Int. J. Agricult. Stat. Sci. Vol. 17, Supplement 1, pp. 2153-2162, 2021 www.connectjournals.com/ijass
DocID: https://connectjournals.com/03899.2021.17.2153 ISSN : 0973-1903, e-ISSN : 0976-3392

ORIGINAL ARTICLE

EVALUATION OF THE EFFICIENCY OF SOME BIOAGENTS AND THEIR INTERACTION WITH THE FUNGICIDE TOPSIN-M IN THE CONTROLLING EGGPLANT ROOT ROT DISEASE CAUSED BY *FUSARIUM OXYSPORUM*

Yehya A. Salih * and Baidaa Abdul-Ridha Abdul-Hussein Al-Mansoury

Department of Plant Protection, College of Agriculture, University of Basrah, Iraq.
E-mail: yehya.salih@uobasrah.edu.iq

Abstract: This study was conducted to evaluate the efficiency of the bioagents *Trichoderma harzianum* and *T. longibrachiatum* and the fungicide Topsin-M and their interaction in controlling root rot disease of eggplant caused by *Fusarium oxysporum*. The results of the laboratory experiment showed the antagonistic ability of the two bioagents factors *T.harzianum* and *T.longibrachiatum* in inhibiting the growth of pathogenic fungi by dual culture method, where the antagonism was of degree 1 and 2, respectively according to the Bell scale. The results of the laboratory experiment also showed that the fungicide Topsin-M inhibited the growth of pathogenic fungi by 100% when used at the recommended concentration of 1 $g.L^{-1}$, while the fungicide inhibited the bioagents *T.harzianum* and *T.longibrachiatum* by 16.63 and 26.80%, respectively. In the field experiment, the results showed that there were significant differences in the infection severity with the pathogenic fungus, so the least infection severity was observed in the treatment that contained the pathogenic fungus with one of the bioagents *T.harzianum* and *T.longibrachiatum* separately and the fungicide Topsin-M (Fo + Th + TM and Fo + Tl + TM), which amounted to 18.33 and 24.00%, respectively, compared to the treatment of pathogenic fungi (Fo), which amounted to 61.67%. The above two treatments also achieved a significant increase in plant height, reaching 77.33 and 74.67 cm, respectively. Also, these two treatments led to an increase in the fresh weight of the shoot and root systems, which amounted to 530, 515, 70 and 67 g, respectively compared to the treatment of pathogenic fungus, which amounted to 380 and 30.7g, respectively and the average dry weight of the shoot and root systems also increased in these two treatments to 79.58, 76.17, 17.50 and 16.92g, respectively compared to the treatment of pathogenic fungi, which amounted to 42.75 and 7.83g, respectively. The results of the experiment also explained that the largest leaf area and the largest yield of the plant were recorded in the same two treatments, as the leaf area reached 781.3 and 729 cm^2, respectively and the yield reached 1864 and 1762 gm, respectively.

***Key words*:** Biological control, Topsin-M, *Trichoderma harzianum*, *Trichoderma longibrachiatum*, *Fusarium oxysporum*.

Cite this article

Yahya Ashoor Salih and Baidaa Abdul-Ridha Abdul-Hussein Al-Mansoury (2021). Evaluation of the efficiency of some bioagents and their interaction with the fungicide Topsin-M in the controlling eggplant root rot disease caused by *Fusarium oxysporum*. *International Journal of Agricultural and Statistical Sciences.* DocID: https://connectjournals.com/03899.2021.17.2153

1. Introduction

Eggplant (*Solanum melongena)* was considered as one of the main vegetable crops, which belongs to the family Solanaceae. Its importance has increased especially in recent years, along with crops belonging to the same family. This crop is affected by many diseases and pests that cause great yield losses [Singh *et al*. (2012). Root rot disease caused by the fungus *Fusarium oxysporum* is one of those diseases that cause economic damage to eggplant and other crops all around the world. Many methods have been used to control this disease, including the use of chemical pesticides, but because of the risks to human and animal health and the environment and their high economic cost. This has led to the interest of agricultural and research institutions in finding alternative means instead of chemical pesticides. Therefore, in recent years, microorganisms have been used as bioagents for

***Author for correspondence** **Received** *May 18, 2021* **Revised** *June 29, 2021* **Accepted** *August 26, 2021*

controlling plant diseases, including *Trichoderma* spp. because they have many mechanisms to control pathogens and reduce infection. Studies have indicated that the combination of chemical pesticides and bioagents increase and enhance disease control and provide integrated management of diseases transmitted through soil and seeds. The tolerance of bioagents to different doses of fungicides led to the possibility of using them in integrated pest management [Anand *et al*. (2009)]. Abd-El-Khair *et al*. (2019) indicated that the combination of the bio agent *T. harzianum* and the fungicide Topsin-M reduced the incidence of seedling death and root rot caused by *F. oxysporum* more than using either of them alone. Some studies were conducted on the diseases of the shoot system of eggplant in Basrah Province but no study was conducted on the diseases of the root system of the crop, so this study aimed to evaluate the efficiency of some bioagents and their interaction with the fungicides Topsin-M in the controlling eggplant root rot disease in Basrah Province.

