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Effect of Mineral Fertilizers and Biofertilization on some Soil Properties and Faba Bean Productivity under Saline Soil Conditions

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ABSTRACT

In winter 2016/2017 and 2017/2018 seasons, two field experiments were carried out in Gelbana village, at private farm, North Sinai Governorate, Egypt to evaluate the effect of partial substitution of 50% and 25% of NPK addition rates recommended by the Ministry of Agriculture (40 kg N fed.$^{-1}$, 100 kg P_2O_5 and 70 kg K_2O fed.$^{-1}$) using biofertilization with *Rhiobium radiobacter* sp. Strain (fixed nitrogen), *Bacillus megatherium* as (dissolving phosphate bacteria) and *Bacillus circulans* (enhancing potassium availability) on some soil properties and faba bean yield, yield attributes and chemical composition content of two faba bean (*Vicia faba* L.) cultivars. *i.e.*, Nubaria 1 and Masr 3. 100-seed weight gave the highest value (98.85 g) when plants of Misr 3 treated with full NPK fertilizer recommended dose + biofertilization. Pod yield, N, P and K content as well as N, P and K-uptake by faba bean (Nubaria1) seeds gave the maximum values under Biofertilization + full NPK fertilizer recommended dose (full-RD). Seed yield and protein content were increased significantly and gave the highest values due to the treatment of Biofertilization + 75% of NPK fertilizer (¾RD) for Nubaria1 plants. Highest yield efficiency (97.25%) was obtained due to Biofertilization + 75% of NPK fertilizer (¾RD) for Misr3 plants. Fertilized treatments in combination with biofertilization decreased values of soil EC and pH and increased soil available N, P and K content after harvest when plants treated with Biofertilization + full NPK-RD which was ascendant to the other treatments and gave the highest with Misr 3 variety.

Keywords: Bio inoculation, NPK fertilization, faba bean, saline soil.

INTRODUCTION

Soil salinity is known to cause considerable yield losses in most crops, thereby leading to reduced crop productivity (Chaum *et al.*, 2011). The salinity-induced crop yield reduction takes place due to a number of physiological and biochemical functions in plants grown under salinity stress which have been listed in a number of comprehensive reviews on salinity effects and tolerance in plants (Jamil *et al.*, 2011 and Krasensky and Jonak, 2012).

Scientists have been vying for the last many decades to overcome the problem of salinity by employing a variety of strategies. The various strategies currently under exploitation was improvement in salinity tolerance of crops through exogenous application of different types of organic and biological fertilizers which help in inhibitory of the adverse effect of salinity (Ehteshami *et al.*, 2007).

One of the important vegetable crops in Egypt is faba bean (*Vicia faba* L.) which the total cultivated area of bean plants is (60000 feddan) produces about 28530 tons (FAO, 2010). Its seeds exhibit high levels of protein (28–36 % of seed dry matter). Faba bean has the highest average reliance on N_2 fixation for growth of the major cool season grain legumes. Several 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. (Erik *et al.*, 2010).

Nitrogen fertilizer utilization has played a significant role in increases of crop yield, Modhej *et al.* (2008). Mahdi *et al*. (2010) found that the N_2 Fixation in faba bean in the range of 69 – 100 kg N fed.$^{-1}$ with nitrogen to the system of 35 kg N fed.$^{-1}$ when only grain was removed. Seed inoculation with nitrogen fixers could improve growth, yield and yield attributes of faba bean (El-Kholy *et al.*, 2010). N increases the nutrients uptake, capacity of photosynthesis assimilation in building metabolites, its translocation and accumulation in the sink (Fathi *et al.*, 2003).

Phosphorus (P) is one of the major plant growth limiting nutrients although it is abundant in soils in both inorganic and organic forms. Phosphate solubilizing micro-organisms (PSMs) are ubiquitous in soils and could play an important role in supplying P to plants in a more environmentally friendly and sustainable manner.

Phosphorus is usually supplied to the plant in many different forms some of which are manufactured, i.e., phosphoric acid and calcium super phosphate, while some others are common in nature such as rock phosphate, Abou El-Yazeid and Abou-Aly (2011). Supplied 46.5 % kg P_2O_5 /fed led to increase of number of pods, seeds/ plant , weight of pod/plant, seed and straw yield /plant and /fed , biological yield /fed , protein (%) as well as protein /fed. may be attributed to the physiological role of P on the meristematic activity of plant tissues and consequently increasing plant growth, also, its function as a part of enzyme system having a vital role of the synthesis of other foods from carbohydrate, Fouda (2017).

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Potassium (K) is one of the principle plant nutrients and plays a vital role as macronutrient in plant growth and sustainable crop production, usual absorbed by plants in larger quantities (Pettigrew, 2008 and Bukhsh *et al.*, 2009).

While involved in many physiological processes, potassium is impact on water relations, photosynthesis, assimilate transport and enzyme activation can have direct consequences on crop productivity. It maintains turgor pressure of cell which is essential for cell expansion (Bukhsh *et al.,* 2010). The role of potassium element in metabolism and many processes needed to sustain and promote plant vegetative growth and development. Moreover, K plays a major role in many physiological and biochemical processes such as cell division and elongation and metabolism of carbohydrates and protein compounds, Taha *et al.* (2016).

