#### 5. Summary

Salinity is one of the most severe biotic stresses limiting crop growth and productivity. It is one of the main factors that limits the spread of plants in their natural habitats. In view of important role of some plant growth promoting rhizobacteria which have positively influence plants vitality and the ability of the plants to cope with a biotic stress conditions specially salinity, for this purpose, a succession of laboratory and pots experiment were designed and carried out. Obtained results can be summarized in the following.

### 5.1. Isolation and purification of plant growth promoting rhizobacteria (PGPR)

This part concerning to isolation and purification of some plant growth promoting rhizobacteria from salt–affected soil samples that were collected from different location in Egypt. One hundred and sixty-five (165) bacterial isolates were obtained and consequently used for the succeeding experiments.

#### 5.2. Screening of salt-tolerant isolates for prospective PGPR features

#### 5.2.1. Salt-tolerant rhizobacterial assessment

#### 5.2.2. Salt tolerant bacteria as PGPR

Subsequently, the most salt-tolerant rhizobacterial isolates in the current study were chosen for further studies. Fifty-two isolates that showed salt-tolerance at 15% sodium chloride were used for the secondary screening which was carried out to investigate the ability of chosen isolates concerning the production of indole acetic acid (IAA), gibberellins, siderophores, hydrogen cyanide (HCN) and ammonia. Moreover, phosphate and potassium solubilization as well as N<sub>2</sub>-fertilization were considered.

#### 5.2.2.1. Indole acetic acid production by salt-tolerant isolates under different NaCl concentration

IAA by the chosen fifty-two isolates were evaluated as a representative of auxins. Data presented that all PGPR isolates gave the highest values of IAA production under 0% concentration sodium chloride and the production was decreased with the increasing of sodium chloride concentration.

At concentration of 8% sodium chloride, isolates STB6, STB33, STB105, STB121, STB156, STB158, STB160, STB162 and STB165 were highly efficient for IAA production and produced amounts ranged from 4 - 8  $\mu$ g/mL. The maximum amount of IAA was produced from isolates STB60 and STB63 being 35.8 and 34.7  $\mu$ g/mL in sodium chloride free medium, respectively. While, the lowest produced amount of IAA was from isolate STB122 being 1.47  $\mu$ g/mL.

### **5.2.2.2** Gibberellins productions by salt-tolerant isolates under different NaCl concentration

All examined isolates showed good capability of gibberellins production in sodium chloride free medium. Isolates STB1, STB13, STB15, STB33, STB42, STB46, STB121, STB151, STB153, and STB165 showed highly efficient for gibberellins production with amounts ranged from 33.26 - 48.5  $\mu g/mL$ . Moreover, these isolates showed high gibberellins production under all studied salt concentrations compared with other isolates.

Data showed that isolates STB33, STB34, STB41, STB51 and STB91 gave lower amounts of gibberellins production under 8% NaCl concentration with amounts ranged from  $0.025-0.12~\mu g/mL$ , while isolates STB1, STB13, STB42,STB 121and STB151 gave higher amounts under 8% NaCl concentration with amounts ranged from 18.69 - 28.45  $\mu g/mL$ . The amount of gibberellins productions decreased by increasing salt concentration. The maximum mean of gibberellins production was under 0% concentration of NaCl being 48.46  $\mu g/mL$ . While, the lowest mean of gibberellins production was under 8% NaCl being 0.025  $\mu g/mL$ .

### **5.2.4.** Siderophores production by salt-tolerant isolates under different NaCl concentration

Data of siderophores production by chosen isolates emphasized that most of the examined isolates were able to produce siderophores under NaCl concentrations. At 2% sodium chloride, 75% of the examined rhizobacterial isolates were able to grow in presence of 8-hydroxyquinoline that indicating

siderophores production. While, all isolates didn't grow at 6% and 8% except isolates STB34, STB74, and STB160 which continued to grow. Moreover, isolates STB6, STB15, STB73, STB74, STB121, STB158 and STB165 exhibited higher growth rate although increasing sodium chloride concentration up to 4%. Whereas, isolate STB34 alone has grown at 8%. Regarding catecholate-type siderophores, data showed that production of catecholate (based on the color intensity) was detected by isolates STB6, STB15, STB33, STB37, STB105, STB153, STB157 and STB58, up to 8% sodium

### 5.2.2.3. Hydrogen cyanide (HCN) production by salt-tolerant isolates under different NaCl concentration

Data revealed that 69.2% of the examined isolates exhibited capability of HCN production. The highest production rate was found in sodium chloride free medium While, HCN production rate decreased with increasing the salinity up to 8% sodium chloride. 40.4% of the examined isolates showed high production rate of HCN under 8% sodium chloride.

