International Journal of Design & Nature and Ecodynamics
Vol. 20, No. 11, November, 2025, pp. 2593-2603
Journal homepage: http://iieta.org/journals/ijdne

# Biocontrol of Tomato Fusarium Wilt by Native *Trichoderma harzianum* and Mycorrhizae in Combination with Chemical Fungicide

Rusul Abdulatef Abdulhussein Altamemi[1*], Zeinab A. M. Al-Tememe[2], Amira Mougou[1]

[1] The National Agronomic Institute of Tunis (INAT), University of Carthage, Tunis 1082, Tunisia
[2] Department of Animal Production, Faculty of Agriculture, Karbala University, Karbala 56001, Iraq

Corresponding Author Email: rusulaltimimi7@gmail.com



https://doi.org/10.18280/ijdne.201110

**Received:** 11 September 2025
**Revised:** 20 October 2025
**Accepted:** 26 October 2025
**Available online:** 30 November 2025

***Keywords:***
*Fusarium oxysporum, Trichoderma harzianum, PCR, Iraq*

**ABSTRACT**

Biocontrol using the fungus *Trichoderma harzianum* demonstrated high efficacy against *Fusarium oxysporum*. In laboratory tests, the inhibition rate reached 84.73%. Under greenhouse conditions, the germination rate and seedling mortality rate were 69.33% and 12.70%, respectively, compared to the *F. oxysporum* control group, which recorded 31.00% germination and 92.33% seedling death. Polymerase chain reaction (PCR) was used to confirm the molecular identity of the *T. harzianum* isolate. In untreated control tomato plants, the infection rate reached 82.34%, with a disease severity index of 0.76. Application of mycorrhizal fungi significantly reduced the infection rate and severity to 35.97% and 0.36, respectively. The pathogenic *F. oxysporum* isolate was originally obtained from infected tomato plants for further pathological studies. Evaluation of different treatments indicated significant improvements in plant growth and a reduction in disease incidence. Treatment with fungicide Beltanol in *F. oxysporum* contaminated soil notably improved plant performance: the average fresh weight of plants, fruit yield, and dry weight reached 39.19 g, 49.5 g, and 31.50 g, respectively, with an average plant height of 61.11 cm, compared to the infected control group, which recorded 14.51 g, 18.9 g, 11.36 g, and 32.45 cm, respectively. The combined treatment of *F. oxysporum* + Beltanol + *T. harzianum* + mycorrhizal fungi was the most effective, yielding the highest fresh weight (54.4 g), dry weight (43.58 g), and plant height (63.58 cm). These results highlight the synergistic effects of biological and chemical control methods in reducing disease severity and promoting significant tomato plant growth. A factorial randomized complete block design (R.C.B.D.) was used, and the data were statistically analyzed using SAS software.

## 1. INTRODUCTION

Fusarium species associated with tomato are grouped into various phylogenetic species complexes, including non-pathogenic species (useful as biocontrol agents) as well as polyphagous (*Fusarium solani*) and specific pathogens (*Fusarium oxysporum* f. sp. *lycopersici*) of tomato [1]. *Fusarium oxysporum* is a complex and important species within the genus Fusarium, and is the main cause of Fusarium wilt disease in many economically important agricultural and horticultural crops, such as tomatoes, cruciferous vegetables, cotton, bananas, and melons [2].

*Trichoderma* spp. produces a wide range of highly effective biological substances and enzymes, and it is widely used as a bio-control agent against many plants' pathogenic fungi [3, 4].

*Trichoderma* spp. belongs to Deuteromycotina, Hyphomycetes class, Moniliales order, Moniliaceae family; it has a sexual phase classified under Division Ascomycotina, Hypocreales order, Hypocrea genus [5]. The most important traits of species affiliated with *Trichoderma* spp. are the speed of growth on industrial growing media and the production of huge numbers of small-sized conidia of green or white colors, which are located at the end of multi-branched conidiophores. *Trichoderma* is able to grow, reproduce, and spread in various environments. It grows in almost all types of soil and on decomposing plants, and it is rare for it to parasitize plants [6]. Several studies have demonstrated the important role of biocontrol fungi in reducing the severity of infection with many root diseases and seedling drop resulting from pathogenic soil-borne fungi and fungus-like pathogens such as *R. solani*, *Verticillium dahliae*, *Phytophthora* spp., *Fusarium* spp., and *Pythium* spp. [7].

Arbuscular mycorrhizae has attracted the attention of scholars and researchers in the field of biofertilization and biological control, due to its ability to stimulate plant growth and productivity, as well as its capacity to limit the growth and pathogenicity of many pathogens [8, 9]. Moreover, it was found recently that these fungi are also able to induce plant resistance against many pathogens. What reinforces this hypothesis is the cellular and chemical defenses that appear in the plant in the presence of the pathogen. In fact, Cordier et al. [10] found that the presence of *Glomus mosseae* inside the root cells of tomato enhances the ability of the plant to reduce the spread of the fungus-like pathogen *Phytophthora parasitica*

inside the root cells by increasing the thickness of the infected cell wall through the deposition of pectin and callus.

Fungicides have been used as one of the important chemical means with the aim of controlling many plant diseases by inhibiting the growth of fungi or killing pathogens, which leads to a reduction in the rate of fungal infection. Studies [1, 11] mentioned the importance of chemical control to reduce plant pathogens, which is one of the most common and widely used methods to reduce disease progression.

Studies have shown that treatment with the chemical fungicide Beltanol reduced the severity of pear root rot caused by the fungus *Fusarium solani*. Beltanol, at a concentration of 1 ml/L, was also used to control root rot caused by the fungus *Rhizoctonia solani* in bean roots, and it successfully inhibited the growth of the pathogenic fungus *R. solani* on PDA media and in semi-closed field environments [12].

Although many fungicides have been used to control Fusarium wilt, their continued use is difficult due to the emergence of resistant isolates and their negative effects on the natural environment, agricultural systems, and human health [13].

Therefore, the current study aims to find effective and environmentally safe alternatives for disease control, focusing on the use of local *Trichoderma* spp. fungi and mycorrhizal fungi to combat Fusarium wilt and promote the growth of tomato plants.

## 2. MATERIAL AND METHOD

### 2.1 Isolation and characterization of *Trichoderma* species naturally associated with tomato, and testing their disease-suppressive potential

One gram of well-mixed soil was taken and added to 9 ml of sterile distilled water in a test tube. The suspension was then shaken vigorously for 30 seconds to ensure proper mixing.

Subsequently, a series of serial dilutions was performed.

1 M sample was taken from the fifth dilution and plated onto a PDA (Potato Dextrose Agar) medium. The sample was spread evenly across the medium's surface by stirring.

The plates were incubated for 2-3 days at a temperature of 25 ± 2°C [14]. Following incubation, purification of the isolated colonies was performed.

$$Percentage\ of\ frequency = \frac{The\ number\ of\ times\ fungi\ appear}{The\ number\ of\ total\ colonies} \times 100 \quad (1)$$

According to research [15], tests have been done on antagonistic ability for the identification of the most active isolates

### 2.2 Isolation of the Trichoderma from soil

1. Collecting soil samples for the purpose of isolating the fungus *Trichoderma* spp.

Soil samples were collected randomly from tomato greenhouses at a depth of 10-15 cm from the area near the rhizosphere, a quantity of approximately 5-10% samples per greenhouse was taken and the samples were placed in polyethylene sterile bags, then it was transferred to the Laboratory of the College of Agriculture / University of Karbala for the purpose of isolating the fungus.

