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Effect of Bio – NP Fertilizer and Different Doses of Mineral N and P Fertilizers on Growth, Yield Productivity and some Biochemical Constituents of Wheat, Faba bean and Onion Plants

[1]Salim, B. B. M. and [2]A. Abou El-Yazied

[1]*Agric. Botany Dept., Fac. Agric., Ain Shams Univ., Shoubra El-Kheima, Cairo, Egypt*
[2]*Hort. Department, Fac., Agric., Ain Shams Univ, Shoubra El-Kheima Cairo, Egypt*

ABSTRACT

Field experiments were conducted at private farm, Qalyubia governorate, Egypt during two of successive winter seasons 2011/2012 in clay loam soil, six treatments linked with each crop were applied: 100% recommended dose of N and P fertilizer (control), recommended dose of N and P fertilizer + 300 kg Bio N- P fertilizer, 75% of recommended dose N and P + 300 kg Bio N- P fertilizer, 50% of recommended dose N and P + 300 kg Bio N- P fertilizer, 25% of recommended dose N and P + 300 kg Bio N-P fertilizer and 300 kg Bio N-P fertilizer alone. Results revealed that using 50% of recommended dose N and P fertilizer plus 300 kg Bio N- P fertilizer gave the highest values of plant height, number of spikes, spikes weight and weight of 1000 grains, grains yield and straw yield of wheat plants as compared to the control and rest treatments. As for faba bean plants, results indicated that the 75% of recommended dose N and P fertilizer plus 300 kg Bio N- P fertilizer produced the highest significant increment in plant height, pods weight, pods number, the weight of 100 seeds, and seeds yield but not significant in number of branches per plant and straw yield of faba bean plant. The highest values of faba bean yield were achieved by treatment of 75% of recommended dose N and P + 300 kg bio-NP. According to onion plant, Application of 100 % recommended dose of N and P fertilizer plus 300 kg Bio N- P fertilizer produced a significant increase in plant height, bulb diameter and bulbs yield per/fed as compared to the control and rest treatments. In general, the highest values of onion growth parameters and bulbs yield were recorded by application of the 100% recommended dose of N and P fertilizer plus 300 kg Bio N- P fertilizer and 75% of recommended dose N and P fertilizer + 300 kg bio-NP treatments as compared to the rest treatments. Using of 100 % recommended dose of N and P fertilizer plus 300 kg Bio N- P fertilizer treatment gave the highest concentrations of N, P and protein in leaves of wheat and faba bean. Same results for N and protein concentration were found in onion plants which received 100 % recommended dose of N and P fertilizer plus 300 kg Bio N- P fertilizer but the highest P concentration showed in onion plants treated with 50% of recommended dose N and P fertilizer plus 300 kg Bio N- P fertilizer.

Key words: Wheat, *Triticum aestivum* L., Faba bean, *Vicia fabae* L., Onion, *Allium cepa*, Biofertilizers, Plant growth promoting rhizobacteria, Bio- NP fertilizer

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crops in Egypt and increasing wheat production is an essential national target to fill the gap between production and consumption (Tawfik *et al.,* 2006). Legumes seeds protein is the natural supplement to cereal grain protein. They also provide fat and carbohydrates. Moreover, legumes are high in bone building minerals and vitamins essential for good health (Porres *et al*., 2003). Faba bean contains a high protein content compared to other legumes amounting to 33% (Elsheikh *et al*., 2000). Onion (*Allium cepa* L.) is one of the most important vegetable crops grown in Egypt, not only for local consumption but also for exportation (Devlin and Witham, 1986).

Maintaining soil fertility and using plant nutrients in sufficient and balanced amounts is one of the key factors to increase crop yield (Diacono *et al*., 2013). Like, nitrogen (N) is the most important nutrient supplied to most non-legume crops, including wheat. The most important role of N in the plant is its presence in the structure of protein and nucleic acids, which are the most important building and information substances of every cell. In addition, N is also found in chlorophyll that enables the plant to transfer energy from sunlight by photosynthesis. Thus, N supply to the plant will influence the amount of protein, amino acids, protoplasm and chlorophyll formed. Moreover, it influences the cell size, leaf area and photosynthetic activity (Namvar *et al*., 2012; Daneshmand *et al*., 2012; Piccinin *et al*., 2013; Diacono *et al*., 2013).

