# ANTAGONISTIC ACTIVITY OF PLANT GROWTH-PROMOTING RHIZOBACTERIA TO CONTROL RHIZOCTONIA SOLANI FUNGUS THAT CAUSES BLACK SCURF DISEASE IN POTATO

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**ABSTRACT**

**The experiment was carried out in the season 2021-2022 at College of Agricultural Engineering Sciences / University of Baghdad, For testing the potential for the plant growth-promoting Rhizobacteria (PGPR) in controlling the Rhizoctonia solani fungus that causes black scurf disease in potatoes. All fungal isolates obtained a very good efficacy in the reduction seed germination percentage (0, 6.7, 15.6 and 17.7%, respectively) in comparison with control (97.7%). The results showed that the Rs1 and Rs4 fungi isolates are the most pathogenic as it recorded 5 and 4.2 respectively. Bl.4 bacterial isolate recorded the highest inhibitory ability of fungus growth (47.6%) with significant differences compared with Bl.5 and Bl.3, while Bl.2 achieved 5.53%. Based on the isolation and nucleotide sequencing, the highest inhibitory ability by bacteria isolated from soil (Bl.4) was achieved by *Bacillus licheniformis*. The results also showed the effectiveness of the immersion method with bacteria in reducing the severity of infection of the vegetative and root systems with *R. solani*, with reduction values of 26.67 and 30.00%, respectively, and significant differences compared with the control treatment (83.33, 88.33% respectively).**

**Keywords: rhizoctonia, bacillus licheniformis, pgpr, field study.**



## الفعالية التضادية للبكتيريا الجذرية المعززة لنمو النبات في مقاومة الفطر Rhizoctonia solani المسبب لمرض القشرة السوداء على البطاطا

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**المستخلص**

**اجريت التجربة خلال الموسم 2021 – 2022 في كلية علوم الهندسة الزراعية / جامعة بغداد ، لتقييم قدرة البكتريا الجذرية المعززة لنمو النبات ( PGPR) في مكافحة الفطر *Rhizoctonia solani* المسبب لمرض القشرة السوداء على البطاطا. سجلت جميع عزلات الفطر فاعلية عالية في خفض النسبة المئوية لانبات البذور ( 0 ، 6.7 ، 15.6 ، 17.7 % على التتابع )، مقارنة بمعاملة السيطرة التي بلغت 97.7% .اظهرت النتائج ان عزلة Rs1 و عزلة Rs4 هي الاكثر امراضية اذ سجلت 5 و 4.2 على التتابع . سجلت بكتريا Bl.4 اكثر قدرة تثبيطية لنمو الفطر بلغت 47.6 % وبفروق معنوية واضحة عن عزلات البكتريا Bl.5 و Bl.3 بينما بلغت بكتريا Bl.2 5.53 %. أظهرت نتائج العزل والتتابع النيوكلوتيدي ان البكتريا المعزولة من الترب Bl.4 والتي حققت اعلى قدرة تثبيطية هي *Bacillus licheniformis* . أوضحت النتائج فعالية طريقة التغطيس بالبكتريا في خفض شدة الإصابة وللمجموعين الخضري والجذري بالفطر *R.solani* بلغت 26.67 و 30.00 % على التتابع مقارنة بمعاملة السيطرة التي بلغت 83.33 و88.33 % بالتتابع للمجموع الخضري والجذري وبفروق معنوية.**

**كلمات مفتاحية: Rhizoctonia، *Bacillus licheniformis*، PGPR، دراسة حقلية**



**Received:17 /2/2023, Accepted:7/5/2023, Published: August,2025.**

## INTRODUCTION

Potato is a vegetable crop of significant economic importance worldwide due to its high nutritional value (24, 34, 44). The cultivation of potatoes has shown substantial growth in Iraq (42). These plants are susceptible to various pathogens disease (6,10,19), including the *R. solani* fungus, which causes black scurf (28). This disease is a widespread issue affecting potato cultivation globally and poses a threat to a wide range of plant species across different families (15 ,25, 35, 49, 51). The disease symptoms manifest on the mutated stems beneath the soil surface, inhibiting seedling emergence above ground. This condition reduces the number of stems per plant compared to healthy plants and causes the formation of stone bodies on tubers' outer surface, diminishing their commercial value (4). Various methods have been employed to manage these diseases, including pesticides, which have led to the development of pathogen-resistant strains and environmental contamination due to pesticide use (16, 40, 47). Therefore, studies have tended to use other methods that are safer on the environment as alternatives to pesticides, such as the use of Plant Growth-promoting Rhizobacteria (PGPR )bacteria(7,36). These studies confirmed its high effectiveness in reducing or eliminating pathogens (12) due to its anti-fungal compounds(14), ability to produce plant hormones, nitrogen fixation, provision of inorganic phosphate(39), and ability to reduce unfavorable environmental influences (8, 9).

PGPR bacteria possess the capability to produce various enzymes, such as chitinases, glucanases, proteases, and lipases, which break down the cell walls of fungal pathogens (29). Additionally, these bacteria can generate auxin, enhancing nutrient absorption and boosting root system growth (20). Research by *B. licheniformis* demonstrated its efficacy in inhibiting the growth of *Pestalotiopsis karstenii*, *Glomerella cingulate*, *Pestalotia diospyri*, and *Botrytis cinerea* fungi on PDA medium, with inhibition rates of 50.5%, 39.9%, 38.5%, and 37.4%, respectively (50). This study aims to explore the potential use of PGPR bacteria in controlling the *Rhizoctonia solani* fungus responsible for black scurf disease in potatoes.

## MATERIALS AND METHODS

**Isolation and molecular identification of *R. solani* fungus:** Samples were collected from the potato fields planted in Salah Al-Din, Al-Yousifiyah, Al-Nahrawan, and Al-Taji regions, Middle Iraq. Plant samples with disease symptoms were characterized by the presence of dark brown areas and ulcers on the stems near the soil surface, along with leaf discoloration such as pallor and purple hues. The samples were individually placed in polyethylene bags, labeled with collection location and date, and then transported to the plant pathology laboratory for pathogen isolation. The pathogen was isolated from the affected samples by extracting pieces from the diseased area and the surrounding tissue, which were cut into small 0.5 cm segments and superficially sterilized with a 0.5% sodium hypochlorite solution for 2 minutes. The pieces underwent four washes with sterile distilled water, followed by drying and then transferred to plates containing potato dextrose agar (PDA) culture medium. An antibiotic was incorporated into the PDA medium at a concentration of 50 mg $L^{-1}$ to inhibit bacterial growth. Subsequently, the plates were incubated for 4 days at 25 ± 2°C. The identified fungi were purified and morphologically characterized using taxonomic keys. Once the pathogenic fungus was isolated, the isolates were cultivated in a liquid potato dextrose broth medium. The mycelium was harvested, transferred to a crushing mortar, and crushed into a powder with liquid nitrogen. DNA extraction Kit (TransGenBiotech, China) was used to extract fungal DNA, and specific primer used for the ITS-1 region was: Forward (5′- TCCGTAGGTGAACCTGCGG -3′) and ITS-4 Reverse (5′ TCCTCCGCTTATTGATATGC-3′) for ribosomal S18 gene. It is specific for the detection of filamentous fungi. The extracted DNA was electrolyzed on agarose gel (1%), containing ethidium bromide dye for (10 minutes) at a voltage of 100 V, which was then lowered to 70 V for 60 minutes. DNA band was detected using UV light. PCR-Master mix was prepared using TransGenBiotech, China, diagnostic Kit. DNA amplification was performed using PCR thermocycler for 40 cycles, which included the following process:

Denaturation step for 3 min at 94˚C , followed by 30s at 94˚C , Annealing step with 35 cycles at 58˚C FOR 30S, extension for 1 min at 72˚C , and final extension with 1 cycle for 5 min at 72˚C . The result of the PCR reaction (DNA product) was sent to the Alpha DNA Company (Canada) for DNA sequencing.

