BIOFERTILIZATION AND ORGANIC MANURING EFFICIENCY ON GROWTH AND YIELD OF POTATO PLANTS (*Solanum tuberosum* L.)

By

R.A. Zaghloul

Agric Botany Department, Faculty of Agriculture, Moshtohor
Zagazig University Banha Branch, Egypt

ABSTRACT

The aim of this research was to study the possibility of biofertilizers using and organic manuring (biogas fertilizer) instead of chemical fertilizers for potato growth and production. Two field experiments were carried out at the Vegetables Experimental Farm of Fac. Agric Moshtohor, Zagazig Univ. during two successive spring seasons of 2001 and 2002. *Azotobacter chroococcum* and *Azospirillum lipoferum* were used as nitrogen fixing bacteria. While, *B. megaterium var. phosphaticum* was used as phosphate solubilizing bacteria. Biogas manure and ammonium sulphate were used as organic and inorganic fertilizers, respectively. Biogas manure was added at a rate of 6 ton/fed. (90 kg N/ fed.) as well as using ammonium sulphate at the same level of nitrogen.

Obtained results indicated that the highest records of evoluted CO_2 were observed in biogas manure treatments. Whereas, the highest records of N_2-ase activity were observed in rhizosphere of potato plants inoculated with asymbiotic N_2- fixers. Inoculation of potato tubers with phosphate solubilizing bacteria combined with various treatments under investigation increased CO_2 evolution and N_2-ase activity.

Inoculation of potato tubers with *Azotobacter & Azospirillum* and a half dose of ammonium sulphate receiving gave higher records of NH_4- N and $NO_3 - N$ in rhizosphere soil than the application of full dose from ammonium sulphate. Biogas manure amendment showed the highest records of N and P in rhizosphere soil.

Growth characteristics of potato plants were significantly increased with biogas manure application in combination with potato tuber inoculation with phosphate solubilizing bacteria. Potato tuber inoculated with *Azotobacter* & *Azospirillum* combined with *B.megaterium var. phosphaticum* showed the highest records of carbohydrate content in tubers. Insignificant difference in tuber yield/ fed. was observed between asymbiotic N_2 – fixing bacteria and biogas manuring treatments.Generally, tuber yield/fed. was higher with *Azotobacter & Azospirillum* and biogas manuring treatments than ammonium sulphate application. Therefore, the use of biofertilizers or organic manuring may be recommended as a substitute for chemical fertilizers in potato crop production especially for exportation.

Key words: potato, *A. chroococcum*, *A. lipoforum*, *B. megaterium* var. *phosphaticum*, biogas manure.

1. 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. Nitrogen and phosphatic chemical fertilizers are commonly used in production of vegetable crops. Application of such chemical fertilizers to the soil causes some problems especially for exportation. It is well known that the nitrogenous fertilizers are 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**). Also, immobilization of phosphorus is the most important problem of phosphatic fertilization in Egypt and this is 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 a particular interest to avoid the previously mentioned problems. Biofertilizers application with a half dose of chemical nitrogen fertilizer proved to be an efficient tool in increasing available nutrients in soil as well as growth performance and yield of cultivated crops is improved. Several investigators indicated that inoculation with *Azotobacters* and *Azospirilla* improved growth and yield of potato crop(**Mahendran** ***et al*****, 1996; Zahir and Muhammed, 1996; Zahir** ***et al*****, 1997; Jadhav** ***et al*** **1998, Mahendran and Kumar, 1998 and El-Ghinbihi and Fetouh, 2001).**

With regard to the effect of organic manuring on plant growth and microbial activity. **Abdel- Magid** ***et al*** **(1996); Zaghloul** ***et al*** **(1996) and Neweigy** ***et al*** **(1997)** reported that the addition of organic manures to the soil encouraged proliferation of soil microorganisms, increased microbial populations and activity of microbial enzymes i.e Dehydrogenase, Urease and Nitrogenase. In addition, **Sood (1993); Sharma (1993); Karadogan (1995); Merghany(1998) and El- Fakhrani (1999)** found that organic manuring had a significant stimulative effect on growth characters and tuber yield of potato plants when compared with application of the same level of nitrogen from inorganic nitrogen fertilizers. Concerning the effect of dual inoculation with asymbiotic N_2- fixing bacteria and phosphate solubilizing bacteria on potato growth, **Abdel- Ati** ***et al*** **(1996); El- Gamal (1996) and Mahendran and kumar (1998)** found that dual inoculation of potato with asymbiotic N_2- fixers i.e *Azotobacters* or *Azospirilla* and *phosphate* solubilizing bacteria improved growth performance, dry matter, carbohydrates content and tuber yield of potato. Also, **Mahendran and Chandramani (1998)** reported that dual inoculation of potato with the above - mentioned microorganisms increased soil available N,P and K.

Therefore, the present investigation was designed to evaluate the effect of biofertilization and organic manuring on growth and yield of potato.

2. MATERIALS AND METHODS

Two field experiments were carried out during the spring seasons of 2001 and 2002 at the Agricultural Research and Experimentation Center (vegetables farm) of Fac. Agric; Moshtohor, Zagazig Univ. to study the effect of nonsymbiotic N_2- fixing bacteria, biogas manure and ammonium sulphate application either individually or combined with phosphate solubilizing bacteria on growth and yield of potato cv. Diamant. Physical and chemical analyses of the experimental soil are shown in Table (1). Also, the chemical analysis of biogas manure is shown in Table (2).

