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EFFECT OF MICROBIAL INOCULATION ON REDUCTION OF COWPEA (*Vigna unguiculata*, L. Walp) CHEMICAL FERTILIZERS UNDER NEWLY RECLAIMED SOILS CONDITION IN EGYPT

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ABSTRACT

This research was conducted during the two summer seasons of 2009 and 2010 at South Tahrir, Experimental Farm of Horticulture Research Institute, Agriculture Research Center (A R C), Beheira, Governorate, Egypt. This study carried out to evaluate the possibility of using different rates of biofertilizer instead a part of chemical fertilizers for optimal nutrition and some attributes of cowpea. The chemical fertilizers were as four levels of recommended fertilization doses of N, P and K fertilizers i.e. 0, 50, 75 and 100 %/ fed. Five treatments of microbial inoculations (biofertilizer) i.e. without microbial inoculation (B0), inoculations with *Azospirillum* sp. and *Trichoderma* sp. for nitrogen (B1), *Bacillus megaterium* for phosphorus (B2), *Pseudomonas fluorescens* for potassium (B3) and the mixtures of all previous of microbial inoculations (B4).

The obtained results indicated that there were gradually significant increases due to fertilize cowpea plants with 75 % from the recommended fertilization on various obtained characters. Also, treating seeds of cowpea with the mixtures of all microbial inoculations of *Azospirillum* sp. and *Trichoderma* sp., as well as *Bacillus megaterium* and *Pseudomonas fluorescens* markedly enhanced effect on cowpea growth, total dry seed yield (ton/ fed.) and pod characters, nitrogen (%), phosphorus (%), potassium (%) content in leaves as well as protein (%) content in seeds.

The interactions between application of the 75 % recommended fertilization and treating cowpea seeds by mixtures of all microbial inoculations helped in producing the most pronounced effects of all vegetative growth characters, total dry seed yield (ton/ fed.), pod characters, nitrogen, phosphorus, potassium content (%) in leaves and protein content (%) in seeds. Seed inoculations with individual *Azospirillum* sp. and *Trichoderma* sp. obtained the superiority treatment for vegetative growth characters and nitrogen (%) in leaves and protein content (%) in seeds. Seed inoculations with individual *Bacillus megaterium* and individual *Pseudomonas fluorescens* obtained the most effective treatment for total dry seed yield (ton/ fed.), pod characters, phosphorus (%) and potassium content (%) in leaves, respectively when compared to the control untreated plants.

INTRODUCTION

Cowpea (*Vigna unguiculata*, L. Walp) is a member of family fabace and considered as one of the important vegetable crops in Egypt, seeds represent a chief source of protein and carbohydrates content. Also, cowpea it can grow easily in the new reclaimed lands. The protein in cowpea seed is rich in amino acids, lysine and tryptophan compared to cereal grains (Santos, 2000). Nutrients are required by plant in adequate quantities for metabolic regulation, production of new tissues as well as development. They are structural components of metabolic and prototoplasm structure (Adelusi and Aileme, 2006).

Plant nutrition is one of the most important factors that increase plant production. Nitrogen is one of the major elements for plants growth and development that has an important role in plant nutrition and therefore is the yield-limiting factor for plant growth in many areas especially in low organic soils. Nitrogen is required in large amounts for plant growth, since it is the basic constituent of many chemical compounds, including proteins and nucleic acids (Rashid *et al.*, 1999 and Najafva *et al*., 2008). In this connection, El-Bably and El-Waraky (2006) suggested that application of N fertilizers increased vegetative growth character as well as yield and its components of cowpea plant fertilized with 30 Kg/ fed. produced the greatest pod yield. On the other hand, Abayomi *et al.* (2008) stated that phosphorus does not only increase seed yields but also nodulation and thus N fixation. Phosphorus application influences the contents of other nutrients in cowpea leaves and seeds (Kang and Nangju, 1983). Phosphorus has also been reported to increase the number of leaves and fruits per plant, as well as earliness of flowering and yields. However, inorganic P-fertilizers are often expensive and not readily available to resource – poor farmers.

Furthermore, P-fertilizer can be fixed into unavailable forms to plants such as Fe and Al oxides in tropical soils (Sample *et al*., 1980). Application of inorganic P fertilizers may thus not be the most viable option to alleviate P – deficiency for improving cowpea production. Effects of P on nodulation (Ankomah *et al*., 1995) and yield (Sanginga *et al*., 2000) of cowpea have been previously reported to vary with genotypic. Moreover, plants fertilized with high levels of phosphorus survived successive periods of drought. These took root growth that allowed them to explore the large amount of water existing in the root zone in addition to possessing a greater capacity to extract water due to greater hydraulic conductivity of root (Chiulele, 2003). Sivak and Walker (1986) mentioned that phosphorus application adds the assimilative capacity of the plants by increasing the leaf area or photosynthetic activity, ensuring an effect on the roots. Benton (1998) recorded that potassium is required for the accumulation and translocation of newly formed carbohydrates. Potassium plays a key role, since it is involved in metabolic processes, such as the synthesis of proteins, enzyme activation, membrane transport processes, charge balance and the generation of turgor pressure (Dorais *et al.,* 2001 and Achilea *et al*. 1999) working on tomato plants, recorded that, potassium is found a free state in cell vacuole, so it plays a great role in water absorption and movement within the plant, consequently, affects cell turgidity as well as leaf expansion, also, it affects carbohydrates biosynthesis and mobilization from part to another.

