

RESPONSE OF FABA BEAN GROWN IN SANDY SOIL TO RHIZOBIA INOCULATION AND NON SYMBIOTIC EFFECTIVE MICROORGANISMS AND/OR N-FIXERS

Tantawy, Eman A.; A. A. M. Ragab; Nemat I. Ghayad and Sh. M. Abd El-Rasoul

Soils, Water and Environ. Res. Inst., Agric. Res. Center, Giza, Egypt.

ABSTRACT

In a farm trail to improve faba bean crop yield and to reduce the productivity cost, inoculation with EM and Compomax (2) beside Rhizobia were done. Plants inoculated with EM combined with Rhizobia scored highest values of N, P and K, plant uptake and plant dry matter at 45day, as well as this treatment gave the highest seed yield and potassium plant uptake at harvest.

Inoculation with Compomax scored the highest nitrogen content of seed and in soil at harvest. In general inoculation increased the soil microbial counts at both 45 days period and at harvest. Increasing of mineral nitrogen decreased the soil microbial counts, total counts and fungi at harvest compared to 45 day.

This work evoked that mineral nitrogen could be reduced to 50% of the recommended dose for bean with the use of EM and Compomax besides Rhizobie inoculation.

Keywords: Rhizobia, N-fixers (Compomax), Effective microorganisms (EM), Nitrogen levels, bean and sandy soil.

INTRODUCTION

Economically, broad bean (*Vicia faba* L.) is still very important in Egypt. It is constant that beans inoculated with Rhizobia because of the symbiotic relationship between them which leads to reduce the mineral nitrogen demands for beans by 60 to 70 %. This reduction in mineral nitrogen is compensated by nitrogen fixed with Rhizobia (Alaa El-Din and Hassan, 1985). In recent decade the foliar sprays of Effective microorganisms (EM) has paid much attention from many agricultural scientists because of the thought that EM application enhanced the harvest and increased uptake of mineral elements (Abd El-Rasoul *et al.*,2004). Also Compomax which contains composite biofertilizers of *Azospirillum*, *Azotobacter* and *Bacillus*, in addition to that microorganisms are nitrogen fixers they also considered as plant growth promoting rhizobacteria (PGPR) by many authors Antoun *et al.*,1998 and Dileep-kumar,(1999) they reported that PGPR could fix nitrogen and produce phytohormones, siderophores and hydrogen cyanide which enhancing nutrient uptake and produce a healthy plant resistant to the pathogenic agent.

The present work is designed to study the effect of the application of effective microorganisms, Compomax (a mixture of nitrogen fixing bacteria) compared with Rhizobia under different nitrogen levels on bean cultivated in sandy soil. Bean plant nodulation status, NPK uptake and microbial community situation in soil were estimated after 45 days from sowing, as well as the determination of seed yield components, NPK uptake for seeds and straw and microbial community situation in soil at harvest.

MATERIALS AND METHODS

A field experiment was carried out at experimental farm of Agricultural research station, Ismailia, Agric Res. Center (ARC) Egypt during winter season 2003-2004, under sprinkler irrigation system to study the effect of bio-fertilizers; Effective microorganisms (EM) and N-fixers (Compomax 2) in combination with inoculation with Rhizobia under two levels of nitrogen (full N recommended dose and 1/2 full N recommended dose) on broad bean plant. Some biological, physical and chemical properties of the investigated soil and water irrigation were determined and are shown in (Table 1).

Table (1): Chemical characteristics of the studied soil (0-30 cm) and irrigation water

Sample	EC dS/m	pH	Soluble ions (meq/l)								SAR
			Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	CO ₃ ⁼⁼	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁼⁼	
Soil	0.33	7.68	1.61	1.28	1.02	0.18	--	1.53	1.92	0.64	0.85
Water	0.41	7.96	1.24	1.76	1.29	0.14	--	0.52	1.92	1.99	1.06
Some physical characteristics of the studied soil (0-30 cm).											
Particle size distribution %						Texture class	Organic matter %	CaCO ₃ %			
Coarse sand	Fine sand	Silt	Clay								
31.82	61.61	1.22	5.35	Sandy			0.44	1.42			
Total count bacterial, fungi, actinomycetes and nitrogen fixers before planted of the studied soil.											
Bacterial count x10 ⁶	Fungi x10 ³	Actinomycetes x10 ³	Total nitrogen fixers 10 ³								
2.00	6.00	13.00	2.00								

The plot area was 10.5 m² (3x3.5 m), and the experiment was designed in a split split-plot design with three replicates, where the bio-fertilizers are allocated in the main plots as follow:

- 1- Control without biofertilization.
- 2- Effective micro-organisms (EM) by spraying 4L/fed two times, once every month starting from cultivation.
- 3- Compomax: mixture of free nitrogen fixers bacteria are sprayed two times, one every month from planting.

The inoculation with Rhizobia treatments were allocated in the sub plots as follows:

- a) Without inoculation.
- b) Inoculation with Rhizobia.

Nitrogen fertilizers were randomly distributed in the sub-sub plots as follows:

- I) Full dose recommended (30 kg N/fed).
- II) Half dose recommended (15 kg N/fed).

The recommended doses of phosphorus and potassium fertilizers were added uniformly at the rates of 30 kg P₂O₅ /fed as super phosphate and 48 kg K₂O/fed as potassium sulphate before cultivation. Nitrogen was added in the form of ammonium sulphate at the rates of 30 kg N/fed as basal dose for bean in two equal split doses (after 30 and 60 days from cultivation).

Traditional symbiosis Rhizobia was a composite of two Rhizobial strains from *Rhizobium Leguminosarum* biovar *viceae* viz ARC 207 and Icarda 441, which, were supplied by the unit of biofertilizers. Depart. Of Agric. Microbiol. Soil, Water, and Environ., Res. Inst. ARC, Giza. The strains were grown and maintained on yeast extract mannitol agar media (Vincent 1970), and a broth culture contains approximately 10⁸ cell/ml of either strain. Equal portion of both strains were mixed with fine peat neutralized with 5% CaCO₃ (2:1 w/v) and the moisture content of the final product was adjusted at 50%. The peat-based inocula were mixed with bean seed and placed in shadow to dry before sowing.

For enumeration the microbial community, the dilution plate method was used in soil after 45th day from bean seed planting and at harvest for counting total bacteria, actinomycetes, fungi and total nitrogen fixing bacteria using the media of Bridson (1978); Küster & Williams, (1966); Martin,(1950) and Watanabe & Barraquie (1979), respectively.

The recommended agricultural practices were carried out along the season. Both bean crop quality and quantity were assessed after 45th day and at harvest. At 45th day from sowing, plants were gently uprooted without tearing the root as possible and nodules were separated from the root and then, counted. Plants and nodules were oven dried at 70 °c to determine their constant dry weight in grams.

Straw and seed samples were also oven dried ground and digested according to Thomas *et al.*, (1967), then subjected to the determination of NPK contents as described by Van Schouwenburg (1968). Available nutrients in the soil after 45 days period and bean harvesting were extracted as described by Jackson (1973), i.e. nitrogen by 2N potassium chloride, Phosphorus by 0.5M sodium bicarbonate and potassium by 1N ammonium acetate.

All obtained data were subjected to statistical analysis according to Snedecor and Chachoran (1930), where mean values were compared using L.S.D at 5% level.

RESULTS

1-Effect of bio-fertilization and N-levels on faba bean plant parameters and NPK-uptake at 45 days:

1.1. Biofertilizer (as foliar spray) effect:

Data in Table (2a) show that bean number and dry weight of nodules after 45 days period increased significantly by addition of effective microorganisms (EM) and Compomax (2) individually compared to the control treatment. The highest values were obtained by using Compomax technology as a foliar spray, since it recorded increments of 45.58 and 33.33 % over the control treatment, respectively. Concerning, plant dry weight, data recorded in

Table (2a) show that significant increased in plant dry weight obtained by using effective microorganisms (EM) individually, It recorded increments of 30.81 % over the control treatment. Also, data illustrated in Table (4a) show that spraying of both effective microorganisms (EM) and N-fixer (Compomax) individually had significantly increased N, P and except K uptake by bean plant compared with control treatment.

Table (2a): Effect of bio-fertilization on number of nodule, dry weight of nodule, plant dry weight and NPK-uptake (g/plant) at 45 days

Bio-fertilization	Number of nodule/plant	Dry weight of nodule g/plant	Plant dry weight g/plant	NPK uptake (g/plant) at 45 days		
				N	P	K
Control	73.50a	0.15a	30.12b	1.08b	0.12b	0.50a
EM	92.75b	0.16b	39.40a	1.41a	0.16a	0.59a
Compomax (2)	107.00c	0.20c	35.57ab	1.15ab	0.16a	0.59a
L.S.D at 0.05	6.34	0.023	5.62	0.28	0.030	n.s

1.2. Inoculation effect:

Table (2b) clearly shows positive effect of Rhizobia inoculation on bean number and dry weight of nodules. However, the number and dry weight of nodules in Rhizobia inoculated treatments, increased by 22.61 and 40 %, respectively when compared to uninoculated ones. Concerning, plant dry weight and NPK-uptake at 45 days, data presented in Table (2b) show that seed inoculation with Rhizobia, had significantly increased N uptake only by bean plant (22.94%) in comparison to uninoculated control.

