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## Impact of PGPR bacteria against crown rot disease on wheat

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### Abstract

A field experiment was conducted at the Tuwaittha Research Station of the Ministry of Science and Technology during the season 2013-2014 to evaluate the effect of PGPR bacteria, including *Bacillus*, *Pseudomonas*, *Azotobacter*, *Azospirillum*, and *Streptomyces* against crown rot disease on wheat caused by *Fusarium graminearum*. *Pseudomonas fluorescens*, *Streptomyces*, *Azotobacter*, *Azospirillum*, *Bacillus subtilis*, *Bacillus pumillus* and bacterial mixture that cultivated in contaminated soil with fungus led to a significant increase in the plant length (69.91, 77.01, 69.78, 77.93, 67.78, 74.33, 81.16 cm) respectively, also in the dry weight for shoots and roots (2.14, 2.44, 2.08, 2.67, 2.12, 2.97, 3.24 g) and (0.243, 0.330, 0.260, 0.336, 0.236, 0.343, 0.410 g) respectively. *P. fluorescens*, *Streptomyces*, *Azotobacter*, *Azospirillum*, *B. subtilis*, *B. pumillus* and bacterial mixture led to a significant increase in number of grains in the spike (33.41, 35.16, 33.66, 36.13, 33.25, 35.25, 40.58) respectively and weight of 1000 grains (30.87, 32.71, 30.88, 35.80, 30.86, 32.71, 38.20 g) respectively and protein content in the grains (9.28, 9.87, 9.32, 9.95, 9.50, 9.88, 10.12%) respectively, the bacterial mixture, *Azospirillum*, *Streptomyces* and *B. pumillus* led to a significant reduction in disease incidence rate reached (25.25, 30.08, 31.5 and 37.16%) respectively, while the bacterial mixture, *Azospirillum*, *Streptomyces*, *B. pumillus*, *P. fluorescens*, *B. subtilis* and *Azotobacter* led to a significant reduction in disease severity reached (20.02, 22.81, 27.82, 29.17, 44.33, 49.08, 49.81%) respectively. *Azospirillum*, *Streptomyces*, *B. pumillus*, and bacterial mixture led to a significant increase in the percentage of germination (90, 90, 91, 94%) respectively. *Azospirillum* was superior in the area of disintegration circle 19.62 mm<sup>2</sup>.

**Keywords** *Fusarium graminearum*, *Azotobacter*, *Azospirillum*, *Streptomyces*, *Bacillus subtilis*, and *Bacillus pumillus*

### Introduction

Plant diseases are affecting on growth and development of plants, consequently, lead to a decrease in production (Salim et al., 2017a). *Fusarium graminearum* is the most aggressive species against wheat plants through pathogenicity tests and causes extensive damage through losses in grain yield and grain contamination with mycotoxins, which are toxic to human beings and animals (Cuilin Yi, 2001). *F. graminearum* attacks a wide range of plant species: cereals, beans, peas, sunflowers, tomatoes, soybeans, and radishes and affects all vegetative and reproductive organs of plants, causing crown

rot, seedling blight, head blight, and foot rot in cereals. The infection incidence leads to reduce grain weight, causing important yield losses from 30% to 70% under epidemic conditions (Miedaner, 1997). Symptoms of disease seem as brown water spots on the spikes, which extends up and down and development of infection with available of appropriate condition, besides the effect of the fungus on the production quantity (Cook, 1981; Murray and Brennan, 2009; Obanora et al., 2013). The continuous application of fungicides has negative effects on human health and the environment that leads to new strains of pathogens that are more resistant

(Khalil et al., 2005). It has become necessary to adopt eco-friendly approaches to improving crop health and yield. Many species of bacteria are called Plant Growth Promoting Rhizobacteria (PGPR), which stimulate germination, improve plant growth and increase its production. (Kloepper et al., 1980, Nihorimber et al., 2011). Improving growth criteria by PGPR bacteria may be either directly by analyzing the organic matter in the soil and providing the plant with the necessary nutrients as well as making some elements more ready for the plant (Adesemoy et al., 2008, Gholami et al., 2009), and some bacteria secrete phytohormones and growth regulators that stimulate roots formation and increase their absorption of water and nutrients (Salisbury, 1994). Or improve the growth criteria may be indirectly by inhibiting of bacteria for pathogen and preventing it from attacking the roots, through the production of antibiotics as well as some of them can directly parasitize on the pathogen and its secretions enzymes that analyzing the cell walls (Bakker et al., 1991; Van Loon et al., 1998). Or through activation of bacteria to plant defense mechanisms, which may be localized in the entry area of the pathogen or may be systemic away from the injured area (Walterse et al., 2005). In the present study, the effectiveness of bacterial strains (*Bacillus*, *Pseudomonas*, *Azotobacter*, *Azospirillum*, and *Streptomyces*) was testing against *F. graminearum*.

