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POTENTIAL OF SOME PLANT GROWTH-PROMOTING RHIZOBACTERIAL STRAINS AS BIOCONTROL AGENTS AGAINST FUSARIUM WILT DISEASE IN CUCUMBER

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ABSTRACT:

Herein, three *Fusarium; oxysporum* strains were isolated from infected cucumber-wilt roots and showed disease severity with the percentages of 73%, 66%, and 51%. The *F. oxysporum* exhibited the highest wilt disease was selected to investigate the biocontrol efficacy of plant growth-promoting rhizobacteria. To achieve this goal, three rhizobacterial strains were isolated and identified as *Achromobacter xylosoxidans* Ck2, *Bacillus haynesii* Ck5, and *Bacillus paramycoides* Ck7. These strains showed efficacy to inhibit the growth of *F. oxysporum in-vitro* with percentages of 56.2±0.2, 51.3±0.3, and 46.2±0.4% for Ck2, Ck5, and Ck7, respectively. These rhizobacterial strains showed nitrogen-fixing activity assayed by acetylene reduction and phosphate-solubilizing with values of (446.9±1.6, 310.8±1.9, and 213.6±1.2 n-moles-C_2H_4/mL/24h) and (183.6±1.1, 192.3±1.3, and 34.29±1.2 µg mL^{-1}) for Ck2, Ck5, and Ck7, respectively. Moreover, these strains showed plant growth-promoting properties including ammonia, hydrogen cyanide (HCN), siderophores, hydrolytic enzymes, and phytohormones production. Under greenhouse conditions, the rhizobacterial strains Ck2, Ck5, and Ck7, and their consortium had the efficacy to protect cumber from wilt disease caused by *F. oxysporum* with percentages of 67.6, 63.2, 58.7, and 75.7%, respectively. Whereas the highest growth performance and the highest wilt protection (79.1%) of *F. oxysporum*-infected cucumber was attained in presence of bacterial consortium under field conditions.

Key Words: *Fusarium oxysporum*, *Cucumis sativus* L., rhizobacterial strains, plant growth-promoting, phytohormones, biocontrol agent, and phytopathogen.

INTRODUCTION

Chemical fertilizers and pesticides are required to provide the essential nutrients for plant growth and to combat phytopathogens through different agronomic practices **(George *et al.,* 2013).** Excessive use of chemical fertilizers results in deterioration of soil properties, imbalances in nutrient

ratios, poor quality of agricultural products, and destruction of the ecological biosystem of environment **(Fouda *et al.,* 2021 and Saied *et al.,* 2021)**. There are several ways to deal with this enormous problem, such as changing resource usage, exploring renewable resources, and converting abiotic resources into infinite biological resources **(Alkahtani *et al.,* 2020 and Khalil *et al.,* 2021b).**

Plant growth-promoting rhizobacteria (PGPRs) are a group of microorganisms capable of colonizing the plant rhizosphere and capable of fixing nitrogen **(Puri *et al.,* 2016)**, dissolving organic and inorganic phosphorus **(Tsegaye *et al.,* 2017)**, secretion of auxin and antibiotics **(Spaepen and Vanderleyden, 2011)**, antagonizing pathogens **(Pal *et al.,* 2001)**, and promoting plant growth **(Hafeez *et al.,* 2006)**. PGPR can promote plant growth by several direct or indirect mechanisms **(Egamberdieva *et al.,* 2010)**. PGPR directly converts unavailable nitrogen and phosphorus sources into nutrients that can be absorbed and utilized by plants, and they secrete indole-3-acetic acid (IAA), gibberellin (GA), and cytokinin (CTK) to directly promote plant growth **(Soliman *et al.,* 2021)**. Moreover, PGPR colonize plant rhizosphere and inhibit or reduce soil-borne diseases **(Khalil *et al.,* 2021b)**. PGPRs also improve plant defenses by conferring resistance in plants, thereby reducing the effects of disease on plant growth, development and yield **(Verma *et al.,* 2015)**. Therefore, the use of PGPR in agricultural production plays an important role in ensuring the sustainable development of modern agriculture while reducing the use of chemical fertilizers and pesticides, preventing the emergence of pests and diseases, and achieving the goal of increasing production.

Cucumber (*Cucumis sativus* L.) is an economically important vegetable crop worldwide, and Fusarium wilt of cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum* (FOS) has become the major limiting factor in the cucumber continuous cropping system **(Raza *et al.,* 2017 and Islam *et al.,* 2018).** FOS is a soil pathogen that invades the vascular system of cucumbers and ultimately causes growth failure, yellowing and leaf necrosis and plant death **(Gordon, 2017)**. The pathogen *Fusarium oxysporum* was ranked the fifth in a survey of the international community of the top 10 fungal plant pathogens **(Dean *et al.,* 2012)**. All strains of *F. oxysporum* are saprophytic and can grow and survive for long periods in soil and in the rhizosphere of many plant species **(Wang *et al.,* 2021a)**. *Fusarium* wilt strains are highly host specific and are classified into more than 120 classes. Traditionally several control strategies for controlling *Fusarium* wilt disease in cucumber have been proposed, including the use of fungicides, crop rotations and resistant varieties. However, these approaches are not environmentally friendly, economical, or reliable **(Raza *et al.,* 2017 and Han *et al.,* 2019).**

Biological control is an interesting alternative method for protecting crops from *Fusarium* wilt. Many species of microorganisms such as *Bacillus* spp., *Pseudomonas* spp., *Trichoderma* spp., *Streptomyces* spp. *and Acinetobacter spp.* have been shown to effectively control FOC (**Raza *et al.,* 2017 ; Salem *et al.,* 2019 and Kumar** *et al.,* **2021).** The combined use of two or more biological control candidates has been used for many years in the control of various important plant diseases (**Jangir *et al.,* 2019 and Hansen *et al.,* 2020**). These methods have been shown to be more effective than using only beneficial microorganisms (**Jangir *et al.,* 2019**). Several studies have demonstrated the possibility of suppressing cucumber *Fusarium* wilt in continuous cropping systems using single antagonistic activity (**Cao *et al.,* 2011 and Han *et al.,* 2019**).

The current study aims to establish an efficient strategy to control cucumber-wilt disease caused by *Fusarium oxysporum* f. sp. *cucumerinum*. To achieve this goal, pathogenic *F. oxysporum* was isolated from the infected root of cucumber and identified based on cultural characteristics and microscopic examination. The highest pathogenic strain of *F. oxysporum* was selected based on their efficacy to produce maximum disease severity under greenhouse experiments. After that, the rhizobacterial strains were isolated from soil samples collected from around the healthy cucumber roots and identified using amplification and sequencing of 16S rRNA. The plant growth-promoting traits of identified rhizobacterial strains include nitrogen fixation, ammonia production, phosphate solubilization, hydrolytic enzymes activity, siderophore and HCN production, secretion of various phytohormones were investigated. Finally, the synergistic effects between rhizobacterial strains and selected *F. oxysporum* were investigated under greenhouse and field experiments.

MATERIALS AND METHODS

1. Isolation of *Fusarium* pathogen from infected cucumber

Fusarium oxysporum strains were isolated according to Kleczewski and Egel (**Kleczewski and Egel, 2011**). Infected cucumber plant roots with typical disease symptoms were collected from North Sinai governorate, Egypt. For fungal isolation, small segments of diseased tissues along with some healthy portions (5x5 mm^2) were cut by a sterilized razor and sterilized their surface with 2% sodium hypochlorite (NaOCl) for 2 minutes. Surface sterilized plant tissues were rinsed with sterilized distilled water for removing the last trace of sodium hypochlorite solution, dried on filter paper, and placed on Petri plates containing 15 mL of potato dextrose agar medium (PDA). Three to four pieces of sterilized tissues were placed in each Petri plate and incubated for 7 days at 25±2 °C. The obtained fungal isolates were identified using morphological culture and microscopic examination according to standard keys (**Booth, 1971 ; Nelson *et al.,* 1983 and Kleczewski and Egel, 2011**). The pure fungal isolates were inoculated on a PDA slant and incubated at 25±2 °C for 7 days, followed by being kept in a refrigerator for further study.

