5. SUMMARY

It is well known that the ability of N_2-fixers and phosphate solubilizers to produce and release various metabolites which stimulate plant growth is considered to be one of the most important factors in soil fertility .

Therefore, the beneficial effects of N_2-fixers and phosphate solubilizers on plant growth enhancement can be attributed not only to N_2-fixation and phosphate solubilizing, but also to the production of plant growth regulators such as auxins, gibberellins and cytokinins.

The aim of this study is the isolation and identification of some microorganisms able to produce plant growth regulators (PGRs), study the effect of some environmental and nutritional factors affecting the PGRs production to determine the optimum conditions for PGRs production.

Finally, study the efficiency of tomato inoculation with plant growth promoting rhizobacteria on growth performance and controlling of tomato root diseases caused by soil borne fungi .

The obtained results can be summarized as follows :

The first part:

5.1. Isolation of plant growth promoting rhizobacteria

One hundred isolates were isolated from the rhizosphere of different crops namely wheat, rice, maize, banana, clover, water grass, rose and bean. The rhizosphere of cereal crops such as wheat,

rice and maize mostly contained higher PGPR isolates rather than the rhizosphere of other investigated crops .

The isolates (one hundred) were examined qualitatively for PGRs (auxins) production by using Salkowski's reagent method . High amounts of auxins were observed with the isolates from rhizosphere of cereal crops .

Fifteen isolates among the examined isolates were highly efficient for auxins production. Therefore, these isolates were screened in subsequent experiment to select the more potent isolates.

5.2. Screening of the more potent bacterial isolates

Fifteen isolates which produced high amounts of PGR_S (auxins) production were screened by culturing on specific media.

All tested bacterial isolates (fifteen isolates) produced considerable amounts of indoles .The bacterial isolates (number 19 and 44) were the more potent for indoles production at different incubation periods.

Therefore , these two isolates were chosen to identify and use in further studies as the effect of some environmental and nutritional factors on plant growth regulators (PGR_s) production .

5.3. Identification of the more potent isolates

The two more potent isolated microorganisms were enable to produce (PGRs) were purified and subjected to detailed morphological and physiological studies .

From the morphological characters, staining properties, spore formation and physiological properties presented, it was clear that the two bacterial isolates could be identified as *Azotobacter chroococcum* and *Bacillus megaterium* var. *Phosphaticum*.

5.4. Effect of some environmental conditions on PGRs production

5.4.1. Effect of different incubation temperatures

Obtained results clearly indicated that both *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* produced considerable amounts of PGRs under different incubation temperatures.

The optimum incubation temperature for highest production of auxins, gibberellin (GA_3) and cytokinins were reduced at 32°C and 30°C for *A. chroococcum* and *B. megaterium* var. *phosphaticum*, respectively.

The recorded amount of indole butyric acid (IBA) was higher than indole acetic acid (IAA) .This result was observed for both *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum*. Kinetin was the highest recorded compound among the produced cytokinins.

Bacillus megaterium var. *phosphaticum* produced higher amounts of zeatin at all incubation temperatures compared to that produced by *Azotobacter chroococcum*. While, *Azotobacter chroococcum* produced higher amounts of cytokinins ((9R)BAP, (9G)BAP and IP) at all incubation temperatures rather than those produced by *Bacillus megaterium* var. *phosphaticum* .

5.4.2. Effect of different incubation periods

The highest amounts of phytohormones (PGRs) were obtained by *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* after four and two days , respectively.

Gibberellic acid amount produced by *Azotobacter chroococcum* was higher than that produced by *Bacillus megaterium* var. *phosphaticum.*

5.5. Effect of some nutritional factors on PGRs production

5.5.1. Effect of carbon sources

Mannitol and glucose were the best carbon sources for PGRs production by *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* , respectively. Also, the produced amounts of IBA was higher than IAA with all investigated carbon sources.

Azotobacter chroococcum produced higher amounts of zeatin and kinetin as compared to those produced by *B. megaterium* var. *phosphaticum*. While, *B. megaterium* var. *phosphaticum* produced higher amounts of (9R) benzyl adenine and (9G) benzyl adenine as compared with those produced by *Azotobacter chroococcum* .