Table (2b): Effect of inoculation on number of nodule, dry weight of nodule, plant dry weight and NPK-uptake (g/plant) at 45 days

Rhizobia inoculation	Number of nodule/plant	Dry weight of nodule g/plant	Plant dry weight g/plant	NPK uptake (g/plant) at 45 days		
				N	P	K
Inoculation	100.33a	0.21a	35.22a	1.34a	0.15a	0.58a
Uninoculation	81.83b	0.15b	34.84a	1.09b	0.14a	0.54a
L.S.D at 0.05	6.50	0.023	n.s	0.23	n.s	n.s

1.3. Nitrogen levels effect:

Regarding to the effect of applied nitrogen levels, data in Table (2c) show the significant increases in bean number and dry weight of nodules in response to 1/2 N full dose treatments, as compared to N full dose. The corresponding increases were 23.06 and 33.33 %, respectively. Data in Table (2c) showed that plant dry weight, N, P and K uptake by bean plant at 45 days were insignificantly affected by the use of full N dose as compared to 1/2 N doses.

Table (2c): Effect of N-levels on number of nodule, dry weight of nodule, plant dry weight and NPK-uptake (g/plant) at 45 days

Nitrogen levels	Number of nodule/plant	Dry weight of nodule g/plant	Plant dry weight g/plant	NPK uptake (g/plant) at 45 days		
				N	P	K
full	81.67a	0.15a	36.14a	1.24a	0.15a	0.56a
½ full	100.50b	0.20b	33.92a	1.19a	0.14a	0.56a
L.S.D at 0.05	5.23	0.011	n.s	n.s	n.s	n.s

1.4. Interaction effect:

The interaction between the sources of biofertilizers *i.e.* EM and Compomax and Rhizobia inoculation had a positive significant effect on the bean number and dry weight of nodules compared to control treatment, (Table 3a). The highest mean values were 112.5 nodules /plant (15.38% over the control) and nodules dry weight of 0.24 g/plant (9.1% over the control). Both increases resulted from the combination of Compomax and Rhizobia inoculation treatment. Concerning, plant dry weight and NPK-uptake at 45 days, Data also showed insignificant differences between these interaction treatments, Tables (3a, 3b, 3c and 3d).

Table (3a): Effect of interaction between bio-fertilization and inoculation on number of nodule, dry weight of nodule, plant dry weight and NPK-uptake (g/plant) at 45 days

Bio-fertilization	Rhizobia inoculation	Number of nodule/plant	Dry weight of nodule g/plant	Plant dry weight g/plant	NPK uptake (g/plant) at 45 days		
					N	P	K
Control	Inoculation	97.50	0.22	31.52	1.37	0.40	1.85
	Uninoculation	49.50	0.08	28.72	0.78	0.36	1.45
EM	Inoculation	91.00	0.17	39.29	1.39	0.43	1.59
	Uninoculation	94.50	0.19	39.51	1.44	0.41	1.41
Compomax (2)	Inoculation	112.50	0.24	34.86	1.25	0.46	1.55
	Uninoculation	101.50	0.17	36.29	1.05	0.47	1.79
L.S.D at 0.05		13.15	0.05	n.s	n.s	n.s	n.s

As indicated in Table (3b) significant difference were recorded for the interaction between biofertilizers *i.e.* EM and Compomax and nitrogen levels on bean number and dry weight of nodule. The highest per cent increases of nodules number/piant (28.16) and dry weight of nodules (15.79) over the control, which, treatment were due to the treatments of Compomax plus ½ N dose, while the respective increases of 18.39 % (number of nodules) and 5.26% (dry weight of nodules) were due to EM plus ½ N dose treatment.

The interaction between the inoculation with Rhizobia and nitrogen levels doses had an insignificant effect on bean number and dry weight of nodules, plant dry weight and NPK-uptake, Table (3c).

Table (3b): Effect of interaction between bio-fertilization and N-levels on number of nodule, dry weight of nodule, plant dry weight and NPK-uptake (g/plant) at 45 days

Bio-fertilization	Nitrogen levels	Number of nodule/plant	Dry weight of nodule g/plant	Plant dry weight g/plant	NPK uptake (g/plant) at 45 days		
					N	P	K
Control	full	60.00	0.11	32.48	1.14	0.39	1.66
	½ full	87.00	0.19	27.76	1.01	0.37	1.64
EM	full	82.50	0.16	39.24	1.40	0.41	1.44
	½ full	103.00	0.20	39.56	1.43	0.42	1.55
Compomax (2)	full	102.50	0.19	36.70	1.18	0.48	1.62
	½ full	111.50	0.22	34.45	1.12	0.45	1.73
L.S.D at 0.05		11.41	1.97	n.s	n.s	n.s	n.s

Table (3c): Effect of interaction between inoculation and N-levels on number of nodule, dry weight of nodule, plant dry weight and NPK-uptake (g/plant) at 45 days

Rhizobia inoculation	Nitrogen levels	Number of nodule/plant	Dry weight of nodule g/plant	Plant dry weight g/plant	NPK uptake (g/plant) at 45 days		
					N	P	K
Inoculation	full	89.67	0.18	36.02	1.30	0.45	1.67
	½ full	111.00	0.24	34.42	1.38	0.41	1.65
Uninoculation	full	73.67	0.13	36.26	1.18	0.42	1.47
	½ full	90.00	0.17	33.42	1.00	0.41	1.62
L.S.D at 0.05		n.s	n.s	n.s	n.s	n.s	n.s

Regarding to the interaction between source of biofertilizers, inoculation with Rhizobia and nitrogen levels, data in Table (3d) showed that the highest values of bean number and dry weight of nodules were obtained by the interaction between biofertilizers with both control + Rhizobia + ½ N dose and N-fixers (Compomax) + Rhizobia + ½ N dose treatment where both recorded 118 nodules /plant. 118 nodules /plant, 0.29 and 0.25 g/plant for nodules numbers and dry weight, respectively. However, the effective microorganisms (EM) were superior in plant dry weight at 45 days with both of EM + Rhizobia + ½ N dose and EM + full N dose without inoculation, which gave the highest values of, 40.48 and 40.39 g plant⁻¹, respectively. N-fixers (Compomax) + Rhizobia + full N dose came in the second order and recorded 37.15 g plant⁻¹.

Table (3d): Effect of interaction between bio-fertilization, inoculation and N-levels on number of nodule, dry weight of nodule, plant dry weight and NPK-uptake (g/plant) at 45 days

Treatments			Number of nodule/plant	Dry weight of nodule g/plant	Plant dry weight g/plant	NPK uptake (g/plant) at 45 days		
Bio-fertilization	Rhizobia inoculation	N-levels				N	P	K
Control	+	full	77.00	0.15	33.72	1.37	0.14	0.59
		½ full	118.00	0.29	29.32	1.38	0.12	0.57
	-	full	43.00	0.08	31.24	0.91	0.12	0.49
		½ full	56.00	0.09	26.20	0.65	0.09	0.36
EM	+	full	85.00	0.16	38.09	1.42	0.18	0.68
		½ full	97.00	0.17	40.48	1.35	0.15	0.56
	-	full	80.00	0.15	40.39	1.37	0.14	0.43
		½ full	109.00	0.23	38.63	1.50	0.18	0.68
Compomax (2)	+	full	107.00	0.22	36.24	1.09	0.17	0.51
		½ full	118.00	0.25	33.47	1.40	0.15	0.54
	-	full	98.00	0.15	37.15	1.26	0.18	0.66
		½ full	105.00	0.19	35.42	0.84	0.16	0.64
L.S.D at 0.05								
N x Inoculation x Bio-fertilization			6.64	0.02	n.s	n.s	n.s	n.s

2-Effect of bio-fertilization and N-levels on faba bean yield and its components:

2.1. Biofertilizer (as foliar spray) effect:

Data recorded in Table (4a) show that bean yield and its components were increased significantly by addition of effective microorganisms (EM) individually compared to the control treatment. The highest values of total yield, seed and straw yields were obtained by using effective microorganisms (EM), since it recorded increments of 36.10, 6.59 and 53.32 % over the control treatment, respectively.

Due to the effect of using Compomax (applied as spray on plant and soil) on bean yield and its components, data in Table (4a) show a significant increase in weight of 100 seed. The percentage increase was 4.47 % over the control treatment.