2. Investigation of the pathogenicity of *F. oxysporum* strains.

The pathogenicity tests were achieved at the garden of Desert Research Center (D.R.C.), EL-Qantara Sharq, North Sinai Governorate, Egypt (31°00'21.6" N and 32°33'48.1" E) under greenhouse conditions. The physical and chemical analyses of soil used in the greenhouse experiment were shown in Table 1. The pathogenicity test was achieved using cucumber cultivar hybrid namely (Vector) collected from Agricultural Research Center, Giza, Egypt. The inoculum of *F. oxysporum* was prepared by culturing the fungus on potato dextrose agar (PDA) medium for 5 days at 25±2 °C. The spore suspension was prepared using 20 mL sterilized distilled water and adjust the conidial concentration at 1 x 10^5 / mL using a haemocytometer **(Boedo *et al.,* 2012)**. Cucumber seeds were surface sterilized with 1.0% sodium hypochlorite for 1.0 min and then thoroughly washed with tap water. Three surface-sterilized cucumber seeds were sown in a plastic pot containing one kg of soil that was previously infected with prepared *F. oxysporum* spore suspension. On the other hand, three surface-sterilized seeds were also sown in a plastic pot containing soil without fungal spore suspension and served as a positive control. Five pots were used for each treatment. The pots were irrigated when it was necessary and adding organic fertilization as recommended by the Ministry of Agriculture and Land Reclamation, Cairo, Egypt.

Table 1. Physical and chemical analysis for soil and organic fertilizer used in the severity disease assay, pot, and field experiment.

Physical analysis		Soluble anions (meq L^{-1})	
Coarse sand (%)	56.6	HCO_3^-	3.4
Fine Sand (%)	31.1	Cl^-	6.4
Silt (%)	7.9	SO_4^{--}	5.9
Clay (%)	4.5	Analysis of Organic fertilization	
Soil texture	Loamy sand	Density (kg/m^3)	675
Chemical characters		Relative humidity	46.7
pH	7.9	EC mmhos/cm at 25°C	13.6
EC (Ds/m)	0.3	PH (1- 10 H_2O)	8.4
O.M. (%)	0.6	Organic matter	50
O.C. (%)	0.3	Organic carbon	30
T.N. (%)	0.5	N (%)	1.8
C/N ratio	11.5	P (%)	0.9
Soluble Cations (meq L^{-1})		K (%)	4.6
Ca^{++}	4.7	Fe (ppm)	3320
Mg^{++}	2.5	Zn (ppm)	93.5
Na^+	8.5	Cu (ppm)	49.1
K^+	0.8	Mn (ppm)	257

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

3. Assessment of the wilt-disease severity.

The percentages of cucumber-wilt disease caused by *F. oxysporum* were assessed after 60 days of planting. Disease severity was recorded by visual observation of the disease symptoms with reference to the

uninfected control. Disease index data were obtained and recorded according to the scale ranging from 0 to 4 **(Saha *et al.,* 2012)**. Symptom severity was graded into four disease classes as follows: 0 (No disease or wilt), 1 (1 - 25% of leaves withered), 2 (26 - 50% of leaves withered/traces of stem rot), 3 (51 - 75% of leaves withered/stunted growth/ stem rot) and 4 (76-100% of leaves withered/ damping off/ wilting/ seedling death). Based on the previous classes, the disease severity was calculated according to the following equation (1).

$$\text{Disease incidence} = \frac{\text{Disease class index x number of diseased plant in class}}{\text{Total investigated plant x maximum disease index}} \text{ x } 100$$

4. Isolation of rhizobacteria strains.

Five grams of rhizosphere soil surrounded the healthy cucumber roots were suspended into 20 mL sterile distilled water in a conical flask. The flask was shaken for 10 min followed by performing a serial dilution up to 10^{-6}. Approximately 1.0 mL of the final dilution was spread on a nutrient agar (NA) plate and incubated for 48 h at 35 ± 2°C. The inoculated plates were checked daily to picked-up the observed bacterial colonies onto a new NA plate. The purified bacterial isolates were streaked onto NA slant and kept in a refrigerator for further study **(Abdo *et al.,* 2021).**

5. Selection of the most potent bacterial isolates

The biocontrol of *F. oxysporum* using rhizobacterial isolates was assessed by the Dual-culture assay method **(Mahgoub *et al.,* 2021).** Briefly, the purified rhizobacterial isolate was spotted on the PDA plate at three equidistant points and incubated at 35 ± 2 °C for 24 h. After that, a fungal disk (5 mm) of *F. oxysporum* was inoculated in the center of PDA plates previously inoculated with rhizobacterial strain. The control was designated as a PDA plate containing a disk of *F. oxysporum* in absence of bacterial inoculation and incubated under the same conditions. The inoculated plates were incubated at 28 ± 2 °C for five days. The efficacy of rhizobacterial strain to inhibit fungal growth was calculated as inhibition percentages (%) as follows:

$$\text{Growth inhibition percentages (\%)} = \frac{C_1 - C_2}{C_1} \text{ x } 100 \quad (2)$$

Where C_1 is the average diameter of fungal growth in absence of rhizobacterial strain, C_2 is the average diameter of the fungal colony in the presence of rhizobacterial strain.

6. Identification of the most potent bacterial isolates.

The most potent bacterial isolates designated as CK2, CK5, and CK7 were identified based on sequencing and amplification of the 16S rRNA gene. The genomic DNA was extracted according to the modified method by **Miller *et al.,* (1999).** 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'-GGTTACCTTGTTACGACTT-3') were used to PCR amplification of the 16S rDNA fragment. The PCR tube containing PCR buffer (1 x), $MgCl_2$ (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 (**Alsharif *et al.,* 2020**). The sequences obtained in the current study were deposited at GeneBank under accession numbers LC610746, LC592037, LC592038 for strains Ck2, Ck5, and Ck7 respectably.

7. Plant-growth promoting traits of the selected rhizobacterial strains.

7.1. Nitrogen fixation.

The qualitative nitrogen-fixing ability of the selected rhizobacterial strains, Ck2, Ck5, and Ck7 was assessment via inoculation onto nitrogen-free Ashby's medium (containing glucose, 5 g; mannitol, 5 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $Na_2MoO_4 \cdot 2H_2O$, 5mg; K_2HPO_4, 0.9 g; KH_2PO_4, 0.1 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; $CaCO_3$, 5 g; agar-agar, 15 g; distilled water, 1000 mL; pH 7.3). The inoculated media was incubated at 35 ± 2 °C for 72 h. The ability of bacterial strains to grow on the nitrogen-free Ashby's media indicates a positive nitrogen-fixing strain (**Kizilkaya, 2008**).

On the other hand, the quantitative assessment was investigated via the assessment of nitrogenase activity by the acetylene reduction method. In this method, the rhizobacterial strain was inoculated in a test tube closed by a rubber stopper and containing 10 mL of nitrogen-free Jensen's semi-solid medium and incubated for 72 h at 35 ± 2°C. After that, one mL of acetylene gas was injected into the air of the headspace and incubated for 24 h at 35 ± 2°C. Finally, one mL of gas sample existing in the tube headspace was

assayed for ethylene production (C_2H_4) using Hewlett-Packard 5890 gas chromatography Series 2 plus. The nitrogenase activity was assessed as a mol number of C_2H_4/mL/24h [n-mol C2H4/mL/24h] (**Muangthong *et al.,* 2015**). The experiment was performed in triplicate.

7.2. Phosphate solubilization

The qualitative evaluation of the inorganic phosphate solubilizing by rhizospheric bacterial strains was performed according to the standard method of **Patel *et al.,* (2017)**. The overnight bacterial strain was streaked onto Pikovskaya's agar medium (contains yeast extract, 0.5 g; dextrose,1.0 g; $Ca_3(PO_4)_2$, 0.5 g; $(NH_4)_2SO_4$, 0.5 g; KCl, 0.2 g; $MgSO_4$, 0.1 g; $MnSO_4$, 0.1 g; $FeSO_4$, 0.1 g; Agar,15 g; distilled H_2O,100 mL) and incubated at 35 ± 2 °C for 3–4 days. The appearance of a clear zone around the bacterial growth indicated a positive result for phosphate solubilization.

The quantitative phosphate solubilization was analyzed by measuring the pH value and phosphorus concentration at specific time intervals (2-10 days). Briefly, Pikovskaya's broth medium complemented with 0.5% $Ca_3(PO_4)_2$ was inoculated with rhizobacterial strain and incubated at 35 ± 2 °C for 10 days on a rotary shaker at 180 *rpm*. Five mL was withdrawn each day and centrifuged at 10,000 *rpm* for 10 min. The available soluble phosphate in the supernatant was detected via measuring the optical density at 700 nm (spectrophotometer-Jenway 6305 UV) using the phosphomolybdate method**.** 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 (**Soliman *et al.,* 2021**).

