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Response of Faba Bean to Combined Application of Growth –Promoting Rhizobacteria and Cyanobacteria

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ABSTRACT

This study shows the effect of some growth –promoting rhizobacteria (*Bacillus subtilis* and *Pseudomonas fluorescens*) either individually or as a mixture of both inoculum of *Nostoc muscorum and Anabaena oryzae* on faba been (*Vicia faba*) yield as well as N, P and K contents. Soil biological activities in terms of dehydrogenase and nitrogenase activities, CO_2 evolution, total bacteria as well as cyanobacteria counts were considered as well. The experiment was conducted at El- Ismailia Agric. Res .station, Agric. Res.Center (ARC), El-Ismailia Governorate, Egypt, in the winter season of 2011/2012. The response of faba been to inoculation with the plant growth promoters in presence of *Rhizobium* inoculation compared to control (100 % N) revealed that the highest significant effect on seed weights was found with *Pseudomonas fluorescens*. Also, *Pseudomonas fluorescens* with 75% N increased the number and weight of pods over control and all other treatments. Biofertilization with *Bacillus subtilis*, *Pseudomonas fluorescens* and mixture of both together with cyanobacteria showed significant effect on NPK % of bean seeds compared to the control of 100 %N. All parameters of the tested soil biological activity under different treatments of growth promoting rhizobacteria were higher than those of the control. It is recommended to use the growth promoting rhizobacteria (*Bacillus subtilis*, *Pseudomonas fluorescens*) individually without N fertilizer and cyanobacteria *(Nostoc muscorum and Anabaena oryzae*) in the presence of *Rhizobium* to save a part of mineral N fertilizer required for bean plant production.

***Key words*:** Faba bean, growth promoting rhizobacteria (*Bacillus subtilis*, *Pseudomonas fluorescens*), cyanobacteria (*Nostoc muscorum , Anabaena oryzae*)

Introduction

Faba bean (*Vicia faba)* crop is the most important food protein source for majority of Egyptian population. Thus, faba bean cultivation in the newly reclaimed soils is considered an important way to increase its productivity (El-Sherif *et al. 2013)*.

Cyanobacteria are used as growth promoting biofertilizer for plants due to their capacity to secrete bioactive substances such as auxins, gibberellins, cytokinins, vitamins, polypeptides, amino acids, antibiotics, and organic acids, which promote plant growth and development (Ragaa, 2013*)*. Plant growth promoting rhizobacteria (PGPR) are among the most effective soil bacteria, they are able to colonize root surface, as a result of some signal communications with the host plant. PGPR represent a wide variety of soil bacteria which, when grown in association with a host plant, show potential influence to improve nodulation of legumes when co-inoculated with *Rhizobium* and stimulate the growth of their host plant (Asad and Vafa, 2011).

Munees and Mohammad (2011) found that the beneficial rhizobacteria facilitate the plant growth through N_2 fixation, solubilization of insoluble phosphorus, production of siderphores and production of phytohormones, lowering of ethylene concentration, production of antibiotics and antifungal metabolites and induced systemic resistance. The application of plant growth promoting rhizobacteria as bio-inoculants may be a feasible preference to chemical fertilizers to increase the productivity of various crops.

The present work was conducted to study the effect of *Bacillus subtilis* and *Pseudomonas fluorescens* either individually without N fertilizer and cyanobacteria *(Nostoc muscorum and Anabaena oryzae* as a mixture of both) or in the presence of *Rhizobium* on nutrient uptake and yield of faba bean grown in sandy soil.