## Material and Methods

### Isolation of *Fusarium graminearum* and preparation of fungal vaccine

Ten plants from each district were selected, then the crown of the wheat stem was cut into small pieces to length (2 mm) and sterilized superficially with 1% sodium hypochlorite solution for 3 minutes and washed twice with sterile distilled water to remove chlorine, the pieces were dried on sterile filter paper and transferred to the medium of potato dextrose agar (PDA) with added 100 µg of the antibiotic (tetracycline) in Petri dishes (9 cm) and incubated at 25±2 °C for three days, pieces of mycelium were transferred by a sterile needle to PDA medium in Petri dishes (Barnett and Hunter, 1972). The pathogenicity of *Fusarium graminearum* was carried out and the fungus was diagnosed by the method of Polymerase chain reaction (PCR). Whereas fungal vaccine was prepared by the following method, where the wheat bran was mixed with corn and water in a ratio of 3: 7: 3 (weight: weight: volume) and distributed in glass vials 150 ml at a rate of 50 g / vial and the medium was sterilized by autoclave at a temperature of 121 °C and a pressure of 1.5 kg/cm<sup>2</sup> for 20 minutes, then the medium was inoculated in each vial with mycelium pieces of a pure culture of *F. graminearum* on PDA medium (5 days old) and incubated at 28 ± 2 °C for 14 days with daily stirring. The inoculation was used at 2 g / kg of soil in the pot experiment and 3 g / m in the field experiment.

### Isolation and diagnosis of bacteria strains

Bacteria were isolated according to the method described by (Wollum, 1982 ; Collee et al., 1996; Macfaddin , 2000), where wheat plants were uprooted, then the soil around the roots was

collected and milled well, taking 10 g of soil and adding 90 ml of Peptone water that consist of 5 g peptone, 5 g Tryptone, 5 g Sodium Chloride and 1liter water, then shaking the mixture in an electric shaker at a speed of 120 r / min for 30 minutes and leave to stagnate, taking 1 ml of the supernatant and add to 9 ml of the saline solution (NaCl) at a concentration of 0.85% to obtain the second decimal dilution then third decimal dilution, taking 0.1 ml from dilution (10<sup>-3</sup>) and disseminated to petri dishes contain the elective medium for each type of bacteria such as (*Bacillus*, *Pseudomonas*, *Azotobacter*, *Azospirillum*, and *Streptomyces*) and incubate of the petri dishes at 30 ° C for 48 hours, the bacterial isolates were preserved in slant cultures contain of Nutrient Agar medium (NA) at 4°C, Nutrient broth medium (NB) was used for multiplication and development of bacteria and other laboratory tests. The bacterial species were diagnosed in the microbiology laboratory based on the characteristics of the growing colonies on their electoral medium such as their color, shape, convexity, luminosity, edge, viscosity, cell shape, and their response to gram stain and the collect method and spore formation using light microscopy (Bergey's Manual, 1985).

### Field experiment

The field experiment was carried out at the Tuwaitha Research Station of the Ministry of Science and Technology during the season 2013-2014. The land was well plowed and leveled, then divided into three sectors, each sector includes 16 treatments, each treatment has 4 lines, length of line 3 m, and the distance between the lines 50 cm. The treatments were distributed randomly to each sector according to a complete randomized block design CRBD. The fungus vaccine *F. graminearum* was added to the lines at a rate of 3 g / m. wheat seeds (cv. Sham 2) were immersed in a bacterial suspension containing 10<sup>7</sup> cells/ml with the addition of gum Arabic at a rate of 2% for 30 minutes and planted in lines at a rate of 25 seeds/line with three replicates per treatment. The germination percentage was calculated 10 days after planting, also the percentage of disease incidence and disease severity in the crown area of the plants after 4-8 weeks.

The following Disease index was adopted for crown rot:

0 =healthy plant

1= 1 - 25% brown color in the crown area.

2= 26 - 50% brown color in the crown area.

3= 51 - 75% brown colors in the crown area and the coloring extends to the roots.

4 = 76 - 100% the whole infection of plants.

$$\text{Disease severity \%} = \frac{\text{Disease grade} \times \text{number of plants in each grade} \times 100}{\text{Total number of plants} \times \text{highest disease grade}}$$

At the end of the season, the characteristics of growth and productivity were taken, the weight of 1000 grains, length of

the plant, and the number of seeds per spike, dry weight of root and shoot, and protein ratio of seeds. The experiment included the following treatments:

1-Seeds without bacteria in non-contaminated soil with <i>F.graminearum</i> .
2- Seeds with <i>P. flourescens</i> in not contaminated soil with <i>F.graminearum</i> .
3- Seeds with <i>Streptomyces</i> in not contaminated soil with <i>F.graminearum</i> .
4- Seeds with <i>Azotobacter</i> in not contaminated soil with <i>F.graminearum</i> .
5- Seeds with <i>Azospirillum</i> in not contaminated soil with <i>F.graminearum</i> .
6- Seeds with <i>B. subtilis</i> in not contaminated soil with <i>F.graminearum</i> .
7- Seeds with <i>B. pumillus</i> in not contaminated soil with <i>F.graminearum</i> .
8- Seeds with bacteria mixture in not contaminated soil with <i>F.graminearum</i> .
9- Seeds with <i>P. flourescens</i> in contaminated soil with <i>F.graminearum</i> .
10- Seeds with <i>Streptomyces</i> in contaminated soil with <i>F. graminearum</i> .
11- Seeds with <i>Azotobacter</i> in contaminated soil with <i>F. graminearum</i> .
12- Seeds with <i>Azospirillum</i> in contaminated soil with <i>F.graminearum</i> .
13- Seeds with <i>B. subtilis</i> in contaminated soil with <i>F. graminearum</i> .
14- Seeds with <i>B. pumillus</i> in contaminated soil with <i>F. graminearum</i> .
15- Seeds with bacteria mixture in contaminated soil with <i>F. graminearum</i> .
16-Seeds without bacteria in contaminated soil with <i>F.graminearum</i> .

