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RESPONSE OF FABA BEAN (*Vicia faba* L.) PLANTS TO DIFFERENT SOURCES AND RATES OF NITROGEN FERILIZATION IN NEWLY RECLAMED SOILS

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ABSTRACT

Effects of application of different nitrogen sources i.e., ammonium nitrate (AN) with different rates of nitrogen (0, 20, 30 and 40 kg N fed.$^{-1}$) and anhydrous ammonia (AA) as well as biofertilizer (*Rhizobium radiobacter* sp.) as PGPR strain either alone or in a combination with AN rates on faba bean (*Vicia faba* L., cv. Nubaria 1) were studied on a loamy sand soil at the Experimental Farm of Ismailia Agriculture Research Station, Egypt during the two successive winter seasons of 2013/2014 and 2014/2015. The results could be summarized as follow: Inoculation with (*Rhizobium radiobacter* sp.) + 40 kg N fed.$^{-1}$ as ammonium nitrate led to highest increases for number of pod plant^{-1}, seed yield, pod yield and seed Mn content , inoculation with (*Rhizobium radiobacter* sp.) + 30 kg N fed.$^{-1}$ as ammonium nitrate led to increase for P and K contents in seeds. Similar increments for 100-seed weight, protein content along with N, Fe and Zn contents of grain were recorded with anhydrous ammonia at rate of 40 kg N fed.$^{-1}$ as ammonium nitrate. The uptake of macro and micronutrients significantly increased in combined treatment of (bio+N_{40}) . In soil after harvest, inoculation with (*Rhizobium radiobacter* sp.) + 40 kg N fed.$^{-1}$ as ammonium nitrate led to highest decreases of both soil pH and EC but increases of available P, K, Fe and Zn contents. The highest values of available N was recorded due to inoculation with (*Rhizobium radiobacter* sp.) + 30 kg N fed.$^{-1}$ as ammonium nitrate , available Mn increased with anhydrous ammonia at the rate of 40 kg N fed.$^{-1}$ as ammonium nitrate.

Keywords : Ammonium nitrate, anhydrous ammonia **,** faba bean, loamy sand soil *Rhizobium radiobacter* sp.

INTRODUCTION

The legume seeds of faba bean (*Vicia faba* L.) are grown world-wide as a protein source for food and feed. At the same time, faba bean offers ecosystem services such as renewable inputs of nitrogen (N) into both crops and soil via biological N_2 fixation, and a diversification of cropping systems. Even though the global average grain yield has almost doubled during the past 50 years, the total area sown to faba beans has declined by 56% over the same period. Faba bean has the highest average reliance on N_2 fixation for growth of the major cool season legumes. As a consequence, the N benefit for following crops is often high; studies have demonstrated substantial savings (up to 40–80 kg N fed.$^{-1}$) in the amount of N fertilizer required to maximize the yield of crops grown after faba bean. Faba bean may prove to be a key component of future arable cropping systems where declining supplies and high prices of fossil energy are likely to constrain the affordability and use of fertilizers (Erik *et al.* 2010).

Anhydrous ammonia (82%N) is a liquid under high pressure and must be injected at least six inches deep into a moist soil because it becomes a gas once it is released from the tank. In soil, ammonia reacts with water to form the ammonium (NH_4^+) ion, which is held on clay and organic matter. Anhydrous ammonia is generally the cheapest source of N, however, the method of application is less convenient and requires more power to apply than most other liquid or dry materials. Another N form of ammonium nitrate is substantially cheaper than others, and their use may be justified on economic grounds provided, they do not adversely affect the yield or quality of the grain (Osman *et al*. 2013).

Biological fertilizers contained beneficial microorganisms each one is produced for a certain purpose such as nitrogen fixationas well as releasing the ions of phosphate, potassium and iron, etc. These microorganisms are usually established around the root and assist the plant to absorb the elements (Wu *et al.* 2005). Inoculation of faba bean plants with specific active rhizobial strains is the main factor for enhancing growth and productivity of this crop (Abo El-Soud *et al.* 2003). Rhizobia are soil-inhabiting bacteria that fix nitrogen from atmosphere to form ammonia, so called biological N_2-fixation (BNF) process (Giller, 2001).

The current study aim to evaluate the integrated effect of anhydrous ammonia and bio-inoculation with *Rhizobium radiobacter* sp strain individually or in combination with different rates of ammonium nitrate as a partial substitution of recommended mineral-N fertilizer for faba bean production and quality under loamy sand conditions as related to the achieved amelioration of some soil properties which were also taken into consideration.

MATERIALS AND METHODS

To study the effect of nitrogen fertilizer sources and rates on soil fertility and faba bean productivity; two field experiments were conducted for a loamy sand soil under sprinkler irrigation system using faba bean (*Vicia faba* L. cv. Nubaria 1) in 2013/2014 and 2014/2015 seasons at Ismailia Agricultural Research Station, Ismailia Governorate, Egypt, located at lat. 30° ٣٥' ٣٠", long. 32° 14' 50" E. Treatments involved four rates from ammonium nitrate, 33% N (AN), 0, 20, 30 and 40 kg N fed.$^{-1}$,anhydrous ammonia, 80% N (AA) and bio-fertilizer of N-fixation PGPR bacteria (bio) and the interaction among them.Table (1) shows properties for soil of the experiment (0 – 30 cm).

Table (1): Some physical and chemical properties of the experimental soil .