Phosphorus (P) is the second major nutrient for plant growth as it is an integral part of different biochemical like nucleic acids, nucleotides, phospholipids and phosphoproteins. Phosphate compounds act as

Corresponding Author: Salim, B.B.M., Department of Agricultural Botany, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.
E-mail: dr.bahaa_badry@yahoo.com

"energy currency" within plants (Russell, 1981; Tisdale *et al*., 1985). P in plants is deficient in most of our soils, which reduce crop production (Memon, 1996; Wissuwa *et al*., 1998). P is a major nutrient, especially for legumes. It is considered the second essential nutrient element for both plants and microorganisms. The problem of phosphorus addition to soil is the amount of available P for plant is usually low.

So, phosphate dissolving bacteria and soil microorganisms can play an important role in improving plant growth and phosphate uptake efficiency by releasing phosphorus from rock or tri-calcium phosphate. Many researchers showed positive effect of phosphorus fertilization on faba bean Hamed (2003). Soil microbes, are a necessary part of soil ecosystem and can handle the following important functions in the soil (Bohme and Bohme, 2006; Abbas zadeh *et al*., 2010) : 1) recycling soil nutrients available in organic form, 2) enhancing soil nutrient availability and hence uptake by plant, 3) improving soil structure by producing different biochemicals, 4) and alleviating soil stresses on plant growth and yield productivity.

Biofertilizers are inputs containing microorganisms which are capable of mobilizing nutritive elements from non-usable form to usable form through biological processes, they include mainly the nitrogen fixing, phosphate solubilizing and plant growth– promoting microorganisms (Goel *et al*., 1999). Increasing and extending the role of biofertilizers can reduce the need for chemical fertilizers and decrease adverse environmental effects. They can play a significant role in fixing atmospheric N and production of plant growth promoting substances. Therefore, in the development and implementation of sustainable agricultural techniques, biofertilization has great importance role in alleviating environmental pollution and deterioration of nature (Saini *et al*., 2004; Namvar *et al*., 2012; Rana *et al*., 2012). Some of investigations have suggested that integrated nutrient management strategies involving inoculation of grains with *Azotobacter sp*. and *Azospirillum sp*. in combination with chemical fertilizers improving both growth and yield of crops, i. e. wheat (Saini *et al*., 2004; Piccinin *et al*., 2013).

Phosphate solubilizing bacteria have the ability to increase available P for plants through production of organic acids (Mehana and Farag, 2000), The microorganisms involved in P solubilization can enhance plant growth by increasing the efficiency of biological nitrogen fixation, enhancing the availability of other trace elements and by production of plant growth promoting substances (Gyaneshwar *et al*, 2002). Also, phosphate solubilizing microorganisms play a key role in the plant metabolism and crop productivity through increasing P availability and uptake of native soil phosphorus by converting insoluble phosphates to soluble forms by producing various organic acids (Raja *et al*., 2002).

Bio- NP fertilizer gave a significant increment in yield and yield components of sesame plants Bio-NP ensures better nitrogen consumption, which is essential to plant growth. The *Azospirillum* bacteria and "tikbaw" converts the airborne nitrogen into ammonia. Ammonia penetrates to the root zone and make the necessary 50% nitrogen needs of the plant available for root consumption. Also, bio-NP changes unavailable $Ca_3\ (PO_4)_2$ to available form in the soil through the activity microorganism (Ahmed *et al*., 2015). In china, Guo and Guo (2000) found that application of G-typed bio-fertilizer (GBF, which contain a large amounts of bacteria) could reduce the need for chemical fertilizers and improving yield.

The purpose of this research was to study the efficiency of Bio- NP fertilizer alone or in combination with different amounts of mineral nitrogen and phosphorus fertilizers on enhancing growth, yield and some biochemical constituents in wheat, Faba bean and onion plants, these plants are selected due to their variation for using nitrogen and phosphorus.

Material and Methods

Cultivation

Two field experiments in private farm at Qalyubia governorate were carried out during the two successive winter seasons 2011 and 2012 to study the effect of bio-NP fertilizer treatments on growth, yield components and some biochemical constituents of wheat, faba bean and onion plants.

Wheat cultivar sakha 93 kindly obtained from Agricultural Research Center, Ministry of Agric., Dokki, Giza, Egypt, wheat grains were sown at 1st November in both seasons. The experiment was arranged in complete randomized block design with five replicates was used and plot area was 12 m^2. Wheat grains were sown by hand drilled in rows 20 cm a part at the rate of 60 kg grains fed 1Nitrogen fertilizer was applied as ammonium nitrate (33.5 percent N) into three portions, 20 % before, 40% and 40% with the first and second irrigations plus calcium super phosphate (15.5% P_2O_5) was applied one portion before sowing plus potassium sulphate by rate 50 kg / fed with the first irrigation.

