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Partial Replacement of Integrated Mineral Fertilizer through Biofertilization to Maximize Economic Yield of Faba Bean under Saline Soil Conditions

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Abstract : In 2014/2015 and 2015/2016 seasons, two field experiments were conducted at private farm, El-Qantra Sharq, Ismailia 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 fad.$^{-1}$, 100 kg P_2O_5 and 70 kg K_2O fed.$^{-1}$) using biofertilization with *Rhiozbium radiobacter* sp strain, *Bacillus megatherium* as (dissolving phosphate bacteria) and *Bacillus circulans* (enhancing potassium availability) on faba bean (*Vicia faba* L., cv. Masr 3) phosphate dissolving bacteria . 100-seed weight, N, P and K content as well as K-uptake by faba bean seeds gave the maximum values under Biofertilization + full NPK fertilizer recommended dose (full-RD). Pod and seed yields as well as seed protein content and protein yield as well as N and P-uptake by seeds were increased significantly and gave the highest values due to the treatment of Biofertilization + 75% of NPK fertilizer recommended dose (NPK-RD). Fertilized treatments decreased values of soil EC and pH and increased soil available N, P and K content after harvest. The treatment of Biofertilization + 75% NPK-RD was superior to other treatments.
Key words: Bio inoculation, NPK fertilization, faba bean, saline soil.

Introduction

The grain legume (pulse) faba bean (*Vicia faba* L.) is 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 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 grain legumes. As a consequence the N benefit for following crops is often high, and 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. 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[1].

Soil salinity is known to cause considerable yield losses in most crops, thereby leading to reduced crop productivity [2]. 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 [3,4]**.** Scientists have been varying for the last many decades to overcome the problem of salinity by employing a variety of strategies. Of the various strategies currently under exploitation, improvement in salinity tolerance of crops through exogenous application of different types of organic and biological fertilizers which help in inhibiting of the adverse effect of salinity [5]**.**

Nitrogen (N) is one of the major nutrients for various plants, especially wheat and other cereals for the producing the economic yields. Its essential role may be attributed to one or all of these reasons: 1) N is constituent of all proteins and nucleic acids and hence of all protoplasm [6], 2) N enhanced the meristematic activities consequently, increasing the cell size that manifested in internodes elongation,[7] and 3) N increases the nutrients uptake, capacity of photosynthesis assimilation in building metabolites, its translocation and accumulation in the sink[8].

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 [9]**.**

Potassium (K) is one of the principle plant nutrients and plays a vital role as macronutrient in plant growth and sustainable crop production, usually absorbed by plants in larger quantities [10,11]**.** 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[12]**.**

Biofertilizers are products containing living microorganisms, which have an ability to convert nutrients from unavailable to available forms for plants by biologic processes [13]**.** 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 [14]**.** 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 [15]**.** 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 [16,17]**.**

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: In order to study the possibility of substituting, partially the amount required of integrated mineral fertilizer (NPK) for fertilization of faba bean plants and its effect on yield, yield quality and some macronutrient contents. Two field experiments in randomized complete block design with three replicates were conducted at private farm in Al-Qantra Sharq, Isamilia Governorate, Egypt during the successive winter seasons of 2014/2015 and 2015/2016.

Soil: A representative soil sample (0 – 30 cm) was taken before planting to determine some physical and chemical properties according to [18,19] and results are shown in Table (1).

Bacterial inoculants: The biofertilizers were inocula 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 produced by the Egyptian Ministry of Agriculture and are inocula 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 .

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^+**	59.53
Sand %	83.58	**K^+**	0.96
Textural class	**Loamy Sand**	**Ca^{++}**	8.94
EC (dSm^{-1}) in soil paste extract	8.37	**Mg^{++}**	14.27
		Cl^-	47.20
pH [Soil suspension 1:2.5]	8.06	**HCO_3^-**	7.58
Organic matter (g kg^{-1})	5.50	**$SO_4^{=}$**	28.92
$CaCO_3$ (g kg^{-1})	10.11	**CO_3^{--}**	nil

Available macro and micronutrients (mg kg^{-1} soil)

N	P	K	Fe	Mn	Zn	
38.98	3.69	189	2.55	1.20	0.66	

Critical limits of nutrients in soil (mg kg^{-1} soil)*

	N	P	K	Fe	Mn	Zn
Low	< 40.0	< 5.0	< 85.0	< 4.0	< 2.0	< 1.0
Medium	40 -80	5 -10	85 - 170	4 - 6	2 - 5	1 - 2
High	> 80.0	> 10.0	> 170	> 6.0	> 5.0	> 2.0

* After Page *et al.* (1982)

(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.

