Egypt. J. Phytopathol., Vol. **46**, No. **1**, pp. 235-255 (2018)

Controlling Maize Late-Wilt and Enhancing Plant Salinity Tolerance by Some Rhizobacterial Strains

El-Shabrawy, E.M.[*], Heba Shehata Sh.[**]
[*] *Plant Pathology Research Institute, A.R.C.*
[**] *Soil, Water and Environment Research Institute, A.R.C.*

The effects of plant growth promoting rhizobacteria (PGPR) on decreasing maize late wilt disease caused by *Cephalosporium maydis*, enhancing plant tolerance to salinity stress and plant growth parameters were studied. *In vitro*, four out of the seven *Pseudomonas fluorescens* isolates and three out of nine isolates of *Bacillus subtilis* showed the potency to cease the growth of all target pathogens. Significant reduction in disease incidence was recorded under greenhouse and field conditions in seeds treated by PGPR strains either individually or in combination. PGPR seed coated treatments carried on biochar were the best which reduced the disease to 72% in normal soil and 70% in saline soil under greenhouse conditions, whereas, it gave 82.8% disease reduction in normal soil and 79.3% in saline soil in the field. This treatment promoted seed germination percentage, plant height, dry weight and chlorophyll levels more than other treatments. Also, seed treatments with rhizobacteria decreased the proline induction compared with untreated plants in normal and saline soil due to decrease of salinity stress by PGPR especially that carried on biochar. Under field conditions in normal and saline soil, rhizobacteria seed coating carried on biochar either alone or in combination significantly decreased disease infection and enhanced the plant yield compared with control.

Keywords: *Bacillus subtilis*, biochar, *Cephalosporium maydis,* Maize and rhizobacteria

Maize (*Zea mays* L.) is considered as one of the most important cereal crops in Egypt. Late wilt, caused by *Cephalosporium maydis* Samra, Sabet & Hingorani, is one of the most economical diseases of maize in Egypt; serious economic losses from late wilt have been reported in Egypt where 100% infection occurs in some fields (Samra *et al*., 1962). Furthermore, the yield losses reached up to 40% (El-Shafey and Claflin, 1999). It is well known that maize is highly sensitive to salinity which reduces crop productivity by 6-19%. In general, biochemical, physiological, morphological and anatomical characteristics of crop species is directly affected by soil salinity (Ashraf, 2004; Ashraf & Harris, 2004; Chinnusamy *et al*., 2005; Parida and Das, 2005). There are many reports which show that salinity induces water deficit in many crop species such as corn, sunflower, potato and soybean (Katerji *et al*., 1998; Katerji *et al*., 2004). A primary response in salt stressed plants is a decrease in plant water potential, resulting in decreased water use efficiency, leading to the overall toxic damages and yield reduction (El-Hendawy *et al.*, 2005; Mansour *et al*., 2005).

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots (Ahmad *et al*., 2008). The enhancement of crop plant growth using PGPR is documented (Reed and Glick, 2004) and these organisms have been used to reduce plant stress associated with phytoremediation strategies for metal contaminated soils (Reed and Glick, 2005). PGPR enhance plant growth through reducing ethylene production, allowing plants to develop longer root better establish during early stages of growth (Glick *et al.,*1998); (ii) enhancing a symbiotic nitrogen fixation (Khan, 2005) or indirectly affecting symbiotic N2 fixation, nodulation or nodule occupancy (Fuhrmann and Wollum,1989); (iii) producing or changing the concentration of plant growth regulators like indole acetic acid (IAA) (Ahmad *et al*., 2008); (iv) raising the solubilisation of nutrients with consequent increase in the supply of bio-available phosophorous and other trace elements for plant uptake (Glick, 1995); (v) production of phytohormones such as auxins, cytokinins and gibberellins (Glick, 1995); and (vi) synthesis of antibiotic and other pathogen-depressing substances such as siderophores, cyanide and chelating agents that protect plants from diseases (Kamnev and Lelie, 2000). These organisms can also increase plant tolerance to flooding (Grichko and Glick, 2001), salt stress (Mayak *et al.,* 2004a) and water deprivation (Mayak *et al*., 2004b).

Biochar defined as a product of thermal degradation of organic based feedstock, through the process of pyrolysis, which requires elevated temperature and the absence of oxygen (Lehmann and Joseph, 2009). Biochar is using as a carrier for beneficial microorganisms and applying it as a biofertilizer or controlling soil borne diseases. Furthermore, biochar is renewable, locally available and inexpensive; have good water holding capacity, good aeration properties, and sustain growth and survival of bacteria over time. Additionally, it is non-toxic, environmentally friendly, easily manufactured, sterilized and manageable in the field (Khavazi *et al*., 2007). Moreover, it is simply converted to powder, mixable and packageable and able to adhere to the seed. Also, growing interest in biochar is associated with its carbon sequestration ability. Biochar is a promising means of reducing the atmospheric CO_2 concentration (Xu *et al.*, 2012). Furthermore, biochar has gained much attention as a soil amendment (Lehmann *et al.,* 2011). Several studies provide evidence that biochar-amended soils have an impact on plant resistance to pathogens. Matsubara *et al.* (2002) demonstrated that charcoal amendments had a suppressive effect on the soil borne pathogen *Fusarium* sp. They found that charcoal produced from coconut fiber suppressed Fusarium crown and root rot, and increased arbuscular mycorrhizal (AM) colonization of asparagus seedlings.

The aim of this work was to assess the effect of plant growth promoting rhizobacteria (PGPR) as seed treatments carrying on biochar on *Cephalosporium maydis* the causal organism of late wilt disease, salinity stress and the growth parameters of *Z. mays* in *vivo* and in *vitro*

Materials and Methods

Fungal and rhisobacterial isolates:

The stalk-rot complex pathogens of maize; *Fusarium oxysporum, F.verticillioides*, *Rhizoctonia solani*, *Cephalosporium maydis*, *Macrophomina phasiolina* and *Acremonium strictum* were obtained from culture type collection of Maize and Sugar Crops Dis. Dept., Plant Pathol. Res. Inst., ARC, Giza. Rhizosphere-rhizoplane colonizing PGPR (*Pseudomonas fluorescens* and *Bacillus subtilis*) were isolated from rhizosphere of maize plants at seedling and mature growth stages grown in the fields. Samples were taken from plants grown in saline soil of the fields of Agricultural Experiment Station at Sahl El-Hossainiya (Sharkiya) governorate and Sakha (Kafr El-Sheikh) governorate and a field located at El-Fayum governorate. Isolation of these strains of bacteria was done according to the method described by Meera and Balabaskar (2012). To identify the bacterial isolates, certain biochemical tests were conducted according to Breed *et al*. (1957). On the basis of colony morphology and fluorescence under UV (365nm), many colonies were randomly selected and further purified by streaking on King`s B medium (KB). Purified isolates were kept on original (KB) slants at 2^0C and Nutrient agar for *Bacillus subtilis* isolates.

