

Biological Effect of Controlling *Rhizoctonia Solani*, The Fungus that Causes Cucumber Root Rot Disease

Kifah Hadi Radhi

Ministry of Education, Babel Education, Iraq

Kfahhady5@gmail.com

Abstract: This study aimed to identify the fungal pathogen responsible for cucumber root rot disease in Babylon province and to study the biological management of this disease. The roots of diseased cucumber plants were used to isolate the fungus *Rhizoctonia solani*. where the pathogenicity tests were conducted for twelve isolates on the Cabbage seeds. Each isolate showed its high pathogenicity in inhibiting the germination of Cabbage seeds. Then, pathogenicity tests were conducted for the strongest four pathogenic isolates in infecting cucumber plants, namely R.s11, R.s10, R.s7, and R.s5. Moreover, the antagonistic potential of *Bacillus subtilis* was likewise tested, and the fungus *Trichoderma harzianum* and *Penicillium gresiofulvum* with the pathogenic fungus *R.solani*, The study findings showed that inhibition percentage was 100%, 92%, and 90%, respectively. Finally, the lath house The results demonstrated how well biological control methods inhibited the harmful fungus *R.solani*.

Key points: *Rhizoctonia solani*, *Trichoderma harzianum*, *Bacillus subtilis*, *Penicillium gresiofulvum*.

Introduction

Cucumis sativus L. is a summer vegetable crop that is a member of the Cucurbitaceae family of plants. In addition to being one of the key crops in protected agriculture, this crop is cultivated in Iraq in the spring and fall. The fruits have a high water content (estimated at 95.1%), and each 100 g of cucumbers has 15 calories, 0.9 g of protein, and 3.4 g of carbs. Additionally, according to (1,2), there are 25 mg of calcium, 27 mg of phosphorous, 1.1 mg of iron, 160 mg of potassium, 250 IU of vitamin A, and 11 mg of ascorbic acid. Cucumbers are exposed to a range of significant plant diseases, and one of the most important diseases that have spread in recent years widely and dangerously in homes and protected fields is Root Rot disease and wilt of infected plants. Root diseases are complex plant diseases, because their causes are found in the soil, which contains organisms that control it, and they have overlapping relationships with it and the surrounding environment. Problem of root rot diseases is one of the serious problems facing crops, especially vegetables at the global level, and what increases its danger is that it grows far from the human perspective, and many of them has a diverse family group. They have a lot of the ability to resist unsuitable environmental conditions and can stay in the soil and plant residues for a long time. Cucumbers are infected with many root diseases caused by soil fungi such as *Fusarium*, *Pythium*, *Rhizoctonia*, and *Phytophthora* (3,4) indicated the effectiveness of biological resistance components in controlling root rot causes. However, the importance of the fungus *harzianum*.T was a biological control element because it possesses a number of mechanisms, including mycoparasitism, competition, antibiosis, induced resistance, and inactivation of pathogen enzymes. It also works to increase the availability of the elements for the plant, increase the plant's tolerance to environmental stress conditions, and decompose complex organic materials into simple components that are easily absorbed from the plant. Numerous investigations demonstrated that the fungus *Trichoderma*

qualified to producing a wide range of antibiotics, including Emodiu Chrysophancol, Pachybas, Gliotoxin, Trichodermol, and Trichodermin. These antibiotics inhibit the development of several fungal diseases, including *R. solani* and *Fusarium*. It also helps to create a strong root that can withstand drought. Plant hormones like IAA can be produced by the fungus. The fungus *T.harzianum* produces the enzymes B.glucanase, Chitinase, Protease, and Xylanase in the culture medium. (5,6) concluded that the biological resistance fungus *Penicillium gresiofulvum* reduced the disease severity of pear seedlings infected with the fungus *F. solani* that causes pear wilt disease and its deterioration to 8.33%. In comparison, the disease severity was 22.91% in the control treatment, which led to an increase in root and shoot weight and length of pear seedlings. *B. subtilis* bacteria also works to produce effective antibiotics against plant pathogenic fungi such as Bacilin, Bacitracin, Subtiline, Subtenolin, and Bacillomycin. It works likewise with these antipoetic to control fungal pathogens, it is the induction of plant resistance through contact between the plant and the non-pathogenic microorganism of the plant. Then, it induces the plant to increase its resistance to diseases without changing the plant's genes and may induce some chemicals such as salicylic acid, (7,8). In the same field, (9,10) found the high activity of *B. subtilis* bacteria in inhibiting soil fungi that include *F.oxysporum* and *R. solani* that cause cucurbit diseases. Finally, (11) indicated the high activity of *B. subtilis* bacteria in inhibiting *R. solani* that cause Pepper root rot disease.

