Assesment the Effect of some Bio-Control Agents and OrganiCul-ITM for Controlling Root-rot and Damping-off Diseases of Tomato *Lycopersicon esculentum* Mill Caused by *Rhizoctonia solani* Kühn

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Abstract — This study was carried out under green house condition in Al-Najaf province to evaluate the efficiency of some of bio-control fungi and abacteria individually (*Trichoderma harzianum, Trichoderma viridi , Chaetumium globosum,Bacillus subtilis,Pseudomonas fluorescens,Azotobacter vinelandii*) and incombination as OrganiCul-ITM,commertial products(*Trichoderma harzianum,Trichoderma viridi, Bacillus subtilis,Pseudomonas fluorescens, Azotobacter vinelandii*) to protect tomato plants against *Rhizoctonia solani* ,the causative agent of root rot and seedling damping-off diseases.
The antagonistic and promotional abilities of *T.harzianum* , *T. viride, ,C.globosum, B. subtilis,P. fluorescens, A.vinelandii* were achieved against *R. solani* in vitro in dual culture assay and in green house.Antagonistic test showed that *T. harzianum* isolate was significantly reduced the mycelial diameter of *R. solani* which reach to 16.10 mm on potato dextrose agar (PDA) medium incompare with *R. solani* Kuhn isolate that was 90 mm, so the inhibition percentage of mycelial growth of *R. solani* Kuhn by *T. harzianum* was 82.21%.
OrganiCul-ITM gave asignificant differences in seeds germination percentage of tomato in green house pots which arise to 89.7%, whereas reached to 18.7 % for treated seeds with *R. solani* in compare with control treatment that amounted 79.6 % . *T. harzianum* also revealed high ability in germination percentage of tomato seeds .
Treating tomato seeds with OrganiCul-ITM gave significant results than individual treatments in reducing the infection of root rot and damping off diseases which reached to 5.4,6.6 % ,respectivelyin compared with that in untreated one in greenhouse soil artificially infested with a *R.solani* Kuhn .

Index Terms— *Rhizoctonia solani, Trichoderma* spp., *Bacillus subtilis*,Tomato ,anti fungus, OrganiCul-ITM , Soil borne pathogens, Biocontrol, Damping –off.

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1 Introduction

Tomato (*Lycopersicon esculantum* .Mill), which follow solanaceae family ,is the most popular and economic vegetable crops in whole parts of the world for its high nutritional value and richness in Vitamins A and C and realy for its multiplicity uses [1].
Agricultural crops are exposed to many different plant diseases [2],tomatoes attacked by the fungus *Rhizoctonia solani* ,the most important pathological soil causative fungi of seedling damping-off, root rots diseases and reduce both yield quality and quantity [3],[4],[5],[6].*R. solani* is one of the phytopathogens that attack tomatoes cultivated under greenhouse conditions, causing root and crown rot [7].
Root rot disease caused by *Rhizoctonia solani* Kuhn is the most destructive disease of tomato [8]. [9].Damping-off diseases are commonly encountered in the greenhouse and are primarily caused by the pathogen *Rhizoctonia solani* Kuhn [10] .
The controlling challenges for these diseases are decreasing pesticide residues in the plants and soil[11]. As well as the resistance of pathogens to pesticides are creating a need for new alternative biological methods to combate fungal diseases [12].

Biological control for soil-borne pathogens by antagonistic microorganisms is one of significant challenges due to difficulties to be controlled with specific fungicides [13], and it was usually have not any toxic residues in food chains, safe,while chemicals fungeicides meight results in soil contamination or other harmful effects [14]. Additionally, Biological control of soil-borne plant pathogens is a potential alternative to the use of chemical pesticides, which have already been proved to be harmful to the environment [15]. Currently many researches had been done using the biological