Materials and Methods

A field experiment was conducted in Ismailia Agricultural Research Station, Agric. Res. Center (Latitude 30° 35′ 41.901″ N and Longitude 32° 16′ 45.843″ E), Egypt, during the winter season of 2011-2012 to study the effect of inoculation with *Bacillus subtilis* and *Pseudomonas fluorescens* either individually or as a mixture, as well as cyanobacteria mixture of *Nostoc muscorum* and *Anabaena oryzae.* All the bacteria and cyanobacteria strains were kindly provided from Agric. Microbiol. Res Dept., Soils, Water and Environ. Res. Inst., ARC, Giza, Egypt. *Rhizobium* inoculation (*Rhizobium leguminosarum*) along with 15 kg N/fed ($NH_4 NO_3$: 33.5 % N) were applied for all treatments except control as recommended by the Ministry of Agriculture and Land

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Reclamation, Egypt. The experimental plot area was 10.5 m^2. Faba bean seeds variety Giza 6 were sown in rows at 30 cm apart and 50 cm between rows that received nitrogen at the rate of 60 kg fed^{-1} (full N dose) as control in the form of ammonium nitrate (33.5%N) applied in three split equal doses after bean seedling development, and 75% N (45 kg N/ fed) in some treatments. Potassium sulphate (48% K_2O) or the rate of 50 kg/ fed and super phosphate (15 % P_2O_5) or the rate of 200 kg /fed were applied during soil preparation. Faba bean plants were treated with growth promoting rhizobacteria after 21, 42 and 52 days from sowing on the soil at the rate of 20L and 30L culture/ fed for both bacteria and cyanobacteria suspensions, respectively.

Preparation of bio-stimulant inoculants:

Bacillus subtilis and *Pseudomonas fluorescens* were grown on king's medium (king *et al*., 1954) and gently agitated on a rotary shaker incubator at 30°C± 2 to reach the log phase (10^7cfu ml^{-1}) after 48 hours. While cyanobacterial strains (*Nostoc muscorum* and *Anabaena oryzae*) were grown on BG11 medium (Rippka *et al.*, 1979). The cultures were incubated in a growth chamber under continuous illumination (3000 lux) at 25°C± 2 for 30 days. Both bacterial and cyanobacterial cultures were homogenized to prepare the bio-stimulant suspensions that will be applied either to soil or for seed soaking before sowing according to the applied treatments.

The experiment comprised the following treatments:

1) Control (recommended dose 60 kg N/fed)
2) *Bacillus subtilis* (B1)
3) B1 + 75% N (45 kg N/fed)
4) *Pseudomonas fluorescens* (B2)
5) B2 + 75%N
6) A mixture of *Nostoc muscorum* and *Anabaena oryzae* (Cyano. Mix)
7) Cyano. Mix + 75% N
8) B1+B2
9) B1+B2 + 75% N
10) A mixture of B1 + B2 + Cyan. Mix (Mixture)
11) Mixture +75% N

At harvest, faba bean plants in plots were collected to determine seed yield and its components, total N, P and K contents in bean pods (Black, 1982).

Statistical analysis: Data were analyzed for the least significant differences at $P<0.05$ (Gomez and Gomez, 1984).

Soil samples were collected to determine some biological, chemical and physical properties (Table 1). in terms of total bacterial counts (Allen, 1959), total cyanobacterial counts (Allen and Stainer, 1968), carbon dioxide evolution (Gaur *et al*., 1971), dehydrogenase activity (DHA) (Casida *et al*., 1964) and nitrogenase activity (Dart *et al*.,1972). Data in Table (2) show some growth promoting substances produced by *Bacillus subtilis*, *Pseudomonas fluorescens, Nostoc muscorum* and *Anabaena oryzae*. The determined materials were fractioned and quantified by HPLC apparatus (Hewlett-Pakard 1050) as described by Kowalczyk and Sandberg(2001).

Table 1: Mechanical, chemical and biological properties of the investigated soil.

Mechanical and chemical properties								
Coarse sand %	Fine sand %	Silt %	Clay %	Textural class	Ca CO_3 %	Organic matter %	pH (1:2.5)	E C (dSm^{-1}) in soil paste extract
45.20	39.50	9.34	5.96	Sandy	2.4	0.12	7.68	0.37
Soluble ions in soil paste extract (meq l^{-1})								
Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}	
0.97	0.87	1.51	0.45	-	1.42	1.02	1.36	
Available macronutrients (mg Kg^{-1})								
N		P			K			
45		7			25			