#### 5.2.2.4 Ammonia production by salt-tolerant isolates under different NaCl concentration

Data showed that 34.6 % of the examined rhizobacterial isolates exhibited the ability to produce ammonia under saline stress up to 4% sodium chloride. Ammonia production was negatively affected by high salinity by all investigated isolates, whereas ammonia was not produced at concentrations of 6% and 8% sodium chloride. Isolates STB6, STB121and STB165 were found to be good producers of ammonia at concentration of 4% sodium chloride.

### 5.2.2.5 Phosphate solubilization by salt-tolerant isolates under different NaCl concentration

The capability of chosen fifty-two rhizobacterial isolates for inorganic phosphate solubilization was qualitatively and quantitatively evaluated under saline stress. Data emphasized that 69.2% of examined rhizobacterial isolates were capable of solubilizing phosphate under saline stress with superiority for isolates STB6, STB121and STB165. Depending on the quantitative evaluation, it could be stated that, phosphate solubilization rate was decreased with increasing sodium chloride concentration up to 8% indication salt tolerance for the examined rhizobacterial isolates.

### 5.2.2.7 Silicate solubilization ability by salt-tolerant isolates under different NaCl concentration

Silicate solubilization was qualitatively and quantitatively evaluated under saline stress. Data revealed that 71.15% of examined isolates (including isolates STB6, STB73, STB121 and STB165) were capable of solubilizing silicate under saline stress. The highest silicate solubilization rate was obtained from isolate STB32 and STB63 followed by STB64 and STB105 up to 8% sodium chloride.

#### 5.2.2.8 Atmospheric nitrogen $(N_2)$ fixing ability by salt-tolerant isolates under different NaCl concentration

The ability of the more potent PGPR rhizobacterial isolates (which were isolated on N-free medium) to fix atmospheric nitrogen was quantitatively measured. Data showed that 68.75 % of the examined rhizobacterial isolates have the ability to fix atmospheric nitrogen while 31.25% of the examined isolates showed negative results for nitrogen fixation.

Furthermore, isolates STB1and STB121 gave the highest values of nitrogenase activity in sodium chloride free medium being 55.2 and 48.0 N moles  $C_2H_4$  / 100 ml culture, respectively. Nitrogenase activity decreased with increasing sodium chloride concentration up to 4%. At concentration of 6% and 8% no nitrogenase activity was found for all examined isolates.

# 5.3. Over-all beneficial effects by the more potent rhizobacterial isolates under different salt concentrations.

From the previous results, isolates STB6, STB74 and STB165were chosen according to their superiority for indole acetic acid (IAA), Gibberellins, siderophores, hydrogen cyanide (HCN) and ammonia production.

Moreover, nitrogen fixation, phosphate and potassium solubilization were considered. Consequently, these isolates were identified and used for the succeeding application experiments.

#### 5.4. Bacterial isolates identification using 16S rRNA sequences

The most potent isolates were chosen and identified by 16S rRNA gene sequence analysis to ascertain their taxonomic positions.

The FASTA homology showed that the 16S rRNA gene sequences of the selected isolates had 99, 97 and 99% nucleotide similarity with that of *Paenibacillus polymyxa*, *Ochrobactrum intermedium* and *Enterobacter cloacae* strains, respectively.

#### 5.5. Survival and vitality of PGPR strains in different carriers

The high bacterial population can be maintained by the application of enriched carriers which supports their growth and activities. Three different carriers namely, compost, peat moss and sawdust were used to investigate the effect of carrier on the population and the activity of the rhizobacterial strains of *Paenibacillus polymyxa*, *Ochrobactrum intermedium* and *Enterobacter cloaca*.

