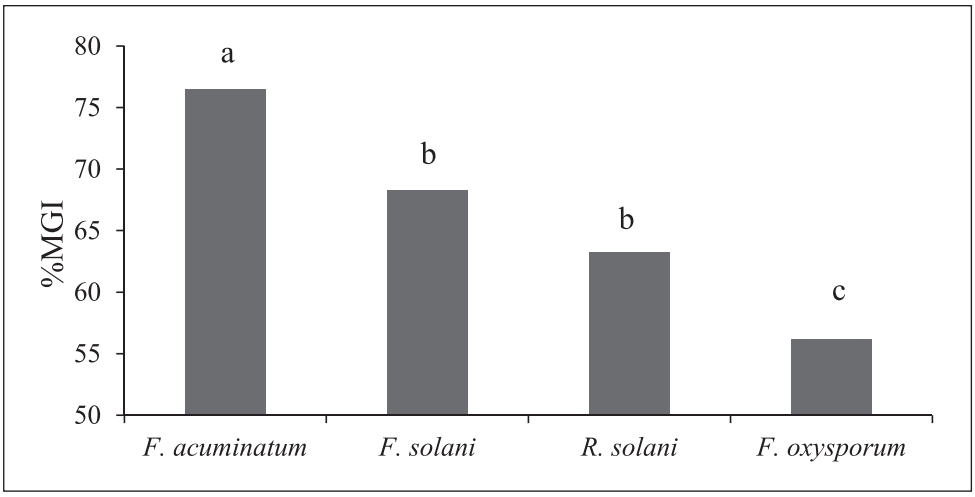

Fig. 2. The percentage of inhibition of *T. harzianum* against the isolated pathogenic fungi causing damping-off to tomato seedlings.

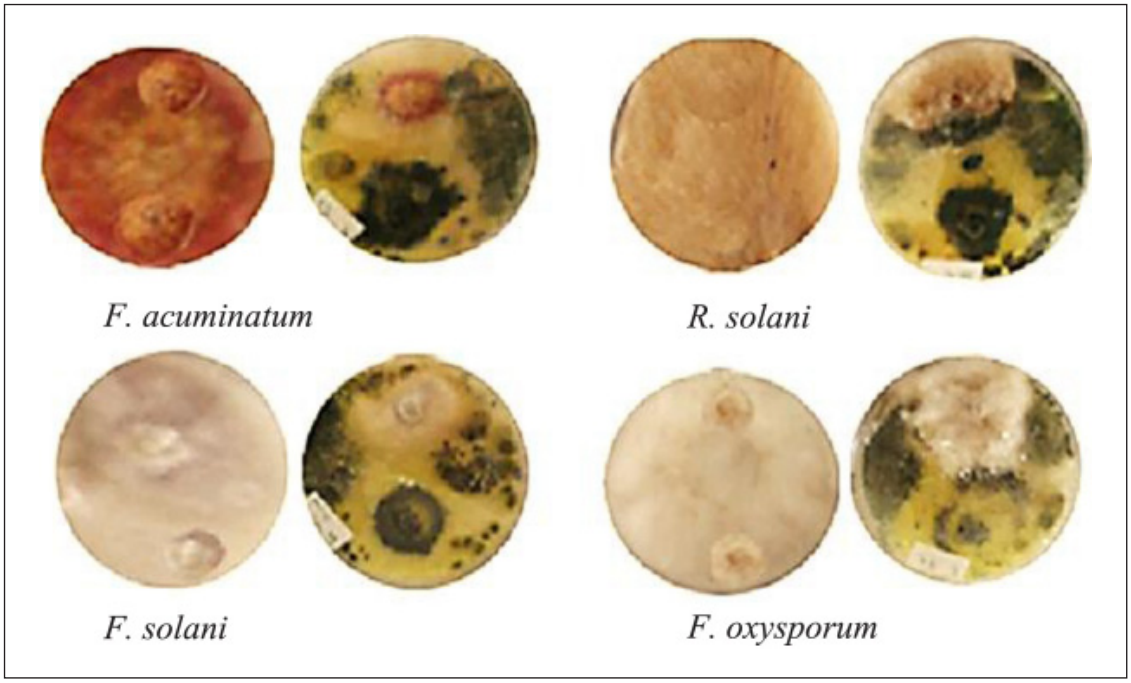

Fig. 3. Antagonistic activity of bio-agent *T. harzianum* against isolated fungi, (left) the fungus alone and (right) the fungus with the bio-agent.

