Evaluation of the Efficiency of Biocontrol Agents, Including *Bacillus paramycoides, Bacillus subtilis, Trichoderma longibrachiatum, Talaromyces oumae-annae,* and *Trichoderma harzianum,* to Control Pepper Root Rot Disease Caused by *Rhizoctonia solani* in Babil Province, Iraq

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Abstract

The study aimed to isolate and identify the causative agent of root rot disease in pepper plants and to evaluate the efficacy of certain biocontrol agents against the pathogen under laboratory and field conditions.The results from the wooden shelter experiments indicated that the treatments used in the study, which included the fungi *T. longibrachiatum, T. oumae-annae, T. harzianum*, and the bacteria *B. paramycoides* and *B. subtilis*, significantly reduced the negative impact of the pathogenic fungus *R. solani*. These treatments provided substantial protection to pepper plants against root rot disease, resulting in a significant reduction in disease severity at varying rates. The combination treatment of *T. longibrachiatum* and *B. paramycoides* was the most effective, reducing disease severity to 6.44% compared to 74.33% in the pathogen-only treatment.

Field experiment results confirmed the findings from the wooden shelter experiments. The addition of biocontrol agents *B. paramycoides, B. subtilis, T. longibrachiatum, T. oumae-annae, and T. harzianum* significantly mitigated the adverse effects of *R. solani*, providing good protection to some pepper plants from root rot disease and resulting in a significant reduction in disease severity at varying rates. The combination treatment of *T. longibrachiatum* and *B. paramycoides* again showed superior performance, reducing disease severity to 6.77% compared to 84.22% in the pathogen-only treatment, positively affecting the growth rate of both the vegetative and root systems, as well as the fresh and dry weights of the plants and roots. This combination treatment achieved the highest values in growth parameters of pepper plants, including length and the fresh and dry weights of both the vegetative and root systems.

Keywords: *Talaromyces oumae-annae, Bacillus paramycoides, Trichoderma longibrachiatum, Rhizoctonia solani, Trichoderma harzianum, Bacillus subtilis*.

1- Introduction:

Pepper (*Capsicum annuum*), a member of the Solanaceae family, is an important vegetable crop cultivated in many countries worldwide. The original habitat of pepper is Central and South America, from where it spread to Asia, Africa, and Mediterranean countries (33). Fungi, particularly soil-dwelling pathogens, are significant disease agents that infect roots, the crown area, and stem bases, thereby inhibiting the absorption of water, nutrients, and salts from the soil(11) . These pathogens cause substantial economic losses by reducing both the quantity and quality of yields. Notable among these fungi are *Rhizoctonia*

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spp., *Fusarium* spp., and *Pythium* spp., which form persistent structures like oospores, sclerotia, and chlamydospores that can endure harsh environmental conditions and become active when suitable conditions arise, leading to primary infections during crop planting (38).

Recently, there has been increasing interest in biocontrol strategies that utilize non-pathogenic microorganisms to inhibit soil-borne pathogens without affecting the remaining microbial communities. These biocontrol agents employ various mechanisms such as direct parasitism on pathogens, competition for nutrients, and the production of secondary metabolites like antibiotics and mycotoxins (41) . Among the effective biocontrol fungi, *Trichoderma* spp. are known for their ability to enhance root growth and development, improve resistance to environmental stresses, and increase crop yields and nutrient uptake (35). Additionally, *Penicillium* spp. have shown in numerous studies to interact positively with crop roots to promote root growth(39,23) . Some species of *Penicillium* are recognized for their antagonistic activity against pathogens through antibiotic production and the induction of systemic resistance in plants by activating multiple plant defense signals(21).

The genus *Bacillus* has also garnered significant attention from researchers worldwide for its role in biological control programs aimed at managing various plant pathogens affecting agricultural crops (8,40) . Given the widespread occurrence of pepper root rot caused by the pathogen *R. solani* in **farms and fields** in Babil Province, this research aims to evaluate the effectiveness of several biocontrol agents, specifically *Talaromyces oumae-annae, Bacillus paramycoides, Trichoderma longibrachiatum, Trichoderma harzianum, Bacillus subtilis*, in managing this disease.

2- Materials and Methods:

2-1- Isolation and Identification of the Pathogenic Fungus *Rhizoctonia solani.*

Samples were collected from pepper plants showing symptoms of infection from four different field sites in Babil Province. In each site, infected plants were randomly selected from the intersections of the field’s quadrants. The plants were uprooted and placed in polyethylene bags, then transported to the laboratory. The next day, fungal isolation was performed. The roots were washed under running water to remove soil particles and cut into segments of approximately 1 cm each. These segments were surface-sterilized using a 2% sodium hypochlorite solution for 2-3 minutes, then rinsed in sterile distilled water for two minutes and thoroughly dried on sterile filter paper inside a laminar flow hood. Four plant segments were placed on each plate containing 25-20 mL of Potato Dextrose Agar (PDA) medium supplemented with 250 mg/L tetracycline as an antibiotic, after sterilizing the medium in an autoclave at 121°C and 1.5 kg/cm² for 15-20 minutes. The plates were incubated at 25±2°C for four days. The fungal growth from the infected plant segments was examined, and small portions of each fungal colony were transferred to fresh PDA plates and incubated at 25±2°C for seven days.

2-2- Antagonistic Ability Test of *Bacillus paramycoides, Bacillus subtilis, Trichoderma longibrachiatum, Talaromyces oumae-annae,* and *Trichoderma harzianum* against *R. solani* on PDA Medium.

The antagonistic ability of *B. paramycoides* and *B. subtilis* against the pathogenic fungus *R. solani* was tested on Nutrient Agar (NA) medium using the serial dilution method (10^{-1} to 10^{-8}) of a 72-hour-old bacterial suspension.