Design and Treatment: The experimental design was a randomized complete block with three replicates. The area of each plot was 50 m^2 (5 X 10 m) and included 20 rows 50 cm apart, two plants per hill and 25 cm between hills. Faba bean (*Vicia faba* L., cv. Masr 3) seeds as the test crop were supplied from Food Legumes Department, Field Crop Research Institute, Agriculture Research Center, Giza, Egypt**.** The transplants were set up into the field on November 22th and 25th in the two successive seasons 2014/2015 and 2015/2016, 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; o 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), while, the second group was mixed with suitable amount of Arabic gum solution 15%, as adhesive material, 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. Phosphorus (P) fertilizer was added to all plots before sowing at a rate of 100 kg fed.$^{-1}$ as superphosphate (15% P_2O_5). Potassium sulphate (40% K_2O) was applied in two equal doses after 21 and 45 days from sowing. Other standard agricultural practices for growing faba bean were carried out as recommended by the Ministry of Agriculture. The experiment included two factors as follows:

1. NPK addition rate, (AR):

(a) No fertilizer; (b) ½ RD; (c) ¾ RD and (D) Full RD.

2. Bio inoculation (B)

(a) Without and (b) with

Therefore there were 8 treatments which represent, 4 (AR) X 2 (bio).

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. Protein content (%) = N content (%) X 6.25
5. Protein Yield (kg fed.$^{-1}$) = Protein content (%) X seed yield (Mg fed.$^{-1}$) X 10
6. Seed nutrient uptake (kg fed.$^{-1}$)=nutrient content (%) X seed yield (Mg fed.$^{-1}$) X 10

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 [20,21]**.** Crude protein in faba bean seeds was calculated by multiplying total N-content by the converting factor 6.25 [22]**.**

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 [18]**.**

Statistical analysis: Results were statistically analyzed using COSTAT 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 significance of the difference between individual means [23]**.**

Results and Discussion

Soil pH and Soil Salinity (EC_e)

Concerning the effect of the treatments on soil pH, in the rhizosphere of grown faba bean, data in Table 2 reveal that all treatments receiving NPK fertilizer with or without biofertilization showed a slight decrease effect on soil pH from 8.04 to 7.99 due to bio inoculation in combination with integrated NPK fertilizer addition and 8.05 to 8.02 due to integrated NPK fertilizer addition without bio inoculation. [24] 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 and active microorganisms released from the addition of bio-fertilizer. Because nitrification of ammonium is an acid forming reaction, the net effect will be a lowered pH. Also, the positive relationship between soil and bio-fertilizers in reduces the hazards of soil salinity and enriches nutrients in soil [25]**.** These results are similar to those obtained by [26,27]**.** These results are in agreement with those obtained by [28,29]**.**

The lowest pH value 7.99 was achieved due to the treatment bio + full (RD) of NPK. As for soluble salts data show that the values were decreased due to the addition treatments. This trend can be due to *Rhizobium* producing phyto-hormones such as indole acetic acid, cytokinines and organic acid which had an effect that 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 [30,31]**.** 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 [32]**.**

The lowest EC value (6.75 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: control > (½ RD) ≥ (¾ RD) > full (RD) as for NPK fertilizer rates and the order was: without bio > with bio as for bio inoculation effect. These results are in agreement with those obtained by [33] who reported that soluble salts decreased when bio-fertilizers were applied alone or in combination with N-fertilizer.

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

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
No	8.04	8.05	7.83	8.82	**8.33a**	45.58	41.83	**43.71c**	3.86	3.76	**3.81d**	200	195	**198c**
½ (RD)	8.02	8.03	7.25	8.23	**7.74b**	50.29	44.21	**47.25**	3.98	3.81	**3.90c**	208	203	**206b**
¾ (RD)	8.00	8.02	7.05	8.02	**7.53b**	53.82	45.18	**49.50**	4.04	3.87	**3.96b**	212	206	**209b**
Full (RD)	7.99	8.02	6.75	7.67	**7.21c**	56.73	48.40	**52.57**	4.08	3.97	**4.03a**	217	211	**214a**
Grand	**--**	**--**	**7.22**	**8.18**		**51.61**	**44.91**		**3.99**	**3.85**		**209a**	**204b**	
F-test			R: ** B: **			R: ** B: **			R: ** B: **			R: ** B: **		
			RxB: NS			RxB: NS			RxB: NS			RxB: 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.$^{-1}$).