**Pathogenicity test of *R. solani* isolates:**

The pathogenic potential of the fungal isolates was assessed following the procedure outlined in reference (45). A nutrient medium composed of Water agar at a 2% concentration was prepared. After the medium solidified, a 0.5 cm diameter disc was extracted from the periphery of the colony of each isolate aged 7 days. These discs were then inoculated in the center of the dish and incubated at 25 ± 2 °C for 3 days until the colony expanded to reach a size of 7 cm. Radish seeds were obtained and subjected to a 2-minute superficial sterilization using a sodium hypochlorite solution. The radish seeds were thoroughly rinsed with sterile distilled water and then dried. A total of fifteen radish seeds were arranged in a circular pattern at the perimeter of the fungal culture dish. Each isolate was tested in three separate dishes, while an additional three dishes contained only radish seeds as a control. The dishes were placed in an incubator at 25 ± 2 °C for a period of 7 days. The evaluation time was determined based on the germination of seeds in the control dishes. Disease severity was assessed using the Disease Severity Index (DSI), which ranges from 0 to 5.

The mean length of mold on stems caused by fungus was assessed for each isolate on the following scale based on chromatic length:

- Scale 1: ≤ 1 mm
- Scale 2: 1 < 3 mm
- Scale 3: 3 < 5 mm
- Scale 4: 5 < 7 mm
- Scale 5: ≤ 7 mm

Pathogenicity was evaluated by converting the chromatography scale to the Disease Severity Index (DSI) as follows:

- Non-pathogenic isolate: 0 - 0.3
- Low virulent isolate: 0.4 - 1.9
- Moderately virulent isolate: 2 - 2.9
- Pathogenic isolate: 3 - 3.9
- Strongly virulent isolate: 4 - 5

**Isolation and purification of PGPR bacteria from the Rhizosphere:** Soil samples were collected from an area around the roots of potato plants, that were selected on the basis of the good vegetative growth of plants compared with other plants from (Al-Yousifiyah region). Soil samples and the root system of healthy plants were transferred in polyethylene bags to the laboratory. The bacteria were isolated by (adding 1 g of the selected soil samples to 9 ml of sterile distilled water in test tubes), mixing well , and making serial dilutions from $1x10^{-1}$ to $1x10^{-8}$. A volume of 1 ml of the last three dilutions prepared above was taken to inoculate test tubes containing 9 ml of liquid culture medium (Nutrient broth), which was prepared, and sterilized with an autoclave device at a 28 ± 2 °C ,for 2 days. The tubes were examined by observing the bacterial growth and the inoculum was taken from the tubes and spread on the surface of a petri dish containing solid medium(Nutrient agar). The samples were incubated at 28 ± 2°C for 24 hours. Bacteria were extracted from plant roots using a direct culture method after cutting the roots into 0.5 cm pieces. These pieces were treated with a 0.5% sodium hypochlorite solution for 2 minutes for superficial sterilization, followed by washing with sterile distilled water three times. After drying, the pieces were transferred to culture dishes, incubated, and purified on fresh medium to obtain pure colonies. The pure isolates were stored in the refrigerator until needed.

**Identification of bacterial isolates by 16S rDNA sequence analysis:** Genomic DNA was isolated using the cell boiling method. The 16S rRNA gene amplification was conducted with specific primers: primer 5'-AGAGTTTGATCCTGGCTCAG- 3' and forward 5'- GGTTACCTTGTTACGACTT- 3' (13). The reaction took place in a 100 µl reaction mixture comprising 1.5 mM of MgCl2, 0.2 mM of each dNTP, 25 pmoles of forward and reverse primers, 50 ng of DNA template, and 5 U of Taq DNA polymerase with its reaction buffer. A 30-cycle reaction was carried out at 94°C for 1 min, 62°C for 30 s, and 72°C for 90 s, followed by a final extension of 10 min at 72°C. According to the supplying company's instructions, the contents of the reaction mixture were mixed prior to placing the tube in the Thermocycler. A mixture of 200

µl TE (Tris and EDTA) and 3800 µl sterile water was prepared, followed by mixing 10 µl of this solution with 10 µl of DNA fragment. Subsequently, 200 µl of the resulting mixture was aliquoted into series of tubes, mixed, and allowed to incubate at room temperature for 5 minutes. The recorded values were obtained directly from the device.

**Antagonistic ability test of bacteria against pathogenic fungi:** Two methods were used to test the antagonistic ability; the first is the method of (43), which involved creating a line of bacterial growth, for bacteria grown on nutrient broth at age of 48 hours on the PDA culture medium, at a distance of 2 cm from the edge of the dish. A disk with a diameter of 0.5 cm (extracted from the periphery of the pathogenic fungus colony at 5 days of age raised on PDA culture medium) was positioned 3.5 cm away from the dish's second edge. Three repetitions were carried out for each bacterium. The dishes were kept in incubation at a temperature of 27±2°C. The percentage of inhibition was calculated six days later using the following formula.

**Inhibition (%) = (A / A + B) x 100**

A = Distance between the bacteria line and the end of the fungal growth.

B = Fungal expansion toward the bacterial line.

The second method was used for the two isolates with the highest inhibition rates of the fungus. It was conducted by adding 1 ml of bacterial inoculum(48h) grown on nutrient broth medium to dishes containing PDA medium before it solidified. Stirring was performed in a capillary motion to homogeneously distribute the bacterial inoculum with the medium. The dishes were inoculated with a disc of 0.5 cm diameter from the edge of the pathogenic fungus colony (at age of 5 days). The control dishes were left without adding the bacterial inoculum. An average of 3 replicates was applied for each treatment. Incubation was then performed , at 27 ± 2 °C for 6 days , after which the inhibition percentage was calculated according to the following equation: Inhibition %= {Growth diameter in the control treatment - Growth diameter in the treatment} / Growth diameter in the control treatment* 100.

**Determination of effective dilutions (highest effective dilution) of bacterial inoculum in inhibiting the growth of pathogenic fungi:**

A range of dilutions of the bacterial inoculum was created by transferring 1 ml of the prepared bacterial inoculum (obtained by mixing 4 g of nutrient broth with 250 ml of distilled and sterilized water using an autoclave at 121 °C and 1.5 Kgcm-2 pressure, and then inoculated with bacterial isolate grown on nutrient agar culture medium for 24 hours and incubated for two days) into a sterile test tube containing 9 ml of sterile distilled water. Dilutions ranging from $10^{-}1$ to $10^{-}10$ were subsequently prepared. Then petri dishes with a diameter of 9 cm were prepared containing the PDA culture medium. Before the solidification of the medium, it was inoculated with the bacterial suspension for all dilutions at a rate 1 $mldish^{-1}$with stirring dishes in a capillary motion after adding the suspension to homogeneously distribute the inoculum within the dishes at 3 dishes for each dilution, and 3 dishes were left for control. After the medium solidified, a 0.5 cm diameter disk was placed in the center of each dish. This disk was taken from the edge of a colony of the pathogenic fungus growing on PDA medium that was 5 days old. The dishes were then incubated at 25 ± 2 °C for three days. Subsequently, the inhibition percentage was determined using the following equation.

Inhibition %= Growth diameter in the control treatment - Growth diameter in the treatment / Growth diameter in the control treatment* 100.

The highest effective dilution in inhibiting the pathogenic fungi was ($10^{-8}$ ) with an inhibition rate of 64.4%.

