Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2019, Vol. 3 (2), 229-246
https://doi.org/10.29329/ijiaar.2019.194.9

Original article

Occurrence and Biological Control of Cucumber Damping Off Disease under Protected Cultivation in Sulaimani , Iraq [1]

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Abstract

Occurrence and cucumber damping-off disease incidence was determined in Sulaimani plastic houses in 2014 revealed from overall disease incidence of 6.82%. The highest incidence and severity reached to 23.7% and 5.0 respectively in Kharajian. While the lowest incidence and severity was detected in Arabit (0.2% and 0.6 respectively). Disease symptoms include pre and post-emergency damping-off of cucumber seedlings. Twelve fungal pathogens were isolated from roots and crown of infected seedlings and plants that explore typical damping-off and root rot symptoms. *Rhizoctonia solani* was the most frequently isolated fungi followed by *Pythium aphanidermatum*, *Fusarium solani* and *Pythium sp*. Morphology and characteristics of *R. solani* and *P. aphanidermatum* match with the original described characters of the fungi. The optimum growth temperature for *P. aphanidermatum* was 30°C and for *R. solani* was between 25-30°C. Pathogenicity test revealed that *R. solani* significantly surpassed all other treatment except *P. aphanidermatum* by inciting 53.3% pre and 66.4% post-emergency damping-off followed by *P. aphanidermatum* that incited 43.6% and 56.3% pre and post emergency damping-off respectively. *T. harzianum* showed high antagonistic ability against both pathogens. Antagonistic ability degree of *T. harzianum* reached to 37.02 against *P. aphanidermatum* and 32.00 against *R. solani*. The bio-control bacterial *Bacillus subtilis, Rhizobacteria, Streptomyces coelicolor* showed high efficiency in controlling the disease. *Rhizobacteria* and *S. coelicolor* completely inhibit *R. solani* growth at 10^{-1} bacteria dilution and significantly surpassed all other treatments. dilution 10^{-1} from all the used bacteria were significantly more efficient against *P. aphanidermatum.* This dilution was contain 21.4 × 10^7 cell forming unit in each milliliter (CFU/ml) in *B. subtilis,* 28 × 10^7(CFU/ml) in *Rhizobacteria,* 29.5 × 10^7 (CFU/ml) in ***S.*** *coelicolor*, 32.2× 10^7(CFU/ml) in *Pseudomonas flouresence* and 22.6 × 10^7 (CFU/ml) in *Azotobacter chroococcus*.

Keywords: *Cucumis sativus*, biological control, fungal diseases, Iraq

Received: 20 August 2018 * **Accepted:** 13 April 2019 * **DOI:** https://doi.org/10.29329/ijiaar.2019.194.9

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[1] A part of this study was presented at the International Agricultural, Biological and Life Science Conference, Edirne, Turkey, September 2-5, 2018.

INTRODUCTION

Cucumber *(Cucumis sativus L.*), belonging to the *Cucurbitaceae* is one of the most important vegetable crops in Iraq planted both in spring and fall and consumed fresh or it may intervene in fruit pickles industry (Hassan, 1991). Cultivation area of cucumber reached to 2427 thousand hectares with annual production of 45764 thousand tons. China is the first large producer in the world (FAO, 2011). While Iraq produce 495616 ton including 334000 ton of cucumber from Kurdistan region (FAO, 2011).

Many fungal diseases infected cucumber, Damping-off disease incited by *Pythium* spp and *Rhizoctonia* spp. remain the major problem facing cucumber growers that can reduce seedling stands and canopy uniformity, resulting in considerable yield loss, and damage to cucumber in many areas of the world (Kwok et al., 1987; Hultberg et al., 2000 and Gravel et al., 2007). Among *Pythium* and *Rhizoctonia* spp that can cause damping-off of cucumber, *P. aphanidermatum* (Edson) Fitzp., *P. ultimum* and *P. irregulare* are the most widely reported world wide (Punja and Yip, 2003). *Pythium* and *Rhizoctonia* spp are important soil-borne plant pathogens that can seriously affect the host plant. *Pythium* disease occurs wherever the plant is growing including seedling damping-off, stem and root rot of young plants and feeder root necrosis in the field (Shew, 1991).

Pythium and *Rhizoctonia* cause pre-and post-emergence damping-off of cucumber. Pre-emergence, infected cucumber seeds are associated with poor-quality and low vigor. Post-emergence infection could infect hypocotyls at the soil line as well as the zone between stem and root. Cucumber seedlings often develop a darkened or shriveled stem appearance. Water-soaked lesions constrict the base of the stem so that it usually collapses. The top of the plant may appear healthy when it falls over by quickly wilts and dies. The roots may decayed and appeared as a weft of very fine metallic-colored threads over the surface of the lesion. In high moisture, *Pythium* has produced a white, cottony growth on the lesion (Uzuhashi et al., 2010).