Table 6. Effect of *T. harzianum* filtrate on the inhibition of mycelial growth of the isolated fungi

Fungi	Mycelial Growth Inhibition* (MGI%)	
	Control	50 ppm	100 ppm
F. solani	0.00 h	40.00 f	55.35 d
F. oxysporum	0.00 h	58.00 c	64.17 b
F. acuminatum	0.00 h	35.75 g	45.73 e
R. solani	0.00 h	65.50 b	71.32 a

*Data are means of four replicates; means followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test.

Table 7. The percentage of mycelium growth inhibition of *T. harzianum* influenced by different concentrations of plant extracts

Plant extracts	Concentration (mg ml^{-1})	MGI* (%)
H. triquetrifolium	0	0.00 h
	20	0.58 h
	30	1.76 h
	40	2.34 h
	45	4.70 g
E. billardieri	0	0.00 h
	20	59.70 f
	30	62.64 e
	40	76.46 b
	45	77.06 b
C. rotundus	0	0.00 h
	20	66.46 d
	30	72.05 c
	40	79.41 ab
	45	80.58.a

*Data are means of four replications; means followed by same letter within columns are not significantly different at (P≤0.05) according to Duncan's Multiple Range Test.

glycoside, alkaloid, saponins, sesquiterpenoids and essential oil (Trivedi *et al.,* 1984, Jeong *et al.,* 2000; Raut & Gaikwad, 2006; Sharma & Singh, 2011b). The mode of action of essential oils is to disrupt the cell membranes of the plant pathogens (Cowan, 1999). On the other hand, *H. triquetrifolium* have been recorded the minimum mycelial inhibition (4.7%) of *T. harzianum* even in the high concentration of the extract (45 mg ml^{-1}). Therefore, this plant extract could safely be used in the unified manager of the pathogenic fungi, because of less effect on the bio-agent and huge effect on the pathogenic fungi.

Effect of two selected fungicides on the fungal mycelial growth of *T. harzianum*

Two fungicides Vitavax 200 and Keenol were selected in this experiment, and their impact on the mycelial growth of the *T. harzianum* were examined (Table 8). All the concentrations of the fungicides (50,100,150 and 200 ppm) had the similar effect on the mycelial inhibition growth, because of the huge impact of these fungicides, it is impossible to use these two fungicides in the combination of the integrated control method (plant extracts + fungicide + bio-agent). Keenol (the active ingredient is Chinosol) link with heavy elements, then configures complex, which is difficult to be absorbed by the pathogen (Al-Jburi, 2002). Gowder *et al*. (2006) found the maximum inhibition rate (100%) when they used carbendazim 0.1 and 0.2%, followed by 96.88 and 88.44% inhibition of growth of *T. harzianum* with thiophanate methyl at concentrations 0.1 and 0.2% respectively.

Management of tomato seedling damping-off in the greenhouse

Treatments (*T. harzianum* + *H. triquetrifolium*) and (*H. triquetrifolium* + Keenol) were revealed a complete prevention of the disease incidence caused by *F. oxysporum*, *F. solani*, *F. acuminatum* and *R. solani* (Table 9). The tomato seedlings were health and vigor since plant height was 30cm after 50 days of planting compared to 12.5cm for the control. The combination between *H. triquetrifolium* and Keenol for *F. oxysporum* were resulted in the highest shoots and root weights (10.05 and 0.65 g) respectively, compared with (0.95 and 0.11g) for the control respectively. Similar results recorded for *F. solani*, *F. acuminatum* and *R. solani*. Application of the two combination treatments (*T. harzianum* + *H. triquetrifolium*) and (*H. triquetrifolium* + Keenol) gave the highest value for each parameter of each fungus mentioned above.