7.3. Ammonia production

The production of ammonia (NH_3) by rhizobacterial strains was investigated by Nessler's reagent. The fresh bacterial culture was inoculated into 10 mL peptone water and incubated for 72h at 35°C, following this, 0.5 mL of Nessler's reagent was added to the inoculated tube. The development of yellow color with a varying degree was considered as a positive result for ammonia production. The results were recorded as +, ++, +++ according to the formed color intensity (**Fouda *et al.,* 2015**).

7.4. Hydrolytic and oxidative-stress enzymatic activities

The efficacy of rhizobacterial strains to producing amylase, protease, cellulase, and catalase was tested using mineral salt (MS) agar media (containing g L^{-1}: $NaNO_3$, 5; KH_2PO_4, 1; K_2HPO_4, 2; $MgSO_4.7H_2O$, 0.5; KCl, 0.1; $CaCl_2$, 0.01; $FeSO_4.7H_2O$, 0.02; agar, 15; distilled H_2O, 1L) supplemented with specific substrate, depending on the enzyme being tested. The rhizobacterial strain was spotted on the center of the MS agar plate supplemented with 1 % of gelatin, soluble starch, and carboxy-methylcellulose (CMC) for protease, amylase, and cellulase respectively. The inoculated plates were incubated at 35 ± 2˚C for 24 h. The plates without bacterial spotting were running with the experiment as a negative

control. At the end of the incubation period, the plates were flooding with specific reagents (1 % iodine for amylase and cellulase, whereas acidic mercuric chloride for protease), and the appearance of a clear zone around the bacterial colony was considered a positive result for extracellular enzymes. The results were recorded as a diameter of a clear zone (mm) **(Khalil *et al.,* 2021a)**.

For catalase activity, the overnight rhizobacterial cells were added to a clean slide using an inoculation loop and mixed with several drops of 3% hydrogen peroxide (H_2O_2). The catalase-positive results were recorded as the formation of oxygen bubbles **(Tiwari and Singh, 2017)**.

7.5. Siderophore production

For siderophore production, King's B medium (containing g L^{-1}: protease peptone, 20; K_2HPO_4, 1.5; $MgSO_4$, 1.5; Agar, 15; distilled H_2O, 1000 mL) was prepared and amended with a complex mixture of (chrome azurol S (CAS)/Fe^3/hexadecyl trimethyl ammonium bromide) as an indicator dye. The dye indicator was added to King's B with a percent of 1:15. The rhizobacterial strains were streaked on King's B plates supplemented with dye indicator and incubated at 28 ± 2 °C for 7 days. The appearance of orange or fluorescent yellow color around the bacterial colony was recorded as a positive siderophore production **(Schwyn and Neilands, 1987)**.

7.6. HCN production

The ability of rhizospheric bacterial isolates to produce hydrogen cyanide (HCN) was investigated on modified nutrient agar (NA) media amended with 4.4 g glycine/L. The bacterial strains are streaked on the modified NA plates followed by adding filter paper (Whatmann No. 1) saturated with 2% sodium carbonate in 0.5% picric acid solution on top of each plate. After that, the plates were sealed and incubated at 35 ± 2 °C for 96h. The appearance of orange to red color on the filter paper indicates the HCN production by the rhizobacterial isolates **(Ahmad *et al.,* 2008)**.

7.7. IAA production

The qualitative production of IAA was assessed as follows: the rhizobacterial strains are inoculated into nutrient broth media supplemented with different concentrations of tryptophan (0, 1, 2, and 5 mg mL^{-1}) and incubated for 15 days at 35 ± 2˚C. After the incubation period, the inoculated culture was centrifuged at 3000 *rpm* for 30 min. After that, the obtained supernatant (2 mL) was mixed with two drops of orthophosphoric acid and 4 mL of Salkowski's reagent (containing: 300 mL conc. sulfuric acid: 500 mL distilled H_2O: 15 mL 0.5 M $FeCl_3$). The formation of pink color indicates successful IAA production. The color intensity was measured at 530 nm using a T60 UV-Visible spectrophotometer **(Alkahtani *et al.,* 2020).** Using a standard IAA graph, the amount of the formed IAA was

calculated to select the best concentration of tryptophan that can be used for further study.

7.8. Detection of other phytohormones

The production of other phytohormones (gibberellic acid (GA3), abscisic acid (ABA), benzyl, kinten, and ziaten) by rhizobacterial strains was evaluated using high-performance liquid chromatography (HPLC) analysis. The rhizobacterial strain was grown in Ashby's broth media and incubated at 35 ± 2˚C for 7 days. At the end of the incubation period, 100 mL of inoculated media was centrifuged to remove cell pellets and collected supernatant which was mixed with 1 mg of Butylated Hydroxy Toluene to avoid the oxidation of the phytohormones. After that, the previous mixture was centrifuged at 960 rpm for 10 min and collected the supernatant was subjected to extraction according to the method of **Ünyayar *et al.,* (1996)**. The yield of the previous extraction was running on TLC with a solvent of isopropanol/ammonia/distilled water (10:1:1 v/v/v). The GA_3, ABA, Ziaten, and Benzyl bands are observed at 254 nm by UV-Vis spectroscopy and detect the R_f value according to standard for each hormone. The detected bands are scraped from the TLC sheet and dissolved in pure methanol and subjected to HPLC analysis.

8. **Biocontrol of *cucumber*-wilt caused by *F. oxysporum* using rhizobacterial isolates.**

8.1. Pot experiment.

The pot experiment was conducted at Desert Research Center, North Sinai Governorate, Egypt under greenhouse conditions (photoperiod of 12:12 h dark: light and temperature of 25 to 30 °C). The experiment was achieved using a completely randomized design with triplicates for each treatment. The hybrid cucumber seeds (Vector) were obtained from Agricultural Research Center, Giza, Egypt, and subjected to surface-sterilization with 5% sodium hypochlorite solution for 2 minutes followed by washing thrice with sterile distilled H_2O before the experiment. The inoculum of rhizobacterial strains was prepared by growing each strain separately or in the consortium in the nutrient broth media at 35 ± 2 °C for 48 hours. Each bacterial culture was centrifuged at 10,000 *rpm* for 10 minutes to collect the cell pellets which were washed twice with sterile distilled H_2O. The collected cell pellets were re-suspended in 100 mL of a sterile 0.1 M phosphate buffer (pH 7.0) to get a final concentration of 1.5×10^9 CFU/mL. The five conducted treatments were: cell pellets of rhizobacterial strain Ck2 suspended in phosphate buffer, cell pellets of Ck5 suspended in phosphate buffer solution, cell pellets of Ck7 suspended in phosphate buffer solution, cell pellets of bacteria consortium (CK2, Ck5, and Ck7) suspended in phosphate buffer solution, and phosphate buffer solution without bacterial cell served as a control. The surface-sterilized cucumber seeds were subjected to pre-germination to check their health and

divided into five groups, each group containing four similar seed germination and immersed into various treatments for five hours. After that, the treated seeds were picked-up and sowed into a pot containing two kilograms of sterilized soil (the soil analysis was shown in Table 1). After one week of seed planting, the soil was treated with 100 mL (1.0×10^5 spores/mL) of the most pathogenic *F. oxysporum* f.sp *cucumerinum* strain. After treatment, all pots were covered with polyethylene bags for 24 h to maintain high humidity. The plants were watered regularly with tap water and checked each week for disease development. Disease severity percentages were estimated after 60 days post-sowing and the percentages of reduction in the incidence of *Fusarium*-wilt were calculated as follows **(Xue *et al.*, 2009)**: Disease reduction (%) =

$$\frac{\text{Disease incidence in the control - Disease incidence in the treated plant}}{\text{Disease incidence in the control}} \times 100$$

8.2. Field experiment

A. Experimental Design.

The field study was achieved at the EL-Qantara Sharq Experimental Station, Desert Research Center (D.R.C.), North Sinai Governorate, Egypt (31°00'21.6" N and 32°33'48.1" E). The design of the field study was a split-plot design and irrigated with drip irrigation. Before planting, the soil was mixed well with organic fertilizers (**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 calcium super phosphate (15.5 % P_2O_5) as phosphorus fertilizer, ammonium sulfate (20.5 % N) as nitrogen fertilizer, and potassium sulfate (48 % K_2O) as potassium fertilizer.