Faba bean cultivar Giza 834 were cultivated at 1st November in both seasons, The experimental unit consisted five ridges four meter in length and 64 cm apart, plot area was 15.4 m^2 The distance between plants was 20 cm. Nitrogen fertilizer was applied as ammonium nitrate (33.5 percent N) into two equal portions, before the first and second irrigations. plus calcium super phosphate (15.5% P_2O_5) was applied one portion before sowing plus potassium sulphate by rate 50 kg / fed with the first irrigation. The experiment was arranged in complete randomized block design with five replicates was used.

Onion seedling cultivar Giza- red were transplanted at 20 November in both seasons, Seedlings were planted on ridges of 58 cm width and 4 m in length at 10 cm apart. Each plot included 5- redges and the plot area was 11.6 m^2. Nitrogen fertilizer was applied as ammonium nitrate (33.5 percent N) into two equal portions, before the first and second irrigations plus calcium super phosphate (15.5% P_2O_5) was applied one portion before cultivation plus potassium sulphate by rate 50 kg /fed with the first irrigation. The experiment was arranged in complete randomized block design with five replicates was used and plot area was.

The physical and chemical properties of the experimental soil site (30 depths) were analyzed in (Table A).

Table A: Mechanical and chemical analysis of the experimental soil

pH	EC ds/m	$CaCO_3$ %	Cation meq / l			Anion meq / l		
			Ca^{++}	Mg^{++}	Na^{+}	HCO_3^-	CL^-	SO_4^{--2}
7.81	1.60	1.50	7.60	2.50	1.78	3.10	3.25	1.54
N	P	K	Sand %		Silte %	Clay %	Soil texture	
(ppm)								
90	85	387	36		44	20	Clay loam	

Central Soil and Plant Analysis Laboratory, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Treatments were as follows related or linked with each cultivated crop in this study

A. 100% recommended dose of N and P fertilizer (control)
B. 100%recommended dose of N and P fertilizer + 300 kg Bio- NP fertilizer (El-MOWAFFER*- BIO^R)
C. 75% recommended dose of N and P fertilizer + 300 Bio- NP fertilizer (El-MOWAFFER*- BIO^R)
D. 50% recommended dose of N and P fertilizer + 300 kg Bio- NP fertilizer (El-MOWAFFER*- BIO^R)
E. 25% recommended dose of N and P fertilizer + 300 kg Bio- NP fertilizer (El-MOWAFFER*- BIO^R)
F. 300 kg Bio-NP fertilizer (El-MOWAFFER*- BIO^R)

Table B: Amount of minerals N and P fertilizers application in different plants (Wheat, Faba bean and Onion) kg/fed.

Mineral N and P % of recommended dose	Ammonium nitrate (33.5 % N)			Calcium super phosphate (15.5 % P_2O_5)		
	Wheat	Faba bean	Onion	Wheat	Faba bean	Onion
100 %	224	100	150	200	150	500
75%	168	75	112.5	150	112.5	375
50 %	112	50	75	100	75	250
25 %	56	25	37.5	50	37.5	125

All additions of mineral fertilizers were as recommended by Ministry of Agriculture and Reclamation, Egypt.

Bio-NP fertilizer treatment was applied in the form of El-MOWAFFER*- BIO^R is a new generation of phosphate – nitrogen biologically active fertilizer, registered in the Egyptian Ministry of Agriculture& Land Reclamation (MALR), under No. 7703/2004 it contains rock phosphate with specialized active strains of microorganisms (Symbiotic/Faculative- Aerobic/ Anaerobic) of nitrogen fixer and phosphate dissolving bacteria (*Azotobacter spp., Bacillus polymyxa* and *Bacillus megatherium var. phosphaticum*), 3g/100 kg Boron active with 200g/100 kg P_2O_5 and 4000g/100 kg Calcium also bio-stimulator), this new sustainable release phosphate-nitrogen ground fertilizer supplies plants with phosphorus, nitrogen. Produced by Union for Agricultural Development company, Egypt (UAD) in one dose before the sowing.

Samples

Wheat samples: wheat plants were harvested at 1st May in both seasons to study some of growth and yield parameters including, plant height, spikes number/m^2 , spikes weights/m^2 , the weight of 1000 grains (g), grains yield per/fed (ton/fed) and straw yield (ton/fed).

Faba bean samples: plants were harvested at 15 April in both seasons to study some of growth and yield parameters including, plant height, no. of branches/row , no. of pods/row , pods weight kg/m^2, the weight of 100 seeds (g), seeds yield per/fed (ton/fed) and straw yield (ton/fed).

