Research Article

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Implication of plant growth-promoting rhizobacteria of *Bacillus* spp. as biocontrol agents against wilt disease caused by *Fusarium oxysporum* Schlecht. in *Vicia faba* L.

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Abstract: Out of seven *Fusarium* spp. isolated from infected faba bean roots, two *Fusarium oxysporum* were selected and showed faba bean-wilt disease severity with percentages of 68% and 47% under greenhouse conditions. The *F. oxysporum* showed the highest wilt disease was selected to complete the current study. Three rhizobacterial strains were isolated and identified as *Bacillus velezensis* Vb1, *B. paramycoides* Vb3, and *B. paramycoides* Vb6. These strains showed the highest *in-vitro* antagonistic activity by the dual-culture method against selected *F. oxysporum* with inhibition percentages of 59 ± 0.2 , 46 ± 0.3 , and $52 \pm 0.3\%$ for Vb1, Vb3, and Vb6, respectively. These rhizobacterial strains exhibit varied activity for nitrogen-fixing and phosphate-solubilizing. Moreover, these strains showed positive results for ammonia, HCN, and siderophores production. The phytohormones production (indole-3-acetic acid, ABA, benzyl, kintin, ziatin, and GA₃) and secretion of various lytic enzymes were recorded by these strains with varying degrees. Under greenhouse conditions, the rhizobacterial strains Vb1, Vb3, Vb6, and their consortium can protect faba bean from wilt caused by *F. oxysporum* with percentages of 70, 60, 65, and 82%, respectively. Under field conditions, the inoculation with the rhizobacterial consortium (Vb1+Vb3+Vb6)

significantly increases the growth performance of the *F. oxysporum*-infected faba bean plant and recorded the highest wilt protection (83.3%).

Keywords: *Fusarium* wilt; *Fusarium oxysporum*; plant growth-promoting; *Vicia faba*; greenhouse experiment; field experiment.

Introduction

Increased human populations combined with restricted agricultural land availability have a significant impact on food security. By 2050, the world's population is predicted to exceed 9 billion people, resulting in a 70% rise in food demand [1]. Agricultural production has increased since the green revolution and farmers have relied on chemical fertilizers to ensure economic stability [2]. However, its deleterious effects on environmental ecology and human cannot be denied. Chemical fertilizers can aid plant growth, but do not play a role in improving soil properties [3]. They are responsible for degrading the soil by lowering its water holding capacity, rising salinity, and creating nutritional disparities [4]. Due to bioaccumulation and biomagnification of various harmful substances, their excessive usage pollutes the ecosystem [5, 6]. Natural radionuclides such as mercury, cadmium, and lead as well as various chemicals insecticides are absorbed by plants through the soil and eventually enter the food chain [7, 8]. Stomach cancer, vector-borne infections, and methemoglobinemia have all been linked to the accumulation of these toxins in newborns and humans [9]. Given all the negative impacts of chemical inputs and the growing public concern about them, alternative soil fertilization options are urgently needed. The global

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challenge is how can current agricultural practices meet the argent food demand without dependence on higher inputs of inorganic chemical fertilizers [10]. Microorganisms are an example of a viable option that can boost plant development and output while also preserving soil fertility. This is accomplished by taking advantage of plant-microbe interactions in the rhizosphere [11].

Plant growth-promoting rhizobacteria (PGPR) are an essential component of organic farming, they live in the rhizosphere and play a crucial role in soil productivity, plant growth, and disease suppression [12]. *Pseudomonas*, *Klebsiella*, *Azoarcus*, *Enterobacter*, *Azospirillum*, *Azotobacter*, *Rhizobium*, *Bacillus*, and *Serratia* are the most common described PGPR genera [11, 13]. Their diversity varies depending on soil type and circumstances, plant type, and nutrient accessibility in the rhizosphere [14]. Although various soil microorganisms are plant growth-promoting, not all strains belonging to a specific genus and species have equivalent hereditary makeup and metabolic competencies [15]. PGPRs are commercially made available in the form of microbial formulations called bio-fertilizers which can be applied directly onto seeds or introduced into the plant rhizosphere wherein they colonize and provide nutritive benefits to the host plant [16]. They are a safe alternative to conventional chemical fertilizers and provide sustainable agricultural production worldwide. Biofertilizer treatment results in improved plant growth in terms of seed germination, shoot and root development, increased biomass, and reduced disease incidence [17].

Faba bean (*Vicia faba* L.) is an important and useful legume that provides food and feed to humans all over the world. It is rich in high-quality protein, energy, fiber, and micronutrients especially Fe, Zn, and pro-vitamin A; and possesses compounds capable of preventing disease and promoting health. This legume is affected by various biotic and abiotic stresses which severely limited its yield. Considering the world's growing population and the necessity for food crops that are best suited to humanity's health, legumes will be in great demand, including common beans, mainly because of their nutritional value. In recent years, the area planted with beans has increased due to its various advantages, including profitability, rotational crop with cereals, atmospheric nitrogen-fixation, and high protein food. The factors that induce the inhibition of fungal invasion of plant roots are complex and poorly understood. A variety of agroecological factors such as the growth stage of the host plant, soil properties, and agronomic practices can influence root colonization by the fungus. *Fusarium oxysporum*, causing *Fusarium* wilt in many crops [18, 19].

Fusarium wilt caused by *Fusarium oxysporum* *Schlechtend.Fr. f. sp. Phaseoli* (Fop) is one of the most important bean diseases, being present in all crop production areas worldwide [20]. *Fusarium* wilt symptoms include foliar chlorosis, premature defoliation of lower leaves, red-brown necrosis of vascular tissue, wilting, and plant death [21]. Fop infects bean plants through wounds, natural openings, or intact roots, preferentially at the junctions of the lateral roots and the taproot [20]. During the infection, hyphae grow intracellularly in the root cortex until they reach the xylem vessels [20, 22]. The development of disease symptoms and degree of colonization in xylem vessels varied greatly among genotypes. Therefore, checking how far upward the pathogen is in the vessels from the roots might be useful to confirm the resistance of inoculated bean plants. This study focuses on the application of soil bacteria as a biocontrol agent against wilt disease caused by *Fusarium oxysporum* Schlecht. in *Vicia faba* L. To end with this goal, pathogenic soilborne fungus of *Fusarium oxysporum* was isolated from the naturally infected bean plants. Rhizospheric soil bacteria were isolated from the soil of healthy bean plants, bacteria that showed better plant growth-promoting capacity along with their antagonistic characteristics were then chosen to be applied as biocontrol agents against wilt disease caused by *Fusarium oxysporum* under greenhouse and field experiments.

Material and methods

Isolation of *Fusarium* pathogen from infected faba bean

The *Fusarium oxysporum* was isolated from roots of naturally infected faba bean plants showing symptoms of wilt disease. The infected plants were collected from the North Sinai governorate, Egypt. The infected roots were subjected to surface sterilization by 2% sodium hypochlorite for 2 min, rinsed twice with sterilized distilled water, and kept for drying under aseptic conditions. The sterilized roots were cut into small pieces (0.5 cm) and immersed on Petri dishes containing potato dextrose agar (PDA) media (containing g L⁻¹: potato, 200; dextrose, 20; agar, 15; 1.0 L distilled H₂O) and incubated for seven days at 25±2°C. The inoculated plates were examined for pure colonies and a single spore was sub-cultured onto new PDA plates.

