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# Integration Effect of *Enterobacter cloacae* and *Paenibacillus polymyxa* with Mineral Fertilizers on Nutrients Uptake, Productivity and Soil Borne Pathogens of Maize and Wheat Plants

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## ABSTRACT

Two experiments were carried out; the first one was conducted *in vitro* to assay antifungal activity of *Enterobacter cloacae* strain LC07192 and *Paenibacillus polymyxa* against *Fusarium verticillioides*, the ear rot pathogen of maize plants and *Bipolaris sorokiniana*, the black point pathogen of wheat. The second experiment was conducted in the field at the private farm in Al-Dakahlia governorate to evaluate too the effect of previous mentioned bacterial strains as phosphate and potassium solubilizing bacteria in utilizing fertilizers either chemical ″super phosphate, potassium sulfate″ or natural alternative minerals ″rock phosphate, feldspar″ on growth, diseases incidence, yield characters and chemical composition of maize and wheat grown in a sequence seasons during 2019-2020. In addition, the available of nutrients post-harvest of maize and wheat. Results showed that *in vitro*, two bacterial strains showed a pronounced reduction of mycelial growth of both pathogens. Under field condition, the significant increase in growth and yield of maize were presented by the combination between two bacterial strains with classical soluble fertilizers. Also, the greatest NPK uptake and protein content of grains recorded with the same treatment. What worth to be mentioned for later crop (wheat), combined between bacterial strains and alternative natural minerals gave significant enhance in the different parameters. On other hand, two bacterial strains had high potential to be used as biocontrol agents, because dual inoculation whether with chemical or with natural mineral fertilizers reduces both ear rot disease incidence and severity of maize plants and the same pronounced decrease of black point index, black point grains and black point seed/spike disease characters of wheat. It worth to be mentioned that when treating soil with rocks combined bacterial strains increased available P and K in the soil post-harvest of maize and enhanced P and K availability post-harvest of wheat.

***Keywords:*** Residual effect, rock phosphate, feldspar, *E. cloacae, P. polymyxa,* biocontrol, maize ear rot, wheat black point.

## 1. Introduction

Maize is one of the important cereal crops in the world's and Egypt; it is the third most important staple food crop both in terms of area and production after wheat and rice. Egypt is one of the major producing Arab countries, contributing an area of 9.948 million hectares, with a total annual production of 7.450 million tons (FAO, 2019). Wheat (*Triticum aestivum* L.) is considered a strategic crop in Egypt. It is the major staple crop produced in Egypt but its production doesn't meet the current demand. The area grown in Egypt was estimated at 1.412 million hectares, yielding 9.0 million tons of wheat grains (FAO, 2019).

Maize and wheat like other crops in Egypt suffers from the alkalinity of the soil which enhances the transformation of available P after a short period of application to tri-calcium phosphate which is unavailable to plants (Zayed, 2005). Consequently, to achieve optimum crop yields, soluble phosphate

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excess fertilizer has been applied at rates that caused excess of phosphorus application. On the other hand, large amounts of K chemical fertilizers are used to maximize crop yield per unit area and to compensate K-decreases in soils due to crop uptake (Shams and Fekry, 2014).

The use of natural alternative fertilizers as rock phosphate (RP) and feldspar as potassium material (RK) may be more useful and environmentally more feasible than soluble P and K (Abdel-Kader and Saleh, 2017). The alternative use of natural elements compounds improving soil physical and chemical properties as well as increasing water uptake and nutrient availability (Eman *et al.,* 2010). On other hand, they are not readily available to plants because they are released slowly and their use as fertilizers often gaves insignificant yield increases of current crops (Zapata and Roy, 2004). So, great attention has been directed towards using the bio-fertilizer (microbial inoculation) as phosphate-solubilizing bacteria, *Enterobacter cloacae* for increasing crop yields, to convert insoluble phosphate in rocks into soluble forms available for plant growth (Shaaban *et al.,* 2015a), through acidification, chelation and exchange reactions and produces strong organic acids in the periplasm (Dey *et al.,* 2021). Therefore, increasing the bioavailability of P and K in soils by inoculation of plant growth-promoting rhizobacteria (PGPR) or combined inoculation with rock materials may lead to increase P and K uptake and plant growth (Shaaban *et al.,* 2015b).

The application of minerals, containing P and K as rock phosphate and feldspar, in combination with the P and K solubilizing bacteria provides the growing plants with a continuous supply of phosphorus and potassium for the best plant growth rate (Han and Lee, 2005). Soil inoculation with potassium releasing bacteria and the soil feldspar was applied solo or integrated might provide a faster and continuous supply of K for improved plant growth, yield and quality (Abou-el-Seoud and Abdel-Megeed, 2012). So, the application of biofertilizers is eco-friendly, relatively cheap, nontoxic, and act as bio-control agent. Thus, usage of biofertilizers possesses significant potential to increase plant yield. In addition reducing the dependence on chemical fertilizers as well as providing a step forward toward sustainable agriculture (Salah *et al.,* 2020 and Fasusi *et al.,* 2021).

Among PGPR, the genus *Enterobacter* is found in a wide range of diverse environments reported to secrete plant growth hormones. Inoculation of *E. cloacae* showed solubilization of phosphate, potassium and zinc (Ramesh *et al.,* 2014 and El-Saied *et al.,* 2020). Principle mechanism in soil for mineral phosphate and potassium solubilization is lowering of soil pH by microbial production of organic acids and mineralization of organic P by acid phosphatase (Khan *et al.,* 2009). Also, isolates of E.*cloacae* are known to be bio-control agents (Kazerooni *et al.,* 2020) *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*), the type species of *Paenibacillus*, is considered to be a plant growth-promoting rhizobacterium (PGPR) with a broad host plant range (Timmusk *et al.,* 2013) and *P. polymyxa* is widespread in the soil and is widely used in agriculture (Park *et al.,* 2012). Being a bio-controlling species *Bacillus polymyxa* provides protection to various plants (Katare *et al.,* 2020).

More than 365 pathogens attach maize plant (Hussain *et al.,* 2016). Fusarium ear rot [*Fusarium verticillioides* (Sacc.) Nirenberg and *F. proliferatumis* (Matsushima) Nirenberg] is the most destructive abundant disease associated with maize grains worldwide (Leslie and Summerell, 2006). It is characterized by discolored and a reduced number of grains, yield as well as the quality of the seeds (Gai *et al.,* 2018). Both pathogens can be survived in infected maize seed without causing apparent symptoms or killing seed tissues (by producing toxic molecules and lytic enzymes) and subsequently transmitted to growing seedlings causing blights and root, stem and ear rot diseases. Under field conditions, the pathogen is systemically transmitted easily through infected seeds to maize growing seedlings by transmitting through stalk up to the ear (Thompson and Raizada, 2018). Furthermore, mycotoxins have harmful effects on human health, poultry and animals as well as enhance fungal virulence that infecting seedlings of some maize genotypes (Li *et al.,* 2019).

