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Isolation of Endophytic Bacteria Associated with *Salvadora persica* Trees and Evaluation of Their Promoting Effects on Onion Plants

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Abstract - This experiment was conducted during the two seasons of 2021/ 2022 and 2022/ 2023 at the faculty of agriculture and Natural Resources private, Aswan University, Aswan governorate, Egypt to isolate and characterize some endophytic bacteria from *Salvadora persica* roots and study their effects on the plant growth, yield and chemical compositions of onion plants. Freshly roots of *S. persica* were collected from tree grown in Al-Gaafra village, Daraw, Aswan governorate. Two bacterial endophytic were isolated and the 1st isolate was identified as *Bacillus subtilis* and the 2nd isolate SEndo 2 (it will be identified later). Numbers of endophytic bacteria were isolated, identified, and tested for their ability to plant growth promotion. Out of 8 clones 2 clones (*Bacillus subtilis* and SEndo 2) were identified based on their good plant growth promotion for different tests, for phosphate solubilization, nitrogen fixation, Gram typing of bacteria, motility, and IAA production. The two isolates successfully produced indole acetic acid in quantities ranged from 1.4 to 2.1 $\mu g \cdot mL^{-1}$. Two bacterial isolates of SEndo 2 (Gram-negative bacteria), *Bacillus subtilis* (Gram-positive bacteria) were screened for plant growth-promoting activities revealing that the isolates showed production of IAA, and capacities to solubilize phosphate, but only *Bacillus subtilis* was able to fix nitrogen and fix nitrogen. The field experiment investigated the effect of two isolated which were selected to study of the influence inoculation endophytic bacteria on the growth and productivity of onion plants. A field trial as randomized complete-blocks (RCB) with 4 replicates in the two seasons was carried out in the farm of Faculty of Agriculture and Natural to investigate the response of onion crop (cv. improved Giza 6) to inoculation with individual and composite inoculums of plant growth-promoting bacteria. Onion inoculation treatments showed a significant effect on the growth, productivity and chemical compositions during the two seasons. The effect of different inoculation treatments can be arranged in descending order on increasing onion plant height as follows: mix of SEndo 2 and *Bacillus subtilis* > *Bacillus subtilis* > SEndo *2*> control in the different periods for both seasons.

Keyword: *Salvadora persica***,** endophytic bacteria**,** *Bacillus subtilis***,** plant growth promotions.

Introduction

Many bacteria collectively referred to as Plant Growth Promoting Rhizobacteria colonize of many plant species and some of which invades inner tissues without causing any symptoms **[1],** termed as endophytes, have beneficial effects on host plant growth, which is manifested in

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improving plant growth and reducing susceptibility to diseases **[2].** These bacteria exhibit properties of phosphorus solubilization and siderophore production, which allow host plants to efficiently uptake phosphorus- and iron-derived nutrients, respectively. Also, some endophytes may interfere with the biosynthesis of phytohormones as auxin and ethylene. In this connection, **[3]** indicated that IAA produced by endophytes improves total pool of hormone along with plant IAA. **[4]** revealed that about 300,000 plant species are thought to be a host to one or more endophytes. The endophytic bacteria can not only improve growth of the host plant, but also help the host to tolerate stress conditions, produce allelopathic effects against other competing plant species and then enable plants to have better growth against biotic and abiotic challenges and competition by other plants **[5]**.

Meswak or Arak tree (*Salvadora persica* L.) of the family Salvadoraceae is an evergreen tree, 4-6 m tall with a short trunk; has a white bark and smooth green leaves. **[6-8]. [9]** indicated that many endophytic bacterial isolated from *Salvadora persica* roots such as *Citrobacter sp*., *Pantoea agglomerans* , *Pseudomonas oryzihabitans*, *Serratia marcescens*, *Enterobacter aerogenes* and *Bacillus sp*. Moreover, *S. persica* has many pharmacological activities were reported experimentally, including antimicrobial, antioxidant, analgesic, anthelmintic, anti-inflammatory, and antiulcer activities **[10].** According to **[11],** the endophytic microbe of *Arcangelisia flava* plant has the potential as the antimicrobial compound producer by the microbes.

Numerous reports exist concerning the useful endophytic bacteria in the plant growth promotion like wheat, rice, canola, potato, tomato, and many more **[12-13].** Most of these reports involve the possible growth promotion potential of endophytes isolated from the same plants. Moreover, other studies have proved the growth promotion effect of endophytic bacteria on non-host plants **[14].** Some researchers have pointed out that endophytes are only able to promote plant growth in the plants that are very closely related to their natural host **[15].** However, endophytic bacteria have been shown several beneficial effects on their plant host directly or indirectly. They can benefit plants directly by helping plants in getting the nutrients, and induce plant growth by modulating growth related hormones, which can help plant grow better under normal and stressed conditions **[16].** Indirectly, endophytic bacteria induce plant growth by discouraging phytopathogens using mechanisms like antibiotic and lytic enzyme production, nutrient unavailability for pathogens, and priming plant defense mechanisms and then protecting plants from future attacks by pathogens **[17]. [18]** reported that *Burkholderia phytofirmans* strain PsJN is a plant growth promoting bacteria, able to establish both rhizosphere and endopyhtic populations in different variety of plants, like potato, tomato, maize, and grapevines; it stimulates plant growth and plant immune defense in many of its host plants.