B- Treatments and samples analysis.

Before the field study, the cucumber seeds were subjected to surface sterilization and pre-germinated to select seeds of the same radical length. The inoculum of four treatments (Ck2, Ck5, Ck7, and consortium) was prepared as mentioned in the greenhouse experiment. The cucumber seeds with similar radical length were incubated with bacterial suspension for 5 h. followed by picked-up and planting into the soil. After one week of planting, approximately 20 mL of *F. oxysporum* suspension (1.0×10^5 spores/mL) was added beside the seedling roots. The split-plot was divided as follows: (1) positive control (seeds planting without any treatment), (2) infected control (seeds planting into soil infected with *F. oxysporum*), (3) seeds treated with Ck2 in presence of *F. oxysporum*,(4) seeds treated with Ck5 in presence of *F. oxysporum*, (5) seeds treated with Ck7 in presence of *F. oxysporum*, (6) seeds treated with the bacterial consortium (Ck2 + Ck5) in presence of *F. oxysporum*, (7) seeds treated with the bacterial consortium (Ck2 + Ck7) in presence of *F. oxysporum*, (8) seeds treated with the bacterial consortium

(Ck5 + Ck7) in presence of *F. oxysporum*, (9) seeds treated with the bacterial consortium (Ck2 + Ck5 + Ck7) in presence of *F. oxysporum.*

At harvest, the plant height (cm), number of branches per plant, fresh weight, and dry weight of plant (g) were recorded. Moreover, the wilt disease severity and the reduction of incidence percentages were also recorded after 60 days of planting **(Ma *et al.,* 2021)**.

9. Statical analysis

The layout of the experiment was a split-plot design. The experiment included three replicates for each treatment. Data were analyzed statistically using the SPSS v17 statistics package. 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 $p < 0.05$.

Results and Discussion

1. *Fusarium oxysporum*, isolation and identification.

Cucumber (*Cucumis sativus* L.) is a common vegetable for human edible products and useful fibers belonging to the family of *Cucurbitaceae*. Cucumber probably originated in the foothills of the Himalayas and has been cultivated for at least 3,000 years. Among common phytopathogenic fungi is *Fusarium oxysporum* because of their ability to exist in the soil as saprophytes or chlamydospores for several years, making attempts to its control is difficult **(Stoddard *et al.,* 2010).** *Fusarium*-root and stem rot of cucumbers caused by *Fusarium oxysporum* f.sp. *radicis cucumerinum* are considered the main reasons for loss of yield **(Vatchev, 2015)**. In the current study, we explore a promising eco-friendly tool to control *Fusarium*-wilt disease**.** Therefore**,** three *Fusarium oxysporum* strains were isolated from the naturally infected root of cucumber collected from Sinai governorate, Egypt, and identified using cultural characteristics and microscopic examination according to standard keys

The pathogenic severity of the obtained three *F. oxysporum* strains to cause cucumber-wilt disease was estimated under greenhouse conditions. Data analysis showed that the percentages of wilt disease severity due to infection with three strains of *F. oxysporum* were 73 %, 66 %, and 51 % as compared with control. The strain that causes the highest cucumber-wilt disease was selected as the most pathogenic strain to complete the current study.

2. Isolation of rhizobacterial strains and select the most potent.

The synergistic relationships between different rhizobacterial strains and plant roots have a positive effect on the growth performance of plants, yield, as well as enhancing the soil quality. These positive effects because of the efficacy of rhizobacterial strains to secrete secondary metabolites that help in nitrogen fixation, phosphate solubilization, reduce the effect of biotic and abiotic stresses, increase the nutrients availability, biocontrol of plant

pathogens, induced systematic resistance (ISR) that increase the plant immunity **(Ismail *et al.,* 2021)**. In the current study, nine bacterial isolates were obtained from healthy cucumber rhizospheric soil and showed the varied degree to suppress the growth of *F. oxysporum* stain by *in-vitro* dual-culture assay with values of 35.01 ± 0.3 % - 56.2 ± 0.2 %. Among nine rhizobacterial isolates, three isolates designated as Ck2, Ck5, and Ck7 showed the highest efficacy to suppress the growth of *F. oxysporum* with percentages of 56.20±0.2, 51.33±0.3, and 46.20±0.4, respectively (**Fig. 1**). The *in-vitro* dual culture is the most common assay used for preliminary study, in which the antagonistic activity was detected through measuring the inhibition percentage of radial mycelial growth towards the inoculated bacterial strains **(Lee *et al.,* 2017)**. In the same line, Islam and co-authors reported that 35 rhizobacterial strains were isolated from cucumber rhizosphere soil and showed varied activity to inhibit the growth of *F. oxysporum* f. sp. *cucumerinum* **(Islam *et al.,* 2018)**. Out of these 35 bacterial isolates, one isolate designated as BA5 showed high activity to suppress the mycelial growth of *F. oxysporum* with an inhibition percentage of 58.4%.

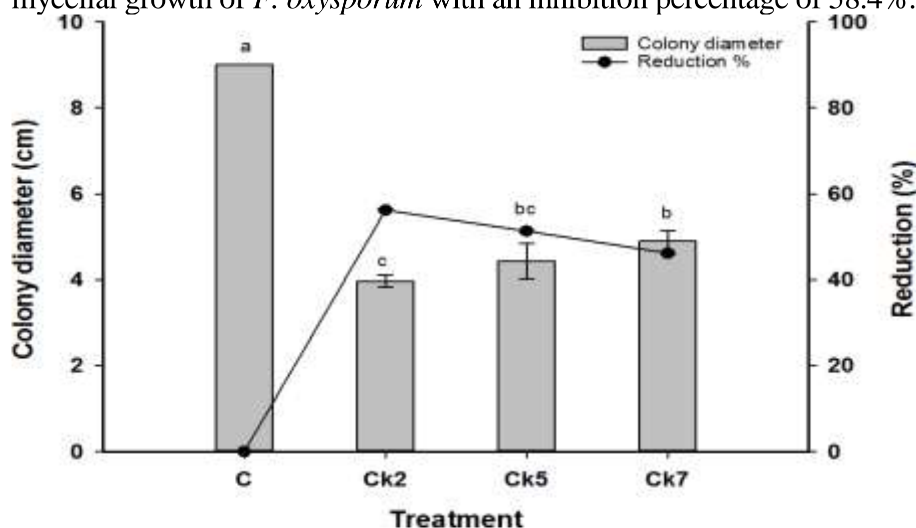

Figure 1. *In-vitro* antagonistic activity of the rhizobacterial isolates against the highest pathogenic *F. oxysporum* showing the colony diameter and reduction percentages for *F. oxysporum* in the presence of rhizobacterial isolates (CK2, Ck5, and Ck7).

3. Identification of the most potent bacterial isolates.

In the current study, the three rhizobacterial isolates, Ck2, Ck5, and Ck7 that showed high potency to suppress the growth of *F. oxysporum* were identified using amplifications and sequencing of the 16S rRNA gene. The sequence gene analysis revealed that the isolates Ck2 have a similarity percentage of 99.7 % with *Achromobacter xylosoxidans*, whereas the isolates Ck5 and CK7 have similarity percentages of 99.7 % and 99.9 % with *Bacillus*

haynesii, and *Bacillus paramycoides* respectively (**Fig. 2**). The *A. xylosoxidans* was previously isolated from the rhizosphere soil of *Jatropha curcas* plant and showed plant growth-promoting properties **(Vyas *et al.,* 2018)**. Also, *Achromobacter* strain was isolated from the rhizosphere of *Manihot esculenta* plant subjected to draught stress **(Zapata *et al.,* 2021)**. Moreover, *Achromobacter xylosoxidans MM1* showed a 50% reduction in tomato-wilt incidents caused by *F. oxysporum f. sp. lycopersici* **(Moretti *et al.,* 2008)**. Compatible with our study, *Bacillus* spp. are used to manage the cucumber-wilt disease caused by *F. oxysporum* f. sp. *cucumerinum* **(Yang *et al.,* 2014 and Wang *et al.,* 2021a)**. *Bacillus* spp. is considered the most common rhizobacterial strain used to suppress the growth of phytopathogenic fungi because of their rapid growth and various secondary metabolites used in biocontrol investigation **(Fira *et al.,* 2018)**. To date, this is the first report that utilized *A. xylosoxidans*, *B. haynesii*, and *B. paramycoides* in biocontrol of cucumber-wilt disease caused by *F. oxysporum* f. sp. *cucumerinum.*