Kernel black-point disease has become one of the most serious problems of wheat, causing great losses in both yield and quality of grains (Fernandez and Conner, 2011). The disease characterized by symptoms of common root rot, seedling blight, leaf spot, head blight and black-point, with a grain loss ranged from 24 to 27% has been recorded in susceptible wheat cultivars (Bhandari *et al.,* 2003). The causes of the appearance of black pointed grain are most often caused by a complex of species including mainly *Alternaria* species and *Bipolaris sorokiniana* (Sacc.) Shoem (Khlebova *et al.,* 2019 and Masiello *et al.,* 2020). Moreover, other fungi belonging to *Aspergillus*, *Cladosporium*, *Curvulavia*, *Fusarium*, *Penicillium* and *Stemphylium* genera can participate in the disease complex and all together these species can induce the expression of the symptoms of black point (Abdullah and Atrosh, 2014&

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Ramires *et al.,* 2018). Among these genera, *Fusarium* and *Alternaria* associated with wheat kernels were reported to produce mycotoxins, toxic secondary metabolites that can be accumulated in colonized tissues (Ramires *et al.,* 2018 and Masiello *et al.,* 2020). Under epidemic conditions, up to 82% of the prevalence of these pathogens in agrocenoses of cereal crops, with its ability to retain their viability in the soil for more than 5 years was reported (Khlebova *et al.,* 2019). So, searching for sustainable alternative bio-agent that achieves more than one goal, which includes promotion of plant productivity and biological control of plant pathogens, is highly needed.

The present study aims to use sustainable alternative bio-agents that achieve more than one goal, including promotion of growth and productivity as well as biological control of important fungal pathogens (*B. sorokiniana* and *F. verticillioides*) of wheat and maize grown in a sequence under influence of mineral fertilizers, whether chemical or alternative natural.

## 2. Materials and Methods

### 2.1. Microorganisms

A highly pathogenic isolates of *Bipolaris sorokiniana* (Sacc) Shoem from a wheat plant causing black point disease as well as *Fusarium verticillioides* (Sacc.) Nirenberg from a maize plant causing ear rot disease was obtained from the Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

*Enterobacter cloaceae* strain LC07192 was selected due to high efficiency in phosphate and potassium solubilizing capacity based on a previous study (El-Saied *et al.,* 2020). *Paenibacillus polymexa* provided from the Microbiology Dept. Soils, Water and Environment Research Institute, Agricultural Research Center (ID: 60019332), Giza 12112, Egypt.

### 2.2. Natural alternative fertilizers

Rock phosphate and feldspar were kindly obtained from Soils, Water and Environment Research Institute, Agric. Res. Center, Giza, Egypt.

### 2.3. Seeds

Maize (*Zea mays* L., cv. Giza 10) and wheat (*Triticum aestivum* L., cv. Giza 168) were obtained from the Ministry of Agriculture and Land Reclamation, Egypt.

### 2.4. Nitrogenase (N-ase) assay.

Nitrogenase activity of *Paenibacillus polymyxa* was measured by the acetylene reduction assay method, after growing on selective Norris-glucose, nitrogen to ensure its ability to fix atmospheric nitrogen based on the method described by Lin *et al.,* (2002).

### 2.5. Antagonistic activity of the bioagents.

Assessment of the antifungal activity of *E. cloacae* LC07192 and *P. polymyxa* was explored against *Bipolaris sorokiniana* and *Fusarium verticillioides* pathogens using the dual culture plate technique. A 5 mm diam. disc, taken from 7 d culture of each of the tested pathogen was placed 1 cm from the edge of each potato dextrose agar (PDA) plate, and a loop of *E. cloacae* LC07192 or *P. polymyxa* was streaked 2 cm from the opposite edge of the plate. PDA plates each inoculated only with the fungal disc served as experimental controls. The test was performed in triplicate. The plates were incubated at 25°C and the inward linear growth of the pathogen was measured after 8 d. The test was ended when fungal growth completely covered the control plates. Fungal growth inhibition was calculated using the following equation: R1 – R2 Growth Inhibition (%) = × 100 R1 where R1= inward linear growth in the control plate, and R2= inward linear growth in the dual culture plate.

### 2.6. Field experiment

A field experiment was carried out at a private farm, Belqas city, Al-Dakahlia Governorate, Egypt, during two successive seasons, summer season 2019 with maize and winter season 2020 with wheat to study the efficiency of bacterial strains in utilizing fertilizers either chemical or natural alternative minerals in soil on growth, diseases incidence, yield and nutrient content of maize plant as the first crop and assay their residual effect on wheat plant as the second crop.

**2.7. Experimental soil and cultivation**

The soil sample was collected from the experimental field at the beginning of the experiment. The chemical and physical analyses were carried out according to the procedures outlined by Richards (1954) and Jackson (1958). Analyses were performed both before planting and after harvesting maize and wheat. Soil is sandy clay loam in texture containing 29.7% clay, 37.9% silt and 32.4% sand. The EC in soil past = 1.84 $dSm^{-1}$. pH in water suspension (1:2.5) = 7.86, organic matter = 0.90% and saturation percentage (SP) = 53.5%. Available N, P and K were 43.5, 13.08 and 355mg $kg^{-1}$, respectively.

Maize and wheat plant were cultivated in a completely randomized design with plot dimensions of 10.5 $m^2$. Each treatment was replicated three times. Concerning maize, the grains were sown on 20 May in 2017. For wheat, the grains were sown on 5 November 2017 after harvest maize.

**2.8. Treatments and experimental design**

All treatments had received both organic fertilizers as farmyard manure (FYM) at a rate of 20 $m^3$/ fed during the soil preparation. Nitrogen was added as ammonium nitrate (33.5%) at (150and 120 kg ammonium nitrate fertilizer/ fed.) for maize and wheat, respectively.

Phosphorous fertilizer was added in two forms**;** superphosphate (15.5% $P_2O_5$) was applied at 150 kg/fed., Rock phosphate (22.5% $P_2O_5$) was applied at 100 kg/fed. Also, potassium was added in two forms; potassium sulfate (48% $K_2O$) was applied at 48 kg/fed, and feldspar (11% $K_2O$) was applied at 220 kg /fed. Phosphorous and potassium fertilizers were applied at one time just at the sowing of the first crop (maize). Accordingly, the experiment was designed as follow; control (T1); chemical fertilizers (superphosphate + potassium sulfate) (T2); chemical fertilizers (superphosphate + potassium sulfate) with inoculation by bacterial strains (T3); natural fertilizers (rock phosphate + feldspar) (T4) and natural fertilizers (rock phosphate + feldspar) with inoculation by bacterial strains (T5). The agricultural practices and irrigation were done according to the recommendations of the Ministry of Agriculture.

**2.9. Preparation of seeds and grains with inoculants**

Healthy and homogenous size maize and wheat grains were wetted by adhesive agent (5% Arabic gum) then air-dried for 30 minutes in shade after that inoculated by the solution of bacterial strains using a liquid culture from *E. cloacae* and *P. polymyxa. E. cloacae*. The biofertilizers inoculation in liquid culture was added two times, the 1st one with sowing and the second was two months later (according to treatments). After the harvest of maize crops, the soil was prepared for the wheat plant. After sowing grains of wheat, just nitrogen fertilizer was applied in form of ammonium nitrate (33.5%N) two times before first and second irrigation. Biofertilizers inoculation in liquid culture was added two times, the 1st one with sowing wheat grains and the second was two months later (according to treatments).

