Control Pepper Fusarium Wilting by biocontrol agent *Trichoderma harzianum* and Chelated Iron Fe-EDDHA

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**Abstract:**

Sweet pepper (*Capsicum annuum* L.) is an economically important vegetable crop. Wilt disease caused by *Fusarium oxysporum* f. sp. *capsici* is a specific pathogen that affects the pepper. Four isolates of *F. oxysporum* f. sp. *capsici* Fo3, Fo6, Fo7 and Fo8 were obtained from diseased pepper plants that were collected from different pepper fields in Baghdad. Fo6 isolate that has high pathogenicity to pepper seeds, *Trichoderma harzianum* (Th) was tested in vitro against *F. oxysporum* f. sp. *capsici* showed a high inhibition rate for the isolate Fo6, the concentration of chelated iron Fe-EDDHA 0.5% reduced the radial growth of Fo6 while did not affect the growth of Th. In pots experiment, the treatment Fo6+Th+Fe showed a significant reduction in the incidence and severity of root rot and wilt diseases. The treatment Th+Fe achieved a highly significant increase in fresh and dry root and vegetative weight 63.36, 130.56, 4.55 and 10.26g respectively, compared with control (without pathogenic) reaching 11.10, 54.83, 1.30 and 3.70g respectively. Moreover, in greenhouse the treatment Fo6+Th+Fe has reduced the incidence and severity of disease 3.33 and 1.67% respectively, and the treatment Th+Fe gave an increase in the total chlorophyll content 104 SPAD compared with the control 70.4 SPAD.

**Keywords:** Fe-EDDHA, Fusarium wilt, Fusarium oxysporum, Pepper, Trichoderma harzianum.

**Introduction:**

Sweet pepper (*Capsicum annuum* L.) is an economically important seasonal vegetable crop all over the world due to its high nutritional value. Wilt diseases and root rot are among the most important diseases that affect the pepper crop, caused by many fungi, including *Fusarium oxysporum*, which causes economic losses to the crop and causes the damping-off seedlings in the early stages of growth. It also causes root rot and vascular wilt of large plants. Several methods have been used to combat these diseases, including chemicals that cause environmental pollution and disruption of biodiversity and biologically. *Trichoderma harzianum* is one of the factors that has been widely used in biological control programs because of its possession of a number of mechanisms in control such as antagonism, competition and parasitism, as well as induction of systemic resistance. It was also found that the use of nutrients solution does not affect the development of diseases, but also contributes to creating a suitable environment in the soil, such as adjusting PH or controlling humidity, so the application of integrated nutrition to plants is one of the main components in sustainable agriculture and has more effectiveness to combat plant diseases is less costly and environmentally friendly and thus reduces disease or makes it at a controlled level. Chelated iron is one of the most nutrients as it has an effect on activating plant immunity and has an important role in plant response to pathogen interactions in terms of secretion of phenolic compounds and pathogenic proteins and its effect on photosynthesis and chlorophyll content. Accordingly, it was found that improving the soil surrounding the roots of the plant is reflected in a way that leads to the inhibition of many pathogens or reaching the best ways to control diseases, the study aimed to: Isolation and identification of the pathogen accompanying the
symptoms of wilting and root rot from infected pepper plants and evaluation of the effect of the biological agent T. harzianum, chelated iron Fe-EDDHA and the interaction between them in controlling the disease.

Materials and Methods:
Isolation and identification of the pathogen

Samples of pepper plants of Carisma variety that showed symptoms of wilt were collected from different pepper greenhouses in Baghdad (Al-Yusufiyah, Abu Ghraib) for the season 2020-2021. Isolation of the pathogen was carried out by cutting the roots and parts of the stem length of 0.5-1 cm and washing with tap water and sterilizing with NaClO solution for 2 min, then rinsing with sterile water. Four pieces were planted in petri dish containing Potato Dextrose Agar (PDA). The plate was incubated at 25±2°C. After three days, the isolates were purified by taking 0.5 cm from the hyphal of emerging fungi and transferred to PDA plates and incubated, the isolates were identified based of morphological characteristics by Asst. Prof. Dr. Bushra Subair Abdulsada from College of Agricultural Engineering Sciences, University of Baghdad.

