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Survey of Microbes Colonizing the Root System of Some Growing Plants on Uhud Mountain in Almadinah Almunawwarah

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Abstract

Plant-microbe associations are crucial in rhizosphere ecosystem processes, as positive interactions between plants, mycorrhizal fungi, and plant-growth-promoting rhizobacteria (PGPR) increase crop yield and serve as natural alternatives to chemical pesticides for controlling plant diseases. This study aimed to isolate microbes from three plants: *Solenostemma argel*, *Heliotropium arbainense,* and *Suaeda vermiculate* from Uhud Mountain in Al-Madinah Al-Munawara. Most isolates showed promising results for PGP traits under *in vitro* conditions, both culturally and biochemically. Bacterial isolates belonged to two phyla, Firmicutes and Actinomycetota, while fungal strains belonged to three phyla: Ascomycota, Deuteromycota, and Zygomycota. The efficiency of isolated microbes in producing antimicrobial agents against pathogenic microbes was assessed by measuring their zone of inhibition using agar plug diffusion and agar-well diffusion methods. Results showed that the maximum zone of inhibition was noted in *Bacillus* isolate C13 against *Penicillium* (30.3 ± 0.6 mm) in primary screening, while isolate C8 (42.7 ± 8.0 mm) was the most active against *Aspergillus* in secondary screening. Evaluating fungal isolates' antagonistic activity revealed the highest activity for isolate A2 (76.7 ± 10.4). This study demonstrated that employing microbes as biological control factors against plant pathogens could be an effective strategy for numerous agricultural advancements.

Keywords: Disease control; *Heliotropium arbainense*; Uhud Mountain; *Solenostemma argel*; *Suaeda vermiculate*

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1. Introduction

Al-Madinah Al-Monawara (Al-Madinah) is a city in western Saudi Arabia, 150 kilometers east of the Red Sea and 400 kilometers from Makkah. Mountains range in height from 800 to 1500 meters above sea level and surround the city to the north, south, and west. The tallest of these mountains is Al Waira, followed by Uhud (1087 m) [1]. Among the mountains in the area, Uhud Mountain stands out as one of the most important historical sites in the Arab Peninsula. No thorough floristic study has been done due to Uhud Mountain's rugged terrain. Due to the significant elevation variations, mountainous regions exhibit more incredible plant species richness and variety than lowland regions [2].

The rhizosphere has been described to include three zones: the endorhizosphere, as portions of the root cortex and endodermis where microbes and mineral ions reside in the apoplastic space between cells; the rhizoplane, as the middle zone next to the root epidermal cells and mucilage; and the ectorhizosphere, as the outermost zone which extends from the rhizoplane out into the bulk soil [3]. Roots regulate microbial activity in the rhizosphere of the soil by releasing root exudates. Microbes can directly or indirectly accelerate plant growth by preventing illness [4]. Plant-microbe interactions in the rhizosphere are crucial in various ecosystem processes, including carbon sequestration and nutrient cycling [5]. Positive plant-microbe interactions include symbiotic relationships with plant-growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi that exist as endophytes or epiphytes. These interactions were shown to benefit plants in a variety of ways, including disease control [6-8], increased nutrient uptake and availability [9, 10], increased resistance to biotic stresses [11, 12], and abiotic stresses [13, 14], each of which led to increased plant productivity [15]. Therefore, the present study is designed for the following:

1. Isolation of microbes from the rhizosphere and the soil around some growing plants collected from Uhud Mountain in Almadinah Almunawwarah.

2. Identification of the isolated microbes morphologically and biochemically.

3. Study some biotechnological impacts of the isolated microbes, like the production of enzymes and antimicrobial activities.

4. Study some plant growth parameters of the isolated microbes, like phosphate solubilization and nitrogen fixation.

2. Materials and methods

2.1. Isolation of tested isolates colonizing root system of tested plants

2.1.1. Sampling of plants and collection of rhizosphere soil samples

Samples were collected from Uhud Mountain in Al-Madinah Al-Munawara in Saudi Arabia; plant A (24°32'25.0" N 39°36'06.5" E), which had sandy soil, and plant B and plant C (24°32'22.6" N 39°36'09.5" E), which also had sandy soil. The view from the locations where plants were sampled is represented in Figure 1. Rhizosphere soil samples were obtained from three selected plant specimens that were identified with the help of Dr. Safia Abdullah Biology Department, College of Science, Taibah University. These samples were carefully placed in sterile bags and then transferred to the laboratory.

2.1.2. Isolation of microorganisms in the rhizosphere of the tested plants

The isolation procedure was initiated within 24 hours after sample collection. The procedure outlined by Vlassak *et al.* [16] was followed for microbial isolation. In this experimental procedure, 10 grams of rhizosphere soil associated with roots of each plant sample were collected and placed in a conical flask containing 90 milliliters of saline solution with a concentration of 0.85%. The specimen was agitated for 15 minutes using a vortex apparatus, after which successive soil suspensions were diluted. From the prepared dilutions, 0.1 ml was spread onto sterilized petri plates containing nutrient agar (NA), potato dextrose agar (PDA), and Sabouraud dextrose agar (SDA) medium, which were prepared according to the ready-made manufacturer's directions (HIMEDIA) by dissolving 13 g, 39 g, and 65 g of commercially formulated media separately in 1 liter of distilled water. Then, the mixtures were sterilized at 121°C for 20 minutes and incubated for one day at 37°C for bacteria and 5-7 days at 28 ± 2 °C for fungi. The resulting colonies were purified using the streaking plate method on the previously mentioned medium. Actinomycetes isolates were purified on starch nitrate agar media [17] containing the following composition (g/L): starch 20; KNO_3 2; NaCl 0.5; K_2HPO_4 1; $MgSO_4.7H_2O$ 0.5; $CaCO_3$ 3; $FeSO_4.7H_2O$ 0.01; agar 20; and distilled water up to 1L. Finally, the resulting colonies were transferred to slant agar for short-term preservation.

Figure 1: View the locations where plants were sampled from Uhud Mountain in Al-Medina Al-Munawara.

2.1.3. Microbiological media

Initially, the preliminary assessments involved using Gram's staining technique, examination of spore formation, and growth on MacConkey agar. The MacConkey agar composition per liter of distilled water included peptone 17 g, lactose 10 g, bile salts 1.5 g, proteose peptone 3 g, NaCl 5 g, neutral red 0.03 g, crystal violet 0.001 g, and agar 3.5 g, adjusted to a pH of 7.1 ± 0.2. The catalase test was conducted using hydrogen peroxide. For starch hydrolysis, the test was performed on starch agar media, with a composition per liter of distilled water: meat extract 3 g, starch 2 g, the peptic digest of animal tissue 5 g, and agar 15 g, adjusted to a pH of 7.2 ± 0.1. The Mannitol fermentation test was conducted on mannitol salt agar, formulated with the following components per liter of distilled water: enzymatic digest of casein 5.0 g, enzymatic digest of animal tissue 5.0 g, beef extract 1.0 g, D-mannitol 10.0 g, NaCl 75.0 g, phenol red 0.025 g, and agar 15.0 g and pH adjusted to 7.4 ± 0.2. Carbohydrates fermentation on phenol red broth, which contains per liter of distilled water: proteose peptone 10 g, HM peptone B 1 g, NaCl 5 g, and phenol red 0.018 g, pH to 7.4 ± 0.2 including addition of 5g of glucose and sucrose separately. Citrate utilization on Simmons citrate agar, which contains per liter of water: $MgSO_4 \cdot 7H_2O$ 0.2 g, K_2HPO_4 1 g, sodium citrate 2 g, NaCl 5 g, $NH_4H_2PO_4$ 1 g, bromothymol blue 0.08 g, and agar 15 g, pH 6.8 ± 0.2. Tests were performed using standard methods by Cappuccino and Sherman [18]. Casein hydrolysis was conducted on Skim milk SM agar media contains per liter of distilled water: SM powder 28 g, tryptone 5 g, dextrose (glucose)1g, yeast extract 2.5 g, and agar 15 g, pH 7.0 ± 0.2. Proteolytic bacteria hydrolyze casein to form soluble nitrogenous compounds indicated

as clear zones surrounding the colonies [19]. A urease test was conducted on Urea Agar Base which contains per 950 ml of water: peptone 1g, dextrose (glucose) 1g, sodium chloride 5g, potassium dihydrogen phosphate 0.8 g, phenol red 0.012 g, disodium hydrogen phosphate 1.2 g, and agar 15 g, final pH 6.8 ± 0.2. The medium was heated to boiling to ensure complete dissolution, followed by autoclaving at 10 lbs pressure (115°C) for 20 minutes. After cooling to 45-50°C, the total volume was aseptically completed to 1 liter by adding 50 ml of sterile 40% urea solution [20]. Finally, lipid hydrolysis was performed by inoculating the bacterial isolates on the basal culture media for lipase production, which contain (g/L): glucose (10), NaCl (5), K_2HPO_4 (0.3), KH_2PO_4 (1), $CaCl_2$ (2), peptone (10), yeast extract (5), $MgSO_4.7H_2O$ (0.2), $(NH_4)_2SO_4$ (2), and olive oil 2% (v/v), pH 7.0 ± 0.2. Then, the plates were incubated at 37°C for 24 hours. After the period of incubation, the plates were flooded with copper sulfate 20% for determination of lipase activity, and green to blue precipitate was observed as a positive result for lipid hydrolysis activity [21].

2.2.Determining the colony forming unit (CFU)

CFU was calculated for tested isolates by multiplying the appropriate dilution factor by the number of colonies in each sample.
The CFU was calculated only for the countable colonies (30-300) using the formula:
CFU/g = (number of colonies x dilution factor)

2.3.Cultural, morphological, and biochemical characterization of the tested isolates

The isolates were characterized by evaluating their cultural and biological features. Different biochemical parameters were performed to identify the bacterial isolates.

2.3.1. Identification of the tested isolates

The general morphology of bacteria was observed from young cultures (24 h) grown on nutrient agar plates. Bacterial films were prepared, stained using gram staining and spore staining, and examined using a microscope with an oil immersion objective to determine microscopic appearance [22]. According to Bergey's Manual of Systematic Bacteriology, the tested isolates were identified up to the genus level according to morphological and physiological characteristics [23]. These isolates are most probably *Bacillus*.

The tested Actinomycetota were identified by examination of morphological and physiological characteristics up to genus level using the keys described in Bergey's Manual of Systematic Bacteriology and the International Streptomyces Project (I.S.P) [24].

Morphological characters of the selected isolates were studied by inoculating into sterile media like, glycerol yeast extract agar, oatmeal agar, mineral agar, and starch casein agar. The media was sterilized and poured into sterile Petri dishes. After solidification, selected isolates were streaked aseptically and incubated at 30 °C for 7 d. Morphological characters

such as colony characteristics, pigment production, absence or presence of aerial and substrate mycelium was observed. The arrangement of spores and sporulating structures were examined microscopically by using cover slip culture method by inserting sterile cover slip at an angle of 45 °C in the starch casein agar medium. A loop full of isolates was taken from 7 d old culture media, inoculated at the insertion place of the cover slip and incubated at 30 °C for 7 d. The cover slip was carefully removed by using sterile forceps and placed upward on a clean glass slide. The bacterial growth on the cover slip was fixed with few drops of absolute methanol for 15 min and washed with tap water then flooded with crystal violet reagent for 1 min followed by washing and blot drying. Finally, the cover slip was examined under the microscope by using oil immersion lens (100 ×) [25].

The tested fungi were identified based on macroscopic (on SDA and PDA) and microscopic characteristics (hypha, reproductive structures, and diameter) according to Humber (1997) up to genus level [26].

Antibiotic sensitivity

Bacterial isolates were tested against 6 different antibiotics: KF 30 µg, PG 10 units, E 15 µg, AP 10 µg, CD 2 µg, and TS 25 µg (MASTRING-S) by the Kirby-Bauer disk diffusion method [27]. Mueller-Hinton medium was prepared for inoculation with freshly prepared bacteria and incubated for 24 h at 37°C. Then, the inhibition zones were measured with a ruler using a mm scale. The experiment involved carrying out triplicate tests to determine the inhibitory zone's average diameter.

2.4. Plant growth-promoting parameters of microbial isolates

2.4.1. Phosphate solubilization

Phosphate solubilization activity of microbial isolates was detected by inoculating isolates on Pikovskayas's agar, which contains glucose (10 g), $(NH_4)_2SO_4$ (0.5 g), $Ca_3(PO_4)_2$ (5 g), NaCl (0.2 g), $MgSO_4.7H_2O$ (0.1 g), KCl (0.2 g), $FeSO_4.7H_2O$ (0.002 g), yeast extract (0.5 g), $MnSO_4.H_2O$ (0.002 g), agar (10 g), H_2O (1000 ml), pH of 7.0, and incubated at 30°C for 48 h for bacteria and at 27 ± 2°C for 5-7 days for fungi. Following this, a clear region surrounding the colony was deemed a positive indication of phosphate solubilization [28].

2.4.2. Nitrogen fixation assay on Jensen's medium

Isolated bacteria were screened on Jensen's medium for nitrogen fixation activity [29]. The composition of Jensen's medium per liter of distilled water was as follows: sucrose (20 g), $CaCO_3$ (2 g), K_2HPO_4 (1 g), NaCl (0.5 g), $FeSO_4$ (0.1 g), Na_2MoO_4 (0.005 g), $MgSO_4.7H_2O$ (0.5 g), agar (20 g), pH 6.9, and incubated at 30°C for two days. The positive result is indicated by the presence of growth.

2.5. Detection of the antagonistic effect of isolated bacteria against plant pathogenic fungi

The present study employed three pathogenic fungi: *Aspergillus* sp., *Alternaria* sp., and *Penicillium* sp. The pathogenic fungal isolates were obtained from a culture collection from the College of Science, Department of Biology, University of Taibah.

2.5.1. Primary screening (microbial antagonism plate assays)

Each isolate was streaked into a nutrient agar plate and incubated at 37°C for 24-48 h, as per the modified method described in [30]. A 10 mm agar plug with actively growing bacteria (24 h old culture on NA) was placed on the plate containing the fungal growth. Following 5-7 days of incubation, antagonism was determined as the distance between the isolate and fungal growth.