Bacterial populations, dehydrogenase activity, the pH values and the moisture contents were evaluated at 30 days interval up to six months of storage.

#### 5.5.1. Rhizobacterial populations in different carriers

Data revealed that, using of peat moss either singly or combined with sawdust (50:50%) as a carrier gave higher survival and population records for the rhizobacterial strains. The highest population of PGPR strains was observed with peat moss (100%) as a carrier.

The population was decreased with increasing the incubation period up to 90 days. The obtained data showed that the survival rate of PGPR strains in all peat moss treatments was better than that in compost. As expected, using of sawdust solely (100%) showed the lowest population rates and survival for all examined rhizobacterial strains.

#### 5.5.2. Determination of dehydrogenase activity in different carriers

According to dehydrogenase activity values, peat moss exhibited a good capability as a carrier for the survival of the rhizobacterial strains, *Paenibacillus polymyxa*, *Ochrobactrum intermedium* and *Enterobacter cloaca*.

Moreover, compost exhibited a good dehydrogenase activity when used with sawdust (50:50%) as a carrier for the survival of the rhizobacterial strains, *Ochrobactrum intermedium* and *Enterobacter cloaca*.

### 5.5.3. Determination of moisture contents and the pH values in different carriers treatment.

Obtained data observed that the moisture content, gradually decreased with increasing of storage period in all carriers treatments. A slight decline in pH was recorded in all carriers' treatments up to 90 days of storage. While, an increase of the pH values was observed for all treatments after 120 days of storage.

The highest moisture content was observed after 180 days with peat moss-based inoculum. While, lowest moisture content was observed with sawdust 100%.

# 5.6. Interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and humic acid on growth performance and yield of squash

This experiment was carried out under greenhouse conditions to evaluate the efficiency of the more potent PGPR strains (*Paenibacillus polymyxa* GQ375783.1), *Ochrobactrum intermedium* MG309678.1 and *Enterobacter cloacae* MG309676.1) combined with inorganic fertilization and foliar application with humic acid on growth performance of squash during growing season 2017.

# 5.6.1. Interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and humic acid on some microbial enzymes activity in soil

Dehydrogenase DH, alkaline phosphatase and nitrogenase activities were periodically estimated as a guide of respiration rate and total microbial activity in soil, mineralization processes of organic phosphorus substrates and N<sub>2</sub>-fixers activity, respectively.

Data showed that dehydrogenase (DH), phosphatase (p-ase) and nitrogenase (N<sub>2</sub>-ase) activities in saline soil that inoculated with salt-tolerant PGPR strains were increased in comparison with un-inoculated one. Additionally, all treatments sprayed with humic acid achieved an increase of DH and P-ase activities compared to non-sprayed ones. Furthermore, data emphasized that soil fertilization with chemical fertilizers gave the lowest values of DH and P-ase activities at all determination periods. While, the soil inoculation with PGPR strains combined with NPK 50% gave the highest records of DH and P-ase.

Concerning N<sub>2</sub>-ase activity, data showed that increase of chemical fertilizers led to decrease nitrogenase activity. While inoculation plants with salt-tolerant PGPR strains recorded increase of N<sub>2</sub>-ase activity than uninoculation ones. Moreover, soil inoculated with salt- tolerant PGPR combined with 50% NPK gave higher values of N<sub>2</sub>- ase activity than that fertilized with 75% NPK. As expected, the lowest N<sub>2</sub>-ase activity values were observed in soil fertilized with chemical fertilizers individually (without inoculation) followed by soil inoculated with salt- tolerant PGPR strains

combined with 75% NPK. The highest significant N<sub>2</sub>-ase activity values were observed in soil inoculated with salt-tolerant PGPR strains combined with 50% chemical fertilizers and humic acid spraying.

Generally, DH, phosphatase and nitrogenase activity were increased with the increasing of periods to reach their maximum values at 30 days (flowering stage) and decreased thereafter at 45 days.