2. Materials and Methods

2.1 Isolation and phenotypic and molecular identification

Samples were taken from the roots of eggplant plants that showed symptoms of root rot disease from several areas in Basrah Province, including Qurna, Medaineh, Al-Haritha, Shatt Al-Arab, Al-Zubayr, Al-Lehais, Abu Al-Khasib and Safwan Al-Karma-the field of the College of Agriculture, University of Basrah. The roots of the affected plants were washed with tab water to remove the soil attached to them and the affected plant parts were cut to a length of 1 cm, then sterilized with 10% sodium hypochlorite (NaOCl) solution of the commercial preparation for 2-3 minutes and then washed with sterile distilled water to remove traces of sterilization, then dried on filter paper. Four pieces were planted in each Petri dish containing steril PDA medium to which the antibiotic Chloramphenicol (250 mg.l^{-1}) was added. The plates were incubated at 25±2°C for seven days, then the pathogenic fungi isolates were purified, glass slides were prepared from them and stained with lactophenol. They were identified based on the phenotypic and microscopic characteristics. The morphological identification was confirmed based on the molecular technology of the isolate of the studied fungus, since the DNA of the fungus was extracted using DNA extraction solutions and according to the manufacturer's Mini Kit Fungus Protocol (Geneaid Company).

The pathogenicity test

The pathogenicity of *F. oxysporum* was tested in W.A (Water Ager) sterilized medium with an autoclave at a temperature of 121°C and a pressure of 15 pounds/ inh^2 was prepared and the antibiotic Cholramphenacol (250 mg.L^{-1}) was added to it. The platas was inoculated by taking a 0.5 cm diameter disc by a sterile cork from near the edges of the seven days colony of pathogenic fungus and the disc was placed in the center of each plate while the control was left without adding pathogen. Three plates were used for each treatment. The plates were incubated in the incubator at a temperature of 25±2°C for three days and after confirming the occurrence of growth, the eggplant seeds (Barcelona cultivar) which superficially sterilized with a solution of sodium hypochlorite (NaOCl) 10% of the commercial preparation were placed in the plates circularly at a distance of 1 cm from the edge of the plate and at a rate of 10 seeds/plate. In the incubator at a temperature of 25±2°C and after seven days the percentage of germination was calculated was calculated according to the following equation:

% Germination percentage = Number of germinated seeds/Total number of seeds × 100

2.2 Antagonism efficiency test for bioagents *T.harzianum* and *T.longibrachiatum* against pathogenic fungus *F. oxysporum*

The dual culture method was used to test the ability of the biocagents *T.harzianum* and *T.longibrachiatum* for antagonism with pathogenic fungus *F. oxysporum.* A petri dish containing sterile PDA medium was divided into two equal parts and then the center of the first section was inoculated with a disc (0.5 cm diameter) from the seven days colonies. Of each of the bioagents *T.harzianum* and *T.longibrachiatum* and the second section was inoculated with a disc (0.5 cm diameter) from the seven days colony of pathogenic fungus *F. oxysporum*, while the control treatment was inoculated with the pathogen alone. The plates were incubated under temperature of 52±2°C. After the growth of pathogenic fungus in the comparison treatment reached the edge of the plate, the degree of antagonism was calculated according to the scale consisting of five degrees and as follows:

Degree	Description
1	The bioagent covers the whole plate.
2	The bioagent covers two-thirds of the plate.
3	The bioagent factor and the pathogenic fungus each cover half of the plate.
4	Pathogenic fungus cover two-thirds of the plate.
5	Pathogenic fungus cover all the plate.

A bioagent is effective when the antagonism degree is 1 or 2.

2.3 Testing the efficiency of Topsin-M in inhibiting the growth of *F. oxysporum* on PDA medium

The PDA medium was prepared and placed in a 250 ml flask at an average of 100 ml for each one and sterilized by autoclave at a temperature of 121°C and a pressure of 15 bound/inh^2 for a period of 30 minutes. After the medium's temperature reached an appropriate degree, the fungicide Topsin-M was added at the recommended concentration (1 gm.L^{-1}) and the flask was shaken well for homogeneity of the fungicide with the culture medium. The medium was poured into sterile Petri dishes and three dishes were used for each treatment. After solidification of the medium, the center of each plate was inoculated with a disc (0.5 cm diameter) from the edge of the seven days colonies of *F. oxysporum* and the bioagents *T.harzianum* and *T.longibrachiatum* separately. The control treatment was left without adding any fungicide Three replicates were prepared for each treatment. The inoculated plates were incubated in the incubator at a temperature of 25±2°C. After the growth in the control treatment reached the edge of the plate, the radial growth of the fungal colonies was measured by taking the average of two perpendicular diameters passing through the center of the plate from the back. The percentage of inhibition was calculated according to the Abbott equation as follows:

$$\% \text{ Inhibition} = \frac{\textit{fungal growth averageincontrol treatment} - \textit{fungal growth averageintreatment}}{\textit{fungal growth averageincontrol treatment}} \times 100$$

2.4 Preparation of the fungal inoculum of the pathogenic fungus *F. oxysporum* and the bioagents *T.harzianum* and *T.longibrachiatum*

The inoculum of the pathogenic fungus *F. oxysporum* which was isolated from the roots of infected eggplant plants and the bioagents *T.harzianum* and *T.longibrachiatum* which was obtained from Basil Y. Mehdi Plant Protection Department, College of Agriculture, University of Basrah were prepared according Dewan (1989) method, using seeds of local millet *Panicum miliaceum* L. the seeds were soaked in water for 6 hours and then washed well to remove impurities from them. The seeds were placed in 250ml flask at a rate of 150g/flask and then sterilized with an autoclave at a temperature of 121°C and a pressure of 15 pounds/inh^2 for one hour. Then the flasks were cooled and inoculated with of five discs per each flask from each of the pathogenic fungi and the bioagents separately taken from the edges of their seven days colonies. The flasks were incubated at 25±2°C for fourteen days with shaking them every 2-3 days for distribution of the inoculumon all seeds.