Onion plant samples: plants were harvested at 1st May in both seasons to study some of growth and yield parameters including, plant height, bulbs diameter, total soluble solids (T.S.S%), bulbs yield (ton/fed) and straw yield (ton/fed).

Determination of biochemical constituents.

Plants leaves were taken for nutritional studies (including N and P). 0.1 gram samples of ground plant materials were wet digested using (H_2SO_4/H_2O_2) mixture as described by Cottenie (1980). Total nitrogen percentage (N) was determined in the dried leaves according to the method described by A.O.A.C. (1975) and the crude protein concentration was calculated by multiplying total nitrogen concentration by factor of 6.25. Total phosphorus (P) in plant was determined calorimetrically Watanabe and Olsen (1965). Total soluble solids (TSS %) in onion bulbs were determined by using Carl Zies refractometer.

Statistical analysis

The statistical analysis of data was done by SAS (2006). Tukey test for separation between means using the following model $Y_{ij} = \mu + Trt_i + e_{ij}$ at 0.05% probability.

Results

Wheat plant

Data presented in Table (1) shows the effect of Bio- NP (El-MOWAFFER*- BIO[R]) treatments on growth and yield parameters including, plant height, spikes number/m^2, spikes weights/m^2, the weight of 1000 grains (g), grains yield per/fed (ton/fed) and straw yield (ton/fed) of wheat cultivar Sakha 93 cv. in both seasons. These data revealed that plant height was insignificantly effect by all treatments. The highest values of plant height were observed with using 50% recommended dose of N and P fertilizer plus 300 kg Bio- NP. Both of 75% or 100% recommended doses of N and P fertilizer plus 300 kg bio- NP fertilizer (El-MOWAFFER*- BIO[R]) treatments stimulate plant height as compared to the control while, the lowest significant value it resulted from the application of 300 kg bio- NP fertilizer (El-MOWAFFER*- BIO[R]) alone.

The highest values of spikes number/m^2 and spikes weight/m^2 were observed with 50% recommended dose of N and P fertilizer + 300 kg Bio- NP as compared to the control and rest treatments in both seasons but the lowest significant values were observed by 300 kg bio- NP fertilizer treatment .

Application of 50% recommended dose of N and P fertilizer plus 300 kg Bio- NP increased grains yield per/fed (ton/fed) and straw yield (ton/fed) as compared to the control (100 % recommended dose of mineral N and P fertilizer) and using 300 kg El-MOWAFFER*- BIO[R] alone. The highest value of grains and straw yields per/fed (ton/fed) were observed by 50 % recommended dose of N and P fertilizer+ 300 kg El-MOWAFFER*- BIO[R] treatment as compared to the control and the rest treatments in both seasons.

Table 1: Effect of Bio- NP and different doses of mineral N and P fertilizers treatments on wheat growth and yield (combined analyses of 2011and 2012 seasons).

Treatments	Plant height (cm)	Spikes		1000- grain weight (g)	Yield (ton/fed.)	
		Number/m^2	Weight (kg) /m^2		Straw	Grains
100% recommended dose of N+ P	98.50	385.2D	1.26	46.57A	5.51	3.28
100% recommended dose of N + P +300 kg bio-NP fertilizer	99.00	404.6B	1.32	47.24AB	5.51	3.53
75% recommended dose of N+ P +300 kg bio-NP fertilizer	99.00	395.0C	1.27	46.24AB	5.58	3.24
50% recommended dose of N+ P +300 kg bio-NP fertilizer	99.40	421.2A	1.41	48.55A	6.05	3.41
25% recommended dose of N+ P +300 kg bio-NP fertilizer	98.00	376.2E	1.21	45.91AB	5.32	3.14
300 kg bio-NP fertilizer	97.40	355.8F	1.18	45.22B	5.13	3.09
MSD Treatments at 0.5	NS	2.742	NS	2.742	NS	NS

NS= Non Significant

Faba bean plant

Data presented in Table (2) shows the effect of Bio- NP fertilizer (El-MOWAFFER*- BIO[R])treatments on growth and yield parameters including, plant height, branches number, pods number per row, pods weights/row, the weight of 100 seeds (g), seeds yield per/fed (ton/fed) and straw yield (ton/fed) of faba bean plants. The results indicated that the combination between 100 %, 75 % and 50 % of recommended doses of N and P fertilizer plus 300 kg Bio N P fertilizer treatments gave stimulating effect in plant height, pods weight, the weight of 100 seeds and seeds yield (ton/fed) and straw yield (ton/fed) of faba bean plant when compared to other treatments.

