

CONTENTS

	Page
1. INTRODUCTION.....	1
2. REVIEW OF LITERATURE.....	4
2.1. Role of phosphorus in plants.....	4
2.2. Amount and forms of phosphorus in soil.....	4
2.3. Effect of inoculating phosphate dissolving microorganisms on leguminous crops.....	6
2.4. Effect of the interaction between phosphate dissolving microorganisms and N ₂ -fixers on leguminous crops.....	8
2.5. Effect of inoculating phosphate dissolving microorganisms on cereal crops.....	11
2.6.. Effect of the interaction between phosphate dissolving microorganisms and nitrogen fixers on cereals crops.....	13
3. MATERIALS AND METHODS.....	16
3.1. Materials.....	16
3.1.1. Soil samples.....	16
3.1.2. Planting.....	16
3.1.3. Microbial inoculum.....	20
3.1.4. Cultivation media used.....	20
3.1.4.1. Modified Bunt and Rovira medium (Abdel-Hafez, 1966).....	20
3.1.4.2. Soil extract agar medium (Allen, 1959).....	21
3.1.4.3. Yeast extract-mannitol agar medium (Allen, 1959).....	21
3.1.4.4. Abdel-Malek and Ishac (1968).....	21
3.1.4.5. Döbereiner medium (1978).....	22
3.2. Methods.....	23
3.2.1. Soil analysis.....	23
3.2.1.1. Soil particle size determination.....	23
3.2.1.2. Organic carbon determination.....	23
3.2.1.3. Exchangeable bases estimation.....	23
3.2.1.4. Electrical conductivity determination.....	23
Layout of pot experiments.....	23
3.2.2. Plant measurements.....	23
3.2.2.1. Plant growth.....	23
3.2.2.1.1. Plant height.....	23
3.2.2.1.2. Number of tillers.....	23
3.2.2.1.3. Spike length.....	24

	Page
3.2.2.2. Yield and yield components.....	24
3.2.2.2.1. Grain and seed yield.....	24
3.2.2.2.2. Straw yield.....	24
3.2.2.2.3. Grain and seed index.....	24
3.2.3. Plant analysis.....	25
3.2.3.1. Determination of nitrogen content.....	25
3.2.3.2. Determination of phosphorus content.....	25
3.2.3.3. Determination of potassium.....	25
3.2.4. Microbiological measurements.....	25
3.2.4.1. Preparation of the cultural medium.....	25
3.2.4.2. Inoculum preparation.....	26
3.2.4.3. Seeds and grains inoculation.....	26
3.2.4.4. Determination of total viable count.....	26
3.2.4.5. Determination of phosphate dissolving bacteria.....	26
3.2.4.6. Determination of symbiotic N-fixing bacteria.....	26
3.2.4.7. Determination of non-symbiotic N-fixing bacteria.....	27
II.4.7.1. Measurement of <i>Azotobacter spp.</i>	27
II.4.7.2. Measurement of <i>Azospirillum spp.</i>	27
3.3. Statistical analysis.....	27
4. RESULTS AND DISCUSSION.....	28
4.1. Faba bean plant.....	28
4.1.1. Microbiological values of faba bean rhizosphere as affected by inoculation with <i>Bacillus megatherium</i> var. <i>phosphaticum</i> and fertilization with P ₂ O ₅	28
4.1.1.1. Pot experiments.....	28
4.1.1.1.1. The count of phosphate dissolving bacteria.....	28
4.1.1.1.2. The count of total viable bacteria.....	31
4.1.1.1.3. The count of <i>Rhizobium spp.</i>	31
4.1.1.1.4. The count of <i>Azotobacter spp.</i>	35
4.1.1.1.5. The count of <i>Azospirillum spp.</i>	38
4.1.1.2. Field experiments.....	41
4.1.1.2.1. The count of phosphate dissolving bacteria.....	41
4.1.1.2.2. The count of total viable bacteria.....	44
4.1.1.2.3. The count of <i>Rhizobium spp.</i>	47
4.1.1.2.4. The count of <i>Azotobacter spp.</i>	47
4.1.1.2.5. The count of <i>Azospirillum spp.</i>	52

