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EFFECT OF PARTIAL SUBSTITUTION OF MINERAL-N BY BIOFERTILIZATION ON GROWTH, YIELD AND YIELD COMPONENTS OF POTATO

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ABSTRACT

The present investigation was carried out during two successive fall seasons of 2005/2006 and 2006/2007 on potato (cv. Claudia) grown at a private Farm, Etsa, Fayoum, Egypt, to study the effect of partial substitution of mineral-N by biofertilization on growth, yield, yield components and chemical composition of potato plants. Mineral-N fertilizer was added to the soil as ammonium nitrate (33.5% N) at the rates of 0, 100, 200, 300 and 400kg fed.$^{-1}$ which represent; control, 25%, 50%, 75% and 100% from the recommended fertilizer rate. Mineral-N fertilizer was applied either alone or in combination with biofertilizer (namely, *Azotobacter chroococcum* and *Azospirillium brasilense* [1**:**1]) representing ten treatments. The obtained results indicated that biomass fresh weight plant^{-1} was positively affected, however, plant height increased but not significantly by the application of mineral-N fertilizer either alone or in combination with the biofertilizer in the two successive seasons. The highest increase was obtained by 300 and 400Kg fed.$^{-1}$ mineral-N fertilizer when combined with biofertilizer as compared to the other treatments in both seasons. Also, total yield fed.$^{-1}$ and its components recorded the highest values with the same treatment which gave marked increase in the two seasons. Total yield, yield plant^{-1}, No. tubers plant^{-1}, mean tuber weight significantly increased with every increment of nitrogen added either alone or with biofertilizer, and the superiority of the compared treatments. The treatments 300 and 400kg fed.$^{-1}$ mineral-N with biofertilizer recorded the highest values and the deference between the two treatments was insignificant. The same trend was recorded with tuber size and the superiority was recorded with the combination with biofertilizer. Marked increases were detected in the large tuber size treated with biofertilizer. The application of 300 or 400Kg ammonium nitrate fed.$^{-1}$ in combination with biofertilizer, gave a significant increase in N, P and K (in both leaves and tubers) as compared to 300 or 400Kg fed.$^{-1}$ alone, except P content of leaves. Biofertilization in combination with either 300 or 400kg fed.$^{-1}$ significantly decreased (NO_2) and (NO_3) concentrations in leaves and NO_3 in tubers in both seasons as compared to the 300 or 400kg fed.$^{-1}$ mineral-N singly. Thus, the application of biofertilizer (*Azotobacter* and *Azospirillium)* in combination with 300kg fed.$^{-1}$; ammonium nitrate is recommended to improve growth and yielding capacity (appr. 17.75% and 17.08%, for both seasons, respectively as compared to the recommend dose [400kg ammonium nitrate fed.$^{-1}$]). Moreover, this treatment also improved some chemical constituents and in the same time, it lowered production cost (through reducing mineral-N fertilizer by about 25%).

Key Words: Potato- Biofertilization – Nitrogen – Vegetative growth – yield – Chemical composition.

INTRODUCTION

In Egypt, potato (*Solanum tuberosum* L.) is considered as one of the most important vegetable crops grown for local consumption and exportation. Potato crop is normally fertilized with a high amount of mineral-N fertilizers which, may have hazard effect in environmental pollution and may produce poor quality crop. Recently, research work is oriented to evaluate biofertilizer as a new sources for plant nutrition. Several reports indicated that the inoculation of some plants with biofertilizers alone or in combination with mineral fertilizers improved plant growth, yield and chemical composition (**Ibrahim and Abdel-Razik (1999); Abdel-Mouty *et al.*, 2002 and Gadallah and El-Masry (2006)**). Therefore, it seems that biological fertilization is a virgin field and more research work should be carried out to evaluate biofertilizers as a source for plant nutrition that might substitute mineral fertilizers. The main goal of this research is to study the relation between biofertilizer and mineral-N fertilizer application and their effect on growth, yield, yield components and chemical constituents of potato plants.

MATERIALS AND METHODS

Two field experiments were conducted in a private Farm at Etsa, Fayoum, Egypt using potato plants (cv. Claudia), during two growing fall seasons of 2005/2006 and 2006/2007, on 13th and 5th October, respectively. The tuber seeds were supplied by the Potato Producers Co-operative Society. The most important physical and chemical characters of the selected soil were determined (Table 1) according to **Wilde *et al*., (1985)**.

Table (1): Physical and chemical characters of the selected soil before planting in both seasons

Properity	2005/2007	2006/2007	Properity	2005/2006	2006`2007
Particle size analysis			**Chemicals**		
Clay %	57.2	56.1	**pH (1:2.5 suspension)**	7.22	7.29
Silt %	28.4	27.9	**ECe (dS m^{-1})**	2.04	2.13
Sand %	14.4	13.0	**Organic matter%**	1.11	1.12
Soil texture	Clay	Clay	**$CaCO_3$%**	5.49	5.03
Macro-and microelements (ppm):					
N	440.00	470.00	**Zn**	26.90	30.10
K	370.00	377.00	**Mn**	8.00	7.81
P	170.30	160.80	**Cu**	0.65	0.70
Fe	72.00	80.00			

Ten treatments were arranged as 0, 100, 200, 300 and 400Kg ammonium nitrate (33.5%N) fed.$^{-1}$ as a source of mineral-N fertilizer (which represents, control, 25%, 50%, 75% and 100% from the recommended fertilization rate) either alone or in combination with the mixture of biofertilizer (*Azotobacter chroococcum* (AT) and *Azospirillium brasilense* (AZ) [1:1]) beside biofertilizer alone.