NS, not significant; the values followed by different letters are significantly different at $P\leq 0.05$.
*,** Significant at P< 0.05 and 0.01, respectively.

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 ameliorative effect in each of the studied soil content of some available macronutrients (*i.e.,* N, P and K mg/kg) as shown in Table 2 . The data showed that a progressive significant increases in all the studied available macronutrients upon treating the soil with bio-fertilizer in combination with NPK-mineral 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 [34], beside the beneficial effects of bacteria on reducing soil pH by secreting organic acids (*e.g.,* acetic, propionic, fumaric and succinic) which leading to change 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 [35]**.** The present results are in agreement with those of [36] 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. Available N ranged between 41.83 to 48.40 mg kg^{-1} without bio addition and 45.58 to 56.73 mg kg^{-1} for bio addition in combination with different NPK-mineral rates. Available P ranged between 3.76 to 3.97 mg kg^{-1} without bio addition and 3.86 to 4.08 mg kg^{-1} for bio addition in combination with different NPK-mineral rates. Available K ranged between 195 to 211 mg kg^{-1} without bio addition and 200 to 217 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 dose could be arranged according to their effects in the following descending order: full (RD) > (¾ RD) > ½ (RD) > no fertilizer for available P content and full (RD) > (¾ RD) ≥ ½ (RD) > no fertilizer for available N and K contents. As for bio addition, the order was: bio > without bio for available N, P and K contents.

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 significant. Highest 100-seed weight of faba bean plants was obtained when plant treated with full (RD) of NPK-mineral fertilizer in combination with bio inoculation. As for NPK rate effect, the order followed the descending order: full (RD) > (¾ RD) > ½ (RD) > no fertilizer. Regarding the response to bio fertilization, the order was as follow: bio > without bio.

The highest value (89.90 g) was obtained due to the treatment full (RD) NPK + bio which increased by 35.12% compared with the plants which did not receive fertilizers.

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

Rate of NPK (R)	100-seed weight (g)			Pod yield (Mg fed.$^{-1}$)			Seed yield (Mg fed.$^{-1}$)		
	Bio inoculation (B)								
	With	Without	Mean	With	Without	Mean	With	Without	Mean
No fertilizer	75.39	66.46	**70.93d**	0.591	0.492	**0.542c**	0.330	0.290	**0.310d**
½ (RD)	80.49	70.67	**75.58c**	0.939	0.726	**0.843b**	0.817	0.588	**0.703c**
¾ (RD)	84.15	73.03	**78.59b**	1.156	0.851	**1.004a**	0.912	0.771	**0.842a**
Full (RD)	89.80	77.86	**83.83a**	1.098	0.894	**0.996a**	0.899	0.700	**0.799b**
Grand mean	**82.46a**	**72.01b**		**0.946a**	**0.741b**		**0.740a**	**0.587b**	
F-test	R: ** B: **			R: ** B: **			R: ** B: **		
	RxB: NS			RxB: **			RxB: **		

Pod and Seed Yields

According 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. 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 [37]. When investigating the role of phosphorus and potassium in plants, [38]concluded that phosphorus is part of the molecular structure of several vitally important compounds, notably nucleic acids. In addition, phosphorus plays an indispensable role in photosynthesis and respiration and is also essential for cell division and for the development of meristem tissues. Also, K is essential for enzyme energy, seed formation, seed quality, stress tolerance and crop maturity.

Growth promoting substances (phytohormones) which would be produced by these organisms play a key role in plant growth and promote seed germination and root elongation. Root development and proliferation of plants in response to biofertilizer activities enhance water and nutrients uptake [39,40]**.** 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 [41].These results agree with those obtained by [42,33,43]**.**

With respect to the statistical analysis, a descending order characterized the effects of NPK addition rates on pod and seed yields as follows: (¾ RD) ≥ full (RD) > ½ (RD) > no fertilizer for pod yield and (¾ RD) > full (RD) > ½ (RD) > no fertilizer for seed yield, respectively. As for the main effect of bio inoculation; the order was: with bio > without bio, respectively and was found true for pod and seed yield. Data also show that, the treatment of Biofertilization + (¾ RD) of NPK-mineral fertilizer was superior to the other treatments. The increases over the control treatment for pod yield were 95.60%, 85.79% and 58.88% as well as 176%, 172% and 148% for seed yield due to NPK–mineral fertilizer rates (¾ RD), full (RD) and ½ (RD), respectively under bio inoculation. These results are well supported by the findings of [35,44] . These results were also in agreement with [45] who reported that rhizobia and microorganisms naturally produce auxins, cytokinins, absicic acids, rhiboflavin, lipo-chito-oligo saccharides and vitamins. These molecules promote cell division and cell elongation which could induce plant growth.