Biofertilizers are products containing living microorganisms, which have an ability to convert nutrients from unavailable to available forms for plants by biologic processes (Vessey, 2003). Microorganisms that allow more efficient nutrients use or increase nutrients availability can provide sustainable solutions for present and future agricultural practices. Phosphate - dissolving microorganisms represent one of such biofertilizers and play an important role in supplying plants with phosphorus. They bring about a number of transformations increasing the solubility of inorganic phosphorus. Some bacteria such as *Bacillus megaterium* provide plants with growth promoting substances and play major role in phosphate solubilization. Also, N_2-fixing microorganisms render gaseous N_2 available for plants, particularly legumes (Fares and Khalil, 2003). Several bacteria that are associated with the roots of crop plants could induce beneficial effects on their hosts and often are collectively referred to plant growth promoting Rhizobacteria (PGPR) (WHO, 2002). In recent years, biofertilizers have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yields through environmentally better nutrient supplies (Wu *et al.,* 2005). Soil chemical and biological characteristics were improved by bio fertilizer. Moreover, due to the use of low doses of chemical fertilizers, agricultural production will be free from contaminants (EL- Habbasha *et al.,* 2007 and Salimpour *et al.,* 2010).

The current study aim at investigating bio-inoculation with *Rhizobium radiobacter* sp strain, *Bacillus megaterium* and *Bacillus circulans* as a partial substitution of recommended mineral-NPK fertilizer to faba bean under saline conditions as related to the achieved amelioration of some soil properties which were taken into consideration in this study.

MATERIALS AND METHODS

The field work: Two field experiments were conducted in private farm at Gelbana village, North Sinai Governorate, Egypt during the successive winter seasons of 2016/2017 and 2017/2018. In order to study the possibility of substituting, partially the amount required of integrated mineral fertilizer (NPK) for fertilization of faba bean plants yield, yield quality and some improve saline soil properties.

Soil sample : A representative soil sample (0 – 30 cm) was taken before planting to determine some physical and chemical properties according to Page *et al.* (1982) and Klute (1986) and results are shown in Table (1).

Table 1. Physical and chemical properties of soil of the experiment

Property		Value	Property	Value
Particle size distribution				
Clay	%	10.20	Soluble ions (mmolc L^{-1})	
Silt	%	6.22	Na^+	90.00
Sand	%	83.58	K^+	0.66
Textural class		Loamy Sand	Ca^{++}	13.67
EC (dSm^{-1}) in soil paste extract		12.86	Mg^{++}	24.27
			Cl^-	85.00
pH [Soil suspension 1:2.5]		8.06	HCO_3^-	10.58
Organic matter (g kg^{-1})		6.50	$SO_4^=$	33.02
$CaCO_3$ (g kg^{-1})		9.66	CO_3^{--}	nil
Available macro and micronutrients (mg kg^{-1} soil)				
N	P	K	Fe	Mn	Zn
39.98	3.99	182	2.86	2.85	0.89

(1) Extractants of available nutrients: NH_4HCO_3-DTPA (P, K, Fe, Mn and Zn), KCl (N)

(2) Texture according to the international soil texture triangle.

Bacterial inoculants: The biofertilizers were inoculate of N_2-fixing microorganisms "NFM" *Rhizobium radiobacter* sp strain (salt tolerant PGPR); P-dissolving bacteria "PDB" (*Bacillus megatherium* var phosphaticum) and enhancing K availability "EKA" (*Bacillus circulans* sp strain). All Bio-fertilizer Production Unit, Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt. and are inoculate in forms of organic, peat like substances to treat seeds at a rate of 600 g material per the amounts of seeds are required for one feddan wetted with 300 ml of adhesive liquid (Arabic gum). Seeds were thoroughly mixed with the inoculants solution in the shade for 2 h before planting, then sown immediately and covered with soil in order to minimize the exposure to the sun. More biofertilization was added 3 times at 30, 45 and 60 days through liquid sprays on soil at a rate of 20L of the inoculant suspension / 400 L water fed.$^{-1}$, feddan = 4200 m^2 .

Design and Treatment: A split-split plot design with three replicates, having a plot area 5 X 10 m (50 m^2), was used. Faba bean varieties were assigned to the main plots, the sub plots included NPK recommended rates while; bio fertilization was randomly distributed in the sub-sub plots. Each sub-sub plot consisted of 20 rows 50 cm apart, two plants per hill and 25 cm between hills. Faba bean seeds (*Vicia faba* L.) cv. Nubaria 1 and Masr 3 supplied from Food Legumes Department, Field Crop Research Institute, Agriculture Research Center, Giza, Egypt were sown after soil preparation. Seeding snow was carried out on 20th and 25th Nov. for the first and second season, respectively. Harvest was done on 26th and 29th of April for the first and second season, respectively.

