

The effect of *Trichoderma longibrachiatum* Against Damping-off Pathogens on Different Varieties of Cucumber in Vitro

Ghufran Mohsin Bashah and Akeel Emad Mohammed

Faculty of Agriculture – University of Kufa – Republic of Iraq.

Corresponding author Email: akeele.alzamily@uokufa.edu.iq

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Abstract

The study was carried out to evaluate the effect of *Trichoderma longibrachiatum* against damping-off pathogens on four commercial cultivars of cucumber in vitro. Various sites of Najaf province including (Abasia, Quzwenia, Haydaria, Manathera and Meshkab) were surveyed to detect the spread of studied disease. Pathogenic fungi were isolated from roots and stem parts of infected cucumber plants that collected from studied sites then diagnosed morphologically. The pathogenicity test was conducted on fungal isolates and the susceptibility of four different cucumber varieties (Superina F1, Beit alpha F1, Cucumber short and Hybrid cucumber) to these isolates was tested in laboratory with the presence of the fungal bio-control *T. longibrachiatum*. One isolate of *Fusarium* sp. and another isolate of *R. solani* found to be very virulence which increase the percentage of damping off on seedlings up to 90 and 100% respectively compare to control treatment of the four studied varieties that recorded 100, 95, 90 and 85 respectively and reduced vegetative and root growth indicators of cucumber plants (the weight and the length of plumule, the weight and the length of radicle) and these isolates were chosen to complete the later studies. The diagnosis of these two isolates then confirmed by (PCR) to *F. solani* and *R. solani* as both isolates were registered in National Center for Biotechnology Information (NCBI) under ON394603 and ON398997 accession numbers respectively. Results showed that *T. longibrachiatum* treatment was increased studied indicators when it was added alone or with other treatments to the soil of plastic pots and provided a protection for cucumber plants from the infection by the disease. The susceptibility of the four studied different cucumber varieties to *F. solani* and *R. solani* showed that Cucumber short and Beit alpha F1 varieties were more susceptible to the infection by damping off pathogens compare to moderate susceptibility in Hybrid cucumber, while Superina F1 showed less susceptibility which clearly indicated in the studied vegetative and root growth indicators of cucumber plants.

Keywords: *F. solani*, *R. solani*, damping-off, cucumber, susceptible cultivars.

Introduction

Cucumber (*Cucumis sativus* L.) is one of 118 genera vegetable crop that belongs to Cucurbitaceae (3), and it cultivated in different regions worldwide including Iraq as it cultivated twice a year either in open field or protected agriculture (19). It is believed that the original place of cucumber is Asia specifically China when it cultivated 100 years B.C. then in the nineteenth century it spread in France (18). Cucumber ranks fourth place after tomato, cabbage and onion in Asia and the second in Europe after tomato (24). It contains 8.2% carbohydrates, 4.0% protein, 3.0% mineral elements, 1.0% fat and little amounts of vitamins as well as it has low calories and used in salads and pickles (25). Cucumber fruits are containing Cucubitacin which helps to protect from cancer, reduce high blood pressure, protect the skin and prevent constipation (9). The area that grown by cucumber in each season is estimated about 95281 dunam in Iraq with total productivity of 242614 ton in 2020 (Central Organization for Statistics in Iraq 2020).

The crop is exposed to many plant diseases as it attacked by bacteria, viruses and fungi particularly *Fusarium solani*, *Rhizoctonia solani*, *Macrophomina phaseolina* pathogens (the causal agents of damping-off disease) (2) which are difficult to control as it endemic in soil, its wide range of hosts, it survive in soil for long periods and its resistance to unfavorable conditions (22). Several methods were used to control damping-off disease including chemical method as it gives quick and decisive results, however, due to the excessive use of these pesticides, negative effects have appeared on human health, non-target

organisms and the environment in general (21; 23). Due to these negative effects, researchers attempt to find alternative methods to control plant pathogens such as the use of disease-resistant plant varieties as it one of the most promising and desirable method due to its safe use, costless and reducing chemical control hazards (17). Resistant varieties also are one of the basic plant pathogens control as these varieties have special genes against some pathogens which prevent disease or impede its development (8).