The experimental treatments were arranged as follows:

1. Control: Untreated plants.
2. 100 kg fed^{-1} mineral-N fertilizer
3. 200 kg fed^{-1} mineral-N fertilizer.
4. 300 kg fed^{-1} mineral-N fertilizer.
5. 400 kg fed^{-1} mineral-N fertilizer
6. (AT+AZ): The mixture of *Azotobacter chroococcum* (AT) and *Azospirillium brasilense* (AZ) [1:1]
7. 100 Kg fed^{-1} mineral-N fertilizer + (AT+AZ).
8. 200 Kg fed^{-1} mineral-N fertilizer + (AT+AZ).
9. 300 Kg fed^{-1} mineral-N fertilizer + (AT+AZ).
10. 400 Kg fed^{-1} mineral-N fertilizer + (AT+AZ).

Preparation of inocula

Modified Ashby's medium (**Hegazi and Niemela, 1976**) was used to grow the *Azotobacter chroococcum* and Dobereiner medium for *Azospirillium brasilense* (**Dobereiner *et al.*, 1976**). The strains (*A. chroococcum* FN 33 and *A. brasilense* FN 17) were isolated and identified in the microbiology laboratory, Faculty of Agriculture, Fayoum University from the soil in which the experiments were performed. Isolates and inoculates were prepared immediately before inoculation. At the logarithmic growth phase, the cultures were centrifuged at 1000 rpm and the cell pellets were washed three times with sterile phosphate buffer (100 mM, pH=7.0). The washed cells were resuspended in the same buffer to the final concentration of about $4x10^8$ cfu/ml and used for tuber seeds inoculation.

Tuber seeds inoculation

Tuber seeds were immersed in Arabic Gum solution (16%) as a sticking agent and then inoculated with *Azotobacter chroococcum* FN 33 and *Azospirillium brasilense* FN 17 mixed in equal quantities (1:1). Inoculated tuber seeds were allowed to air dry in shade before planting according to **Allen (1971)**.

The different treatments were arranged in complete randomized blocks design with four replications. The experimental plot was 17.5 m^2. Each plot was planned to consist of 5 rows; 5m long and 70cm width and every two plots were separated from each other with one row. Tubers seeds were planted on 13^{th} and 5^{th} October in 2005 and 2006, respectively at interrow spacing of 25cm.

The rates of ammonium nitrate were applied in three equal doses, added after 6, 8 and 10 weeks from planting. Phosphorus and potassium fertilization was applied at the rate of 75kg P_2O_5 and 96kg K_2O fed^{-1}, respectively. Calcium superphosphate and potassium sulphate were the P and K sources, each in turn. Phosphorus fertilizer was applies to the soil before planting, while potassium was applied through two equal doses after 7 and 9 weeks from planting. The general agricultural practices were applied as recommended for commercial potato production.

Data recorded

a) Vegetative growth characters: After 90 days from planting a random sample consisted of 8 plants, from each experimental unit, was chosen from the two outer rows. Plants were carefully taken and immediately carried to the laboratory to determine plant height and biomass fresh weight.

b) Yield potential: At harvest time, ten random plants were taken to determine tuber yield plant^{-1} and number of tubers plant^{-1}. Mean tuber weight were calculated, tuber yield of three inner rows of every plot were taken and weighted and tuber yield fed.$^{-1}$ were calculated, and divided to three size grades; more than 60mm, 30-60mm and less than 30mm in diameter.

c) Chemical analysis

1) Leaf samples for chemical determination, were taken from the fourth upper leaf of potato stems of eight randomly selected plants, after 90 days from planting, were collected, washed with tap water, rinsed three times with distilled water and dried at 70°C in a forced-air oven till constant weight. The dried leaf samples were finely ground and a weight of 0.2g of the fine powder were digested using a mixture of sulphoric and perchloric acids. The following determinations were performed:
   1. Leaf N content was estimated using the Microkjeldahal apparatus as described in **A. O. A. C (1995).**
   2. Nitrite and nitrate were determined (mg kg^{-1} dry matter of leaves), 0.5g dried material of leaves was shaken in 20ml distilled water for 30min., then filtered **(Bar-Akiva, 1974)**. An aliquot of the same extract was also analyzed for nitrite using sulphanilic acid and α-naphthylamine method **(Chapman and Pratt, 1961)**. Another aliquot of the extract was analyzed for nitrate using phenol disulfonic acid method **(Page *et al.*, 1982)**.
   3. Leaf P content was colourimetrically estimated according to the Stannous molybdate chloride method as illustrated in **A. O. A. C (1995)**.
   4. Leaf K content was photometrically measured using Flamphotometer as mentioned by **Wilde *et al.* (1985)**.