Preparing the field for cultivation

The field experiment was conducted in the Horticulture and Forestry Department, Directorate of Basrah Agriculture in a greenhouse with dimensions of 32 × 9 m. The soil was tilled, leveled and divided into rows, with a height of 30 cm, pit a distance of 1 m between one row and another and a distance of 50 cm between one pit and another. The drip irrigation system was used and service operations were conducted for the soil and for the plant. Each treatment was conducted with three replicates and the experiment included seven treatments as follows:

1- Control treatment (Control).
2- Treatment of the pathogenic fungus *F. oxysporum* alone (Fo)
3- Treatment of pathogenic fungus + biocontrol agent *T. harzianum* (Fo + Th).
4- Treatment of pathogenic fungus+ biocontrol agent *T. longibrachiatum*(Fo + Tl)
5- Treatment of pathogenic fungus+ fungicide Topsin-M (Fo + TM)
6- Treatment of pathogenic fungus + *T. harzianum* + Topsin-M (Fo + Th + TM)
7- Treatment of the pathogenic fungus + Topsin-M + *T. longibrachiatum* (Fo + Tl + TM)

The inoculum (1% w/w) of the bioagents *T. harzianum* and *T. longibrachiatum* was added. After three days, the pathogenic *F. oxysporum* inoculum was added at the same average and after a day of adding the pathogenic fungus inoculum, Topsin-M was added into the soil at the recommended concentration with irrigation. Then one-month eggplant seedlings (Barcelona cultivar), were planted, at an average of two seedlings per pit.

2.5 Study of the effect of bioagents *T. harzianum* and *T. longibrachiatum* and the fungicide Topsin-M on the infection severity with the pathogenic fungus *F. oxysporum*

The infection severity with the pathogenic fungus *F. oxysporum* on eggplant roots at the end of the season (four months) in the treatments mentioned in the previous paragraph was calculated according to four-degree scale as follows:

0 = The roots are healthy

1 = Slight discoloration on the roots and yellowing of a number of leaves

2 = Full root discoloration with overall yellowing of the leaves

3 = The discoloration extends from the roots to the bases of the stems

4 = Plant death

The infection severity for each treatment was calculated according to Mckinney's equation (1923) which found in Al-Waely (2004) as follows:

$$\% \text{ Infection severity} = \frac{(\text{Number of plants in deg ree } 0 \times 0) + - - - + (\text{Number of plants in deg ree } 4 \times 4)}{\text{Number of plants tested} \times \text{highest infection degree}} \times 100$$

2.6 Study of the effect of bioagents *T. harzianum* and *T. longibrachiatum* and Topsin-M on plant growth indicators.

Measurements of plant growth indicators were taken from three plants in each experimental unit taken randomly. These measurements included the height of plants (cm), fresh and dry weight of the shoot and root systems (gm), leaf area (cm^2) and total plant yield (gm/ plant).

Statistical analysis

Laboratory results were analyzed by using a Completely Randomized Design (CRD),while the field experiment was conducted according to a Randomized Complete Block Design (RCBD) and all averages were compared using the least (LSD) significant difference test at a probability level of 1% for laboratorial experiments and 5% for field experiment [Al-Rawi and Khalaf Allah (1980)]. All statistical analyses were conducted by using Genstat discovery edition program.

3. Results and Discussion

3.1 Isolation and identification of the fungus *F. oxysporum*

The results of isolation and identification showed the presence of the fungus *F. oxysporum* in all samples collected from eggplant plants that showed symptoms with an occurrence percent of 25.92 %. The phenotypic diagnosis showed that the fungus has a septate mycelium, the macroconidia were spindle or falcate shaped and three septate. The macroconidia were oval or spherical-shaped carried on short phialides. The clamydospores also observed, they have a rough wall and with two cells (Fig. 1). These features are consistent with Leslie and Summerell (2006). The results of the molecular diagnosis showed that the isolate of *F. oxysporum* was in compatible with the global isolates registered according to the available information in the National Center for Biotechnology Information (NCBI) and the Gen Bank, with rates ranging between 97.72-99.40% (Fig. 2).

Pathogenicity test

The results (Table 1) showed that the pathogenic fungus *F. oxysporum* reduced the percentage of germination of eggplant seeds significantly compared to the control treatment, it reduced the germination

Table 1: Effect of the pathogenic fungus *F. oxysporum* and the biocontrol agent *T. harzianum* and *T. longibrachiatum* on the germination percentage of eggplant seedin plates.

Treatments	%Germination*
F. oxysporum	23.3
T.harzianum	96.7
T. longibrachiatum	96.7
Control	93.3
L.S.D. 0.01	31.63

* Each number represents an average of three replicates.