Property				Value	Property		Value
Particle size distribution							
Clay %				10.15	Soluble ions (mmolc L^{-1})		
Silt %				6.27	Na^+		9.07
Sand %				83.58	K^+		0.78
Textural class(2)				Loamy Sand	Ca^{++}		5.90
EC (dSm^{-1}) in soil paste extract				1.86	Mg^{++}		2.85
					Cl^-		6.85
pH [Soil suspension 1:2.5]				8.03	HCO_3^-		1.89
Organic matter (g kg^{-1})				4.81	$SO_4^=$		9.86
$CaCO_3$ (g kg^{-1})				12.51			
Available macro and micronutrients (mg kg^{-1} soil) (1)							
N	P	K	Fe	Mn	Zn		
40.98	2.75	189	1.67	0.76	0.65		

(1) Extractants of available nutrients: NH_4HCO_3-DTPA (P, K, Fe, Mn and Zn),
(2) Texture according to the international soil texture triangle.

A randomize complete block design with three replicates was followed. Compost was added to the all plots at a rate of 5 megagram, Mg fed.$^{-1}$ (megagram; 1 Mg = 10^6 g). The area of each plot was 50 m^2 (5 X 10 m) and included 20 rows 50 cm apart, two plants per each hill and 25 cm between hills. Faba bean (*Vicia faba* L., cv. Nubaria 1) seeds supplied from Food Legumes Department, Field Crop Research Institute, Agriculture Research Center, Giza, Egypt. Sowing dates were November 25th and 30th in the two successive seasons 2013/2014 and 2014/2015, respectively. Plants were thinned to one plant per hill after 21 days from planting.

Biofertilizer was prepared from *Rhizobium radiobacter* sp strain (plant growth promoting rhizobacteria, PGPR) deposited in Gene bank under number of HQ395610 Egypt by Bio-fertilizer Production Unit, Department of Microbiology, Soils, Water and Enviro. Res. Inst., Agric. Res. Center, Giza, Egypt. At sowing , the seeds of faba bean were inoculated with *Rhiobium radiobacter* sp. strain. The bio-fertilizer was applied at a rate of 100 g for each 25 kg seeds wetted with 300 ml of adhesive liquid (Arabic gum). Seeds were thoroughly mixed with the inoculants in the shade, then sown immediately and covered with soil in order to minimize *Rhizobia* exposure to the sun ;finally, bio-fertilizer at the rate 20 L of the inoculants suspension /400 L water fed.$^{-1}$,(feddan = 4200 m^2) was added within irrigation system three times after 21, 45 and 65 days after sowing (DAS).

Compost manure was prepared according to (Nasef *et al.,* 2009) using 5 Mg fed.$^{-1}$ of some crop residues (rice straw, maize stover and faba bean straw), air dried and piled into 5 – 10 layers, each about 50-cm thick, An amount of 300 kg/weight of farmyard manure was added to each pile to enhance microorganism activity. Piles were moistened with a sufficient quantity of water (about 60%). Every 21 days the piles were turned over until well decomposed. After 63 days, the compost was well decomposed and ready for use. The compost manure was mixed thoroughly with the soil one month before sowing. The final product was chemically analyzed according to Brunner and Wasmer (1978). Tables 2 show some properties of the compost used in the experiment.

Table (2): Some properties of compost.

Moisture content (%)	EC dS m^{-1} 1:10	pH 1:2.5	O.C ($g\ kg^{-1}$)	O.M	C/N ratio	Total macro-nutrients			Total micro-nutrients		
						N	P	K	Fe	Mn	Zn
						($g\ kg^{-1}$)					
									($mg\ kg^{-1}$)		
20	4.12	7.81	354	610	19.3	18.3	9.61	22.8	253	152	27.3

The experiment included two factors as follows:

1. N-Sources (S): (a) Mineral ammonium nitrate(AN); (b) Anhydrous ammonia (AA) and (c) Bio-fertilizer *Rhizobium radiobacter* sp as N_2 fixator (bio).
2. N-fertilization rates (R): (a) without N-addition (N_0), (b) 20 kg N fed.$^{-1}$ (N_{20}), (c) 30 kg N fed.$^{-1}$ (N_{30}) , (d) 40 kg N fed.$^{-1}$ (N_{40}).

Therefore, there were 12 treatments which represent, 3 (N-sources) X 4 (nitrogen rates). Phosphorus (P) fertilizer was added to all plots before sowing at a rate of 100 kg fed.$^{-1}$ as super phosphate (15.5 % P_2O_5) . Potassium sulphate (48 % K_2O) was applied as soil application at a rate of 75 kg fed.$^{-1}$ in two equal splits, 21 and 45 days after sowing respectively.

Nitrogen (N) fertilizer was added in the form of ammonium nitrate in three equal splits, 21, 45 and 40 days from sowing respectively; anhydrous ammonia, with full dose, was injected directly into the moderately moister soil at 15cm depth with 30 cm spacing between points of injection before 5 days from planting according to the injection technique previously used by Farrag *et al.* (2011). Agricultural practices for growing faba bean were carried out as recommended by the Ministry of Agriculture.

Methods of Analysis

At maturity, the middle three rows of each plot were harvested and air dried to determine the following characteristics:

1. No. pod *per* plant
2. 100-seed weight
3. Pod yield (Mg fed.$^{-1}$)
4. Seed yield (Mg fed.$^{-1}$)

Laboratory analysis

Dried seeds were milled to fine powder and then digested with a mixture of concentrated sulfuric and perchloric acids for nutrients determination. The analyses of both soil and plant samples were carried out using the methods described by Chapman and Pratt (1961) and Jakson (1973). Crude protein in faba bean seeds was calculated by multiplying total N-content by the converting factor 6.25 (Hymowitz, *et al.,* 1972).