[19] studied diversity of bacterial endophytes from different agricultural crops and reported that total 50 bacterial endophytes were isolated from the leaves of various crop plants. Some Gram positive and some Gram negative bacteria were isolated. They added that conformation of isolates was done on basis of biochemical characteristics and Enzymes such as Catalase, Amylase, Gelatinase, Protease, Lipase, Chitinase. These isolates were characterized for the plant growth promoting as phosphate solubilization, production of ammonia, IAA, production of phenolic acid and root formation of carrot slices, mataki and chana plants. Therefore, this study is aimed to isolate and characterize some endophytic bacteria from *Salvadora persica* roots and study their ability for plant growth and yield promoting of onion plants.

Material and methods

Plant sampling and bacterial endophytes isolation:

Roots of *Salvadora persica* L. (family Salvadoraceae) was collected from tree grown in Al-Gaafra village, Daraw, Aswan governorate during the two seasons of 2021/ 2022 and 2022/ 2023. The plant roots were washed by running tap water and subsequently in sterile distilled water, and then surface sterilized by ethanol 70% for 1 min, sodium hypochlorite 2.5% for 5 min, ethanol 70% for 30 s, and finally washed in sterile distilled water 3 times. The last washing water was plated onto nutrient agar, Czapek Dox (CD) agar, and potato dextrose agar (PDA) media. Bacterial endophytes were isolated from the sterilized plant roots **[20].** The bacterial growth from the internal tissues or crushed segments were checked for purity, transferred to fresh cultural slants and stored at 4 ^{0}C for further study.

Screening of plant growth-promoting properties of bacterial endophytes:

Bacterial endophytic isolates were screened for phosphate solubilization by procedure of **[21]** using Pikovskaya medium and bromophenol blue as indicator. Identification of isolates was done by Vitek 2 compact system according to **[22]**. To test the antibacterial activity of endophytes, bacterial isolates were cultured in nutrient broth medium for 6 days at 35 ± 2 C on a shaker at 180 rpm. The crude fermentation broth were blended thoroughly and centrifuged at 4000 rpm for 5 min. Liquid supernatants were extracted with an equal volume of ethyl acetate thrice. The organic solvent extract was then evaporated and the crude extracts were dissolved in dimethyl sulfoxide and used for antibacterial screening assay by well diffusion method **[23]**.

Gram staining:

Gram typing of bacteria was determined by the potassium hydroxide (KOH) method. One drop of 3% KOH solution was placed on a clean microscope slide and isolates of the endophytic bacteria were emulsified to the drop. After stirring continuously for 60 s, the reaction of gram-negative was indicated when the organisms became thick and stringy, and formed long strands within the first 30 s. Gram-positive organisms did not alter the suspension **[24]**.

Phosphate solubilization activity:

The mineral phosphate solubilization activity was assayed by inoculating bacteria on agar medium plates (Table 2) containing insoluble phosphate (glucose, 10 g L−1; NH4Cl, 5 g L−1; NaCl, 1 g L−1; MgSO4.7H2O, 1 g L−1; Ca3(PO4)2, 0,8 g L−1; pH 7.2). The capacity of solubilizing mineral phosphate was confirmed by the presence of a zone of the clearance around the colonies after 48 h at 30 ◦C **[25]**.

The N- fixation:

The N- fixation capacity was determined by using the N free semi-solid medium NFb **[26]**. The presence of the bacterial pellicle in the medium surface after incubation for 2 days at 28 ◦C was the indication of bacterial ability to fix N.

Determination of Indole Acetic Acid (IAA) production:

Indole Acetic Acid (IAA) production by the isolated endophytes was estimated qualitatively and quantitatively, through the using of amended Luria- Bertani agar medium (LB). For qualitative determination of IAA, the assay of **[27]** was adopted. For quantitative assay of IAA, the method was described by **[28]** was applied.

Siderophore production:

The cultured bacterial strains were spotted on the Chromeazurol S agar plate **[29].** Development of yellow orange hallow zone around the bacterial spot has been considered as positive indication for Siderophore production.

The field experiment:

The present study was conducted during the two consecutive seasons of 2021/2022 and 2022/2023 in the farm of Faculty of Agriculture and Natural Resources, Aswan University, Aswan, Egypt. The physical and chemical properties of the soil under study were shown in Table 1. For mineral fertilizer, 208 kg ammonium nitrate (NH_4NO_3 33.5% N), 266 (kg) calcium super phosphates (15.5% P_2O_5) and 50 kg potassium sulphate (48% K_2O)/ fed were applied. Seedlings of the onion were transplanted on November 20th, 2021 and 2022. Onion cultivar (Improved Giza 6) was used in this study. The source of this cultivar is Shandaweel Research Station, Agricultural Research Center, Sohag, Egypt.

The experiment was conducted in randomized complete-blocks (RCB) with 4 replicates in the two seasons.

Table 1. Physical and chemical properties of the soil under study.

