Effect of the bioagents *Trichoderma harzianum* and *T.longibrachiatum* on tomato fusarium wilt disease caused by *Fusarium oxysporum* f.sp. *lycopersici* and some plant growth parameters under greenhouse conditions

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Abstract

This study was conducted to know the effect of the bioagents*Trichoderma harzianum* and *T.longibrachiatum* against fusarium wilt disease on tomato plant caused by the pathogenic fungus *Fusariumoxysporum* f.sp*. lycopersici*. The results of the pathogenicity test showed that *F. oxysporum*f.sp*. lycopersici*is a pathogenic fungus,as the percentage of disease severity of tomato seeds reached 31.71%. The results also showed that the percentage of germination of tomato seeds increased to 100% and 96%, when they treated with *T.harzianum* and *T.longibrachiatum* respectively, compared with the treatment of pathogenic fungus *F.oxysporum* f.sp. *lycopersici*, which amounted to 46%. The greenhouse experiment revealed that*T.harzianum* and *T.longibrachiatum* when interacted with the pathogenic fungus *F.oxysporum* f.sp. *lycoperisici*(T.h + F.o.l and T.l +F.o.l) decreased the disease severity to 7.81and 9.05% compared to the pathogenic fungus. alone which amounted to 68.31% . The results also indicated that the treatments of T.h +F.o.l and T.l + F.o.l led to increase the plant height up to 134.0 and 126.0 cm respectively, compared to the pathogenic fungus alone which was 102.0 cm. Also, it was noticed that the treatment of T.h. + F.o.l gave the highest increase in the dry weight of the shoot and root systemsreached 3279.0 and 146.2 gm respectively, which differed significantly from the pathogenic fungus treatment, which amounted to727.0 and 46.4gm respectively, followed by the treatment of T.l + F.o.l which reached 2147.0 and 107.6 gm respectively.Finally, it was founded that the treatment of T.h + F.o.l gave the highest increase in the yield of fruits, which amounted to 3722.0 g / plant, followed by the treatment of T.l +F.o.l which reached 1666.0 gm/ plant, so they significantly differedfrom the pathogenic fungus treatment which was 1105.0 gm / plant.

DOI Number: 10.14704/nq.2022.20.10.NQ55956 **NeuroQuantology 2022; 20(10): 9816-9826**

***e*ISSN**1303-5150

Introduction

Tomato(*Lycopersicon esculentum*Mill) is a vegetable crop commonly used in most parts of the world. It is characterized by its high nutritional value, because it contains carbohydrates, proteins, fats and some mineral elements such as phosphorous, calcium, iron and some vitamins including A, C, B1 and B6 (Matloob *et al*, 1989).Tomato like other vegetable crops, suffers from many diseases, including fusarium wilt disease caused by the pathogenic fungus *Fusarium oxysporum* f.sp. *lycopersici*, which belongs to the phylum Deuteromycota, form classHyphomycetes, form order Moniliales, and form family Tuberculariaceae (Leslie and Summerell, 2006). This pathogenic fungus has a significant effect on the fresh and dry weight of the shoot and root systems when compared with healthy plants and significantly reduces the yield of the crop(Ghali *et al*, 1986). It transmits by seeds and infection occurs on one side of the plant, so, the progression of the infection leads to the death of whole plant (Anil and Raj, 2015). Al-Asadi (2016) and Al-Husseini (2020) found that this fungus may negatively affect the growth parameters of tomato plants, such as the number of fruits, the number of branches, the weight of the fruit yield, the height of the plant, and the fresh weight of the shoot and root systems. Different methods have been followed to control the disease, including chemical and biological methods. The chemical pesticides have an essential role in significant reducing the risk of infection by pathogenic fungi (Dias, 2012 and Schreinemachers *et al*., 2016), but they effect on environment, nontarget organisms and the health of the workers and consumers of agricultural commodities contaminated with them. Therefore, the researchers and scientists seek to search for safer and less dangerous methods for human health, the environment and non-target organisms, to be an alternative to chemical pesticides or reduce their use due to the risks they cause. In recent years, the use of microorganisms in the field of biological control has been widely and consciously used.*Trichoderma*is one of the most used fungi in this field for several reasons, including its rapid reproduction, high production of spores, easily isolation, tolerance to extreme environmental conditions and possesses several modes of action, so it has proven its high efficiency in controlling the plant diseases (Decal *et al*., 1995 ; Harman, 2000; Harman *et al*. 2004). It was found from many studies, that*Trichoderma* spp. has been used for controlling different pathogens and plant diseases and led to decrease disease severity and increase the plant growth parameters significantly (Fayyadh*et al*.2012; Salih and Al-Maerich,2016;Salih and Mansoor,2019; Mahde *et al*., 2019; Salih and Al-Mansoury,2021;Al-Mansoury and Salih, 2022). Given the importance of tomato fusarium wilt disease and the importance of finding alternative control methods to control the disease and reduce the environment pollution, this studywas carried out to know the effect of the bioagent factors such as *Trichodermaharzianum* and *T.longibrachiatum* against tomato fusarium wilt disease under greenhouse conditions.