Biological control of plant diseases also used widely to control plant pathogens particularly endemic organisms in soil such as *Trichoderma* spp. to protect plant due to its multiple mechanisms against pathogens including the competition for food and place, antagonistic, parasitism, secretion of enzymes and induction of resistance in plants (15; 10). Therefore, the current study aimed to evaluate the effect of *T. longibrachiatum* against damping-off pathogens on four commercial cultivars of cucumber in vitro.

Materials and Methods

Isolation of pathogens

A field survey in several sites (Abasia, Quzwenia, Haydaria, Manathera and Meshkab) in Najaf province was conducted in summer season 2021. Pathogenic fungi were isolated from roots and stem parts of cucumber plants that collected from studied sites. 0.5 to 1cm pieces of roots and stem were cut and washed well then sterilized with 2% sodium hypochlorite for 3 minutes, after that, it washed with sterile water several times to remove traces of sterilization and put in Petri plates on P.D.A medium then incubated for three

days at $25 \pm 2^\circ\text{C}$, until the colonies of the fungus became clear. Isolates were purified and re-cultured on 9-cm sterile Petri plates containing potato dextrose agar (P.D.A) and kept at 4°C until used.

Identification of fungi

Fungal isolates were diagnosed morphologically under the microscope based on the structure of their hyphae, colour of colonies, spores and the nature of growth on culture media followed by molecular diagnoses using polymerase chain reaction (PCR) and Internal transcribed spacer (ITS) to confirm their identification.

Bio-control agent used in this study

Trichoderma longibrachiatum isolate was obtained from Plant Pathology postgraduate laboratory/ Faculty of Agriculture/University of Kufa. This isolate was used in previous studies and showed high control of some pathogens when it registered in GenBank (NCBI) under MZ021580 accession number.

Cucumber seeds used

Four different cucumber varieties (Superina F1, Beit alpha F1, Cucumber short and Hybrid cucumber) were obtained from local markets in Najaf province and used in this study.

The effect of *T. longibrachiatum* on the growth of *F. solani* and *R. solani* that cause damping-off disease on cucumber in vitro

The experiment was carried out in Plant Pathology Laboratory/ Department of Plant Protection/ Faculty of Agriculture/University of Kufa. Loamy-sandy soil was divided into parts, the first part was not sterilized and the second part was autoclaved at 121°C and 1.5bound/ench² pressure for 1 h and next day the soil sterilization was repeated under the same conditions for 1 h.

200g of autoclaved and non-autoclaved soil was put in small plastic pots and inoculated with 2g/pot of *Panicum miliacem* seeds contaminated with the pathogenic fungi *F. solani*, *R. solani* and *T. longibrachiatum*. Pots were watered for three days to activate fungi in the soil. Seeds of cucumber varieties (Superina F1, Beit alpha F1, Cucumber short and Hybrid cucumber) were sterilized with 10% of commercial concentration of sodium hypochlorite for 2 minutes then washed with sterilized distilled water to remove traces of chemical solution. 3 seeds of each variety of cucumber were planted in each pot (Table 1), after 10 days, the percentage of seed germination and seedling damping off, the weight and the length of plumule, the weight and the length of radicale were calculated.

Table 1. The distribution of experimental treatments.

Treatments	Superina F1	Hybrid cucumber	Biet alpha F1	Cucumber short
T1	R1R2 R3	R1R2 R3	R1R2 R3	R1R2 R3
T2	R1R2 R3	R1R2 R3	R1R2 R3	R1R2 R3
T3	R1R2 R3	R1R2 R3	R1R2 R3	R1R2 R3
T4	R1R2 R3	R1R2 R3	R1R2 R3	R1R2 R3
T5	R1R2 R3	R1R2 R3	R1R2 R3	R1R2 R3

T6	R1R2 R3	R1R2 R3	R1R2 R3	R1R2 R3
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As the treatments were arranged as follows:

T1: Soil + *T. longibrachiatum*.