In vitro antifungal activity of bacterial strains:

The antifungal activity of rhizobacterial isolates was evaluated against five pathogens involved in the stalk-rot complex of maize on PDA + 0.2% yeast extract (PDAY) medium by using the dual culture technique described by Dennis and Webster (1971). Bacterial isolates were streaked at one side of Petri dishes 9-cm-diam.containing 15-20 ml of PDAY medium. Discs (6-mm-diam.), taken from 7-day-old cultures of *F. oxysporum, F. verticillioides*, *R. solani*, *C. maydis*, *M. phasiolina* and *A. strictum* were placed at the opposite side of Petri dishes perpendicular to the bacterial streak and incubated at 27+2 ºC for 7 days. Four replicate plates were prepared for each isolate. Petri dishes inoculated with fungal discs alone served as control. The inhibition zone was measured as the distance between the fungal colony margin and the area of antagonist growth after 7 days. Inhibition zones or cessation of the fungal growth were used as criteria for the positive effect as follows: (-): no inhibition zone, (+) = ≤ 5 mm inhibition zone; (++) = 5 to 10 mm inhibition zone; (+++) = ≥ 10mm inhibition zone and (++++): no fungal growth and bacteria fill the plate.

Production of antifungal metabolites:

Various metabolites produced by the antagonistic bacteria used in the present study were estimated. The antagonistic bacteria were *P. fluorescens* (PSR-2) and *B. subtilis* (BSR-9). Siderophores were semi quantified using chrome azurole S (CAS) medium following the method of Schwyn and Neilands (1987). Production of hydrogen cyanide (HCN) was detected as described by Castric and Castric (1983). Chitinase activity was assayed by the method described by Renwick *et al.* (1991).

Greenhouse experiment:

These experiments were carried out for studying the effect of PGPR on controlling maize late wilt, the target pathogen in stalk rot complex; also the plant growth parameters were studied. The experiment was done under greenhouse conditions at the Maize and Sugar Crops Dis. Dept., Plant Pathol. Res. Inst., ARC, Giza, following the soil infestation technique as described by Samra *et al*. (1966), during summer of 2017 growing season. Autoclaved clay loam soil (normal soil) and saline soil-brought from a field of Sahl El-Hossainiya were used. Maize seeds (cv. Boushy), susceptible to *C. Maydis* was used.

Inoculum preparation:

Fungal growth for inoculation was prepared by growing *C. maydis* isolate on autoclaved sorghum grains in 500 ml glass bottles and incubation at 27 ± 2ºC for 2 weeks until sufficient growth of the fungus was obtained (El-Shafey *et al.*, 1979). Contents of the bottles were poured out and mixed to get homogenized inoculum, and then inoculua were used for soil infestation at the rate of 30g /kg soil. Concerning preparation of rhizobacterial inocula, two full loops of highly antagonistic *B. Subtilis* and/or *P. fluorescens* isolates from 3-day-old cultures grown on nutrient agar and/or King's B media, respectively, were transferred aseptically into conical flasks (250 ml) containing 100 ml of broth medium and incubated at 27± 2ºC for 48h on a rotary shaker (150 rev min^{-1}). Bacterial cells were removed by centrifugation at 10000 rpm for 10 min at 4ºC. The pellet was re-suspended in small quantity of sterile distilled water. Serial decimal dilutions were done to obtain 10^8cfu/ml for each isolate (Cavaglieri *et al.,* 2005).

Preparation the mixture of PGPR strains:

Mixture of equal volumes from each rizhobacterial culture (*P. fluorescens* and *B. subtilis*) at log phase (10^8cfu/ml) was prepared for seed treatment before planting in greenhouse and field experiments.

Seed and soil treatment methods:

Seed soaking technique:

Maize seeds were soaked in the prepared bacterial suspension at the rate of 100 seed /100 ml in 250 ml Erlenmeyer flasks. Control seeds were soaked in sterile distilled water. Flasks were incubated at 25ºC on a rotator shaker at (70 rpm) for 6 h. After incubation, excess inoculums were removed and seeds were left to air dry for 30 min. at room temperature and immediately planted in the infested-potted soil (Cavaglieri *et al.,* 2005).

Seed coating technique:

Seed coating was done using the method described by Bardin *et al.* (2004). Seeds were soaked for 15min. in 1% methyl cellulose (MC) solution at the rate of 3ml per 100 seeds. Thereafter, seeds were removed and placed in plastic bags then mixed with bacterial suspension at the rate of 5 ml per 100 seeds. Bags were inflated with air and shaken vigorously. Seeds were directly planted in the potted soil. Seeds coated with sterile distilled water acted as control.

Soil drench technique:

Soil drenching was carried out according to Jenana *et al.* (2009) and modified as follows: potted soil was thoroughly drenched at sowing time with 100 ml of prepared solution from bacterial suspension. Soil drenching was weekly repeated for two times starting from the planting time. Control soil was drenched by sterile distilled water.

Biochar preparation from rice straw:

Rice straw was cut using milling machine to a diameter about 1mm. After that, the sample was dried in a vacuum oven at 80 ^{o}C for 24 hours. Slow pyrolysis experiments of rice straw were carried out at 300 ^{o}C for 4 hour (El-Adely *et al.,* 2015). The physicochemical properties of biochar were determined at Soils, Water and Environment Research Institute – Giza where, pH (7.0), carbon (63.8%), N/P/K (2.55%, 0.65 and 1.18, respectively), cation exchange capacity (CEC) 32.15 coml /kg and ash 16.3.

Application with PGPR:

Greenhouse experiment:

P. fluorescens and *B. subtilis* isolates were applied in four methods, *i.e* seed soaking, seed coating, soil drench and seed coating with carrier biochar either individually or in combination. All treatments were done in normal soil as well as saline soil. Eight treated seeds were sown in each pot (25-cm-diam.). Untreated seeds were used as control in infested soil normal and/or saline. Three pots were used as replicates for each treatment. Pots were distributed in greenhouse under ambient conditions. Disease incidence and vegetative growth were recorded 95 days after sowing. Meanwhile, seed germination percentage was recorded 10 days after sowing and determinations of chlorophyll and proline were done at seedling stage.

Analysis of plant growth parameters:

Germination (%), plant height (cm), fresh and dry weight (g/plant), proline (ppm/g Fw) and chlorophyll (mg/g Fw) were determined. Seed germination percentage was recorded 10 days after sowing; meanwhile, samples used for determination of chlorophyll and proline were obtained at seedling stage. After three months of planting in greenhouse, all treated plants in each of the four pots were separately uprooted, thoroughly washed under running tap water to remove soil particles from roots. The morphological characteristics were measured and dry weight was determined after air-drying the fresh material for five days.

Total chlorophyll and proline content determinations:

The total chlorophyll content was determined in fresh leaves using the method described by Saric *et al.,* (1976). Proline content in fresh root tissue was determined spectrophotometrically following the ninhydrin method described by Bates *et al.,* (1973) using L-proline as a standard.

Field experiment:

A local maize variety Boushy, sensitive to infection with late-wilt disease, was chosen for the experiment. PGPR treatments, as mentioned in greenhouse experiment, except soil drench treatment were studied under field conditions in disease nursery at Gemmiza Agricultural Experiment Station, Gharbiya, Egypt, and

Agricultural Experiment Station at Sahl El-Hossainiya (Sharkiya) during summer of 2018. Untreated seeds were used as control. Three replicate plots, each of 20 plants were used for each treatment. Disease incidence as infection percentage was recorded 95 days after sowing. Maize grain yield parameters were evaluated during harvest period.