	Page
4.1.2. Growth and yield of faba bean plant as affected by inoculation with <i>Bacillus megatherium</i> var. <i>phosphaticum</i> and fertilization with P ₂ O ₅	56
4.1.2.1. Pot experiments	56
4.1.2.1.1. Plant height and number of tillers	56
4.1.2.1.2. Yield and yield components	63
4.1.2.2. Field experiments	66
4.1.2.2.1. Plant height and number of tillers	66
4.1.2.2.2. Yield and yield components	69
4.1.3. Values of NPK of faba bean as affected by inoculation with <i>Bacillus megatherium</i> var. <i>phosphaticum</i> and fertilization with P ₂ O ₅	74
4.1.3.1. Pot experiments	74
4.1.3.1.1. Nitrogen content	74
4.1.3.1.2. Phosphorus content:	78
4.1.3.1.3. Potassium content:	82
4.1.3.2. Field experiments:	85
4.1.3.2.1. Nitrogen content	85
4.1.3.2.2. Phosphorus content	89
4.1.3.2.3. Potassium content	92
4.2. Wheat plant	97
4.2.1. Microbiological values of wheat rhizosphere as affected by inoculation with <i>Bacillus megatherium</i> var. <i>phosphaticum</i> and fertilization with P ₂ O ₅	97
4.2.1.1. Pot experiments	97
4.2.1.1.1. The count of phosphate dissolving bacteria	97
4.2.1.1.2. The count of total viable bacteria	100
4.2.1.1.3. The count of <i>Rhizobium</i> spp.	102
4.2.1.1.4. The count of <i>Azotobacter</i> spp.	102
4.2.1.1.5. The count of <i>Azospirillum</i> spp.	107
4.2.1.2. Field experiments	110
4.2.1.2.1. The count of phosphate dissolving bacteria	112
4.2.1.2.2. The count of total viable bacteria	112
4.2.1.2.3. The count of <i>Rhizobium</i> spp.	116
4.2.1.2.4. The count of <i>Azotobacter</i> spp.	120
4.2.1.2.5. The count of <i>Azospirillum</i> spp.	120

	Page
4.2.2. Growth and yield of wheat plant as affected by inoculation with <i>Bacillus megatherium</i> var. <i>phosphaticum</i> and fertilization with P ₂ O ₅	124
4.2.2.1. Pot experiments	124
4.2.2.1.1. Plant height and number of tillers.....	124
4.2.2.1.2. Yield and yield components.....	129
4.2.2.2. Field experiments	133
4.2.2.2.1. Plant height and number of tillers.....	133
4.2.2.2.2. Yield and yield components.....	137
4.2.3. Values of NPK of wheat as affected by inoculation with <i>Bacillus megatherium</i> var. <i>phosphaticum</i> and fertilization with P ₂ O ₅	142
4.2.3.1. Pot experiments	142
4.2.3.1.1. Nitrogen content.....	142
4.2.3.1.2. Phosphorus content	145
4.2.3.1.3. Potassium content.....	148
4.2.3.2. Field experiment.....	154
4.2.3.2.1. Nitrogen content.....	154
4.2.3.2.2. Phosphorous content	154
4.2.3.2.3. Potassium content.....	157
5. SUMMARY AND CONCLUSION.....	161
6. REFERENCES.....	172
ARABIC SUMMARY	

5. SUMMARY AND CONCLUSION

The effect of soil inoculation with phosphate dissolving bacteria, *Bacillus megatherium* var. *phosphaticum* was studied in pots and field experiments under different levels of P_2O_5 / fed such as 0, 5, 10, 15, 20 and 25 for faba bean and 0, 5, 10, 15 and 20 P_2O_5 / fed with wheat. Clayey soils were used for cultivation of faba bean seeds variety improved Giza 3 and wheat grains cultivars Sakha 69. The cultivation of these experiments was conducted during the two successive seasons of 1998/1999 and 1999/2000. Soil microbial densities, P-dissolving bacteria, total bacterial count, *Rhizobium spp.*, *Azotobacter spp.* and *Azospirillum spp.* were periodically determined during different plant growth periods. Growth features of both tested plants, yield and yield components and their content of N, P and K were also determined during different plant growth periods.