2. Isolation of *Trichoderma* spp. fungus from soil (Dilution plate method)

Samples were prepared after sifting them with a fine sieve to get rid of impurities and stones stuck in them. Soil samples of 10 grams were taken and placed in a 500 ml beaker containing 90 ml of sterile distilled water to obtain a stock solution [16] were then put on a magnetic stirrer for 20 minutes to mix the soil well with water. A volume of one ml of the soil solution was added to nine ml of sterile distilled water and mixed for one minute to homogenize the solution to obtain a dilution of $10^{-1}$. The process was repeated until reaching the sixth dilution, 0.1 ml each taken from the last three dilutions ($10^{-6}$, $10^{-5}$, and $10^{-4}$) and injected into Petri dishes containing the solidified nutrient medium Potato Dextrose Agar (PDA), and added antibiotic chloramphenicol. Dishes were stirred horizontally and in a circular manner to ensure homogenization of the soil on the surface of the nutrient medium. Three replicate dishes were used for each soil dilution. The dishes were incubated (25 ± 2°C) for 5-7 days with continuous checking of any fungal growth [17]. Fungal colonies were purified by transferring hyphal tips from the growing colonies into new Petri dishes containing fresh PDA medium with added chloramphenicol. The plates were incubated again under the same conditions as previously described.

Isolated fungi were identified based on their taxonomic characters mentioned in the research [18].

### 2.3 Contrast ability test in vitro

To analyze the effect of selected biocontrol agents (*T. harzianum*, *T. asperellum*, and *T. viride*) against the target pathogen *Fusarium oxysporum* f. sp. *lycopersici*, a double-cropping method was adopted on PDA medium.

Medium plates were divided into two equal parts using a stationary pen. The center of the first half was inoculated with a 5 mm diameter disc taken from a fresh colony of the pathogenic fungus, while the center of the second half was inoculated with a similar disc taken from the edge of a fresh colony of the resistant biocontrol fungus.

In the third treatment, the center of the plate was inoculated with two discs: one from the pathogenic fungus and the other from the resistant fungus, to study the interaction between them.

Three replicates were performed for each double-cropping treatment, and the plates were then incubated at 25 ± 2°C. The results were observed after seven days of incubation according to the scoring scale shown in Table 1 [16].

**Table 1.** Measurement [16]

| Grade Awarded | Rated Observations |
|---|---|
| 1 | The biocontrol agent completely covers all the dishes |
| 2 | The biocontrol agent covers 2/3 of the dish |
| 3 | The biocontrol agent and the target pathogen both cover half the dish |
| 4 | The target pathogen covers 2/3 of the dish |
| 5 | The target pathogen covers the entire dish |

Fungal agents rated within grades 1 and 2 have a high biocontrol capacity. The most pathogenic isolate of *F. oxysporum* was obtained from infected tomato plants and identified through detailed phenotypic and microscopic examination.

The percentage of inhibition was determined by measuring the radius of the pathogenic fungus's growth and comparing it

to the growth of the bioresistant fungi in the control treatment.

Fungi were inoculated 1 cm from the edge of a Petri dish for each isolate separately to assess the effectiveness of the biocontrol agent in reducing pathogen growth.

The percentage was calculated using the Abbott equation [19], as follows:

$$Percentage\ of\ inhibition = \frac{Average\ colony\ radius\ in\ Control - Average\ colony\ radius\ in\ Treatment}{Average\ colony\ radius\ in\ Control} \times 100 \quad (2)$$

### 2.4 Contrast ability test in greenhouses

In the greenhouse experiment, a mixture of sandy soil and peat moss at a ratio of 1:3 (w/w) was used as the culture medium. This experiment was carried out in plastic pots in the greenhouses of the College of Agriculture - University of Karbala, where the soil and peat moss were mixed and sterilized using an autoclave device at a temperature of 121°C and a pressure of 15 psi for 60 minutes. The next day, the sterilization process was repeated under the same conditions mentioned previously. The sterilized soil was placed in 3 kg plastic pots at a rate of three replicates for each isolate. The treatment was left without adding the pathogenic fungus (control treatment), and the *Trichoderma* spp. fungus was added to the soil at a rate of two grams per kilogram of soil. The biological agent was added to the soil before planting. As for the pathogenic fungus, it was added to the soil at a rate of 1 gram per kilogram of soil and added to the soil before planting (the *F. oxysporum* fungus was grown on a medium of millet). In the final treatment, the pathogenic fungus *F. oxysporum* and *Trichoderma* spp. were added. Surface-sterilized tomato plant seeds were disinfected with sodium hypochlorite solution (15% free chlorine) and sown in the prepared pots at a rate of 10 seeds per pot.

The experiment was conducted with three replicates for each individual treatment. Pots were distributed in the greenhouse. The temperature ranged from 20 to 25°C. According to the modern commercial design, it was completed as follows:

1. Control treatment – without any fungus.
2. Treatment with *F. oxysporum* only.
3. Treatment with *Trichoderma* spp. and *F. oxysporum*.

The percentage was calculated two weeks after planting according to the following equation:

$$Germination\ percentage = \frac{Number\ germinated\ seeds}{Total\ number\ of\ seeds} \times 100 \quad (3)$$

The percentage of seedling death was calculated four weeks after planting according to the following equation:

$$Percentage\ of\ seedling\ death = \frac{Number\ of\ dead\ seedling}{Total\ number\ of\ esssgling} \times 100 \quad (4)$$

### 2.5 Morphological characterization of *Trichoderma* spp.

Three selected isolates of the fungus were characterized based on the phenotypic traits of their colonies and microscopic examination after culture on Potato Dextrose Agar medium (PDA) and using the following taxonomic keys (Table 2). Then, the polymerase chain reaction technique was used at the In-Center Asco Learning/Baghdad/Iraq to molecularly identify the isolate under study.

**Table 2.** Primer sequences

| Primer Name | Sequence (5′→3′) | Target Region | Function |
|---|---|---|---|
| ITS1 | TCCGTAGGTGAACC TGCGG | Upstream of ITS1 | Forward Primer |
| ITS4 | TCCTCCGCTTATTG ATATGC | Downstrea m of ITS2 | Reverse Primer |

**Table 3.** Reaction components

| Component | Volume (µL) |
|---|---|
| Template DNA (Genomic DNA) | 1.0 |
| Forward Primer | 1.0 |
| Reverse Primer | 1.0 |
| Taq DNA Polymerase 5 U/ µL | 0.2 |
| dNTP Mix (10 mM) | 0.5 |
| $MgCl_2$ (usually 50 mM stock) | 1.5 |
| 10XPCR Buffer | 2.5 |
| Nuclease-Free Water | Up to 25.0 |
| Total Reaction Volume | 25.0 |

### 2.6 Experiment in the greenhouse

In the greenhouse experiment, a mixture of sandy soil and peat moss was used in a ratio of 1:3 v/v. This experiment was carried out in plastic pots in the greenhouses of the College of Agriculture - University of Karbala, where the soil and peat moss were mixed and sterilized using an autoclave device at a temperature of 121°C and a pressure of 15 pounds for two inch for 60 minutes, and on the next day, the sterilization process was repeated again under the same conditions mentioned previously. The sterilized soil was planted in three kg plastic pots at a rate of three replicates for each isolate The treatment was left without adding the pathogenic fungus (control treatment), and the *T. harzianum* fungus was added to the soil at a rate of two grams per kilogram of soil and the biological agent was added to the soil before planting as for the pathogenic fungus, it was added to the soil at a rate of 1 gram for kg of soil and added to the soil before planting, In the final treatment, the pathogenic fungus *F. oxysporum* and *T. harzianum* were added. Surface-sterilized tomato plant seeds were disinfected with sodium hypochlorate solution (15% free chlorine) and sown in the prepared pots at a rate of 10 seeds/per pot.

The experiment was conducted with three replicates for each individual treatment; Pots were distributed in the greenhouse. The temperature ranged from 20 to 25°C, and the growth period was two and a half to three months. According to the Modern commercial design, it was completed as follows:

1. Control treatment - without any fungus.
2. Treatment with *F. oxysporum* only.
3. Treatment with *T. harzianum* and *F. oxysporum*.

