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# Detection of *Fusarium* Wilt Strains in Tomato and Role of Some Biological Factors in Inducing Systemic Resistance

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***Abstract:*** The detection of *Fusarium oxysporum* f. sp *lycopersici* strains causing tomato wilt disease showed the role of humic acid and *Trichoderma harzianum* in inducing systemic resistance. The *F. O.* f. sp *lycopersici* was isolated from many regions of Basrah province-Iraq from wilted tomato plants, the severity of the infection was 35%. The isolates F3, F4, F5 and F6 whose genomes were associated with the uniinitiator belonged to *F. O.* f. sp *lycopersici* (F). The isolates of F5 and F6 were identified to strain 1 and isolate F3 and F4 were identified to strain 3 which was the new recorded in Basrah. The biological agent *T. harzianum* (Th), together with the humic acid (H) and their interaction in the severity of the infection, was less severe in the FThH treatment (0%), followed by the FTh. The maximum height was in non-contaminated soil and in ThH, Th and H, was 91.71, 86.33 and 83.48 cm, respectively. In contaminated soil, the best plant height was in ThH (88.74 cm). The dry shoot system weight was maximum in ThH. The induction of resistance in plants showed the highest concentration of peroxidase in FThH. The polyphenol oxidase enzyme showed an increase in biological agent and humic acid (ThH) in soil contaminated with pathogen (1.908 units $ml^{-1}$) followed by Th and H which was significantly different from the other treatments.

***Keywords***: *Fusarium* wilt, Systemic resistance, Biological control, *Lycopersicon esculentum* Mill

Tomato (*Lycopersicon esculentum* Mill) is considered to be widely cultivated crops about all over the world and recognized by their high nutrient value since this crop contain carbohydrates, proteins, fats, and some mineral elements like phosphorous, calcium, iron, and some vitamins such as A, C, B1 and B2. Vascular wilt diseases include fusarium wilting in tomatoes which is caused by *F. O.* f. sp *lycopersici* and is one of the factors which limit planting this crop. Multiple methods have been used for the purpose of eliminating the effect of the pathogen from the plant like use chemical fungicides and excessive use leads to many problems including the environment pollution and the appearing of the resistant races as well as their direct effect on the health of mankind and animals and their killing of fishes and birds and their effect on the soil fertility through their effect on soil microbes. Houssien et al (2010) concluded that the efficacy of the systematic resistance in the tomato plant against the fusarium wilting disease. Humic acids have a positive effect on the assimilations of nutrients by the plant and the micronutrients transmission in particular. The group of amino acids in humic acids can assimilate the negative phosphate ion and improve its readiness for the plant (Lutzow et al 2006). In addition, humic acids discourage the activity of enzyme (IAA oxidase) which leads to the increase of the activity of oxen which in turn plays a role in the stimulation of the growth of the plant and roots. In view of the importance the fusarium wilting disease on tomato crops and the importance of finding alternative resistance methods instead of the chemical fungicides. This study aims at identifying *F. O.* f. sp *lycopersici* strains by isolating the pathogen and diagnosing by PCR and efficacy of humic acid and *T. harizanium* on management of this disease.

## MATERIAL AND METHODS

**Isolation and diagnosis the pathogen:** Samples of wilted tomato plants were brought to the lab from many regions in the province of Basrah and the infected plant parts (stem and branches) were washed with running water, cut (1 cm) and then sterilized with sodium hypochlorite solution (NaOCl) at 1% concentration for one minute .These were then washed with distilled water to remove the traces of sodium hypochlorite solution and dried with filter paper Whatman1 and placed in the petri dishes containing solid PDA media which added antibiotic chloramphenicol (250mg.l-1 ). This was replicated with three dishes which had five pieces per dish and then incubated at 25 ± 2°C for 7days (Al – Waily 2004).

**Pathogenicity:** *F. oxysporum* was grown on millet grains and incubated at 25 ± 2°C for 14 days and then it was added to 10g/pot which contained sterilised soil and manure (1: 3). The severity of infection was calculated after four weeks on pathological severity scale (Decal et al 1997),

| Degree | Infection |
|---|---|
| 0 | no infection |

1 Light yellowing and wetness of the first two leaves
2 Yellowing and wetting a number of lower leaves
3 Extreme wilt and plant death

The pathogen was re-isolated from the plants that showed the infection for the purpose of calculating the severity of the disease.