2) Random samples of tubers after complete drying for at least 96 hours at 70°C in forced-air oven, were chosen. The dried tuber samples were finely ground and weights of 0.2g of the dried fine powder were prepared for the following chemical determinations: tuber N, P, K, nitrite and nitrate contents were determined using the same analytical methods used for the determination of leaf samples. Starch percentage (in tuber fresh weight) was determined as described by **Malik and Singh (1980)**. Tuber carbohydrate percentage was colourimetrically estimated using the method exhibited by **Michel *et al.* (1956).**

Statistical analysis

All data of the two seasons were subjected to the statistical analysis according to the used design. The least significant difference test (LSD) at p = 0.05 level was used to verify the differences between treatments as mentioned by **Snedecor and Cochran (1980)**.

RESULTS AND DISCUSSION

Vegetative growth characters

The results in Table (2) clarify that plant height and biomass fresh weight plant^{-1} were affected by both mineral-N and biofertilizer treatments in both seasons. Biofertilizer alone showed a significant increase in biomass fresh weight plant^{-1} in both seasons as compared to the control treatment. The increases in biomass fresh weight plant^{-1} were: 42.37% and 36.52% in the first and the second seasons, respectively. While plant height showed insignificant increases in both seasons.

Table (2): Influence of bio-and mineral-N fertilization on some growth characters of potato plants in both seasons of 2005/2006 and 2006/2007.

Treatments		Plant height (cm)		Biomass fresh Weight plant^{-1} (g)	
Bio-F	**Mineral-N (Kg fed.$^{-1}$)**	**2005/2006**	**2006/2007**	**2005/2006**	**2006/2007**
Control		31.40	33.80	330.4	360.1
	100	32.20	34.80	453.1	480.6
	200	33.90	36.00	517.6	540.0
	300	35.60	37.40	571.3	570.7
	400	36.20	38.40	603.9	593.2
AT+AZ		33.80	34.60	470.4	491.6
AT+AZ	**+ 100**	34.90	35.80	567.3	570.1
AT+AZ	**+ 200**	36.10	37.40	621.2	617.9
AT+AZ	**+ 300**	39.70	41.60	658.8	690.3
AT+AZ	**+ 400**	40.00	41.90	671.4	693.8
L.S.D. 5%		**4.62**	**4.44**	**17.9**	**21.3**

Control = Untreated plants
AT = *Azotobacter chroococcum* **AZ** = *Azospirillium brasilense*

Data presented in Table (2) show that, during both seasons, the inoculation of tuber seeds with biofertilizer at any used rate of mineral-N fertilizer (100, 200, 300 and 400Kg ammonium nitrate fed.$^{-1}$), caused an insignificant increase in plant height comparing with the corresponding mineral-N treatments. While the biomass fresh weight plant^{-1} recorded a great positive increases in the both seasons, the treatments of any rate of mineral-N fertilizer in combination with biofertilizer compared with the corresponding mineral-N alone treatments (100, 200, 300 and 400Kg ammonium nitrate fed.$^{-1}$) showed a significant increase by 25.20%, 20.02%, 15.32% and 11.18%, respectively in the first season and 18.62%, 14.43%, 20.96% and 16.96%, respectively in the second season.

In general, the differences between the combined treatment of biofertilizer plus 300Kg fed.$^{-1}$ was superior or equal to 100Kg fed.$^{-1}$ mineral-N in both seasons.

However, the plants fertilized with 400Kg fed.$^{-1}$ combined with biofertilizer were insignificantly differed from those fertilized by 300Kg fed.$^{-1}$ combined with biofertilizer in all studied growth characters in both seasons. These indicate that bio-fertilization could compensate the reduction of the mineral-N level. This insignificance could be attributed to the higher efficiency of biofertilizer under lower nitrogen rate.

The positive effect of mineral-N fertilization on growth parameters of potato plants may be attributed to the role of nitrogen in protoplasm formation and all proteins, amino acids, nucleic acid, many enzymes as well as energy transfer materials; ADP and ATP **(Russel, 1973)**, thus stimulating nutritional status of the plant **(Medani *et al*., 2000)**.The beneficial effect of inoculation of tuber seeds with the mixture of biofertilizer on growth parameters may by due to its ability to release some plant promoting substances, mainly IAA, gibberellic acid and cytokinin like substances which stimulate plant growth **(El-Merich *et al*., 1997)**, increasing the water and mineral uptake from the soil, also led to an increase in root surface area, root hairs and root elongation as affected by Azotobacter **(Sundaravelu and Muthukrishinan, 1993)**, and increasing the ability to convert N_2 to NH_4 and thus make it available to plants **(Hanafy *et al*., 1997)**. The positive effects on plant growth might be attributed to the enhancing effect of biofertilizers on the availability of nutrients and its uptake. The obtained results are in harmony with those recorded by **Ashour *et al.* (1997), Ibrahim and Abdel-Razik (1999) and Gadallah and El-Masry (2006)**.