The obtained results can be summarized in the following:

I. Faba bean (*Vicia faba*, L.):

I.1. Microbiological measurements:

I.1.1. Pot experiments:

The count of bacterial populations was increased from 72.5×10^3 to 123.8×10^4 when using 25 kg P_2O_5 /fed for phosphate dissolving bacteria in case of uninoculation and inoculated case, respectively.

For the total viable bacterial count in rhizosphere increased to reach the maximum value of 110×10^6 cfu/g under inoculation at the 2nd month when using 10 and 25 kg P_2O_5 / fed. These values were 92 and 105×10^5 cfu/g under uninoculation.

In case of *Rhizobium spp.*, a considerable increase in their mean densities was observed. The increases recorded were from 39.17×10^3 cfu/g in uninoculated soil to 63.3×10^5 cfu/g in case of inoculated case.

The counts of *Azotobacter spp.* in rhizosphere of faba bean plant increased to reach the maximum value being 95×10^3 cfu/g soil in case of inoculation at the 2nd month and fertilized with 20 and 25 kg P₂O₅/fed. These values were 68 and 64×10^2 cfu/g in case of uninoculation.

Regarding the count of *Azospirillum spp.*, the maximum value of densities was found to be 93×10^3 cfu/g at the 2nd month of inoculated treatment and fertilized with 15 and 25 kg P₂O₅/fed. These values were 52 and 64×10^2 cfu/g in case of uninoculated treatment.

I.1. 2. Field experiments:

The mean value of P-dissolving bacterial count increased from 39.8×10^3 cfu/g unfertilized soil to reach 57.5×10^3 cfu/g soil fertilized with 25 kg P₂O₅ / fed in case of uninoculation. These values became 53.0×10^4 and 70.5×10^4 with inoculated treatment and the same conditions of fertilization.

The lowest total counts was found to be 30×10^5 cfu/g were obtained under uninoculated treatments at the 1st and 4th month in case of 0.0 and 5 kg of P₂O₅/fed. The highest value in case of inoculation was 66×10^6 cfu after the 2nd month with 20 kg P₂O₅/fed.

At the later stages, the counts of *Rhizobium spp.* sharply dropped and reached their lowest values being 14×10^3 cfu/g at the 4th month without P addition and uninoculated treatments. After the 2nd month when using 25 kg P₂O₅/fed, the count was 105 cfu/g of inoculated soil.

For *Azotobacter spp.*, the mean count was increased from 27.5×10^2 cfu/g without P to reach 30.8×10^2 cfu/g by using 25 kg P₂O₅ / fed. These values became 37.0 and 46.3×10^3 in case of inoculation.

Regarding the count of *Azospirillum spp.*, at the later month, the counts sharply dropped and reached their lowest values of 11×10^2 cfu/g at the 4th month without P addition and uninoculated treatments. In case of inoculation, the highest values were 80×10^3 cfu/g soil using 20 and 25 kg P₂O₅/fed.

I.2. Faba bean features:

I.2.1. Pot experiments:

Growth characters:

Obtained results showed that plant height was increased with P-dissolving bacteria. The highest value of plant height was 108.88 cm in the 1st season, while it was 107.25 cm in the 2nd season. Both of these values were recorded in case of using 25 kg P₂O₅/fed and bacterial inoculation. These values were 105.63 and 105.0 cm in the 1st and 2nd seasons in case of uninoculated treatment..

Also, the number of tillers / plant were increased in both growing seasons. The number of tillers / plant were increased to reach the highest values at the level of 25 kg P₂O₅/fed either in case of inoculation or uninoculation one.

Yield and yield components:

Obtained data showed that the number of seeds in pod and 100-seed weight were gradually increased with bacterial inoculation. The maximum value of 100-seed weight was found to be 61.00 and 63.72 g in case of uninoculation in the 1st and 2nd seasons, respectively. These values were 63.13 and 64.60 g in the 1st and 2nd season for inoculation when using 25 and 20 kg P₂O₅/fed.

The highest value of seed yield was achieved when using 25 kg P₂O₅ and without P-dissolvers to be 36.4 g/pot and 42.9 in the 1st season and in the 2nd season, respectively. These values were 40.47 and 44.10 in case of inoculation in 1st and 2nd seasons, respectively. The same trend was obtained with straw yield. The mean value of seed yield in the two seasons is 42.29 g/pot in case of inoculated treatment.