Concerning the interaction effect between nitrogen fertilizer rates and bio inoculation on faba bean yield and its components, data presented in Table 3 reveal that, the interaction gave significant values of all parameters. Except for 100-seed weight, the interaction was insignificant.

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. Increasing under salinity stress than the inoculated plants with bioferilizers and high rate of NPK fertilizer might be caused by the induction or activation of proline syntheses from glutamate or decrease in its utilization in protein syntheses or enhancement in protein turnover. Thus, proline may be the major source of energy and nitrogen during immediate post stress metabolism and accumulated proline apparently supplies energy for growth and survival, thereby inducing salinity tolerance [46]**.**

The treatments of control without NPK fertilizer or bio inoculation increased proline content and gave the highest value (234.7 □ g g^{-1} dwt) in the descending order of no fertilizer > ½ (RD) > (¾ RD) > full (RD).

Table 4. proline (µg g^{-1} dwt), protein content (%) and protein yield (kg fed.$^{-1}$) of faba bean as affected by bio and NPK– fertilizers

Rate of NPK (R)	Proline (µg g^{-1} dwt)			Protein content (%)			Protein yield (kg fed.$^{-1}$)		
	Bio inoculation (B)								
	With	Without	Mean	With	Without	Mean	With	Without	Mean
No fertilizer	221.7	234.7	**228.2**	19.81	17.42	**18.62**	65.37	50.52	**57.95d**
½ (RD)	179.0	191.3	**185.2**	20.56	18.31	**19.44**	168.0	107.7	**137.8c**
¾ (RD)	153.3	183.0	**168.2**	21.27	18.53	**19.90**	194.0	142.9	**168.4a**
Full (RD)	109.0	166.7	**137.8**	21.17	18.92	**20.04**	190.3	132.4	**161.4b**
Grand mean	**165.8**	**193.9**		**20.70**	**18.29**		**154.4**	**108.4**	
F-test	R: ** B: **			R: ** B: **			R: ** B: **		
	RxB: **			RxB: NS			RxB: **		

Seed protein content and protein yield

Data pertained the effect of studied factors on protein content are presented in Table 4. With respect to amendments, data showed that significant differences could be detected within treatments using NPK-mineral fertilizer and bio inoculation on protein content and protein yield. Concerning the effect of NPK-fertilization rates, the results revealed no significant differences among the ¾ (RD) and full (RD) addition rates for protein content, wherein ¾ (RD) achieved the greatest protein yield. [42] pointed out that protein content in wheat grain increased with high rates of mineral N fertilizer up to 100 kg N fed.$^{-1}$. [47] reported 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 [48]. The increase in protein content could be attributed to the integrated effect of NPK fertilizer plus bio effect of microorganisms such as N_2 fixing bacteria (salt tolerant PGPR strains of N_2-fixer bacteria) and phosphorus dissolving bacteria on increasing the available contents of nutrients as a storehouse for plant growth against the adverseable conditions as well as maximizing the biological yield and grain quality of faba bean [35]. Also, exudation of plant growth regulators such as auxins, gibberellin and microorganisms [13] contribute to such positive effect.

The maximum value of protein content (21.27%) was recorded in the plants treated with ¾ (RD) of NPK- mineral fertilizer + bio fertilization which recorded 22.1% increase over the control treatment (without NPK fertilizer or bio addition). The highest value of protein yield (192 kg fed.$^{-1}$) was also obtained due to ¾ (RD) of NPK- mineral fertilizer + bio fertilization causing 284% increase over the control treatment (without NPK fertilizer or bio addition).

The interaction between NPK fertilizer rates and biofertilization treatments had no significant effect on protein content while showed significant impact on protein yield. [49] found that bio-mineral fertilization was more effective in increasing protein content of peanut plants as compared with the individual mineral fertilization. These results are in agreement with those obtained by [50,51].