**Estimation of polyphenol oxidase enzyme in resistance:** This enzyme was estimated by the method of Thomas and Janie (1986) for the determination of the enzymatic activity of poly phenol oxidase by the quantitative method. This method was followed by estimating the increase in absorption at 470 nm using Spectrophotometer where this absorption is caused by the catechol oxidation as an enzyme substrate. Then the change in the absorption was recorded during 5 minutes at 30$^{0}$C, and then the readings were recorded after taking three replicates for each reading. The number of enzymatic units ml$^{-1}$ of the enzyme solution were calculated from the following equation:-

$$\text{Number of enzyme units / ml} \frac{\text{Change in light absorption during 5 minutes * Dilution}}{\text{Time}} * 0.001$$

**Estimation of peroxidase enzyme in resistance - Induced of tomato plants**

Absorption was read directly in spectrophotometer with a wave length of 470nm with three replicates per treatment.

Enzymatic activity (absorption unit / gr soft root) = reading of the apparatus

$$\text{Model weight} * \frac{\text{Size taken for reading}}{\text{Extraction volume}}$$

**Polymerase Chain Reaction (PCR-RFLP)**

**Extraction of DNA of *F. o.* f sp. *lycopersici:*** The extraction solution CTAB buffer was prepared (Kereny et al 1999) by putting 10 g of CTAB in a glass beaker and then 350 ml distilled water was added it. The solution was re-stirred for few seconds and then heat in the microwave for one minute .Re-stirred and mix for another 1-2 minutes, then return to microwave for 1 minute .Then added 50 ml of Tris - HCl 1 molar (pH = 8) and allow Tris to dissolve by continuing mixing. 10 ml EDTA 0.5 molar was added to the solution to maintain pH less than eight. NaCl was added and continued mixing and then the solution was transferred to an inserted glass cylinder and the volume was made to 500 ml with distilled water to which 1% of mercaptoethanol was added.

**Electrophoresis:** The success of DNA extraction was determined by the use of electrophoresis technology on Agaros gel, where the migration gel basin is submerged in the main basin containing 1X TBE solution. The DNA was mixed and then bromophenol blue dye which was then injected into the drill and after the end of the injection process connects the electrodes to the power supply and stabilizes the power supply on 80 volts and 65 mA and gel is left until the bromophenol blue dye from the drill to the other side is solidified. After the end the migration process was checked and the gel in the UV device observes the DNA nested bundles which are interacted with ethidium bromide tincture**.**

## RESULTS AND DISCUSSION

**Isolation and diagnosis of *F. oxysporum* and pathogenicity:** The colonies of fungi were characterized by lumbar growth or sparse or abundant fungal yarn with the emergence of rings surrounding the mushroom center, colonies of this mushroom appear pale orange and sometimes appear in violet or the pale purple from the back of the dish, In addition to the fungus, there are three types of spores, which are large conidic spores that are short to medium in length straight to the sickle shape and small conidial spores in large numbers It is characterized by its semi-oval shape, which originates on the phyllides and often has a basal foot cell, the spines are spherical and sometimes curved. These spores consist of a group of short-term assemblies and chlamydia spores that appear either in single cells or in short-term or interstitial chains similar results were reported by Pitt et al (1997).

**Pathogenicity of the *F.o*.f sp. *lycopersici* on tomato plant:** The pathogen severity of the *F.o*.f sp. *lycopersici* was 35% because the pathogen has a high ability to infect tomato plants, where the symptoms of the disease were characterized on the plants diseased by yellowing of leaves and wilting of the plant. This may be attributed to the production of many toxic substances, including fusaric acid and fusaric acidordyhed and lycomarasmine, which inhibit the process of formation of tylosate in the wooden vessels as well as the effect of the process of cellular respiration as a result of its association with the iron element and its interaction with oxidase enzymes in the process of breathing (Dawar 2007). The yellowing of the leaves of the plant was due to the production of cellulose-decomposing enzymes and pectin that break down cell walls (Agrios 1997).

**Molecular diagnosis:** Molecular classification technologies adopted RFLP-PCR and sequencing to diagnose fungal isolates isolated from tomato plants and from different areas of Basra Governorate. The results of molecular diagnostics indicate that DNA extraction by using CTAB Buffer method from all isolates of *F. o*. f. sp *lycopersici* proved the efficacy in subsequent tests. The clear DNA bundles using the electrophoresis technique on acarose gel (% 2) indicated that the extraction method used has demonstrated its efficiency in obtaining a large amount of genome for *F.o.* f sp. *lycopersici* as compared with other methods such as the use of the

cactus method and the Lysis Buffer method. In addition to being an economical and inexpensive method also gives very good results. These observations agreed with many studies that confirmed the efficiency of the CTAB Buffer method in extracting DNA from the fungi (Kerenyi et al 1999, Pant et al 2009, Karthikeyan et al 2010).