Plants were thinned to one plant per hill after 21 days from planting. Faba bean seeds were divided into two groups. The first group without inoculation was sowing at integrated mineral NPK fertilizer (IMF) at rates of; 0 kg NPK fed.$^{-1}$ (no fertilizer) to represent control treatment, (20 kg N fed.$^{-1}$, 50 kg P_2O_5 fed.$^{-1}$ and 35 kg K_2O fed.$^{-1}$) as ½ recommended dose (½ RD), (30 kg N fed.$^{-1}$, 75 kg P_2O_5 fed.$^{-1}$ and 55 kg K_2O fed.$^{-1}$) as ¾ recommended dose (¾ RD) and (40 kg N fed.$^{-1}$, 100 kg P_2O_5 fed.$^{-1}$ and 75 kg K_2O fed.$^{-1}$) as full recommended dose (Full RD). The second group was mixed with suitable amount of Arabic gum solution 15%, as adhesive material, and then thoroughly mixed with bacterial inoculants at rate of 10g / kg faba bean seeds. Urea (46% N) was the source of mineral nitrogen fertilizer, which was applied at three equal doses after 21, 35 and 50 days of faba bean planting and applied the bacterial fixed N_2 at a rate 20 L mixed with 200 L water /fed for three times 21, 45 and 65 days from planting. Phosphorus (P) fertilizer was added to all plots before sowing at a rates 50 , 75 and 100 kg P_2O_5 fed.$^{-1}$ as superphosphate (15% P_2O_5) and applied of P-dissolving bacteria "PDB" (*Bacillus megatherium* var phosphaticum) at a rate 25 L bacterial mixed with 200 L water /fed was applied into two period 33 and 45 days from planting.

Potassium sulphate (40% K_2O) was applied in two equal doses after 21 and 45 days from sowing and applied with enhancing K availability "EKA" (*Bacillus circulans* sp strain) at a rate 20 L bacterial mixed with 200 L water /fed for two doses after 22 and 45 days from planting. Other standard agricultural practices for growing faba bean were carried out as recommended by the Ministry of Agriculture. The experiment included three factors as follows:

1. **NPK addition rate, (R):**

(a) 0 RD; (b) ½ RD; (c) ¾ RD and (D) Full RD.

2. **Bio inoculation (B):**

(a) With and (b) without

3. **Variety (V):**

(a) Nubaria 1 (b) Masr 3

Therefore there were 16 treatments which represent, 4 (R) X 2 (B) X 2 (V).

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

1. 100-seed weight
2. Pod yield, mega gram fed.$^{-1}$ (Mg fed.$^{-1}$); 1 Mega gram $=10^6$ g = 1000 kg = tonne
3. Seed yield (Mg fed.$^{-1}$)
4. Yield efficiency (%) = (grain yield / pod yield) x 100
5. Protein content (%) = N content (%) X 6.25
6. Seed nutrient uptake (kg fed.$^{-1}$)= nutrient content (g kg^{-1}) X seed yield (Mg fed.$^{-1}$)

Laboratory analysis: Sufficient amounts of dried seed was milled to a fine powder and then digested with a mixture of concentrated sulfuric and perchloric acids for nutrient determination. The analyses of plants and soil were carried out using the methods described by Chapman and Pratt (1961) and Jackson (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, pH and available N, P and K contents according to the some methods used for analyzing the initial soil 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 of the difference between individual means (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Soil pH and Soil Salinity (EC_e)

Concerning the effect of all the treatments on soil pH, in the rhizosphere of grown faba bean, data reveal that all treatments receiving NPK fertilizer with or without biofertilization showed a slight decrease effect on soil pH from 8.04 to 7.95 due to bio inoculation in combination with integrated NPK fertilizer addition and 8.05 to 8.01 due to integrated NPK fertilizer addition without bio inoculation (Table 2). Nasef *et al.* (2009) found that, applied biofertilizer reduced soil pH due to various acids (amino acids such as glycine and cysteine as well as humic acid) or acid forming compounds and active microorganisms released from the addition of bio-fertilizer.

Also, as a result of the nitrification of ammonium and the production of organic acids, the end result is to reduce the acidity number of the soil. The positive relationship between soil and bio-fertilizers in reduces the effect of soil salinity and increases nutrients in soil (Rashed, 2006). These results are in a harmony with those obtained by Abdel Lattif (2007) and Poraas *et al*., (2009). These results are in agreement with those obtained by Shaban *et al.* (2012) and Helmy *et al.* (2013a).

The lowest pH 7.95 was found due to treatment of bio + full (RD) of NPK.

As for soluble salts data show that the values were decreased by the addition treatments. *Rhizobium* producing phyto-hormones like indole acetic acid, cytokinines and organic acid which decreases salinity stress in the rhizosphere. Such products reduce the deleterious effect of Na-salts, and improve soil structure, increasing aggregate stability and drainable pores. Consequently, these created conductive pores enhancing the leaching process of soluble salts through irrigation fractions. Shaban and Omar (2006) and Ashmaye *et al.* (2008). This would improve soil conditions for plant growth. Improvement in porosity and aggregation may have occurred due to the applied biofertilizer and hence enhanced the leaching of salts (Zaka *et al.*, 2005).

The lowest EC value (6.34 dSm^{-1}) was recorded under the treatment of full (RD) NPK fertilizer + bio inoculation. The treatments could be arranged according to their effects on reducing EC of soil in the following descending order: full (RD) > (¾ RD) > (½ RD) > control as for NPK fertilizer rates and the order was: with bio > without bio as for bio inoculation effect.