In the last decade biofertilizers were used extensively as an eco-friendly approach to minimize the use of chemical fertilizers, improve soil fertility status and enhance crop production by their biological activity in the rhizosphere. Bio-organic farming today has become of great importance for sustainable agriculture, limiting deterioration of the agricultural lands and environment, producing safer crop products for human and animal consumption and increasing possibility of biological control of harmful insects and soil-borne pathogens (Gomaa and Abou-Aly, 2001). These microorganisms are able to promote proper circulation of plant nutrients, which are capable of mobilizing important nutritional elements in the soil from non-usable to usable form by the crop plants through their biological processes and reduce the need for chemical fertilizers. Biological nitrogen fixation is a key factor to sustain agricultural productivity. Increasing crop yield cannot be fulfilled unless we carefully make use of biological nitrogen fixation. Since the nodulation is solely a physiological process, it can be induced by using different plant growth regulators. In this process nitrogenfixing bacteria enter plant through its root hairs where the nodules will be formed. These nodules can provide a niche for the bacteria to fix atmospheric nitrogen. They not only fix atmospheric nitrogen but also produce certain plant growth promoting hormones (Frankenberger and Arshad, 1995). These bacteria belong to several genera including *Azotobacter*, *Azospirillum*, *Bacillus*, *Arthrobacter*, *Enterobacter*, *Pseudomonas*, *Alcaligenes* and *Klebsiella* *Azotobacter* has been reported to fix not only atmospheric nitrogen but also produce plant growth regulators which antagonize plant pathogens *Azotobacter* is a free living nitrogen-fixing bacterium. It is used as a biofertilizer in growing many crop plants.

In this connection, phosphate solubilizing microorganisms play a key role in the plant metabolism and crop productivity. They have been reported to increase the availability and uptake of native soil phosphorus by converting insoluble phosphates to soluble forms by producing various organic acids (Raja *et al*., 2002). Free living nitrogen fixing bacteria such as *Azotobacter* and *Azospirillum* have the ability not only to fix nitrogen but also to release certain phytohormons of GA_3, IAA and cytokinins which increased the surface area per unit root length and enhanced root hair branching with an eventual increase in the uptake of nutrients from soil nature which could stimulate plant growth, absorption of nutrients and photosynthesis process (Abdel-Latif *et al*., 2001).

This research was conducted to study the possibility of using different kinds of biofertilizers, instead of a part of chemical fertilizers and environmental pollution for optimal nutrition of cowpea and its some attributes. Also, to integrating chemical fertilizers levels of N, P, and K with 75 % from the recommended fertilization and microbial inoculations in both individually or in combination has been found to be quite promising not only in maintaining higher growth and productivity but also in improving pod characters and quality.

MATERIALS AND METHODS

The experiment was carried out at South Tahrir, Experimental Farm of Horticulture Research Institute, Agriculture Research Center (A R C), Beheira, Governorate, Egypt during the two summer seasons of 2009 and 2010.

Soil samples were taken from 30 cm soil surface to determine the physical and chemical properties of the experimental soil presented in Table 1.

Table 1: Physical and chemical properties of the experimental soil during the two lat summer seasons 2009 and 2010 seasons.

Seasons	Physical properties				Chemical properties									
	Sand (%)	Silt (%)	Clay (%)	Texture	EC dSm^{-1}	PH	Soluble cation (meg $^{1-1}$)				Soluble anion (meg $^{1-1}$)			Total N %
							P^{++}	Co^{++}	Mg^{++}	K^{+}	Hco_3^{-}	Cl^{-}	So_4^{-}	
2009	94	4	2	Sand	0.21	8.9	0.16	0.72	0.57	0.20	0.55	0.42	0.57	0.010
2010	92	5	3	Sand	0.17	8.8	0.15	0.75	0.54	0.19	0.57	0.43	0.56	0.008

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Layout of the experiment and treatments:

The experiment contained 20 treatments, were combination between NPK levels and some biofertilizer treatments as follows:

A. N, P and K levels:

1- 0 % NPK.
2- 50 % recommended dose NPK Kg /fed.
3- 75 % recommended dose NPK Kg /fed.
4- 100 % recommended dose NPK Kg /fed.

B. Biofertilizer treatments:

1-B0 = uninoculated treatment (control).
2-B1 = *Azospirillum* sp. and *Trichoderma* sp. for nitrogen.
3-B2 = *Bacillus megaterium* for phosphorus.
4-B3 = *Pseudomonas fluorescens* for potassium.
5-B4 = mixtures of all previous.

These treatments were arranged in split plot design with three replications. N, P and K levels treatments were assigned at random in the main plots, while sub plots were devoted to biofertilizer treatments. Nitrogen fertilizer application added as a form of ammonium sulphate (20.6 % N) half of the recommended dose of nitrogen fertilizer added at three weeks after sowing and the equal dose after two weeks from the previous one. Phosphorus fertilizer application added as a form of superphosphate (15.5 % P_2O_5). Potassium fertilizer application added as a form of potassium sulfate (50 % K_2O). Both phosphorus and potassium fertilizers application added (as a one dose) before sowing. The recommended fertilization doses used as (61.8 Kg N, 31 Kg P_2O_5 and 37.5 Kg K_2O / fed.). The experimental unite area was 15 m^2 and contain 3 drippers lines, with 5 m length and 1 m width. Cowpea seeds (cv. Kaha-1) were sown on 7th and 11th of April in 2009 and 2010, respectively in rows at both sides at 20 cm distance between hills. Two microbial inoculated seeds were sown and covered by sand soil in a hill using drip irrigation system. All other agricultural practices were conducted for this region as recommended by the Ministry of Agriculture in Egypt whenever were necessary.

Inoculation preparation:

The Bacterial strains *Azospirillum* sp. and *Trichoderma* sp. (B1) for nitrogen, *Bacillus megaterium* (B2) for phosphorus and *Pseudomonas fluorescens* (B3) for potassium were kindly obtained from the Organic Agric. Research Unit, Department of Environmental Biotechnology, GEBRI, Sadat City, Minufiya University, Egypt. The nodule isolates and the reference strains were grown for 72 h in Yeast Mannitol Broth (YMB) according to the method mentioned by **Vincent, 1970**. Bacterial suspension prepared from 3-day-old culture was injected into sterilized vermiculite then left to dry before filling with peat moss media to inoculation with seeds. The inoculation rate was adding at 50 % for seed inoculation and 50 % at 15 days after planting.

The following characters were recorded:

1- Vegetative growth characters:

Three plants were taken randomly from each replicate at flowering and pod setting stage (50 days after sowing) to measure the following vegetative characters i.e. plant height (cm), number of branches and leaves, fresh and dry weights/plant (g).