**Estimation of DNA:** The amount of DNA extracted from all isolates of the *F. o.* f. sp *lycopersici*, which were measured on the 593 and 583 nanometers, were of good quantity and for all isolates as the DNA concentration.

**Polymerase chain reaction of PCR-RFLP technology:** The use of prefixes mentioned has given positive results through the success of the amplification process with all the isolates of the pathogen *F. o*. f. sp *lycopersici* isolated from the stems of tomato plants infected with fusarium wilt and succeeded in distinguishing between isolates, as polymorphic bands and non-genetically shaped packages appeared monomorphic band.

**Prefix OPA- 01:** The number of total packages obtained by using this prefix 03 packs, as shown in the figure of this number, included 21 genetically formed packets with a genetic variation of 63% and the most were from field isolation (8 packs) and isolation of Safwan was with smallest number of packets( two packages). The field isolation also recorded the most number of genetically shaped packages, which are 8 packages, while the minimum packaged groups were recorded at the isolation of Khor Al-Zubayr (two packages) and the maximum package was recorded at the field isolation (0933 base pair) .

**Effect of different treatments on plant height in contaminated and non-contaminated soil:** These results agreed with many studies that indicated the role of biological factors in increasing plant lengths and the number of branches through many mechanisms including stimulating growth in the plant , increasing the rigidity of the plant cell wall and increasing nutrients and make them dissolved and ready to be easily absorbed by the plant and its role in competing for the nutritional requirements pathogens with several pathogens (Yedidia et al 1998, Vazquez et al 1998). The increase in plant lengths and the number of branches was due to the role of biogenical factors in stimulating plant growth. *T. harzianum* has a role in increasing growth by supplying phosphorus and regulating ethylene production in the roots (Haupt 2007, Martinez-Medina et al 2010)

**Effect of different treatments on the dry shoot system weight in a contaminated and non- contaminated soil:** The highest mean dry shoot system weight of the tomato plant was in ThH (27.24 g,) followed by Th and H at 26.45 and 25.38 g respectively which was significantly higher than the control (18.12 g). In a soil that is not contaminated with the pathogen dry shoot system weight of Tomato plant was 25.78 g, which was significantly higher than the control (17.17 g) The Th and H treatments recorded 24.93 and 24.17 g respectively. The highest vegetative dry weight rate in ThH and H in contaminated and non-contaminated soil with the pathogenic fungus 26.51 and 25.69 g followed by the treatment of H, where the rate of treatment was 24.78

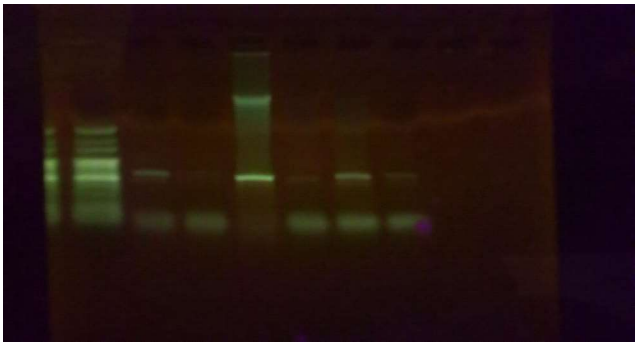

**Fig. 1.** Electrophoresis of the genome *F. o*. f. sp *lycopersici* for all d isolates

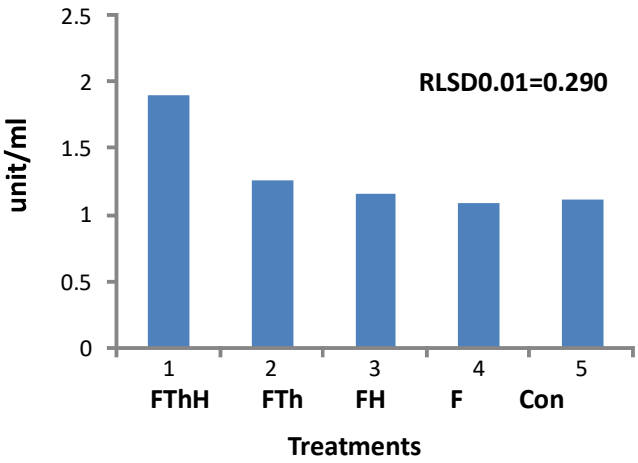


**Fig. 2.** Effect of different treatments on the concentration of peroxidase enzyme in fusarium wilt infected plants

**Table 1.** Effect of different factors on plant length (cm) contaminated and non - contaminated soil in the field

| Treatment | Height (cm) | | Average |
|---|---|---|---|
| | With *F.o.* | Without *F.o.* | |
| ThH | 88.74 | 91.72 | 90.23 |
| Th | 81.94 | 90.71 | 86.33 |
| H | 80.25 | 86.71 | 83.48 |
| Control | 61.37 | | 66.07 |
| Average | 87.08 | 84.98 | |