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One milliliter of each dilution was transferred to 9 cm Petri dishes containing PDA medium, and the plates were swirled to distribute the suspension evenly. Four replicates were prepared for each dilution (10^{-4} to 10^{-8}). A 5 mm disc from a 7-day-old *R. solani* culture was placed at the center of each plate. Four plates without bacterial addition served as the control. The plates were incubated at 26±1°C for 15 days. The growth rate of the pathogenic fungus and the percentage of inhibition were calculated using the Montealegre equation (29):

%Fungal Growth Inhibition= (Average Diameter of Fungal Colony in Control−Average Diameter of Fungal Colony in Treatment /Average Diameter of Fungal Colony in Control) x 100

The antagonistic abilities of the biocontrol fungi *Trichoderma longibrachiatum, Talaromyces oumae-annae, Trichoderma harzianum* were tested against the R. solani isolate using the double culture technique on 9 cm Petri dishes containing PDA medium. The medium was sterilized using a steam autoclave for 15-20 minutes. Each Petri dish was divided into two equal halves by an imaginary line. A 0.5 cm fungal disc of the 7-day-old *R. solani* (R4) was placed in the center of one half, while a 0.5 cm disc from the edge of a colony of each biocontrol fungus *T. longibrachiatum, T. oumae-annae, T. harzianum* was placed in the center of the other half. All plates were incubated at 25±2°C. Once the pathogen in the control treatment reached the edge of the plate, the percentage of inhibition was calculated using the Montealegre formula mentioned above.

2-3- Evaluation of Biocontrol Agents in Reducing Disease Severity and Enhancing Pepper Plant Growth in a Plastic House and Wooden Shelter Conditions

The pathogenicity test for the *R. solani* isolate was conducted on pepper seedlings under wooden shelter conditions at the Al-Musayyib Project - Al-Musayyib Technical College in 2024. The experiment used a mixture of loamy soil and peat moss in a 2:1 ratio, sterilized with commercial formalin at 20 mL/L water (40% formalin concentration). The soil was treated with 3 liters of formalin solution per cubic meter of soil, sprayed onto the soil piled on nylon sheets, mixed thoroughly, and covered with transparent nylon for seven days under sunlight. After seven days, the soil was aerated for three days and then distributed into 7 kg plastic pots (4). Each pot was planted with 30-day-old pepper seedlings, with three replicates for each treatment, and placed inside a plastic house. Standard agricultural practices, including irrigation and fertilization, were followed. After six weeks, the treatments were applied as follows:

1. Control (untreated) 2. *Trichoderma longibrachiatum* (T.l) 3. *Trichoderma harzianum* (T.h) 4. *Talaromyces oumae-annae* (P.o) 5. *Bacillus subtilis* (B.s) 6. *Bacillus paramycoides* (B.p) 7. *Rhizoctonia solani* (R.s) 8. R.s + T.l 9. R.s + T.h 10. R.s + P.o 11. R.s + B.s 12. R.s + B.p 13. R.s + Beltanol (fungicide) 14. T.l + T.h 15. P.o + T.l 16. B.s + T.l 17. T.l + B.p 18. R.s + T.h + T.l 19. R.s + P.o + T.l 20. R.s + B.s + T.l 21. R.s + B.p + T.l 22. P.o + T.h 23. B.s + T.h 24. B.p + T.h 25. R.s + P.o + T.h 26. R.s + B.s + T.h 27. R.s + B.p + T.h 28. B.s + P.o 29. B.p + P.o 30. R.s + B.s + P.o 31. R.s + B.p + P.o .

The bacterial suspension, carried on kaolin, was added to the soil at a rate of 5 g/1 L water per pot. Biocontrol fungi *T. harzianum, T. longibrachiatum, T. oumae-annae,* carried on a medium of corn grit, wheat bran, and water in a 3:7:3 ratio, were added at 5 g per

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treatment as required. Beltanol fungicide was applied at 1 mL/L water, with two sprays ten days apart, starting seven

days before the pathogen was introduced. After seven days of applying the biocontrol agents and

fungicide, the pathogenic fungus grown on millet seeds was added. Observations were recorded after two months, with the disease severity calculated using the following scale:

0 = No infection. 1 = Mild infection, wilting symptoms include 1-25% of the plant's leaves. 2 = Moderate infection, wilting symptoms include more than 25-50% of the plant's leaves. 3 = Severe infection, wilting symptoms include more than 50-75% of the plant's leaves. 4 = Very severe infection, plant death, wilting symptoms include more than 75-100%.

The infection severity was calculated according to Mickenny (28) as follows:

of the roots are infected, 5=plant death. Disease Severity (%) = ((Plants in 1 degree ×1+… Plants in 5 degree ×5)/ all plants ×5) ×100%.

The averages of height, soft and dry weight of the vegetative and rootstocks of the pepper plant were calculated for all treatments

2-4- Evaluation of the efficiency of biological control agents with the fungi *T. longibrachiatum, T. harzianum, T. oumae-annae* and the bacteria *B. subtilis* and *B. paramycoides* on the infection severity of the pathogenic fungus *R. solani* and some growth parameters of the pepper plant in the greenhouse and under field conditions.

This experiment was conducted in Babylon Province in the greenhouse area of the Musayyib project for the 2023-2024 agricultural season. The soil was plowed, leveled, and softened with the addition of DAP fertilizer, divided into six beds with a width of 60 cm and a distance of 80 cm between beds. The beds were fertilized with moderate amounts of decomposed and fermented organic manure, and a drip irrigation system was installed. A completely randomized block design (R.C.B.D) was used for this experiment, with three replicates for each treatment and three plants per replicate. Previously prepared seedlings of 30 days old were planted, with a distance of 50 cm between each plant. After 45 days from planting in the greenhouse, treatments were applied as in the shade experiment.