Table 1. Physical and chemical analyses of the experimental soil.

Parameters	Unit	Seasons	
		2001	**2002**
A. Mechanical analysis			
Coarse sand	(%)	**3.62**	**3.15**
Fine sand	(%)	**23.12**	**25.18**
Silt	(%)	**26.50**	**27.12**
Clay	(%)	**46.76**	**44.55**
Textural class		**Clay loam**	**Clay loam**
B. Chemical analysis			
Organic matter	(%)	**1.52**	**1.67**
Total nitrogen	(%)	**0.26**	**0.28**
Total phosphorus	(%)	**0.14**	**0.18**
Total potassium	(%)	**0.31**	**0.34**
pH		**8.31**	**8.11**

Table 2. Chemical analysis of biogas manure.

Parameters	Unit	Seasons	
		2001	2002
Organic matter	(%)	61.30	58.21
Organic carbon	(%)	32.55	33.76
Total nitrogen	(%)	1.48	1.56
Total phosphorus	(%)	0.81	0.86
Total potassium	(%)	1.20	1.28
C:N ratio		24.02	21.64
Iron	(ppm)	48.1	45.5
Zinc	(ppm)	36.2	32.1
Copper	(ppm)	23.3	27.6
Manganese	(ppm)	16.5	21.3

Mechanical analysis was estimated according to **jackson (1973)**. While, chemical analysis of soil and biogas 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 UF5 and *Azospirillum lipoferum* Mn3 strains were provided from the unit of Biofrtilization , Fac. of Agric., Ain Shams Univ., Cairo, Egypt. While, *Bacillus megaterium var phosphaticum* (pure local strain) was provided from Microbiology Dept. Soil & Water and Environment Res. Inst., Agric., Res. Center, Giza, Egypt.

2.3. Inocula preparation

For preparation of *A. chroococcum* and *A. lipoferum* inocula, modified Ashby's medium **(Abdel- Malek and Ishac, 1968)** and semi-solid malate medium **(Dobereiner, 1978)** were inoculated with *A. chroococcum* and *A. lipoferum*, respectively then incubated at 30° C and 32°C for 7 days, respectively. Also, **Bunt and Rovira medium (1955) modified by Abdel- Hafez (1966)** was inoculated with *B. megaterium var. Phosphaticum* then incubated at 30° C for 7 days.

Biogas manure was added to the soil before sowing at a rate of 6 ton/ fed. (90kg N/fed). Chemical phosphorus fertilizer at a rate of 31kg P_2O_5 in form of calcium superphosphate (15.5% P_2O_5) was applied during preparation of the soil for all treatments. Nitrogen and potassium fertilizers were added at rates of 90 kg N/ fed. and 96 kg K_2O / fed. in forms of ammonium sulphate (20.5% N) and potassium sulphate (48%K_2O) in three equal doses at 15,30 and 60 days after emergence.

2.4. Inoculation process

Pieces of potato tuber were successfully washed with water and air dried. Thereafter, they were soaked in cell suspension of a mixture (1:1) from *A. chroococcum* and *A. lipoferum* (1ml contains about $8X10^7$ viable cells) for 30 minutes. Tubers of control treatment was treated with the same manner but using N- deficient medium instead of bacterial cultures.

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

2.5. Experimental design

Treatments were distributed in a randomized complete block design with three replicates. This experiment included the following treatments:

1- Control (without any addition).
2- *A.chroococcum* & *A.lipoferum* inoculum (1:1) + a half dose of inorganic N- fertilizer (hdn).
3- A full dose of biogas manure, 6 ton/fed. (90 kg N/fed.).

4- A full dose of ammonium sulphate (90kgN/fed).
5- Control + *B. megaterium* (PSB) inoculum.
6- *A. chroococcum* & *A.lipoferum*+ hdn+ PSB.
7- A full dose of biogas manure+ PSB.
8- A full dose of ammonium sulphate+ PSB.

2.6. Cultivation Process

Cultivation process was performed at January 20th by sowing uninoculated or inoculated tuber pieces in ridges 5m long and 70 cm a part. Other field practices for potato growing were followed according to the recommendations of the Ministry of Agriculture.

2.7. Sampling and determinations

Representative soil samples from rhizosphere of potato plants were taken at 7, 15, 30, 60, and 90 days from sowing. The samples were microbiologically analyzed for densities of *Azotobacters, Azospirilla.*, phosphate solubilizing bacteria, CO_2 evolution and N_2-ase activity. Also, rhizosphere soil samples were chemically analyzed at 15, 30, 60, and 90 days for ammoniacal and nitrate nitrogen, total nitrogen and phosphorus.

2.7.1.A. Microbiological analysis

A.1. Densities of *Azotobacters* and *Azospirilla* were determined on modified Ashby's medium **(Abdel- Malek and Ishac, 1968)** and Semi-solid malate medium **(Dobereiner, 1978)**, respectively using the most probable number technique **(Cochran, 1950)** whereas, the density of inorganic phosphate dissolvers was determined on **(Bunt and Rovira medium, 1955** modified by **Abdel- Hafez, 1966)** using the plate count method.

