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ORIGINAL ARTICLE

EVALUATION OF THE EFFICIENCY OF THE BIOAGENT *TRICHODERMA LONGIBRACHIATUM* AGAINST ROOT ROT DISEASE OF CUCUMBER PLANT CAUSED BY THE FUNGUS *FUSARIUM SOLANI*

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Abstract: This study was conducted to evaluate the efficiency of the bioagent *Trichoderma longibrachiatum* in controlling cucumber root rot disease caused by *Fusarium solani*. The pathogenic fungus was isolated from the roots of cucumber plant infected with root rot disease. The pathogenicity of *F. solani* was tested in Petri dishes and it was found that it reduced the germination percentage to 50% compared to the control treatment, which was 90%. The results of dual culture showed that the bioagent *T. longibrachiatum* inhibited the growth of *F. solani* and the degree of antagonism reached 1 according to Bell scale. The results of the field experiment which conducted in the greenhouse showed that the bioagent *T. longibrachiatum* led to increase the germination percentage of cucumber plants, reaching 96.7%, which significantly differed from the pathogenic fungus treatment, which amounted to 63.3%. The results indicated that the bioagent *T. longibrachiatum* significantly reduced the percentage of disease severity to 20.33%, compared with the treatment of the pathogenic fungus *F. solani* alone, which reached 65.67%. bioagent *T. longibrachiatum* also increased the plant height significantly up to 177 cm, compared to control treatment and pathogenic fungus treatment, which were 142 and 116 cm, respectively. It was also found that bioagent *T. longibrachiatum* when it was interacted with the pathogenic fungus led to increase the plant height up to 167 cm, compared with the treatment of pathogenic fungi alone, which was 116 cm. Fresh and dry weight of shoot and root systems also increased significantly in the treatment of *T. longibrachiatum* compared to the treatment of pathogenic fungus, followed by the treatment of the interaction between *T. longibrachiatum* and the pathogenic fungus treatments. The results also revealed the efficiency of *T. longibrachiatum* in increasing plant productivity represented by the number of fruits, the average weight of the fruits and the fruit yield per plant, which were significantly differed from the pathogenic fungus treatment alone.

***Key words*:** Root rot, Cucumber, *Fusarium solani*, *Trichoderma longibrachiatum*.

Cite this article

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1. Introduction

Cucumber (*Cucumis sativius* L.), which belongs to family Cucubitaceae is one of the most important vegetable crops in Iraq and the world and its original habitat is India. Cucumber has many nutritional benefits, where its fresh fruits contain vitamins and elements such as Fe, K, P, Ca and a high percentage of water in addition to its medicinal uses. It is widely cultivated in Iraq at spring and autumn seasons, as it is grown in greenhouses and open fields. Cucumber is exposed to many diseases, including fungal, bacterial and viral diseases, in addition to insect pests and nematodes. Root rot disease caused by *Fusarium solani* is one of the important fungal diseases on this plant and considered as the most important determinants of crop cultivation in most countries over the world [Brasileiro *et al*. (2004)]. Several methods and strategies have been used to control this disease, such as fungicides, as they have a direct effect on the pathogen or enter the plant tissues and turn into toxic substances for the pathogen. However, pesticides effect on human health and the environment in addition to their high cost.

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Therefore, microorganisms have been used to control plant pathogens, which in recent years have received global attention through using them directly or using their formulation for controlling the diseases or inducing the systemic resistance. The bioagent are considered as control is a safe alternative to chemical pesticides, more effective and less harmful to the environment and non-target organisms. Many researchers have pointed out the importance and ability of bioagent *Trichoderma* spp. in stimulating the systemic resistance of plants against various pathogens, through the plant's secretion of the enzyme peroxidase as well as an increase in the production of the chitinase enzyme by the resistant fungus [Yeledhalli *et al*. (2012)].