Materials and methods :

Isolation of the fungus *Rhizoctonia solani* from the diseased cucumber plants' roots

To get rid of all the plankton and contaminants on the surface of the roots, the fungus was separated from the roots of the infected cucumber plants and then rinsed under running water (tap water) for fifteen minutes. The roots were then surface sterilized by soaking them in a 2% sodium hypochlorite solution for three minutes after being sliced into tiny pieces that ranged in length from five to ten millimeters.

These parts were cleaned with sterile water and then placed onto sterile filter paper to drain any remaining water. It was then moved using sterile forceps to a 9 cm Petri plate filled with sterile PDA culture material, where it was kept for 20 minutes at 121°C and 1.5 kg/cm² Placing four pieces per plate in an autoclave equipment. For four days, the plates were kept at 25 ± 2°C in the incubator.

The fungal colony was purified and kept in test tubes with PDA culture media after it had grown on the medium. Using the taxonomic keys unique to the fungus, the sex and species of the fungi connected to the roots of the infected cucumber plants were determined.

Pathogenicity tests on Cabbage seeds.

Using local cabbage seeds, pathogenic fungal isolates linked to cucumber plant roots were examined.

Twelve fungal isolates produced from acetate were examined for pathogenicity using the methodology established by (12). Water Agar was made by adding 20 g to a liter of water that had been autoclave sterilized at 121 °C and 1.5 kg/cm² for 20 minutes. Petri plates with a 9 cm diameter were prepared to hold agar culture material. After the medium solidified, the plates were infected in the middle with a disc diameter of 5 mm from the boundaries of the culture and from the cultures of fungi that had grown on the culture media at the age of 7 days. Tetracycline antibiotic was added to this agar.

After that, the plates were incubated for three days at a temperature of 25 ± 2. Subsequently, 25 sterilized local cabbage seeds per plate were sowed in a circular motion close to the plate's boundaries using a 2% sodium hypochlorite solution. Each isolate was replicated on four plates, in addition to the pathogenic fungus-free control treatment. Seven days after planting, the plates were incubated at the same temperature, and the percentage of germination was determined using the following formula:

$$\text{Germination percentage} = \frac{\text{number of germinated seeds}}{\text{total number of seeds}} \times 100$$

Effect of isolates of the pathogenic fungus *Rhizoctonia solani* on infecting cucumber plants

Some isolates of the pathogenic fungus *R. solani*, specifically R.s11, R.s10, R.s7, and R.s5, which demonstrated high pathogenicity in the prior test, were used in this investigation. Following the preparation of 500 cm³ glass flasks, 100 g of seeds were put into each one, 300 cm³ of water was added, and the seeds were immersed for six hours to cultivate the pathogenic isolates on local millet *Panicum miliaceum* seeds. Additionally, an autoclave was used to sanitize the extra water for 20 minutes at 121 °C and 1.5 kg/cm². They were then left for the second day before being sterilized in the same manner.

Following cooling, three 5-mm-diameter discs from the vicinity of the isolates' culture on PDA culture media at 7 days of age were used to inoculate each flask of millet seeds with the pathogenic fungus. The flasks were then incubated at 25 ± 2 °C for 14 days, shaking every two to three days for 10 minutes to guarantee aeration and dispersion of the fungus inoculum to the seeds. (13).

Sterilized plastic pots measuring 12 cm in diameter, 12 cm in height, and 1.5 kg in capacity were used in the experiment, which was conducted using a Completely Randomized Design (CRD) with four repetitions. At a rate of 1% w/w, isolates of pathogenic fungus were introduced to a loamy soil that included two grains of sand and one peat moss. Five cucumber seeds were planted in each pot a week later, along with a control treatment free of harmful fungus. The pots were then watered and monitored.

The seedlings were removed after a month, when the disease's symptoms started to show, and the pathological index below was used to quantify the disease's incidence and severity:

0= A green branch growing naturally and a white root that showed no signs of infection.

1= Vegetative development is inhibited by 25% and more than 0–25% of the root is light brown.

2= Vegetative development is lowered by 50% and more than 25–50% of the root is dark brown.

3 = Vegetative development is inhibited by 75% and more than 50-75% of the root is dark brown.