5.5.2. Effect of DL- tryptophan concentrations

Production of auxins, gibberellic acid and cytokinins was increased with increasing the tryptophan concentration under investigation .

The highest amounts of auxins, gibberellic acid and cytokinins produced by the two strains were obtained with addition

of 10^{-3} molar tryptophan . While, the lowest amounts of PGRs were observed with addition of 10^{-8} molar tryptophan .

5.5.3. Effect of adenine concentrations

The highest amounts of PGRs produced by *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* strains were obtained when the concentration of adenine reached 10^{-5} and10^{-4} molar , respectively.

At the optimum adenine concentration *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* produced higher amounts of IBA rather than IAA. *Azotobacter chroococcum* gave higher amount of gibberellic acid rather than that given by *Bacillus megaterium* var. *phosphaticum* .

Azotobacter chroococcum gave higher values of cytokinin compounds rather than those produced by *Bacillus megaterium* var. *phosphaticum* strain at most applied concentrations of adenine.

5.6. Effect of optimal conditions on PGRs production

The optimal conditions gave the highest amounts of PGRs as compared with the other individual treatments. This result is logic and was anticipated .

Bacillus megaterium var. *phosphaticum* produced higher amounts of auxins rather than *A. chroococcum*. While, *A. chroococcum* produced higher amounts of gibberellic acid that produced by *B. megaterium* var. *phosphaticum.*

Respecting the cytokinins production , results showed that zeatin and (9R) benzyl adenine were produced with higher amounts

by *A. chroococcum* compared to the amounts produced by *B. megaterium* var. *phosphaticum* .

The second part:

5.7. Efficiency of inoculation with PGPR on tomato growth performance

and controlling the root diseases

5.7.1. Antagonistic activity of PGPR

The two tested PGPR strains showed higher suppression (inhibition) against the two pathogenic fungi i.e *Fusarium oxysporum* f.sp *lycopersici* and *Fusarium solani* .

Clear zones around PGPR growth were showed . Such clear zones are likely to be due to the production of antibiotic substances by PGPR strains (*Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum*) . Siderophores and cyanogens are the main compounds produced by most PGPR strains .

5.7.2. Effect of inoculation on damping-off and survival plants

Tomato seedlings inoculated with *B. megaterium* var. *phosphaticum* gave lower percentage of damping-off rather than the inoculated with *A. chroococcum*.

Tomato inoculation with the mixture of *A. chroococcum* and *B. megaterium* var. *phosphaticum* appeared lower percentage of damping–off than those inoculated with either *A. chroococcum* or *B. megaterium* var. *phosphaticum* individually.

The lowest percentages of survival plants of tomato were observed with the treatments of soil infested with either *F.*

oxysporum f.sp *lycopersici* or *F. solani* .Whereas , the highest percentage of survival plants was observed with the treatment of tomato inoculated with the mixture of *A. chroococcum* and *B. megaterium* var. *phosphaticum* in presence of soil infested with *F. solani*.

Un-sterilized soil treatments gave lower percentages of damping-off rather than sterilized ones .

5.7.3. Effect of inoculation on DHA activity

Tomato inoculation with the mixture of *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* gave high values of DHA as compared with individual inoculation treatments .

Tomato inoculation with PGPR (*Azotobacter chroococcum* or *Bacillus megaterium* var. *phosphaticum*) in combination with soil infestation with either *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly increased DHA compared to un-inoculated ones.

5.7.4. Effect of inoculation on phosphatase activity

Tomato inoculation with PGPR (*B. megaterium* var. *phosphaticum*) significantly increased the phosphatase activity rather than that inoculated with *A. chroococcum* .

Dual inoculation with PGPR gave significant increase in phosphatase activity rather than the individual inoculation with either *Azotobacter chroococcum* or *Bacillus megaterium* var. *phosphaticum* .

5.7.5. Effect of inoculation on nitrogenase activity .

Tomato inoculation with *Azotobacter chroococcum* only significantly increased N_2–ase activity as compared to other investigated treatments.

