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# Biocontrol Potential of Endophytic Bacteria Against Cereal Cyst Nematode, *Heterodera avenae*

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## ABSTRACT

The effects of five endophytic bacterial strains, namely *Pantoea* sp., *Pseudomonas stutzeri*, *Klebsiella* sp., *Brevundimonas diminuta*, and *Bacillus cereus* were tested as plant growth promoters and biocontrol agents against cereal cyst nematode *Heterodera avenae* under laboratory and greenhouse conditions. The main PGP-properties, namely the production of IAA, HCN and ammonia as well as the ability to solubilize-P were examined under *in vitro* conditions. Additionally, greenhouse experiment were performed in two seasons (2021/2022 and 2022/2023) at Ismailia Agricultural Research Station to evaluate the effectiveness of endophytic strains as a biocontrol agents against cereal cyst nematode. According to the laboratory experiment, all of the tested endophytic bacteria were apparently able to trigger PGP-properties. However, *B. cereus* followed by *Ps. stutzeri* and *Brev. diminuta* appears to be superior to the other endophytic strains in the production of IAA, HCN, and ammonia as well as the ability to solubilize phosphate. While, *Klebsiella* sp. strains displayed the lowest capacity in this concern. Results of greenhouse experiment revealed that all treatments induced a significant ($P \leq 0.05$) decline in *H. avenae* population, compared to the control. A percentages reduction in number of *H. avanae* white female cysts, were observed in treated roots after 60 days of nematode inoculation with all strains compared with control treatment. Treatment *B. cereus* recorded (91.5 and 93%) and *Ps. stutzeri* (84.4, and 87%) in both seasons, respectively. However, an increased number of white female cyst development was observed in the control treatment in both tested seasons. The same results were observed in the reduction of the reproduction factor. Also, the results showed that all inoculation treatments significantly increased plant growth and yield parameters of wheat over control treatment. This trend was true in both growing seasons. Among bio-agents, the maximum increase in 100 grain weight 104% (2021/22), and 96% (2022/23) were recorded with *B. cereus*, followed by *Ps. stutzeri* 91% (2021/22), and 78% (2022/23). Almost a similar trend was observed for the root, and shoot dry weights and wheat yield characters. The data acquired demonstrated a considerable increase in shoot N, P, and K content at all bacterial inoculation treatments, along with comparable growth characteristics outcomes for wheat plants.

***Keywords:*** Biological control, *Triticum aestivum* L., Cereal cyst nematode *Heterodera avenae,* Rhizobacteria and endophytic bacteria.

## 1. Introduction

Wheat (*Triticum aestivum* L.) is a strategic cereal crop utilized as a key source of protein and carbohydrates in the human diet. Additionally, grains include trace levels of lipids, fiber, minerals, and vitamins (Arzani and Ashraf, 2017). Based on the worldwide yearly output of 760 million tonnes harvested from 219 million hectares, it supplies about one-fifth of the calories and protein for approximately 4.5 billion people in 94 countries (Atris *et al*., 2023). Roughly 3.39 million feddan are planted in Egypt, with an annual output of about 9.28 million tonnes and an average yield of 2.74 tonnes

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per feddan (FAOSTAT, 2016). Wheat is of particular significance in Egypt since local production is insufficient to meet yearly needs; consequently, boosting wheat output through improving unit land area productivity and the cultivated area is Egypt's top priority to close the gap between wheat production and consumption (Gharib *et al*., 2016).

Every year, a significant percentage of food commodity losses are attributed to plant illnesses brought on by different types of pests or pathogens. It is generally established that the constant and indiscriminate use of chemical pesticides to eradicate plant diseases harms soil ecology, plant ecology and human health through hazardous chemical residues. A few modern, sustainable, and eco-friendly disease management techniques with particular reference to wheat crops, plant growth-promoting bacteria are a much more effective and superior alternative to chemical nematocides (Singh *et al*., 2021). One of the most significant soil-borne diseases that infects cereal crops all over the globe is the cereal cyst nematode *H. avenae*. In certain infected crops, *H. avenae* may reduce wheat yields by anywhere between 10% and 100% (Yang *et al*., 2019).

While a synthetic nematicide is often used in contemporary intensive agriculture to manage this nematode and protect wheat yields (Zhang *et al*., 2016), this practice is unsustainable in the long run since it is costly and pollutes the surrounding environment (Qiu *et al*., 2020). Additionally, intensive agriculture practices based on the continual use of mineral fertilizers have reduced soil biodiversity and made CCN infestations worse (Hu *et al*., 2014 amd Matute *et al*., 2018). Therefore, adopting an ecologically friendly perspective on fertilization in crop management is crucial for creating genuinely sustainable agroecosystems since it might lower soil pollution as well as the harm caused by CCN (cereal cyst nematode).