Identification of *Fusarium* spp.

The *Fusarium* spp. cultured were identified based on cultural followed by morphological characteristics and confirmed by macro- and micro-conidial structure [23-25].

Pathogenicity test for *F. oxysporum*.

The pathogenicity test of two stains of *F. oxysporum* was achieved at EL-Qantra Sharq Experimental Station of Desert Research Center (D.R.C.), North Sinai Governorate, Egypt (31°00'21.6" N and 32°33'48.1" E) as greenhouse experiment. The pathogenic capabilities were tested using Maryot-2 faba bean cultivar collected from Agricultural Research Center, Giza, Egypt. The *Fusarium* inocula were prepared by inoculation of each fungal strain into potato dextrose broth media and incubated at 25±2°C for 15 days. The sandy-loam soil used in this experiment was autoclaved, added to sterilized pots (2 kg/ pot), and mixed thoroughly with fungal inoculation (2%). The physicochemical analysis of used soil is illustrated in Table 1. The seeds of collected faba bean were surface sterilized using 2% sodium hypochlorite for 4 min., washed in distilled water. Each pot was sown with three sterilized faba bean seeds. Pots without *F. oxysporum* inoculation and sown with sterilized faba bean seeds were running with the experiment as a control. The experiment was achieved in triplicates. The greenhouse condition was standardized throughout the experiment at a temperature between 18 to 24°C. The plants were irrigated as necessary and observed for diseases development.

Disease severity assessment.

The wilting percentage caused by each *F. oxysporum* was assessed after 60 days from planting, based on leaf yellowing and/or root discoloration. The symptoms of typical wilt due to *Fusarium* infection are numerically assessed according to their severity in ranging between 0 to 5 as follows: (0) mean no wilt symptoms appeared; (1) mean approximately from 1 to 20% of vascular plant systems are light brown and the plant leaves appear as yellow; (2) mean approximately from 20 to 40% of vascular plant systems are light brown and the plant leaves appear as yellow; (3) mean approximately from 40 to 60% of vascular plant systems are dark brown and the plant leaves appear as yellow; (4) mean approximately from 60 to 80% of vascular plant systems are dark brown and the plant leaves appear as yellow; (5) mean approximately from

80 to 100% of the leaves are yellowish and the vascular systems are dark brown or completely dead plants.

The disease severity was calculated according to the following equation [26]:

$$\text{Disease severity} = \frac{\sum[(N0) + (N1) + (N2) + (N3) + (N4) + (N5)]}{5T} \quad (1)$$

Where: (N) means the number of plants corresponding to the symptom grade, (0), (1), (2), (3), (4), and (5); (5T) mean the total number of plants (T) multiplied by maximum symptom grade (5).

Isolation of bacterial rhizospheres.

To isolate different bacterial species from rhizosphere soil of healthy faba bean plant, approximately 10 g of collected rhizosphere soil was mixed with 100 mL sterilized distilled water and stirred for 10 min. After that, the serial dilution was made up to 10⁻⁷ and inoculate 0.1 mL of final dilution into nutrient agar media. The inoculated plates were incubated at 35 ± 2°C for 24 h. The different morphological bacterial colony was re-streaked onto new nutrient agar plates to confirm the purity. The purified isolates are inoculated onto nutrient agar slant and kept in a refrigerator at 4°C for further study.

Selection of the most potent bacterial isolates

The most potent bacterial isolates were selected based on their highest efficacy to inhibit the growth of *Fusarium oxysporum* by using the *in-vitro* dual culture assay method [27]. Each rhizospheric bacterial isolate was inoculated at three equidistant points on the PDA plate and incubated at 35 ± 2°C for 24 h. At the end of the incubation period, a disk (5 mm) of a 7-days-old culture of *F. oxysporum* was put in the center of bacterial inoculated PDA plates. The control was designated as a PDA plate containing a disk of *F. oxysporum* without bacterial inoculation and running with the experiment under the same conditions. The plates were incubated at 28 ± 2°C for five days. The fungal growth inhibition percentage was calculated using the following equation:

$$\text{Growth inhibition (\%)} = \frac{R1 - R2}{R1} \times 100 \quad (2)$$

Where R1 is the radial growth of *F. oxysporum* in absence of bacterial isolate (control), while R2 is the radial growth

Table 1: Physical and chemical characters of soil and organic fertilizer used in the current study.

Soil analysis		Organic fertilizer analysis	
Characters	Results	Characters	Results
Physical characters		Density (kg/m ³)	670
Coarse sand (%)	56.58	Relative humidity	46.70
Fine Sand (%)	31.04	EC mmhos/cm at 25°C	13.56
Silt (%)	7.93	PH (1- 10 H ₂ O)	8.40
Clay (%)	4.45	Organic matter	50.00
Soil texture	Loamy sand	Organic carbon	29.00
Chemical characters		N (%)	1.68
pH	7.86	P (%)	0.59
EC (Ds/m)	0.29	K (%)	4.78
O.M. (%)	0.59	Fe (ppm)	3310
O.C. (%)	0.34	Zn (ppm)	94.80
T.N. (%)	0.3	Cu (ppm)	48.15
C/N ratio	11.3	Mn (ppm)	264
Soluble Cations (meq L⁻¹)			
Ca ⁺⁺	4.9		
Mg ⁺⁺	2.2		
Na ⁺	8.3		
K ⁺	0.9		
Soluble anions (meq L⁻¹)			
HCO ₃ ⁻	3.1		
Cl ⁻	6.7		
SO ₄ ⁼⁼	5.7		

EC is electrical conductivity; O.M. denotes the organic matter percent; O.C. denotes the organic carbon; T.N denotes the total nitrogen; C/N denotes the carbon to nitrogen percent.

of *F. oxysporum* in presence of bacterial isolate. The experiment was achieved in triplicate for each bacterial isolate.

Identification of the most potent bacterial isolates.

The most potent bacterial isolates designated as Vb1, Vb3, and Vb6 were identified based on sequencing and amplification of the 16S rRNA gene. The genomic DNA was extracted according to the modified method [28]. Briefly, a separate bacterial colony was picked up by a sterile toothpick and suspended in 50 µL of sterilized deionized

water. The cell suspension was put in a water bath for 10 min at 97°C, after that, the suspension was centrifuged for 10 min at 15000 rpm to recover the upper layer that contains the DNA. The intensity of DNA in the collected layer was calculated by measuring its absorbance at 260 nm by UV-spectrophotometer. A bacterial universal primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTACCTTGTTACGACTT-3') were used to PCR amplification of the 16S rDNA fragment. The PCR tube containing PCR buffer (1 x), MgCl₂ (0.5 mM), Tag DNA polymerase (2.5 U, QIAGEN Inc.), Deoxynucleoside triphosphate (dNTP, 0.25 mM), universal primer (0.5 µM), and bacterial DNA (5 ng). The PCR cycling conditions were 94 °C for three minutes, followed by 30 cycles at 94°C for

30 seconds, 55°C for 30 seconds, 72°C for 60 seconds, and final extension at 72°C for ten minutes. The forward and reverse sequencing for PCR products was achieved using Applied Biosystem's 3730xl DNA Analyzer technology at Sigma company, Cairo, Egypt.