2.5.2. Secondary screening by modified agar-wells diffusion method

The isolates were added to the nutrient broth and incubated at 37°C for a duration ranging from 24 to 48 hours. Next, the cell-free supernatant (CFS) was acquired through centrifugation at 6000 rpm for 15 minutes using an MPW-350R centrifuge. A 0.2 ml portion of the activated fungal suspension was then gently spread across the surface of the nutrient agar. In the agar-wells diffusion technique, wells were created in the pour plates using a sterile cork borer with a 10 mm diameter, and these wells were subsequently filled with 200 µl of the CFS previously prepared [31]. Afterward, all the plates were allowed to stand at room temperature for 2 hours. They were then incubated under aerobic conditions at 27 ± 2°C for 5 to 7 days. The resultant inhibition zones around the wells were measured in millimeters, and their measurements were duly recorded.

2.6. Antagonistic activity of isolated fungi against plant pathogenic fungi

To assess the antagonistic efficacy of fungal isolates towards phytopathogenic fungi, a 10 mm diameter disc of the antagonistic isolates derived from the periphery of a 6-day-old culture was positioned on one side of Petri dishes with potato dextrose agar (PDA). The plates were incubated at 27 ± 2°C for one day. Subsequently, individual agar plates were prepared, and a disc of 10 mm in diameter containing phytopathogenic *Alternaria*, *Aspergillus*, and *Penicillium* cultures aged 7 days was carefully put on each plate, ensuring that the disc was positioned opposite to the isolates. The plates were incubated at 28 ± 2C° for 6 days. The growth of the phytopathogens compared to the antagonist growth was visually observed at the end of the incubation period. Each fungus was inoculated alone as a control [32].

3. Results and discussions

3.1. Isolation of tested isolates colonizing root system of tested plants

A total of 51 isolates (38 of bacteria and 13 of fungi) were isolated on nutrient agar, starch nitrate agar, potato dextrose agar, and Sabouraud dextrose agar medium from the rhizosphere soil of Mount Uhud in Al-Madinah Al-Munawara in Saudi Arabia. They revealed that 38 bacteria isolates were divided into seven from plant A, 13 from plant B, and 18 from plant C, and 13 fungi were divided into nine from plant A, one from plant B, and three from plant C, after excluding similar isolates.

3.2. Determining the colony forming unit (CFU)

The CFU for each rhizosphere isolate revealed that plant C soil (2790) has the highest number of colonies, followed by plant A soil (570), and finally plant B soil (300).

3.3. Cultural and microscopic characterization of tested isolates

3.3.1. Bacterial isolates

Color, size, texture, elevation, margin, opacity, form, Gram stain reactivity, and cell shape characteristics were used to identify all isolates, as summarized in Tables 1, 2, and 3. The findings indicated that the isolates exhibited a diverse array of cultural traits. At the same time, some isolates were identified as spore-forming.

Table 1: Cultural and microscopic characteristics of bacterial isolates from the rhizosphere soil of *Suaeda vermiculata*

Isolate	Color	Margin	Size	Texture	Form	Opacity	Elevation	Gram reaction	Cell shape	Spore formation
A1	white	Undulate	Medium	smooth	circular	Opaque	flat	+	short bacilli	**+**
A2	white	Undulate	small	smooth	circular	transparent	umbonate	+	short bacilli	-
A3	pink	Entire	very small	smooth	circular	Opaque	raised	+	short bacilli	**+**
A4	white	Undulate	medium	smooth	circular	Opaque	flat	+	short bacilli	**+**
A5	creamy	Undulate	medium	smooth	irregular	Opaque	flat	+	short bacilli	-
A6	white	Undulate	small	rough	irregular	Opaque	flat	+	short bacilli	**+**
A7	white	Entire	small	smooth	circular	transparent	convex	+	short bacilli in chain	-

Table 2: Cultural and microscopic characteristics of bacterial isolates from the rhizosphere soil of *Heliotropium arbainense*

Isolate	Color	Margin	Size	Texture	Form	Opacity	Elevation	Gram reaction	Cell shape	Spore formation
B1	pink	Undulate	large	rough	irregular	opaque	raised	+	short bacilli	+
B2	creamy	Entire	very small	smooth	circular	transparent	raised	+	short bacilli	+
B3	white	Entire	large	rough	irregular	opaque	flat	+	short bacilli	-
B4	creamy	Undulate	medium	rough	irregular	opaque	raised	+	short bacilli	-
B5	creamy	Undulate	medium	smooth	irregular	opaque	convex	+	long bacilli	-
B6	white	Undulate	medium	rough	irregular	opaque	raised	+	short bacilli	-
B7	pale yellow	Undulate	small	smooth	circular	transparent	convex	+	long bacilli	+
B8	apricot	Undulate	large	smooth	irregular	opaque	convex	+	short bacilli	+
B9	pink	Undulate	very small	smooth	circular	transparent	raised	+	long bacilli	+
B10	creamy	Undulate	medium	smooth	irregular	transparent	raised	+	short bacilli	-
B11	orange	Filiform	medium	smooth	circular	opaque	raised	+	long bacilli	-
B12	creamy	Undulate	large	smooth	irregular	opaque	convex	+	long bacilli	-
B13	orange	Undulate	large	smooth	irregular	opaque	convex	+	short bacilli	-

Table 3: Cultural and microscopic characteristics of bacterial isolates from the rhizosphere soil of *Solenostemma argel*

Isolate	Color	Margin	Size	Texture	Form	Opacity	Elevation	Gram reaction	Cell shape	Spore formation
C1	white	undulate	very small	smooth	irregular	opaque	convex	+	short bacilli	-
C2	creamy	undulate	very small	smooth	circular	transparent	raised	+	short bacilli	+
C3	white	undulate	small	smooth	irregular	opaque	convex	+	short bacilli	-
C4	white	Entire	very small	smooth	circular	transparent	convex	+	short bacilli	-
C5	apricot	undulate	medium	smooth	irregular	opaque	convex	+	short bacilli	+
C6	white	undulate	medium	smooth	circular	opaque	convex	+	short bacilli	-
C7	creamy	undulate	large	smooth	irregular	opaque	convex	+	short bacilli	-
C8	creamy	undulate	small	smooth	irregular	opaque	convex	+	short bacilli	-
C9	pale yellow	Entire	medium	smooth	circular	transparent	raised	+	short bacilli	-
C10	creamy	undulate	medium	smooth	circular	opaque	convex	+	short bacilli	-
C11	creamy	Entire	very small	smooth	circular	opaque	raised	+	short bacilli	-
C12	creamy	undulate	medium	smooth	irregular	opaque	convex	+	short bacilli	-
C13	creamy	undulate	large	smooth	circular	opaque	raised	+	short bacilli	-
C14	apricot	Entire	large	rough	circular	opaque	flat	+	short bacilli	-
C15	white	undulate	large	smooth	irregular	opaque	convex	+	short bacilli in chain	-
C16	creamy	undulate	large	smooth	irregular	opaque	flat	+	short bacilli	-
C17	white	undulate	large	smooth	irregular	opaque	raised	+	short bacilli in chain	-
C18	pink	undulate	large	smooth	irregular	opaque	raised	+	short bacilli	-

On applying Gram's staining, all bacterial isolates from the three plants revealed a positive reaction (Figure 2).

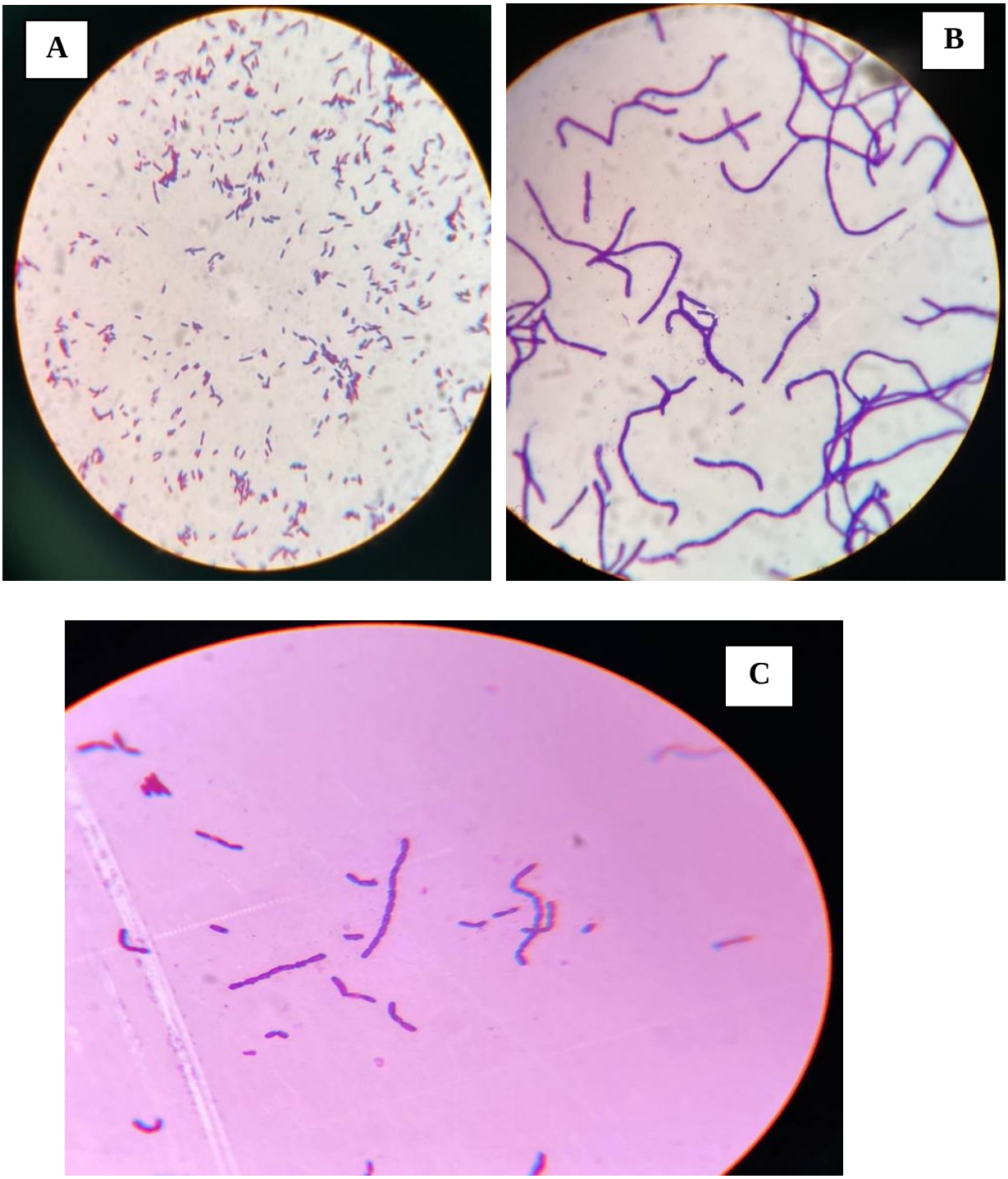

Figure 2: Gram staining for the tested bacterial isolates from tested plants after incubation in nutrient agar medium for 24h at 37°. Isolate C16 (A), isolate B11 (B), and isolate A7 (C).

3.3.2. Fungal isolates

The fungal isolates obtained from the various plant rhizosphere soils were identified macroscopically and microscopically as *Aspergillus*, *Fusarium*, *Penicillium*, and *Rhizopus* species. The microscopic features of the isolates are presented in (Figure 3). *Aspergillus* species had septate hyphae, hyaline conidiophores and radial conidial head bearing the spores (isolates A2, A4, A5, A9, B1 and C2). *Penicillium* species appeared as septate hyphae with conidiophores and secondary branches (metulae). The metulae bear flasked shaped phialides with unbranched chains of round conidia (A3 isolate). *Fusarium* species showed septate hyphae, sickle- shaped macroconidia (isolates A1, A6, A7, A8 and C1). *Rhizopus* with a deeply cottony texture of the colony having a white to gray-brown color on the top, pale white color on the reverse and Brown coloured branched sporangiophores occurring in clusters at the tip of the hyphal tubes. The spores are rounded with flattened bases (C3 isolate).