Available macronutrients (N, P and K)

The effects of NPK-mineral fertilizer added as either solely or combined with bio-fertilizer (*Rhizobium radiobacter*, *Bacillus megatherium* and *Bacillus circulans*) to the experimental soil plots under cultivation with faba bean caused a pronounced ameliorated effect in each of the studied soil content of available N, P and K as shown in Table 2 . The data showed that a progressive significant increases in all the studied available macronutrients upon treating the soil with biofertilizer in presence of NPK fertilizer, particularly at the applied 100% NPK-mineral fertilizer (Full RD) as compared to the treatment of no NPK-fertilizer.

The superiority of applied 100% NPK-mineral + bio inoculation is mainly attributed to its ability to release some plant promoting substances, mainly indolic acetic acid (IAA), gibberellic acid and cytokinin like substances which stimulate plant growth (Gomaa and Abou Aly, 2001), beside the beneficial effects of bacteria on reducing soil pH by organic acids (*e.g.*, acetic, propionic, fumaric and succinic) which leading to change of nutrients to available forms ready for uptake by plants. Also, the latter conditions led to enhance the microbial activity in soil, which accelerate the decomposition of organic matter and maximize soil content of nutrients Ewees and Abdel Hafeez (2010). The present results are in agreement with those of Ewees and Osman (2013) who found that treating the soil with bio-fertilizer in combination with N-mineral fertilizer caused progressive significant increases in all the studied available macronutrients and micronutrients nutrients. Available N ranged between 41.8 to 53.6 mg kg^{-1} without bio addition and 45.6 to 57.7 mg kg^{-1} for bio addition in combination with different NPK-mineral rates. Available P ranged between 3.76 to 4.06 mg kg^{-1} without bio addition and 3.86 to 4.12 mg kg^{-1} for bio addition in combination with different NPK-mineral rates. Available K ranged between 195 to 209 mg kg^{-1} without bio addition and 200 to 222 mg kg^{-1} for bio addition in combination with different NPK-mineral rates. The soil treated with bio + full (RD) of NPK gave the highest values of available N, P and K contents.

The used NPK-mineral fertilizer followed the following descending order: full (RD) > (¾ RD) > ½ (RD) > no fertilizer. As for bio addition and faba bean vaiety, the order was: bio > without bio and Nubaria1 > Masr, respectively. This was found true for available N, P and K in soil after harvest without significant effect between the two varieties for available K.

Table 2. Soil pH, EC and available macronutrient contents (mg kg $^{-1}$ soil) at harvest as influenced by bio and NPK– fertilizers

Varity (V)	Rate of NPK (R)	pH		EC			Available-N			Available-P			Available-K		
		Bio inoculation (B)													
		With	Without	With	Without	Mean	With	Without	Mean	With	Without	Mean	With	Without	Mean
Nubaria 1	0 (RD)	8.04	8.05	7.83	8.82	8.33	45.6	41.8	43.7	3.86	3.76	3.81	200	195	198
	½ (RD)	8.02	8.03	7.25	8.23	7.74	50.3	44.2	47.2	3.98	3.81	3.90	208	203	205
	¾ (RD)	8.00	8.02	7.05	8.02	7.53	53.8	45.2	49.5	4.04	3.87	3.96	212	206	209
	Full (RD)	7.99	8.02	6.75	7.67	7.21	56.7	48.4	52.6	4.08	3.97	4.02	217	211	214
	Mean	---	---	7.22	8.18	7.70	51.6	44.9	48.3 b	3.99	3.85	3.92 b	209	204	207
Misr 3	0 (RD)	8.04	8.04	7.81	8.96	8.39	46.6	43.0	44.8	3.98	3.90	3.94	201	200	200
	½ (RD)	8.02	8.03	7.29	8.75	8.02	51.3	45.8	48.6	4.05	3.98	4.01	209	201	205
	¾ (RD)	7.98	8.02	6.79	8.32	7.56	54.7	48.1	51.4	4.09	4.01	4.05	214	204	209
	Full (RD)	7.95	8.01	6.34	8.00	7.17	57.7	53.6	55.6	4.12	4.06	4.09	222	209	216
	Mean	---	---	7.06	8.51	7.78	52.6	47.6	50.1 a	4.06	3.99	4.02 a	211	204	208
Mean of Bio				7.14 a	8.35 b		52.1 a	46.3 b		4.03 a	3.92 b		210 a	204 b	
Mean of Rate				0	8.36 d		0	44.2 d		0	3.88 d		0	199 d	
				½	7.88 c		½	47.9 c		½	3.96 c		½	205 c	
				¾	7.54 b		¾	50.5 b		¾	4.00 b		¾	209 b	
				Full	7.19 a		Full	54.1 a		Full	4.06 a		Full	215 a	
F-test				V: NS	R:**	B:**	V: **	R:**	B:**	V: **	R:**	B:**	V: NS	R:**	B:**
				VxR: NS	VxB:**		VxR:NS	VxB: NS		VxR: NS	VxB: **		VxR: NS	VxB: NS	
				RxB: NS	VxRxB: NS		RxB:*	VxRxB: NS		RxB: NS	VxRxB: NS		RxB: NS	VxRxB: NS	

½ (RD), ½ recommended dose (20 kg N fed.$^{-1}$, 50 kg P_2O_5 fed.$^{-1}$ and 35 kg K_2O fed.$^{-1}$); (¾ RD), ¾ recommended dose (30 kg N fed.$^{-1}$, 75 kg P_2O_5 fed.$^{-1}$ and 55 kg K_2O fed.$^{-1}$) and Full (RD), full recommended dose (40 kg N fed.$^{-1}$, 100 kg P_2O_5 fed.$^{-1}$ and 75 kg K_2O fed.$^{-1}$).