The biological control agents *B. subtilis* and *B. paramycoides*, carried on their respective media, were added to the soil in the root zone near the crown area at a rate of 5 grams per 1 liter of water. After one week, the inoculum of the biological agent fungi *T. longibrachiatum, T. harzianum, T. oumae-annae*, carried on a medium of corn grit + wheat bran + water in a ratio of 3:7:3, was added to the soil at a rate of 10 grams. The chemical treatment with Beltanol pesticide was performed at a rate of 1 gram per liter of water for two sprays, ten days apart, with the first spray occurring seven days before the addition of the pathogenic fungus for the treatments requiring it. The pathogenic fungus *R. solani* treatment was added seven days after inoculation with treatments, carried on millet seeds at a rate of 20 grams. After inoculating with the experiment treatments, irrigation, fertilization, and pest control for insects, whitefly, aphids, and spiders were performed according to the guidelines of the Iraqi Ministry of Agriculture for pepper cultivation in Iraq. Results were obtained four months after planting by calculating the percentage of root rot disease severity according to the scale used in the paragraph and measuring the length, fresh weight, and dry weight of the shoot and root systems of the pepper plant.

2-5 Statistical Analysis:

The Completely Randomized Design (CRD) was applied for laboratory experiments and greenhouse experiments, while the Randomized Complete Block Design (RCBD) was used for field experiments(5). Data were analyzed using the Statistical Analysis System (SAS, 2012), and the results were compared using the Least Significant Difference (L.S.D) test at a probability level of 0.05.

4-Results and Discussion

4-1 Isolation and Identification of the Pathogenic Fungus *Rhizoctonia solani*:

The results in Table (1) indicate the isolation of fungi from infected plant parts (roots and areas of the stem near the roots) taken from samples collected from pepper fields. The presence of root rot disease in pepper plants was found across all surveyed areas in Babylon Province, with infection rates ranging from 13% to 32%. The highest infection rate was recorded in the Al-Azawiyah field, which reached 32%, followed by the Al-Muhawil field at 23%. The high infection rates in these areas are attributed to the fact that these regions are dedicated to pepper cultivation, where the crop is planted annually and repeatedly.

Microscopic examination revealed four isolates of the fungus *R. solani*. These isolates showed significant variation in their growth rate, formation of sclerotia, and mycelial density, in addition to differences in colony color, which ranged from brown to whitish-brown. The presence of septate mycelium with brown to whitish-brown color, short and numerous cells, was noted. The mycelium exhibited many branches, which grew at right angles to the main hyphae, with constriction of the branching cells at the point of origin and the formation of transverse septa in the branches near the point of origin. The absence of asexual spores or conidia was observed, which is consistent with the findings of many researchers (42, 12).

Table (1) Field survey of pepper plant root rot disease for some fields in Babil Governorate for the agricultural season 2023-2024.

sample number	Location	Cultivars	Field area/dunum	Infection rate (%)
1	Babylon / Haidari	Olympus	1	13
2	Babylon/ Mahaweel	Karisma	8	23
3	Babylon/Jableh	Karisma	10	20
4	Babylon/Al Azzawiya	Karisma	1.5	32

2.

Testing the Antagonistic Ability of Bacteria *B. paramycoides* and *B. subtilis*, and Fungi *T. longibrachiatum*, *Talaromyces oumae-annae*, *T. harzianum*, and the Fungicide Beltanol in Inhibiting the Isolate of the Pathogenic Fungus *R. solani* on PDA Medium:

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The results of this test, shown in Table (2), demonstrated a high antagonistic ability among the biological control agents, which included bacteria *B. paramycoides* and *B. subtilis*, and fungi *T. longibrachiatum*, *Talaromyces oumae-annae*, *T. harzianum*, compared to the control treatment, which was 0.0%, and the use of the fungicide against the pathogenic fungus in the laboratory. The fungi used in biological resistance showed high antagonistic ability four days after the pathogenic fungus was cultured until it reached the edge of the plate. The treatment with the fungus *T. longibrachiatum* showed the highest inhibition rate at 98.8%.

The fungus species *Trichoderma* spp. is a significant agent in the biological control of pathogens. This fungus has various antagonistic properties against pathogens and is important for improving plant growth and production, as well as having the ability to induce resistance (18, 47). Most species of *Trichoderma spp.* exhibit high efficiency against pathogens and have several mechanisms, such as competition for space and nutrients, and parasitism. The antagonistic organism directly removes nutrients from the pathogen, and the antagonism mechanism involves producing metabolic substances that inhibit pathogens from plants (10, 43). This was followed by the treatment with bacteria *B. paramycoides*, which showed a high inhibition rate of 96.6%, then the treatment with the fungus *T. oumae-annae*, which showed a high inhibition rate of 94.4%. The treatment with the fungus *T. harzianum* showed a good inhibition rate of 93.3%, and the treatment with bacteria *B. subtilis* showed a good inhibition rate of 91.1%.

Table (2) illustrates the test of the antagonistic ability of some biological agents, represented by bacteria *B. paramycoides, B. subtilis,* fungi *T. longibrachiatum, Talaromyces oumae-annae, T. harzianum,* and the fungicide Beltanol in inhibiting the isolate of the pathogenic fungus *R. solani* under laboratory conditions.