Materials and methods

Isolation and identification of the pathogenic fungus

Samples of tomato plant affected by fusarium wilt disease were collected. The roots and stem ends of these plants were cut into pieces in length of 2 cm for each piece, sterilized with sodium hypochlorite solution(NaOCl) at a concentration of 10% of the commercial solution for 2-3 minutes, washed with sterile distilled water and dried with filter paper Whatman No.1. Five pieces were circularly put in each Petri dish(9cm diameter) containing sterile PDA medium with

an antibiotic chloramphenicol (250 $mg.1^{-1}$). The dishes were incubated in the incubator at a temperature of 25±2 °C for seven days and then re-purified by taking 0.5 cm disc from the edge of the colony and putting it in another Petri dish containing sterile PDA medium. The dishes were again incubated at a temperature 25±2°C for seven days. After that, the fungus was identified according to the taxonomic characteristics based on Booth (1971) and Leslei and Summerell (2006).

Preparation of the inoculum of the pathogenic fungus *F.oxysporum* f.sp.*lycopersici* and the bioagents *T. harzianum and T. longibrachiatum*

The inoculum of the pathogenic fungus which was isolated from the infected plants and the bioagents *T.harzianum* and *T.longibrachiatum* which were obtained from Baidaa A.A.AL-Mansoury, Plant Protection Department, Agriculture College, University of Basrah was prepared byusing the seeds of local millet *Panicum miliaceum* L. The seeds were washed well to remove impurities and dust, and soaked with water for about six hours, spread on filter papers and left to dry slightly at laboratory temperature. The seeds were placed in 250 ml flasks at a rate of 100 g per each flask and moistened with a little sterile distilled water. The flasks were autoclaved at a temperature of 121°C and a pressure of 15 pounds/inch² for an hour and re-sterilized again for an hour. Each flask was inoculated with five discs (0.5 cm diameter)taken from the edge of seven days colony of the pathogenic fungus and the two bioagents separately. The flasks were incubated at 25±2°C for 14 days, with shaking them every 2-3 days to ensure the homogeneous distribution of fungi on the whole seeds.

Pathogenicity test

In this experiment, plastic pots of 5 kg capacity were used containing a mixture of soil and peatmoss at a ratio of 3:1 were used, the mixture was autoclaved at a temperature of 121°C and a pressure of 15 pounds/inch² for one hour. After that, the soil was inoculated with the pathogenic fungus *F.oxysporum* f.sp.*lycopersici* carried on millet seeds at a rate of 1%w/w. (Dewan, 1989). Six pots were contaminated with the pathogen and three potsleft without pathogen (as control) were prepared and left for three days with irrigation as needed.Four-week-old Aya cultivar seedlings of tomato were transferred to all pots with two seedlings per each one with continual irrigation. After six weeks the disease severity percentage was calculated according to the scale prepared by Al-Asadi, 2016 as follow :

0= healthy plants
1=Slight yellowing of the leaves
2= Yellowing leaves with slight wilting
3= Whole plant wilting