Table (4a): Effect of bio-fertilization on yield of bean and its components

Bio-fertilization	Total yield Kg/fed	Seed yield Kg/fed	Foliage yield Kg/fed	100 seed weight (g)
Control	1640.00 b	604.21 a	1035.79 b	88.65 b
EM	2232.08 a	644.00 a	1588.08 a	88.76 b
Compomax (2)	1495.83 b	528.00 a	967.83 b	92.61 a
L.S.D at 0.05				
	246.61	n.s	302.40	3.58

2.2. Inoculation effect:

No significant effect was detected due to seed inoculation with any used biofertilizers compared to uninoculated seeds (Table 4b)

Table (4b): Effect of inoculation on yield of bean and its components

Rhizobia Inoculation	Total yield Kg/fed	Seed yield Kg/fed	Foliage yield Kg/fed	100 seed weight (g)
Inoculation	1782.50 a	578.62 a	1203.88 a	90.09 a
Uninoculation	1796.11 a	605.52 a	1190.59 a	89.93 a
L.S.D at 0.05	n.s	n.s	n.s	n.s

2.3. Nitrogen levels effect:

Nitrogen levels (Table 4c) show significant increases in total bean yield and foliage yield in response to full N dose treatments, as compared to ½ full N dose. The corresponding increases were 14.00 and 16.29 %, respectively.

Table (4c): Effect of Nitrogen levels on yield of bean and its components

Nitrogen levels	Total yield Kg/fed	Seed yield Kg/fed	Foliage yield Kg/fed	100 seed weight (g)
Full	1906.39 a	618.99 a	1287.40 a	91.36 a
½ full	1672.22 b	565.15 a	1107.07 b	88.65 a
L.S.D at 0.05	210.21	n.s	161.66	n.s

2.4. Interaction effect:

The interaction between the bio-fertilizer *i.e.* EM, Compomax (2) and Rhizobia inoculation exhibited a positive significant effect on seed yield only (Table 5a). In spite of that the highest values of seed yield were obtained by using seed inoculation with Rhizobia in combinations with EM, the percentage of increase of seed yield was (12.78 %) over the inoculated control compared with those obtained by Rhizobia inoculation combined with compomax (2) which recorded a decrease of 49.92 % compared to inoculated control treatment.

Table (5a): Effect of interaction between bio-fertilization and inoculation on yield of bean and its components

Bio-fertilization	Rhizobia Inoculation	Total yield Kg/fed	Seed yield Kg/fed	Foliage yield Kg/fed	100 seed weight (g)
Control	Inoculation	1750.00	621.10	1128.90	88.60
	Uninoculation	1530.00	587.32	942.68	88.70
EM	Inoculation	2314.17	700.47	1613.70	89.20
	Uninoculation	2150.00	587.53	1562.47	88.32
Compomax	Inoculation	1283.33	414.30	869.03	92.46
	Uninoculation	1708.33	641.70	1066.63	92.77
L.S.D at 0.05		n.s	162.58	n.s	n.s

Positive significant differences were detected due to the effect of interaction between biofertilizers *i.e.* EM and Compomax and nitrogen levels on seed yield and weight of 100 seed. The highest percentage increase of seed yield of 4.46 over the control treatment was due to the treatments of EM plus ½ N dose, while the increase of 100 seed weight was due to control plus full N dose treatment (Table 5b).

Table (5b): Effect of interaction between bio-fertilization and N-levels on yield of bean and its components

Bio-fertilization	Nitrogen levels	Total yield Kg/fed	Seed yield Kg/fed	Foliage yield Kg/fed	100 seed weight (g)
Control	full	1905.00	666.87	1238.13	93.66
	½ full	1375.00	541.55	833.45	83.64
EM	full	2172.50	591.40	1581.10	87.77
	½ full	2291.67	696.60	1595.07	89.75
Compomax (2)	full	1641.67	598.70	1042.97	92.65
	½ full	1350.00	457.30	892.70	92.57
L.S.D at 0.05		n.s	188.67	n.s	8.02

The interaction between the inoculation with Rhizobia and the different nitrogen levels showed a positive significant effect on total yield and foliage yield, Table (5c). The highest mean values of total yield and foliage yield were 2026.11 and 1384.27 kg fed⁻¹ respectively, which were obtained by the interaction between Rhizobia inoculation + full N dose treatment.

Table (5c): Effect of interaction between inoculation and N-levels on yield of bean and its components

Rhizobia inoculation	Nitrogen levels	Total yield Kg/fed	Seed yield Kg/fed	Foliage yield Kg/fed	100 seed weight (g)
Inoculation	Full	2026.11	641.84	1384.27	91.20
	½ full	1538.89	515.40	1023.49	88.97
Uninoculation	Full	1786.67	596.13	1190.53	91.52
	½ full	1805.56	614.90	1190.66	88.33
L.S.D at 0.05		382.01	n.s	323.69	n.s

Regarding to the interaction between source of biofertilizers, inoculation with Rhizobia and nitrogen levels, data in Table (5d) showed that the highest values of total yield and foliage yield were obtained by the interaction between biofertilizers with EM + *Rhizobia* + full N dose, where both recorded 2495 and 1793.67 kg/fed, respectively.

Table (5d): Effect of bio-fertilization, Inoculation and N-levels on yield of bean and its components

Treatments			Total yield Kg/fed	Seed yield Kg/fed	Foliage yield Kg/fed	100 seed weight (g)
Bio-fertilization	Rhizobia inoculation	N-levels				
Control	+	full	1950.00	672.40	1277.60	94.30
		½ full	1550.00	569.80	980.20	82.90
	-	full	1860.00	661.33	1198.67	93.02
		½ full	1200.00	513.30	686.70	84.38
EM	+	full	2495.00	701.33	1793.67	86.17
		½ full	2133.33	699.60	1433.73	92.23
	-	full	1850.00	481.47	1368.53	89.37
		½ full	2450.00	693.60	1756.40	87.27
Compomax (2)	+	full	1633.33	551.80	1081.53	93.12
		½ full	933.33	276.80	656.53	91.79
	-	full	1650.00	645.60	1004.40	92.18
		½ full	1768.67	637.80	1128.87	93.35
L.S.D at 0.05						
N x Inoculation x Bio-fertilization			460.36	n.s	411.56	n.s

3-Effect of bio-fertilization and N-levels on NPK uptake by bean plant:

3.1. Effect of biofertilizers on NPK uptake by bean plant:

Data illustrated in Table (6a) showed that spraying any of effective microorganisms (EM) and Compomax (2) both individually did not increased significantly N, P and K uptake by seed, while they increased significantly by foliage spray on bean plant compared with the control treatment. The highest values of N and K were obtained by using EM, while the highest values of P were obtained by using Compomax (2) as a foliar spray, since it recorded increments of 42.52, 117.43 and 78.3 % over the control treatment, respectively.

Table (6a): Effect of bio-fertilization on NPK-uptake (Kg fed⁻¹) in bean seeds and foliage

Bio-fertilization	Macronutrients uptake (Kg fed ⁻¹) by seed			Macronutrients uptake (Kg fed ⁻¹) by foliage		
	N	P	K	N	P	K
Control	20.65 a	4.23 a	5.02 a	8.16 b	4.01b	3.50b
EM	23.65 a	4.20 a	5.53 a	11.63a	4.55b	7.61a
Compomax (2)	23.27 a	3.62 a	4.53 a	7.60 b	7.15a	6.62a
L.S.D at 0.05	n.s	n.s	n.s	2.12	1.13	1.51

3.2. Effect of inoculation with Rhizobia on NPK-uptake by bean plant:

Data present in Table (6b) show that seed inoculation with Rhizobia recorded no significant increases of NPK uptake by seed and foliage of bean plant except for P-uptake by seed, which had significantly increased along with uninoculated treatment compared to the inoculated ones.

Table (6b): Effect of inoculation on NPK-uptake (Kg/fed) in bean seeds and foliage

Rhizobia inoculation	Macronutrients uptake (Kg fed ⁻¹) by seed			Macronutrients uptake (Kg fed ⁻¹) by foliage		
	N	P	K	N	P	K
Inoculation	21.92 a	3.76 a	4.94 a	9.34 a	5.50a	6.17a
Uninoculation	23.12 a	4.28 b	5.12 a	8.92 a	4.98a	5.66a
L.S.D at 0.05	n.s	0.451	n.s	n.s	n.s	n.s

3.3. Effect of nitrogen levels on NPK-uptake:

Data in Table (6c) showed that P-uptake by seed and N and P uptake by foliage of bean plant increased significantly with addition full N dose as a compared to ½ full dose. Since, the increases were about 20.6 % (P) for seeds and 14.45 % (N), 27.11 % (P) for foliage.

Table (6c): Effect of N-levels on NPK-uptake (Kg/fed) in bean seeds and foliage

Nitrogen levels	Macronutrients uptake (Kg fed ⁻¹) by seed			Macronutrients uptake (Kg fed ⁻¹) by foliage		
	N	P	K	N	P	K
full	23.80 a	4.39 a	5.28 a	9.74 a	5.86a	5.81a
½ full	21.24 a	3.64 b	4.77 a	8.51 b	4.61b	6.01a
L.S.D at 0.05	n.s	0.558	n.s	1.20	0.99	n.s

3.4. Interaction effects:

The effect of interactions between biofertilizer (EM and Compomax), inoculation with Rhizobia and nitrogen levels are presented in Tables (7a, 7b, 7c and 7d).