**Tests of the ability of bacteria to stimulate the growth Nitrogen fixation (estimation of nitrogen ratio in seeds)**

The nitrogen ratio was estimated by using the Micro Kjeldahl method in the Food manufacturing Laboratory belongs to the Ministry of Science and Technology. The seeds were ground well by using a mill and put 0.5 g of seeds powder in a Kjeldahl tube, then added 6 g of potassium sulfate mixture (K<sub>2</sub>SO<sub>4</sub>) consisting of 200 g of potassium sulfate (K<sub>2</sub>SO<sub>4</sub>), 20 g of aqueous copper sulfate (CuSO<sub>4</sub>.5H<sub>2</sub>O) and 2 g of selenium. The mixture was heated at 350-375 ° C for 1.5 hours to obtain a clear solution, after the solution became cool, 25 ml of distilled water and 40 ml of Sodium Hydroxide solution N10 were added to the solution in the Kjeldahl tube through the distillation apparatus, then the Kjeldahl tube was transferred to the nitrogen distillation apparatus.10 ml of boric acid was added in a glass beaker 100 ml and place the beaker under the condensation tube in the distillation apparatus. When the distilled solution in the receiving apparatus reached 70 ml, stops the distillation. The distillate solution with 0.01 standard sulfuric acid was added to a burette 100 ml and the process continued until the color changed from green to pink and the total nitrogen ratio was calculated according to the following formula:

$$N\% = 1.4 / S \times N \times (B-T)$$

T = ml of the consumed sulfuric acid by the process of model titration.

B = ml of the consumed sulfuric acid by the process of control model titration.

N = standard of sulfuric acid.

S = weight of the used model.

The protein content in seeds was estimated according to the formula:

$$\% \text{ Protein} = \% \text{ nitrogen} \times 6.25 \text{ (Coefficient Factor)}$$

**The ability of bacteria to dissolve phosphorus**

PKV agar medium was used to carry out this test, which consists of Glucose 10 g, Tricalcium phosphate 5 g, Ammonium sulphate 0.5 g, Potassium sulphate 0.2 g, Magnesium sulphate 0.1 g, Yeast extract 0.5 g, Manganese sulphate 0.002 g, Ferrous sulphate 0.002 g, Agar 20 g (Pikovskaya, 1948). The ingredients were dissolved in 1000 ml of distilled water and sterilized at a temperature of 121 °C and a pressure of 1.5 kg/cm<sup>2</sup> for 20 minutes, the medium was poured into Petri dishes 9 cm and the center of each dish was inoculated with a swab from a bacterial colony of the tested bacteria. The dishes were incubated at 23 °C for 10 days. The diameter of the disintegration area circle, which represents the large circle around the bacterial vaccine, and the diameter of the small circle which represents the bacterial growth was measured, the area of the circle was calculated:

$$\text{Diameter of disintegration circle} / \text{mm} = \text{diameter of large circle} - \text{diameter of the colony}$$

$$\text{Area of disintegration circle in mm}^2 = \text{area of large circle} - \text{area of a small circle}$$

**Results and Discussion**

The results in Table (1) showed that cultivation of seeds in contaminated soil with *F. graminearum* led to a significant reduction in the plant growth characteristics, the plant length reached 51.85 cm compared to 88.71cm for plants resulting from the planted seeds in not contaminated soil with fungus, a similar decrease in the dry weight for shoots and roots, where reached 1.55 g and 0.137 g, compared to 4.06 g 0.53 g respectively, the treatments of *P. fluoreescens*, *Streptomyces*, *Azotobacter*, *Azospirillum*, *B. subtilis*, *B. pumillus* and bacterial mixture that cultivated in contaminated soil with fungus led to a significant increase in the plant length (69.91, 77.01, 69.78, 77.93, 67.78, 74.33, 81.16 cm) respectively, also in the dry weight for shoots and roots (2.14, 2.44, 2.08, 2.67, 2.12, 2.97, 3.24 g) and (0.243, 0.330, 0.260, 0.336, 0.236,0.343, 0.410 g) respectively. The plant growth characteristics in contaminated soil with *F. graminearum* were decreased due to roots infection with the fungus, therefore the roots unable to absorb water and nutrients. (Nourozian and Etebarian, 2006, Hajieghrari, 2009, Hamissi et al., 2011,