Some studies pointed to the efficacy of biological control with a half dose of recommended fungicide (Papavizas, 1985). The filtrate of *T. harzianum* is working on the inhibition of enzymes excreted by the pathogenic fungi for encouraging some of the material for plant growth (Dewan *et al.*, 1994 and Harman, 2000). Using bio-control agents also work on increasing the weights and heights of the plants (Chang *et al.*, 1986). Its ability to increase plant growth comes from the excreted materials for the growth of the organization which has a significant role in improving plant growth and

Table 8. Mycelial growth inhibition of *T. harzianum* using different concentrations of fungicides

Fungicides	Mycelial Growth Inhibition (MGI %)*			
	50 ppm	100 ppm	150 ppm	Mean
Vitavax 200	90.58c	94.11b	100.00 a	96.17b
Keenol	100.00a	100.00a	100.00 a	100.00a
Means	95.29b	97.05b	100.00 a	

*Data are means of four replicates, means followed by same letter within columns are not significantly different at (P≤0.05) according to Duncan's Multiple Range Test.

Table 9. Effect of integral treatments on the occurrence of tomato seedling damping-off and plant vigor

Treatments	*F. oxysporum*				*F. solani*				*F. acuminatum*				*R. solani*			
	% Damping-off	Plant height (cm)	Plant vigor		% Damping-off	Plant height (cm)	Plant vigor		% Damping-off	Plant height (cm)	Plant vigor		% Damping-off	Plant height (cm)	Plant vigor	
			Shoot (g)	Root (g)			Shoot (g)	Root (g)			Shoot (g)	Root (g)			Shoot (g)	Root (g)
H. triquetrifolium	37.50 b	18.38 d	1.91 g	0.46 c	32.50 b	18.25d	1.82 e	0.4 d	35.00 b	18.5 e	1.96ef	0.34 d	30.00b	18.50c	2.35 e	0.38 c
Vitavax 200	25.00 c	18.00 d	3.70 d	0.34 d	17.50 c	17.50 e	2.37 d	0.3 f	22.50 c	17.5 f	2.98 d	0.32 d	12.50 c	18.50c	2.64 d	0.32de
Keenol	22.00 c	22.25 b	4.87 c	0.53 b	15.00 c	21.25 c	4.04 c	0.5 c	20.00 c	20.0 d	3.93 b	0.25 e	20.00 c	20.00b	3.00 c	0.38 c
T. harzianum	12.00 d	21.00 c	2.57 f	0.25 e	10.00 c	21.5 bc	2.21 d	0.4 d	15.00 c	21.3 c	2.07 e	0.42c	12.50 c	20.50b	2.60de	0.36cd
T. harzianum + *H. triquetrifolium*	0.00 e	30.25 a	9.85 b	0.64 a	0.00 d	30.00 a	9.86 b	0.7 a	0.00 d	30.5 a	10.85a	0.69 a	0.00 d	30.25a	10.70a	0.67 a
H. triquetrifolium + Vitavax 200	10.00 d	21.00 c	2.71 e	0.28 e	10.00 c	22.00 b	2.23 d	0.4 e	15.00 c	22.0 b	3.22 c	0.46 b	15.00 c	20.50b	3.29 b	0.44 b
H. triquetrifolium + Keenol	0.00 e	30.00 a	10.05 a	0.65 a	0.00 d	30.50 a	10.5 a	0.64 b	0.00 d	30.8 a	10.9 a	0.72 a	0.00 d	30.50a	10.81a	0.70 a
Control	77.50 e	12.50 e	0.950 h	0.11 f	82.50 a	15.750 f	1.64 f	0.22 g	77.50 a	16.75g	1.80 f	0.21 f	77.50 a	17.50d	1.70 f	0.32 e

Data are means of four replicates; means followed by the same letter within a column are not significantly different at ($P \leq 0.05$) according to Duncan's Multiple Range Test.

increasing the productivity or anti-materials that reduce the harmful growth of the pathogen (Windham *et al*., 1986 and Dewan *et al.,* 1994).

