1. Introduction

Tomato (Lycopersicon esculentum Miller), Solanaceae is one of the most important vegetable crops in all over the world, because of its high nutritive value and excellent source of vitamin A and it plays an important role in maintaining the human health being a rich source of lycopene, ascorbic acid and phenols; tomato is used in treatment of cancer (Giovannucci, 1999; George et al., 2004); it is economically attractive and the area under cultivation is increasing daily. The five countries recorded top productions of tomato were United States, China, Turkey, Italy and India. Iraq production of 913493 tons in 2005 (F.A.O., 2007). Although tomato is commercially grown across the globe; there is no place where the plant is free of disease, one of the main constraints to tomato production is damage caused by pathogens, including viruses, bacteria, nematodes and fungi, which cause severe losses in production (Barone and Fruscianete, 2007). Seed health is an important factor in the control of diseases, since an infected seed is less viable, has low germination, reduced vigor and reduced yield (Van Gastel et al., 1996). Various methods for controlling such diseases have been investigated including the use of resistant varieties, cultural practices, plant volatile compounds, plant extracts and biological control, one of the most important bio-control agents is Trichoderma spp. that is the most frequently isolated soil fungi and present in
plant root ecosystems (Harman et al., 2004). The antagonistic activity of the genus Trichoderma spp. to F. solani and R. solani has been widely demonstrated in the soilless mix (Lewis et al., 1998). B. subtilis would make an effective biological control agent by testing it against the damping-off of tomato seedlings caused by Rhizoctonia solani (Asakaand Makoto, 1996). Isolation of pathogenic fungi from tomato seeds and seedlings are Rhizoctonia solani (4 isolates), Acremonium sp., Fusarium solani, Fusarium oxysporum and Fusarium proliferatum caused damping-off disease (damping-off disease are usually refers to the disintegration of stem and root tissues at and below the soil line, the plant tissues become water-soaked and mushy, and the seedling wilts and falls over). The objective of this study is control of tomato damping-off disease by biological control using biocontrol agents of Bacillus subtilis and Trichoderma harzianum.

Materials and Methods

Dual culture technique

Mycelial plugs 5mm of T. harzianum fungus was inoculated in the right half of plates contained solidified PDA (Potato Dextrose Agar) and 5mm mycelial discs for each of pathogenic fungi R. solani (four isolates), Acremonium sp., F. solani, F. oxysporum and F. proliferatum with an equal distance of 4cm between both plugs of the pathogen and biocontrol agent. Control treatments inoculated with each pathogenic fungus only. Plates were incubated at 25±2°C for 7 days, whereas cultures of Rhizoctonia solani inoculated for 3 days. Each treatment replicated three times, pathogenic growth was recorded after contact with the antagonists.

The degree of antagonism was rated according to Bell, et al (1982) 5-point rating scale as follows:

1 = Antagonist overgrew the pathogen and covered the entire medium.
2 = Antagonist overgrew at least 2/3 of the medium surface.
3 = Antagonist and pathogen each colonized about 1/2 of the medium surface.
4 = Pathogen colonized overgrew at least 2/3 of the medium surface and withstood encroachment by antagonist.
5 = Pathogen completely overgrew the antagonist and occupied the entire medium surface.

Effect of Bacillus subtilis on the growth of pathogenic fungi:

B. subtilis was obtained from Plant Protection Department/College of Agriculture/University of Salahaddin. Petri dishes containing PDA were inoculated with B. subtilis culture (48 hrs. old) grown on PDA by placing four drops on the outline of perpendicular lines at a distances of 1 cm from the dish edge, plates were incubated at 25 °C for 48 hours (Paulitz et al., 1992). The same plates were also inoculated with mycelial disc (0.5cm) of pathogenic fungi (4 days old) in the center, control treatments included plates inoculated with pathogens alone. All plates were incubated at 25 °C for 7 days and calculated after full growth of pathogenic in control, the percentage of inhibition was measured according to following formula:

\[
\% \text{ Inhibition} = \frac{R1 - R2}{R1} \times 100
\]

R1 = the growth rate of the pathogenic fungus at control treatment.
R2= the growth rate of the pathogenic fungus at dual culture.
Effect of Biocont-T., Bacterial agent on disease suppression in greenhouse:

The control of disease applied in the greenhouse of Grdarasha/Erbil Research directorate during March, 2015, the all following experiments received the results pre and post emergence damping-off after 40 days.