**2.10. Sampling and collecting data**

**I. External morphology**

Different morphological characteristics of maize and wheat plants at vegetative stages (60days) were measured i.e., plant height (cm); number of leaves/plant; stem diameter of maize and number of tillers/ plant of wheat; Leaf area ($cm^2$) Leaf area/plant in $dm^2$ measured according to Alessi and Power (1975) using the following formula: Leaf area (LA) = leaf length x maximum leaf width x 0.75. Leaf area in $dm^2$ of three plants was summed and the leaf area/plant was calculated. Fresh and dry weight (g)/plant, the samples including stems and leaves were dried in an oven at 70ºc until a constant weight (Black, 1965).

**II. Photosynthetic pigments content**

Photosynthetic pigment content were determined by taking three plant samples randomly from each plot for determination chlorophyll a, chlorophyll b and carotenoid (mg/g f. wt) on the 4th leaf from the plant apex. According to Saric *et al.* (1976)

**III. Disease's assessment**

Maize disease rating by ear rot disease was recorded, 90 days after sowing. Infection by ear rot symptoms was recorded as the percentage of infected leaf area (% average lesion size) under heavy natural infection. Criteria expressing the severity of infection as described by Hilu and Hooker (1963) and El-Shafey (1970) was used. Ear rot disease incidence was determined by counting the number of infected ears of a random sample of 50 plants in 5 different positions according to Multitu *et al.,* (2003). Severity was scored using a scale where, 0= 0% infection, 1= 1-10%, 2=11-25%, 3=26-50%, 4=51-75% and 5=76-100% infection (Bigirwa *et al.,* 2007).

During the winter season, observations on wheat seedling emergence and seedlings mortality per square meter were recorded after 2 and 3 weeks, respectively of sowing by counting the total number of seedlings in four linear meters selected randomly from the middle five rows of each plot. The number of spikes per square meter was noted before harvest following the same procedure as used for seedling emergence and post-emergence mortality. After harvest, data on black point infection of seed were recorded. Black pointed grains per spike were recorded from 20 spikes selected randomly in each plot. The percentage of black pointed grains was calculated from 400 grains of a composite sample taken from each plot. The grains were indexed for black point infection using a 0-5 scale suggested by Gilchrist, (1985).

**IV. Yield and yield components**

At the harvesting stage, plants were collected from each plot to determine yield and its components. For maize, no. grains/plant, no. of rows/ear, length of the ear & grain and straw yields (g/plant) were determined. For wheat, the number of spikes/m$^2$, number of grains/spike, wt. of 1000 grain (gm), length of the spike (cm), grain yield (g/m$^2$) and straw yield (kg/m$^2$) were measured. The biological yield (ton/fed.) and harvest index for the two crops.

**VI. Chemical constituents in grains**

- Nitrogen content in grains (g/100g D. wt) was determined in the digested solution by the modifed microkjeldahl method as described by Jones *et al. (*1991).
- Phosphorus content in grains (g/100g D. wt) was estimated spectrophotometrically by model no. UV2100 S/N: BH 16041603003according to Peters *et al,* (2003).
- Potassium content in grains (g/100g D. wt) was determined by photometrically by JENWAY PFP7 model according to Peters *et al.,* (2003).
- Determination of nutrient uptake (kg/fed) of nitrogen, phosphorus and potassium (kg/fed.) was calculated by multiply N, P and K % by grain yield (kg/fed.) (Chapman and Pratt, 1961).
- Crude protein in grains was calculated by multiplying N content by 6.25 factor for maize (A.O.A.C. 1970) and by 5.71 for wheat (Bishni and Hughes, 1979).
- Determination of total carbohydrates in grains (g/100g D. wt) was estimated using the anthrone method (Sadasivam and Manickam, 1996).

**2.11. Statistical analysis**

The statistical analysis software; CoStat version 6.4 (CoHort Software) was used for the analysis of variance (ANOVA) of the data, comparison among means was carried out using Duncan's new multiple range test at probability (*P*) level $\leq$ 0.05(CoStat, 2005). Trials of the field experiments were arranged in a complete randomized block design.

## 3. Results

### *3.1. In vitro* study

**I. Antagonistic activity of the *E. cloacae* and *P. polymyxa***

*In vitro***,** two antagonistic bacterial isolates, *E. cloacae* and *P. polymyxa* obtained from the soil rhizosphere were tested in dual culture assay against maize *Fusarium verticillioides* (FV) and wheat *Bipolaris sorokiniana* (BS) pathogens (Fig. 1). Both antagonistic bacteria strongly inhibited the growth of the two target pathogens, with different degrees. *P. polymyxa* recorded the highest reduction of BS and FV pathogens (27.06 and 29.33%, respectively). *E. cloacae* treatment came in the second rank in reduction of BS or FV pathogens (22.94 and 24.56%, respectively). *Enterobacter cloacae* was found to have the ability to solubilize complex phosphate and potassium. *Paenibacillus polymyxa* on the other side was found to fix atmospheric nitrogen. Testing nitrogen fixation test revealed nitrogen activity of 49 nmol $C_2H_4$ /100ml/h.

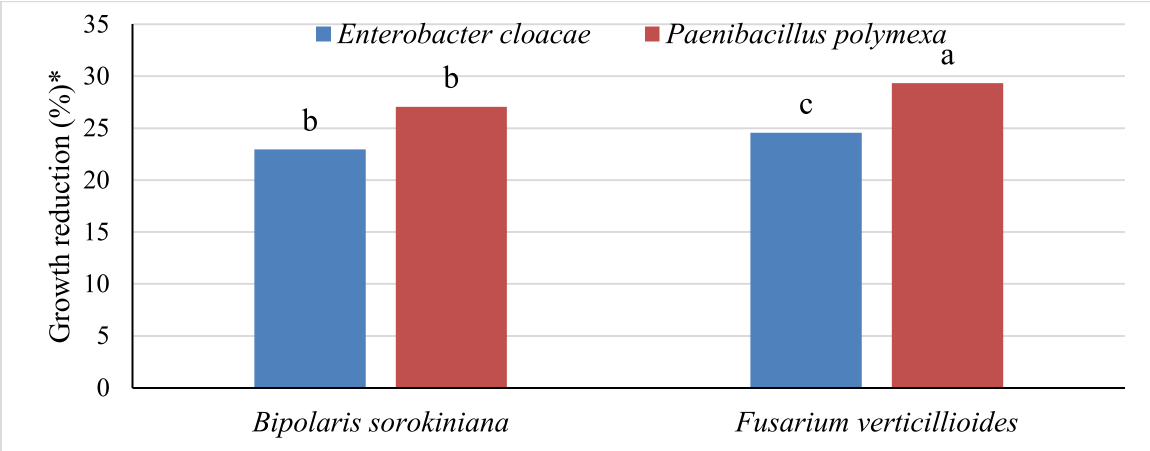


**Fig. 1:** Growth of *Fusarium verticillioides* (maize) and *Bipolaris sorokiniana* (wheat) pathogens as affected by *Enterobacter cloacae* and *Paenibacillus polymyxa* in a dual culture test

*Growth inhibition of each fungal pathogens (%) =Radius growth of each pathogen in the direction of antagonistic bacteria/radius of growth in the absence of antagonistic bacteria

Columns superscripted by the different letters are significantly different using Duncan's Multiple Range Test at *P*-value of ≤.0.05.