**Calculating the numerical density of bacteria:** The direct method of counting bacterial colonies in the dishes was followed. A series of decimal dilutions, from $10^{-1}$ to $10^{-8}$, was prepared by transferring 1 ml of the bacterial culture grown on nutrient broth at age of 2 days to a series of test tubes. A volume of 1 ml of the last dilution ($10^{-8}$) was distributed in a petri dish containing nutrient agar. The dish was stirred in a capillary motion to ensure the homogenous distribution of the bacterial inoculum on the medium. Three replicates were made and incubated at 28 ± 2 °C for 24 hours , after which the number of bacterial colonies

was calculated according to the following equation :

**No. of colonies in (ml) of the original sample = No. of colonies x Reciprocal of the dilution**

Accordingly, the numerical density of the bacterial colony was found to be 72 x $10^{-8}$CFU $ml^{-1}$.

**Biocontrol of black scurf disease in potato caused by *Rhizoctonia* fungus in the field:**

The experiment was carried out by following the randomized complete block design (RCBD). Soil prepration were carried out and then the experiment field was divided as a line of 2 m long, where the distance between lines was 85 cm. with in area of 100 $m^2$, The experiment included the following treatments:

T1 = Tubers in soil contaminated with pathogenic fungi (*R.solani*).

T2 = Tubers treated by soaking with bacteria before planting with fungi.

T3 = Tubers in soil treated with bacteria in the irrigation before planting with fungi.

T4 = Treatment with Rezolex pesticide at a concentration of 1g/l (the active substance is tolkophos methyl).

Each treatment was applied in 3 replicates. The fungal inoculum was loaded on Millet seeds and added to all treatments at a concentration of 2 g $Kg^{-1}$ soil ( soil was weighed from the same field specifically the place of planting tubers by one Kg and 2 g of the fungi inoculum was added and it was distributed regularly in the tuber planting cradle prepared for planting) . The soil was moistened and, after three days, potato tubers (Alpida cultivar) were sterilized with 10% of sodium hypochlorite for 3 minutes, washed with sterile water, and then planted at a rate of 3 tubers per treatment. As for the treatments that need the bacterial inoculum only, two methods were used, the first method was by adding it to the soil at age of 24 hours, at a concentration of (72 x $10^{-8}$CFU $ml^{-1}$ ) at a rate of 100 ml /Kg soil after 3 days of inoculum with pathogenic fungus, and 3 days after adding of bacterial inoculum the potato tubers were planted. As for the second method, the tubers were soaked in the bacterial inoculum at the same concentration for an hour, and then the tubers were planted, while the control treatment includes sterilized soil without fungal inoculum. The results were recorded by calculating the values of the infection severity for the vegetative and root systems, fresh weight and dry weight, and production. The percentage of infection severity was calculated according to the following pathological scale:

A- Roots system:

0 = No root rot symptoms.

1= Appearance of ulceration, or obvious discoloration (1-34%).

2 = Appearance of ulceration or obvious discoloration (35-50%).

3 = Appearance of ulceration or obvious discoloration (51-79%).

4 = Appearance of ulceration or obvious discoloration (80-100%).

B- Vegetative system:

0 = Plant without symptoms.

1 = A spot on the stem with a diameter of 24mm.

2 = A spot on the stem with a diameter of 25-50 mm.

3 = A spot or spots of more than 50 mm.

4 = Presence of spots that completely surround the stem with a diameter of less than (24 mm).

5 = Presence of spots that completely surround the stem with a diameter of more than ( 24 mm).

**Statistical analysis**

The data was evaluated using a completely randomized design (CRD), while the field experiment was structured based on a randomized complete block design. Significance testing among the means was conducted using the least significant difference test at a 0.05 level of significance.

## RESULTS AND DISCUSSION

**Isolation and identification of fungi**

The results of the morphological and microscopic tests of the fungus isolated from the infected plants showed that four isolates Rs1, Rs2, Rs3 and Rs4 were obtained. They are characterized by white colonies (Figure 1) that tending to develop light or dark brown color with age. They also show divided mycelium with branching near the terminal septum of the mycelium cells, narrowing of the branches and the formation of barriers near the area of their emergence were also observed, along with the formation of Blister cells, which belong to the *R. solani* fungus. These results are congruous with previous results from (31,32).

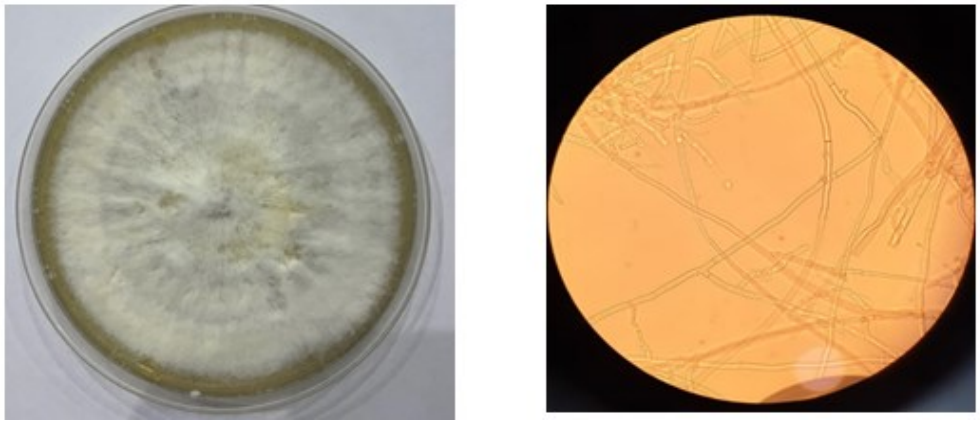

**Figure 1. a- Fungal growth on PDA medium in 25˚C for 7 days, b- Mycelium pathogenicity potential test of *R. solani* fungal isolates**

The results demonstrated in (Figure 2) show that the isolates recorded a high pathogenicity potential, which was tested by measuring the length of the discoloration distance on seedlings, as described by Sneh et al.( 2004). The results showed that the Rs1 and Rs4 isolates are the most pathogenic, as they scores of 5.0 and 4.2, respectively, followed by Rs3 isolate (3.6) and Rs2 (3.0).

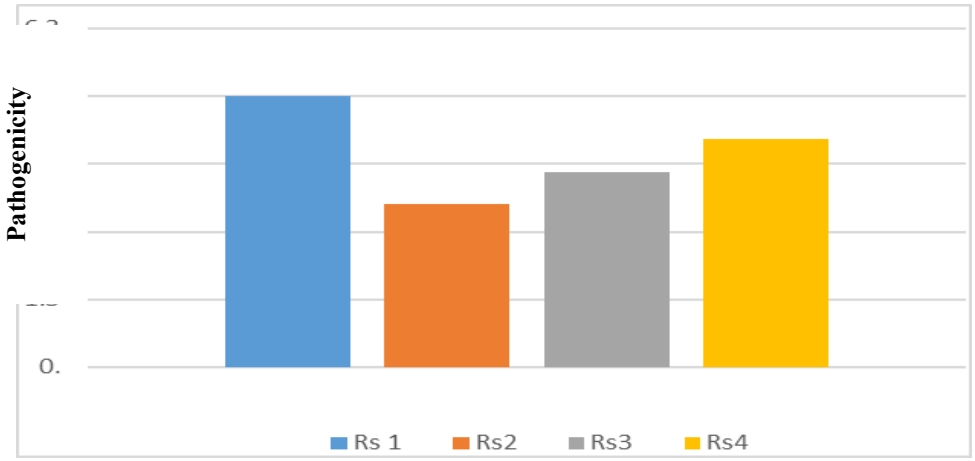


**Figure 2. Pathogenicity of *R. solani* isolates.**

The results in Table 1 show that all the four isolates achieved high effectiveness in reducing the percentage of seed germination, as they recorded 0, 6.7, 15.6 and 17.7%, respectively, compared with the control treatment which recorded 97.7% (Figure 3).