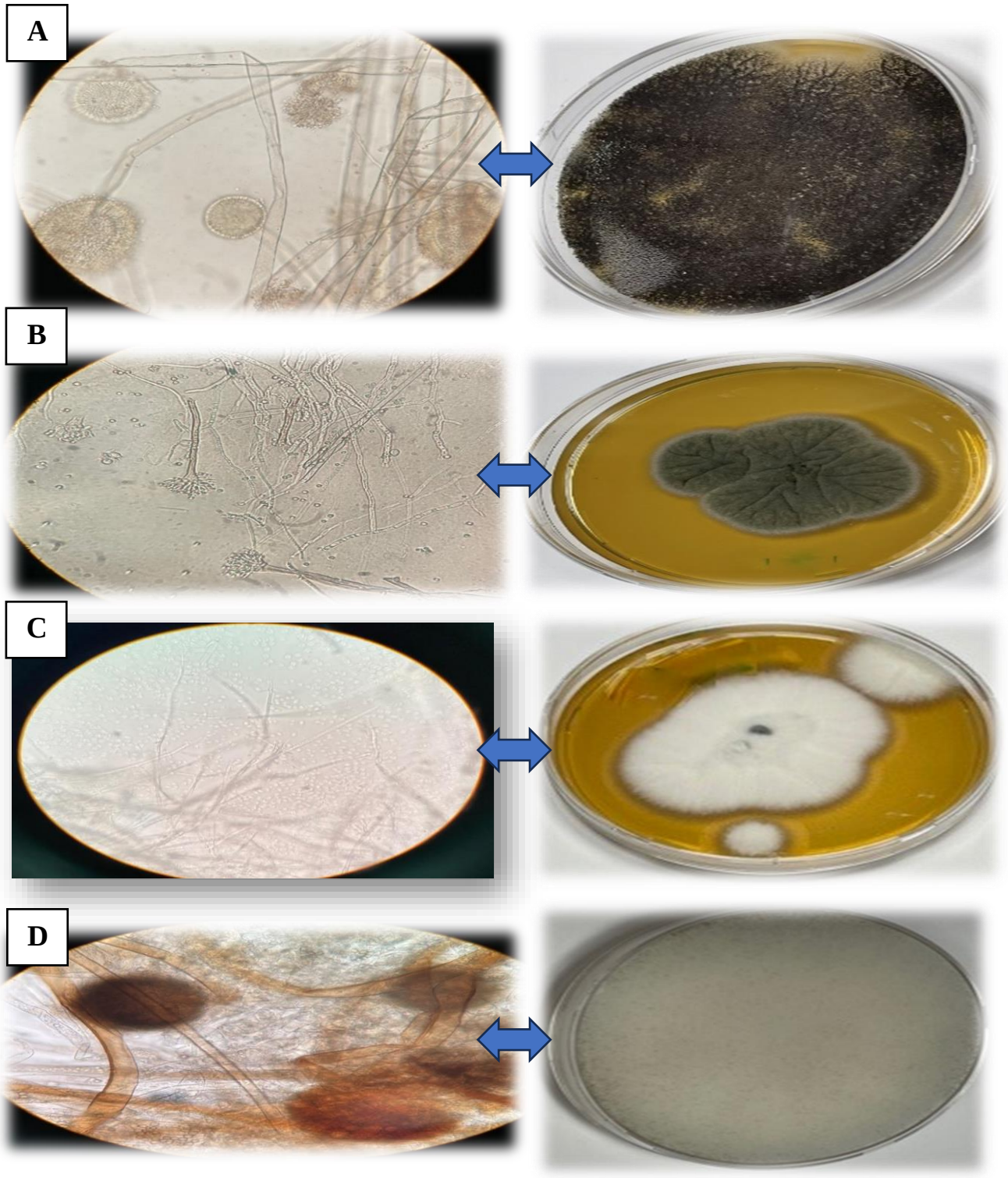

Figure 3: Microscopic and macroscopic characteristics of the tested fungal isolates from tested plants after incubation on Sabouraud agar medium for 5 days at 27 ± 2°C. *Aspergillus* (A), *Penicillium* (B), *Fusarium* (C), and *Rhizopus* (D).

3.4. Biochemical characterization of tested isolates

The biochemical examination of the isolated bacteria was displayed in Tables 4, 5, and 6 and illustrated in Figures 4 and 5. All isolated bacteria were positive in the catalase test and found acid-producing when tested for mannitol fermentation. However, all isolated bacteria had a negative reaction to lactose fermentation when cultured on MacConkey agar. In most isolates, 30 out of 38 were capable of hydrolyzing urea, followed by 26 capable of breaking down starch, 21 were degraded casein, and nine isolates were capable of hydrolyzing lipids. Most isolates have used glucose and sucrose as energy sources, whereas a few have used citrate. Therefore, bacterial isolates belonged to two phyla, Firmicutes and Actinomycetota, after isolates were identified culturally and biochemically.

Table 4: Biochemical parameters of bacterial isolates from the rhizosphere soil of *Suaeda vermicular*

Isolate	Urease test	Lipid hydrolysis	Sucrose fermentation	Glucose fermentation	Mannitol fermentation	Starch hydrolysis	Casein hydrolysis	Catalase test	Citrate utilization	Growth on MacConkey agar
A1	+	+	+	+	+	+	+	+	+	-
A2	+	+	+	+	+	-	-	+	+	-
A3	-	+	+	+	+	+	+	+	-	-
A4	+	+	+	+	+	+	-	+	+	-
A5	+	+	+	+	+	+	-	+	+	-
A6	-	+	+	+	+	-	+	+	-	-
A7	+	-	+	+	+	+	+	+	-	-

*For sugar fermentation tests + reaction means bacterial isolates produce acid only.

Table 5: Biochemical parameters of bacterial isolates from the rhizosphere soil of *Heliotropium arbainense*

Isolate	Urease test	Lipid hydrolysis	Sucrose fermentation	Glucose fermentation	Mannitol fermentation	Starch hydrolysis	Casein hydrolysis	Catalase test	Citrate utilization	Growth on MacConkey agar
B1	+	-	+	+	+	+	-	+	+	-
B2	+	-	-	-	+	+	-	+	-	-
B3	+	-	-	+	+	+	+	+	+	-
B4	+	-	+	+	+	-	-	+	-	-
B5	+	-	+	+	+	+	+	+	-	-
B6	+	-	+	-	+	+	+	+	-	-
B7	-	-	+	+	+	+	-	+	-	-
B8	-	-	+	+	+	+	+	+	-	-
B9	+	-	+	+	+	-	-	+	-	-
B10	+	-	+	+	+	-	-	+	-	-
B11	-	-	+	+	+	+	-	+	+	-
B12	+	+	+	+	+	+	+	+	-	-
B13	+	-	+	+	+	+	-	+	-	-

*For sugar fermentation tests + reaction means bacterial isolates produce acid only.

Table 6: Biochemical parameters of bacterial isolates from the rhizosphere soil of *Solenostemma argel*

Isolate	Urease test	Lipid hydrolysis	Sucrose fermentation	Glucose fermentation	Mannitol fermentation	Starch hydrolysis	Casein hydrolysis	Catalase test	Citrate utilization	Growth on McConkey agar
C1	+	-	+	+	+	+	-	+	-	-
C2	+	-	+	+	+	+	-	+	-	-
C3	+	-	+	-	+	-	-	+	-	-
C4	+	-	+	+	+	-	+	+	-	-
C5	+	-	+	-	+	-	+	+	-	-
C6	+	-	+	+	+	-	+	+	-	-
C7	+	-	+	+	+	+	+	+	-	-
C8	+	-	+	+	+	+	-	+	-	-
C9	-	+	+	+	+	-	-	+	-	-
C10	-	+	+	+	+	-	+	+	-	-
C11	+	-	+	+	+	+	+	+	-	-
C12	+	-	+	+	+	-	+	+	-	-
C13	-	-	+	+	+	+	-	+	-	-
C14	+	-	+	+	+	+	+	+	-	-
C15	+	-	-	+	+	+	+	+	-	-
C16	+	-	+	+	+	+	+	+	-	-
C17	+	-	+	+	+	+	+	+	+	-
C18	+	-	+	+	+	+	+	+	+	-

*For sugar fermentation tests + reaction means bacterial isolates produce acid only

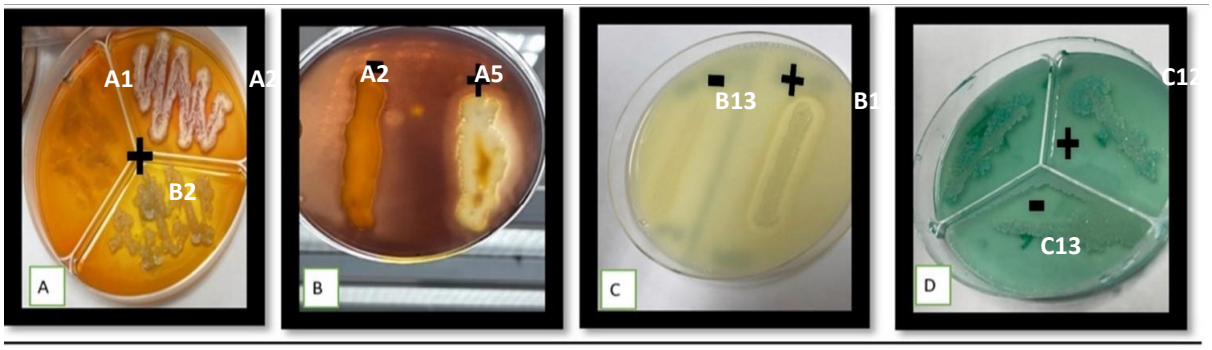

Figure 4: Results for different biochemical tests applied for identifying the tested bacterial isolates after incubation for 24h at 37°C. Positive to mannitol fermentation for isolate A1, A2, and B2 (A), positive to starch hydrolysis test for isolate A5 and negative for isolate A2 (B), positive to casein hydrolysis test for isolate B12 and negative for isolate B13 (C), positive to lipid hydrolysis test for isolate C12 and negative for isolate C13 (D).

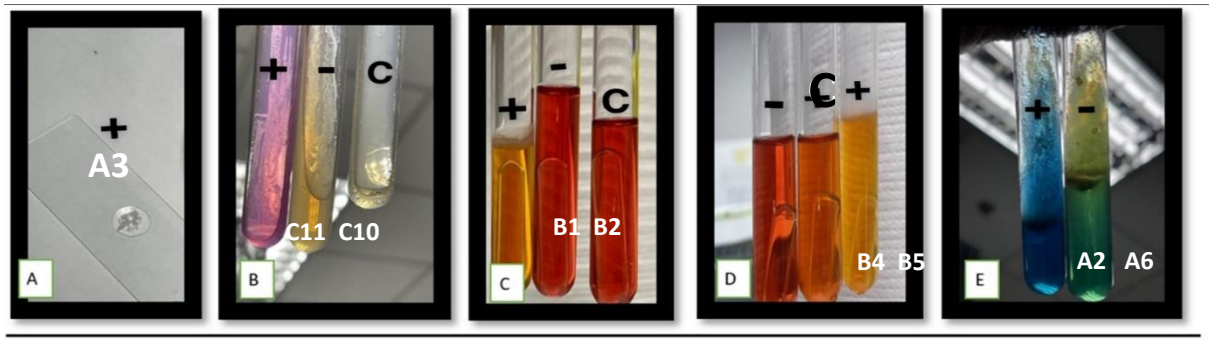

Figure 5: Results for different biochemical tests applied for identifying the tested bacterial isolates after incubation for 24 h at 37°C. Positive to catalase test for isolate A3 (A), positive to urease test for isolate C11 and negative for isolate C10 (B), positive to glucose fermentation by production of acid for isolate B1 and negative for isolate B2 (C), positive to sucrose fermentation by production of acid for isolate B4, B5. (D), positive to citrate utilization for isolate A2 and negative for isolate A6 (E). *C: Negative control

3.4.1 Antibiotic sensitivity

The results for testing antibiotic sensitivity against 6 different antibiotics showed that all isolates were sensitive to cotrimoxazole (Tables 7, 8, and 9).

Table 7: Antibiotic sensitivity of bacterial isolates from *Suaeda vermiculata*

Isolate	Phylum	AP	CD	E	PG	KF	TS
A1	Firmicutes	15 ± 1 (S)	R	17 ± 2(S)	R	22 ± 1.7(S)	31 ± 2(S)
A2	Firmicutes	18 ± 5(S)	15 ± 1(S)	R	13 ± 1.2(S)	19 ± 2(S)	29 ± 1.5(S)
A3	Firmicutes	R	22 ± 1.5(S)	17 ± 1.5(S)	R	R	29 ± 1.2(S)
A4	Firmicutes	23 ± 1.5(S)	19 ± 2.6(S)	31 ± 2.6(S)	24± 1.5(S)	32 ± 1.5(S)	30 ± 0 (S)
A5	Firmicutes	24 ± 1.7(S)	17 ± 1(S)	32 ± 2.1(S)	24± 0 (S)	29± 0 (S)	31± 1(S)
A6	Firmicutes	R	17 ± 4(S)	R	R	R	30.7± 1.2(S)
A7	Firmicutes	18 ± 1.5(S)	R	31± 1.5(S)	16± 1.7(S)	26.3± 2.9(S)	26.3± 2.3(S)

*R: Resistant, S: Sensitive

Table 8: Antibiotic sensitivity of bacterial isolates from *Heliotropium arbainense*

Isolate	Phylum	AP	CD	E	PG	KF	TS
B1	Actinobacteria	R	R	21± 0.6(S)	R	18± 1.2(S)	26± 3.6(S)
B2	Firmicutes	R	22± 0.6(S)	20 ± 4.3(S)	15± 1.5(S)	15± 4.3(S)	20 ± 1.5(S)
B3	Firmicutes	R	R	23 ± 0. 6(S)	R	16± 1.5(S)	25± 4.3(S)
B4	Firmicutes	19 ± 1(S)	R	28± 1(S)	13± 2.6(S)	17± 1.7(S)	32± 6.5(S)
B5	Firmicutes	23 ± 1(S)	23 ± 2(S)	27.3 ± 1.5(S)	20 ± 1(S)	34± 1.5(S)	33 ± 0 (S)
B6	Firmicutes	R	R	22 ± 0.6(S)	R	15 ± 1(S)	24± 1.7(S)
B7	Firmicutes	R	R	R	R	R	25± 2(S)
B8	Firmicutes	R	25 ±1.5(S)	R	R	R	31 ± 1(S)
B9	Firmicutes	R	R	R	R	R	28 ± 1(S)
B10	Firmicutes	23 ± 2(S)	18 ± 1(S)	15 ± 2.1(S)	18 ± 1(S)	23± 1.5(S)	33 ± 7(S)
B11	Actinobacteria	24 ± 1(S)	23 ± 2(S)	19 ± 2(S)	20 ± 1(S)	24± 1.5(S)	33 ± 7.5(S)
B12	Actinobacteria	R	18 ± 1(S)	30 ± 1.5(S)	R	34± 5(S)	32 ± 1(S)
B13	Actinobacteria	12.7 ± 1.2(S)	R	25 ± 1.2(S)	R	R	29 ± 0 (S)