### 3.2. In field study

### I. Growth parameters

Regarding the effect of mineral fertilization either chemical ″superphosphate, potassium sulfate″ or natural ″rock phosphate, feldspar″ either applied alone or in combination with microbial inoculation *(Enterobacter cloacae* and *Paenibacillus polymyxa)* on vegetative growth at 60 days of maize growth as the first crop and wheat plants as the second crop. Data presented in (Table 1) revealed that all the investigated parameters were significantly increased with the mineral application when compared with the control treatment.

**Table 1:** Growth parameters of maize and wheat grown in a sequence as influenced by mineral fertilizers (chemical and natural) with or without biofertilizers.

| Treatments | Maize plant (first crop) | | | | | |
|---|---|---|---|---|---|---|
| | Stem (cm) | Leaves (No.)/plant | Diameter of stem (cm) | Leaf area ($cm^2$) | F.wt ear leaf (g/plant) | D. wt ear leaf (g/plant) |
| **T1** | 96.00 e | 13.50 e | 1.32 d | 503.63 e | 12.19 e | 3.89 d |
| **T2** | 123.0 c | 14.50 c | 1.71 b | 689.06 c | 13.44 c | 4.17 c |
| **T3** | 167.5 a | 16.00 a | 2.30 a | 826.20 a | 15.60 a | 5.74 a |
| **T4** | 108.5 d | 14.00 d | 1.50 c | 518.00 d | 12.81 d | 4.03 cd |
| **T5** | 137.5 b | 15.00 b | 1.82 b | 732.38 b | 13.94 b | 4.50 b |
| | **Wheat plant (second crop)** | | | | | |
| **Treatments** | **Stem (cm)** | **Leaves (No.)/plant** | **No. of tillers** | **Leaf area ($cm^2$)** | **F.wt shoot (g/plant)** | **D. wt shoot (g/plant)** |
| **T1** | 77.50 d | 16.50 d | 4.00 d | 29.25 e | 11.73 e | 3.17 e |
| **T2** | 79.00 c | 20.00 c | 4.75 c | 31.50 d | 15.00 d | 3.70 d |
| **T3** | 83.50 b | 24.00 b | 6.00 b | 37.50 c | 17.50 b | 4.55 c |
| **T4** | 84.00 b | 24.50 b | 6.00 b | 38.25 b | 16.80 c | 4.95 b |
| **T5** | 88.75 a | 27.50 a | 7.25 a | 42.07 a | 20.05 a | 6.07 a |

For each plant, the means of each criterion followed by the different letters within each column are significantly different using Duncan's Multiple Range Test at *P*-value of ≤.0.05.

T1. Control; T2. Chemical fertilizers "super phosphate +potassium sulfate"; T3. Chemical fertilizers "super phosphate + potassium sulfate"+ inoculation with bacterial; T4. Natural fertilizers "rock phosphate+feldspar"; T5. Natural fertilizers "rock phosphate + feldspar"+ inoculation with bacterial strains.

For maize, the application of biofertilizers with chemical mineral fertilizers (superphosphate + potassium sulfate) increased stem length by 74.48%, number of leaves/plant by 18.52%, the diameter of stem by 74.24%, leaf area by 64.05%, fresh and dry weight of ear leaf by (27.97 and 47.56%), respectively. Referring to wheat plants, the highest values of growth parameters enhanced with biofertilizers combined with natural alternative fertilizers ″rock phosphate+ feldspar″ as a residual by (14.52, 66.67, 81.25, 43.83, 70.93 and 91.48%) For stem length, number of leaves/plant, number of tillers, leaf area, fresh and dry weight (g)/plant, respectively compared with control.

**II. Photosynthetic pigments**

Results tabulated in (Table 2) showed that mineral fertilizers either chemical or natural applied solely or combined with bacterial strains had a positive effect on photosynthetic pigments of maize and wheat comparing with control. For maize, application of chemical fertilizers with biofertilizers increased by (36.85, 35.97, 36.51 and 180.51%) for chl a, chl b, total chlorophyll and carotenoid, respectively over control. Concerned with wheat, application alternative natural fertilizers with biofertilizers were increased by 65.46% for chl a, 94.39% for chl b, 76.80 for total chlorophyll, 112.95% for carotenoid compared with control.

**Table 2:** Photosynthetic pigment of maize and wheat grown in a sequence as influenced by mineral fertilizers (chemical and natural) with or without biofertilizers.

| Treatments | Chl a (mg/g) | Chl b (mg/g) | Total chlorophyll (mg/g) | Carotenoid (mg/g) |
|---|---|---|---|---|
| | | **Maize plant** | | |
| **T1** | 0.426e | 0. 278 d | 0.704 e | 0.118 e |
| **T2** | 0.504 c | 0.317 c | 0.821 c | 0.263 c |
| **T3** | 0.583 a | 0.378 a | 0.961 a | 0.331 a |
| **T4** | 0.449d | 0.307 c | 0.756 d | 0.221 d |
| **T5** | 0521 b | 0.339 b | 0.860 b | 0.297 b |
| | | **Wheat plant** | | |
| **T1** | 0.304 d | 0.196 e | 0.5 00 e | 0.193 d |
| **T2** | 0.323 d | 0.233 d | 0.556 d | 0.274 c |
| **T3** | 0.451 c | 0.271 c | 0.722 c | 0.309 b |
| **T4** | 0.477 b | 0.310 b | 0.787 b | 0.320 b |
| **T5** | 0.503a | 0.381 a | 0.884 a | 0.411 a |

For each plant, the means of each criterion followed by the different letters within each column are significantly different using Duncan's Multiple Range Test at *P*-value of $\leq$.0.05.

T1. Control; T2. Chemical fertilizers "super phosphate +potassium sulfate"; T3. Chemical fertilizers "super phosphate + potassium sulfate"+ inoculation with bacterial; T4. Natural fertilizers "rock phosphate+feldspar"; T5. Natural fertilizers "rock phosphate + feldspar"+ inoculation with bacterial strains.

**III. Yield parameters**

The data given in (Table 3) represent the response of the yield parameters to the mineral application either classical "soluble fertilizer" or alternative natural along with or without biofertilizers. Concerning maize, the data clearly showed that the yield parameters were significantly increased with the combined application of biofertilizers and chemical mineral fertilizers compare with control. Where it increased the number of ears/plant by 50%, number of seeds/plant by 242.24%, number of rows/ear by 24% and length of the ear by 32.35%. Moreover, it enhanced by (50.50, 23.32, 36.02 and 10.64 %) for grain yield/fed, straw yield/fed, biological yield/fed and harvest index, respectively, compares with control treatment. Biofertilizers together with natural mineral fertilizers (rock phosphate + feldspar) recorded the highest number of spikes/$m^2$ by (41.15%), the number of grains/spike by (65.93%) and weight of 1000 grains by (20.83%). The same treatment gave increasing by (33.58, 23.13, 26.87 and 5.09%) for grain yield (ton/fed.), straw yield (ton/fed), biological yield (ton/fed.) and harvest index, respectively compared with control. From the above-mentioned results, it can be noticed that fertilization with natural mineral fertilizers to maize enhanced yield parameters of the second crop (wheat).