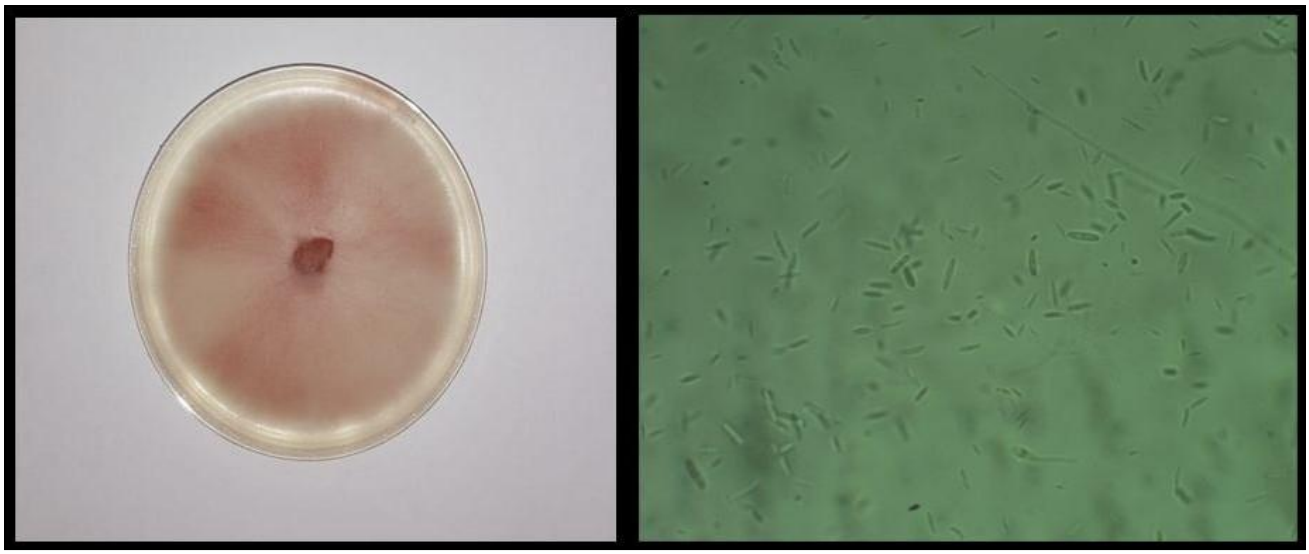

Fig. 1: The colony and the conidia of the pathogenic fungus *F. oxysporum* which isolated from eggplant roots infected with root rot disease in Basrah Province

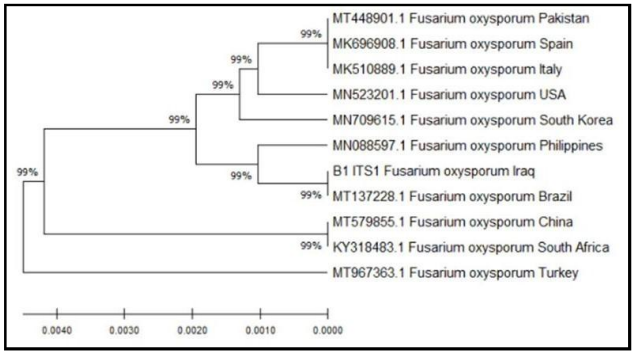

Fig. 2: The genetic tree of the pathogenic fungus *F. oxysporum* isolated from the roots of eggplant infected with root rot disease

percentage to 93.3% compared to the control treatment in which the germination percentage reached, with significant differences among them. This result is in agreement with Kareem and Al-Araji (2017) who indicated that the pathogenic fungus *F. oxysporum* caused the death of eggplant seedlings by 83.3% before emergence and 90% after emergence. The low percentage of eggplant seed germination is due to some isolates of *Fusarium* spp. Which have the ability to produce pectin and cellulose degrading enzymes such as Polygalactoronase enzyme in addition to productoxic substances such as Fusaric acid, Lycomarasmine and Dehydrofusaric acid and blockage the vascular with mycelium and conidia produced by the fungus [Srinivas *et al*. (2019)]. Table 1 also showed that treatment with the two bioagents *T.harzianum* and *T. longibrachiatum* achieved germination percentages and 96.7%, respectively, with significant differences from the treatment of pathogenic fungi, which amounted to 23.3%. These results agreed with several studies that indicated the effectiveness of the biocontrol agent *Trichoderma* spp. in increasing the germination percentages of many vegetable crops, including eggplant and reducing the incidence of root rot disease [Nahar *et al*. (2018), Al-Abbad (2020)].

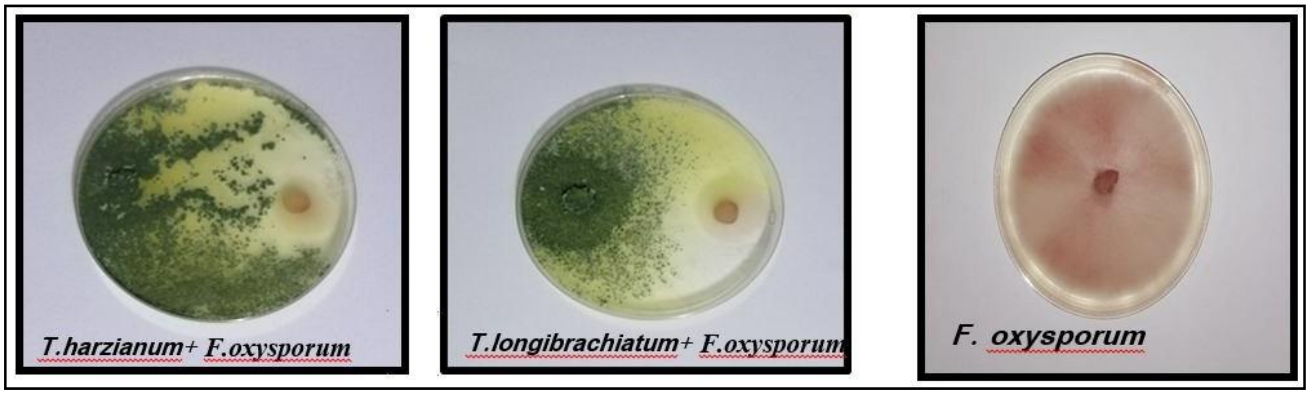

Fig. 3: Antagonism test for biocontrol agent *T.harzianum* and *T.longi brachiatum* against pathogenic fungus *F. oxysporum* on PDA medium

3.2 Test of antagonism efficiency of the two bioagents *T.harzianum* and *T.longibrachiatum* against the pathogenic fungus *F. oxysporum*.

