

## **Integrated Effect of Bio-organic Manures and Mineral Ertilizers on Potato Productivity and the Fertility Status of a Calcareous Soil**

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**Abstract:** The aim of this research was to study the possibility of using bio- organic manures instead of mineral fertilizers for increasing potato growth production and soil fertility status as well as improving soil physical, chemical and biological properties. A field experiment was carried out at Bahig village, Behera Governorate, Egypt during two successive seasons of 2008 and 2009. *Azotobacter chroococcum* and *Azospirillum lipoferum* were used as nitrogen fixing bacteria, as well as *B. megaterium* var. phosphaticum was used as phosphate dissolving bacteria. Chicken manure and ammonium sulphate were used as organic and inorganic fertilizers, respectively, however chicken manure was added at the rate of (7 ton/fed 90kg N/fed) as well as using ammonium sulphate at the same level of nitrogen. The obtained results indicated that the highest values of CO<sub>2</sub> evolution was observed in chicken manure treatments. Whereas the highest value of N<sub>2</sub>-ase enzyme activity were observed in rhizosphere of potato plants inoculated with asymbiotic N<sub>2</sub>-fixers + a half dose of ammonium sulphate. Inoculation of potato tubers with phosphate dissolving bacteria combined with other applied treatments increased CO<sub>2</sub> evolution and N<sub>2</sub>-ase activity. A higher values of NH<sub>4</sub>-N and NO<sub>3</sub>-N in rhizosphere soil were obtained at soil treated with chicken manure followed by inoculation of potato tubers with *Azotobacter* & *Azospirillum* and a half dose of ammonium sulphate as compared to soil treated with full dose of ammonium sulphate. Growth parameters of potato plants were significantly increased with chicken manure in combination with potato tuber inoculated with phosphate dissolving bacteria. Potato tubers inoculated with *Azotobacter* & *Azospirillum* + a half dose of ammonium sulphate combined with *B. megaterium* var. phosphaticum showed the highest value of carbohydrate contents in the tubers. There is a significant increase in tuber yield (ton/fed) as a result of applying biofertilizers + a half dose of ammonium sulphate or amendment of soil by chicken manure or fertilized with ammonium sulphate compared with control without a significant differences between them. Inoculation of potato tubers with phosphate dissolving bacteria generally caused significant increase in tuber yield/fed. Generally, tuber yield/fed was higher with *Azotobacter* & *Azospirillum* and chicken manure treatments than ammonium sulphate application. The results indicated that the application of chicken manure or inoculation with N<sub>2</sub>-fixing bacteria in combination with inoculated potato tubers with phosphate dissolving bacteria improved soil fertility status and availability of macro and micronutrients in the rhizosphere of potato plants. Therefore, the use of biofertilizers or organic manure may be recommended as a substitute for half dose of chemical fertilizer in potato crop production especially for exportation.

**Key words:** *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus megaterium*, chicken manure, potato, N<sub>2</sub>-ase enzyme, dehydrogenase enzyme, N, P, K, Fe, Mn, Zn.

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### **INTRODUCTION**

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops cultivated in Egypt for local consumption and exportation. Increasing the quality of potato for exportation is the main aim of potato growers. It ranks after wheat, rice and maize as the fourth most important crop for human consumption (Ewing, 1997). Nitrogen and phosphatic chemical fertilizers are commonly used in the production of vegetable crops. Applications of such chemical fertilizers to the soil cause some problems especially for exportation. It is well known that the nitrogen is lost via nitrate reduction, denitrification and ammonia volatilization. Moreover, some nitrogenous fertilizers can be leached to the surface and underground water causing environmental pollution (Attia, 1990).

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Also, immobilization of phosphorus is the most important problem of phosphatic fertilization in Egypt and this due to soil alkalinity. Taking the economical point into account, the high prices of chemical fertilizers may increase the production costs of potato producers. Therefore, the use of biofertilizers and organic manures is of a particular interest to avoid the previously mentioned problem. Biofertilizers application with a half dose of chemical nitrogen fertilizer proved to be an efficient tool increasing available nutrients in soil as well as growth performance and yield of cultivated crops is improved.

Microorganisms are important in agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers as much as possible (Dilfuza 2007). Bacteria inoculants are able to increase plant growth, speed up seed germination, improve seedling emergence, responses to external stress factors and protect plant against diseases (Lugtenberg *et al.*, 2002). Plant growth promoting bacteria may be important for plant nutrition by increasing N and P uptakes by the plants and playing a significant role as PGPR in the bifertilization of crops (Cakmakci *et al.*, 2005). (Shaban and El-Sherief, 2007) reported that Plant growth promoting bacteria have potentiality to convert nutritional elements from unavailable to available form through biological processes.

Several investigators indicated that inoculation with *Azotobacter* and *Azospirillum* improved growth and yield of potato crop (Mahendran *et al.*, 1996; Mahendran and Kumar 1998) and El-Ghinibihi and Fatouh (2001). Shaban and Omer (2006) reported that adding organic manure as a soil amendment was more benefit for soil fertility status, due to it attains more pronounced contents of macro- (N, P and K) and micronutrients (Fe, Mn, Zn and Cu), which was consequently reflected on plant growth and yield (Ismaiel, 2002 and Mahdy, 2003). This is due to such strains can actively colonize plant roots and improved its growth and yield by direct or indirect mechanisms. Moreover, bio-fertilizers, particularly N-fixing bacteria, were suggested to reduce the used N-mineral fertilizer quantities and produce clean and healthy crops (Mantripukhri, 2006). Abbas *et al.* (2006) stated that bio-fertilization through seed or grain inoculation *Azotobacter* or *Azospirillum* as well as applying organic manure could minimize the dose of N-mineral required to be applied, which is a profitable from the economical point of view, and effective in reducing chemical pollution of soil with N.