control as viable and reliable practice against many soil borne pathogens[16]. *Trichoderma* , *Chaetomium* , *Pseudomonas* and *Bacillus* genera are most feasible biocontrol microorganism suppress several pathogens like *Rhi*zoctonia solani , which reduction the incidence of tomato root rot and damping-off diseases caused by *Rhizoctonia solani*. Furthermore, [17] reported that soil application with *Trichoderma harzianum* was significantly reduced incidence of tomato damping-off disease caused by *Rhizoctonia solani*. The most important factors used in this kind of biocontrol agents against the causes of the pathogens is *Trichderma harzianum* [18], *Trichoderma harzianum* protected the bean seedlings against pre-emergence damping off infection, reduced the disease severity and increased the plant growth in the presence of *R. solani* pathogen [19]. Fungi of the genus *Trichoderma* are important biocontrol agents of several soil borne phytopathogens and use different mechanisms for the control of phytopathogens which include mycoparasitism, competition for space and nutrients, secretion of antibiotics and fungal cell wall degrading enzymes [20]. In addition, *Trichoderma* could have a stimulatory effect on plant growth as a result of modification of soil conditions[21].

Another example of the biological control agents is the Gram positive bacterium *Bacillus subtilis*, which has been found to possess bio-control potential for a variety of phytopathogenic fungi [22]Also,the mechanisms by which *B. subtilis* reduces plant diseases include antagonism of fungal pathogens, by competing for niche and nutriments, by producing fungi-toxic compounds, and stimulating the defensive capacities of the host plant[23] . *Trichoderma* spp, in combination with *bacillus* spp were used to suppress *Rhizoctonia solani* in tomatoes seedlings [24] .

2-MATERIAL AND METHODS

The laboraterial experiments studies was carried out in Faculty of Science laboratories - Kufa University,while the field experiments were conducted under greenhouse and field conditions at the experimental farm of agriculture extention center in Al- Najaf province at 2014 season.

Tomato seeds (*Lycopersicon esculantum* .Mill) cv. Wigdan was obtained from Agricultur Centre, Al-Najaf, Iraq, Seeds were placed on sterile cotton and filter paper that was moistened with sterile distilled water in Petri dishes and incubated at 25 ºC [25] .This test was showed no infected tomato seeds.

2-1-PHYSICAL AND CHEMICAL PROPERTIES

Measurement of physical and chemical properties of the soils ,that were collected from the study region for a depths of 0-30 cm included

1-Soil Texture: Estimated according to [26].

2- ECe and pH:-Estimated according to [27] .

2-2- Isolation of Pathogens and bio-agents fungus:

Rhizoctonia solani was isolated from naturally infected tomato plants, showing damping off and root rot symptoms, cultivated in Al-Najaf, Iraq. It was microscopically identified on the basis of cultural and microscopic characteristics

Trichoderma harzianum and *Trichoderma veridi* isolates obtained from the Department of the plant protection –Kufa University ,*Chaetumium globosum* was isolated from rice plant rhizosphere located in Rice research institute . The isolated fungi was identified on the basis of cultural and microscopic morphological characters according to the key given by [28].

Bacillus subtilis and Pseudomonas fluorescens strain were obtained from Biology department, Faculty of Science, Babylon University, The fungus were identified according to approved taxonomic keys Pathogencity of the isolate toward tomato plant was estimated . The fungi of were used *in vitro*, in pots and in field experiments.

The commercial product OrganiCul-ITM(*Trichoderma harzianum , Trichoderma viridi, Pseudomonas fluorescens , Bacillus subtilis, Azospirillium brasillense, Azotobacter vinelandii*) was obtained as commercial products and tested in the green house.

2-3-Preparation of bio-agents inoculums:

The bio agents fungi (colony forming unit, cfu) suspension of each *T.harzianum ,T. viride* and *C. globosum* fungus was prepared in sterile distilled water from 7-days-old-culture on potato dextrose agar (PDA)[29],[30].The fungal inoculum was harvested by flooding the culture with sterile distilled water and then rubbing the culture surface with a sterile glass rod. The fungal propgules concentration in each suspension was determined by counting using a haemocytometer slide (Adjusted at 108 cfu / ml) [31].