Statistical analyses:

Data were subjected to statistical analysis, according to the method described by Steel and Torrie (1990), whereas the differences between treatments were tested by calculating L.S.D. at 5% level.

Results

Isolation and identification of Rhizobacterial strains:

Plant growth promoting rhizobacteria (PGPR) isolation was done from rhizosphere of maize plants, these isolates were subjected to identification using morphological and biochemical characterization and fluorescence under UV (365nm). Identification process indicated that a number of seven isolates of *Pseudomonas fluorescens* and nine isolates of *Bacillus subtilis* were isolated from maize plants rhizosphere. Isolates were further tested for their antifungal activities. The biochemical tests were performed for further confirming the identification of the effective native isolates of *P. fluorescens* and *B. subtilis*. All isolates of *P. fluorescens* produced similar results with regard to Gram staining (negative) and starch hydrolysis (negative). Also, they were positive for gelatin liquefaction, catalase test, oxidase test and fluorescent pigmentation. On the other hand, it was found that all *B. subtilis* isolates were positive for gram staining, spore forming, starch hydrolysis, catalase test and citrate test. Meanwhile, they were negative for oxidase test and nitrate reduction test.

Antagonistic effect of rhizobacterial strains against some soil fungi:

Results, in Table 1 indicate that most of the tested *Pseudomonas* isolates efficiently inhibited the radial growth of the virulent maize pathogens tested. It was observed that *B. subtilis* (BFR1, BFR2, BSR8) filled the plate during 48h of incubation at 27±2 ˚C, giving 100% growth inhibition against *C. maydis* followed by *B. subtilis* (BFR3, BSR9). *P. fluorescens* (PSR3, PSR2) recorded 100% inhibition against *C .maydis*. It was observed that seven antagonistic organisms; namely (BFR-2, BFR-3, BSR-8, PFR-9, PSR-1, PSR-2 and PSR-3) showed the potency to cease the growth of all target pathogens. On the other hand, isolate BKR-6 was unable to affect any of the tested pathogens (*F. oxysporum, F. verticillioides*, *R. solani*, *M. phasiolina*, *C. maydis*, and *A. strictum*) involved in the stalk-rot complex.

Table 1. *In vitro* antagonistic effects of *P. fluorescens* and *B. subtilis* against some soil fungi on PDAY medium

No.	Rhizobacterial strains	Antagonistic effects against pathogens					
		R. solani	***M. phasiolina***	***F. oxysporium***	***F. verticillioides***	***C. maydis***	***A. streactum***
BFR-1	*B. subtilis*	+	+	-	-	++++	+
BFR-2	*B. subtilis*	++	+	+++	++	++++	++++
BFR-3	*B. subtilis*	-	-	+	++	+++	-
BKR-4	*B. subtilis*	-	-	+	+	++	+
BKR-5	*B. subtilis*	-	-	-	-	+	-
BKR-6	*B. subtilis*	-	-	-	-	-	-
BSR-7	*B. subtilis*	++	+	-	-	+	+
BSR-8	*B. subtilis*	++	+	++	+++	++++	+++
BSR-9	*B. subtilis*	+++	+	++	+++	+++	+++
PFR-1	*P. fluorescens*	++	+	+	+	+++	+++
PSR-2	*P. fluorescens*	+	-	+	+++	++++	+++
PSR-3	*P. fluorescens*	-	+	+	+	+++	++
PSR-4	*P. fluorescens*	-	-	-	++	+	-
PKR-5	*P. aurgenosa*	-	-	-	+	+	+
PKS-6	*P. mendocin*	-	-	+	+	+	-
PFS-7	*P. putida*	-	-	-	+	+	+

Culture assay: + represents 0-5 mm, ++ represents 5-10 mm, +++ represents 10-15 mm, ++++ represents >15 mm wide zones.

Antifungal metabolites produced by rhizobacterial strains:

P. fluorescens (PSR2) had the ability to produce siderophore and chitinase better than *B. subtilis* isolate (BFR-9) (Table, 2). It produced same levels from hydrogen cyanide (HCN) and B-1, 3-gluecanase as *B. subtilis.*

Table 2. Antifungal metabolites produced by rhizobacterial strains

Treatment	Siderophore	HCN	Chitinase	*β*-1,3-gluocanase
P. florescence (PSR2)	+++	++	++	+
B. subtitles (BFR-9)	++	++	+	+

Greenhouse experiment:

A highly antagonistic rhizobacterial strains *i.e.* (*B. subtilis* BSR-9 and PSR-2) previously tested for it antagonistic effect against maize stalk rot complex pathogens were used against *C. maydis* the causal organism of maize late wilt, also the main component of stalk-rot complex in the greenhouse experiment. Results in Table 3 show that, seed treated with rhizobacterial strains significantly reduced the infection percentage with late wilt compared to control treatment, the infection percentage in normal soil was lower than saline soil. Generally *P. fluorescens* was highest in reducing infection percentage than *B. subtilis*. Also, treatment of rhizobacterial strains together gave high disease reduction percentage in both normal and saline

soils. It was observed that the best method for treating seeds with rhizobacteria to control infection was seed coating plus biochar as a carrier. Meanwhile, soil drench was the lowest effective method in this respect. Data in Table 3 also reveal that seeds coated with biochar as a carrier of both rhizobacterial strains inocula scored the highest reduction in disease incidence, being 72%, followed by *P. fluorescens* carried on biochar application reached to 66.7% in normal soil. Whereas, in saline soil, maize seeds coated with biochar mixed with the two bacteria tested gave 70% reduction in infection followed by biochar application with *P. fluorescens* 65%. On the other hand, seeds soaked in both bacteria came after seed coating treatment in reducing the infection where the highest reduction was 55.5% in normal soil with *P. fluorescens* treatment followed by 47.5% reduction in saline soil. Soil drenching treatment was the lowest effective method for controlling late wilt disease, whereas, it never gave more than 45% reduction.

Table 3. Effect of two rhizobacterial strains tested on maize late wilt in normal and saline soils under greenhouse conditions during 2016 growing season

Treatment	Late wilt Infection (%)				Reduction (%)			
	Seed soaking	Seed coating	Soil drench	Inocula Carried on Biochar	Seed soaking	Seed coating	Soil drench	Inocula Carried on Biochar
Normal soil								
P. fluorescens	28.6	25.0	35.7	21.4	55.5	61.1	44.5	66.7
B. subtilis	35.7	28.6	39.3	28.6	44.5	55.5	38.9	55.5
P+B*	32.2	21.4	36	17.9	49.9	66.7	44	72
Control	64.3	64.3	64.3	64.3	-	-	-	-
Saline soil								
P. fluorescens	37.5	28.6	39.3	25	47.5	60	45	65
B. subtilis	42.9	32.1	44.6	28.6	40	55	37.5	60
P+B	39.3	28.6	40.4	21.4	45	60	43.4	70
Control	71.4	71.4	71.4	71.4	-	-	-	-
LSD 0.05%	Treat. = 0.536			Methods = 0.519		TxM = 1.467		

P+B* (*P. fluorescens* + *B. subtilis*)

Effect of two rhizobacterial strains tested on some plant growth parameters of maize:

Data illustrated in Table 4 show that, seed treatments with rhizobacterial strains significantly promoted plant growth compared to control either in normal or saline soil. No significant differences were found among treatments and/or methods of application in their effects on seed germination percentages, whether in normal or saline soil. But, the percentage of seed germination was higher in seed coated with biochar inocula and seed coated only with the two rhizobacteria tested either alone

or together where it reached 96.3% in normal soil and 92.6% in saline soil compared with the control 70.4 and 66.7%. All treatments significantly increased plant height comparable to check plants either in normal or in saline soil. There were significant differences between seed treatments carried out using *P. fluorescens* either alone or combined with *B. subtilis* meanwhile, biochar inocula recorded the highest increase in maize plant height measurement in normal soil (107 and 102.7 cm, respectively). Treatment with *B. Subtilis* showed no significant difference between methods of application.