The results of the antagonism experiment (Fig. 3) showed that the bioagents *T.harzianum* and *T.longibrachiatum* have a high antagonistic ability against the pathogenic fungus *F. oxysporum* in PDA media according to the scale of, the degree of antagonism reached 1 and 2 for each of them respectively, so this means that these fungi are effective antagonistic factoris. This result agreed with many studies that confirmed the antagonistic ability and role of *Trichoderma* species in inhibiting the growth of the pathogenic fungus *F. oxysporum* in laboratory experiments [Al-Abbad (2020), Shihab and Abood (2019)]. The reason for the biocontrol agent *Trichoderma* to have a high antagonism against pathogens is due to direct parasitism on the mycelium of the pathogenic fungus and production of cell wall degrading enzymes such as B-1, 3 glucanase, chitinase and protease or competition [Agrawal and Kotasthane (2012)].

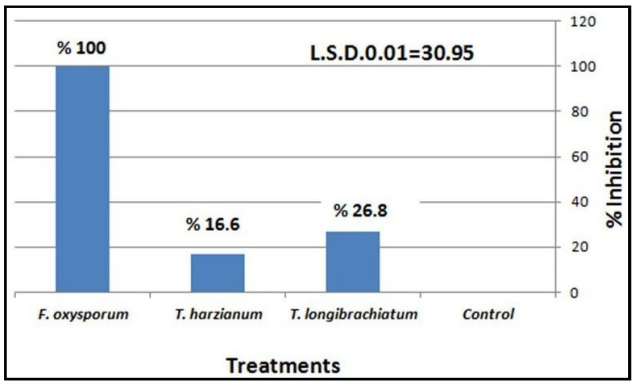

Fig. 4: Effect of the fungicide Topsin-M on the growth of the pathogenic fungus *F. oxysporum* and the bioagents *T.harzianum* and *T.longibrachiatum* on PDA medium

3.3 Testing the effect of the fungicide Topsin-M on the growth of pathogenic fungus *F. oxysporum* and the bioagents *T.harzianum* and *T.longibrachiatum* on PDA medium

The results (Figs. 4 and 5) showed that the use of Topsin-M at a concentration of 1 $g.L^{-1}$ led to inhibit the growth of pathogenic fungus by 100% compared to the control treatment, which had a 0% inhibition rate, while it inhibited the growth of bioagents *T.harzianum* and *T.longibrachiatum* by a percentage of 16.63 and 26.80%, respectively compared to the percentage of pathogenic fungus inhibition when using the same concentration, which reached 100%. The result inhibition