Molecular identification of the pathogen

The isolates were purified by the single spore method, the isolate was grown on PDA plates and incubated. After 7 days, DNA extraction was performed according to the method of Velarde Felix using standard equipment by the Korean company Bioneer, Then, a polymerase chain reaction (PCR) was performed using primers ITS1-Fwd (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4-Rev (5′-TCCCTCGCTTATTGATATGC-3′). Amplifications were performed with a first cycle of 5 min at 95°C for Pre-denaturation, 35 cycle of 30 sec at 95°C for denaturation, 35 cycle of 30 sec at 60°C for Annealing, 35 cycle of 45 sec at 72°C for Extension and, a final extension at 72°C for 5 min with 1 cycle. The products of the DNA amplification process were detected using the electrophoresis method, and then the amplification products were sent to the Korean Macrogen company. Similarities with the nucleic acid sequences of the studied isolates were searched and compared with the nucleotide sequences in the GenBank using the program BLAST.

Pathogenicity test of isolates on pepper plant seeds

The pathogenicity of four isolates was tested according to the method Bolkan and Butler. PDA plates were prepared and local pepper seeds were planted after being sterilized with sodium hypochlorite solution for 2 min then rinsed with sterile water. Twenty seeds were planted in PDA plates with four replications. After three days, the center of each plate was inoculated with each isolate separately, by placing a 0.5 cm from a fungal colony and four plates without inoculation served as control and incubated. The percentage of seed germination was calculated according to the following equation:

\[ \text{% Germination seeds} = \frac{\text{No.of germinated seeds}}{\text{Total No. of seeds}} \times 100 \]

One isolate was selected, which gave the highest rate of seed decay.

Antagonism test of Trichoderma harzianum against Fusarium oxysporum f. sp. capsici in vitro

The biocontrol agent T. harzianum was purified from the local commercial preparation supplied by the Ministry of Agriculture/Plant Protection Department, using the dilution method, Then the antagonism was tested against the isolate F. oxysporum using the dual culture method, the antagonism was evaluated according to the five-point scale by bell.

Assessment of Fe-EDDHA against Fusarium oxysporum f. sp. capsici and T. harzianum in vitro

Flasks were prepared to contain PDA sterile media, and then iron was added to the nutrient medium in three concentrations 0.5, 1 and 2% (w/v), and sterilized by autoclaved and poured into petri dishes. The center of each dish was inoculated with 5mm of the pathogenic with four replications for each concentration, also the center of four dishes was inoculated with T. harzianum, and four dishes without iron as a control, then incubated at 25±2°C. The colony growth rate and the inhibition rate for F. oxysporum and T. harzianum were calculated after seven days, according to the following equation:

\[ \text{% Inhibition} = \frac{\text{The rate colony diameter of control} - \text{The rate colony diameter of treatment}}{\text{The rate colony diameter of control}} \times 100 \]

Assessment of T. harzianum and Fe-EDDHA against Fusarium oxysporum f. sp. capsici in pots

Soil and peat mixture 2:1 was sterilized by autoclaved and placed in plastic pots 5 kg then soil was infested with a pathogen at the rate of 1% (w/w) that was grown on local millet seeds (Panicum miliaceum). After 2 days the pots were planted with five pepper seedlings of Carisma
variety for each pot and irrigated. After three days, commercial formulation of *T. harzianum* was added at 1% (w/w) and chelated iron Fe-EDDHA was added at 0.5% (w/v) depending on test of Fe-EDDHA in vitro, each treatment included four replicates and as follows:

A: Pathogen only (Fo6).

B: Fo6 + *T. harzianum* (Fo6+Th).

C: Fo6 + Fe-EDDHA (Fo6+Fe).

D: Fo6 + *T. harzianum* + Fe-EDDHA (Fo6+Th+Fe).