Mickenny (1923) equation cited from Al-Waily (2004) was applied to calculate the disease severity percentage as follow:

$$\%Disease\ Severity = \frac{Sum(Infected\ plants\ number\ in\ each\ degree \times Degree\ number)}{Total\ infected\ plants \times Highest\ degree} \times 100$$

Effect of the bioagents *T. harzianum and T. longibrachiatum* and the pathogenic fungus *F.oxysporum* f.sp. *lycopersici*on the percentage of tomato seed germination in plates

The water agar medium (AW) was used for testing the effect of the *Trichoderma harzianum and T.longibrachiatum*and the pathogenic fungus*F.oxysporum* f.sp. *lycopersici* on tomato seed germination. The plates with water agar were inoculated with 0.5 cm diameter disc taken from the edge of seven days colony of each fungus by a sterile cork borer, with lifting a comparison treatment without inoculation. Three replicates were used for each treatment. All plates were incubated at a temperature of 25±2° C for three days. After three days, ten tomato seeds sterilized with sodium hypochlorite solution with a concentration of 10% of the commercial solution for 2-3 minutes, were placed in each

plate circularly at a distance of 1 cm from the edge of each plate. All plates were incubated at a temperature of 25±2°C. After seven days, the percentage of germination was calculated according to the following equation:

$$\%Germination = \frac{Number\ of\ greminated\ seeds}{Total\ number\ of\ seeds} \times 100$$

Effect of the bioagent fungi on the fusarium wilt disease and some plant growth parameters in the greenhouse

The greenhouse experiment was carried out at the Agricultural Research Station / College of Agriculture / University of Basrah in a plastic house with dimensions of 32 x 9 m. The soil was plowed, leveled, smoothed and divided into three rows (30 cm height and 50 cm wide) with 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 applied, and service operations were conducted for the soil and the plant.Each treatment was carried out with three replicates. The experiment included ten treatments with planting seedlings method at the age of 40 days. The bioagents*T.harzianum* and *T.longibrachiatum*werefirstly added at a rate of 1% w/w per pit.After three days, the pathogenic fungus*F.oxysporum* f.sp.*lycopersici* was added at the same rate. After another three days, the 40-day-old tomato seedlings were planted at a rate of three seedlings per each pit. The disease severity percentage and some plant growth parameters including the plant height (cm) the dry weight of shoot and root systems (gm) and the plant yield (gm/plant) were calculated a after four months. The experiment included the following treatments:

1- Treatment of the pathogenic fungus *F.oxysporum* f.sp.*lycopersici* (F.o.l)
2- Treatment of the bioagent *T. harzianum* (T.h)
3- Treatment of thebioagent*T. harzianum* + the pathogenic fungus (T.h + F.o.l)
4- Treatment of the bioagent*T. longibrachiatum* (T.l)
5- Treatment of the bioagent*T. longibrachiatum* + the pathogenic fungus (T.l + F.o.l)
6- Control treatment (uncontaminated soil).

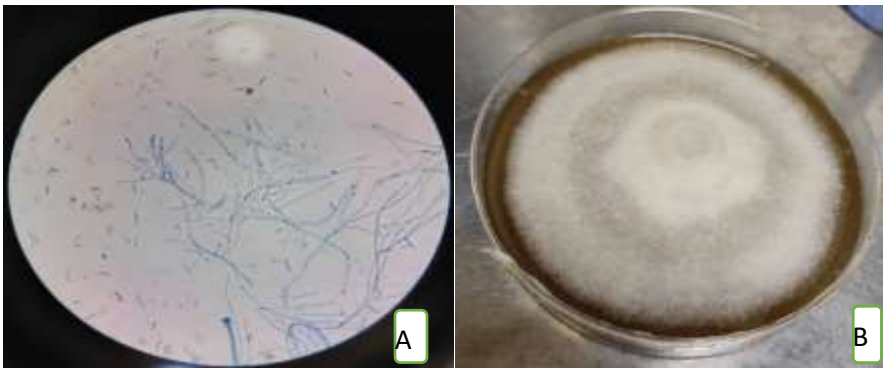
Statistical analysis

The laboratorial experiments were analyzed by using a CompletelyRandomized Design (CRD), whereas the field experiments were carried out according to a Randomized Complete Block Design (RCBD). All averages were compared by using the least significant difference test (LSD) at a probability level of 0.01 for the laboratorial experiments and 0.05 for the field experiments and all treatments were repeated for three times (Al-Rawi and Khalaf Allah, 1980). All statistical analyses were carried out by using GenStat Discovery Edition program.