2- Total dry seed yield (ton/ fed.) and pod characters:

A random sample of 10 dry pods at harvesting time from each replicate were taken to determine the following data: average pod length (cm), average pod diameter (cm), average number of seeds per pod, average weight of 100 seeds (seed index). Dry pods of all plants per plot were harvested and thrashed manually and total dry seed yield (kg)/plot were determined to calculate total yield as ton/fed.

3- Chemical composition in leaves and protein content (%) in seeds:

Fresh weight of foliage and seeds of cowpea were dried in an electric forced-air oven at 70^{o}C to constant weight then fractionated and sifting. The fine powder (0.2 g) of dry sample was digested in a mixture of sulphuric and perchloric acids according to Piper (1947) to estimate total nitrogen in both foliage and seeds but phosphorus and potassium in foliage only (N, P and K) as wet digestion. Total nitrogen (%) was determined by using the modified “Micro-Kheldahl” method apparatus of Parnas and Wagner as described by Pergl (1945). Total protein % was calculated in seeds by multiplying nitrogen content (%) by 6.25. Phosphorus (%) was estimated spectrophotometrically as described by King (1951). Potassium (%) was determined using the Flamephotometr as described by Brown and Jackson (1955).

4-Statistical analysis:

All data were subjected to the statistical analysis of variance and treatment means were compared according to the Least Significant Differences (L S D at 0.05) test method as described by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

1-Vegetative growth characters of cowpea:

1-a- Effect of chemical fertilizers levels (N, P and K):

Data illustrated in Table 2 exhibited as plant height, number of branches and leaves as well as fresh and dry weights/ plant obtained significant increases on vegetative growth characters with increasing the fertilization levels up to 75 % from the recommended fertilization level which include the best characters i.e. tallest plants (39.4 - 44.8 cm), the highest number of branches (4.2 – 4.9) and leaves (25.3 – 28.9), heavier fresh (70.9 – 81.9 g) and dry (16.2 - 20.2 g) weights/ plant in the 1st and 2nd season, respectively when compared with two the other levels of fertilizers and control. On the other hand, the lowest recorded of the previous characters achieved from zero chemical fertilization level (control).

Table 2: Effect of chemical fertilizers levels of N, P, K and microbial inoculations on plant height (cm)/ plant, number of branches)/ plant, number of leaves/ plant, fresh and dry weights/ plant of cowpea plants during the two summer seasons of 2009 and 2010.

Characters / Treatments	Plant height (cm)/ plant		Number of branches/ plant		Number of leaves/ plant		Fresh weight (g)/ plant		Dry weight (g)/ plant	
N, P and K levels	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**
0 %	27.3	31.2	3.3	3.7	15.9	17.5	45.6	53.6	9.74	12.32
50 %	33.3	36.9	3.7	4.0	22.5	25.3	47.9	59.2	10.30	13.46
75 %	39.4	44.8	4.2	4.9	25.3	28.9	70.9	81.9	16.87	20.66
100 %	36.5	41.2	3.7	4.5	22.1	25.9	66.2	76.5	13.91	17.51
L S D at 5 %	**2.47**	**2.03**	**0.74**	**0.91**	**1.50**	**1.81**	**2.19**	**2.76**	**0.98**	**1.03**
Microbial inoculations	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**
B0	32.3	37.1	3.2	3.8	19.8	22.8	54.2	64.5	11.85	15.18
B1	35.4	39.6	4.0	4.7	21.7	25.4	59.8	70.8	12.94	16.56
B2	33.3	37.3	3.7	4.2	20.8	23.6	56.1	66.9	12.44	15.45
B3	33.5	36.3	3.6	3.8	20.3	22.8	55.6	64.9	12.51	15.49
B4	36.2	42.5	4.3	4.8	24.7	27.4	62.3	71.9	13.79	17.26
L S D at 5 %	**1.78**	**1.65**	**0.47**	**0.53**	**1.17**	**1.77**	**2.78**	**3.14**	**0.63**	**0.69**

B0= without microbial inoculation- B1= *Azospirillum* sp. and *Trichoderma* sp. B2= *Bacillus megaterium*. B3= *Pseudomonas fluorescens*. B4= mixtures of all previous.

Concerning the effect of chemical fertilizers of nitrogen, phosphorus and potassium application on cowpea plants, the results are going in the same line with those obtained by many authors as: Ferreira (2004) postulated that increments of phosphorus allowed the growth of roots which resulted in increased number of leave. Singh *et al*. (2006) indicate that cowpea response (cv. Narendra Lobia-2) to P fertilizer (0, 25, 50 or 75 Kg/ ha.) at P 50 Kg/ha. gave the highest number of leaves per plant, vine length, number of nodules per plant. El-Waraky and Kasem (2007) explained that increasing applied N rate up to 40 Kg N /fed. led to significant increases in plant height, number of leaves and branches of cowpea seeds cv. Kafer El-Sheikh – 1. Moreover, Farahvash *et al*. (2010) suggested the adding nitrogen chemical fertilizers (without urea, 50, 100 and 150 Kg/ha. urea). Nitrogen had significant effect only on leaf number per plant by application of 50 and 150 Kg/ha. urea of cowpea. Azarpour *et al*. (2011) mentioned that the maximum plant height of cowpea was recorded from usage of 45 Kg/ha. pure nitrogen from urea fertilizer.