Soil Sample

Top soil samples (0-30 cm) were collected from all the experimental plots at the maximum growth stages, air dried, crushed and sieved through a

2 mm sieve and analyzed for soil EC and pH along with available N, P, K, Fe, Mn and Zn contents according to the same methods used for analyzing the initial soil according to Black (1965) and Page *et al.,* (1982).

Statistical analysis

Results were statistically analyzed using COSTATC software. The ANOVA test was used to determine significantly ($p \leq 0.01$ or $p \leq 0.05$) treatment effect and Duncan Multiple Range Test was used to determine significantly the difference between individual means (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Soil properties

Soil pH

Data illustrated in Fig 1 show that the decrease of soil pH was noticed in soil treated with biofertilizer in combination with AN at the rate 40KgN/Fed (7.88) as compared with those treated by either AA or biofertilizer alone. In fact, certain forms of nitrogen such as NH_4 and NO_3 tend to reduce soil pH. The bacteria that fixed N_2 dissolved P and available K led to decrease in soil pH when added alone or in combination with chemical fertilizers, The obtained data may be explained on the base of some products of transformation mineral fertilizers added to the soil; such product have acidic effect. During microbial reactions, acidity (H^+) is released, which will ultimately decrease soil pH .In this regard, Nasef *et al.* (2009) reported that the applied bio-fertilizer resulted in reduction of soil pH due to various acids (amino acids such as glycine and cysteine as well as humic acid) or acid forming compounds along with active microorganisms released from the addition of bio-fertilizer.

Schmitt and Rehm (1993) stated that application of all the N-fertilizer sources, significantly reduced soil pH as compared to the control treatment, nitrification of ammonium being an acid forming reaction with net effect being a lower pH. These results are in agreement with those later obtained by Shaban *et al.* (2012) and Costa, et al., (2008) who found that the soil pH decreased with the applied of nitrogen fertilizer.

Total soluble salts (EC dSm^{-1})

Data illustrated in Fig (1) also show that the obtained values of EC were decreased with the increase of AN-rate in combination with bioferilizer as compared with combined AA and bioferilizer .The lowest EC values (1.25, 1.24 and 1.18 dSm^{-1}) were recorded with the treatments of AN, at the rate of 40 kg N $fed.^{-1}$, AA, at N_{40} and bio + N_{40}, respectively. These results could be related to the influence of bioferilizer on total porosity and improving soil aggregation and thus possible moving salts in soil under irrigation water (Zaka *et al*.,2005) . The used N sources could be arranged according to their effects on reducing EC of soil in the following descending order: bio > AA > AN. As for AN rates, the order was: $N_{40} > N_{30} > N_{20} > N_0$. Of course , acidity possibly produced from added AN or AA could be affecting in reducing the the concerned EC through facilitating the salt leaching .Helmy *et al.* (2013)

reported that soluble salts decreased when the bio-fertilizers were applied alone or in combination with N-fertilizer.

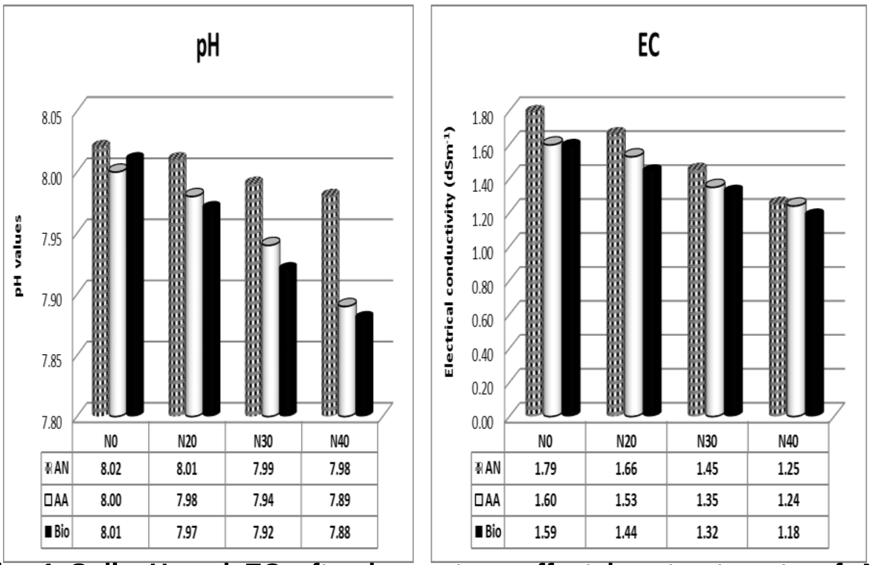

Fig. 1 Soil pH and EC after harvest as affect by treatments of AN Ammonium nitrate; AA anhydrous ammonia and Bio, N0, No N addition; N20, 20 Kg N fed.$^{-1}$; N30, 30 Kg N fed.$^{-1}$ and N40, 40 Kg N fed.$^{-1}$

Available macronutrients (N, P and K)