No.	Transactions	Colonizer diameter (cm)	% Scaling ratio
1	B.p + R.s	0.3	96.6
2	T.L + R.s	0.1	98.8
3	T.O + R.s	0.5	94.4
4	Pathogenic fungi alone	9.0	0.0
5	T.H+R.S	0.6	93.3
6	B.S+R.S	0.8	91.1
7	Beltanol + R.S	0.0	100
8	L.S.D	0.151	2.294

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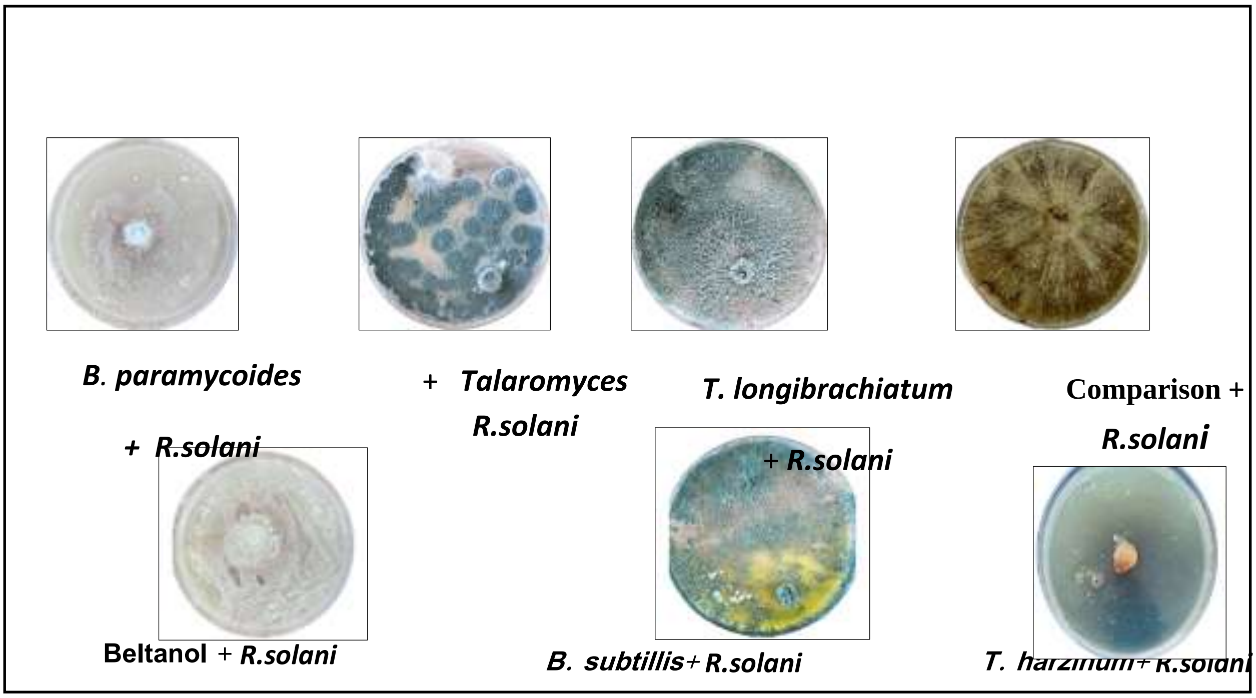
R.s= ***Rhizoctoni solani*** **،** ***Bacillus paramycoides*** **=B.p ،** ***Trichoderma longibrachiatum*** **= T.l،** ***Talaromyces oumae-annae*** **=T.o ،** ***Bacillus subtillis*** **= B.s ،*****Trichoderma harzinum*** **= T.h *.**



R.s= ***Rhizoctoni solani*** **،** ***Bacillus paramycoides*** **=B.p ،** ***Trichoderma longibrachiatum*** **= T.l،** ***Talaromyces oumae-annae*** **=T.o ،** ***Bacillus subtillis*** **= B.s ،*****Trichoderma harzinum*** **= T.h *.**

Figure (1) illustrates the antagonistic ability of the *bacteria B. paramycoides ,B. subtilis,* the fungi *T. longibrachiatum, T. oumae-annae, and T. harzianum*, and the fungicide Beltanol in inhibiting the isolate of the pathogenic fungus *R. solani* compared to the control treatment on PDA medium.

3. Evaluation of the Efficiency of Biological Control Agents Represented by the Fungi *T. longibrachiatum, T. harzianum, and T. oumae-annae, and the Bacteria B. subtilis and B. paramycoides* in Reducing the Severity of Infection by the Pathogenic Fungus *R. solani* and Some Growth Parameters of Pepper Plants in the Greenhouse and Under Shade Conditions:

The results in Table (3) show that the biological control agents represented by the fungi *T. longibrachiatum, T. harzianum, T. oumae-annae,* and the bacteria *B. subtilis* , *B. paramycoides* significantly reduced the severity of root rot disease in pepper plants caused by the pathogenic fungus *R. solani* compared to the control treatment (plant alone). The combined treatment of the

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biological agents *T. longibrachiatum* and *B. paramycoides* in the presence of the pathogenic fungus *R. solani* was superior to the other treatments, achieving a reduction in infection severity to 6.44%, compared to the percentage of infection severity in the treatment with the pathogenic fungus alone, which was 74.33%. The reason for the superiority of the combined treatments in reducing infection severity and increasing plant growth indicators is that the interaction between microorganisms provides better results due to the utilization of the various mechanisms these organisms possess against plant pathogens. Additionally, the cooperative effect between the biological control agents used in the experiment may stimulate systemic resistance in the plant against pathogens (13, 25).