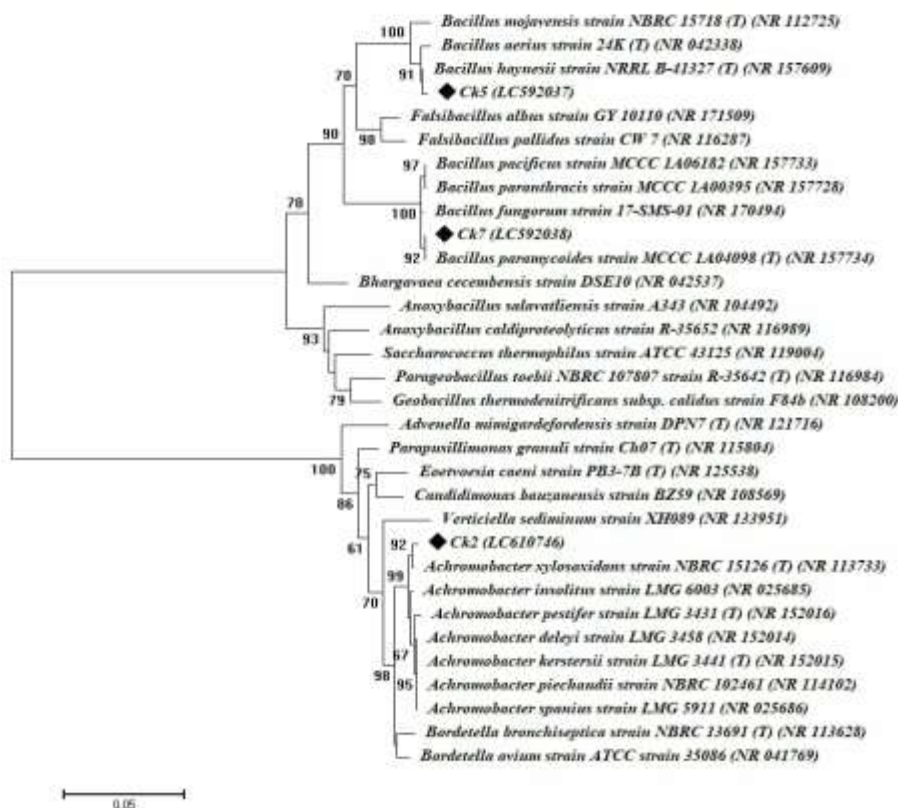

Figure 2. Phylogenetic tree of 16S rRNA gene sequences of the three rhizobacterial isolates with comparing the sequence with those deposited in the NCBI. The analysis was conducted with MEGA 6.1 using the neighbor-joining method with bootstrap value (1000 replicates).

4. Plant growth-promoting properties of rhizobacterial strains.

The potency of rhizobacteria strains to incorporate in the management of plant disease caused by various phytopathogens can be correlated with their plant growth-promoting properties. Among these properties, secretion of hydrolytic enzymes, nitrogen fixations, nutrient competitions, siderophores production, phosphate-solubilization, production of various phytohormones, and ammonia production **(Beneduzi *et al.,* 2012)**. The growth of selected rhizobacterial strains on nitrogen-free Ashby's medium indicates their ability to nitrogen fixation qualitatively. To confirm this potentiality, nitrogenase enzyme activity was assessed by the acetylene reduction method. The gas chromatography data analysis showed that the maximum nitrogenase activity was recorded for *A. xylosoxidans* Ck2 with the value of 446.88 ± 1.63 n-mole C_2H_4/ml/24h, followed by *B. paramycoides* Ck7 and *B. haynesii* Ck5 with values of 310.8 ± 1.98 and 213.6 ± 1.19 n-mole C_2H_4/ml/24h **(Table 2)**. In the same context, the efficacy of rhizobacterial species associated with the root of *Lasiurus sindicus* and *Calligonum polygonoides* to fixing nitrogen were quantitatively estimated by measuring the nitrogenase activity by an acetylene reduction method **(Gothwal *et al.,* 2007)**. Also, the qualitative screening for nitrogen fixation by rhizobacterial strain *B. amyloliquefaciens* B2 that used in biocontrol of cucumber-wilt disease caused by *F. oxysporum f. sp. cucumerinum* was achieved by their ability to grow on nitrogen-free Ashby's medium **(Wang *et al.,* 2021a)**.

Table. 2. Characterization of selected rhizosphere bacterial strains Ck2, Ck5, and Ck7 as plant growth-promoting.

Test	Rhizosphere bacterial isolate		
	Ck2	Ck5	Ck7
Nitrogen fixation (n mole C_2H_4/ml/24 h.)	446.88 ± 1.63 [a]	213.6 ± 1.19 [c]	310.8 ± 1.98 [b]
Phosphorus solubilization (clear zone (mm))	10.9±0.10[b]	11.8±0.13 [a]	4.8±0.04 [d]
Ammonia production	+++	+++	++
Amylase (mm)	13.3±0.7 [a]	0.00	11.3±0.9 [b]
Cellulase (mm)	29.0±1.5 [a]	26.0±4.6 [a]	32.0±0.0 [a]
Protease (mm)	15.7±2.3 [a]	19.0±0.6 [a]	17.7±0.3 [a]
Catalase	+	+	+
Siderophore	++	+++	++
HCN	++	+++	+

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

Solubilization of inorganic phosphate and production of ammonia is considered anther mechanisms used by rhizospheric bacterial strains to biocontrol of phytopathogens and increase plant growth **(Li *et al.,* 2016).** The major amount of phosphorous that exists in the soil is in insoluble forms and cannot be directly assimilated by plants, resulting in restricted plant

growth. Therefore, solubilization and mineralization of phosphorus carried out by phosphate solubilizing bacteria is an important plant growth-promoting trait (**Tandon *et al.,* 2020**). This process requires various steps including the production of various organic acids that are used in mineralization (dissolve the mineral complexes) followed by the secretion of phosphatase or phytase enzymes to break down the insoluble phosphates (solubilization) (**Ku *et al.,* 2018**). Various rhizobacterial strains such as *Pseudomonas*, *Arthrobacter*, *Bacillus*, *Flavobacterium, Microbacterium*, *Rhodococcus*, *Burkholderia, Serratia, and Enterobacter* have showed efficacy in solubilizing phosphate to enhance the yield of various crops **(Eid *et al.,* 2021). Ditta *et al.*, (2018)** have reported that the inoculation of rock phosphate with phosphate solubilizing microorganisms (PSM) and organic fertilizers (OF) increases the stability of soil aggregate by a value of 37% and enhance phosphate solubilization with an increase of 2.4-fold as compared with uninoculated (control). **Ditta et al., (2018)** also showed the planting of chickpea in presence of rock phosphate inoculated with PSM and OF increases the shoot length, dry weight of nodules/plant, nodule numbers/plant, pods numbers/plant yield with values of 17%, 13%, 23%, 15%, 15% respectively as compared with control (planting of chickpea in absence of rock phosphate, PSM, and OF). In the current study, the activity of rhizobacterial strains Ck2, Ck5, and Ck7 was checked using Pikovskaya agar media, the appearance of a clear zone around the bacterial growth indicates their activity to phosphate solubilizing. Data analysis showed that the maximum diameter of the clear zone (11.8±0.13mm) was formed around the growth of *B. haynesii* Ck5 followed by *A. xylosoxidans* Ck2 and *B. paramycoides* Ck7 with clear zones of 10.9±0.10 mm and 4.8±0.04 mm respectively (**Table 2**). The diameter of formed clear zones are directly proportional with the amount of liberated phosphate as reported previously (**Soliman *et al.,* 2021**).

The amount of liberated P in Pikovskaya broth media was calculated quantitatively from second days to tenth days using the phosphomolybdate method. The qualitative assay is compatible with the quantitative assay that recorded the highest P-liberation was recorded for CK5 followed by Ck2 and Ck7, respectively. Analysis of variance showed that the amount of liberated P by bacterial isolates Ck5 was 120.9±3.2 µg mL^{-1} on 2 day and reached 192.3±1.3 µg mL^{-1} on the tenth day (**Figure 3**). The lowest amount of phosphate solubilization is recorded by bacterial strain Ck7 which was 38.42±2.4 µg mL^{-1} after 2nd days and decreased to 37.29±1.2 µg mL^{-1} after ten days as compared with control which recorded 25.64±0.59 µg mL^{-1} and 26.31±0.49 µg mL^{-1} after two and ten days respectively, (**Figure 3**). Overall, the rhizosphere microorganisms that have the efficacy to phosphate solubilization are considered a promising tool instead of chemical fertilization to improve the crop yield.