Data presented in Fig 2, show that available N, P and K (mg kg^{-1}) contents were more affected at the combined treatment of AN at the rate N_{40} with AA and biofertilizer. Available N ranged between 45.12 for the rate of N_0 to 54.0 mg kg^{-1} for AN at the rate N40 addition, 48.0 to 58.1 mg kg^{-1} for AA alone and AA+N40 addition and 48.8 to 59.1 mg kg^{-1} for bio alone and bio+N40 addition . Available P ranged between 2.77 to 2.93 mg kg^{-1} for the rate of N_0 and AN at the rate of N40 addition, 2.83 to 2.94 mg kg^{-1} for AA alone and AA+N40 addition and 2.77 to 2.95 mg kg^{-1} for bio alone and bio+N40 addition . Available K ranged between 190 to 197 mg kg^{-1} for the rate of N_0 and AN at the rate N40 addition, 195 to 205 mg kg^{-1} for AA alone and AA+N40 addition and 198 to 210 mg kg^{-1} for bio alone and bio+N_{40} addition. The soil treated with bio + N_{40} gave the highest values of available P and K contents, the soil treated with bio + N_{30} having the highest value for available N content. The used different sources of N fertilizer could be arranged according to their effects in the following descending order: bio > AA > AN for available N and K contents but AA > bio > AN for available P content. As for N addition rates, the order was: $N_{40} > N_{30} > N_{20} > N_0$ for available N, P and K contents. In this regard, Mustafa *et al*.(2006) found that available phosphate in soil was significantly increased by seed inoculation with *Bacillus megatherium* . Shaban and Omar (2006) added that soil available N increased as the level of mineral N increased especially with bioferilizer (*Azospirillum brasilense NO*40).

The production of organic and inorganic acids as a result of the microorganism's activities must have contributed to a decrease in soil pH which produces more chelating ions, leading to an increase in available forms of elements in the rhizosphere zone. These results are in agreement with those obtained by Ewees and Abdel Hafeez (2010).

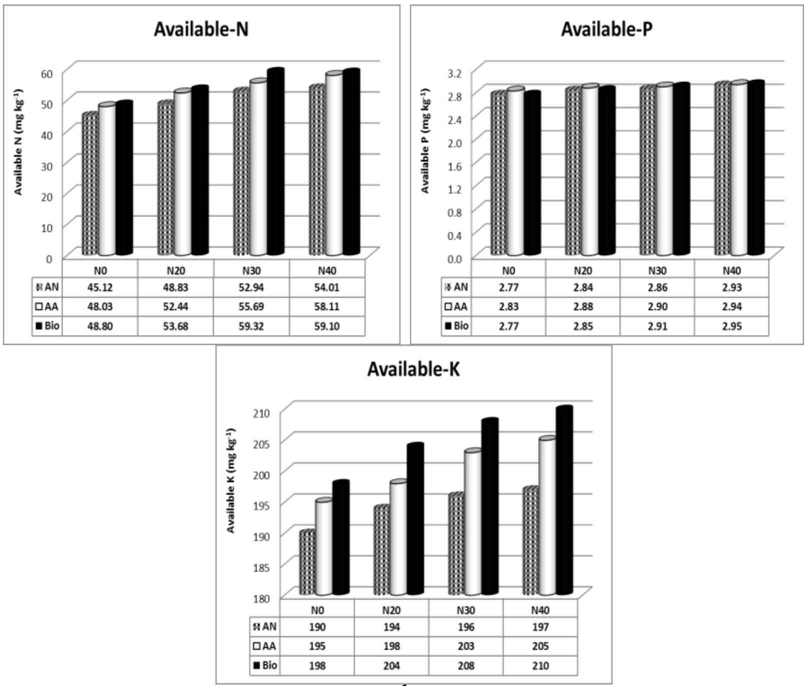

Fig. 2. N, P and K contents (mg Kg^{-1}) in soil after harvest as affect by applied treatments

Available micronutrients (Fe, Mn and Zn)

The concentrations of available Fe, Mn and Zn in soil at the end of the experiment increased due to application of N- sources as well as higher AN rates (Fig 3). The highest available Fe and Zn values (2.02 and 0.89 mg kg^{-1}) were respectively, obtained due to treatment of bio + 40 kg N fed.$^{-1}$ AN. This may be due to the vital role of biofertilizer which contains microorganisms that make these nutrients more available in the soil. In addition, bacteria cause some elements such as Fe, Mn and Zn to release in available forms in soil through breakdown of organic materials in the soil. Shaban et al (2012) indicated that the application of bio-fertilizers resulted in decreasing soil pH and increased available content of Fe, Mn and Zn. The highest available Mn (0.98mg Kg^{-1}) was obtained under the treatment AA +40 Kg N fed^{-1} as AN. Tisdale *et al.,* (1999) reported that anhydrous ammonia reaction with water produces OH^-. This OH^- should dissolve and hydrolyze certain fractions of soil organic matter and increase the availability of nutrients in soil.