Table (7a): Effect of interaction between bio-fertilization and inoculation on NPK-uptake (Kg fed⁻¹) in bean seeds and foliage

Bio-fertilization	Rhizobia inoculation	Macronutrients uptake (Kg/fed) by seed			Macronutrients uptake (Kg fed ⁻¹) by foliage		
		N	P	K	N	P	K
Control	Inoculation	20.74	3.70	5.04	9.35	2.52	2.51
	Uninoculation	20.57	4.76	5.00	6.96	5.49	4.49
EM	Inoculation	27.25	4.69	6.20	12.35	3.25	7.82
	Uninoculation	20.04	3.71	4.87	10.90	5.86	7.41
Compomax	Inoculation	17.79	2.89	3.58	6.32	10.72	8.18
	Uninoculation	28.75	4.36	5.49	8.89	3.58	5.07
L.S.D at 0.05		6.61	1.20	1.40	n.s	2.41	2.59

Positive significant differences were recorded for the interaction between biofertilizer and inoculation with Rhizobia on NPK-uptake in seed and PK-uptake in foliage, while N-uptake in foliage was insignificant (Table 9a). The percentage increases were 39.77 (N) for seed by using Compomax (2) + inoculation with Rhizobia and 23.02 (K) for seed by using EM with +

inoculation with Rhizobia over the control treatment. While, the percentage increases were 325.4 (P) and 225.9 (K) for foliage by using Compomax + inoculation with Rhizobia over the control treatment.

Table (7b) show significant differences for the effect of interaction between biofertilizer and nitrogen levels on N-uptake in seed and PK-uptake in foliage, while PK-uptake in seed and N-uptake in foliage were insignificant. The percentage increases were 15.92 (N) for seed and 92.7 (P) for foliage by using Compomax with full N dose compared to the control treatment and 248.73 (K) for foliage by using EM with ½ full N dose over the control treatment.

Table (7b): Effect of interaction between bio-fertilization and N-levels on NPK-uptake (Kg fed⁻¹) in bean seeds and foliage

Bio-fertilization	Nitrogen levels	Macronutrients uptake (Kg fed ⁻¹) by seed			Macronutrients uptake (Kg fed ⁻¹) by foliage		
		N	P	K	N	P	K
Control	full	23.05	4.86	5.50	8.28	4.93	4.25
	½ full	18.26	3.59	4.54	8.03	3.09	2.75
EM	full	21.64	4.08	5.32	12.09	3.17	5.64
	½ full	25.65	4.32	5.75	11.16	5.94	9.59
Compomax	full	26.72	4.23	5.02	8.36	9.50	7.55
	½ full	19.81	3.02	4.04	6.34	4.80	5.70
L.S.D at 0.05		7.78	n.s	n.s	n.s	2.12	2.39

The effect of interaction between the inoculation with Rhizobia and nitrogen levels revealed a positive significant effect on PK-uptake for seed and P-uptake for foliage, while N-uptake in seed and NK-uptake in foliage was insignificant (Table 7c). The percentage increases were 2.77 (P), 10.78 (K) for seed and 50.64 (P) for foliage, which were obtained by the interaction between Rhizobia inoculation with full N dose.

Table (7c): Effect of interaction between inoculation and N-levels on NPK-uptake (Kg fed⁻¹) in bean seeds and foliage

Rhizobia inoculation	Nitrogen levels	Macronutrients uptake (Kg fed ⁻¹) by seed			Macronutrients uptake (Kg fed ⁻¹) by foliage		
		N	P	K	N	P	K
Inoculation	full	24.80	4.45	5.55	10.03	7.05	6.45
	½ full	19.04	3.07	4.32	8.65	3.94	5.89
Uninoculation	full	22.81	4.33	5.01	9.45	4.68	5.18
	½ full	23.44	4.22	5.23	8.38	5.27	6.13
L.S.D at 0.05		n.s	0.92	1.14	n.s	1.88	n.s

Owing to the effect of the triple interaction between source of biofertilizers, inoculation with Rhizobia and nitrogen levels, data in Table (7d) showed that the NPK-uptake in seed were insignificant, while the positive significant results of nitrogen uptake by foliage were obtained when using

EM + inoculation by Rhizobia and full N dose. The highest values of P-uptake by foliage were obtained with the use of Compomax (2) + inoculation with Rhizobia and full N dose and the highest values of K-uptake by foliage were obtained by EM + uninoculation and ½ full N doses treatments.

Table (7d): Effect of bio-fertilization, inoculation and N-levels on NPK-uptake (Kg fed⁻¹) in bean seeds and foliage

Treatments			Macronutrients uptake (Kg fed ⁻¹) by seed			Macronutrients uptake (Kg fed ⁻¹) by foliage		
Bio-fertilization	Inoculation	N-levels	N	P	K	N	P	K
Control	+	full	24.21	4.37	5.51	7.92	2.30	1.79
		½ full	17.26	3.02	4.56	10.78	2.74	3.23
	-	full	21.89	5.36	5.49	8.63	7.55	6.71
		½ full	19.25	4.16	4.52	5.29	3.43	2.27
EM	+	full	25.81	4.84	6.45	13.09	3.05	7.17
		½ full	28.68	4.55	5.95	11.61	3.44	8.46
	-	full	17.48	3.32	4.19	11.09	3.28	4.11
		½ full	22.61	4.09	5.55	10.71	8.43	10.71
Compomax (2)	+	full	24.39	4.14	4.69	9.08	15.79	10.38
		½ full	11.18	1.63	2.46	3.55	5.65	5.97
	-	full	29.05	4.33	5.36	8.64	3.21	4.72
		½ full	28.45	4.40	5.61	9.14	3.95	5.42
L.S.D at 0.05								
N x Inoculation x Bio-fertilization			n.s	n.s	n.s	2.01	1.21	1.36

4- Effect of bio-fertilization and N-levels on soil available NPK at 45 days and after bean harvest:

4.1. Effect of biofertilizers on soil available NPK:

In respect to available NPK amounts remained in soil after both 45 days and after bean harvesting, data illustrated in Table (8a) showed that spraying both effective microorganisms (EM) and Compomax both individually increased significantly available N and K in soil after 45 days and P, K after harvest, while available-P after 45 days and available N after harvest were insignificant. The highest values of available-N amount after 45 days were obtained by using Compomax as a foliar spray and the highest available- K values were obtained by using EM, since it recorded increments of 191.54 and 27.92 % over the control treatment, respectively. The highest value available-P after harvest was obtained by using EM as a foliar spray, since it recorded in increment of 27.14 % over the control treatment, respectively.

Table (8a): Effect of bio-fertilization on soil available NPK (ppm) at 45 days and after bean harvesting

Bio-fertilization	Available NPK (ppm) at 45 days			Available NPK (ppm) at harvest		
	N	P	K	N	P	K
Control	65.83a	8.17a	136.18b	138.88a	2.80b	77.22a
EM	169.87b	5.96a	174.20a	133.17a	3.56a	61.39b
Compomax (2)	191.92c	9.76a	143.78ab	145.75a	3.03b	65.93b
L.S.D at 0.05	19.02	n.s	35.96	n.s	0.33	9.93

4.2. Effect of inoculation with rhizobia on soil available NPK:

Data present in Table (8b) show significant increases due to the inoculation with Rhizobia on available-N at 45 days and on available NPK after harvesting. While both available P and K exhibited positive significant responses to this treatment at 45 days period. These results were in comparison with those obtained by control treatments.

Table (8b): Effect of inoculation on NPK (ppm) at 45 days and harvest of the studied soil

Rhizobia inoculation	Available NPK (ppm) at 45 days			Available NPK (ppm) at harvest		
	N	P	K	N	P	K
Inoculation	145.78a	6.61a	134.09a	135.17a	2.99a	70.36a
Uninoculation	139.17a	9.32b	168.68b	143.37a	3.27a	66.01a
L.S.D at 0.05	n.s	2.15	26.79	n.s	n.s	n.s

4.3. Effect of nitrogen levels on soil available-NPK:

Data in Table (8c) show that available NPK after 45 days and after bean harvesting did not increased significantly due to the use different N levels except for P-available after 45days, which, increased significantly with addition full N dose as compared to ½ full doses. Since, the increase value was 47.98 % (P).

Table (8c): Effect of N-levels on soil available NPK (ppm) at 45 days and after bean harvesting

Nitrogen levels	Available NPK (ppm) at 45 days			Available NPK (ppm) at harvest		
	N	P	K	N	P	K
Full	140.22a	9.50a	156.43a	133.22a	3.08a	68.37a
½ full	144.72a	6.42b	146.34a	145.31a	3.18a	67.99a
L.S.D at 0.05	n.s	1.70	n.s	n.s	n.s	n.s

4.4. Interaction effects:

The effect of interaction between biofertilizer, inoculation with Rhizobia and nitrogen are presented in Tables (9a, 9b, 9c and 9d).