	Soil Texture	Sand %	Silt %	Clay %
Physical properties	Sandy	94.65	2.31	3.04

	pH	EC (ds/m)	Soluble cations meq/ L				Soluble anions meq/ L		
Chemical properties			Na^+	K^+	Ca^{++}	Mg^{++}	CO^{-3}	HCO^{-3}	Cl^-
	8.10	0.25	13.20	7.25	2.02	0.45	0.00	4.50	3.45

Endophytic bacteria inoculation and treatments:

Two bacterial isolates of SEndo 2 (T2), *Bacillus subtilis* (T3), and (T4) formed a mix of T2-T3 were selected for their better PGP activities in order to test their effects on growth and production of onion plant. The onion seedlings were surface disinfected by soaking in 2.5% sodium hypochlorite for 5 min, 70% ethanol for 1 min, and then washed by sterile distilled water for 5 times. Bacterial pure cultures of T2, T3 and T4 were grown in nutrient broth at 35 ± 2 C on a shaker at 180 rpm. The bacterial cultures were diluted in sterilized distilled water to reach final concentration of $10^{6–8}$ CFU mL^{-1} **[20].** Onion's seedlings were coated with peat-based inocula of the endophytic bacteria treatments for 1 hr using Arabic gum as adhesive material. Inoculation was performed on the same day of sowing and was dried in the shadow before planting. After planting, the inoculated plots received a liquid bacterial culture as a boost inoculum every 15 days for each season and 4 times.

Treatment plot consisted of 10 rows each row was 3 m long and 0.5 m wide. Onion seedlings were planted on 7 cm apart to reach a total plant density of 300 plant/ plot.

Data Collection:

Data on the growth, yield and yield components of onion was recorded from three central rows; plants were selected randomly for each replicate of the four treatments. However, data for the phonological, yield and its attributes parameters of onion crop were collected. The measurements were days to maturity, plant height (cm), number of leaves/ plant, neck diameter (cm), bulb diameter (cm), average bulb weight (g), marketable and total bulb yield. Nutritional analysis of plant samples were total soluble solids, N, P, and K percentages.

The obtained data were subjected to statistical analysis according to the technique of analysis of variance (ANOVA) for the factorial experiment in complete randomized block design as reported by **[30].** Least significant of difference (LSD) method was used as described by **[31]**.

Results

The current investigation was carried out for the isolation, characterization and the plant promotion effects on onion of endophytic bacteria from *Salvadora persica*. A total of 8 different bacterial clones were isolated from the *S. persica* roots, out of the 8 clones, 2 clones were identified based on their good PGPR activities. The bacteria were isolated from roots, and were identified and then analyzed for various traits consistent with plant growth promotion in vitro and field experiments. Number of bacterial isolates recovered from *Salvadora persica* roots along with average cell counts recovered on LB agar growth media as shown in Table 2. The obtained results in this study revealed that *Salvadora persica* roots harbor an abundance of *Bacillus subtilis* and SEndo 2 (we couldn't idebtify SEndo 2 and we will fix it later). The absence of the two bacterial colonies on control plates confirmed that isolates obtained are endophyte. The two isolates were tested for number of important properties (Table 3). *Bacillus subtilis* and SEndo 2 isolates were Gram-positive and negative, respectively. Also *B. subtilis* was positive in motility, while SEndo 2 was negative. Based on the preliminary phenotypic properties, results revealed that the two isolates act as plant growth promotion. The two isolates are entire in their margins and able to produce IAA, however its concentration in *Bacillus subtilis* higher than those in SEndo 2 as shown in color concentration. Only *Bacillus subtilis* was able to fix nitrogen, indicating its ability to produce protease. The two strains formed a clear halo zone around the spot inoculation of Pikovskaya's medium, indicating phosphate solubilization activity.

Table 2. Number of bacterial isolates recovered from *Salvadora persica* roots along with average cell counts recovered on LB agar growth media.

Source	**Direct bacteria Isolation**
Total bacteria isolates	13×10^6
Bacillus subtilis	8×10^6
SEndo 2	5×10^6

Table 3. Morphological and plant growth promotion traits of endophytic bacteria associated *Salvadora persica*.

Endophytic bacteria	***Bacillus subtilis***	**SEndo 2**
Family name	Bacillaceae	Enterobacteriaceae
IAA (($\mu g \cdot mL^{-1}$)	2.1	1.4
Gram stain	+	-
Shape	long rod	short rod
Margin	entire	entire
Motility	-	+
IAA production	+	+

P solubilization	+	+
Siderophore production	+	+
N fixation activity	+	-

The two different bacterial clones were isolated from the *Salvadora persica* roots, identified based on their good PGPR activities as *Bacillus subtilis* and SEndo 2. After enumeration of isolated bacteria, two endophytic bacteria i.e. *Bacillus subtilis* and SEndo 2 were selected on the basis of colony size, morphology, shape, and color and growth pattern (Fig. 1). Highest cell counts were on LB agar 8×10^6 cfu/gfw and 5×10^6 cfu/gfw for *Bacillus subtilis* and SEndo 2, respectively.