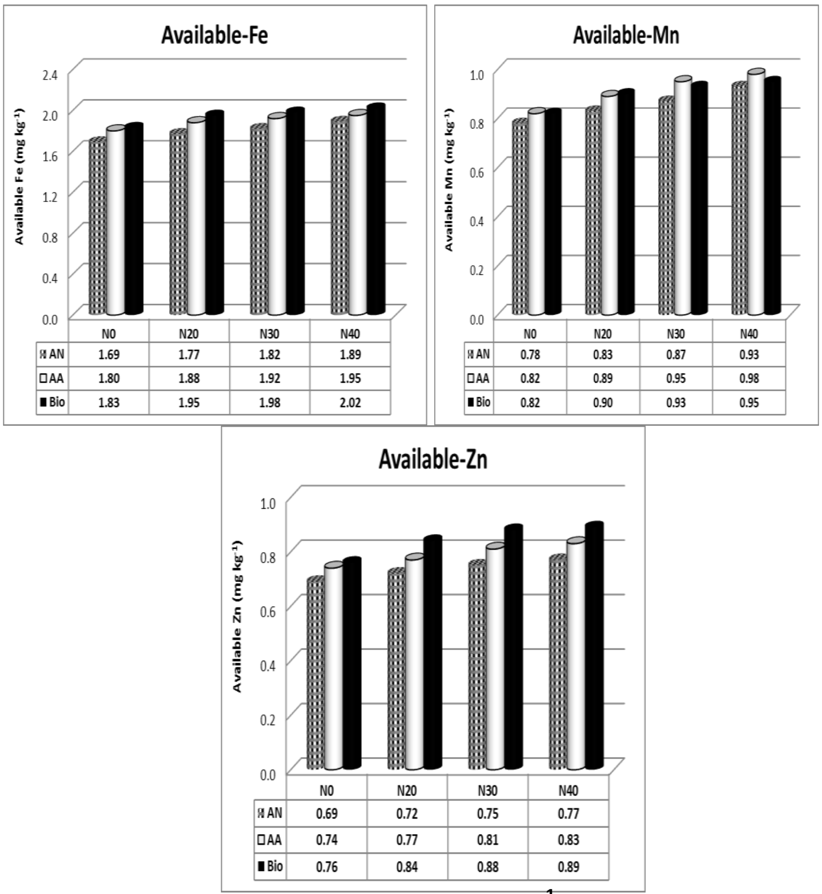

Fig. (3) Available Fe, Mn and Zn contents (mg Kg^{-1}) in soil after harvest as affect by applied treatments.

Effect of Fertilization on Yield and Yield Components.

Number pods plant^{-1}

Number of pod plant^{-1} of the studied faba bean plants is shown in (Table 3). Regarding the statistical analysis, data show that defferent sources of N fertilization were significantly favorable .Concerning the response to nitrogen sources, the main effect followed a descending of: bio+AN > AA > AN. The highest value of number of pods per plant (25.0) was obtained due to the treatment bio + 40 kg N fed.$^{-1}$ as AN which increased by 47.1% compared with the plants treated with either bio or AN only. As for AN rate, there was the following descending order: N_{40} > N_{30}> N_{20}

100-seed weight

100-seed weight of faba bean plants is shown in (Table 3) . Data show that N fertilization sources and rates significantly increased 100-seed weight while, there was no significant difference between the applied N sources. As for N rate effect, there was the following descending order: $N_{40} > N_{30} > N_{20} > N_0$. Regarding the response to N-sources, the main effect followed a descending of: AA = bio+AN > AN. The highest value (75.61 g) was obtained due to the treatment AA at 40 kg N fed.$^{-1}$ which increased by 21.8% compared with control plants

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Table 3. Effect of applied treatments on some growth parameters and yield

N-rate (Kg fed.$^{-1}$)	N-source							
	Control	**AA**	**Bio**	**Mean**	**Control**	**AA**	**Bio**	**Mean**
	No. pod plant^{-1}				100-seed weight (g)			
0	9.67	13.33	17.00	13.33 d	56.72	62.08	61.78	60.19 d
20	12.33	17.33	19.67	16.44 c	62.84	66.75	65.07	64.89 c
30	14.33	20.00	22.33	18.89 b	66.46	69.70	71.97	69.38 b
40	15.00	23.00	25.00	21.00 a	69.12	75.61	74.39	73.04 a
Mean	12.83 c	18.42 b	21.00 a		63.79 b	68.54 a	68.30 a	
F-test	S:**	R:**	SxR: NS		S:**	R:**	SxR: NS	
	Seed yield (Mg fed.$^{-1}$)				Pod yield (Mg fed.$^{-1}$)			
0	0.247	0.578	0.601	0.475 d	0.457	0.646	0.703	0.602 d
20	0.365	0.661	0.725	0.584 c	0.541	0.752	0.924	0.739 c
30	0.378	0.688	0.829	0.632 b	0.587	0.824	0.987	0.799 b
40	0.589	0.789	0.905	0.761 a	0.670	0.893	1.012	0.858 a
Mean	0.395 c	0.679 b	0.765 a		0.564 c	0.779 b	0.907 a	
F-test	S:**	R:**	SxR: *		S:**	R:**	SxR: NS	

AN, Ammonium nitrate; AA, Anhydrous Ammonia and biofertilizer (*Rhizobium radiobacter* sp.)
NS, not significant. The values followed by different letters are significantly different at P≤ 0.05.
***,** Significant at P< 0.05 and 0.01, respectively.**

Seed and Pod Yields.

Data presented in (Table 3), showed that seed and pods yields significantly increased by using different rates of AN along with AA and biofertilizer either alone or as interacted with AN rate. The favorable effect of nitrogen fertilizer may be due to N stimulation of plant growth, which would increase the amount of light energy intercepted by leaves with encouragement for photosynthetic pigments and photosynthesis which finally increase synthesized metabolites and consequently development of leaves and seeds (Wortman *et al.,* 2011). Concerning the effect of bioferilizer, it produces growth promoting substances (phytohormones) which play a key role in plant growth and promote seed germination and root elongation. Root development and proliferation of plants of course in response to biofertilizer activities, water and nutrients uptake should be enhanced (Kandil *et al.,* 2011 and Joshi *et al.,* 2012). These results agree with those obtained by Siam *et al.* (2013) and Piccinin *et al.* (2013).

A descending order characterized the effects of N fertilization sources on seed and pod yields as follows: bio+AN_{40} > AA > AN. As for the main effect of N-fertilization rates; the order was: N_{40} > N_{30} > N_{20} > N_0, respectively.

Concerning the interaction effect between the rates of AN with AA and biofrtilizer on faba bean yield and its components, data presented in (Table 3) reveal that the maximum seed and pod yields (0.905 and 1.012 Mg fed.$^{-1}$, respectively) were achieved due to application of bio + 40 kg N fed.$^{-1}$ as AN and the corresponding increments over control were 50.6 and 44.0%, respectively. The interaction gave non-significant values of all parameters; for the seed yield, however, the interaction was significant. These results are in agreement with those of Matiru and Dakora (2004), who reported that rhizobia naturally produce auxins, cytokinins, absicic acids, rhiboflavin, lipo-chito-oligosaccharides and vitamins. These molecules promote cell division and cell elongation which could induce plant growth.