Also , soil infestation with either *Fusarium oxysporum* f.sp *lycopersici* or *Fusarium solani* in combination with the mixture of two studied PGPR showed higher records of N_2-ase activity than that inoculated with *Azotobacter chroococcum* only .

Higher records of N_2-ase activity in case of dual inoculation with PGPR could be attributed to the synergistic effect of both *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* .

5.7.6. Effect of inoculation with PGPR on tomato growth characters

Soil infestation with either *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly decreased the growth characters of tomato .

Growth characters of tomato were significantly increased with the inoculated treatments with PGPR compared to un-inoculated ones.

Tomato inoculation with PGPR either individually or dually combined with soil infestation with pathogenic fungi *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly increased the growth characters compared to un-inoculated treatments with PGPR .

5.7.7. Effect of inoculation with PGPR on macro-nutrients content of tomato shoots

Tomato inoculation with the mixture of PGPR significantly increased the macro-nutrients content (N, P and K) of tomato shoots compared to the individual inoculation with either *Azotobacter chroococcum* .or *Bacillus megaterium* var. *phosphaticum*.

Soil infestation with either *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly decreased the macro-nutrients content of tomato shoots .

5.7.8. Effect of inoculation with PGPR on endogenous phytohormones

Inoculation of tomato with the mixture of *Azotobacter chroococcum* and *Bacillus megaterium* var. *phosphaticum* gave higher records of endogenous phytohormones rather than the individual inoculation with either *Azotobacter chroococcum* or *Bacillus megaterium* var. *Phosphaticum* .

Soil infestation with either *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly decreased the phytohormones in tomato leaves.

5.7.9. Effect of inoculation with PGPR on photosynthetic pigments

Soil infestation with either *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly decreased the photosynthetic pigments (chlorophyll a , b and carotenoids) in tomato leaves .

Soil infestation with the pathogenic fungi in presence of tomato inoculation with PGPR significantly increased the photosynthetic pigments in tomato leaves compared to soil infestation with pathogenic fungi in absence of tomato inoculation with PGPR .

5.7.10. Effect of inoculation with PGPR on peroxidase and polyphenol oxidase

Soil infestation with either *F. oxysporum* f.sp *lycopersici* or *F. solani* significantly decreased the peroxidase and polyphenol oxidase activities in tomato plants. Tomato inoculation with PGPR significantly increased the peroxidase and polyphenol oxidase activities in tomato plants compared to the un-inoculated ones .

Tomato inoculation with PGPR combined with soil infestation with pathogenic fungi significantly increased the activity of peroxidase and polyphenol oxidase as compared to soil infestation with pathogenic fungi only.

5.7.11. Effect of inoculation with PGPR on N and P content in tomato rhizosphere

Rhizosphere of tomato infested with either *F. oxysporum* f.sp *lycopersici* or *F. solani* showed low values of N and P .

Tomato inoculation with the mixture of the two studied strains significantly increased the available macro-nutrients content (N and P) in soil as compared with the inoculation by each strain individually.

The highest records of available N and P in tomato rhizosphere were observed with the treatment of tomato inoculation

with the mixture of the two studied strains in presence of either *F. oxysporum* f.sp *lycopersici* or *F. solani*.

In view of the obtained results, it was clearly that the soils of Egypt are rich in plant growth promoting rhizobacteria, which are able to produce sensible amounts of auxins, gibberellins and cytokinins.

Results also indicated that these microorganisms have inhibition effect on plant pathogenic fungi , especially root rot and wilt fungi. Antagonistic activity of PGPR is likely to be due to the ability of these microorganisms to produce antifungal agents such as siderophores and hydrogen cyanide.

Moreover, obtained results of this study indicated that the inoculation with PGPR enhanced plant growth. Beneficial effects of these microorganisms could be attributed to their ability to fix atmospheric nitrogen or phosphate solubilizing besides their ability to produce plant growth promoting regulators and to antagonize pathogenic fungi .

Summing up, the obtained results from the current study could be recommended that the inoculation with PGPR can be used as alternative of chemical control. Since, the application of fungicides will be reduced , and consequently the hazardous effects on human and environment will avoided or reduced.