1-b- Effect of microbial inoculations (biofertilizer):

As shown in the same Table 2, the microbial inoculations of cowpea seed with the mixtures of all microbial inoculations of *Azospirillum* sp. and *Trichoderma* sp. for nitrogen, *Bacillus megaterium* for phosphorus and *Pseudomonas fluorescens* for potassium brought about significant increased of vegetative growth characters i.e. plant height, number of branches and leaves and both fresh and dry weights/ plant, followed by individual inoculation with microbial of *Azospirillum* sp. and *Trichoderma* sp. for nitrogen in the 1^{st} and 2^{nd} season, respectively. On the other hand, insignificant differences produced with microbial inoculations of each *Bacillus megaterium* and *Pseudomonas fluorescens* when compared with the control uninoculations plants. These results are come to the same conclusion by Yousry *et al.* (1987) who decided that inoculation of pea (*Pisum sativum*) plants with *Bacillus megatherium* increased plant dry matter by 10.9 %, while the combined application of *B. megatherium* and P-fertilizer increased dry matter by 19.7 %. Singh *et al*. (2006) studied microbial inoculation *Rhizobium* inoculated to seeds at 0.5 Kg/ha, VAM (vesicular arbuscular mycorrhiza) inoculated to soil at 5 Kg/ ha, or *Rhizobium* + VAM), and found That the inoculation of *Rhizobium* + VAM resulted in the highest number of leaves, vine length and number of nodules per plant of cowpea cv. Narendra Lobia-2. El-Waraky and Kasem (2007) pointed out that inoculation of cowpea seeds cv. Kafer El-Sheikh - 1 with biofertilizer (Halex–2: a biofertilizer containing a mixture of non symbiotic N_2-fixing bacteria of the genera *Azospirillum*, *Azotobacter* and Klebsiclla) significantly increased in plant height, number of leaves and branches. Farahvash *et al*. (2010) referred that biofertilizers of cowpea (without biofertilizers, *Azotobacter*, nitroxine and nitroxine + *Azotobacter*) caused significant effect on leaf number and plant height.

1-c- Effect of interactions between chemical fertilizers levels and microbial inoculations:

There were significant interactions between the chemical fertilizers levels and microbial inoculations for plant height, number of branches and leaves as well as both fresh and dry weights/plant Table 3. The best characters obtained with adding the fertilization dose of 75 % the recommended of N, P, K and the mixtures of all microbial inoculations followed by individual microbial inoculation with *Azospirillum* sp. *Trichoderma* sp. for nitrogen when compared to the control untreated plants. Similar results were also reported by Kahlon and Sharanappa (2006) were found that application of 50 Kg P_2O_5 /ha. along with a phosphorus-solubilizing bacterium (PSB; *Bacillus megaterium* var. phosphaticum) and vesicular arbuscular mycorrhiza (VAM; *Glomus mosseae*) resulted in higher dry matter accumulation in leaves, stem and total dry matter accumulation. Higher number of nodules, dry matter of nodules and affectivity of nodules noticed with 50 Kg P_2O_5 /ha. along with PSB and VAM. El-Waraky and Kasem (2007) mentioned that applied N at any rate and inoculation of cowpea seeds cv. Kafer El-Sheikh - 1 with biofertilizer (Halex–2) increased plant height, number of leaves and branches. Also, the combination of 40 Kg N/ fed. Plus (Halex–2) was the best treatment for improving most vegetative growth characters.

Table 3: Effect of the interaction between chemical fertilizers levels of N, P, K and microbial inoculations on plant height (cm), number of branches, number of leaves/ plant, fresh and dry weights/ plant of cowpea plants during the two summer seasons of 2009 and 2010.

Characters / Treatments		Plant height (cm)/ plant		Number of branches/ plant		Number of leaves/ plant		Fresh weight (g)/ plant		Dry weight (g)/ plant	
N, P and K levels	Microbial inoculations	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
0 %	B0	24.9	29.3	2.7	2.9	14.0	16.3	42.2	50.5	8.63	11.41
	B1	28.7	31.7	3.7	4.0	16.7	18.7	47.5	55.8	10.30	12.70
	B2	26.5	31.0	3.3	3.7	15.7	17.0	45.2	53.2	9.66	11.90
	B3	27.0	29.7	3.0	3.3	16.0	17.0	43.8	51.3	9.58	12.08
	B4	29.4	34.3	4.0	4.7	17.0	18.3	49.1	57.1	10.55	13.53
50 %	B0	29.7	31.7	2.9	3.0	19.7	20.3	42.9	52.7	9.17	11.95
	B1	34.4	39.3	4.1	4.7	23.7	27.3	50.8	61.7	10.58	13.82
	B2	32.5	36.3	3.7	4.3	22.0	25.0	47.1	60.1	10.04	13.14
	B3	33.6	34.3	3.7	3.7	21.3	24.0	45.2	54.9	10.39	13.21
	B4	36.6	43.0	4.3	4.0	26.0	29.7	53.4	66.4	11.32	15.20
75 %	B0	35.0	41.3	3.3	4.0	22.0	23.7	61.5	73.1	14.47	18.33
	B1	41.6	45.3	4.4	5.7	25.3	30.7	75.2	88.4	17.39	22.24
	B2	39.8	42.3	4.1	4.7	24.0	28.0	68.7	80.9	16.73	19.90
	B3	37.8	43.0	4.0	4.3	22.3	26.7	69.2	78.3	16.50	19.88
	B4	43.0	52.0	5.1	5.7	32.7	35.3	79.7	88.9	19.22	22.94
100 %	B0	39.7	46.1	4.0	5.3	23.7	30.7	70.4	81.6	15.15	19.03
	B1	37.0	42.0	3.7	4.3	21.0	25.0	65.7	77.3	13.50	17.50
	B2	34.6	39.3	3.5	4.0	21.3	24.3	63.5	73.4	13.32	16.87
	B3	35.4	38.0	3.5	4.0	21.7	23.3	64.3	75.1	13.52	16.77
	B4	36.0	40.7	3.9	5.0	23.0	26.3	66.9	75.2	14.07	17.37
L S D at 5 %		**3.67**	**3.20**	**0.87**	**1.10**	**2.34**	**3.50**	**5.25**	**6.08**	**1.20**	**1.36**

B0= without microbial inoculation- B1= *Azospirillum* sp. and *Trichoderma* sp. B2= *Bacillus megaterium*. B3= *Pseudomonas fluorescens*. B4= mixtures of all previous.

Farahvash *et al*. (2010) found significant effect of interactions between the chemical nitrogen and biofertilizers on leaf dry weight of cowpea. The tallest plants were obtained from urea and nitroxine, while the shortest plants belonged to *Azotobacter* and *Azotobacter* + nitroxine. The highest leaf dry weight was observed for 100 Kg /ha. chemical N + *Azotobacter* and 150 Kg/ ha. + nitroxine, while the lowest value belonged to 150 Kg/ha. urea + nitroxine + *Azotobacter*.