The next best treatment was the combined treatment of the fungi *T. oumae-annae* and *T. longibrachiatum* with the pathogenic fungus *R. solani*, which achieved an infection severity of 8.66%, compared to the control treatment with the pathogenic fungus alone. The reason is that *Trichoderma* spp. fungi can be exploited as fungal biocontrol agents worldwide to manage soil-borne diseases, enhance plant growth, and stimulate systemic resistance in plants to improve their disease resistance (20). The reduction in infection severity in treatments containing the fungus *T. oumae-annae* is due to its production of several important enzymes such as manganese peroxidase and lignin peroxidase, which play a significant role in stimulating systemic resistance in plants (16).

The treatment with the biocontrol fungus *T. longibrachiatum* and the pathogenic fungus *R. solani* showed an infection severity of 12.22%, compared to the control treatment with the pathogenic fungus alone. The fungus *Trichoderma spp.* is an important agent in the biological control of pathogens. This fungus has various antagonistic properties against pathogens and is important for improving plant growth and production, as well as its ability to stimulate resistance (18, 47).

The next treatment was the biocontrol bacteria *B. paramycoides* with the pathogenic fungus *R. solani*, which showed an infection severity of 12.99%, compared to the control treatment with the pathogenic fungus alone. The genus *Bacillus* spp. is an efficient biocontrol agent against many plant pathogens, including fungi that are highly sensitive to these bacteria. This is due to their production of various antimicrobial compounds that affect the growth of microorganisms harmful to plants, making them highly efficient in inhibiting the metabolic activities of competitive organisms, including soil-dwelling fungi, and they also stimulate plant growth and productivity both qualitatively and quantitatively (17, 24).

The treatment with the biocontrol fungus *T. oumae-annae* and the pathogenic fungus *R. solani* showed an infection severity of 13.22%, compared to the control treatment with the pathogenic fungus alone. The fungus produces several bioactive compounds that cause physiological changes in fungal cells, preventing their growth and producing many compounds and enzymes such as carbohydrates, polyamines, amino acids, peptides, lectins, and enzymes that have high antagonistic ability against many fungi. One of the most important compounds produced by this fungus *P. commune* is the clavin alkaloids, which are biologically active and important secondary metabolites (16).

The treatment with the biocontrol fungus *T. harzianum* and the pathogenic fungus *R. solani* showed an infection severity of

15.77%, compared to the control treatment with the pathogenic fungus alone. The fungus *T. harzianum* is a biocontrol agent with high efficacy against pathogenic fungi. It enhances the roots of the host plant and helps facilitate the absorption of minerals such as iron in the root zone (50). The fungus *T. harzianum* produces other antifungal compounds such as harzianolide, 6-pentyl-pyrone (6-PP), harzianic acid, and aspinolide. Harzianic acid is an iron chelator that participates in the removal of heavy metals like iron, enhancing the host plant by absorption through the root and exhibiting antifungal activity, stimulating plant defenses, and providing protection against pathogens (46, 2).

The treatment with the biocontrol bacteria *B. subtilis* and the pathogenic fungus *R. solani* reduced the infection severity to 16.88%, attributed to the ability of *B. subtilis* bacteria to degrade fungal hyphae by secreting several degrading enzymes such as protease, lipase, amylase, and chitinase, which play a role in breaking down chitin, the main component of the cell walls of most fungi (30, 3).

The treatment with the fungicide Beltanol significantly reduced the infection severity to 13.88%, compared to the control treatment with the pathogenic fungus alone. The effect of the chemical fungicide Beltanol on fungi is due to its active ingredient, sulfate hydroxyquinoline-8, which binds with metals and heavy elements such as iron, sulfur, and copper, leading to the death of the pathogen because these elements are necessary for the pathogen's survival (2).

The results in Table (3) show that the biological control agents represented by the fungi *T. longibrachiatum, T. harzianum,* and *T. oumae-annae,* and *the bacteria B. subtilis* and *B. paramycoides* significantly improved growth parameters of pepper plants compared to the control treatment (pathogenic fungus alone). The combined treatment of *T. longibrachiatum* and *B. paramycoides* in the presence of the pathogenic fungus achieved high growth parameter values for pepper plants, with plant height, fresh and dry weight of the shoot and root systems reaching 39.21, 21.46 cm, 68.86, 18.86, 21.46, 14.36 g, respectively. This was followed by the combined treatment of the fungi *T. longibrachiatum* and *T. oumae-annae*in the presence of the pathogenic fungus, which showed significant increases in growth parameters, with plant height, fresh and dry weight of the shoot and root systems reaching 36.06, 19.33 cm, 65.66, 18.63, 19.96, 13.52 g, respectively, compared to the control treatment, which reached 12.36, 5.83 cm, 15.06, 5.41, 6.37, 2.47 g, respectively.

The reason for the superiority of the combined treatments in reducing the percentage of infection severity by the pathogenic fungus is that the interaction between biological control agents achieves better results. Each biological agent may use different mechanisms to combat the pathogen, and by combining these mechanisms from each biological control agent, there will be greater suppression of the pathogen and better results than using the biological control agent alone (13). This result agrees with many studies that have shown that the interaction between biological control agents is more effective in reducing the percentage of infection severity by plant pathogens compared to using a biological agent alone (37, 48, 25).

Regarding the treatments with biological control agents alone in the presence of the pathogenic fungus, the treatment with *T. longibrachiatum* showed the highest growth parameter values for pepper plants compared to the control treatment, with plant height,

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fresh and dry weight of the shoot and root systems reaching 34.66, 14.73 cm, 52.76, 18.36, 15.17, 9.85 g, respectively. This is because this biocontrol fungus plays a role in promoting plant growth, improving vegetative and root growth indicators, and increasing production both quantitatively and qualitatively. Most types of this fungus can live in plant cells without causing negative damage to the plant but rather have a role in promoting growth and development, such as increasing metabolism and energy production by producing a wide range of enzymes, particularly those that degrade the cell walls of the pathogenic fungus, in addition to inducing systemic resistance in the plant (36). The treatment with the bacteria *B. paramycoides* with the pathogenic fungus showed high growth parameter values for pepper plants compared to the control treatment, with plant height, fresh and dry weight of the shoot and root systems reaching 32.56, 14.53 cm, 50.66, 16.06, 15.06, 9.06 g, respectively. This is attributed to the ability of *B. paramycoides* bacteria to enhance plant growth and stimulate systemic resistance by secreting various antimicrobial compounds, such as lipopeptides, antibiotics, enzymes, and volatile organic compounds, and also competing for space and nutrients (7).