Many researchers succeed in biological control of *Pythium* diseases using *Trichoderma* spp. particularly damping-off tobacco disease (Devaki et al., 1992), cotton (Cook, 1993), and root rot of sugarcane (Padmanaban and Alexander, 1982). *Phytophthora* sp. could attack many plants including tobacco, durian, Tangerine (Ownley et al., 2003). Many reports refereed to the successes in the biological control of plant diseases, such as control of black shank of tobacco caused by *P. parasitica var*. *nicotianae* (Ownley et al., 2003) and control of root and stem rot of durian and tangerine caused by *P. palmivora* and *P. parasitica*, respectively. Shameem (2006) has reported that *Trichoderma* spp. could control root rot of cotton incited by *Rhizoctonia* sp., damping-off of tobacco (Cole and Zvenyika, 1988), and *Rhizoctonia* black scurf of potato (Beagle-Ristaino and Papavizas, 1985). *Trichoderma* spp. provide high efficacy to control of charcoal rot of peanut caused by *R. bataticola*. Some other studies conducted in Iraq pointed out to the susceptibility of Cucurbitaceac plants to severe infections with wilt disease incited by *Pythium aphanidermatum* particularly in the commercial cucumber cultivar (El- Behadli and

Al-Azawi, 1979). Thus developing of new integrated control program is important to raise cultivation area and yield of the crops. The current study is conducted to determine occurrence of cucumber damping-off disease in Sulaimani plastic houses and possibility of biological of the disease.

Materials and Methods

Disease Survey

Disease survey was conducted in 200 cucumber plastic houses from Feb. to May, 2014, 200 infected seedling samples were collected from 21 locations from Byawella, Anab, Khurmal, Zameqy, Sirwan, Grde Naze, Saidsadeq, Gamish Tepa, Kani speca, Kharajian, Raziana, Arabet, Sulaimani Research Center, Zhalla, Bazian, Raparen, Zaraeen, Tanjaro and Kani Panka. Infected seedling samples and the soil surrounding the roots were collected mixed and placed in plastic bags then transferred to the lab., Damping-off percent in each plastic house was calculated according to (Stanghellini and Phillips, 1975). While disease severity in each plant was measured according to a 0-5 scale developed by (Jaber, 1996).

Isolation and identification of the pathogens

The infected samples were washed with tap water; Small pieces of the infected roots and 0.5-1cm of the crown were cut, sterilized with 1% Sodium Hypochlorite for 2min then washed with distilled sterilized water and dried on sterile filter papers. Five pieces of the samples were distributed in PDA plates and incubated at 25 ± 2°C, after 3d the developing mycelia were purified by replacing the hyphal tip of each colony in PDA plates and incubated further for 3-5d. Identification of the pathogens was conducted according to the standard taxonomic keys for each pathogen (Barnett and Hunter, 1998; Domsch et al. 2007). The diagnosed pathogens were preserved on PDA slants in the refrigerator for further studies.

Pathogenicity test

Pathogenicity test was conducted using water culture test (Al-Maaroof, 1984). Flasks with 250ml sterile water were inoculated with previously prepared inoculum of each pathogen at a rate of 1/5 cultures of 3-4d colony/flask after mixing. Two cucumber seedlings with two leaves were fired in each flask after uprooted from previously sowed pots, the roots washed and submerged below water level in the flask. PDA free of inoculum used in the control treatment. The experiment was carried out by three replicates. The flask was incubated in growth chamber at 25±2ºC until symptoms appearance. The seedling was considered susceptible if symptom developed within 24-96h using the following scale: +++= wilt symptom appear within 48h, ++= wilt symptom appear within 72h, += wilt symptom appear within 96h, -=No any symptom appear within the experiment period. The pathogens were re-isolated from the infected seedlings to prove Koch's postulate (Watanabe, 1984)

Soil culture test was conducted by seed sterilization with 1% sodium hypochlorite for 2min dried on sterilized filter papers, then planted in plastic pots contains 500g of sterile silt loam soil. Artificial inoculation was conducted by adding 0.5 colony of 3-4d of each pathogen/pot. Mixed thoroughly with the soil, irrigated and covered with polyethylene bags for 3d. PDA alone used in control treatment. Five cucumber seeds were planted in each pot after sterilization with 1% Sodium Hypochlorite for 2min; the experiment was carried out using RCBD with 3 replicates. The percentage of pre-emergence death of seedling was calculated after germination of all the seeds in the control treatment. The post- emergence death of the seedlings was counted ten days later from seedling emergence according to (Jaber, 1996).