Biological properties	
Dehydrogenase activity (µg TPFg^{-1} dry soil Day^{-1})	10.58
Nitrogenase activity (µ mole C_2H_4 g $soil^{-1}hr.^{-1}$)	1.78
Phosphatase activity (mg of *p*-NP 100 g $soil^{-1}$ h^{-1})	0.98
CO_2 evolution (mg100g $soil^{-1}$ day^{-1})	14.71
Bacterial counts (10^5 cfu g $soil^{-1}$)	13.00
cyanobacterial counts (10^2 cfu g $soil^{-1}$)	7.00

Results and Discussions

The plant growth promoting rhizobacteria (PGPR) *Bacillus subtilis, Pseudomonas fluorescens* and cyanobacteria (*Nostoc muscorum, Anabaena oryzae)* produced indole acetic acid and gibberellic acid. Table (2) that play a Key role in improving growth of many plants when applied as biofertilizers, these finding agree with Aref (2011).

Table 2: Extracellular growth regulators analyses.

Strains	Indol acetic acid	Gibberellic acid
	mgl^{-1}	
Bacillus subtilis	112	427
Pseudomonas fluorescens	163	509
Nostoc muscorum	1823	2859
Anabaena oryzae	1118	8839

Results in table (3) revealed that reducing the chemical N fertilizer to 75% of the recommended dose gave 1736 kg/fed yield of seeds with cyanobacterial mixture while *Pseudomonas fluorescens* (B2) gave 1838 kg/fed yield of seeds Also, (B2) +75%N gave the significant estimates of weight and number of nodules (4.21 g, 97.0) compared with control (1.56 g, 58.0) .These results show that *Pseudomonas fluorescens* did satisfy a part of nitrogen needed and increased seed weights. Asad and Vafa (2011) found that co-inoculation with *Pseudomonas* spp. and *Rhizobium* spp. has been reported to enhance nitrogen fixation and plant biomass in various leguminous species including faba bean. Haiquan *et al*. (2013) mentioned that the mechanisms of PGPR mediated enhancement of plant growth and yields in many crops are as follows: ability to produce 1-aminocyclopropane-1-carboxylates(ACC) deaminase to reduce the level of ethylene in roots of the developed plants and thereby increasing the root length and growth; ability to produce hormones like indole acetic acid , gibberellic acid and cytokinins; asymbiotic nitrogen fixation; solubilization of mineral phosphates and mineralization of other nutrients and control of phytopathogenic microorganisms

Table 3: Impact of rhizobacteria and cyanobacteria inoculants and mineral N on faba bean yield components in sandy soil.

Treatment	Weight of 100-seeds(g)	Weight of seeds (kg/ Fed)	Weight of straw (ton/ Fed)	Weight of nodules (g/ plant)	No of nodules/plant
Control	106.30	1717	1.457	1.56	58.00
B1	98.34	1572	0.835	3.67	94.33
B1+ 75%N	96.27	1101	0.485	2.56	87.00
B2	99.40	1838	0.672	3.59	84.00
B2+ 75%N	104.20	1652	0.868	4.21	97.00
Cyano.Mix	86.07	1586	0.774	2.65	77.00
Cyano. Mix +75%N	100.83	1736	0.933	2.72	90.00
B1+B2	102.70	1465	1.456	3.54	94.33
B1+B2 +75%N	64.80	1418	0.831	2.21	68.00
Mixture	99.91	1446	1.344	3.03	91.00
Mixture +75% N	104.50	1437	0.700	3.79	95.00
LSD at o.o5	5.18	108	0.176	0.14	5.19

B1: *Bacillus subtilis* B2*: Pseudomonas fluorescens* Cyano.Mix : *(Nostoc muscorum +Anabaena oryzae)* Mixture : *(Bacillus subtilis + Pseudomonas fluorescens+ Nostoc muscorum +Anabaena oryzae*).