The obtained sequences were analyzed using BLAST as compared with those deposited in the GeneBank database. Multiple sequence alignment was performed using ClustalX 1.8 software package and a phylogenetic tree was constructed by the neighbor-joining method using MEGA (Version 6.1) software. The confidence level of each branch (1000 repeats) was tested by bootstrap analysis. The sequences obtained in the current study were deposited at GeneBank under accession numbers LC571587, LC571588, LC 571589 for strains Vb1, Vb3, and Vb6 respectively.

Characterization of the most potent rhizospheric bacterial strains as plant growth-promoting

Nitrogen fixation

A- Qualitative screening

The primary screening to investigate the efficacy of the most potent rhizospheric bacterial strains to nitrogen-fixing was achieved through streaking on nitrogen-free malate medium (containing g L⁻¹: yeast extract, 0.1; KH₂PO₄, 0.4; NaCl, 0.1; MgSO₄·7H₂O, 0.2; FeCl₃, 0.1; Na₂MoO₄·2H₂O, 0.2; biotin, traces; 1.0 L distilled H₂O) supplemented with bromothymol blue as an indicator [29]. The inoculated plates were incubated at 35 ± 2°C for 48 h. The producing blue color around bacterial growth is designated as a positive nitrogen fixer. The results were recorded as positive or negative based on blue color formation.

B- Quantitative bioassay using (acetylene reduction assay)

Nitrogenase activity was determined separately by the method of acetylene reduction technique given by [30]. A number of tubes (3) containing medium were inoculated with single colonies from each different isolates and incubated for 72h at 30°C. After that, the tubes were closed with sub seal, ten percent of pure acetylene (C₂H₂) was injected into the tubes which were incubated for 4 h, and then 0.1 of gas samples were withdrawn for the determination of C₂H₄ formed using Hewlett-Packard 5890 gas chromatography Series 2 plus, fit with two flames; Ionization and electron capture detectors, electron pressure control system (EPC) and Hp-Plot AL₂O₃ capillary

column (cross-linked AL₂O₃) (50 m x 0.53 mm* 15.0 mm film thickness) and attached with HP computer unit and printer. The applied temperatures were 170°C, 120°C, and 200°C for injector, column, and detector, respectively. The carrier gas (nitrogen) at flow rate 10 ml/min, hydrogen and air for flame ionization detector were at a rate of 30 and 300 ml/min, respectively. Standard pure ethylene was diluted with air and acetylene and mixed in special containers to obtain concentrations ranging from 100 to 1000 ppm; this was used as a reference for calculating the ethylene concentration in the sample. Results were calculated as ethylene produced/ml liquid culture /day.

Phosphate solubilization

A- Qualitative screening

The efficacy of rhizospheric bacterial strains to solubilizing phosphate was primary screening according to Jasim *et al.* [31] using Pikovskaya agar media (containing g L⁻¹: glucose, 10; Ca₃(PO₄)₂, 5; (NH₄)₂SO₄, 0.5; NaCl, 0.2; MgSO₄·7H₂O, 0.1; KCl, 0.2; FeSO₄·7H₂O, 0.002; yeast extract, 0.5; MnSO₄·2H₂O, 0.002; agar, 15; 1.0 L distilled H₂O). Briefly, the bacterial strain was inoculated as spot on the center of Pikovskaya agar plate and incubated at 35 ± 2°C for 48 h. The results were recorded as the diameter of the clear zone formed around each bacterial growth. The experiment was achieved in triplicates.

B- Quantitative screening

The quantitative phosphate solubilization was analyzed by measuring pH values and P concentration at interval times (2 - 10 days). Briefly, Pikovskaya's broth medium supplemented with 0.5% Ca₃(PO₄)₂ was inoculated with rhizospheric bacterial strain and incubated at 35 ± 2°C for 10 days on a rotary shaker at 180 rpm. Five ml samples were taken daily and centrifuged at 10,000 rpm for 10 min. The phosphomolybdate method was used for the determination of available soluble phosphate in the culture supernatant using a spectrophotometer (Jenway 6305 UV) measuring at O.D. 700 nm. The concentration of P was calculated from the slope of the standard curve of P. Also, the pH of the broth medium was measured daily with a digital pH meter [32].

Ammonia production

The ability of rhizospheric bacterial strains to produce ammonia was assessed in peptone water broth. Each

bacterial strain was inoculated into tubes containing 10 mL peptone water and incubated at $35 \pm 2^\circ\text{C}$ for 48 h. At the end of the incubation period, approximately, 0.5 mL of Nessler's reagent was added to culture broth media. The development of yellow color was recorded as +, ++, +++ based on the color intensity [33].

Extracellular Enzymatic Activities

The productions of extracellular enzymes (amylase, cellulase, protease, and catalase) of the rhizospheric bacterial strains were investigated by inoculating the isolates in a mineral salt (MS) agar media (containing g L⁻¹: NaNO₃, 5; KH₂PO₄, 1; K₂HPO₄, 2; MgSO₄·7H₂O, 0.5; KCl, 0.1; CaCl₂, 0.01; FeSO₄·7H₂O, 0.02; agar, 15; distilled H₂O, 1L) supplemented with specific additives, depending on the enzyme being tested. The MS agar media without bacterial inoculation was running with the experiment as a negative control. After incubation at $35 \pm 2^\circ\text{C}$ for 24 h, specific reagents were added and the size of the clear zone (mm) surrounding the bacterial colony was measured, indicating extracellular enzymatic activities. All assays were performed in triplicates. For amylolytic and cellulolytic activities, the bacterial strains were inoculated onto MS agar medium supplemented with 1% soluble starch and 1% carboxy-methylcellulose (CMC) respectively. After the incubation period, the plates were flooded with 1% iodine. For protease activity, the MS agar medium containing 1% gelatin was used to determine the proteolytic activity of the bacteria. After incubation period, gelatin degradation was checked using acidic mercuric chloride as an indicator [34, 35].

For catalase activity, the pure bacterial colony from young culture (18-24h.) was put into a glass slide and mixed with a few drops of 3% H₂O₂. The appearance of oxygen bubbles indicates the presence of catalase activity [36].

Siderophore production

Secretion of siderophore was qualitatively analyzed using king's B agar medium containing chrome azurol S as an indicator dye, Fe³⁺ solution, and hexadecyltrimethylammonium bromide (HDTMA). The fresh bacterial culture was spotted onto a king's B agar plate and incubated at 28°C for 72 h. The appearance of an orange halo around the bacterial growth indicates a positive siderophore production [37].

HCN production

The purified rhizospheric bacterial isolate was tested for HCN production through inoculation on king's B agar medium amended with glycine [38]. After that, a Whatman No. 1 filter paper was soaked in a solution of (2% sodium carbonate mixed with 0.05% picric acid solution) and put on the lid of inoculated king's B petri dish. The plates were sealed with parafilm and incubated at $28 \pm 2^\circ\text{C}$ for 48 h. The change in the color of the filter paper from deep yellow to reddish-brown color indicates a positive result for HCN production.

IAA production

A- Qualitative screening.

The most potent rhizospheric bacterial isolates were screened for qualitative IAA production. Briefly, the bacterial isolates are inoculated into nutrient broth media amended with different concentrations of tryptophan (0, 1, 2, and 5 mg mL⁻¹) and incubated at $35 \pm 2^\circ\text{C}$ for 15 days. At the end of the incubation period, the inoculated culture was centrifuged at 3000 rpm for 30 min. After that, two mL of the obtained supernatant was mixed with 2 drops of orthophosphoric acid and 4 mL of Salkowski's reagent (300 mL concentrated Sulfuric acid: 500 mL distilled water: 15 mL 0.5 M FeCl₃). The development of pink color indicates successful IAA production. The intensity of the formed color was measured at 530 nm using a spectrophotometer (T60 UV-Visible spectrophotometer) [39]. The amount of IAA was estimated via a standard IAA graph to detect the best tryptophan concentration for IAA production.