Foroutan, 2012) reported that infection of the crown and root area with the fungus will impact water and nutrients absorption so that the plant becomes weak. Improving plant growth characteristics may be caused by an increase of nutrient elements for the plant through the degradation of organic matter in the soil, which is representing the main role of growth-enhancing bacteria (Elshanshoury, 1995). Some bacteria species in the soil such as *Azospirillum* are fixing atmospheric nitrogen and converting to formulas which absorb by plants, other bacteria act to metabolize root secretions from carbohydrates and provide the plant with nitrogen to manufacture amino acids (Elshanshoury, 1995, El-Sirafy et al., 2006). Several studies indicated that bacteria secrete organic acids such as Lactic acid, Malic acid, Citric acid that dissolve phosphorous (Jeffries et al., 2003, Khan et al., 2007, Adesemoye and Kloepper, 2009). Other studies reported that the bacteria release effective groups from the secondary metabolite products lead to the chelate of linked ions with phosphates and convert to dissolve (Goldstein, 1986, Arshad and Frankenberger, 1998). (Rajkumar et al., 2010) indicated that the produced siderophores by some growth-enhancing bacteria act as chelating compounds and dissolve phosphorous. Improving plant growth may be through the production of bacteria to plant hormones such as IAA, Indole-3-acetic acid, auxins, cytokinin, and gibberellins, all of which are stimulating growth. It has been indicated that bacterial species of the genus *Azospirillum* produce plant hormones (Steenhoudt et al., 2000). Some species of bacteria *Bacillus* and *Pseudomonas* produce growth regulators that help in roots forming and increasing of surface area for the roots, thereby increasing their

absorption of water and nutrients. It is known that IAA hormone stimulates the formation of roots, helps in the division of cells and increase their size (Salisbury, 1994 Ahmed et al., 2005, Joseph et al., 2007). Salim et al., (2019) reported that using of bio-fertilizers (*Azotobacter chroococcum* & *Pseudomonas fluorescens*) led to improvement of growth traits and yield of wheat through development the activity of root system and producing of hormones. Bacteria improves growth criteria indirectly by inhibiting pathogens. This inhibition may be directly due to secretion of bacteria to antibiotics that prevent growth of pathogen (Nourozian et al., 2006), and it may be through the direct parasitism on the pathogen and its secretion to the external enzymes that break down the cell walls of the pathogen such as the Chitinase, B-1, 3-glucanase (Bakker et al., 1991, Bharathi et al., 2004, Gilck, 2012). It is not excluded that some bacteria act to break down fungal toxins that produced from pathogens as secondary metabolite compounds. Other studies indicated a high ability of *Streptomyces* bacteria to parasitize on the fungal pathogens (Zhao et al., 2011, Saleh et al., 2013). Such antagonists can compete with pathogens for nutrients, inhibit pathogen growth by secreting antibiotics, or reduce pathogen populations through parasitism (Salim et al., 2017b). The ability of some bacteria to secrete chelated siderophores for iron leads to inhibition of pathogens that need iron, and efficacy of *Pseudomonas* in this field has been indicated (Loper and Buyer 1991, Loper and Gross, 2007). Inhibition of the pathogen growth by the bacteria can be indirectly by stimulating self-defensive plant methods called Induced Systemic Resistance (ISR) (Van Loon, 2007, Shahraki et al., 2009).

**Table 1. Effect of PGPR bacteria on plant length and dry weight of plants**

NO	Treatments	Plant length (cm)	Dry weight of plants (g)	
			Shoot	Root
1	Untreated seeds with bacteria and fungus	88.71	4.06	0.530
2	Treated seeds with <i>P. flourescens</i>	91.80	4.30	0.600
3	Treated seeds with <i>Streptomyces</i>	95.91	5.02	0.736
4	Treated seeds with <i>Azotobacter</i>	90.25	4.28	0.616
5	Treated seeds with <i>Azospirillum</i>	95.66	5.07	0.780
6	Treated seeds with <i>B. subtilis</i>	91.33	4.32	0.640
7	Treated seeds with <i>B. pumillus</i>	94.33	5.16	0.763
8	Treated seeds with bacteria mixture	103.41	5.92	0.836
9	Treated seeds with <i>P. flourescens</i> + fungus	69.91	2.14	0.243
10	Treated seeds with <i>Streptomyces</i> + fungus	77.01	2.44	0.330
11	Treated seeds with <i>Azotobacter</i> + fungus	69.78	2.08	0.260
12	Treated seeds with <i>Azospirillum</i> + fungus	77.93	2.67	0.336
13	Treated seeds with <i>B. subtilis</i> + fungus	67.78	2.12	0.236
14	Treated seeds with <i>B. pumillus</i> + fungus	74.33	2.97	0.343
15	Treated seeds with bacteria mixture + fungus	81.16	3.24	0.410
16	Untreated seeds with bacteria + fungus	51.85	1.55	0.137
	LSD 0.05	9.315	0.120	0.032

Each number represents the rate of three replicates, each replicate has 4 lines, and each line has 10 plants.