Biological control of the pathogens

The bio-control agent *T. harzianum* was used (commercial product bio count-T, Al-Baraka, Jordan). Viability of the product was tested before using in the experiments. Bio-control ability of the fungi was tested against *P. aphanidermatum* and *R. solani* by double culturing both of them on 9cm potato sucrose Agar media (PSA) plates. The center of each half of the plate was inoculated with 0.5cm disk of 7d old fungal growth, while the other half center inoculated with similar disk of the 3d pathogenic fungi and incubated at 25±2ºC. The experiment was carried out using RCD with 3 replicates. Growth diameters were measured each 24h until the plates was full. Inhibition calculated by counting the fungi colony diameter, compared to the fungi colonies developing in the control. Inhibition percentage of the fungal growth was calculated, according to AL-Qarghuli (1999).

Antibiosis of the bacterial strains against *P. aphanidermatum* and *R. solani*

Viability of the bacterial bio-agent strains *B. subtilis*, *S. coelicolor*, *P. flouresence*, *A. chroococcus*, and *Rhizobacteria* were tested on Nutrient Agar and incubated at 37±2ºC for 24 to 48h before use, the isolates transferred from N.A to N.B media and incubated at 37°C for 48h, three series dilutions 10^{-1}, 10^{-2} and 10^{-3}, of each bacteria was prepared. One ml of each bacterium placed in 9ml of distilled water using sterile micropipette to prepare the first dilution then, 1ml from the first dilution placed in 9 ml of sterile distilled water to prepare the second dilution and so on for the third dilution. One ml of each dilution placed in 9 cm sterile Petri dish containing PSA and smeared by L. shape. Each Petri dish was inoculated with 0.5 cm disc of the fungal growth t from the edge of 3d old colonies and placed in the center of PSA plate. The experiment was conducted using RCD with 3 replicates, 3 plates/replicate, other replicates were left without inoculation, the plates incubated at 25±2ºC for 3d. Growth rate percentage for *P. aphanidermatum* and *R. solani* was calculated according to AL-Qarghuli (1999).

The effective concentrations of the bacterial isolates on growth inhibition of *P. aphanidermatum* and *R. solani*

Series dilutions of *B. subtilis, S. coelicolor* and ***Rhizobacteria*** was prepared by adding 1ml of the Bacterial stocks to 9ml sterile distilled water test tubes. A series of dilutions 10^{-1} to 10^{-3} were prepared as mentioned before. 27 PSA plates (9cm) were inoculated with the bacterial inoculums from all the dilutions at a rate of (1ml/dish), 3 plates/replicate for each dilution, the other 3 plates were left without inoculation as control. All the plates were incubated at 25±2ºC for 24h. Each plate was inoculated with 0.5cm of the fungal growth taken from the edge of 3d old fungal colonies growth to the center of PSA plates using RCD with three replicates. The plates were incubated at 25±2ºC for 3d. % inhibition of the fungal growth was calculated as mentioned before.

Population density account of the bacteria

After obtaining the mitigation dilution (10^{-1}) of *B.subtilis, S. coelicolor* and *Rhizobacteria* against *P. aphanidermatum* and *R. solani*, three 9cm NA plates were inoculated with 0.5 ml bacteria/plate. The inoculated plates were moved in a circular motion to ensure uniform distribution of the bacterial inoculums on the surface of the media and incubated at 37±2ºC for 24h. Number of colonies in each dish was calculated and the average extracted by hit upside dilution to account population density of each bacteria according (Abdul Wahid et al., 1996).

Population density of the bacterial units = number of colonies × inverted dilution.

Plastic house experiment

Efficiency of different bio-control agent's in protection of cucumber infection with P. aphanidermatum and R. solani was evaluated in the field according to the laboratory results. The experiment was carried out under plastic house conditions at Horticulture Dept., College of Technical Agric., Halabja using RCBD with 3 replicates. Sterilized plastic pots with 1kg soil used for each treatment. The experiment included the following treatment, T1= cultivation in sterile soil; T2= Artificial inoculation with P. aphanidermatum at a rate of 0.5 plate colony content/pot; T3= Artificial inoculation with R. solani at a rate of 0.5 plate colony content/pot; T4= Application of the bio-control agent T. harzianum to the inoculated soil treatment at a rate of 0.10g/pot; T5= Soil treatment with B. subtilis at a concentration of 214 × 106 CFU/ml at a rate of 30ml/pot, the fungal pathogen added after 4d according to Al-Dulaimi, 2000. T6= Soil treatment with S. coelicolor at a concentration of 295×106 CFU/ml at a rate of 30ml/pot, the fungal pathogen added after 4d according to Al-Dulaimi, 2000; T7= Soil treatment with Rhizobacteria at a concentration of 28×107 CFU/ml at a rate of 30ml/pot, the fungal pathogen added after 4d according to (Al-Dulaimi, 2000). Local cucumber seeds were surface sterilized with 1% sodium hypochlorite for 2 min, then washed with sterile water and planted at a rate of 5

seeds/pot. Three pots were used for each treatment. Disease percent for each treatment was calculated as mentioned before. After 30d of fungal inoculation the plants were carefully removed from the soil and the roots washed with tap water to remove the attached soils. Results were analyzed statistically and the treatment arithmetic means were compared according to Duncan's Multiple Range Test (DMRT) at 0.05 level.