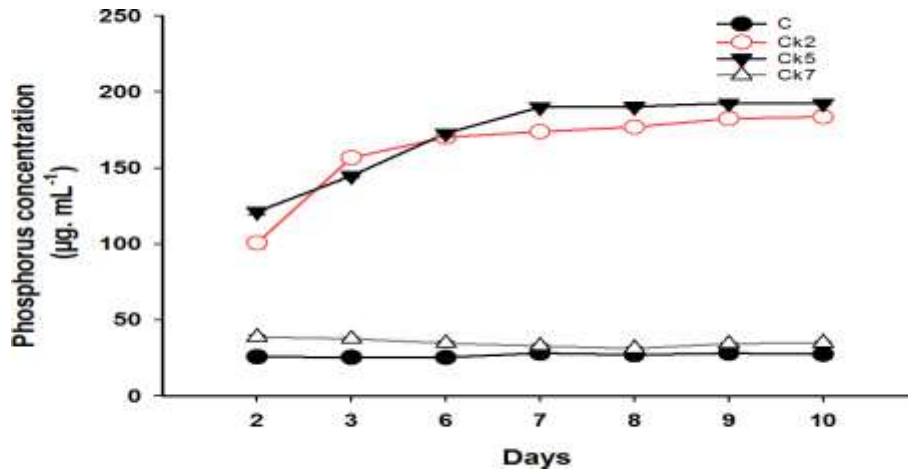

Figure 3. The availability of P in Pikovskaya broth media inoculated by rhizobacterial strains Ck2, Ck5, and Ck7 at interval times.

The obtained data revealed that all rhizobacterial strains can produce ammonia with varying degrees depending on the color intensity formed after the addition of Nessler's reagent to inoculated peptone water media (**Table 2**). The highest productivity (depending on brownish color intensity) was noticed for Ck2 and Ck5 followed by Ck7. The positive impacts of ammonia concentration on plant growth can be summarized in the synthesis of nitrogen-containing molecules, enhancing the length of shoot, root, and plant fresh weight. Also, ammonia can increase the resistance of plant towards various phytopathogens as reported previously **(Passari *et al.,* 2016).**

For hydrolytic enzymes and oxidative stress related enzymes, the rhizobacterial strains *A. xylosoxidans* Ck2, *B. haynesii* Ck5, and *B. paramycoides* Ck7 possess the efficacy to secret amylase, cellulase, protease, and catalase except for the bacterial strains Ck5 lack the efficacy to secrete amylase enzyme (**Table 2**). Data analysis showed that the highest clear zone formed around bacterial strain Ck2, Ck5, and Ck7 was recorded for cellulase enzymes with values of 29.0±1.5, 26±4.6, and 32±0.0 mm respectively. Polymers such as cellulose, lignin, chitin, and starch can be decomposed by the action of hydrolytic enzymes, ultimately producing valuable minerals that enhance plant growth **(Nannipieri, 2010)**. Also, the secretion of hydrolytic enzymes by rhizosphere microbes can be used to degrade the cell wall components of phytopathogens and hence protect the plants from infections **(Egamberdieva *et al.,* 2010)**. The various rhizobacterial species such as *Bacillus, Enterobacter, Pseudomonas, Acinetobacter* have shown the inhibitory action against different pathogenic fungi in-vitro **(Egamberdieva *et al.,* 2008 and Soliman *et al.,* 2021)**. The toxic-free radicals formed under different stresses can be scavenged by the

catalase enzyme and hence indirectly promote plant growth **(Kumar *et al.,* 2012)**.

Iron is an essential element for plants and in most cases exists in the form of Fe^{3+} that tends to form insoluble oxyhydroxides and hydroxides, and hence makes Fe^{3+} inaccessible for plants and microorganisms **(Rajkumar *et al.,* 2010)**. Some rhizobacterial strains have a mechanism through which can obtain inaccessible iron. This mechanism involves the synthesis of various forms of low-molecular iron chelators known as siderophores. Siderophores have a high ability to bind with ferric (Fe^{3+}) iron and are reduced to ferrous (Fe^{2+}) iron in the bacterial cell membrane. Within the rhizosphere, plants can absorb Fe^{2+} from the soil *via* several mechanisms such as uptake of iron-siderophore complexes, chelation, and iron release, or by ligand exchange reaction **(Chandran *et al.,* 2021)**.

The siderophore-producing rhizobacteria can support plant growth *via* direct mechanism through the availability of iron to plants and inaccessible to phytopathogens. The production of siderophores by *Bacillus pumilus* 8N-4 was utilized as a mechanism to promote plant growth. Inoculation of the wheat plant by 8N-4 strain leads to significant increases in root length, increase biomass production, and enhanced growth parameters **(Hafeez *et al.,* 2006)**. Also, the production of siderophores by *Pseudomonas* and *Bacillus* spp. were used to biocontrol of various phytopathogenic fungi such as *Pythium, Fusarium*, and *Aspergillus* spp. **(Trapet *et al.,* 2016 and Ali *et al.,* 2020)**. Rhizobacterial strains producing siderophores were used to suppress the growth of phytopathogens *F. graminearum, F. moniliforme,* and *Macrophomina phaseolina* that infect maize and peanuts **(Pal *et al.,* 2001)**. In the present study the selected rhizobacterial strains Ck2, Ck5, and Ck7 are recorded as positive siderophores-producers (Table 2). The production was confirmed through the change of King's B medium to orange color surrounding the rhizobacterial growth.

Another mechanism used by rhizobacteria to suppress the phytopathogen and hence promote plant growth is the secretion of volatile metabolites known as hydrogen cyanide (HCN). The HCN has the efficacy to impeding the electron transport chain that is required for energy supply to plant pathogens that ultimately lead to cell death **(Abd El-Rahman *et al.,* 2019).** Data showed that the rhizobacteria Ck2, Ck5, and Ck7 possess the ability to form varying degrees of reddish-brown color on the filter paper which is loaded on growth plates that indicates the HCN production (**Table 2**).

Indole-3-acetic acid (IAA) is a natural auxin synthesized by plants and plant growth-promoting microorganisms. Approximately 80% of rhizobacteria that produce auxins can colonize the plant seeds or plant root system and produce IAA. The main functions of IAA are summarized in promoting cell division, enhancing cell differentiation and cell extension, stimulating germination processes, controlling in root development and

vegetative growth, mediating the plant response to lights and gravity, influencing chlorophyll biosynthesis, enhancing the production of various metabolites, enhancing the photosynthesis process, and increase the tolerance of plants to different environmental stresses **(Spaepen and Vanderleyden, 2011 and Eid *et al.,* 2021)**. Various rhizosphere bacteria such as *Rhizobium, Pseudomonas, Bradyrhizobium, Bacillus, Agrobacterium, Klebsiella,* and *Enterobacter* are characterized by their high ability to produce auxins as compared with those non-rhizosphere **(Kuzmicheva *et al.,* 2017)**.

The potency of rhizobacterial strains Ck2, Ck5, and Ck7 to produce IAA were analyzed in broth media supplemented with various concentrations of L-tryptophan (0, 1, 2, and 5 mg mL^{-1}) as a starter for IAA production after 15 days. Data analysis showed that the IAA production was tryptophan concentration-dependent. As shown in **Figure** 4A, the lowest IAA production was recorded for bacterial strain Ck5 followed by Ck2 and Ck7 with values of 1.1±0.1 µg mL^{-1}, 2.3±0.5 µg mL^{-1}, and 4.1±0.3 µg mL^{-1} respectively in absence of L-tryptophan. These values are increased to 101.3±3.2 µg mL^{-1}, 55.21±1.12 µg mL^{-1}, and 78.68±0.99 µg mL^{-1} in presence of 5 mg mL^{-1} tryptophan. In the same context, the production of IAA by *Bacillus siamensis* in liquid media is tryptophan-dependent. The maximum IAA production (9.89 µg mL^{-1}) was achieved in media containing 250 ppm tryptophan after 96h as compared with media without tryptophan **(Suliasih and Widawati, 2020)**.