Results and discussion

Isolation and identification of the pathogenic fungus *F.oxysporum* f.sp. *lycopersici*

The results showed that the fungus *F.oxysporum* f.sp. *lycopersici*was isolated from the root and the end stem of the infected plants with fusarium wilt disease.The fungus hada white to purple cottony growth, septate mycelium and three types of spores: microconidia, macroconidia and chlamydospores (Fig.1). The result agreed with several studies confirmed that the pathogenic fungus *F.oxysporum* f.sp. *lycopersici* is the main cause of tomato fusarium wilt (El.Kazzaz *et al*. 2008 and Charoenporn *et al*. 2010).



Fig(1) The pathogenic fungus *F.oxysporum* f.sp. *lycopersici* isolated from infected tomato plants
A- The Colony B- Hyphae and conidia (Magnification power of 40x)

Pathogenicity test

The results of the pathogenicity test(Figure2) elucidated that the isolated fungus *F.oxysporum*f.sp.*lycopersici*was significantly pathogenic compared to control treatment.It reducedthe percentage of germination of tomato seeds to 63.3% compared to the control treatment, which amounted to 86.6%. The results agreed with Al-Hamdani (2006) who found that the pathogenic fungus *F. oxysporum* f.sp. *lycopersici* has a high ability to cause wilt disease, seed rot and damping-off. Symptoms of the disease represented by yellowing leaves and wilting of the plant. This is due to the fact that the fungus secretes a lot of toxic substances, including lycomarasmine and fusaric acid, which effect on the plant growth and the process of cellular respiration, as a result of the union of iron element with oxidase enzymes in the process of breathing (Pitt, 2000; Dawar *et al*., 2007; Fayyadh and Abbas, 2018).

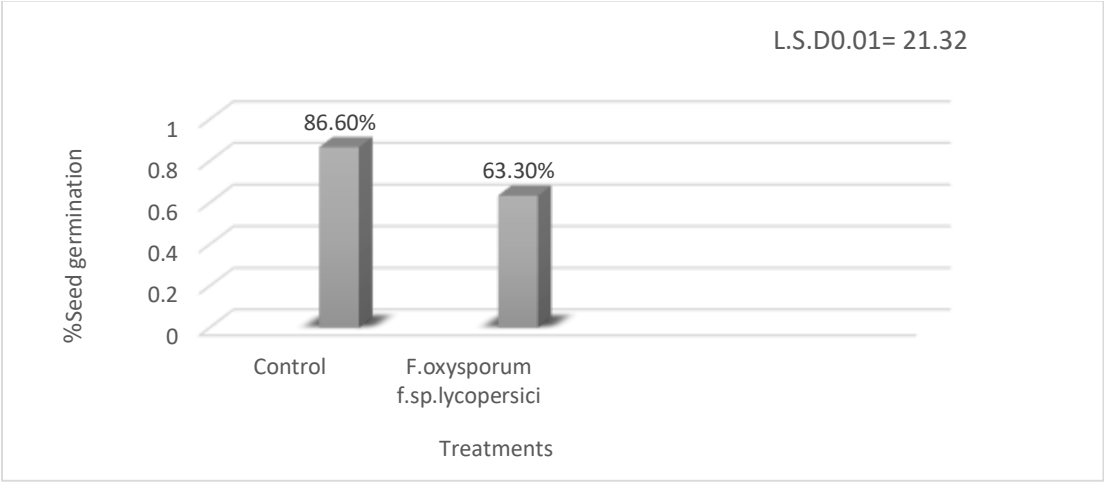


Fig (2) Effect of the pathogenic fungus *F.oxysporum* f.sp.*lycopersici* on the percentage of germination of tomato seeds
T.h= *Trichoderma harzianum* ; T.l=*T.longibrachiatum*; F.o.l= *Fusarium oxysporum* f.sp.*lycopersici*