*****R: Resistant, S: Sensitive

Table 9: Antibiotic sensitivity of bacterial isolates from *Solenostemma argel*

Isolate	Phylum	AP	CD	E	PG	KF	TS
C1	Firmicutes	21 ± 1(S)	21 ± 3.6(S)	32± 0 (S)	27 ± 2(S)	28 ± 1.5(S)	33 ± 1(S)
C2	Firmicutes	15 ± 0 (S)	R	30.7 ± 1.5(S)	R	21 ± 2(S)	29.3 ± 2.1(S)
C3	Firmicutes	12 ± 1.5(S)	18.7 ± 3.5(S)	18.3± 0. 6(S)	R	R	32 ± 2(S)
C4	Firmicutes	14 ± 0 (S)	R	30.3 ± 1.5(S)	12± 0 (S)	20 ± 0 (S)	24.3 ± 0.6(S)
C5	Firmicutes	R	R	R	R	15± 1(S)	32 ± 0 (S)
C6	Firmicutes	R	R	26 ± 1.5(S)	R	31 ± 1.5(S)	28 ± 1.5(S)
C7	Firmicutes	18 ± 1(S)	R	27 ± 1(S)	R	34± 1.5(S)	30 ± 1.5(S)
C8	Firmicutes	20 ± 0 (S)	15 ± 2(S)	26 ± 1(S)	R	35 ± 2.6(S)	31 ± 0 (S)
C9	Firmicutes	18 ± 2(S)	R	20 ± 3.1(S)	18± 1.5(S)	25 ± 0 (S)	35 ± 0 (S)
C10	Firmicutes	R	R	26 ± 1(S)	R	R	33 ± 2(S)
C11	Firmicutes	R	R	R	R	20 ± 1(S)	32 ± 1(S)
C12	Firmicutes	R	R	R	R	29 ± 1.5(S)	32 ± 1.5(S)
C13	Firmicutes	15 ± 3.6(S)	17 ± 4.2(S)	30 ± 0.6(S)	R	15 ± 2(S)	30 ± 0 (S)
C14	Firmicutes	15 ± 3.6(S)	17 ± 1(S)	30 ± 2(S)	R	35 ± 3.6(S)	30 ± 1(S)
C15	Actinobacteria	R	R	22 ± 1(S)	R	20 ± 1(S)	26 ± 0 (S)
C16	Actinobacteria	29 ± 4 (S)	22 ± 1(S)	31 ± 3.6(S)	26 ± 2(S)	31 ± 1(S)	34 ±1(S)
C17	Actinobacteria	21 ± 5(S)	19 ± 0.6(S)	30 ± 5.1(S)	20 ± 1(S)	37 ± 0 (S)	32 ± 2(S)
C18	Actinobacteria	18 ± 7.6(S)	19 ± 7.9(S)	32 ± 10.3(S)	20 ± 0 (S)	39 ± 1(S)	31 ± 1(S)

*****R: Resistant, S: Sensitive

3.5. Plant growth-promoting parameters of bacteria

Figure 6 shows the two parameters characterizing PGPR, nitrogen fixation and phosphate solubilization, and the results are presented in Tables 10, 11, and 12. The results show that 23 out of 38 isolates were positive for the two PGP parameters tested: 12 isolates in *Solenostemma argel*, nine isolates in *Heliotropium arbainense,* and two isolates in *Suaeda vermiculata.*

Table 10: Plant growth-promoting parameters of bacterial isolates from the rhizosphere soil of *Suaeda vermiculata*

Isolate	Nitrogen fixation	Phosphate solubilization	Phylum
A1	+	-	**Firmicutes**
A2	-	+	**Firmicutes**
A3	+	-	**Firmicutes**
A4	+	+	**Firmicutes**
A5	+	+	**Firmicutes**
A6	+	-	**Firmicutes**
A7	+	-	**Firmicutes**

Table 11: Plant growth-promoting parameters of bacterial isolates from the rhizosphere soil of *Heliotropium arbainense*

Isolate	Nitrogen fixation	Phosphate solubilization	Phylum
B1	+	+	**Firmicutes**
B2	+	+	**Firmicutes**
B3	+	+	**Firmicutes**
B4	+	-	**Firmicutes**
B5	+	+	**Firmicutes**
B6	+	+	**Firmicutes**
B7	+	+	**Firmicutes**
B8	+	-	**Firmicutes**
B9	+	+	**Firmicutes**
B10	+	+	**Firmicutes**
B11	+	-	**Actinomycetota**
B12	+	+	**Actinomycetota**
B13	+	-	**Actinomycetota**

Table 12: Plant growth-promoting parameters of bacterial isolates from the rhizosphere soil of *Solenostemma argel*

Isolate	Nitrogen fixation	Phosphate solubilization	Phylum
C1	+	+	**Firmicutes**
C2	+	-	**Firmicutes**
C3	+	+	**Firmicutes**
C4	+	-	**Firmicutes**
C5	+	+	**Firmicutes**
C6	+	+	**Firmicutes**
C7	+	+	**Firmicutes**
C8	+	+	**Firmicutes**
C9	-	+	**Firmicutes**
C10	+	+	**Firmicutes**
C11	+	-	**Firmicutes**
C12	+	+	**Firmicutes**
C13	+	+	**Firmicutes**
C14	+	-	**Firmicutes**
C15	+	+	**Actinomycetota**
C16	+	+	**Actinomycetota**
C17	+	+	**Actinomycetota**
C18	+	-	**Actinomycetota**

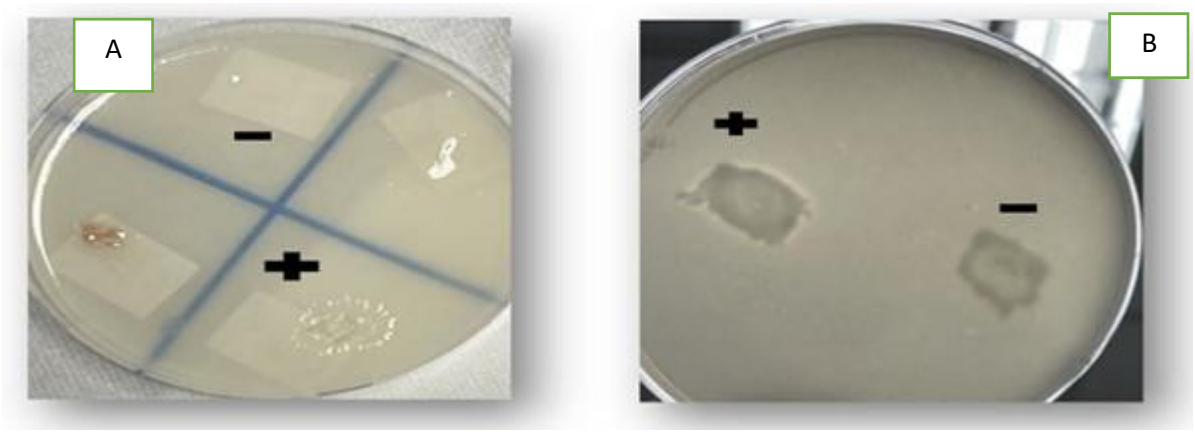

Figure 6: PGPR parameters for the tested bacterial isolates after 24-48h incubation at 30°C. Positive to nitrogen fixation for isolate A4 and negative for isolate A2 (A), positive to phosphate solubilization for isolate A2, and negative to isolate A1 (B).

3.5.1. Phosphate solubilization abilities of fungal isolates

The clear zone became visible in the three isolates of *Aspergillus* sp. and one isolate of *Penicillium* sp., but there was no clear zone in the other fungal isolates (Figure 7).

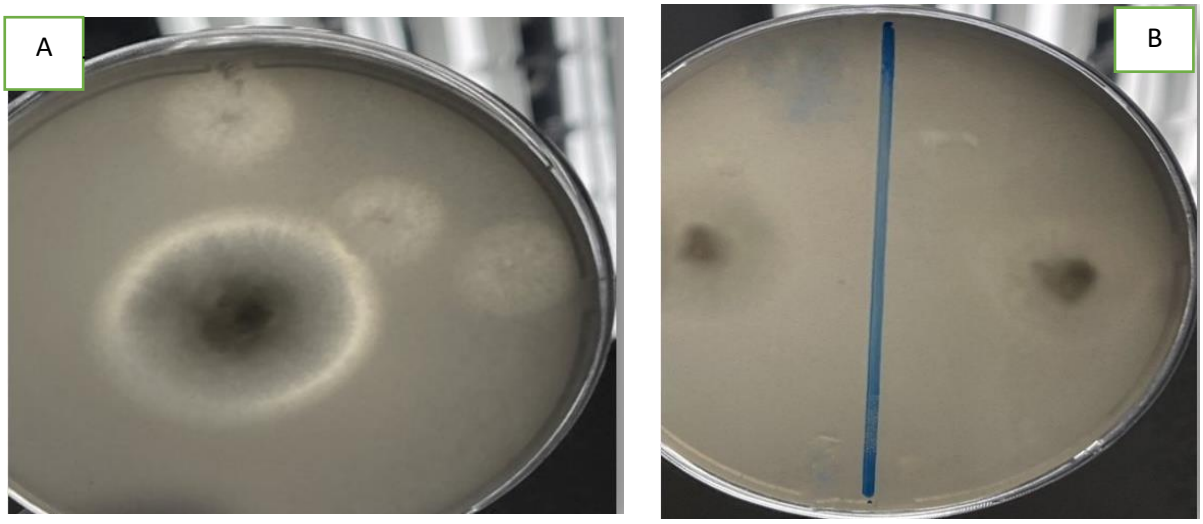

Figure 7: Phosphate solubilization abilities of the tested fungal isolates after incubation for 5-7 days at 27 ± 2°C. Positive to phosphate solubilization for *Aspergillus* isolates A2 (A) and negative to phosphate solubilization for *Fusarium* isolate C1 (B).

3.6. Detection of the antagonistic effect of isolated bacteria against plant pathogens

3.6.1. Primary screening

The agar-plug diffusion technique demonstrated antifungal activity; each isolate was streaked into a nutrient agar plate where NA was chosen rather than PDA or SDA; this was due to a preliminary test that showed that NA was the best medium for fungal and bacterial growth. From the total of 38 bacterial isolates investigated, only a few out of 24 isolates demonstrated inhibition zones towards three species of phytopathogenic fungi. This observation is further supported by the graphical representations in Figures 8, 9,10, and 11. On the other hand, four bacterial isolates did not exhibit any antifungal activity, as seen by the absence of noted inhibition zones. The maximum zone of inhibition was noted against *Penicillium* at 30.3 ± 0.6 mm, followed by *Alternaria* at 30.3 ± 1.5 mm. Therefore, the decision to select isolates for secondary screening was based on the outcomes obtained from this procedure.

4.6.2. Secondary screening

During the secondary screening phase, only ten bacterial isolates had antifungal activity against only two types of plant pathogenic fungi, *Aspergillus* and *Alternaria.* However, these isolates did not demonstrate any antagonistic effect against *Penicillium*. The isolate C8 exhibited the highest activity against *Aspergillus* (42.7 ± 8.0 mm), while C9 was the most active against *Alternaria* (29 ± 2.6 mm), as presented in Figures 12 and 13.

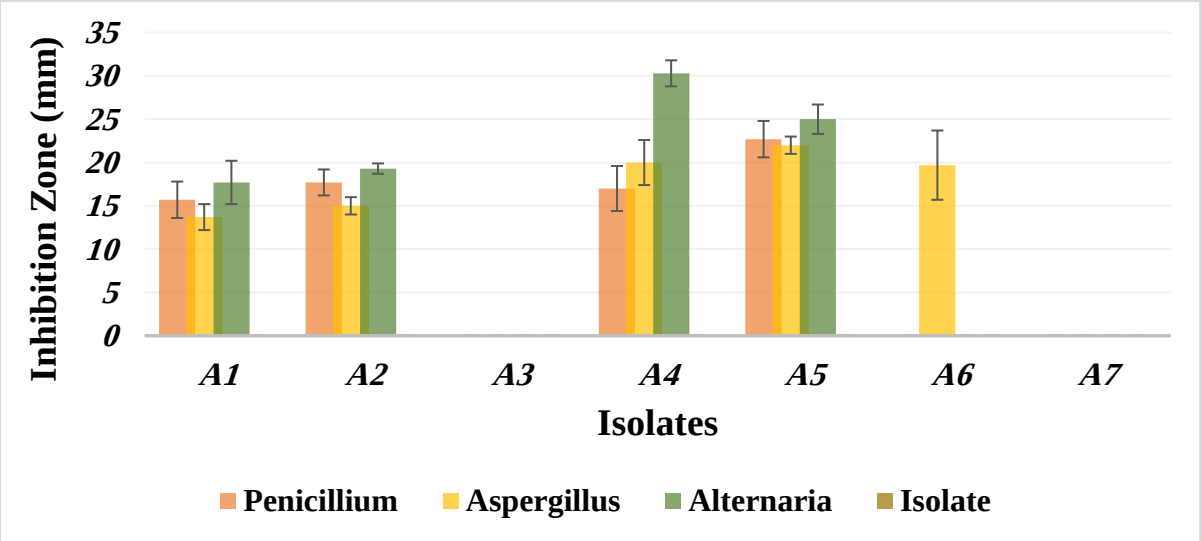

Figure 8: Antifungal activity of bacterial isolates from the rhizosphere soil of *Suaeda vermiculate* against three phytopathogenic fungi: *Aspergillus*, *Alternaria*, and *Penicillium.*