The application of 75% recommended dose of N and P fertilizer plus 300 kg bio-NP fertilizer recorded the highest values of plant height, number of branches per plant, pods weight, pods number/row, 100 seeds weight, seeds yield/fed and straw yield/fed of faba bean plants in both seasons as compared to the rest treatments. But the lowest values of all studied growth parameters and yield components of faba bean plants were resulted by using 300 kg bio- NP fertilizer individually in both seasons.

Onion plant

Data presented in Table (3) shows the effect of Bio- NP (El-MOWAFFER*- BIO[R]) treatments on growth and yield parameters including, plant height, bulb diameter, bulbs yield per/fed (ton/fed) and straw yield (ton/fed) onion plants. Application of 100% recommended dose of mineral N and P fertilizer plus 300 kg bio-NP fertilizer treatment recorded the highest values of plant height, bulb diameter and bulbs yield (ton/fed) of onion plants on the other hand the highest values of total soluble solids resulted by 100% recommended dose of N+ P fertilizer +

300 kg bio-NP fertilizer and 75% recommended dose of N+ P fertilizer +300 kg bio-NP fertilizer treatments as compared to the control and rest treatments at harvest.

The lowest values of onion growth parameters and yield components produced by using 300 kg bio-NP fertilizer (El-MOWAFFER*- BIO^{R}) treatment alone without any addition of recommended doses of mineral N and P fertilizers.

Table 2: Effect of Bio- NP and different doses of mineral N and P fertilizers treatments on faba bean growth and yield (combined analyses of 2011and 2012 seasons).

Treatments	Plant height (cm)	Branches No.	Pods		100 seed weight (g)	Yield (ton/fed)	
			No./row	Weight (kg) /row		Straw	Seeds
100% recommended dose of N+ P	122.00AB	3.00	684.00C	1.53C	64.36BC	3.99	1.63B
100% recommended dose of N + P +300 kg bio-NP fertilizer	120.67A-C	3.00	690.33AB	1.66B	65.24A-C	4.23	1.68AB
75% recommended dose of N+ P +300 kg bio-NP fertilizer	123.67A	3.33	694.00A	1.77A	67.11A	4.47	1.81A
50% recommended dose of N+ P +300 kg bio-NP fertilizer	122.00AB	3.00	688.33BC	1.63B	66.19AB	4.10	1.52BC
25% recommended dose of N+ P +300 kg bio-NP fertilizer	118.67BC	3.00	672.0D	1.48D	63.74BC	3.98	1.44C
300 kg bio-NP fertilizer	116.67C	3.00	649.0E	1.47D	62.60C	3.68	1.432C
MSD Treatments at 0.5	4.238	NS	5.089	0.030	2.742	NS	0.175

NS= Non Significant

Table 3: Effect of Bio- NP and different doses of mineral N and P fertilizers treatments on onion growth and yield (combined analyses of 2011and 2012 seasons).

Treatments	Plant height (cm)	Diameter (cm)	TSS %	Yield (ton/fed)	
				Straw	Bulbs
100% recommended dose of N+ P	76.20A-C	95.60BC	13.20	1.00C	16.20AB
100% recommended dose of N + P +300 kg bio-NP fertilizer	78.30A	104.3A	13.45	1.13B	18.77A
75% recommended dose of N+ P +300 kg bio-NP fertilizer	77.40AB	98.20B	13.45	1.35A	16.66AB
50% recommended dose of N+ P +300 kg bio-NP fertilizer	77.00AB	96.40BC	13.30	1.03C	16.28AB
25% recommended dose of N+ P +300 kg bio-NP fertilizer	75.50BC	95.10C	12.80	1.00C	15.97B
300 kg bio-NP fertilizer	73.50C	92.00D	12.80	0.99C	14.26B
MSD Treatments at 0.5	2.742	2.7425	NS	0.0451	2.705

NS= Non Significant

Data in Table (4) showed that, using 100% recommended dose of mineral N and P fertilizer + 300 kg bio-NP fertilizer treatment led to increase P concentration in leaves of wheat and faba bean but resulted by 50 % recommended dose of mineral N and P fertilizer + 300 kg bio-NP fertilizer in onion plants when compared to control plants and other treatments.

Table 4: Effect of Bio- NP and different doses of mineral N and P fertilizers treatments on nitrogen, phosphorus and protein (%) contents in leaves of wheat, faba bean and onion (combined analyses of 2011and 2012 seasons).