The infection percentage of tomato plants was calculated and surveyed regardless of the size of the infection using the following equation:

$$Percentage\ of\ infection(\%) = \frac{The\ number\ of\ infected\ plants}{The\ Total\ number} \times 100 \quad (5)$$

A satisfactory index was established according to the scale of research [20]. It consists of five grades, and according to McKinney [21], the degree of infection is estimated according to the appearance of the infection, based on the following evidence (Table 3).

**Table 3.** A satisfactory index scale shows a pathological scale of five grades

| Infection Severity Rating | Percentage of the Infected Part of Tomato Plants |
|---|---|
| 0 | Healthy (no disease symptoms). |
| 1 | There is no wilting of the plant, but a slight discoloration of the vessels and yellowing of the leaves occurs. |
| 2 | Complete discoloration of the roots from inside, complete yellowing of the leaves, drooping and falling, and slight wilting. |
| 3 | The discoloration extends from the roots to the bases of the stems and becomes pale with the death of some of its vegetative parts. |
| 4 | Plant death. |

The degree of infection is estimated according to the appearance of the infection.

The severity of the infection was calculated according to the equation [22] as follows:

$$Disease\ severity = \frac{Number\ Pants\ Category\ (0) + \cdots + number\ of\ plants\ Category\ (4)}{Total\ number\ tested\ Top\ category\ index} \times 100 \quad (6)$$

### 2.7 Isolation of mycorrhizal fungi from the soil

For isolating the mycorrhizal fungus (locally), samples were taken from the soil surrounding the roots of the experimental plants (randomly from the rhizosphere) using the method of perpendicular diagonals by creating an imaginary rectangle, Samples were collected from the four corners of the rectangle and from a diagonal intersection area and at a depth of (5.5 - 25 cm) of the rhizosphere, Samples were collected from different soils from fields planted with the experimental plants, The samples were mixed together separately in order to obtain a homogeneous and representative sample for the entire field. The samples were placed in sterile nylon bags and recorded on them (location and host plant), and kept in the laboratory until use in the future for isolation purposes. The wet testing method previously described was followed study [23], for the purpose of isolating spores and later diagnosing them. This method consisted of weighing 250 grams of soil and placing it in a 2.5-liter glass beaker to which one liter of running water was added. The mixture was stirred well and left for 1-2 minutes to allow the soil particles to fall to the bottom of the beaker. The suspension was then passed through A set of sieves with diameters of 177, 90, 50, and 35 microns. The contents of the sieve (50 and 35 microns) were collected separately in 100 ml glass beakers by using a slow stream of water, then placing 10 ml of the suspension in a dish for the purpose of examination using a dissection microscope at 10× magnification, the spores were picked up with a Pasteur pipette and placed in a watch bottle. In the presence of a little water, the examination was repeated at 40× magnification, and the diagnosis was made based on the spore's phenotypic characteristics, which included the color, the size, the shape, and the diameter of the hyphae attached to the spore and the nature of the outer layer of the spore [24]. The diagnosis was made based on the morphological characteristics of the spore, based on the taxonomic key published in reference [25]. In order to obtain pure cultures of the mycorrhizal fungus, single spores of the isolate under study were captured and inoculated with three-day-old millet seedlings that had been previously grown under laboratory conditions in plastic dishes containing a filter paper, the seedlings were inoculated by picking up the spores with forceps or a pipette and transferring them to the roots of the seedlings which were then planted in sterilized sandy soil previously sterilized at 121℃ and a pressure of 1.2 $kg/cm^2$ for an hour, The sterilization process was repeated for three consecutive days [26]. The plastic pots were placed in the greenhouse (Karbala University / College of Agriculture), and after two months, the roots were examined to confirm whether the infection had occurred. The development of these seedlings continued for three months until they were ready as an inoculum for use in subsequent field experiments.

### 2.8 Increased mycorrhizal inoculation

Plastic pots taking of five kg plastic pots capacity containing a mixture of previously sterilized soil and peat moss in a ratio of 1:2 were used They were added by 50 grams of the inoculum under the surface layer of the potting soil, Another 50 grams of the inoculum was mixed with the top layer of the potting soil and the local millet seeds that had previously been planted, The outer surface was sterilized with sodium hypochlorate at a concentration of 1% of the commercial solution for three minutes, and 25 seeds were planted in one pot [27]. The seeds dwindled to 10 after a week of planting. Seeds with the root and shoots were removed six weeks after germination, and samples from the soil and roots were then examined. under a microscope to ensure that the roots are infected with mycorrhizae after staining them with a dye acid fuchsin method according to study [28] which was mentioned that after washing the roots well with running water, they were cut into small pieces of one cm length and placed in 20 ml glass vials, a 10% KOH solution was added to it, and then the roots were placed in a water bath at 70℃ for 15 minutes, then the solution was poured and the roots were washed with distilled water three times, and a 1% hydrogen peroxide solution ($H_2O_2$) was added to it, the roots were left for 10 minutes at room temperature, after which the solution was poured off and the roots were washed well with distilled water, 1% hydrochloric acid was added to it, and the roots were left in the solution for three minutes at room temperature. Then the acid was poured without washing the roots, and finally the roots were stained with Acid fuchsin dye, which was prepared in the laboratory according to research [29] as follows:

63 ml glycerin, 875 ml lactic acid, and 63 ml distilled water.

After adding the dye, the roots were placed in a water bath at 70℃ for 30 minutes. Then, the root pieces were transferred to a glass slide at a rate of 10 pieces per slide, and they were examined under a light microscope.

The cultivation and soil sterilization processes were carried out in the same way as mentioned when testing Trichoderma fungi in the greenhouse.

The experiment was carried out by making three replicates per individual treatment. Pots were distributed in the plastic house according to the Modern commercial design. It was completed as follows:

1) Control treatment - without any fungus.
2) Treatment with Mycorrhizal only.

3) Treatment with *T. harzinum* only.
4) Treatment with *F. oxysporum* only.
5) Treatment with *F. oxysporum* and Mycorrhizal.
6) Treatment with *F. oxysporum* and Beltanol.
7) Treatment with *F. oxysporum* and *T. harzinum*.
8) Treatment with *F. oxysporum*, Beltanol, and Mycorrhizal.
9) Treatment with *F. oxysporum* and Beltanol, and *T. harzinum*.
10) Treatment with *F. oxysporum* and Mycorrhizal, and *T. harzinum*.
11) Treatment with *F. oxysporum* and Beltanol, and *T. harzinum*, and Mycorrhizal.

Beltanol: The active ingredient: 8-hydroxyquinoline sulphate 50% w/v (500 g/L), manufacturer: Probelte, specific concentration used in this study: 1 ml per liter of water.

### 2.10 Statistical analysis

A factorial randomized complete block design (R.C.B.D.) was used. The results were analyzed using SAS software, with four replications for each treatment. Mean differences were tested using the Least Significant Difference (LSD) test at a probability level of 0.05.

## 3. RESULTS AND DISCUSSION

### 3.1 Isolation and characterization of *Trichoderma* spp. naturally associated with tomato and testing their disease-suppressive potential

A total of 23 *Trichoderma* spp. isolates were obtained and distributed over the survey regions. The most virulent (*Trichoderma* spp.) isolates were identified for subsequent testing.

### 3.2 Screening of their disease-suppressive potential and elucidation of their antifungal compounds

Three species of the genus (Figure 1) *Trichoderma* were obtained from the isolation process: *T. harzianum, T. asperellum,* and *T. viride*. *T. harzianum* was identified for use in field experiments because it is better than two fungi, *T. asperellum* and *T. viride,* in inhibiting fungal growth of *F. oxysporum*; the inhibition rate was recorded at 84.73% (Table 4).