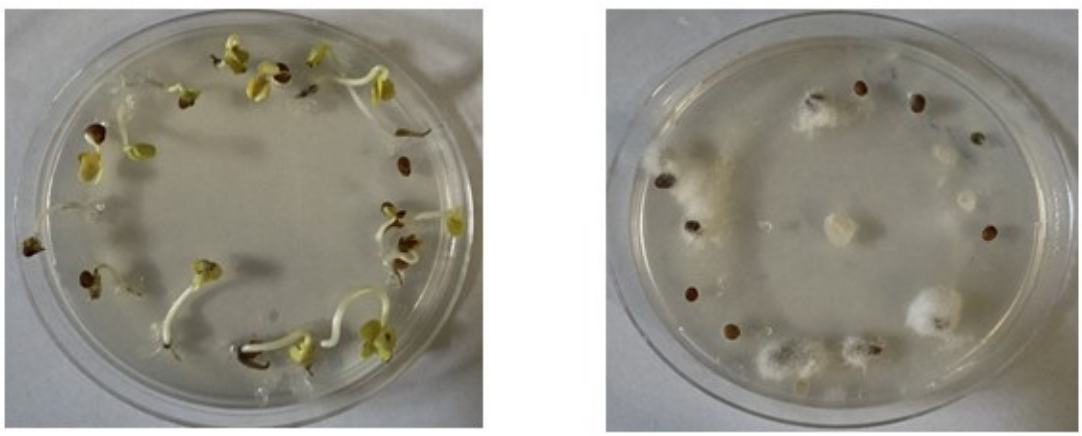

**Figure 3. Pathogenicity potential test on radish seeds: a- Control treatment, b- Rs1 isolate treatment**

The difference in the pathogenicity of isolates might be due to the difference in their ability to produce pectin-degrading enzymes and cellulose and proteolytic enzymes that affect preventing germination or rotting of seeds (2,17). These results are in agreement with those of (3) which confirmed the difference in the pathogenicity potential of Rhizoctonia isolates from infected plants of different regions.

**Table 1. Results of the pathogenicity potential test of *R. solani* fungal isolates.**

| No | Treatment | Percentage of seed germination (%) |
|---|---|---|
| 1 | Rs1 | 0.00 |
| 2 | Rs2 | 17.75 |
| 3 | Rs3 | 15.6 |
| 4 | Rs4 | 6.7 |
| 5 | control | 97.7 |
| L.S.D 0.05% | | 23.25 |

**each number represents the average of 3 replicates**

**Molecular characterization of the pathogenic fungus isolate:**

The electrophoresis results of fungus-extracted DNA on agarose gel revealed the presence of a single band with a molecular weight of 550bp (Figure 4) for the fungal isolate. This identification was accomplished by utilizing transcriptional spacer primers (ITS1 and ITS4). This outcome validated the effectiveness of the primers in amplifying the DNA of *R. solani* fungus species.

**Studying the nucleotide sequence of *R. solani* fungi:** The nucleotide sequencing results shown in Table 2, illustrate that the isolate of *R. solani* recorded identicality ratio of 99% with the global isolates found in the NCBI Global Gene Bank, where two sites of variation of the mutational type were found. The nucleotide sequences of the fungus have been deposited in the World Genebank Organization under accession number OL587651.1 and have become a reference for Iraq, Middle East and the world.

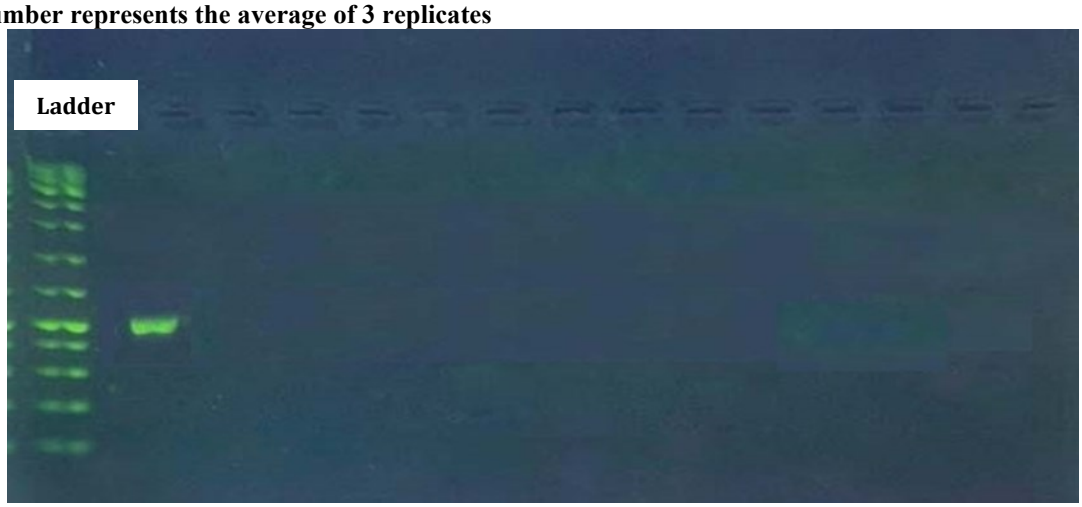


**Figure 4. Electrophoresis of *R. solani* isolate**

**Table 2. Identicality ratio of *R.solani* isolate with global isolates**

| Gene: 18S ribosomal RNA gene | | | | | |
|---|---|---|---|---|---|
| Type of substitution | Location | Nucleotide | Sequence ID with compare | Source | Identities |
| Transition | 227 | G\A | ID: MN749476.1 | *Rhizoctonia solani* | 99% |
| Transition | 341 | C\T | | | |

**Isolation and purification of PGPR bacteria from the Rhizosphere:** The results of the isolation step showed that five isolates were obtained, three isolates from the roots (Bl. 1, Bl. 2 and Bl. 3) and two obtained by the dilution method(Bl. 4 and Bl. 5). Many studies confirmed the possibility of isolating a number of bacterial genera that stimulate the growth of plants that and colonize the area around the roots (1, 27).

**Antagonistic ability test of bacteria against pathogenic fungi:** The results in Table 3 reveal that all bacterial isolates achieved various inhibition rates on fungus. Bl.4 bacteria was significantly superior and recorded the highest inhibitory ability (47.6%), followed by Bl.1 (42.9%), Bl.5 (24.05), and and Bl.3 (18.57%), while Bl.2 recorded a slight inhibitory ability (5.53%), compared with the control treatment which recorded 0% (Figure 5).The difference in the effectiveness of bacteria in inhibiting the growth of fungi may be due to their different abilities in the amount of metabolites produced and the rate of production. The enzymes, such as chitinase, proteases, and B-1,3-glucanse, as well as organic compounds and antibiotics, act on degrading the walls of fungus cells, also, bacteria compete with pathogenic fungi on the growth factors(nutrients), the bacteria species degrade the chitin that is responsible for the formation of fungal hyphae, they also cause abnormal growth of fungi and thus inhibit fungal growth( 5,30,48).