# 5.6.2. Interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and humic acid on some oxidative enzymes

Data revealed that inoculation squash with salt- tolerant PGPR strains combined with different doses of chemical fertilizers gave higher values of plants oxidative enzymes (Peroxidase and polyphenol oxidase) than that uninoculated ones. Also, inoculated squash showed increase in abovementioned enzymes by increasing dose of inorganic fertilizers, regarding to spraying plants with humic acid, data showed that an increase of peroxidase and polyphenol oxidase activities in plants sprayed with humic acid than that non-sprayed plants. Similar trend of results were observed in all investigated treatments.

In addition, the lowest records of peroxidase and polyphenol oxidase activity were obtained in squash fertilized with full dose of chemical fertilizers. While, the highest values of peroxidase and polyphenol oxidase activity were observed in squash that inoculated with salt- tolerant PGPR strains combined with 50%NPK.

# 5.6.3. Interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and humic acid on available N, P and K

Data showed that the soil inoculated with salt- tolerant PGPR strains achieved an increase available of N, P and K compared to un-inoculate one. In addition, soil inoculated with salt- tolerant PGPR strains combined with 25% NPK gave lower records of available N, P and K than that amended with either 50% or 75% of chemical fertilize.

Foliar application of humic acid gave significant higher available of N, P and K in comparison with non- sprayed plants Similar trend of results were observed in all investigated treatments. Furthermore, the lowest values of available N, P and K were observed in soil inoculated with salt- tolerant PGPR strains combined with 25%NPK and without sprayed by humic acid. While, the heights records were observed in soil inoculated with salt- tolerant PGPR strains combined with 50% chemical fertilizers and sprayed with humic acid.

# **5.6.4.** Interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and humic acid on N, P and K uptake

Data showed that squash inoculated with salt - tolerant PGPR strains gave an increase of N, P and K uptake compared to that un-inoculated and treated with chemical fertilizers individually. Also, data cleared that squash inoculated with salt- tolerant PGPR combined with 50% of chemical fertilizers gave higher values of N, P and K uptake than that fertilizer with 75% followed by 25% NPK, in presence of PGPR inocula.

Foliar application of squash with humic acid gave significant higher values of N, P and K uptake in comparison with non- sprayed plants. Moreover, the heights records of N, P and K uptake were obtained in squash inoculated with salt- tolerant PGPR strains combined with 50% chemical fertilizers. While, the lowest records of N, P and K uptake in squash was observed in treatment fertilized with full dose chemical fertilizers solely.

# 5.6.5. Interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and humic acid on growth characteristics of squash

Squash inoculation with salt- tolerant PGPR strains have positive effective on growth characteristics i.e. plant height, plant fresh weight, root and shoot dry weight, number of branches, leaves and flowers, root and Shoot length (cm) compared to squash un- inoculated. Also, data showed that inoculation of squash with salt- tolerant PGPR strains in combination with 50% NPK gave higher records of growth characteristics than that fertilized with 25% NPK followed by 75% NPK. While, squash growth characteristics were significantly decreased when fertilized with full dose of NPK alone.

In general, squash sprayed with humic acid gave significant higher values of growth characteristics than non-sprayed treatments. The lowest values of growth characteristics were observed in squash plants inoculated with salt-tolerant PGPR strains combined with 25% of chemical fertilizers without foliar spraying by humic acid. Whereas, inoculation of squash with PGPR strains combined with 50% NPK and sprayed with humic acid gave the highest records of above mentioned parameters. The beneficial effect of the tested PGPR on growth characteristics may be attributed to their ability to produce IAA,

gibberellins, solubilization of phosphate and potassium as well as their ability to fix atmospheric nitrogen, which were previously confirmed in this study.

# **5.6.6.** Interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and humic acid on yield and yield components of squash

Inoculation of squash with salt-tolerant PGPR strains significantly increased yield and yield components in comparison with that un-inoculated plants. Moreover, inoculation of squash plants with salt- tolerant PGPR strains in combination with 50% NPK gave higher values of above mention criteria than plants fertilization with 75% NPK followed by 25% NPK.

