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Plant Growth-Promotion Activities of Endophytic Bacteria Associated with The Medicinal Plant *Areva javanica* (Family: Amaranthaceae)

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ENDOPHYTES are ubiquitous microorganisms that reside within plant tissues and contribute to plant growth and well-being. However, knowledge about plant growth-promoting endophytes, particularly in medicinal plants, remains limited. This study aimed to isolate and characterize putative endophytic bacteria from *Aerva javanica* in the Albaha region of Saudi Arabia. Endophytic bacteria were isolated from the roots, stems, leaves, and inflorescences of the plant. The selected isolates exhibited plant growth-promoting traits such as indole-3-acetic acid (IAA) production, ammonia formation, and phosphate solubilization. Additionally, they demonstrated varying capacities to produce lytic enzymes and hydrogen cyanide (HCN), suggesting potential antifungal properties. The isolates also exhibited tolerance to different stress conditions. Partial sequencing of the 16S rRNA gene was used for bacterial identification, and NCBI BLAST analysis revealed that the isolates belonged to the genera *Micrococcus*, *Enterobacter*, *Pseudomonas*, *Delftia*, and *Bacillus*. These findings highlight the potential of endophytic bacteria to enhance plant growth and protect crops from soil-borne diseases.

Keywords: Areva javanica, Endophytes, Stress, Plant growth-promotion.

1. Introduction

Medicinal plants and their valuable secondary metabolites have widespread applications in agriculture and industry due to their economic significance. Recent research has focused on the endophytic microbiota, exploring their presence within medicinal plant tissues (Alzahrani et al., 2022; Lin et al., 2024). This investigation contributes to a better understanding of colonization, diversity, and the functional roles of endophytic bacteria. The composition of endophytic communities is influenced by various environmental factors and host-related properties, including the plant's genetic traits, developmental stage, and phenology (Goulart et al., 2019). A diverse range of bacterial species has been identified as endophytes, including Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes (Tarabulsi et al., 2024). Medicinal plants provide a complex environment shaped by diverse abiotic and biotic factors, which facilitate the colonization of specific endophytes. However, due to varying nutritional requirements at different growth stages, the physiological characteristics of plants change throughout their life cycle (El-Shabasy et al., 2023; Rat et al., 2021). Endophytes play a crucial role in promoting plant growth and overall health (Tarabulsi et al., 2024; Vardumyan et al., 2024). These beneficial microorganisms enhance plant resilience through both direct and indirect mechanisms (Noemi and Everlon, 2022). Plant growth-promotion and protection scientists use endophytic bacteria as bio-agents to facilitate sustainable agriculture. Particularly, endophytic microorganisms can produce a diverse array of metabolic compounds such as hydrogen cyanide (HCN) and lytic enzymes. Production of the secondary metabolite HCN is well-documented among prokaryotes especially within antagonistic *Pseudomonas* species. Hydrogen cyanide synthesis and antifungal activity of the bio-control strain *P. fluorescens* from Greenland highly depend on the growth medium. The known biocontrol strains *P. aeruginosa* and *P. protegens* (previously fluorescens) have revealed that the HCN synthesis in *Pseudomonas* is encoded by three biosynthetic genes, hcnA, hcnB, and hcnC, regulated in an operon structure. Hydrogen cyanide in the rhizosphere not only suppresses plant pathogens but also regulates phosphate availability. Therefore, endophytes could be exploited as 'beneficial microorganisms' in a new strategy to improve plant health and productivity. This includes nitrogen fixation, inorganic phosphate solubilization, secretion of siderophores, and production of IAA (Tarabulsi et al., 2024; El-Shabasy et al., 2023). *Aerva javanica* (Family: Amaranthaceae) is a perennial herbaceous plant known

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for its therapeutic advantage. This plant species exhibits a high level of adaptation to survive in the unique environment in the south of Saudi Arabia (El-Tayeh et al., 2021; Nagei et al., 2021; Wu et al., 2021). In a study by Suleiman (2019), the plant organs' ethanolic extract revealed antimicrobial activities against six human pathogens, and different extracts of *A. javanica* exhibited antibacterial activities. *Enterobacter cloaceae* isolated from *A. javanica* roots as an endophytic bacterial strain could perform ACC deaminase activity, nitrogen fixation, and ammonia production (Singh and Jha, 2015). The strain demonstrated tolerance to salinity up to NaCl 6% (w/v), as well as the ability to grow under stress conditions of temperature (50°C) and pH (11). In another investigation, the endophytic fungus *Cercospora* sp. PM018, isolated from the same plant, produced palmitic acid and stearic acid as antibacterials (Mookherjee et al., 2020). In this context, **t**his study aimed to isolate and identify putative endophytic bacteria from the native herbaceous plant *Aerva javanica* and evaluate their potential for plant growth promotion, antifungal activity, and stress tolerance.

2. Materials and Methods

2.1. Sampling and microbial identification

Areva javanica plant (Family: Amaranthaceae) samples were collected from Shada Al-Asfal Mountain (19°44'07.7"N 41°22'52.5" E) in Albaha region, Jeddah, Saudi Arabia (Fig. 1). The plant species identification was confirmed and recorded in the herbarium of the Biological Sciences Department at King Abdul-Aziz University. Twelve plant samples were carefully uprooted from the site, transported to the laboratory in sterile zipper bags, and stored at 4°C until use. Endophytic bacteria were isolated from fresh plant samples within 48 hours following the method described by Qin et al. (2009). Plant organs were washed five times with distilled water and allowed to dry in a laminar flow. After drying, the plant samples underwent a five-step surface sterilization procedure. The plant organs were rinsed in 5% sodium hypochlorite (NaOCl) for 5 minutes, followed by 2.5% sodium thiosulfate ($Na_2S_2O_3$) for 5 minutes, and then 75% ethanol for 1 minute. Next, the plants were rinsed in 10% sodium bicarbonate ($NaHCO_3$) for 5 minutes. Finally, the samples were washed in sterile distilled water to remove disinfectant residues (Qin et al., 2009). Small segments of all plant organs were placed directly on the agar media (starch nitrate agar, starch casein agar, tap water yeast extract agar, humic acid-vitamin agar, inorganic salts-starch agar, and potato dextrose agar). The cultures were incubated at 28°C for 1-2 weeks, the putative endophytic bacterial isolate was purified on tryptic soy agar to get pure isolates with three replicates of each colony assessed (Musa et al., 2020; Phongsopitanuna et al., 2020).