With respect to organic manure, Akalan (1983) pointed out that addition of organic manure is one of fundamental processes to minimize the nutrients losses from soil by means leaching and denitrification. So, sustainable farming by using such organic manure in agriculture is considered to be a strategy to preserve the environment and prevent chemical pollution. Also, organic fertilization is very important not only for providing the plants with their nutritional requirements without having any undesirable impacts on the environment but also for improving physical, chemical and biological properties of the soil (Abdel-Hamid *et al.*, 2004 and El-Ghamry *et al.*, 2005).

With regard to the effect of organic manure on plant growth and microbial activity, Abdel-Magid *et al.*, (1996) Mahmoud (2006) and Bakry *et al.*, (2009) reported that the addition of organic manures to the soil encourage proliferation of soil microorganisms, increased microbial populations and activity of microbial enzymes i.e. dehydrogenase, urease and nitrogenase. In addition, El-Fakharani (1999) and Abdel-Ghani and Bakry (2005) found that organic manure had a significant effect on growth characters and tuber yield of potato plants when compared with application of the same level of nitrogen from inorganic nitrogen fertilizers. El-Mancy *et al.*, 2008 studied the combined effect of organic and mineral fertilizers on yield of potato grown under coarse-textured soil conditions; they found that chicken manure was more effective than farm yard manure when either the organic manure applied singly or in combination with chemical NPK fertilizers. Concerning the effect of dual inoculation with a symbiotic N<sub>2</sub>-fixing bacteria and phosphate dissolving bacteria on potato growth, Abdel-Ati *et al.*, (1996) and Mahendran and Kumar (1998) found that dual inoculation of potato with the asymbiotic N<sub>2</sub>-fixers i.e. *Azotobacter* or *Azospirillum* and phosphate dissolving bacteria improved growth performance, dry matter, carbohydrate content and tuber yield of potato. Also, Mahendran and Chandramani (1998) reported that dual inoculation of potato with the abovementioned microorganisms increased soil availability to N, P, K, Zn, Mn and Fe.

The present study was investigated to evaluate the role of biofertilizer and organic manure on growth, yield of potato and improving availability of elements in the studied soil.

## MATERIALS AND METHODS

A field experiment was carried out during seasons of 2008 and 2009 at Baheig village, Marrioute sector, Behera Governorate to study the effect of non-symbiotic N<sub>2</sub>-fixing bacteria, chicken manure and ammonium sulphate application either individually or combined with phosphate dissolving bacteria (PDB) on growth and yields of potato (cv. Diamant) as well as their effects on improving of nutrients availability and physical properties of the studied soil.

**Physical and Chemical Characteristics of the Experimental Soil Are Shown in Table (1):****Table 1:** physical and chemical characteristics of the studied soil.

Soil characteristic			
Soil physical properties	Values	Soil chemical properties	Values
Particle size distribution %		EC (dS/m)	1.66
Sand	50.14	Soluble Ions (meg./L)	
Silt	13.75	Ca <sup>++</sup>	6.87
		Mg <sup>++</sup>	5.27
Clay	36.11	Na <sup>+</sup>	3.74
		K <sup>+</sup>	0.97
Texture class	Sand clay	CO <sub>3</sub> =	0.0
		HCO <sub>3</sub>	3.16
Bulk density	1.37	Cl <sup>-</sup>	3.91
Total porosity	44.59	SO <sub>4</sub> =	9.78
Field capacity (v/v) %	39.61	pH (1: 2.5 soil water ssp.)	7.93
Wilting point (v/v) %	23.15	Organic method %	0.65
Available water (v/v) %	16.46	CaCO <sub>3</sub> %	36.49
Infiltration rate (cm h <sup>-1</sup> )	0.6	Total N ( % )	0.231
		Available nutrients (ppm):	
Saturated hydraulic conductivity	0.4	P	6.20
		N	33.38
		K	204
Sp %	46.50	Fe	6.26
		Mn	0.53
		Zn	0.59

**Table 2:** Characteristics of chicken manure used in study.

Soil characteristic	First season 2007/2008	Second season 2008/2009
pH (1:10 organic manure: water susp.)	8.16	8.63
EC dSm <sup>-1</sup> (1:10organic manure: water susp.)	1.89	1.72
CaCO <sub>3</sub> %	0.77	0.77
Organic matter %	47.36	47.49
O.C %	26.70	27.11
Total N %	1.36	1.33
C/N ratio	16.36	15.90
Bulk density, g cm <sup>-1</sup>	00.38	00.99
Available P %	0.339	0.337
Available K %	1.22	1.201
Total Fe %	0.0091	0.0082
Available Mn (ppm)	72.23	71.94
Available Zn (ppm)	84.46	84.66
Available Cu (ppm)	11.60	12.10
Available Pb (ppm)	12.31	12.35

Mechanical and physical analyses were estimated according to Jackson (1973). While, chemical analysis of the soil and chicken manure was estimated according to Black *et al.*, (1982).