Data in Table 4 reveal that significant increase in dry weight of grown plants was recorded compared with check plants, either in normal or saline soil. Seeds coated with *P. fluorescens* and a mixture of *P. fluorescens* and *B. subtilis* with or without biochar resulted in higher dry weights.

Regarding to proline concentration in plant tissues, data in Table 4 indicated that control plants in normal soil had a higher proline concentration 0.74 ppm/g Fw than plants inoculated with PGPR. In saline soil, the proline levels increased significantly in all treatment, but control plants had the highest concentration of proline (1.87 ppm/g Fw). Treatments of *P. fluorescens* and /or *B. subtilis* with the carrier biochar showed the lowest levels of proline. Also, seed treatments with *P. fluorescens* and/or *B. subtilis* individually achieved low levels of proline more than combination treatments except with the carrier biochar. The highest levels of proline were obtained in soil drench with any of the two tested rhizobacteria either alone or in double combination followed by seed soaking treatment in normal and/or saline soil.

On the other hand, results in Table 4 show that plants grown from seed treated with any of the two tested bacteria had chlorophyll levels significantly higher than control (11 mg/g FW) this was obvious in saline soil. The treatments of mixed PGPR mixer under any method of application gave the highest levels of chlorophyll content followed by *P. fluorescens* in both soils, where PGPR mixed treatment gave 19.1 mg/g Fw followed by seed coating treatment (18.2 mg/g Fw) in normal soil, the same treatments gave 14.7mg/g Fw and 13.7mg/g Fw, respectively in saline soil compared with plant grown in normal soil control (8.17 mg/g Fw).

Effect of rhizobacetrial strains on maize late wilt and yield under field conditions:

Data in Table 5 reveal that seed treatment with rhizobacteria (PGPR) by soaking, coating or carrying on biochar, significantly reduced maize infection with late wilt compared with check plants under normal or saline soil (55.3% infection in normal soil and 60% in saline soil). However, the combined treatment of *P. fluorescens* and *B. subtilis* with biochar gave the highest reduction in late wilt infection, being 82.8% in normal soil and 79.3% in saline soil, followed by seed coating with *P. fluorescens* and/or *B. subtilis*. On the other hand, the lowest treatment in reducing disease incidence was seed soaking in both normal and saline soil, giving 59.7 and 62.8% reduction in infection percentage, respectively.

Table 4. Effect of two rhizobacterial strains on maize plant growth parameters under greenhouse conditions in normal and saline soils during 2016 growing season

Treatment		Germination %		Plant height		Dry weight		Proline ppm/g Fw		Chlorphyll mg/g Fw	
		N. soil	S. soil	N. soil	S. soil	N. soil	S. soil	N. soil	S. soil	S. soil	N. soil
P. florescence	Seed soaking	81.5	74.1	92.0	86.0	26.6	21.3	0.42	0.53	13.2	11.3
	Seed coating	92.6	81.5	100.0	87.0	29.3	24.0	0.27	0.45	15.8	12.2
	Soil drench	85.2	70.4	87.3	87.0	26.2	18.5	0.45	0.55	12.07	8.9
	Biochar inocula	96.3	85.2	102.7	93.7	32.4	27.0	0.17	0.37	16.7	12.8
B. subtilis	Seed soaking	77.8	74.1	90.0	85.0	30.1	21.7	0.48	0.81	11.3	10.1
	Seed coating	96.3	88.9	96.7	90.3	32.2	24.3	0.33	0.56	14.07	10.1
	Soil drench	81.5	77.8	90.3	85.0	28.0	22.0	0.49	0.97	11.2	9.4
	Biochar inocula	96.3	88.9	95.0	92.0	34.1	25.3	0.30	0.54	14.2	12.3
P. florescence + *B. subtilis*	Seed soaking	88.9	85.2	92.7	90.0	29.6	25.7	0.54	0.98	13.3	12.7
	Seed coating	96.3	88.9	102.3	96.7	35.3	26.3	0.46	0.58	18.3	13.7
	Soil drench	85.2	88.9	94.0	88.0	27.3	27.3	0.58	0.98	14.1	12.2
	Biochar inocula	96.3	92.6	107.0	99.0	36.7	29.9	0.43	0.60	19.1	14.8
Biochar		77.8	74.1	89.0	85.0	22.5	18.2	0.70	1.22	13.3	9.1
Control		80.4	68.7	80.0	78.7	18.5	13.3	0.74	1.87	11.00	8.2
L.S.D. 0.05		11.043	10.787	6.386	7.906	8.893	2.898	0.088	0.206	0.838	1.08

Table 5 shows that seed treatments with rhizobacterial strains (PGPR) either alone or in combination significantly improved crop production compared to check plants. Combination of rhizobacterial strains with biochar caused makeable increments in maize yield parameters compared with individual treatments. All treatments significantly increased yield of the grown plants in comparison to check plants (17.8ardb/fed in normal soil and 14.3 ardb/fed in saline soil).

The same trend was observed regarding seed treatment with combination of rhizobacterial strains with biochar, *P. fluorescens* mixed with *B. subtilis* in normal and saline soils produced the highest yield, being 27.8 and 24.3ard/fed, respectively. In contrast, plants treated with *B. subtilis* alone produced the lowest yield (19.5 and 17.6 ardb/fed in normal and saline soils, respectively).

Seeds coated with biochar alone significantly reduced infection compared with control but not significantly increased the yield. There was a significant difference between methods of application under treatments.

Table 5. Effect of two rhizobacetrial strains on maize late wilt and yield under field conditions at Gemmeiza and Sahl Elhsinia during 2016 growing season

Treatment		Gemmeiza (Normal soil)			Sahl Elhsinia (Saline soil)		
		% Inf.	% Red.	Yield ardb/fed	% Inf.	% Red.	Yield ardb/fed
P. florescence	Seed soaking	22.3	59.7	21.8	22.3	62.8	18.4
	Seed coating	13.3	75.9	23.2	17.5	70.8	19.8
	Biochar inocula	12.5	77.4	26.5	15.2	74.7	23.5
B. subtilis	Seed soaking	19.4	64.9	19.5	23.8	60.3	17.6
	Seed coating	16.5	70.2	22.3	19.5	67.5	22.3
	Biochar inocula	14.5	73.8	24.6	16.4	72.7	23.6
P. florescence + B. subtilis	Seed soaking	18.2	67.1	23.5	16.8	72.0	20.5
	Seed coating	10.3	81.4	27.6	12.6	79.0	22.9
	Biochar inocula	9.5	82.8	27.8	12.4	79.3	24.3
Biochar inocula		46	28.6	18.3	45	25.0	16.7
Control		55.3		17.8	60		14.3
L.S.D. 0.05%		3.287		3.892	4.961		4.074

Discussion

The present study was carried out to control maize late wilt disease and evaluate plant salt tolerance using plant growth-promoting bacteria (PGPR) and biochar as a carrier. The PGPR strains *i.e., Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated *in vitro* and *in vivo* to manage maize late wilt caused by *Cephalosporium maydis* and enhancement of maize growth under salinity stress. Four methods of application were carried out *i.e.,* seed soaking, seed coating, soil drench and seed coating with biochar carrier.