E: Fe-EDDHA (Fe).

F: *T. harzianum* (Th).

G: Fe-EDDHA + *T. harzianum* (Fe+Th).

H: Plant only (Control).

After (30) days, the infection rate was calculated using the following equation:

\[
\text{% infection} = \frac{\text{No. of infected plants}}{\text{Total No. of plants}} \times 100
\]

And the disease severity was calculated using the scale of Five degrees as follows:

0= healthy plant, 1= 1-25% of the roots are light brown, 2= 25-50% of the roots are dark brown, 3= 50-75% of the roots are dark brown, 4= 75-100% of the roots are dark brown and plant dead. Also the wet and dry root and vegetative weight was calculated.

**Assessment of *T. harzianum* and Fe-EDDHA against *Fusarium oxysporum* f. sp. *capsici* in greenhouse**

A field experiment was carried out in greenhouses/ college of agricultural engineering sciences/ university of Baghdad for the year 2021-2022. The soil was prepared and all the necessary agricultural operations were performed, and the experiment was designed according to a completely randomized block design (RCBD) with three replications. Pepper seedlings of Carisma variety were planted and it was drip irrigated, then roots were contaminated with the pathogen inoculum at 1% (w/w), after 3 days the commercial formulation of *T. harzianum* was added at 1% (w/w), and chelated iron Fe-EDDHA was added to the roots at a concentration of 0.5% (w/v), and the same treatments were performed in the pots experiment (Fo6, Fo6+Th, Fo6+Fe, Fo6+Th+Fe, Fe, Th, Fe+Th and Control).

After 45 days, the disease incidence and severity of infection were calculated as in the equations mentioned in the previous experiment, and the chlorophyll content of plants was calculated before and after adding the mentioned factors to the field by SPAD device with four replications for each treatment.

**Results and Discussion:**

**Isolation and identification of the pathogen**

The results of isolation from diseased plants showed that four isolates were diagnosed based on morphological characteristics Table 1. The colonies’ growth appeared on the plate in a white to pink color, and they were observed under the microscope, Microconidia spherical in shape and Macroconidia crescent shape, also seen as Chlamydospore and isolates were distinguished as belonging to the genus *Fusarium* according to the taxonomic keys.

**Table 1. Isolates and Collection area.**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Collection area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fo3</td>
<td>Al-Yusufiyah</td>
</tr>
<tr>
<td>Fo6</td>
<td>Al-Yusufiyah</td>
</tr>
<tr>
<td>Fo7</td>
<td>Abu Ghraib</td>
</tr>
<tr>
<td>Fo8</td>
<td>Abu Ghraib</td>
</tr>
</tbody>
</table>

**Molecular diagnosis of the pathogen isolates**

The results of the electrophoresis on agarose gel showed the appearance of a clear band for each isolate Fig. 1, which had a molecular weight of 550bp, and the results of the sequences of nucleotide for the genomic region ITS1/ITS4 showed the local isolates conformity the global isolates registered in the GeneBank with a matching ratio of 98-99%, and the isolates Fo3, Fo6, Fo7 and Fo8 were deposited in the gene bank and the bank number for each isolate was given as follows: OP315634, OP315631, OP315632 and OP315633.
Figure 1. Electrophoresis of DNA amplification products with a molecular weight of 550 bp (70 volts for 50 min) of the regions ITS1-Fwd and ITS4-Rev of the isolates *Fusarium oxysporum*, Lanes 1-4: isolates from disease pepper plant *F. oxysporum f. sp. capsici* Fo3, Fo6, Fo7 and Fo8, Lane 5: Marker 100 bp DNA Ladder.

Pathogenicity test of isolates on pepper plant seeds

The results showed of testing the pathogenicity of isolates on seeds, Table 2 and Fig.2 That all isolates reduced the percentage of germination for local pepper seeds with significant differences, the isolate Fo6 significantly superior in reducing the germination rate seeds, which was 15.00% compared with control 100%, and isolate Fo3 recorded the highest germination rate of 30%. Followed by isolate Fo7 which recorded 25%, and then Fo8 which recorded 20%. These differences between the isolates in their ability to reduce the rate of germination are due to the difference isolates in their ability to produce mycotoxins such as Fusaric acid, Trichothecenes, Zearalenones and Enniatins which affect seeds and cause failure to germination. Based on this experiment was selected the isolate Fo6 was for later experiments as it is the most pathogenicity.