Effect of the bioagents *T. harzianum and T. longibrachiatum* and the pathogenic fungus *F.oxysporum fsp lycopersici on* the percentage of tomato seed germination

Figure (3) revealed that bioagents *T.harziannum* and *T.longibrachiatum* had no negative effect on the percentage of tomato seed germination, which amounted to 100%

and 96%, respectively, compared with the control treatment, which amounted to 100%. While the pathogenic fungus *F.oxysporum* f.sp. *lycopersici* reduced the percentage of seed germination to 46%. The reason is due to the fact that the bioagents fungi is non-pathogenic to plants and possess mechanisms of parasitism on the pathogenic fungi in addition to promoting the plant growth (Bjorkman *et al*., 1998). While the pathogenic fungus decreased the percentage of seed germination significantly because of it is ability to produce enzymes and toxic compounds (Agrios, 2005).

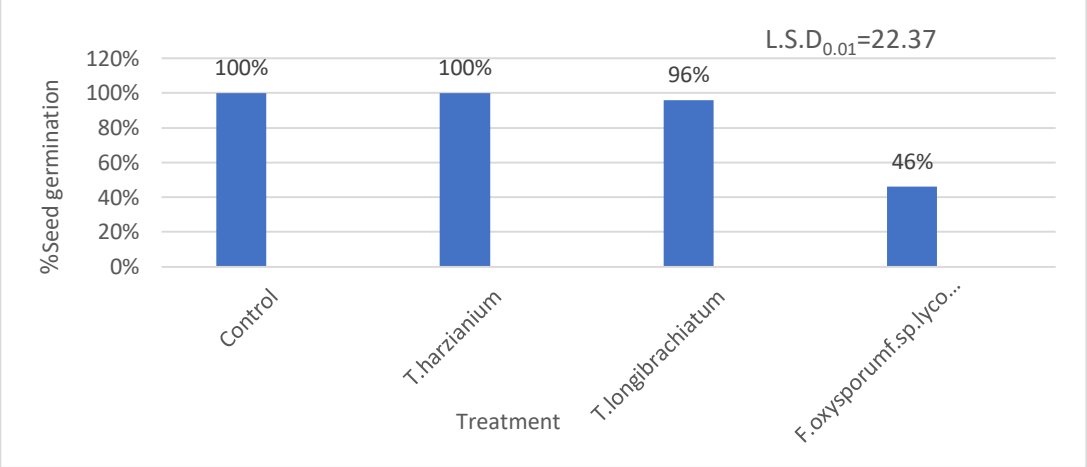


Fig (3) Effect of the bioagents *T.harzianum* and *T.longibrachiatum* and the pathogenic fungus *F.oxysporum*f.sp.*lycopersici* on the percentage of tomato seedsgermination
T.h= *Trichoderma harzianum* ; T.l= *T.longibrachiatum* ; F.o.l= *Fusarium oxysporum* f.sp.*lycopersici*

Effect of the bioagents *T. harzianum* and*T. longibrachiatum* on the disease severity with the pathogenic fungus *F.oxysporum* f.sp. *lycopersici*

The results (Figure4) showed that the bioagents *T.harzianum* and *T. longibrachiatum*significantly reduced the percentage of disease severity to 7.81% and 9.05% respectively, when they interacted with the pathogenic fungus compared to the pathogenic fungus alone which was 68.31%. This result was agreed with Ibrahim (2016), Salih and Mansoor (2019), Salih and Al-Mansoury (2021) and Al-Mansoury and Salih (2022), who found that the bioagents *T.harzianum* and *T.longibarchitum*reduced the disease severity by the pathogenic fungi when they interacted with them. The reason is due to the role of the bioagent *T.harzianum* and *T.longibarchitum* in reducing the severity of infection with the pathogenic fungus *F.oxysporum f.sp. lycopersici* by reducing the secretions of the toxic compounds and the production of degrading enzymes that are responsible for antagonism against the pathogens (Barari, 2016 ; Awad *et al*., 2017; Sallam *et al.,* 2019).