Effect of Fertilization on Yield and Yield Components of faba bean.

100-seed weight

Data in Table 3 demonstrate the effect of NPK-mineral fertilization without and with bio inoculation with (*Rhizobium radiobacter, Bacillus megatherium* and *Bacillus circulans*) as a partial substitute for mineral NPK on faba bean yield and its component. Such data reveal that application of all fertilizer treatments proved to be significantly. Highest 100-seed weight (98.85 g) of faba bean plants was obtained when plant treated with full (RD) of NPK-mineral fertilizer in combination with bio inoculation for Misr 3 variety which increased by 48.7% compared with the plants which did not receive fertilizers. As for NPK rate effect, the descending order was: full (RD) > (¾ RD) > ½ (RD) > no fertilizer. Regarding the response to bio fertilization, the order was as follow: bio > without bio. Misr 3 variety was superior as compared with Nubaria 1 variety. Application of bio-phosphors was significantly increased 100-seed weight, without chemical phosphorus, while the effect of mineral phosphate biofertilizer was not significantly affected 100-seed weight, Shahram and Peyman (2016). Reda and Badr (2015) showed that inoculation with Rhizobium increased significantly the weight of 100 seeds faba bean under saline soil.

Pod and Seed Yields

Due to the data in Table 3, pod and seed yield of faba bean were significantly increased as a result of applying different NPK-fertilization rates with or without bio inoculation under the two faba bean varieties. 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 and increase photosynthetic pigments and photosynthesis, and in turn increase synthesized metabolites and consequently leaves and seeds (Wortman *et al.,* 2011). As for the role of phosphorus and potassium in plants, El-Kabbany and Darwish (2002) concluded that phosphorus is part of the molecular structure of some vitally important compounds, notably nucleic acids. Also, plays an important role in photosynthesis, respiration and cell division. On the other hand, K is essential for enzyme energy, seed formation, seed quality, stress tolerance and crop maturity. (Kandil *et al.*, 2011 and Joshi *et al.*, 2012) found that phytohormones play a key role in plant growth and promote seed germination and root elongation thus enhance water and nutrients uptake.

Table 3. 100-seed weight (g), seed and pod yields (Mg fed.$^{-1}$) as well as yield efficiency of faba bean as affected by NPK fertilizers and bio inoculation

Varity (V)	Rate of NPK (R)	100-seed weight			Pod yield			Seed yield			Yield efficiency		
		Bio inoculation (B)											
		With	Without	Mean	With	Without	Mean	With	Without	Mean	With	Without	Mean
Nubaria 1	0 (RD)	75.39	66.46	70.93	0.691	0.492	0.592	0.530	0.390	0.460	76.70	79.27	77.98
	½ (RD)	80.49	70.67	75.58	1.292	0.726	1.009	1.051	0.588	0.819	81.35	80.99	81.17
	¾ (RD)	84.15	73.03	78.59	1.356	0.853	1.105	1.212	0.768	0.990	89.38	90.60	90.04
	Full (RD)	89.80	77.86	83.83	1.364	0.894	1.129	1.199	0.700	0.949	87.90	78.30	83.10
	Mean	82.46	72.01	77.23b	1.176	0.741	0.958b	0.998	0.612	0.805b	83.83	82.29	83.06 a
Misr 3	0 (RD)	82.27	70.99	76.63	0.758	0.636	0.697	0.466	0.378	0.422	61.48	59.43	60.46
	½ (RD)	92.94	77.08	85.01	1.166	1.060	1.113	0.965	0.640	0.803	82.76	60.38	71.57
	¾ (RD)	95.77	79.25	87.51	1.199	1.137	1.168	1.166	0.956	1.061	97.25	84.08	90.66
	Full (RD)	98.85	80.96	89.90	1.210	1.183	1.197	1.157	1.052	1.105	95.62	88.93	92.27
	Mean	92.46	77.07	84.76a	1.084	1.004	1.044a	0.939	0.757	0.848a	84.28	73.20	78.74 b
Mean of Bio		87.5a	74.5b		1.130a	0.872b		0.968a	0.684b		84.05a	77.75b	
Mean of Rate		0	73.8 d		0	0.644 d		0	0.441 c		0	69.2 d	
		½	80.3 c		½	1.061 c		½	0.811 b		½	76.37 c	
		¾	83.1 b		¾	1.137b		¾	1.026 a		¾	87.72 b	
		Full	86.9 a		Full	1.163 a		Full	1.027 a		Full	90.28 a	
F-test		V: **	R:**	B:**	V: **	R:**	B:**	V: **	R:**	B:**	V: **	R:**	B:**
		VxR: *	VxB:**		VxR:NS	VxB: **		VxR: **	VxB: **		VxR: **	VxB: **	
		RxB: **	VxRxB: NS		RxB:**	VxRxB: **		RxB: **	VxRxB: **		RxB: **	VxRxB: **	

Also, increased yield due to biofertilization reflected the ability of PDB and NFM in increasing phosphatase activity (for PDB) and increasing N (for NFM) as well as producing growth regulating hormones (Sobh *et al*., 2000). These results agree with those obtained by, Siam *et al.* (2013), Helmy *et al.* (2013b) and Piccinin *et al.* (2013).