Yield and its components

It could be noticed from the data illustrated in Table (3) that potato yield and its components were affected by both mineral-N and biofertilizer treatments in both seasons. Concerning the effect of biofertilizer, the results shows that, the inoculation of tubers seeds with the biofertilizer singly exhibited a significant increase in mean tuber weight, yield plant^{-1}, yield fed^{-1}, tuber yield [(>60mm) and (60-35mm)] diameter in both seasons as compared to the control treatment. The increases in the mentioned yield and its components as stated before were: 72.07%, 72.79%, 70.37%, 202.44% and 70.34%, respectively in the first season, and 61.62%, 61.76%, 58.98%, 175.00% and 61.82%, respectively in the second one. However, the number of tubers plant^{-1} was increased insignificantly in both seasons. On the other hand the tuber yield (<35mm) diameter in the second season and tuber dry matter% in both seasons were decreased significant. The reduction was -5.34% for tuber dry matter% in the first season, and -14.77% and -3.87% for tuber yield (<35mm) diameter and tuber dry matter% in the second season.

Data in Table (3) clearly showed that inoculation with biofertilizer and fertilization with any level of ammonium nitrate (100, 200, 300 and 400Kg fed.$^{-1}$) gave highly significant yield increases as well as its components except tuber yield (<35mm) in diameter and tuber dry matter% in both seasons as compared to plants given only a corresponding mineral-N fertilizer. In this respect, the treatment of 100Kg fed.$^{-1}$ in combination with biofertilizer showed a significant increase by 2.16%, 24.66%, 27.35%, 35.25%, 40.12% and 50.83%, respectively in the first season. While the increments, in the second one, reached: 2.60%, 29.35%, 32.71%, 38.49%, 43.25% and 53.18%, respectively more than the treatment of 100Kg fed.$^{-1}$ only for number of tubers plant^{-1}, mean tuber weight, yield plant^{-1}, yield fed^{-1}, tuber yield (>60mm) and (60-35mm) diameter. The treatment of 200Kg fed.$^{-1}$ in combination with biofertilizer showed a significant increase by 4.82%, 18.42%, 25.47%, 26.47%, 38.24% and 21.73%, respectively in the first season and 4.81%, 15.21%, 23.21%, 23.90%, 31.92% and 22.31%, respectively in the second one as compared with 200Kg fed.$^{-1}$ treatment alone. The increase in the above mentioned yield and its components as stated before which obtained by the treatment of 300Kg fed.$^{-1}$ combined with biofertilizer as compared to the treatment of 300Kg fed.$^{-1}$ alone in both seasons reached 7.01%, 14.36%, 22.39%, 23.22%, 30.75% and 20.55%, respectively in the first season and 5.71%, 13.49%, 19.98%, 20.62%, 28.35% and 16.83%, respectively in the second season. The increase in the above mentioned yield and its component parameters obtained by the treatment of 400Kg fed.$^{-1}$ combined with biofertilizer as compared to the treatment of 400Kg fed.$^{-1}$ alone in both seasons reached 7.08%, 6.73%, 14.29%, 19.06%, 24.36% and 14.94%, respectively in the first season and 7.17%, 5.34%, 12.89%, 18.90%, 23.80% and 14.96%, respectively in the second season. While the tuber yield (<35mm) diameter and tuber dry matter%, decreased with increasing levels of mineral-N or the mixture of biofertilizer and any used level of mineral-N fertilizer (100, 200, 300 and 400Kg ammonium nitrate fed.$^{-1}$) in both seasons.

Thus, the combination between the treatments of mineral-N and biofertilizer had a great positive effect on yield and its components as compared to the treatments of mineral-N alone. However, plants treated with 400Kg fed.$^{-1}$ combined with biofertilizer caused an insignificant increase in yield and its components in both seasons compared with the treatment of 300Kg fed.$^{-1}$ combined with the biofertilizer.

The increases in the recorded yield and its components except tuber yield (<35mm) in diameter and tuber dry matter% in both seasons as compared to the control treatment of potato plants is expected with mineral-N fertilizer when combined with biofertilizer could be attributed to the major functions of nitrogen on enzymes, photosynthesis and endogenous hormones synthesis, which consequently affect plant growth and its yield **(Marschner, 1995 and Hanafy *et al*., 1997)**. In this respect, **Ashoure *et al.* (1997)** found that inoculating potato tuber seeds with Nitrobein (biofertilizer) significantly increased total yield and average tuber weight plant^{-1}. The favourable effect of biofertilizer treatments could be attributed to its enhancing effect on plant growth characters which could be reflected on potato yield and its components and inoculating potato tuber seeds with Halex2 (biofertilizer) significantly increased average yield plant^{-1}, total yield fed.$^{-1}$ and percentage of large and medium tuber size grades, while increasing level of Halex2 decreased small sized tubers (**Ibrahim and Abdel-Razik, 1999**). Moreover, the present results appeared to be in close agreement with previous results obtained by **Abdel-Mouty *et al.* (2002) and Gadallah and El-Masry (2006).**

Table (3): Influence of bio-and mineral-N fertilization on yield and its components of potato plants in both seasons of 2005/2006 and 2006/2007.