Results and Discussion

Survey results in cucumber plastic houses in Sulaimani province revealed that the overall mean incidence of damping-off and root rot disease reached to 6.82% (Table1). The highest disease incidence 23.7% was detected at Kharajian followed by 19.6% at Raziana and 17.5 % at Kanispeca, while the lowest disease incidence 0.2% was detected at Arbet and Byawella, while no any disease symptoms appeared in Halabja, Sirwan, Grde Naze, Bakrajo and Tanjro plastic houses. The highest disease severity was detected in Kharajan, Raparen and Tanjaro plastic houses while the lowest disease severities were detected in Arbet and Khurmal. Damping-off symptoms appeared on the seedling includes pre-emergence and post-emergence damping-off. In the pre-emergence damping-off, the infected seeds are decayed and failed to germinate which resulted in poor stands after many days of planting. The post-emergence damping-off is most often observed in seed flats or among transplants. Symptoms appeared on the stems near the soil surface (crown). The affected area of the stem took a water-soaked appearance and sometimes became constricted. Eventually, the stems were unable to maintain structural support of seedlings, which usually collapse and the seedlings die within 24 to 48h. Disease symptoms also include chlorosis and wilt of cucumber seedling leaves. The low disease incidence in Sulaimani may be attributed to the time of survey that was conducted from February to March, where the environmental conditions were not suitable to the pathogen activity since both fungi require 25-30 ºC for infection process. Also the fungal population may be less in spring than autumn due to the decomposition of organic matter, which turns the soil pH to the acid side, which is not suitable for the pathogen activity. Furthermore cucumber cultivation in plastic houses have been practiced in Sulaimani since 2008, thus the pathogen need more time to colonize the soil and increase population in the absences of effective control programs. No disease appearance in Halabja, Sirwan, Grdhe Naze, Bakrajo and Tanjro plastic houses is a good indication for temperature requirements of the pathogen, since these areas are more colder than others where the mean temperature range was between 11.57-12ºC out the plastic houses during February and March, and the humidity ranged between 44.5-55.6% during the survey time. Cucumber seedling infection with P. aphanidermatum happens when the temperature ranges between 20-32ºC (Ben-Yephet and Nelson, 1999). While R. solani can be grown in a wide temperature ranges between 8-36 ºC, but the optimum temperature for its growth is between 25-30ºC (Agrios, 1997).

Isolation and identification of accompanies fungi with the infected plants

Twelve fungal and one bacterial pathogens were isolated from the root and crown of infected cucumber seedlings and plants that explored typical symptoms of damping-off and root-rot symptoms. *R. solani* was the main frequent isolated fungi from cucumber plants followed by *P. aphanidermatum, Fusarium solani, Pythium* sp and *Alternaria solani*. While other pathogens were less frequent during their isolation from cucumber plant during February to March.

Identification of the isolated pathogens from cucumber plant was conducted using the specific identification keys used for the pathogen (Domsch et al., 2007, Barnett and Hunter, 1998).

Table 1. Disease incidence and severity of damping-off in cucumber plastic house at different locations in Sulaimani during 2014.

Location	No. of plastic house	% Mean Disease incidence	% Mean Disease severity
Halabja	35	0.0	0.0
Byawella	15	0.3	1.0
Anab	20	1.9	1.3
Khurmal	5	0.9	0.7
Zameqy	5	4.3	1.3
Sirwan	15	0.0	0.0
Grde Naze	5	0.0	0.0
Said sadiq	5	7.4	3.3
Gamish Tepa	5	6.1	3.2
Kani speca	10	17.5	4.1
Kharajian	15	23.7	5
Raziana	20	19.6	4.4
Arbet	5	0.2	0.6
Bekrajo	5	0.0	0
Zhalla	5	7.2	3.2
Bazian	5	13.6	3.1
Raparen	10	15.4	5
Zaraeen	5	10.2	3
Tanjaro	5	0.0	0.0
Kani Panka	5	8.1	5
Total	200	6.82	5

Pathogenicity test

Pathogenicity test of the frequent isolated fungi from cucumber seedling using water culture test, revealed that *R. solani*-1 was more pathogenic than the other pathogens, where damping-off symptoms appeared within 48h on cucumber seedlings (Table2) followed by *R. solani*-2, *P. aphanidermatum*-1

and *P. aphanidermatum*-2 that incited damping-off symptoms on cucumber seedling after 72h of the inoculation, the shoot began wilting, then drying and discoloring of the root system in brown with reduction in the overall size of the roots.