**Table 3. Antagonistic ability of bacterial species against pathogenic fungi**

| No | Treatment | Inhibition percentage (%) |
|---|---|---|
| 1 | Bl.1 | 42.90 |
| 2 | Bl.2 | 5.53 |
| 3 | Bl.3 | 18.57 |
| 4 | Bl.4 | 47.60 |
| 5 | Bl.5 | 24.05 |
| 6 | control | 0.00 |
| | L.S.D 0.05% | 11.72 |

**- Each number represents the average of 3 replicates**

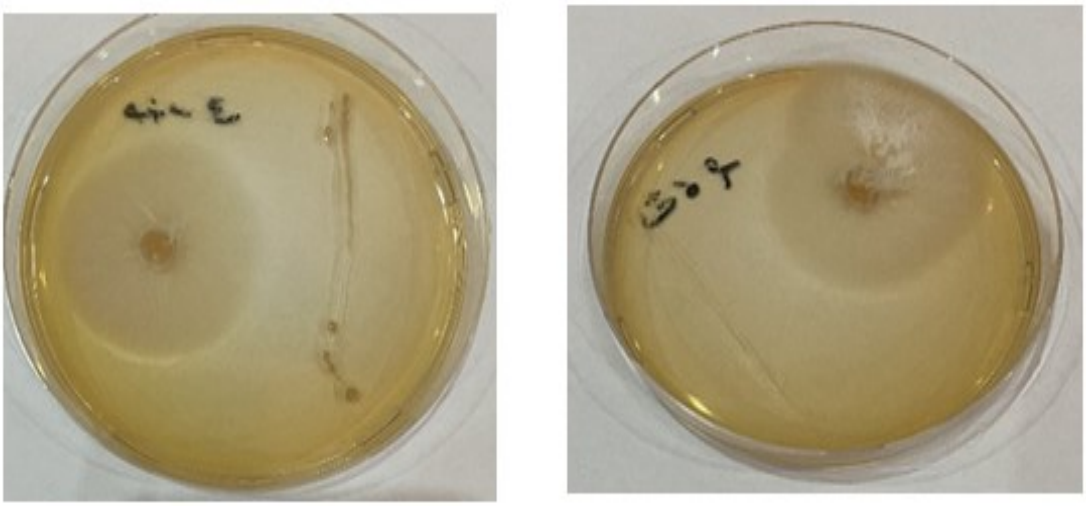

**Figure 5. Antagonistic ability of bacteria against the pathogenic *R. solani* fungus**

Bacterial species has proven its ability to inhibit a number of pathogens, such as *B. licheniformis* which is recorded different inhibition rates of fungi *Phaeocremonium aleophilum*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Phytophthora infestans* (86, 84.7, 84.4 and 83%, respectively) in the laboratory (33). (11) Demonstrated the effectiveness of *B. licheniformis* in inhibiting the growth of *Aspergillus* species (*A. carbonarius*, *A*. niger, *A. flavus*, *A. parasiticus* and *A. ochraceus*) , and the inhibition rates were 60, 50, 49, 47 and 44%, respectively, whereas a rate of 53% was recorded for the *Penicillium verrucosum* fungus in the laboratory.

**Table 4. Antagonistic ability of the two bacterial isolates cultured by media the poisoning method**

| No | Treatment | Inhibition percentage (%) |
|---|---|---|
| 1 | Bl.1 | 74.00 |

| | | |
|---|---|---|
| 4 | Bl.4 | 77.40 |
| 6 | control | 0.00 |
| | L.S.D 0.05% | 11.39 |

The results in Table 4 and (Figure 6,7) showed that Bl. 4 and Bl. 1 bacterial isolates were significantly superior, and achieved higher -Numbers represent the average of **3** replicates inhibition rates of . The tests were performed on the culture media by adding 1 ml of the bacterial inoculum to dishes containing PDA medium before solidification, at 77.4 and 74.0%, respectively, compared with the control treatment which achieved 0%.

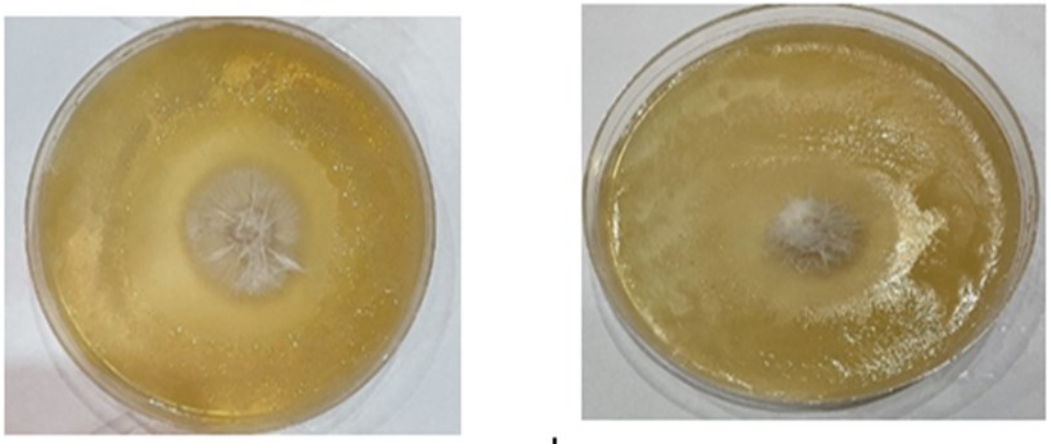

**Figure 6. Antagonistic ability of the two bacterial isolates by the adoption of the medium method**

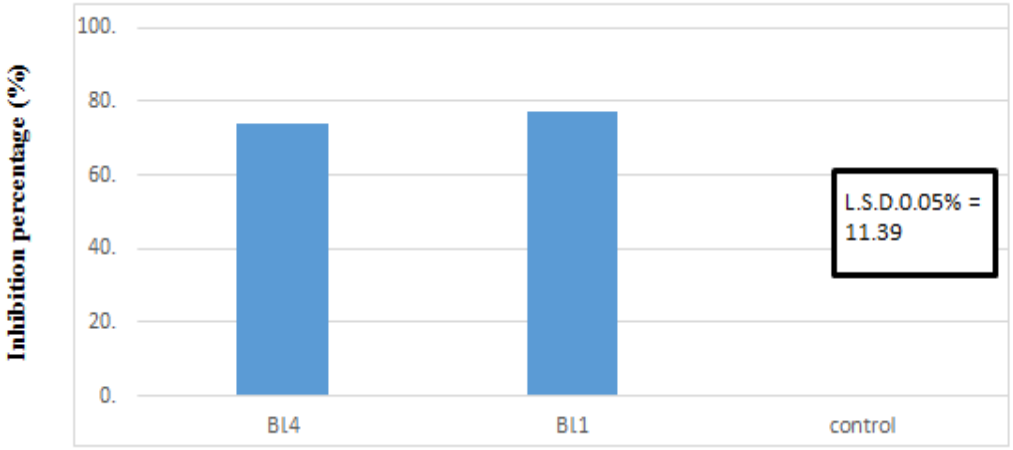


**Figure 7.Inhibitory ability of the two most inhibiting bacterial isolates of *R. solani***

**Molecular diagnosis of the bacterial isolates:** The results of electrophoresis (Figure 8) of the DNA extracted from the two bacterial isolates that achieved the highest inhibitory ability against the pathogenic *R. solani* fungus on agarose gel showed the presence of one band with a molecular weight of 1250 bp for the two bacterial isolates, as indicated by using 16s RNA transcriptional spacer primers.

This result confirmed the ability of these primers to amplify the DNA of bacterial species.