Macronutrient content and uptake

It can be seen from results presented in Tables 5 and 6 that the 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. The differences among the treatments were significant for P and K contents while there was no significant difference between ¾ (RD) and ½ (RD) treatments for N-content. Also, the treatment consisting of full (RD) of NPK fertilizer + bio was superior for increasing the contents of N, P and K as compared to the other treatments.

Table 5. N, P and K content (%) of faba bean seeds as affected by bio and NPK– fertilizers

Rate of NPK (R)	N-content			P-content			K-content		
	Bio inoculation (B)								
	With	Without	Mean	With	Without	Mean	With	Without	Mean
No fertilizer	3.17	2.79	**2.98c**	0.48	0.42	**0.45d**	2.51	2.36	**2.43d**
½ (RD)	3.29	2.93	**3.11b**	0.52	0.44	**0.48c**	2.57	2.41	**2.49c**
¾ (RD)	3.35	2.96	**3.16b**	0.56	0.48	**0.52b**	2.63	2.50	**2.56b**
Full (RD)	3.39	3.03	**3.21a**	0.60	0.52	**0.56a**	2.69	2.54	**2.61a**
Grand mean	**3.30a**	**2.93b**		**0.54a**	**0.46b**		**2.60a**	**2.45b**	
F-test	R: ** B: **			R: ** B: **			R: ** B: **		
	RxB: NS			RxB: NS			RxB: **		

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 2) through secreting chelating substances (such as organic acids) which are important for solubilizing sparingly soluble inorganic compounds to more available forms for plants uptake [39,52]. 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 protein production, NO_3- uptake and transport within the plant and amino acid transport [53].

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, involve in various metabolic processes which have direct impact on vegetative and reproductive phases of plants These results coincide with the results of [54,47,55,56].

Table 6. N, P and K uptake (kg fed.$^{-1}$) of faba bean seeds as affected by bio and NPK– fertilizers

Rate of NPK (R)	N-uptake			P-uptake			K-uptake		
	Bio inoculation (B)								
	With	Without	Mean	With	Without	Mean	With	Without	Mean
No fertilizer	10.46	8.09	**9.28d**	1.58	1.22	**1.40c**	8.28	6.83	**7.55d**
½ (RD)	26.88	17.23	**22.06c**	4.25	2.59	**3.42b**	21.00	14.17	**17.58c**
¾ (RD)	30.55	22.82	**26.69a**	5.11	3.70	**4.40a**	23.99	19.28	**21.63a**
Full (RD)	30.48	21.21	**25.84b**	5.39	3.64	**4.52a**	24.18	17.78	**20.98b**
Grand mean	**24.59a**	**17.34b**		**4.08a**	**2.79b**		**19.36a**	**14.52b**	
F-test	R: ** B: **			R: ** B: **			R: ** B: **		
	RxB: **			RxB: **			RxB: **		

The main effect of NPK fertilizer rate treatments showed a descending increase in the order: full (RD) > (¾ RD) > ½ (RD) > no fertilizer for seed P and K contents and full (RD) > (¾ RD) ≥ ½ (RD) > no fertilizer for N content.

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

As for N, P and K uptake by faba bean seeds, the main effect of NPK fertilizer rate can be arranged in the following descending order: (¾ RD) > full (RD) > ½ (RD) > no fertilizer for N and K uptake as well as full (RD) ≥ (¾ RD) > ½ (RD) > no fertilizer for P uptake. The highest N and P uptake (30.55 and 5.11 kg fed.$^{-1}$), respectively were obtained due to (¾ RD) of NPK fertilizer + bio giving increases of 278% and 319% over the control (un treated plants) while, the K uptake of plants treated with full (RD) of NPK fertilizer + bio was the highest and the value was (24.18 kg fed.$^{-1}$) representing 254%increase over the control (un treated plants).

Conclusion

Efficient plant nutrition should ensure both enhanced and sustainable agricultural production and safeguard the environment. Chemical and microbial fertilizer has its advantages and disadvantages in terms of nutrient supply, soil quality and crop growth. As well, biological fertilization with N_2 fixing bacteria, phosphorus solubilizing bacteria and potassium dissolving bacteria are of great importance in increasing crop production and saving mineral fertilizers. Moreover, inoculation of plants grown in salt affected soils with Salt-tolerant microorganisms offered them tolerance against salinity, thereby increased their production. 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.