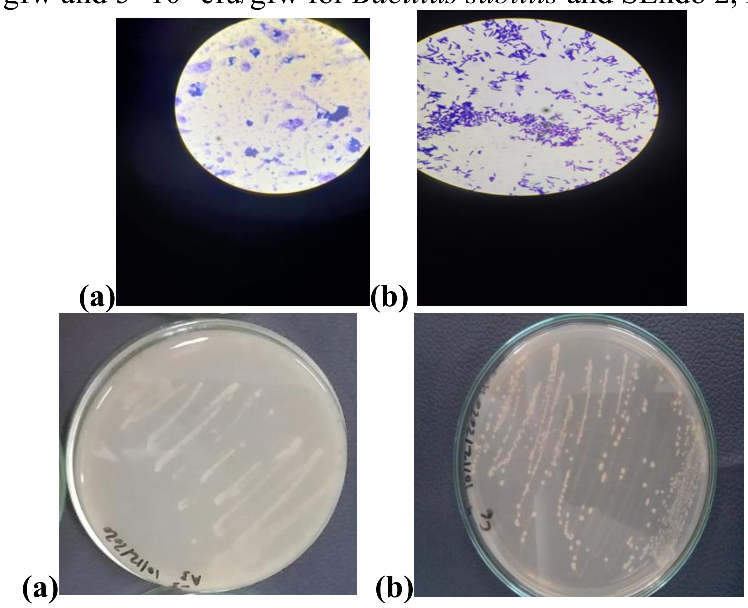

Fig. 1. The two bacterial endophytes, SEndo 2 (a). and *Bacillus subtilis* (b).

To screen for ability of phosphate solubilizing of the two bacterial endophytes, spot-on-lawn technique was employed and phosphate solubilizing ability of bacterial endophytes (Clear zones) as shown in Fig. 2. Results showed that the two isolates i.e. *Bacillus subtilis* and SEndo 2 possessed phosphate solubilizing ability.

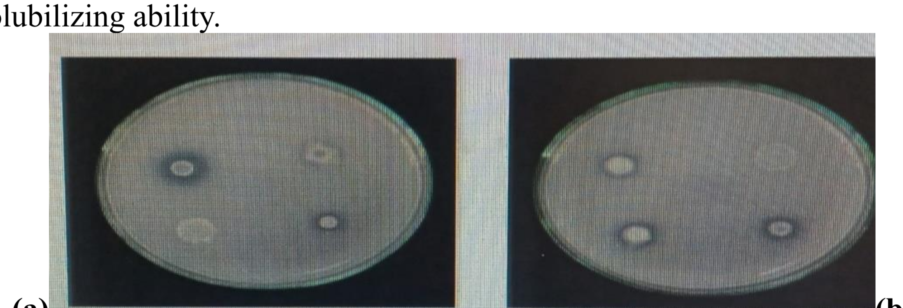

Fig. 2. Phosphate solubilizing ability of the two bacterial endophytes, *Bacillus subtilis* (a) and *SEndo 2* (b).

The two isolates i.e. *Bacillus subtilis* and SEndo 2 produced IAA. The higher IAA concentration (2.1 $\mu g\cdot mL^{-1}$) was attained with *Bacillus subtilis*, while the lower concentration (1.4 $\mu g\cdot mL^{-1}$) with SEndo 2 as shown in Table 3 and Fig. 3.

Fig.3. Indole acetic acid (IAA) production of SEndo 2 (left) and *Bacillus subtilis* (right).

Field experiment:

1- *Onion plant height:*

Endophytes isolates i.e. SEndo 2, *Bacillus subtilis* and that checked for their abilities as plant growth promotion were retained for onion inoculation in the field experiment. Onion inoculation treatments showed a significant effect on plant height during the two seasons (Table. 4). Different inoculation treatments resulted in significant increment in plant height 65, 80, 95 and 110 days after transplanting compared to the control for both seasons. However, the tallest onion plants were registered with inoculation by the two endophytic bacteria as mixture compared to other treatments. The effect of different inoculation treatments can be arranged in descending order on increasing onion plant height as follows: mix of SEndo 2 and *Bacillus subtilis* > *Bacillus subtilis* > SEndo 2 > control in the different periods for both seasons.

2- *Neck diameter**:***

Regarding neck diameter of onion plants, a significant difference in this trait was observed between the inoculation treatments (Table 5). The nick diameter was higher in plants inoculated by endophytes than un-inoculated control. The highest values of nick diameters 65, 80, 95 and 110 days after transplanting were recorded with plants inoculated by mix of SEndo 2 and *Bacillus subtilis*, followed by *Bacillus subtilis* treatments compared to the other treatment for both seasons. Meanwhile, lower nick diameter was recorded with un-inoculated control, followed by SEndo 2 in both seasons.

3- *Number of leaves/ plant:*

Number of leaves per plant of onion 65, 80, 95 and 110 days after transplanting as affected by inoculation treatments were shown in Table 6. Lowest values were noticed in un-inoculated plants (T1), followed by those inoculated by SEndo 2 (T2). Compared to the un-inoculated onion plants, a significant increase in the number of leaves per plant was observed in onion plants inoculated by mix of SEndo 2 and *Bacillus subtilis* (T4), followed by those inoculated alone by *Bacillus subtilis* (T3) for different periods in both seasons.