I.2.2. Field experiments:

Growth characters:

Data showed that the plant height was significantly increased with P-dissolving bacterial inoculation. The mean values of plant height were 106.2 and 112.5 cm in the 1st season in case of uninoculation and inoculated case, respectively. While these values were 104.0 and 106.7 cm in the 2nd season for uninoculated and inoculated treatments, respectively.

Regarding the number of tillers / plant, obtained values were significantly increased at 25 kg P₂O₅/fed. The values of tillers numbers / plant under the inoculation were increased from 3.75 without P₂O₅ addition to 4.75 at 25 kg P₂O₅/fed for both seasons. These values were 2.50, 2.75 and 3.75, 4.00 without inoculation for the 1st and the 2nd seasons, respectively.

Yield and yield components:

The highest value of pod seeds was recorded with 20 kg P₂O₅/fed being 3.48 in the 1st season, while it is 4.10 in the 2nd season when using 25 kg P₂O₅/fed without bacterial inoculation. These values became 4.10 and 4.34 in case of inoculation for the 1st and the 2nd seasons, respectively.

Concerning the 100-seeds weight as affected by the phosphorous application under the inoculation treatments, the values of 100-seed weight were gradually increased in case of bacterial inoculation over the other case of uninoculation.

Seed and straw yields were significantly affected by the inoculation under the used phosphorus levels.

I.3. Values of NPK of faba bean:

I.3.1. Pot experiments:

Nitrogen:

In case of the bacterial inoculation, the lowest values of N % were found to be 3.5, 3.8, and 0.4 without P-fertilization in case of uninoculation for shoots, seeds and straw, respectively. The highest values of N content were achieved with the treatments of 25 kg P₂O₅/fed and seed inoculation with phosphate solubilizing bacteria being 3.8, 4.2 and 0.5 for shoots, seeds and straw, respectively.

Phosphorus:

Concerning the P-content, the highest values of P% without inoculation were 0.49, 0.61 and 0.17 when using 25 kg P₂O₅/fed for shoots, seeds and straw in the 1st season, respectively. In case of inoculation, P% values were 0.53, 0.63, 0.54 and 0.19 when using the phosphate dissolving bacteria with 25 kg P₂O₅/fed in the first season for shoots, seeds and straw, respectively.

Potassium:

For measured values of K, the highest values of K% were found in the treatment of 25 kg P₂O₅/fed. The lowest values of K% content were 3.6, 0.61 and 1.14 were recorded in the 2nd season without phosphorous

fertilization and uninoculation with P-dissolving bacteria. The highest mean values of K% were achieved due to applying 25 kg P₂O₅/fed and without seed inoculation to be 3.8, 0.86 and 1.32 for shoots, seeds and straw in the 2nd season, respectively. These values were 3.8, 0.9 and 1.34%, when the treatments having 25 kg P₂O₅/fed in case of inoculated treatment in the 2nd season, respectively.

I.3.2. Field experiments:

Nitrogen:

For nitrogen content, the highest values of N% were found to be 3.98, 4.13 and 0.68 in case of using 25 kg P₂O₅/fed in the 1st season without inoculation for shoots, seeds and straw, respectively. The bacterial inoculation increased the N-content for all studied plant organs in both seasons. These values became 4.02, 4.21 and 0.72 for shoots, seeds and straw under inoculation in the 1st season.

Phosphorus:

Concerning the phosphorus content, the highest values of P% were achieved under the treatments of 25 kg P₂O₅/fed without seed inoculation with P-solubilizing bacteria being 0.43, 0.52, and 0.14 in the second season for faba bean shoots, seeds and straw, respectively. These values became 0.46, 0.53 and 0.15 under inoculation in the 2nd season for the above mentioned parameters.

Potassium:

Regarding the potassium content, the highest values of K% were achieved under the treatments of using 25 kg P₂O₅/fed without seed inoculation. These values were determined to be 4.4, 0.65, and 1.52 for shoots, seeds and straw, respectively. These values increased to 4.46, 0.67 and 1.54 under inoculation in the 1st season.