The selected plant, are well known as a folk medicine for their highest health values as they contain phenolic compounds, for example, *H. triquetrifolium* provides Hypericin (Apaydin *et al*., 1999). This plant usually known as antibacterial and antifungal potent. Furthermore, a combination of plant extracts alone or with a half dose of fungicides, though the overall purpose is to inhibit fungal growth. The increased global chemophobia and reduced efficiency of chemicals due to pathogen resistant strains have forced producers to evaluate the contents of sustainable agriculture (Conforti *et al*., 2002). *Trichoderma* produces a plethora of secondary metabolites with its bio-activity, including in this antibiotic group, which are natural products able to inhibit microbial growth (Vinalea *et al*., 2008).

CONCLUSION

The highest occurrence of damping-off of tomato was in Kanimasyan and caused by *F. solani*, *F. oxysporum*, *F. equiseti*, *F. acuminatum*, *F. compactum*, *R. solani*, *M. phaseolina* and *P. lycopercisi*. The most virulent isolates of fungi were *R. solani* and *F. oxysporum*. The fungicide – Keenol- was completely inhibited tested fungi, extracts of *C. rotundus* resulted in a high mycelial growth of the pathogens. The combinations (*H. triquetrifolium* + Keenol) and (*T. harzianum* + *H. triquetrifolium*) were the best integrated management of the damping-off pathogenic fungi; *R. solani*, *F. oxysporum*, *F. solani and F. acuminatum* since the infected tomato seedlings was 0.00%.

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REFERENCES

Abawi, G.S. and Martin, S.B. 1985. *Rhizoctonia* foliar blight of cabbage in New York State. *Plant Disease* **69**: 158-161.

Abou-Jawdeh, Y., Sobh, H. and Salameh, A. 2002. Antimycotic activities of selected plant flora, growing wild in Lebanon against phytopathogenic fungi. *Journal of Agricultural and Food Chemistry* **50**: 3208-3213.

Agrios, G.N. 1997. Plant pathology. (4th edition) Academic Press, New York. 606 pp.

Ahmed, R.M. 2007. The inhibitory effect of some extracts of indigenous plant at some location in Iraqi Kurdistan on rice Pathogenic Fungi, M.Sc. thesis. Field Crops, University of Sulaimani.

Akrami, M., Golzary, H. and Ahmadzadeh, M. 2011. Evaluation of different combinations of *Trichoderma* species for controlling *Fusarium* rot of lentil. *African Journal of Biotechnology* **10(4)**: 2653-2658.

AL-Adil, K.M. and Abid, M.K. 1979. Pesticides in plant protection. Published by the University of Mosul, 397 pp. (In Arabic).

Al-Ani, Q.M., Adham, A. and Ahmed, M.T. 2003. Effect of some plant extracts on the growth of some pathogenic bacteria and fungi. *Anbar Journal of Agricultural Sciences* **1**: 8-14. (In Arabic).

Ali, R.M.S. 2007. Biological and protective studies of Damping -off and Root Rot Disease of Forest seedling in Sulaimani governorate Nurseries. M.Sc .Thesis, University of Sulaimani, 139 pp. (In Arabic)

AL-Jburi, H.H.S. 2002. Effect of inhibitor growth Culture and some plant extracts to infection of board bean plants with rot root causes. M.Sc. thesis, College of Agriculture, University of Baghdad. (In Arabic).

Amini, J. and Sidovich, D.F. 2010. The effects of fungicides on *Fusarium oxysporum* f. sp. *lycopersici* associated with *Fusarium* Wilt of Tomato, *Journal of Plant Protection Research* **50(2)**: 172-178.

Apaydin, S., Zeybek, U., Ince, I., Karamenderes, G., Ozturk, C. and Tuglular, B. 1999. *Hypericum triquetrifolium* Turra extract exhibits anti-nociceptive activity in the mouse. *Journal of Ethnopharmacology* **67(3)**: 307-312.

Bae, Y.S. and Knudsen, G.R. 2007. Effect of sclerotial distribution pattern of *Sclerotinia sclerotiorum* on biocontrol efficacy of *Trichoderma harzianum*. *Applied Soil Ecology* **3**: 150-158.

Barnett, H.L. and Hunter, B.B. 1972. Illustrated genera of imperfect fungi. (3rd edition). Burgess publishing company, 241 pp.