The antifungal activity of isolated PGPR were tested against stalk- rot complex pathogens (*F. oxysporum, F. verticillioides*, *R. solani*, *C. maydis*, *M. phasiolina* and *A. strictum*) using dual culture method. Results indicated that four out of the seven *P. fluorescens* isolates and three out of nine isolates of *B.subtilis* showed the potency to cease the growth of all pathogens. Also, it was observed that *B. subtilis* completely inhibited the growth of *F. oxysporum, F. verticillioides*, *C. maydis*, *M. phasiolina* and *A. strictum*. This finding agreed with that suggests by Nalisha *et al.* (2006), Kim *et al.* (2008) reported that *B. subtilis* produces antifungal compounds

and suppressed radial growth of *S. rolfsii*. In fact, antibiosis and competition for space and nutrients are generally the mode of antagonism observed with *Bacillus* species (Edwards *et al.,* 1994). Most *Bacillus* spp. produces many kinds of antibiotics such as bacillomycin, fengycin, mycosubtilin, and zwittermicin, which are effective on suppressing growth of target pathogens *in vitro* and/or *in situ* (Kim *et al.,* 2008). On the other hand, *B. subtilis* grew rapidly and colonized the surface of the medium and suppressed fungal growth. These results are in accordance with the findings of Mavrodi *et al.* (2001) who reported that *P. fluorescens* antagonism is related to mechanisms more diverse than those found for *Bacillus.* Effects are attributed to production of siderophores, volatile compounds, antibiotics (*e.g.,* pyrrolnitrin, tensin, and pyoluterin), cell-wall degrading molecules besides extracellular chitinase, and protease enzymes activity as well as competition and induced systemic resistance.

A greenhouse experiment was conducted to evaluate the influence of a highly antagonistic rhizobacterial strains as well as inoculated biochar on controlling late wilt disease and corn growth characteristics in saline and normal soil. *B. subtilis* BSR-9 and *P. fluorescens* PSR-2, the most antagonistic strains, were used in treating seeds against *C. maydis*. Results indicated that rhizobacterial strains significantly reduced infection percentage in normal and saline soils under any method of application tested compared to control treatment. The reduction in the disease incidence was higher in seed treated with rhizobacterial strains together and seed coating plus biochar as a carrier method followed by *P. fluorescens* carried on biochar in normal and saline soils. This finding agreed with many investigations used biochar as a carrier for beneficial microorganisms (Matsubara *et al.* 2002, Khavazi *et al.*, 2007). It is renewable, locally available and inexpensive; have good water holding capacity, good aeration properties, and sustain growth and survival of bacteria over time. Additionally, the right inoculants carrier should be non-toxic, environmentally friendly, easily manufactured, sterilized and manageable in the field (Khavazi *et al.*, 2007). Moreover, it is important that the carrier be simply converted to powder, mixable and packageable and able to adhere to the seed. It should easily release bacteria into the soil (Smith 1992). Also, growing interest in biochar is associated with its carbon sequestration ability. Biochar is a promising means of reducing the atmospheric CO2 concentration (Xu *et al.,* 2012). Furthermore, biochar has gained much attention as a soil amendment (Lehmann *et al.,* 2011). Several studies provided evidence that biochar-amended soils have an impact on plant resistance to pathogens. Matsubara *et al.* (2002) demonstrated that charcoal amendments had a suppressive effect on the soil borne pathogen *Fusarium* sp. They found that charcoal produced from coconut fiber suppressed Fusarium crown and root rots, and increased arbuscular mycorrhizal (AM) colonization of asparagus seedlings. Also, results indicated that significant reduction in the disease incidence was recorded by seed treatments with rhizobacterial strain *P. fluorescens*, either individually or in combination with *B. subtilis*. Moreover, it was observed that seed coated with biochar as a carrier was the best method for treating seeds with both rhizobacteria. These results are in harmony with those reported by many researchers (El-Assiuty *et al.,* 1991; El-Mehalowy *et al.,* 2004). Similarly, Hamza *et al.* (2013) reported that the formulations of *B. subtilis*, *B. pumilus*, *P. fluorescens* and

Epicoccum nigrum caused noticeably reduction in maize late wilt under field conditions. Ebrahim (2010) found that the most effective antibiotics (DAPG, PLT, PCA and PLN) and several enzymes (lipase and protease) as well as siderophores (pyoverdine and pyochelin) are produced by *P. fluorescens,* which showed antagonistic activity toward *R. solani* and significantly inhibited root infection when coated onto sugar beet seeds. Also, Bacillus-based biological control agents have modes of action that include antibiosis, parasitism, colonization, competition and induced systemic resistance (Jacobsen *et al.,* 2004).

Concerning plant growth parameters under greenhouse conditions, results indicated that seed treatment with rhizobacterial strains especially that carried on biochar promoted growth of maize plants, resulted in high percentage of germination and significantly increased plant height, dry weight, chlorophyll and decreased proline production in comparison to control whether in normal or saline soil. These results are in harmony with those reported by Zeng and Zhang (2010) they reported that seed coating is a technique that might combine several components, such as nutrient elements, fertilizers, plant growth regulators and pesticides at much lower rates than the traditional application, by applying them to seed with adhesive agents to increase seed performance. Also, they found that seed-coating does not inhibit the germination process; on the contrary, plant fresh weight, germination energy, germination percentage, germination index and vigor index of coated seeds were significantly higher than in uncoated seeds. Likewise, Gholami *et al.,* (2009) found that maize seeds coated with gum Arabic as an adhesive and rolled in a suspension of bacteria (PGPR) with perlite significantly increased the seed germination and seedling vigour of maize. In additions seeds treated with a talc-based formulation with PGPRs resulted in better yield than untreated seeds. They reported that plant height, leaf area, seed dry weight, 100-seed weight and shoot dry weight were significantly higher for coated seeds than for controls. Similarly, Alagawadi and Gaur (1988) showed that seeds soaked in liquid culture of rhizobia or *Pseudomonas straiata* produced plants with more dry matter, grain and straw yield of chickpea than untreated seeds. Moreover, the combined inoculation of these two bacteria gave better results. Moreover, biochar is the carbonaceous residue left in the pyrolysis process. Several studies have highlighted its benefit for mitigation of global climate change and as an effective strategy to manage soil quality and crop productivity (Renard *et al*., 2012). Amending soil with biochar has increasingly attracted widespread attention for its chemical stability, ideally suited for sequestrating C in soil, rapidly increases soil fertility and plant growth by supplying and retaining nutrients while simultaneously improving the physical and biological properties of the soil (Woolf *et al*., 2010, Uzoma *et al*., 2011).

