Int. J. Agricult. Stat. Sci. Vol. 18, Supplement 1, pp. 2027-2032, 2022
DocID: https://connectjournals.com/03899.2022.18.2027
www.connectjournals.com/ijass
ISSN: 0973-1903, e-ISSN: 0976-3392

ORIGINAL ARTICLE

STUDY OF EFFECT OF BIOAGENT *TRICHODERMA LONGIBACHIATUM* ON CUCUMBER ROOT ROT DISEASE CAUSED BY THE PATHOGENIC FUNGI *RHIZOCTONIA SOLANI* AND *FUSARIUM OXYSPORUM* IN POTS

Huda H. A. Al-Abbas* and Yehya A. Salih

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

Abstract: The study aimed to investigate the effect of the bioagent *Trichoderma longibrachiatum* on cucumber root rot disease. Two pathogenic fungi named *Rhizoctonia solani* and *Fusarium oxysporum* were isolated from the roots of cucumber plants infected with root rot disease. The pathogenicity of these fungi was tested in pots and observed that pot of them are pathogenic fungi. The results showed that the germination percentage was significantly reduced to 46.7% and 50% in response to the treatment with *R. solani* and *F. oxysporum,* respectively compared to control treatment which was 80%. The results also showed that the disease severity increased in the treatment of *R. solani* which reached 72.7%, followed by 62% with the treatment of *F. oxysporum* compared to control treatment which was 86.7%. Furthermore, the results indicated that *R. solani* and *F. oxysporum* led to increase damping off to 40.4 % and 22 %, respectively compared to the control treatment (0 %). It was also found that the exydates of *R. solani* and *F. oxysporum* significantly reduced the percentage of germination to 33.3 % and 43.3%, respectively compared to the control treatment which was 86.7%. Moreover, the interaction between *T. longibrachiatum* and each of two pathogenic fungi *R. solani* and *F. oxysporum* significantly reduced disease severity to 20.33 and 22.33%, respectively compared to the pathogenic fungi alone which were 80.33 and 69.67% ,respectively.

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

Cite this article

Huda H. A. Al-Abbas and Yehya A. Salih (2022). Study of Effect of Bioagent *Trichoderma longibachiatum* on Cucumber Root Rot Disease Caused by the Pathogenic Fungi *Rhizoctonia solani* and *Fusarium oxysporum* in pots. *International Journal of Agricultural and Statistical Sciences.* DocID: https://connectjournals.com/03899.2022.18.2027

1. Introduction

Cucumber (*Cucumis sativius* L.) belongs to the family Cucurbitaceae is considered to be an important crop in Iraq and the world. The origin habitat of this plant is India [Al-Bayati *et al*. (2012)]. Cucumber contains high level of vitamins and nutrients such as Fe, K, P and Ca, and a high percentage of water as well as its medicinal uses. Cucumber is infected with many fungal diseases like root rot and damping off which is caused by *Rhizoctonia solani* and *Fusarium oxysporum* resulting in a high economic losses [Muriungi *et al*. (2014)]. Various methods have been used to control these diseases such as fungicides which either have direct effects on pathogens or indirectly through entering plant tissues and turn into toxic substances against the causative pathogen. However, pesticides have some negative health effects on human, environment in addition to high cost [Salih and Al-Mansoury (2021), Qin *et al*. (2011)]. Therefore, biological control is considered as the best and safe method to control the pathogens. This method has recently been receiving worldwide attention through the direct use of microorganisms against plant pathogens or indirectly through different compounds produced by microorganisms. The antifungal activities of these compounds suppress the pathogen or stimulate the systemic resistance against the pathogen. The biological control is safe alternative method with high efficiency, less environmentally harmful and less harmful to non-target organisms. The *Trichoderma* species are commonly used as bioagent microorganisms against seed, root and foot rot, because they have various mode

***Author for correspondence** **Received** September 08, 2021 **Revised** February 24, 2022 **Accepted** April 02, 2022

of actions for controlling soil borne fungi that cause different diseases [Dumaresq *et al*. (2016)].