Table (3) evaluates the efficiency of biological control agents represented by the fungi *T. longibrachiatum, T. harzianum,* and *T. oumae-annae,* and *the bacteria B. subtilis* and *B. paramycoides* in reducing the severity of infection by the pathogenic fungus R. solani and some growth parameters of pepper plants in the greenhouse and under shade conditions.

No	treatments	severity of infection %	Vegetative growth length (cm)	Vegetative weight (g)		root system length (cm)	Root system weight (g)	
				fresh	dry		fresh	dry
1	T.l	0.0	39.46	36.36	18.16	19.82	17.62	9.97
2	T.h	0.0	34.46	33.66	15.76	14.83	13.36	7.30
3	T.o	0.0	37.56	34.56	16.66	16.33	14.76	8.36
4	B.s	0.0	32.51	32.26	13.97	13.96	12.86	6.41
5	B.p	0.0	38.16	34.86	17.06	17.38	15.96	9.08
6	Control	34.66	27.07	29.86	11.52	11.13	9.96	3.86
7	R.s	74.33	12.36	15.06	5.41	5.83	6.37	2.47
8	R.s+T.l	12.22	34.66	52.76	18.36	14.73	15.17	9.85
9	R.s+T.h	15.77	27.16	44.36	13.46	12.33	11.17	6.46
10	R.s+T.o	13.22	33.76	48.16	14.97	13.60	12.96	6.86
11	R.s+B.s	16.88	30.07	41.96	14.30	11.53	10.72	5.16
12	R.s+B.p	12.99	32.56	50.66	16.06	14.53	15.06	9.06
13	+Beltanol R.s	13.88	25.16	29.42	12.19	9.83	9.86	7.76
14	T.l +T.h	0.0	44.86	53.96	27.63	18.38	18.86	13.76
15	T.o+T.l	0.0	41.06	53.26	30.76	19.53	19.56	14.74

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16	B.s+T.l	0.0	37.16	51.06	25.26	16.83	16.50	12.31
17	B.p+T.l	0.0	46.66	55.97	32.06	20.03	26.06	16.06
18	R.s+T.h+T.l	9.44	34.06	59.46	17.76	17.83	17.05	12.76
19	R.s+T.o+T.l	8.66	36.06	65.66	18.63	19.33	19.96	13.52
20	R.s+B.s+T.l	10.33	31.26	57.76	16.76	17.73	15.96	11.86
21	R.s+B.p+T.l	6.44	39.06	68.86	18.86	21.53	21.46	14.36
22	T.o+T.h	0.0	35.46	45.56	17.36	15.73	15.61	11.36
23	B.s+T.h	0.0	32.16	43.76	15.97	12.73	13.96	10.56
24	B.p+T.h	0.0	35.06	46.66	19.36	16.82	16.96	12.86
25	R.s+T.o+T.h	25.66	29.16	39.76	15.41	15.71	12.86	10.97
26	Rs+Bs+T2	27.44	25.86	37.46	14.08	12.83	10.17	10.26
27	R.s+B.p+T.h	23.22	29.76	44.86	19.52	18.83	14.65	13.86
28	B.s+T.o	0.0	33.06	45.97	21.19	14.17	18.05	13.36
29	B.p+T.o	0.0	38.46	55.43	25.85	15.16	19.06	15.63
30	R.s+B.s+T.o	19.88	32.76	47.86	16.74	14.94	14.16	9.52
31	R.s+B.p+T.o	16.55	35.86	49.86	20.76	16.05	16.16	11.85
32	L.S.D	2.721	2.501	0.135	0.209	0.202	0.058	0.331

R.s= *Rhizoctoni solani* ، *Bacillus paramycoides* =B.p ، *Trichoderma longibrachiatum* = T.l، *Talaromyces oumae-annae* =T.o ، *Bacillus subtillis* = B.s ،*Trichoderma harzinum* = T.h *.

The fungus *T.oumae-annae* exhibited high efficiency in growth parameters for pepper plants compared to the control treatment, where the length and fresh and dry weight of both shoot and root were 33.76, 13.60 cm, 48.16, 14.97, 12.96, 6.86 g respectively. This is attributed to *T.oumae-annae* being a Plant Growth Promoting Fungus (PGPF), as it has the ability to provide numerous benefits to plants by promoting growth, increasing yield, and protecting against pests and pathogens through the release of enzymes, bioactive compounds, or antibiotics against plant pathogenic fungi. Among the important types of PGPF are *Trichoderma* spp., *Pinicillium* spp., and *Gliocladium* spp(44).

Treatment with the biocontrol fungus *T. harzianum* in the presence of the pathogenic fungus showed a significant increase in growth parameters such as plant length, root length, and shoot and root fresh and dry weight, compared to treatment with the pathogenic fungus alone. Biological factors demonstrate a high capability in protecting pepper plants from fungal pathogens due to possessing various chemical compounds known as antibiotics(19).