The number of grains in the spike, the weight of 1000 grains, and the protein content in the grains were significantly decreased for the planted seeds in contaminated soil with *F. graminearum*, where reached 25.63 grains/spike, 16.17 g, and

8.12% respectively, compared to 41.33 grains/spike, 37.98 g, and 11.49% for previous characteristics in the planted seeds in not contaminated soil with fungus (Table 2), whereas the treatments of *P. flourescens*, *Streptomyces*, *Azotobacter*,

*Azospirillum*, *B. subtilis*, *B. pumillus* and bacterial mixture that cultivated in contaminated soil with fungus led to a significant increase in several grains in the spike (33.41, 35.16, 33.66, 36.13, 33.25, 35.25, 40.58 ) respectively and weight of 1000 grains (30.87, 32.71, 30.88, 35.80, 30.86, 32.71, 38.20 g) respectively and protein content in the grains (9.28, 9.87, 9.32, 9.95, 9.50, 9.88, 10.12%) respectively. Infection of the plant by fungus in the crown area caused the crown and root rot, which prevented the absorption of nutrients and led to weak growth and incomplete of grains in the spikes and some of the

grains were empty, thus led to a reduction in their number and weight and protein content. this agrees with (Nourzian and Etabarian, 2006, Hajieghrari, 2009, Hamissi et al., 2011, Foroutan, 2012, Khalifa, 2013). It is noted from the overall results that the bacterial mixture was more efficient in improving the growth criteria than using bacteria individually, thus leads to the conclusion that using many biological agents with different mechanisms will be more efficient than one mechanism.

**Table 2. Effect of PGPR bacteria on number of grains/ spike, weight of 1000 grain and protein in grains**

No	Treatments	Number of grains/ spike	Weight of 1000 grain	% Protein in grains
1	Untreated seeds with bacteria and fungus	41.33	37.98	11.49
2	Treated seeds with <i>P. fluorescens</i>	44.33	40.01	11.75
3	Treated seeds with <i>Streptomyces</i>	47.98	44.45	12.24
4	Treated seeds with <i>Azotobacter</i>	43.08	40.43	11.80
5	Treated seeds with <i>Azospirillum</i>	47.40	45.18	12.19
6	Treated seeds with <i>B. subtilis</i>	41.28	40.53	11.76
7	Treated seeds with <i>B. pumillus</i>	44.83	44.24	11.89
8	Treated seeds with bacteria mixture	51.80	47.13	12.24
9	Treated seeds with <i>P. fluorescens</i> + fungus	33.41	30.87	9.28
10	Treated seeds with <i>Streptomyces</i> + fungus	35.16	32.71	9.87
11	Treated seeds with <i>Azotobacter</i> + fungus	33.66	30.88	9.32
12	Treated seeds with <i>Azospirillum</i> + fungus	36.13	35.80	9.95
13	Treated seeds with <i>B. subtilis</i> + fungus	33.25	30.86	9.50
14	Treated seeds with <i>B. pumillus</i> + fungus	35.25	32.71	9.88
15	Treated seeds with bacteria mixture + fungus	40.58	38.20	10.12
16	Untreated seeds with bacteria + fungus	25.63	16.67	8.12
	LSD 0.05	7.449	10.713	1.862

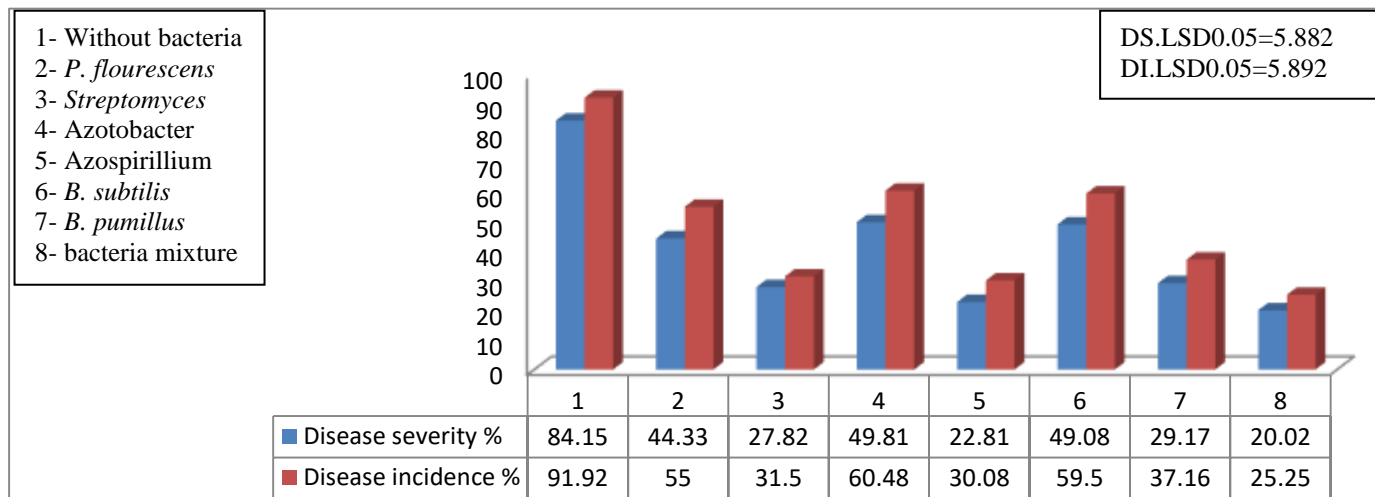
Each number represents rate of three replicate, each replicate has 4 lines, and each line has 10 plants