Treatments		Yield per plant(g)		No. of tubers plant^{-1}		Mean tuber weight (g)		Yield (ton/fed.)		Tuber yield (>60mm) diameter (ton/fed.)		Tuber yield (60-35mm) diameter (ton/fed.)		Tuber yield (<35mm) diameter (ton/fed.)		Tuber dry matter %	
Bio-F	Mineral-N (Kg fed.$^{-1}$)	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007
Control		342.2	361.4	9.18	9.21	37.27	39.24	4.86	5.12	0.82	0.88	2.63	2.75	1.41	1.49	22.68	22.48
	100	583.1	570.2	9.26	9.25	62.97	61.64	7.66	7.56	3.29	3.26	3.01	2.99	1.36	1.31	22.37	22.36
	200	732.7	760.4	9.34	9.36	78.45	81.24	10.01	10.42	5.10	5.42	3.82	3.90	1.08	1.10	21.48	21.86
	300	791.4	812.3	9.41	9.46	84.10	85.87	10.98	11.25	6.31	6.42	3.99	4.16	0.68	0.67	21.00	21.07
	400	857.8	871.9	9.46	9.48	90.68	91.97	11.49	11.59	7.02	7.06	3.95	4.01	0.52	0.52	20.72	20.54
AT+AZ		591.3	584.6	9.22	9.22	64.13	63.42	8.28	8.14	2.48	2.42	4.48	4.45	1.32	1.27	21.47	21.61
AT+AZ	+ 100	742.6	756.7	9.46	9.49	78.50	79.73	10.36	10.47	4.61	4.67	4.54	4.58	1.21	1.22	22.64	21.93
AT+AZ	+ 200	919.3	936.9	9.79	9.81	92.90	93.60	12.66	12.91	7.05	7.15	4.65	4.77	0.96	0.99	22.05	21.79
AT+AZ	+ 300	968.6	974.5	10.07	10.00	96.18	97.45	13.53	13.57	8.25	8.24	4.81	4.86	0.47	0.47	21.76	21.66
AT+AZ	+ 400	980.4	984.3	10.13	10.16	96.78	96.88	13.68	13.78	8.73	8.74	4.54	4.61	0.41	0.43	21.62	21.50
L.S.D. 5%		20.42	23.79	0.19	0.24	3.22	3.70	0.82	0.65	0.34	0.41	0.21	0.22	0.16	0.17	0.47	0.25

Control = Untreated plants **AT** = *Azotobacter chroococcum* **AZ** = *Azospirillium brasilense*

Chemical constituents

In both seasons of the study, the results presented in Tables (4 and 5) show that the treated plants with biofertilizer (AT+AZ) alone recorded a significant increase in the content of N, P and K in leaves and tubers, except the content of P in leaves increased as insignificantly compared to the control treatment in both seasons.

Concerning the effect of the combination between bio-and mineral-N fertilization treatments on N, P and K contents. The data in Tables (4 and 5) indicate that N and K contents in leaves and tubers were significantly increased with inoculation of tuber seeds, and by increasing the rates of mineral-N level reaching their maximum increase by the treatment of 400Kg fed.$^{-1}$ in both seasons as compared to the corresponding mineral-N alone, except P content in leaves which insignificantly increased by those treatments. However, the differences between the treatments of 400Kg fed.$^{-1}$ and 300Kg fed.$^{-1}$ combined with (AT+AZ) were insignificant. This insignificance could be referred to higher efficiency of biofertilizer (AT+AZ) under lower nitrogen level **(Mehrotra and Lehri, 1997)**. **Hanafy *et al*. (1997)** suggested that the addition of biofertilizers increases the ability to convert N_2 to NH_4 and thus make it available to plant. Also, many investigators showed that inoculation of biofertilizers increased N concentration in potato **(Abd El-Ati *et al*., 1996; Ibrahim and Abdel-Razik, 1999)** and **(Gadallah and El-Masry, 2006)** in onion plants.

Table (4): Influence of bio-and mineral-N fertilization on N, P, K, NO_2 and NO_3 (mg g^{-1} dry matter of leaves) in both seasons of 2005/2006 and 2006/2007.

Treatments		N mg g^{-1}		P mg g^{-1}		K mg g^{-1}		NO_2 mg g^{-1}		NO_3 mg g^{-1}	
Bio-F	Mineral-N (Kg fed.$^{-1}$)	2005/2006	2006/2007	2005/2006	2006/2007	2005/2006	2006/2007	2005/2006	2006/2007	2005/2006	2006/2007
Control		3.46	3.53	0.55	0.54	4.06	4.37	0.092	0.099	0.983	1.005
	100	3.69	3.62	0.56	0.54	5.52	5.56	0.103	0.108	1.039	1.018
	200	3.78	3.71	0.54	0.52	6.46	6.51	0.117	0.121	1.096	1.079
	300	4.02	4.12	0.51	0.52	7.53	7.44	0.136	0.129	1.222	1.257
	400	4.11	4.17	0.51	0.50	7.70	7.68	0.138	0.140	1.242	1.266
AT+AZ		3.68	3.73	0.57	0.55	5.72	5.68	0.087	0.094	0.944	0.943
AT+AZ	+ 100	3.86	3.84	0.56	0.54	6.08	6.17	0.100	0.106	1.001	0.994
AT+AZ	+ 200	4.07	4.12	0.54	0.52	7.41	7.36	0.107	0.109	1.052	1.043
AT+AZ	+ 300	4.22	4.19	0.52	0.50	7.91	8.04	0.121	0.115	1.131	1.126
AT+AZ	+ 400	4.31	4.30	0.52	0.49	8.45	8.52	0.127	0.124	1.188	1.181
L.S.D. 5%		0.19	0.12	n.s.	n.s.	0.22	0.17	0.013	0.011	0.068	0.063