Treatments	Wheat			Faba bean			Onion		
	N	P	Protein	N	P	Protein	N	P	Protein
100% recommended dose of N+ P	1.783	0.450B	11.145	3.05AB	1.20	19.06AB	2.02AB	0.783B	12.62
100% recommended dose of N + P +300 kg bio-NP fertilizer	1.97	1.267A	12.292	3.58A	1.32	22.39A	2.25A	1.20A	12.73
75% recommended dose of N+ P +300 kg bio-NP fertilizer	1.783	0.533B	11.145	3.00AB	1.27	18.75AB	2.03AB	1.177A	12.71
50% recommended dose of N+ P +300 kg bio-NP fertilizer	1.743	0.527B	10.893	3.10AB	1.27	19.37AB	2.10AB	1.97A	13.12
25% recommended dose of N+ P +300 kg bio-NP fertilizer	1.733	0.513B	10.835	2.75B	1.25	17.19B	1.88AB	1.67A	11.77
300 kg bio-NP fertilizer	1.700	0.497B	10.620	2.73B	1.25	17.08B	1.82B	1.20A	11.35
MSD Treatments at 0.5	NS	0.177	NS	0.723	NS	4.5316	0.4032	0.3154	NS

NS= Non Significant

Treated wheat plants with 100% recommended dose of N+ P fertilizer + 300 kg bio-NP fertilizer gave the highest significant concentrations of P when compared to the rest treatments, same treatment gave the highest values in N and protein contents. The lowest values in N and protein contents resulted when applied 300 kg bio-NP fertilizer for wheat plants, the lowest value of P was found in control plants which received 100% recommended dose of N+ P fertilizer.

Application of 100% recommended dose of N and P fertilizers plus 300 kg bio-NP fertilizer gave the highest significant values of N and protein concentration but the lowest concentration in nitrogen and protein produced when using 300 kg bio-NP fertilizer treatment in faba bean plants.

The highest concentration of phosphorus and protein in onion leaves produced when using 50% recommended dose of N and P fertilizer +300 kg bio-NP fertilizer treatment. Onion plants which received 300 kg bio-NP fertilizer alone gave the lowest concentration of nitrogen and protein in leaves of onion. The highest significant value of N concentration in onion leaves resulted by 100% recommended dose of N and P fertilizer plus 300 kg bio-NP fertilizer.

Discussion

Phosphorus problem in Egyptian alkaline soils is in insoluble form due to fixation. In this context the release of soluble phosphorus from fixed and insoluble forms in the soil aided by microorganisms. This process gives a sustainable supply of available phosphorus; as phosphate solubilization occurs due to some organic acids released by specialized bacterial strains, especially the 2- ketogluconic – hydroxylic acid which is the main way that enables microorganisms to release P from insoluble forms and make it available for plants like faba bean plant. Co_2 is released by transpiration; then forms carbonic acid with water in the soil; resulting in a higher buffering capacity to the soil pH. Acids react with the phosphate rock transforming tri-calcium phosphate to di-calcium phosphate then finally mono-calcium phosphate, the available form for plants (Kennedy and Tchan 1992; Pol, 1998; Mehana and Farag, 2000).

Therefore, using El-MOWAFFER*- BIO^R with different doses of mineral N and P fertilizer providing sufficient N and P under adverse conditions it contains rock phosphate with specialized active strains of microorganisms (Symbiotic/Faculative- Aerobic/ Anaerobic) of nitrogen fixer and phosphate dissolving bacteria (*Azotobacter spp., Bacillus polymyxa* and *Bacillus megatherium var. phosphaticum*) with fully adapted against environmental changes and stress conditions and enhanced with bio-stimulants.

Nitrogen fixers secrete growth promoting substances like GA_3 and IAA which enhance root proliferation and growth of plants this is very benefit to enhance the growth and development of wheat and onion plants (Hartmann *et al.,* 1983; Gomaa, 1995; Rabie *et al*., 1995).

Enhancement of crop yields of cereals by inoculation with nitrogen fixing bacteria was observed in many experiments (Bhattarai and Hess 1993; Ozturk *et al*. 2003). Yield increases obtained in inoculated plants were attributed to the production of plant growth substances by the root-colonizing bacteria (Kennedy and Tchan 1992).

The stimulating effect of biofertlizers in studied plants was found to be similar with those obtained by (Khattab and Gomaa, 2003; Mohamed and Gomaa, 2005) they stated that vegetative growth parameters increased in the biofertilizer treatments compared to control. The increase in grains yield/fed., might be due to that the role of bio-NP fertilizer on enhancing metabolic process such as net assimilation rate that resulted increased growth parameters and yield components, or may be due to the activation of the growth micro flora including many plant growth stimulators. The highest values of wheat plant height were observed with using mix of the three bacteria *Azotobacter chroococcum* + *Bacillus megatherium* + *Bacillus circulans,* mycorrhiza and potassium silicate (Salim *et al*., 2011).