Also, results indicated that squash plants sprayed with humic acid recorded significant increase of yield and yield components compared to non-sprayed plants. This result may be due to humic acid increase the uptake of some nutritional elements.

It is important to mention that inoculation of squash with salt-tolerant PGPR strains combined with 50% NPK gave the highest records of number, weight of fruits per plant, total protein and T.S.S. being 3.33,128 g, 14.45 mg and 7.6% respectively. The lowest records of number, weight of fruits per plant, total protein and soluble solids (T.S.S) were obtained when squash inoculation with PGPR strains combined to 25% NPK.

# 5.7. interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and *Saccharomyces* extract on pepper growth performance and yield

This experiment was carried out under greenhouse conditions to evaluate the efficiency of the more potent PGPR strains (*Paenibacillus polymyxa* (GQ375783.1), *Ochrobactrum intermedium* (MG309678.1) and *Enterobacter cloacae* (MG309676.1) combined with chemical fertilization and spraying with *Saccharomyces* extract as a foliar application on growth performance and yield of pepper during growing season of 2017.

# 5.7.1. Interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and *Saccharomyces* extract on some microbial enzymes in soil

Data showed that the lowest records of DH and P-ase activities were observed when soil inoculated with salt-tolerant PGPR strains combined with 25% NPK from chemical fertilizers and non-sprayed with *Saccharomyces* extract as a foliar. Additionally, inoculation soil with salt-tolerant PGPR strains

with 75% NPK gave higher values of DH and P-ase activities than that amended with 50% NPK followed by soil fertilized with full dose of NPK alone.

Moreover, data indicated that DH, P-ase and N<sub>2</sub>-ase activities gave high significant records when soil inoculated with salt-tolerant PGPR strains in comparison with un-inoculated one. Furthermore, the heights significant records of DH and P-ase activities were obtained in soil inoculation with salt-tolerant PGPR strains combined with 75% NPK from chemical fertilizers. Additionally, all treatments that sprayed with *Saccharomyces* extract achieved increase of DH, P-ase and N<sub>2</sub>-ase activities compared to non-sprayed ones.

Concerning  $N_2$ -ase activity, data emphasized that the lowest values of  $N_2$ -ase in soil fertilized with chemical fertilizers individually. Moreover, soil inoculated with salt- tolerant PGPR strains combined with 25% NPK gave higher values of  $N_2$ - ase activity than that fertilized with either 50% or 75% NPK from chemical fertilizers.

Rhizosphere of pepper which inoculated with salt-tolerant PGPR strains recorded increase of  $N_2$ -ase activity than rhizosphere of un- inoculated plants. The highest significant values of  $N_2$ -ase activity were observed in soil inoculated with salt-tolerant PGPR strains combined with 25% chemical fertilizers and sprayed with *Saccharomyces* extract as a foliar.

Generally, DH, phosphatase and nitrogenase activity were increased with the increasing of periods to reach their maximum values at 30 days (flowering stage) and decreased thereafter at 60 days.

# 5.7.2. Interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and *Saccharomyces* extract some oxidative enzymes

Data in showed that the lowest values of peroxidase and polyphenol oxidase activities were obtained in pepper plants that fertilized with chemical fertilizers alone and non-sprayed with *Saccharomyces* extract.

In additional, inoculation of pepper plants with salt- tolerant PGPR strains combined with 75% NPK gave higher values of abovementioned enzymes than that inoculated with PGPR strains in combination with 50% NPK. Furthermore, pepper plants were inoculated with salt- tolerant PGPR strains combined with different doses of chemical fertilizers gave an increase values of plants oxidative enzymes compared to that amended with chemical fertilizers solely and uninoculated with PGPR strains.

In addition, the highest significant values of pepper plants inoculated with salt- tolerant PGPR strains combined with (75%) NPK. Spraying plants with *Saccharomyces* extract, data showed an increase of peroxidase and polyphenol oxidase activities compared to non-sprayed ones.

# 5.7.3. Interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and *Saccharomyces* extract on growth characteristics of pepper

Data indicated that the lowest records of growth characteristics were observed when pepper plants fertilized with chemical fertilizers solely and without foliar spraying with *Saccharomyces* extract. In addition, inoculation of pepper with salt- tolerant PGPR strains combined with 25% NPK gave lower records of growth characteristics than that fertilized with either 50% or 75% NPK.