2. Materials and Methods

2.1 Isolation and identification of the pathogen *Fusarium solani*

The infected cucumber plants that showed symptoms of root rot disease were collected and brought to the laboratory. The crown area and the roots of the affected plants were washed with tap water to remove the clay, then they were cut into small pieces 1-1.5 cm. Then superficially sterilized for 2-3 minutes with a commercial sodium hypochlorite solution (NaOCl) at a concentration of 10%, the pieces were washed with distilled water to remove traces of sterilization and dried by sterilized filter paper (Whatman No. 4). Then, they were put in Petri dishes with a diameter of 9 cm, containing sterile PDA culture medium supplemented with the antibiotic Chloramphenicol (250 mg/L) at an average of 5 pieces per each dish with three replicates. Then the dishes were incubated at a temperature of 25±2°C for four days. The grown fungi were isolated, identified according to phenotypic and microscopic characteristics based on the characteristics.

2.2 Pathogenicity test of *Fusarium solani* on cabbage seeds

The pathogenicity of the *F.solani* which isolated from the roots of the cucumber plant was tested on sterilized water agar 2% with Chloramphenicol (250 mg/L). The dishes were inoculated with a 0.5 cm diameter disc taken from the edge of a seven-day-age colony of *F. solani*. The dishes were incubated at a temperature of 25±2°C for three days, after that the cabbage seeds (golden acre cultivar) were superficially sterilized with 10% sodium hypochlorite solution for two minutes and dried on sterilized filter paper and put in the plate circularly near the edge, at an average of 10 seeds per each plate and with three replications, leaving a control treatment containing cabbage seeds without treating with pathogen. The dishes were placed in the incubator at a temperature of 25±2°C for 7 days, after that the percentage of germination was calculated as the following equation:

$$\text{Germination percentage} = \frac{\text{The number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

2.3 Antagonism test between the bioagent *T. longibrachiatum* and *Fusarium solani*

The antagonism between the bioagent *T. longibrachitum* and the *F. solani* was tested. A petri dish containing sterile PDA was divided into two equal parts and then the center of the first part was inoculated at a distance of 1 cm from the edge of the dish with a 0.5 cm diameter disc from the seven days colony of *T. longibrachitum*, the second part was inoculated at a distance of 1 cm from the edge of the dish with 0.5 cm diameter disc from the seven days colony of *F.solani* with a control treatment that was inoculated with a disc of diameter of 0.5 cm of the pathogen alone and with three replications for each treatment. All plates were incubated at a temperature of 25±2°C. The degree of antagonism was calculated after the growth in the control treatment reached the edge of the plate. The antagonism degree was calculated according to the scale of Bell *et al*. (1982), which consists of five degrees, as follows:

1- The bioagent covers the whole plate, including the pathogen.
2- The bioagent covers two-thirds of the plate.
3- The bioagent covers half of the plate.
4- The pathogen covers two-thirds of the plate.
5- The pathogen covers the whole plate.

The bioagent was considered as an effective agent if the degree of antagonism was 1 or 2.

2.4 Preparation of the fungal inoculation

The inoculum of the pathogen *F. solani* that was isolated from infected plants and the bioagent *T. longibrachiatum* were prepared by using an appropriate amount of seeds of local millet *Panicum miliaceum*. The seeds were washed with tap water to remove impurities and dust from them, then soaked for six hours, they dried with filter paper. The seeds were

distributed in 250 ml flasks with an amount of 100 gm per each flask and a little water was added to it to moisten them. The flask were blocked with cotton plugs and autoclaved at a temperature of 121°C and a pressure of 15 pounds/ing^2 for 20 minutes. The flasks were taken out to cool, then they were inoculated with the bioagent and pathogen, each individually by adding five 0.5 cm diameter disc of seven days colony of each one. The flasks were incubated at a temperature of 25±2°C for two weeks, shaking them every 2-3 days to ensure distribution of the inoculum on all seeds