One of the most promising alternatives to chemical pesticides is the biocontrol of plant infections, which involves the use of microorganisms or substances of biological origin. Biocontrol is environmentally benign and can protect plants from a variety of diseases (Anckaert *et al*., 2021) through a variety of processes, including conflict for resources and space, parasitism, antibiosis, and enhancing host plant defenses (Köhl *et al*., 2019). The beneficial bacterial strains that can produce phytohormones and function as effective biostimulants in sustainable agriculture include those from the genera *Bacillus* and *Pseudomonas*, which are principally responsible for the production of IAA, Gibberellic acid, and Abscisic acid (Odoh, 2017). It is suggested that microbial secretion of phytohormones replaces existing traditional chemical additions, producing finished products with improved bioactivity and purity, but also at a significantly lower cost (Shi *et al*., 2017). A wide variety of bacteria that are connected with plants, including some commercially significant plant diseases and other helpful species used as biocontrol agents of plant pathogens, can be found in the genus *Pantoea*. The biocontrol agents from this genus that are best understood typically create one or more antimicrobial compounds that increase the effectiveness of biocontrol (Smits *et al*., 2019). The effects of tested endophytic bacteria (*Pantoea sp., Pseudomonas stutzeri, Klebsiella sp., Brevundimonas diminuta,* and *Bacillus cereus*) as biocontrol agents against the cereal cyst nematode,*H. avenae* on wheat were studied under greenhouse conditions. Additionally, research on their chemical pathways for boosting plant development.

## 2. Materials and Methods

### 2.1. Isolation of bio-agent

The endophytic bacterial strains were obtained from Kandil *et al*. (2018)

### 2.2. Assay of PGP-related properties *in vitro*:

#### 2.2.1. Qualitative HCN estimation:

The Cyanide Detection Solution (CDS), which consists of 2 g of picric acid and 8 g of $Na_2CO_3$ dissolved in 200 ml of sterilized distilled water, was used to detect the production of cyanide after the Lorck (1948) assay was carried out to qualitatively determine the HCN produced by endophytic strains.

#### 2.2.2. Phosphate solubilization production assay:

The endophytic strains were tested for their ability to solubilize phosphate using tricalcium phosphate in Pikovskaya medium as described by Jasim *et al*. (2013).

**2.2.3. Qualitative production of ammonia:**

The endophytes produced $NH_3$ *in vitro* using qualitative technique in peptone water according to the procedures described by Ahmad *et al*. (2008).

**2.2.4. Indole acetic acid estimation:**

The endophytic strains were tested for their ability to produce IAA as a qualitative determination as described by Bric *et al*. (1991).

**2.3. Inocula preparation:**

Luria Bertani broth medium (L.B) Bertani (1951) using sterile growth media, bacterial cell suspensions were spectrophotometrically standardized to a population density of $10^9$ cells $ml^{-1}$ at 640 nm. Each endophytic bacterium was grown and maintained in its LB medium to a density of not less than $10^8$ cells $ml^{-1}$ in the broth culture. Every bacterial liquid culture was mixed separately with sterilizing fine peat moss, previously neutralized with 5% $CaCO_3$ (2:1 w/v) with a moisture content of the final product. Wheat seeds were coated with peat-based inocula of the endophytic bacteria using Arabic gum as adhesive material. After planting, the inoculated pots received a liquid bacterial culture.

**2.4. Greenhouse Experiments:**

The experiments were conducted at Ismailia Agricultural Research Station, Ismailia Governorate, located by coordinates Latitude 30° 35′ 41. 901″ N and Longitude 32° 16′ 45. 843″ E, Egypt, throughout two growing winter seasons of 2021/2022 and 2022/2023. The effectiveness of five endophytic bacterial strains (*Pantoea* sp., *Pseudomonas stutzeri*, *Klebsiella* sp., *Brevundimonas diminuta*, and *Bacillus cereus*) was tested as biocontrol agents against cereal cyst nematode (*Heterodera avenae*) on wheat under greenhouse conditions.

**2.5. Screening test:**

Seeds of susceptible wheat cultivar cv. Giza171 Abdel-Baset and Dawabah (2020) was obtained from the Field Crops Research Institute, Agricultural Research Center, Egypt. Seeds were sown in clay pots (4 kg soil) filled with steam-sterilized sand and clay mixture. The main physical and chemical characteristics of used soil were carried out according to the methods documented by Alef and Nannipieri (1995) and the obtained data were recorded in Table (1).

**Table 1:** Some physical and chemical properties of the studied soil

| Physical properties | | | |
|---|---|---|---|
| **Particle size distribution (%)** | | | **Texture grade** |
| **Sand** | **Silt** | **Clay** | |
| 87.51 | 4.21 | 8.28 | Sandy |

| Chemical properties | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Soluble cations and anions in saturated soil extract (meq/L)** | | | | | | | | **Available nutrients (mg/kg)** | | |
| | | **Cations** | | | | **Anions** | | | | | | |
| **PH (1:2.5)** | **EC dS/m** | **$Ca^{++}$** | **$Mg^{++}$** | **$Na^+$** | **$K^+$** | **$Cl^-$** | **$HCO^-_3$** | **$CO^=_3$** | **$SO^=_4$** | **N** | **P** | **K** |
| 7.27 | 0.18 | 0.80 | 0.45 | 0.28 | 0.18 | 0.25 | 0.33 | -- | 1.13 | 13.20 | 4.38 | 163.1 |

**2.6. Preparation of nematode inoculum:**

At the end of the growing season, samples of *H. avenae*-infected wheat plants were taken from a heavily infested wheat field in the Ismailia governorate. On the roots of these plants, newly produced brown cysts were collected and preserved in dry sand that had undergone steam sterilization. Using the sieve and flotation procedure Shepherd (1970), cysts were removed from the sand during the second season and crushed in sterilized water to retrieve eggs and freshly hatched second-stage juveniles ($J_2$).