The results in Fig (1) showed that the cultivation of wheat seeds in contaminated soil with fungus *F. graminearum* resulted in infection of plants with crown rot disease with ratio 91.92%, and the treatment of seeds with bacteria before planting and then planting in contaminated soil with the fungus resulted in a significant reduction in the disease incidence, the treatments of bacterial mixture, *Azospirillum*, *Streptomyces* and *B. pumillus* were distinguished in a significant reduction in disease incidence rate reached (25.25, 30.08, 31.5 and 37.16%) respectively, compared to the treatments *P. fluorescens*, *B. subtilis* and *Azotobacter* (55.00, 59.5 and 60.48% ) respectively, the decrease in the disease incidence rate was accompanied by a similar decrease in the disease severity , where reached (20.02, 22.81, 27.82, 29.17, 44.33, 49.08, 49.81%) in the treatments of bacterial mixture, *Azospirillum*, *Streptomyces*, *B. pumillus* , *P. fluorescens*, *B. subtilis* and *Azotobacter* respectively, compared to the control treatment (seeds not treated with bacteria in contaminated soil with fungus) 84.15%. whereas some minor infections occurred in the produced plants from the treated wheat seeds with bacteria that cultivated in non-contaminated soil with fungus between 0-1.83%, with disease severity ranging between 0-1.25% depending on the type of

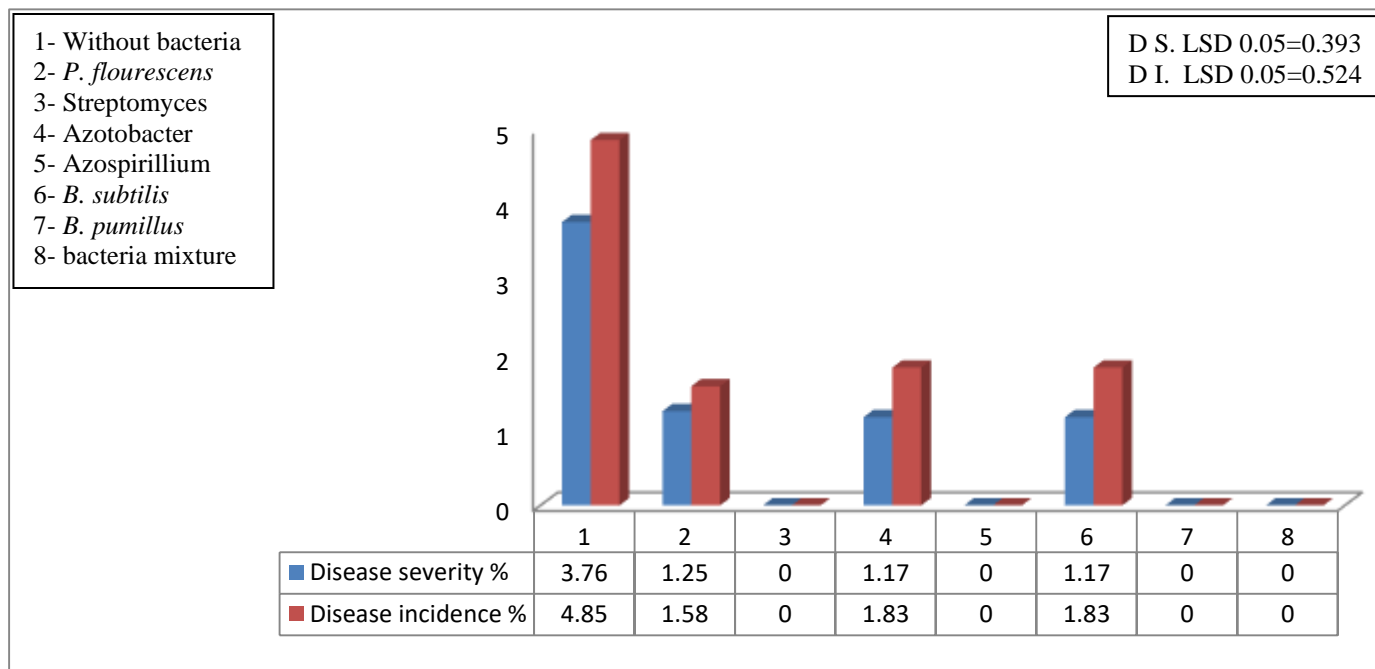
bacteria in (Fig 2). This is normal as the field soil is not sterile and contains many microorganisms, including pathogens, which may be among the species of *Fusarium*. Decrease of disease incidence and disease severity in plants resulting from the treated seeds with bacteria may be due to the direct impact of bacteria on the fungus *F. graminearum* in the soil through competition on the location, also the bacteria cover the seeds and multiply on the roots later, then acts as a barrier against the pathogen and some of these bacteria possess the ability to displace the pathogen through breaking down the organic compounds in the soil and acquiring nutrient elements. Perhaps the greatest effect of these bacteria on pathogens through their secretion of antibiotics, which is related directly to their reproduction on the roots, which causes inhibition of the pathogen. (Whipps, 2001, Haas and Keel, 2003).It is not excluded that one of the mechanisms of the direct impact of bacteria on the pathogen through its parasitism on fungal mycelium and its secretion of enzymes that degrade the cellular wall such as Chitinase, Protease, and B-1,3-glucanase that lead to killing the fungus (Chen et al., 2000), and Chitinase enzyme is a guide to the biological activity of bacteria from PGPR against pathogenic fungi such as *Botrytis cinerea* (Frankowski et al., 2001),