2.5 Field experiment

The field experiment was conducted at the Agricultural Research Station, College of Agriculture, University of Basrah, on February 8, 2021 in a plastic house with dimensions of 10 × 40 m. The soil was prepared, plowed and divided into three rows with a long of 36 meters for each one, with a distance of 1 m between one row and another, with three replications each. A replicate contains five plants, the distance between each pit and the other is 40 cm. Drip irrigation system was used. The bioagent *T. longibrachiatum* was added with a rate of 1% w/w per each pit and left for three days with continuous moisterning of the soil. Then the pathogen *F. solani* was added at an average of 1% w/w per each pit. A control treatment free of both pathogen and bioagent was also prepared. The irrigation was continued for three days. After that, each pit was planted with cucumber seeds (GANAA F1 variety), at an average of 5 seeds per each pit. The experiment included the following treatments

1- The pathogen *F. solani* alone (F.s).
2- The pathogen *F.solani* + the bioagent *T. longibrachiatum* (F.s + T.l).
3- The bioagent *T. longibrachiatum* (T.l) alone.
4- Control treatment (control).

The disease severity was calculated after two months according to the disease index of four degrees followed by Al-Hasnawi (2017).

0- The plant is healthy and the root system is white in colour.
1- Discoloration of 1-25 of the root with a light brown color.
2- Discoloration of 26-50 of the root with a dark brown color.
3- Discoloration of 51-75 of the root with a dark brown color with yellowing of the leaves.
4- Discoloration of 76-100 of the root with a dark color with the death of the plant.

Then Mickenny's equation (1923) was applied as follows:

$$\text{Disease severity \%} = \frac{(\text{Number of plants in a degree of } 0 \times 0) + \ldots + (\text{Number of plants in a degree of } 4 \times 4)}{\text{Number of tested plants} \times 4} \times 100$$

2.6 Plant height

At the end of the season, the height of the plants for each experimental unit was calculated from the place where the plant contacts the soil. The final average of the height was calculated at the end of the season.

2.7 Fresh weight of shoot and root systems

At the end of the season, the weight of the shoot and root systems was calculated by using a sensitive scale after uprooting the plants from the soil and removing the clay from the roots by tap water and separating the shoot system from the root system.

2.8 Dry weight of shoot and root systems

The shoot and root systems were dried by an oven device at a temperature of 70°C for 48 hours, then the dry weight of each one was calculated using a sensitive scale.

2.9 Number of fruits/plant

The number of fruits per each plant was calculated from the beginning of the harvest up to end of the season, then the average of the total fruits per each plant was calculated according to the following equation

$$\text{Average number of total fruits/plant} = \frac{\text{Total number of fruits per experimental unit}}{\text{Number of plants in each experimental unit}}$$

2.10 Weight of the single fruit

At the end of the season, the average weight of the single fruit was calculated by dividing the average total yield of the plant in each experimental unit by the number of the harvested fruits according to the following equation:

$$\text{Average weight of single fruit (g)} = \frac{\text{Total treaments yield (g)}}{\text{The number of fruits in each experimental unit}}$$

2.11 Fruit yield per plant (g/plant)

This fruit yield per one plant was calculated by multiplying the number of fruits by the average weight of the fruit.

2.12 Statistical analysis

The laboratory experiments were conducted according to the Complete Random Design (CRD) and the field experiments according to the Randomized Complete Block Design (RCBD) and the significant differences between the means were compared with LSD test (Least Significant Difference) at the probability level of 0.01 for the laboratory experiments and 0.05 for the field experiments [Al-Rawi and Khalafallah (1980)]. The results were analyzed according to the GenStat **eriscovary** Edition program and the Microsoft Excel program.

3. Results and Discussion

3.1 Isolation and identification of the pathogenic fungus *Fusarium solani*

The results of isolating the *F. solani* after purification and studying its phenotypic and diagnostic characteristics showed that the isolated fungus was *F. solani* (Fig. 2). The colony is white to creamy color on the PDA medium, reverse is pale with appearance of areas of violet color. Microconidia are called spindle-shaped or ovoid, undivided, produced from long phialides, Macroconidia are straight and septate with 3-4 septa (Fig. 1). These characteristics are consistent with what was mentioned by Khan and Siddiquei (2021) and AL-Bayati (2019).