Seed Quality

Seed protein content

Data pertained the effect of studied treatments on protein content are shown in Table (4). With respect to applied treatments, data showed that no significant differences could be detected within treatments using AA and bio+AN. Concerning the effect of N-fertilization rates, the results revealed no significant differences among the AN addition rates, wherein 40 kg N fed.$^{-1}$ achieved the greatest protein yield. Siam, *et al.* (2013) pointed out that protein content in wheat grain increased with high rates of mineral N fertilizer up to 100 kg N fed.$^{-1}$. Namvar and Teymur (2013) added that inoculation with *Azotobacter sp.* and *Azospirillium sp*. increased wheat grains protein content by 10%. The current results are in agreement with those reported by Abedi *et al.* (2010) and Rana *et al.* (2012).

Table (4): Effect of treatments on seed protein along with N, P and K contents

N-rate	N-source							
	Control	**AA**	**Bio**	**Mean**	**Control**	**AA**	**Bio**	**Mean**
	Protein content (g kg^{-1})				N-content (g kg^{-1})			
0	14.61	17.63	17.36	16.53 b	23.37	28.20	27.77	26.45 b
20	18.65	19.19	19.37	19.07 a	29.83	30.70	31.00	30.51 a
30	16.85	19.69	19.71	18.75 a	26.97	31.50	31.53	30.00 a
40	19.29	19.81	19.38	19.49 a	30.87	31.70	31.00	31.19 a
Mean	17.35 b	19.08 a	18.95 a		27.76 b	30.53 a	30.33 a	
F-test	S:**	R:**	SxR: NS		S:**	R:**	SxR: NS	
	P-content (g kg^{-1})				K-content (g kg^{-1})			
0	03.03	03.47	04.77	03.76 d	20.50	21.10	21.07	20.89 d
20	03.57	04.00	05.27	04.28 c	21.07	21.87	22.33	21.76 c
30	03.97	04.33	05.43	04.58 b	21.50	22.00	22.80	22.10 b
40	04.73	05.07	05.13	04.98 a	22.27	22.30	22.53	22.37 a
Mean	03.83 c	04.22 b	05.15 a		21.33 c	21.82 b	22.18 a	
F-test	S:**	R:**	SxR: **		S:**	R:**	SxR: **	

In general, the highest increase in protein content (35.7%) was recorded in the plants treated with AA at rate of 40 kg N fed.$^{-1}$. The second highest (32.6%) was in plants treated with of bio + N_{40} as AN.

The increase in protein content could be attributed to the integrated effect of nitrogen plus bio effect of microorganisms such as N_2 fixing bacteria which increase available nutrients for plant growth and accordingly maximizing the biological yield and grain quality of barley (Ewees and abdel Hafeez, 2010). Also, exudation of plant growth regulators such as auxins, gibberellin as well as microorgnisms contribute to such positive effect (Vessy, 2003) . These treatments can be arranged due to their effect on protein content in the following order: AA > bio+AN > AN.

Macronutrients content

Data in Table (4) also show that N, P and K contents increased significantly due to addition of AN, as well as either AA or biofertilizer and combinations with AN. Treatment consisting of bio + N_{30} as AN was superior for increasing the contents of P and K and the treatment of AA at N_{40} was superior for increasing the content of N as compared to the other treatments. This promoting effect could be related to the N supplementary effect of N_2 fixing bacteria (used as bio N -fertilizer) to plants due to their ability to fix free molecular atmospheric nitrogen as well as the role of these bacteria in improving the availability of soil elements Table (4) through secreting chelating substances (such as organic acids) which are important for solubilizing sparingly soluble inorganic compounds to more available forms for plants uptake (Kandil *et al.,* 2011 and Daneshmand *et al.,* 2012).

On the other hand, it is well known that nitrogen fertilizers influence the content of photosynthetic pigments, the synthesis of the enzymes taking part in the carbon reduction, the formation of the membrane system of chloroplasts, etc. Thus the increase in growth and yield owing to the application of N-fertilizers maybe attributed to the fact that these nutrients being important constituents of nucleotides, proteins, chlorophyll and enzymes, involved in various metabolic processes which have direct impact on vegetative and reproductive phases of plants (Osman *et al.* 2013). These results coincide with the results of Abbas *et al.* (2011), Namvar and Teymur (2013) and Diacono *et al.,* (2013).

The main effect of AN, AA and bio-fertilizer treatments showed a descending increase in the order: (bio+AN_{30} > AA > AN) for seed P and K contents but (AA+N_{40} > bio+AN > AN) for N content. The effect of N-fertilization rate, on increasing P and K contents in seeds followed the order: (N_{40} > N_{30} > N_{20}> N_0) with no significance difference for N content. The percentages response of N, P and K content by faba bean seeds over the treatments received all N as compost (control) at 5 Mg fed.$^{-1}$ were 15.6, 22.6 and 5.65%, respectively.

Macronutrients total content:

Results in Table (5) illustrate the total content of macronutrients in seeds of faba bean under the different nitrogen sources and different rates. The data showed that N, P and K increased with increasing the rate of AN. The highest values of N, P and K total contents in seeds of faba bean were recorded by bio-fertilizer followed by anhydrous ammonia compared with mineral ammonium nitrate fertilizer. Concerning the interaction between the sources and the rates, the obtained results recorded that N, P and K gave significant response with the combined treatments between bioferilizer and

N_{40} as ammonium nitrate. The corresponding relative increases for mean values of N total content in seeds of faba bean were 38.76 %, 49.77 % and 84.10 % for the rate of 20, 30 and 40 kg N fed-1 respectively. Concerning, the relative increase of mean values P faba bean as affected by different rates were, 38.42 %; 60.23% and 103.67 % respectively, compared with control. The relative increase of mean values K were 28.31%; 41.16 % and 71.08% as affected by different rates of mineral nitrogen 20, 30 and 40 kg N fed^{-1} respectively compared with control.