T2: Soil + *F. solani*.

T3: Soil + *R. solani*.

T4: Soil + *F. solani* + *T. longibrachiatum*.

T5: Soil + *R. solani* + *T. longibrachiatum*.

T6: Soil + *F. solani* + *R. solani* + *T. longibrachiatum*.

Statistical analysis

Randomized Complete Design (C.R.D) was used to arrange the experiment. The least significant difference (L.S.D.) was used to compare means at 5% level of significance ($P > 0.05$). Data were analyzed using the statistical program Genstat (version 12 Vsn Interrectional, Hemel Hempstead, UK).

Results and Discussion

Isolation and identification of pathogenic fungi

Results of current study indicated that all *F. solani* and *R. solani* was spread in all studied sites, while, *Macrophomina phaseolina* isolates were appeared lightly in some of these sites. The spreading of damping-off pathogens may attributed to the cultivation of cucumber repeatedly during the year or planting other vegetables belonging to the cucurbit family in the same fields which leads to the accumulation of pathogenic fungi spores in the soil for a long time, in addition to its tolerance to unfavorable conditions especially high temperatures (22). Results of the morphological isolation showed that

17 isolates of *Fusarium* spp. 5 isolates of *Rhizoctonia solani* and one isolate of *Macrophomina phaseolina* were obtained (12; 1). Isolates obtained grown on P.D.A medium showed variation in some phenotypic characteristics including growth speed and differences in colony shapes and colours. Molecular diagnoses of the very virulence isolates using polymerase chain reaction (PCR) indicated that these isolates are belonging to *F. solani* and *R. solani* as both isolates were registered in National Center for Biotechnology Information (NCBI) under ON394603 and ON398997 accession numbers respectively.

The pathogenicity test of damping-off disease pathogens on the four studied varieties of cucumber in pots that contain sterilize and non-sterilize soils and measuring plant growth indicators of

Results of pots experiment showed that there were no significant differences between the isolated fungi compare to control treatment. Pathogenicity test in plastic pots contained sterilized and non-sterilized soils showed different level of pathogenicity of these isolates on seedlings

of cucumber as one isolate of *Fusarium* spp. and another isolate of *R. solani* found to be very virulence which increase the percentage of damping off on seedlings up to 90 and 100% respectively compare to control treatment of the four studied varieties that recorded 100, 95, 90 and 85 respectively. The reason for the variation in the pathogenicity of *Fusarium* spp. isolates may attribute to the mycotoxins such as Javanicin and Fusaric acid that secrete by these isolates which kill cucumber seeds and inhibit enzymes (13; 4). While, the variation in the pathogenicity of *R. solani* isolates may be attribute to the secretion of metabolic substances that have a toxic effect and lead to failure germination (14). Table 2 results showed that *T. longibrachiatum* treatment was significantly affected the average of plumule weight and gave an increasing in it amounted 0.50g compare to control treatment (soil only) which gave 0.31g

followed by *F. solani* + *T. longibrachiatum*, *R. solani* + *T. longibrachiatum* and *F. solani* + *R. solani* + *T. longibrachiatum* treatments that gave 0.19, 0.18, and 0.12g respectively. While, *R. solani* and *F. solani* treatments were gave the lowest average of plumule weight amounted 0.00 and 0.05g. The outcome also indicated that there were significant differences between studied varieties as Superina F1 variety was exceeded other varieties in the average of plumule weight when it achieved 0.23g followed by Hybrid cucumber, Beit alpha F1 and Cucumber short that recorded 0.18, 0.16 and 0.18g respectively. The interaction showed significant differences between treatments, varieties and the soil type as the treatment that contains *T. longibrachiatum* was significantly exceeded in plumule weight of Superina F1 variety and gave 0.63g compare to 0.35g in control.