F. solani incited light damping-off symptoms on cucumber seedlings after more than 96h while no any symptoms appeared on *Pythium* sp and *Alternaria solani* inoculated seedling as well as the control treatment. The most pathogenic fungi were introduced in field experiment tests.

Table 3 shows pathogenicity test of the most frequent isolated fungi on cucumber seedlings using soil culture method. *R. solani*-1 significantly surpassed all other fungi except *P. aphanidermatum*-1, in their pathogenicity test, the fungi caused 53.3% pre-emergence damping-off of the seedlings after 7d and 66.4% of the seedling after 14d, followed by *P. aphanidermatum* which incited 43.6% of seedling damping-off after 7d and 56.3% after 15d, while *R. solani*-2, *P. aphanidermatum*-2 and *F. solani* surpassed control treatment though they showed low pathogenicity on cucumber seedlings. No significant differences were detected between *F. oxysporium* and *A. solani* with the control treatment, which means they are not pathogenic.

Morphology and characteristic of the pathogenic fungi

R. solani colony color was white in the beginning of the growth then turned to different levels of brown color. The colony produced small round brown sclerotia with zonation appearance in the colonies. Microscopic test of the fungi colonies showed that the mycelium is septate with abundant branch, which forms a right angle with the main hyphae and the existence of clear, cross sectional barriers near the branching points. No any distinctive structures such as sexual or asexual spores were found in the colony while Barrel cluster cells where found in chain in the places of sclerotia formation. These characteristics match with the original characters of the fungus (Staplers and Anderson, 1996).

Microscopic examination of the *P. aphanidermatum* colony shows presence of non- septate hyphae and the finger like sporangia that is the characteristic of the fungus. The sexual organs, oogonia, antheridia, and oospores also produced by *P. aphanidermatum.* Oogonia mostly terminal and spherical oospores was a pleroetic. The colony color on P.D.A media was white cottony, the fungal characteristic are matching with the original characteristic of the fungus mentioned in Waterhouse key (Waterhouse, 1967).

Table 2. Pathogenicity test of the most frequent isolated fungi from the root and stem of cucumber seedlings using water culture method.

Treatment	Pathogenicity test*
R.solani -1	+++
-2 *R.solani*	++
-1 *P. aphanidermatum*	+++
-2 *P. aphanidermatum*	++
Fusarium solani	+
Fusarium oxysporium	+
Pythium sp	-
Alternaria solani	-
Control	-

*+++= wilt symptom appearance within 48 hours.

++= wilt symptom appearance within 72 hours.

+= wilt symptom appearance within 96 hours.

-=No any symptom appearance within the experiment period.

Table 3. Pathogenicity test of the most frequent isolated fungi from the roots and stems of cucumber seedling using soil culture method.

Treatment	% Pre-emergence damping-off after 7 day	% Post-emergence damping-off after 15 day
R. solani -1	53.3	66.4
R. solani -2	27.3	33.0
P. aphanidermatum-1	43.6	56.3
P. aphanidermatum-2	33.3	37.6
Fusarium solani	19.6	23.3
Fusarium oxysporium	6.7	13.6
Alternaria solani	0.0	0.0
Control	0.0	0.0
L.S.D (0.05)	10.3	13.7

Antibiosis ability T. harzianum against R. solani and P. aphanidermatum

Result of (Table 4) shows that T. harzianum has high antagonistic activity against the pathogenic fungi R. solani and P. aphanidermatum. Antagonistic ability degree against P. aphanidermatum and R.

solani reached up to 37.02 and 32.00 respectively after 4 days of inoculation according to the standard scale used for this purpose. No significant differences were detected between combination of *T. harzianum* with both of *R. solani* and *P. aphanidermatum* treatments at different time intervals (Table 5). These results confirm the findings of other previous researchers (Al-Khafaji, 1985; Sharif et al., 1988). The antagonism ability of the bio-control agent *T. harzianum* refers to various mechanisms effects one of which may be through direct parasitism by wrapping mycelium of the bio-control agent *T. harzianum* on the pathogen hyphae (Sivan and Chet, 1989) or due to its ability to secrete antibiotics and certain analytic enzymes for the cell walls of the pathogenic fungus such as Protease enzymes, B-1,3-glucanase and Chitinase (Limon et al., 1999; Aziz et al., 1993; Harman, 1996) Another approach may be due to combination of both mechanisms secretion antibiotic and analytic enzyme on food and space (Saad, 2001).