Osmolytes synthesis induced by abscisic acid hormone can also protect plants against drought stress. Notably, osmolytes can decrease the hydric potential of the cell and thereby help it to avoid losing water. Proline is one type of osmolyte, and changed like abscisic acid hormone depending on salt concentration as reported by many investigators (Paleg *et al*., 1983; Ashraf and Foolad 2005) they showed that proline synthesis can be seen as a symptom of stress in plants, taking into account their pathway of induction. It has also been reported that plants that were inoculated

with PGPR (*Pseudomonas* sp.) showed a decrease in proline synthesis when they were under water stress (Tiwari *et al.,* 2016). This is in agreement with our results, that plants treated by rhizobacterial strains together with seed coating plus biochar as a carrier method followed by *P. fluorescens* carried on biochar in normal and saline soils may have a lower level of stress in saline conditions than control plants. Bogges *et al.* (1977) determined that water stress prevents the oxidation of proline, resulting in higher proline levels. In addition, other researchers working with *Bacillus megaterium* BOFC15 and *Arabidopsis* found that spermidine improves drought tolerance in plants, which was associated with altered levels of ABA (Zhou *et al.,* 2016).

Total chlorophyll contents of plants grown from seeds treated by PGPR strains were significantly higher than control. The mixture of bacteria with biochar in seed coating recorded the highest increase in chlorophyll levels in the grown plants compared to other treatments and control in both soil types. This finding is in agreement with Aly *et al.* (2008) they demonstrated that cyanobacteria strains applied either as seed-soaking or foliar, significantly increased the chlorophyll content in sugar beet leaves. Also, they pointed to the role of PGPR as phytohormone sources which increase the chlorophyll content. Grattan and Grieve (1994) suggested that PGPR can reduce the harmful effect of NaCl on N uptake, the essential nutrient component of chlorophyll molecule structure in saline soil.

Under field conditions, seed treatments with rhizobacterial strains significantly decreased disease and improved maize ear and yield characters. The enhancing effects of seed inoculation with rhizobacteria on shoot dry weight and yield of maize were reported by many researchers (Pandy *et al.,* 1998; Shaharoona, *et al.,* 2006). Results of this investigation are similar to those obtained by Vikram (2007) and Gholami *et al.* (2009) as they stated that auxins produced by rhizobacteria can influence plant growth, including seed germination and root development which improve uptake of essential nutrients thus increasing plant growth. Gholami *et al.* (2009) suggested that the increased synthesis of hormones like gibberellins trigger the activity of specific enzymes that promote early germination, such as α-amylase, which have brought an increase in availability of starch assimilation. Our results revealed that plants grown from treated seeds with rhizobacterial strains together with seed coating plus biochar as a carrier method followed by biochar application with *P. fluorescens* in normal and saline soils are in agreement with many studies that shown positive effects of biochar on plant growth in tropical climates (Major *et al*., 2010). Many chemical and physical characteristics of biochar, such as high porosity, sorption capacity and high water holding capacity (WHC), might create suitable habitats for microorganisms, promoting their activities (Thies and Rillig, 2009). Also, Vidhyasekaran and Muthamilan (1996) treated chickpea seeds with a talc-based formulation of three *P. fluorescens* strains to control of chickpea wilt. The results showed that field emergence, chickpea yield and suppression of disease were improved by seed treatment. Research has demonstrated that biochar application can significantly improve crop productivity (Chan *et al.,* 2007).

In conclusion, usage of seed coating of rhizobacterial strains (*e.g*. *B. subtilis* and *P. fluorescens*) individually or together plus biochar as a carrier minimized late wilt disease, salinity stress effect and enhanced maize growth parameters.

References

Ahmad, F.; Ahmad, I.; and Khan, M.S. 2008. Screening of free living rhizosphericbacteria for their multiple plant growth promoting activities. *Microbiological Research*, **163**:173-181.

Alagawadi, A.R. and Gaur, A.C. 1988. Associative effect of Rhizobium and phosphate solubilizing bacteria on the yield and nutrient uptake of chickpea. *Plant and Soil* **105** 241-246.

Aly, M.H.A.; Abd El-All, Azza A.M. and Mostafa, Soha, S.M. 2008. Enhancement of sugar beet seed germination, plant growth, performance and biochemical compounds as contributed by algal extracellular products. *J. Agric. Sci., Mansoura Univ.* **33**(12); 8429-8448.

Ashraf, M. 2004. Some important physiological selection criteria for salt tolerance in plants. *Flora,***199**: 361-376.

Ashraf, M. and Harris, P.J.C. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci*., 166: 3-16.

Ashraf, M. and Foolad, M.R. 2005. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.,* **59**: 206–216.

Bardin, S.D.; Huang, H.C. and Moyer, J.R. 2004. Control of Pythium damping-off of sugar beet by seed treatment with crop straw powders and a biocontrol agent. *Biological Control,* **29**:453-460.

Bates, L.S.; Waldren, R.P. and Tear, I.D. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39, 205-207.

Bogges, S.F.; Aspinnal, D. and Paleg, L.G. 1977. Inhibition of Proline Oxidation by Water Stress. *Plant Physiol*., **59**: 930–932.

Breed, R.S.; Murray, E.G.D. and Smith, N.R. 1957. "*Bergey's Manual of Determinative Bacteriology* ". 7th Ed. The Williams and Wilkins Combany, U.S.A., 1130 p.

Castric Kathleen, F. and Castric, P. A. 1983. Method for rapid detection of cyanogenic bacteria. *Appl. Environ. Microbial.* **45**: 701-702.

Cavaglieri, L.; Orlandoa, J.; Rodríguez, M.I.; Chulzeb, S. and Etcheverry, M. 2005. Biocontrol of *Bacillus subtilis* against *Fusarium verticillioides in vitro* and at the maize root level. *Res. Microbiol*., **156**:748–754.

Chan, K.Y.; Van Zwieten, E. L.; Meszaros, I.; Downie, A. and Joseph S. 2007. Agronomic values of green waste biochar as a soil amendment. *Australian Journal of Soil Research*. **45**: 629–634.

Chinnusamy, V. A.; Jagendorf and J. K. Zhu. 2005. Understanding and improving salt tolerance in plants. *Crop Sci*., **45**: 437-448.

Dennis, C. and Webster, J. 1971. Antagonistic properties of species-groups of *Trichoderma.* I . Product ion of nonvolatile antibiotics. *Trans. Br. Mycol. Soc*., **57**:25-39.

Ebrahim, E.M.T. 2010. Relation of Fluorescent Bacteria in Controlling Some Root Diseases of Sugar Beet. Ph.D. Thesis, Fac. Sci., Bani-Seuf Univ., Egypt, 195p.

Edwards, S.G.; McKay, T. and Seddon, B. 1994. Interaction of Bacillus species with phytopathogenic fungi – Methods of analysis and manipulation for bicontrol purposes. In:" *Ecology of Plant Pathogens*". (Blakeman J.P. and Williamson, B. Eds.). CAB International, Wallingford, Oxon, UK., pp. 101-118.