Table 2. The effect of *F. solani*, *R. solani* on plumule weight (g) of cucumber varieties in the presence of *T. longibrachiatum* after 10 days of planting

Treatments	Superina F1		Hybrid cucumber		Beit alpha F1		Cucumber short		Fungus effect
	Non-sterilize	sterilize	Non-sterilize	sterilize	Non-sterilize	sterilize	Non-sterilize	sterilize	
Control (soil only)	0.35*	0.31	0.33	0.27	0.31	0.24	0.33	0.26	0.31
<i>T.longbrachiatum</i>	0.63	0.45	0.55	0.45	0.51	0.38	0.53	0.36	0.50
<i>R.solani</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>F.solani 1</i>	0.11	0.06	0.10	0.07	0.00	0.00	0.00	0.00	0.05
<i>T.longbrachiatum</i> + <i>R.solani</i>	0.31	0.28	0.14	0.03	0.18	0.15	0.18	0.17	0.18
<i>T.longbrachiatum</i> + <i>F.solani 1</i>	0.21	0.20	0.19	0.18	0.21	0.09	0.28	0.17	0.19
<i>T.longbrachiatum</i> + <i>R.solani</i> + <i>F.solani 1</i>	0.18	0.14	0.18	0.05	0.14	0.06	0.10	0.07	0.12
Variety effect	0.23		0.18		0.16		0.18		

Soil effect	Sterilize 0.16		Non-sterilize 0.22	
	L.S.D 5%			
	Varieties 0.02902	Fungi 0.03838	Soils 0.02052	Interaction 0.10857

*Each number in the table represents the average of three replicates.

Results of Table 3 showed that *T. longibrachiatum* treatment was significantly affected the average of plumule length as it amounted 5.30cm compare to control treatment (soil only) which gave 4.37cm followed by *R. solani* + *T. longibrachiatum*, *F. solani* + *T. longibrachiatum* and *F. solani* + *R. solani* + *T. longibrachiatum* treatments that gave 3.19, 2.98, and 1.99cm respectively. While, *R. solani* and *F. solani* treatments were gave the lowest average of plumule length amounted 0.00 and 0.71cm. The

outcome also indicated that there were significant differences between studied varieties as Superina F1 variety was exceeded other varieties in the average of plumule length when it achieved 3.10cm followed by Hybrid cucumber, Beit alpha F1 and Cucumber short that recorded 2.67, 2.45 and 2.38cm respectively. The interaction showed significant differences between treatments, varieties and the soil type as the treatment that contains *T. longibrachiatum* was significantly exceeded in plumule length of all varieties.

Table 3. The effect of *F. solani*, *R. solani* on plumule length (cm) of cucumber varieties in the presence of *T. longibrachiatum* after 10 days of planting

Treatments	Superina F1		Hybrid cucumber		Beit alpha F1		Cucumber short		Fungus effect
	Non-sterilize	sterilize	Non-sterilize	sterilize	Non-sterilize	Sterilize	Non-sterilize	sterilize	
Control (soil only)	4.6*	4.33	5	4.33	4.33	4.17	3.83	4.33	4.37
<i>T.longbrachiatum</i>	6.33	5	5.43	5	5.5	4.5	6.43	4.17	5.30
<i>R.solani</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>F.solani</i> 1	1.67	1.33	1.67	1	0.00	0.00	0.00	0.00	0.71
<i>T.longbrachiatum</i> + <i>R.solani</i>	5.1	3.77	3.67	2.37	3.43	2.67	2.53	2	3.19
<i>T.longbrachiatum</i> + <i>F.solani</i> 1	3.23	3	2.43	2.43	3.1	3	3.67	3	2.98
<i>T.longbrachiatum</i> + <i>R.solani</i> + <i>F.solani</i> 1	3.17	1.83	2.33	1.67	2.27	1.33	2.33	1	1.99
Variety effect	3.10		2.67		2.45		2.38		
Soil effect	sterilize 2.37				Non-sterilize 2.93				
L.S.D 5%	Varieties 0.3252		Fungi 0.4301		Soils 0.2299		Interaction 1.2166		

*Each number in the table represents the average of three replicates.