**2.1. Potato Tubers:**

Certified potato tubers (cv. Diamant) were obtained from the General Authority for producers and Exporters of Horticultural crops, Cairo, Egypt

**2.2. The Used Microorganisms:**

*Azotobacter chroococcum* AC and *Azospirillum lipoferum* MN strain were provided from the unit of Biofertilization, Fac. of Agric., Alexandria Univ., Cairo, Egypt. While *Bacillus megaterium* var. phosphatium was provided from the Microbiology Dept. Soils, Water and Environment Res. Inst., Agric. Res. Centre, Giza, Egypt.

**2.3. Inocula Preparation:**

For the preparation of *A. chroococcum* and *A. lipoferum* inocula, Modified Ashby's medium (Abdel-Malek and Ishak, 1968) and semi-solid malate medium (Döbereiner, 1978) were inoculated with *A. chroococcum* and *A. lipoferum* respectively. Also, Bunt and Rovira (1955) medium modified by Abdel-Hafez (1966) was inoculated with *B. megaterium* var. phosphaticum then incubated at 30 °C for 7 days.

#### 2.4. Organic and Chemical Fertilizers:

Organic manure used in study (chicken manure was added to the soil 21 days before sowing at rate of 7 ton/fed (90 kg N/fed). The chemical and physical analyses of organic manure used are shown in Table (2).

Chemical phosphorus fertilizer at rate of 35 kg P<sub>2</sub>O<sub>5</sub> in the form of calcium superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>) was added during preparation of the soil for all treatments. Nitrogen and potassium fertilizers were added at rates of 90 kg N/fed and 96 kg N/fed in forms of ammonium sulphate (20.5% N) and potassium sulphate (48% K<sub>2</sub>O), respectively in three equal rates at 15, 30 and 60 days after emergence.

#### 2.5. Inoculation Process:

Pieces of potato tuber were successfully washed with water and air dried. Thereafter, they were soaked in cell suspension of mixture (1:1) from *A. chroococcum* and *A. lipoferum* (1ml contain 8x10<sup>7</sup> viable cell) for 30 minutes. Tubers of control treatment were treated with the same manner but using N-deficient medium instead of bacteria cultures.

Potato tuber pieces of all treatments were divided into two parts, the first part was planted without inoculation with phosphate dissolving bacteria whereas, the second part was planted after soaking in cell suspension of *B. megaterium* var. phosphaticum (1 ml contain 10<sup>8</sup> viable cells) for 30 minutes. Sucrose solution (30%) was added as an adhesive agent prior to inoculation.

#### 2.6. Experimental Design:

Treatments were distributed in a split plot design with three replicates, where the inoculation with *B. megaterium* var. phosphaticum was allocated in the main plots. Treatments with bio or mineral or organic fertilizer were randomly distributed in the sub-plots. These treatments are:

1. Control (without any addition)
2. *A. chroococcum* & *A. lipoferum* inoculum (1:1)+ a half rate of recommended nitrogen dose as ammonium sulphate (1/2 of RND).
3. Chicken manure, 7 ton /fed (90 kg N/fed).
4. Ammonium sulphate (90kg N/fed).
5. Control + *B. megaterium* (PDB) inoculum.
6. *A. chroococcum* & *A. lipoferum* + (1/2 of RND) + PDB
7. Chicken manure , 7 ton /fed (90 kg N/fed) + PDB
8. Ammonium sulphate + PDB

#### Cultivation Process:

Cultivation process was performed in January 23<sup>rd</sup>, for two seasons, by sowing uninoculated or inoculated tuber pieces in ridges 5 m long and 70 cm a part. Other field practices for potato growing were followed according to the recommendation of the Ministry of Agriculture.

#### 2.7. Sampling and Determinations:

Representative soil samples from rhizosphere of potato plants were taken at 30, 60 and 90 days from sowing. The samples were microbiologically analyzed for densities of *Azotobacter*, *Azospirilla*, and phosphate dissolving bacteria, CO<sub>2</sub> evolution and N<sub>2</sub>-ase activity (average of two growing seasons). Also, rhizosphere soil samples were chemically analysed at the same periods (30, 60 and 90 days) for ammonical and nitrate nitrogen, total nitrogen and phosphorus.

##### 2.7.1. A. Microbiological Analysis:

###### A.1:

Densities of *Azotobacter* and *Azospirilla* were determined in the rhizosphere of potato plants on modified Ashby's medium (Abdel-Malek and Ishak, 1968) and Semi-solid malate medium (Döbereiner, 1978), respectively using the most probable number technique (Cochran, 1950) whereas, the density of phosphate dissolving bacteria was estimated by Bunt and Rovira medium, 1955 modified by Abdel-Hafez, 1966 using the plate count method.

###### A.2:

Carbon dioxide evolved by soil microorganisms was estimated according to Page *et al.*, (1982).

###### A.3:

Nitrogenase activity was estimated according to Hardy *et al.*, (1973).

### **2.7.2. Chemical Analysis of Soil:**

#### **B.1:**

Ammonical and nitrate nitrogen were determined according to Bremner and Keeny (1965).

#### **B.2:**

Total nitrogen was estimated according to A.O.A.C. (1980), whereas, total phosphorus was estimated according to A.P.H.A (1992).

### **2.7.3. Growth Parameters:**

After 70 days from sowing, plant high, dry matter of shoot system, leaf number/plant and branch number/plant were estimated as average of two growing seasons.

### **2.7.4. Chemical Analysis of Plants:**

Total nitrogen and phosphorus were periodically determined (average of two growing seasons) in dried leaves at 30, 60 and 90 days from sowing. After harvesting, number of tubers/kg, percentage of total carbohydrate in tubers and tuber yield/fed were estimated. Total carbohydrate was estimated according to Michel *et al.*, (1956).