3.2 Pathogenicity test of *Fusarium solani* on cabbage seeds

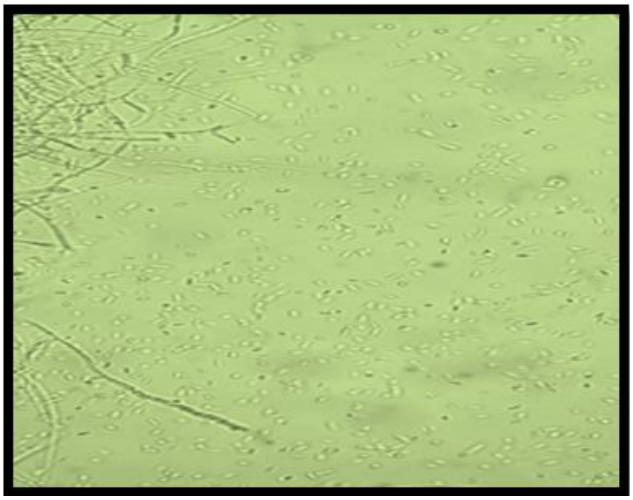
The results showed the ability of *F. solani* to inhibit the growth of cabbage seeds (GOLDEN ACRE IMP cultivar), where the percentage of seeds germination decreased to 50% compared to the control treatment, which was 90% (Fig. 3). These results agreed with Karim (2012) regarding the ability of the *F. solani* to reduce the percentage of germination of cabbage seeds. The reason for this may be due to the ability of *F. solani* to produce many toxins, including Fusarubin, Javanicin, Anhydrofusarudin, Protenoneons and Polypetide, which have toxic effects and play an important role in the fungus pathogenicity

3.3 Antagonism test between the bioagent *T. longibrachiatum* and the pathogen *Fusarium solani*

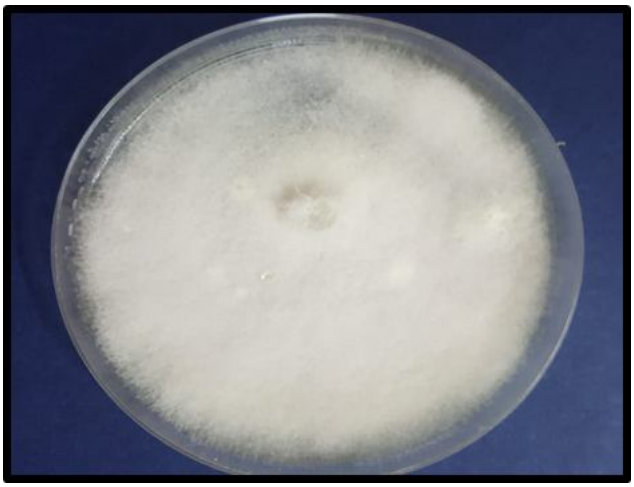
The results of the antagonism experiment (Fig. 4) showed that the bioagent *T. longibrachiatum* has a high antagonistic ability against the pathogen *F. solani*. The degree of antagonism reached 1 according to the scale of Bell *et al*. (1982), where the bioagent covered the whole plate. This result agreed with the study carried out by Al-Abbad (2020) about the high antagonistic ability *T. longibrachiatum* against the pathogens. The antagonistic ability of this fungus is due to different tools including direct parasitism, by wrapping the mycelium of the bioagent around the pathogen hyphae, ability to produce antibiotics and some enzymes that degrade the cell walls of the pathogen such as Protease, β-1,3-glucanase, Chitinase or through the ability to compete for food and space.