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**Table 3:** Yield parameters of maize and wheat grown in a sequence as influenced by mineral fertilizers (chemical and natural) with or without biofertilizers.

| Treatments | Maize plant | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **No. of ear/plant** | **No. of seeds/plant** | **No. of rows/ear** | **Length of ear (cm)** | **Grain yield (g)/plant** | **Straw yield(g)/plant** | **Grain yield (ton/fed.)** | **Straw yield (ton/fed.)** | **Biological yield (ton/fed.)** | **Harvest index** |
| **T1** | 1.00 c | 303.00 e | 12.50 e | 17.00 e | 107.5e | 122.65e | 3.01d | 3.43d | 6.44d | 46.71d |
| **T2** | 1.20 b | 963.00 c | 14.25 c | 19.50 c | 137.56c | 137.89c | 3.85b | 3.86b | 7.71b | 49.94b |
| **T3** | 1.50 a | 1037.00 a | 15.50 a | 22.50 a | 161.77a | 151.23a | 4.53a | 4.23a | 8.76a | 51.68a |
| **T4** | 1.00 c | 439.00 d | 14.00 d | 18.50 d | 120.12d | 130.45d | 3.36c | 3.65c | 7.01c | 47.94c |
| **T5** | 1.40 a | 987.00 b | 14.50 b | 20.00 b | 139.00b | 139.15b | 3.89 b | 3.90b | 7.79b | 49.97b |
| | **Wheat plant** | | | | | | | | | |
| **Treatments** | **Number of spikes / ($m^2$)** | **Number of grains / (spike)** | **Wt. of 1000 grains (g)** | **Length of spike (cm)** | **Grain yield /$m^2$(g)** | **Straw yield/$m^2$ (kg)** | **Grain yield (ton/fed.)** | **Straw yield (ton/fed.)** | **Biological yield (ton/fed.)** | **Harvest index** |
| **T1** | 678 e | 45.5 e | 48.00 e | 5.50 | 638 e | 1.08 d | 2.68e | 4.54d | 7.22 e | 37.14 d |
| **T2** | 717 d | 53.0 d | 49.50 d | 6.00 | 731 d | 1.19 c | 3.07d | 5.00c | 8.07 d | 38.05 c |
| **T3** | 815 c | 64.0 c | 54.00 c | 7.00 | 776.91c | 1.25 b | 3.26c | 5.25 b | 8.51 c | 38.33 b |
| **T4** | 823 b | 67.0 b | 55.50 b | 7.50 | 789.56b | 1.27 b | 3.32b | 5.33b | 8.65 b | 38.34 b |
| **T5** | 957 a | 75.5 a | 58.00 a | 8.50 | 851.5 a | 1.33 a | 3.58a | 5.59 a | 9.16 a | 39.03 a |

For each plant, the means of each criterion followed by the different letters within each column are significantly different using Duncan's Multiple Range Test at *P*-value of ≤.0.05.
T1. Control; T2. Chemical fertilizers "super phosphate + potassium sulfate"; T3. Chemical fertilizers "super phosphate + potassium sulfate"+ inoculation with bacterial; T4. Natural fertilizers "rock phosphate+feldspar"; T5. Natural fertilizers "rock phosphate + feldspar"+ inoculation with bacterial strains.

### IV. Disease's assessment

Data in (Fig. 2) show that all of the tested chemical or natural fertilizers in the presence or absence of bacterial strains reduced DI and DS along the tested period during maize growing season. Among the tested applications, natural fertilizers and chemical fertilizers dual with bacterial strains (11.62 and 11.26%, respectively) followed by sole natural and chemical fertilizers (12.37 and 12.18%, respectively) were found to be the best treatments in reducing DI as compared to the negative control (13.36%). However, a similar trend was recorded in DS parameters by chemical fertilizers + bacterial strains application (1.26), followed by natural fertilizers dual with bacterial strains and sole natural fertilizers applications (1.5 and 1.7, respectively) as compared to the negative control (2.22).

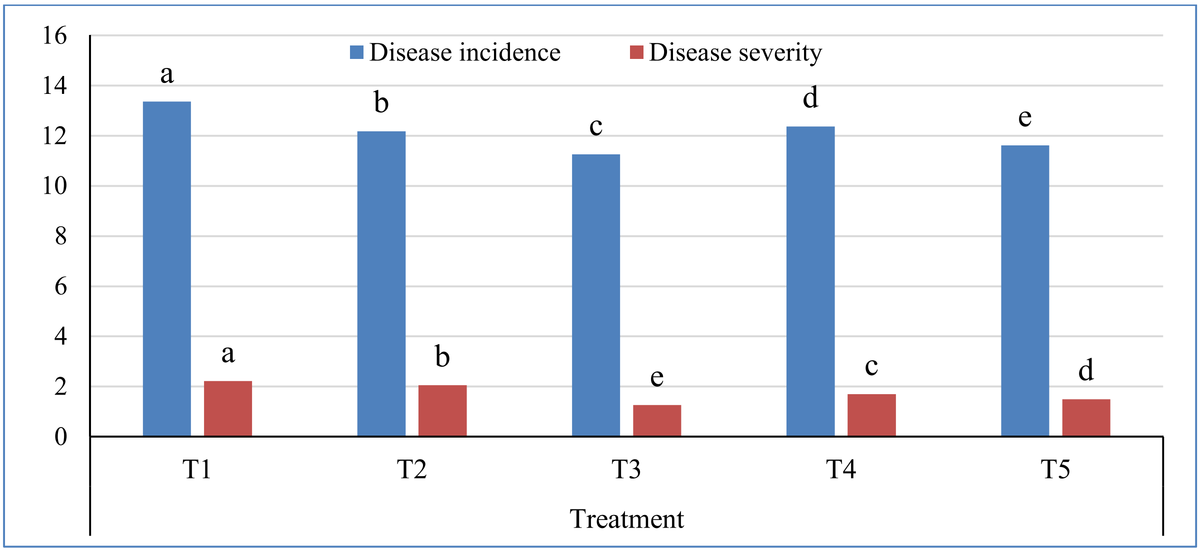


**Fig. 2:** Effect of seed bacterial treatments on ear rot incidence and severity of maize under field conditions

For each criterion, columns superscripted by the different letters are significantly different using Duncan's Multiple Range Test at *P*-value of ≤0.05. Where, T1= Control, T2= Chemical fertilizers (super phosphate +potassium sulfate), T3= Chemical fertilizers (super phosphate + potassium sulfate) + bacterial strains, T4= Natural fertilizers (rock phosphate feldspar) and T5. Natural fertilizers (rock phosphate + feldspar) + bacterial strains.

Data in (Fig. 3) show a significant ($P \leq 0.05$) reduction in the infection percentage of black point disease as a response to the residual effect of previous maize crop treatment by bacterial strains. Moreover, such disease reduction, however, was greatly marked in seedling mortality percentage when treatments of chemical fertilizers + bacterial strains, sole natural fertilizers, or its dual with bacterial strains were applied (2.10, 1.77 and 1.58%, respectively) as compared to control treatment (4.92%). The same pronounced decrease in the level of black point index percentage was recorded by a dual application of natural fertilizers + bacterial strains, being 3.41%, followed by the sole natural fertilizers and chemical fertilizers + bacterial strains applications (3.85 and 4.30%, respectively). However, a similar trend recorded in the percentages of both black point grains and black point seeds/spike characters as a response to chemical fertilizers + bacterial strains, sole natural fertilizers or it's dual with bacterial strains applications (10.86, 10.48 & 10.44% and 3.96, 3.95 & 3.54%, respectively) as compare to negative controls (13.17 and 5.12%, respectively).

