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COMPARATIVE STUDY OF DIFFERENT BIOFERTILIZER MIXTURES ON FABA BEAN AND WHEAT YIELD IN NEWLY-RECLAIMED SOILS

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ABSTRACT

The present work was carried out on faba bean and wheat crops growing in sandy loam highly calcareous soil and sandy soil at West Nubaria region during the winter seasons of 2000/2001 and 2001/2002, to study the effect of interactions between mycorrhizal fungi (AM), phosphate dissolving bacteria (PDB) and *Rhizobium leguminosarum* biovar *viceae* (R) or *Azotobacter chroococum* (A) on growth, yield and nutrient uptake by faba bean and wheat plants. The experiments were lied out in a split-plot design with four replications. The main plots were for mineral fertilizers (two levels) and sub-plots were for inoculation treatments (eight inoculation treatments).

The results clearly showed that inoculation with *Rhizobium leguminosarum* or *Azotobacter chroococum* singly or in combination with AM or PDB and (AM + PDB) markedly enhanced the yield and all the measured yield components of faba bean and wheat plants, as well as nutrients uptake by plants under calcareous and sandy soil conditions compared to uninoculated or inoculated plants by AM and PDB alone or the dual inoculation with (AM + PDB). Nodulation of faba bean plants was positively affected by inoculation with *Rhizobium* and/or any other inocula mixed with *Rhizobium*. Faba bean and wheat dry weight was increased by about 25% due to inoculation with mixtures of AM + R + PDB or AM + A + PDB as compared to uninoculated plants or any other inoculation treatment.

Nitrogen uptake by inoculated faba bean and wheat plants with *Rhizobium* and *Azotobacter* and/or their mixture with AM and PDB was increased by 3.97 and 6.38%, with relative increase in seed yield by 11.28 and 3.72% as compared to uninoculated or inoculated plants with AM or PDB and (AM + PDB) for calcareous soil, respectively. While, it was increased by 8.95 and 44.1% with relative increase in seed yield by 16.42 and 6.85% for sandy soil. Inoculation with mycorrhiza or phosphate dissolving bacteria singly and with any of their combinations increased P-supply which led to improve P-uptake by faba bean and wheat plants as compared to uninoculated or inoculated plants with *Rhizobium* and *Azotobacter*, it was increased by 1.91 and 3.57% for calcareous soil and by 2.28 and 1.94% for sandy soil, respectively.

In general, a highly response of faba bean or wheat plants to the inoculation mixed was observed to be higher than that for single inoculation for the two tested soil types. This indicated that there was a positive interaction between the two and/or three inocula for the welfare of faba bean and wheat plants.

Keywords: Biofertilizer, mixture inocula, *Rhizobium leguminosarum*, *Azotobacter chroococum*, arbuscular mycorrhiza, *Bacillus megaterium*.

INTRODUCTION

As the Egyptian population continues to increase, it is necessary to increase land reclamation to close the gap between agriculture production and food demand. The newly-reclaimed lands in West Nubaria region are mainly calcareous and sandy soils. Poor soil fertility and nutrients deficiency in these soils are very common and could be the main constraints for agriculture production in some cases (El-Fayoumy, 1990). The application of mineral fertilizers to calcareous soils proved to be ineffective and pose a great potential for environmental pollution. Recently, there is a general trend all over the world to increase and promote new agriculture practices for cleaner crop production. One of the main practices to achieve this goal is to expand alternative agriculture including organic farming and to promote and increase the use of biofertilizers. This practice will minimize mineral fertilizers application and will prevent environmental pollution. Biofertilizers are cheaper, cleaner and safer to use for providing essential elements for plants. Moreover, it became an important factor to increase the availability of phosphorus and micronutrients to correct their deficiencies in calcareous soils.

The positive interaction between plants and rhizosphere micro-organisms can improve plant nutrition, nitrogen fixation, plant tolerance to environmental stresses and biologically controlled pathogens. There has been great interest and effort to develop an effective nitrogen fixing system for non-leguminous field crops such as wheat, barley and corn.

The mechanisms or functions of different types of biological fertilizers are not well established. It has also been shown that external mycorrhizal fungi hyphae are able to take up nitrogen both as NH_4^+ and NO_3^- and translocate N to the host plant in considerable amounts (Johansen *et al.*, 1994 and Tobar *et al.*, 1994). Despite this, it has not been clearly established the plant growth can be improved by N transport via external hyphae of AM fungi. The ability of external hyphae to absorb mineral N in large amounts could potentially change the plant depletion pattern upon AM-fungal colonization. The effect may be mediated partially by direct hyphal transport mechanisms, but also by the changes in the root system architecture and root longevity that are sometimes observed following colonization (Amijee *et al.*, 1989 and Hooker *et al.*, 1992). The inoculation with phosphate solubilizing bacteria and AM fungi singly or in combination significantly increased plant dry matter of maize as compared with the uninoculated treatment. Comparatively, mycorrhizal inoculation was more effective in increasing plant dry matter than inoculation with phosphate solubilizing bacteria. This indicates that AM fungi was most efficient in increasing P uptake and other nutrients from the soil than were phosphate solubilizing bacteria. However, dual inoculated plants with the two endophytes had significantly greater dry matter accumulation than uninoculated treatments or singly inoculated with phosphate solubilizing bacteria (Attia and Badr El-Din, 1999).

Inoculation of *Glomus mosseae* with *Bradyrhizobium japonicum* increased the shoot dry weight, nodulation, accumulated nitrogen and phosphorus as well as nitrate reductase activity in soybean plants (Shalaby and Hanna, 2000).