Data in Table (4) revealed that the treatments B1+B2 gave significant nitrogen concentrations of faba bean seeds followed by B1 and the mixture B1+B2+Mix of cyanobacteria with increases of 0.38 , 0.35 and 0.35 % respectively compared to control of 0.26%. Moreover, results indicated that the highest P concentration in response to the tested treatments is 2.19% at B2 compared to control of 1.06 %. Asad and Vafa (2011) found that PGPR can increase P availability to plants through solubilizing insoluble phosphates and this improved P nutrition, which in turn increases the biological nitrogen fixation and availability of other nutrients for plants. In respect to K concentration, the highest significant level in seeds was due to the treatment B1+75%N (1.21%) compared to control (0.52%) Abbas Zadeh *et al*. (2010) found that PGPR are able to enhance the availability of different nutrients including N, P and micronutrients. For example, *Rhizobium* spp., in symbiosis with their legume host plants, and *Azospirillum* in non-symbiotic association with some plants, can fix atmospheric N_2 and 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. PGPR can also increase Fe solubility and hence uptake by plant. El-Sherif *et al*. (2013) found that inoculation with cyanobacteria increased the availability of NPK and consequently their contents in seeds and straw of faba bean. Many investigators proved that the rhizosphere organisms promote uptake of minerals by roots which is parallel to the increase in the volume of the root system resulted by the increase of root number, thickness and length. These

changes in root system in response to the use of biofertilizer enable more nutrients availability and plant uptake leading to increased crop yield and its quality (Harish *et al*., 2010).

Table 4: Nitrogen, phosphorus and potassium concentrations of faba bean seeds as affected by rhizobacteria, cyanobacteria and mineral N Fertilization.

Treatments	Faba bean seeds N, P and K concentration (%)		
	N	P	K
Control	0.26	1.08	0.52
B1	0.35	1.20	0.72
B1+ 75%N	0.33	1.25	1.21
B2	0.27	2.19	0.81
B2+ 75%N	0.29	0.95	0.81
Cyano. Mix	0.29	1.30	0.65
Cyano. Mix+75%N	0.29	1.16	0.58
B1+B2	0.38	1.19	0.63
B1+B2 +75%N	0.29	1.25	0.86
Mixture	0.35	1.34	0.65
Mixture +75% N	0.31	1.34	0.60
LSD 0.05%	0.02	0.05	0.04

B1: *Bacillus subtilis* B2*: Pseudomonas fluorescens* Cyano.Mix: (*Nostoc muscorum +Anabaena oryzae)* Mixture : *(Bacillus subtilis + Pseudomonas fluorescens+ Nostoc muscorum +Anabaena oryzae*).

Soil biological activity:

The numbers of total cyanobacteria (Fig.1) were the highest in the treatment of biofertilizer mixture +75 % N (T4) and cyanobacteria +75% N (T7) being 10 x10^2 cfu g $soil^{-1}$ and 8x10^2 cfu g $soil^{-1}$ respectively .Results also revealed that all tested soil biological activities that obtained in response to the different biofertilizers applied were higher than those of control (T1) with counts of 2x10^2 cfu g dry $soil^{-1}$. *Pseudomonas fluorescens* (B2)(T4) showed the highest bacterial counts of 40x10^5 cfu g $soil^{-1}$ (Fig. 2) compared to the control of 2 x10^5 cfu g $soil^{-1}$.