4= Vegetative development is inhibited by more than 75% and more than 75–100% of the root is dark brown.

The following formula was used to determine the percentage of illness severity. (14): -

$$\text{disease severity} = \frac{(\text{Number of plants of grade } 0 \times 0) + \dots + (\text{Number of plants of grade } 4 \times 4)}{\text{Total tested plants} \times \text{highest infection grade}} \times 100$$

Then, sections were taken from the roots of infected cucumber plants and sterilized with 2% sodium hypochlorite, and cultured on a PDA medium. After examination and identification, the same pathogenic fungus *R. solani* was observed.

Antagonistic potential tests

Efficiency test of *Bacillus subtilis* isolates in inhibiting the isolate of the pathogenic fungus (s5 R.) *Rhizoctonia solani* on PDA culture media

The antagonistic potential of *B. subtilis* was tested against the isolate of the pathogenic fungus (R.s5) *R. solani* on the PDA culture medium. (15) procedure was followed by adding the bacterial suspension with four drops with a diameter of 4 cm around the colony of pathogenic fungi. This suspension was placed in the center of the plate with a diameter of 5 mm and by 4 replicates and leaving 4 plates as a comparison treatment without the addition of bacteria after 7 days the inhibition percentage of pathogenic fungi was calculated according to the following equation.

$$\text{Inhibition} = \frac{\text{average diameter of the control colony} - \text{average diameter of the treatment colony}}{\text{the average diameter of the control colony}} \times 100$$

Determination of the effective concentration of *B. subtilis* in influencing the growth of the pathogenic fungus (*R.s5*) *R. solani* on PDA culture media

A series of *B. subtilis* inoculum dilutions of concentration 10^{-1} - 10^{-10} were prepared by taking 1 ml of the bacterial inoculum grown on liquid culture media Nutrient Broth by a medical syringe. The dilution was transferred to a test tube containing 9 ml of sterilized distilled water. After conducting the dilutions 10^{-1} - 10^{-10} , 44 Petri dishes with a diameter of 9 cm containing PDA medium were prepared, and 40 plates were inoculated with bacterial inoculum at a rate of 4 plates for each dilution. (15) procedure was used when adding the bacterial inoculum in the form of 4 spots on equal dimensions from the circumference of a circle with a diameter of 4 cm passing from the center of the inoculated dish with a disc of a diameter of 5 mm taken from the edge of the colony of pathogenic fungus *R. solani* (*R.s5*) grown on PDA medium at the age of 7 days. Four plates were left without inoculation with the bacterial suspension and sterile distilled water was added to it for comparison. The plates were incubated in the incubator under 1 ± 25 degrees for 7 days. Then, the amount of inhibition was calculated by calculating the diameter of the mushroom colony and comparing it with the mushroom colony growing in the control treatment. The highest inhibitory dilution of 10^{-5} was determined for *B. subtilis*, and the percentage of fungal growth inhibition was calculated according to the following equation:

$$\text{Inhibition} = \frac{\text{average diameter of the control colony} - \text{average diameter of the treatment colony}}{\text{the average diameter of the control colony}} \times 100$$

Calculating the population density of *B. subtilis* bacteria

Four 9-cm-diameter Petri plates filled with sterile PDA media were made following the previous step's acquisition of the greatest inhibitory dilution of *B. subtilis* 10^{-5} from the bacterial inoculum of the pathogenic fungus *R. solani*. A 10^{-5} bacterial solution containing 1 ml/dish of *B. subtilis* bacteria was used to inoculate the plates. The number of colonies in each plate was counted after 48 hours of incubation at $25 \pm 2^\circ\text{C}$, and the colony rate was then multiplied by the reciprocal of effective dilution, as per Clark (1965) It was found that the number of colonies of *B. subtilis* was 25×10^6 .

Efficiency test of *Trichoderma harzianum* and *Penicillium gresiofulvum* in inhibiting the isolation of the pathogenic fungus (*s5 R.*) *Rhizoctonia solani* on PDA culture media

The isolate of the pathogenic fungus (*R.s5*) *Rhizoctonia solani*, which was previously found to be the most effective pathogenic isolate, was used to investigate the antagonistic potential of *Trichoderma harzianum* and *P. gresiofulvum*. The twofold culture approach was used for this test. A 5 mm drop disc of the biological resistance fungus cultured on PDA culture medium at 7 days of age was injected in the center of the first section of a 9 cm diameter Petri dish that contained PDA culture media with an imagined diameter. The pathogenic fungus *R. solani*, which was cultivated on PSA culture medium at a 7-day age, was inserted into the middle of the second section. Four replicates were used in the experiment |as a control treatment for each fungus, where the plates were kept for seven days at $25 \pm 2^\circ\text{C}$ in the incubator. The degree of fungal growth extension for each colony was used to estimate the antagonism. Additionally, the antagonist was calculated using the following methodology developed by (16,17):

Grade 1 - The biological control fungus covers the entire plate area.