Table 2. Pathogenicity of isolates on pepper plant seeds*.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fo3</td>
<td>30</td>
</tr>
<tr>
<td>Fo6</td>
<td>15</td>
</tr>
<tr>
<td>Fo7</td>
<td>25</td>
</tr>
<tr>
<td>Fo8</td>
<td>20</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>L.S.D 0.05</td>
<td>10.30</td>
</tr>
</tbody>
</table>

*Each No. represents an average of four replicates.

Figure 2. Pathogenicity of isolates on seeds in the media PDA.

Antagonism test of *Trichoderma harzianum* against *Fusarium oxysporum f. sp. capsici* in vitro

The results of the experiment showed that the biocontrol agent *T. harzianum* (Th) gave a high antagonistic ability against isolate Fo6 in the PDA media, Fig. 3, reaching a degree of 1 which is the degree for biological agent grow more than 3/4 of the plate, and this is consistent with what was mentioned in. Accordingly, it was used in next experiments.
Assessment of Fe-EDDHA against *Fusarium oxysporum* f. sp. *capsici* and *T. harzianum* in vitro

The results of the experiment showed that the three concentrations used 0.5, 1 and 2% gave significantly different results Fig. 4, as iron showed a decrease in the growth of the pathogenic isolate Fo6, and the concentration recorded 0.5% the highest significant difference in reducing the colony growth rate, which amounted 3.25cm and an inhibition rate of 63.89%, followed by the two concentrations 1 and 2% and without significant difference between them, as the growth rate of the fungal colony of the pathogenic fungus was 4.75 and 5.81cm respectively, and an inhibition rate of 47.22 and 35.44% respectively, compared with control in which the growth rate of the fungal colony reached 9cm and an inhibition rate of 0%. While there were no significant differences in the growth rate of the fungal colony of the biological agent *T. harzianum* in the three iron concentrations which amounted 8.90, 8.93 and 9 cm respectively, and no significant differences were recorded in the percentage of inhibition which amounted to 1.11, 0.78 and 0% respectively compared with control. These results were consistent with what was mentioned Segarra26 who stated that the availability of iron in the nutrient medium may affect the growth of mycelium inside the dish because of its toxicity that affects the growth of pathogenic fungi, and these results are in agreement with what was stated by Wiebe27. It was noticed a decrease in the lateral growth of *Fusarium* with the addition of a small concentration of chelated iron to the nutrient medium, and *T. harzianum* was not affected when iron was added and reduced the growth of the pathogen in all concentrations of iron tested, and the reason for this is that the fungus *Trichoderma* has the ability to produce Siderophores better than *F. oxysporum* and therefore the effect of iron is less on it and also increases its competitiveness as a biocontrol agent against pathogens28.
Assessment of *T. harzianum*, Fe-EDDHA against *Fusarium oxysporum* f. sp. *capsici* in pots

The results of the study showed that all the treatments used gave significant differences in the disease incidence and the severity of infection Table 3, according to the pathological evidence of Sun Fig. 5 and significant differences in the wet and dry roots and vegetative weight of pepper plants infected with the pathogenic isolate *F. oxysporum* (Fo6), compared with the treatment of the pathogen agent alone and without the pathogen, the Fo6+Th+Fe treatment achieved the highest significant difference in reducing the disease incidence and the severity of infection, which amounted 15.00 and 5.00% respectively, compared to the control treatment Fo6 in which the percentage and severity of infection reached 100 and 76.25% respectively, and were equal the two treatments Fo6+Th and Fo6+Fe significantly reduced the incidence of infection, which amounted to 20.00% and the severity of infection reached 10.00 and 12.50% respectively. These results are consistent with what was stated by Duc9 who stated that the decrease in the disease incidence and the severity of infection in the presence of more than one factor is due to the synergistic action between them that stimulated plant growth in addition to stimulating the systemic resistance of the plant and thus contributed to the suppression of the pathogen. These results also give us an indication that the addition of iron with the biological control agent *T. harzianum* stimulates the plant to increase the resistance of the pathogen by activating the defense enzymes in the host and iron plays a key role in the production of reactive oxygen species (ROS) which in turn interact with hormone signals, thus stimulating molecular and cellular responses that strengthen cells against pathogens10.