Sclerotiumrolfsii (Ordentlich et al., 1988), and Phytophthora (Kim et al., 2008). The effect of bacteria in reducing of infection and disease development may be through its activation of the plant's defensive mechanisms, called induced systemic resistance (ISR), which is characterized by the

accumulation of jasmonic acid (JA) and ethylene in the plant as induction signals that work to stimulate resistance in the plant for a wide range of pathogens (Bakker et al., 2007).



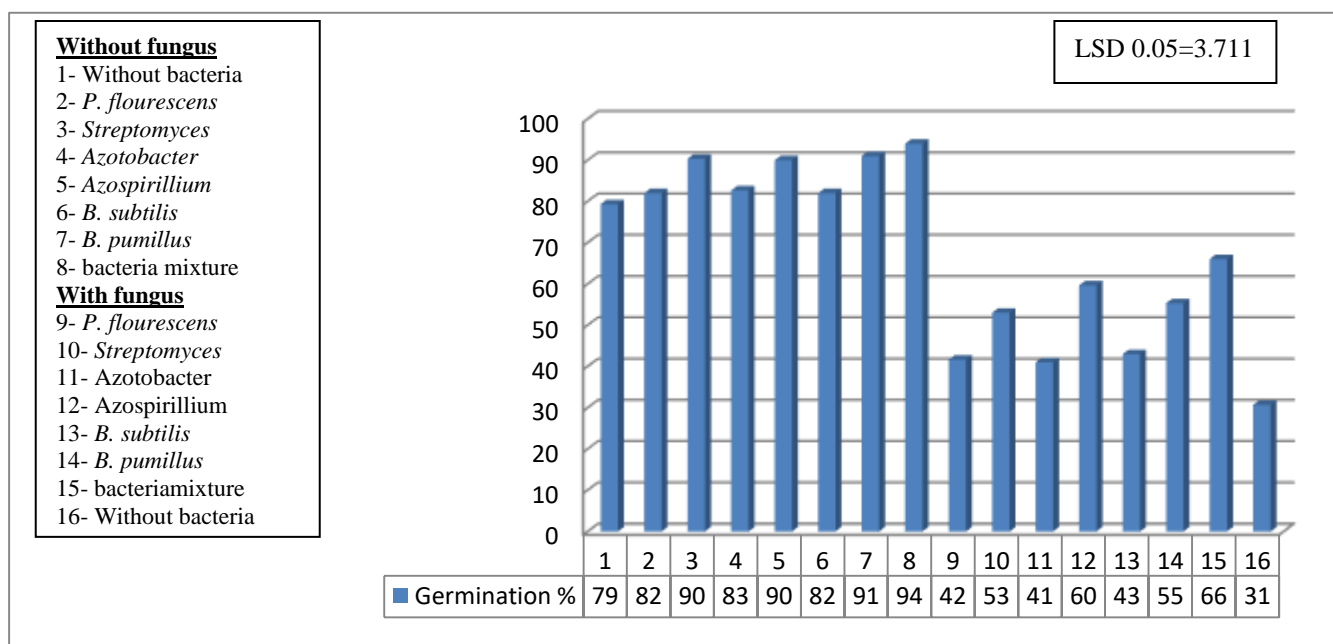
**Fig 1. Effect of PGPR bacteria on disease incidence and disease severity of crown rot on wheat plants cultivated in contaminated soil with fungus** (Each number represents the rate of three replicates, each replicate represents the transplant line and each line contains 25 seeds)



**Fig 2. Effect of PGPR bacteria on disease incidence and disease severity of crown rot on wheat plants cultivated in not contaminated soil with fungus** (Each number represents the rate of three replicates, each replicate represents the transplant line and each line contains 25 seeds)

The results of Fig (3) indicate that significant increases in seeds germination rates in not contaminated soil with pathogenic fungus compared to other treatments in contaminated soil with pathogenic fungus, the treatments of *Azospirillum*, *Streptomyces*, *B. pumillus* and bacterial mixture that cultivated in not contaminated soil with fungus led to a significant increase in the percentage of germination (90, 90, 91, 94%) respectively. The high germination rate in treated