**2.7. Treatments, fertilization and experimental design:**

The bacterial strains were added five times. $1^{st}$ was coated in wheat seeds Weller and Cook (1983). $2^{nd}$ application with nematode inoculations. Then, treatments were added every 10 days after inoculation as 20 L/Fed for each treatment. A comparison treatment with the nematicide oxamyl 24% L, Vydate® (N', N'- dimethyl-N-((methylcarbamoyl) oxy)-1- thiooxamimidate) was added at the rate of 4 L/Fed as recommended two days after inoculation. While untreated- infected plants were served as control. Plants were thinned to 3 seedlings per pot 7 days following germination, and each pot was inoculated with *H. avenae* at the rate of 25000 eggs + $J_2$/pot pipetted in 3 holes around the base of each seedling at a depth of about 5–10 cm. Water was given to the plants as required.

Phosphorus was added before sowing as calcium superphosphate (15.5% $P_2O_5$) at the rate of 200 kg/fed (0.8 g/pot). Potassium was added as potassium sulphate (48% $K_2O$) at the rate of 100 kg/fed (0.4 g/pot) after 15 and 30 days from sowing in equal two doses. While, nitrogen fertilizer at the rate of 100 kg N/fed (1.95 g fertilizer/pot) in the form of ammonium sulphate (20.5% N) was applied in three equal doses at10, 20 and 30 days from sowing.

The pots were arranged in a complete randomized design (CRD) and repeated twelve times for each treatment over the wheat growing seasons (2021/2022 and 2022/2023). A white female's root growth was seen under a stereomicroscope 60 days after the *H. avenae* inoculation. Comparing each therapy to the control treatment, the reduction percentage (percent) of cysts of white females was determined.

Plants were gently uprooted after each experiment, and the roots were cleaned of soil. Nematode cysts were removed from the roots and potting soil, selected out and then crushed in an appropriate amount of water to release eggs and $J_2$s. $J_2$s were also extracted from the potting soil and counted Shepherd (1986). The nematode reproduction factor (Rf), which is equal to Pf/Pi, was computed after the final nematode population (Pf) was calculated as no.of eggs plus cysts and$J_2$/pot Abdel-Baset and Dawabah (2020).

**2.8. Sampling:**

After 45 days from sowing, wheat plants were uprooted from each pot to assay the content of N, P and K of wheat shoots. The oven dried plant materials were wet digested by using a mixture of pure $HClO_4$ and $H_2SO_4$ at a ratio of 1:1. Total nitrogen was determined using the micro-Kjeldahel method, phosphorus was determined Spectrophotometrically using ammonium molybdate and stannus chloride reagents, while potassium was determined using Flamephotometer as described by Van Schouwenburg (1968).

The experiment ended 120 days following nematode inoculation. The growth parameters shoot length and dry weight of both roots and shoots, as well as dry weight of spikes, 100 grain weight and spikes length were recorded. The roots of the plants were cleaned of dirt and the nematode reproduction factors were calculated. Comparing the experimental group to the control group, increasing percentages (%) were recorded.

**2.9. Statistical analysis**

Data were analyzed using one-way ANOVA by MSTAT-C software ver. 2.10. Comparisons of means were performed by Duncan's multiple range test at $p \leq 0.05$ Duncan (1955).

## 3. Results

**3.1. The ability of the tested bacterial strains to exhibit some PGP-properties:**

The bacterial strains were examined in the laboratory for their preliminary qualitative assay of IAA, HCN and $NH_3$ production capacity as well as phosphate solubilization.

**3.1.1. Indole acetic acid (IAA) production qualitatively:**

The preliminary qualitative assay was performed using LB-tryptophan agar to detect the ability of the strains to produce IAA (Table 2 and Fig. 1). The color developed in LB- tryptophan agar medium treated with Salkowski reagent as a result of IAA production by all strains *Pantoea* sp., *Ps. stutzeri, Klebsiella* sp., *Brev. diminuta* and *B.cereus* were the most IAA-producing indicated by the intense reaction color by these strains when grown on LB tryptophan agar in comparison with control in their plate cultures treated with Salkowski reagent. The five bacterial strains showed a pink to red color with little variation in intensity. However, *B. cereus* and *Pantoea sp.* outperformed other rhizobacteria tested in this regard.

### 3.1.2. Hydrogen cyanide (HCN) production qualitatively

Another interested trait of PGPR's is the ability to produce biocide compounds, including cyanide, results in Table (2) and Fig. (1) revealed a positive reaction indicating the formation of HCN as authenticated by a color change from yellow to dark brown. *Pantoea* sp., *Ps.* *Stutzeri, Klebsiella* sp., *Brev. diminuta* and *B. cereus* were more active HCN producers compared with the control. However, *B. cereus* and *Brev. diminuta* was superior compared to other tested strains in this concern.