3.4 Field experiment

Effect of the bioagent *T. longibrachiatum* on the percentage of germination, the percentage of disease severity and plant height in the presence of *F. solani* causing root rot disease of cucumber: The results of the field experiment (Table 1) showed that the bioagent *T. longibrachiatum* led to increase the percentage of germination up to 96.7%, which was significantly excelled on the treatment of the *F. solani,* which amounted to 63.3. The results also showed that the disease significantly decreased when the bioagent interacted with the *F. solani* (F.s.+T.l). It reached 20.33% compared with the *F. solani* treatment alone, which amounted to 65.67%, with highly significant differences. This result agreed with Al-Behadli (1994) and Salih and Mansoury (2021), who indicated the role of Trichoderma in reducing the disease severity of the *F. solani* by reducing pathogen secretions of toxic substances and through the production of degarding enzymes that are responsible for the antagonistic ability against the pathogen. It was also found that the bioagent led to increase the plant heights reaching 177 cm, which was significantly excelled the control treatment and the pathogenic fungus treatment which were 142 and 116 cm, respectively, followed by the treatment of the interaction between the bioagent and the pathogenic fungus (F.s. + T.l.), reaching 167 cm, which significantly differed from the pathogenic fungus alone treatment which was 116 cm. This result was in agreement with Salih and Mansoury (2021), who indicated that the bioagent *T. harzianum* led to increase the plant height significantly compared to the pathogen *F. solani*. The



Fungus Conidia



Fungus colony

Fig. 1: Fungus colony and conidia of *Fusarium solani* isolated from cucumber roots on PDA culture medium

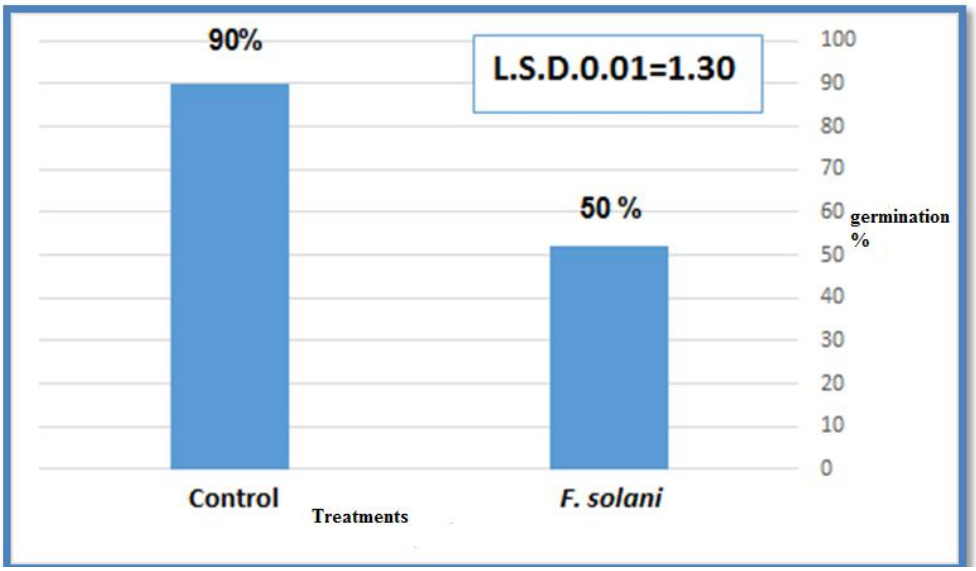
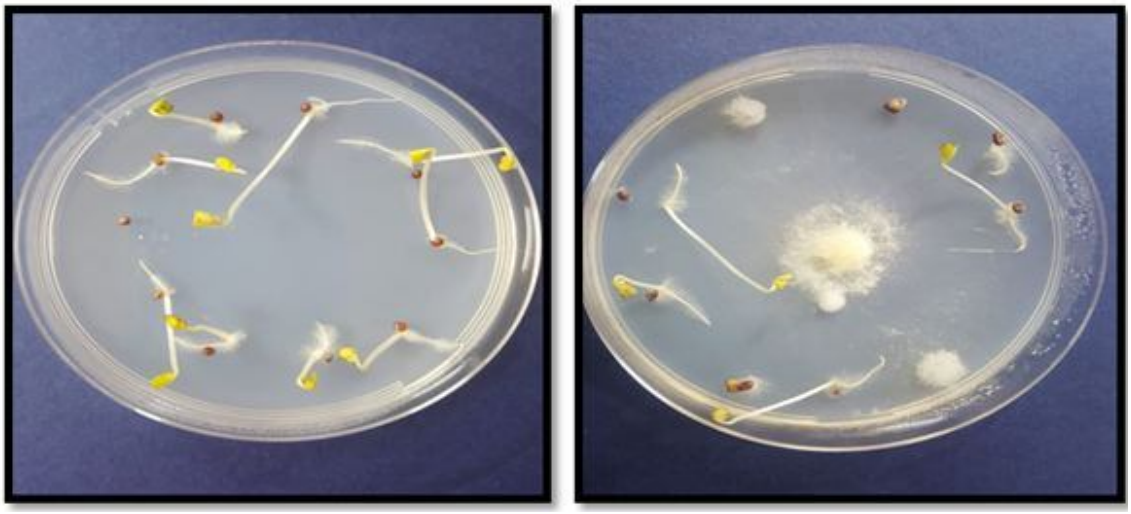