B- Quantitative assay

For quantitative IAA assay, the rhizospheric bacterial isolates were inoculated in nutrient broth media supplemented with 5 mg mL⁻¹ of tryptophan (the best concentration of tryptophan for IAA production based on qualitative screening) and incubated at $35 \pm 2^\circ\text{C}$ for 14 days. Approximately two mL of inoculated culture was withdrawn after the 2nd day up to the 14th day with 2 days interval and centrifuged at 3000 rpm for 30 minutes. One mL of the supernatant was mixed with 1 drop of orthophosphoric acid and 2 mL of Salkowski's reagent. The intensity of development of pink color was measured at 530 nm using a spectrophotometer [39]. The amount of IAA produced was estimated by the standard IAA graph to detect the best days for IAA production.

Detection of other phytohormones

The three most potent rhizospheric bacterial isolates were screened for their ability to produce other phytohormones including gibberellic acids (GA_3), abscisic acid (ABA), benzyl, kintin, and ziaten. The bacterial isolates were grown in Ashby's medium (containing g L⁻¹: mannitol, 10; sucrose, 5; K_2HPO_4 , 0.5; NaCl, 0.2; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCO_3$, 0.5; $CaSO_4$, 0.1; $FeCl_3$, 0.001; $MnSO_4 \cdot 2H_2O$, 0.001; $Na_2MoO_4 \cdot 2H_2O$, 0.002; 1.0 L distilled H_2O) and incubated at $30 \pm 2^\circ C$ for 7 days. Then the ability of the growing cultures to produce phytohormones is well determined quantitatively by high-performance liquid chromatography (HPLC) as previously reported [40].

Biocontrol of *Fusarium* wilt in *Vicia faba* plant using rhizosphere bacterial isolates

Greenhouse experiment.

A. Experimental Design.

The pot experiment using a completely randomized design and three replicates for each treatment was achieved to investigate the efficacy of rhizospheric bacterial isolates (Vb1, Vb3, and Vb6) to reduce the incidences of *Fusarium* wilt in faba bean plants under greenhouse conditions. The soil used in the greenhouse experiment was collected from EL-Qantra Sharq Experimental Station of Desert Research Center (D.R.C.), North Sinai Governorate, Egypt (Table 1). The Maryot-2 faba bean cultivar and their surface sterilization were achieved as mentioned in the pathogenicity test.

B. Bacterial inoculations

The three bacterial species were inoculated into nutrient broth media separately or in a consortium and incubated for 24 h at $35 \pm 2^\circ C$. The sterilized faba bean seeds were subjected to pre-germination to check their health and divided into five groups, each group containing four similar germination seeds were selected. Four groups were soaked in 100 ml of bacterial culture (6×10^9 cell/ml) that were grown individually or in a consortium, whereas the fifth group of pre-germinated seeds was soaked in the un-inoculated bacterial culture as a control. All soaked pre-germinated seeds are incubated for 6 h at $35 \pm 2^\circ C$ and sown in pots filled with 2 kg of sterilized soil. Each pot receives 3 pre-germinated seeds. The most pathogenic *F. oxysporum* was inoculated onto 100 ml of PD broth media and incubated at $25 \pm 2^\circ C$ for 15 days. At the end of the

incubation period, the inoculated PD broth media was centrifuged, and the obtained pellets were resuspended in 100 ml distilled water (adjusted at 5×10^5 conidia/ml) which was used as an inoculum for soil after one week of planting. The plants were irrigated as necessary and daily observed to check the infection. Disease severity percentages were estimated after 60 days post-sowing [41]. The percentages of reduction in the incidence of *Fusarium* wilt were calculated as follow:

$$\text{Reduction percentages (\%)} = \frac{A - B}{A} \times 100 \quad (3)$$

Where A is disease incidence of positive control, B is disease incidence of treatment.

Field experiment.

A. Experimental Design.

The field trials were conducted at the EL-Qantra Sharq Experimental Station of Desert Research Center (D.R.C.), North Sinai Governorate, Egypt. The physical and chemical analysis of soil was represented in Table 1. The field experimental design was achieved using a split-plot design, in which the distance between rows was 50 cm, while the distance between different plants on the same row was 30 cm and the distance between different treatments was 1.0 m. The experiment was carried out using 6 replicates for each treatment with an area of 3 m for every plot. The drip irrigation was used as an irrigation system during the experiment. The organic fertilization was added for the first time before planting and mixed well with the soil. The chemical and physical composition of organic fertilizer is shown in **Table 1**. On the other hand, inorganic fertilization was added (full doses) as recommended by the Ministry of Agriculture and Land Reclamation, Cairo, Egypt. The inorganic fertilizer consists of ammonium sulfate (20.5% N) as nitrogen fertilizer, calcium superphosphate (15.5% P_2O_5) as phosphorus fertilizer, and potassium sulfate (48% K_2O) as potassium fertilizer. The inorganic fertilization was added at three periods as follows: immediately after planting, after 30 days, and after 40 days of planting.

B- Inoculation, planting, and samples analysis

Before the experiment, the faba bean seeds were subjected to surface sterilization and pre-germination to select the seeds with the same radical length. The three bacterial

isolates (Vb1, Vb3, and Vb6) were inoculated into nutrient broth media and incubated at $35 \pm 2^\circ\text{C}$ for 24 h. At the end of the incubation period, the selected seeds were incubated with bacterial suspension (10^9 CFU mL^{-1}) for 6 h which was picked up and planted into the soil. After one week of planting, approximately 20 ml of *Fusarium oxysporum* suspension (5×10^5 conidia/ml) was added beside the seedling roots. The different treatments were conducted as follows: (1) seeds without any treatment (healthy control), (2) seeds planting into soil infected with *F. oxysporum* (infected control), (3) seeds treated with Vb1 and planting in soil infested with *F. oxysporum*, (4) seeds treated with Vb3 and planting in soil infested with *F. oxysporum*, (5) seeds treated with Vb6 and planting in soil infested with *F. oxysporum*, (6) seeds treated with the bacterial consortium (Vb1 + Vb3) and planting in soil infested with *F. oxysporum*, (7) seeds treated with the bacterial consortium (Vb1 + Vb6) and planting in soil infested with *F. oxysporum*, (8) seeds treated with the bacterial consortium (Vb3 + Vb6) and planting in soil infested with *F. oxysporum*, (9) seeds treated with the bacterial consortium (Vb1 + Vb3 + Vb6) and planting in soil infested with *F. oxysporum*.

After 60 days of sowing, the plant height (cm), number of pods per plant, fresh weight, and dry weight of plant (g) were also recorded. Moreover, the disease root rot/wilt severity and reduction percentages were recorded as mentioned above after 60 days of planting [41].

Statistical analysis

The layout of the experiment was a split-plot design. The experiment included three replicates for each treatment. Data were subjected to statistical analysis by a statistical package SPSS v17. The mean difference comparison between the treatments was analyzed by analysis of variance (ANOVA) and subsequently by Turkey's HSD (honestly significant difference) test at $p < 0.05$.