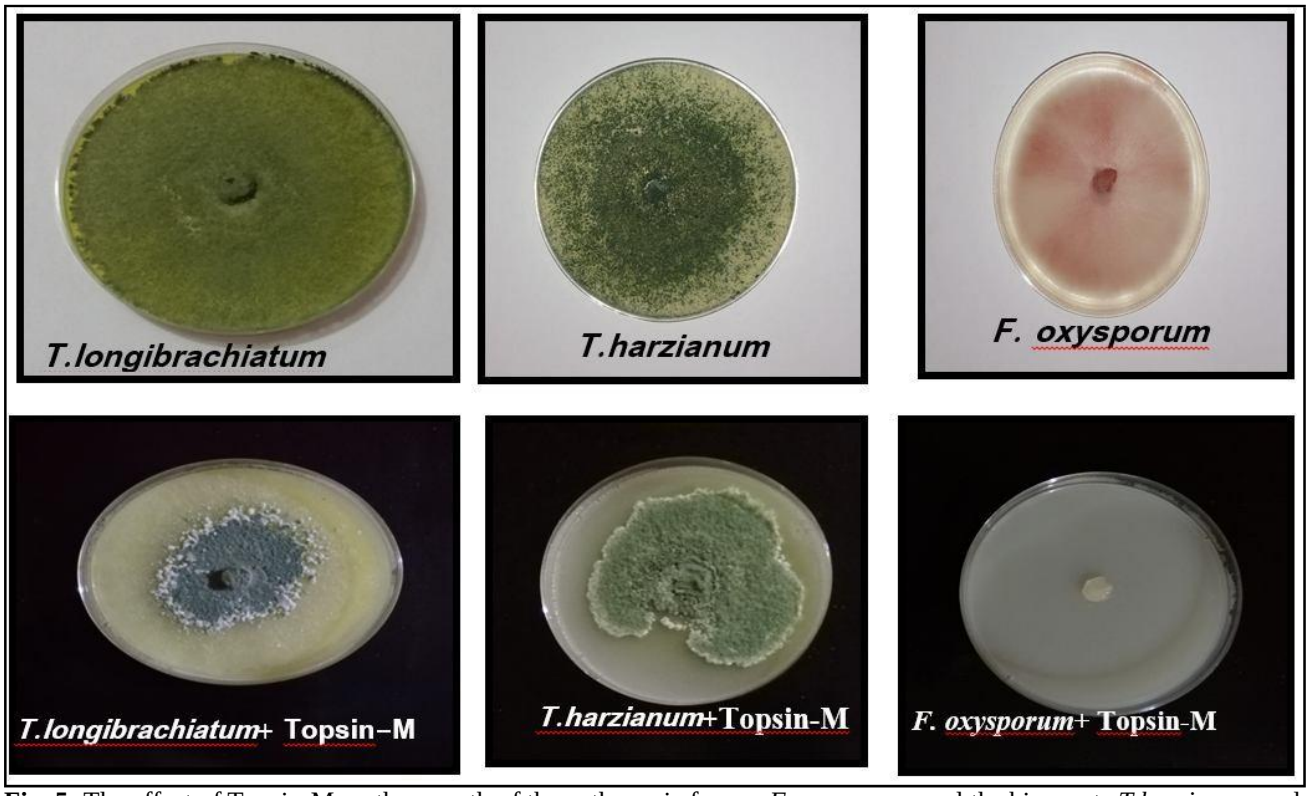

Fig. 5: The effect of Topsin-M on the growth of the pathogenic fungus *F. oxysporum* and the bioagents *T.harzianum* and *T.longibrachiatum* on PDA medium

of the pathogenic fungus agreed with several studies that demonstrated the efficiency of Topsin-M in inhibiting the pathogenic fungus on PDA [Yadav *et al*. (2018)], whereas the result of the bioagent agreed with Salih and Mansoor (2019) who found that Topsin-M did not inhibit the bioagent factor *T.harzianum* on PDA medium.

3.4 Study of the effect of the bioagents *T.harzianum* and *T.longibrachiatum* and the fungicide Topsin-M on the infection severity with pathogenic fungus *F. oxysporum*

The results explained (Table 2) that the lowest severity of infection with pathogenic fungus *F. oxysporum* in the greenhouse was achieved in the treatment containing the biocontrol agent *T.harzianum* and the fungicide Topsin-M in the presence of the pathogenic fungus (Fo + Th + TM) which amounted to 18.33%, followed by the treatment containing the bioagent *T.longibrachiatum* and Topsin-M in the presence of *F. oxysporum* (Fo + Tl + TM) which amounted to 24.00%. These two treatments were significantly differed from the pathogenic fungus (Fo) treatment, which amounted to 61.67%. This result agreed with Awad *et al*. (2017) and Sallam *et al*. (2019) who indicated the role of *T.harzianum* and *T.longibrachiatum* in reducing the infection severity of *F. oxysporum* by reducing toxic effect of pathogen and producing the degrading enzmes for the cell wall of pathogen. This is due to the synergistic action between the fungicide Topsin-M and bioagent that reduced the damage caused by the pathogen *F. oxysporum* [Mahmood *et al*. (2015)].

Table 2: Effect of the bioagents *T.harzianum* and *T.longibrachiatum* and Topsin-M on the infection severity with *F. oxysporum* causing the root rot eggplant.

Treatments	severity Infection%
Control	0
Fo	61.67
Fo+TM	33.33
Fo+Tl	40.67
Fo+Th	37.00
Fo+Tl+TM	24.00
Fo+Th+TM	18.33
L.S.D.0.05	3.93

* Each number represents an average of three replicates.
Th = *Trichoderma harzianum*, Tl = *Trichoderma longibrachiatum*, Fo = *Fusarium oxysporum*, TM = Topsin-M.

3.5 tudy of the effect of the bioagents *T.harzianum* and *T.longibrachiatum* and the fungicide Topsin-M on different growth indicators of eggplant

Fresh and dry weight of shoot and root systems

It was shown from Table 3 that the highest average of fresh weight and dry weight of the shoot system of a single plant at the end of the season occurred in the two treatments Fo + Th + TM and Fo + Tl + TM, which reached 530, 515, 79.58 and 76.17 g, respectively, followed by the treatments Fo + Th and Fo + Tl which were 490.7, 483.3, 69.75 and 68.25gm, respectively. So, all of these treatments were significantly differed from pathogenic fungus treatment which reached 380 and 42.75 g, respectively, which gave the lowest fresh and dry weight of the shoot system. It was also noticed from the same table that the highest average of fresh and dry weight of the root system of a single plant at the end of the growing season was recorded in the same above treatments Fo + Th + TM and Fo + Tl + TM, which reached 70, 67.7, 17.50 and 16.92g, respectively which were significantly differed from the control treatment, which amounted to 43.3 and 10.83g, respectively and the treatment of the pathogenic fungus, which amounted to 30.7 and 7.83g, respectively. This study was in agreement with Nahar *et al*. (2018) who found that the bioagent *T. harzianum* led to increase the growth of eggplant seedlings. Also, this increase is due to the synergistic effect of the fungicide, which led

Table 3: Effect of the bioagents *T.harzianum* and *T.longibrachiatum* and the fungicide Topsin-M on the fresh and dry weight of the shoot and root systems of eggplant in the greenhouse.

Tretments	dry Weight average(g)*		Fresh Weight average(g)*	
	Root system	Shoot system	Root system	Shoot system
Control	10.83	68.58	43.3	484.7
Fo	7.83	42.75	30.7	380
Fo + TM	10.42	62.08	41.7	460
Fo + T1	11.08	68.25	44.3	483.3
Fo + Th	15	69.75	60	490.7
Fo + T1 + TM	16.92	76.17	67.7	515
Fo + Th + TM	17.50	79.58	70	530
L.S.D.0.05	3.64	7.48	14.59	40.49

* Each number represents an average of three replicates.
Th = *Trichoderma harzianum*, Tl = *Trichoderma longibrachiatum*, Fo = *Fusarium oxysporum* ,TM = Topsin-M.

to the inhibition of the pathogen and the ability of the bioagent to stimulate root growth and increase its ability to absorb nutrients through the secreted enzymes. Also, this significant increase in the fresh and dry weight of the root system may be due to the positive effect of the bioagent *T. harzianum* by providing protection to the roots by forming colonies around the roots. It also increases the size of the root system and the hardness of the roots and it may has a direct effect on the biosynthesis processes of the plant [Jamal-Uddin *et al.* (2020)].