CD (p=0.05)
Treatment = 3.13, Contaminated and uncontaminated = 1.92 , Interaction = 1.56

compared to the control treatment, which was 17.65, while the average dry weight of vegetative in contaminated and non-contaminated soil was 23.01 and 24.29 g. The average dry weight of the vegetative group of tomato plants obtained in the treatment soil not contaminated with the pathogen differed significantly from treatments contaminated with pathogenic fungi, as the dry weight of the vegetative group of tomato plants occurred in the treatment ThH (25.78 g), which was distinguished by highly significant differences from the control treatment (17.17 g) followed by the treatments Th and H (24.93 and 24.17 g, respectively). The vegetative dry weight in ThH and H with soil contaminated and not contaminated with the pathogenic fungus 26.51 and 25.69 g followed by treatment H (24.78) as compared to the control (17.65) while the average vegetative dry weight in contaminated and unpolluted soil was 23.01 and 24.29g.

**Determination of peroxidase enzyme** There was increased in peroxidase enzyme in the treatment with humic acid (HTh) in the plants cultivated in soil contaminated with the pathogen, as the enzymatic activity reached 1.297 units $g^{-1}$ net weight, which followed by Th (1.099 units $g^{-1}$ wet weight,) which also differed significantly from other treatments, followed by humic acid (H) (0.990 units $g^{-1}$ wet weight) compared to the control treatment (0.357 units $g^{-1}$ wet weight).

**Determination of polyphenol oxidase enzyme:** The polyphenol oxidase enzyme (Fig. 3) showed an increase in humic acid (ThH) in soil contaminated with pathogenic fungi (1.908 units $ml^{-1}$) and was significantly superior to other treatments. This was followed by biological agent Th and acid

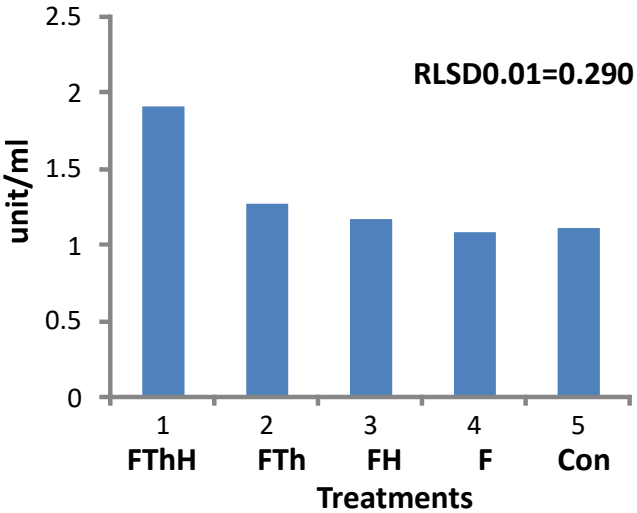


**Fig. 3.** Effect of different treatments on the enzyme polyphenol oxidase in tomato plant as planted in soil contaminated with pathogen

**Table 2.** Effect of different treatments on dry shoot system weight on contaminated and non-contaminated soil

| Treatment | Dry weight (g) in soil | | Average |
|---|---|---|---|
| | With *F.o.* | Without *F.o.* | |
| ThH | 25.78 | 27.24 | 27.51 |
| Th | 24.93 | 26.45 | 25.69 |
| H | 24.17 | 25.38 | 24.78 |
| Control | 17.17 | 18.12 | 17.65 |
| Average | 22.31 | 24.29 | |

CD (p=0.05)
Treatment = 1.09, Contaminated and uncontaminated = 0.67 , Interaction = 0.55

Humic acid (H) as, compared to the control and pathogen (F) treatment (0.118 and 0.951 units $ml^{-1}$ respectively). Barcelo et al (1996) indicated the ability of the biological agent *Trichoderma* spp. to induce resistance to many plant pathogenic fungi as a result of increased peroxidase secretion in the plant. Rodrigues (2011) indicated the increase of peroxidase and polyphenol oxidase in the roots of infected tomato plants induce resistance against many pathogens.

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