Table 4 results showed that *T. longibrachiatum* treatment was significantly affected the average of radicale weight and gave an increasing in it amounted 0.30g compare to control treatment (soil only) which gave 0.19g followed by *F. solani* + *T. longibrachiatum*, *R. solani* + *T. longibrachiatum* and *F. solani* + *R. solani* + *T. longibrachiatum* treatments that gave 0.11, 0.10, and 0.04g respectively. While, *R. solani* and *F. solani* treatments were gave the lowest average of radicale weight amounted 0.00 and 0.03g. The outcome also indicated that there were significant differences between studied varieties as Superina F1 variety was exceeded other varieties in the average of radicale weight when it achieved 0.14g followed by Hybrid cucumber, Beit alpha F1 and Cucumber

short that recorded 0.11, 0.10 and 0.09g respectively. The interaction showed significant differences between treatments, varieties and the soil type as the treatment that contains *T. longibrachiatum* was significantly exceeded in radicale weight of Superina F1 variety in non-sterilize soil and gave 0.44g compare to 0.25g in control (soil only) followed by Hybrid cucumber, Cucumber short and Beit alpha F1 when it gave 0.23, 0.20 and 0.22g respectively. While, the lowest average of radicale weight was recorded in *R. solani* and *F. solani* in all studied varieties for both types of soils.

Table 4. The effect of *F. solani*, *R. solani* on radicale weight (g) of cucumber varieties in the presence of *T. longibrachiatum* after 10 days of planting

Treatments	Superina F1		Hybrid cucumber		Beit alpha F1		Cucumber short		Fungus effect
	Non-sterilize	sterilize	Non-sterilize	sterilize	Non-sterilize	sterilize	Non-sterilize	sterilize	
Control (soil only)	0.25*	0.13	0.23	0.14	0.22	0.16	0.20	0.16	0.19
<i>T.longbrachiatum</i>	0.44	0.3	0.29	0.26	0.27	0.26	0.28	0.26	0.30
<i>R.solani</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>F.solani</i> 1	0.07	0.08	0.06	0.06	0.00	0.00	0.00	0.00	0.03
<i>T.longbrachiatum</i> + <i>R.solani</i>	0.19	0.16	0.09	0.03	0.18	0.07	0.05	0.06	0.10
<i>T.longbrachiatum</i> + <i>F.solani</i> 1	0.13	0.11	0.15	0.11	0.10	0.09	0.12	0.11	0.11
<i>T.longbrachiatum</i> + <i>R.solani</i> + <i>F.solani</i> 1	0.08	0.03	0.04	0.02	0.07	0.01	0.03	0.04	0.04
Variety effect	0.14		0.11		0.10		0.09		
Soil effect	sterilize 0.09				Non-sterilize 0.13				
L.S.D 5%	Varieties 0.01985		Fungi 0.02626		Soils 0.01404		Interaction 0.07428		

*Each number in the table represents the average of three replicates.

Table 5 results showed that the treatments of *T. longibrachiatum* was significantly exceeded other treatments in radicale length as it recorded 4.7cm followed by *R. solani* + *T. longibrachiatum*, *F. solani* + *T. longibrachiatum* and *F. solani* + *R. solani* + *T. longibrachiatum* which gave radicale length amounted 2.5, 2.3 and 1.5cm respectively compare to 3.9cm in control treatment that contains soil only. While, *R. solani* and *F. solani* treatments were gave the lowest average of radicale length amounted 0.00 and 0.06cm. There were significant differences between varieties when Superina F1 variety was exceeded other varieties in the average of radicale length when it achieved 2.7cm followed by Hybrid cucumber, Beit alpha F1 and Cucumber short that recorded 2.3, 2.00 and 1.93cm respectively. The interaction between treatments, varieties and soils showed significant differences as *T. longibrachiatum* treatment in Superina F1 variety planted in sterilized and non-sterilized soils and gave 5.67 and 5cm respectively compare to 4.5 and 4.2 in control. *T. longibrachiatum* treatment in Hybrid cucumber and Cucumber short varieties planted in non-sterilized soil was exceeded and gave 4.77 and 4.67cm respectively compare to control followed by *T. longibrachiatum* treatment Hybrid cucumber and Cucumber short planted in sterilized soil as it recorded 4.8 and 3.83cm respectively compare to control followed by (*R. solani* + *T. longibrachiatum*) (*F.*