Result and Discussions

Isolation and identification of pathogenic *Fusarium* spp.

Faba bean (*Vicia faba* L.) is considered one of the most important legume crops, grown overall in the world to be used as human food and animal feed [42]. The significant losses in faba bean yield can be related to fungal infections which cause root rot, damping-off, and

wilt diseases [43]. The most common fungal species that destroy faba bean growth and hence cause significant yield losses are *Fusarium* species [44]. Therefore, seven *Fusarium* spp. were isolated from the naturally infected root of faba bean collected from Sinai governorate, Egypt. The obtained *Fusarium* spp. were identified based on morphological and cultural characteristics as *Fusarium proliferatum*, *Fusarium subglutinans*, *Rhizoctonia solani*, *Fusarium solani* (two isolates), and *Fusarium oxysporum* (two isolates).

Fusarium oxysporum is considered the main phytopathogen that causes *Fusarium* wilt and can be remain to exist in the soil for several years without a main host; therefore, it is difficult to control the disease caused by *Fusarium* [45]. In the current study, the pathogenic severity for two *F. oxysporum* strains to cause wilt disease in faba bean was estimated under greenhouse experiment. Data analysis showed that the percentages of disease severity due to infection with two strains of *F. oxysporum* were 68% and 47% as compared with control. Therefore, we select the strain that causes the highest *Fusarium* wilt for further tests.

Isolation and selection of the most potent rhizospheric bacterial isolates

The interactions between rhizospheric bacterial species and the root system of plants have a positive impact on plant health, yield, and soil quality. These positive impacts can be attributed to the high efficacy of rhizospheric bacterial strains and secondary metabolites to increase the phosphate solubilization, nitrogen fixation, increase the availability of nutrients, ameliorate the biotic and abiotic stresses, biological control of phytopathogens, enhance the plant immunity through induced systematic resistance (ISR) [46]. In the current study, out of 23 bacterial species isolated from rhizosphere soil of healthy faba bean plant, seven bacterial isolates exhibited the efficacy to inhibit the growth of *F. oxysporum* using in-vitro dual culture assay with varying degrees ranging between 39.06 ± 0.3 and 59.1 ± 0.2 . Among seven rhizospheric bacterial isolates, three isolates designated as Vb1, Vb3, and Vb6 showed the highest efficacy to inhibit *F. oxysporum* growth with reduction percentages of 59.1 ± 0.2 , 46.4 ± 0.3 , and 52.5 ± 0.3 , respectively (Fig. 1). The in-vitro dual culture is a common assay method for preliminary investigation, in which the antagonistic activity was detected through measuring the inhibition percentage of radial mycelial growth towards the inoculated bacterial strains [47]. Consistent with our study, out of 35 rhizospheric bacterial isolates, five

Figure 1: Antagonistic activity of the most potent bacterial isolates Vb1, Vb3, and Vb6 against highest pathogenic *F. oxysporum* strain by in-vitro dual culture assay. Bars with the different letters are significantly different ($p \leq 0.05$). Error bars indicate means $\pm$ SE ($n = 3$).

isolates exhibit *F. oxysporum* mycelial growth inhibition with varying percentages, and the bacterial isolate BA5 was the promising rhizospheric bacterial isolate to inhibit the mycelial growth with a percent of 58.3% [48]. Also, out of 100 microbial isolates obtained from rhizosphere soil samples, the bacterial isolate NJN-6 which was identified as *Bacillus amyloliquefaciens* showed the highest inhibition percentage towards *F. oxysporum* [49].

Identification of the most potent bacterial isolates

The three bacterial isolates designated as Vb1, Vb3, and Vb6 that exhibit high antagonistic activity with *F. oxysporum* were undergone identification based on amplification and sequencing of the 16S rRNA gene. The sequence analysis showed that the isolates Vb1 have a similarity percentage of 99.7 with *Bacillus velezensis*, whereas the isolates Vb3, and Vb6 have similarity percentages of 99.9 and 99.8% with two isolates of *Bacillus paramycoides*, respectively (**Fig. 2**). Similarly, the five rhizospheric bacterial isolates that showed high efficacy against *Fusarium* wilt, root rot, and crown diseases in tomatoes were identified using 16S rRNA as *Acinetobacter calcoaceticus* (AcDB3), *B. amyloliquefaciens* (BaMA26), *B. siamensis* (BsiDA2), *B. subtilis* (BsTA16), and *B. thuringiensis* (BtMB9) [50]. Accordingly, the *Bacillus* spp. are predominant rhizospheric bacterial species used to biocontrol of phytopathogenic fungi [47, 49, 50]. This phenomenon can be attributed to their different secondary metabolites and rapid growth which enables them to high integrate into biocontrol studies [51]. Recently, the cell immobilization

of *B. velezensis* NH-1 was used to biocontrol of *Fusarium* wilt caused by *F. oxysporum* in cucumber [52]. Although the *B. velezensis* is a common bacterial species used in biocontrol, this is the first report for using it and *B. paramycoides* for biocontrol of *Fusarium* wilt caused by *F. oxysporum* in faba bean.

Characterization of the rhizospheric bacterial strains as plant growth-promoting

The antagonistic activity of rhizospheric bacterial species towards phytopathogenic fungi can be attributed to their plant growth-promoting traits. Among these traits, the efficacy of rhizospheric bacteria to producing hydrolytic enzymes, nutrient competitions, nitrogen fixations, siderophores production, phosphate solubilization, phytohormones production, and ammonia production [53]. The preliminary screening of three selected rhizospheric bacteria showed their efficacy to fixed nitrogen by producing blue color around the bacterial colony grown on nitrogen-free malate medium. The quantification nitrogen-fixing assay was achieved through the acetylene reduction method. Data on solid media (qualitative assay) are compatible with those recorded from liquid media (quantitative assay) after being analyzed by gas chromatography. Data analysis showed that the highest nitrogen-fixing was recorded for bacterial isolate Vb1 with the ethylene production value of 391.4 ± 1.5 n mole C_2H_4 /ml/24h, followed by Vb3 and Vb6 with values of 351.8 ± 1.2 and 258.7 ± 1.01 n mole C_2H_4 /ml/24h (**Table 2**). Similarly, the nitrogen-fixing efficacy of rhizospheric bacterial species associated with *Calligonum polygonoides* and *Lasiurus indicus* was assessed qualitatively via color change and quantitatively via acetylene reduction assay [29].

Another mechanisms utilized by rhizospheric microbes to enhance plant growth and then compete with phytopathogens are phosphate solubilization and ammonia production [54]. Among macronutrients required by a high amount for enhancement of plant growth is phosphorus ion, which exists in most cases as an insoluble form. Rhizospheric bacteria have the ability to convert phosphorus from insoluble to soluble form through different mechanisms such as the production of enzymes and /or organic acids [55]. In the current study, the appearance of a clear zone around the bacterial growth on Pikovskaya agar media indicates their success in phosphate solubilizing. The diameter of clear zones was 1.3 ± 0.02 , 9.4 ± 0.05 , and 9.9 ± 0.12 mm for Vb1, Vb3, and Vb6, respectively (**Table 2**) which correlated with the amount of liberated phosphate. The quantitative assay is depending

Figure 2: Phylogenetic analysis of 16sr RNA sequences of the bacterial isolates with the sequences retrieved from NCBI. The analysis was conducted with MEGA 7 using the neighbor-joining method with bootstrap value (1000 replicates).