In the greenhouse, the germination rate and seedling death rate for the treatment (T23+*F. oxysporum*) were recorded as 69.33% and 12.70%, respectively, according to Table 5.

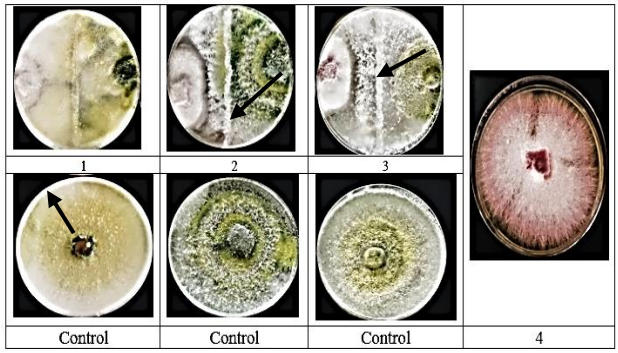



**Figure 1.** The antagonistic ability of (1) *T. harzianum*, (2) *T. asperellum,* (3) *T. viride*, and (4) *F. oxysporum*

The contrasting ability of the fungus has been shown in *Trichoderma* spp. (Table 4) against *F. oxysporum*. The highest inhibition rate, 84.73%, was recorded for isolate number 23. The lowest inhibition rate was recorded for isolate number 1, which reached 27.3%.

The results shown in Table 5 indicate that fungal isolates *Trichoderma* spp. play an important role in reducing seed rot and seedling death, but at varying rates, where the germination rate ranged between 28.66%-69.33%. While the comparison infected with the fungus recorded a germination rate of *F. oxysporum* 31.00%, with a significant difference. The seedling death rate ranged between 12.70%-30.33%.

The germination and seedling death percentages for plants treated with *F. oxysporum* were recorded at 31.00% and 92.33%, respectively. Compared to the control, which recorded a germination rate of 92.23% and a seedling death rate of 0.00.

The biocontrol agent *T. harzianum* showed high antagonistic capacity against *F. oxysporum* under study under laboratory conditions, reaching 1.0 on the Bell scale, which is the highest compared to the *T. asperellum* and *T. viride*. In addition to the comparison treatment, it is known that the bioresistant fungi *Trichoderma* spp. generally possess various mechanisms, including parasitism on pathogenic fungal hyphae, competition for the nutritional environment, and the production of some antibiotics that have an inhibitory effect on some of the pathogenic fungal enzymes, and their ability to produce a number of toxic chemical compounds such as gliotoxin, trichothecin, and viridin [30]. This is likely due to the production of vital antifungal enzymes that degrade the cell walls of pathogenic fungi, such as proteases, esterases, phosphatases, and others [31]. Or as a result of the secretion of inhibitory substances against the pathogenic fungus, thus reducing it [32], in addition to the clumping and adhesion of the spores of the bio-resistant fungus to the fungal hyphae of the pathogenic fungus, leading to its decomposition [33].

**Table 4.** Percentage of inhibition of isolates *Trichoderma* spp. against *F. oxysporum*

| *Trichoderm* Isolation Number | Percentage of Inhibition | *Trichoderm* Isolation Number | Percentage of Inhibition | *Trichoderm* Isolation Number | Percentage of Inhibition |
|---|---|---|---|---|---|
| 1 | 27.3 | 9 | 79.62 | 17 | 56.20 |
| 2 | 53.70 | 10 | 61.10 | 18 | 76.53 |
| 3 | 51.85 | 11 | 77.77 | 19 | 51.84 |
| 4 | 82.44 | 12 | 53.78 | 20 | 37.44 |
| 5 | 48.34 | 13 | 57.40 | 21 | 68.69 |
| 6 | 79.22 | 14 | 68.72 | 22 | 81.47 |
| 7 | 29.3 | 15 | 62.95 | 23 | 84.73 |
| 8 | 62.95 | 16 | 70.63 | | |

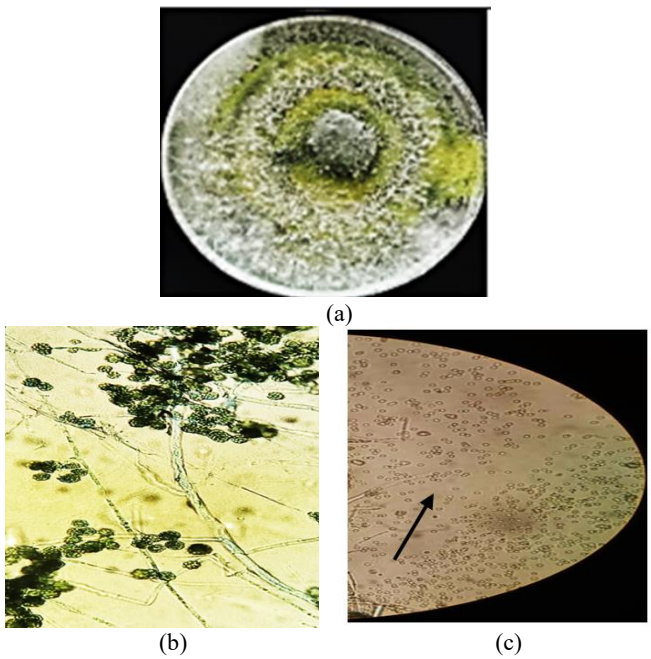
$LSD_{0.05} = 7.621$

**Table 5.** Effect of *Trichoderma* spp. on germination rate and seedling death rate of tomato seeds and seedlings treated with *F. oxysporum* under greenhouse conditions

| S | Treatment | Germination Rate, % | Seedling Death Rate, % |
|---|---|---|---|
| 1 | Control | 92.23 | 0.00 |
| 2 | *F. oxysporum* | 31.00 | 92.33 |
| 3 | T1+*F.oxysporum* | 39.23 | 30.33 |
| 4 | T2+*F.oxysporum* | 48.66 | 23.33 |
| 5 | T3+*F.oxysporum* | 43.66 | 24.66 |
| 6 | T4+*F.oxysporum* | 61.33 | 28.65 |
| 7 | T5+*F.oxysporum* | 55.33 | 26.66 |
| 8 | T6+*F.oxysporum* | 56.66 | 24.33 |
| 9 | T7+*F.oxysporum* | 37.66 | 31.33 |
| 10 | T8+*F.oxysporum* | 44.66 | 23.80 |
| 11 | T9+*F.oxysporum* | 56.33 | 28.66 |
| 12 | T10+*F.oxysporum* | 44.66 | 25.55 |
| 13 | T11+*F.oxysporum* | 44.66 | 21.57 |
| 14 | T12+*F.oxysporum* | 43.00 | 25.13 |
| 15 | T13+*F.oxysporum* | 40.45 | 22.33 |
| 16 | T14+*F.oxysporum* | 51.66 | 28.66 |
| 17 | T15+*F.oxysporum* | 44.66 | 23.80 |
| 18 | T16+*F.oxysporum* | 49.66 | 23.00 |
| 19 | T17+*F.oxysporum* | 43.33 | 22.66 |
| 20 | T18+*F.oxysporum* | 54.33 | 28.66 |
| 21 | T19+*F.oxysporum* | 43.66 | 27.66 |
| 22 | T20+*F.oxysporum* | 28.66 | 23.66 |
| 23 | T21+*F.oxysporum* | 51.66 | 25.66 |
| 24 | T22+*F.oxysporum* | 63.33 | 27.66 |
| 25 | T23+*F. oxysporum* | 69.33 | 12.70 |
| | $LSD_{0.05}$ | 6.615 | 16.43 |

### 3.3 Morphology of *Trichoderma harzianum*

The *T. harzianum* colony began as white, gradually turning dark green with a cottony texture. The spore secretion from the colony was largely liquid, covering the entire surface of the plates. The colony reached approximately 9 cm in diameter after three days of incubation at 25–27°C.