Also the results showed there are significant differences in the wet and dry roots and vegetative weight total, Table 3 and Fig. 6, the treatment Fo6+Th+Fe recorded a high significant difference in the increase in the wet weight of the root and vegetative total, which amounted to 40.63 and 125.70g respectively, compared with control treatment Fo6 which recorded 5.63 and 125.70g respectively, and fo6+Th+Fe treatment recorded an increase in the dry weight of the root and vegetative total, which amounted 4.20 and 9.23g respectively, compared with Fo6 treatment which recorded 0.63 and 1.56g respectively, and Th+Fe treatment recorded the highest value the wet and dry weight of the root and vegetative total was 63.36, 130.56, 4.55 and 10.26g respectively, compared with control treatment (Plant only) which reached 11.10, 54.83, 1.30 and 3.70g respectively, and these results were in agreement with what mentioned11,32 they showed that the reason for the increase in weights in the presence of *T. harzianum* and Fe-EDDHA is due to the ability of these factors to use different strategies they possess against pathogens, such as stimulating the plant to produce growth regulators, which have a very important role in plant growth, It works to organize levels of plant hormones such as Auxins, Cytokinins, Gibberellins and Ethylene33.

<table>
<thead>
<tr>
<th>Treatment No.</th>
<th>Treatment code</th>
<th>disease incidence %</th>
<th>severity of infection %</th>
<th>wet weight of the root (g)</th>
<th>Dry weight of the root (g)</th>
<th>wet weight of the vegetative (g)</th>
<th>Dry weight of the vegetative (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Fo6</td>
<td>100.00</td>
<td>76.25</td>
<td>5.63</td>
<td>0.63</td>
<td>22.7</td>
<td>1.56</td>
</tr>
<tr>
<td>B</td>
<td>Fo6+Th</td>
<td>20.00</td>
<td>10.00</td>
<td>34.73</td>
<td>2.56</td>
<td>105.56</td>
<td>8.36</td>
</tr>
<tr>
<td>C</td>
<td>Fo6+Fe</td>
<td>20.00</td>
<td>12.5</td>
<td>16.26</td>
<td>1.50</td>
<td>67.36</td>
<td>4.97</td>
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<tr>
<td>D</td>
<td>Fo6+Th+Fe</td>
<td>15.00</td>
<td>5.00</td>
<td>40.63</td>
<td>4.20</td>
<td>125.70</td>
<td>9.23</td>
</tr>
<tr>
<td>E</td>
<td>Th</td>
<td>0.00</td>
<td>0.00</td>
<td>39.40</td>
<td>3.40</td>
<td>117.46</td>
<td>9.00</td>
</tr>
<tr>
<td>F</td>
<td>Fe</td>
<td>0.00</td>
<td>0.00</td>
<td>36.33</td>
<td>2.63</td>
<td>89.06</td>
<td>5.63</td>
</tr>
<tr>
<td>G</td>
<td>Th+Fe</td>
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<td>0.00</td>
<td>63.36</td>
<td>4.55</td>
<td>130.56</td>
<td>10.26</td>
</tr>
<tr>
<td>H</td>
<td>Control</td>
<td>0.00</td>
<td>0.00</td>
<td>11.10</td>
<td>1.30</td>
<td>54.83</td>
<td>3.70</td>
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<td>L.S.D 0.05</td>
<td></td>
<td>13.08</td>
<td>7.721</td>
<td>4.96</td>
<td>0.561</td>
<td>6.08</td>
<td>0.989</td>
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</tbody>
</table>

*Each No. represents an average of four replicates.*
Figure 5. The scale of the infection, A: 0= healthy plant, B: 1= 1-25% of the roots are light brown, C: 2= 25-50% of the roots are dark brown, D: 3= 50-75% of the roots are dark brown, E: 4= 75-100% of the roots are dark brown and plant dead.