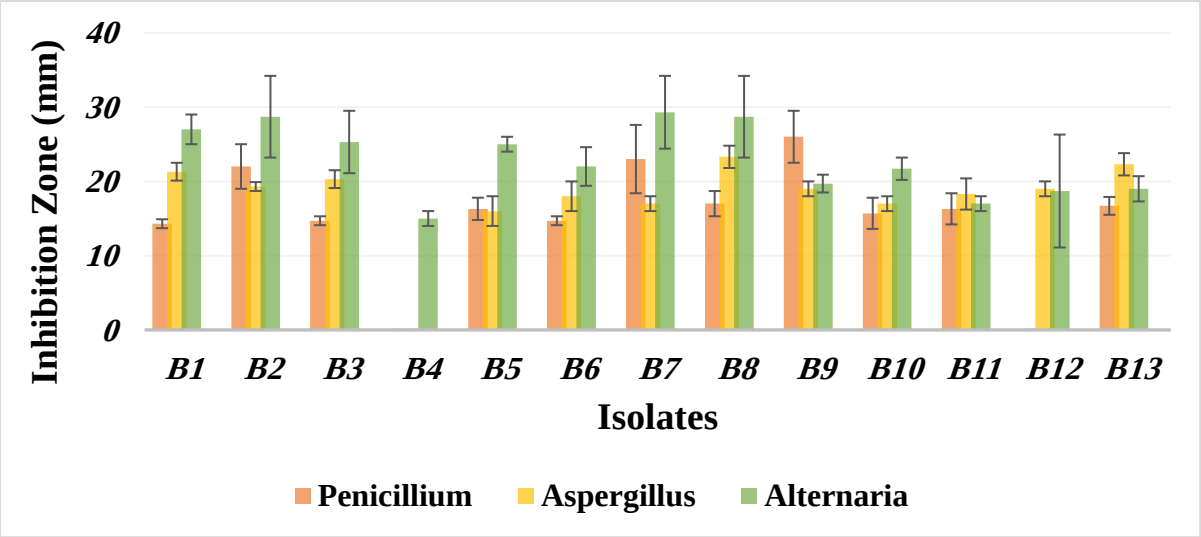

Figure 9: Antifungal activity of bacterial isolates from the rhizosphere soil of *Heliotropium arbainense* against three phytopathogenic fungi: *Aspergillus*, *Alternaria*, and *Penicillium.*

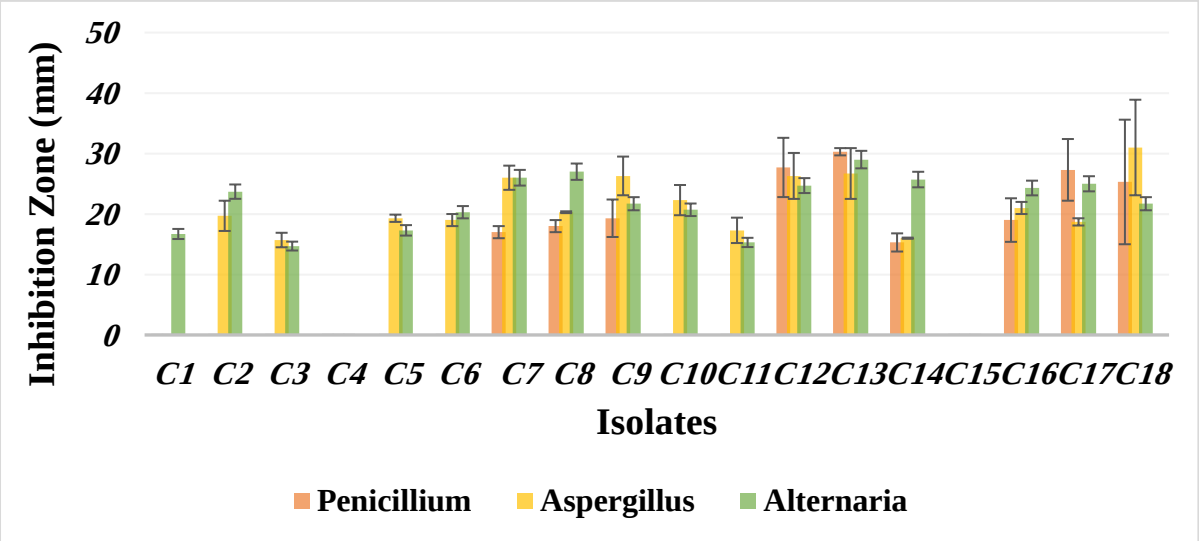

Figure 10: Antifungal activity of bacterial isolates from the rhizosphere soil of *Solenostemma argel* against three phytopathogenic fungi *Aspergillus*, *Alternaria*, and *Penicillium*

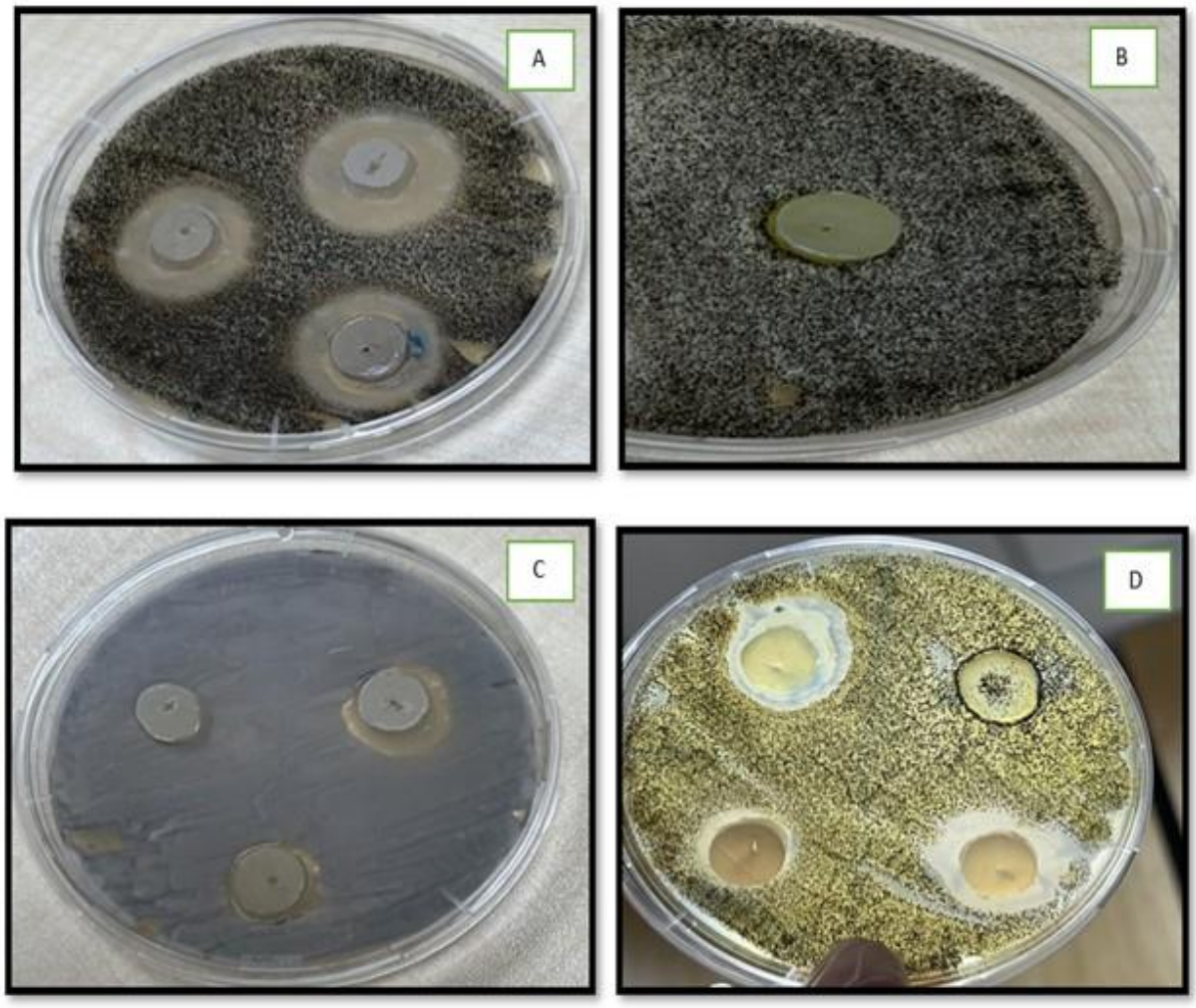

Figure 11: Antagonistic effect of the tested bacterial isolates against three phytopathogenic fungi *Aspergillus*, *Alternaria*, and *Penicillium*, by agar-plug diffusion method. (A) isolates B6, C6 and 8 against *Aspergillus*, (B) isolate C15 against *Aspergillus*, (C) isolates B6, C6 and 8 against *Penicillium* and (D) isolates C4, 9, 10, and 11 against *Alternaria.*

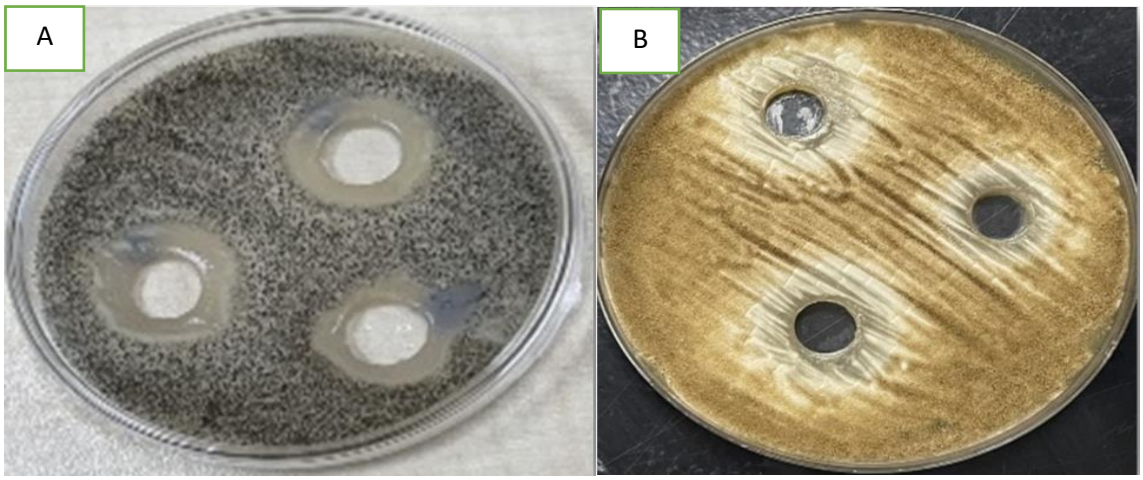

Figure 12: Antagonistic effect of the tested bacterial isolates against three phytopathogenic fungi by agar-well diffusion method: *Aspergillus*, *Alternaria*, and *Penicillium*. (A) isolates B1, 3 and 9 against *Aspergillus* and (B) isolates B6, C6, and 8 against *Alternaria*.

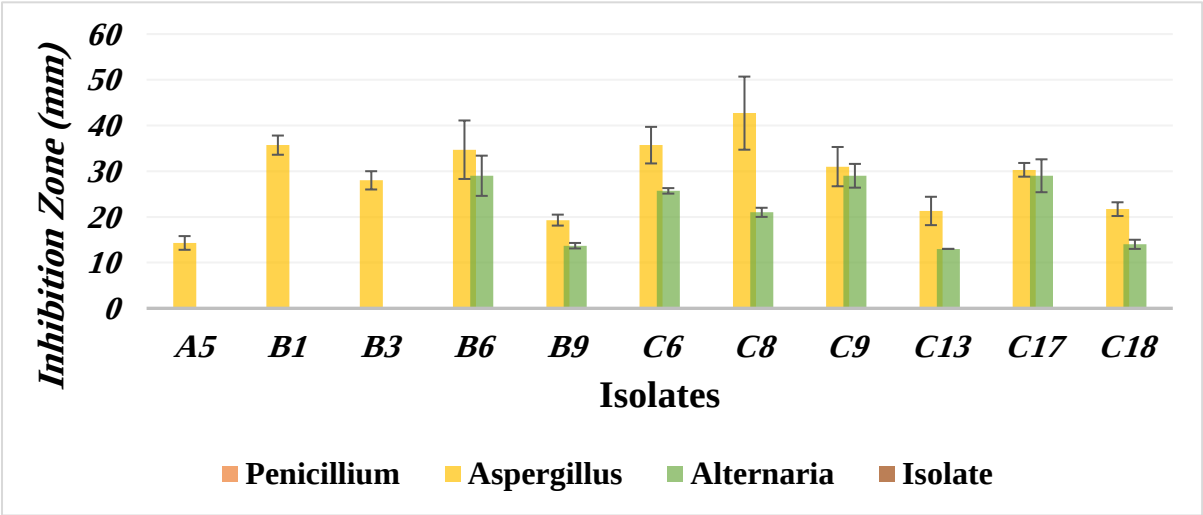

Figure 13: Antifungal activity of the tested bacterial isolates from tested plants against pathogenic fungi: *Aspergillus*, *Alternaria*, and *Penicillium*.

4.6.3. Estimation of antagonistic efficiency of fungal isolates from tested plants against phytopathogenic fungi

The results showed varying degrees of growth suppression by the phytopathogens. The highest activity was observed in the A2 isolate (76.7 ± 10.4), which exhibited a long growth diameter compared with *Penicillium* growth **(**20 ± 1.7), meaning that the isolate reduced the growth area of phytopathogens. Among 13 fungi isolates, only one isolate (A4) exhibited antagonistic activity against *Alternaria*, as seen in Figures 14, 15, 16, and 17.