A.2. Carbon dioxide evoluted 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.B. Chemical analysis of soil

B.1. Ammoniacal 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 height, dry matter of shoot system, leaves number/plant and branches number/ plant were estimated.

2.7.4. Chemical analysis of plant

Total nitrogen and phosphorus were periodically determined in dried leaves at 15, 30, 60, and 90 days from sowing.

At 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)**

2.8. Statistical analysis

Analysis of variance (ANOVA) of data obtained from growth characters, yield and yield component were carried out according to **Snedecor and Cochran (1989)**. The differences between the means values of various treatments were compared by Duncan's multiple range test **(Duncan's, 1955)**.

3. RESULTS AND DISCUSSION

3.1. Effect of biofertilization and organic manuring on microbial densities in rhizosphere of potato plants.

Data in Table (3) show the periodical changes of *Azotobacters*, *Azospirilla* and phosphate solubilizing bacteria in rhizosphere of potato plants. Microbial densities of the abovementioned bacterial groups gradually increased with the increasing of growth period to reach their maximum records at 60 days and decreased thereafter. The same trend of results was observed in all treatments. Rhizosphere of potato plants inoculated with *Azotobacter* & *Azospirillum* and receiving a half dose of ammonium sulphate contained higher densities of

Azotobacters and *Azospirilla* than either biogas manure or ammonium sulphate application. Such 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); Shatokhina and Khristenko (1996) and Saleh *et al* (1998).** Regarding the densities of phosphate solubilizing bacteria, obtained data show that rhizosphere of potato plants amended with biogas manure contained the highest densities of p-solubilizing bacteria compared to other treatments. Irrespective of control, the lowest densities of p-solubilizing bacteria was observed in the treatment of ammonium sulphate application. The same trend of results was observed at various determination periods. This result is in accordance with **Abdel-Magid *et al* (1996) and Neweigy *et al* (1997).**

It is worthy to notice that the rhizosphere of potato plants inoculated with phosphate solubilizing bacteria contained higher densities of *Azotobacters*, *Azospirilla* and phosphate dissolvers compared to the uninoculated ones. This was true in all treatments and different determination periods. These results are in harmony with **Zaghloul *et al* (1996) and Saad and Hammad (1998).**

3.2. Effect of biofertilization and organic manuring on CO_2 evolution and N_2-ase activity in rhizosphere of potato plants.

Data presented in Table (4) indicate that carbon dioxide evoluted by soil microorganisms gradually increased throughout the experimental period to reach their maximum values at 60 days and decreased thereafter. The same trend of results was obtained in both growing seasons and various investigated treatments. The highest records of evoluted carbon dioxide was observed in biogas manure treatment compared to other treatments when applied alone. As well, this trend of results was obtained in case of biogas manure application combined with phosphate solubilizing bacteria inoculum. .Rhizosphere of potato plants inoculated with *Azotobacter* & *Azospirillum* showed higher values of carbon dioxide evolution than either uninoculated one or ammonium sulphate application. Inoculated treatments with phosphate solubilizing bacteria showed higher values of CO_2 evolution than uninoculated treatments. This was observed in both growing seasons as well at all determination periods. This result may explain the role of *B.megaterium var. phosphaticum* in supplying different soil microorganisms and plants with their available phosphorus requirements and consequently increase the activity of soil microorganisms. It is clear from data presented in Table (4) that the highest records of N_2-ase activity were recorded in the rhizosphere of potato plants inoculated with asymbiotic N_2-fixers and supplemented with a half dose of inorganic N-fertilizer. Also, rhizosphere of potato plants supplemented with biogas manure showed higher records of N_2- ase activity than the application of full dose from ammonium sulphate. These results are in agreement with **Mahendran *et al* (1996); Zahir *et al* (1997) and Saleh *et al* (1998).** They reported that biofertilization of plants with asymbiotic N_2-fixers increased N_2-ase activity compared to unbiofertilized ones. Also, they found that increasing the dose of inorganic N- fertilizer resulted a decreasing in N_2-ase activity. Moreover, it could be concluded that inorganic N- fertilization exhibited a negative effect on biological N_2 fixation. Nevertheless, low doses of N- fertilizer promoted the response of cultivated plants to inoculation with asymbiotic N_2- fixers **(El-Demerdash, 1994 and Jadhav *et al* 1998).** They reported that half of the recommended dose of added inorganic N-fertilizer can be saved by asymbiotic N_2-fixers inoculation. Such trends support the obtained results in the current study.

It is obvious from data recorded in Table (4) that when tuber of potato plants inoculated with phosphate solubilizing bacteria, the N_2-ase activity values were increased compared to uninoculated ones. Generally, carbon dioxide evolution and N_2-ase activity values were higher during the second season than the first one. These differences between the two seasons are likely to be due to the differences in the climatic conditions.

Table 3. Azotobacters, Azospirilla and inorganic phosphate solubilizing bacteria densities (number/g dry weight of soil) in rhizosphere of potato plants. (The recorded densities represent the average of the two growing seasons).