Grade 2 - The biological control fungus covers two-thirds of the plate area and the pathogenic fungus covers one-third of the plate area.

Grade 3 - The biological control fungi and pathogenic fungi cover half of the plate.

Grade 4 - The biological control fungi cover one third of the plate area and the pathogenic fungus covers two-thirds of the plate area.

Grade 5- The pathogenic fungus covers the entire plate area.

Efficiency test of biological control agents *B. subtilis*, *Trichoderma harzianum* and *Penicillium gresiofulvum* in protecting cucumber plants from infection with the pathogenic fungus (*R.s5*) *Rizoctonia solani* and their effect on some growth traits under lath house conditions

On January 25, 2022, a private farm in Babylon Governorate, Al-Mahaweel District, inside District 3 / Bad'a Al-Nasiriyah, and plot number 1/2 was used for the lath house experiment.

As previously noted, local millet seeds were used to cultivate the pathogenic fungus (*R.s5*) *Rizoctonia solani*, while nutrient broth culture medium was used to cultivate the biological bacterium *B. subtilis*. The seeds of the native millet were used to cultivate the bio-resistant fungus *Penicillium gresiofulvum* and *Trichoderma harzianum*. Cucumber seedlings were planted in sterilized plastic pots measuring 20 cm in diameter, 20 cm in height, and 5 kg in capacity. The experiment was conducted using a completely random design (CRD), with four replicates for each treatment and two seedlings per pot. Among the therapies were the following ones:

1. Control treatment (soil not contaminated with pathogenic fungi)
2. Treatment of the pathogenic fungus (*R.s5*) *Rizoctonia solani* alone
3. Treatment of *Bacillus subtilis* alone.
4. Treatment of the biological fungus (*T.h*) *Trichoderma harzianum* alone.
5. *Penicillium gresiofulvum* (*P.g*) alone.
6. Treatment of biological bacteria *B.s* with *T.h* fungi without the pathogenic fungus.
7. Treatment of the biological bacteria *B.s* with the fungus *P.g* without the pathogenic fungus.
8. Treatment of the biological *T.h* fungus with the biological fungus *P.g* without the pathogenic fungus.
9. Treatment of the biological bacteria *B.s* with the fungus *T.h* with the fungus *P.g* without the pathogenic fungus.
10. Treatment of the biological bacteria *B.s* with the pathogenic fungus *R.s5*.
11. Treatment of the biological fungus *T.h* with the pathogenic fungus *R.s5*.
12. Treatment of the fungus *P.g* with the pathogenic fungus *R.s5*.
13. Treatment of the biological bacteria *B.s* with the fungus *T.h* with the pathogenic fungus *R.s5*.
14. Treatment of the biological bacteria *B.s* with the fungus *P.g* with the pathogenic fungus *R.s5*.
15. Treatment of the biological fungus *T.h* with the biological fungus *P.g* with the pathogenic fungus *R.s5*.
16. Treatment of biological bacteria *B.s* with the fungus *T.h* with the fungus *P.g* with the pathogenic fungus *R.s5*.

The pathogenic fungus (*R.s5*) *Rizoctonia solani* was added at a ratio of 1% w/w in special treatments, and a suspension of biological bacteria (*B.s*) *B. subtilis* inoculum was added from a 72-hour-old culture with irrigated water of concentration 25×10^6 CFU/ml in their treatments to the point of saturation. Similarly, the biological fungi were added to their treatments at a rate of 1% w/w before adding the pathogenic fungus (*R.s5*) *R. solani* for 7 days (15). After completing the implementation of the experiment, the treatments were irrigated and subjected to follow-up, and after 40 days the disease incidence and severity were estimated using the pathological index according to the (18,19) equation. The growth parameters of cucumber plants were calculated from the plant height and the fresh and dry weight of the root and shoot.