The interaction between biofertilizer and inoculation with rhizobia on soil available NPK after 45 days and after harvesting had no significant effect on all treatment parameters, Table (9a).

Table (9a): Effect of Interaction between bio-fertilization and inoculation on available NPK (ppm) at 45 days and after bean harvesting

Bio-fertilization	Rhizobia inoculation	Available NPK (ppm) at 45 days			Available NPK (ppm) at harvest		
		N	P	K	N	P	K
Control	Inoculation	71.17	6.28	133.90	132.67	2.91	73.38
	Uninoculation	60.50	10.06	138.45	145.10	2.69	81.05
EM	Inoculation	181.33	5.73	141.70	142.00	3.44	65.63
	Uninoculation	158.00	6.19	206.70	124.33	3.67	57.15
Compomax (2)	Inoculation	184.83	7.80	126.68	130.83	2.62	72.05
	Uninoculation	199.00	11.72	160.88	160.67	3.44	59.82
L.S.D at 0.05		n.s	n.s	n.s	n.s	n.s	n.s

Table (9b) indicated that there were positive significant differences for available PK after 45 days and available-N after bean harvesting in response to the interaction between biofertilizer and N-levels, while N-available after 45 days and PK-available after harvesting were insignificant. The percentage increases were 17.91 (P) due to the use of Compomax combined with full N dose and 49.01 (K) for the use of EM + ½ full N dose over the control treatment after 45 days, while the highest value of available-N after harvest was obtained by using the control treatment + ½ full N dose (156.77).

Table (9b): Effect of interaction between bio-fertilization and N-levels on soil NPK (ppm) at 45 days and bean harvesting

Bio-fertilization	Nitrogen levels	Available NPK (ppm) at 45 days			Available NPK (ppm) at harvest		
		N	P	K	N	P	K
Control	Full	65.17	10.61	141.05	121.00	2.79	79.73
	½ full	66.50	5.74	131.30	156.77	2.81	74.70
EM	Full	177.00	5.40	152.75	127.33	3.42	60.03
	½ full	162.33	6.53	195.65	139.00	3.70	62.75
Compomax (2)	Full	178.50	12.51	175.50	151.33	3.04	65.33
	½ full	205.33	7.01	112.07	140.17	3.03	66.53
L.S.D at 0.05		n.s	6.19	52.12	27.49	n.s	n.s

The interaction between inoculation with Rhizobia and nitrogen levels on soil available NPK after 45 days and at harvest did not significantly increase (Table 9c).

Regarding to the triple interaction between source of biofertilizers, inoculation with Rhizobia and nitrogen levels, data in Table (9d) showed that the soil available-NPK after 45 days and after bean harvesting had not significantly increased except for available-PK after 45 days, which, had significantly increased. While the highest value of available-P was obtained by the use of Compomax + uninoculation with Rhizobia + full N dose (16.61 ppm), the highest value of available-K (226.2 ppm) was obtained with EM + uninoculation + ½ full N doses treatment.

Table (9c): Effect of interaction between inoculation and N-levels on NPK (ppm) at 45 days and harvest of the studied soil

Rhizobia inoculation	Nitrogen levels	Available NPK (ppm) at 45 days			Available NPK (ppm) at harvest		
		N	P	K	N	P	K
Inoculation	Full	141.56	5.57	133.47	131.78	3.06	70.59
	½ full	150.00	5.64	134.72	138.56	2.93	70.12
Uninoculation	Full	138.89	11.43	179.40	134.67	3.10	66.14
	½ full	139.44	7.21	157.96	152.07	3.43	65.87
L.S.D at 0.05		n.s	n.s	n.s	n.s	n.s	n.s

Table (9d): Effect of bio-fertilization, inoculation and N-levels on soil available- NPK (ppm) at 45 days and after bean harvesting

Treatments			Available NPK (ppm) at 45 days			Available NPK (ppm) at harvest		
Bio-fertilization	Inoculation	N-levels	N	P	K	N	P	K
Control	+	full	70.00	8.06	149.50	113.67	2.78	75.33
		½ full	72.33	4.51	118.30	151.67	3.03	71.43
	-	full	60.33	13.15	132.60	128.33	2.79	84.13
		½ full	60.67	6.96	144.30	161.37	2.58	77.97
EM	+	full	155.67	6.26	118.30	143.00	3.22	64.33
		½ full	176.00	3.23	165.10	145.00	3.29	66.93
	-	full	167.33	4.53	187.20	110.67	3.24	55.73
		½ full	148.67	7.85	226.20	138.00	4.11	58.57
Compomax (2)	+	full	168.00	8.41	132.60	137.67	2.78	72.10
		½ full	201.67	7.20	120.77	124.00	2.46	72.00
	-	full	189.00	16.61	218.40	165.00	3.29	58.57
		½ full	209.00	6.83	103.37	156.33	3.60	61.07
L.S.D at 0.05								
N x Inoculation x Bio-fertilization			n.s	3.94	31.97	n.s	n.s	n.s

5 - Effect of bio-fertilization and N-levels on total bacterial count, fungi, actinomycetes and total nitrogen fixers at 45 days and after harvesting of the studied soil:

5.1. Effect of biofertilizers:

Data recorded in Table (10a) show that soil micro-organisms counts were significantly affected by addition of effective microorganisms (EM) individually compared to the control treatment. The highest values of total bacterial count, fungi and total nitrogen fixers after 45 days were obtained by using effective microorganisms (EM), since it recorded increments of 154.79, 35.59 and 233.33 % over the control treatment, respectively.

Regarding to the effect of Compomax (used as spray on plant and soil) on soil micro-organisms counts, data in Table (10a) show that the significant percentage increases were 15.25 and 109.52 % for fungi and total nitrogen fixers over the control treatment, respectively.

Table (10a): Effect of bio-fertilization on total bacterial count, fungi, actinomycetes and total nitrogen fixers at 45 days of the studied soil

Bio-fertilization	Bacterial count x10 ⁶	Fungi x10 ³	Actinomycetes x10 ³	Nitrogen fixers x10 ³
Control	3.34b	14.75a	18.25 a	5.25 a
EM	8.51a	20.00b	18.63 a	17.50 b
Compomax (2)	3.53b	17.00c	17.00 b	11.00 c
L.S.D at 0.05	0.64	1.91	1.15	1.21

5.2. Effect of inoculation with rhizobia:

Data present in Table (10b) show that, soil micro-organisms counts had significantly increased due to Rhizobia inoculation except for fungi, which had significantly increased on uninoculated treatment compared to the inoculated ones. The highest values of total count, actinomycetes and total nitrogen fixers after 45th days in the inoculated treatments had increased by 66.23, 13.37 and 121.43 %, respectively compared to uninoculated treatment.

Table (10b): Effect of inoculation on total bacterial count, fungi, actinomycetes and total nitrogen fixers at 45 days of the studied soil

Rhizobia Inoculation	Bacterial count x10 ⁶	Fungi x10 ³	Actinomycetes x10 ³	Nitrogen fixers x10 ³
Inoculation	6.40a	16.00a	19.08 a	15.50 a
Uninoculation	3.85b	18.50b	16.83 b	7.00 b
L.S.D at 0.05	0.42	1.08	0.78	1.15

5.3. Effect of nitrogen levels:

Data in Table (10c) showed that total bacterial count increased significantly by the addition of full N dose as a compared to ½ full N dose, while fungi showed significant increases with the use of the ½ N full dose treatment, as compared to N full dose. Since, the increase values were 17.62 % (total bacterial count) and 17.94 % (fungi).

Table (10c): Effect of Nitrogen levels on total bacterial count, fungi, actinomycetes and total nitrogen fixers at 45 days of the studied soil

Nitrogen levels	Bacterial count x10 ⁶	Fungi x10 ³	Actinomycetes x10 ³	Nitrogen fixers x10 ³
full	5.54a	15.83a	17.67 a	10.83 a
½ full	4.71b	18.67b	18.25 a	11.67 a
L.S.D at 0.05	0.29	1.19	n.s	n.s

5.4. Interaction effects:

The effect of interaction between biofertilizer (EM and Compomax), inoculation with Rhizobia and nitrogen are presented in Tables (11a, 11b, 11c and 11d).

Table (11a), detected that interaction between biofertilizer and inoculation with Rhizobia had positively affected the soil micro-organisms counts after 45 days. The highest values of total bacterial count and total nitrogen fixers were obtained by Rhizobia inoculation in combination with EM. The percentage increases were 88.93 and 166.67 % over the inoculated control. While the highest values of fungi count was achieved with the treatment of EM without Rhizobia inoculation. The percentage increase was 68.97 % over the control without Rhizobia inoculation. Also data in Table (11a) revealed that both fungi and total nitrogen fixers were treated with Rhizobia inoculation + Compomax had achieved higher count than those obtained by the control treatment with Rhizobia inoculation. The corresponding percentage increases were 16.67 and 50.