Treatments	Days after sowing									
	7	15	30	60	90	7	15	30	60	90
	Non inoculated with phosphate solubilizing bacteria					Inoculated with phosphate solubilizing bacteria				
	Azotobacters ×10^5									
Control	10.30	24.50	45.30	48.20	31.30	22.20	36.40	52.10	62.30	36.30
Azoto. &Azos. + hdn	34.20	81.40	110.1	140.0	95.40	42.30	86.30	120.2	186.4	160.3
Biogas manure	18.20	62.60	74.20	90.10	43.20	38.80	80.20	88.30	96.60	78.60
Ammonium sulphate	12.01	40.40	53.20	82.02	38.40	36.20	44.10	56.50	82.20	70.40
	Azospirilla ×10^4									
Control	8.40	23.40	38.10	41.20	26.20	24.10	36.50	52.20	60.40	43.30
Azoto.& Azos. + hdn	40.20	78.60	92.10	120.1	82.10	60.20	71.30	86.30	98.30	81.40
Biogas manure	36.20	61.30	70.20	90.60	63.40	56.30	68.20	80.20	112.1	86.40
Ammonium sulphate	25.50	42.20	62.10	81.30	52.20	43.20	56.60	78.10	91.10	73.40
	phosphate solubilizing bacteria×10^5									
Control	12.60	20.40	28.40	42.20	38.10	18.70	32.40	40.50	54.00	42.50
Azoto.& Azos. + hdn	16.40	40.20	48.40	68.00	62.30	28.20	72.20	90.30	116.2	72.30
Biogas manure	20.20	48.60	62.00	90.30	80.00	32.40	96.30	110.2	128.0	91.40
Ammonium sulphate	14.40	28.20	40.20	72.40	60.30	21.10	36.30	56.00	81.30	56.60

Azoto., Azotobacter.
Azos., Azospirillum.

hdn., half dose of nitrogen.

Table 4. Carbon dioxide evolution and nitrogenase activity in rhizosphere of Potato plants during the two growing seasons.

Treatments	Days after sowing																			
	7		15		30		60		90		7		15		30		60		90	
	CO$_2$ evoluted (µg/g dry soil/ hr.)										N$_2$-ase activity (n moles C$_2$H$_4$/g dry soil/hr.)									
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
	Non inoculated with phosphate solubilizing bacteria																			
Control	8.16	10.2	14.02	18.60	21.2	23.3	48.01	50.10	30.12	32.1	12.2	14.1	18.2	16.3	23.1	26.4	28.5	31.10	21.4	26.3
Azoto. &Azos. + hdn	15.1	18.2	28.64	32.30	48.1	52.2	65.20	68.10	36.80	40.3	28.3	30.6	36.2	40.1	61.2	72.1	98.3	105.1	72.1	74.2
Biogas manure	22.1	26.4	49.29	38.30	62.6	70.3	103.10	109.1	82.40	86.8	24.6	25.5	30.4	33.1	48.2	51.1	69.2	73.10	54.3	58.5
Ammonium sulphate	12.6	16.1	22.91	26.40	51.4	58.2	58.20	62.60	61.30	65.2	20.3	18.6	34.4	36.2	46.1	44.3	61.4	65.20	42.1	45.1
	Inoculated with phosphate solubilizing bacteria																			
Control	11.2	13.3	18.16	20.3	31.2	36.1	51.30	56.40	46.60	42.10	22.6	25.5	26.1	30.2	43.2	46.1	48.50	51.30	30.4	36.2
Azoto.& Azos. + hdn	18.4	21.2	45.83	43.2	68.1	76.3	106.1	110.2	81.40	86.20	36.5	41.6	52.5	63.3	82.2	96.1	148.1	161.2	89.4	45.6
Biogas manure	26.1	32.4	51.56	56.2	82.3	93.1	153.1	161.2	106.4	116.2	32.4	35.2	41.2	46.3	63.1	65.5	121.1	130.3	81.4	88.5
Ammonium sulphate	17.5	23.6	32.08	40.2	57.1	62.3	70.12	82.40	63.40	68.30	28.3	32.4	36.3	38.2	52.3	56.3	82.40	85.30	63.2	66.5

Abbreviations: as those stated in Table (3) •

S1: The first season. **S2: The second season**

3.3. Effect of biofertilization and organic manuring on nitrogen forms in rhizosphere of potato plants.

Data recorded in Table (5) show the periodical changes of NH_4-N and NO_3-N in rhizosphere of potato plants. Obtained data show that ammoniacal and nitrate nitrogen gradually increased with the progression of growth period and reaching their maximum records at 60 days. This was true in all applied treatments. Biogas manure application increased ammoniacal and nitrate nitrogen in rhizosphere of potato plants.

Inoculation of potato tuber at sowing with *Azotobacter* & *Azospirillum* and a half dose of ammonium sulphate supplementation gave higher records of NH_4-N and NO_3-N than the application of full dose from ammonium sulphate. Similar results were obtained by **Zaghloul *et al* (1996) and Neweigy *et al* (1997)** who reported that ammoniacal and nitrate nitrogen content were higher in case of biofertilization with asymbiotic N_2-fixers than inorganic N-fertilization in rhizosphere soil of wheat and sorghum, respectively.

From data presented in Table (5) it is worthy to mention that the inoculation of potato tuber with *B.megaterium var. phosphaticum* in combination with various investigated treatments increased NH_4-N and NO_3-N values in comparison with uninoculated ones. This trend of results was observed in both growing seasons and various determination periods. This result may explain the role of p-dissolvers in availability of phosphorus and micro-elements and consequently different soil microbial activity were increased.