On the other hand,in order to the effect of different sources on the relative increase of mean values of N, P and K total content in seeds were 84.97 % and 111.11 % for N; 82.98 % but 149.53 % for P and 74.59 % and 100.59 % for K regarding with applied anhydrous ammonium and bio-fertilizer compared with ammonium nitrate , respectively.

Table (5): Effect of treatments on N, P and K total content (kg $fed.^{-1}$) in seeds

N-rate	N-source							
	Control	**AA**	**Bio**	**Mean**	**Control**	**AA**	**Bio**	**Mean**
	N				P			
0	5.73	16.29	16.69	12.90 d	0.747	2.001	2.865	1.871 d
20	10.92	20.28	22.49	17.90 c	1.306	2.643	3.822	2.590 c
30	10.16	21.66	26.15	19.32 b	1.503	2.976	4.516	2.998 b
40	18.20	25.00	28.06	23.75 a	2.791	3.996	4.646	3.811 a
Mean	11.25 c	20.81b	23.35 a		1.587 c	2.904 b	3.962 a	
F-test	S:**	R:**	SxR: *		S:**	R:**	SxR: *	

	K			
	Control	AA	Bio	Mean
0	5.04	12.20	12.66	9.96 d
20	7.70	14.44	16.22	12.78 c
30	8.14	15.13	18.92	14.06 b
40	13.13	17.59	20.39	17.04 a
Mean	8.50 c	14.84 b	17.05 a	
F- test	S **	R **	SX R **	

It is evident that total contents of N, P and K in seeds of faba bean could be arranged according to their uptake in the following orders: Bio-fertilizer > anhydrous ammonium> ammonium nitrate. These results are in harmony with those obtained by Massoud et al., (2004) who suggested that inoculation with N_2-fixer bacteria increased uptake of N, P and K by pea plants. Helmy et al (2013) added that the seeds inoculation by bio-fertilization + 40 kg N ha^{-1} AN treatment was superior for increasing the uptake of N, P and K in seeds of pea. On the other hand, Osman et al (2013) found that anhydrous ammonia increased significantly values of N, P and K contents of rice grain compared to urea fertilizer form in two seasons.

Micronutrient contents in seeds.

Values of seed Fe, Mn and Zn contents of faba bean seeds as affected by application of AN rates , AA and biofertilization each either alone and in combinations with AN are shown in Table (6). Values followed a pattern similar to that shown by the macronutrient where they increased significantly by the addition of the aforementioned fertilization treatments. AA at the rate of 40 kg N $fed.^{-1}$ treatment bieng most effective; treatment of bio + N_{40} was most effective on the content of Mn as compared to the other treatments. The percentages response of Fe, Mn and Zn content by faba bean seeds over the control treatment were 5.42, 19.0 and 12.7%, respectively. These findings are in agreement with those reported by Nasef *et al*. (2009) who reported that application of bio-fertilizer combined with mineral N fertilizer caused pronounced increases in Fe, Mn and Zn contents as a result to increasing soil available micronutrients contents during two seasons under rice cropping. These increases may be attributed to the role of organic sources in improving these micronutrients availability which was likely attributed to several reasons: *i*) Releasing of these nutrients through microbial decomposition of organic matter ; *ii*) Enhancing the chelation of metal ions by fulvic acid, organic legends and / or other organic function groups which may promote the mobility of metal from solid to liquid phase in soil environment; *iii*) Lowering the redox statues of iron and manganese, leading to reduction of Fe^{3+} & Mn^{4+} to Fe^{2+} and Mn^{2+} and / or transformation of insoluble chelated forms into more soluble ions.

Table (6): Effect of treatments on seed Fe, Mn and Zn contents

N-rate	N-source							
	Control	**AA**	**Bio**	**Mean**	**Control**	**AA**	**Bio**	**Mean**
	Fe-content (mg kg^{-1})				Mn-content (mg kg^{-1})			
0	81.88	91.04	90.86	87.93 b	50.83	54.92	51.09	52.28 d
20	90.17	93.80	92.20	92.06 a	58.53	63.41	54.79	58.91 c
30	90.65	94.78	92.88	92.77 a	62.39	64.02	61.13	62.51 b
40	91.21	95.14	93.43	93.26 a	63.96	65.73	65.84	65.18 a
Mean	88.48 b	93.69 a	92.34 a		58.93 b	62.02 a	58.21 b	
F-test	S:**	R:**	SxR: *		S:**	R:**	SxR: NS	

	Zn-content (mg kg^{-1})			
	AN	AA	Bio + AN	Mean
0	26.47	29.71	30.52	28.90 c
20	30.40	32.52	31.96	31.63 b
30	31.58	33.71	33.26	32.85 a
40	32.13	33.85	33.64	33.21 a
Mean	30.15 b	32.45 a	32.35 a	
F-test	S:**	R:**	SxR: NS	

Micronutrients content in seeds.