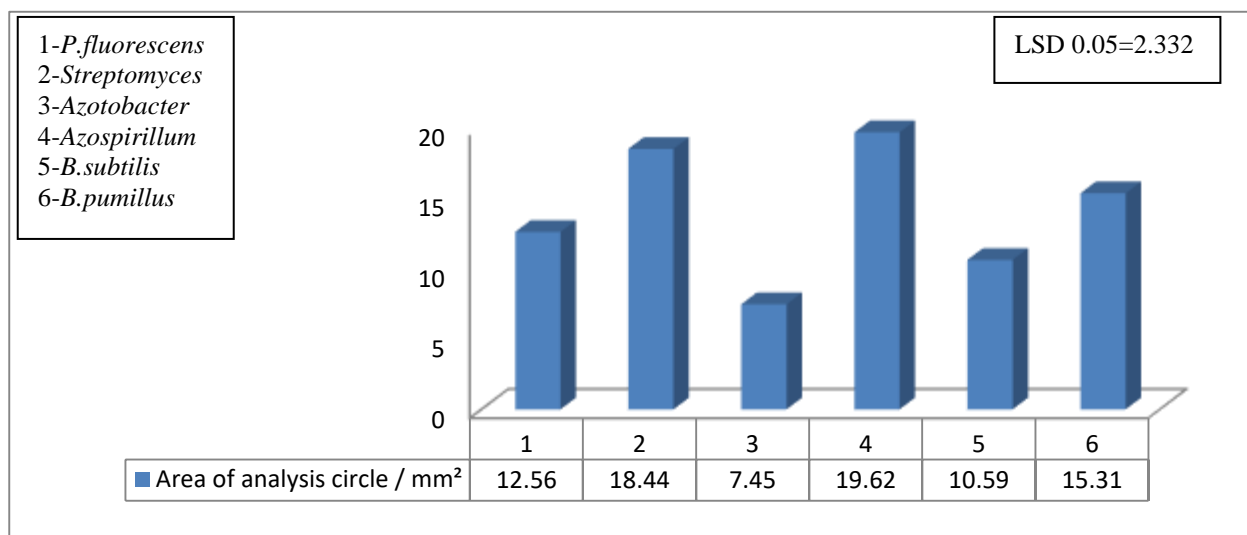
seeds with bacteria may be due to its secretion of plant hormones and auxins that have the property of stimulating and accelerate the seeds germination. Numerous studies have indicated the ability of PGPRs to secrete hormones and auxins, including IAA and cytokinines that plant-stimulating and improving of growth criteria (Kravchenko et al., 1991, Nezarat and Gholami, 2009, Al-Dulaimi, 2013, Dhiab, 2013 Saeed, 2013).



**Fig 3. Effect of PGPR bacteria on germination percentage % of wheat seeds** (Each number represents the rate of three replicates)

It is clear from the results in Fig (4) that all used bacteria in this study possess the ability to dissolve phosphorous in varying degrees and *Azospirillum* was superior in area of disintegration circle 19.62 mm<sup>2</sup> (Fig 5), followed by *Streptomyces* 18.44 mm<sup>2</sup>, *B. pumillus* 15.31 mm<sup>2</sup> compared to the disintegration areas of bacteria *P. fluorescens*, *B. subtilis*, *Azotobacter* 12.56, 10.59, 7.45 mm<sup>2</sup>, respectively. Numerous studies have indicated that some PGPR bacteria can dissolve linked phosphorous, called Phosphate Solubilizing Microorganism (PSM), and make it ready for absorption by plants, including species of the genus *Bacillus*, *Pseudomonas*, *Rhizobia* (Adesemoye and Kloepper, 2009). *Bacillus megaterium* is the most efficient bacterial species in dissolving phosphorous (Chen et al., 2008).

PSMs act to dissolve phosphorous by secreting organic acids with low molecular weights, including gluconic acid, Lactic acid, 2- ketoglucanic, Malic acid, citric acid, and glyoxylic acid that dissolve insoluble phosphate and Lower the pH around the cells. Organic acids act as ion-chelating materials by release the hydroxyl and carboxyl groups that chelate the phosphate-related ions and the phosphates become dissolved (Rodriguez and Fraga, 1999, Khan et al., 2007, Gamalero and Glick, 2011), also the produced siderophores compounds by some bacterial species of PGPR possess the ability to chelate the metallic elements and dissolving of phosphorous (Glick, 1995, Wu, et al., 2005, Chen et al., 2008).



**Fig 4. The ability of PGPR bacteria to dissolve phosphorus on the culture medium** (Each number represents 5 replicates)





**Fig 5. The ability of *Azospirillum* to dissolve phosphorus on the culture medium**

## Conclusions

The treated seeds with PGPR bacteria, including *P. fluorescens*, *Streptomyces*, *Azotobacter*, *Azospirillum*, *B. subtilis*, *B. pumillus*) before planting as single or combined resulted in improving of wheat growth characteristics and reducing of *F. graminearum* infection.

## Authors' contributions

All authors have contributed significantly to the conception and design of the study, the interpretation of data, and the drafting and revision of the manuscript. All authors read and approved the final manuscript.

## Conflict of Interest

The authors hereby declare no conflict of interest.

## Consent for publication

The authors declare that the work has consent for publication.

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