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 [57]. It could be recommended that salt tolerant plant growth promoting rhizobacteria (PGPR) should be used to face the problem of salinity or excessive NPK-mineral use for the faba bean plants.

References

1. Erik, S. J., Mark, B. P. and Henrik, H.N. (2010). Faba Beans in Sustainable Agriculture. A review, Faba bean in cropping systems. Field Crops Research. 115 (3):203–216.
2. Chaum S., Pokasombat, Y. and Kirdmanee, C. (2011). Remediation of salt-affected soil by gypsum and farmyard manure − Importance for the production of Jasmine rice. Aust. J Crop Sci., 5: 458-465.
3. Jamil A., Riaz, S., Ashraf, M. and Foolad, M.R. (2011). Gene expression profiling of plants under salt stress. Crit. Rev. Plant Sci., 30: 435-458.
4. Krasensky, J. and Jonak, C. (2012). Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. J. Exp. Bot., 63: 1593-1608.
5. Ehteshami, S.M.R., Aghaalikhani, M., Khavazi, K. and Chaichi, M.R. (2007). Effect of phosphate solubilizing microorganisms on quantitative and qualitative characteristics of maize (Zea mays L.) under water deficit stress. Pakistan J. Biological Sci., 10(20): 3585-3591.
6. Russel, E. W. (1973). Soil conditions and plant growth.10 th Ed., Longman Group Ltd., London.
7. Osman, A.S., El- Shahat, R.M. and Seyam, H.M. (2000). Response of wheat to fertilization treatments of nitrogen, azolla and micro-nutrients. Fayoum J. Agric. Res. and Dev., 14 (2): 68 – 74.
8. Fathi, A. I., Ismail,B.A., Eisa, S.A.I. and Easa, M.O. (2003). Effect of nitrogen, sulphur and boron fertilization on growth, yield and quality of canola. Egypt. J. Soil Sci., 18(8): 317 – 329.
9. Abou El-Yazeid, A. and Abou-Aly, H.E. (2011). Enhancing growth, productivity and quality of tomato plants using phosphate solubilizing microogranisms. Australian J. Basic and Appl. Sci., 5(7): 371 – 379.
10. Pettigrew, W.T. (2008). Potassium influences on yield and quality production for maize, wheat, soybean and cotton. Physiolgia Plantarum, 133: 670-681.
11. Bukhsh, M.A.A., Ahmed, R., Ishaque, M. and Malik, U. (2009). Response of maize hybrids to varing potassium application in Pakistan. Paki. J. Agric. Sci., 46(3): 179–184.
12. Bukhsh, M.A.A., Ahmed, R., Malik, A.U., Hussain, S. and Ishaque, M. (2010). Agrophysiological traits of three maize hybrids as influenced by varying potassium application. Life Sci. Int. J., 4(2): 1487–1496.
13. Vessey, J.K. (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil. 255:571–586.
14. Fares, C.N. and Khalil, M.F. (2003). Effect of biofertilizers and mycorrhizal fungi on nutrient uptake and growth of soybean and maize plants cultivated individual or intercropping system. Egypt. J. Appl. Sci., 18(5B): 774 – 788.
15. Wu, S., Cao, Z., Li, Z., Cheung, K. and Wong, M. (2005). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125: 155- 166.
16. El-Habbasha, S.F., Hozayn, M. and Khalafallah, M.A. (2007). Integration effect between phosphorus levels and bio-fertilizers on quality and quantity yield of faba bean (*vicia faba l.*) in newly cultivated sandy soils. Research Journal of Agri. and Biol. Sci., 3(6): 966-971.
17. Salimpour, S., Khavazi K, Nadian, H., Besharati, H. and Miransari, M. (2010). Enhancing phosphorous availability to canola (Brassica napus L.) using P solubilizing and sulfur oxidizing bacteria. Australian Journal of Crop Science 4(5): 330-33.
18. Page, A.L., Miller, R.H. and Keeney, D.R. (1982). "Methods of Chemical Analysis". Part 2: Chemical and microbiological properties (Second Edition). American Society of Agronomy, Inc. and Sci. Soc. of America, Inc. Publishers, Madison, Wisconsin U.S.A.
19. Klute, A. (1986) "Methods of Analysis". Part 1, Soil Physical Properties. ASA and SSSA, Madison, WI
20. Chapman, H.D. and Pratt, P.F. (1961). "Methods of Analysis for Soils, Plants and Water". Agric. Publ. Univ., of California, Riverside.
21. Jackson, M.L.(1973). Soil Chemical Analysis. Prentice Hall India.
22. Hymowitz, T.F., Collins, P. and Walker, W.M. (1972). Relationship between the content of oil, protein and sugar in soybean seed. Agron. J., 64: 613-616.
23. Gomez, K.A. and Gomez, A.A. (1984). Statistical Procedures for Agricultural Research. John Willey and Sons, New York, USA.
24. Nasef, M.A., Shaban, Kh.A. and Abdel Hameed, A.F. (2009). Effect of compost and bio-fertilizer application on some chemical soil properties and rice productivity under saline soil condition. J. Agric. Mansoura Univ., 34 (4): 2609- 2623.