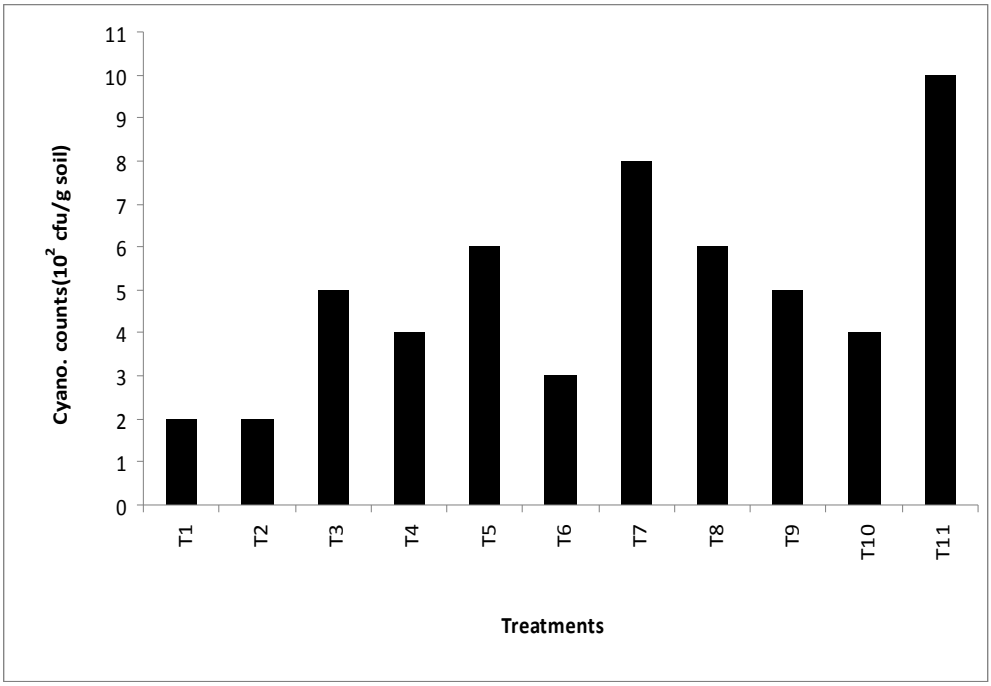

Fig. 1: Cyanobacterial counts in rhizosphere of faba bean plant.

T1) Control (rcomended dose 60 kg N/fed), T2) Bacillus subtilis (B1), T3) B1 + 75% N (45 kg N/fed), T4) Pseudomonas fluorescens (B2) , T5) B2 + 75%N, T6) A mixture of Nostoc muscorum and Anabaena oryzae (Cyano. Mix) , T7) Cyano. Mix + 75% N , T8) B1+B2 , T9) B1+B2 + 75% N , T10) A mixture of B1 + B2 + Cyan. Mix (Mixture) ,T11) Mixture +75% N

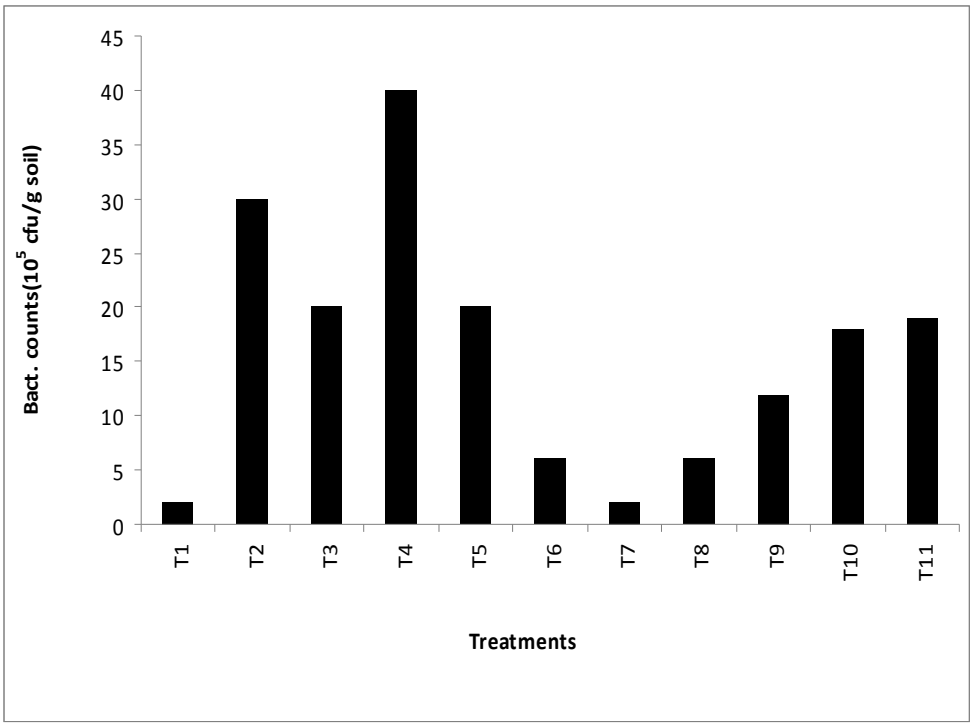

Fig. 2: Bacterial counts in rhizosphere of faba bean plant.