### **2.7.5. Available Nutrients Content:**

After harvesting of potato plants, available phosphorus was determined according to Olsen and Sommers, (1982) available potassium (Soltanpour and Schwab 1977) and available Zn, Fe and Mn according to Lindsay and Novell, (1978).

### **2.8. Statistical Analysis:**

All data obtained of both the two studied seasons were averaged in a mean value for each of the tested plant and soil parameters, and then statistically analyzed by using L.S.D. at 0.05 as described by Snedecor and Cochran (1989). The differences between the mean values of various treatments were compared by Duncan's multiple range tests (Duncan's 1950).

## **RESULTS AND DISCUSSION**

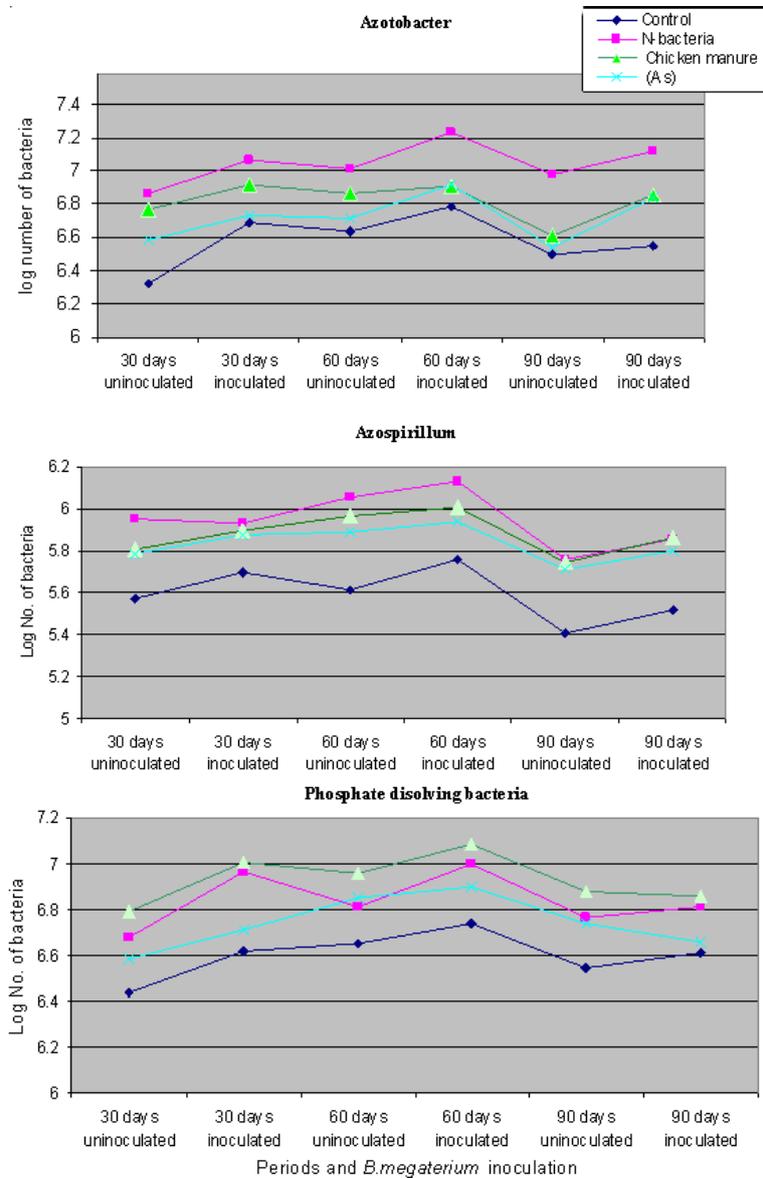
### **3.1. Effect of Biofertilizers and Organic Manure on Microbial Densities in the Rhizosphere of Potato Plants:**

Data illustrated in Fig. (1) show the periodical changes of *Azotobacter*, *Azospirillum* and phosphate dissolving bacteria with rhizosphere of potato plants. Microbial densities of the abovementioned bacteria group gradually increased with the increasing growth period to reach their maximum values at 60 days, the flowering stage which is the most active period in plant growth. These distinguished increases through this stage indicated that the bacterial numbers positively influenced by the plant root exudates and surplus of nutrients. Afterwards, the population turned to decrease to reach maturity stage. The same trend was observed in all treatments. Rhizosphere of potato plants inoculated with *Azotobacter* & *Azospirillum* and receiving a half dose of ammonium sulphate gave higher densities of *Azotobacter* or *Azospirillum* than their chicken manure or ammonium sulphate application. It is well known that *Azotobacter* or *Azospirillum* not only provide nitrogen, but also produce a variety of growth-promoting substances.

These substances stimulate at least to some degree, the production of root exudates which in turn affect their numbers. The results may indicate that the introduced inoculum has the ability to survive and colonize the root zone of potato plants. Similar results were obtained by Abdel-Ati *et al.*, (1996) and Saleh *et al.*, (1998).