There are several studies about using the bioagents *T. harzianum* and *T. longibrachiatum* against root rot and damping – off diseases on okra and eggplants were recently carried out at Basrah province [Salih and Al-Mansoury (2021)]. Despite the importance of root rot disease in cucumber plant no study is yet reported on using the bioagent *T. longibrachiatum* against this disease in Iraq. Thus, this study was suggested to detect of the effect of *T. longibrachiatum* on cucumber root rot disease caused by *R. solani* and *F. oxysporum*

2. Materials and Methods

2.1 Isolation and identification of the pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum*

The infected cucumber plants with root rot disease were collected from several regions in Basrah governorate during the agricultural season 2021. The crown and roots of the infected plant were washed to remove the stuck mud. Plants were cut into small pieces of 1-1.5 cm, followed by surface sterilization with 10% commercial sodium hypochlorite solution (NaOCl) for 2-3 minutes. All pieces were washed with distilled water to remove traces of sterilization. Sterile filter paper (Whatman No.4) was used to dry pieces before planting in Petri dishes (9 cm diameter), containing sterile PDA medium supplemented with an antibiotic Chloramphenicol (250mg/L). Three plates were used with five pieces in each one. Then plates were incubated at a temperature of 25±2°C for four days. After that, the fungi were purified and identified according to phenotypic and microscopic characteristics.

2.2 Pathogenicity test of the pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum*

This experiment was conducted in pots. Pots were filled with mixture of soil and peat moss at a ratio of 1:3 (soil: peat moss). The mixture was autoclaved at 121°C and 15 pounds/inch^2 for an hour, the sterilization procedure was repeated again on the second day. The fungal inoculum *R. solani* and *F. oxysporum* was added to the soil mixture at a rate of 1 % weight. The soil mixture and the inoculum were put in a polyethylene bag and mixed well to homogenize the inoculum with the soil. Then they put in pots (0.5 kg) and irrigated with water for three days. After that, cucumber seeds (GANAA F1) were sterilized superficially with 10% sodium hypochlorite solution for 2-3 minutes. Ten seeds per pot and three replicates were applied while the control treatment was left without adding any pathogenic fungus. Pots were irrigated and incubated at 28 ± 2°C under suitable growth and light conditions. So, the plants were irrigated whenever it needed. The percentage of germination was calculated after one week of planting according to the following equation:

$$\text{Germination \%} = \frac{\textit{Number of the germinated seeds}}{\textit{Total number of seeds}} \times 100$$

After four weeks damping off was calculated according to the following equation:

$$\text{\% Damping off} = \frac{\textit{Number of deadly infected seedings}}{\textit{Number of germinated seedings}} \times 100$$

The disease severity was calculated (after about 6 weeks) according to the test disease as follows:

Disease index of the fungus *R. solani*

0 = The plant is healthy, and the root system is white in color.

1= Root discoloration yellowish- brown and ulceration less than 10 mm in diameter around the stem.

2= Root discoloration dark brown and ulceration 11-20 mm in diameter around the stem.

3=Reddish brown ulceration surrounding the stem.

4= Death of plant.

Disease index of the fungus *F.oxysporum*

0 = Plant is healthy.

1= Yellowing of lower leaves.

2 = Wilting of lower leaves and yellowing of upper leaves.

3= Death of lower leaves and wilting of upper leaves.

4= Plant death completely.

Then Mickenny's equation (1923) cited from Al-Waily (2004) was used to calculate the disease severity as follows:

$$\text{\% Disease severity} = \frac{(\textit{Number of plant on degree } 0\times 0) + \ldots + (\textit{Number of plant on degree } 4\times 4)}{\text{Total number of plant tested} \times 4} \times 100$$

2.3 Test of the effect of the pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum* and bioagent *Trichoderma longibrachiatum* exudates on cucumber seeds germination in plates

In this experiment, flasks (250 ml) were used with 150 ml of prepared liquid culture medium (PD Broth) supplemented with Chloramphenicol (250 mg /l). The flasks were sterilized at 121°C and 15 pound/inch^2 for 20 minutes. Each flask was inoculated with 5 pieces of 0.5 cm diameter of the fungal isolates. Inoculums were taken from the edges of seven-days-age colony. Flasks were incubated at 25±2°C for 14 days with shaking every 48 hours during the incubation period. The cultures were filtered using Whatman No.1 filter paper, the suspension was then passed through fine filter with diameter of 0.22 micrometers (Millipore filter) to obtain sterile suspension. Ten cucumber seeds were sterilized with 10% hypochlorite solution and put circularly near the edge of each petri dish contain filter papers wetted with 6 ml of each of pathogenic and bioagent exudates. Control treatment was left without adding any exudate. Three replicates were prepared for each treatment. The plates were incubated under 25 ± 2°C for 8 days with adding exudate continuously as it needed. The percentage of germination was calculated according to the following equation:

$$\text{Germination \%} = \frac{\textit{Number of the germinated seeds}}{\textit{Total number of seeds}} \times 100$$