### 3.1.3. Ammonia production ($NH_3$).

Data in Table (2) and Fig. (1) showed that all rhizobacterial strains had a positive reaction to Nessler's reagent illustrated by a color change from brown to yellow as a result of ammonia production. *Pantoea* sp., *Ps.* *Stutzeri, Klebsiella* sp., *Brev. diminuta* and *B. cereus* produced deep brown color indicating higher production of ammonia compared with control. In addition, there is a relative superiority of *B. cereus* and *Brev. diminuta* compared to other strains.

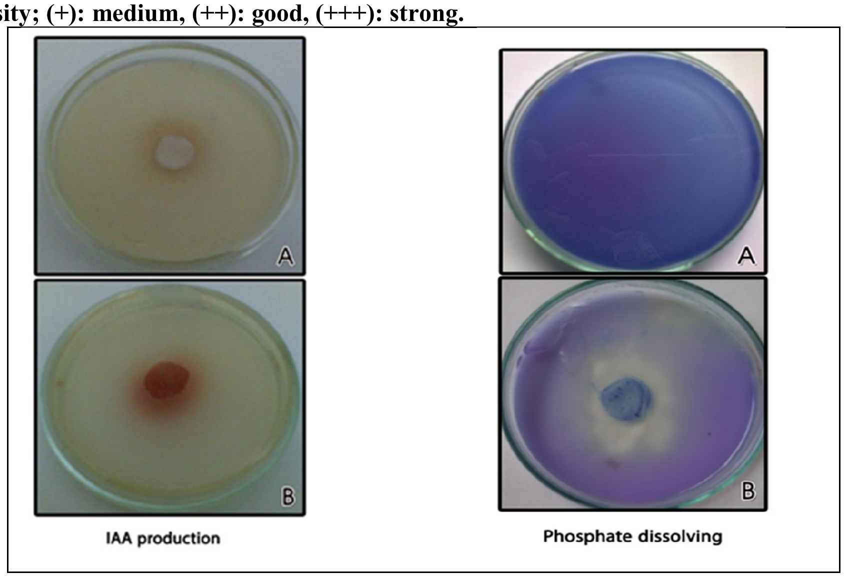
### 3.1.4. Phosphate solubilization qualitatively

Data in Table (2) and Fig. (1) showed positive reactions among the 5 strains (*Pantoea* sp., *Ps. stutzeri, Klebsiella* sp., *Brev. diminuta* and *B. cereus*) regarding their phosphate-solubilization capacity compared with control recorded the highest reaction illustrated in deep blue color on Pikoviskaya's agar plus bromophenol blue. However, the capability of the rhizobacterial strains to solubilize phosphate decleared that *Ps. Stutzeri* followed by *B.cereus* was more effective, while other strains displayed the lowest capacity in this respect.

**Table 2:** The ability of rhizobacteria to produce plant growth promoters and biocontrol substances

| No | Strains | HCN | $NH_3$ | P-solubilization | IAA |
|---|---|---|---|---|---|
| 1 | *Pantoea* sp. | + | + + | + | + + + |
| 2 | *Ps. stutzeri* | + + | + | + + + | + |
| 3 | *Klebsiella* sp. | + | + + | + | + + |
| 4 | *Brev. diminuta* | + + + | + + + | + | + + |
| 5 | *B. cereus* | + + + | + + + | + + | + + + |

**Color intensity; (+): medium, (++): good, (+++): strong.**



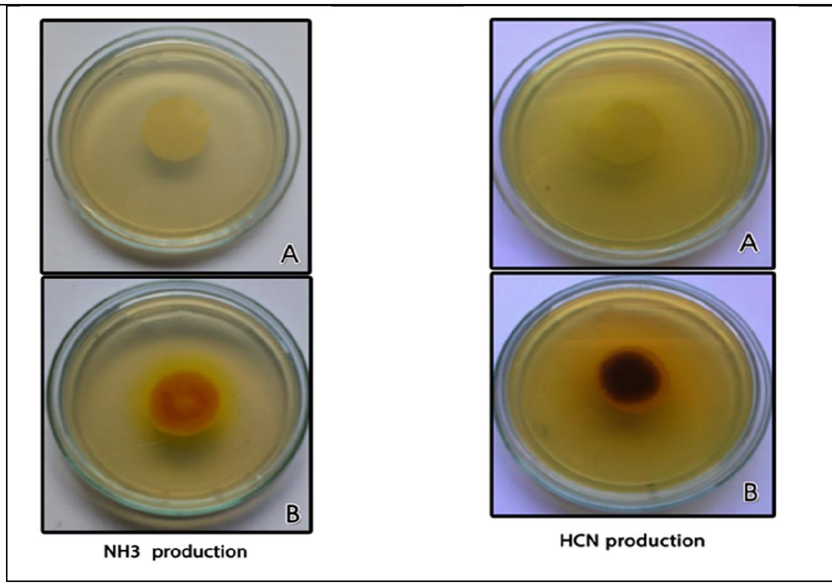



**Fig. 1:** Production of some plant growth promoters and biocontrol substances by the bacterial strains (A: negative reaction, B: positive reaction)

### 3.2. Effects of bacterial strains on some wheat growth and yield parameters, infected with *H. avanae* under greenhouse conditions:

Wheat plant characters, i.e., shoot length and dry weight of roots and shoots as well as spikes dry weight, 100 grain weight, and spikes length after 120 days from sowing as affected by rhizobacterial inoculation are presented in Table (3).