Table 2: Characterization of selected rhizosphere bacterial strains Vb1, Vb3, and Vb6 as plant growth-promoting.

Test	Rhizosphere bacterial isolate		
	Vb1	Vb3	Vb6
Nitrogen fixation (n mole C ₂ H ₄ /ml/24 h.)	391.44±1.46 ^a	351.84±1.17 ^a	258.72±1.01 ^b
Phosphorus solubilization (clear zone (mm))	1.3±0.02 ^d	9.4±0.05 ^a	9.9±0.12 ^a
Ammonia	+	++	+
Amylase (mm)	14±0.0 ^a	0.00	5.33±0.88 ^b
Cellulase (mm)	36±0.0 ^a	28.33±3.84 ^b	30±1.53 ^b
Protease (mm)	13±2.08 ^a	17.67±2.40 ^a	17.67±0.88 ^a
Catalase	-	+	+
Siderophore	+	+	+
HCN	+	++	++

Values within the same row with different letters are significantly different ($p \leq 0.05$), values are means $\pm$ SD ($n = 3$), -, +, ++ = negative, low, strong.

on the amount of liberated organic acid detected by the phosphomolybdate method. The results showed that the highest phosphate solubilizing was achieved by bacterial isolate Vb6 followed by Vb3 and Vb1. Also, the difference between the amount of liberated phosphate recorded by bacterial isolates Vb6 and Vb3 are non-significant which recorded $91.5 \pm 1.2 \mu\text{g ml}^{-1}$ and $91.1 \pm 1.3 \mu\text{g ml}^{-1}$ respectively at 2nd days and reached $152.2 \pm 1.3 \mu\text{g ml}^{-1}$ and $151.8 \pm 1.3 \mu\text{g ml}^{-1}$ at tenth days (**Fig. 3**). The lowest amount of phosphate solubilization is recorded by bacterial strain Vb1 which was $41.82 \pm 0.75 \mu\text{g ml}^{-1}$ after 2nd days and decreased to $37.73 \pm 0.62 \mu\text{g ml}^{-1}$ after tenth days as compared with control which recorded $25.64 \pm 0.59 \mu\text{g ml}^{-1}$ and $27.31 \pm 0.49 \mu\text{g ml}^{-1}$ after second and tenth days, respectively (**Fig. 3**). According to the obtained data, the quantitative assay is compatible with those reported by the qualitative assay (formed clear zone). The production of low molecular weight organic acid is considered the main mechanism for phosphate solubilization by different bacterial species [56].

The obtained data highlighted that all rhizospheric bacterial isolates have the ability to produce ammonia with varying degrees according to color intensity after adding Nessler's reagent (**Table 2**). The highest intensity for brownish color was reported for isolate Vb3 followed by Vb1 and Vb6. Ammonia production enhanced plant growth by the synthesis of biomolecules containing nitrogen. Also, ammonia can increase the root and shoot length, enhance the fresh weight of the plants, and increase the efficacy of the plant against phytopathogens attacks [57].

The extracellular hydrolytic enzymes secreted by rhizospheric microbes are important for the decomposition of different polymers such as chitin, cellulase, starch, and lignin which ultimately to mineralization to beneficial minerals such as P, N, and S [58]. The secretion of these hydrolytic enzymes by different rhizosphere microbes has a critical role in the suppression of phytopathogenic fungi through the degradation of their cell wall [59, 60]. For example, hydrolytic amylase, lipase, cellulase, pectinase, and protease enzyme are released by rhizospheric bacterial species such as *Bacillus*, *Pseudomonas*, and *Enterobacter* are showed inhibitory action against phytopathogenic fungi [61]. In the current study, the rhizosphere bacterial strains *B. velezensis* Vb1, *B. paramycoides* Vb3, and *B. paramycoides* Vb6 possess the efficacy to secrete amylase, cellulase, protease, and catalase except the bacterial strains Vb3 and Vb1 lack the efficacy to secrete amylase and catalase (**Table 2**). Data analysis showed that the highest clear zone formed around bacterial strain was recorded for cellulase enzymes with values of 36 ± 0.0 , 28.3 ± 3.8 ,

Figure 3: The amount of P-liberated by rhizospheric bacterial strains Vb1, Vb3, and Vb6 in liquid media.

and $30 \pm 1.53 \text{ mm}$. Catalase enzymes promote plant growth by the indirect mechanism through scavenging the free radicals released because of biotic and abiotic stresses [36].

Siderophores have a critical role in the biocontrol of soilborne fungi as reported previously [62]. The inhibitory effect of siderophores against phytopathogenic fungi due to converting Fe^{3+} (inorganic form) to Fe^{2+} (organic form), and hence making it available for plant and unavailable for phytopathogens [63]. The selected bacterial strains Vb1, Vb3, and Vb6 are recorded as siderophores producers through the appearance of an orange halo around the bacterial growth. Similarly, *Bacillus siamensis*, *B. subtilis*, and *B. amyloliquefaciens* are characterized by their efficacy in producing siderophores which help in the biocontrol of *Fusarium* wilt caused by *F. oxysporum* [50]. Also, the efficacy of *Pseudomonas aeruginosa* JAS-25 to colonizing the roots, secretion of antibiotics, and antagonistic activity against pathogenic fungi can be related to their potentiality to siderophores production [64]. Hydrogen cyanide (HCN) is defined as a volatile secondary metabolite secreted by various rhizosphere bacterial species. It is characterized by its efficacy in suppression of soilborne pathogenic fungi through blocking the electron transport system and hence disrupting the supply of the cell by energy which ultimately to cell death [65]. Data showed that all selected rhizosphere bacterial strains have the ability to produce HCN with different degrees due to the color change of filter paper from deep yellow to reddish-brown (**Table 2**). Recently, various rhizosphere bacterial species are recorded as positive for HCN production such as *Aeromonas*, *Pseudomonas*, *Bacillus*, *Alcaligenes*, and *Rhizobium* [66].

Figure 4: Phytohormones production by three rhizospheric bacterial species Vb1, Vb3, and Vb6. A) denote the qualitative production of IAA at different concentrations of tryptophan (0, 1, 2, and 5 mg mL⁻¹); B) denote the quantitative production of IAA at different interval times in presence of 5 mg mL⁻¹ tryptophan, C) denote the production of ABA, Benzyl, Kinten, and Ziaten phytohormones detected by HPLC analysis, D) denote the production of GA₃ detected by HPLC. Bars with the different letters are significantly different ($p \leq 0.05$). Error bars indicate means $\pm$ SE ($n = 3$).