II. Wheat (*Triticum aestivum* L.):

II.1. Microbiological measurements:

II.1.1. Pot experiments:

Data showed that the count of P-dissolving bacteria in rhizosphere of wheat plant increased to reach the maximum value being 120×10^4 cfu/g. This in case of bacterial inoculation with 15 kg P_2O_5 /fed at the 2nd month. The lower count (30×10^2 cfu/g) was recorded in case of uninoculated treatment without P-fertilization at the 1st month.

The total colony counts also increased to reach the maximum value being 130×10^5 cfu/g under inoculation treatments and using 20 kg P_2O_5 /fed after the 2nd month. It is worthy to note that the inoculation with P-dissolving bacteria leads to increase the number of other bacterial groups in plant rhizosphere.

The increases of *Rhizobium spp.* mean values recorded were from 32.4×10^2 cfu/g of uninoculated soil to 58.1×10^4 cfu/g of inoculated soil. The maximum value of *Rhizobium spp.* density was found to be 95×10^4 cfu/g soil was observed after the 2nd month of inoculated treatments and 20 kg P_2O_5 /fed.

The counts of *Azotobacter spp.* in wheat rhizosphere increased to reach the maximum value of 70×10^3 cfu/g soil under inoculation case after the 2nd month with 20 kg P_2O_5 /fed, while it was 25×10^2 cfu/g soil of uninoculated treatment.

The maximum value of *Azospirillum spp.* density of 65.0×10^3 cfu/g soil was observed after the 2nd month of inoculated treatment with 20 kg P_2O_5 /fed treatment. This value was 40×10^2 cfu/g uninoculated case. At the later months of uninoculated treatments, the counts of

Azospirillum spp. reached their lowest values of 10×10^2 cfu/g soil after the 4th month without P addition.

II.1.2. Field experiments:

The P-dissolving bacteria in wheat rhizosphere increased to reach the maximum value of 85×10^4 cfu/g soil under inoculation condition after the 2nd month. This value was 70×10^2 cfu/g uninoculated treatment. On the other hand, the lowest counts was found to be 30.0×10^2 cfu/g soil in case of uninoculated treatments after the 1st and 4th month.

The count of total viable bacteria in wheat rhizosphere increased to reach the maximum values of 70.0×10^5 cfu/g soil under inoculation conditions after the 2nd month with 20 kg P_2O_5 /fed. The lowest counts were found to be 30.6×10^4 cfu/soil in case of uninoculated treatments after the 1st month.

In case of uninoculation, the count of *Rhizobium spp.* reach its maximum value after the 2nd month of planting with 20 kg of P_2O_5 /fed. This value was found to be 65×10^2 cfu/g soil. The counter part number in case of inoculation experiments was 80×10^4 under the same conditions.

In wheat rhizosphere, the counts of *Azotobacter spp.* increased to reach the maximum value of 83×10^3 cfu/g soil fertilized with 15 kg P_2O_5 /fed under inoculation condition after the 2nd month. This value was 40×10^2 cfu/g uninoculated case under the same conditions

The mean increases under zero P_2O_5 were found to be from 17.5×10^2 cfu/g of uninoculated soil to 34.3×10^3 cfu/g of inoculated soil. The maximum value of *Azospirillum spp.* densities was 78×10^3 cfu/g soil,

which was observed after the 2nd month of inoculated and 10 kg P₂O₅/fed. While, it was 35 x 10² cfu/g of uninoculated soil.

II.2. Wheat features:

II.2.1. Pot experiments:

Growth characters:

Data showed that plant height was greatly affected by the bacterial inoculation. By using *Bacillus megatherium* var. *phosphaticum*, the maximum values of plant height were achieved with 20 kg P₂O₅/fed being 101.75 and 101.38 cm in the 1st and 2nd seasons, respectively. These values were 97.5 and 94.3 cm in case of uninoculated case.

Regarding the number of tillers as affected by inoculation of wheat, data showed that the number of tillers greatly affected. Since they moved from 4.0 and 4.3 to 5.4 and 4.7 in case of uninoculated and inoculated case and from 1st and 2nd seasons, respectively.

Yield and yield components:

The highest value of spike length (cm), spike weight (g), 1000-grain weight (g), grain yield (g/pot) and straw yield (g/pot) were found when using 20 kg of P₂O₅/fed. By using bacterial inoculation, the obtained values in both seasons are 9.57, 9.60; 2.23, 2.02; 42.11, 42.68; 61.28, 61.38; and 103.13, 123.25 for above mentioned parameters for the 1st and the 2nd seasons, respectively,.