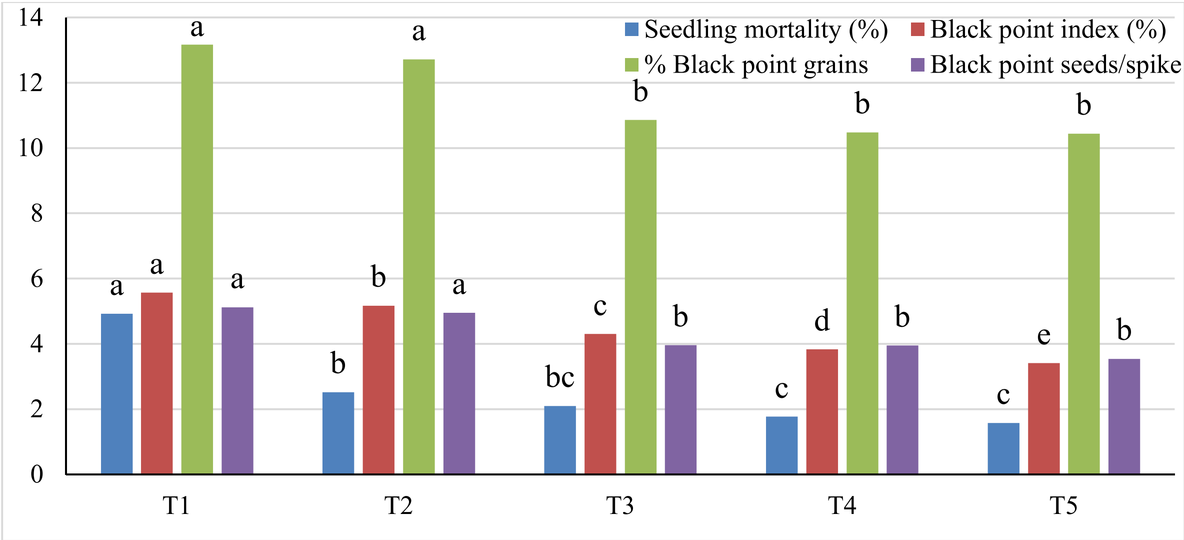


**Fig. 3:** Effect of seed bacterial treatments on black point incidence of wheat under field conditions

For each criterion, columns superscripted by the different letters are significantly different using Duncan's Multiple Range Test at *P*-value of ≤0.05. Where, T1= Control, T2= Chemical fertilizers (super phosphate +potassium sulfate), T3= Chemical fertilizers (super phosphate + potassium sulfate) + bacterial strains, T4= Natural fertilizers (rock phosphate feldspar) and T5. Natural fertilizers (rock phosphate + feldspar) + bacterial strains.

### V. Chemical constituents in grains

Data showed in (Table 4) cleared the application of different mineral fertilizers either chemical or natural and biofertilizers *(Enterobacter cloaceae* and *Paenibacillus polymyxa*). It was noticed that chemical fertilizers applied with bacterial strains, significantly increased chemical constituents for maize. Where, it recorded increased uptake by (78.49, 339.87 and 214.71%) for nitrogen, phosphorous and potassium, respectively compared with control. And there are no significant differences between values of protein in inoculated treatments. While, it increased by 27.80 % for carbohydrates over control.

**Table 4:** Chemical constituents in grains of maize and wheat grown in a sequence as influenced by mineral fertilizers (chemical and natural) with or without biofertilizers.

| Treatment | Nitrogen | | Phosphorous | | Potassium | | Crude Protein (%) | Total carbohydrate (%) |
|---|---|---|---|---|---|---|---|---|
| | N (%) | N uptake kg/fed. | P (%) | P uptake kg/fed. | K (%) | K uptake kg/fed. | | |
| | | | | **Maize plant** | | | | |
| **T1** | 1.56 c | 46.95e | 0.13 d | 3.913 e | 0.22 d | 6.622 e | 9.75 c | 44.21 e |
| **T2** | 1.62 b | 62.39c | 0.27 b | 10.400 c | 0.31 b | 11.94 c | 10.13b | 51.00 c |
| **T3** | 1.85 a | 83.80 a | 0.38 a | 17.212 a | 0.46 a | 20.84 a | 11.56a | 56.50 a |
| **T4** | 1.60bc | 53.81d | 0.20 c | 6.727 d | 0.28 c | 9.42 d | 10 bc | 47.17 d |
| **T5** | 1.81 a | 70.44 b | 0.29 b | 11.287 b | 0.32 b | 12.45 b | 11.31a | 52.12 b |
| | | | | **Wheat plant** | | | | |
| **T1** | 1.68 c | 45.017e | 0.33 d | 8.84 d | 0.42 e | 11.25d | 9.66 c | 43.22 e |
| **T2** | 1.73 b | 53.11 d | 0.47 c | 14.43c | 0.51 d | 15.66c | 9.95 b | 47.60 d |
| **T3** | 2.10 a | 68.52 b | 0.53 b | 17.29b | 0.57 c | 18.60b | 12.08 a | 53.34 c |
| **T4** | 1.75 b | 58.03 c | 0.55 b | 18.24b | 0.60 b | 19.90b | 10.06 b | 54.12 b |
| **T5** | 2.12 a | 75.82 a | 0.61 a | 21.82a | 0.65 a | 23.25a | 12.19 a | 58.19 a |

For each plant, the means of each criterion followed by the different letters within each column are significantly different using Duncan's Multiple Range Test at *P*-value of ≤0.05.
T1. Control; T2. Chemical fertilizers "super phosphate +potassium sulfate"; T3. Chemical fertilizers "super phosphate + potassium sulfate"+ inoculation with bacterial; T4. Natural fertilizers "rock phosphate + feldspar"; T5. Natural fertilizers "rock phosphate + feldspar"+ inoculation with bacterial strains.

Referring to wheat, application natural fertilizers with biofertilizers gave the highest uptake for nitrogen, phosphorous and potassium uptake by (68.43, 146.83 and 106.67%) respectively, over control. While inoculated treatments recorded insignificant differences in protein values. Values increased by 34.64% for carbohydrate compared with control.

**VII. N, P and K availability in soil post-harvest of maize and wheat**

Results presented in (Table 5) cleared those applications of natural fertilizers (rock phosphate & feldspar) consistently increased available P and K nutrients in the rhizosphere over the control in both crops. Application of rocks with bacterial strains increased available P and K in the soil post-harvest of the maize by (64.54 and 27.99%), respectively compares with control. On other hand, sole application rocks to maize (as the first crop) enhanced the availability of P by (49.65%) and K by (16.44%) in the soil post-harvest of the wheat (as the second crop). Concerning nitrogen availability, it did not affect by different applications either chemical or natural with or without inoculation.