Meanwhile, *B. subtilis* and *Pseudomonas fluorescens* aninoculum was prepared and counted by plate count technique (108 CFU / ml) as maintained by[32].

2-4Evaluation of antagonistic activity of bio-agents

2-4-1-In dual culture technique (*in vitro*):

The antagonistic effect of each bio-agents individually against *R. solani* pathogens *in vitro* was evaluate using the dual culture technique [33]. Each bio-agents and *R.solani* were cultured, separately, on PDA medium for 7 days at 25ºC. Disc (5mm- diameter) from each bio- control fungus was inoculated on surface of PDA medium in side of Petri dish. A disc (5 mm - diameter) of *R. solani* was inoculated at equal distance of the opposite side of Petri dish. Petri dishes were inoculated with each pathogenic fungus only as control. Three Petri dishes for each bio-control - pathogenic fungus

treatment, as well as the control, were used as replicates. The inoculated Petri dishes were incubated at 25 ºC at 7 days. Antagonistic effect of bio-agents as decrease of the mycelial growth of pathogenic fungi, was determined using the following formula. Antagonistic effect =A-B/Ax100[34], [35]. Where, A: The diameter of mycelial growth of pathogenic fungus in control and B: The diameter of mycelial growth of pathogenic fungus with bio-agents.

2-4-2-Test pathogenicity and incentive for fungi on seed germination of tomato seedling in pots.

Pathogencity of studied fungi toward tomato plants (cv. Wigdan) was estimated [36]. The most aggressive isolate of each pathogenic fungus was used in pot experiments.Tomato seeds were mixed thoroughly with 2 ml of the bacterial suspension(108cfu/ml) or fungal spore suspension(106conidia/ml) in 0.1 % carboxy methyl cellulose in Petri dish. Seeds were air dried for 30 min and planted directly.

% Seed germination = No. of emerged seeds*100 / Total number of seeds (1)

2-5-Disease assessment:

Antagonistic effects of these bioagents against the soil borne pathogens that cause damping-off and root-rot diseases, were tested in a greenhouse in pots under artificially infestation conditions.

Fungus was grown on barley grain medium (200 g of grain/500 ml flasks) at 25 ºC for 15 days. The sandy loam soil used for the test was then infested with each fungus at a rate of 3% by weight and 20 cm diameter pots were filled with 5 kg of the inoculated soils and placed on benches in a greenhouse at 28 ± 3 °C. The soil of the control pots was mixed with the same amount of non inoculated autoclaved barley medium. There were three pots per treatment arranged according to a completely randomized design. five seeds of tomatoes were sown in each pot. After one month,the pots were thinned to three plants per pot. Disease incidence for pre-emergence damping-off and root rot at 30 and 60 days after sowing, respectively, were assessed as follows:

$$\% \text{ Damping off)} = \frac{\text{No. of non-emerged seeds}}{\text{No. of sown seeds}} * 100 \quad (2)$$

$$\% \text{ Root-rot} = \frac{\text{No. of plants with root-rot}}{\text{Total no. of emerged plants}} * 10 \quad (3)$$

2-6-Statistical analysis

The studied treatments were in three replicates (the laboratory experiment were designed as C.R.D ,while field experiment were designed as R.C.B.D , treatment means has been compared according to Fisher's Least Significant Difference (L.S.D) at 0.05[37] .

The experiment treatments were :-

laboratory experiment treatments

1- *R. solani* + *B. subtilis*
2- *R. solani* +*P. fluorescens*
3- *R. solani* +*A. vinelandii*
4- *R. solani* + *T. viride*
5- *R. solani* + *T. harzianum*
6- *R. solani* + *C.globosum*
7- Control (*R. solani*)

field experiment treatments

1- *R. solani* + *B. subtilis*
2- *R. solani* +*P. fluorescens*
3- *R. solani* +*A. vinelandii*
4- *R. solani* + *T. viride*
5- *R. solani* + *T. harzianum*
6- *R. solani* + *C.globosum*
7- *R. solani* + OrganiCul-ITM
8- *R. solani*
9- Control(pure soil)

3-Results and Discussion

3-1-Physical and Chemical Properties:

The results appeared that the soil texture in studied fields was sandy loam, , The bulk density values for soils in the surface horizon was 1.38 gm. Cm $^{-3}$, while the value of pH and salinity ECe for samples were 7.56, 2.49 ds.m^{-1} respectively. table (1), so these good values for pH and salinity belong to the appropriate management processes ,this is Compatible with what referred by [38] .