(a) (b)

Fig. 1. The plant sample and the study zone: (a) *Aerva javanica* plant sample in the field; (b) a map of Shada Al-Asfal Mountain location.

Individual colonies from the Tryptic Soy Agar (TSA) cultures were picked using a sterile toothpick and transferred into an Eppendorf tube containing 25 µL of Mastermix solution, which consisted of 50 units/mL of Taq polymerase, 400 µM dNTPs, and 3 mM $MgCl_2$. Additionally, the reaction mixture included 19 µL of ultrapure PCR water, 2 µL of the forward primer, and 2 µL of the RNA template (Weiland, 1997; Nxumalo et al., 2020). The reaction conditions were initiated by a denaturation step at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, and elongation at 72°C for 2 min (Altschul et al., 1990). These conditions were used to amplify a partial 16S rDNA fragment using the bacterial universal primers 27f (5′ AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′). PCR outcomes were sent to Macrogen, Inc. (Seoul, Korea) for sequencing. The obtained sequences were matched to similar

sequences in their 16S rRNA using the NCBI nucleotide search (BLAST), based on the GenBank database (Islam et al., 2009).

2.2. Phosphate solubilization

The isolates were inoculated onto Pikovskaya's agar plates supplemented with tricalcium phosphate and incubated at 28°C for 24 h. The experiment was performed in three replicates. Phosphate solubilization was detected by a clear zone surrounding a developing colony according to Nxumalo et al. (2020). The formula used to compute the phosphate solubilization index (PSI) was as follows:

PSI = [colony diameter (mm) + halo zone diameter (mm)] / colony diameter (mm).

2.3.Indole-3-acetic acid (IAA) production

The determination of IAA production was conducted using the standard approach established by Ndeddy and Babalola (2016). The strains were introduced into a solution of tryptic soy broth supplemented with 2 mg/ml of L-tryptophan and subjected to incubation on an orbital shaker of 150 rpm at 28°C for 72 h. The culture broths underwent centrifugation of 10,000 rpm for 10 min. Following centrifugation, 1 ml of the supernatant was combined with 2 ml of Salkowski reagent. The presence of a pink color indicates the formation of IAA. The investigation was conducted in triplicate to confirm the reaction.

2.4. Ammonia production

The ammonia generation by endophytic isolates was evaluated using the method described by Islam et al. (2009). 10 µl of recently prepared strain culture were introduced into test tubes containing 10 ml of peptone water broth and placed in an incubator at 28 C for 24 hours. The cultures were placed on a rotary shaker (150 rpm), after incubation, 1 ml of Nessler's reagent was introduced into each test tube, and any color change was recorded. A shift in the media's hue to yellow or brown indicates a beneficial outcome for ammonia production. Three replicates were conducted to reduce the sampling bias.

2.5. Antifungal activities and HCN production

The bacterial isolates were evaluated for their capacity to inhibit the growth of three phytopathogens, *Lasiodiplodia theobromae*, *Penicillium chrysogenum*, and *Aspergillus flavus*. This was achieved via the dual-culture technique that detected the percentage of radii of growth inhibition. A mycelial plug (5 mm) was used to inoculate each phytopathogen on one side of a Petri dish of 20 ml tryptic soy agar (TSA), with the plug being positioned 20 mm from the edge. Each endophytic bacterial isolate was inoculated on the opposite side. After seven days of incubation, the antagonism of the selected bacterial isolates against the phytopathogens was assessed by measuring the radius of the phytopathogen colony using the specific formula:

The percentage (%) of radial growth inhibition (PIRG) is calculated as $(R_1 - R_2/R_1) \times 100$

The hydrogen cyanide (HCN) release was detected using picric acid reagent (Pitiwittayakul et al. 2021; Mohamed et al. 2022). The bacterial cultures were streaked individually on TSA media supplemented with 0.4% (w/v) glycine. The sterile filter paper was dipped in picric acid solution (0.5%) dissolved in sodium carbonate (2%) and was attached to the ceiling of Petri plates. The plates were sealed with parafilm and incubated at 28°C for 24 h (Pranay et al. 2019). Visually, the change in the color of the filter paper from yellow to reddish-brown indicates a favorable finding.

2.6. Production of extracellular enzymes

The selected isolates were tested for their capacity to produce the hydrolytic enzymes amylase, protease, gelatinase, lipase, esterase, and cellulase on TSA media supplemented with 1% starch, skimmed milk, gelatin, Tween 80, Tween 20, and carboxymethylcellulose (CMC), respectively (Ndeddy and Babalola, 2016; Pitiwittayakul et al., 2021; Mohamed et al., 2022). The bacterial inocula were streaked on the corresponding media and incubated for 72 h at 28 C with three replicates for each tested enzyme. Catalase activity was identified by adding 1 ml of a 3% hydrogen peroxide (H_2O_2) solution to the bacterial colonies according to the method described by Pitiwittayakul et al. (2021). The enzyme index (EI) is defined as the ratio of the diameter of the halo to the diameter of the colony.

EI was detected using the following equation:

EI = Diameter of hydrolysis zone (mm) / Diameter of colony (mm). The productivity level was categorized as low ($0<EI\leq2$); moderate ($2<EI\leq3$); and high ($EI>3$).

2.7. Abiotic stress tolerance

The endophytic bacteria were exposed to different levels of salinity (NaCl), heavy metals (Cu^{2++} and Co^{2++}), high and low-temperature degrees, and pH values. The salinity resistance test was assessed by detecting bacterial growth on TSA cultures containing the concentrations 0.5%, 2.5%, 5%, 7.5%, and 10% of NaCl (Mahdi et al., 2020). The isolates were subjected to heavy metal tolerance stress by inoculating TSA plates that were supplemented with heavy metal ($CuSO_4$ and $CoSO_4$) at the different concentrations 4, 10, 20, 30, 50, 100, 150, 200, 300, 500, 700, 1000, 1250, and 1500 ppm (Mahdi et al., 2020). The temperature stress resistance by endophytic bacteria was evaluated at temperatures ranging from 4 to 60 C. The pH values ranged from 4 to 11 pH. The maximum tolerance concentration (MTC) in all tests was detected (Abdel-Rahman et al., 2021; Kumar et al., 2016; Perelomov et al., 2020).