Result and discussion

1. Isolation of the fungus *Rhizoctonia solani* from the roots of cucumber plants.

The fungus was isolated from twelve cucumber growing areas in Babylon Governorate as listed in Table (1). The results showed that all areas of the study were infected with the fungus *Rhizoctonia solani*, as the disease severity ranged between 15% - 58% and the highest disease severity reached 58% in the Nile region. The reason for the high disease severity was the repeated cultivation of the crop in the same field for several years, which led to the colonization of the fungus in the area. Along with the lack of service provided to the crop (personal contact with some farmers), the lowest disease severity was 15% in the Jableh area.

Table (1) Areas of isolating the fungus *Rhizoctonia solani* from the regions of Babylon Governorate and the disease severity with the fungus

Seq.	Place where the sample was taken	Isolate symbol	Disease severity %
1	Al-hasswa	R.s1	20
2	Al-mashroaa	R.s2	18
3	Jableh	R.s3	15
4	Al-Musayyab	R.s4	25
5	Nile	R.s5	58
6	Hashemite	R.s6	31
7	Al Muhaweel	R.s7	34
8	Midhatiyah	R.s8	40
9	Al-qasim	R.s9	39
10	Abi gharq	R.s10	50
11	Al-sada	R.s11	46
12	Nahea alemam	R.s12	43

2. Pathogenicity tests

➤ Pathogenicity tests of isolates of the pathogenic fungus *Rhizoctonia solani* on Cabbage seeds

According to Table (2), all of the tested fungal isolates significantly decreased the proportion of cabbage seeds that germinated; the percentage varied from 0% to 30% when compared to the 100% control treatment. On the other hand, isolates of the pathogenic fungi R.s5 and R.s7, R.s10, and R.s11 had nil germination because they were very pathogenic, while the isolate of the fungus F.s3 had the greatest germination rate, which was 30%. These findings align with the research conducted by (9).

The field experimental results showed that all fungal isolates were pathogenic, but there was a discrepancy in the percentage of their effect on germination. The poisonous chemicals released by fungal isolates, as well as the variations in their amount and quality, might be the cause of this. Additionally, they have the capacity to secrete pectolytic enzymes, such as polygalacturonase, while non-pathogenic fungal isolates are either unable to secrete this enzyme or have minimal activity in doing so (20). Additionally, it has the capacity to release ligninolytic enzymes, such as ligninase and peroxidase, which are found in the host's cell wall and play a crucial role in infection and the dissemination of mushroom poisons and associated enzymes within those cells (21).

Table (2) Pathogenicity of isolates of the pathogenic *Rhizoctonia solani* fungus on Cabbage seeds

Isolate symbol	% germination	Isolate symbol	% germination
R.s1	20	R.s8	20
R.s2	10	R.s9	10
R.s3	30	R.s10	0
R.s4	10	R.s11	0
R.s5	0	R.s12	20
R.s6	10	Control	100
R.s7	0	LSD	1.8

* Each number represents 4 replicates. * R.s=*Rhizoctonia solani*

Effect of isolates of the pathogenic fungus *R. solani* on infecting cucumber plants

The illness incidence and severity of the cucumber plant varied significantly among the investigated isolates of the pathogenic *R. solani*, according to Table (3)'s findings. At 100% and 100%, respectively, the isolate R.s5 achieved the maximum disease incidence and severity in cucumber seedlings. In contrast to the control treatment, where the illness incidence and severity were 0% and 0%, respectively, it had an impact on the inhibition of cucumber seed germination and growth. The amount of enzymes and poisons released by each isolate varies, which accounts for the variation in illness incidence and severity among isolates (22).

Table (3) The effect of isolates of the pathogenic fungus *R. solani* on infecting cucumber plants

Seq.	Isolate symbol	Disease incidence%	Disease severity%
1	R.s5	100.00	100.00
2	R.s7	90.00	85.00
3	R.s10	80.00	73.00
4	R.s11	85.00	80.00
6	Control	0.00	0.00
LSD at 5%		3.10	1.74

* Each number represents 4 replicates.* R.s=*Rhizoctonia solani*

3. Antagonistic potential tests

➤ Efficiency test of *Bacillus subtilis* isolate in inhibiting the isolate of the pathogenic fungus (*R.s5*) *Rhizoctonia solani* on PDA culture media

With a 100% inhibition rate compared to the control treatment's zero inhibition, the laboratory test findings in Table (4) demonstrated that *B. subtilis* had a strong antagonistic potential against the isolate of the pathogenic fungus *R.solani* (*R.s5*) on the culture media.