Regarding the densities of phosphate dissolving bacteria, as well known it have the capability to bring insoluble P in soil into soluble form by producing organic acids. The obtained data show that rhizosphere of potato plants amended with chicken manure and received *B. megaterium* gave the highest densities of P-dissolving bacteria compared with other treatments. This may be not only due to the vital role of organic materials in enriching microbial communities but also to the preinoculation of potato tubers received *B. megaterium* before planting. Irrespective of control, the lowest densities of P- dissolving bacteria was observed in the treatments of ammonium sulphate application. These results are in accordance with Abdel-Magid *et al.*, (1996). It is note worthy to mention that the rhizosphere of potato plants inoculated with phosphate dissolving bacteria for any applied treatment led to higher densities of studied bacteria as compared to the uninoculated treatments, especially that treatment fertilized by chicken manure. Mahmoud (2006) and Bakry *et al.*, (2009) reported that the addition of organic manures to the soil encourage proliferation of soil microorganisms.

These results assured the vital role of relationship between both of *B. megaterium* and  $N_2$ -fixing bacteria which used in this study. This was true in all treatments.



**Fig. 1:** Periodical changes of *Azotobacter*, *Azospirillum* and phosphate dissolving bacteria density in rhizosphere of potato plants as affected by biofertilizer, chicken manure and chemical N fertilizer.

**3.2. Effect of Biofertilizers and Organic Manure on  $CO_2$  Evolution and  $N_2$ -ase Activity in Rhizosphere of Potato Plants:**

Data presented in Table (3) indicate that the carbon dioxide evolved (mean of two growing seasons) by soil microorganisms gradually increased throughout the experimental periods to reach their maximum values at 60 days, the flowering stage which is the most active period in plant growth and decreased thereafter. This trend was observed in various treatments. All these increases over the control in carbon dioxide as a result of any treatment (B) or as a result of inoculation with phosphate dissolving bacteria (A) or their interaction were significant. Irrespective of phosphate dissolving bacteria (A), the highest record of evolved carbon dioxide was observed in case of chicken manure treatment followed by that plants inoculated with  $N_2$ -fixing bacteria and supplemented with a half dose of inorganic N-fertilizer as compared to other treatments.



### 3.3. Effect of Biofertilizers and Organic Manure on Nitrogen Forms in the Rhizosphere of Potato Plants:

Data presented in Table (4) show the periodical change of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  in rhizosphere of potato plants. The obtained data show that the ammonical and nitrate nitrogen gradually increased with the progression of growth periods and reaching the maximum records to 60 days. It was true in all applied treatments that organic manure application significantly increased ammonical and nitrate nitrogen to the maximum values in rhizosphere of potato plants.

Inoculation of potato tubers at sowing with *Azotobacter* & *Azospirillum* and a half dose of ammonium sulphate supplementation gave higher records of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  than the application of full dose from ammonium sulphate. Similar results were obtained by Neweigy *et al.* (1997) and Nasef *et al.*, (2010) who reported that ammonical and nitrate nitrogen content were higher in the case of biofertilization with asymbiotic  $\text{N}_2$ -fixers than the inorganic N-fertilization in rhizosphere soil of wheat and sorghum. Also, Massoud *et al.*, (2005) pointed out that the Rhizobium bacteria inoculation increased  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  concentration in soil as compared with uninoculated treatments.

From the data presented in Table (4), it is note worthy to mention that the inoculation of potato tubers with *B. megaterium* var. phosphaticum in combination with the various investigated treatments significantly increased  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  values in comparison with uninoculated ones. This trend was observed on all determinations periods. These results may explain the role of P-dissolvers in the availability of phosphorus and micro-elements, and consequently different microbial activities were increased.

**Table 4:** Ammonical and nitrate concentration (ppm) of different periods in the rhizosphere of potato plants (average of two growing seasons).

Inoculation with phosphate dissolving bacteria (A)									
Ammonical nitrogen ( $\text{NH}_4\text{-N}$ ) concentration (ppm)									
Treatments (B)	30 days after planting			60 days after planting			90 days after planting		
	Un*	In**	Mean	Un*	In**	Mean	Un*	In**	Mean
Control	14.30	19.00	16.65c	19.10	23.00	21.05d	17.30	15.00	16.20d
Azoto & Azos +1/2RND	21.60	29.30	25.45b	37.00	45.60	41.30b	21.00	32.00	26.50b
chicken manure	27.70	31.60	29.65a	41.00	55.50	48.25a	23.00	33.10	28.05a
Ammonium sulphate (As)	23.30	28.50	25.90b	31.00	35.00	33.00c	20.00	26.70	23.32c
Mean	21.73b	27.10a	--	32.03b	39.78a	--	20.33b	26.68a	--
L.S.D at 5%	A	1.345			2.730			0.577	
	B	1.150			0.837			0.936	
	AB	1.646			1.176			1.324	
Nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) concentration (ppm)									
Control	16.10	31.00	23.55d	29.10	21.00	25.05d	12.30	19.10	15.70d
Azoto & Azos +1/2RND	40.50	55.00	47.75b	50.13	38.00	44.07b	25.10	37.00	31.05b
chicken manure	40.16	60.10	50.13a	61.41	40.00	50.71a	32.16	43.00	37.58a
Ammonium sulphate (As)	28.59	45.00	36.79c	42.16	26.00	34.00c	23.10	28.00	25.55c
Mean	31.38b	47.78a	--	31.25b	45.70a	--	23.17b	31.78a	--
L.S.D at 5%	A	0.620			1.404			3.234	
	B	2.28			1.215			1.286	
	AB	n.s.			1.719			1.820	