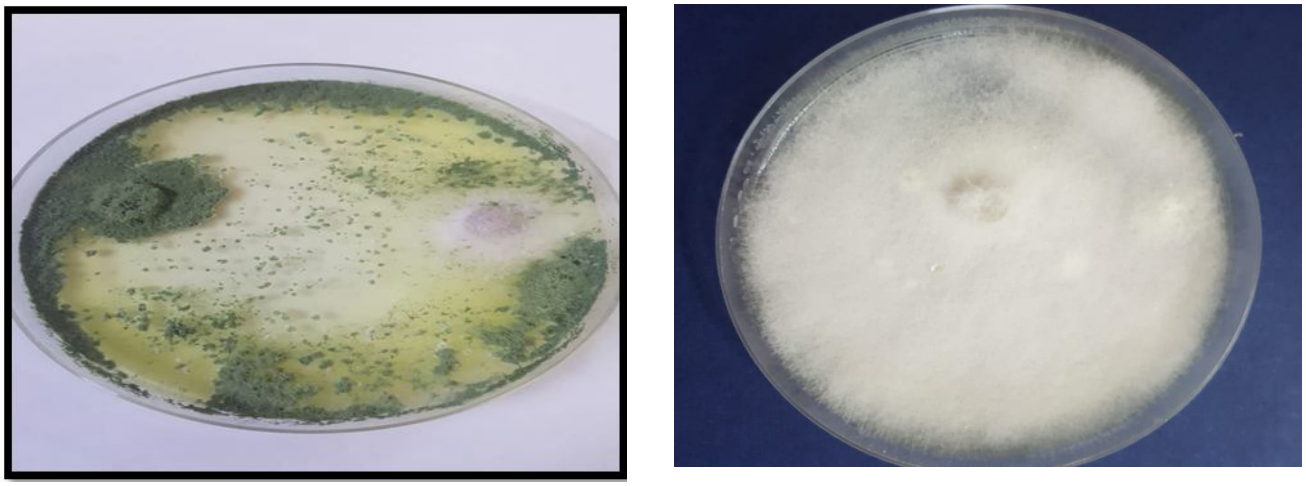
Fig. 2: Effect of *Fusarium solani* on the cabbage seeds germination percentage on PDA medium



Fusarium solani

Control

Fig. 3: Effect of *Fusarium solani* on the percentage of germination of cabbage seeds in plates



F. solani* + *T. longibrachiatum ***F. solani***

Fig. 4: Antagonism test between the bioagent *T. longibrachiatum* and *F. solani*

Table 1: The effect of the bioagent *T. longibrachiatum* and the pathogenic fungus *F. solani* on the percentage of germination, disease severity, and plant height in the presence of *F. solani* causing root rot disease of cucumber.