4- *Bulb diameter:*

The result presented in Table 7 showed that the inoculation of onion had tremendously increased the bub diameter as compared to control on the 65^{th}, 80^{th}, 95^{th} and 110^{th} day after onion transplanting in both seasons. Analysis of variation had registered a significant difference ($P < 0.05$) among inoculation treatments for bub diameter. The higher bulb diameter was registered with mix of SEndo 2 and *Bacillus subtilis*, followed by *Bacillus subtilis* inoculation alone. While the lowest values of bulb diameter were observed with un-inoculated plants and that inoculate by SEndo 2 alone on the different periods for both seasons.

5- *Days to maturity:*

The results presented in Table 8 clearly indicate the influence of endophytic bacteria on days to maturity of onion plants. The result revealed that inoculation treatments had significantly increased the number of days to maturity ($P < 0.05$). Among the inoculation treatments, plants inoculated with mix of SEndo 2 and *Bacillus subtilis*, followed by *Bacillus subtilis* alone had recorded higher number of days to maturity than the other treatments. The least number of days to maturity was attained due to un-inoculated plants and those inoculated by SEndo 2 alone in the two studied seasons.

6- *Average bulb weight:*

The average bulb weight recorded for onion plants at harvest (110 day) obtained is presented in Table 8. The result indicated that the different inoculation treatments had increased the bulb weight as compared to un-inoculated control. Among the used inoculations, mix of SEndo 2 and *Bacillus subtilis*, followed by *Bacillus subtilis* alone were more influential in improving the bulb weight of the onion plants. Result analyses of bulb weight records suggest that there significant differences among treatments for bulb weight in both seasons.

7- *Marketable bub yield:*

Changes in marketable bulb yield are shown in Table 8. A significant increment was observed in marketable bulb yield obtained from inoculated plants. However, plants inoculated with mix of SEndo 2 and *Bacillus subtilis*, followed by *Bacillus subtilis* alone had more pronounced effect on the marketable bulb yield. In general, effect was minimal in the marketable bulb yield obtained from plants treated with SEndo 2 alone. The least values of marketable bulb yield in the two studied seasons were as result of un-inoculated control.

8- *Total yield:*

Onion inoculation treatments showed a significant effect on total yield during the two seasons (Table 8). Different inoculation treatments led to significant increment in total yield of bulbs compared to the control for both seasons. However, the higher bulb yield was obtained with inoculation with the two endophytes as mixture compared to other treatments. The effect of different inoculation treatments can be arranged in descending order on increasing total yield as follows: mix of SEndo 2 and *Bacillus subtilis* > *Bacillus subtilis* > SEndo 2 > control in the different periods for both seasons.

9- *Chemical compositions:*

Changes in total soluble solids, N, P and K percentages are shown in Figs.4-7. A significant increment was observed in these traits measured from inoculated onion. However, plants inoculated with mix of SEndo 2 and *Bacillus subtilis*, followed by *Bacillus subtilis* had more pronounced effect on these chemical compositions. In general, effect was minimal in total soluble solids, N, P and K percentages obtained from plants treated with SEndo 2 alone. The least values of the studied traits in the two studied seasons were due to un-inoculated control.

Table 4. Plant height (cm) of onion plants 65, 80, 95 and 110 days after transplanting.

Treat.	60 days		80 days		95 days		110 days	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
T1	30.33 ± 1.45	28.67 ± 1.20	41.33 ± 0.88	40.00 ± 0.58	46.33 ± 0.88	44.67 ± 1.20	57.33 ± 1.76	55.67 ± 2.03
T2	47.00 ± 1.53	41.00 ± 1.73	62.67 ± 0.87	61.00 ± 0.57	67.33 ± 0.88	64.00 ± 0.58	76.33 ± 0.88	73.67 ± 2.03
T3	56.00 ± 0.58	59.33 ± 0.67	68.33 ± 0.86	67.67 ± 1.76	72.67 ± 0.88	71.00 ± 0.58	79.67 ± 0.88	79.67 ± 1.45
T4	62.67 ± 1.45	63.00 ± 0.58	73.00 ± 0.58	71.00 ± 0.58	76.33 ± 0.88	75.33 ± 0.88	83.00 ± 0.58	85.33 ± 0.88
Average	49.00 ± 3.70	48.00 ± 4.23	61.33 ± 3.67	59.92 ± 3.66	65.67 ± 3.52	63.75 ± 3.56	74.08 ± 3.04	73.58 ± 3.43
LSD 5%	4.85	4.30	2.92	2.83	3.26	2.75	3.81	6.34

Table 5. Neck diameter (cm) of onion plants 65, 80, 95 and 110 days after transplanting.