Some researchers have pointed out that *T. harzianum* secretes enzymes including: cellulase that analyzes the cell walls of *P. aphanidermatum*, which consists mainly of cellulose, which is used as a source of food by the fungus. The fungus also secretes pectinase, which analyzes pectin that involves in the cell walls composition of *R. solani* (Alwan, 2005; Kamal Al-Din, 2008).

Efficiency of some bacterial bio-control agents against *P. aphanidermatum*.

Result of biological control of *P. aphanidermatum* by using some bacterial strains showed that all the bacterial dilutions were significantly surpassed the control treatment (Table 6). *B. subtillus* showed high antagonistic activity and significantly surpassed all other treatments and caused complete inhibition of the fungal growth at 0.1ml dilution followed by *A. chroococcus, P. flouresence, S. coelicolor,* and *Rhizobacteria* which inhibit *P. aphanidermatum* growth by 80%, 79%, 78% and 67% compared with the control treatment.

Bacterial concentrations in the dilution 10^{-1} for all the used bacteria were significantly more effective against *P. aphanidermatum.* Accordingly the numerical density of the used bacteria *B. subtilis, S. coelicolor* and *Rhizobacteria* were 21.4×10^7, $28 \times 10^7$and 29.5×10^7 colony formation units/ml respectively. This dilution was selected for further studies which contain 21.4×10^7 cells forming unit/ milliliter (CFU/ml) in *B. subtilis*, 28×10^7(CFU/ml) in *Rhizobacteria*, 29.5×10^7(CFU/ml) in *S. coelicolor*, 32.2×10^7(CFU/ml) in *P. flouresence* and 22.6×10^7(CFU/ml) in *A. chroococcus*.

Table 4. Antagonistic degree of *T. harzianum* against the causal agents of cucumber damping-off disease

Fungal pathogen	Antagonistic degree
P. aphanidermatum	37.02
R. solani	32.00

Table 5. Antagonistic activity of *T. harzianum* against *R. solani* and *P. aphanidermatum*

Treatment	Colony diameter (mm)*				
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	Mean
P. aphanidermatum + T. harzianum	12.33^{g}	20.00^{f}	20.00^{f}	20.00^{f}	18.08^{c} (69%)
T. harzianum	23.64^{g}	43.68^{f}	68.38^{e}	83.44^{a}	54.78^{b}
P. aphanidermatum	27.67^{f}	48.67^{c}	78.60^{b}	82.00^{a}	58.27^{a}
Mean	19.23^{d}	41.60^{c}	53.90^{b}	62.48^{a}	43.71^{f}
R. solani+ T. harzianum	15.00^{g}	22.00^{f}	22.00^{f}	22.00^{f}	20.30^{c} (66.2%)
T. harzianum	23.64^{h}	43.68^{f}	68.38^{e}	83.44^{d}	54.78^{b}
R. solani	21.73^{f}	61.13^{c}	73.33^{b}	84.00^{a}	60.05^{a}
Mean	20.12^{d}	42.27^{c}	54.57^{b}	63.14^{a}	45.04^{f}

*Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at $P < 0.05$.

*Each number is a mean of three replicates.

Table 6. Efficiency of various concentrations of bacteria on growth rate of *P. aphanidermatum* on P.S.A media at 25 ± 2 ºC.

Bacterial Bio-agent	Bacteria dilution	Colony diameter (mm) * *P. aphanidermatum*				Mean growth rate (mm)
		24hrs.	48hrs.	72hrs.	96hrs.	
Rhizobacteria	10^{-1}	0.00^{h}	13.08^{uv}	24.6^{mnop}	41.7^{f}	19.84^{h}
	10^{-2}	2.66^{z}	20.66^{qr}	29.44^{jk}	33.34^{j}	21.52^{fg}
	10^{-3}	8.26^{x}	27.24^{jklm}	44.00^{ef}	52.04^{d}	32.88^{d}
B. subtilis	10^{-1}	0.00^{h}	0.00^{h}	0.00^{h}	0.00^{h}	0.00^{j} (100%)
	10^{-2}	2.8^{z}	16.9^{st}	24.36^{nop}	45.38^{ef}	22.36^{gh}
	10^{-3}	11.86^{vw}	37.28^{gh}	57.56^{c}	61.84^{c}	42.14^{b}
A. chroococcu	10^{-1}	0.00^{h}	5.12^{yx}	16.88^{st}	26.8^{klmn}	12.2^{i} **(**80%)
	10^{-2}	0.00^{h}	$14.66t^{uv}$	29.68^{j}	45.84^{e}	22.5^{f}
	10^{-3}	4.12^{z}	$15.1t^{u}$	45.08^{e}	63.54^{b}	31.9^{d}
S. coelicolor	10^{-1}	0.00^{h}	3.6^{z}	9.58^{wx}	38.98^{g}	13.04^{i} (78%)
	10^{-2}	12.66^{uv}	23.38^{op}	38.06^{gh}	58.00^{c}	33.02^{d}
	10^{-3}	19.1^{rs}	27.94^{jkl}	57.36^{c}	70.04^{a}	43.6^{b}
P. flouresence	10^{-1}	0.00^{h}	6.94^{xy}	19.18^{rs}	25.58^{lmno}	12.92^{I} **(**79%)
	10^{-2}	4.68^{yz}	22.00^{pq}	35.58^{hi}	46.66^{e}	27.24^{e}
	10^{-3}	14.18^{tuv}	23.9^{op}	41.84^{f}	53.2^{c}	34.74^{c}
Mean	-	5.36^{d}	17.18^{c}	31.54^{b}	44.58^{a}	30.82
(control) *P. aphanidermatum*	0	27.67^{jkl}	48.67^{e}	78.60^{a}	82.00^{a}	59.27^{a}