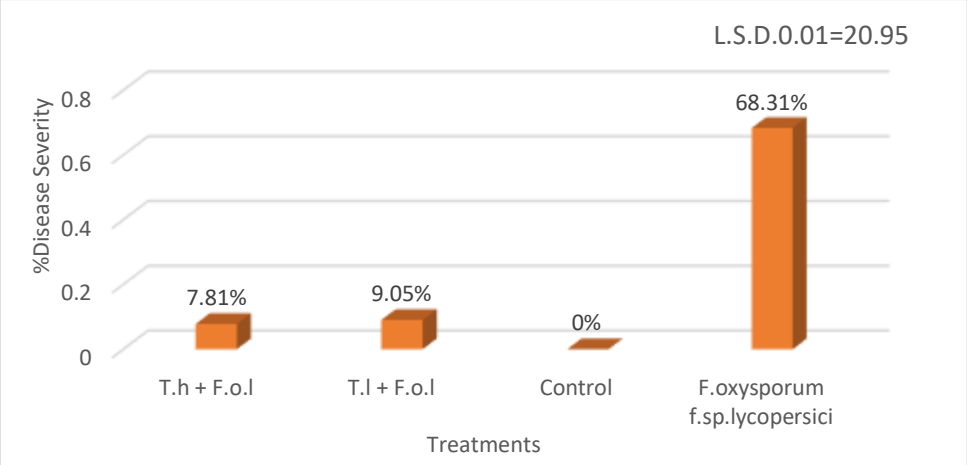


Fig (4) Effect of the bioagents *T.harzianum* and*T.longibrachiatum* onthe percentage of disease severity of the pathogenic fungus *F.oxysporum* f.sp. *lycopersici*
T.h= *Trichoderma harzianum* ; T.l= *T. longibrachiatum* ; F.o.l= *Fusarium oxysporum* f.sp.*lycopersici*

Effect of bioagents *T. harzianum* and *T. longibrachiatum* and the pathogenic fungus *F.oxysporum f.sp. lycopersici* and their interaction on the plant height

It was found from the results (Table 1) that the bioagents *T.harzianum and T.longibrachiatum* led to increase the plant height to142.0 and 137.0cm respectively, which outperformed by high significant differences from the control treatment and the pathogenic fungus treatment which were 109.0 and 102.0cm respectively, followed by the treatments of T.h+F.o.l and T.l+F.o.l, which amounted to 134.0 and 126.0 cm respectively. So, all these treatments differed significantly from the control treatment and the pathogenic fungus treatment. The results agreed with Singh *et al*. (2019) who showed that the fungus *Trichoderma* spp. have a role in promoting plant growth through the production of some substances that cause the dissolution of phosphate and production of hydrogen cyanide. The results also agreed with Salih and Mansoor (2019) and Salih and Al-Mansoury (2021) who found that the bioagents *T.harzianum* and *T.longibrachiatum*led to increase the plant height significantly compared to the pathogenic fungus treatment. The reason for the increase in the height of plants may be due to the role of the bioagents in stimulating the plant and increasing nitrogen absorption from the soil in addition to inhibition the pathogenic fungus enzymes and promote the plant growth (Yedidia *et al*., 2001; Munir *et al*., 2014; Garcia *et al*., 2016).