Un\*= Uninoculated treatment

In\*\*= Inoculated treatment

A= Inoculation factor

B= treatment factor

Azoto= Azotobacter

Azos= Azospirillum

RND= recommended nitrogen dose

### 3.4. Effect of Biofertilizers and Organic Manure on Total Nitrogen and Phosphorus in the Rhizosphere Potato Plants:

Data in Table (5) indicate that total nitrogen and phosphorus percentages in the rhizosphere of potato plants gradually decreased with the progression of growth period and reached their minimum records at 60 days and increased thereafter. In most cases, these differences in nitrogen and phosphorus percentages were significant as a result of inoculation with phosphate dissolving bacteria (A) or treatments with different N- sources (B) or their interaction. The decrease of nitrogen and phosphorus content of soil could be attributed to nutrients uptake by potato plants especially at flowering periods, at 60 days after planting where it is the most active period in plant growth. Potato rhizosphere for soil amended with chicken manure showed the highest nitrogen and phosphorus content and these results were observed at all estimation periods. These increases as a result of applying chicken manure, which are in accordance with those obtained by Mahdy, 2003 who stated that organic manure attains more pronounced contents of macro- (N, P and K) and micronutrients (Fe, Mn, Zn and Cu), which was consequently reflected on plant growth and yield. Biofertilization with *Azotobacter* & *Azospirillum* showed higher values of nitrogen and phosphorus percentages in the rhizosphere of potato than fertilization with ammonium sulphate.

These results are in agreement with the findings of Mahendran and Kumar (1998) who reported that biofertilization application increase nutrient contents in soil in comparison with inorganic N-fertilizers.

Also, the data presented in Table (5) emphasize that inoculation of potato tuber with *B. megaterium* var. phosphaticum in combination with asymbiotic N<sub>2</sub>-fixers or organic manure or ammonium sulphate significantly increased, in most cases, total nitrogen and total phosphorus in the rhizosphere of potato plants. Such results may explain the synergetic effect of phosphate dissolving bacteria. This result is accordance with Mahendran and Chandmani (1998) who reported that dual inoculation of potato tubers with *Azospirillum* and phosphate dissolving bacteria increased soil N and P availability as improved potato yield and tuber quality.

**Table 5:** Changes in total nitrogen and total phosphorus in the rhizosphere of potato plants (Average of two growing seasons).

Inoculation with phosphate dissolving bacteria (A)									
Total nitrogen (%)									
Treatments (B)	30 days after planting			60 days after planting			90 days after planting		
	Un*	In**	Mean	Un*	In**	Mean	Un*	In**	Mean
Control	0.051	0.053	0.052c	0.028	0.045	0.036	0.043	0.044	0.043c
Azoto & Azos +1/2RND	0.053	0.066	0.059b	0.033	0.040	0.037	0.046	0.057	0.052b
Chicken manure	0.055	0.073	0.064a	0.036	0.041	0.038	0.053	0.067	0.061a
Ammonium sulphate (As)	0.053	0.058	0.056bc	0.026	0.041	0.034	0.039	0.054	0.047c
Mean	0.053b	0.063a	--	0.031b	0.042a	--	0.046b	0.055a	--
L.S.D at 5%	A				0.011				0.003
	B				n.s.				0.004
	AB				0.005				0.006
Total phosphorus (%)									
Control	5.28	6.10	5.71c	4.32	5.39	4.96d	8.13	6.12	7.64c
Azoto & Azos +1/2RND	7.44	7.82	7.63b	8.33	7.35	7.84b	8.91	9.02	8.96b
Chicken manure	8.16	9.78	8.45a	9.80	7.84	8.82a	11.12	12.05	11.61a
Ammonium sulphate (As)	8.16	6.96	7.74b	6.37	6.37	6.37c	6.90	6.90	6.90d
Mean	7.24	7.96	--	7.35a	6.76b	--	8.77	8.52	--
L.S.D at 5%	A				0.294				n.s.
	B				0.196				0.294
	AB				0.294				0.441

Un\* = Uninoculated treatment  
 In\*\* = Inoculated treatment  
 A = Inoculation factor  
 B = treatment factor  
 Azoto= Azotobacter  
 Azos= Asozpirillum  
 RND= recommended nitrogen dose

**3.5. Effect of Biofertilizers and Organic Manure on Some Growth Parameters of Potato Plants:**

It is obvious from the data in Table (6) that growth characters, i.e. plant high, leaf number/plant, branches number and shoot dry matter/plant after 70 days from sowing are significantly increased with treatments used.

Chicken manure treatment led to the greatest values of the studied growth parameters which recorded 68.8 cm for plant height, 16.43 leaf / plant, 4.03 branches / plant and 34.4 gm Shoot dry matter / plant. These increases were more attributed to the vital role of organic manure, which applied to correct and improve the physical, chemical and biological properties of the soil and this consequently encourage the plant growth parameters. Similar results were observed by Merghany (1998) and El-Fakharani (1999), who found that organic manure had a significant effect on growth characters of potato plants when compared with the application of the same level of nitrogen obtained from inorganic N-fertilizers. Irrespective of chicken manure treatment, the values of growth parameters were higher in the asymbiotic N<sub>2</sub>-fixers treatments than ammonium sulphate application. This may be due to the production of growth regulators such as auxins, cytokinins and gibberellins by asymbiotic N<sub>2</sub>-fixers bacteria which affect the production of root biomass and nutrients uptake (Fulchieri and Frioni, 1994, Jaday *et al.*, 1998 and Massoud *et al.*, 2008).

**Table 6:** Effect of organic, mineral and biofertilization treatments on some growth parameters of potato plants after 70 days from sowing (Average of two growing seasons).