The ability of rhizosphere bacterial strains to produce auxin and cytokinin is a promising tool to study the profound effect of these strains on plant growth [67]. Indole-3-acetic acid (IAA) is considered the main auxins synthesized by plants and has a profound role in leaf formation, root initiation, embryo development, phototropism, fruit development, geotropism, abscission. It has a positive impact on the root length, increases root branches, root laterals, and root hair that facilitate the nutrient uptake from the surrounding environment [68]. The efficacy of three rhizospheric bacterial strains Vb1, Vb3, and Vb6 to produce IAA were screened qualitatively at broth media in the absence and presence of L-tryptophan (0, 1, 2, and 5 mg mL⁻¹) as a precursor for IAA after 15 days. Data represented in **Fig. (4A)** showed that the maximum

IAA production in absence of L-tryptophan was achieved for bacterial isolate Vb6 with a value of 5.2 ± 1.03 $\mu\text{g mL}^{-1}$ and this concentration was increased up to 67.9 ± 3.3 $\mu\text{g mL}^{-1}$ at 5 mg mL⁻¹ L-tryptophan. Moreover, the IAA production by bacterial strains Vb1 and Vb3 was increased from 2.1 ± 0.2 and 2.8 ± 0.4 $\mu\text{g mL}^{-1}$ in absence of L-tryptophan to 81.3 ± 1.9 and 58.2 ± 4.3 $\mu\text{g mL}^{-1}$ in presence of 5 mg mL⁻¹ tryptophan respectively (**Fig. 4A**). Compatible with our study, the IAA production by *B. siamensis* was increased in liquid media supplemented with L-tryptophan as compared with those without L-tryptophan, and the highest IAA production was achieved at 250 $\mu\text{g mL}^{-1}$ [69].

According to qualitative assay, the concentration of 5 mg mL⁻¹ L-tryptophan is the best for higher IAA production and hence it was selected to investigate the quantitative

IAA assay. Analysis of variance showed that the maximum IAA concentration was recorded at 10, 14, and 14 days for rhizospheric bacterial strains Vb1, Vb3, and Vb6 with values of 61.4 ± 3.7 , 25.9 ± 1.4 , and 59.6 ± 0.5 $\mu\text{g ml}^{-1}$, respectively.

Furthermore, the rhizospheric bacterial strains were quantitative screening for their efficacy to produce various phytohormones using HPLC analysis. Data analysis showed that the successful rhizospheric bacterial species Vb1, Vb3, and Vb6 to produce ABA, Benzyl, Kinten, Ziaten, and GA_3 with values of (0.04 ± 0.01 , 0.03 ± 0.01 , and 0.02 ± 0.01 mg/100 ml), (1.53 ± 0.1 , 0.23 ± 0.05 , and 0.22 ± 0.03 mg/100 ml), (0.34 ± 0.01 , 0.96 ± 0.01 , and 0.27 ± 0.01 mg/100 ml), (0.54 ± 0.01 , 0.67 ± 0.01 , and 0.38 ± 0.01 mg/100 ml), and (14.45 ± 1.6 , 13.88 ± 0.9 , and 7.80 ± 0.8 mg/100 ml), respectively (Fig. 4 B and C). The ABA has an important role in improving plant adaptation under various stresses and regulating plant growth [70]. Also, Gibberellic acid is another phytohormone that has a critical role in ameliorating different stress, directly affects plant growth, plant yield, nitrogen metabolism, and mineral nutrition [71]. The efficacy of rhizosphere bacteria strains to secrete different phytohormones are mentioned in different published studies [70, 72, 73]. Based on the above plant growth-promoting traits of three rhizosphere bacterial strains can be use of these strains as bioinoculant to increase plant growth and hence increase the tolerance of a plant to phytopathogens infection.

Biocontrol of Fusarium wilt in *Vicia faba* L. plant using rhizosphere bacterial isolates.

Greenhouse experiment.

The efficacy of plant growth-promoting rhizobacteria Vb1, Vb3, and Vb6 to reduce the wilt disease severity caused by *F. oxysporum* was investigated under greenhouse conditions. Data analysis showed that the treatment of *Vicia faba* (faba bean) seeds with rhizosphere strains have the efficacy to protect the plant from Fusarium wilt with percentages of 70.0, 60.13, and 65.2% for Vb1, Vb3, and Vb6 respectively (Table 3). Interestingly, the treatment of seeds with bacterial consortium decreases the Fusarium wilt disease severity with a percentage of 82% (Table 3). Similarly, the rhizospheric bacteria strain *Bacillus thuringiensis* strain BtMB9 and *Acinetobacter calcoaceticus* strain AcDB3 have the efficacy to decrease the Fusarium wilt disease severity in tomato, whereas the bacterial combination can highly decrease disease severity than separate one [50]. The obtained results indicated that the rhizosphere soil of the

Table 3: Effect of rhizospheric bacterial strains Vb1, Vb3, and Vb6 on disease severity and protection of faba bean plants infected with *Fusarium oxysporum* under greenhouse conditions.

Treatments	<i>Fusarium</i> wilt severity	Protection (%)
Un infected faba bean plant (control)	0.00 ^e	100 ^a
<i>B. velezensis</i> Vb1 + <i>F. oxysporum</i>	30.0 $\pm$ 2.0 ^c	70.00 ^c
<i>B. paramycoides</i> Vb3 + <i>F. oxysporum</i>	39.9 $\pm$ 1.8 ^a	60.13 ^e
<i>B. paramycoides</i> Vb6 + <i>F. oxysporum</i>	34.8 $\pm$ 3.1 ^b	65.20 ^d
Mix (Vb1 + Vb3 + Vb6) + <i>F. oxysporum</i>	18.0 $\pm$ 2.0 ^d	82 ^b

Values within the same column with different letters are significantly different ($p \leq 0.05$), values are means $\pm$ SD ($n = 3$).

same plant is the proper source to isolate bacterial species used in antagonists [74]. Although some investigators reported that the antagonistic bacterial species used in the control of phytopathogens can come from rhizosphere soil of different plants [75]. Previous studies reported the high efficacy of *Bacillus* spp. to be used in biocontrol of Fusarium wilt disease caused by *F. oxysporum* under greenhouse conditions [50, 76]. Furthermore, *Bacillus siamensis* has the efficacy to decrease the disease severity of tobacco brown spots caused by *Alternaria alternata* under greenhouse conditions [77]. Also, rhizosphere *Bacillus amyloliquefaciens* was used to significantly decrease the Fusarium wilt disease severity caused by *F. oxysporum* under greenhouse experiment [78]. To the best of our knowledge, this is the first report that uses the three rhizosphere bacterial strains *B. velezensis* and *B. paramycoides* to control Fusarium wilt caused by *F. oxysporum* under greenhouse conditions.

Field experiment

Data represented in Table 4 showed the potency of *B. velezensis* Vb1, *B. paramycoides* Vb3, and *B. paramycoides* Vb6 to improve *F. oxysporum*-infected faba bean plants either separately or in a consortium. Inoculation of faba bean seeds with rhizospheric bacterial strains and planting into *F. oxysporum*-infected soil causes a significant increase in plant heights, the number of pods/plant, and fresh and dry weight as compared with those planting into infected soil without bacterial inoculation. For instance, inoculation with separate bacterial species

Table 4: The morphological characteristics and wilt disease severity of faba bean plant due to treatment with three rhizospheric bacterial strains Vb1, Vb3, and Vb6 (individually or in a consortium) against *F. oxysporum*.