2.8. Statistical analysis

Data were statistically analyzed using the Statistical Package of the Social Sciences (SPSS) Software Windows (version 20.0). One-way analysis of variance (ANOVA) was done where appropriate. The results were consdered statistically significant at $P \leq 0.05$.

3. Results

3.1. Isolation of endophytic bacteria

The control Petri dishes of isolation had no growth, indicating the efficacy of the five-step surface sterilization. A total of 1129 bacterial colonies were isolated from the roots, stems, leaves, and inflorescences of healthy Aerva javanica plants, obtained from Shada Alasfal Mountain in the Albaha region of Saudi Arabia. The roots and stems exhibited the greatest abundance of bacterial colonies (359 and 311 colonies respectively), while the inflorescences displayed only 199 colonies. Tryptic soy agar (TSA) and starch casein agar (SCA) were the most favorable for putative endophytic bacteria isolation, with 225 and 191 colonies, respectively. However, humic acid-vitamins agar (HVA) exhibited growth of (only 128 colonies) the lowest number of endophytic bacteria (Fig. 2). Out of all the isolated putative endophytic bacteria, only six bacterial isolates were selected for this study. These isolates were dominant across all parts of *Aerva javanica*, based on their morphological features, Gram stain results, and direct microscopic examination. Microbial identification was further confirmed using molecular genetic techniques.

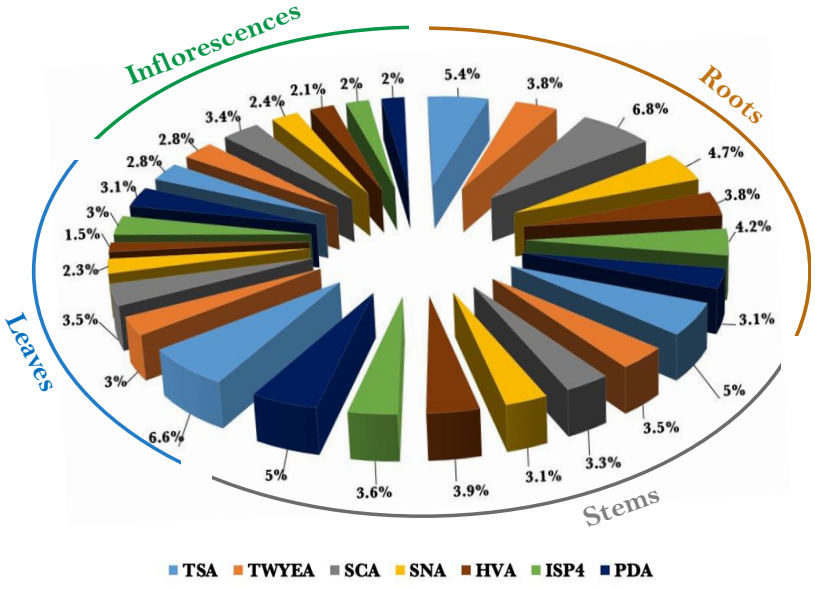

Fig. 2. The total percentage of the appeared bacterial colonies on different isolation media for each plant organ of *Aerva javanica*.

3.2. Molecular identification of the selected endophytic bacteria

Six bacterial isolates were selected for further investigation based on the variations seen among their colonies. These bacterial isolates BAB1, BAB2, BAB3, BAB4, BAB5, and BAB6 were identified genetically (Table 1). The results showed that BAB1 had a 99% similarity to the sequence of *Micrococcus* sp. Mcap_H18, BAB2 had a 99% similarity to the sequence of Enterobacter cloacae HSNJ4, BAB3 had a 99% similarity to the sequence of *Pseudomonas aeruginosa* AAII-2, BAB4 had a 99% similarity to the sequence of Delftia sp. lp09, BAB5 had a 99% similarity to the sequence of *Bacillus licheniformis* VTM1R80, and BAB6 had a 99% similarity to the

sequence of *Bacillus parabrevis* strain NAP3. The fractional 16S rRNA gene sequences obtained were compared to the succession accessible in the GenBank database using the BLAST browser (NCBI) to identify the isolated endophytic bacterial strains. Fig. 3 shows the phylogenetic tree for the six isolated endophytic bacterial strains from the medicinal plant *Aerva javanica* (naturally growing in Shada Al-Asfal Mountain, Saudia Arabia) based on comparisons of partial 16s rRNA sequences.

Table 1. Identification of the active endophytic bacteria strains isolated from *Aerva javanica* plant in Shada Al-Asfal Mountain area.

Isolate	Species	Percent of Similarity	Accession Number
BAB1	*Micrococcus* sp. Mcap_H18	100	KP640586.1
BAB2	*Enterobacter cloacae* HSNJ4	100	KY463446.1
BAB3	*Pseudomonas aeruginosa* AAII-2	100	LN558606.1
BAB4	*Delftia* sp. lp09	99	KR673339.1
BAB5	*Bacillus licheniformis* VTM1R80	100	KP245794.1
BAB6	*Brevibacillus parabrevis* NAP3	99	KJ872854.1

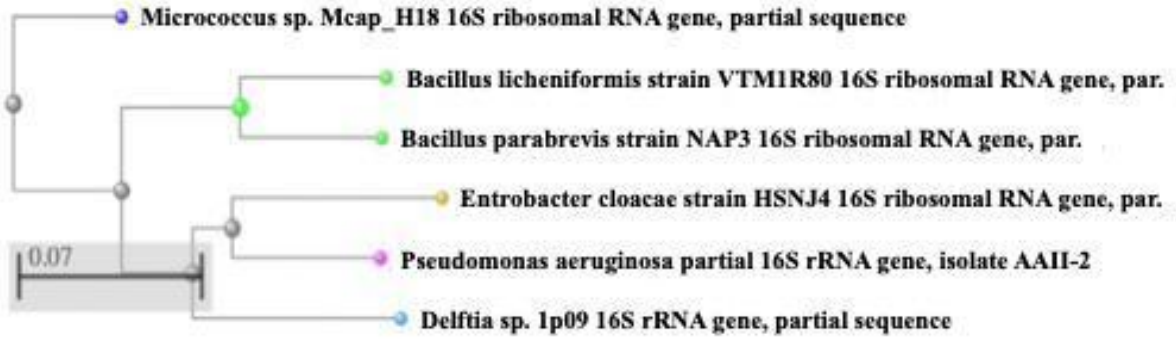

Fig. 3. Phylogenetic analysis of the 16S rRNA sequences of the bacterial isolates was conducted according to database from NCBI.