Treatments (B)	Plant Height (cm)			Leaf number/plant			Branches number/plant			Shoot dry matter (g/plant)		
	Un*	In**	Mean	Un*	In**	Mean	Un*	In**	Mean	Un*	In**	Mean
Control	37.00	58.0	47.5 d	7.80	10.90	9.35 d	1.40	3.15	2.28c	8.05	13.35	10.7d
Azoto & Azos +1/2	43.50	79.5	61.5 b	11.50	15.30	13.40b	3.15	4.60	3.88a	25.17	35.60	30.4b
Chicken manure	56.00	81.5	68.8 a	15.65	17.20	16.43a	3.40	4.65	4.03a	31.40	37.30	34.4a
Ammonium sulphate (As)	38.50	72.5	55.5 c	10.65	13.90	12.30c	2.45	4.15	3.30b	18.04	34.05	26.1c
Mean	43.8 b	72.8 a	--	11.40b	14.33a	--	2.60 b	4.14 a	--	20.7b	30.08a	--
L.S.D At 5%	A				0.342				0.031			0.456
	B				0.304				0.168			0.762
	AB				0.429				0.237			1.078

Un\* = Uninoculated treatment  
 In\*\* = Inoculated treatment  
 A = Inoculation factor  
 B = treatment factor  
 Azoto= Azotobacter  
 Azos= Asozpirillum  
 RND = recommended nitrogen dose

Regarding to the interaction between different N-sources used and inoculation with phosphate dissolving bacteria, the highest values of potato growth performance were observed in the applied chicken manure treatment combined with potato tubers inoculated with phosphate dissolving bacteria (PDB). These increases are more attributed to the effective role of chicken manure and high densities of phosphate dissolving bacteria as shown by Fig (1) as well as increasing ammonical and nitrate nitrogen contents in the rhizosphere soil (Table 4) when potatoes tubers inoculated with PDB. In general, growth parameters of potato plants were higher in all investigated treatments which were inoculated with phosphate dissolving bacteria as compared to uninoculated ones.

### 3.6. Effect of Biofertilizers and Organic Manure on Total Nitrogen and Phosphorus Uptake:

Data in Table (7) showed the periodical changes in nitrogen and phosphorus uptake by potato plants. Obtained data show that nitrogen and phosphorus uptake were increased with the increasing growth period and reached their maximum values at 60 days, however, all applied treatments gave significant increases in these parameters.

**Table 7:** Changes in total nitrogen and total phosphorus uptake of potato leaves (Average of two growing seasons)

Inoculation with phosphate dissolving bacteria (A)									
Total nitrogen (%)									
Treatments (B)	30 days after planting			60 days after planting			90 days after planting		
	Un*	In**	Mean	Un*	In**	Mean	Un*	In**	Mean
Control	1.61	1.69	1.65 c	2.35	3.29	2.82 b	1.71	2.65	2.18d
Azoto &Azos +1/2RND	2.54	2.52	2.53 a	2.75	4.63	4.16 a	2.63	3.81	3.22a
Chicken manure	2.21	2.71	2.46 a	3.81	4.50	4.16 a	2.51	3.51	3.01b
Ammonium sulphate (As)	1.91	2.36	2.14 b	3.47	3.81	3.64 a	2.01	2.96	2.49c
Mean	2.07b	2.32a	--	3.10 b	4.06 a	--	2.22b	3.23a	--
L.S.D at 5%	A	0.062			0.356			0.062	
	B	0.065			0.395			0.079	
	AB	0.092			0.596			0.113	
Total phosphorus (%)									
Control	0.161	0.182	0.172 d	0.230	0.225	0.228 d	0.211	0.225	0.218c
Azoto &Azos +1/2RND	0.351	0.511	0.431 b	0.490	0.581	0.536 b	0.421	0.511	0.466a
Chicken manure	0.361	0.594	0.478 a	0.560	0.674	0.618 a	0.401	0.532	0.467a
Ammonium sulphate (As)	0.281	0.361	0.321 c	0.350	0.531	0.440 c	0.317	0.417	0.367b
Mean	0.20 b	0.412 a	--	0.408 b	0.503a	--	0.338b	0.421a	--
L.S.D at 5%	A	0.006			0.012			0.006	
	B	0.007			0.006			0.004	
	AB	0.009			0.009			0.006	

Un\*= Uninoculated treatment  
In\*\*= Inoculated treatment  
A= Inoculation factor  
B= treatment factor

Azoto= Azotobacter  
Azos= Asozpirillum  
RND= recommended nitrogen dose

These increases were correspondent with the decreases of available nitrogen and phosphorus contents in the rhizosphere soil of potato plants (Table 5). The highest values of nitrogen and phosphorus uptake were observed in asymbiotic N<sub>2</sub>-fixars inoculated treatments or in treatment used of chicken manure. Mahendran and Chandramani (1998) and Massoud *et al.*, (2008) found that bio-inoculation with N<sub>2</sub>-fixing bacteria increased N, P, and K uptake by plants. These data are confirmed by Lifshitz *et al.* (1987) who reported that bio-inoculation was resulted in an increase in immobilization of insoluble nutrients followed by enhancement of uptake by the plants.

The increase of nutrient uptake by potato tubers in treatment fertilized by organic manure can be attributed to their role in plant growth as a source of all necessary macro and micronutrients in available forms and also to their high values of Azotobacters, Azospirilla and phosphate dissolving bacteria densities in chicken manure treatment as compared to ammonium sulphate application which was previously discussed in Fig (1). This result is in accordance with that obtained by El-Shikha *et al.*, (2005).