*Values followed by the same letter are not significantly different according to Duncan's Multiple Range at P< 0.05.

*Each number is a mean of three replicates.

Efficiency of some bacteria bio-control agents against *R. solan*

Table (7) shows the significant effect of the bio-control bacterial agent on growth of R. solani comparing with the control in spite the concentration. Rhizobacteria and S. coelicolor completely inhibit R. solani growth at 10^{-1} bacteria dilution and significantly surpassed all other treatments followed by B. subtilis which inhibit 78% of the fungal growth, while P. flouresence and A. chroococcus resulted in inhibition of 65% and 44.5% of the fungal growth respectively. Dilution 10^{-1} of all the bio-agent bacteria's was more effective against R. solani and significantly surpassed all other used dilutions in

reduction of the fungal growth. Effect of the bio-agent bacteria Streptomyces on growth rate of R. solani may be attributed to the ability of the bacteria to produce more than 50 different types of antibiotics including streptomycin, neomycin, chloramphenicol and tetracycline which may protect cucumber plants from infection of damping-off disease caused by *R. solani* plants under greenhouse conditions (Sadeghi et al., 2009).

Seed treatment with the bacteria reduced seedlings infection with damping off disease under natural or artificially inoculated soils. Biological control trials revealed increase in root system of the treated plants in comparing with untreated control. Soil actinomycetes, especially *Streptomyces* have wide range activity against plants diseases.

According to (Beattie, 2006), bacteria that reduce the incidence or severity of plant diseases are bio-control agents, while those that appear antagonistic activity toward the pathogen are defined as antagonistic adversaries. The following rizospheric environment and bacterial antagonistic activities can highlight synthesis of Hydrolytic enzymes, such as chitinases, glucanases, proteases, lipase, which can analyze pathogenic cells (Neeraja et al., 2010, Maksimov et al., 2011).

Biological control of cucumber damping-off disease in the plastic house

Efficiency of fungal and bacterial biological control agents in controlling cucumber damping-off in the field is represented in (Table 8) *T. harzianum* and *B. subtilis* significantly controlled cucumber damping-off incited by *P. aphanidermatum* by 100% comparing with the control 1, no significant differences were detected between both treatments with the negative control (Untreated soil). *T. harzianum, S. coelicolor* and *Rhizobacteria* were highly effective and significantly decreased damping-off of cucumber seedlings incited by *R. solani* by 100% compared with the positive control 2. No significant differences were detected betwen *T. harzianum* against *R. solani* with the negative control (Untreated soil).

Soil borne pathogens may be efficiently controlled by the bio-control bacteria due to the bacteria ability to secret different types of antibiotics: Pyrrolnitrin, 2,4-Diacetylphloroglucinol and Siderophores (Bakker et al., 2003; Mavrodi et al., 2001). It also may be by high competition for food between the causal agent and the biological control agents. This leads to exclusion of the pathogen from root surfaces, which represent an appropriate environment for the pathogen (Suslow, 1982). Zhou and Paulitz (1993) found that *B. subtilis* reduced vesiculation and germination of the zoospores through competition for food and changes in cation levels such as calcium, which affects vesiculation and germination of zoospores.

Table 7. Efficiency of different bacterial concentrations on growth rate of *Rhizoctonia solani* on P.S.A media at 25 ± 2°C.