3.3. Direct PGP activities of *A. javanica* bacterial putative endophytes

The isolated endophytic bacterial strains were evaluated for plant growth-promotion traits, including phosphate solubilization, indole-3-acetic acid (IAA) production, and ammonia generation. Of the bacterial isolates, 50% exhibited phosphate solubilization ability, while 66.7% showed IAA production. Notably, only one isolate demonstrated a complete lack of ammonia production capability. Among the isolates, BAB6 exhibited the highest potential for phosphate solubilization, with an effectiveness index (EI) of 2.75. Additionally, BAB1, BAB4, and BAB6 showed high IAA production, while BAB2 and BAB5 exhibited the highest ammonia production (Table 2).

Table 2. Phosphate solubilization index (PSI), IAA release, ammonia formation, and HCN production activities by the endophytic bacteria strains.

Bacterial Isolate	Phosphate Solubilization Index	IAA Production	Ammonia Production		HCN Production	
			Color	Growth Rate	Color	Growth Rate
Micrococcus sp. Mcap_H18	1.00	-	Light Yellow	+	Reddish Brown	+++
Enterobacter cloacae HSNJ4	1.00	++	Brownish Yellow	+++	Light Brown	+
Pseudomonas aeruginosa AAII-2	3.65	++	Light Yellow	+	Dark Brown	++
Delftia sp. lp09	1.00	++	Yellow	-	Dark Brown	++
Bacillus licheniformis VTM1R80	3.38	-	Brownish Yellow	+++	Light Brown	+
Brevibacillus parabrevis NAP3	3.75	+	Yellowish Brown	++	Dark Brown	++

3.4. Indirect PGP activities of *A. javanica* bacterial putative endophytes

All the tested bacterial isolates were positive for HCN production, with the BAB1 strain showing the highest production of HCN (Table 2). All endophytic bacterial strains displayed antagonistic effects against the pathogenic fungi, *Aspergillus flavus*, *Lasiodiplodia theobromae*, and *Penicillium chrysogenum*. However, only one bacterial isolate could not suppress the growth of *L. theobromae*. *Bacillus licheniformis* VTM1R80 exhibited the highest antifungal properties (80.3 % inhibition) against *L. theobromae*. *Pseudomonas aeruginosa* AAII-2 also showed a strong inhibitory effect towards *L. theobromae* (64.4%). However, *Micrococcus* sp. Mcap_H18 demonstrated any inhibition activity of 8.0%, 35.6%, and 29.9% to *L. theobromae*, *P. chrysogenum*, and *A. flavus*, respectively. The endophytic bacterial strain *Pseudomonas aeruginosa* AAII-2 caused 52.9% inhibition to the phytopathogenic fungus *Penicillium* chrysogenum and 29.2 % inhibition to *A. flavus*. For *Aspergillus flavus*, all bacterial strains showed limited inhibition percentages ranging from 22.9% to 31.9% (Table 3).

Table 3. Antagonistic activity of the endophytic bacteria strains against phytopathogenic fungi, showing colonies diameter (mm) and Percentage of inhibition (%).

Isolates	*Lasiodiplodia theobromae*		*Penicillium chrysogenum*		*Aspergillus flavus*	
	Colony Diameter (mm)	Percentage of Inhibition	Colony Diameter (mm)	Percentage of Inhibition	Colony Diameter (mm)	Percentage of Inhibition
Control	44.0±3.0a	0.0%	29.0±3.0a	0.0%	48.0±3.0a	0.0%
Micrococcus sp. Mcap_H18	43.7±1.5a	8.0%	18.7±2.1bc	35.6%	33.7±2.5bc	29.9%
Enterobacter cloacae HSNJ4	40.3±1.2a	8.3%	27.3±1.5b	5.7%	37.0±1.7bc	22.9%
Pseudomonas aeruginosa AII-2	15.7±2.1d	64.4%	13.7±2.1bc	52.9%	34.0±1.7bc	29.2%
Delftia sp. lp09	27.3±2.1c	37.9%	23.7±2.1bc	18.4%	35.5±2.1bc	25.7%
Bacillus licheniformis VTM1R80	8.7±3.5e	80.3%	18.7±2.1c	35.6%	32.7±2.1c	31.9%
Brevibacillus parabrevis NAP3	36.0±1.0b	18.2%	29.0±2.0b	0.0%	37.0±2.0b	22.9%

The endophytic bacteria were investigated for their tendency to synthesize the hydrolytic enzymes, amylase, protease, gelatinase, cellulase, lipase, and esterase. The enzymatic index (EI) for the endophytic bacterial strain was considered, in the case of, the ability to produce at least three of the six subjected enzymes (Table 4). All strains were able to produce amylase enzymes. Four strains produced protease, gelatinase, and esterase enzymes. However, only two strains showed lipolytic activity, and three strains exhibited cellulolytic activity. Certain strains demonstrated exceptional enzymatic efficiency. *Micrococcus* sp. Mcap_H18 strain realized the highest amylase and gelatinase production of 1.38 EI and 4.94 EI, respectively. *Pseudomonas aeruginosa* AAII-2 showed the highest protease and esterase production, with EI of 2.59 and 2.00, respectively. On the other hand, *Bacillus licheniformis* VTM1R80 demonstrated the highest cellulase production (2.0 EI). The endophytic bacteial strains *Pseudomonas aeruginosa* AAII-2 and *Delftia* sp. lp09 exhibited high EI readings (0.96 and 1.00, respectively) for lipases biosynthesis.

Table 4. The enzymes activity index of the endophytic bacteria isolated from *Areva javanic*.