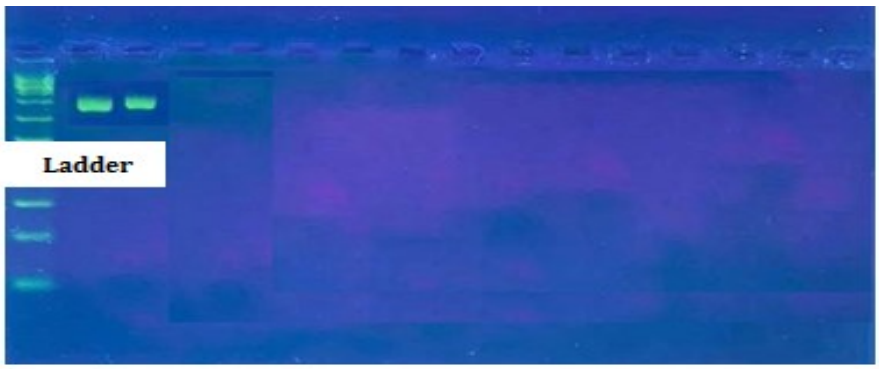


**Figure 8. Electrophoresis results of two bacterial isolates**

**Table 5. The identicality ratio of the two bacterial isolates with global isolates**

| Type of substitution | Location | Nucleotide | Sequence ID with compare | Source | Identities |
|---|---|---|---|---|---|
| Transition | 291 | G\A | ID: KC914624.1 | *Pseudomonas aeruginosa* | 99% |
| Transition | 314 | T\C | | | |
| Transition | 161 | T\C | ID: MT043736.1 | *Bacillus licheniformis* | 99% |
| Transition | 248 | A\G | | | |
| Transition | 468 | C\T | | | |
| Transvertion | 515 | G\T | | | |

The results of isolation and nucleotide sequencing Table 5, show that the Bl.4 species which achieved the highest inhibitory ability was *Bacillus licheniformis*, while that for Bl.1 was *Pseudomona saeruginosa*. Both species recorded an identicality ratio of 99% with the global isolates found in the NCBI Global Genebank. Three sites of mutational-type and one site of reversal-type for *B. licheniformis*, along with two sites of mutational-type for *Pseudomonas aeruginosa*, were found. The nucleotide sequences of *B. licheniformis* have been deposited in the World Genebank Organization under accession number OL442684.1 and it has become a reference for Iraq, Middle East, and the world.

**Biocontrol of black scurf disease in potato caused by *Rhizoctonia* in the field:** The results listed in Table 6 show the effectiveness of *Bacillus licheniformis* in reducing the negative effects of the *R. solani* fungus (Figure 9) . The bacterial species provided protection for plants compared with control treatment. The soaking method of potato tubers reduced the infection severity of the shoot and root systems of *R.solani* by 26.67 and 30.00%, respectively compared with the irrigation method (51.67 and 33.33%, respectively), while the reduction values reached 51.67 and 23.33%, respectively in the pesticide treatment. The results showed the significant effectiveness of the two methods and the pesticide in reducing the infection severity for the root and vegetative systems compared with the control treatment (83.33 and 88.33%, respectively). Also, the results showed the role of the bactria in increasing the fresh and dry weight of the plant by the two methods; 1117.97 and 77.67 g, respectively, by the soaking method and 766.7 and 68.67 g, respectively, by the irrigation method, compared with the control treatment ( 566.97 and 48.00 g, respectively). The increase in production was observed for all treatments; the soaking method recorded the highest mean (2000.00 g) compared with the control treatment (666.67 g). The effectiveness of the soaking method might be due to stimulating the systemic resistance and the direct contact with the compounds used, compared with that of the irrigation method which leads to the loss of a large percentage through soil particles. The effectiveness of the pesticide could be due to its ability to affect the synthesis of lipids and the transport process, as well as its effect on the fungal cell membrane, which facilitates the inhibition of the growth of

the fungus in the plant and thus improves its growth (18, 41, 52). Many studies have shown the positive role of bacteria in reducing the incidence of pathogens, as well as improving the growth parameters of plants, and induction of resistance in plants (26,37). The effectiveness of the bacteria might be due to their ability to produce amino acids, vitamins, and hormones, enhance nutrients in the soil, improve photosynthesis, and inhibit the growth of pathogens (22,23). (46) Indicated that *B.licheniformis* was effective in inhibiting fungi, increasing root number and seedling fresh weight, as well as increasing the leaves content of chlorophyll and antioxidants in Arabidopsis treated with bacteria. (21) reported that *B. licheniformis* increased yield to 17.9 $Kgtree^{-1}$ and productivity by 3.2 times compared with the control treatment.

**Figure 9. Field experments**

**Table 6. Evaluation of the efficiency of bacteria in reducing the infection severity caused by *R. solani* in potato**

| Treatment | Infection severity of the shoot system | Infection severity of the root system | Wet weight (g) | Dry weight (g) | Productivity (g) |
|---|---|---|---|---|---|
| 1 Bl4 (soaking) | 26.67 | 30.00 | 1117.97 | 77.67 | 2000.00 |
| 2 Bl4 (irrigation ) | 51.67 | 33.33 | 766.7 | 68.67 | 1166.67 |
| 3 Pesticide (irrigation ) | 51.67 | 23.33 | 1000.00 | 79.00 | 1483.33 |
| 4 Control | 83.33 | 88.33 | 566.97 | 48.00 | 666.67 |
| L.S.D.0.05% | 10.40 | 16.06 | 191.30 | 5.17 | 114.10 |

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## DECLARATION OF FUND

The authors declare that they have not received a fund.

## REFERENCES

1. Abbas, A. ; S.U. Khan ; W.U. Khan ; T.A. Saleh; M.H.U. Khan; S. Ullah and M. Ikram.2019 . Antagonist effects of strains of Bacillus spp. against *Rhizoctonia solani* for their protection against several plant diseases:Alternatives to chemical pesticides. Comptes Rendus Biologies. 342(5-6):124-135. https://doi.org/10.1016/j.crvi.2019.05.002

2. Abdelghany, M. M. A., ; M. Kurikawa ; M. Watanabe ; H. Matsui ; M. Yamamoto; Y. Ichinose and Y. Noutoshi .2022 . Surveillance of pathogenicity of *Rhizoctonia solani* Japanese isolates with varied anastomosis groups and subgroups on Arabidopsis thaliana. Life. 12(1): 76. https://doi.org/10.3390/life12010076

3. Abdul-Karim, E. K. and H.Z.Hussein.2025. Efficiency of Aqueous and Alcoholic Extract of *Calotropis Procera* in Resisting the Fungus *Rhizoctonia solani*, the Causative Agent of Black Scurf Disease on Potatoes. Polish Journal of Environmental Studies.34(3):2513-2523. https://doi.org/10.15244/pjoes/188063.

4. Aydın, M. H. 2022 . *Rhizoctonia solani* and its biological control. Türkiye Tarımsal Araştırmalar Dergisi. 9 (1): 118 135. https://doi.org/10.19159/tutad.1004550

5. Baard, V. ; O.O. Bakare ; A.I. Daniel ; M. Nkomo ; A. Gokul ; M. Keyster and A. Klein.2023 . Biocontrol potential of *Bacillus subtilis* and *Bacillus tequilensis* against four Fusarium species. Pathogens. 12(2): 254. https://doi.org/10.3390/pathogens120202544.