With respecting to the statistical analysis, a descending order characterized the effect of NPK addition rates on pod and seed yields as follows: full (RD) > (¾ RD) > ½ (RD) > no fertilizer for pod yield and full (RD) ≥ (¾ RD) > ½ (RD) > no fertilizer for seed yield, respectively. As for the main effect of bio inoculation; the order was: with bio > without bio. Also, Misr 3 > Nubaria 1 and this was found true for pod and seed yield. Data also show that, the highest values (1.364 and 1.212 Mg fed.$^{-1}$) were obtained owing to treatments of Biofertilization + (full RD) of NPK-mineral fertilizer for pod yield and Biofertilization + (¾ RD) of NPK-mineral fertilizer for seed yield, respectively for Nubaria 1 variety. The increases over the control treatment were 177% for pod yield and 210% for seed yield. These results are well supported by the findings of Matiru and Dakora, (2004)Otieno *et al.* (2007) and Ewees and Abdel Hafeez (2010).

Data presented in Table 3 show that as concerning to the interaction effect between nitrogen fertilizer rates and bioinoculation on faba bean yield and its components, , the interaction gave significant values of all parameters for seed yield and yield efficiency.

Yield efficiency

Seed yield efficiency, which is the ratio of seed yield to pod yield at maturity varied between 59.43%–97.25% as shown in Table 3. Variability in yield efficiency could be attributed to variability in yield components, (Ayaz, 2001). Highest yield efficiency was found when Misr 3 plants treated with Biofertilization + (full RD) of NPK-mineral fertilizer. Results show following descending order: Nubaria 1 > Misr 3 ; Biofertilization > without bio and full (RD) > (¾ RD) > ½ (RD) > no fertilizer.

Seed Quality

Proline content

As for proline content, data in (Table 4) reveal that values significantly decreased by application of NPK-mineral fertilizer at different rates solely and their combinations with bio fertilization for the two faba bean varieties of Nubaria1 and Misr3. Increasing under salinity stress than the inoculated plants with bioferilizers and high rate of NPK fertilizer might be caused by activation of proline syntheses from glutamate or decrease in its utilization in protein syntheses or enhancement in protein turnover. Proline also consider the source of energy during immediate post stress metabolism and supplies energy for growth and survival, thereby inducing salinity tolerance (Gad 2005).

Seed protein content

Table 4 showed the effect of studied factors on protein content and data showed that significant differences could be detected within treatments using NPK-mineral fertilizer and bio inoculation on protein content. As for the effect of NPK-fertilization rates, the results revealed slightly differences among the ¾ (RD) and full (RD) addition rates for Nubaria1, wherein ¾ (RD) with biofertilization gave the highest protein yield. Siam, *et al.* (2013) reported that fertilization with mineral N fertilizer up to 100 kg N fed.$^{-1}$ increased wheat grain protein content. The results are in a harmony with those obtained by Namvar and Teymur (2013), Abedi *et al.* (2010), Rana *et al.* (2012), Kandil *et al.*, (2011), Daneshmand *et al.*, (2012), Abbas *et al.* (2011) and Diacono *et al.*, (2013). The increase in protein content may be due to the effect of NPK fertilizer and bio effect of N_2 fixing bacteria (salt tolerant PGPR strains of N_2-fixer bacteria) and phosphorus dissolving bacteria in increasing nutrients content for plant growth (Ewees and abdel Hafeez, 2010), exudation of plant growth regulators i.e, auxins and gibberellin (Vessy, 2003).

Maximum value of protein content (21.3%) was reported in the plants treated with ¾ (RD) of NPK- mineral fertilizer + bio fertilization which recorded 22.4% increase over the control treatment (without NPK fertilizer or bio addition) for Nubaria1.

The interaction between NPK fertilizer rates and biofertilization with faba bean varieties treatments had significant effect on protein content. Mabrouk (2002) found that bio fertilization was effective in increasing protein content of peanut plants than the individual addition of mineral fertilization. These results are in agreement with Hussein (2007) and Omran *et al.*, (2009).

Table 4. praline content (µg g^{-1} dwt), protein content (%), N-content (%) and N-uptake (kg $fed.^{-1}$) of faba bean seeds as affected by NPK fertilizers and bio inoculation