3.4. Effect of biofertilization and organic manuring on total nitrogen and phosphorus in rhizosphere of potato plants.

Data in Table (6) indicate that total nitrogen and phosphorus percentages in rhizosphere of potato plants gradually decreased with the progression of growth period and reached their minimum records at 60 days and increased thereafter. The decrease of nitrogen and phosphorus content of soil could be attributed to nutrients uptake by potato plants especially at early growth periods. Rhizosphere soil of potato plants amended with biogas manure showed the highest nitrogen and phosphorus content and this result was observed at all estimation periods.

In general biofertilization with *Azotobacter* & *Azospirillum* showed higher values of nitrogen and phosphorus percentages than fertilization with ammonium sulphate. These results are in agreement with the findings of **Mahendran *et al* (1996); Zahir *et al* (1997) and Mahendran and Kumar (1998)** who reported that biofertilizers application increase nutrients content in soil in comparison with inorganic N-fertilizers.

Also, data recorded in Table (6) emphasize that inoculation of potato tuber with *B.megaterium var. phosphaticum* in combination with asymbiotic N_2-fixers, biogas manure and ammonium sulphate increased nutrients content (N and P) in soil compared to uninoculated treatments. Such results may explain the synergistic effect of phosphate solubilizing bacteria. This result is in accordance with **El-Gamal (1996); Mahendran and Chandramani (1998)**. They reported that dual inoculation of potato tuber with *Azospirillum* and phosphate solubilizing bacteria increased soil N and P availability as well as improved potato yield and tuber quality.

3.5. Effect of biofertilization and organic manuring on some growth characters of potato plants.

It is obvious from data in Table (7) that growth characteristics i.e plant height, leaves number/'plant and branches number/plant were significantly increased with biogas manure application in combination with potato tuber inoculated with *B.megaterium var. phosphaticum*. This finding was observed in both growing seasons. The high records of potato growth performance which were observed in biogas manure treatments can be explained by their high densities of phosphate solubilizing bacteria (Table, 3) and the increase of ammoniacal and nitrate nitrogen content in rhizosphere soil (Table, 5). Similar results were observed by **Merghany (1998) and El-Fakhrani (1999).** They found that organic manuring had a significant stimulative effect on growth characters of potato plants when compared with application of the same level of nitrogen from inorganic N-fertilizers.

Table 5. Ammoniacal and nitrate nitrogen concentration in rhizosphere of potato plants during the two growing seasons.

Treatments	Days after sowing															
	Ammoniacal nitrogen (ppm)								Nitrate nitrogen (ppm)							
	15		30		60		90		15		30		60		90	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
	Non inoculated with phosphate solubilizing bacteria															
Control	8.60	9.2	14.6	18.1	26.2	30.1	16.4	14.6	9.80	10.2	13.2	15.2	18.3	20.1	12.4	14.1
Azoto. &Azos. + hdn	21.3	28.1	41.0	44.0	48.0	52.0	30.0	28.0	11.4	13.8	19.0	23.0	36.0	38.0	21.0	24.0
Biogas manure	29.6	31.2	46.0	43.0	63.0	65.0	36.0	33.0	19.6	21.2	30.0	28.0	41.0	44.0	20.0	22.0
Ammonium sulphate	14.6	16.2	28.0	30.0	41.0	43.0	26.0	24.0	15.1	22.1	26.0	24.0	35.0	32.0	18.0	21.0
	Inoculated with phosphate solubilizing bacteria															
Control	12.6	14.0	19.0	21.0	32.0	34.0	20.0	18.0	12.6	14.0	16.0	18.0	21.0	23.0	15.1	14.5
Azoto.& Azos. + hdn	28.0	32.1	48.0	51.0	58.0	62.0	38.0	36.0	15.6	16.8	24.0	28.0	43.0	46.0	30.0	32.0
Biogas manure	33.1	36.3	50.0	53.0	61.0	66.0	40.0	42.0	23.1	30.0	36.0	32.0	52.0	56.0	29.0	33.0
Ammonium sulphate	16.8	18.2	32.0	34.0	46.0	49.0	26.0	28.2	21.3	26.5	32.0	28.0	39.0	36.0	27.2	29.1

Abbreviations: as those stated in Table (3)
S1: The first Season
S2: The second season

Table 6. Periodical changes in total nitrogen and phosphorus in rhizosphere of potato plants during the two growing seasons.

Treatments	Days after sowing															
	15		30		60		90		15		30		60		90	
	Total nitrogen (%)								Total phosphorus (%)							
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
	Non inoculated with phosphate solubilizing bacteria															
Control	0.200	0.229	0.210	0.214	0.175	0.181	0.190	0.181	0.110	0.116	0.101	0.112	0.074	0.091	0.102	0.118
Azoto. &Azos. + hdn	0.305	0.326	0.215	0.226	0.140	0.151	0.175	0.196	0.182	0.193	0.144	0.156	0.125	0.138	0.179	0.186
Biogas manure	0.375	0.381	0.235	0.240	0.105	0.118	0.198	0.224	0.233	0.240	0.175	0.173	0.161	0.165	0.200	0.228
Ammonium sulphate	0.245	0.250	0.230	0.226	0.110	0.126	0.165	0.188	0.144	0.152	0.126	0.131	0.122	0.118	0.135	0.142
	Inoculated with phosphate solubilizing bacteria															
Control	0.232	0.238	0.220	0.196	0.116	0.121	0.165	0.178	0.130	0.136	0.122	0.128	0.096	0.110	0.118	0.122
Azoto.& Azos. + hdn	0.355	0.362	0.280	0.261	0.158	0.163	0.211	0.236	0.173	0.182	0.153	0.161	0.141	0.148	0.172	0.180
Biogas manure	0.418	0.426	0.312	0.260	0.187	0.168	0.242	0.286	0.337	0.361	0.184	0.190	0.153	0.163	0.230	0.242
Ammonium sulphate	0.265	0.271	0.250	0.231	0.155	0.172	0.195	0.220	0.153	0.171	0.136	0.143	0.126	0.121	0.145	0.138