T. harzianum acts as a competitor against fungal pathogens, especially under nutrient-limiting conditions(6،26). It has a high capacity to induce Systemic Acquired Resistance (SAR)(14) ، and secrete enzymes that degrade the cell walls of pathogenic fungi, such as Protease, which degrades soil nutrients(45). Similarly, treatment with the biocontrol bacterium *B. subtilis* in the presence of the pathogenic fungus showed a

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significant increase in growth parameters compared to treatment with the pathogenic fungus alone. *B. subtilis* plays an active role in the Rhizosphere region by inhibiting pathogens, competing for nutrients, inducing plant systemic resistance, and secreting enzymes like Protease, Lipase, Amylase, and Chitinase, which degrade fungal hyphae.(34).

4-4 The evaluation of the efficacy of biological control agents, including fungi *T. longibrachiatum, T. harzianum, T. oumae-annae,* and *bacteria B. subtilis and B. paramycoides,* against the severity of infection by the pathogenic fungus *R. solani* and some growth parameters of pepper plants in plastic greenhouse and field conditions.

The results are shown in Table (4)that these agents significantly reduced the severity of root rot disease caused by *R. solani* compared to the control treatment. The combination treatment of *T. longibrachiatum* + *B. paramycoides* was particularly effective, achieving a reduction in severity of infection to 6.77% compared to 84.22% in the control treatment. This superiority of combination treatments in reducing infection severity and increasing plant growth indicators highlights the potential of microbial interactions in enhancing plant health The interaction between microorganisms gives The best results were achieved due to the utilization of various mechanisms possessed by these organisms against plant pathogens. Additionally, the cooperative effect between the biological resistance factors used in the experiment may contribute to stimulating the systemic resistance of pepper varieties selected against the pathogenic fungus (13, 25). Following these were treatments with the biological fungi *T.oumae-annae* and *T. longibrachiatum* against the pathogenic fungus *R.solani*, where the infection severity was 7.22% compared to the control treatment with the pathogenic fungus alone. This was followed by treatment with *T. longibrachiatum* along with the pathogenic fungus *R.solani*, resulting in an infection severity of 15.33% compared to the control treatment. Subsequently, treatment with the biological bacteria *B. paramycoides* along with the pathogenic fungus *R.solani* showed an infection severity of 16.55%, followed by treatment with *T.oumae-annae* along with the pathogenic fungus *R.solani*, resulting in an infection severity of 18.66%. Moreover, treatment with the biological fungus T. harzianum alone with the pathogenic fungus *R.solani* resulted in an infection severity of 20.55%. Additionally, treatment with the bacterial species *B.subtillis* along with the pathogenic fungus R.solani showed a reduction in infection severity to 25.99%. This reduction can be attributed to the ability of *B.subtilis* to produce numerous antibiotics such as Subtenolin, Bacillin, Bacillomycin, and Bactracin, which degrade the cytoplasm of fungal hyphae and distort fungal hyphal tips (9, 29). The pesticide treatment with Beltanol achieved a significant reduction in infection severity to 12.11%. The efficacy of Beltanol in reducing the percentage of infection severity is attributed to its high effectiveness in affecting pathogenic fungi, thereby reducing the severity of resulting infections. Furthermore, it forms chelating compounds with copper in plant tissues, facilitating its passage into the fungal cells, where it is released and leads to the killing of the pathogen (27).

The positive effects of biological agents on the significant increase in the length and fresh and dry weight of both shoot and root biomass of pepper plants compared to the treatment with the pathogenic fungus alone were evident. All interaction treatments between biological agents and the pathogenic fungus resulted in significant increases in shoot and root length and weight of the pepper plants. Treatment with *T. longibrachiatum* + *B. paramycoides* in the presence of the pathogenic fungus showed high growth parameters for pepper plants, with shoot and root lengths and weights reaching 68.72 cm, 37.06 cm, 394.06 g, and 98.03 g, respectively. This was followed by treatment with the fungal combination *T. longibrachiatum* + *T.oumae-annae*, which also recorded significant growth increases.

The reduction in root biomass due to the effect of pathogenic fungal agents negatively affected shoot biomass leading to a decrease in yield(32,31,49). Interactions between biological resistance factors were shown to increase yield compared to using a single biological resistance factor. The significant increase in growth parameters of selected eggplant varieties in interaction treatments was attributed to the synergistic effect between the biological resistance mechanisms possessed by these bacteria and fungi.

When used individually in the presence of the pathogenic fungus, the biological agent *T. longibrachiatum* showed the highest growth values for pepper plants compared to the control treatment. This was due to its ability to produce Indol asitc acid (IAA), which enhances root growth, leading to an increase in root mass and colonization area for beneficial microbial plant colonization, thus enhancing nutrient uptake(2). Enzymes that degrade the cell walls of pathogenic fungi, in addition to inducing systemic resistance in plants (36).

The treatment of *B. paramycoides* with the pathogenic fungus gave high efficiency for the growth parameters of pepper plants compared to the control treatment in which the length and soft and dry weight of the vegetative and root groups were 60.38, 34.76 cm, 323.96, 88.03, 32.86 and 21.06 g.The *T. oumae-annae* mushroom treatment gave high efficiency for the growth parameters of pepper plants compared to the control treatment in which the length and dry weight of the vegetative and root groups were 57.85, 33.36 cm, 333.56, 87.06, 30.06, and 14.16 g, respectively. The treatment of the biofungus *T. harzianum* in the presence of the pathogenic fungus gave a significant increase in growth parameters such as plant height, root length, soft and dry weight of the vegetative and root groups 54.19, 30.60 cm, 320.26, 85.44, 28.86 and 17.06 g, respectively, compared to the pathogenic fungus treatment alone, and the *B. subtillus* treatment gave a significant increase in growth parameters.In the presence of the pathogenic fungus, there was a significant increase in growth parameters such as plant height, root length, soft and dry weight of the vegetative group and the root group .5147, 28.86 cm, 323.96, 84.83, 27.71, 15.66 g, respectively. Biological agents are characterized by a high ability to protect pepper plants from infection by pathogenic fungi due to its possession of many chemical compounds known as antibiotics (19).