It is clear that, treatments inoculated with salt- tolerant PGPR strains have positive effective on growth characteristics i.e. plant height, plant fresh weight, root and shoot dry weight, number of branches, leaves and flowers, root and shoot length (cm) compared to un- inoculated plants.

Furthermore, inoculation with PGPR strains combined with 50% NPK and spraying with *Saccharomyces* extract gave the highest significant records of above mentioned parameters of pepper.

## 5.7.4. Interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and *Saccharomyces* extract on N, P and K uptake

Inoculated pepper with salt-tolerant PGPR strains combined with 25% chemical fertilizers gave the lowest records of N, P and K uptake and non-sprayed with *Saccharomyces* extract. Also, data showed that inoculation treatments with salt- tolerant PGPR strains combined with 50% of chemical fertilizers gave higher values of N, P and K uptake than that fertilized with 75% NPK followed by 25%.

In addition, pepper inoculated with salt-tolerant PGPR strains gave higher records of N, P and K uptake compared to with that un-inoculated ones. Furthermore, the heights records of N, P and K uptake were observed in pepper inoculated with salt- tolerant PGPR strains combined with 50% chemical fertilizers and sprayed with *Saccharomyces* extract. Foliar application of pepper plants with *Saccharomyces* extract gave significant higher values of N, P and K uptake in comparison with non- sprayed plants.

# 5.7.5. Interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and *Saccharomyces* extract on available N, P and K

Inoculation soil with salt-tolerant PGPR strains combined with 25% chemical fertilizers gave the lowest records of available N, P and K and non-sprayed with *Saccharomyces* extract. Also, results cleared that soil inoculated with salt-tolerant PGPR strains combined with 50% NPK gave higher records of available N, P and K than that amended with either 25% or 75% of chemical fertilizers. Moreover, inoculation with salt-tolerant PGPR strains achieved an increase of available N, P and K compared to un-inoculate treatments.

Foliar application with *Saccharomyces* extract and inoculation with salttolerant PGPR strains combined with 50% NPK gave the highest significant of available N, P and K in comparison with non-sprayed plants.

# 5.7.6. Interaction effect among salt-tolerant PGPR strains, inorganic fertilizers and *Saccharomyces* extract on yield and yield components

Data emphasized that the lowest records of number, weight of fruits per plant, vitamin C and soluble solids T.S.S. were observed when pepper inoculation with salt-tolerant PGPR strains combined with 25% chemical fertilizers. Moreover, inoculation of pepper with salt- tolerant PGPR strains in combination with 50% NPK gave higher values of above mentioned criteria than plants fertilization with 75% NPK followed by 25% NPK.

Generally, results showed that yield and yield components of pepper records were significantly increased when inoculation with salt-tolerant PGPR strains than un-inoculated plants. The highest records of yield and yield components was obtained when treatments inoculated with salt-tolerant PGPR strains combined with 50% chemical fertilizers. Significantly higher plant and grain N, P, and K content were observed in biofertilizer combined with N, P (75 %) and K.

In view of the obtained results, it could be concluded that the Egyptian salt-affected soils are a good source of plant growth promoting rhizobacteria, which have the ability of phytohormones production, siderophores, ammonia and CN production, silicate and phosphate solubilization and nitrogen fixation,.

Peat moss and compost-sawdust (50:50%) exhibited a good capability as a carrier for the survival of the rhizobacterial strains, *Paenibacillus polymyxa*, *Ochrobactrum intermedium* and *Enterobacter cloaca*.

Moreover, obtained results of this study indicated that the inoculation with PGPR in combination with half dose of inorganic fertilizers and humic acid or *Saccharomyces* extract as foliar application enhanced plant growth and increased yield under saline stress.

In view of the obtained results from the current study, it could be recommended that the inoculation of salt-affected soils with PGPR can be used as a biofertilizers and biostimulants for vegetable crops to enhance growth performance and productivity under saline stress.