Biocont-T. a commercial formula of (Trichoderma harzianum):

Three concentrations (0, 5 and 10) g of Biocont-T/kg seeds were examined with 2Kg sterilized soil in pots (20 cm diameter). The soil was infested with R. solani, F. solani, F. oxysporum and Acremonium sp., grown on millet seeds at a rate of 0.5% before two days of sowing. Ten sterilized tomato seeds (GS-12) in 1% sodium hypochlorite (NaOCl) for 2 min., and washed twice with sterilized distilled water for 2 min., treated with (5 and 10) g of Biocont-T, then sown in infested soil for each pot and watered. The treatment replicated three times.

Effect of Bacillus subtilis bacteria:

B. subtilis bacterial were screened in greenhouse to control plant disease and B. subtilis were grown on petri dishes contained lysogeny Broth medium (LB medium) at 20 ºC for 24-48 h. A well-isolated colony 12 hours old from medium was suspended in 5ml distill water using sterilized loop. The suspension was adjusted to 0.5 Mcfarland (Basri and Fan, 2005). Hundred tomato seeds were immersed with bacterial suspension, after 2-5 min. excess liquid were removed before thickening them overnight under a sterile air stream in advance of being planted in the greenhouse (Amein and Weber, 2002).

Results and Discussion

Effect of Trichoderma harzianum on the mycelial growth of pathogenic fungi:

Results in Table (1) showed that T. harzianum inhibited the mycelial growth of examined fungi with considerable degrees according to rating scale described by (Bell et al., 1982). The results confirmed that T. harzianum had a noticeable antagonistic activity against all tested fungi particularly Rhizoctonia solani (isolate 4), since the antagonist overgrew the pathogen entirely, while R.solani (isolate 1) observed in the scale 2 when antagonist overgrew at least 2/3 of the medium surface (Fig. 2-A).

Table (1): Effect of Trichoderma harzianum on the mycelial growth of pathogenic fungi according to Bell scale

<table>
<thead>
<tr>
<th>Pathogenic Fungi</th>
<th>Bell scale</th>
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<tbody>
<tr>
<td>Rhizoctonia solani (1)</td>
<td>2</td>
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<tr>
<td>Rhizoctonia solani (2)</td>
<td>1</td>
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<tr>
<td>Rhizoctonia solani (3)</td>
<td>1</td>
</tr>
<tr>
<td>Rhizoctonia solani (4)</td>
<td>3</td>
</tr>
<tr>
<td>Acremonium sp.</td>
<td>1</td>
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<tr>
<td>Fusarium solani</td>
<td>1</td>
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<tr>
<td>Fusarium oxysporum</td>
<td>1</td>
</tr>
<tr>
<td>Fusarium proliferatum</td>
<td>1</td>
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<tr>
<td>Control (pathogenic fungi only)</td>
<td>5</td>
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</tbody>
</table>

The potential of Trichoderma spp. as biocontrol agent phytopathogenic fungi on several crops is well known particularly Fusarium spp. and Rhizoctonia solani (Poddar et al., 2004; Rojo et al., 2007; Dhahir, 2013).

This results was compatible with studies stated that the ability of T. harzianum in the inhibition growth of R. solani and other fungi due to different mechanisms such as production of toxic metabolites or due to direct parasitism on fungal mycelium (Barakat et al., 2007), or production of enzymes like B-1, 3 glucanases and
chitinases which break down the fungal cell wall (Shalini and Kotasthane, 2007).

Effect of *Bacillus subtilis* on the mycelial growth of pathogenic fungi:

The results illustrated that the bacteria *B. subtilis* was completely inhibited the growth of pathogens, *Rhizoctonia solani* (isolate 2) and *F. oxysporum* inhibited with 92.33%. (Fig. 1, 2-B), The antagonistic of *B. subtilis* refer to production of many antibiotics such as subtiline, bactracin, iturin and surfactin or to inducing cell wall degrading enzymes such as endochitinase, proteases and B-1, 3-glenases which break down the fungal cell wall (Page *et al.*, 1982; Muis and Quimio, 2006).