Table (11a): Effect of interaction between bio-fertilization and Rhizobia inoculation on total bacterial count, fungi, actinomycetes and total nitrogen fixers at 45 days of the studied soil

Bio-fertilization	Rhizobia inoculation	Bacterial count x10 ⁶	Fungi x10 ³	Actinomycetes x10 ³	Nitrogen fixers x10 ³
Control	Inoculation	5.33	15.00	23.00	9.00
	Uninoculation	1.35	14.50	13.50	1.50
EM	Inoculation	10.07	15.50	15.75	24.00
	Uninoculation	6.95	24.50	21.50	11.00
Compomax (2)	Inoculation	3.80	17.50	16.50	13.50
	Uninoculation	3.25	16.50	15.50	8.50
L.S.D at 0.05		0.98	2.73	1.81	2.37

Positive significant differences were recorded for the interaction between biofertilizer and nitrogen levels on soil micro-organisms counts after 45 days (Table 11b). The percentage increases over the control treatment after 45 days were 263.48, 53.85, 20 and 485.71 for total bacterial count, fungi, actinomycetes and total nitrogen fixers, respectively due to EM + full N dose.

Table (11b): Effect of interaction between bio-fertilization and N-levels on total bacterial count, fungi, actinomycetes and total nitrogen fixers at 45 days of the studied soil

Bio-fertilization	Nitrogen levels	Bacterial count x10 ⁶	Fungi x10 ³	Actinomycetes x10 ³	Nitrogen fixers x10 ³
Control	full	2.82	13.00	17.50	3.50
	½ full	3.87	16.50	19.00	7.00
EM	full	10.25	20.00	21.00	20.50
	½ full	6.77	20.00	16.25	14.50
Compomax (2)	full	3.55	14.50	14.50	8.50
	½ full	3.50	19.50	19.50	13.50
L.S.D at 0.05		0.84	2.90	1.75	2.00

The interaction between the inoculation with Rhizobia and nitrogen levels doses had a positive significant effect on soil micro-organisms counts,

except fungi count after 45 days were insignificant (Table 11c). The percentage increases were 32.93 (actinomycetes), 149.93 (total nitrogen fixers), which, were obtained by interaction between Rhizobia inoculation + ½ full N dose treatment. While, the highest increase of total bacterial counts was 174.32 %, which, were obtained by interaction between Rhizobia inoculation + full N doses.

Table (11c): Effect of interaction between inoculation and N-levels on total bacterial count, fungi, actinomycetes and total nitrogen fixers at 45 days of the studied soil

Rhizobia inoculation	Nitrogen levels	Bacterial count x10 ⁶	Fungi x10 ³	Actinomycetes x10 ³	Nitrogen fixers x10 ³
Inoculation	full	8.12	14.33	17.33	14.33
	½ full	4.68	17.67	20.83	16.67
Uninoculation	full	2.98	17.33	18.00	7.33
	½ full	4.74	19.67	15.67	6.67
L.S.D at 0.05		0.59	n.s	1.31	1.72

Table (11d): Effect of bio-fertilization, inoculation and N-levels on total bacterial count, fungi, actinomycetes and total nitrogen fixers at 45 days of the studied soil

Treatments			Bacterial count x10 ⁶	Fungi x10 ³	Actinomycetes x10 ³	Nitrogen fixers x10 ³
Bio-fertilization	Inoculation	N-levels				
Control	+	full	4.67	12.00	21.00	6.00
		½ full	6.00	18.00	25.00	12.00
	-	full	0.97	14.00	14.00	1.00
		½ full	1.73	15.00	13.00	2.00
EM	+	full	16.60	16.00	18.00	28.00
		½ full	3.53	15.00	13.50	20.00
	-	full	3.90	24.00	24.00	13.00
		½ full	10.00	25.00	19.00	9.00
Compomax (2)	+	full	3.10	15.00	13.00	9.00
		½ full	4.50	20.00	24.00	18.00
	-	full	4.00	14.00	16.00	8.00
		½ full	2.50	19.00	15.00	9.00
L.S.D at 0.05						
N x Inoculation x Bio-fertilization			0.54	n.s	0.97	1.21

Regarding to the triple interaction between source of biofertilizers, inoculation with Rhizobia and nitrogen levels (biofertilizers & inoculation & N-levels), data in Table (11d) showed that the micro-organisms counts in soil after 45 days increased significantly except for fungi which had insignificantly increased. The positive results of total bacterial count (16.6 x10⁶) and total nitrogen fixers (28 x10³) were due to the application of EM + inoculation by Rhizobia and full N dose treatment. While the highest count of actinomycetes (25 x10³) was obtained by the use of control + inoculation with Rhizobia and ½ full N doses treatments.

6. Effect of bio-fertilization and N-levels on soil total bacterial count, fungi, actinomycetes and total nitrogen fixers after bean harvesting of the studied soil:

6.1. Effect of biofertilizers:

Data recorded in Table (12a) show that soil micro-organisms counts increased significantly by the addition of effective microorganisms (EM) individually compared with the control treatment. The highest values of total bacterial count, fungi, actinomycetes and total nitrogen fixers after bean harvesting were obtained by using effective microorganisms (EM), since it recorded increments of 373.85, 65.56, 56.94 and 283.33 % over the control treatment, respectively.

With view of the effect Compomax (as spray on plant and soil) on soil micro-organisms counts, data in Table (14a) show that the significant percentage increases were 32.45 and 93.33 % for fungi and total nitrogen fixers over the control treatment, respectively.

Table (12a): Effect of bio-fertilization on soil total bacterial count, fungi, actinomycetes and nitrogen fixers after harvesting

Bio-fertilization	Bacterial count x10 ⁸	Fungi x10 ³	Actinomycetes x10 ⁴	Nitrogen fixers x10 ³
Control	0.65 a	7.55 a	18.00 b	7.50 a
EM	3.08 b	12.50 b	28.25 a	28.75 b
Compomax (2)	0.53 a	10.00 c	14.25 b	14.50 c
L.S.D at 0.05	0.18	2.37	3.98	2.03

6.2. Effect of inoculation with Rhizobia:

Data presented in Table (12b) show that the soil micro-organisms counts had significantly increased with uninoculation with Rhizobia treatment except for actinomycetes which was insignificantly increased, the values of total bacterial count, fungi and total nitrogen fixers after bean harvesting increased by 146.34, 14.68 and 7.16 %, respectively, compared with the Rhizobia inoculated treatment.

Table (12b): Effect of inoculation on soil total bacterial count, fungi, actinomycetes and nitrogen fixers after bean harvesting

Rhizobia Inoculation	Bacterial count x10 ⁸	Fungi x10 ³	Actinomycetes x10 ⁴	Nitrogen fixers x10 ³
inoculation	0.82 a	9.33 a	19.50 a	16.33 a
Uninoculation	2.02 b	10.70 b	20.83 a	17.50 b
L.S.D at 0.05	0.09	1.32	n.s	1.00

6.3. Effect of nitrogen levels:

Data in Table (12c) showed that total bacterial count and total nitrogen fixers bacteria increased significantly by the application of ½ full N dose compared to full N dose while actinomycetes showed significant increases due to the N full dose treatments compared to ½ N full dose. Since, the corresponding increases values were 17.69 % (total bacterial count), 20.68 (total nitrogen fixers) and 14.18 % (actinomycetes).

Table (12c): Effect of Nitrogen levels on total bacterial count, fungi, actinomycetes and nitrogen fixers after bean harvesting

Nitrogen levels	Bacterial count $\times 10^6$	Fungi $\times 10^3$	Actinomycetes $\times 10^4$	Nitrogen fixers $\times 10^3$
full	1.30 a	10.03 a	21.50 a	15.33 a
½ full	1.53 b	10.00 a	18.83 b	18.50 b
L.S.D at 0.05	0.13	n.s	1.63	0.86

6.4. Interaction effects:

The effects of interaction between biofertilizer inoculation with Rhizobia and nitrogen are presented in Tables (13a, 13b, 13c and 13d).

Significant differences were recorded due to the interaction effect between biofertilizer and inoculation with Rhizobia on soil micro-organisms counts after bean harvesting. The highest values of total count, actinomycetes and total nitrogen fixers were obtained by uninoculated treatment in combinations with EM. The percentage increases were 614.29, 217.39 and 560 % over the uninoculated control.