Table (4) Examine *B. subtilis*' ability to inhibit the pathogenic fungus isolate *R.solani* (R.s5) in PDA culture medium.

Treatment	Growth of a Diagonal of <i>R.solani</i>	% inhibition
<i>B.subtilis</i>	0	100
Control	9	0.00
LSD at 5%	1.41	2.00

* Each number represents 4 replicates.

➤ **Determination of the effective concentration of each of *B. subtilis* in the effect on the growth of the pathogenic fungus (*R.s5*) *R. solani* on PDA culture media**

The results of the laboratory examination in Table (5) also showed that the antagonistic potential of a series of dilutions of *B. subtilis* against the isolate of the pathogenic fungus *R.solani* (*R.s5*) that the highest inhibitory dilution was 10^{-5} with an inhibition rate of 88%, while the inhibition rate was Zero in the control treatment.

Table (5) Test of a series of dilutions of *B. subtilis* bacteria in inhibiting the isolate of the pathogenic fungus (*R.s5*) *R. solani* on PDA culture media

Concentration	Growth of a Diagonal of <i>R.solani</i>	% inhibition
10^{-1}	0.50	94.00
10^{-2}	0.65	93.00
10^{-3}	0.76	92.00
10^{-4}	0.95	89.00
10^{-5}	1010	88.00
10^{-6}	2.00	78.00
10^{-7}	2.90	68.00
10^{-8}	3.85	57.00
10^{-9}	4.70	48.00
10^{-10}	5.30	41.00
Control	9.00	00.00
LSD at 5%	0.40	1.46

* Each number represents 4 replicates.

➤ **Calculation of the population density of *B. subtilis* bacteria.**

After obtaining the lowest inhibitory dilution of *B. subtilis* 10^{-5} from the bacterial inoculum of the pathogenic fungus *R.solani*. The number of colonies in each plate was counted and the colony rate of bacteria was multiplied by the reciprocal of effective dilution (23). It was found that the number of colonies of *B. subtilis* was 25×10^6 .

➤ **Efficiency test of *Trichoderma harzianum* and *Penicillium gresiofulvum* in inhibiting the isolation of the pathogenic fungus (*s5 R.*) *Rhizoctonia solani* on PDA culture media**

With a high percentage of 92%, the results of Table (6) show how well the biological resistance component *Trichoderma harzianum* inhibits the isolation of the pathogenic fungus (*R.s5*) *Rhizoctonia solani*. But with the comparative therapy, the proportion of inhibition was zero. With an inhibition rate of 90% compared to the control treatment, which had no inhibition rate, the same table also shows how well the biological fungus *Penicillium gresiofulvum* inhibited the isolate of the pathogenic fungus (*R.s5*). These findings aligned with those of several studies, including (24).

Table (6) Efficiency test of *Trichoderma harzianum* and *Penicillium gresiofulvum* in inhibiting the isolation of *Rhizoctonia solani* (s5 R.) on PDA culture media

Treatment	Growth of a Diagonal of R.solani	% inhibition
<i>Trichoderma harzianum</i>	0.70	92.00
<i>Penicillium gresiofulvum</i>	0.88	90.00
Control	9.00	0.00
LSD 5%	0.80	1.10

* Each number represents 4 replicates.

4. Efficiency test of biological control agents *B. subtilis*, *Trichoderma harzianum*, and *Penicillium gresiofulvum* in protecting cucumber plants from infection by the pathogenic fungus (*Rs5*) *Rizoctonia solani* and their effect on some growth characteristics under lath house conditions