Isolate	Production of Enzymes by (Enzyme Index)						Catalase Production*
	Amylase Production	Protease Production	Gelatinase Production	Lipase Production	Esterase Production	Cellulase Production	
***Micrococcus* sp. Mcap_H18**	1.38a	0d	4.98a	0d	1.32c	1.14b	+++
***Enterobacter cloacae* HSNJ4**	1.06c	1.14c	1.49c	0d	0d	0d	+
***Pseudomonas aeruginosa* AAII-2**	1.09b	2.6a	3.4b	0.94b	2.02a	1.1bc	+++
***Delftia* sp. lp09**	1.09b	1.44bc	0d	1.13a	1.45b	0d	+
***Bacillus licheniformis* VTM1R80**	1.35a	0d	0d	0d	1.18bc	2a	++
***Brevibacillus parabrevis* NAP3**	1.16ab	1.72b	3.37b	0d	0d	1c	++

* +: low production rate, ++: moderate production rate, +++: high production rate

Abiotic stress resistance of the isolated endophytic bacterial strains was detected by observing their development at different levels of sodium chloride, temperatures, pH, and heavy metals. Most of the endophytic strains demonstrated the capability to thrive in the presence of 2.5% salt, except for E. cloacae HSNJ4 and B. parabrevis NAP3, which exhibited no ability to grow under salinity stress. *Micrococcus* sp. Mcap_H18 and *Bacillus licheniformis* VTM1R80 reached their maximum growth rates of 7.5% and 10% respectively (Table 5). Regarding temperature stress, only three strains were able to grow at 20°C, even though all bacterial strains were capable of growing within the range of 28-37°C. In addition, *E. cloacae* HSNJ4, *P. aeruginosa* AAII-2, *B. licheniformis* VTM1R80, and *B. parabrevis* NAP3 tolerated temperatures up to 50°C (Table 5). The bacterial strains were investigated further for their ability to thrive in low and high-pH milieu. All strains could grow at a minimum pH of 6, while their tolerance to higher pH levels varied. For example, *E. cloacae* HSNJ4 and *B. parabrevis* NAP3 tolerated up to pH of 9 and 10, respectively. At the same time, the other isolates grew up to a pH of 11 (Table 5).

Table 5. Stress tolerance by the selected bacteria at different stress conditions of salinity, pH, high and low temperatures.

Stress tolerance	condition	Bacterial Isolates					
		Micrococcus sp. Mcap_H18	*Enterobacter cloacae* HSNJ4	*Pseudomonas aeruginosa* AAII-2	*Delftia* sp. Lp09	*Bacillus lichen-iformis* VTM1R80	*Brevibacillus parabrevis* NAP3
Salinity	0.5%	+++	+++	+++	+++	+++	+++
	2.5%	+++	-	++	++	+++	-
	5%	++	-	-	-	+++	-
	7.5%	+	-	-	-	++	-
	10%	-	-	-	-	+	-
	12.5%	-	-	-	-	-	-
	15%	-	-	-	-	-	-
pH	pH4	-	-	-	-	-	-
	pH5	-	-	-	-	-	-
	pH6	+++	++	++	++	+++	+++
	pH7	+++	+++	+++	+++	+++	+++
	pH8	+++	+++	+++	+++	+++	+++
	pH9	+++	++	+++	+++	+++	++
	pH10	+++	-	+++	+++	+++	+
	pH11	++	-	+++	++	++	-
Temperature	4 °C	-	-	-	-	-	-
	10 °C	-	-	-	-	-	-
	20 °C	-	-	+	++	+	-
	28 °C	+++	+++	+++	+++	+++	+++
	37 °C	+++	+++	+++	+++	+++	+++
	45 °C	-	+++	+	-	++	++
	50 °C	-	++	-	-	-	-
	55 °C	-	-	-	-	-	-
	60 °C	-	-	-	-	-	-

+: low production rate, ++: moderate production rate, +++: high production rate

The endophytic bacteria strains demonstrated resistance to copper sulfate and cobalt sulfate, which were used in heavy metal stress tests. *B. licheniformis* VTM1R80 was the lowest resistant strain to copper sulfate, with MIC at 200 ppm Cu^{2++}. In contrast, *E. cloacae* HSNJ4 and *P. aeruginosa* AAII-2 showed higher resistance up to 300 ppm Cu^{2++}. Nevertheless, *Micrococcus* sp. Mcap_H18, *Delftia* sp. lp09, and *B. parabrevis* NAP3 exhibited remarkable resistance to Cu^{2++} concentrations at 500, 700, and 1000 ppm, respectively, (Table 6). However, the endophytic bacterial strains exhibited higher resistance to cobalt sulfate than copper sulfate. For example, *B. parabrevis* NAP3 exhibited MIC at 1500 ppm of cobalt sulfate, followed by *Delftia* sp. lp09 and *Micrococcus* sp. Mcap_H18 with concentrations of 1250 and 1000 ppm, respectively. At 500 ppm, both *P. aeruginosa* AAII-2 and *B. licheniformis* VTM1R80 exhibited their maximum tolerance to cobalt sulfate. *E. cloacae* HSNJ4 MIC was resistant showing higher MIC of cobalt sulfate at 700 ppm (Table 6).

Table 6. Heavy metal stress tolerance by the selected bacteria to different concentrations of copper and cobalt.

Heavy metal	Con. ppm	Bacterial Isolates					
		Micrococcus sp. Mcap_H18	*Enterobacter cloacae* HSNJ4	*Pseudomonas aeruginosa* AAII-2	*Delftia* sp. Lp09	*Bacillus lichen-iformis* VTM1R80	*Brevibacillus parabrevis* NAP3
Copper Sulphate	4	+++	+++	+++	+++	++	+++
	10	+++	+++	+++	+++	++	+++
	30	+++	+++	++	+++	++	+++
	50	+++	+++	++	+++	++	+++
	100	+++	+++	++	+++	+	+++
	150	+++	+++	+	+++	+	+++
	200	+++	+++	+	+++	+	+++
	300	++	+	+	++	-	+++
	400	++	-	-	++	-	+++
	500	+	-	-	++	-	+++
	700	-	-	-	+	-	++
	1000	-	-	-	-	-	+
	1250	-	-	-	-	-	-
	1500	-	-	-	-	-	-
Cobalt Sulphate	4	+++	+++	+++	+++	+++	+++
	10	+++	+++	+++	+++	+++	+++
	30	+++	+++	+++	+++	+++	+++
	50	+++	+++	+++	+++	+++	+++
	100	+++	+++	+++	+++	+++	+++
	150	+++	++	++	+++	++	+++
	200	+++	++	++	+++	++	+++
	300	+++	++	++	+++	++	+++
	400	+++	++	++	+++	++	+++
	500	++	++	+	+++	+	+++
	700	++	+	-	+++	-	+++
	1000	+	-	-	++	-	++
	1250	-	-	-	+	-	+
	1500	-	-	-	-	-	-