The enhanced growth parameters by biofertilizer application may be attributed to microorganisms such as *Pseudomonus, Azotobacter, Azospirillium* and mycorrhiza can secrete growth promoting factor, i.e., gibberellins, cytokinins like substances and auxins (Hartmann *et al.,* 1983; Gomaa, 1995; Rabie *et al*., 1995; Akbari *et al*., 2007). Existence of microbial communities like *Azotobacter sp*. and *Azospirillum sp*. in the rhizosphere promotes the growth of the plant through the cycling and availability of nutrients, increasing the health of roots during the growth stage by competing with root pathogens and increasing the absorption of nutrients and water (Vessey, 2003; Zorita and Canigia, 2009; Daneshmand *et al*., 2012). Secretion of vitamins and amino acids, auxin and fixing atmospheric nitrogen by *Bacillus*, *Azotobacter* and *Azosprillum* are among the direct mechanisms of increasing root development and plant growth (Akbari *et al*., 2007).

Now due to increasing of costs of N- fertilizers and the dangers of increasing soil salinity and pollution of the agricultural environment as well as significant increase in nitrate accumulation in several vegetable crops including leafy crops, it is likely to further limit the application of N (Hamida and El-Komy, 1996).

Moreover PGPR including *Bacillus* spp., *Pseudomonas fluorescence* and *P. putida* are able to enhance P availability, by production of organic acids and phosphatase enzymes through producing siderophores. Moreover, N-fertilizers in the presence of biofertilization increased the decomposition and mineralization rate of organic fertilizers as well as the availability of nutrients for plant growth. These results are in agreement with those obtained by Gadhia and Mane (1995) and Zahran *et al*., (2002) on wheat plant. In addition, phosphate solubilizing bacteria individually significantly increased yield, seed moisture, ash and fat in faba bean. *Rhizobium* and phosphate solubilizing bacteria significantly increased yield and seed quality (moisture, crude protein, fat, crude fiber and ash content) and decreased seeds carbohydrate content of faba bean plants. A synergetic effect was observed when the two types of microorganisms were combined which produced comparable increments in yield were obtained resulting from either microbial fertilizers (nitrogen fixing and/or phosphate solubilizing bacteria) or chemical fertilizers (nitrogen and phosphorus fertilizers) Rugheim and Abdelgani (2012). On the other hand, using biofertilizers can subside for a certain amount of mineral N, particularly in the production of safe food Wessam (2001).

Namvar and Khandan (2013) indicated that wheat (*Triticum aestivum* L.) yield components and protein content of grains had a strong association with the N fertilization, biofertilizer inoculation and weed interference. Higher rates of N fertilization and biofertilizer (*Azotobacter* sp. and *Azospirillum* sp.) inoculation increased plant height, spike number per unit of area, grains number per spike, 1 000-grains weight, grain yield, biological yield and grain protein content. Salim *et al*. (2013) concluded that the application of biofertilizers, micronutrients and potassium silicate had stimulate effect in wheat growth parameters and yield components so led to enhance salt tolerance in wheat cultivars.

The favorable effect of nitrogen in the presence of applied biofertilizers may be due to release some of nutrients and improving of soil conditions for plant growth. Yield increases obtained in inoculated plants were attributed to the production of plant growth substances by the root-colonizing bacteria (Kennedy and Tchan 1992).

Moreover many investigators reported that the vegetative growth of onion plants and minerals uptake were increased with increasing the levels of NPK-fertilizers (Singh *et al*., 1989; Rizk 1997; EL-Desuki and Sawan, 2001; EL-Desuki *et al*. 2006). Moreover that the total bulbs yield and bulbs quality were improved by increasing the levels of NPK fertilizers (Haggag *et al*., 1986; Sato, 1988; Setty *et al*.,1989; and EL-Desuki, 2004). The role of nitrogen fertilizer and bio-fertilizers (nitrobeine) application on enhancing growth and yield of onion plant may be due to increasing the availability of nitrogen to onion plant absorption which increasing the accumulation of nitrate on onion bulbs (EL-Desuki and Sawan, 2001; EL-Desuki, 2004; EL-Desuki *et al*., 2006). These effects could be ascribed to the increases in root surface area, root hairs and root elongation as affected by *Azotobacter sp.* as mentioned by Sundaravelu and Muthukrishinan (1993).