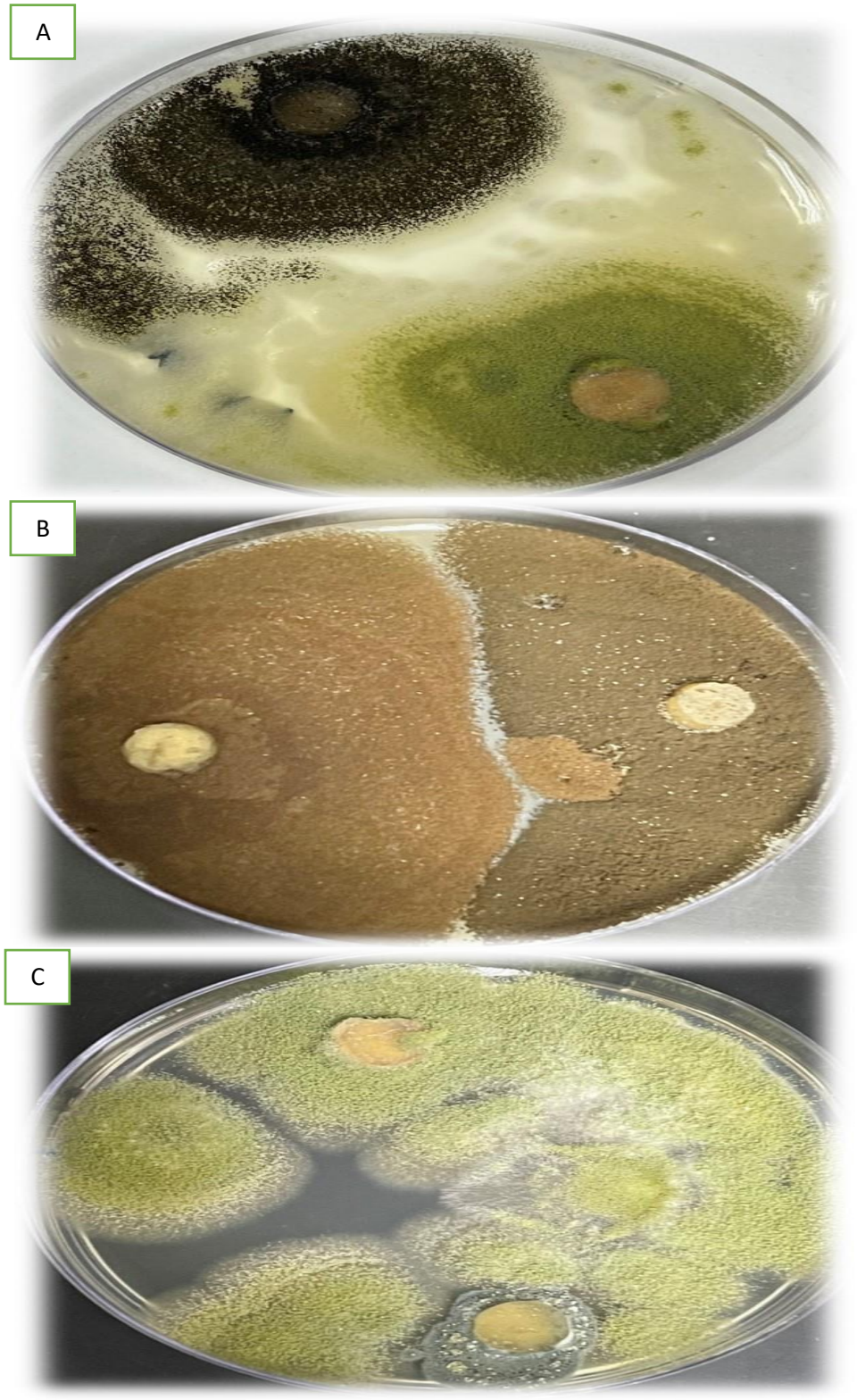

Figure 14: Antagonistic activity of the tested fungal isolates from tested plants against phytopathogenic fungi: *Aspergillus*, *Alternaria*, and *Penicillium*. (A) isolate A4 against *Aspergillus*, (B) isolate A9 against *Alternaria*, and (C) isolate A4 against *Penicillium*.

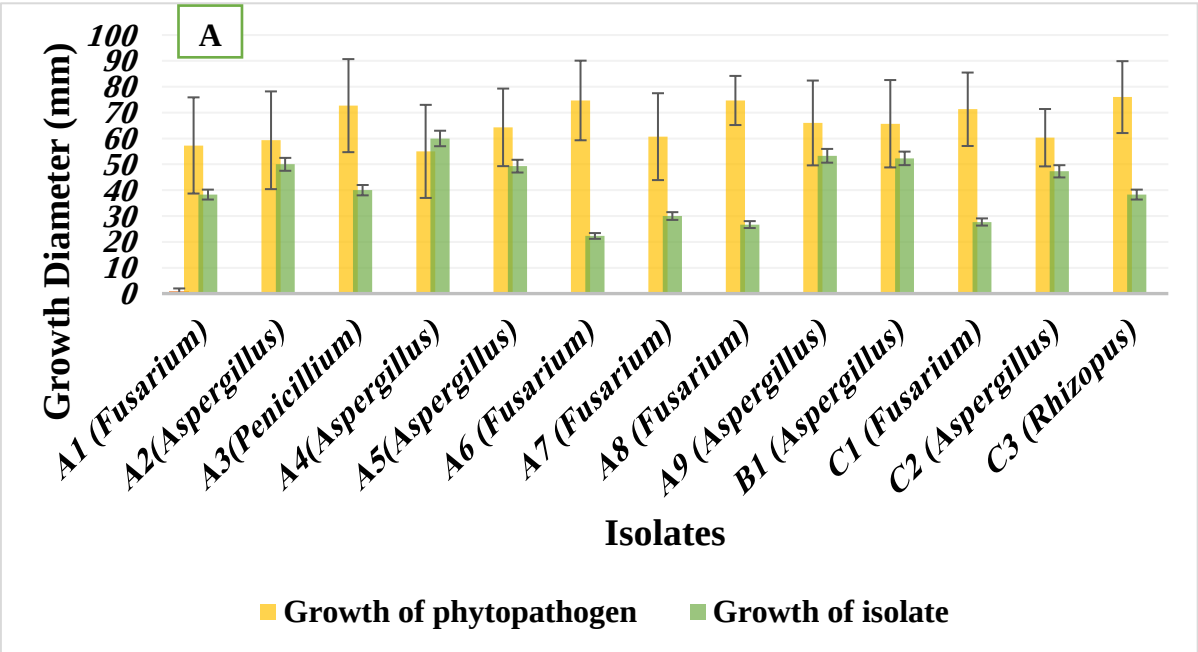

Figure 15: Antagonistic activity of the tested fungal isolates from tested plants against *Alternaria.*

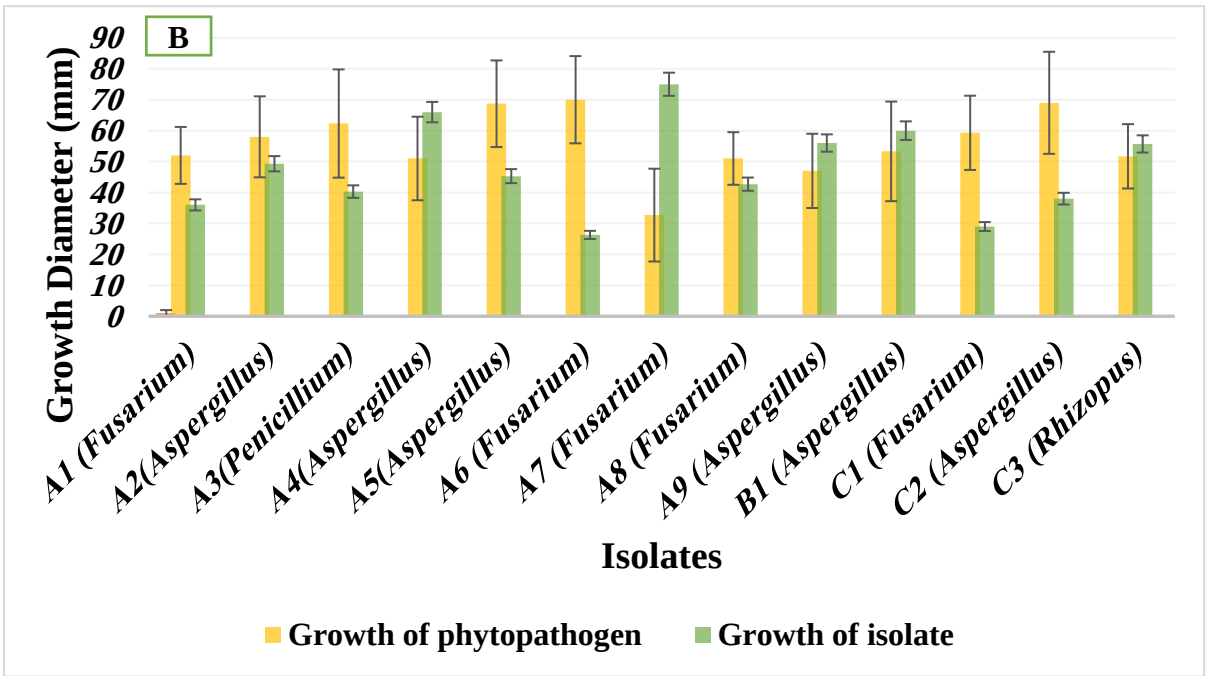

Figure 16: Antagonistic activity of the tested fungal isolates from tested plants against *Aspergillus.*

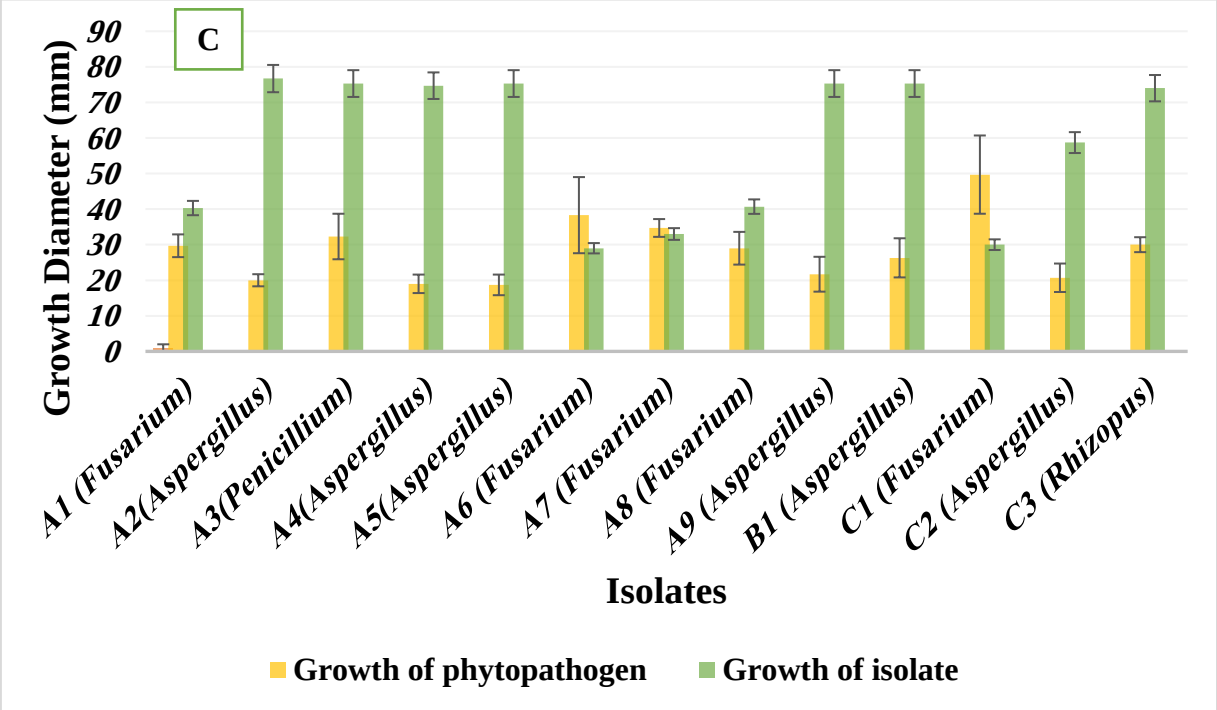

Figure 17: Antagonistic activity of the tested fungal isolates from tested plants against *Penicillium.*

4. Discussion

The present study showed that all tested bacterial isolates were Gram-positive. This is due to environmental factors such as temperature, moisture, and pH. Various environmental conditions, including temperature and moisture, impact the upper soil layers. For example, arid, semi-arid, and desert soils frequently experience high temperatures. Some observations have shown that temperatures in temperate soils from middle latitudes vary from 50 to 70 °C [33], whereas in desert regions, temperatures of 90°C have been seen [34]. Elevated temperatures are often associated with reduced water levels in the soil [35]. It was found that a *Pseudomonas* strain exhibited the highest extracellular enzyme activity in conditions of significant water activity, as conducted in an investigation by Gomez *et al.* [36]. However, a desiccation-resistant bacterium (*Deinococcus*) and a soil thermophilic isolate (*Parageobacillus*) showed the highest extracellular enzyme activity under dry conditions (specifically, water activity levels that varied from 0.5 to 0.8).

Conversely, in our current study, Firmicutes dominated with 30 isolates that belonged to the genus *Bacillus*, followed by Actinomycetota with 8 isolates. *Bacillus* species are essential

members of the microbial flora that coexists with various crops. Many authors identify *Pseudomonas* as the dominant genus in the rhizosphere owing to its faster growth rate under favorable environmental conditions than *Bacillus* [37].

Bacillus spp. predominates due to its ability to use the nutrients provided by the plant via exudates efficiently. Additionally, various investigations have reported that *Bacillus* strains have produced substances that inhibit the growth of other microorganisms [38]. The lifestyles of all members of Bacillaceae are defined by their ability to form endospores. This phenomenon allows them to survive under unfavorable conditions for long periods, a few reaching thousands of years [39]; this explains the presence of these *Bacillus* spp. in the Uhud Mountain. In contrast, an earlier investigation [40] identified *Bacillus* as the prevailing genus within the rhizosphere of *Elaeagnus angustifolia L*. In similar studies performed on pea plants by Nazir *et al.* [4], the genera *Bacillus, Pseudomonas, Micrococcus,* and *Azotobacter* were isolated. Their study recorded more strains (80 isolates) of rhizobacteria, and Gram-positive *Bacillus* dominated the rhizobacterial communities.

Our study observed the growth of actinomycetes. Similar phyla were also isolated in similar studies conducted on other plants, such as maize [41], grape [42], and peanut [43]. Most actinomycetes have significant therapeutic effects. They rely on the soils of plant rhizospheres for survival, where they can aid in preventing the infection of plants' roots by harmful bacteria. Some of them possess the capacity to produce antibiotics, siderophores, antimicrobial enzymes, and plant growth-boosting chemicals. They are also capable of phosphate solubilization and compete for food and space with plant pathogens [44]. These plant growth-promoting PGP microorganisms have the added benefit of acting as biofungicides. They can be used in conjunction with inorganic fungicides or as a substitute to control fungicide resistance among plant diseases and to minimize the number of fungicide applications per year, which would otherwise cause substantial deterioration of soil health [45].