6. Cao, J.; B. Liu ; X. Xu ;X. Zhang ;C. Zhu Y. Li and X. Ding .2021 . Plant endophytic fungus extract ZNC improved potato immunity, yield, and quality. Frontiers in Plant Science. 12:707256. https://doi.org/10.3389/fpls.2021.707256

7. Fathi, F. ; R. Saberi-Riseh and P. Khodaygan .2021 . Survivability and controlled release of alginate-microencapsulated *Pseudomonas fluorescens* VUPF506 and their effects on biocontrol of *Rhizoctonia solani* on potato. International Journal of Biological Macromolecules. 183:627-634. https://doi.org/10.1016/j.ijbiomac.2021.04.159

8. Glick, B. R. 2012. Plant growth-promoting bacteria: mechanisms and applications. Scientifica.2012:1-12 [CrossRef] https://doi.org/10.6064/2012/963401

9. Glick, B.R.; D.M.Penrose; and J. Li .1998. A model for the lowering of plant ethylene concentrations by plant growth promoting bacteria. Journal Theoretical Biology. 190(1): 63–68. [CrossRef]. https://doi.org/10.1006/jtbi.1997.0532

10. González-Hernández, A. I. ; R. Pérez-Sánchez ;J. Plaza and M.R. Morales-Corts .2022 . Compost tea as a sustainable alternative to promote plant growth and resistance against *Rhizoctonia solani* in potato plants. Scientia Horticulturae.300:111090. https://doi.org/10.1016/j.scienta.2022.111090

11. Hassan, Z. U.; R. Al Thani; H. Alnaimi; Q. Migheli and S. Jaoua. 2019. Investigation and Application of *Bacillus licheniformis* Volatile Compounds for the Biological Control of Toxigenic *Aspergillus* and *Penicillium* spp. ACS Omega.4: 17186-17193. https://doi.org/10.1021/acsomega.9b01638

12. Hirozawa, M. T.; M.A. Ono ; I.M.D.S. Suguiura ; J.G. Bordini and E.Y.S. Ono .2023 . Lactic acid bacteria and Bacillus spp. as fungal biological control agents. Journal of Applied Microbiology.134(2):lxac083. https://doi.org/10.1093/jambio/lxac083

13. Jebur, H. A. and J. M. Auda . 2020. Evalution of antimicrobial activity of partialpurified bacteriocin from local isolate of *Bacillus licheniforims* HJ2020 MT192715.1 .Iraqi Journal of Agricultural Sciences. 2022:53(5):1174-1183 https://doi.org/10.36103/ijas.v51i6.1191

14. Jeong, M.H.; Y.S. Lee ; J. Y. Cho ; Y. S. Ahn ; J.H.Moon; H.N. Hyun ;G.S. Cha and K.Y. Kim .2017. Isolation and characterization of metabolites from *Bacillus licheniformis* MH48 with antifungal activity against plant pathogens. Microbial Pathogenesis.110: 645–653.[CrossRef]. https://doi.org/10.1016/j.micpath.2017.07.027

15. Kareem, T. A. and M. S. Hassan .2013 . Molecular characterization of *Rhizoctonia solani* isolated from pepper plants in Iraq by using PCR. Diyala Agricultural Sciences Journal,5(2):45-54. https://journal.djas.uodiyala.edu.iq/index.php/dasj/article/view/2740 .

16. Kalia, A. and S.K. Gosal . 2011 . Effect of pesticide application on soil microorganisms. Archives of Agronomy and Soil Science.57(6):569-596. https://doi.org/10.1080/03650341003787582

17. Kankam, F. ; S. Larbi-Koranteng and J. Adomako .2021 . Rhizoctonia disease of potato: Epidemiology, toxin types and management. Egyptian Journal of Phytopathology. 49(1):197-209. https://doi.org/10.21608/ejp.2021.72057.1028

18. Kataria, H. R. ; H. Singh and U. Gisi .1989 .Interactions of fungicide-insecticide combinations against *Rhizoctonia solani* in vitro and in soil. Crop protection. 8(6): 399-404.https://doi.org/10.1016/0261-2194(89)90064-1

19.Kiptoo, J. ; A. Abbas ;A.M. Bhatti ;H.M. Usman ; M.A. Shad ;M. Umer… and S. Iqbal .2021. *Rhizoctonia solani* of potato and its management: a review. Plant Protaction .5(3):157-169. https://doi.org/10.33804/pp.005.03.3925

20.Kwon, J. H.; S. J. Won ; J. H. Moon ; C.W.Kim and Y.S. Ahn . 2019.Control of fungal diseases and increase in yields of a cultivated jujube fruit (*Zizyphus jujuba* Miller

var. *inermis* Rehder) orchard by employing Lysobacter antibioticus HS124. Forests.10(12): 1146. [CrossRef] https://doi.org/10.3390/f10121146

21. Kwon, J. H.; Won, S. J.; Moon, J. H.; Lee,U.K.;Park,Y.S.;Maung,C.E.H.;Ajuna,H.B. and Y, S. Ahn, 2021. *Bacillus licheniformis* PR2 Controls Fungal Diseases and Increases Production of Jujube Fruit under Field Conditions. Horticulturae.7(30):49. https://doi.org/10.3390/horticulturae7030049 1

22. Lan, Q.; Y. Liu ; R. Mu ;X. Wang ;Q. Zhou ;R. Islam... and Y. Tian .2024 . Biological control effect of antagonistic bacteria on potato black scurf disease caused by *Rhizoctonia solani*. Agronomy, 14(2): 351. https://doi.org/10.3390/agronomy14020351

23.Lastochkina, O.; L. Pusenkova; D. Garshina; C. Kasnak; R. Palamutoglu; I. Shpirnaya, and I. Maksimov.2022 . Improving the biocontrol potential of endophytic bacteria *Bacillus subtilis* with salicylic acid against *Phytophthora infestans*-caused postharvest potato tuber late blight and impact on stored tubers quality. Horticulturae. 8(2): 117. https://doi.org/10.3390/horticulturae8020117

24.Liu, J. ; Z. Sun ; Y. Zou ;W. Li ; F. He; X. Huang, and X. Wu .2021 . Pre-and postharvest measures used to control decay and mycotoxigenic fungi in potato (*Solanum tuberosum* L.) during storage. Critical Reviews in Food Science and Nutrition. 62(2): 415-428.https://doi.org/10.1080/10408398.2020.1818 18688

25. Majumdar, R. ; C.A. Strausbaugh ; P.J. Galewski ; R. Minocha and C.W. Rogers .2022 . Cell-Wall-Degrading enzymes-related genes originating from rhizoctonia solani increase sugar beet root damage in the presence of *leuconostoc mesenteroides*. International Journal of Molecular Sciences.23(3): 1366. https://doi.org/10.3390/ijms23031366

26.Marzouk, T. ; M. Chaouachi ;A. Sharma ;S. Jallouli ;R. Mhamdi ;N. Kaushik and N. Djébali .2021 . Biocontrol of Rhizoctonia solani using volatile organic compounds of solanaceae seed-borne endophytic bacteria. Postharvest Biology and Technology. 181:111655. https://doi.org/10.1016/j.postharvbio.2021.111655

27.Maslennikova, V. S.; V.P. Tsvetkova ;E.V. Shelikhova ;M.P. Selyuk ; T.Y. Alikina ; M.R. Kabilov and I.M. Dubovskiy .2023 . *Bacillus subtilis* and *Bacillus amyloliquefaciens* mix suppresses rhizoctonia disease and improves rhizosphere microbiome, growth and yield of potato (Solanum tuberosum L.). Journal of Fungi.9(12):1142. https://doi.org/10.3390/jof9121142

28. Mat Razali, N. ; S.N. Hisham I.S. Kumar ; R.N. Shukla ; M. Lee ; M.F. Abu Bakar and K. Nadarajah .2021 . Comparative genomics: insights on the pathogenicity and lifestyle of *Rhizoctonia solani*. International journal of molecular sciences.22(4): 2183. https://doi.org/10.3390/ijms22042183

29. Maung, C. E. H.; T. G.Choi ; H. H. Nam; K. Y. Kim. 2017. Role of *Bacillus amyloliquefaciens* Y1 in the control of Fusarium wilt disease and growth promotion of tomato. Biocontrol Science Technology. 27(12):1400–1415.[CrossRef]. https://doi.org/10.1080/09583157.2017.1406064

30. Mehmood, S.; Z. Khatoon ;I. Amna, Ahmad ; M.A. Muneer ;M.A. Kamran ... and M.F.H. Munis .2023 . Bacillus sp. PM31 harboring various plant growth-promoting activities regulates Fusarium dry rot and wilt tolerance in potato. Archives of Agronomy and Soil Science. 69(2): 197-211. https://doi.org/10.1080/03650340.2021.1971654

31. Misawa, T. and D. Kurose. 2019. Anastomosis group and subgroup identification of *Rhizoctonia solani* strains deposited in NARO Genebank, Japan. Journal of General Plant Pathology.85(4):282-294. Doi: 10.1007/s10327-019-00848-8

32. Nasimi, Z. ; J. Barriuso ; T. Keshavarz and A. Zheng .2024 . Molecular, physiological, and biochemical properties of sclerotia metamorphosis in *Rhizoctonia solani*. Fungal Biology Reviews. 48: 100351. https://doi.org/10.1016/j.fbr.2023.100351

33 .Nigris, S. ; E. Baldan ; A. Tondello, ;F. Zanella; N. Nicola Vitulo; G. Gabriella Favaro ;V. Guidolin ; N. Bordin ; A. Telatin E. Barizza ; S. Marcato; M. Zottini; A. Squartini ; G. Valle and B. Baldan, .2018.