Different microbial genera such as *Pseudomonas, Proteus, Bacillus, Klebsiella,* and *Xanthomonas* are characterized by cytokinin producers. Cytokinins have a critical role in plants such as stimulating cell division, enhancing the development of roots, nutrients, and minerals uptake through the formation of root hair, expansion of cells, and plant tissue differentiation **(Amara *et al.,* 2015)**. Several higher plants, rhizosphere microbes, and endophytic microorganisms have the potentiality to produce gibberellins (GA) to be involved in different plant processes such as germination of seed, stem elongation, cell division, flowering, fruit set, and delayed aging in various plant parts **(Bottini *et al.,* 2004).** Various species of rhizobacteria such as *Enterococcus faecium, Bacillus* spp., *Promicromonospora, Pseudomonas* spp. have been characterized by gibberellins production **(Tsukanova *et al.,* 2017)**. The main role of abscisic acid (ABA) is in unfavorable environments such as water stress, salt stress, and cold stress. Plants and associated microbes are producing ABA to combat the negative impacts of various environmental stresses. For example, in a water stress environment, ABA helps the plants to overcome water loss by stomatal closure. Therefore, the presence of ABA in the rhizosphere microbes and followed uptake and transportation by plants are a very important step to plant growth under various-stressed environments **(Tsegaye *et al.,* 2017)**.

In the current study, the efficacy of rhizobacterial strains Ck2, Ck5, and Ck7 to producing GA3, ABA, Benzyl, Kinten, and Ziaten were investigated using HPLC analysis. Data analysis showed that the successful formation of ABA (0.04±0.01, 0.04±0.01, and 0.03±0.01 mg/100mL), Benzyl (0.23±0.01, 0.66±0.01, and 0.21±0.01 mg/100mL), kinten (0.36±0.01, 0.32±0.00, and 0.25±0.01 mg/100mL), ziaten (0.21±0.01, 0.43±0.01, and 0.36±0.01 mg/100mL), and GA_3 (14.29±0.48, 12.43±0.79, and 9.28±1.07 mg/100mL) for Ck2, Ck5, and Ck7 respectively, (**Figure 4 B and C**). Based on plant growth-promoting traits, it can be concluded that these strains (Ck2, Ck5, and Ck7) can be used as biofertilizers to improve plant growth and then enhance the tolerance of a plant to phytopathogens infection.

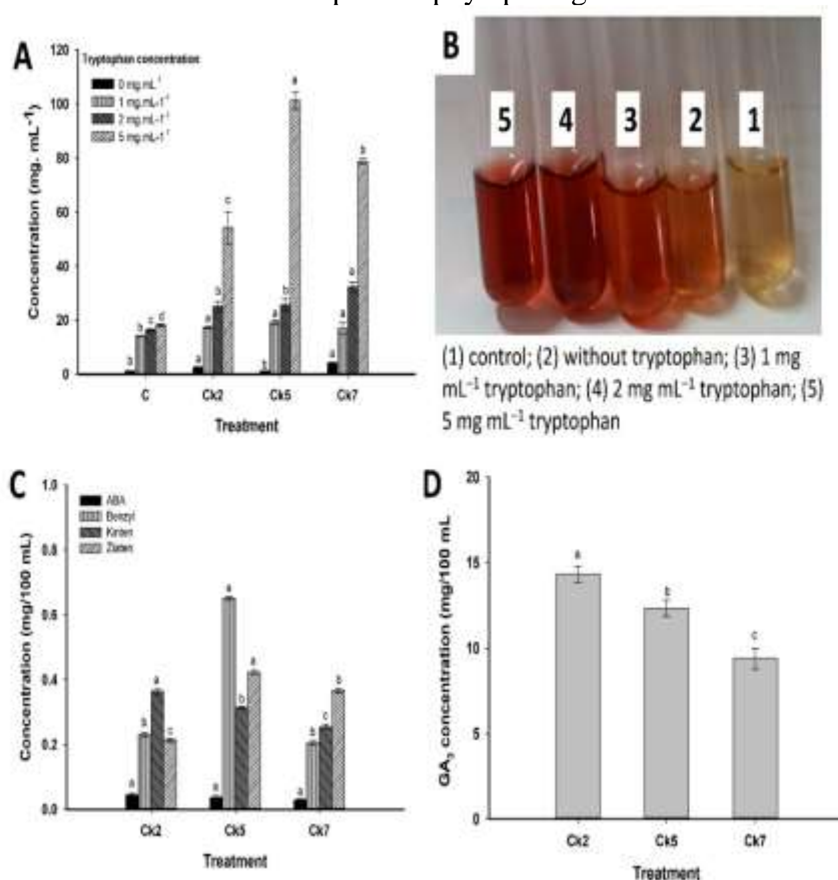

Figure 4. Phytohormones production by three rhizobacterial strains Ck2, Ck5, and Ck7 (A) is the qualitative production of IAA at different concentrations of tryptophan (0, 1, 2, and 5 mg mL^{-1}); (B) is the color change after adding Salkowski's reagent in presence and absence of tryptophan; (C) is the production of ABA, Benzyl, Kinten, and Ziaten; (D) is the production of GA3 detected by HPLC analysis.

5. Biocontrol of cucumber-wilt caused by *F. oxysporum* using rhizobacterial strains:

5.1. Pot experiment

The activity of plant growth-promoting rhizobacteria Ck2, Ck5, and Ck7 to reduce the severity of cucumber-wilt disease caused by *F. oxysporum* was investigated under greenhouse conditions. Data showed that the inoculation of cucumber seeds with bacterial strains Ck2, Ck5, and Ck7 have the potency to reduce the Fusarium wilt severity with percentages of 67.3, 63.2, and 58.7 %, respectively (**Table 3**). Interestingly, the highest reduction in the wilt disease severity was conducted for the bacterial consortium (Ck2+Ck5+Ck7) with a value of 75.7% (**Table 3**). Compatible with our study, the incidence of cucumber-wilt disease caused by *F. oxysporum* f. sp. *cucumerinum* was significantly reduced with percentages of 48, 22, and 63 % due to treatment with *B. amyloliquefaciens* strain B2, *Pleurotus ostreatus* strain P5, and their consortium (B2+P5), respectively under greenhouse conditions **(Wang *et al.,* 2021a)**. Also, the disease severity of *Fusarium* tomato-wilt was significantly decreased due to treatment with the bacterial consortium (*B. thuringiensis* BtMB9 + *Acinetobacter calcoaceticus* AcDB3) than separate treatment **(Khalil *et al.,* 2021b)**.

The combination between two or more rhizosphere microorganisms in a biocontrol study is considered an efficient tool to control soil-borne pathogens **(Jangir *et al.,* 2019)**. The maximum synergistic activity of bacterial consortium can be attributed to the fact that the biocontrol depends on various mechanisms which can be complemented with each other in the case of consortium treatments **(Wang *et al.,* 2021a)**. This phenomenon was confirmed in the present investigation due to various activities with varying degrees of rhizobacterial strains as plant growth-promoting. In the current study, various biocontrol mechanisms for *A. xylosoxidans* Ck2, *B. paramycoides* Ck7, and *B. haynesii* Ck5 have been reported such as siderophore and HCN production, secretion of hydrolytic enzymes, oxidative-stress related enzymes, ammonia production, and phytohormones production. To date, this is the first report using *A. xylosoxidans*, *B. paramycoides*, and *B. haynesii* in biocontrol of cucumber-wilt disease caused by *F. oxysporum* f. sp. *cucumerinum*

Table 3. Effect of rhizobacterial strains Ck2, Ck5, and Ck7 on the severity of cucumber-wilt disease caused by *F. oxysporum* under greenhouse conditions.

Treatments	*Fusarium* wilt severity	Protection (%)
Un infected cucumber plant (control)	0.00	100
A. xylosoxidans Ck2 + *F. oxysporum*	32.4±1.2	67.6
B. haynesii Ck5 + *F. oxysporum*	36.8±1.3	63.2
B. paramycoides Ck7 + *F. oxysporum*	41.3±1.5	58.7
Consortium (Ck2 + Ck5 + Ck7) + *F. oxysporum*	24.3± 2	75.7

5.2. Field experiment

The activity of *A. xylosoxidans* Ck2, B. *haynesii* Ck5, and *B. Paramycoides* Ck7 either alone or in a consortium to ameliorate infection with *F. oxysporum* f. sp. *cucumerinum* that causes cucumber-wilt disease are shown in Table 4 under filed conditions. Analysis of variance revealed that the inoculation of cucumber seeds with rhizobacterial strains and planting into soil infested with *F. oxysporum* causes a significant increase in the growth performance of cucumber (plant heights, branches per each plant, and fresh and dry weight) compared with cucumber seeds planting into infected soil in absence of rhizobacterial inoculation. Generally, the planting of cucumber in soil infected with *F. oxysporum* in absence of rhizobacterial strains causes a suddenly dropping in all morphological characteristics as compared with those planted in presence of rhizobacteria. The number of branches per plant was highly decreased in cucumber planting into infected soil in absence of rhizobacteria (8.6±2) as compared with either healthy plant (in absence of *F. oxysporum* and bacterial inoculation) (15.3±2) or presence of bacterial inoculation. Moreover, the number of branches per plant in presence of rhizobacterial strains is significantly increased compared with the healthy plant.