Several enzymes, including amylase, caseinase, and lipase, have been identified in bacteria isolated from aggressive mountain regions. These enzymes exhibited thermostability and demonstrated tolerance to a diverse range of pH levels and other challenging circumstances essential to their use in industry applications [46]. Our data revealed that 26 out of 38 isolates can hydrolyze starch, followed by 21 degraded caseins, and nine isolates can hydrolyze lipids.

The bacteria isolated were tested under *in vitro* conditions for their plant growth-promoting traits. The rhizospheric soil contained abundant total bacteria, followed by N_2-fixing and phosphate-solubilizing bacteria. Additional study on biological N_2 fixation and P solubilization is crucial for energy conservation. Therefore, adding potential microorganisms to soils may improve crop productivity by preserving a healthy microbial population and facilitating atmospheric N_2 fixation while producing more soluble P through solubilizing insoluble P from the soil [47].

Agronomists have extensively researched the colonization of plant root systems or soil through seed bacterization. The primary contributing factor to this phenomenon may be attributed to the secretion of nutrients by organisms inhabiting the soil environment, often referred to as the rhizosphere. Additionally, plant roots release signal molecules known as root exudates, which further contribute to this process. These chemicals are actively involved in the bacterial construction of the rhizosphere, hence influencing colonization [48].

Bacteria employ various techniques to support their antagonistic activity against other microbes, including synthesizing toxins, peptides, antibiotics, bacteriocins, and enzymes that inhibit their competitors' growth [49]. In another test to measure the efficacy of the bacterial abilities and their impact on plant growth, bacterial isolates were tested to detect their antifungal and antibacterial abilities against phytopathogenic fungi and human pathogenic bacteria.

To ascertain the presence of bacteria with antagonistic properties against *Aspergillus*, *Alternaria*, and *Penicillium*, thirty-eight (38) bacterial isolates were examined. The primary screening results, conducted using the agar-plug diffusion method, revealed that thirty-four (34) of these isolates exhibited varying degrees of antagonism towards these three phytopathogenic fungi. Four isolates showed no antagonistic activity against phytopathogenic fungi.

The growth inhibition of phytopathogens may be due to hyperparasitism, antibiosis [50], or the production of chitinase and B-1,3-glucanase enzymes, which degrade the cell wall of the pathogens [51]. Muhammad and Amusa [52] reported that two distinct mechanisms mediated the interactions between the antagonist and the pathogen. One aspect involves the synthesis of biologically active metabolites, while the other relates to the fast proliferation of

biocontrol agents on a wet agar surface, resulting in a reduction of pathogen development. The chitinolytic enzyme, one of the cell wall-degrading enzymes, is crucial in displaying antagonism by breaking down chitin, a significant structural component of the cell wall. The additional extracellular enzymes, 1,3-glucosidase, cellulase, and protease, are additionally responsible for hyphal lysis and preventing phytopathogen proliferation [53]. Chitinases are the primary defense mechanism produced by Actinomycetota against fungi [54]. Actinomycetes produced extracellular antifungal metabolites, particularly chitinase and β -1,3 glucanase, which in *F. oxysporum* and *S. rolfsii* caused a swelling of the hyphae, aberrant morphologies, and cell wall lysis, preventing the growth of fungi [55, 56].

In the secondary screening, eleven isolates were chosen to be tested against phytopathogenic fungi using the agar-well diffusion method. Most of them belonged to *Bacillus* spp., which can develop diverse secondary metabolites with distinct structures and functions [57]. Various studies have shown that *Bacillus* species are one of the most abundant sources of bioactive natural products; they have a broad spectrum of antimicrobial activity due to their use as antifungal agents [58] and antibacterial agents [59].

Our results indicated that all eleven isolates exhibited antagonistic effects against *Aspergillus,* but none showed antagonistic activity against *Penicillium*. The agricultural application of plant protection, specifically targeting soil-borne fungal infections, is a significant study area within a particular discipline. This field focuses on adapting to the soil environment, enhancing the ability to compete effectively against other microorganisms [60].

As for evaluating the antagonistic activity of fungal isolates against tested phytopathogens, more significant differences were observed. Isolates that produced more antagonistic compounds against phytopathogens presented the highest growth area compared to pathogens' growth. Among the thirteen isolates tested, only one isolate, A4, produced compounds capable of reducing the growth of all phytopathogenic fungi. However, twelve out of thirteen fungal isolates did not reduce the growth of *Alternaria* sp. This finding is consistent with recent research by Lou *et al.* [61] that emphasizes the capacity of specific *Alternaria* isolates to generate bioactive compound metabolites that exhibit antifungal properties. Among thirteen isolated fungi, ten exhibited antagonistic activity against *Penicillium* sp., while five isolates reduced the growth of *Aspergillus* sp. Simultaneously, the highest activity was observed in the A2 isolate (76.7 ± 10.4), which exhibited a long growth diameter compared with *Penicillium* growth (20 ± 1.7), meaning that the isolate reduced the growth area of

phytopathogens. Many of the fungal isolates detected in our research are ascomycetes, recognized for their ability to generate antimicrobial substances [62]. Certain species of *Aspergillus* and *Fusarium* have been the subject of organic compound profiling, with Morath *et al.* [63] and Siddiquee *et al.* [64] having successfully isolated antifungal components.

5. Conclusion

In conclusion, using microbes as biological control factors for plant pathogens could be a promising technique for several agricultural improvements, as shown in this study. However, extensive research is required to ascertain the same antagonistic effect on other crops *in vivo*.

6. Acknowledgments

I extend my sincere appreciation to Prof. Ebaa Ebrahim El-Sharouny for her invaluable guidance and supervision.

7. Conflict of Interest

The authors declare no conflict of interest.

References

[1] Alghamdi, A.G., A.A. Aly, and H.M. Ibrahim, *Assessing the environmental impacts of municipal solid waste landfill leachate on groundwater and soil contamination in western Saudi Arabia.* Arabian Journal of Geosciences. 14(5) (2021) DOI: 10.1007/s12517-021-06583-9.

[2] Obaid, W.A., T. Boutraa, and U.K. Abdel-Hameed, *Floristics and soil characteristics of Ohud mountain, Al-Madinah Al-Munawarah, Western Saudi Arabia.* Journal of Mountain Science. 17(10) (2020): 2405-2417 DOI: 10.1007/s11629-020-6016-4.

[3] Sharma, P., M.M.M. Sharma, A. Malik, M. Vashisth, D. Singh, R. Kumar, B. Singh, A. Patra, S. Mehta, and V. Pandey, *Rhizosphere, Rhizosphere Biology, and Rhizospheric Engineering*, in *Plant Growth-Promoting Microbes for Sustainable Biotic and Abiotic Stress Management*. 2021, Springer International Publishing. p. 577-624.

[4] Nazir, U., M.Y. Zargar, Z.A. Baba, S.A. Mir, F.A. Mohiddin, and N.A. Bhat, *Isolation and characterization of plant growth promoting rhizobacteria associated with pea rhizosphere in North Himalayan region.* International Journal of Chemical Studies. 8(1) (2020): 1131-1135 DOI: 10.22271/chemi.2020.v8.i1o.8401.

[5] Korenblum, E., H. Massalha, and A. Aharoni, *Plant-microbe interactions in the rhizosphere via a circular metabolic economy.* The Plant cell. 34(9) (2022): 3168-3182 DOI: 10.1093/plcell/koac163.

[6] Jamil, F., H. Mukhtar, M. Fouillaud, and L. Dufossé, *Rhizosphere Signaling: Insights into Plant-Rhizomicrobiome Interactions for Sustainable Agronomy.* Microorganisms. 10(5) (2022): 899 DOI: 10.3390/microorganisms10050899.

[7] Summuna, B., S. Gupta, and P.A. Sheikh, *Plant Growth and Health Promoting Plant-Microbe Interactions*, in *Plant Health Under Biotic Stress*. 2019, Springer Singapore. p. 253-260.

[8] Khatoon, Z., S. Huang, M. Rafique, A. Fakhar, M.A. Kamran, and G. Santoyo, *Unlocking the potential of plant growth-promoting rhizobacteria on soil health and the sustainability of agricultural systems.* Journal of Environmental Management. 273 (2020): 111118 DOI: 10.1016/j.jenvman.2020.111118.

[9] Shabir, T., Z.A. Baba, S.A. Dar, and A.M. Husaini, *Improving Plant Growth and Quality of Plant-Products: An Interplay of Plant- microbe Interaction.* International Journal of Current Microbiology and Applied Sciences. 9(11) (2020): 3759-3766 DOI: 10.20546/ijcmas.2020.911.451.

[10] Wang, Z., H. Yang, Y. Ma, G. Jiang, X. Mei, X. Li, Q. Yang, J. Kan, Y. Xu, T. Yang, J. Lin, and C. Dong, *WGCNA analysis revealing molecular mechanism that bio-organic fertilizer improves pear fruit quality by increasing sucrose accumulation and reducing citric acid metabolism.* Frontiers in plant science. 13 (2022): 1039671-1039671 DOI: 10.3389/fpls.2022.1039671.

[11] Borah, P., N. Gogoi, S.A. Asad, A.J. Rabha, and M. Farooq, *An Insight into Plant Growth-Promoting Rhizobacteria-Mediated Mitigation of Stresses in Plant.* Journal of Plant Growth Regulation. 42(5) (2022): 3229-3256 DOI: 10.1007/s00344-022-10787-y.

[12] Lyu, D., J. Zajonc, A. Pagé, C.A.S. Tanney, A. Shah, N. Monjezi, L.A. Msimbira, M. Antar, M. Nazari, R. Backer, and D.L. Smith, *Plant Holobiont Theory: The Phytomicrobiome Plays a Central Role in Evolution and Success.* Microorganisms. 9(4) (2021): 675 DOI: 10.3390/microorganisms9040675.

[13] Selvakumar, G., P. Panneerselvam, and A.N. Ganeshamurthy, *Bacterial Mediated Alleviation of Abiotic Stress in Crops*, in *Bacteria in Agrobiology: Stress Management*. 2011, Springer Berlin Heidelberg. p. 205-224.

[14] Zolla, G., D.V. Badri, M.G. Bakker, D.K. Manter, and J.M. Vivanco, *Soil microbiomes vary in their ability to confer drought tolerance to Arabidopsis.* Applied Soil Ecology. 68 (2013): 1-9 DOI: 10.1016/j.apsoil.2013.03.007.

[15] Drost, S.M., M. Rutgers, M. Wouterse, W. de Boer, and P.L.E. Bodelier, *Decomposition of mixtures of cover crop residues increases microbial functional diversity.* Geoderma. 361 (2020): 114060 DOI: 10.1016/j.geoderma.2019.114060.

[16] Vlassak, K., L. Van Holm, L. Duchateau, J. Vanderleyden, and R. De Mot, *Isolation and characterization of fluorescent Pseudomonas associated with the roots of rice and banana grown in Sri Lanka.* Plant and Soil. 145(1) (1992): 51-63 DOI: 10.1007/bf00009541.

[17] Waksman, S.A., *Strain specificity and production of antibiotic substances. X. characterization and classification of species within the Streptomyces Griseus Group.* Proceedings of the National Academy of Sciences of the United States of America. 45(7) (1959): 1043-1047 DOI: 10.1073/pnas.45.7.1043.

[18] Cappuccino, J.G. and N. Sherman, *Microbiology: a laboratory manual*. 1992: Pearson Higher Ed.

[19] Sharma, A.K., V. Sharma, J. Saxena, B. Yadav, A. Alam, and A. Prakash, *Isolation and Screening of Extracellular Protease Enzyme from Bacterial and Fungal Isolates of Soil.* International Journal of Scientific Research in Environmental Sciences. 3(9) (2015): 334-340 DOI: 10.12983/ijsres-2015-p0334-0340.

[20] Christensen, W.B., *Urea Decomposition as a Means of Differentiating Proteus and Paracolon Cultures from Each Other and from Salmonella and Shigella Types.* Journal of bacteriology. 52(4) (1946): 461-466 DOI: 10.1128/jb.52.4.461-466.1946.

[21] Soleymani, S., H. Alizadeh, H. Mohammadian, E. Rabbani, F. Moazen, H.M. Sadeghi, Z.S. Shariat, Z. Etemadifar, and M. Rabbani, *Efficient media for high lipase production: one variable at a time approach.* Avicenna journal of medical biotechnology. 9(2) (2017): 82.

[22] Madigan, M.T., J.M. Martinko, and J. Parker, *Brock biology of microorganisms*. Vol. 11. 1997: Prentice hall Upper Saddle River, NJ.

[23] Sneath, P.H., N.S. Mair, M.E. Sharpe, and J.G. Holt, *Bergey's manual of systematic bacteriology. Volume 2*. 1986: Williams and Wilkins.

[24] Shirling, E.B. and D. Gottlieb, *Methods for characterization of Streptomyces species.* International Journal of Systematic Bacteriology. 16(3) (1966): 313-340 DOI: 10.1099/00207713-16-3-313.

[25] Gebreyohannes, G., F. Moges, S. Sahile, and N. Raja, *Isolation and characterization of potential antibiotic producing actinomycetes from water and sediments of Lake Tana, Ethiopia.* Asian Pacific journal of tropical biomedicine. 3(6) (2013): 426-435 DOI: 10.1016/S2221-1691(13)60092-1.

[26] Humber, R.A., *Fungi: identification*, in *Manual of techniques in insect pathology*. 1997, Elsevier. p. 153-185.

[27] Bauer, A.W., W.M.M. Kirby, J.C. Sherris, and M. Turck, *Antibiotic Susceptibility Testing by a Standardized Single Disk Method.* American Journal of Clinical Pathology. 45(4_ts) (1966): 493-496 DOI: 10.1093/ajcp/45.4_ts.493.

[28] Nautiyal, C.S., S. Bhadauria, P. Kumar, H. Lal, R. Mondal, and D. Verma, *Stress induced phosphate solubilization in bacteria isolated from alkaline soils.* FEMS Microbiology Letters. 182(2) (2000): 291-296 DOI: 10.1111/j.1574-6968.2000.tb08910.x.

[29] Jensen, H. *Notes on the biology of Azotobacter*. in *Proceedings of the Society for Applied Bacteriology*. 1951. Wiley Online Library.