Treat.	60 days		80 days		95 days		110 days	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
T1	1.10 ± 0.06	1.17 ± 0.03	1.30 ± 0.06	1.37 ± 0.09	1.50 ± 0.06	1.60 ± 0.06	1.80 ± 0.06	2.00 ± 0.06
T2	1.40 ± 0.06	1.60 ± 0.06	1.70 ± 0.06	1.90 ± 0.12	1.90 ± 0.06	2.10 ± 0.12	2.33 ± 0.03	2.50 ± 0.06
T3	1.67 ± 0.09	1.70 ± 0.06	2.10 ± 0.06	2.10 ± 0.06	2.23 ± 0.09	2.37 ± 0.09	2.60 ± 0.06	2.53 ± 0.12
T4	2.20 ± 0.06	2.10 ± 0.06	2.50 ± 0.05	2.30 ± 0.06	2.60 ± 0.06	2.50 ± 0.06	2.80 ± 0.06	2.63 ± 0.09
Average	1.59 ± 0.13	1.64 ± 0.10	1.90 ± 0.14	1.92 ± 0.11	2.06 ± 0.13	2.14 ± 0.11	2.38 ± 0.12	2.42 ± 0.08
LSD 5%	0.26	0.21	0.22	0.32	0.22	0.32	0.10	0.33

Table 6. No. leaves/ plant of onion plants 65, 80, 95 and 110 days after transplanting.

Treat.	60 days		80 days		95 days		110 days	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
T1	5.00 ± 0.58	6.00 ± 0.58	7.00 ± 0.58	8.00 ± 0.58	8.00 ± 0.58	9.00 ± 0.58	9.00 ± 0.58	10.33 ± 0.88
T2	8.00 ± 0.58	7.67 ± 0.33	10.00 ± 0.58	11.00 ± 0.58	11.00 ± 0.58	12.00 ± 0.58	13.00 ± 0.58	13.33 ± 0.88
T3	9.33 ± 0.58	8.67 ± 0.67	12.00 ± 0.58	12.67 ± 1.20	13.33 ± 0.88	13.67 ± 1.20	14.67 ± 1.20	15.67 ± 1.20
T4	10.00 ± 0.58	9.33 ± 0.88	13.00 ± 0.58	13.00 ± 0.58	14.33 ± 0.88	15.00 ± 0.58	16.67 ± 1.20	18.00 ± 0.58
Average	8.08 ± 0.62	7.92 ± 0.47	10.50 ± 0.73	11.17 ± 0.68	11.67 ± 0.80	12.42 ± 0.75	13.33 ± 0.94	14.33 ± 0.94
LSD 5%	1.73	1.10	2.00	2.77	2.40	2.77	2.73	2.75

Table 7. Bulb diameter (cm) of onion plants 65, 80, 95 and 110 days after transplanting.

Treat.	60 days		80 days		95 days		110 days	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
T1	3.37 ± 0.09	3.20 ± 0.12	4.20 ± 0.12	4.10 ± 0.06	4.70 ± 0.12	4.40 ± 0.06	5.40 ± 0.15	5.27 ± 0.12
T2	4.20 ± 0.06	4.30 ± 0.06	5.27 ± 0.09	5.47 ± 0.13	7.30 ± 0.15	7.57 ± 0.09	8.00 ± 0.06	8.20 ± 0.26
T3	4.50 ± 0.06	4.57 ± 0.09	6.23 ± 0.12	6.37 ± 0.12	8.27 ± 0.13	8.03 ± 0.22	9.00 ± 0.49	8.87 ± 0.32
T4	4.90 ± 0.06	4.87 ± 0.03	6.63 ± 0.09	6.60 ± 0.06	8.60 ± 0.12	8.40 ± 0.06	9.33 ± 0.15	9.27 ± 0.19
Average	4.24 ± 0.17	4.23 ± 0.22	5.58 ± 0.29	5.63 ± 0.30	7.22 ± 0.46	7.10 ± 0.48	7.93 ± 0.48	7.90 ± 0.48
LSD 5%	0.22	0.22	0.31	0.33	0.48	0.47	0.97	0.87

Table 8. Days to maturity, average bulb weight (g), marketable bub yield (ton/ fed) and total yield (ton/ fed) of onion plants.

Treat.	Days to maturity		Average bulb weight		Marketable bub yield		Total yield	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
T1	103.33 ± 1.76	107.67 ± 1.20	72.67 ± 1.76	73.33 ± 1.20	8.93 ± 0.09	9.20 ± 0.06	11.20 ± 0.06	11.23 ± 0.12
T2	114.33 ± 1.76	114.33 ± 0.67	82.67 ± 1.45	86.00 ± 1.15	10.70 ± 0.06	10.60 ± 0.06	12.97 ± 0.24	13.30 ± 0.06
T3	118.67 ± 0.88	120.67 ± 0.33	88.67 ± 0.88	90.00 ± 1.15	12.13 ± 0.12	12.40 ± 0.06	14.67 ± 0.09	14.73 ± 0.09
T4	122.33 ± 1.20	125.00 ± 0.58	96.67 ± 1.76	99.67 ± 2.19	14.17 ± 0.0 9	14.40 ± 0.06	15.90 ± 0.12	16.00 ± 0.12
Average	114.67 ± 2.24	116.92 ± 2.00	85.17 ± 2.72	87.25 ± 2.92	11.48 ± 0.58	11.65 ± 0.59	13.68 ± 0.54	13.82 ± 0.54
LSD 5%	3.51	2.13	5.62	5.70	0.35	0.22	0.52	0.35