Barone A. and Frusciante, L. 2007. Molecular marker-assisted selection for resistance to pathogens in tomato, Marker-Assisted Selection, Current status and future perspectives in crops, livestock, forestry and fish. pp. 153-164.

Baxter, A.P. and Van der Linde, E. 1999. Collecting and preserving fungi. *Asafrinet Manual for Mycology*, 99 pp.

Boskani, J.H.S.I. 2008. The effect of some wild plant extracts in controlling chickpea *Ascochyta* Blight and *Fusarium* Wilting in Sulaimaniyah Governorate, Ph.D thesis, College of Agriculture, University of Baghdad. 92 pp. (In Arabic).

Bowers, J.H. and Locke, J.C. 2004. Effect of Formulated Plant Extracts and Oils on Population Density of *Phytophthora nicotinae* in soil and control of *Phytophthora* Blight in the greenhouse. *Plant Disease* **88**: 11-16.

Bruin, G.C.A. and Edgington, L.V. 1981. Adaptive resistance in Peronosporales to metalaxyl. *Canadian. Journal of Plant Pathology* **3**: 201-206.

Conforti, F., Statti, A., Tundis, R., Menichini, F. and Houghton, P. 2002. Antioxidant activity of ethanolic extract of *Hypericum triquetrifolium* Turra aerial part. *Fitoterapia* **73**: 479-483.

Cardozo, F. 2004. Composition of essential oil from the leaves of *Eryngium foetidum* L. from the Venezuelan Andes. *Journal of Essential Oil Research* **15**: 120-125.

Chang, Y.C., Baker, R., Kleifeld, O. and Chet, I. 1986. Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum.* Plant Disease **70**: 145-148.

Chapagain, B.P., Wiesman, Z. and Tsror, L. 2006. *In vitro* study of saponin-rich extracts against prevalent phytopathogenic fungi. *Industrial Crops and Products* **26**: 109-115.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews* **12(4)**: 564-582.

Dennis, C. and Webster, J. 1971. Antagonist properties of species group of *Trichoderma*. hyphal interaction. *Transactions of the British Mycological Society* **57**: 263-269.

Dewan, M.M., Ghisalberti, E.L., Rawland, C. and Sivasithamparam, K. 1994. Reduction of symptoms of take-all of wheat and Rye grass seedlings by the soil-borne fungus *Sordaria fenicola. Applied Soil Ecology* **1**: 45-51.

El-Haidery, A.A.J. 2007. Isolation and Identification some of the pathogens of Okra seed decay and damping-off and their control by different techniques of *Trichoderma harzianum* Rifai. M.Sc. thesis of University of Kufa, 122 pp.

El-Mougy, N.S., Abdel-Kader, M.M., Abdel-Kareem, F., Embabi, E.I., El-Mohamady, R. and Abed El-Khair H. 2011. Survey of fungal diseases affecting some vegetable crops and their rhizospheric soil borne microorganisms, *Research Journal of Agriculture and Biological Sciences* **7(2)**: 203-211.

F.A.O. 2007. FAO Stat, Corn production in 2005. Available online: http://faostat.fao.org/site/340/default.aspx.

Garret, S.D. 1970. Pathogenic root infecting fungi. Cambridge Univ. Press, 294 pp.

Gergis, M.M., Al-Ani, R.A. and Al-Hiti, A.A. 1992. Plant Diseases. Ministry of higher education and scientific research, University of Baghdad, College Agriculture. 1st edition, 504 pp.

Giovannucci, E. 1999. Tomatoes, tomato based products, lycopene and cancer. Review of the epidemiologic literature. *Journal National Cancer Institute* **91**: 317-331.

Gowder, S.B., Ramesh Babu, H.N., Nargund, V.B. and Krishnappa, M. 2006. Compatibility of fungicides with *Trichoderma harzianum. Agricultural Science Digest* **26(4)**: 279-281.

Haleem, R.A.J. 2001. *Fusarium* crown rot and root of tomato and their control in Dohuk governorate. M.Sc. thesis, College of Agriculture, University of Dohuk, 124 pp. (In Arabic).