Abbreviations: as those stated in Table (3) .

The records of growth parameters were higher in the asymbiotic N_2-fixers treatments than ammonium sulphate application. This may be due to the production of growth regulators such as auxins, cytokinins and gibberillins by asymbiotic N_2-fixing bacteria which affect the production of root biomass and nutrients uptake **(Fulchieri and Frioni, 1994; Jadhav *et al* 1998 and El-Ghinbihi and Fetouh, 2001).** The same trend of results was obtained in both growing seasons.

In general, growth parameters of potato plants were higher in all investigated treatments which inoculated with phosphate solubilizing bacteria compared to uninoculated ones.

3.6. Effect of biofertilization and organic manuring on total nitrogen and phosphorus.

Data in Table (8) show the periodical changes in nitrogen and phosphorus content in potato plants. Obtained data show that total nitrogen and phosphorus content was increased with the increasing of growth period and reached their maximum values at 60 days. The increase of nitrogen and phosphorus content was correspondent with the decrease of nitrogen and phosphorus content in rhizosphere soil of potato plants (Table, 6). Inoculation of potato tubers with asymbiotic N_2-fixing bacteria gave higher records of total nitrogen in potato plants compared to either biogas manure or ammonium sulphate application. The highest records of nitrogen content was observed in asymbiotic N_2-fixers inoculated treatments in combination with *B.megaterium var. phosphaticum.* **El-Gamal (1996) and Mahendran and Chandramani (1998)** found that dual inoculation of potato tubers with *Azospirillum* and phosphate solubilizing bacteria increased N, P and K content of potato plants. Biogas manure application either solely or in combination with phosphate solubilizing bacteria showed higher records of total phosphorus in comparison with other investigation treatments. Also, biogas manure treatments gave higher values of nitrogen and phosphorus content compared to ammonium sulphate application. The increase of such nutrients content in biogas manuring can be attributed to the high records of *Azotobacters, Azospirilla* and phosphate solubilizing bacteria densities in biogas manure treatment compared to ammonium sulphate application which previously discussed in Table (3). These results are in harmony with **Sharma (1993); Karadogan (1995) and Merghany (1998).**

Generally, inoculation of potato tubers with phosphate solubilizing bacteria in combination with various investigation treatments improved nitrogen and phosphorus content in potato plants. This was observed at all determination periods as well as in both growing seasons.

3.7 Effect of biofertilization and organic manuring on dry matter, tuber numbers per kg, carbohydrate content and tuber yield.

Data in Table (9) indicate that dry matter per plant was significantly increased in biogas manure application in combination with *B.megaterium var. phosphaticum* inoculum. *Azotobacter & Azospirillum* inoculum in combination with phosphate solubilizing bacteria significantly decreased the number of tubers/kg. It is worthy to mention that the decrease of tubers number /kg is desirable character for consumers.

Irrespective of control, the highest number of tubers/kg was observed with ammonium sulphate application.

Respecting the effect of different investigated treatments on carbohydrate content in potato tubers, obtained data show that potato tuber inoculated with *Azotobacter & Azospirillum* combined with *B.megaterium var. phosphaticum* showed the highest records of carbohydrate content. Biogas manure application showed higher values of carbohydrate content than ammonium sulphate application. This result was consistent in case of biogas manure application either alone or in combination with phosphate solubilizing bacteria. These results are confirmed with those obtained by **Merghany (1998) and El-Fakhrani (1999)** who found that the organic manuring significantly increased the total amount of carbohydrates in dry matter of potato tubers in comparison with inorganic N-fertilization.

Data in Table (9) also show that dual inoculation of potato tubers with *Azotobacter & Azospirillum* and phosphate solubilizing bacteria significantly increased tuber yield/fed. compared to other investigated treatments. The increase of tubers yield/fed. of potato plants due

Table 7. Effect of biofertilization and organic manuring on some growth characters of Potato plants.