2- Total dry seed yield (ton/fed.) and pod characters of cowpea:

2-a- Effect of chemical fertilizers levels (N, P and K):

Data presented in Table 4 revealed that the 3rd fertilization level (75 % recommended dose) i.e. 46.4 N, 23.3 P_2O_5 and 28.1 K_2O pure Kg/ fed. produced significantly the heaviest total dry seed yield (ton/ fed.) of cowpea pod, length (cm), number of seeds/ pod and 100 seed weight (g) as compared to control treatment (zero level fertilization treatment) and the other two fertilization levels. On the other hand, pod diameter (cm) had insignificant effect when compared to the control treatment. These results also are in agreement with Tyiem and Chieng (2003), Mazaheri and Hoseini (2003) on cowpea, Anuja *et al*. (2006) suggested that different levels of phosphorus and potassium (50, 60 and 70 Kg/ha.) supplied with a constant rate of N (20 Kg/ha.). Maximum yield was obtained under P at 70 Kg/ ha. + K at 70 Kg/ ha. of cowpea plant.

Table 4: Effect of chemical fertilizers levels of N, P, K and microbial inoculations on pod length (cm), pod diameter (cm), number of seeds/ pod, 100 seed weight (g) and total dry seed yield (ton/ fed.) of cowpea plants during the two summer seasons of 2009 and 2010.

Characters / Treatments	Pod length (cm)		Pod diameter (cm)		No. of seeds/ pod		100 seed weight (g)		Total dry seed yield (Ton/fed.)	
N, P and K levels	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**
0 %	11.30	12.06	0.69	0.74	7.82	8.29	11.28	11.09	0.500	0.531
50 %	11.77	12.60	0.73	0.77	8.20	8.69	11.76	12.53	0.587	0.647
75 %	12.90	13.95	0.79	0.81	9.64	10.37	13.50	14.10	0.654	0.717
100 %	12.24	12.93	0.75	0.78	8.58	9.01	12.22	12.86	0.624	0.673
L S D at 5 %	**0.36**	**0.32**	**N.S.**	**N.S.**	**0.25**	**0.27**	**0.41**	**0.38**	**0.045**	**0.039**
Microbial inoculations	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**
B0	11.67	12.60	0.73	0.76	8.03	8.74	11.75	12.50	0.569	0.616
B1	11.79	12.61	0.73	0.76	8.13	8.75	11.88	12.53	0.573	0.619
B2	12.08	12.95	0.74	0.78	8.65	9.16	12.20	12.70	0.590	0.644
B3	12.31	13.06	0.75	0.79	8.85	9.42	12.53	13.20	0.613	0.659
B4	12.41	13.21	0.76	0.78	9.15	9.37	12.61	13.35	0.612	0.673
L S D at 5 %	**0.33**	**0.29**	**N.S.**	**N.S.**	**0.17**	**0.21**	**0.27**	**0.24**	**0.032**	**0.026**

B0= without microbial inoculation- B1= *Azospirillum* sp. and *Trichoderma* sp. B2= *Bacillus megaterium*. B3= *Pseudomonas fluorescens*. B4= mixtures of all previous.

Singh *et al*. (2006) indicate that cowpea response (cv. Narendra Lobia-2) to P fertilizer (0, 25, 50 or 75 Kg/ha.) at P 50 Kg/ha. gave the highest number of pods per plant, pod length, pod diameter, pod weight and pod yield. El-Waraky and Kasem (2007) defined that increasing applied N rate with 30 and 40 Kg N /fed. significantly increased seed yield/ fed., number of pods /plant, number of seeds per pod and weight of 100-seeds of cowpea. (Abayomi *et al.*, 2008) concluded that nitrogen fertilizer had a positive effect on yield and yield components of cowpea. Farahvash *et al*. (2010) decided that the highest number of pods per plant was obtained by adding 50 kg/ha. urea to cowpea plants. Uarrota (2010) postulated that increasing phosphorus levels (as form of superphosphate) from 20 to 40 Kg/ha. positively increase yield of grain and number of pods per plant of cowpea cv. IT at high level of 40 Kg/ha. compared to the control (zero level Kg/ha. phosphorus).

Moreover, Azarpour *et al*. (2011) assumed that the maximum seed yield, number of pod per plant, number of seeds per pod, pod length, seed length and seed width were recorded from use of 45 Kg/ ha. pure nitrogen from source of urea of cowpea.

2-b- Effect of microbial inoculations (biofertilizer):

It is obvious from the data in the same Table that, the maximum total dry seed yield (ton/ fed.) of cowpea was noticed significant increased from seed inoculated with either mixture (B4) of microbial inoculation i.e. *Azospirillum* sp. and *Trichoderma* sp. (B1), *Bacillus megaterium* (B2) and *Pseudomonas fluorescens* (B3) or individually with *Pseudomonas fluorescens* in the two seasons and *Bacillus megaterium* in the 2nd season only as well as pod characters. Individual seed microbial inoculated of *Bacillus megaterium* obtained non-significant effect in the 100 seed weight (g) in the 2nd season only. Pod diameter (cm) had non-significant effect in the two seasons. On the other hand, seed inoculation with *Azospirillum* sp. and *Trichoderma* sp. tendency to increase yield and pod characters but this increment had non-significant different when compared to the control (un-inoculation seeds). The results are in contradiction with those reported by Singh *et al*. (2006) studied that response of cowpea cv. Narendra Lobia-2 to microbial inoculation (*Bradyrhizobium* inoculated to seeds at 0.5 Kg /ha., VAM [vesicular arbuscular mycorrhiza] inoculated to soil at 5 Kg/ha., or *Rhizobium* + VAM). The inoculation of *Bradyrhizobium* + VAM number of pods per plant, pod length, pod diameter, pod weight pod yield. El-Waraky and Kasem (2007) reported that inoculation of cowpea seeds cv. Kafer El-Sheikh - 1 with biofertilizer (Halex–2) led to significant increases in total seed yield/ fed., number of pods /plant and weight of 100-seeds of cowpea. Farahvash *et al*. (2010) stated that significant effect of biofertilizer (without biofertilizers, *Azotobacter,* nitroxine and nitroxine + *Azotobacter*) on seed yield of cowpea. Musa *et al.* (2011) postulated that inoculation seed of cowpea with *Bradyrhizobium* strain significantly increased the seed yield.