Figure 6. A: Fusarium oxysporum f. sp. capsici, B: Fusarium oxysporum f. sp. capsici and Trichoderma harzianum and Fe-EDDHA, C: Control, D: Fusarium oxysporum f. sp. capsici and Fe-EDDHA.

Assessment of T. harzianum and Fe-EDDHA against Fusarium oxysporum f. sp. capsici in greenhouse

The results of the field study showed that there were significant differences in reducing the disease incidence and the severity of infection in the field after adding the factors to plants infected with the pathogenic isolate F. oxysporum (Fo6), Table 4 and Fig.7, the Fo6+Th+Fe treatment gave the lowest disease incidence and the severity of infection amounted 3.33 and 1.67% respectively, compared with control (Fo6) which recorded 100 and 84.17% respectively. The treatments Fo6+Th and Fo6+Fe were significantly equal in reducing the disease incidence of infection as it reached 13 and 10% respectively, and the infection severity reached 7.67 and 7.50% respectively. These results are in agreement with Dar44 who showed that the application of T. harzianum effectively controls a large number of soil-born fungi including F. oxysporum and shows good parasitic activity, Therefore, it can be used effectively as a biocontrol agent against many plant pathogens, as it prevents pathogen invasion through parasitism, antibiotic production, competition and decomposition of pathogenic fungal hyphae and production of organic metabolites such as Enodin and Chitinase and volatile inhibitors such as Acetaldehyde and stated.
Wang\textsuperscript{36} that the effect of iron in reducing the disease incidence and severity of plant infection comes from its ability to enhance or interfere with antifungals, or through its effect on the virulence of the pathogen and host defense, and that its role in immunity of plants mentioned Aznar by affecting the hormones Salicylic acid, Jasmonic acid and Ethylene, which are essential in induction of acquired resistance.

The results also showed significant differences in all content of chlorophyll in the field, Table 4, as it was found that the addition of factors caused an increase in the total chlorophyll content in pepper plants. The Fo6+Th+Fe treatment showed its superiority over the rest of the treatments in the presence of the pathogen, and recorded the content of chlorophyll 71.4 SPAD, compared with the control (Fo6) which recorded a decrease in the content of chlorophyll amounted to 45.0 SPAD, and the Th+Fe treatment gave a significant and obvious increase in the content of chlorophyll, as it recorded 104.6 SPAD, with a high significant difference from the comparison treatment (Con), in which the content of chlorophyll reached 70.4 SPAD. The above results indicate that the addition of T. harzianum with chelated iron Fe-EDDHA led to an increase in the chlorophyll ratio as a result of an increase in the activity of enzymes stimulating plant growth and an increase in the absorption of nutrients that support photosynthesis. This result is consistent with what. Ramirez-Pimentel they mentioned that the addition of T. harzianum caused a significant increase in the total chlorophyll content and an increase in the signals of Salicylic acid (SA), a plant hormone that participates in the regulation of photosynthesis reactions, chlorophyll content and transpiration rate, and that the biological fungi caused an increase in the amount of phenolic compounds and the relative content of chlorophyll, and the effect of Addition of chelated iron Fe-EDDHA increases the content of chlorophyll as it is mainly and effectively involved in the photosynthesis process, and plays a key role in the redox mechanism through its effect on cytochromes, a proteins containing (heme) which are with a central iron atom in its core as a catalyst that participates in the transport chain electron and respiration\textsuperscript{37}, in addition, micronutrients can act as a cofactor for some enzymatic activities especially for enzymes participation in pathways that produce photosynthetic pigments\textsuperscript{38} and combining beneficial microorganisms with micronutrient fertilizers it’s a better way to enhance iron bio fortification and improve crop quality\textsuperscript{39}.