Treatments	Germination * %	% Disease severity	Plant height (cm*)
F.s.	63.3	65.67	116
F.s.+T.l.	76.7	20.33	167
T.l.	96.7	0	177
Control	83.3	0	142
L.S.D.0.05	**8.40**	**4.013**	**27**

*Each number represents an average of three replicates
Trichoderma longibrachiatum = T.l. *Fusarium solani* = *F.s.*

Table 2: Effect of the bioagent *T. longibrachiatum*, the pathogenic fungus *F. solani* and the interaction between them on the fresh and dry weight of the shoot and root systems of cucumber.

Treatments	Fresh weight (g)		Dry weight (g)	
	Shoot system	Root system	Shoot system	Root system
F.s.	102.7	2.53	14.63	0.42
F.s.+T.l.	171.4	4.31	22.36	0.77
T.l.	241.7	6.81	26.90	0.97
Control	156.7	3.96	19.72	0.52
L.S.D.$_{0.05}$	81.9	1.75	10.61	0.64

*Each number represents an average of three replicates *Trichoderma longibrachiatum* = T.l. *Fusarium solani* = F.s.

remarkable increase in plant heights is due to the role of the bioagent in increasing plant growth through preparation of phosphorous and regulating ethylene production in roots [Martinez-Medina *et al*. (2010)].

Table 3: Effect of the bioagents *T. longibrachiatum* and the pathogenic fungus *F.solani* and the interaction between them on the yield of cucumber plant.

Treatments	Average number of fruits*fruit/ plant	Average of fruit weight (g)*	Average of plant yield (g)*
F.s.	2.333	127.700	325
F.s.+T.l.	3.167	146.100	402
T.l.	4.010	164.400	500
Control	3.500	149.900	372
L.S.D.$_{0.05}$	0.558	26.21	114.7

*Each number represents an average of three replicates *Trichoderma longibrachiatum* = T.l. *Fusarium solani*=*F.s*

3.5 Fresh and dry weight of shoot and root systems

It was shown from Table 2 that the best bioagent *T. longibrachiatum* led to increase the fresh weight of shoot and root systems of cucumber up to 241.7 and 6.81 g, respectively, with a high significant difference from the control treatment which was 156.7 and 3.96 g, respectively and the pathogenic fungus treatment which was 102.7 and 2.53 g, respectively, followed by the treatment of the interaction between the bioagent and the pathogenic fungus (F.s.+T.l.), which amounted to 171.4. and 4.31 g, respectively, which differed significantly from the treatment of pathogenic fungus alone, which amounted to 102.7 and 2.53 g, respectively. It was also found that the bioagent T. *longibrachiatum* led to dry weight of shoot and root systems of cucumber up to 26.90 and 0.97 g, respectively and the pathogenic fungus treatment significantly excelled and gave 14.63

and 0.42 g, respectively. The results agreed with Salih and Mansoury (2019), who found that the bioagent *T. longibrachiatum* led to increase the fresh and dry weight of shoot and root systems of okra significantly.

3.6 Plant yield

Table 3 elucidated that the bioagent was significantly different from *T. longibrachiatum* lwhich ed to increase the average number of fruits up to 4.010 fruits /plant followed by the treatment F.s. + T.l., which amounted to 3.167 fruits/plants, they were significantly differed from the pathogenic fungus treatment which was 2.333 fruits /plant. It was also found from the same table that *T. longibrachiatum* led to increase the fruit weight significantly, which reached 164.400 g, compared to the pathogenic fungus treatment which was 127,700 g. on the other hand, *T. longibrachiatum* achieved a significant increase in plant yield, as the average yield reached 500 g, which differed from the pathogenic fungus and amounted to 325 g. These results agreed with Abbas (2020), who indicated the role of bioagents in improving the plant growth by providing phosphate ions continuously in the early stages of crop growth. So, this leads to increase the yield of the crop represented by the number of fruits, the weight of the fruit and the plant yield. The results also agreed with Salih and Mansoury (2021), who found that the bioagent *T. longibrachiatum* led to increase fruit weight, fruit number and plant yield significantly compared to the pathogen *F. solani*.

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