Plant height

It was observed from the results Table 4 that the highest average of the height of a single plant at the end of the growing season was achieved in the treatment Fo + Th + TM, which reached 77.33 cm, followed by the treatment Fo + Tl + TM, which reached 74.67 cm in height.These two treatments were significantly differed from the control treatment without pathogenic fungus and the pathogenic fungus treatment which were 68.67 and 53 cm, respectively. In addition to that, all other treatments were significantly differed from the pathogenic fungus treatment which was 53 cm. This result is in agreement with Kareem and Al-Araji (2017) who found that the treatment with *T.harzianum* led to increase the height of the eggplant to 19 cm compared to the control treatment which was 14.67 cm and the pathogenic fungus treatment which was 11.67 cm. The result also agreed with Singh *et al*. (2019) who elucidated that *Trichoderma* spp. have a role in promoting plant growth through the production of some substances that dissolve phosphates and the production of Sidrophores, Hydrogen cyanide and the enzyme Protease. The reason for the increase in plant height in the presence of the bioagent *T. harzianum* may be due to the fungus exudates which inhibit the enzymes of the pathogenic fungus, as well as its producing of some substances which promote plant growth.

Table 4: Effect of the bioagents *T.harzianum* and *T.longibrachiatum* and the fungicide Topsin-M on plant height, leaf area and plant yield of eggplant in the greenhouse.

Treatments	Plant yield (g/plant)*	Leaf area (cm^2)*	Plant height (cm)*
Control	1451	624	68.67
Fo	966	403.2	53
Fo + TM	1222	595.5	65.67
Fo + T1	1420	593.5	70.33
Fo + Th	1483	670	69.83
Fo + T1 + TM	1762	729	74.67
Fo + Th + TM	1864	781.3	77.33
L.S.D.0.05	151.9	63.09	3.20

* Each number represents an average of three replicates.
Th = *Trichoderma harzianum*, Tl = *Trichoderma longibrachiatum*, Fo = *Fusarium oxysporum* ,TM = Topsin-M.

Leaf area

The results in Table 4 showed that there were statistically significant differences in the leaf area of the different experimental treatments and the two treatments Fo + Th + TM and Fo + Tl + TM were the best treatments, as the leaf area in them reached 781.3 and 729 cm^2, respectively, which were significantly differed from the control treatment and pathogenic fungus treatment which were 624 and 403.2cm^2, respectively. It was also noticed that the treatments Fo + Th and Fo + Tl led to increase the leaf area up to 670.0 and 593.5cm^2 , respectively, which were significantly differed from the pathogenic fungus treatment (403.2cm^2). This result agreed with Hewedy *et al*. (2020) who showed that the bioagent. *Trichodrma* produces large quantities of organic acids that dissolve phosphates and calcium in the soil, which increases soil fertility and makes these elements available for absorption by the plant and this reflects positively on plant growth. The result also agreed with Mei *et al*. (2019) who showed that the treatment with *T. harzianum* increased the leaf area of the plant compared to treatments without it. This result also agreed with Khan *et al*. (2013) who elucidated that the bioagent produces plant growth regulators, which stimulate the plant growth. This may be due to the ability of *T. harzianum* and *T. longibrachiatum* for having several mechanisms, including parasitism, competition for food and space, stimulation of growth and induction of systemic resistance [Kareem *et al*. (2016)].

Plant yield

It was found that the highest rate of production per plant at the end of the growing season was obtained in the two treatments Fo + Th + TM and Fo + Tl + TM, which reached 1864 and 1762 g/plant, with significant differences from the control treatment which was 1451 g/plant and the pathogenic fungus treatment which was 966 g/plant. Whereas all other treatments were significantly differed from the pathogenic fungus treatment, which gave the lowest average of production

per plant, as it reached 966 g/plant (Table 4).

The efficacy of treatments containing the bioagent *T. harzianum* and the fungicide Topsin-M in increasing the average of production is consistent with what was found by Faruq *et al*. (2014) who indicated the ability of the bioagent *T. harzianum* to suppress the pathogenic fungus, stimulate plant growth and increase the average of eggplant production.

References

Abd-El-Khair, H., Elshahawy, I.E. and H.E. Haggag (2019). Field application of Trichoderma spp. combined with thiophanate-methyl for controlling Fusarium solani and Fusarium oxysporum in dry bean. *Bulletin of the National Research Centre*, **43(1)**, 1-9.

Agrawal, T. and A.S. Kotasthane (2012). Chitinolytic assay of indigenous *Trichoderma* isolates collected from different geographical locations of Chhattisgarh in Central India. *Springerplus*, **1**, 1-10.

Al-Abbad, B.Y.M. (2020). Evaluation of the efficiency of some polymeric materials for producion a bioformulation of the fungus *Trichoderma longibrachiatum* for laboratorial controlling of some tomato diseases. *Master Thesis*, College of Agriculture, University of Basrah, Iraq.