On the other hand, the treatment of cucumber seeds with the rhizobacterial consortium (Ck2+Ck5+Ck7) in presence of fungal pathogen significantly increased the fresh and dry weight with values of 50.7±2.1 g and 24.6± 1.7 g, respectively, followed by treatment with the consortium (Ck2+ Ck5) with values of 47.3±1.2 g and 22.3±2.2 g, respectively. The lowest fresh (18.3±3.0 g) and dry (7.3±1.5 g) weight were observed for cucumber planting in infected soil in absence of rhizobacterial strains (Table 4). Similarly, the presence of rhizobacterial strains *B. pumilus, Pseudomonas alcaligenes,* and *Rhizobium* sp. causes significant improvement in morphological characteristics (fresh weight, dry weight, plant height, and amounts of pods) of lentils that planted in soil infected with *Fusarium oxysporum* (**Akhtar *et al.,* 2010**).

In the current study, the severity percentage of cucumber-wilt after 60 days of planting in soil infected with *F. oxysporum* and in the absence of rhizobacterial strains was 68.3±1.2% (Table 4). As shown the presence of rhizobacterial strains that have plant growth-promoting properties can significantly decrease the incidence of wilt disease. For example, the presence of bacteria consortium (Ck2 + Ck5 + Ck7) has the efficacy to protect cucumber plant from wilt with percentages of 79.1%, followed by bacterial treatment (Ck2 + Ck5) that reduce the cucumber-wilt with a percentage of 73.3%. Also, the treatment with separate rhizobacterial strains can decrease the severity of the cucumber-wilt disease. For example, the disease severity decreased with the percentage of 68.1% after treatment with *A. xylosoxidans* Ck2 followed by *B. haynesii* Ck5 and *B. paramycoides* Ck7

with percentages of 65.3 % and 61.7 % (Table 4). The most common rhizobacterial strains have the efficacy to decrease the severity of wilt disease caused by *F. oxysporum* was belonging to *Bacilli* group due to their different inhibition mechanisms such as synthesis of volatile compounds, enhance the induce systematic resistance (ISR) in the plant, and rapidly root colonization, besides other plant growth-promoting traits **(Wang *et al.*, 2021b)**. In the current study, the combination between *Bacillus* spp. and *Achromobacter* sp. can complement the suppressive mechanisms from each other and improve the resistance of plant toward wilt disease infection.

Table 4. The morphological characteristics and wilt disease severity of cucumber plant due to treatment with three rhizospheric bacterial strains Ck2, Ck5, and Ck7 (individually or consortium) against *F. oxysporum*

Treatment	Plant height (cm)	Branches / plant	Fresh weight (g)	Dry weight (g)	Wilt severity (%)	Protection (%)
Healthy plant	**89.67±2.0**	**15.3±2.0**	**37.0±1.5**	**14.8±3.0**	**0.0±0.0**	**100**
Infected plant	**52.0±1.5**	**8.6±2.0**	**18.3±3.0**	**7.3±1.5**	**68.3±1.2**	**31.7**
Ck2+ *F. oxysporum*	**103.3±1.0**	**18.7±3.0**	**45.3±3.1**	**19.0±1.0**	**31.9±1.7**	**68.1**
Ck5 + *F. oxysporum*	**98.0±1.2**	**17.7±2.0**	**44.0±1.5**	**17.3±2.6**	**34.7±0.6**	**65.3**
Ck7 + *F. oxysporum*	**93.0±2.6**	**16.3±2**	**42.7±2.5**	**16.0±1.0**	**39.3±1.2**	**61.7**
(Ck2+ Ck5) + *F. oxysporum*	**118.67±0.6**	**23.0±1.0**	**47.3±1.2**	**22.3±2.2**	**26.7±1.3**	**73.3**
(Ck2+ Ck7) + *F. oxysporum*	**100.67±1.0**	**18.0±2.0**	**43.5±2.1**	**18.3±0.6**	**30.7±1.0**	**69.3**
(Ck5+ Ck7) + *F. oxysporum*	**113.0±1.5**	**21.0±1.0**	**46.7±1.0**	**20.0±0.6**	**28.3±0.9**	**71.7**
(Ck-mix) + *F. oxysporum*	**126.3±2.1**	**24.6±2.0**	**50.7±2.1**	**24.6± 1.7**	**20.9±2.2**	**79.1**

Healthy plant meaning planting of cucumber in soil without rhizobacterial strains and *F. oxysporum*; infected plant meaning planting of cucumber in *F. oxysporum*-infected soil in absence of rhizobacterial strains; Ck2 is *A. xylosoxidans;* Ck5 is *B. Haynesii*, and Ck7 is *B. paramycoides.*

CONCLUSIONS

In the current study, a promising eco-friendly tool to control Fusarium-wilt disease in cucumber plants was conducted. Rhizobacterial strains that were isolated from healthy cucumber rhizospheric soil; showed the highest efficacy to suppress the disease severity of *F. oxysporum*. The potential capacity of rhizobacterial strains as a biocontrol agent against fungal phytopathogen could primary based on the ability to secrete secondary metabolites that help in nitrogen fixation, phosphate solubilization, reduce the effect of biotic and abiotic stresses, increase the nutrients availability, and produce different phytohormones such as indole-3-acetic acid, ABA, Benzyl, Kinten, Ziaten, and GA3. Prospects concerning rhizobacterial strains with ability to induce systematic resistance and increase the plant immunity should be further investigated.

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إستخدام بعض سلالات البكتريا الجذرية المحفزة لنمو النبات للمكافحة الحيوية ضد مرض الذبول الفيوزارمى في الخيار

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اولا : تم عزل 3 سلالات من فطر الفيوزاريم اوكسيسبورم من جذور نباتات الخيار المصابة بالذبول والتى اظهرت شده إمراضية بنسب 51% و 66% و 73% وتم اختيار الفطر المسبب لأعلي قدرة إمراضية للمضي قدما فى هذة الدراسة.

ثانيا : تم عزل مستحثات حيوية (سلالات بكتريا جذرية محفزه لنمو النبات) من تربة نباتات الخيار السليمة والتي أتضح عند تعريفها بالتعبير الجينى أنها (اكرومورباكتر زيلواوكسيدانس و باسيلس هينسي وباسيلس باراميكويد) وقد اظهرت هذه السلالات قدره تثبيطيه لنمو فطر الفيوزاريم بنسب 56.2% و 51.3% و 46.2% علي التوالي كما اظهرت هذة السلالات البكتيريه قدرة علي تثبيت النيتروجين وإذابة الفوسفات بقيم تتراوح (446.9±1.6, 310.8±1.9, and 213.6±1.2 n−moles−C_2H_4/mL/24h) (183.6±1.1, 192.3±1.3, and 34.29±1.2 µg mL^{-1}) للعزلات Ck7,Ck5,Ck2 علي التوالي كما أظهرت هذة السلالات البكتيرية خصائص معززة لنمو النبات تشمل إنتاج الأمونيا وسيانيد الهيدروجين والسيدروفوروالانزيمات التحلل المائى و انزيم الكتاليز والفيتوهرمون.

ثالثا : أظهرت هذة السلالات البكتيرية منفردة اومجتمعة قدرة عالية علي حماية نباتات الخيار من الإصابة بالذبول الفيوزارمى بقيم تتراوح بين 67.6% و 63.2% و58.7% و75.7% علي التوالي تحت ظروف تجربة الصوبة. كما تم تسجيل اعلي انخفاض في شدة الإصابة بالمرض نتيجة التلقيح بخليط من سلالات البكتريا الجذريه والتي اظهرت كفاءة في زيادة نسبة الحماية للنبات الى 79% تحت ظروف التجربة الحقليه.