El-Adly, R. A.; Yossef, A.M.; Modather, F. Hussein, Enas, A. I. and Dalia M. A.. 2015. Biogrease based on biochar from rice straw and waste cooking oil. *International Journal of Advances in Pharmacy, Biology and Chemistry*. **4**(1), 91-97.

El-Assiuty, E.M.; El-Hamahmy, A.A. and El-Sharkawy, A.Y. 1991. *Bacillus subtilis*, *Pseudomonas fluorescens* and *Verticillium tricorpus* as biological agents against late-wilt of maize. *Egypt. J. Appl. Sci*., **6**:824-829.

El-Hendawy, S.E., Y. Hu, G.M. Yakout, A.M. Awad, S.E. Hafiz and U. Schmidhalter. 2005. Evaluating salt tolerance of wheat genotypes using multiple parameters. *Europ. J. Agron*., **22**:243-253.

El-Mehalowy, A. A.; Hassanein, N.M.; Khater, H.M.; Daram El-Din, E.A. and Youssef, Y.A. 2004. Influence of maize root colonization by *Rhizosphere actinomycetes* and yeast fungi on plant growth and on the biological control of late wilt disease. *Int. J. Agric. Biol.*, **6**:599-605.

El-Shafey, H. A.; Abd-El-Rahim, M.F. and Refaat, M.M. 1979. A new *Cephalosporium* wilt disease of grain sorghum in Egypt. *Proc. 3rd Egypt, Phytopathology Congress*, pp. 513-532.

El-Shafey, H.A.; and Claflin. L.E. 1999. Late wilt. In: *"Compendium of Corn Diseases*". D.G. White (Ed.), *3rd*. St. Paul: APS Press, pp. 43-44.

Fuhrmann, J. J. and Wollum, A.G. 1989. Nodulation competition among *Bradyrhizobium japonicum* strains as influenced by rhizosphere bacteria and iron availability. *Biology and Fertility of Soils.* **7**: 108-112.

Gholami A.; Shahsavani S.; and Nazarat S. 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *International Journal of Biology and Life Science*. **5**(1), 35-40.

Glick, R.; Penrose, D. M.; Li, J. 1998. A model for the lowering of plant ethylene concentrations by plant growth promoting bacteria. *Journal of Theoretical Biology*. **190**: 63-68.

Glick, B.R. 1995. The enhancement of plant growth by free living bacteria. *Can. J. of Microbiol*., **41**:109-114.

Grattan, S.R. and Grieve, C.M. 1994. Mineral nutrient acquisition and respons by plants grown in saline environments. In: hand book of. "*Plant and Crop Stress*". Pessarakli. M. (Ed.). Marcel Dkker, New York, pp. 203-226.

Grichko, V.P. and Glick, B.R. 2001. Flooding tolerance of transgenic tomato plants expressing the bacterial enzyme ACC deaminase controlled by the 35S, rol Dor PRB-1b promoter. *Plant Physiology and Biochemistry*. **39**:19-25.

Hamza, A.M.; El-Kot, G.A. and El-Moghazy, S. 2013. Non-traditional methods for controlling maize late wilt disease caused by *Cephalosporium maydis*. *Egypt. J. Biological Pest Cont*., **23**(1):87-93.

Jacobsen, B.J.; Zidack, N.K. and Larson, B.J. 2004. The role of Bacillus-based biological control agents in integrated pest management systems: Plant diseases. *Phytopathology*, **94**:1272-1275.

Jenana, R.K.B.; Haouala, R.; Triki, M.A.; Godon, J.; Hibar, K.; Khedher, M.B. and Henchi, B. 2009. Composts, compost extracts and bacterial suppressive action on *Pythium aphanidermatum* in tomato. *Pak. J. Bot*., **41**(1):315-327.

Kamnev, A.A. and Lelie, D., 2000. Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. *Bioscience Reports*. **20**: 239-258.

Katerji, N.; van Hoorn, J.W.; Hamdy A. and Mastrorilli, M. 2004. Comparison of corn yield response to plant water stress caused by salinity and by drought. *Agric. Water Mange*., **65**: 95-101.

Katerji, N.; van Hoorn, J.W.; Hamdy, A.; Mastrorilli, M. and Karam, F. 1998. Salinity and drought, a comparison of their effects on the relationship between yield and evapotranspiration. *Agric. Water Manage*., **36**: 45-54.

Khan, A.G. 2005. Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *Journal of Trace Elements in Medicine and Biology*. **18**: 355-364.

Khavazi, K.; Rejali F.; Seguin P.; and Miransari M. 2007. Effects of carrier, sterilization method, and incubation on survival of *Bradyrhizobium japonicum* in soybean (*Glycine max* L.) inoculants. *Enzyme and Microbial Technology*. **41**: 780-784.

Kim, W.G.; Weon, H.Y. and Lee, S.Y. 2008. *In vitro* antagonistic effects of Bacilli isolates against four soilborne plant pathogenic fungi. *Plant Pathol. J*., **24**(1):52-57.

Lehmann J.; Rillig M.C. Thies J.; Masiello, C.A.; Hockaday, W.C.; and Crowley, D. 2011. Biochar effects on soil biota. A review. *Soil Biology and Biochemistry*. **43**:1812-1836.

Lehmann, J. and Joseph, 2009. Biochar for Environmental Management: Science and Technology. Earthscan, London, 85-105.

Major, J.; Rondon, M.; Molina, D.; Riha, S. J.; and Lehmann, J. 2010. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant Soil*, **333**: 117–128.

Mansour, M.M.F.; Salama, K.H.A.; Ali, F.Z.M. and Hadid, A.F.A. 2005. Cell and plant responses to NaCl in *Zea mays* L. cultivars differing in salt tolerance. *Gen. Appl. Plant Physiol*., **31**: 29-41.

Matsubara, Y.; Hasegawa, N. and Fukui, H. 2002. Incidence of Fusarium root rot in asparagus seedlings infected with arbuscular mycorrhizal fungus as affected by several soil amendments. *J. of the Japanese Society for Horticultural Science*. **71**: 370-374.

Mavrodi, O.V.; McSpadden, G.B.B.; Mavrodi, D.V.; Bonsall, R.F.; Weller, D.M. and Thomashow, L.S. 2001. Genetic diversity of *phl*D from 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp.. *Phytopathology*. **91(**1):35–42.

Mayak, S.; Tirosh, T.; Glick, B.R.; 2004a. Plant growth-promoting bacteria that confer resistance in tomato plants to salt stress. *Plant Physiology and Biochemistry*. **42**: 565-572.

Mayak, S.; Tirosh, T.; Glick, B.R.; 2004b. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Science*. **166**: 525-530.

Meera, T and Balabaskar, P. 2012. Isolation and characterization of *Pseudomonas fluorescens* from rice fields. *Int. J. Food, Agric., Veterinary Sci*., **2**(1):113-120.

Nalisha, I.; Muskhazli, M. and Nor Farizan, T. 2006. Production of Bioactive Compounds by *Bacillus subtilis* against *Sclerotium rolfsii. Malays. J. Microbiol*., **2**(2):19-23.