Generally, inoculation of potato tubers with phosphate dissolving bacteria (BDP) in combination with various treatments improved nitrogen and phosphorus uptake by potato plants. This was observed at all determinations periods where BDP led to availability of phosphorus as well as the synthesis of auxins, cytokinins and gibberellic acid like-substances which enhanced the plant growth, absorption nutrients and photosynthesis process.

### 3.7. Effect of Biofertilizers and Organic Manure on Tuber Number per Kg, Total Carbohydrate (%) and Tuber Yield:

Data presented in Table (8) show that investigated treatments caused significant differences in tubers number/ kg, carbohydrate content % and total yield ton/fed.

With respect to tubers number/ kg, results indicate that the *Azotobacter* & *Azospirillum* inoculation treatment significantly decreased the number of tubers/kg followed by chicken manure then ammonium treatment, which represented 8.09, 8.44 and 10.7 tubers / kg. It is worthy to mention that the decrease of tuber number/kg is a desirable character for consumers.

**Table 8:** Effect of organic manure, mineral and biofertilization on dry matter, tuber number/kg carbohydrate content and tuber yield (Average of two growing seasons).

Treatments (B)	Tubers number/Kg			Total Carbohydrate %			Total yield ton/fed		
	Inoculation with phosphate dissolving bacteria (A)								
	Un*	In**	Mean	Un*	In**	Mean	Un*	In**	Mean
Control	11.15	12.2	11.7a	26.85	32.00	29.43d	8.35	10.16	8.94b
Azoto & Azos +1/2 RND	8.31	7.86	8.09c	50.15	64.30	57.23a	14.76	18.72	16.70a
Chicken manure	7.81	9.06	8.44c	42.20	61.70	51.95b	13.24	18.72	16.47a
Ammonium sulphate (As)	9.75	11.65	10.7b	37.65	54.55	46.10c	13.36	18.14	15.75a
Mean	9.26b	10.19a	--	39.21b	53.13a	-	11.86	16.58	--
L.S.D At 5%									
	A				3.637			1.198	
	B				0.975			0.839	
	AB				1.379			1.699	

Un\* = Uninoculated treatment  
 In\*\* = Inoculated treatment  
 A = Inoculation factor  
 B = treatment factor

Azoto = Azotobacter  
 Azos = Azospirillum  
 RND = recommended nitrogen dose

As for the effect of different investigated treatments on total carbohydrate in potato tubers, the obtained data show that potato tubers inoculated with *Azotobacter* & *Azospirillum* combined with *B. megaterium* var. phosphaticum showed the highest value of total carbohydrate. This result isn't in accordance with that obtained by Abou-zeid (1993) who found that total soluble carbohydrate % decreased due to inoculation of two wheat varieties with *A. brasilense* (strains Sp7 and Sp 245), while it increased for rice grain non-significantly. Organic manure application showed higher values of total carbohydrate than ammonium sulphate application. This result was associated with the case of chicken manure application either alone or in combination with phosphate dissolving bacteria. These results are in agreement with those obtained by Merghany (1998) and El-Fakharani (1999) who found that the organic manure significantly increased the total carbohydrates in potato tubers in comparison with inorganic N-fertilization.

Regarding potatoes Total yield (ton/fed), Data in Table (8) indicate that applying asymbiotic N<sub>2</sub>-fixing bacteria or organic or inorganic N-fertilizer led to significant increases in yield over control, with insignificant differences between them. Also, the data in the same table show the importance of bio-inoculation of phosphate dissolving bacteria in increasing this parameter, where it was significant increase. The increase of tuber yield/fed of potato plants due to bio-inoculation with asymbiotic N<sub>2</sub>-fixers (*Azotobacter* & *Azospirillum*) could be attributed to the capability of the organisms to fix nitrogen which could be taken by the growing plants. Similar results were observed by Mahendran *et al.*, (1996), Mahandran and Kumar (1998), El-Ghinbihi and Fetouh (2001), El-Etr *et al.*, (2004), and Massoud *et al.*, (2008) who reported that potato tubers inoculated with asymbiotic N<sub>2</sub>-fixers in combination with *B. megaterium* var. phosphaticum gave the highest tuber yield/fed.

These results are also in the line with those obtained by Aly (2003) who stated that some bacteria such as *Azotobacter chroococcum* and *Paenibacillus polymyxa* are capable to produce some hormones which induces the proliferation roots and root hair that increase nutrient absorbing surfaces as well as produce organic acids, which solublize inorganic and organic forms of mineral elements, and consequently increase stems and leaves then the straw and grain yields.

Generally, from the data presented in Table (8) it can be concluded that the inoculation of potato tubers with phosphate dissolving bacteria in combination with either asymbiotic N<sub>2</sub>-fixers or organic manure as well as inorganic fertilization improved tuber yield/fed of potato plants.

### 3.8. Effect of Biofertilizers and Organic Manure on Availability of Some Macro and Micro Nutrients after Harvesting Tubers Plant:

Data presented in Table (9) show the available nutrients content (N, P, K, Fe, Zn and Mn) after harvesting potato plants, the obtained results indicated that the abovementioned nutrients significantly increased by inoculation of potato tubers at sowing with phosphate dissolving bacteria under all the studied treatments compared to uninoculated plants. The highest values of available nutrients were observed when the organic manure was inoculated with phosphate dissolving bacteria. Also, using of any nitrogen fertilizer, inoculation with *Azospirillum* and *Azotobacter*, chicken manure or ammonium sulphate caused significant increases in the studied macro and micro nutrients.



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