Harman, G.E. 2000. Myths and dogmas of biocontrol changes and perception derived from research on *Trichoderma harzianum* strain T-22. *Plant Disease* **84(4)**: 377-393.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M. 2004. *Trichoderma* species opportunistic, avirulent plant symbionts. *Microbiology* **2**: 43-56.

Heather, W.A., Pratt, B.H. and Shin, T.Y. 1977. Pre- and post- emergence damping – off of seedlings of *Pinus* spp. by *Phytophthora cinnamoni* and *P. drechsleri*. *Australian Journal of Botany* **25**: 385-393.

Hiller, K. and Friedrich, M. 1974. The antimycotic effect of *Astrancia, Eryngium creticum* and *Sancula* saponins. *Pharmazie* **29(12)**: 787-788.

Ikediugwu, F.E.O. and Ejale, A.U. 1980. Root surface mycoflora cassava (*Monihot esculenta*) and postharvest rot of the tubers. *Mycopathologia* **71**: 67-71.

Ismael, J.H.S. and Salih, R.M. 2015. Integrated management of tomato damping-off disease in Bekrajo, Sulaimani Governorate, Kurdistan Region, Iraq, paper accepted for publication in; *Jordan Journal of Agricultural Sciences*.

Jayasuriya, H., Clark, A.M. and McChesney, D.J. 1991. New antibacterial filicinic acid derivatives from *Hypericum drummondii*. *Journal of Natural Products* **54**: 1314-1320.

Jeong, S.J., Miyamoto, T., Inagaki, M., Kim, Y.C., Higuchi, R. and Rotundines, A.C. 2000. Three novel sesquiterpene alkaloids from *Cyperus rotundus*. *Journal of Natural Products* **63**: 673-675.

Jiskani, M.M., Pathan, M.A., Wagan, K.H., Imran, M. and Abro, H. 2007. Studies on the control of tomato damping-off disease caused by *Rhizoctonia solani* Kuhn. *Pakistan Journal of Bot*any **39(7)**: 2749-2754.

Karumi, Y., Onyeyilli, P.A. and Ogugbuaja, V.O. 2004. Identification of active principles of *M. balsamina* (Balsam apple) leaf extract. *Journal of Medical Sci*ences **4(3)**: 179-182.

Krebs, C.J. 1978. Ecology: The Experimental Analysis of Distribution and Abundance. Harper and Row Publisher, NewYork, 678 pp.

Khan, M.W., Khan, A.M. and Saxena, S. 1973. The fungi and nematodes associated with roots of cauliflower. *Indian phytopathology* **26**: 303-309.

Knight, D.E. 1960. Studies on *Didimylla lycopersici* Kleb., the causal fungus of stem rots disease of tomatoes *Transaction of the British Mcological Society* **43**: 519-522.

Kucuk C. and Kivance, M. 2004. *In vitro* antifungal activity of strains of *Trichoderma harzianum*. *Turk. Journal Biol*ogy **28**: 111-115.

Montealegre, J.R., Reyes, R., Perez, L.M., Silva, R. P. and Besoain, X. 2003. Selection of bio-antagonistic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. *Electronic Journal Biotechnology* **6**: 115-127.

Moustafa, S.E.S. and Khafagi, Y.S. 1992. Reaction of certain tomato cultivars to *Fusarium* wilt and root rot diseases caused by *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani. Assiut Journal of Agricultural Sciences* **23**: 199-207.

Nawar, S.L. 2008. Control of root-rot of green bean with composted rice straw fortified with *Trichoderma harzianum. American-Eurasian Journal of Agricultural and Environmental Sci*ences **3(3)**: 370-379.

Ozbay, N. and Steven, E.N. 2004. *Fusarium* crown and root rot of tomato and control methods, *Plant Pathology Journal* **3(1)**: 9-18.

Papavizas, G.C. 1985. *Trichoderma and Gliocladium*: biology, ecology and the potential for biocontrol. *Annual Review of Phyto-pathology* **23**: 23-54.

Phllips, D.H. 1959. The destruction of *P. lycopersici* kleb in tomato haulm composts. *Annual Applied Biol*ogy **47**: 240-253.