**Table 5:** Available nutrients in the soil postharvest of maize and wheat grown in a sequence as influenced by mineral fertilizers (chemical and natural) with or without biofertilizers.

| Treatments | After harvest maize plant | | |
|---|---|---|---|
| | **Nitrogen (ppm)** | **Phosphorous (ppm)** | **Potassium (ppm)** |
| **T1** | 44.50 a | 10.18 e | 368.77 e |
| **T2** | 41.50 c | 14.50 c | 432.57 c |
| **T3** | 35.00 e | 12.30 d | 395.40 d |
| **T4** | 43.47 b | 15.03 b | 448.50 b |
| **T5** | 36.50 d | 16.75 a | 472.00 a |
| | **After harvest wheat plant** | | |
| **T1** | 42.75 a | 8.56 e | 320.35 e |
| **T2** | 41.50 b | 11.08 c | 337.00 c |
| **T3** | 33.60 c | 10.00 d | 330.50 d |
| **T4** | 43.50 a | 12.81 a | 373.00 a |
| **T5** | 31.50 d | 12.15 b | 368.80 b |

For each plant, the means of each criterion followed by the different letters within each column are significantly different using Duncan's Multiple Range Test at $P$-value of $\leq$.0.05.

T1: Control ; T2. Chemical fertilizers "super phosphate +potassium sulfate"; T3. Chemical fertilizers "super phosphate + potassium sulfate"+ inoculation with bacterial; T4. Natural fertilizers "rock phosphate + feldspar"; T5. Natural fertilizers "rock phosphate + feldspar"+ inoculation with bacterial strains.

## 4. Discussion

Our data indicate that each of *E. cloaceae* and *P. polymyxa* has a strong inhibitory effect against the linear growth of *B. sorokiniana* and *F. verticillioides* pathogens. These results are compatible with (Zhang *et al.,* 2018) who reported that *P. polymyxa* ShX301, among five isolated strains, showed the highest antagonistic activity against spore germination and mycelial growth of *Verticillium dahliae*, a causal agent of cotton wilt disease. Previous data showed different antagonistic activity, ranging from 75.00 to 59.52%, against five tested fungal pathogens, including *Sclerotinia sclerotiorum* ZH25, *Fusarium oxysporum* f. sp. *niveum* ZH4, *Botrytis cinerea* ZH256, *Colletotrichum truncatum* ZH26, *Rhizoctonia solani* (AG 1-1A) HZ36 and *Phytophthora capsici* ZH56 (A2 mating type), causal agents of different diseases in crops and vegetables. Additionally, several biological studies showed the antifungal activity of *E. cloacae* isolates against the growth of different soil-borne phytopathogens such as *Pythium ultimum*, *Fusarium moniliforme* and *Fusarium oxysporum* (Hinton and Bacon, 1995 and Suárez-Estrella *et al.,* 2007).

Generally, it can be concluded that the superiority may be due to the fast effect of NPK in chemical form for plant growth (Kabesh *et al.,* 2009). What is more, RP and K materials (alternative natural mineral fertilizers) are cheaper sources of P and K; however, most of them are not readily available to a plant because the minerals are released slowly and their use as fertilizer often causes insignificant yield increases of current crop (Zapata and Roy, 2004), so such natural mineral fertilizers are more useful for wheat, as a later crop. So, it is necessary to use biofertilizers. *Enterobacter cloacae* have phosphate and potassium solubilizing properties (El-saied *et al.,* 2020). Also, it is known to be biocontrol agent (Kazerooni *et al.,* 2020). So, affect accelerate growth parameters, yield criteria, nutrient concentration. In addition, reduces disease incidence. *Paenibacillus polymyxa* on the other side considered bio-controlling agents (Katare *et al.,* 2020) and is regarded as free-living nitrogen-fixing bacteria; therefore it was subjected to nitrogenase activity test to ensure its ability to fix atmospheric nitrogen. The positive nitrogenase activity of *Paenibacillus polymyxa* is in line and supported by the previous studies on such bacterium and thus, such strain might play an important role in protein biosynthesis, either by direct nitrogen supply (through fixation of nitrogen) or indirectly by the accumulation of nitrite and its subsequent increased the plant yield (Hussein and Arafa, 2009 and Yazdani *et al.,* 2009). It was found that there is no antagonistic activity between *Paenibacillus polymyxa* and *Enterobacter cloaceae* (El-saied *et al.,* 2020) and this is attributed to they did not produce antibacterial metabolites (Glick *et al.,* 1999). Therefore, both bacteria could be used together as a single complimentary formula.

Bio-inoculation with chemical mineral fertilizers recorded the best results for maize. These results agreed with Borham *et al.* (2017) on wheat and Yassen *et al.* (2019) on maize plants. On other hand, the application of biofertilizers in conjunction with natural mineral fertilizers (RP and RK) to maize improved significantly the growth parameters of wheat as a second crop at the vegetative growth stage (60 days). The inoculation with biofertilizers through insoluble rock phosphate enhanced plant vigor and nutrient uptake and caused a dramatic increase in plant growth of wheat crop (Shams El-Deen *et al.,* 2020). This may be due to the ability of bacteria to produce hormones, especially IAA (Sheng and Huang, 2001). In these connections, *P. polymyxa* were correlated with its nitrogen fixation, soil phosphorus solubilization and producing plant growth regulating hormones such as indole-acetic acid and cytokinins (Spaepen *et al.,* 2007 & Lal and Tabacchioni, 2009). Phosphorus and nitrogen are known to play an important role in the molecular structure of nucleic acids, DNA and RNA resulting in increased protein synthesis and protoplasm formation with increasing in vegetative growth (El-Shanshoury, 1995). This is attributed to creating favorable conditions for bacteria for the root system to absorb and translocate water and nutrients to the green parts of the plant and promoting photosynthetic activities that result in denser vegetative growth (Zaki *et al.,* 2019).

Bio-inoculation with chemical mineral fertilizers recorded favored higher accumulation of chlorophyll a, b and carotenoid contents of maize that matched with previous findings (Borham *et al.,* 2017) on wheat and (Yassen *et al.,* 2019) on maize plants. On other hand, synergetic effects of biofertilizers and natural alternative fertilizers matched with (Shaaban *et al.,* 2015b) on the wheat plant. Bacterial inoculation reduced chlorophyll loss and stimulated synthesis of chlorophyll through encourages pyridoxal enzymes formation that plays an vital role in α-amino levulinic acid synthetase as a primary compound in chlorophyll synthesis (Ramadan *et al.,* 2003) or promotion of cytokinins (Gaballah, 1995). By increasing absorption and translocation of essential metal ions by bacterial inoculation, which leads to acceleration of metabolic rates related to the synthesis of these constituents (Costa-Santos *et al.,* 2021).