(a)

(b) (c)

**Figure 2.** *T. harzianum* (a) colony on PDA, (b) mass of conidia under microscope (400x), (c) conidia

Under microscopic examination, *T. harzianum* spores were smooth-walled, spherical to ovoid, often green and sometimes glassy, and clustered at the edges of the flasks. The sporophores branched into tufts, containing widely spaced, often irregularly curved flask-shaped spores (Figure 2). These results are consistent with those reported in the study [19].

The most inhibitory isolate was diagnosed molecularly using PCR. Nucleotide sequence analysis of the PCR product from the fungus isolated in this study demonstrated that, using BLAST, this isolate was identified as *T. harzianum*, as shown in Figure 3.

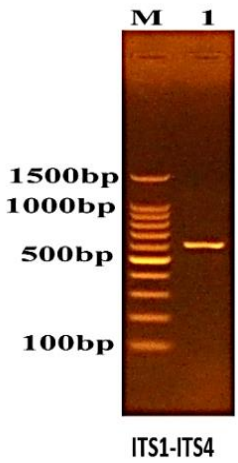



**Figure 3.** Agarose gel electrophoresis of amplified PCR product (550 bp)

The nearest-neighbor tree represents the genetic relationship between the fungi isolated in this study of the resistant fungus *T. harzianum* and other isolates of the same fungus previously recorded at the National Center for Biotechnology Information (NCBI), as shown in Figure 4.

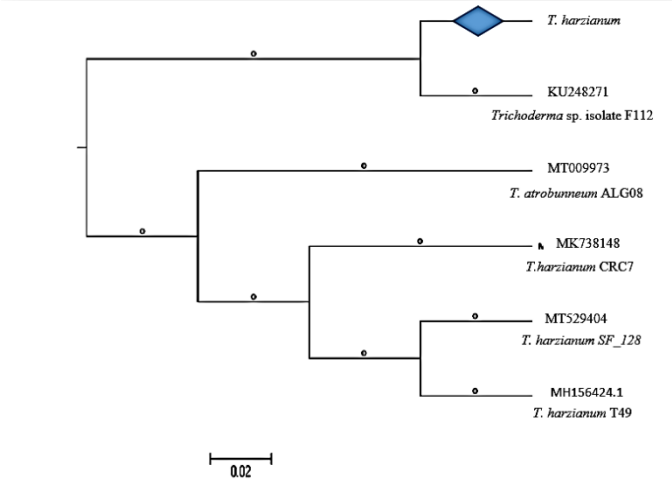



**Figure 4.** Neighbor-joining tree of *Trichoderma harzianum* isolate

### 3.4 Effect of using mycorrhizal fungus on the percentage of infection and disease severity

Table 6 and Figures 5 and 6 show, respectively, that the infection rate and disease severity in tomato plants treated with the pathogen *F. oxysporum* reached 82.34% and 0.76%, respectively, and also show a significant difference. The data also highlights the important role of mycorrhizal fungi in reducing both the infection rate and severity, as they recorded 35.97% and 0.36%, respectively.

**Table 6.** Effect of biological control using mycorrhizal fungus on the percentage and severity of infection by *F. oxysporum* of tomato plants

| Treatments | Disease Severity (%) | Percentage of Infection % |
|---|---|---|
| Control | 0.0 | 0.0 |
| Fol | 0.76 | 82.34 |
| M+ Fol | 0.36 | 35.97 |

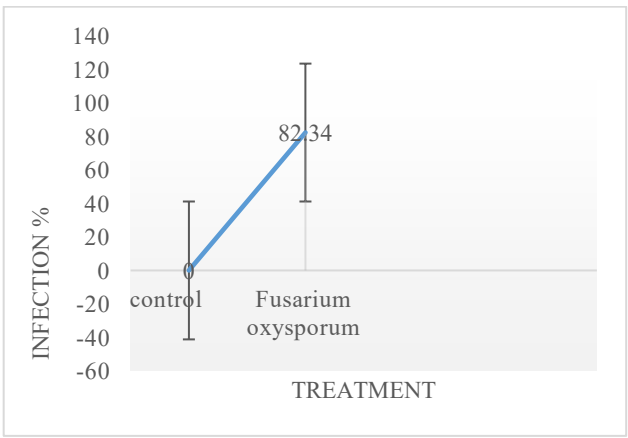



**Figure 5.** The effect of infection with the fungus *F. oxysporum* in tomato plants on the percentage of infection in the greenhouse

Here, the role of biological factors in stimulating systemic resistance becomes clear through the increase of enzymes (peroxidase, polyphenol oxidase, chitinase beta-glucan, and phenylenediamine), phenols, and the reduction of dead cells, all of which are reflected in the decrease in the incidence and severity of infection [34].

The isolate under study showed high efficiency in reducing the incidence and severity of infection. This can be attributed to the wide host range of the mycorrhizal fungus and its ability to colonize plant roots and thus improve plant growth by increasing the availability and absorption of nutrients (Figure 7).

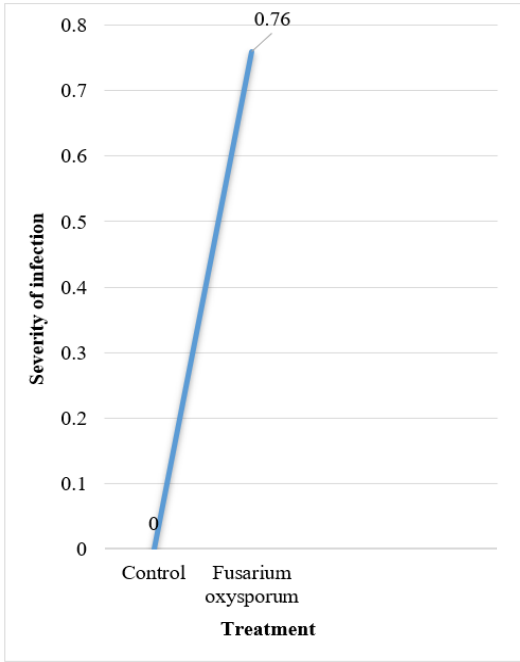



**Figure 6.** The effect of infection with the fungus *F. oxysporum* in tomato plants on the severity of infection in the greenhouse

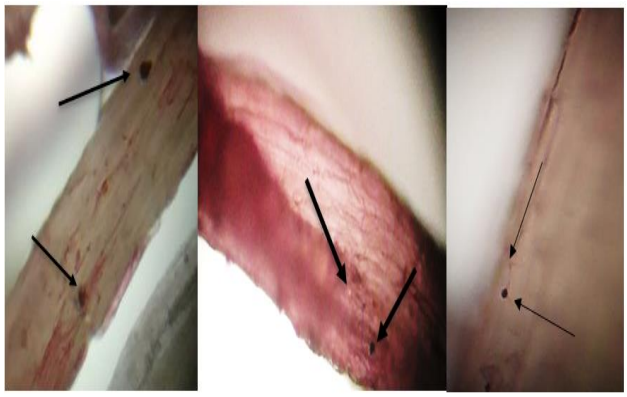


**Figure 7.** Presence of mycorrhizae in the roots (vesicles, suckers)

### 3.5 Evaluation of the efficiency of the chemical pesticide Beltanol, the *T. harzianum* and mycorrhizal against the pathogenic fungus *F. oxysporum*, and some growth parameters of tomato plants under greenhouse conditions