2.4 Pots experiment

This experiment was carried out in the pots putting in a plastic house at the Research Station of the College of Agriculture, University of Basrah. The soil and peat moss were sterilized with formalin and used with a rate of 1:3 (soil: peat moss). A 40% formalin stock solution was prepared by diluting it to a ratio of 1/50 and added to the soil with a rate of 3 liters per 1 m^3 [Tawajen (1975)]. A polyethylene sheet was used to cover the soil for three days, then before the soil exposed to air for seven days to remove the formalin residues. After that, the sterilized soil was placed in plastic pots, contain 3 kg soil for each one. The bioagent *T. longibrachiatum* was added in an average of 1% w/w of grown fungus on millet seeds and left for three days with irrigation. After that, the pathogenic fungi *R. solani* and *F. oxysporum* grown on millet seeds at a rate of 1% w/w were mixed with the soil thoroughly and irrigated for other three days. Ten superficially sterilized cucumber seeds (GANAA F1) were planted per each pot. All treatment were performed with three replicates.

The experiment involved the following treatments:

1. The pathogenic fungus *R.solani* alone.
2. The bioagent *T. longibrachiatum* + *R. solani.*
3. The pathogenic fungus *F. oxysporum* alone.
4. The bioagent *T. longibrachiatum*+*F. oxysporum.*
5. The bioagent *T. longibrachiatum* alone.
6. Control.

The percentage of disease severity and plant growth parameters such as plant height, fresh and dry weight of the shoot and root systems were calculated in the end of experiment.

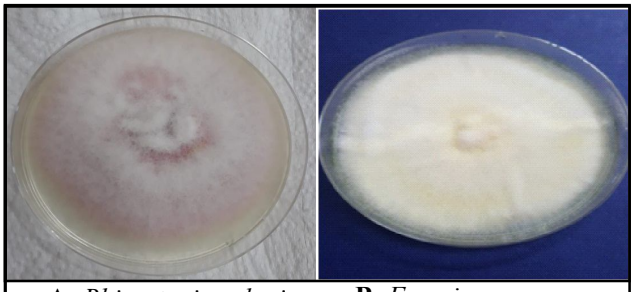
2.5 Statistical Analysis

All laboratorial experiments were conducted according to the Completely Randomized Design (CRD), while pot experiments were carried out according to Randomized Complete Block Design (RCBD). The mean differences among the averages were compared with least significant difference (LSD) under probability level of 0.01 for the laboratorial experiments and 0.05 for pot experiments. All treatments were repeated for three replicates [Al-Rawi and Khalaf Allah (1980)]. All statistical analyses were conducted by GenStat Discovery Edition and Microsoft Excel programs.

3. Results and Discussion

3.1 Isolation and identification of pathogenic fungi

Two pathogenic fungi named *Rhizoctonia solani* and *Fusarium oxysporum* were isolated from the root of cucumber plants infected with root rot disease which collected from different region of Basrah governorate represented by Al-Zubair, Safwan, Shatt Al-Arab, Abu Al-Khasib and Al-Medaineh during the growing season of 2021. Fig. 1 and Table 1 showed that *R. solani* has been isolated from most surveyed regions (4 regions),



A- *Rhizoctonia solani* **B-** *Fusarium oxysporum*

Fig. 1: Colonies of the pathogenic fungi *Rhizoctonia solani* (A) and *Fusarium oxysporum* (B) isolated from the roots of cucumber plants infected with root rot disease on PDA culture medium

Table 1: Fungi isolated from the roots of cucumber plants infected with root rot disease collected from different regions of Basrah governorate during the agricultural season 2021.

Regions	Pathogenic fungi
Al-Zubair	*R. solaniF. oxysporum*
Al-Medaineh	*F. oxysporum*
Abu Al-khaseeb	*R. solani*
Safwan	*R. solani*
Shatt Al-Arab	*R. solani*

while *F. oxysporum* has been isolated from only two regions included Al-Zubair and Al-Medaineh. Their morphological and microscopic characteristics were identical with parameters.

3.2 Pathogenicity test of Pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum* pathogens

The results in Table 2 showed that the germination percentage was significantly decreased in response to the treatment with *R. solani* and *F. oxysporum* to 46 and 50%, respectively in comparison with control treatment (86.7%). Furthermore, these fungi led to significant increase in disease severity amounted to 72.7 and 62.1%, respectively. Also they led to increase damping off significantly, reached to 40 and 22%, respectively. These results are agreed with many previous studies conducted by Salih and Al-Mansoury (2021), who indicated that *R. solani* and *F. oxysporum* have led to increase the disease severity in cucumber, eggplant and okra plants. Many plant pathogens are virulent for the host plant resulting in light or severe infection. However, there are three metabolic activities in microorganisms that are important in pathogenicity including secretion of lytic enzymes, toxins and growth regulators, so the pathogenicity may vary depending on the mode of action of these metabolites, either collectively or individually [Agrios (2005)].