The results of Table (7) indicate that there are significant differences for all treatments of biological resistance factors, whether alone or interacting with each other, in raising the growth parameters of cucumber plants when compared to the control treatment in terms of plant height, fresh and dry weight of the root and shoot. Where it can be observed the superiority of the treatment of the interaction of biological agents with bacteria *B. subtilis*, *Trichoderma harzianum*, and *Penicillium gresiofulvum* over the rest of the treatments. The plant height and the fresh and dry weight of the shoot and root reached (12 and 20 cm), (25 and 72 g), and (8.00 and 24.10g), respectively. In comparison, the control treatment recorded (10 and 16 cm) and (17 and 49 g), and (6.60 and 16.20 g) for plant height, the fresh and dry weight of the root and shoot reached, respectively. These agents worked to increase the availability of the elements for the plant and increase the plant's tolerance to environmental stress conditions and decompose complex organic materials into simple components that are easily absorbed by the plant. This result is similar to what was stated by (24) and (11). Table (7) indicates that there are significant differences for all treatments of biological resistance factors, whether alone. Otherwise interacting with each other when interacting with the pathogenic fungus *Rizoctonia solani* in raising the growth parameters of cucumber plants when compared to the treatment of pathogenic fungi alone in terms of reducing the disease incidence and severity. It led to raising the growth parameters of cucumber plants in terms of plant height, and fresh and dry weight of the root and shoot. It can be noticed that the superiority of the treatment of the interaction of biological components with *B. subtilis*, *Trichoderma harzianum*, and *Penicillium gresiofulvum* when interacting with the pathogenic fungus *Rizoctonia solani* over the rest of the treatments. The disease incidence and severity reached (9.00 and 5.00%), respectively, which was reflected in the growth parameters of plants Cucumbers in terms of plant height, fresh and dry weight of the shoot and root were (9.4 and 15.5) cm, (16.5 and 40.00) g, and (6.00 and 13.10) g, respectively. In comparison, the treatment of the pathogen alone, as the disease incidence and severity were (100 and 80%), respectively. Furthermore, the plant height and the fresh and dry weight of the root and shoot were (4.10 and 7.80) cm and (8.10 and 15.40) g, and (2.80 and 5.20) g, respectively. These biological elements worked on using their different mechanisms from antagonism, competition, parasitism, (11).

Table (7) Efficiency test of biological control agents *B. subtilis*, *Trichoderma harzianum*, and *Penicillium gresiofulvum* in protecting cucumber plants from infection by the pathogenic fungus (*Rs5*) *Rizoctonia solani* and its effect on some growth characteristics under lath house conditions

Seq.	Treatment type	disease incidence %	Severity %	Length		Fresh weight		Dry weight	
				Root	Shoot	Root	Shoot	Root	Shoot
1	The pathogenic fungus R.s5	100.00	80.00	4.00	7.80	8.10	15.40	2.80	5.20
2	<i>subtilis B.</i>	0.00	0.00	10.30	16.80	19.00	58.00	6.10	19.80

3	T. harzianum	0.00	0.00	10.70	17.10	20.00	60.00	6.20	20.00
4	P. gresiofulvum	0.00	0.00	10.20	16.30	18.00	55.00	6.00	18.10
5	T.h +B.s	0.00	0.00	11.50	18.60	23.00	69.00	7.80	23.00
6	T.h +P.g	0.00	0.00	11.10	18.00	22.00	67.00	7.20	22.20
7	B.s +P.g	0.00	0.00	10.80	17.80	21.00	63.00	7.00	21.00
8	T.h + B.s +P.g	0.00	0.00	12.00	20.00	25.00	72.00	8.20	24.10
9	B.s +R.s	18.00	16.00	8.50	13.70	14.60	27.00	4.70	9.10
10	+R.s T.h	15.00	8.00	8.70	14.00	15.20	29.00	5.30	9.50
11	+R.s P.g	20.00	13.00	8.30	13.20	14.20	25.00	4.20	8.30
12	T.h +B.s + R.s	10.00	6.00	9.20	15.00	16.30	37.00	5.90	12.60
13	T.h +P.g+ R.s	12.00	7.00	9.00	14.80	16.10	34.00	5.70	11.10
14	B.s +P.g + R.s	13.00	7.00	8.90	14.50	15.80	32.00	5.30	10.80
15	T.h + B.s +P.g+ R.s	9.00	5.00	9.4	15.5	16.50	40.00	6.00	13.10
16	Control	0.00	0.00	10.00	16.00	17.00	49.00	6.60	16.20
	LSD 5%	1.70	1.16	0.52	0.78	0.82	1.12	0.52	0.52

* Each number represents 4 replicates.