The results in Table 7 showed that all treatments achieved a significant increase in the growth parameters of tomato plants, represented by fresh and dry weights and plant lengths, compared to the control treatment of the *F. oxysporum* alone. In the same table, the combination of *F. oxysporum* with Beltanol, *T. harzianum*, and mycorrhizal fungi (F+B+T+M) resulted in the highest values for the measured growth

parameters among all treatments, highlighting the effect of the tested control factors. The average fresh weight of plants and fruits, and the dry weight of plants were 43.86, 54.4, and 43.58 g, respectively. The average plant height reached 63.58 cm, significantly higher than the *F. oxysporum*-only treatment, which had the lowest average fresh weight of plants and fruits, and dry weight was 14.51, 18.9, and 11.36 g, respectively, and the length of tomato plants (vegetative and root groups) reached 32.45 cm. On the one hand, adding mycorrhizal to a contaminated *F. oxysporum* inoculum achieved a significant increase in the fresh weight of plants and fruits and the dry weight of plants, 33.01, 48.5, and 26.48 g, respectively. The length of the plants (vegetative and root groups) reached 59.88 cm compared to the control treatment of the pathogenic fungus *F. oxysporum* alone. Regarding the treatment of adding the biological fungus *T. harzianum* to soil contaminated with the fungus *F. oxysporum* inoculum, it achieved a significant increase in all growth standards under study for tomato plants. The average fresh weight of plants and fruits, and dry weight of plants reached 38.57, 49.8, and 32.38 g, respectively. The length of the plants (vegetative and root groups) reached 61.70 cm compared to the control treatment of the pathogenic fungus *F. oxysporum* alone. The treatment with Beltanol added to soil contaminated with *F. oxysporum* inoculum achieved a significant increase in the fresh weight of plants and fruits, and the dry weight of plants was 39.19, 49.5, and 31.50 g, respectively. The length of the plants (vegetative and root groups) reached 61.11 compared to the control treatment of the pathogenic fungus *F. oxysporum* alone.

As for the treatments of adding the biological fungus harzianum and mycorrhizal, and the control treatment to which only sterilized millet seeds were added in the field soil not contaminated with the fungus *F. oxysporum* inoculum, each one separately, they all achieved a clear increase in the growth parameters under study for tomato plants, namely the fresh weight of fruits and plants, the dry weight of plants, and the length of plants (vegetative and root groups). The double and triple interaction between treatments was significantly superior to the control treatment contaminated only with the pathogenic fungus *F. oxysporum* inoculum for the traits under study.

**Table 7.** Evaluation of the efficiency of the chemical pesticide Beltanol, and the *T. harzianum* and mycorrhizal against the pathogenic fungus *F. oxysporum,* and some growth parameters of tomato plants under greenhouse conditions

| Treatment | Average Fresh Plant Weight, g/plant | Average Fresh Fruit Weight, g/plant | Average Dry Weight of Plants, g/plant | Average Plant Height, cm |
|---|---|---|---|---|
| Control | 38.61 | 50.3 | 30.45 | 58.39 |
| M | 40.37 | 51.9 | 31.49 | 59.88 |
| T | 41.44 | 53.4 | 32.20 | 61.09 |
| F | 14.51 | 18.9 | 11.36 | 32.45 |
| F+M | 33.01 | 48.5 | 26.48 | 56.99 |
| F+B | 39.19 | 49.5 | 31.50 | 61.11 |
| F+T | 38.57 | 49.8 | 32.38 | 61.70 |
| F+B+M | 39.03 | 48.8 | 32.86 | 61.88 |
| F+B+T | 42.35 | 50.2 | 39.87 | 60.64 |
| F+M+T | 39.51 | 50.7 | 30.28 | 56.99 |
| F+B+T+M | 43.86 | 54.4 | 43.58 | 63.58 |
| $LSD_{0.05}$ | 7.324 | 12.50 | 6.777 | 8.731 |

Beltanol is a chemical insecticide that has the ability to form chelating compounds with copper inside the host tissues, facilitating its passage into the pathogen's cells, and then being released to kill it [35]. The importance of chemical control in reducing plant pathogens can also be demonstrated [11], which is one of the most widely used methods to limit the development of diseases. Beltanol at concentrations of 0, 0.5, and 1 mg/L has shown an effective role in inhibiting *Alternaria alternata*, with superiority of 1000 and 1.0 mg/L concentration, which recorded a 100% rate of tested fungi. Showed that treatment with Beltanol at a concentration of 1 ml/L achieved the highest reduction in the severity of infection by pathogenic fungi [12]. These include the fungi Rhizoctonia solani and *Fusarium solani*. The study showed that the use of Beltanol significantly reduced the severity of infection by Fusarium graminearum [36], which causes root rot and stem canker in beans under woody vegetation conditions. The study also confirmed the effective role of Beltanol in reducing the severity of Fusarium wilt caused by *Fusarium oxysporum* in sumac [37].

*Trichoderma* is an important and highly beneficial fungus in controlling pathogenic fungi. Its advantages include remote sensing, identifying host fungi, speed in attacking plant pathogens, and suppressing their growth. The fungus has enzymes, including chitinase, proteases, and β-1,3-glucanase, which work to decompose the hyphal walls of pathogenic fungi, in addition to their important role in improving plant growth [38].

It has been demonstrated that *Trichoderma* spp. can induce resistance in tomato plants to the pathogen *F. oxysporum*, which works to increase the thickness of root cells, close the intercellular spaces, and prevent the development of the disease by not allowing the fungal hyphae to penetrate the adjacent healthy (uninfected) root cells.

It has also been indicated that arbuscular mycorrhizae play a fundamental role in stimulating the roots of tomato plants to produce metabolites in appropriate quantities and concentrations, giving the roots resistance to these compounds [39], including hydrogen peroxide, and increasing phenol and malondialdehyde (MDA) levels. The activity of enzymes that play a key role in lignification, such as POD, PPO, and PAL, also increases.

It has been demonstrated that mycorrhizal plants gain protection against pathogens compared to non-mycorrhizal plants [40]. Researchers reported an increase in the content of non-enzymatic antioxidants in tomato plants when they were treated with mycorrhiza and the pathogenic fungus *F. oxysporum* [41].

In a study conducted by Manila and Nelson [42], on tomatoes, an increase in mycorrhizal colonies, an increase in tomato biomass, and a decrease in the percentage of disease incidence and severity caused by the fungus *F. oxysporum* f. sp. *lycopersici* were observed. Kareem and Hassan [43] reported that the use of the fungus mycorrhizal and the pathogenic *F. oxysporum* led to a significant increase in phenolic content and

an increase in the activity of the enzymes POD and PPO in the root. Arbuscular mycorrhizal fungi can be used in biological control against pathogenic fungi in greenhouses and the field, as they have shown a significant reduction in the disease index and disease severity [44].

The results showed that the three mycorrhizal fungi reduced the severity of the disease and the length of the brown area affected by the disease, and led to a significant increase in plant height and the fresh and dry weights of the vegetative and root systems. They also outperformed the disease-infected plants in fruit number and weight [45]. Regarding the presence of *T. harzianum* with mycorrhizal fungi, which stimulated increased root colonization by mycorrhizal fungi, the results are consistent with what was reported in the study [46], which showed that the infection rate of eggplant roots with the mycorrhizal fungus *Glomus* spp. increased when it interacted with *T. harzianum*. These results are consistent with studies [47, 48] and also with what was reported in the study [49]. This efficacy is further supported by the accurate identification of the *F. oxysporum* isolate, which was confirmed according to the morphological and taxonomic criteria described in *The Fusarium Laboratory Manual* [50]. So we recommend prioritizing bio-resistance to benefit society and the agricultural sector in the future.

## 4. CONCLUSIONS

The study results demonstrated the high efficacy of *T. harzianum* in controlling *Fusarium oxysporum*. The growth inhibition rate of the pathogenic fungus in laboratory conditions reached 84.73%, confirming its ability to reduce the spread of the disease. The use of mycorrhizal fungi reduced the percentage and severity of infection by pathogenic fungi to 35.97% and 0.36%, indicating their role in enhancing plant resistance. Treatment with the fungicide Beltanol showed a significant improvement in the growth characteristics of infected tomato plants, with an increase in the average fresh and dry weight of plants and fruit weight, as well as plant height (shoot and root), compared to the control group, which showed the lowest rates. The treatment *F. oxysporum* + Beltanol + *T. harzianum* + Mycorrhizal was the best of all. It recorded the highest rates of fresh weight, dry weight, and plant height, indicating a synergistic effect between the different treatments in enhancing the growth and disease resistance of infected plants. Confirmation of *F. oxysporum* as the causative agent of the disease using polymerase chain reaction (PCR) technology, which adds reliability to the study results.