**Table 3:** Effects of bacterial strains on some wheat growth and yield characters infected with *H. avanae* under greenhouse conditions.

| Treatment | Shoot length (cm) | Inc % | Root dry weight (g) | Inc% | Shoot dry weight (g) | Inc % | Spike dry weight (g) | Inc % | Spike length (cm) | Inc % | 100 grain weight (g) | Inc % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Growing season 2021-2022** | | | | | | | | | | | | |
| ***Pantoea* sp** | $59.3^{c}$ | 169.5 | $1^{b}$ | 43 | $2.4^{c}$ | 85 | $2.9^{c}$ | 163.6 | $15.6^{c}$ | 51.4 | $3.8^{c}$ | 72 |
| ***Ps. Stutzeri*** | $71^{a}$ | 222 | $1.2^{a}$ | 71 | $2.7^{ab}$ | 107 | $3.2^{b}$ | 191 | $17^{ab}$ | 65 | $4.2^{b}$ | 91 |
| ***Klebsiella* sp** | $68.3^{a}$ | 210 | $1.1^{ab}$ | 57 | $2.6^{b}$ | 100 | $2.8^{c}$ | 154.5 | $16.3^{bc}$ | 58 | $3.7^{c}$ | 68 |
| ***B. diminuta*** | $66^{b}$ | 200 | $1.2^{a}$ | 71 | $2.6^{b}$ | 100 | $2.6^{d}$ | 136 | $15.3^{c}$ | 48.5 | $3.6^{c}$ | 64 |
| ***B. cereus*** | $71^{a}$ | 222 | $1.2^{a}$ | 71 | $2.8^{a}$ | 115 | $3.5^{a}$ | 218 | $17.6^{a}$ | 70.8 | $4.5^{a}$ | 104 |
| **Oxmyle** | $57^{c}$ | 159 | $1.2^{a}$ | 71 | $2.7^{ab}$ | 107 | $2.6^{d}$ | 136 | $15.6^{c}$ | 51.4 | $4.5^{a}$ | 104 |
| **Nematode only (control)** | $22^{d}$ | -- | $0.7^{c}$ | -- | $1.3^{d}$ | -- | $1.1^{e}$ | -- | $10.3^{d}$ | -- | $2.2^{d}$ | -- |
| **LSD≤ 0.05** | 3.08 | -- | 0.11 | -- | 0.17 | -- | 0.19 | -- | 1.14 | -- | 0.32 | -- |
| **Growing season 2022-2023** | | | | | | | | | | | | |
| ***Pantoea* sp** | $58.3^{c}$ | 187 | $1.1^{ab}$ | 57 | $2.4^{c}$ | 85 | $2.8^{bc}$ | 154 | $14.6^{c}$ | 46 | $3.7^{c}$ | 61 |
| ***Ps. Stutzeri*** | $68.3^{a}$ | 236 | $1.1^{ab}$ | 57 | $2.8^{a}$ | 115 | $3.3^{a}$ | 200 | $16.6^{ab}$ | 66 | $4.1^{b}$ | 78 |
| ***Klebsiella* sp** | $61.6^{b}$ | 203 | $1^{b}$ | 43 | $2.5^{bc}$ | 92 | $2.9^{b}$ | 163 | $15.6^{bc}$ | 56 | $3.9^{c}$ | 70 |
| ***B. diminuta*** | $61.6^{b}$ | 203 | $1.1^{ab}$ | 57 | $2.6^{b}$ | 100 | $2.7^{c}$ | 145 | $15.3^{c}$ | 53 | $3.7^{c}$ | 61 |
| ***B. cereus*** | $69^{a}$ | 240 | $1.2^{a}$ | 71 | $2.8^{a}$ | 115 | $3.5^{a}$ | 218 | $17.3^{a}$ | 73 | $4.5^{a}$ | 96 |
| **Oxmyle** | $58.6^{bc}$ | 188 | $1.2^{a}$ | 71 | $2.6^{b}$ | 100 | $2.7^{c}$ | 145 | $15.6^{bc}$ | 56 | $4.6^{a}$ | 100 |
| **Nematode only (control)** | $20.3^{d}$ | -- | $0.7^{c}$ | -- | $1.3^{d}$ | -- | $1.1^{d}$ | -- | $10^{d}$ | -- | $2.3^{d}$ | -- |
| **LSD≤ 0.05** | 3.1 | -- | 0.13 | -- | 0.16 | -- | 0.19 | -- | 1.14 | -- | 0.22 | -- |

Means are an average of 4 replicates. Means followed by the same letter(s) in a column are not significantly different according to Duncan's multiple range test ($P \leq 0.05$). Increasing percentages (%)=treatment-control/controlX100

Obtained results revealed that all the treatments significantly increased plant growth and yield parameters of wheat over control treatment in both growing seasons (2021/22 and 2022/23). Among tested bio-agents, the maximum increases in 100 grain weight 104% (2021/22), and 96% (2022/23) were recorded with *B. cereus*, followed by *Ps. stutzeri* 91% (2021/22), and 78% (2022/23). Almost a similar trend was observed for root and shoot dry weights in both tested seasons.