Table 2: Effect of the pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum* on the percentage of germination, disease severity and damping off in pots experiment.

Treatments	Germination %	Disease severity %	Damping off %
R. solani	46.7	72.7	40.4
F. oxysporum	50.0	62.1	22.0
Control	86.7	0.0	0.0
L.S.D. 0.01	17.68	15.57	28.59
*Each number represents three replicates			

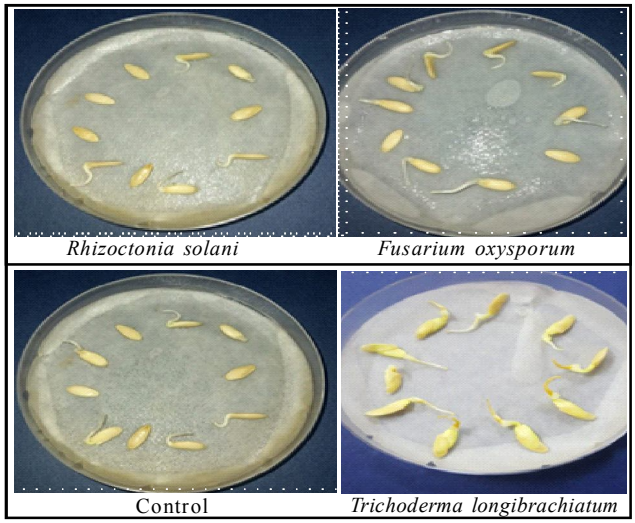


Fig. 2: Effect of the exudates of pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum* and the bioagent *Trichoderma longibrachiatum* on the

3.3 Test of the effect of the pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum* and the bioagent *Trichoderma longibrachiatum* exudates on the of cucumber seeds germination in plates

The results (Fig. 2 & 3) revealed that the treatment with bioagent *T. longibrachiatum* exudate led to increase the germination percentage up to 96.71%, while the exudates of the pathogenic fungi *R. solani* and *F. oxysporum* resulted in a significant reduction in germination percentage 33.3% and 43%, respectively compared to the control and *T. longibrachiatum* treatment which were 86.7% and 96.7%, respectively. These results agreed with results of Al-Outbi (2020) who found that the treatment with the exudate pathogenic fungi *R. solani* led to reduce the germination percentage in okra seeds, while the exudate of *T. longibrachiatum* led to increase the seed germination

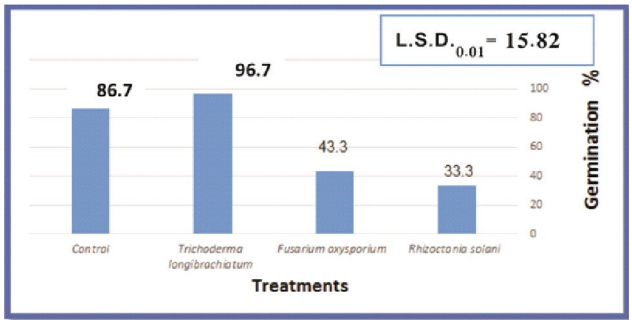


Fig. 3: Effect of the exudates of the pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum* and the bioagent *Trichoderma longibrachiatum* on the germination percentage of cucumber seeds in the plates

Table 3: Effect of the pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum,* the bioagent *Trichoderma longibrachiatum* and the interaction among them on the percentage of disease severityof root rot disease of cucumber and plant height in the post.

Treatments	Disease severity %	Plant height (cm)
R.solani	80.33	11.50
R.solani + T. longibrachiatum	22.23	28.87
F.oxysporum	69.67	13.67
F.oxysporum + T. longibrachiatum	20.33	31.33
T. longibrachiatum	0	58.67
Control	0	31.87
L.S.D.0.05	8.37	4.23
*Each number represents three replicates		

on percentage. The bioagent exudate has the ability to decrease the negative effect of pathogens and consequently increase the percentage of germination. In addition, the bioagent exudate also has the ability to penetrate into the seeds and induce systemic resistance. However, soaking the seeds with the exudate of pathogenic fungi alone led to decay the seeds and cause root rot disease. These pathogenic fungi are considered to be the most common fungi that cause seed decay and reduce the germination due to the secretion of several toxins and enzymes [Diwan and Al-Bahadli (1985)].