Table (1) Effect of the bioagents*T. harzianum and T. longibrachiatum* and the pathogenic fungus *F.oxysporum*f.sp.*lycopersici* and their interaction on the height of tomato plants in the greenhouse

Treatments	Plants height (cm)
Control	109.0
T.harzianum	142.0
T.h+F.o.l	134.0
T.longibrachiatum	137.0

T.l +F.o.l	126.0
Fusarium oxysporum f.sp.*lycopersici*	102.0
$L.S.D_{0.05}$	15.73

T.h= *Trichoderma harzianum* ; T.l= *T. longibrachiatum* ; F.o.l= *Fusarium oxysporum* f.sp.*lycopersici*

Effect of bioagents*T. harzianum* and *T. longibrachiatum* and the pathogenic fungus *F.oxysporum* f.sp. *lycopersici* and their interaction on the dry weight of the shoot and root systems

It was noticed from the results of the experiment (Table2) that the pathogenic fungus F.o.l led to reduce the dry weight of shoot and root systems of the plant which amounted to 727.0 and 46.4 gm respectively, compared to control treatment which was 1122.0gm.The treatment of T.h + F.o.l gave ahighest rate of dry weightof shoot and root systems which reached 3279.0 and 146.2 gm respectively. So, these treatments significantly differed from control and pathogenic fungus treatments which were 1122.0,727.0,70.4 and 46.4 gm respectively, followed by the treatment of *T.harzianum* and *T.longibrachitum*, which amounted to 2916.0, 2492.0,132.1 and 118.0 gm respectively. This result agreed with John *et al*. (2019) in increasing the dry weight of the root system of tomato when the bioagent*T.harzianum* was added to the soil. Also, it agreed with Salih and Al-Mansoury (2021) who found that the bioagents *T.harzianum* and *T.longibrachitum* led to increase the dry weight of shoot and root systems of eggplant when they interacted with *F.oxysporum*.

Table (2) Effect of the bioagents *T. harzianum* and *T. longibrachiatum* and the pathogenic fungus *F.oxysporum*f.sp.*lycopersici* and their interaction on dry weight of shoot and root systems (gm) of tomato plants in the greenhouse

Treatments	Dry weigh (gm)	
	Root system	Shoot system
Control	70.4	1122.0
T.harzianum	132.1	2916.0
T.h+F.o.l	146.2	3279.0
T.longibrachiatum	118.0	2492.0
T.l +F.o.l	107.6	2147.0
F.oxysporum f.sp. lycopersici	46.4	727.0
$L.S.D._{0.05}$	16.68	525.8

T.h= *Trichoderma harzianum* ; T.l= *T. longibrachiatum* ; F.o.l= *Fusarium oxysporum* f.sp.*lycopersici*

Effect ofthe bioagents*T. harzianum* and *T. longibrachiatum* and the pathogenic fungus *F.oxysporum*f.sp. *lycopersici* and their interaction on plant yield

The results (Table 3) revealed that the highest yield per plantwas obtained in the treatment ofT.h + F.o.l, which amounted to 3722.0 gm/plant, followed by the treatment of *T. harzianum*, which amounted to 2777.0 gm/plant, which were significantly differed from the control and the pathogen treatments, which amounted to 1637.0 and 1105.0 gm/plant respectively. The results agreed with Ibrahim (2016), whoreferred to the role of the

*e*ISSN1303-5150

bioagent*T.harzianum* in stimulating the systemic resistance of tomato plants through the production of phenolic compounds and enzymes, promoting plant growth, improving and strengthening the absorption of nutrients and from the soil. The result also came in compatible with Salih and Al-Mansoury (2021) who showed that *T.harzianum* and *T.longibrachiatum*led to increase the plant yield significantly compared to the pathogenic fungus *F.oxysporum*.

Table (3) Effect of the bioagents*T. harzianum* and *T.longibrachiatum* and the pathogenic fungus *F.oxysporum*f.sp.*lycopersici* and their interaction on tomato plant yield

Treatments	Plant yield (g/plant)
Control	1637.0
T.harzianum	2777.0
T. h+F.o.l	3722.0
T.longibrachiatum	2055.0
T.l +F.o.l	1666.0
F.oxysporumf.sp.lycopersici	1105.0
L.S.D	540.9

T.h= *Trichoderma harzianum* ; T.l= *T. longibrachiatum* ; F.o.l= *Fusarium oxysporum* f.sp.*lycopersici*

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