Table (13a): Effect of interaction between bio-fertilization and inoculation on soil total bacterial count, fungi, actinomycetes and nitrogen fixers after harvesting

Bio-fertilization	Rhizobia inoculation	Bacterial count $\times 10^6$	Fungi $\times 10^3$	Actinomycetes $\times 10^4$	Nitrogen fixers $\times 10^3$
Control	Inoculation	0.60	7.00	24.50	10.00
	Uninoculation	0.70	8.10	11.50	5.00
EM	Inoculation	1.15	11.50	20.00	24.50
	Uninoculation	5.00	13.50	36.50	33.00
Compomax (2)	Inoculation	0.70	9.50	14.00	14.50
	Uninoculation	0.35	10.50	14.50	14.50
L.S.D at 0.05		0.24	n.s	5.37	2.73

Table (13b): Effect of interaction between bio-fertilization and N-levels on soil total bacterial count, fungi, actinomycetes and nitrogen fixer after bean harvesting

Bio-fertilization	Nitrogen levels	Bacterial count $\times 10^6$	Fungi $\times 10^3$	Actinomycetes $\times 10^4$	Nitrogen fixers $\times 10^3$
Control	full	0.50	6.60	25.50	6.00
	½ full	0.80	8.50	10.50	9.00
EM	full	2.90	13.00	23.00	25.00
	½ full	3.25	12.00	33.50	32.50
Compomax (2)	full	0.50	10.50	16.00	15.00
	½ full	0.55	9.50	12.50	14.00
L.S.D at 0.05		n.s	n.s	5.03	2.60

Table (13b) revealed that positive significant differences were detected owing to the interaction effect between biofertilizer and nitrogen levels on both actinomycetes and total nitrogen fixers after bean harvesting, while the effect on both total bacterial count and fungi after harvesting were insignificant. The percentage increases were 219.05 and 261.11 in response to EM + ½ full N dose treatment over the control treatment.

The interaction effect between the inoculation with Rhizobia and nitrogen levels doses are shown in Table (13c). A positive significant interaction effect on both actinomycetes and total nitrogen fixers after bean harvesting were detected, while both total bacterial count and fungi after bean harvesting were insignificantly affected. The percentage increase were 4.76 due to the interaction between Rhizobia inoculation with full N dose and 1.85, which, was obtained by the interaction between Rhizobia inoculation + ½ full N doses treatment, respectively.

Table (13c): Effect of interaction between inoculation and N-levels on soil total bacterial count, fungi, actinomycetes and nitrogen fixers after bean harvesting

Rhizobia inoculation	Nitrogen levels	Bacterial count x10 ⁶	Fungi x10 ³	Actinomycetes x10 ⁴	Nitrogen fixers x10 ³
Inoculation	full	0.67	8.67	22.00	14.00
	½ full	0.97	10.00	17.00	18.67
Uninoculation	full	1.93	11.40	21.00	16.67
	½ full	2.10	10.00	20.67	18.33
L.S.D at 0.05		n.s	n.s	3.11	1.60

Table (13d): Effect of bio-fertilization, inoculation and N-levels on soil total bacterial count, fungi, actinomycetes and nitrogen fixers after bean harvesting

Treatments			Bacterial count x10 ⁶	Fungi x10 ³	Actinomycetes x10 ⁴	Nitrogen fixers x10 ³
Bio-fertilization	Inoculation	N-levels				
Control	+	full	0.30	5.00	34.00	8.00
		½ full	0.90	9.00	15.00	12.00
	-	full	0.70	8.20	17.00	4.00
		½ full	0.70	8.00	6.00	6.00
EM	+	full	1.10	12.00	20.00	20.00
		½ full	1.20	11.00	20.00	29.00
	-	full	4.70	14.00	26.00	30.00
		½ full	5.30	13.00	47.00	36.00
Compomax	+	full	0.60	9.00	12.00	14.00
		½ full	0.80	10.00	16.00	15.00
	-	full	0.40	12.00	20.00	16.00
		½ full	0.30	9.00	9.00	13.00
L.S.D at 0.05						
N x Inoculation x Bio-fertilization			0.13	n.s	3.08	n.s

Regarding to the triple interaction between source of biofertilizers, inoculation with Rhizobia and nitrogen levels, data in Table (13d) showed that the micro-organisms counts in soil after bean harvesting increased significantly except for both fungi and total nitrogen fixers which had insignificantly increased. The positive results of total bacterial count (5.3×10^5) and actinomycetes (47×10^4) were obtained when using EM combined with Rhizobia uninoculation and $\frac{1}{2}$ full N dose treatment.

DISCUSSION

The use of biofertilizers and nitrogen fixing bacteria in bean cultivation are well documented. Fanous *et al.*, (2003) reported that biological inocula of EM and N-fixing bacteria as biofertilizer added to bean gave higher rates of nutrient uptake specially nitrogen element. They also recorded that EM enhanced the peanut growth. The same results mentioned by Abd E I-Rasoul *et al.*, (2003). Saleh *et al.* (2000) revealed that Rhizobia inoculation increased significantly the number and dry weight of nodules of faba bean. Alagwadi and Gaur (1988) found that single inoculation with Rhizobia to bean increased the nodulation. Also total N_2 fixed (shoot + seed) was higher in dual inoculation with *Bradyrhizobium jaboricum* combined with *Azospirillum brasiliense* than in Rhizobia solely (Galal, 1997). In present work as compomax (2) contain *Azospirillum*, it was obvious that the inoculation with *Bradyrhizobium jaboricum* in presence of Compomax creates a synergism interrelationship which affected positively the number of nodule showed in treatment of compomax + Rhizobia + half dose of nitrogen.

Abd El-Rasoul *et al* (2003) reported that the use of EM enhanced yield of bean due to greater rates of photosynthesis and dry matter accumulation. They recorded that the high response of bean seed yield to EM application can be explained on the basis that EM increases germination, stimulates the photosynthetic process and enhances the enzymes activities. Microorganisms of EM and N-fixers have a beneficial role in emergence speed of seedling, leaf photosynthesis and diseases resistance and herbs which consequently, produces healthy growth and wealthy yield, Fanous *et al.* (2003). Primavesi (1999) added that EM improved the quality and quantity of crop production. Also results showed that Rhizobia combined with EM was superior and enhanced total and seed yield, which could be resulted from the role of *Rhizobium leguminosarum* that increased the growth and N_2 -fixation by faba bean plants (Abd El-Rasoul *et al.*, 2003). In addition, Saleh *et al.* (2000) reported that inoculation with Rhizobia increased faba bean seed by 8.8% compared to uninoculated treatments.

Due to NPK uptake Abd EL-Rasoul *et al.*(2004), indicated that spraying both EM and nitrogen fixing biofertilizers had increased significantly N, P and K in bean seeds and straw over the control treatments. These results in harmony with those obtained by the present work. Fanous *et al.* (2003) came in line with the results of the current work and reported that EM and compomax contain nitrogen fixing bacteria, yeasts, lactic acid bacteria, photosynthetic bacteria and fungi that multiply and encourage bean plants for uptake nutrients.

With regard to the available NPK in soil, Azam *et al.* (1988) who reported that the atmospheric N₂ –fixed in the soil after the addition diazotrophs is not immediately available for plant uptake because of its immobilization by the microbial biomass of the soil. In a pot experiment executed by Silsbury, (1989) the acetylene reduction assay revealed that chickpea cultivar had accumulated in their shoot about the same amount of N from fixation and in the present work the treatment of Rhizobia + full dose of nitrogen had the second rank in keeping of nitrogen in soil after the application of full N dose which resulted in a large amount of fixed nitrogen into soil.

Shabaev (1986) supposed that N fertilizers increase the nonsymbiotic nitrogen fixation level due to the enhancement of the photosynthetic activity of higher plants, which resulted in an increase of carbon-containing root excretion serving as energy sources for heterotrophic nitrogen-fixing microorganisms, this opinion is confirmed by Sirota (1982) and Umarova (1982). Also microorganisms in Compomax are nitrogen fixing bacteria (Compomax showed superior in N soil content in the present work, in the same time, N₂ fixing bacteria are considered as plant growth promoting regulators (PGPR), which, produces phyto-hormones, siderophores and hydrogen cyanide, that enhancing nutrient uptake and plant growth.

In case of the response of soil microorganisms to the use of EM, the nitrogen fixers besides Rhizobia in bean cultivation, results indicated that the application of EM increased the densities of total bacteria; total nitrogen fixing bacteria and fungi which scored the largest numbers along with EM treatments. Also the results of the current work showed that total count of bacteria decreased at harvest.

Kozyakov *et al.*(2002) indicated that increasing amount of microbial biomass have often been found in rhizosphere after nitrogen addition they explained that by increased amount of root exudation with increasing nitrogen levels. Soderberg (2004) reported the contradictory results when said that plant growth decreased and presumably also root growth when nitrogen levels were increased. Also mentioned that, biomass (in term of total counts of bacteria) and activity do not always have to be correlated furthermore, keeping in mind that plant species and growth conditions were different from a study and other.

In conclusion, it is quite evident in the present study that the use of EM, Compomax and Rhizobia inoculation in bean cultivation may become more beneficial than the use of Rhizobia inoculation only. This in turn that these biofertilizers beside Rhizobia inoculation can save about 50% of mineral nitrogen amounts required for bean which consequently become environmentally more safe.