3.4 Effect of the pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum*, the bioagent *Trichoderma longibrachiatum* and the interaction among them on the percentage of disease severity of cucumber root rot and plant height in pots

The results of pot experiments (Table 3) revealed that effectiveness of the bioagent *T. longibrachiatum* in reducing the disease severity of root rot caused by *R. solani* and *F. oxysporum*. The percentage of disease severity was significantly reduced by 22.33% in the treatment of *T. longibrachiatum* + *R. solani* and by 20.33% in the treatment of *T. longibrachiatum* +*F. oxysporum* compared to the treatments with the two pathogenic fungi alone which were 80.33% and 69.6%, respectively. These results agreed with Salih and Al-Mansoury (2021) who confirmed the efficacy of the bioagents *T. harzianum* and *T. longibrachiatum* in decreasing disease severity by root rot in tomato, okra and eggplant caused by *R. solani* and *F. oxysporum. Kucuk* and Kivanc (2003) also explained that adding the bioagent Trichoderma to the soil, led to inhibit the growth of *R. solani* and *Sclerotium rolfsii* with a percent of 76.6% and 82%, respectively. Table 3 also showed that the treatment with *T. longibrachiatum* led to increase the plant height in the presence of the pathogenic fungi *R. solani* and *F. oxysporum*. up to 28.87 and 31.33 cm, respectively, which significantly differed from the treatments with the pathogenic fungi alone which were 11.50 and 13.67 cm, respectively. Also, it was found from the same table that the bioagent *T. longibrachiatum* led to increase the plant height significantly which reached 58.67 cm compared to control treatment which was 31.87 cm. These results agreed with Salih and Al-Mansoury (2021) who indicated that the bioagents *T. harzianum* and *T. longibrachiatum* led to increase the plant height significantly when they interact with *R. solani* and *F. oxysporum.* The high efficacy of the bioagent attributed to its ability to compete for nutrient and thus reduce the density of other microorganisms, which consequently

Table 4: Effect of the pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum*, the bioagent *Trichoderma longibrachiatum* and the interaction among them on the fresh and dry weight of shoot and root systems of cucumber in the post.

Treatments	Shoot systems		Root system	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
R.solani	1.98	0.240	0.337	0.003
R.solani + T. longibrachiatum	14.62	2.077	0.563	0.136
F.oxysporum	2.16	0.337	0.110	0.013
F.oxysporum + T. longibrachiatum	14.72	2.493	0.650	0.136
T. longibrachiatum	19.34	2.817	1.027	0.293
Control	11.74	1.433	0.313	0.053
L.S.D.0.05	3.29	0.868	0.310	0.061
*Each number represents three replicates				

increase the availability of nutrients in the place on which bioagent can live.

3.5 Effect of the pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum*, the bioagent *Trichoderma longibrachiatum* and the interaction among them on fresh and dry weight of the root and shoot systems of cucumber in the post

Table 4 elucidated that the treatments with *R. solani* and *F. oxysporum* had significantly reduced the fresh and dry weight of shoot system to 1.98 g, 0.240 g, 2.16 g and 0.337 g, respectively compared to control treatments which were 11.74 g and 1.433 g, respectively. The results also revealed that the treatment with *R. solani* and *F. oxysporum* also reduced the fresh and dry weight of root system to 0.337, 0.003, 0.110 and 0.013 gm respectively, compared to control treatments which were 0.313 and 0.053 gm, respectively. It was also noticed that the bioagent *T. longibrachiatum* led to increase the fresh and dry weight of shoot and root systems significantly 19.34, 2.817, 1.027, 0.293 gm, respectively compared to the treatments of pathogenic fungi alone and control which has no pathogen. The results (Table 4) also showed that the treatments of interaction between the bioagent *T. longibrachiatum* and *R. solani* led to increase the fresh and dry weight of shoot and root systems significantly which amounted to 14.62, 2.077, 0.563 and 0.136 gm, respectively compared to treatments of pathogenic fungus alone (*R. solani*) which were 1.98, 0.240, 0.337 and 0.003 gm, respectively. From other hand, the treatments of interaction between the bioagent *T. longibrachiatum* and *F. oxysporum* also led to increase the fresh and dry weight of shoot and root systems significantly, which amounted to 14.72, 2.443, 0.650 and 0.136 gm, respectively compared to treatments of pathogenic fungus alone (*F. oxysporum*) which were 2.16, 0.337, 0.110 and 0.013 gm, respectively.

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