Growth characters / Treatments	Plant height (cm)		Leaves number/plant		Branches number / plant	
	S1	S2	S1	S2	S1	S2
	Non inoculated with phosphate solubilizing bacteria					
Control	38^{h}	41^{h}	8.3^{f}	8.60^{f}	1.6^{d}	1.3^{d}
Azoto.&Azos+hdn	42^{f}	45^{f}	12.6^{c}	11.6^{d}	3.3^{b}	3.0^{b}
Biogas manure	66^{d}	55^{e}	16.6^{a}	16.3^{a}	3.6^{b}	3.3^{b}
Ammonium sulphate	40^{g}	43^{g}	11.9^{d}	10.6^{e}	2.6^{c}	2.3^{c}
	Inoculated with phosphate solubilizing bacteria					
Control	58^{e}	56^{d}	10.3^{b}	10.6^{c}	3.0^{b}	3.0^{b}
Azoto.&Azos+hdn	78^{b}	79^{b}	14.6^{a}	$16.3.^{b}$	4.3^{a}	4.6^{a}
Biogas manure	80^{a}	81^{a}	16.3^{a}	16.6^{b}	4.3^{a}	4.6^{a}
Ammonium sulphate	70^{c}	71^{c}	12.6^{b}	14.3^{c}	4.6^{a}	3.6^{b}

Abbreviations: as those stated in Table (3).

Means followed by the same letter (s) within each column are not significantly from each other at 5% level.

Table 8. Periodical changes in total nitrogen and phosphorus content of potato plants during the two growing seasons.

Treatments	Days after sowing															
	15		30		60		90		15		30		60		90	
	Total nitrogen (%)								Total phosphorus (%)							
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
	Non inoculated with phosphate solubilizing bacteria															
Control	0.86	0.92	1.58	1.62	2.32	2.40	1.74	1.83	0.109	0.118	0.142	0.162	0.210	0.228	0.192	0.210
Azoto. &Azos. + hdn	1.68	1.63	2.40	2.61	3.42	3.70	2.92	2.86	0.212	0.226	0.362	0.381	0.465	0.510	0.338	0.425
Biogas manure	1.34	1.43	1.98	2.24	3.75	3.95	2.30	2.51	0.263	0.281	0.34	0.362	0.627	0.591	0.421	0.434
Ammonium sulphate	0.98	1.06	1.82	1.95	3.20	3.48	1.93	2.12	0.186	0.218	0.276	0.286	0.380	0.376	0.250	0.318
	Inoculated with phosphate solubilizing bacteria															
Control	0.84	0.96	1.62	1.70	2.86	3.12	2.21	2.45	0.141	0.153	0.186	0.192	0.240	0.228	0.200	0.224
Azoto.& Azos. + hdn	1.96	1.81	2.54	2.48	4.10	4.25	3.60	3.86	0.290	0.320	0.381	0.416	0.602	0.590	0.481	0.510
Biogas manure	1.83	1.62	2.12	2.61	3.88	4.12	3.24	3.42	0.311	0.332	0.406	0.431	0.681	0.611	0.492	0.510
Ammonium sulphate	1.12	1.26	1.96	2.26	3.45	3.72	2.83	2.96	0.196	0.236	0.335	0.361	0.568	0.530	0.391	0.428

Abbreviations: as those stated in Table (3) .

to inoculation with asymbiotic N_2-fixer (*Azotobacters & Azospirillum*) could be attributed to the capability of these organisms to fix nitrogen which could be taken by the growing plants. Similar results were observed by **Mahendran *et al* (1996), Mahendran and Kumar (1998)) and El-Ghinbihi and Fetouh (2001)** who reported that potato tubers inoculated with asymbiotic N_2-fixers in combination with *B.megaterium var. phosphaticum* gave the highest tubers yield /fed.

Data in Table (9) also show that insignificant difference in tuber yield/fed. was observed with biogas manuring amendment compared to biofertilization treatment.

Irrespective of control treatment, the lowest tuber yield/fed. was obtained with ammonium sulphate application.

Generally, from data presented in Table (9) it can be concluded that the inoculation of potato tubers with phosphate solubilizing bacteria in combination with either asymbiotic N_2-fixers or organic manuring as well as inorganic nitrogen fertilization improved tuber yield /fed. of potato plants.

Table 9. Effect of biofertilization and organic manuring on the dry matter, tuber number / kg, carbohydrate content and tuber yield.

Parameters	Dray matter g / plant		Tuber number/kg		Carbohydrate content (%)		Tuber yield Ton/fed	
Treatments	S1	S2	S1	S2	S1	S2	S1	S2
	Non inoculated with phosphate solubilizing bacteria							
Control	8.50^{f}	9.00^{h}	12.66^{a}	12.63^{a}	28.1^{h}	30.6^{h}	5.80^{f}	6.20^{e}
Azoto. & Azos. + hdn	28.2^{c}	26.0^{e}	8.42^{d}	8.33^{d}	50.2^{d}	52.6^{d}	10.0^{c}	12.0^{ab}
Biogas manure	33.3^{b}	36.0^{b}	8.66^{d}	8.60^{d}	44.8^{e}	46.9^{e}	10.5^{c}	9.10^{c}
Ammonium sulphate	16.1^{d}	22.0^{f}	10.60^{c}	10.33^{c}	39.4^{f}	41.3^{f}	8.90^{d}	11.2^{b}
	Inoculated with phosphate solubilizing bacteria							
Control	13.0^{e}	13.5^{g}	11.33^{b}	11.66^{b}	33.3^{g}	31.5^{g}	6.40^{c}	6.80^{d}
Azoto. & Azos. + hdn	36.0^{a}	34.0^{c}	7.83^{e}	7.21^{e}	62.4^{a}	63.1^{a}	12.9^{a}	13.4^{a}
Biogas manure	37.0^{a}	38.0^{a}	9.33^{d}	8.90^{d}	60.2^{b}	61.8^{b}	12.2^{a}	12.9^{ab}
Ammonium sulphate	35.0^{ab}	31.6^{b}	$10,33^{c}$	10.61^{c}	52.4^{c}	53.3^{c}	11.2^{b}	12.0^{ab}

Abbreviations: as those stated in Table (3).