2-c- Effect of interactions between chemical fertilizers levels and microbial inoculations:

Results of the interaction effect between chemical fertilizers and microbial inoculations on total dry seed yield and pod characters of cowpea presented in the Table 5 were significant. Trait of pod diameter (cm) exhibited non-significant effect. The highest total dry seed yield (ton/ fed.) of cowpea was recorded from use the mixtures of all microbial inoculations and application of 75 % from the recommended dose of chemical fertilization level which was 0.691 and 0.783 ton/ fed. of total dry seed in both 1st and 2nd season, respectively. These results are consistent with those of El-Waraky and Kasem (2007) stated that combined with (Halex–2) biofertilizer with N fertilizer at the rate of 30 Kg N/ fed. gave a high seed yield, number of pods/plant, number of seeds/ pod and weight of 100- seeds of cowpea. Singh *et al.* (2007) proved *Rhizobium* inoculation, 30 Kg N and 60 Kg P_2O_5/ha. produced significantly higher number of pods/plant, length/pod, seed index, seed and straw yield of cowpea cv. Narendra Lobia over control. Farahvash *et al*. (2010) declared that significant effect of interactions between the chemical nitrogen and biofertilizers. Maximum number of pods per plant belonged to 50 inoculation seeds of cowpea with *Bradyrhizobium* strain plus

N and *Bradyrhizobium* plus P fertilizers, significantly increased seeds yield of cowpea. Musa *et al.* (2011) suggested that inoculation seeds of cowpea with *Bradyrhizobium* strain plus N and *Bradyrhizobium* plus P fertilizers. significantly increased the seeds yield of cowpea.

Table 5: Effect of the interaction between chemical fertilizers levels of N, P, K and microbial inoculations on pod length (cm), pod diameter (cm), number of seeds/ pod, 100 seed weight (g) and total dry seed yield (ton/ fed.) of cowpea plants during the two summer seasons of 2009 and 2010.

Characters / Treatments		Pod length (cm)		Pod diameter (cm)		No. of seeds/ pod		100 seed weight (g)		Total dry seed yield (Ton/ fed.)	
N, P and K levels	Microbial inoculations	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
0 %	B0	10.92	11.66	0.67	0.71	7.4	8.0	10.8	11.5	0.471	0.591
	B1	11.11	11.91	0.70	0.73	7.6	8.0	11.0	11.7	0.480	0.527
	B2	11.49	12.15	0.69	0.73	7.9	8.4	11.4	11.7	0.507	0.533
	B3	11.38	12.22	0.68	0.75	8.0	8.5	11.7	12.2	0.523	0.537
	B4	11.61	12.35	0.71	0.77	8.2	8.6	11.5	12.4	0.520	0.540
50 %	B0	11.33	12.03	0.69	0.73	7.6	8.2	11.0	11.9	0.550	0.585
	B1	11.55	12.44	0.72	0.76	7.9	8.4	11.5	12.3	0.566	0.626
	B2	11.75	12.67	0.73	0.77	8.2	8.7	11.8	12.6	0.584	0.655
	B3	11.93	12.98	0.75	0.80	8.5	9.1	12.1	12.9	0.616	0.672
	B4	12.28	12.88	0.74	0.79	8.8	9.0	12.4	13.1	0.618	0.695
75 %	B0	11.79	13.20	0.75	0.77	8.1	9.1	12.3	13.1	0.590	0.637
	B1	12.63	13.85	0.77	0.79	8.9	10.1	13.1	13.7	0.647	0.688
	B2	13.07	14.07	0.80	0.83	10.1	10.7	13.6	13.9	0.659	0.732
	B3	13.53	14.14	0.80	0.85	10.4	10.8	14.0	14.7	0.682	0.744
	B4	13.47	14.48	0.82	0.82	10.7	11.1	14.5	15.1	0.691	0.783
100 %	B0	12.65	13.50	0.79	0.82	9.0	9.6	12.9	13.5	0.665	0.722
	B1	11.85	12.25	0.73	0.75	8.1	8.4	11.9	12.4	0.600	0.633
	B2	12.02	12.89	0.74	0.78	8.4	8.9	12.0	12.6	0.610	0.655
	B3	12.41	12.91	0.75	0.78	8.5	9.3	12.3	13.0	0.630	0.681
	B4	12.27	13.11	0.76	0.77	8.9	8.8	12.0	12.8	0.617	0.675
L S D at 5 %		**0.61**	**0.45**	**N.S.**	**N.S.**	**0.33**	**0.42**	**0.51**	**0.47**	**0.065**	**0.053**

B0= without microbial inoculation- B1= *Azospirillum* sp. and *Trichoderma* sp. B2= *Bacillus megaterium*. B3= *Pseudomonas fluorescens*. B4= mixtures of all previous.

3- Chemical composition in leaves and protein content (%) in seeds of cowpea:

3-a- Effect of chemical fertilizers levels (N, P and K):

Data illustrated in Table 6 clearly revealed that there were significant increases in the chemical composition in leaves and protein content (%) in seeds of cowpea; due to add the 3rd of chemical fertilization level (75 % of N, P and K) which were superior in those respects as a compared with the rest of the other studied levels. These results are in line with those reported by El-Waraky and Kasem (2007) who stated that applied N rate with 30 and 40 Kg N /fed. significantly increased seed crude protein content of cowpea. Because nitrogen is a constituent of protein, it increases with N content in tissue (Marschner, 1995). Anuja *et al*. (2006) demonstrated that P and K at

50, 60 and 70 Kg/ha. fertilizers were supplied with a constant rate of N (20 Kg/ha.) of cowpea cv. TUX 944. and found that crude protein content increased with increasing levels of P and K. at P: K at 70:70 Kg/ha. Farahvash *et al*. (2010) commented that nitrogen had significant effect on percentage of protein from 100 and 150 Kg/ha. urea in seeds of cowpea.