Table 1:Physical and chemical properties of studied soils.

Properties	Values	Units
Sand	49.7	%
Silt	31.0	%
Clay	19.3	%
Texture	sandy loam	---
Bulk density	1.38	g.cm^{-3}
pH	7.56	---
E.Ce	2.49	dS.m^{-1}

3-2-in vitro test :

The results in figure (1-a) appeared that the fungi *Trichoderma harizianum* ,*Trichoderma. viride* , *Chaetumium globosum* gave 16.10, 18.60,19 .30 mm, were more effective in reducing mycelial growth of *R. solani in vitro* test. than the bacteria *Bacillus subtilis* , *Pseudomonas fluorescens* and *Azotobacter vinelandii* which gave 25.70, 27.30, 28.40 mm respectively.

Also *T. harizianum* gave significant antagonistic effects against *R. solani* ,the percentages of mycelial growth reduction was 82.12% ,while *T. viride* , *C. globosum* ,*B. subtilis* , *P. fluorescens* and *A. vinelandii* were 79.36, 78.56, 71.44 , 69.67, 68.45% respectively. Results showed that the

best growth inhibition against pathogenic fungi was obtained by *T. harzianum* that amounted 82.12, while the lowest one was obtained by *Azotobacter vinelandii* that gave 68.45 figure (1-b).This is agree with previous studies that refer to *T. harzianum* and *B. subtilis* ability to give the best protection to tomato.These results are harmonized with those obtained by [39] ,who reported that *Trichoderma* spp. secreted chitinase and B 1,3 glucanase in supernatants. Also, *B. subtilis* inhibited the mycelial growth, radial growth, spore germination and germ-tubes length [40],[41] demostrates that *B. subtilis* can inhibit effect on fungal pathogens by secretion several antifungal metabolites such as subtilin, bacitracin, bacillin and bacillomycin.

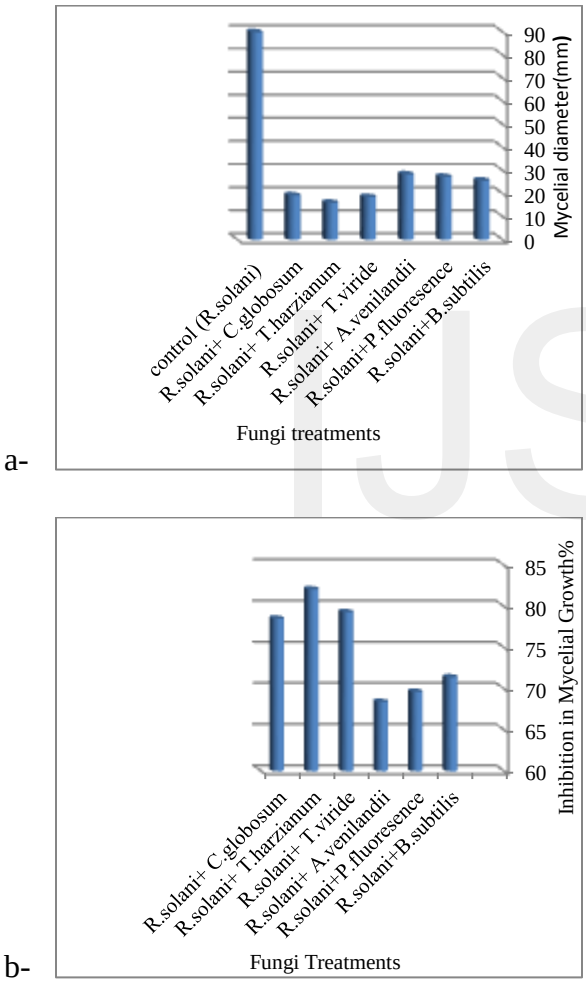


Figure 1: Effect of studied biocontrol agents on (a) radial growth of *R. solani* (b) inhibition in mycelial growth% in dual culture *in vitro* tests.