Data in Table (7) indicated clearly that Fe, Mn and Zn (mg/kg) total contents in seeds of faba bean increased with increasing the rate of mineral N (ammonium nitrate) , the effect of different sources of nitrogen were significant . The interaction between different sources of nitrogen and rates were also significantly affected. The increase in elements total content in the concerned seeds was more pronounced at the highest level with nitrogen combined with bio-fertilizer compared with other treatments. Biofertilizers play a very significant role in improving soil fertility by fixing atmospheric nitrogen, both, solubilizing insoluble soil phosphates and producing plant growth substances in the soil (Venkatashwarlu, 2008) . Helmy et al (2013) reported that the applied of bio-fertilizer + 40 kg N fed^{-1} led to increase Fe, Mn and Zn content in seeds of pea . Abd El-Kader (2002) showed that anhydrous ammonia injected before sowing, gave higher yield and minerals uptake than other nitrogen sources. Siam et al (2012) added that the micronutrients contents increased by increasing rate of anhydrous ammonia (at 140kg N fed^{-1}.) compared with control. These results were attributed to increase in utilization coefficient of ammonia gas as a source of nitrogen form. Also ammonia gas as the nitrogen source may promote changes in the mineral composition of plant.

Table (7): Effect of treatments on Fe, Mn and Zn total contents (g fed.$^{-1}$) by seeds.

N-rate	N-source							
	Control	**AA**	**Bio**	**Mean**	**Control**	**AA**	**Bio**	**Mean**
	Fe				Mn			
0	20.28	52.64	54.58	42.50 d	12.46	31.72	30.71	24.96 d
20	32.94	61.92	66.89	53.92 c	21.45	41.87	39.73	34.35 c
30	34.32	65.11	77.02	58.82 b	23.64	44.03	50.76	39.48 b
40	53.77	75.01	84.54	71.11 a	37.69	51.84	59.60	49.71 a
Mean	35.33 c	63.67 b	70.76 a		23.81 c	42.36 b	45.20 a	
F-test	S:**	R:**	SxR: *		S:**	R:**	SxR: *	

	Zn			
	Control	AA	Bio	Mean
0	6.43	17.17	18.34	13.98 d
20	11.12	21.45	23.18	18.58 c
30	11.99	23.18	27.58	20.92 b
40	18.95	26.70	30.44	25.36 a
Mean	12.12 c	22.12 b	24.89 a	
F test	S **	R **		SxR *

The superiority of bio-fertilization + 40 kg N fed^{-1} treatment could be due to the beneficial role of the bio-fertilizer, microorganisms and their biological activity , in particular , which could help building up the microflora.

CONCLUSION

On the light of the obtained results , it could be suggested that N fertilization affect all the measured traits of faba bean. Among N sources, the inoculation with (*Rhizobium radiobacter* sp.) as PGPR strain was superior followed by anhydrous ammonia. On the other hand, among the nitrogen fertilization levels, the N_{40} treatment, (40 kg N fed.$^{-1}$) in all studies traits was generally superior. Also, the use a combination of biofertilizer and inorganic fertilizers as ammonium nitrate at 40 kg N fed.$^{-1}$ achieved the highest yield without negative effect on seed quality under sandy loam conditions.

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أستجابة نباتات الفول البلدي لصور ومعدلات مختلفة من التسميد النيتروجينى في الأراضي المستصلحة حديثا

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- تم دراسة أستجابة نباتات الفول البلدي صنف نوبارية ١ لمعدلات مختلفة من التسميد النيتروجيني (٠ ، ٢٠ ، ٣٠ و ٤٠ كجم ن فدان-١ نترات الأمونيوم مع الامونيا اللامائية والتسميد الحيوى المثبتة للنيتروجين (الريزوبيم راديوباكترPGPR بالبكتيريا فى ارض سلتية رملية بمحطة البحوث الزراعية بمحافظة الأسماعيلية - جمهورية مصر العربية خلال موسم الشتاء لعامين متتالين هما ٢٠١٣/٢٠١٤ و ٢٠١٤/٢٠١٥ على انتاجية وجودة الحبوب و أمتصاصه لبعض العناصر الكبري و الصغري فى الفول البلدى مع تحسين بعض صفات التربة.
- أظهرت النتائج ان استخدام التسميد الحيوى مع ٤٠كجم نيتروجين من نيترات الامونيوم ادى الى زيادة عدد القرون للنبات الواحدومحصول القرون والحبوب وايضا تركيز المنجنيز بينما استخدام التسميد الحيوى مع المعدل ٣٠كجم نيتروجين من نيترات الامونيوم ادى الى زيادة تركيز الفوسفور والبوتاسيوم فى الحبوب.
- بالاضافة لذلك فقد سجلت النتائج زيادة وزن المائة بذرة وتركيز كل من البروتين ،النيتروجين،الحديد والزنك فى البذوروذلك باستخدام الامونيا الامائية مع ٤٠كجم نيتروجين من نيترات الامونيوم
- ايضا فقد اظهرت النتائج زيادة تواجد كل من العناصر الكبرى والصغرى فى البذور بزيادة معدلات نترات الامونيوم وباستخدام التسميد الحيوى وسجلت اعلى قيم باستخدام التسميد الحيوى مع المعدل ٤٠كجم نيتروجين من نيترات الامونيوم
- كذلك فقد سجلت نتائج التحاليل فى التربة بعد الزراعة ان استخدام التسميد الحيوى مع ٤٠كجم نيتروجين من نيترات الامونيوم ادت الى انخفاض رقم الحموضة ودرجة التوصيل الكهربائى وزيادة تركيز الفوسفور ،البوتاسيوم،الحديد والزنك
- كذلك فقد سجل النيتروجين الميسربها اعلى قيم باستخدام التسميد الحيوى مع المعدل ٣٠كجم نيتروجين من نيترات الامونيوم بينما زاد تركيز المنجنيزالميسر باستخدام الامونيا الامائية مع ٤٠كجم نيتروجين من نيترات الامونيوم