Inoculation tests showed that chemical fertilizers or natural alternative mineral with dual bacterial strains were able to delay foliage symptoms and significantly reduce both disease incidence (DI) and severity (DS) of black point symptoms on maize plants. Data also showed a significant reduction in seedlings mortality percentages, black point grains and black point seeds/spike characters of wheat seedlings. Previous studies indicated that *P. polymyxa* and *E. cloaceae* have the broad-spectrum antagonistic activity related to the antimicrobial compounds secreted by such bacteria, which effectively managed plant diseases caused by fungi, bacteria and nematodes (Timmusk *et al.,* 2009; Weselowski *et al.,* 2016 and Zhang *et al.,* 2018). The results were parallel to Zhang *et al.* (2018) who reported the ability of *P. polymyxa* ShX301strain to decrease the incidence of cotton wilt disease under naturally infected soil by *Verticillium dahliae.*

Several isolates of *Enterobacter cloacae* are known to be bio-control agents for different rots and pre-emergence damping-off of pea, beet, cotton, and cucumber plants incited by *Pythium* spp., as well as of *Fusarium* wilt of cucumber, spinach and some other plant diseases caused by fungal pathogens (Tsuda *et al.,* 2001 and Kazerooni *et al.,* 2020). The antifungal activity of *E. cloacae* against the growth of *Pythium* sp. and *Rhizoctonia solani* may be related to the possible production of volatile compounds such as ammonia (Howell *et al.,* 1988). *P. polymyxa*, has long been known for its ability to produce peptide antibiotics resistant, such as polymyxins, polypeptins, gavaserin, saltavalin, and jolipeptin against bacteria and a series of LI-F antibiotics, gatavalin, and fusaricidins against fungi, Gram-positive bacteria and actinomycetes (Deng *et al.,* 2011 & Lal and Tabacchioni, 2009). Additionally, Kavitha *et al.* (2005) purified a 37-kDa protein from culture filtrates of *Bacillus polymyxa* strain VLB-16 inhibiting mycelial growth of *Pyricularia grisea* and *Rhizoctonia solani*. Similar result was obtained by Deng *et al.* (2011), by a purified 71.9-kDa protein of *P. polymyxa* JSa-9 that exhibiting a broad range of antimicrobial activity against several bacterial and fungal pathogens. Rybakova *et al.* (2016) reported the ability of *P. polymyxa Sb3-1* to effectively suppress the growth of *Verticillium longisporum* directly, and via its volatiles, and some of these antimicrobial volatiles have been identified as 2-nonanone and 3-hydroxy-2-butan (Rybakova *et al.,* 2017).

Data of the current results matched with Yazdani *et al.* (2009), who stated that application of biofertilizers in combination with chemical fertilizer (NPK) gradually improved yield of maize as compared with mineral fertilizers only. Synergetic effects of biofertilizers and chemical fertilizers have also been reported by Borham *et al.* (2017) on wheat Yassen *et al.* (2019) and Nyaera *et al.,* (2019) on maize plants. These increases in yield parameters may be due to an increase in the solubilization of chemical fertilizers, therefore, stimulate the growth and absorption of minerals by plants (Park *et al.,* 2003). As for wheat, consensus effects of biofertilizers and alternative fertilizers have also been matched with the work on some plant species such as potato (El-Sayed *et al.,* 2014) and table beet El-Sayed *et al.,* 2018), wheat (Shams El-Deen *et al.,* 2020). Furthermore, it can be attributed to the increase in nitrogen fixation by bacterial inoculation that improves vegetative growth and finally the yield of plants (Vessey, 2003 and Premsekhar and Rajashree, 2009). Also, production of plant growth hormones by the bacteria and effect root growth and extension positively, leading to more absorption of nutrients, which reflect more growth through nitrogen compounds assimilation, forming growth substances that increase cell division and tissues enlargement, finally, affects the formation of the organs, which was reflected as a high yield production (El-Khawas, 1990).

The current results matched with these of Yassen *et al.* (2019) who reported that phosphorous and potassium content of maize plants gave the highest data with conjunction application of, soluble fertilizer with bacterial strains. Hence, their uptake increased. Regarding wheat, alternative natural fertilizers as a residual with dual strains gave the highest contents from phosphorous and potassium, therefore, lead to the highest P and K uptake. These results agreed with El-Sayed *et al.* (2018) on table beet and Shams El-Deen *et al.* (2020) on wheat. Higher nutrient uptake might be related to higher biomass yield, inoculation with PK solubilizing bacteria produced a beneficial effect on the growth of different plants (Xiao *et al.,* 2017 and El-Saied *et al.,* 2020). Bacterial strains can provide an alternative technology to make K and P available for uptake by plants. In our case, co-inoculation of *E. cloacae* synergistically solubilized RP, which were added into the soil and make them much more available for uptake by the plant (Mahfouze and Sharaf–Eldin, 2007) found that *E. cloacae* as phosphorus solvent bacteria can produce organic acids that increasing solubility and availability of phosphorus to plant. Moreover, it has a considerable role in proving K compounds to plant by storing K in their biomass (a significant quantity of fixed K), which is potentially available to plants (Jones *et al.,* 2003). It has been reported that the production of various extracellular polymers (primarily proteins and polysaccharides) can also be led to the release of K from K-bearing minerals for plant uptake (Shelobolina *et al.,* 2012). Increasing N uptake may be related to the Paenibacillus strain used in this study that might have the capacity to fix atmospheric nitrogen, solubilize the complex phosphate, synthesis of growth hormones and production of antibiotics (Radhakrishnan *et al.,* 2017).

Enhanced total carbohydrate of maize has been matched with previous findings Yassen *et al.* (2019) on maize plants and El-Saied *et al.* (2020) on fennel. On other hand, synergetic effects of biofertilizers and natural alternative fertilizers matched with Shaaban *et al.,* (2015b) on the wheat plant. The importance of biofertilizers in increasing the percentages of total carbohydrate may be due to the role of these biofertilizers on the enzymatic systems responsible for the biosynthesis of these compounds (Hassan, 2009). The results suggest that the synthesis of photosynthetic pigments in leaves is an induced factor for carbohydrate synthesis (Kahil *et al.,* 2017).

Our results demonstrated that the synergistic effects of co-inoculation of PK solubilizing bacteria integrated with the direct application of PK rocks regards to P and K availability after first crop. On other hand, PK rocks treated soil increased both P and K availability in the rhizosphere after second crop. Synergetic effects of biofertilizers and natural alternative fertilizers agreed with Han *et al.* (2006) on pepper and cucumber plants; El-Sayed *et al.* (2018) on table beet and Shams El-Deen *et al.,* (2020) on wheat. A synergistic effect could occur between the co-inoculation of phosphorus and potassium solubilizing bacteria from one side and the direct application of P and K rocks on the other side because these rocks are solubilized slowly (Han *et al.,* 2006) and the presence of bacterial inoculation improve P and K availability in soils by producing organic acids and other chemicals, which stimulate growth and mineral uptake by plants (Park *et al.,* 2003 and Mardad *et al.,* 2013).

## 5. Conclusion

Combining two bacterial strains (*E. cloacae and P. polymyxa*) with classical soluble fertilizers enhanced growth, yield parameters and chemical composition of the first test crop (maize).While dual inoculation combined with natural alternative fertilizers significantly increased growth and productivity of the second crop (wheat) and has positive effect on available nutrient post-harvest of two crops. In addition, the ability of two bacterial strains as safe, environment-friendly and effective means to fight *B. sorokiniana*, the causal agent of black point in wheat and *F. verticillioides*, the causal agent of ear rot in maize plant. So, dual Inoculation of both bacteria is providing a step forward toward sustainable agriculture and lowering excessive use of synthetic fungicides.

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