Al-Rawi, K.M. and A.M. Khalaf Allah (1980). Design and Analysis of Agricultural experiments. Directorate of books for Printing and Publishing, Mosul University, Iraq.

Anand, T., A. Chandrasekaran, S. Kuttalam, T. Raguchander and R. Samiyappan (2009). Management of cucumber (*Cucumis sativus* L.) mildews through Azoxystrobin-Tolerant *Pseudomonas fluorescens*. *J. Agric. Sci. Technol.*, **11**, 211-226.

Awad, M.A., E.Z. Khlifa, M.E. Fouly, A.A. Shahin, H.A.E. Bialy and S.M. Fahmy (2017). Biological Control of Tomato Root-Rots using Trichoderma Spp. Menoufia. *Journal of Plant Protection*, **2(3)**, 167-182.

Dewan, M.M. (1989). Identify and frequency of occurrence of fungi in root of wheat and rye grass and their effect on take all and host growth. *Ph.D Thesis*, University West Australia.

Hewedy, O.A., K.S. Abdel-Lateif and R.A. Bakr (2020). Genetic diversity and biocontrol efficacy of indigenous *Trichoderma* isolates against Fusarium wilt of pepper. *J. Basic Microbiol.*, **60**, 126-135.

Faruq, A.N., M.T. Islam, M.Z.R. Bhuiyan, M. Mamun-ur-Rashid, M.R. Amin and S. Hoque (2014). Efficacy of soil application with Trichoderma harzianum T22 and some selected soil amendments on Fusarium wilt of eggplant (Solanum melongena L.). *Applied Science Reports*, **8(2)**, 69-74.

Jamal Uddin, A.F.M., N. Sabrina, M.A. Husna, M.H. Imam and M. Rakibuzzaman (2020). Bio-efficacy of *Trichoderma harzianum* spore concentrations on tomato production. *Int. J. Bus. Soc. Sci. Res.*, **8(3)**, 124-129.

Kareem, T., O. Ugoji and O. Aboaba (2016). Biocontrol of Fusarium wilt of cucumber with *Trichoderma longibrachiatum* NGJ167 (Rifai). *Br. Microbiol. Res. J.*, **16**, 1-11.

Kareem, H.J. and A.M. Al-Araji (2017). Evaluation of Trichoderma harzianum biological control against Fusarium oxysporum f. sp. melongenae. *Iraqi Journal of Sciences*, **58(4B)**, 2051-2060.

Khan, M.O., N. Shah, S. Shahzad and M.N. Khan (2013). Induce tolerance in *Trichoderma* species against fungicide. Available online at:www.awkum.edu.pk/PJLS.

Leslie, J.F. and B.A. Summerell (2006). Fusarium laboratory workshops-A recent history. *Mycotoxin Research*, **22(2)**, 73-74.

Mei, L.I., L.I.A.N. Hua, X.L. Su, T.I.A.N. Ying,, W.K. Huang, M.E.I. Jie and X.L. Jiang (2019). The effects of Trichoderma on preventing cucumber fusarium wilt and regulating cucumber physiology. *Journal of Integrative Agriculture*, **18(3)**, 607-617.

Mahmood, Y., M.A. Khan, N. Javed and M.J. Arif (2015). Comparative efficacy of fungicides and biological control agents for the management of chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris*. *J. Anim. Plant Sci.*, **25(4)**, 1063-1071.

Nahar, N., M.R. Islam, M.M. Uddin, P. de Jong, P.C. Struik and T.J. Stomph (2018). Reducing damping-off problems in eggplant (Solanum melongena L.): A participatory testing of nursery management in Bangladesh. *Crop Protection*, **112**, 177-186.

Salih, Y.A. and N.M. Mansoor (2019). A study of the effect of bioagent *Trichoderma harzianum* Rifai, the fungicide Topsin-M and their interaction on root rot disease of okra *Abelmoschus esculentus* in the field Basrah. *J. Agri. Sci.*, **32(2)**, 320-336.

Sallam, N.M., A.M. Eraky and A. Sallam (2019). Effect of Trichoderma spp. on Fusarium wilt disease of tomato. *Molecular Biology Reports*, **46(4)**, 4463-4470.

Shihab, K.M. and I.D. Abood (2019). Genetic segregation of tomato trihybrid, double cross and detection of Mi1. 2, Mi-3 resistance genes against root-knot nematode (*Meloidogyne* spp.). *International Journal of Agricultural and Statistical Sciences*, **15(1)**, 153-162.

Singh, A.K., B.P. Bhatt, P.K. Sundaram, C. Naresh, R.C. Bharati and S.K. Patel (2012). Faba bean (*Vicia faba* L.) phenology and performance in response to its seed size

class and planting depth. *International Journal of Agricultural and Statistical Sciences*, **8(1)**, 97-109.

Singh, P., J. Singh, R.S. Rajput and A. Vaishnav (2019). Exploration of multitrait antagonistic microbes against *Fusarium oxysporum* f.sp. *lycopersici. J. Appl. Nat. Sci.*, **11(2)**, 503-510.

Srinivas, C., D. Nirmala and M. Narasimha (2019). *Fusarium oxysporum* f. sp. *lycopersici* causal agent of vascular wilt disease of tomato: Biology to diversity-A review. *Saudi J. Biologic. Sci.*, **26**, 1315-1324.

Yadav, A.S., S. Yadav and R. Singh (2018). Screening of fungicides *in vitro* and seed treatment against Fusarium wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris. J. Pharm. Phytoch.*, **7(3)**, 2908-2911.