Control = Untreated plants
AT = *Azotobacter chroococcum* **AZ** = *Azospirillium brasilense*

Table (5): Influence of bio-and mineral-N fertilization on N, P, K, NO_2 and NO_3 (mg g^{-1} dry matter of tubers) in both seasons of 2005/2006 and 2006/2007.

Treatments		N mg g^{-1}		P mg g^{-1}		K mg g^{-1}		NO_2 mg g^{-1}		NO_3 mg g^{-1}	
Bio-F	Mineral-N (Kg $fed.^{-1}$)	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007	2005/ 2006	2006/ 2007
Control		1.62	1.55	0.059	0.056	3.85	3.87	0.036	0.039	0.417	0.414
	100	1.66	1.65	0.060	0.058	4.15	4.14	0.041	0.042	0.458	0.455
	200	1.70	1.72	0.065	0.062	4.42	4.36	0.046	0.049	0.494	0.499
	300	1.76	1.81	0.071	0.067	4.56	4.48	0.054	0053	0.536	0.561
	400	1.84	1.85	0.074	0.075	5.04	5.13	0.056	0.059	0.552	0.573
AT+AZ		1.72	1.74	0.063	0.061	4.11	4.06	0.039	0.041	0.406	0.403
AT+AZ	+ 100	1.72	1.75	0.067	0.061	4.59	4.28	0.045	0.048	0.443	0.451
AT+AZ	+ 200	1.75	1.77	0.072	0.067	4.76	4.62	0.050	0.052	0.472	0.486
AT+AZ	+ 300	1.81	1.83	0.075	0.074	4.98	4.87	0.055	0.057	0.498	0.503
AT+AZ	+ 400	1.88	1.86	0.078	0.080	5.14	4.96	0.065	0.064	0.526	0.520
L.S.D. 5%		**0.09**	**0.10**	**0.004**	**0.005**	**0.22**	**0.13**	**0.012**	**0.012**	**0.036**	**0.027**

Control = Untreated plants
AT = *Azotobacter chroococcum* **AZ** = *Azospirillium brasilense*

Also, the data show that the tubers of inoculated potato plants by biofertilizer (AT+AZ) only or plus any used rate of mineral-N fertilizer (100, 200, 300 and 400Kg $fed.^{-1}$) contained higher P content in both seasons than that of the control. The enhancing effect of biofertilizer on increasing P content in the tubers could be attributed to the beneficial effects of AT+AZ bacteria on reducing soil pH by secreting organic acids (e.g. acetic, propionic, fumaric and succinic), which brought about the dissolution of bounds forms of P and render them available for growing plants **(Ibrahim and Abd El-Aziz, 1977)**. These results are in agreement with those reported by **Gadallah and El-Masry (2006)** on onion

Moreover, **Hanafy *et al*. (1997)** mentioned that using *Azotobacters* increased root surface, root hairs and root elongation. Thus, factors caused by application of biofertilizers could improve P uptake on onion plant. With regard to K concentration, a significant increase of K concentration was obtained from combination between biofertilizer with the treatments of mineral-N fertilizer as compared to the treatment which only received mineral-N fertilizer in both seasons. These results are in agreement with those reported by **Ibrahim and Abdel-Razik (1999)** on potato and **Gadallah and El-Masry (2006)** on onion.

Data in Tables (4 & 5) showed that in both seasons the rates of 300 and 400Kg ammonium nitrate $fed.^{-1}$ combined with biofertilizer had a significant decrease in NO_2 and NO_3 content of leaves and NO_3 content in fresh weight of tubers of potato plant comparing to plants given only a corresponding mineral-N fertilizer. While the NO_2 content of tubers was insignificantly increased.

The results presented in Table (6) indicate that increasing the amount of mineral-N singly or in combination with biofertilizer significantly decreased total carbohydrates% and starch% in fresh weight of tubers, but the addition of biofertilizer enhances the concentration of total carbohydrates% and starch% in fresh weight of tubers comparing with mineral-N signal. The trend was parallel in both years.

Table (6): Influence of bio-and mineral-N fertilization on total carbohydrates% and starch% in fresh weight of tubers of potato plants in both seasons of 2005/2006 and 2006/2007.