Varity (V)	Rate of NPK (R)	Proline content			Protein content			N-content			N-uptake		
		Bio inoculation (B)											
		With	Without	Mean	With	Without	Mean	With	Without	Mean	With	Without	Mean
Nubaria 1	0 (RD)	222	235	228	19.8	17.4	18.6	3.17	2.79	2.98	16.8	10.9	13.8
	½ (RD)	179	191	185	20.6	18.3	19.4	3.29	2.93	3.11	34.6	17.2	25.9
	¾ (RD)	153	183	168	21.3	18.5	19.9	3.35	2.96	3.16	40.6	22.9	31.8
	Full (RD)	109	167	138	21.2	18.9	20.0	3.39	3.03	3.21	40.7	21.2	30.9
	Mean	166	194	180 b	20.7	18.3	19.5 b	3.30	2.93	3.11b	33.1	18.0	25.6 b
Misr 3	0 (RD)	235	251	243	20.3	18.1	19.2	3.25	2.90	3.08	15.1	11.0	13.1
	½ (RD)	174	196	185	20.6	18.7	19.6	3.29	2.99	3.14	31.8	19.1	25.5
	¾ (RD)	143	184	164	20.8	19.1	20.0	3.33	3.06	3.19	38.8	29.2	34.0
	Full (RD)	127	166	147	21.1	19.5	20.3	3.37	3.12	3.25	39.0	32.8	35.9
	Mean	170	199	185 a	20.7	18.9	19.8 a	3.31	3.02	3.16 a	31.2	23.0	27.1 a
Mean of Bio		168 b	197 a		20.7 a	18.6 b		3.31 a	2.97b		32.2 a	20.5 b	
Mean of Rate		0	236 a		0	18.9 d		0	3.03 d		0	13.4 d	
		½	185 b		½	19.5 c		½	3.13 c		½	25.7 c	
		¾	166 c		¾	19.9 b		¾	3.18 b		¾	32.9 b	
		Full	142 d		Full	20.2 a		Full	3.23 a		Full	33.4 a	
F-test		V: *	R:**	B:**	V: **	R:**	B:**	V: **	R:**	B:**	V: **	R:**	B:**
		VxR: **	VxB:NS		VxR:*	VxB: **		VxR: **	VxB: *		VxR: **	VxB: **	
		RxB: **	VxRxB: *		RxB: NS	VxRxB: NS		RxB: NS	VxRxB: NS		RxB: **	VxRxB: **	

The treatments of control without NPK fertilizer or bio inoculation increased proline content and gave the highest value (251 µg g^{-1} dwt) for Misr 3 variety in the descending order of: no fertilizer > ½ (RD) > (¾ RD) > full (RD); without bio > with bio and Misr3 > Nubaria1.

Macronutrient content and uptake

Data presented in Tables 4 and 5 show that N, P and K content and uptake by faba bean seeds significantly increased owing to application of NPK-mineral fertilizer solely or in combination with bio fertilization for the two faba bean varieties except for K-uptake, the effect of faba bean varieties was insignificant. The treatment consisting of full (RD) of NPK fertilizer + biofertilization was superior for increasing the content and uptake of N, P and K as compared to the other treatments for Nubaria 1 and Misr 3 varieties.

Table 5. P and K content (%) as well as P and K-uptake (kg $fed.^{-1}$) of faba bean seeds as affected by NPK fertilizers and bio inoculation

Varity (V)	Rate of NPK (R)	P-content			P-uptake			K-content			K-uptake		
		Bio inoculation (B)											
		With	Without	Mean	With	Without	Mean	With	Without	Mean	With	Without	Mean
Nubaria 1	0 (RD)	0.48	0.42	0.45	2.54	1.63	2.09	2.51	2.36	2.43	13.3	9.19	11.2
	½ (RD)	0.52	0.44	0.48	5.49	2.60	4.05	2.57	2.41	2.49	27.0	14.2	20.6
	¾ (RD)	0.56	0.48	0.52	6.75	3.67	5.21	2.63	2.50	2.56	31.9	19.3	25.6
	Full (RD)	0.60	0.52	0.56	7.19	3.64	5.42	2.69	2.54	2.61	32.2	17.8	25.0
	Mean	0.54	0.46	0.50 a	5.49	2.89	4.19 a	2.60	2.45	2.53 a	26.1	15.1	20.6
Misr 3	0 (RD)	0.36	0.33	0.35	1.70	1.23	1.47	2.43	2.30	2.37	11.3	8.71	10.0
	½ (RD)	0.45	0.38	0.42	4.38	2.43	3.41	2.50	2.34	2.42	24.1	15.0	19.6
	¾ (RD)	0.48	0.40	0.44	5.56	3.83	4.70	2.53	2.37	2.45	29.5	22.6	26.1
	Full (RD)	0.55	0.43	0.49	6.41	4.53	5.47	2.58	2.41	2.50	29.8	25.4	27.6
	Mean	0.46	0.38	0.42 b	4.51	3.01	3.76 b	2.51	2.36	2.43 b	23.7	17.9	20.8
Mean of Bio		0.50 a	0.42b		5.00 a	2.95 b		2.56 a	2.40 b		24.9 a	16.5 b	
Mean of Rate		0	0.40 d		0	1.78 d		0	2.40 d		0	10.6 d	
		½	0.45 c		½	3.73 c		½	2.46 c		½	20.1 c	
		¾	0.48 b		¾	4.95 b		¾	2.51 b		¾	25.8 b	
		Full	0.53 a		Full	5.44 a		Full	2.55 a		Full	26.3 a	
F-test		V: **	R:**	B:**	V: **	R:**	B:**	V: **	R:**	B:**	V: NS	R:**	B:**
		VxR: *	VxB: NS		VxR:**	VxB: **		VxR: **	VxB: **		VxR: **	VxB: **	
		RxB: *	VxRxB: NS		RxB:**	VxRxB: **		RxB: **	VxRxB: **		RxB: **	VxRxB: **	

The inoculation with phosphate solubilizing bacteria may be the main reason for increasing P content whereas, phosphate solubilizing bacteria solubilize un-available phosphate in soil, which became available for plant uptake. On the other hand, K had a favorable effect on amino acid transport and protein production within the plant (William, 2008).