Riaz, T., Khan, S.N. and Javaid, A. 2010. Management of *Fusarium* corm rot of gladiolus (*Gladiolus grandiflorus* sect. Blandus cv. Aarti) by using leaves of allelopathic plants. *African Journal of Biotechnology* **9(30)**: 4681-4686.

Raut, N.A. and Gaikwad, N.J. 2006. Antidiabetic activity of hydro-ethanolic Extract of *Cyperus rotundus* in alloxan induced diabetes in rats. *Fitoterapia* **77**: 585-588.

Sefidkon, F., Dabiri, M. and Alamshahi, A. 2011. Chemical composition of the essential oil of *Eryngium billardieri* F. Delaroche. *Journal of Essential Oil Research* **16(1)**: 42 (Abstract).

Sharma, S.K. and Singh, A.P. 2011a. Morphological, microscopical and physico- chemical investigations on the rhizomes of *Cyperus rotundus* Linn. *Research Journal of Pharma-ceutical, Biological and Chemical Sciences* **3(6)**: 798-806.

Sharma, S.K. and Singh, A.P. 2011b. Anti-microbial investigations on rhizomes of *Cyperus rotundus* Linn. *Scholars Research Library Der Pharmacia Lettre* **3(3)**: 427-431.

Shekhani, H.K.A. 2008. Isolation and diagnosis of damping off and root rot fungi of cauliflower (*Brassica oleracea* var. *botrytis*) between two sowing dates and their control. M.Sc. Thesis, College of Agriculture, University of Salahadin, 106 pp.

Smith, I.M., Dunez, J., Phillips, D.H., Lelliot, R.A.L.T. and Archer, S.A. 1991. European hand book of plant diseases. 1st edition, Willey – Blackwell, 598 pp.

Srinon, W., Chuncheen, K., Jirattiwarutkul, K., Soytong, K. and Kanokmedhakul, S. 2006. Efficacies of antagonistic fungi against *Fusarium* wilt disease of cucumber and tomato and the assay of its enzyme activity. *Journal of Agriculture and Technology* **2(2)**: 191-201.

Subhan, F., Khan, N. and Sewell, R.D.E. 2009. Adulterant Profile of Illicit Street Heroin and Reduction of its Precipitated Physical Dependence Withdrawal Syndrome by Extracts of St John's Wort (*Hypericum perforatum*). *Phytotherapy Research* **23**: 564-571.

Sultana, N. and Ghaffar, A. 2010. Effect of fungicides, microbial antagonists and oilcakes in the control of *Fusarium solani,* the cause of seed rot, seedling and root infection of bottle gourd, bitter gourd and cucumber. *Pakistan Journal of Bot*any **42(4)**: 2921-2934.

Sunder, A.R., Das, N.D. and Krishnaveni, D. 1995. *In vitro* Antagonism of *Trichoderma* spp. against two fungal pathogens of castor. *Indian Journal Plant Protec*tion **23(2)**: 152-159.

Trivedi, B., Motl, O., Herout, V. and Sorm, F. 1984. Composition of the oil from *Cyperus rotundus*: Structure of patchoulenone. *Coil Czech. Chime. Commune.* **29**: 1675-1688.

Vinalea, F., Krishnapillai, S., Emilil, G.O., Sheridan, R.M., Wooa, L. and Matteo, L. 2008. *Trichoderma* plant pathogen interactions. *Soil Biology.* and *Biochemistry*, **40**: 1-10.

Wiest, A., Grzegorski, D., Goulard, C., Rebuffat, S., Ebbole, D., Bodo, B. and Kenerley, C. 2002. Identification of peptaibols from *Trichoderma virens* and cloning of a peptaibol synthetase. *Journal of Biological Chemistry* **277(23)**: 20862-20868.

Windham, M.T., Elad, Y. and Baker, R. 1986. A mechanism for increased growth induced by *Trichoderma* spp. *Phytopathology* **76**: 518-521.

Zaghloul, R.A., Neweigy, N.A., Hanafy, E.A. and Khalifa, N.A. 2008. Effectiveness of Bio-control agents against tomato soil borne pathogens. *3rd Environment Conference*, *Faculty of Science, Zagazig University*, 123–142.