References

1. Al-Mohammadi, Fadel Musleh, and Abdul-Jabbar Jassem Al-Mishaal.. Vegetable production. For third-grade students, Extension. The University of Baghdad. 223 pages. 1989
2. Shakir Alkhafaji R, Muhsin Khalfa H, Lf Almsaid H. Rat hepatocellular primary cells: A cellular and genetic assessment of the chitosan nanoparticles-induced damage and cytotoxicity. Archives of Razi Institute. 2022 Apr 30;77(2):579-84.
3. Chaudhary AK, Yadav J, Gupta AK, Gupta K. Integrated disease management of early blight (*Alternaria Solani*) of potato. Trop. Agrobiodivers. 2021;2:77-81.
4. khalfa HM, al-msaid HL, abood AH, naji MA, Hussein SK. Cellular genetic expression of purinergic receptors in different organs of male rats injected with cyclophosphamide. InAIP Conference Proceedings 2020 Dec 4 (Vol. 2290, No. 1, p. 020033). AIP Publishing LLC.
5. Harman GE. Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant disease. 2000 Apr;84(4):377-93.
6. Al-Bayati, Esraa Mowaffaq Obaid. Biological and chemical control of the fungus *Fusarium solani* associated with pear roots in Babylon Governorate. Master's thesis. College of Science. Al-Mustansiriya University. 108 p. 2010.
7. Wani AB, Chadar H, Wani AH, Singh S, Upadhyay N. Salicylic acid to decrease plant stress. Environmental Chemistry Letters. 2017 Mar;15(1):101-23.
8. AL-Msaid HL, Waleed AH, AL-Sallami AS. Relationship Between Hyperviscosity and Sex Hormone in Azoospermia and Oligozoospermia Patients Compares with The Control Group. Int J Pharm Qual Assur. 2019;10(4):637-9.
9. Farah S, Sahera N. Antifungal activity of *Pseudomonas aeruginosa* and *Bacillus subtilis* against pathogens of cucurbitaceous fruits. Int. J. Innov. Res. Sci. Eng. Tech. 2016;5:1-5.
10. AL-Msaid HL, Khalfa HM, Rashid AA, Hussain NN. Relationship between Sperm DNA Fragmentation and Interleukin 17 in Patients with Leukocytospermia. Journal of Bioscience and Applied Research. 2024 Nov 21;10(4):809-15.
11. Radhi KH. Biocontrol of the causal agent *Rhizoctonia solani* of pepper root rot disease in babylon governorate. International Journal of Agricultural & Statistical Sciences. 2021 Dec 2;17.

12. Bolkan HA, Butler EE. Studies on heterokaryosis and virulence of *Rhizoctonia solani*. *Phytopathology*. 1974 Jan 1;64(5):13-522.
13. Dewan MM, Sivasithamparam K. Growth promotion of rotation crop species by a sterile fungus from wheat and effect of soil temperature and water potential on its suppression of take-all. *Mycological Research*. 1989 Sep 1;93(2):156-60.
14. Pethybridge SJ, Nelson SC. Leaf Doctor: A new portable application for quantifying plant disease severity. *Plant disease*. 2015 Oct 29;99(10):1310-6.
15. Hassoun, Ibrahim Khalil. Biological and chemical control of the pathogen *Rhizoctonia solani* kuhn. PhD thesis - College of Agriculture - University of Baghdad. 113 p. 2005.
16. Bell DK, Wells HD, Markham CR. In vitro antagonism of *Trichoderma* species against six fungal plant pathogens.
17. Ali LO, Khalfa HM, Al Sahlanee R, Almsaid HL. Histological changes in liver and cardiac rat tissues after exposure to chitosan nanoparticles orally. *Medical Journal of Babylon*. 2023 Jan 1;20(1):215-8.
18. Chiang KS, Liu HI, Bock C. A discussion on disease severity index values. Part I: warning on inherent errors and suggestions to maximise accuracy. *Annals of Applied Biology*. 2017 Sep;171(2):139-54.
19. Dosh RH, Khalfa HM, Al-Rehemi SM, Almsaid HL, Hadi N. IN VIVO ACTIVATION OF P2Y4 PURINERGIC RECEPTORS USING ATP IN RAT EPIDERMAL TISSUE. *Wiadomości Lekarskie* monthly journal. 2022;75(11):2729-33.
20. Papavizas GC. Virulence, host range, and pectolytic enzymes of single-basidiospore isolates of *Rhizoctonia praticola* and *Rhizoctonia solani*.
21. Bruce RJ, West CA. Elicitation of lignin biosynthesis and isoperoxidase activity by pectic fragments in suspension cultures of castor bean. *Plant physiology*. 1989 Nov 1;91(3):889-97.
22. Jetiyanon K. Defensive-related enzyme response in plants treated with a mixture of *Bacillus* strains (IN937a and IN937b) against different pathogens. *Biological control*. 2007 Aug 1;42(2):178-85.
23. Clark FE. Agar-plate method for total microbial count. *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*. 1965 Jan 1;9:1460-6.
24. Al-Bayati AJ. Impact of construction safety culture and construction safety climate on safety behavior and safety motivation. *Safety*. 2021 May 18;7(2):41.