On the other hand, the highest increase in spike dry weight (218%) was recorded with *B. cereus* in two seasons, while the lowest increase was observed with *B. diminuta*, and oxmyle in two growing seasons. Again, *B. cereus* exerted a salient superiority and recorded the highest increase in spike length, at the same time *B. diminuta* achieved the lowest increase compared with the control group.

### 3.3. Effects of endophytic bacterial strains on the N, P and K content of wheat plants infected with the cereal cyst nematode, *H. avanae*:

Table (4) shows the effects of endophytic inoculation on N, P and K content of wheat shoots in both seasons after 45 days of sowing wheat seeds which infected with the cereal cyst nematode (*H. avanae*). Obtained data showed that shoot N, P and K content were significantly increased at all bacterial inoculation treatments and similar results of wheat plant growth characteristics were obtained. The highest content of nitrogen in both seasons was observed from *B. cereus*, followed by *Ps. Sutzeri* in the first season and *Pantoea* sp.in the second one. The results exhibited increment of nitrogen uptake three and two times in the first and second season respectively, compared to the control. However, nematicide oxmyle treatment was the lowest treatment followed by the control treatment. Data in both seasons revealed the superiority of *B. cereus* followed by *Ps. sutzeri* which showed an increase in phosphorus uptake, while, *Klebsiella* sp. became in the third order. The phosphorus uptake was increased four times in both seasons compared to control. On the other hand, nematicide oxmyle treatment was the lowest content of potassium and phosphorus. However, the K-content was increased in *Ps. Stutzeri* strain compared to B. cereus without significant differences in-between.

**Table 4:** Effects of endophytic bacterial strains on the N, P and K content of wheat plants infected with *H. avanae* under greenhouse conditions.

| Treatments | Growing season 2021-2022 | | | Growing season 2022-2023 | | |
|---|---|---|---|---|---|---|
| | N-content ($mg. plant^{-1}$) | P-content ($mg.plant^{-1}$) | K-content ($mg. plant^{-1}$) | N-content ($mg.plant^{-1}$) | P-content ($mg.plant^{-1}$) | K-content ($mg. plant^{-1}$) |
| ***Pantoea* sp.** | 62.5 $^{e}$ | 10.2 $^{bc}$ | 75.3 $^{bc}$ | 89.5$^{a}$ | 9.8$^{cd}$ | 71.8b$^{c}$ |
| ***Ps. stutzeri*** | 96.4 $^{ab}$ | 14.1 $^{a}$ | 85 $^{b}$ | 84.1$^{a}$ | 16.7$^{a}$ | 114.7$^{a}$ |
| ***Klebsiella* sp.** | 87.2 $^{bc}$ | 12.7 $^{ab}$ | 81.4 $^{b}$ | 81$^{a}$ | 11.8$^{bc}$ | 86.6$^{b}$ |
| ***Brev. diminuta*** | 79.5 $^{cd}$ | 10.9 $^{bc}$ | 80.3$^{b}$ | 82$^{a}$ | 12.7$^{b}$ | 78.3$^{b}$ |
| ***B. cereus*** | 104.4 $^{a}$ | 15.3 $^{a}$ | 100 $^{a}$ | 93.3$^{a}$ | 18.6$^{a}$ | 110$^{a}$ |
| **Oxmyle** | 71.3$^{de}$ | 8.7 $^{c}$ | 65.7 $^{c}$ | 78.6$^{a}$ | 7.3$^{de}$ | 53$^{cd}$ |
| **Nematode Only (control )** | 32.1 $^{f}$ | 3.5 $^{d}$ | 32.8 $^{d}$ | 53.7$^{b}$ | 4.8$^{e}$ | 47.6$^{d}$ |
| **LSD ≤ 0.05** | 14.3 | 2.6 | 10.1 | 21.9 | 2.7 | 21 |

Means are an average of 4 replicates. Means followed by the same letter(s) in a column are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

### 3.4. Effects of bacterial strains against cereal cyst nematode, *H.avanae*

Experimental results in Table (5) revealed that cysts per pot decreased significantly in all the treatments as compared to the control treatment in both tested growing seasons. Significant reduction ($P \leq 0.05$) in reproduction factor (RF) of cyst nematode was observed with treatment oxmyle (0.31), and (0.28) in seasons 2021/22 and 2022/23, respectively. The second most effective treatment after oxamyl in reducing RF was *B. cereus*, and these values were not significantly different from those caused by oxmyle treatment in two growing seasons. However, the effects of *B. dementia*, and *Pantoea* sp. were slightly weaker in the two growing seasons. RF of *B. dementia* was (0.88), and (0.83) in seasons 2021/22, and 2022/23, respectively. While, RF of *Pantoea* sp. was (0.82), and (0.79) in both seasons, respectively. Noteworthy, percentage reduction in number of *H. avanae* white female cysts after 60 days of nematode inoculation, was observed in all treatments compared with control treatments onces. The highest values were found with oxmyle treatment (93, and 94%) followed by *B. cereus* (91.5, and

93%) and *P. stutzeri* (84.4 and 87%). However, an increased number of white female cyst was observed in the control treatment in both tested seasons.