25. Rashed, S.H. (2006). Effect of bio and organic fertilization on maize (*Zea mays*). MSc. Thesis, Fac. Agric., Mansoura Univ., Egypt.
26. Abdel Lattif, M.K. (2007). Improving the efficiency of potassium fertilization in some Egyptian soils. Ph.D. Thesis, Fac. Agric., Al-Azhar Univ., Cairo, Egypt.
27. Poraas, M.M.E., Sallam, A.M., Shaban, Kh.A. and Abou El-Defan, T.A. (2009). Using of some soil amendments and N_2-fixer bacteria in improving the quality and yield of wheat grains as well as some characteristics of Sahl El-Tina saline soil (North-Sinai). Egypt. J. Soil Sci., 49 (2): 255 – 270.
28. Shaban, Kh.A., Eman, R. Hamed and Heba, S. Shehata (2012). Soil amendments and their efficacy on soil properties and productivity of barley and faba bean plants under newly reclaimed soil conditions. Egypt. J. Soil Sci., 52(3): 349 – 365.
29. Helmy, A.M., Abdel Fattah, M.K. and Shaban, Kh.A. (2013a). Nitrogen efficiency in barley under saline-sodic conditions as affected by urea, compost and biofertilizer (*Rhizobium radiobacter* sp.). J. Soil Sci. and Agric. Eng., Mansoura Univ., 4(11): 1169 – 1189.
30. Shaban, Kh.A. and Omar, M.N.A. (2006). Improvement of maize yield and some soil properties by using nitrogen mineral and PGPR group fertilization in newly cultivated saline soils. Egypt. J. Soil Sci., 46(3): 329 – 342.
31. Ashmaye, S.H., Shaban, Kh.A. and Abd El-Hader, M.G. (2008). Effect of mineral nitrogen, sulphur, organic and bio-fertilizers on maize productivity in saline soil of Sahl El-Tina. Minufiya J. Agric. Res., 33(1): 195 – 209.
32. Zaka, M.A., Obaid, U.R., Rafa, H.U. and Khan, A.A. (2005). Integrated approach for reclamation of salt affected soils. J. Agric. and Soc. Sci., 1 (2): 94–97.
33. Helmy, A.M., Shaban, Kh.A. and Mona G. Abdel Kader, (2013b). *Rhizobium* biofertilization with or without mineral-N fertilization for a new cowpea cultivar (Kafr El-Sheikh 1) grown under saline conditions. Zagazig J. Agric. Res., 40 (5) 891 – 906.
34. Gomaa, A.O. and Abou-Aly, H.E. (2001). Efficiency of bioferitlization in the presence of both inorganic and organic fertilizers on growth, yield and chemical constituents of anise plant (*Pimpinella anisum* L.). *Proc. of the fifth Arabian Hort. Conf.*, Ismailia, Egypt, March 24-28, pp. 73-80.
35. Ewees, M.S.A. and Abdel Hafeez, A.A.A. (2010). Response of maize grain yield to a partial substitution of N-mineral by applying organic manure, bio-inoculation and elemental sulphur as an alternative strategy to avoid the possible chemical pollution. Egypt J. Soil Sci., 50(1): 141 – 166.
36. Ewees, M. S.A. and A. Sh. Osman (2013). Partial Substitution of Mineral Nitrogen Fertilizers by Bio-Fertilizer to Alleviate the Possible Risks of Chemical Pollution for Broccoli Plants. Egypt. J. Soil Sci., 53(2): 175 – 194.
37. Wortman, S.E., Davis, A.S., Schutte, B.J. and Lindquist, J.L. (2011). Integrating management of soil nitrogen and weeds. Weed Sci., 59: 162 170.
38. El-Kabbany, E.A.Y., Darwish, A.A. (2002). Evaluating the performance of biofertilizer cerealin on wheat under different levels of nitrogen and phosphorus fertilization. Egypt. J. Appl. Sci., 17, 390–402.
39. Kandil, A.A., El-Hindi, M.H., Badawi, M.A., El Morarsy, S.A. and Kalboush, F. A.H. (2011). Response of wheat to rates of nitrogen, biofertilizers and land leveling. Crop and Enviro., 2(1): 46–51.
40. Joshi, R.A., Prasanna, R., Shivay, Y.S. and Nain, L. (2012). Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. Euro. J. Soil Biology, 50: 118 – 126.
41. Giller, K.E. (2001). Nitrogen fixation in tropical cropping systems. K.E .Giller. 2nd Ed., 323-335.
42. Siam, H.S., Shaban, Kh.A. And Safaa, A.M. (2013). Evaluation of applying different mineral nitrogen sources on soil fertility and wheat productivity under saline soil conditions. J. Applied. Sci. Res., 9(4): 3146 – 3156.
43. Piccinin, G.G., Braccini, A.L., Dan, L.G.M., Scapim, C.A., Ricci, T.T. and Bazo, G.L. (2013). Efficiency of seed inoculation with *Azospirillum brasilense* on agronomic characteristics and yield of wheat. Industrial Crops and Products, 43:393–397.
44. Otieno, P.E., Muthomi, J.W., Chemining, G.N. and Nderitu, J.H. (2007). Effect of rhizobia inoculation, farmyard manure and nitrogen fertilizer on growth, nodulation and yield of selected food grain legumes. African Crop Sci. Conference Proceedings, 8: 305 – 312.
45. Matiru, V.N. and Dakora, F.D. (2004). Potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. Afr. J. Biotech., 3(1): 1-7.
46. Gad, N. (2005). Interactive effect of salinity and cobalt on tomato plants. II. Some physiological parameters as affected by cobalt and salinity. Res. J. Agric. Sci., 1: 270-276.