Treatments		Total carbohydrates %		Starch %	
Bio-F	**Mineral-N (Kg fed.$^{-1}$)**	**2005/2006**	**2006/2007**	**2005/2006**	**2006/2007**
Control		47.14	47.41	21.08	21.18
	100	46.27	46.52	20.71	20.04
	200	45.63	45.68	19.94	19.80
	300	45.21	45.36	19.13	18.92
	400	44.67	44.74	18.85	18.73
AT+AZ		46.77	46.35	20.33	20.49
AT+AZ	**+ 100**	46.46	46.22	20.11	20.37
AT+AZ	**+ 200**	46.32	46.28	20.00	20.21
AT+AZ	**+ 300**	45.86	45.44	19.49	19.49
AT+AZ	**+ 400**	45.39	45.03	19.00	19.28
L.S.D. 5%		**0.95**	**1.02**	**0.64**	**0.58**

Control = Untreated plants
AT = *Azotobacter chroococcum* **AZ** = *Azospirillium brasilense*

Thus, the combination between mineral-N and biofertilizer had great positive effect on yield and its components as compared to mineral-N alone. It is worthy to note that, the application of biofertilizer alone or in combination with mineral-N fertilizer (at any level) produced lower values of NO_2 and NO_3 concentration in potato leaves and tubers as compared to the plants which only received mineral-N fertilizer (regardless the level of mineral-N fertilizer), except NO_2 content in tubers. This might be due to the positive relationship between NO_2 and NO_3 concentration in leaves and nitrogen fertilization level. This result confirm the suggestion that several plant species accumulate NO_3 as a result of excess of N uptake **(Hanafy *et al.*, 1997 and 2000)**. The lower leaf NO_3 concentrations in biofertilizer treatments were attributed to the relative decrease in NO_3 uptake and increase in ammonium uptake due to the delayed mineralization and nitrification of the soil **(Matsumoto and Yamagata, 1999)**. **Hanafy *et al*., (1997)** indicated that using biofertilizers combined with 50% mineral-N supply, decrease NO_3 accumulation in Jew's mallow and radish leaves and this decrease may be due to the reduction in mineral-N application level. Also, it might be suggested that, under the effect of biofertilizers, some growth-promoting substances, e.g. auxins, gibberellins and cytokinins could be formed or released **(Hartmann *et al*., 1983)**. These phytohormones, especially cytokinins could be related to nitrate reductase content in plants which led to the reduction in NO_3 concentration. **Knypl (1979)** reported that, cytokinins enhance the activity of nitrate reductase and markedly enhanced the efficiency of nitrate reductase induction in many plants. **Abdin *et al*. (1993)** mentioned that, plant hormones like benzyladinine (as a cytokinin) increase the level of reductase gene expression.

Results of the present study clarify that the increase of chemical constituents by the increase of mineral-N fertilizer may be attributed to nitrogen fertilization which rise the capacity of plants to adsorb nutrients by the increase of root surface $unit^{-1}$ of soil volume as well as the high capacity of plants supplied with nitrogen in building metabolites as a result of nutrient uptake **(Mandour *et al*., 1986)**. Moreover, **Midan (1995)** mentioned that mineral-N fertilizer might promote metabolic processes within the plant, which could reflect a positive effect on chemical composition of pepper fruits but this is dependent on variety, soil fertility and cultivation date. In addition, many investigators explained the importance of biofertilizers in terms of reducing soil pH by secreting organic acids which bring about the dissolution of some bound nutrients and make them available for plants **(Ibrahim and Abd-Aziz, 1977).** The concentration of NO_3 in plant tissues is always in a dynamic state since it represents the differences between rate of N-absorption and rates of translocation and assimilation within the plant. These results confirmed the suggestion that several plants species accumulate NO_3 as a result of excess of N uptake over its reduction **(Hanafy *et al*., 1997 and 2000)**. Moreover, **Rufty *et al*. (1982)** reported that NO_3 is believed to accumulate in a storage pool; presumably in the vacuoles, from which it is not readily available. In the two seasons, bio-N showed lower values of NO_2 and NO_3 concentration in onion bulbs when compared with the plants which only received mineral-N fertilizer (regardless the different levels of mineral-N fertilizer). In addition, less values of NO_2 and NO_3 concentration were obtained by the treatments of bio-N in combination with mineral fertilizer in both seasons. In this respect, **Hanafy *et al*., (1997 and 2000)** suggested that the increments in total soluble sugars concentrations in many plants play a role as an osmoticum and this might be implicated indirectly in decreasing NO_3 accumulation in plants.

The beneficial effect of biofertilizer (AT+AZ) on the detected chemical constituents of potato leaves and tubers may be due to the releasing nitrogen and organic exudates and their role in facilitating the absorption of all nutrients by plants. Meanwhile, the increase in N, P and K uptake by plants could be, in general, due to the roots system size **(Amara and Dahdoh, 1997)**. These results are agreement with the findings of **Mohamed and El-Ganaini (2003).**

Finally, it could by concluded that, the use of only bio-N fertilizers for the production of potato plants is insufficient, so, they must be used together with mineral-N fertilizer. The application of biofertilizer (*Azotobacter* and *Azospirillium)* in combination with 300 kg fed.$^{-1}$; ammonium nitrate) is recommended for improving growth and yielding capacity (appr. 17.75% and 17.08%, for both seasons, respectively as compared to the recommend dose). Moreover, this treatment also improved some chemical constituents and in the same time, it lowered production cost (through reducing mineral-N fertilizer by about 25%) as well as diminishing the environmental pollution by minimizing the harmful effects of using chemical fertilizers on human health.