solani + *T. longibrachiatum* and *F. solani* + *R. solani* + *T. longibrachiatum*) treatments respectively. While, the lowest average of radicale length in was recorded in *R. solani* + *F. solani* treatments in sterilized and non-sterilized soils in all varieties.

Results of current study showed that *F. solani* inhibited cucumber seeds germination which may be attributed to the mycotoxins that secreted by this fungus such as Fusaric acid, Javanicin and Zearalenone which lead to inhibit enzymes function (13; 4), in addition, the fungus is infected seeds before and after the emergence and leads to the rotting of these seeds and cause huge economic losses (6; 10). *R. solani* also inhibited the germination of cucumber seeds due to the secretion of enzymes including Pectinase, Cellukase and Phosphatase that degrade plant cell walls and cause seedlings damping off (16). Adding the bio-control agent *T. longibrachiatum* was increased cucumber growth indicators as the efficiency of *Trichoderma* spp. to control plant pathogens may be attributed to the secretion of plant growth regulators that enhanced plant growth and increase the resistance of plants to unsuitable environmental conditions and pathogens (4; 11). Sarhan et al. (20) were confirmed the efficiency of *T. longibrachiatum* and *T. asperium* to control *M.phaseolina* the causal agent of charcoal rot of sunflower (5).

Table 5. The effect of *F. solani*, *R. solani* on radicale length (cm) of cucumber varieties in the presence of *T. longibrachiatum* after 10 days of planting

Treatments	Superina F1		Hybrid cucumber		Beit alpha F1		Cucumber short		Fungus effect
	Non-sterile	sterile	Non-sterile	sterile	Non-sterile	sterile	Non-sterile	sterile	
Control (soil only)	4.5*	4.2	4.77	4	4.1	3.27	3.5	3.2	3.9
<i>T.longbrachiatum</i>	5.67	5	4.77	4.7	4.1	3.83	4.67	4.8	4.7
<i>R.solani</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>F.solani</i> 1	1.83	1.1	1.1	0.67	0.00	0.00	0.00	0.00	0.06
<i>T.longbrachiatum</i> + <i>R.solani</i>	3.5	3.4	3	1.2	2.83	1.83	2.33	2	2.5
<i>T.longbrachiatum</i> + <i>F.solani</i> 1	2.67	2.33	2.5	2.2	2.33	2	2.33	2	2.3
<i>T.longbrachiatum</i> + <i>R.solani</i> + <i>F.solani</i> 1	2.33	1.33	1.5	1.3	1.67	1	1.33	1.17	1.5
Variety effect	2.7		2.3		1.93		2.0		
Soil effect	sterilize 2.0				Non-sterilize 2.40				
L.S.D 5%	Varieties 0.3105		Fungi 0.4107		Soils 0.2195		Interaction 1.1616		

*Each number in the table represents the average of three replicates.

Conclusion

Damping off on cucumber is one of important diseases that cause destructive losses due to the infection of seeds and seedlings in early stages of growth particularly in favourable conditions of the pathogens. This study suggests the possibility of using Superina F1 variety to eliminate damping off disease on cucumber as it showed less susceptibility to the pathogens of disease. In addition, the use of *T. longibrachiatum* was provided a protection to cucumber plants with

enhancing the vegetative and root growth indicators.

Conflict of interest

The authors have no conflict of interest.

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