3-3- Pot culture experiments in green house.

3-3-1- Seed germination

Evaluation of seed treatments with various bio-agents and OrganiCul-ITM against *R. solani* in pot culture revealed that seed dressing with OrganiCul-ITM treatment significantly improved seed germination reached 89.7% in compare with control 79.6% (Figure 2-a) followed by *T. harzianum* , *T. viride* , *C.globosum* , *B. subtilis* , *P. fluorescens* , *A. vinelandii.* 83.1, 81.2,80.9,79.5,75.7,73.0 % respectively, [42] reported the efficacy of biocontrol agents as seed treatment against *R. solani and F.oxysporum* pathogen,also OrganiCul-ITM treatment achievd asignificant affectd on seedling death that amounted 10.3 % in compare with control treatment that reach 20.4%(Figure 2-b).

The reason for increased germination of seeds could be attributed to the fact that antagonist was seed-borne in present case and *R. solani* soil borne. The biocontrol agents and OrganiCul-ITM seemed to have restricted the growth of pathogen near the seed either by the process of antibiosis or mycoparasitism and thus improved the seed germination that might be due to decreased incidence of pathogenic infection,which proved better results of decreasing of seedling death with biocontrol agents [43].

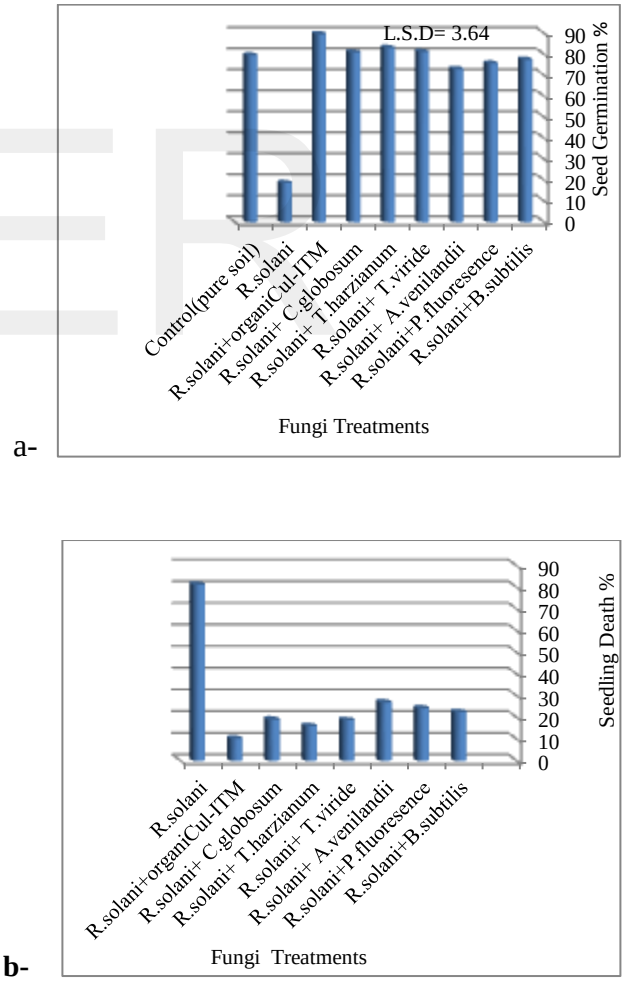
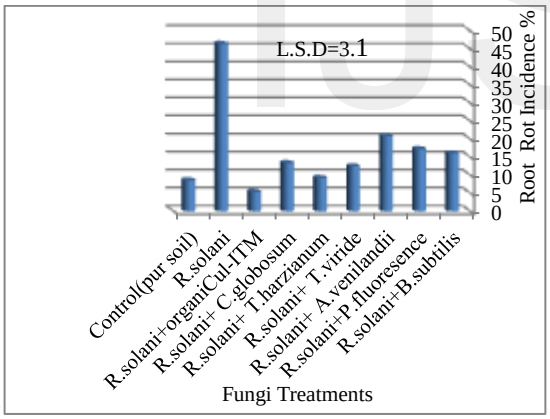