Hassan *et al*. (2006) reported that the biofertilizer rhizobactrin application increased vegetative growth (plant height , number of leaves , leaf area and dry weight), chlorophyll reading, nitrogen, phosphorus and potassium in globe artichoke as well as yield and its components. Zaki *et al*. (2009) mentioned that broccoli plants treated with biofertilizer showed higher vegetative growth parameters, i. e., plant height, leaf number, fresh and dry weight of leaves and total yield as well as N, P and K in tissues of broccoli leaves.

The importance of biological fixation of nitrogen has increased, the beneficial impact of N fixing bacteria at thought to be direct plant growth promotion by the production of plant growth regulators (Noel *et al*., 1996), enhanced access to soil nutrients (Pol, 1998).

Darwish (2003) reported that increasing nitrogen fertilization led to increases in plant height, ear diameter, leaf area, N and protein in plant (Salardini *et al* ., 1992; Raja ,2001) reported that increasing nitrogen fertilization increased significantly the green ear yield and kernel weight. Abou El-Yazied *et al.* (2007) Indicated that increasing nitrogen fertigation levels (100, 120, 140 to 160) kg N/fed and (or) chelated calcium concentration (500 and 1000 ppm Ca EDTA) led to increasing the vegetative growth (leaf area, plant height, fresh and dry weight), chemical composition (total chlorophyll, nitrogen, phosphorus, potassium and calcium in leaves) and yield components (ear length, ear diameter, weight of 1000 seed, un husked ear, husked ear and total yield). Nitrogen is an essential nutrient for plant growth and development involved in vital plant functions such as photosynthesis, DNA synthesis, protein formation and respiration (Blackshaw *et al*., 2005; Rana *et al*., 2012; Diacono *et al*., 2013).

This favorable effect of N may be due its effective role in many biochemical processes within plants as it is necessary for cell protoplasm formation, photosynthesis activity in all plants and necessary for division and merestimic activity in plant organs (Russel, 1973) In addition, nitrogen is essential for synthesis of chlorophyll, enzymes and proteins, phosphorus is essential for root growth, phospho-proteins, phospholipids and ATP, ADP formation but potassium play an important role of promotion of enzymes activity and enhancing the translocation of assimilates and protein synthesis Devlin and Witham, (1986). Phosphorus is a component of high-energy substances such as ATP, ADP and AMP; it is also important for nucleic acids and phospholipids.

The stimulating effect of using Bio- NP fertilizer on nitrogen, phosphorus and total protein concentrations in three studied plants was found to be similar with obtained by (Ahmed *et al*., 2015) on sesame plants .

Khattab and Gomaa(2003) found that applied biofertlizers treatments increased NPK contents in genovese basil. EL-Desuki *et al*., (2006) found also clear relationship between bulbs quality i. e. average bulb weight and TSS, carbohydrates, N, P, K contents were significantly affected by the interaction treatments between levels of minerals fertilizers and bio-fertilizers application, Moreover, the highest values of average bulb weight and TSS, N, P, K contents were recorded with plants received the highest level of minerals fertilizers (100%) with using mixture of nitrobeine and phosphorene.

Appplication of 75% or 50% of the recommended dose of N fertilizer with Rhizobium inoculant or the application of 75% or 50% of the recommended dose of P fertilizer with VAM inoculant resulted in considerable increases on the concentration of N, P, K as well as the percentage of protein in seeds and straw of the two cultivars of faba bean Talaat and Abdallah, (2008).

The highest values of nitrogen concentration in the leaf tissues of celeriac plant were obtained from the dual inoculation with *Azotobacter* and *Azospirillium* coupled with full dose of N followed by the check plants (given full dose of mineral N) (Shehata *et al*., 2010). Salim *et al*., (2011) showed that the *Azotobacter spp*. treatment gave the highest significant values with total nitrogen and proteins while the maximum value of phosphorus concentration was recorded by mycorrhiza treatment in wheat plants.

Biofertilizer inoculation increased grains protein content about 9.53% compared to non-inoculated plants (Namvar and Khandan, 2013). Ahmed *et al*.,(2015) found clear that the highest values of Sesame varieties seeds N, P, K, Mg, Na, Mn and Zn uptake were resulted from the addition of the highest rate of Bio- NP fertilizer (350 kg/fed.).

Finally, using Bio- NP fertilizer (El-MOWAFFER*- BIO^{R}) with 50 % of recommended doses of mineral N and P fertilizers lead to enhance growth and yield of wheat plants. But with 75 % of recommended doses of mineral N and P fertilizer on faba bean and onion plants, results indicated that we can rationalization of mineral N and P fertilizers by 50% or 25 % of the recommended doses with benefit to keep also more clean and safety environment.

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