Paleg, L.G., Stewart, G.R., Bradbeer, J.W. 1983. Proline and Glycine Betaine Influence Protein Solvation. *Plant Physiol*., **75**: 974–978.

Pandy, A.; Sharma, E. and Plani, L.K.S. 1998. Influence of bacterial inoculation on maize in upland farming systems of the Sikkim Himalaya. *Soil. Biol. Biochem*., **30**(3):379-384.

Parida, A.K. and Das, A.B. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicol. Environ. Safe*. **60**:324-349.

Reed, M.L.E.; Glick, B.R.; 2004. Applications of free living plant growth-promoting rhizobacteria. *Antonie van Leeuwenhoek,* **86**: 1- 25.

Reed, M.L.E.; Glick, B.R. 2005. Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. *Can. J. of Microbiol*., **51**: 1061-1069.

Renard, D.; Iriarte, J.; Birk, J.J.; Rostain, S.; Glaser, B. and McKey, D. 2012 Ecological engineers ahead of their time: The functioning of pre- Columbian raised-field agriculture and its potential contributions to sustainability today. *Ecol. Eng.,* **45**: 30-44.

Renwick, A.; Campbell, R. and Coe, S. 1991. Assessment of *in vivo* systems for potential biocontrol agents of *Gaeomannomycesgraminis. Plant Pathology*, **40**(4):524-532.

Samra, A.S.; Sabet, K.A. and Hingorani, M.K. 1962. A new wilt disease of maize in Egypt. *Plant Dis. Reporter*, **46**:481–483.

Samra, A.S.; Sabet, K.A.; Abdel-Rahim, M.F.; El-Shafey, H.A.; Mansour, I.M.; Fadl, F.A.; Dawood, N.A. and Khalil, Ikbal H.I. 1966. Investigations on Stalk-Rot Diseases of Maize in U.A.R. *Ministry of Agric., Egypt, Tech. Bull*., 204 p.

Saric, M.; Kastrori, R.; Curie, R.; Cupina, T. and Gerie, I. 1976. Chlorophyll determination. Univ. Unoven Sadu Parktikum is fizologize Bibjoke, Beagard Hauncna, Anjiga, 215 p.

Schwyn, B. and Neilands, J. B. 1987. Universal chemical assay for the determination of siderophores. *Anal. Bio Chem*. **160**(1):47-56.

Shaharoona, B.; Arshad, M.; Zahir, Z.A. and Khalid, A. 2006. Performance of *Pseudomonas* spp. Containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. *Soil. Biol. Biochem*., **38**:2971–2975.

Smith R.S. 1992. Legume 253inoculants formulation and application. *Can. J. of Microbiol*. **38**: 485-92.

Steel, P.G.D. and Torrie, J.H. 1990. Principles and Procedures of Statistics. McGraw-Hill Book Company, INC., New York, 481p.

Thies J. E. and Rillig, M. 2009. Characteristics of biochar: biological properties. In: Lehmann, J.; Joseph, S. (Eds.), Biochar for Environmental Management: Science and Technology. Earthscan, London, 85-105.

Tiwari, S.; Lata, C.; Chauhan, P.S.; Nautiyal, C.S. 2016. *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. *Plant Physiol. Biochem*., **99**: 108–117.

Uzoma, K.C.; Inoue, M.; Andry, H.; Fujimaki, H.; Zahoor, A. and Nishihara, E. 2011. Effect of cow manure biochar on maize productivity under sandy soil condition. *Soil Use Manag.,* **27**(2), 205-212.

Vidhyasekaran, P. and Muthamilan, M. 1996. Development of Formulation of *Pseudomonas fluorescens* for Control of Chickpea Wilt. *Plant Disease.* **79**(8), 782-786.

Vikram, A. 2007. Efficacy of phosphate solubilizing bacteria isolated from vertisols on growth and yield parameters of sorghum. *Res. J. Microbiol.*, **2**(7):550-559.

Woolf, D.; Amonette, J.E. Street-Perrott, F.A. Lehmann, J. and Joseph, S. 2010 Sustainable biochar to mitigate global climate change. *Nature Communications,* No. 56.

Xu G.; Lv, Y.; Sun, J.; Shao, H.; and Wei, L. 2012. Recent Advances in Biochar Application in Agricultural Soils: Benefits and Environmental Implications. *Clean Soil Water Air* 0, 1-6.

Zeng, D. F.; and Zhang, L. 2010. A novel environmentally friendly soybean coating agent. *Acta Agriculturae Scandinavica* Section B – *Soil and Plant Science*. **60**:545-551.

Zhou, C.; Ma, Z.; Zhu, L.; Xiao, X.; Xie, Y.; Zhu, J.; Wang, J. 2016. Rhizobacterial Strain *Bacillus megaterium* BOFC15 Induces Cellular Polyamine Changes that Improve Plant Growth and Drought Resistance. *Int. J. Mol. Sci*., 17, 976.

Received 07/05/2018;
in revised form 11/06/2018)

مكافحة مرض الذبول المتأخر فى الذرة الشامية ورفع كفاءة النباتات لتحمل الملوحة بواسطة بعض سلالات الريزوبكتريا

السعيد محمد الشبراوى* – هبه شحاته شحاته**
* معهد بحوث أمراض النباتات- مركز البحوث الزراعية- الجيزة - مصر.
** معهد بحوث الاراضى والمياه - مركز البحوث الزراعية – الجيزة

تم دراسة تأثير الريزوبكتيريا المحفزة لنمو النباتات (PGPR) على خفض الاصابة بالذبول المتأخر فى الذرة الشامية المتسبب عن فطر السيفالوسبوريم مايادس، ورفع كفأة تحمل النباتات للملوحه ومؤشرات النمو فى النباتات. فى الدراسة بالمعمل وجد ان اربع عزلات من سبعه من السيدوموناس فلورسينس وثلاثه من تسع عزلات من الباسيلس ساتلاس اظهرت تضادا عاليا للفطريات موضع الدراسه. التقاوى المعاملة بالـ (PGPR) منفردة او خليط والمحمولة على البيوشار بطريقة تغليف التقاوى اظهرت نباتاتها انخفاضا معنويا فى درجة الاصابة بالذبول المتاخر سواء فى الصوبة او الحقل. كما كانت هذه المعاملة افضل المعاملات فى خفض الاصابة بالمرض حيث انخفض بنسبة ٧٢% فى التربة العادية و٧٠% فى التربة الملحية تحت ظروف الصوبة وبينما كانت تحت ظروف الحقل ٨٢.٨% فى الاراض العادية و٧٩.٣% فى الارض المالحه. وقد حفزت هذه المعاملة زيادة نسبة الانبات، طول النباتات، الوزن الجاف و مستوى الكلورفيل الكلى عن باقى المعاملات. كذلك خفضت المعاملة مستويات البرولين المنتج فى النباتات بالمقارنة بالكنترول سواء فى التربة العادية او الملحية نتيجة لخفض تأثيرات الملوحة على النباتات من قبل الـ(PGPR) وبالاخص تلك المحمولة على البيوشار. تحت ظروف الحقل سواء فى التربة العادية او المالحة خفضت الـ (PGPR) المغلفة للتقاوى سواء منفردة اوخليط من البكتيريا من اصابة النباتات بالمرض ورفعت انتاجية النباتات من المحصول.