+: low production rate, ++: moderate production rate, +++: high production rate

4. Discussion

Plant growth-promotion bacteria are promising tools to enhance plant growth and increase crop yields in sustainable agriculture (Gaafar et al., 2021). Endophytic bacteria are valuable bioresources due to their capacity to inhabit the internal tissues of plants through direct contact (Chaturvedi et al., 2016). Plants face ongoing challenges from their surroundings, including living organisms and non-living factors including infections, high and low pH levels, extreme temperatures, and heavy metals. Endophytes can assist these plants through various direct and indirect means, offering possible support (Tarabulsi et al., 2024). The study aimed to isolate and characterize the selected endophytic bacteria from Aerva javanica plant based on their morphological features. The following six plant growth-promoting bacteria were isolated from various parts of *A. javanica*. They were genetically identified as *Micrococcus* sp. Mcap_H18, Enterobacter cloacae, Pseudomonas aeruginosa, *Delftia* sp., *Bacillus licheniformis*, and *Brevibacillus parabrevis*. *Bacillus*, *Enterobacter*, and *Pseudomonas* are the predominant genera of bacterial putative endophytes, they can contribute to plant health through two distinct processes "direct and indirect" (Devi et al., 2017; Khan et al., 2022; Pinto et al., 2022). These strategies involve the active participation of bacteria in promoting plant development (Noemi and Everlon, 2022), e.g. enhancement of nutrient uptake and phytohormone levels in the plant. These interactions directly contribute to the development of the root system, and increase biomass production. Due to these advantages, they can be labeled as biofertilizers (Bamisile, 2018). Initially, the direct actions of PGP have been conducted by screening for the solubilization of phosphate, the formation of ammonia, and IAA production. 50% of the bacterial isolates were capable of exhibiting phosphate solubilization (El-Shabasy et al., 2023). The genera that showed the highest level of productivity were *Bacillus* and *Pseudomonas*. Pinto et al. (2022) have documented comparable findings. Endophytic bacteria can produce indole acetic acid (IAA), an important hormone that regulates plant growth. They do this by using different routes and using tryptophan as the major building block (Rustamova et al., 2022). Our analysis revealed that all the isolates tested, except BAB1 and BAB5 strains, could produce IAA.

Extensive evidence exists to support the fact that the majority of endophytic bacteria are capable of synthesizing IAA (Mukherjee et al., 2017; Khan et al., 2022).

The generation of ammonia is a significant characteristic of bacteria that promotes plant growth. It collects and provides nitrogen to their host plants, enhancing plant development. Several investigations have documented the ammonia generation by endophytic bacteria. All bacterial strains exhibited positive ammonia production in this investigation. The strains have been classified as diazotrophic bacteria due to their capacity to convert gaseous nitrogen into a useful form of ammonia (Ji et al., 2014), the results align with the discoveries made by Khan et al. (2022) and Pinto et al. (2022). All the examined bacterial strains showed positive results for the HCN production test. It was thought that the synthesis of HCN contributes to the stimulation of plant growth by inhibiting plant diseases (Lin et al., 2024; Wu et al., 2021). HCN synthesis is believed to increase the availability of phosphorus by chelating metals, thereby indirectly increasing the availability of nutrients in host plants (Agbodjato et al., 2015; Rijavec and Lapanje, 2016).

Additionally, indirect PGP activities encompass the synthesis of secondary metabolites, such as antimicrobial agents and enzymes. These activities protect plants against biotic threats, such as pests, and abiotic challenges, including salt salinity, extreme pH levels, temperatures, and heavy metal exposure. This work investigated indirect pathways by conducting screenings for antifungal activity against pathogenic fungi, lytic enzymes, and abiotic stimuli such as salt salinity, high and low pH, temperatures, and heavy metals like copper sulfate and cobalt sulfate. These parameters have significant impacts on agricultural output and serve as important limitations. The prevalence of salt salinity is extensive, impacting around 10% of the Earth's land surface, particularly in regions with irrigation (Abdel-Rahman et al., 2021; Singh et al., 2019). Furthermore, fluctuations in temperature can induce several forms of stress in plants, including osmotic damage, desiccation, loss of stomatal control, and decreased efficiency of the photosynthetic machinery (Singh et al., 2020; Al-Ghamdi 2022). Research has demonstrated that a rapid rise in the surrounding temperature of 5-7 degrees Celsius induces heat stress in plants. According to Ljubej et al. (2021), it disrupts photosynthesis, decreases plant water availability, hinders flowering and fruiting, and attracts pests and illnesses. The pH of soil primarily affects the water in the soil and has a crucial role in influencing soil biology, chemistry, and physical processes. These factors directly affect the growth and development of plants and the productivity of crops (Msimbira and Smith, 2020), while there is variation among plants in their ability to withstand severe pH levels, the majority of agricultural plants thrive best when the pH is close to neutral (Al-Ghamdi 2022). Heavy metal contamination of soil is a form of mineral toxicity that has a physiological impact on plants. The presence of abnormally high levels of heavy metals in soils can be attributed to mining activities, the composition of the parent rocks, and the processing of metals. Plant growth is significantly impacted by a high concentration of heavy metals, as it results in toxic effects that hamper the plants' ability to absorb nutrients. This, in turn, leads to damage to the membrane integrity and enzyme activity of the plants' cells (Elsadany et al., 2024; Abdelhady et al., 2024). Endophytic bacteria engage in several interactions with plants, including control of plant diseases through antagonism, which indirectly enhances plant development. Endophytes possessing plant growth-promoting properties are employed as a substitute for chemical pesticides in terms of plant protection (Abdel-Rahman et al., 2021). Our results indicated that every endophytic bacteria strain exhibited an inhibition zone against the used phytopathogenic fungi. These endophytes can act as biocontrol agents by suppressing the growth of pathogenic microbes. Endophytes possess enzymatic capabilities that enable them to defend their host plants by breaking down the cell walls of harmful bacteria, as described by Ben Slama (2019). Endophytes acquire nutrients from plants through the secretion of enzymes. These enzymes enhance plant nutrition and contribute to plant aging by breaking down certain organic compounds within the plants (Singh et al., 2020).

5. Conclusions

The *Aerva javanica* medicinal plant, native to Shada Al-Asfal Mountain, provides an ecological niche for a variety of bacterial endophytes. The isolated putative endophytic bacterial strains exhibited varying activities related to IAA production, ammonia formation, and phosphate solubilization, which are direct traits for plant growth promotion. Additionally, these strains demonstrated the ability to produce hydrogen cyanide (HCN) and several lytic enzymes with antifungal properties. The bacterial strains also showed tolerance to various stress conditions, including pH, temperature, heavy metals, and salinity. These findings suggest the potential of endophytic bacterial strains to protect plant crops from soil-borne pathogens and promote their growth and development. While further research under field conditions is needed to validate these findings, this approach appears to be a promising tool for sustainable agriculture.