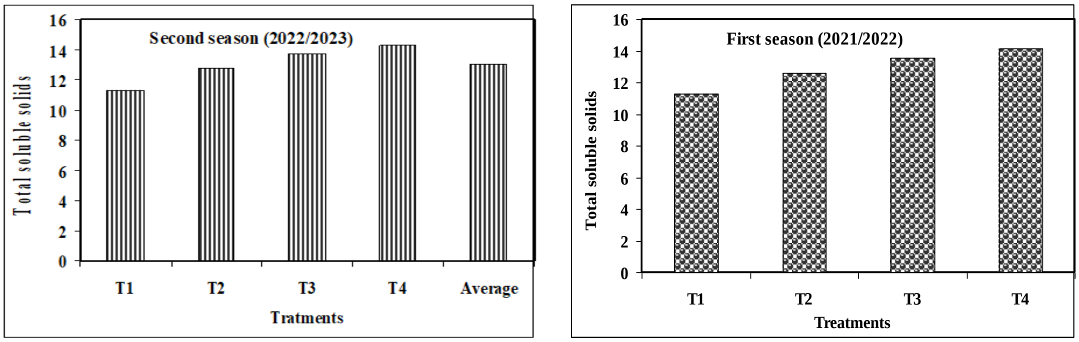

Fig. 4. Total soluble solids (TSS %) of onion bulbs at harvest during the two seasons (2021/2022 and 2022/2023).

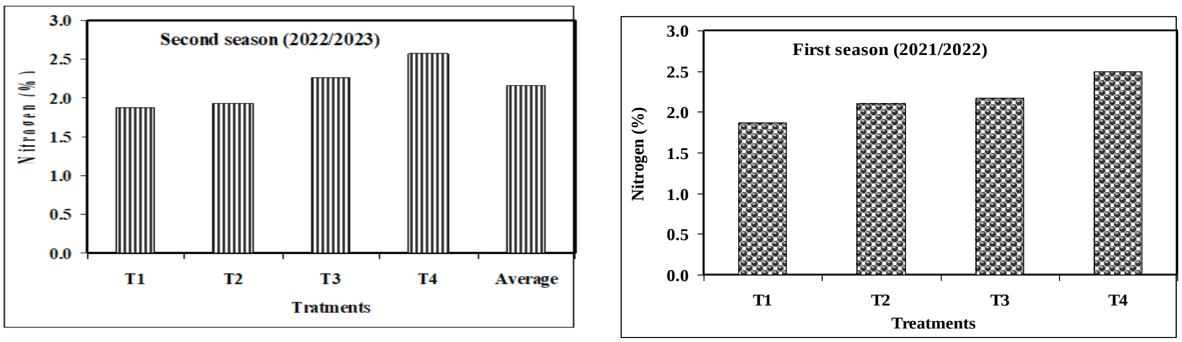

Fig. 5. The percentage of nitrogen of onion bulbs at harvest during the two seasons (2021/2022 and 2022/2023).

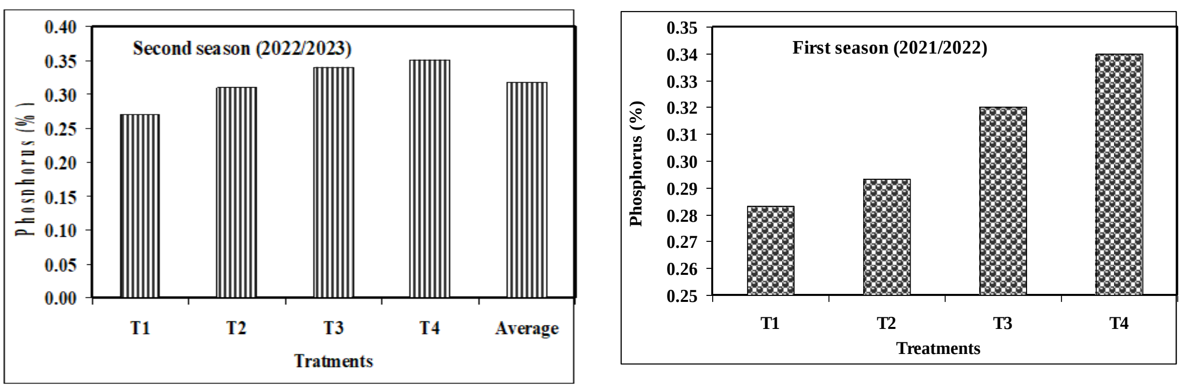

Fig. 6. The percentage of phosphorus of onion bulbs at harvest during the two seasons (2021/2022 and 2022/2023).

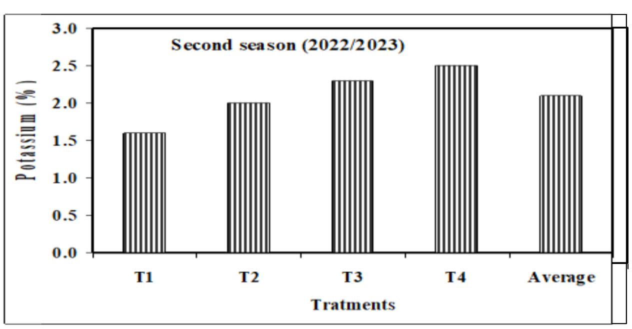

Fig. 7. The percentage of potassium of onion bulbs during the two seasons (2021/2022 and

2022/2023).