### Table 4. The disease incidence and severity of infection, total content of chlorophyll in the greenhouse\textsuperscript{*}

<table>
<thead>
<tr>
<th>Treatment No.</th>
<th>Treatment code</th>
<th>Disease incidence %</th>
<th>Severity of infection %</th>
<th>Total content of chlorophyll (SPAD) Before adding</th>
<th>After adding</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Fo6</td>
<td>100.00</td>
<td>84.17</td>
<td>57.0</td>
<td>45.0</td>
</tr>
<tr>
<td>B</td>
<td>Fo6+Th</td>
<td>13.33</td>
<td>7.67</td>
<td>58.3</td>
<td>61.6</td>
</tr>
<tr>
<td>C</td>
<td>Fo6+Fe</td>
<td>10.00</td>
<td>7.50</td>
<td>64.0</td>
<td>66.8</td>
</tr>
<tr>
<td>D</td>
<td>Fo6+Th+Fe</td>
<td>3.33</td>
<td>1.67</td>
<td>52.0</td>
<td>71.4</td>
</tr>
<tr>
<td>E</td>
<td>Th</td>
<td>0.00</td>
<td>0.00</td>
<td>67.9</td>
<td>74.0</td>
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<tr>
<td>F</td>
<td>Fe</td>
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<td>0.00</td>
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<tr>
<td>G</td>
<td>Th+Fe</td>
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<td>95.1</td>
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<tr>
<td>H</td>
<td>Con</td>
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<td>0.00</td>
<td>71.6</td>
<td>70.4</td>
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<td>L.S.D 0.05</td>
<td>7.31</td>
<td>7.50</td>
<td>9.26</td>
<td>13.09</td>
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</table>

\textsuperscript{*}Each No. represents an average of four replicates.
Conclusions:
Different isolates of *Fusarium oxysporum* f. sp. *capsici* causes pepper wilt. Identification of these isolates was based on morphological characteristic and ITS sequence analysis, PCR amplification by using ITS1/ITS4 primers. The pathogenicity was tested all isolates differed in the disease incidence and severity. Fo6 isolate produced the highest rate of seed inhibition and the highest incidence and severity of wilt disease in pots, The use of biocontrol agent *Trichoderma harzianum* with chelated iron Fe-EDDHA at a concentration of 0.5% alone or in combination led to reducing the disease incidence and severity in pots and greenhouse, Also increased the fresh and dry weight of roots and vegetative part, in addition the chlorophyll content in plants was increased.

Authors’ declaration:
- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed on ethical consideration’s approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Authorship contribution:
A. N. A. A. (Conception, design, acquisition of data, analysis, interpretation).
B. S. A. A. (drafting the MS, revision and proofreading).

References:


الخلاصة:
الفلفل الحلو من المحاصيل الاقتصادية الهامة، يعد مرض الذبول المتسبب عن الفطر Fusarium oxysporum f. sp. capsici من الأمراض المهمة التي تؤثر على الفلفل، حيث ارتفعت نسب وشدة الإصابة بالمرض. تم استخدام عامل المكافحة الاحيائية Trichoderma harzianum ضد الفطر Fusarium oxysporum f. sp. capsici في التجارب، حيث أظهرت المعاملة إффективية عالية ضد العزلة Fusarium oxysporum f. sp. capsici، وتحل في المقابلة 100% من الالتهابات المحمولة في الظروف الطبيعية. ونتيجة لذلك، في التجربة الثانية، ارتفعت نسبة النمو الشعاعي للعزلة Fusarium oxysporum f. sp. capsici بنسبة ٦٣٪ خلال فترة التجربة، مما يثبت فعالية المعادلة Trichoderma harzianum في علاج الالتهابات الفيوزارميا. 

الكلمات المفتاحية: الحديدي المخلبي، الفطر الفيوزارميا، Trichoderma harzianum,