Treatment	Plant height (cm)	Pods/plant	Fresh weight (g)	Dry weight (g)	Wilt severity (%)	Protection (%)
Healthy plant	83.3±2.1 ^f	21.0±1.0 ^d	51.7±1.2 ^e	18.0±2.6 ^d	0.0±0.0 ^g	100 ^a
Infected plant	64.7±1.5 ^e	12.3±1.5 ^e	38.7±2.1 ^f	13.3±1.5 ^e	71.7±2.1 ^a	28.3 ^g
Vb1+ <i>F. oxysporum</i>	88.7±0.6 ^d	23.0±1.0 ^c	57.3±2.1 ^d	22.0±3.0 ^c	32.3±0.6 ^d	67.7 ^d
Vb3 + <i>F. oxysporum</i>	89.0±1.0 ^d	23.7±2.1 ^c	57.7±2.1 ^d	22.7±2.1 ^c	41.7±1.2 ^b	58.3 ^f
Vb6 + <i>F. oxysporum</i>	86.0±1.0 ^{ef}	22.0±2.6 ^{cd}	56.3±3.1 ^d	21.0±3.0 ^c	37.0±1.7 ^c	63.0 ^e
(Vb1+Vb3) + <i>F. oxysporum</i>	93.0±2.6 ^c	24.7±1.5 ^c	60.3±2.5 ^c	23.3±3.2 ^b	29.0±1.0 ^d	71 ^c
(Vb1+Vb6) + <i>F. oxysporum</i>	96.7±1.2 ^b	26.3±1.5 ^b	65.7±1.5 ^b	24.3±2.5 ^b	26.3±0.6 ^e	73.7 ^c
(Vb3+Vb6) + <i>F. oxysporum</i>	97.3±1.5 ^b	28.7±1.6 ^b	66.7±3.1 ^b	26.3±2.1 ^b	31.3±1.2 ^d	68.7 ^d
(V-mix) + <i>F. oxysporum</i>	113.0±2.0 ^a	33.3±2.1 ^a	75.7±1.5 ^a	33.7±1.5 ^a	16.7±1.2 ^f	83.3 ^b

Healthy plant meaning planting of faba bean in soil without rhizobacterial strains and *F. oxysporum*; Infected plant meaning planting of faba bean in *F. oxysporum*-infected soil in absence of rhizobacterial strains; Vb1 is *B. velezensis*; Vb3 is *B. paramycooides*, and Vb6 is *B. paramycooides*. Values within the same row with different letters are significantly different ($p \leq 0.05$), values are means $\pm$ SD ($n = 3$).

enhance plant height with percentages of 21.3% - 24.3%, whereas the bacterial consortium highly improves faba bean plant height with percentages of 28.3% - 48.3% and the highest significant value was recorded for V-mix treatment (Vb1+Vb3+Vb6) as compared with the infected plant (plant sowing in infected soil in absence of bacterial strains). Moreover, the number of pods/ plants were highly reduced in the infected plant (12.3±1.5) as compared with a healthy plant (21.0±1.0). The presence of plant growth-promoting rhizobacterial strains causes a significant increase in the number of pods as compared with healthy plants and the highest number was recorded for the bacterial consortium.

On the other hand, the fresh weight and dry weight of healthy and infected faba bean plants after 60 days of sowing were (51.7±1.2 g and 18.0±2.6 g) and (38.7±2.1 g and 13.3±1.5 g), respectively. The presence of *F. oxysporum* in absence of bacterial rhizospheric strains has a negative impact on all morphological characteristics of faba bean. Inoculation of plants with different bacterial strains Vb1, Vb3, and Vb6 has been significantly improving fresh and dry weight in presence of fungus. For instance, the presence of *B. velezensis* Vb1 improves the fresh weight and dry weight with values of 18.6% and 8.7% respectively, whereas the bacterial consortium (V-mix) increases the fresh weight and dry weight with percentages of 37% and 20.4% respectively in presence of *F. oxysporum* strain (Table 4). The obtained data are in harmony with those reported that the rhizobacterial strains *Rhizobium* sp., *Bacillus pumilus*, and *Pseudomonas alcaligenes* have the efficacy to improve the number of pods, dry weight,

fresh weight, and plant height of lentil in presence of *Fusarium oxysporum* causing wilt disease [79]. Similarly, rhizobacterial strain *Bacillus* sp. SJ-5 was used to biocontrol disease caused by *Fusarium oxysporum* & *Rhizoctonia solani* in soybean plants [80].

The current study showed that the three rhizospheric *Bacillus* spp. have the potency to improve the growth performance of the *F. oxysporum*-infected faba bean plant. This phenomenon can be attributed to antagonistic activity between rhizospheric bacteria and pathogens, producing lytic enzymes, secretion of bioactive inhibitory compounds, or competition between bacteria and pathogenic fungi on essential nutrients [81, 82]. *Bacillus* spp. are considered the most common rhizospheric bacterial strains used to decrease the wilting disease caused by *F. oxysporum* in various plants [83, 84]. The various published studies reported that the *Bacillus* spp. have various suppressive mechanisms including producing volatile substances, induction of systematic resistance, stimulating plant growth, and aggressive plant roots colonization [13, 79].

The planting of faba bean in infected soil with *F. oxysporum* caused 71.7 $\pm$ 2.1 wilt severity after 60 days of planting (Table 4). The inoculation of faba bean seeds with plant growth-promoting rhizospheres causes a significant decrease in wilt disease. For example, the presence of bacteria consortium V-mix (Vb1+Vb3+Vb6) has the efficacy to protect faba bean plant from wilt with percentages of 83.3%, followed by bacterial treatment Vb1+Vb6 that reduce the wilt with a percentage of 73.7%. Not only rhizobacterial consortium can reduce the wilt

disease, but also separate species have the ability. For instance, the presence of *B. velezensis* Vb1 can reduce the wilt disease with a percentage of 67.7% as compared with infected plants followed by *B. paramycoides* Vb6 and *B. paramycoides* Vb3 (Table 4). Un-compatible with our study, Akköprü and Demir reported that the treatment with single rhizobacterial species was more effective than *Glomus intraradices* as mycorrhizal fungi or consortium between mycorrhizae and rhizosphere bacteria to biocontrol of *F. oxysporum* f. sp. *lycopersici* in tomato plant [85]. The obtained results are in harmony with those recorded by Khalil and co-authors, who reported that the rhizospheric bacterial strains *B. siamensis* BsiDA2 and *B. subtilis* BsTA16 have significantly reduced wilt disease in tomatoes caused by *Fusarium oxysporum* spp. *lycopersici* [50]. The rhizospheric *Bacillus* spp. are used by various researchers to biocontrol wilt disease caused by *F. oxysporum* in various plants under greenhouse and field conditions [74, 81]. In the current study, the activity of *Bacillus* spp. to reduce the wilt disease can be attributed to producing siderophores, HCN, hydrolytic enzymes, and ammonia which help in suppressive phytopathogens.

Conclusion

In the current study, seven *Fusarium* spp. were isolated from the infected roots of the *Vicia faba* L. plant collected from Sinai governorate, Egypt. Two *F. oxysporum* strains that caused the highest disease severity of *Fusarium* wilt were selected. Then, three rhizobacterial species of *Bacillus* which showed the highest plant growth-promoting properties and antagonistic activities were isolated from the rhizospheric soil of the healthy faba bean plant. Biocontrol of *Fusarium* wilt in bean plants using the selected rhizobacterial species of *Bacillus* under the greenhouse and field experiments revealed the efficacy of plant growth-promoting rhizobacteria to reduce the wilt disease severity caused by *F. oxysporum*. Species of rhizobacteria being associated with the healthy plant roots, which promote plant growth and induce the plant defense against microbial pathogens should be subjected to further investigation.

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methodology, Resources, Software, Validation, Visualization, Writing-original draft; Amr Mahmoud Abd El-Gwad and Abbas Ahmed El-Ghamry: Conceptualization, Data curing, Investigation, Resources, Validation, Visualization, Writing-original draft.

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