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تأثير الإحلال الجزئى للنيتروجين المعدنى بالتسميد الحيوى على النمو والمحصول ومكوناته للبطاطس

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أجريت هذه الدراسة خلال موسمين متتاليين (2006/2005، 2007/2006) على نباتات البطاطس (صنف كلوديا) بإحدى المزارع الخاصة (أطسا– الفيوم - مصر)، وذلك لدراسة إمكانية إحلال التسميد الحيوى بدلا من التسميد المعدنى النيتروجينى أو جزء منه لنباتات البطاطس. استخدم فى هذه الدراسة نترات الأمونيوم (33.5% نتروجين) كمصدر للسماد النتروجينى المعدنى، وذلك بمعدلات: صفر، 100، 200، 300 و 400 كجم/فدان والتى تمثل: الكنترول، 25%، 50%، 75% و 100% على التوالى من المعدلات الموصى بها لتسميد نباتات البطاطس. أضيف السماد المعدنى إما منفردا أو مع التسميد الحيوى، وهو عبارة عن خليط من الأزوتوباكتر والأزوسبيريلليم بنسبة 1:1.

أوضحت النتائج المتحصل عليها ما يلـــى:

- أدت معاملات التسميد النتروجينى المعدنى سواء بصورة منفردة أو مع التسميد الحيوى إلى حدوث تأثير إيجابى للوزن الطازج للنبات، بينما كانت هناك زيادة غير معنوية فى طول النبات فى كلا موسمى الدراسة.
- أدت معاملات التسميد النيتروجينى المعدنى سواء بصورة منفردة أو مع التسميد الحيوى إلى إحداث زيادة معنوية فى المحصول ومكوناته للدرنات فى كلا الموسمين فيما عدا محصول الدرنات الأقل من 35مم فى القطر والوزن الجاف للدرنات حيث حدث نقص تدرجى بزيادة معدل التسميد النيتروجينى المعدنى.

- أظهرت معاملـة التسـميد النتروجينـى المعـدنى بمعـدل 300 أو 400كجـم نتـرات الأمونيـوم/ فـدان مـع التسـميد الحيـوى إلـى الحصـول علـى أعلـى زيـادة فـى ارتفـاع النبـات، وزن المـادة الطازجـة للنبـات ومحصول الدرنات ومكوناته فى كلا الموسمين.
- كان للتسميد النيتروجينى المعدنى بمعدل 300 أو 400كجم نترات الأمونيوم / فدان مختلطا بالتسميد الحيوى أثر واضح فى زيادة تركيز محتوى الأوراق والدرنات من النتروجين– الفوسفور– البوتاسيوم فى كلا موسمى الدراسة، فيما عدا محتوى الأوراق من الفوسفور حيث لم يحدث أى تأثير معنوى.
- معاملة التسميد النيتروجينى المعدنى بمعدل 300كجم نترات الأمونيوم / فدان + التسميد الحيوى أعطت انخفـاض معنـوى فـى محتـوى كـل مـن النتريـت والنتـرات فـى الأوراق والنتـرات فـى الـدرنات مقارنـة بمعاملة التسميد النيتروجينى 300كجم/ فدان.
- تركيز الكربوهيدرات الكلية والنشا فى الدرنات نقص بزيادة معدلات التسميد النيتروجينى المعدنى سواء أضـيف منفرداً أو مضـاف إليـه التسـميد الحيـوى، ولكـن إضـافة التسـميد الحيـوى يحسـن مـن تركيـز الكربوهيدرات الكلية والنشا فى الدرنات مقارنة باستخدام التسميد النيتروجينى المعدنى منفرداً.
- معاملـة التسـميد النتروجينـى المعـدنى بمعـدل 300كجـم/فـدان + التسـميد الحيـوى كـان لهـا نفـس تـأثير المعاملة 400كجم/فدان + التسميد الحيوى حيث لم يكن هناك أى فرق معنوى بينهما فى موسمى الدراسة.
- وعلى ذلك، فإن استخدام التسميد النتروجينى المعدنى بمعدل 300كجم/ فدان من نترات الأمونيوم فى وجود التسميد الحيوى (مخلوط من بكتريا الأزوتوباكتر و الأزوسبيريلليم) يؤدى إلى تحسين النمو والقـدرة المحصـولية (حيـث زاد المحصـول بمقـدار 17.75% و 17.08% علـى التـوالى فـى كـلا الموسـمين أعلـى مـن معـدل التسـميد الموصـى بـه 400كجـم نتـرات أمونيـوم/ فـدان) والمكونـات الكيميائيـة، عـلاوة علـى خفض تكلفـة الإنتـاج مـن خـلال نقص كميـة السـماد النتروجينـى المعـدنى المسـتخدم (بمقـدار 25%) بالإضـافة إلـى خفـض معـدل تلـوث البيئـة، وعلـى ذلـك فإنـه يوصـى باستخدام المعدل السابق فى تسميد البطاطس تحت الظروف المشابهة لهذه التجربة.