REFERENCES

- Abd El-Rasoul, Sh. M., Mona Hanna, H.; Elham . Aref and F.M. Ghazal (4004): " Cyanobacteria and effective microorganisms as possible biofertilizer in wheat production" J. Agric. Sci. Mansoura Univ.29 (5):2783-2797.
- Abd El-Rasoul, Sh. M.; S. M. El-Saadany, M. M. Hassan and Amira, A. Salem (2003): " Comparison between the influence of some biofertilizers or effective microorganisms and organic or inorganic fertilizers on wheat grown on sandy soil:" Egypt J. Appl. Sci., 18(8)388-408.
- Alla El-Din, M .N. and M. E. Hassan (1985). ICARDA/IIAO NVP six year report, 1979/1985
- Alagwadi, A. R. and A. C. Gaur (1988). Associative effect of Rhizobium and phosphate solubilizing bacteria on the yield and nutrient uptake of chickpea. Plant and Soil 105, 241.
- Antoun, H.; C.J. Bcauchamp; N. Goussard; R. Chabat and R. Latande (1998). Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes; Effect on radishes (*Raphsmus sativus* L.) . Plant and Soil, 204:57-67
- Azam, F.; K. A. Malik and M. I. Sajjad (1986). Uptake by wheat plant and turnover within soil fraction of residual N from legumin plant material and inorganic fertilizer. plant and Soil, 95:97-108
- Bridson, E.Y.(1978). Natural and synthetic culture media. In: CRC Handbook series in Nutrition and food, Section G, vol.III, miloslav Recheigl, Jr.Ed. CRC press Inc., Clevel and USA.
- Dileep-Kumar, B.S.(1999). Fusarial with suprcssion and crop improvement through two rhizobacterial strain in chickpea growing in soil infected with *Fusarium oxysporum* F.Sp ciceris. Biol. Fert. Soils.29:87-91.
- Fanous, N. E.; Sh. M. Abd El-Rasoul and M.M. Hassan (2003): " Sustainable peanut production through integration between Bio, organic and chemical fertilizers". J. Agric Sci. Mansoura Univ. 28(5): 4233-4248.
- Galal, Y.G. M. (1997). Dual inoculation with strains of *Bradyrhizobium japonicum* and *Azospirillum brasilense* to improve growth and biological nitrogen fixation of soybean (*Glycine max* L.). Biol. Fertil. Soils 24,317.
- Jackson, M.L. (1973). Soil Chemical Analysis. Prentice-Hall Ins., Engle Wood Cliffs, U.S.A.
- Kozyakov, Y. S.V. Siniakina; J. Ruehlmann; G. Domanski and K. Stahr (2002). Effect of nitrogen fertilization on below-ground carbon allocation in lettuce. Journal of the Soinece of Food and Agriculture 82, 1432-1441
- Küster, E. and Williams, S. T. (1966). In: Methods for study the ecology of soil micro-organisms. Parkinson, d.; Gvay, T.R. G. and Williams, S.T. (1971): IBP Handbook No.19:1971.
- Martin, P.(1950).Use of acid rose Bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci., 69:215-2332

- Mussa, S.A.I.; M.M.Hanna and F.M.Ghazal (2003). Effect of cyanobacteria-wheat association on wheat growth and yield components. *Egypt J. Biotechnol.* 14:164-174.
- Primavesi, A.M.(1999). Comparison of the influence of EM and other bacterial and mineral products on field beans. Sixth International conference on Kyuesi. Nature Farming Pretoria, Pretoria, south Africa, 23-31 October.
- Salim, S. A., M. A. El-Deeb and A. A. Ragab (2000). Response of faba bean (*Vicia faba* L.) to Rhizobium inoculation as affected by nitrogen and phosphorus fertilization. *Bull. Fac. Agric., Cairo Univ.*, 51:17-30.
- Shabaev, V.P.(1986). The effect of cropping and fertilizer nitrogen rates on nitrogen balance in soil. *Plant and Soil* 91, 249-256.
- Silsbee, J. H. (1989). Nodulation and nitrogen fixation (acetylene reduction) of four cultivars of chickpea. *Aust. J. Exptl. Agric.*, 29,663.
- Sirota L. B. (1982). Nonsymbiotic N-fixation in the rhizosphere of N-fertilized plants. In *Ecological Effects of Agrochemical (fertilizer) Application. Abstracts of All-union workshop on the UNESCO Program Man and Biosphere, Project 9a ONTI NCBI Pushchino* pp 37-38
- Snedecor, G.W and W.G. Chachoran (1980). *Statistical methods* 7th ed.. Iowa State Univ. press, Ames, Iowa, usa PP.255-269.
- Soderberg Katarina H., (2004). The influence of nitrogen fertilization on bacterial activity in the rhizosphere of barley. *Soil Biology and Biochemistry* 36, 195-198.
- Thomas, R.L.; R.W. Shearer and Z.R. Mayer (1967). Comparison of conventional and automated procedures for nitrogen, phosphorus and potassium analysis of plant material using single digestion. *Agron. J.*, 59,pp.240.
- Van Sionouwenburg, J.Ch.(1968). *International Report of Soil and Plant Analysis. Lab of Soil and fertilizer Agric., Univ. of Wageningen, the Netherland.*
- Vincent, D.M.(1970). *Manual for particle study of Root Nodule Bacteria.* Blackwell, Oxford.
- Umarove, M.M. (1982). Nonsymbiotic N-fixation in phytoplankton and its role in the N balance in soil. In *Ecological Effects of Agrochemical (fertilizer) Application. Abstracts of All-union workshop on the UNESCO Program Man and Biosphere, Project 9a ONTI NCBI Pushchino* pp 42.
- Watanabe, I. and W.S. Barraquie (1979). Low levels of fixed nitrogen required for isolation of free living N₂-fixing organisms from rice roots. *Nature*, 277:565-566

استجابة الفول البلدى النامى فى ارض رملية الى التلقيح بالريزوبيا والميكروبات
الحية الدقيقة النافعة ومثبتات النتروجين
ايمان عبد الجيد طنطاوى - عبد العزيز محمد رجب - نعمت امام غياض و
شعبان محمد عبد الرسول
معهد بحوث الاراضى والمياه والبيئة - مركز البحوث لآزراعية - الجزيرة- مصر

يمكن أن تتغير العادات الغذائية المصرية لكن الاعتماد على طبق الفول في مائدة الإفطار سيظل هو الأساس للفرد المصري . ولهذا فان للفول أهمية خاصة في مصر ، ولزيادة محصول الفول وتقليل تكاليف انتاجه تم تلقيح الفول بال EM والبـ Compomax كلقاحات غير تكافلية بالإضافة إلى تلقيحه بالريزوبيا الخاصة به والمستقرة معه منذ عقود .

في هذا البحث تم تلقيح الفول بال EM الذي يحتوي على مجموعه من البكتريا والخمائر والفطريات النافعة التي تساعد على تثبيت النتروجين الجوي لا تكافليا كما تساعد أيضا على تكوين نبات قوي يمكنه مواجهة الظروف المختلفة مع إعطاء إنتاجيه عالية .

وأيضا تم التلقيح بالبـ Compomax حيث يحتوي على مجموعه من مثبتات النتروجين اللاكتكافليه وبعض العناصر الغذائية الكبرى والصغرى وذلك بالإضافة الي التلقيح بالريزوبيا . وكانت أهم النتائج المتحصل عليها:

- الريزوبيا كانت متفوقه في وزن ال ١٠٠ حبه كما أنها أعطت اعلي عدد ووزن للعقد الجذرية ،أيضا الريزوبيا ساندت ال EM بقوة حيث أعطت معها اعلي محصول كلي ومحصول حبوب .

- الـ EM وكذلك الريزوبيا أعطت نتائج متفوقه، فال EM+٢/١ كمية النتروجين الموصي بها أعطت اعلي وزن جاف واعلي محتوى من الـ K ، P ، N للنباتات في عمر ٤٥ يوم .

- الـ EM + الريزوبيا ٢/١ كمية النتروجين الموصي بها أعطت اعلي محصول كلي ومحصول حبوب متقارب جدا وبنون معنوية مع المعامله بالبـ EM + الريزوبيا + كل كمية النتروجين الموصي بها حيث سجلت الأولى ٢٤٥٠ كجم/فدان والثانيه ٢٤٩٥ كجم/فدان للمحصول الكلي وكذلك سجلت الأولى ٦٩٩ كجم/فدان والثانية ٧٠١ كجم/فدان لمحصول الحبوب وبالتالي يمكن تقليل كمية النتروجين المستخدمة للفول .

- الـ Compomax كان متفوقا في محتوى النبات من النتروجين وكذلك محتوى التربه من النتروجين بعد الحصاد وهذا يعود بالنفع علي المحصول التالي .

- التلقيح عامه عمل علي زيادة عدد الميكروبات في الوقت الذي قلت فيه أعداد الميكروبات بزيادة النتروجين المعدني .

- قلت أعداد الميكروبات في الحصاد عنها في ٤٥ يوم ماعدا الأكتينوميستات والبكتريا المثبتة للنتروجين .