## ACKNOWLEDGEMENTS

We extend our thanks and gratitude to the Departments of Animal Production, Horticulture, and Landscape Engineering and the Deanship of the College of Agriculture at the University of Karbala for their assistance in completing this work.

## REFERENCES

[1] McGovern, R. J. (2015). Management of tomato diseases caused by Fusarium oxysporum. Crop Protection, 73: 78-92. https://doi.org/10.1016/j.cropro.2015.02.021

[2] Szczechura, W., Staniaszek, M., Habdas, H. (2013). Fusarium oxysporum f. sp. radicis-lycopersici – The cause of Fusarium crown and root rot in tomato cultivation. Journal of Plant Protection Research, 53(2): 172–176. https://doi.org/10.2478/jppr-2013-0024

[3] Bahadur, A., Dutta, P. (2022). *Trichoderma* Spp.: Their Impact in Crop Di seases Management (8th ed.). IntechOpen. https://doi.org/10.5772/intechopen.101846

[4] Nusaibah, S.A., Musa, H. (2019). A review report on the mechanism of *Trichoderma* spp. as biological control agent of the basal stem rot (BSR) disease of *Elaeis guineensis*. In Trichoderma - The Most Widely Used Fungicide. https://doi.org/10.5772/intechopen.84469

[5] Alexopoulos, C.J., Mims, C.W., Blackwell, M. (1996). Introductory Mycology. John Wiley and Sons, New York.

[6] Kamala, T., Devi, S.I., Sharma, K.C., Kennedy, K. (2015). Phylogeny and taxonomical investigation of *Trichoderma* spp. from Indian region of Indo-Burma biodiversity hot spot region with special reference to Manipur. BioMed Research International, 2015(1): 285261. https://doi.org/10.1155/2015/285261

[7] Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M. (2004). *Trichoderma* species—Opportunistic, avirulent plant symbionts. Nature Reviews Microbiology, 2(1): 43-56. https://doi.org/10.1038/nrmicro797

[8] Dhiab, N.S. (2012). The use of phosphate rock and superphosphate and the addition of fungal and bacterial fertilizers in the growth and yield of potatoes (PhD thesis). University of Baghdad, College of Agriculture.

[9] Ozgonen, H., Akgul, D.S., Erkilic, A. (2010). The effects of arbuscular mycorrhizal fungi on yield and stem rot caused by Sclerotium rolfsii Sacc. in peanut. African Journal of Agricultural Research, 5(2): 128-132.

[10] Cordier, C., Pozo, M.J., Barea, J.M., Gianinazzi, S., Gianinazzi-Pearson, V. (1998). Cell defense responses associated with localized and systemic resistance to Phytophthora parasitica induced in tomato by an arbuscular mycorrhizal fungus. Molecular Plant-Microbe Interactions, 11(10): 1017-1028. https://doi.org/10.1094/MPMI.1998.11.10.1017

[11] Al-Tememe, Z.A.M., Al-Sweedi, T.M.M., Abdullah, K.M., Jawid, C.K., Hamzah, R.M. (2019). Diagnosis of causes of cankers on apricot trees (Prunus armeniaca L.) using PCR technique in some of the Al-Qantara, Al-Baydaa orchards in Karbala, Iraq. IOP Conference Series: Earth and Environmental Science, 388: 012008. https://doi.org/10.1088/1755-1315/388/1/012008

[12] Altememe, Z.A.M., Alssirag, M.A., Bajlan, S.G.S., Al-Amirry, A.T., Kadhim, A.A. (2019). Study on Alternaria alternate, Fusarium chlamydosporum accompanying the Eucalyptus camaldulensis L. trees in some regions of Kerbala province /Iraq and identification of isolate with PCR technique. AIP Conference Proceedings, 2144(1): 040008. https://doi.org/10.1063/1.5123109

[13] Pritesh, P., Subramanian, R.B. (2011). PCR based method for testing Fusarium wilt resistance of tomato. African Journal of Basic & Applied Sciences, 3(5): 219-222. https://idosi.org/ajbas/ajbas3(5)11/5.pdf.

[14] Schaad, N.W., Jones, J.B., Chun, W. (2001). Laboratory Guide for Identification of Plant Pathogenic Bacteria. APS Press, USA.

[15] Bell, D.K., Well, H.D., Markham, C.R. (1982). In vitro antagonism of Trichoderma species against six fungal plant pathogens. Phytopathology, 72(4): 379-382. https://www.cabidigitallibrary.org/doi/full/10.5555/1982