Table 6: Effect of chemical fertilizers levels of N, P K and microbial inoculations on nitrogen (%), phosphorus (%), potassium (%) in leaves and protein (%) in seeds content of cowpea plants during the two summer seasons of 2009 and 2010.

Characters / Treatments	Nitrogen in leaves (%)		Phosphorus in leaves (%)		Potassium in leaves (%)		Protein in seeds (%)	
N, P and K levels	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**
% 0	2.42	2.56	0.159	0.198	1.585	1.794	17.85	19.53
% 50	2.65	2.85	0.185	0.231	1.714	2.113	19.37	20.14
% 75	2.98	3.20	0.252	0.308	2.298	2.413	21.39	21.92
% 100	2.69	3.06	0.208	0.282	2.159	2.156	20.09	21.33
L S D at 5 %	**0.36**	**0.30**	**0.075**	**0.057**	**0.063**	**0.075**	**0.93**	**0.87**
Microbial inoculations	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**
B0	2.53	2.75	0.188	0.240	1.907	2.044	19.30	20.20
B1	2.87	3.17	0.189	0.241	1.923	2.065	20.15	21.38
B2	2.64	2.87	0.217	0.265	1.933	2.083	19.66	20.62
B3	2.66	2.79	0.201	0.258	1.985	2.260	19.50	20.48
B4	2.73	3.01	0.209	0.269	1.948	2.142	19.78	20.97
L S D at 5 %	**0.18**	**0.14**	**0.027**	**0.021**	**0.046**	**0.070**	**0.56**	**0.48**

B0= without microbial inoculation- B1= *Azospirillum* sp. and *Trichoderma* sp. B2= *Bacillus megaterium*. B3= *Pseudomonas fluorescens*. B4= mixtures of all previous.

3-b- Effect of microbial inoculations (biofertilizer):

Data presented in Table 6 show that treating seeds of cowpea with individual of the each of microbial inoculation and the mixtures of all microbial inoculations obtained markedly enhanced effect on nitrogen (%), phosphorus (%) and potassium content (%) in leaves. Individual microbial inoculation of *Azospirillum* sp. and *Trichoderma* sp. followed with the mixtures of all microbial inoculations gave the highest protein content (%) in seeds. These results in agreement with those reported by Ramadan *et al.* (2002) they concluded that nitrogen uptake by cowpea plants were increased by 49.78%. Inoculation by mycorrhiza or phosphate dissolving bacteria increased P-supply which led to improve P uptake by cowpea plants for sandy soils. El-Waraky and Kasem (2007) proposed that inoculation of cowpea seeds with the biofertilizer (Halex–2) significantly increased seed crude protein content of cowpea. Shahaby (1997) generalized that nitrogen fixation bacteria increased total nitrogen content in the Nile valley and reclaimed soil. Phosphate solubilizing bacteria (*Bacillus polymyxa* and *Pseudomonas fluorescens*) release organic and inorganic acids which reduce soil pH leading to change of phosphorus and other nutrients to available forms ready for uptake by plants (Singh and Kapoor, 1999).

3-c- Effect of interactions between chemical fertilizers levels and microbial inoculations and protein content (%) in seeds:

The most favorable treatment of the interaction obtained with the application of 75 % recommended fertilization and inoculation seeds of cowpea with the mixtures of all microbial inoculations (Table 7) helped in producing the most pronounced effects of nitrogen, phosphorus and potassium content (%) in leaves. On the other hand, seed inoculations with *Azospirillum* sp. and *Trichoderma* sp. significantly increased nitrogen and protein content (%) in leaves and seeds, respectively. when compared to the other inoculations and control untreated plants. Also, inoculation seeds of cowpea with the *Bacillus megaterium* and *Pseudomonas fluorescens* significant effect on phosphorus and potassium content (%) in leaves, respectively compared to uninoculated seeds of cowpea.

Table 7: Effect of the interaction between chemical fertilizers levels of N, P and K and microbial inoculations on nitrogen (%), phosphorus (%), potassium (%) in leaves and protein (%) in seeds content of cowpea plants during the two summer seasons of 2009 and 2010.

Treatments / Characters		Nitrogen in leaves (%)		Phosphorus in leaves (%)		Potassium in leaves (%)		Protein in seeds (%)	
N, P and K levels	**Microbial inoculations**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**	**2009**	**2010**
0 %	**B0**	2.13	2.35	0.144	0.186	1.571	1.7403	17.24	18.78
	B1	2.60	2.87	0.143	0.193	1.588	1.751	18.41	19.85
	B2	2.43	2.50	0.168	0.201	1.576	1.801	17.70	19.55
	B3	2.40	2.37	0.161	0.199	1.611	1.881	17.31	19.25
	B4	2.53	2.70	0.177	0.210	1.580	1.796	18.60	20.21
50 %	**B0**	2.41	2.50	0.173	0.202	1.606	1.998	18.64	19.41
	B1	3.02	3.13	0.185	0.224	1.713	2.094	20.12	20.95
	B2	2.62	2.77	0.193	0.253	1.737	2.078	19.68	20.11
	B3	2.53	2.83	0.182	0.233	1.775	2.087	19.40	19.80
	B4	2.67	3.00	0.190	0.245	1.739	2.110	19.01	20.72
75 %	**B0**	2.65	2.87	0.203	0.249	2.012	2.218	19.42	20.17
	B1	3.27	3.53	0.230	0.287	2.253	2.301	21.10	23.15
	B2	3.00	3.20	0.293	0.321	2.289	2.325	21.77	21.95
	B3	3.10	3.17	0.261	0.329	2.444	2.637	21.94	21.78
	B4	2.90	3.23	0.275	0.353	2.491	2.643	21.73	22.56
100 %	**B0**	2.95	3.27	0.233	0.325	2.437	2.281	21.91	22.72
	B1	2.60	3.13	0.198	0.261	2.138	2.114	19.97	21.55
	B2	2.50	3.00	0.215	0.285	2.131	2.127	19.49	20.88
	B3	2.62	2.80	0.201	0.270	2.111	2.237	19.33	21.10
	B4	2.80	3.10	0.195	0.286	1.980	2.020	19.76	20.38
L S D at 5 %		**0.32**	**0.28**	**0.060**	**0.053**	**0.091**	**0.139**	**1.12**	**0.95**

B0= without microbial inoculation- B1= *Azospirillum* sp. and *Trichoderma* sp. B2= *Bacillus megaterium*. B3= *Pseudomonas fluorescens*. B4= mixtures of all previous.