Means followed by the same letter (s) within each column are not significantly different from each other at 5% level.

CONCLUSION

From the obtained results, it can be concluded that potato tuber inoculation with asymbiotic N_2-fixers (*Azotobacter & Azospirillum*) and a half dose of inorganic N-fertilizer supplementation improved growth characters, carbohydrate content and consequently gave higher tuber yield/fed. than using full dose of inorganic N fertilizer. About 50% of the cost of inorganic N-fertilizer can be saved with asymbiotic N_2-fixers inoculation.

Moreover, it is preferable now to use organic manuring and biofertilizers in cultivation. Also, the growth characteristics, carbohydrate content and yield of potato plants inoculated with asymbiotic N_2-fixers combined with phosphate solubilizing bacteria were almostly corresponding to those fertilized with biogas manure combined with phosphate solubilizing bacteria. Therefore, the use of biofertilizers or organic manuring may be recommended as a substitute for chemical fertilization to improve potato productivity and quality to face the local consumption and exportation.

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فعالية التسميد الحيوي والعضوي على نمو وإنتاجية نباتات البطاطس

راشد عبد الفتاح زغلول

قسم النبات الزراعى- كلية الزراعة بمشتهر- جامعة الزقازيق - فرع بنها

ملخص

كان الهدف من هذا البحث هو دراسة إمكانية استخدام الأسمدة الحيوية والتسميد العضوى كبديل للتسميد الكيماوى في إنتاج محصول البطاطس. وأقيمت لهذا الغرض تجربتان حقليتان بمزرعة التجارب بكلية الزراعة بمشتهر خلال موسمى ٢٠٠١، ٢٠٠٢ . إستُخدِمت سلالتين فعالتين من البكتريا المثبتة لآزوت الهواء الجوى هما *Azotobacter chrococcum , Azospirillum lipoferum* كذلك إستُخدِمت سلالة فعالة من البكتريا المذيبة للفوسفات وهى *B.megaterium var. phosphaticum*. تم استخدام سماد البيوجاز كسماد عضوى وسماد سلفات الامونيوم كسماد كيماوى حيث أضيف سماد البيوجاز بمعدل ٦ طن/فدان (٩٠ كجم ازوت/فدان)كذلك تم استخدام نفس المعدل لوحدات الأزوت من سماد سلفات الأمونيوم ولقد أظهرت نتائج هذه الدراسة ما يلى:-

لوحظ عند التسميد بسماد البيوجاز أعلى نشاط للميكروبات ممثلاً فى معدل ثانى أكسيد الكربون المنطلق فى منطقة الريزوسفير. بينما كان أعلى نشاط لإنزيم النيتروجينيز عند تلقيح تقاوى البطاطس عند الزراعة بمخلوط من الأزوتوباكتر والأزوسبيريللام. وعموما فقد أوضحت النتائج أن معدل إنطلاق ثانى أكسيد الكربون وكذلك نشاط إنزيم النيتروجينيز قد ازداد عند التلقيح بالبكتريا المذيبة للفوسفات مختلطة مع المعاملات المختلفة.

عند تلقيح تقاوى البطاطس بالبكتريا المثبتة لآزوت الهواء الجوى (الأزوتوباكتر والأزوسبيريللام) مع إضافة نصف جرعة من سماد سلفات الأمونيوم أعطى ذلك قيماً أعلى من النيتروجين الأمونيومى والنتراتي فى منطقة الريزوسفير مقارنة بالتسميد بجرعة كاملة من سماد سلفات الأمونيوم. أعطى سماد البيوجاز قيماً اعلى للنيتروجين والفوسفور الكلى فى التربة مقارنة ببقية المعاملات. لوحظت فروقاً معنويةً فى صفات النمو التى دُرِسَت حيث إزدادت هذه القياسات معنوياً عند التسميد بسماد البيوجاز وتلقيح التقاوى عند الزراعة بالبكترِيا المذيبة للفوسفات.كذلك أدى التلقيح بالبكتريا المثبتة لأزوت الهواء الجوى مختلطة مع البكتريا المذيبة للفوسفات إلى زيادة معنوية فى محتوى درنات البطاطس من الكربوهيدرات.

لم يشاهد فروق معنوية فى محصول الدرنات/ فدان عند التلقيح بالبكتريا المثبتة للآزوت والتسميد بسماد البيوجاز. وعموما أوضحت هذه الدراسة أن محصول الدرنات/فدان فى حالة التسميد الحيوى أو العضوى أعلى عنه فى حالة التسميد الكيماوي. يمكن في ضوء نتائج هذه الدراسة أن يوصي باستخدام التسميد الحيوي أو العضوي في إنتاج محصول البطاطس كبديل للأسمدة الكيماوية خاصة وأنه يفضل حاليا استخدام هذه الأسمدة في الزراعة سواء كان ذلك للإنتاج المحلي أو للتصدير لان معظم المستوردين يفضلون المنتج الزراعي الناتج من الزراعة العضوية والحيوية.