**Table 5:** Effects of bacterial strains against cereal cyst nematode, *H. avanae* on wheat

| Treatment | Growing season 2021-2022 | | | | Growing season 2022-2023 | | | |
|---|---|---|---|---|---|---|---|---|
| | White female/ plant | Red % | Cyst/ pot | RF | White female/ plant | Red % | Cyst/ pot | RF |
| ***Pantoea* sp.** | 39[c] | 74.6 | 61.6 [bc] | 0.82 | 39.6[b] | 75 | 59.6[b] | 0.79 |
| ***Ps. Stutzeri*** | 24[d] | 84.4 | 35.6 [d] | 0.47 | 20.6[c] | 87 | 32[c] | 0.42 |
| ***Klebsiella* sp.** | 40[c] | 74 | 60[c] | 0.8 | 39[b] | 75.4 | 56.6[b] | 0.75 |
| ***B. diminuta*** | 52[b] | 66 | 66.6[b] | 0.88 | 43.6[b] | 72.5 | 62.6[b] | 0.83 |
| ***B. cereus*** | 13[e] | 91.5 | 29.3 [de] | 0.39 | 11[d] | 93 | 25.6[cd] | 0.34 |
| **Oxmyle** | 10[e] | 93 | 23.6[e] | 0.31 | 9[d] | 94 | 21[d] | 0.28 |
| **Nematode only (control )** | 154[a] | -- | 196[a] | 2.6 | 159[a] | -- | 208[a] | 2.7 |
| **LSD≤ 0.05** | 9.0 | -- | 6.3 | -- | 4.8 | -- | 7.1 | -- |

Means are average of 4 replicates.[1]Pi= initial population density (25000 eggs +$J_2$s/pot). [2]Rf = Reproduction factor = final nematode population (Pf) / Pi. Red percentage (%) was calculated in comparison with the control treatment.

## 4. Discussion

Egypt is situated in a semi-arid region where agricultural land is plagued by soil-borne illnesses and ongoing soil fertility deterioration, both of which influence crop output and the availability of food for humans. In parallel with the present work Eid *et al.* (2021), pointed endophytic bacteria are a promising new area of study; some are utilized as bio-fertilizers and biocontrol agents to boost plant growth and fitness by the manufacture of phytohormones or biofertilizers, or by lowering abiotic and biotic stress tolerance. Due to the diversity of their hosts, endophytic bacteria can be used in several intriguing agricultural and therapeutic applications on different plant species. By creating lytic enzymes like chitinases and cellulases, they can control soil-borne diseases. In this study, some rhizobial and endophytic bacterial (*Pantoea* sp., *Pseudomonas stutzeri*, *Klebsiella* sp., *Brevundimonas diminuta*, and *Bacillus cereus*) that could control cereal cyst nematode, *H. avenae*, and their characterization as an antagonistic mechanism were evaluated as well as, their benefits on the growth of infected wheat plants in two successive seasons. In the present study, all rhizobacterial and endophytic strains were able to exhibit direct PGP- properties like IAA production and phosphate solubilization, which may display several modes of beneficial action. Additionally, its indirect mechanisms suppress nematode populations like HCN and ammonia production. This finding was emphasized by other investigators (Majeed *et al*., 2015 and Sahu *et al*., 2018).

In greenhouse experiments, some data revealed a significant increase in wheat growth and yield characters. All rhizobacterial and endophytic strains increased significantly shoot length, dry weights of shoot and root as well as100 grain weight, spike length, and their dry weights in both tested seasons. The reason for the increase may be attributed to the synthesis of plant auxin IAA, and phosphate metabolism (Abaid-Ullah *et al*., 2015; Abdel-Baset and El-Egami, 2019 and Kadhum *et al*., 2021).

These results are consistent with those reported by Kuklinsky-Sobral *et al*. (2004) who reported that 34% of the endophytic bacteria in association with soybean are IAA-producers, which stands out in the synthesis of key compounds that support plant development. In addition, Shariati *et al.* (2017) reported that the genome of *Pantoea agglomerans* strain P5 revealed genes related to phosphate solubilization, the generation of phytohormones that promote plant growth, and IAA. Moreover, Lemos *et al*. (2022) revealed that the *B. cereus* UFPEDA 1060B strain can solubilize phosphate, furthermore, the production of IAA *in vitro* conditions. The activity of the strain *P. stutzeri* IB-I6C was discovered during the treatment of wheat seeds, according to Gilvanova *et al*. (2022). The strain IB-I6C significantly increased the length and weight of wheat roots after colonizing them under salty circumstances, demonstrating the culture's broad growth range. The discovery of the beneficial effects of the bacterium *Pseudomonas stutzeri* IB-I6C on the development of wheat plants under salt loading coincides with the capacity to manufacture IAA. Additionally, the strain IB-I6C has phosphate-solubilization capability. Thus, the strain *Ps. stutzeri* IB-I6C includes the essential elements for the creation of a potential multifunctional biological agent for agro-biotechnology and soil bioremediation, both under normal circumstances and under technologically contaminated and saline stress.