These results are in general agreement with reported with El-Waraky and Kasem (2007) that combined with (Halex–2) biofertilizer and applied N rate up to 30 Kg N /fed. led to significant increase seed crude protein content of cowpea. Singh *et al.* (2007) proved that *Rhizobium* inoculation, 30 Kg N and 60 Kg P_2O_5/ha., protein content of seed was influenced by *Rhizobium*, nitrogen and phosphorus of cowpea cv. Narendra Lobia. Abdel-Hady (2009) demonstrated that fertilizing cowpea cv. Kaha -1 with combination of FYM + Biofertilizer (Phosphorine + Rhizobacterien) + 1/2 NPK (recommended doses) produced the highest number of branches/plant, highest pod length, highest number of seeds /pod, highest pod filling and protein contents when compared to the control untreated plants. Farahvash *et al*. (2010) recorded that significant effect of interactions between the chemical nitrogen and biofertilizers. The highest percent of protein was obtained from 100 and 150 Kg/ha. chemical N without biofertilizer and the lowest from *Azotobacter* when no N chemical was used. The results indicated that biofertilizers didn't increase protein yield alone of cowpea and they should be applied along with chemical fertilizers.

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تأثير أستخدام اللقاحات الميكروبية على خفض معدلات الأسمدة المعدنية فى نباتات اللوبيا فى الأراضى المستصلحة حديثاً فى مصر

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أجريت تجربتان فى محطة بحوث جنوب التحرير التابعة لمعهد بحوث البساتين ،مركز البحوث الزراعية ، فى محافظة البحيرة ، مصر خلال الموسم الصيفى لعامي 2009 ، 2010 لدراسة تأثير أستخدام اللقاحات الميكروبية المختلفة (التى تؤدى الى خفض جزء من معدلات التسميد الكيماوى الموصى بها من عناصر النتروجين والفوسفور والبوتاسيوم) على المواصفات الخضرية والمحصول وجودة القرون و المحتوي الكيماوي للاوراق والمحتوى من نسبة البروتين % فى نباتات اللوبيا صنف قها 1 (*Vigna unguiculata*, L. Walp.) وذلك تحت نظام الرى بالتنقيط. صممت التجربة بنظام القطع المنشقة مرة واحدة في ثلاث مكررات حيث وضعت معدلات التسميد النتروجينى والفوسفاتى والبوتاسى الاربعة (صفر% - 50 % - 75 % - 100 % من معدل التسميد الموصى به لتسميد نبات اللوبيا) فى القطع الرئيسية (والخمس معاملات للبكتريا وهى كما يلى :-

without microbial inoculation (B0), inoculations with *Azospirillum* sp. and *Trichoderma* sp. for nitrogen (B1), *Bacillus megaterium* for phosphorus (B2), *Pseudomonas fluorescens* for potassium (B3) and the mixtures of all previous of microbial inoculations (B4). حيث وضعت في القطع المنشقة.

وكانت أفضل النتائج التى تم الحصول عليها هى كما يلى :-

٢ -أدت الزيادة التدريجية لمستويات التسميد المختلفة حتى المستوى التسميدى الكيماوى الثالث (75 % من معدلات التسيد الموصى بها) الى زيادة معنوية ملحوظة فى كل من الصفات الخضرية المختلفة والمحصول الكلي الجاف (طن / فدان) لبذور اللوبيا وجودة القرون مع زيادة محتواها من النسبة المئوية من عناصر النتروجين والفوسفور والبوتاسيوم وكذلك زيادة النسبة المئوية لمحتوى البذور من البروتين مقارنة بمعاملة الكنترول (صفر % من معدلات التسيد الموصى بها).

٣ -وجدت فروق معنوية بين معاملات التلقيح الميكروبى المختلفة سواء عند تلقيحها مفردة أو مختلطة مع بذور اللوبيا وذلك في صفات النمو الخضري والمحصول الكلي الجاف للبذور وأيضاً محتوي الاوراق من النيتروجين والفوسفوروالبوتاسيوم حيث زادت النسبة المئوية للبروتين نتيجة التلقيح بالخليط الميكروبى متبوعة بالتلقيح المفرد لبكتريا تثبيت النتروجين مقارنة ببذور نباتات الكنترول غير الملقحة.

٤ - أفضل النتائج التى تم الحصول عليها فى هذه التجربة هى نتائج التفاعل بين التسميد الكيماوى بمعدل 75 % من كمية التسميد الكلية الموصى بها لنبات اللوبيا وكذلك أستخدام مخلوط من اللقاحات الميكروبية المختلفة حيث أدت الى أفضل الصفات الخضرية خاصة الوزن الطازج والجاف للنبات وأعلى محصول كلى من البذور الجافة (طن/ فدان) وأيضاً طول القرن وعدد البذور به ووزن ال 100 بذرة وكذلك زيادة محتوى الأوراق من النسبة المئوية للعناصر الغذائية الكبرى وهى عناصر النتروجين والفوسفور والبوتاسيوم وزيادة النسبة المئوية للبروتين فى بذور اللوبيا مقارنة بنباتات الكنترول غير الملقحة. كما أدى التلقيح المفرد لبذور نباتات اللوبيا بميكروب النتروجين *Azospirillum* sp. and *Trichoderma* sp. الى أعلى زيادة فى صفات النمو الخضرى وزيادة محتوى البذور من عنصر النتروجين والبروتين (%) كذلك أدى التلقيح ببكتريا *Bacillus megaterium* و*Pseudomonas fluorescens* الى أعلى زيادة فى محصول البذور الجافة (كجم/ فدان) وتحسين جودة القرون وزيادة محتواها من عنصرى الفوسفور والبوتاسيوم مقارنة بنباتات اللوبيا غير المعاملة.

قام بتحكيم البحث

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