Bio-agent	Bacteria dilution	Colony diameter (mm) *R. solani*				Mean growth rate
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	
Rhizobacteria	10-¹	0.000^{y}	0.00^{y}	0.00^{y}	0.00^{y}	0.00^{I} (100%)
	10-²	0.000^{y}	6.38^{vwy}	16.06^{qrs}	25.6^{klm}	12.02^{h}
	10-³	3.02^{wxy}	15.58^{qrst}	21.22^{nop}	42.68^{fghi}	20.72^{g}
B. subtilis	10-¹	0.000^{y}	2.26^{xy}	11.58^{tu}	38.66^{ij}	13.12^{h} (78%)
	10-²	2.76^{xy}	15.64^{qrst}	39.34^{hij}	45.92^{fg}	25.9^{f}
	10-³	7.2^{vw}	19.66^{opq}	40.52^{hij}	59.16^{e}	31.64^{e}
A. chroococcus	10-¹	7.06^{vw}	17.86^{pqr}	46.72^{f}	61.56^{d}	33.3^{e} (44.5%)
	10-²	17.04^{pqrs}	26.38^{klm}	43.36^{fgh}	65.94^{c}	38.18^{d}
	10-³	13.34^{stu}	28.88^{k}	45.34^{fg}	81.06^{a}	42.14^{c}
S. coelicolor	10-¹	0.00^{y}	0.00^{y}	0.00^{y}	0.00^{y}	0.00^{I} (100%)
	10-²	14.16^{rstu}	25.44^{klm}	41.06^{ghij}	66.22^{c}	36.72^{d}
	10-³	23.42^{mno}	41.34^{ghij}	61.00^{d}	64.82^{cd}	47.64^{b}
P. flouresence	10-¹	3.34^{wxy}	10.52^{uv}	28.62^{kl}	41.06^{ghij}	20.88^{g} (65%)
	10-²	14.58^{rstu}	24.24^{lmn}	42.00^{ghij}	63.62^{cd}	36.1^{d}
	10-³	26.04^{klm}	37.88^{j}	56.00^{e}	75.36^{b}	48.82^{b}
Mean	-	8.8^{d}	18.16^{c}	32.86^{b}	48.78^{a}	27.14
R. solani (control)	0	21.73^{nop}	61.13^{d}	73.33^{b}	84.00^{a}	60.05^{a}

*Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at P < 0.05.

*each number is a mean of three replicates.

The effects may also be due to the efficiency of the bacteria in induction of systemic resistance and stimulate plant growth; the bacteria reduced the secondary inoculums spread around the roots. It has been found that induced resistance in cucumber is associated with an increase in the deposition of lignin in the infection site (Ongena et al., 1999) as well as increase enzymes activity or phytoalexines such as Peroxidase, Chitinase, B-1, 3-glucanase, Polyphenoloxidase (Ongena et al., 1999; Al-Dulaimi, 2000). It also may be due to the ability of the bacteria to survive and multiply depending on root exudates and thus continuing in their ability to secrete different metabolic compounds (Dowlng and O'Gara, 1994).

High efficiency of the bio-control agent *T. harzianum* in reducing disease percentage and severity of damping-off on cucumber seedlings may be attributed to penetration of *T. harzianum* to the root

system and secreting Peroxides and Chitinase enzymes in the treated plants after 48-72 hours (Yedidia et al., 1998).

It may also directly parasitize on the mycelium of the pathogenic fungus (Sivan and Chet, 1989) or it may be due to its ability to secrete antibiotics of certain analytic enzymes for the cell walls of the pathogenic fungi such as glucanase B-1, 3-, Protease, Chitinase (Mathre et al., 1999; Limon, 1999) or for competition on food (Elad et al., 1980).

The positive effect of the biological control agent on plant growth refers to the bacteria which act on increasing nutrient uptake (Valoon and Bakker, 2003), thus it enhance plant resistance to the disease by producing Salcylic acid, Auxins and Gibberellins, as well as increases of chlorophyll contents in the leaves (Shehata et al., 2000). Bacteria also work on increasing the effectiveness of some enzymes like Peroxidase, Chitinase and Polyphenoloxidase which increases protein content in the treated plants (Ongena et al., 1999). Combination of induced resistance with other resistance mechanisms in the host plant may be more effective.

Table 8. Efficiency of some bio-control agents in disease control of cucumber damping-off in plastic house under sulaimani conditions.

Treatments	% Disease *incidence	% Disease severity
Untreated soil (Negative control)	0.7^{c}	0.7^{c}
P. aphanidermatum (positive control 1)	9.34^{a}	8.95^{a}
R. solani (positive control 2)	8.70^{b}	8.97^{a}
T. harzianum + P. aphanidermatum	0.7^{c}	0.7^{c}
B. subtilis + P. aphanidermatum	0.7^{c}	0.7^{c}
T. harzianum + R. solani	0.7^{c}	0.7^{c}
S. coelicolor + R. solani	0.7^{c}	0.7^{c}
Rhizobacteria + R. solani	0.7^{c}	0.7^{c}

*Values followed by the same letter are not significantly different according to Duncan's Multiple Test at $P < 0.05$.

*Each number is a mean of three replicates, Numbers are transformed according to Arcsine transformation

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