II.2.2. Field experiments:

Growth characters:

In case of inoculation, the increase of P₂O₅ leads to increase the height of wheat plant to the maximum value to be 104.75 cm for both cultivated seasons in case of inoculated treatment. This value was 101.3 in case of uninoculation.

For the number of tillers / plant, obtained values were 5.15 and 5.60 for the two cultivated seasons with bacterial inoculation, while these values were 4.3 and 4.8 without bacterial inoculation and 20 kg P₂O₅/fed..

Yield and yield components:

The obtained values of the spike weight (g) is 2.34g when using 20 kg P₂O₅/fed and bacterial inoculation, The other values were 43.4, 2.5 and 5.3 for 1000-grain weight (g), grain yield (ton/fed) and straw yield (ton/fed) in the 1st season, respectively.

II.3. Values of NPK of wheat:

II.3.1. Pot experiments:

Nitrogen:

The bacterial inoculation maximized the N content under all the P₂O₅ levels. The obtained values were 2.17, 2.19; 2.49, 2.50 and 0.43, 0.41 for wheat shoots in boating stage, grain and straw respectively when using 20 kg P₂O₅ /fed in the 1st and 2nd season. These values were 2.06, 2.03; 2.40, 2.40 and 0.40, 0.39 for the above mentioned parameters and the same conditions.

Phosphorus:

The obtained values of P-content increased with bacterial inoculation to be 0.43, 0.44; 0.35, 0.36 and 0.128, 0.133 in the two seasons for wheat shoots at boating stage, grain and straw at maturity, respectively. These values were 0.40, 0.41; 0.36, 0.33 and 0.13, 0.13 in case of uninoculated treatment for the above mentioned parameters.

Potassium:

The values obtained of uninoculated treatment and 20 kg P₂O₅/fed were 2.73, 2.70; 0.67, 0.69 and 1.56, 1.57 in the two seasons for wheat

shoots at boating stage, grain and straw at maturity, respectively. These values little increased in case of inoculation to be 2.75, 2.78; 0.70, 0.71 and 1.61, 1.61 in the 1st and 2nd season for the above mentioned parameters.

II.3.2. Field experiments:

Nitrogen:

Data obtained showed that the bacterial inoculation increased the N content in plant parts. The values were 1.97, 2.42; 2.16, 2.35 and 0.40, 0.39 in case of bacterial inoculation with 20 kg P₂O₅/fed for shoots at boating stage, grain and straw at maturity in the 1st and 2nd seasons, respectively. These values were 1.93, 2.38; 2.04, 2.29 and 0.38, 0.343 without bacterial inoculation and 20 kg P₂O₅/fed.

Phosphorous:

The values of phosphorus were 0.37, 0.43; 0.35, 0.40 and 0.12, 0.143 in the 1st and 2nd seasons without inoculation and 20 kg P₂O₅/fed for wheat shoots at boating stage, grain and straw at maturity, respectively. These values become 0.398, 0.435; 0.355, 0.418 and 0.125, 0.153 in the 1st and 2nd seasons in case of bacterial inoculation for the above mentioned parameters.

Potassium:

The values of K% increased due to inoculation with P-dissolving to be 2.18, 2.33; 0.82, 0.89 and 1.23, 1.46 in the 1st and 2nd seasons and used 20 kg P₂O₅/fed for shoots at boating stage, grain and straw at maturity, respectively. These values were 2.01, 2.25; 0.755, 0.875 and 1.16, 1.42 for the above mentioned parameters under the same conditions.

CONCLUSION

It can be concluded from the obtained results that the importance of using the biofertilizers specially the phosphate dissolving bacteria which is known as phosphobacterin.

The use of such this biofertilizer leads to increase in the yield and yield components, NPK uptake and the total count of different bacterial groups in rihizosphere such as p-dissolving bacteria, *Rhizobium spp*, *Azotobacter spp*, *Azospirillum spp* and the total viable bacteria as well.

The above mentioned increases of examined parameters may be to the increase of soluble phosphorus available to cultivated plants and/or producing some growth promoters by p-dissolving bacteria and subsequently by the other microbes increased also in plant rhizosphere.