Data in Fig. (3) Indicated that B2 treatment recorded the highest CO_2 evolution values of 122.87 mg 100 g soil / day followed by 95.92 mg 100 g soil / day for the biofertilizer mixture (B1 + B2 + Cyano. Mixture) (T10) compared to 11.61 mg /100 g soil /day for the control treatment.

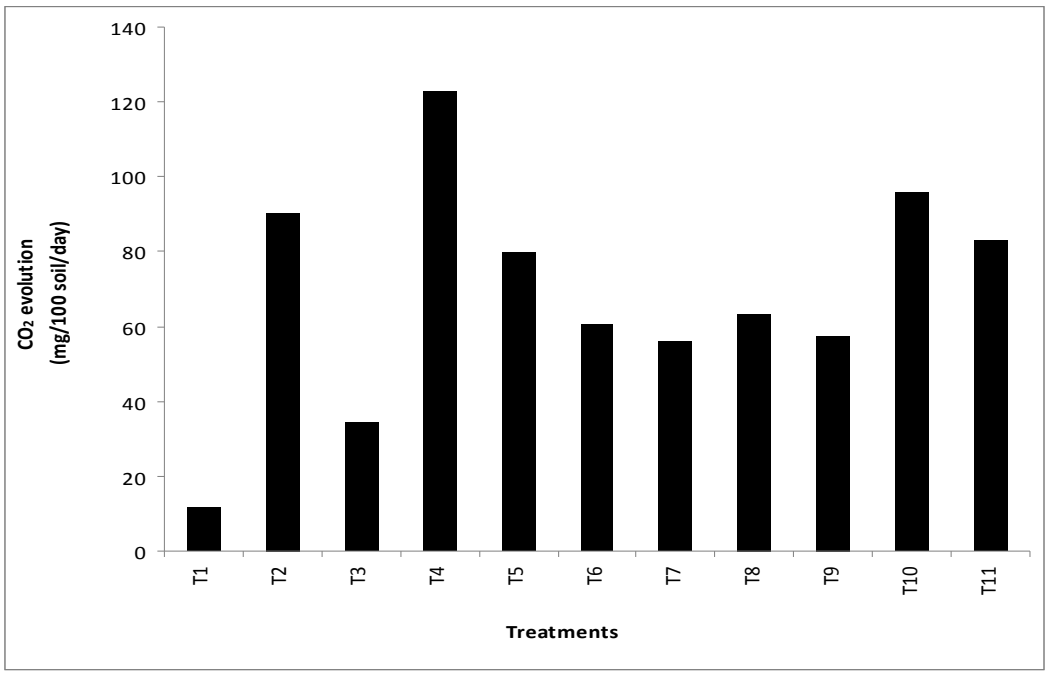

Fig. 3: CO_2 evolution in rhizosphere of faba bean plant.

Dehydrogenase activity (Fig. 4) exhibited the same trend achieved in CO_2 evolution, where the maximum activity was due to B2 (T4) followed by the biofertilizer mixture (B1 + B2 + Cyano Mixture) (T10) of 98.32 and 96.95µg TPF g^{-1} soil day^{-1} compared of 43.52 µg TPFg / soil / day for control treatment.