The maximum values (3.39, 0.60 and 2.69 %) of N, P and K content duo to the treatments were achieved owing to addition of full (RD) of NPK fertilizer + bio for Nubaria1.

As for N, P and K uptake by faba bean seeds, the main effect of the treatments can be arranged in the following descending order: full (RD) > (¾ RD) > ½ (RD) > no fertilizer; bio > without biofertilization and Nubaria 1 > Misr 3. The highest N, P and K uptake (40.7, 7.19 and 32.2 kg fed.$^{-1}$), respectively were obtained due to the same treatment of highest N, P and K content giving increases of 273%, 341% and 250% over the control (un treated plants). The interaction effect between varieties, NPK rates and bio fertilization was significant.

CONCLUSION

From the present data, it can be concluded that biofertilization by *Rhiobium radiobacter* sp strain, *Bacillus megatherium* as (dissolving phosphate bacteria) and *Bacillus circulans* inoculants could be applied to faba bean as a supplement to inorganic NPK-fertilizer. Considerable increase was observed when plants were treated with bio inoculation + 75% NPK-recommended by the Ministry of Agriculture. The improvement of faba bean growth and yield may be attributed to one or more of the following factors:1) availability of more NPK due to N_2-fixation; 2) production of growth promoting substances by microorganisms.; 3) the successful competition of the bacteria, which antagonizes root pathogens (Amara, 2001).

In the present study, faba bean plants grown in saline soil were inoculated with microorganisms isolated from saline soil. In addition, inoculation of faba bean plants in the present investigation saved about ¼ of recommended dose of NPK mineral fertilizer. Thereby, the use of the present inoculum could be valuable in increasing plant yield and seed quality, saving mineral fertilizer and decreasing environmental pollution.

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تأثير الأسمدة المعدنية متحده أو منفردة على بعض صفات التربة وإنتاجية صنفين من الفول البلدي تحت ظروف الأراضي الملحية

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أجريت تجربتان حقليتان في مزرعة بقرية جلبانة– محافظة شمال سيناء – مصر خلال الموسم الشتوي لعامي 2017/2018 و 2016/2017 م وذلك لتقييم تأثير إضافة التسميد الحيوي ببكتريا مثبتة للنيتروجين ، مذيبه للفسفور و بكتريا ميسرة للبوتاسيوم معاً علي خفض التأثير الإجهادي للملوحة بالتربة وإحلالة كبديل بيئي آمن للأسمدة المعدنية الكبري المصنعة بما يعادل 25% و 50% من المعدلات الموصي بها من قبل وزارة الزراعة المصرية وأثر ذلك علي جودة وأنتاجية صنفين من حبوب الفول البلدي هما نوبارية1 و مصر3 (*Vicia faba* L., cv. (Nubaria1 and Masr 3 ومحتواهما من بعض العناصر الغذائية الكبري الممتصة. وأمتدت الدراسة لتقدير محتوي التربة من بعض العناصر الكبري الميسرة ودرجة الحموضة و التوصيل الكهربي بها بعد الحصاد. ويمكن تلخيص أهم النتائج المتحصل عليها كما يلي: أزدادت قيم وزن الـ100 حبة وكانت أعلي قيمة (98.85 جم) قد تحصل عليها عندما عوملت نباتات صنف مصر3 بالجرعة الكاملة الموصي بها من أسمدة (ن، فو و بو) مع التسميد الحيوي. أعلي أنتاجية لمحصول القرون ومحتوي الحبوب من النيتروجين و الفسفور و البوتاسيوم وكذلك الممتص منها كانت لنباتات صنف نوبارية1 مع المعاملة (الجرعة الكاملة الموصي بها من أسمدة ن ، فو و بو) + التسميد الحيوي. محصول الحبوب ومحتواها من البروتين لأعطي أعلي قيم لها عندما عوملت نباتات صنف نوبارية1 بـ 75% من الجرعة الموصي بها من التسميد المعدني المتكامل لأسمدة (ن ، فو و بو) مع التسميد الحيوي. أعلي كفاءة محصولية (97.25%) تم النحصل عليها عندما عوملت نباتات صنف مصر3 بـ 75% من الجرعة الموصي بها من التسميد المعدني المتكامل لأسمدة (ن ، فو و بو) مع التسميد الحيوي. إزداد محتوى النيتروجين و الفسفور و البوتاسيوم الميسر بالتربة بينما أنخفضت قيم التوصيل الكهربي ودرجة الحموضة بالتربة نتيجة لإضافة الأسمدة المعدنية خاصة مع التسميد الحيوي و كانت المعاملة (100% من معدلات الأسمدة النيتروجينية و الفوسفاتية والبوتاسية المعدنية الموصي بها + التسميد الحيوي بالبكتريا المستخدمة مجتمعة معاً) هي المعاملة التي أعطت أعلي النتائج مقارنة بباقي المعاملات و أعطت أعلي قيم لها خاصة مع نباتات صنف مصر3. مما سبق يمكن التوصية بإمكانية إحلال الأسمدة الكبري المعدنية المصنعة في وجود التسميد الحيوي في الأراضي الملحية كبديل طبيعي آمن بيئياً يقلل من مخاطر استخدام الأسمدة المعدنية وتلوث التربة والبيئة المحيطة.