Table (4) likely presents the evaluation of the efficacy of biological control agents, including fungi *T. longibrachiatum, T. harzianum, T. oumae-annae,* and *bacteria B. subtilis and B. paramycoides*, in controlling the severity of infection by the pathogenic fungus *R. solani* and some growth parameters of pepper plants under greenhouse and field conditions.

No	treatments	severity of infection %	Vegetative growth length (cm)	Vegetative weight (g)		root system length (cm)	Root system weight (g)	
				fresh	dry		fresh	dry
1	T.l	0.0	70.39	369.49	92.63	39.15	38.76	17.30
2	T.h	0.0	63.65	344.26	90.44	31.56	35.36	13.86
3	T.o	0.0	61.95	354.56	91.23	38.76	36.46	19.36
4	B.s	0.0	59.46	346.86	90.03	27.96	34.71	12.56
5	B.p	0.0	65.40	360.76	91.73	36.76	37.86	15.96
6	Control	0.0	54.94	290.86	70.83	24.36	29.27	10.56
7	R.s	84.22	29.84	69.06	33.77	12.46	22.56	8.37
8	R.s+T.l	15.33	64.25	340.46	88.93	36.28	33.96	21.81
9	R.s+T.h	20.55	54.19	320.26	85.44	30.60	28.86	17.03
10	R.s+T.o	18.66	57.85	333.56	87.03	33.36	30.06	14.16
11	R.s+B.s	25.99	47.51	293.66	84.83	28.86	27.71	15.66
12	R.s+B.p	16.55	60.38	323.96	88.03	34.76	32.86	21.06
13	Beltanol + R.s	12.11	45.82	223.86	71.633	27.36	27.06	12.59
14	T.l +T.h	0.0	73.18	454.36	102.55	40.06	41.36	23.66
15	T.o+T.l	0.0	69.69	460.56	99.03	38.26	43.76	26.16
16	B.s+T.l	0.0	66.95	403.36	94.13	33.26	37.49	22.48
17	B.p+T.l	0.0	79.94	589.46	145.99	42.86	47.61	29.26
18	R.s+T.h+T.l	11.66	63.04	371.26	95.63	35.36	39.66	18.06
19	R.s+T.o+T.l	7.22	64.64	382.76	96.67	38.86	41.11	19.56
20	R.s+B.s+T.l	19.88	55.98	350.96	95.13	35.27	39.26	16.26
21	R.s+B.p+T.l	6.77	68.72	394.06	98.03	37.06	44.31	623.1
22	T.o+T.h	0.0	67.18	399.56	107.53	30.26	36.27	20.36
23	B.s+T.h	0.0	53.17	369.36	99.93	27.76	33.26	18.16
24	B.p+T.h	0.0	61.61	385.06	3101.6	34.2	43.27	24.56
25	R.s+T.o+T.h	23.77	48.97	335.66	89.44	29.46	40.36	16.76
26	Rs+Bs+T2	25.66	44.48	290.86	83.03	23.46	35.66	16.16
27	R.s+B.p+T.h	20.33	48.28	335.96	92.23	34.06	38.27	18.86
28	B.s+T.o	0.0	42.73	373.26	106.13	30.86	39.86	22.06
29	B.p+T.o	0.0	66.45	415.56	116.13	40.26	45.26	26.56
30	R.s+B.s+T.o	17.55	56.77	367.46	80.63	32.06	37.26	19.81
31	R.s+B.p+T.o	15.33	64.71	389.96	82.44	33.71	41.93	21.56
32	L.S.D	1.846	1.167	1.565	1.889	2.134	2.172	0.311

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R.s= *Rhizoctoni solani* ، *Bacillus paramycoides* =B.p ، *Trichoderma longibrachiatum* = T.l، *Talaromyces oumae-annae* =T.o ، *Bacillus subtillis* = B.s ،*Trichoderma harzinum* = T.h *.

T. harzianum acts as a competitor to fungal pathogens, especially when nutrients are scarce (6, 26). It has a high ability to induce acquired resistance (SAR) (14), and the amazing potential that enables it to secrete enzymes that degrade the cell wall of pathogenic fungi and parasitize them, including protease enzymes that work to colonize nutrients in the soil (45). Also, treatment with the chemical pesticide Beltanol achieved a reduction in the severity of the pathogenic fungus infection of pepper plants treated with it compared to the treatment of the pathogenic fungus alone, which reflected positively on the growth parameters of the pepper plant, and this is consistent with many studies that proved the inhibitory effectiveness of Beltanol for the pathogenic fungus (22, 3). The results of the field experiment confirmed the previous results of the laboratory and potted experiments, as for the interference coefficients between the fungi, the biological resistance factors represented by the fungi *T. longibrachiatum, T. harzianum* and *T. harzianum. T. harzianum, T. oumae-annae, B. subtillis , B. paramycoides* alone without the addition of the pathogenic fungus did not show any infection with the pathogenic fungus R. solani. The table also shows a significant increase in the growth parameters of the pepper plant in the treatments that included only biological resistance factors, whether overlapping or individually, all achieved a significant increase in height and soft and dry weight of total vegetative and root mass.

Conclusion

The results of isolation and identification revealed several isolates from different regions of the pathogenic fungus *Rhizoctonia solani*. The findings demonstrated a significant antagonistic capability among the biocontrol agents compared to the control treatment.

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