Biocontrol traits of *Bacillus licheniformis* GL174, a culturable endophyte of *Vitis vinifera* cv. Glera. BMC Microbiology. 18(1):1-16. https://doi.org/10.1186/s12866-018-1306-5

34. Omaye, J. D. ; E. Ogbuju ; G. Ataguba ; O. Jaiyeoba ;J. Aneke and F. Oladipo .2024 . Cross-comparative review of Machine learning for plant disease detection: Apple, cassava, cotton and potato plants. Artificial Intelligence in Agriculture.12:127-151. https://doi.org/10.1016/j.aiia.2024.04.002

35. Ogoshi, A. 1996 . Introduction - the genus Rhizoctonia. In. Rhizoctonia species: taxonomy, molecular biology, ecology, pathology and disease control. Eds. Sneh B, Jabaji-Hare S, Neate S and Dijst G. Kluwer Academic Publishers, The Netherlands. pp1-9. DOI: 10.1007/978-94-017-2901-7_1

36.Pandey, C. ; D. Prabha ; Y. K. Negi ; D.K. Maheshwari ;S. Dheeman and M. Gupta.2023 . Macrolactin A mediated biocontrol of *Fusarium oxysporum* and *Rhizoctonia solani* infestation on Amaranthus hypochondriacus by *Bacillus subtilis* BS-58. Frontiers in Microbiology. 14: 1105849. https://doi.org/10.3389/fmicb.2023.1105849

37.Papp, O.; T. Kocsis; B. Biró; T. Jung; D. Ganszky; E. Abod and D. Drexler .2021 . Co-inoculation of organic potato with fungi and bacteria at high disease severity of *Rhizoctonia solani* and Streptomyces spp. increases beneficialeffects. Microorganisms.9(10):2028. https://doi.org/10.3390/microorganisms9102028

38.Parmeter, J. R. and H. S. Whitney. 1970. Taxonomy and nomenclature of the imperfect stage In:*Rhizoctonia solani* biology and pathology.(ed). J. R. Parmeter. University of California Barkley.Los Angeless PP:7-19

39.Park, H.G.; Y. S. Lee ; K.Y. Kim,; Y. S. Park; K.H. Park ; T.H.Han ; C.M.Park; Y. S. Ahn. 2017.Inoculation with *Bacillus licheniformis* MH48 promotes nutrient uptake in seedlings of the ornamental plant Camellia japonica grown in Korean reclaimed coastal lands. Horticultural Science and Technology . 35(1):11–20.[CrossRef]. http://dx.doi.org/10.12972/kjhst.20170002

40.Pathak, V. M. ; V.K. Verma ;B.S. Rawat ; B. Kaur ; N. Babu ; A. Sharma ... and J.M. Cunill.2022 . Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review. Frontiers in microbiology.13:962619. https://doi.org/10.3389/fmicb.2022.962619

41.Ramadan, N. A. and A. A. Mahmod. 2005. Chemical Contorl of Pathogenic Sunflower(*Helianthus annuus* L)Seed Borne Fungi.Rafidain Journal of Science.16(7):39-48.https://search.emarefa.net/detail/BIM-363006

42. Rand, K.S. and A. D. K. Alhiyali, 2018. Detection of the seasonal variations of patato sales in wholesale markets of Baghdad province during the period (2010-2015) and forecasting by using seasonal time series models (sarima).The Iraqi Journal of Agricultural Sciences.49(1): 50-57. https://doi.org/10.36103/ijas.v49i1.206

43.Raspor,P.;D .Miklic-Milek; M. Avbelj and N.Cadez.2010.Biotechnol of greymould disease on grape caused by *Botrytis cinerea* with Autochthonous wine yeasts. Food Techology Biotechnology.48(3):336-343. https://hrcak.srce.hr/57565

44. Sinshaw, N. T. ;B.G. Assefa; S.K. Mohapatra and A. M. Beyene. 2022. Applications of computer vision on automatic potato plant disease detection: A systematic literature review. Computational intelligence and neuroscience. (1): 7186687. https://doi.org/10.1155/2022/7186687

45.Sneh, B.; E. Yamoah and A.Stewart. 2004. Hypovirulent Rhizoctonia spp. Isolates from New Zealand soils protect radish seedlings against dampingoff caused by *R. solani*.New Zealand Plant Protection .57:54-58. http://dx.doi.org/10.30843/nzpp.2004.57.6889

46. Sukkasem,P. ; A.Kurniawan; T. C. Kao and H. Chuang. 2018. A multifaceted rhizobacterium *Bacillus licheniformis* functions as a fungal antagonist and a promoter of plant growth and abiotic stress tolerance. Environmental and Experimental Botany. 155(16):541-551. https://doi.org/10.1016/j.envexpbot.2018.08.005.

47. Tudi, M.; H. Daniel Ruan ;L. Wang ;J. Lyu ; R. Sadler ; D. Connell and D. T. Phung.2021 .Agriculture development, pesticide application and its impact on the environment. International journal of

environmental research and public health.18(3):1112. https://doi.org/10.3390/ijerph18031112

48. Win, T. T. ; B. Bo ;P. Malec and P. Fu .2021 . The effect of a consortium of Penicillium sp. and Bacillus spp. in suppressing banana fungal diseases caused by Fusarium sp. and Alternaria sp. Journal of Applied Microbiology. 131(4): 1890-1908. https://doi.org/10.1111/jam.15067

49.Woodhall, J. W. ; L. Brown ;M. Harrington ;M. Murdock ; C.A. Pizolotto ;P.S. Wharton and K. Duellman.2022 . Anastomosis groups of *Rhizoctonia solani* and *binucleate Rhizoctonia* associated with potatoes in Idaho. Plant Disease.106(12):3127-3132. https://doi.org/10.1094/PDIS-12-21-2683-RE

50. Won , S. J.; J. H. Kwon; D. H. Kim and Y. S. Ahn . 2019. The effect of *Bacillus licheniformis* MH48 on control of foliar fungal diseases and growth promotion of camellia oleifera seedlings in the Coastal Reclaimed Land of Korea. Pathogens . 8(1):1-14. https://doi.org/10.3390/pathogens8010006

51. Yang, Y. ;J. Hu ; X. Wei ;K. Huang ;C. Li and G. Yang. 2024. Deciphering core microbiota in rhizosphere soil and roots of healthy and *Rhizoctonia solani*-infected potato plants from various locations. Frontiers in Microbiology.15:1386417. https://doi.org/10.3389/fmicb.2024.1386417

52.Zhang, N.; W. Hu; R. Hou; P. Du; W. Miao ;R. Wang... and H. Xu .2023 . Enhanced fungicidal activity and mechanism of pyraclostrobin nanoparticle with reactive oxygen species responsiveness against *Rhizoctonia solani*. Journal of Cleaner Production.421:138494. https://doi.org/10.1016/j.jclepro.2023.138494