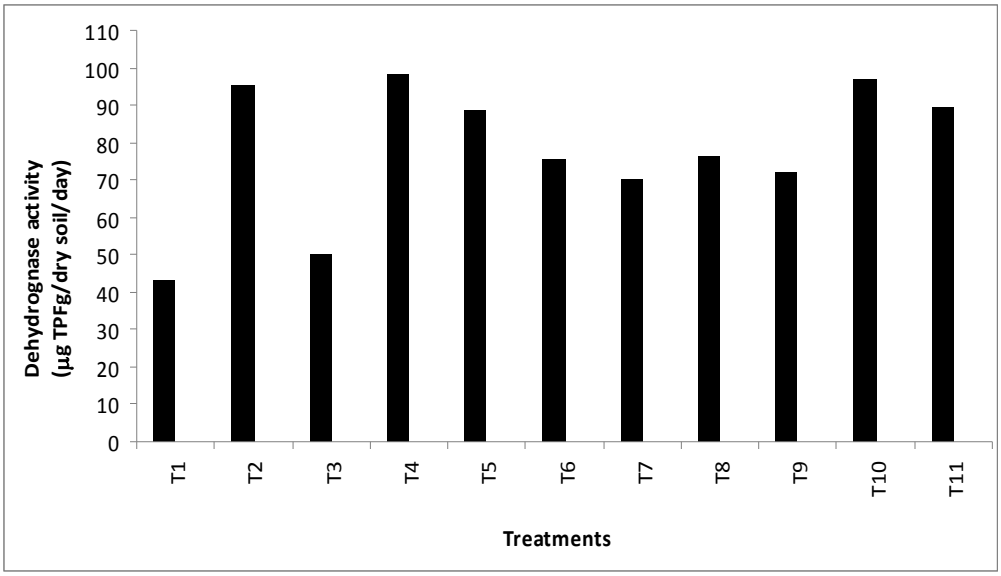

Fig. 4: Dehydrogenase activity in rhizosphere of faba bean plant.

Data in Fig. (5) showed that the use of cyanobacteria in presence of 75% N (T7) surpassed the other tested treatments of 41.07 µ mole C_2H_4 g soil /hr) .El-Sherif *et al. (2013)*. found that cyanobacteria can be incorporated into soil as organic matter and also as a source of enzymes as they produce acid and alkaline extracellular phosphatases that are active in solution or located in the peri-plasmatic space of the cell wall. Both biomass and exopolysaccharides incorporated into soil induce a growth promotion of other microorganisms and increased the activity of soil enzymes that participate in the liberation of nutrients required by plants.

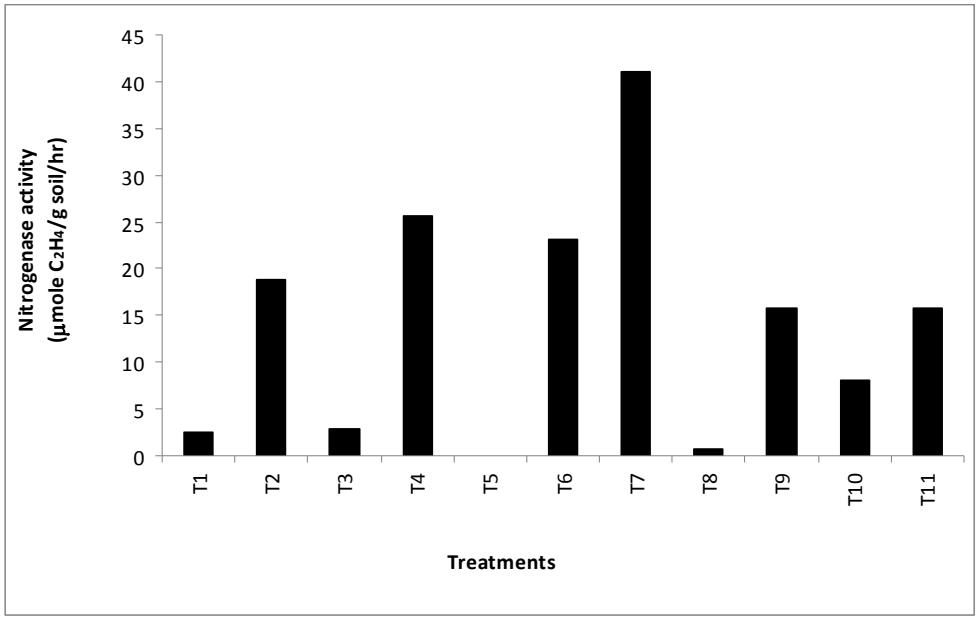

Fig. 5: Nitrogenase activity in rhizosphere of faba bean plant.

Conclusion:

In conclusion, the adoption of plant bio-growth activators in the forms of multifunctional microbial formulations deems compulsory. This actually secure better plant development and more hygienic farm outputs. A rationalized mineral level of necessary plant nutrients simultaneously with the application of these biopreporates is the way for satisfying proper quality and quantity of agricultural products.

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