Figure (1): Inhibition effect of *Bacillus subtilis* on growth of Pathogenic fungi

Control

*T. harzianum*

Figure (2): A- Inhibition of pathogenic fungi by mycelial growth of *T. harzianum* on PDA in *vitro*
Biological control using (Biocont – T. (T. harzianum) and B. subtilis):

Results submitted in Table (2) clarified that Bacillus subtilis reduced incidence pre emergence damping-off to range 6.67-13.33% compared to ranged 30.00-40.00% in control, post emergence damping-off did not shown (Fig. 3), whereas post emergence ranged 0-6.67% in control, there is significant difference between treatments of pre and post emergence and control. These results similar with Morsy et al.,(2009) in reducing of pre and post emergence damping-off by 19.0% and 11.0% respectively. In contrast, the disease incidence reaches to 45.0% and 20.0%, respectively with the inoculum of Fusarium solani through application of Bacillus subtilis. Regardless of defect efficiency B. subtilis may be in reducing pathogens damage. The promotion of tomato growth due to their abilities to produce phytohormones, vitamins and solublizing minerals besides, their role in direct inhibition of pathogen growth (Morsy, 2005; Zaghloul et al., 2007). Biocont – T (T. harzianum) was tested at concentrations of 5g and 10g/kg seeds, pre emergence damping-off incidence ranged between 10.00-16.67% at first concentration between 3.33-13.33% at second concentration. In contrast the disease incidence reaches to ranged between 30.00% - 40.00% with the inoculum of pathogenic fungi through application of Trichoderma harzianum (Table 2) there is significant difference between treatments and control. Emerged plants appeared healthy with no post emergence damping-off incidence at both concentrations, compared to ranged between 0-6.67% in control (Fig. 3).
Shabir and Rubina (2010), observed that the Antagonist of *Trichoderma harzianum* inhibited the mycelial growth of *R. solani* ranged between 85.5 and 83.0 % as compared to control, the inhibition of *R. solani* by *Trichoderma* species could probably be due to the secretion of extracellular cell degrading enzymes such as chitinase B-1, 3- glucanase, cellulose and lectin, which help mycoparasites in the colonization of their host, the inhibition of pathogen may be also been attributed to the production of secondary metabolites by antagonists such as glioviridin, viridin and gliotoxin. One of the most important characteristics necessary for acceptance and effectiveness of biocontrol agents is their ability to survive in the environments other than their origin and colonize plants roots during certain period of time to control plant pathogens (Nemec et al., 1996).

Hervas et al., (1998) studied the efficiency of *B. subtilis* and *T. harzianum* on pathogenic to suppress tomato diseases. Their result showed the combination of *B. subtilis* and *T. harzianum* was effective in suppressing damping-off pre and post emergence but it did not differ significantly from treatments with either of these antagonists alone. The mechanism of *Trichoderma* and *Bacillus* action on pathogens may be attributed to their attacking and binding the pathogens by sugar linkage and begins to secrete extracellular protease and lipase (Cal et al., 2004). Biocont-T (*T. harzianum*) suppressed the deleterious soil microbes by competing at the active sites, reduced disease development, and subsequently stimulated the growth and yield of plants (Ahmed and Upadhyay, 2009).

<table>
<thead>
<tr>
<th>Pathogenic fungi</th>
<th>Pre emergence%</th>
<th>Post emergence%</th>
<th>Control (only fungi)</th>
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<tbody>
<tr>
<td></td>
<td>B. subtilis</td>
<td>T. harzianum</td>
<td>B. subtilis</td>
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<tr>
<td>R. solani</td>
<td>6.6 10.00</td>
<td>10.00</td>
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<td>7.00</td>
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<tr>
<th></th>
<th>Bacillus subtilis (106 cell/ml)</th>
<th>Biocont-T. (T. harzianum) 5 g/kg seeds</th>
<th>Biocont-T. (T. harzianum) 10g/kg seeds</th>
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Figure (3): Effect of Biocont-T. (T. harzianum) and Bacillus subtilis on the suppression of damping-off in greenhouse:

A-control (non-inoculated fungi)  B-Rhizoctonia solani  C- Fusarium solani  D- Fusarium oxysporum  E- Acremonium sp.

References