Figure 2. Effect of seed dressing with various biocontrol agents and OrganiCul-ITM on (a) seed germination and (b) seedlings death of tomato raised in soil inoculated with *R. solani* in the pots.

3-3-2- **Root rot and damping - off incidence and survival plants in green house**

In a greenhouse pot test, all the seed treatments reduced root rot and damping - off incidence by *R. solani* (Figure 3a,3b). OrganiCul-ITM proved most effective in restraining root rot and damping - off incidence significantly to 5.4,6.6.5%. Although *T. harzianum* , *T.viride* and *C.globosum* were superiorly reduced root rot 09.3, 12.5, 13.4% and damping-off 08.6, 10.9, 11.4% respectively over root rot by *B. subtilis* , *P. fluorescence* and *A. vinelandii* 16.1, 17.3.20.8 % and damping-off 13.9,14.8, 15.7%respectively in compare with control which gave 8.6,12.5% and with *R. solani* 46.5,24.6 for root rot and damping- off respectively . So, according to this results ,all this biocontrl agents in componation as commercial product (OrganiCul-ITM) achieved asignificant survival plants that reached 89% followed by *T. harzianum* , *T.viride* and *C.globosum* which gave 83.1,79.4,76.2 % respectively (Figure 3c).The decrease in root rot and damping -off incidence may be attributed to the inhibitory action of seed treatments with bio-control agents on pathogenic growth and multiplication in the rhizosphere,so the antagonists may reduce or ,inhibit pathogenic growth through antibiosis/mycoparasitism mechanisms[45] , [46].



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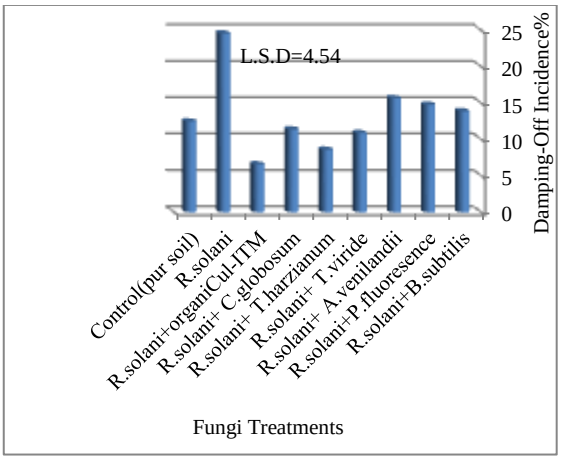

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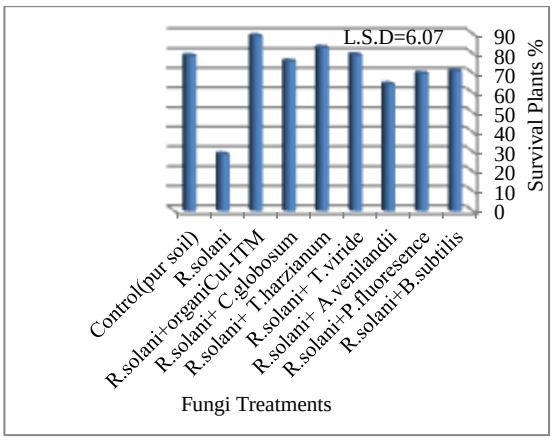

c-

Figure 3:Effects of bioagent treatments on(a) damping-off and (b)root-rot incidence caused by *R. solani* and (c)survival plants of tomato in a greenhouse pot test.

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