1384099.
[16] Khan, A.M. (2021). A Book on Experiments of Microbiology. Women University Press. Jayoti Vidyapeeth Women's University, Jaipur. https://www.jvwu.ac.in/documents/90-%20FINAL-%20Practical%20microbiology%20book%20FINAL.pdf.
[17] Bhimani, A., Sohaliya, N. (2020). Isolation of soil mycoflora from agricultural fields of Narmada District. International Research Journal of Multidisciplinary Scope, 2(1): 50-53. https://doi.org/10.47857/irjms.2021.v02i01.050
[18] Harman, G.E., Kubicek, C.B. (2002). Trichoderma and Gliocladium: Basic Biology, Taxonomy, and Genetics. CRC Press, London. https://www.taylorfrancis.com/books/mono/10.1201/9781482295320/trichoderma-gliocladium-volume-1-kubicek-gary-harman.
[19] Altindag, M., Sahin, M., Esitken, A., Ercisli, S., Guleryuz, M., Donmez, M.F. (2006). Biological control of brown rot (*Monilinia laxa* Ehr.) on apricot (*Prunus armeniaca* L. cv. Hacıhaliloğlu) by *Bacillus*, *Burkholderia*, and *Pseudomonas* application under *in vitro* and *in vivo* conditions. Biological Control, 38(3): 369-372. https://doi.org/10.1016/j.biocontrol.2006.04.015
[20] Gao, T., Yao, H., Song, J., Liu, C., Zhu, Y., Ma, X., Pang, X., Xu, H., Chen, S. (2010). Identification of medicinal plants in the family Fabaceae using a potential DNA barcode ITS2. Journal of Ethnopharmacology, 130(1): 116-121. https://doi.org/10.1016/j.jep.2010.04.026
[21] McKinney, H.H. (1923). Influence of soil temperature and moisture on infection of wheat seedling by Helminthosporium sativum. Journal of Agricultural Research, 26(5): 195-217.
[22] AI-Haider, M.A., AI-Kubaisi, Q.Y. (2025). Climatic water balance for AI-Ruhba District in Najaf Governor–Iraq. Iraqi Journal of Science, 66(6): 2394-2405. https://doi.org/10.24996/ijs.2025.66.6.17
[23] Mwangi, L. (2023). Method for isolation and observation of AMF Spores from soil. CIFOR, World Agroforestry Centre, United Nations Avenue Off Limuru Road, Gigiri, Nairobi, Kenya. https://www.cifor-icraf.org/resources-documents/Method-for-isolation-and-observation-of-Arbuscular-Mycorrhiza-Fungi-Spores.pdf.
[24] Gerdemann, J.W., Nicolson, T.H. (1963). Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society, 46(2): 235-244. https://doi.org/10.1016/S0007-1536(63)80079-0
[25] Mycological Research News. (2007). Mycological Research, 111(3): 253-256. https://doi.org/10.1016/j.mycres.2007.02.009
[26] Khalifa, E.Z. (1991). Biological control of tomato Fusarium wilt by Trichoderma harzianum. Minufiya Journal of Agricultural Research, 16(2): 1247-1259.
[27] Vincent, J.M. (1970). A manual for practical study of nodules bacteria. In IBP Handbook, pp. 125-126.
[28] Phillips, J.M., Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society, 55(1): 158-161. https://doi.org/10.1016/S0007-1536(70)80110-3
[29] Kormanik, P.P., Bryan, W.C., Schultz, R.C. (1980). Procedure and equipment for staining numbers of plant root samples for endomycorrhizal assays. Canadian Journal of Microbiology, 26(4): 536-538. https://doi.org/10.1139/m80-090
[30] Singh, S.P., Singh, H.B., Singh, D.K., Rakshit, A. (2014). Trichoderma-mediated enhancement of nutrient uptake and reduction in incidence of Rhizoctonia solani in tomato. Egyptian Journal of Biology, 16: 29-38. https://doi.org/10.4314/ejb.v16i1.4
[31] Abbas, A., Jiang, D., Fu, Y.(2017). *Trichoderma* spp. as antagonist of *Rhizoctonia solani*. Journal of Plant Pathology & Microbiology, 8(3): 402. https://doi.org/10.4172/2157-7471.1000402
[32] Jiang, L., Pettitt, T.R., Buenfeld, N., Smith, S.R. (2022). A critical review of the physiological, ecological, physical and chemical factors influencing the microbial degradation of concrete by fungi. Building and Environment, 214: 108925. https://doi.org/10.1016/j.buildenv.2022.108925
[33] Hernández-Dorta, A., Jaizme-Vega, M.D.C., Ríos-Mesa, D. (2024). Effect of mycorrhizal symbiosis on the development of the Canary Island tomato variety "Manzana Negra" under abiotic stress conditions. Agriculture, 14(6): 828. https://doi.org/10.3390/agriculture14060828
[34] Abd alsada, A.S., AL-Malik, S. (2024). Effect of interaction between two types of mycorrhizae and vermicompost on soil biological traits and growth of corn plant. Euphrates Journal of Agricultural Science, 16(3): 967-975. https://iasj.rdd.edu.iq/journals/uploads/2025/05/06/46edff4073b43affda6479e03cdf3f9f.pdf.
[35] Juber, K.S., Hassoun, I.K. (2008). Evaluation of the efficiency of some resistance-inducing chemicals and biocontrol agents in controlling potato stem canker disease under glasshouse and field conditions. Arab Society for Plant Protection, 26: 50-57. https://arabjournalpp.org/wp-content/uploads/2021/04/50-57.pdf.
[36] Al-Mansoury, B.A.R., Salih, Y.A.(2022). Evaluation of the efficiency of bio agents *Trichoderma harzianum* and *T. longibrachiatum* and some fungicides and a chemical compound against the fungus *Rhizoctonia sp.* that causes eggplant root rot disease in vitro. Euphrates Journal of Agriculture Science, 13(3): 210-231. https://iasj.rdd.edu.iq/journals/uploads/2025/04/22/cdc78dd6ee11e18ff0b68cc0e04b7ba8.pdf.
[37] Jasim, B.R., Matloob, A.A.H. (2025). Biological control of Fusarium solani and Rhizoctonia solani caused of root rot disease on pepper. Euphrates Journal of Agricultural Science, 17(1): 1038-1050. https://iasj.rdd.edu.iq/journals/uploads/2025/04/30/85db685e6b468077ac0dccaa31279dbd.pdf.
[38] El-Khallal, S.M. (2007). Induction and modulation of resistance in tomato plants against *Fusarium* Wilt disease by bioagent fungi (Arbuscular mycorrhiza) and/or hormonal elicitors (jasmonic acid & salicylic acid): 1 - changes in growth, some metabolic activities and endogenous hormones related to defence mechanism. Australian Journal of Basic and Applied Sciences, 1(4): 691-705.
[39] Akhtar, M.S., Shakeel, U., Siddiqui, Z.A. (2010). Biocontrol of Fusarium wilt by Bacillus pumilus, Pseudomonas alcaligenes, and Rhizobium sp. on lentil. Turkish Journal of Biology, 34(1): 1-7.

https://doi.org/10.3906/biy-0809-12
[40] Hashem, A., Akhter, A., Alqarawi, A.A., Singh, G., Almutairi, K.F., Abd_Allah, E.F. (2021). Mycorrhizal fungi induced activation of tomato defense system mitigates Fusarium wilt stress. Saudi Journal of Biological Sciences, 28(10): 5442-5450. https://doi.org/10.1016/j.sjbs.2021.07.025
[41] Hage-Ahmed, K., Krammer, J., Steinkellner, S. (2013). The intercropping partner affects arbuscular mycorrhizal fungi and *Fusarium oxysporum* f. sp. *lycopersici* interactions in tomato. Mycorrhiza, 23(7): 543-550. https://doi.org/10.1007/s00572-013-0495-x
[42] Manila, S., Nelson, R. (2014). Biochemical changes induced in tomato as a result of arbuscular mycorrhizal fungal colonization and tomato wilt pathogen infection. Asian Journal of Plant Science and Research, 4: 62-68.
[43] Kareem, T.A., Hassan, M.S. (2014). Evaluation of *Glomus mosseae* as biocontrol agents against *Rhizoctonia solani* on tomato. Journal of Biology, Agriculture and Healthcare, 4(2): 15-19.
[44] Abohatem, M., Chakrafi, F., Jaiti, F., Dihazi, A., Baaziz, M. (2011). Arbuscular mycorrhizal fungi limit incidence of Fusarium oxysporum f. sp. albedinis on date palm seedlings by increasing nutrient contents, total phenols and peroxidase activities. The Open Horticulture Journal, 4: 10-16. https://doi.org/10.2174/1874840601104010010
[45] Aboud, H.M., Al-Mosaye, A.H., Abed, R.M. (2014). Interaction of *Bacillus subtilis* and *Trichoderma harzianum* with mycorrhiza on growth and yield of cucumber (*Cucumis sativus* L.). International Journal of Current Research, 6(8): 7754-7758. https://journalcra.com/sites/default/files/issue-pdf/5929.pdf
[46] Singh, M., Mishra, M., Srivastava, D.K., Singh, P.K. (2020). Biological control of Fusarium wilt of tomato by arbuscular mycorrhizal fungi with intercropping. Plant Pathology & Quarantine, 10(1): 1-9. https://plantpathologyquarantine.org/pdf/PPQ_10_1_1.pdf.
[47] Hussein, A.N., AL-Amery, S.M.H., Abdulabbas, H.S., AL-Kinani, M.A.A., Salman, H.S. (2024). The effects of interaction between *Trichoderma* Fungi and Arbuscular Mycorrhizal Fungi on protect and improve the growth of plants. GSC Biological and Pharmaceutical Sciences, 29(3): 276-285. https://gsconlinepress.com/journals/gscbps/sites/default/files/GSCBPS-2024-0488.pdf.
[48] Şirin, U. (2011). Determining the effects of *Trichoderma harzianum* and some mycorrhizal fungi on plant growth and against *Rhizoctonia solani* Kühn in *Lilium* under in vivo conditions. African Journal of Biotechnology, 10(67): 15142-15150. https://doi.org/10.5897/AJB11.2444.
[49] Bhuvaneswari, G., Reetha, S., Sivaranjani, R., Ramakrishnan, K. (2014). Effect of AM fungi and Trichoderma species as stimulants of growth and morphological characters of chilli (*Capsicum annuum* L.). International Journal of Current Microbiology and Applied Sciences, 3(3): 447-455.
[50] Leslie, J.F., Summerell, B.A. (2008). The Fusarium Laboratory Manual. John Wiley & Sons.