Discussion

This work aimed for studying isolation and identification of endophytic bacteria from *Salvadora persica* L. roots as well as their plant growth promotion on onion plants under Aswan conditions. The obtained results indicated that two endophytes bacteria were isolated from *S. persica* roots i.e. SEndo 2 and *Bacillus subtilis*. These isolates previously appeared as endophytes in different plant species like *Bacillus sp*. from *Spharanthus indicus* **[32],** *Bacillus subtilis* from *S. persica* **[9],** *Klebsiella pneumonia*e from *Ipomoea batatas* **[33].** Our results reported that the two isolates able to produce IAA which acts as plant growth promotion. Various studies reported that many endophytic bacteria have the potential to produce IAA. This may be resulted in improving growth promotion of different plants when the plants are colonized with endophytes as bacterial IAA improves total pool of IAA along with the plants IAA **[34].** Indole acetic acid is the most common plant hormone, which increases the growth and production of the plants **[35]** and it also involved in the cell enlargement and division, tissue differentiation, as well as physiological processes **[36].** Our results reveal the ability of both gram negative bacterial isolates (SEndo 2) and bacterial genera as *Bacillus* that belongs to the gram positive group with the ability to produce siderophores **[37] [9].** In this study the two isolates are able to solubilize phosphate; this makes them available to improve crop growth and productivity **[38].** Also, the ability to nitrogen fixation was detected only with *Bacillus subtilis*. The ability of endophytic bacteria isolated from *Pistacia atlantica* trees was evaluated to phytohormone production such as IAA, nitrogen fixation and phosphate solubilization **[39].** They indicated that all strains (*Pseudomonas*, *Stenotrophomonas*, *Bacillus*, *Pantoea* and *Serratia* genus) were able to produce IAA and to solubilize phosphate. The same results were reported for other endophytic bacteria **[40].**

The field experiment for the possibility of bacteria isolates for improving onion growth and productivity clearly indicates that inoculation treatments had significantly increased the growth traits i.e. plant height, neck diameter, number of leaves/ plant and days to maturity. Among the inoculation treatments, plants inoculated with SEndo 2 plus *Bacillus subtilis*, followed by *Bacillus subtilis* alone had recorded higher values of the growth characteristics. Also, the obtained results showed that the inoculation of onion plants had tremendously increased the production parameters of onion plants like bulb diameter, bulb weight, marketable bulb yield and total yield. Maximum values of these traits were also registered with the inoculation by SEndo 2 plus *Bacillus subtilis*, followed by *Bacillus subtilis* inoculation alone. In general, inoculation by the two isolates each as combination between them or as individual led to improve the chemical compositions of onion plants and this was detected in TSS, nitrogen, phosphorus and potassium percentages. Bacterial endophytes can improve plant growth by direct and indirect mechanisms, such as the production of phytohormones, phosphate solubilization, ammonia production, nitrogen fixation, production of siderophores, secretion of lytic enzymes, and HCN, as well as protect the plant against phytopathogens **[41-42].** The obtained results for enhancing growth and productivity of onion plants may be due to the vital role of endophytic bacteria that able to produce phytohormone (IAA), N-fixation, and phosphate solubilization. Many studies showed the beneficial role of endophytic bacteria in improving plant growth. In this respect, **[43]** reported that endophytic bacteria isolated from *Zea mays* enhance plant growth and is an endophytic of a wide variety of plant species. They added that the ability of this diazotrophic endophyte to contribute nitrogen fixation to plants is of interest for agricultural purposes. Numerous isolates as *Bacillus subtilis* from different plants were act as plant growth promotion **[44] [39] [19].** The present results for the vital role of endophytic bacteria in improving plant growth and productivity was in agreed with **[45].** They isolated some of endophytic bacteria from *Thymus vulgaris* i.e. *Bacillus sp.* and investigated their role as bio fertilizers to enhance essential oil contents. They suggested that the higher growth performance of *T. vulgaris* was attained with the presence of the endophytic bacteria.

Conclusions

In this study, endophytic bacteria were isolated from *Salvadora persica* roots grown in Aswan governorate. These endophytic bacterial isolates were classified based on their morphological and biochemical characteristics as *Bacillus subtilis* and SEndo 2 and these isolates could Gram-positive and –negative, respectively, they solubilize phosphate, and produce indole IAA. Only *Bacillus subtilis* has been shown to be able to fix nitrogen. Therefore, the two isolates have the potential to stimulate plant growth. For his point of view, the obtained results pointed out that inoculated with the mix of SEndo 2 and *Bacillus subtilis*, followed by *Bacillus subtilis* had more pronounced effect on the growth and productivity traits of onion plants. Further studies should be carried out of the different plant organs of *S. persica* and different plant species to identify and isolation endophytic bacteria species and to investigate their functions on the growth and productivity of crops.

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