Declarations

Neither this manuscript nor one with substantially similar content under our authorship has been published or is being considered for publication elsewhere**.**

Ethics approval and consent to participate

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References

Abdel-Rahman, H.M., Zaghloul, R.A., Hassan, E.A., El-Zehery, H.R.A. and Salem, A. A. (2021) New strains of plant growth-promoting rhizobacteria in combinations with humic acid to enhance squash growth under saline stress. *Egypt. J. Soil. Sci.*, 61, 129-146.

Abdelhady, M., Abdelghany, T., Mohamed, S., & Abdelhamed, S. (2024). Biological Application of Zinc Oxide Nanoparticles Created by Green Method. *Egypt. J. Soil. Sci.*, 64(3): 757- 775.

Agbodjato, N., Noumavo, P., Baba Moussa, F. (2015). Characterization of potential plant growth promoting rhizobacteria isolated from Maize (Zea mays L.) in central and Northern Benin (West Africa). *Appl. Environ. Soil Sci.* 01-09.

Al-Ghamdi, A. Y. (2022). Phytochemical screening, and *in vitro* antimicrobial potential of *Aerva javanica* leaf extracts, collected from Shada Mountain, Al-Baha, Saudi Arabia. *Novel Res. Microbiol. J.* 6(2), 1515-1529.

Altschul, S., Gish, W., Miller, W. (1990) Basic local alignment search tool. J. Mol. Biol. 215(3) 403–410.

Alzahrani, K., Jastaniah, S., Amasha, R. (2022). Diversity and Colonization of Endophytic Actinomycetes in some Medicinal Plants: Review. *J. Contemp. Med. Sci.* 8(3).

Bamisile, B., Dash, C., Akutse, K. (2018). Fungal endophytes: beyond herbivore management. *Front Microbiol.* 9(11).

Ben Slama, H., Triki, M., Chenari Bouket, A. (2019). Screening of the High-Rhizosphere Competent Limoniastrum monopetalum' Culturable Endophyte Microbiota Allows the Recovery of Multifaceted and Versatile Biocontrol Agents. *Microorganisms* 7(8), 249.

Chaturvedi, H., Singh, V. and Gupta, G. (2016). Potential of bacterial endophytes as plant growth promoting factors. *J. Plant Pathol. Microbiol.* 7, 376.

Devi, K. A, Pandey G, Rawat, A. K. S., Sharma, G. D., Pandey, P. (2017). The Endophytic Symbiont-Pseudomonas aeruginosa Stimulates the Antioxidant Activity and Growth of *Achyranthes aspera* L. *Front Microbiol.* 27(8), 1897.

El-Shabasy, S. A., Hamissa, A. E. M., Talha, N. I., & ElBasuny, A. A. (2023). Effect of Different Phosphate Fertilizers on the Availability of some Heavy Metals in El-Gabal El Asfer Soil under Maize and Wheat Cropping System. *Egypt. J. Soil. Sci.*, 63(3), 267-275.

Elsadany, A., B Belal, E., Ibrahim, S., Abdallah, S., Khafagy, D., & Khalil, A. (2024). Biosorption of some heavy metals from wastewater by *Pseudoanabaena mucicola*. *Egypt. J. Soil. Sci.*, 64(1), pp: 313-323 DOI: 10.21608/EJSS.2024.249891.1689.

El-Tayeh, N., Galal, H., Soliman, M. (2020). Association of Morphological, Ecological, and Genetic Diversity of Aerva javanica Populations Growing in the Eastern Desert of Egypt. *Agronomy* 10, 402.

Gaafar, D. E., Baka, Z. A., Abou-Dobara, M. I. , Shehata, H. S., El Tapey H. M. (2021). Microbial Impact on Growth and Yield of *Hibiscus sabdariffa* L. and Sandy Soil Fertility. *Egypt. J. Soil. Sci.* 61, (2) 259-274.

Goulart, M., Cueva-Yesquén, L., Hidalgo Martinez, K. (2019). Comparison of specific endophytic bacterial communities in different developmental stages of Passiflora incarnata using culture-dependent and culture-independent analysis. *Microbiology* Open 8, e896.

Islam, M., Madhaiyan, M., Deka Boruah, H. (2009). Characterization of plant growth-promoting traits of free-living diazotrophic bacteria and their inoculation effects on growth and nitrogen uptake of crop plants. *J. Microbiol. Biotechnol.* 19(10), 1213–1222.

Ji, S., Gururani, M., Chun, S. (2014). Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. *Microbiol. Res.* 169, 83–98.

Khan, M. S., Gao, J., Zhang, M., Xue, J., Zhang, X. (2022). *Pseudomonas aeruginosa* Ld-08 isolated from Lilium davidii exhibits antifungal and growth-promoting properties. *PLOS ONE* 17(6), e0269640.

Kumar, A., Singh, R., Yadav, A. (2016). Isolation and characterization of bacterial endophytes of Curcuma longa L. *3 Biotech* 6, 60.

Lin, X., Wang, M., Xie, F., Cheng, Y., Yang, L., Gao, J., Li, W., Zhang, X., Tang, T. (2024). Endophytic Bacteria *Enterobacter cloacae* PN7 Promotes Biosynthesis and Accumulation of Saponins in Panax notoginseng. Curr Microbiol. 82(1):41.

Ljubej, V., Karalija, E., Salopek-Sondi, B. (2021). Effects of Short-Term Exposure to Low Temperatures on Proline, Pigments, and Phytochemicals Level in Kale (Brassica oleracea var. acephala). *Horticulturae* 7, 341.

Mahdi, I., Fahsi, N., Hafidi, M. (2020). Plant Growth Enhancement using Rhizospheric Halotolerant Phosphate Solubilizing Bacterium Bacillus licheniformis QA1 and Enterobacter asburiae QF11 Isolated from Chenopodium quinoa Willd. *Microorganisms* 8, 948.

Mohamed, A., Abd El-Megeed, F., Hassanein, N. (2022). Native Rhizospheric and Endophytic Fungi as Sustainable Sources of Plant Growth Promoting Traits to Improve Wheat Growth under Low Nitrogen Input. *J. Fungi* 8, 94.