47. Namvar, A. and Teymur, K. (2013). Response of wheat to mineral nitrogen fertilizer and biofertilizer (*Azotobacter* sp. and *Azospirillium* sp.) inoculation under different levels of weed interference. Ekologija J. 59(2): 85 – 94.
48. Rana, A., Joshi, M., Prasanna, R., Shivay, Y.S. and Nain, L. (2012). Biofertification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. Euro. J. Soil Biology, 50: 118–126.
49. Mabrouk, S.S. (2002). Response of some peanut cultivars to bio and organo-mineral fertilization. Zagazig J. Agric. Res., 29(6): 2071 – 2083.
50. Hussein, M.M.M. (2007). Response of some flax genotypes to bio and nitrogen fertilization. Zagazig J. Agric. Res., 34 (5): 815 – 844.
51. Omran, S.E.H., Mohamed, E.A.I. and El-Gpaly, A.H. (2009). Influence of organic and biofertilization on productivity, viability and chemical components of Flax seeds. Egypt. J. Soil Sci., 49 (1): 49 – 64
52. Daneshmand, N.G., Bakhshandeh, A. and Rostami, M.R. (2012). Biofertilizer affects yield and yield components of wheat. Inter. J. Agric., 2(6): 699–704.
53. William, T.P. (2008). Potassium influences on yield and quality production for maize, wheat, soybean and cotton. Physiologia Plantarum 133: 670 – 681.
54. Abbas, M.H.H., Ahmed, O.A.I., Manal, A.H. El-Gamal and Haythum, M.S. (2011). Integrated effect of mineral nitrogen, bio and organic fertilization on soybean productivity. Egypt. J. Biotech., 39: 43 – 63.
55. Osman E.A.M., EL- Masry, A.A. and Khatab, K.A. (2013). Effect of nitrogen fertilizer sources and foliar spray of humic and/or fulvic acids on yield and quality of rice plants. Advances in Applied Sci. Res., 4(4):174-183.
56. Diacono, M., Rubino, P. and Montemurro, F. (2013). Precision nitrogen management of wheat; a review. Agronomy for Sustainable Development. 33(1): 219 – 241.
57. Amara, M.A.T. (2001). Impact of foliar applica-tion with Molybdenum and biofertilization on the growth and yield of Soybean plants in sandy soil. Desert Inst. Bull. Egypt, 51(1): 111 – 127.

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