[30] Crawford, D.L., J.M. Lynch, J.M. Whipps, and M.A. Ousley, *Isolation and characterization of actinomycete antagonists of a fungal root pathogen.* Applied and environmental microbiology. 59(11) (1993): 3899-3905 DOI: 10.1128/aem.59.11.3899-3905.1993.

[31] Biyari, S. and L. Fozouni, *The Inhibitory Effect of Probiotic Bacteria against Drug - Resistant Candida Species Isolated from the Oral Cavity of the Elderly.* Shiraz E-Medical Journal. 19(6) (2018) DOI: 10.5812/semj.62026.

[32] Zhao, X., D. Hou, J. Xu, K. Wang, and Z. Hu, *Antagonistic Activity of Fungal Strains against Fusarium Crown Rot.* Plants (Basel, Switzerland). 11(3) (2022): 255 DOI: 10.3390/plants11030255.

[33] Gonzalez, J.M., M.C. Portillo, and M. Piñeiro-Vidal, *Latitude-dependent underestimation of microbial extracellular enzyme activity in soils.* International Journal of Environmental Science and Technology. 12(7) (2014): 2427-2434 DOI: 10.1007/s13762-014-0635-7.

[34] McCalley, C.K. and J.P. Sparks, *Abiotic Gas Formation Drives Nitrogen Loss from a Desert Ecosystem.* Science. 326(5954) (2009): 837-840 DOI: 10.1126/science.1178984.

[35] Biederman, J.A., R.L. Scott, M.L. Goulden, R. Vargas, M.E. Litvak, T.E. Kolb, E.A. Yepez, W.C. Oechel, P.D. Blanken, T.W. Bell, J. Garatuza-Payan, G.E. Maurer, S. Dore, and S.P. Burns, *Terrestrial carbon balance in a drier world: the effects of water availability in southwestern North America.* Global Change Biology. 22(5) (2016): 1867-1879 DOI: 10.1111/gcb.13222.

[36] Gomez, E.J., J.A. Delgado, and J.M. Gonzalez, *Influence of water availability and temperature on estimates of microbial extracellular enzyme activity.* PeerJ. 9 (2021): e10994-e10994 DOI: 10.7717/peerj.10994.

[37] Chiniquy, D., E.M. Barnes, J. Zhou, K. Hartman, X. Li, A. Sheflin, A. Pella, E. Marsh, J. Prenni, A.M. Deutschbauer, D.P. Schachtman, and S.G. Tringe, *Microbial Community Field Surveys Reveal Abundant Pseudomonas Population in Sorghum Rhizosphere Composed of Many Closely Related Phylotypes.* Frontiers in microbiology. 12 (2021): 598180-598180 DOI: 10.3389/fmicb.2021.598180.

[38] Akpor, O.B., M.A. Okonkwo, T.A. Ogunnusi, and O.M. Oluba, *Production, characterization and growth inhibitory potential of metabolites produced by Pseudomonas and Bacillus species.* Scientific African. 15 (2022): e01085 DOI: 10.1016/j.sciaf.2021.e01085.

[39] Rizzo, C., E. Arcadi, R. Calogero, A. Ciro Rappazzo, G. Caruso, G. Maimone, A. Lo Giudice, T. Romeo, and F. Andaloro, *Deciphering the evolvement of microbial communities from hydrothermal vent sediments in a global change perspective.* Environmental Research. 240 (2024): 117514 DOI: 10.1016/j.envres.2023.117514.

[40] Ramos, B., J.M. Pozuelo González, N. Acero de Mesa, and F.J. Gutiérrez Mañero, *Seasonal variations of Bacillus isolated from the rhizosphere of Elaeagnus angustifolia L.* Orsis. 13 (1998): 007-16.

[41] Karnwal, A., *Isolation and identification of plant growth promoting rhizobacteria from maize (Zea mays L.) rhizosphere and their plant growth promoting effect on rice (Oryza sativa L.).* Journal of Plant Protection Research. 57(2) (2017): 144-151 DOI: 10.1515/jppr-2017-0020.

[42] Marasco, R., E. Rolli, M. Fusi, A. Cherif, A. Abou-Hadid, U. El-Bahairy, S. Borin, C. Sorlini, and D. Daffonchio, *Plant growth promotion potential is equally represented in diverse grapevine root-associated bacterial communities from different biopedoclimatic environments.* BioMed research international. 2013 (2013): 491091-491091 DOI: 10.1155/2013/491091.

[43] Bouhraoua, D., S. Aarab, A. Laglaoui, M. Bakkali, and A. Arakrak, *Beneficial effect of rhizobacteria inoculation on nutrition and mycorrhization of peanut grown in Morocco.* Int. J. Curr. Microbiol. App. Sci. 4(5) (2015): 748-755.

[44] Jalan, R., B. Pradhan, S. Kumar Singh, A. Das, R.L. Barik, J. Meher, R.R. Mishra, D. Dubey, and B. Chandra Behera, *Isolation and Identification of Antibacterial compound from Actinomycetes isolated from Mangrove soil.* Research Journal of Pharmacy and Technology. (2022): 1461-1466 DOI: 10.52711/0974-360x.2022.00242.

[45] Bashir, A., M.M. Manzoor, T. Ahmad, S. Farooq, P. Sultan, A.P. Gupta, and S. Riyaz-Ul-Hassan, *Endophytic fungal community of Rosa damascena Mill. as a promising source of indigenous biostimulants: Elucidating its spatial distribution, chemical diversity, and ecological functions.* Microbiological Research. 276 (2023): 127479 DOI: 10.1016/j.micres.2023.127479.

[46] Rao, T.E., M. Imchen, and R. Kumavath, *Marine enzymes: production and applications for human health*, in *Advances in food and nutrition research*. 2017, Elsevier. p. 149-163.

[47] Lee, J., N.-Y. Jo, S.-Y. Shim, L.T.Y. Linh, S.-R. Kim, M.-G. Lee, and S.-G. Hwang, *Effects of Hanwoo (Korean cattle) manure as organic fertilizer on plant growth, feed quality, and soil bacterial community.* Frontiers in plant science. 14 (2023): 1135947-1135947 DOI: 10.3389/fpls.2023.1135947.

[48] Yadav, A.N., *Plant Microbiomes for Sustainable Agriculture: Current Research and Future Challenges*, in *Sustainable Development and Biodiversity*. 2020, Springer International Publishing. p. 475-482.

[49] Amer, A., B. Hamdy, D. Mahmoud, M. Elanany, M. Rady, T. Alahmadi, S. Alharbi, and S. AlAshaal, *Antagonistic Activity of Bacteria Isolated from the Periplaneta americana L. Gut against Some Multidrug-Resistant Human Pathogens.* Antibiotics (Basel, Switzerland). 10(3) (2021): 294 DOI: 10.3390/antibiotics10030294.

[50] Nehra, S., R.K. Gothwal, S. Dhingra, A.K. Varshney, P.S. Solanki, P. Meena, P.C. Trivedi, and P. Ghosh, *Mechanism of Antagonism: Hyperparasitism and Antibiosis*, in *Microbial Biocontrol: Sustainable Agriculture and Phytopathogen Management*. 2022, Springer International Publishing. p. 257-277.

[51] Vaghela, B., R. Vashi, K. Rajput, and R. Joshi, *Plant chitinases and their role in plant defense: A comprehensive review.* Enzyme and Microbial Technology. 159 (2022): 110055 DOI: 10.1016/j.enzmictec.2022.110055.

[52] Muhammad S and A. Amusa N, *In-vitro inhibition of growth of some seedling blight inducing pathogens by compost-inhabiting microbes.* African Journal of Biotechnology. 2(6) (2003): 161-164 DOI: 10.5897/ajb2003.000-1033.

[53] Kotb, E., A.H. Alabdalall, A.I. Alghamdi, I.M. Ababutain, S.A. Aldakeel, S.K. Al-Zuwaid, B.M. Algarudi, S.M. Algarudi, A.A. Ahmed, and A.M. Albarrag, *Screening for chitin degrading bacteria in the environment of Saudi Arabia and characterization of the most potent chitinase from Streptomyces variabilis Am1.* Scientific reports. 13(1) (2023): 11723-11723 DOI: 10.1038/s41598-023-38876-2.

[54] Lacombe-Harvey, M.-È., R. Brzezinski, and C. Beaulieu, *Chitinolytic functions in actinobacteria: ecology, enzymes, and evolution.* Applied microbiology and biotechnology. 102(17) (2018): 7219-7230 DOI: 10.1007/s00253-018-9149-4.

[55] Rani, S., J.R. Kumar, M.K. Ahammed, S.K. Rao, S.R.R. Reddy B, and S.J. Devi R, *Biochemical characterization of Actinomycetes isolated from rhizosphere soils of groundnut in Andhra Pradeh.* Ecology, Environment and Conservation. 29(01) (2023): 342-346 DOI: 10.53550/eec.2023.v29i01.049.

[56] Prapagdee, B., C. Kuekulvong, and S. Mongkolsuk, *Antifungal potential of extracellular metabolites produced by Streptomyces hygroscopicus against phytopathogenic fungi.* International journal of biological sciences. 4(5) (2008): 330-337 DOI: 10.7150/ijbs.4.330.

[57] Chaabouni, I., A. Guesmi, and A. Cherif, *Secondary Metabolites of Bacillus: Potentials in Biotechnology*, in *Bacillus thuringiensis Biotechnology*. 2012, Springer Netherlands. p. 347-366.

[58] Tran, C., I.E. Cock, X. Chen, and Y. Feng, *Antimicrobial Bacillus: Metabolites and Their Mode of Action.* Antibiotics (Basel, Switzerland). 11(1) (2022): 88 DOI: 10.3390/antibiotics11010088.

[59] Yilmaz, M., H. Soran, and Y. Beyatli, *Determination of poly-β-hydroxybutyrate (PHB) production by some Bacillus spp.* World Journal of Microbiology and Biotechnology. 21(4) (2005): 565-566 DOI: 10.1007/s11274-004-3274-1.

[60] Samson, R.A., N. Yilmaz, J. Houbraken, H. Spierenburg, K.A. Seifert, S.W. Peterson, J. Varga, and J.C. Frisvad, *Phylogeny and nomenclature of the genus Talaromyces and taxa accommodated in Penicillium subgenus Biverticillium.* Studies in mycology. 70(1) (2011): 159-183 DOI: 10.3114/sim.2011.70.04.

[61] Lou, J., L. Fu, Y. Peng, and L. Zhou, *Metabolites from Alternaria fungi and their bioactivities.* Molecules (Basel, Switzerland). 18(5) (2013): 5891-5935 DOI: 10.3390/molecules18055891.

[62] Zhi-Lin, Y., C. Yi-Cun, X. Bai-Ge, and Z. Chu-Long, *Current perspectives on the volatile-producing fungal endophytes.* Critical Reviews in Biotechnology. 32(4) (2012): 363-373 DOI: 10.3109/07388551.2011.651429.

[63] Morath, S.U., R. Hung, and J.W. Bennett, *Fungal volatile organic compounds: A review with emphasis on their biotechnological potential.* Fungal Biology Reviews. 26(2-3) (2012): 73-83 DOI: 10.1016/j.fbr.2012.07.001.

[64] Siddiquee, S., S.A. Azad, F. Abu Bakar, L. Naher, and S. Vijay Kumar, *Separation and identification of hydrocarbons and other volatile compounds from cultures of Aspergillus niger by GC–MS using two different capillary columns and solvents.* Journal of Saudi Chemical Society. 19(3) (2015): 243-256 DOI: 10.1016/j.jscs.2012.02.007.

الملخص العربي

مسح للميكروبات المستوطنة في جذور بعض النباتات المتنامية على جبل أحد في المدينة المنورة

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الملخص العربي :

الخلفية: يعد ارتباط النباتات و الميكروبات أمرًا حاسمًا في عمليات النظام البيئي للمنطقة المحيطة بالجذور، حيث إن التفاعلات الإيجابية بين النباتات وفطريات الميكوريزا والبكتيريا المحفزة لنمو النبات تزيد من انتاج المحاصيل وتعمل كبدائل طبيعية للمبيدات الكيميائية للسيطرة على أمراض النبات.

الطرق: هدفت هذه الدراسة إلى عزل الميكروبات من ثلاث نباتات: *Solenostemma argel* ، *Heliotropium arbainense* ، و *Suaeda vermiculate* من جبل أحد بالمدينة المنورة.

النتائج: أظهرت معظم العزلات نتائج واعدة لصفات تحفيز نمو النبات في الظروف المختبرية، سواءً عن طريق فحص المزارع والخواص البيوكيميائية. انتمت العزلات البكتيرية إلى شعبتين هما Firmicutes و Actinomycetota ، بينما انتمت السلالات الفطرية إلى ثلاث شعب: Ascomycota ، Deuteromycota ، و Zygomycota. وقد تم تقييم كفاءة الميكروبات المعزولة في إنتاج عوامل مضادة للميكروبات ضد الميكروبات المسببة للأمراض من خلال قياس منطقة تثبيط النمو الميكروبي. أظهرت النتائج أن أكبر منطقة تثبيط كانت في عزلة البكتيريا العصوية *Bacillus* رقم C8 ضد فطر *Penicillium* (٣٠,٣ ± ٠,٦ مم) في الفحص الأولي، بينما كانت العزلة C8 هي الأكثر فعالية ضد فطر *Aspergillus* في الفحص الثانوي (٤٢,٧ ± ٨,٠ مم). وكشف تقييم النشاط المضاد للعزلات الفطرية عن أعلى نشاط للعزلة A2 فطر *Penicillium* (٧٦,٧ ± ١٠,٤).

الاستنتاج: أظهرت هذه الدراسة أن استخدام الميكروبات كعوامل مكافحة بيولوجية ضد مسببات الأمراض النباتية يمكن أن يكون استراتيجية فعالة للعديد من التطورات الزراعية.

الكلمات المفتاحية: السيطرة على الأمراض؛ *Heliotropium arbainense*؛ جبل أحد؛ معلمات تحفيز نمو النبات؛ *Solenostemma argel*؛ *Suaeda vermiculate*