Mookherjee, A., Mitra, M., Kutty, N. (2020). Characterization of endo-metabolome exhibiting antimicrobial and antioxidant activities from endophytic fungus Cercospora sp. PM018. *South African J. Bot.* 134.

Msimbira, L. and Smith, D. (2020). The Roles of Plant Growth Promoting Microbes in Enhancing Plant Tolerance to Acidity and Alkalinity Stresses. *Front. Sustain. Food Syst.* 4, 106.

Mukherjee, A., Bhattacharjee, P., Das, R., Pal, A., Paul, A. K. (2017). Endophytic bacteria with plant growth promoting abilities from *Ophioglossum reticulatum* L. *AIMS Microbiol.* 3(3), 596-612.

Musa, Z., Ma, J., Egamberdieva, D. (2020). Diversity and Antimicrobial Potential of Cultivable Endophytic Actinobacteria Associated with the Medicinal Plant Thymus roseus. *Front. Microbiol.* 11.

Nagei, T., Alshaeri, S., Alsulaimany, F. (2021). The Role of Aerva Javanica (Burm. F.) Juss. Growing in Jazan, Saudi Arabia as Antimicrobial and Coagulating Factor. *Euro. J. Appl. Sci.* 9(5), 456-471.

Ndeddy Aka, R. and Babalola, O. (2016). Effect of bacterial inoculation of strains of Pseudomonas aeruginosa, Alcaligenes feacalis and Bacillus subtilis on germination, growth and heavy metal (Cd, Cr, and Ni) uptake of *Brassica juncea*. *Int. J. Phytorem.* 18, 200–209.

Noemi, C. and Everlon, C. (2022). Endophytic fungi: a tool for plant growth promotion and sustainable agriculture. *Mycology* 13(1), 39-55.

Nxumalo, C., Ngidi, L., Shandu, J. (2020). Isolation of endophytic bacteria from the leaves of Anredera cordifolia CIX1 for metabolites and their biological activities. *BMC Complement Med. Ther.* 20, 300.

Perelomov, L., Sizova, O., Rahman, M. (2022). Metal-Tolerant Bacteria of Wastewater Treatment Plant in a Large City. *Sustainability* 14, 11335.

Phongsopitanuna, W., Sripreechasakc, P., Rueangsawang, K. (2020). Diversity and antimicrobial activity of culturable endophytic actinobacteria associated with Acanthaceae plants. *Science Asia* 46, 288-296.

Pinto, M., Inocente, L., Oliveira, P. (2022). Plant Growth-Promoting (PGP) Traits of Endophytic Bacteria from In *Vitro* Cultivated *Tectona grandis* L.f. *Forests* 13(10), 1539.

Pitiwittayakul, N., Wongsorn, D., Tanasupawat, S. (2021) Characterisation of Plant Growth-Promoting Endophytic Bacteria from Sugarcane and Their Antagonistic Activity against Fusarium moniliforme. *Trop. Life Sci. Res.* 32(3), 97-118.

Pranay, K., Padmadeo, S., Jha, V. (2019). Screening and identification of amylase producing strains of *Bacillus*. *J. Appl. Biol. Biotech* 7, 57–62.

Qin, S., Li, J., Chen, H. (2009). Isolation, diversity, and antimicrobial activity of rare actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. *Appl. Environ. Microbiol.* 75, 6176-6186.

Rat, A., Naranjo, H. D., Krigas, N., Grigoriadou, K., Maloupa, E., Alonso, A. V., Schneider, C., Papageorgiou, V. P., Assimopoulou, A. N., Tsafantakis, N., Fokialakis, N., Willems, A. (2021). Endophytic Bacteria From the Roots of the Medicinal Plant Alkanna tinctoria Tausch (Boraginaceae): Exploration of Plant Growth Promoting Properties and Potential Role in the Production of Plant Secondary Metabolites. Front. Microbiol. 12, 633488. doi: 10.3389/fmicb.2021.633488

Rijavec, T. and Lapanje, A. Hydrogen cyanide in the rhizosphere: not suppressing plant pathogens, but rather regulating a vailability of phosphate. *Front. Microbiol.* 2016, 7, 1785.

Rustamova, N., Wubulikasimu, A., Mukhamedov, N., Gao Y., Egamberdieva, D., Yili A. (2020). Endophytic Bacteria Associated with Medicinal Plant *Vernonia anthelmintica*: Diversity and Characterization. *Curr Microbiol.* 77(8):1457-1465.

Singh, D., Kaur, S., Kumar, A. (2020). In vitro drought tolerance in selected elite clones of Eucalyptus tereticornis Sm. *Acta Physiol. Plant* 42, 1–9.

Singh, R. and Jha, P. (2015). Plant Growth Promoting Potential of ACC Deaminase Rhizospheric Bacteria Isolated from Aerva javanica: A Plant Adapted to Saline Environments. *Int. J. Curr. Microbiol. App. Sci*. 4(7), 142-152.

Singh, R., Singh, T., Pandey, A. (2019). Chapter 1, Microbial Enzymes-An Overview. In Biomass, Biofuels, Biochemicals. *Advances in Enzyme Technology*, Elsevier 1-40.

Suleiman, M. (2019). Ethnobotanical, Phytochemical, and Biological Study of Tamarix aphylla and Aerva javanica Medicinal Plants Growing in the Asir Region, Saudi Arabia. *Trop. Cons. Sci.* 12.

Tarabulsi, M. K., Nagshabandi, M. K., Al Jaouni, S. K. Selim, S. (2024). Utilizing N-Fixing Bacteria to Biomanagement of Heavy Metals for Sustainable Husbandry Future Directions: A Review. *Egypt. J. Soil Sci.* 64, 1339-1353.

Vardumyan, H., Singh, A., Rajput, V. D., Minkina, T., El Ramady, H., Ghazaryan, K. (2024). Additivemediated phytoextraction of copper-contaminated soils using *Medicago lupulina* L. *Egypt. J. Soil. Sci*., 64(2), 599-618.

Wu, W., Chen, W., Liu, S., Wu J., Zhu, Y., Qin L., Zhu, B. (2021). Beneficial Relationships Between Endophytic Bacteria and Medicinal Plants. *Front. Plant Sci.* 12:646146.

Weiland, J. (1997). Rapid procedure for the extraction of DNA from fungal spores and mycelia. *Fungal Genet. Rep*. 44, 60–63.