Many microorganisms associated with plants produce plant hormones as part of their metabolism, and these hormones may be thought of as crucial regulators of plant growth and development Oliveira *et al*. (2003). Bacterial auxin production alters the root system by increasing the quantity and size of adventitious roots Gutierrez *et al*. (2012). One should be aware that the IAA impact might change depending on their concentration. Higher IAA concentrations may prevent callus tissues that promote plant development depending on the kind of plant Ribeiro and Cardoso (2012). It is crucial to identify microorganisms that transform chemical components by secreting organic acids or phosphatases Moreira and Araújo (2013). When they are made accessible to plants, the microbes they create are prominent examples of plant growth promoters, mineralizing organic phosphorus to form soluble phosphate compounds Nahas (2002).

Data on N, P, and K content of wheat shoots indicated that tested strains increased the nutrients concentration in wheat shoots. This might attribute to the ability of the plant growth promoting rhizobacteria to fix nitrogen in association or endophytic manners, solubilization of minerals and/or certain growth promoting substances, which positively affect root development and consequently their function in the uptake of both water and nutrients. In This concern, Suman *et al*. (2020) showed superiority of maize and wheat are used as test crops in *Pantoea* inoculation experiments in glasshouse pots. *Pantoea* sp., an endophyte from maize, and *Pantoea agglomerans*, a rhizospheric isolate from wheat, were discovered to be the most effective in enhancing plant development when compared to prescribed NPK fertilizer control. Increased nutrient uptake by solubilizing insoluble forms, nitrogen fixation, ammonia release, and the synthesis of plant growth phytohormones (IAA, CKs, GAs) that regulate plant physiology are just a few of the action mechanisms that *B. cereus* described as having the capacity to promote plant growth and play a role in sustainable agriculture Lopes *et al*. (2018).

Biocontrol products containing *Bacillus* species are being developed from rhizobacteria for the management of different soil-borne diseases of field crops Gardner *et al*. (2004). The antagonistic effect of tested PGPR may have occurred through their production of nematoxic chemicals such as HCN and $NH_3$, which are toxic to nematodes Abdel-Baset and El-Egami (2019). Results obtained from the application of all endophytic strains (*Pantoea* sp., *Ps. stutzeri*, *Klebsiella* sp., *B. diminuta*, and *B. cereus*) under a greenhouse conditions indicated that all inoculation treatments significantly reduced white females on roots and RF of cereal nematode, *H. avanae* in comparison with a control treatment in two tested seasons. Percentage reduction of the number of *H. avanae* white female cysts was observed in roots, and the Reproduction factor (RF) was decreased with all treatments compared with control treatments. Treatment oxmyle followed by *B. cereus*, and *P. stutzeri* were the most effective ones. These results are in line with those obtained by Ahmed *et al*. (2018) who reported that noteworthy reduction in white female cyst development was observed on roots treated with Avermectin seed coating followed by isolates *B. cereus* XZ 24-2-1, *B. cereus* XZ-33-3, *B. weihenstephansis* MH-58-60-01 and *B. thuringiensis* MH 032-003 as compared to control treatment. Moreover, the The same trend was found by Ahmed (2017) who screened the effect of *Pseudomonas* strains in a greenhouse against wheat cyst nematode *H. avenae*. His results revealed that a noteworthy reduction in white female development was observed for *P. putida* Ps190 (75.2%) followed by *P. fluorescent* Ps104 (67.6%), *P. putida* Ps197 (66.7%), *P. putida* Ps196 (58.2%), and *P. fluorescens* Ps109 (57.7%) as compared to the results of the untreated control treatment.

Our results matched with Zhang *et al*. (2016) who demonstrated that the strain's *B.cereus* (09B18) culture filtrate increased second-stage juvenile ($J_2$) nematode mortality and decreased *in vitro* egg hatch. The effects of treatments with bacterial suspensions of strain 09B18 on the quantity of white female wheat roots were seen in the results of greenhouse and field studies. In greenhouse testing, the rate of decrease was 75.9% by 09B18 and 70.2% by 09X01, therefore, be thought of as potential biocontrol agents. This may be due to the ability (*B.cereus*) to produce hydrogen cyanide (HCN). Numerous metalloenzymes, such as copper-containing cytochrome c oxidases, were shown to be strongly inhibited by the cyanide ion produced from HCN Blumer and Haas (2000). It supports many plant root diseases' suppression and broad-spectrum antibacterial action. Additionally, it has been hypothesized that HCN may harm certain plant-pathogenic nematodes (Gallagher and Manoil, 2001and Insunza *et al*., 2002). It is widely known that HCN, a powerful inhibitor of respiratory pathways, may lead to nematode hypoxia and paralysis Gallagher and Manoil (2001). The root-knot nematode Meloidogyne hapla is thought to be biologically controlled mostly by HCN Lee *et al*. (2011). HCN causes harm by impeding other crucial metalloenzymes including cytochrome c oxidase, the last link in the aerobic respiratory chain Abdel-Baset and El-Egami (2019).

## 5. Conclusion

Promising results using endophytic bacteria as biocontrol agents and plant growth promoting were obtained. So, using these strains improves nutrient availability and uptake which promotes plant growth rate and mitigates the negative effects of cereal cyst nematode (*Heterodera avenae*) on wheat. Future research is needed to study the mode of action of bacterial strains in plants to mitigate the biotic stresses to develop new strains.

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