In the present study, interactions between AM fungi, phosphate dissolving bacteria and *Rhizobium* or *Azotobacter* and their effects on growth, yield and nutrient uptake of faba bean and wheat plants grown in a newly reclaimed calcareous and sandy soils in West Nubaria region were investigated.

MATERIALS AND METHODS

Field experimental sites

Two experimental sites were chosen in West Nubaria region; the first site (El-Amal village) is at km 46 on Alexandria-Cairo desert road, 9 km east of the road (sandy loam highly calcareous soil) and the second site (El-Ashrat Allaf village) is at km 64 on Alexandria-Cairo desert road, 2 km east of the road (sandy soil). The initial soil physicochemical characteristics of the surface layers (0-30 cm) were determined after Page *et al.*(1982) and Klute (1986) and recorded in Table (1).

Table (1): Some initial soil physicochemical characteristics of the experimental soil surface layers (0-30 cm).

Soil characteristics	Calcareous soil site				Sandy soil site			
	2000/2001		2001/2002		2000/2001		2001/2002	
	Faba bean	Wheat	Faba bean	Wheat	Faba bean	Wheat	Faba bean	Wheat
Soil pH (1:2.5)	8.30	8.34	8.27	8.31	8.07	8.05	7.98	8.07
Soil EC, dS m^{-1}*	1.88	1.94	1.97	1.93	0.92	0.97	0.98	0.95
Total $CaCO_3$, %	22.91	23.52	23.36	24.13	8.72	9.14	9.26	9.42
O.M., g kg^{-1}	3.82	3.96	3.90	4.21	1.97	2.26	2.35	2.18
Soil texture	Sandy loam				Sandy			
Available								
macronutrients	51.13	53.26	52.24	49.92	37.21	33.52	28.93	29.78
N, ppm	3.69	4.12	4.26	3.88	2.73	2.18	3.13	2.65
P, ppm	102.4	97.3	114.2	109.5	82.7	91.3	79.4	80.8
K, ppm								

* Saturated soil paste extract.

Biofertilizer sources

1. *Rhizobium leguminosarum* biovar *viceae* (R)

The strain was isolated from nodulated faba bean plants grown in West Nubaria region. Vincent's methods (Vincent, 1970) were used for isolation and characterization of the used root-nodule bacteria ($2x10^7$ cells ml^{-1}).

2. *Azotobacter chroococum* (A)

Free-living N_2-fixing bacteria was isolated from the rhizosphere of wheat plants grown in the Nubaria Agriculture Research Station. Soil suspension was prepared by shaking 1 g of soil in 100 ml sterile distilled water. One ml of this suspension was poured and spread onto agar plates containing Jensen mannitol minimal medium (Jensen, 1951). A loopfull from single colony growing was restreaked to a fresh medium plate. This was repeated at least four times to obtain pure culture ($15x10^8$ cells ml^{-1}).

3. Arbuscular Mycorrhiza (AM)

Mycorrhizal spores used in this study were mixture of *Glomus macrocarpium*. These spores were originally extracted by a wet-sieving and decanting technique (Gerdemann and Nicolson, 1963) from corn rhizosphere and multiplied in pot cultures with onion grown for 3 months in sandy soil (450 spores g^{-1}). The mycorrhizal inoculum consisted of infected root fragments, spores and mycelium.

4. Phosphate dissolving bacteria, *Bacillus megaterium* (PDB)

Bacillus megaterium was obtained from Department of Agricultural Microbiology, NRC, Cairo, Egypt. Five days old culture of *Bacillus megaterium* grown on nutrient broth medium containing 10^8 cells ml^{-1} was used as liquid inoculant. Seeds were mixed well with the suspension of *Bacillus megaterium* before planting.

Field experimental design and treatments application

Two field experiments in each site were carried out during the winter seasons of 2000/2001 and 2001/2002. The experiments were carried out in a split-plot design with four replications. The main plots were for mineral fertilizers and sub-plots were for inoculation treatments. Two mineral fertilizer treatments were used, the first treatment was control without any mineral NPK addition and the second was fertilization with 35.71, 71.43 and 114.24 kg ha^{-1} of N, P_2O_5 and K_2O for faba bean in calcareous soil, respectively, and 71.4, 107.14 and 114.24 kg ha^{-1} of N, P_2O_5 and K_2O in case of sandy soil, respectively. As for wheat, the treatments were 238.1, 107.14 and 114.24 kg ha^{-1} of N, P_2O_5 and K_2O in calcareous soil and 285.71, 107.14 and 114.24 kg ha^{-1} of N, P_2O_5 and K_2O in sandy soil.

Eight inoculation treatments were used in each experiment (Table 2).

Table (2): Inoculation treatments for each crop.

Treatment	Crop	
	Faba bean	Wheat
Uninoculated (Un)	Control	Control
Inoculated		
In_1	Arbuscular mycorrhiza (AM)	(AM)
In_2	*Rhizobium* (R)	*Azotobacter* (A)
In_3	Phosphate dissolving bacteria (PDB)	(PDB)
In_4	AM + R	AM + A
In_5	AM + PDB	AM + PDB
In_6	PDB + R	PDB + A
In_7	AM + PDB + R	AM + PDB + A

Faba bean experiments: Inoculated and uninoculated faba bean seeds, Giza Blanka variety, were sown at the rate of 110 kg ha^{-1} during the second week of October 2000 and 2001. The experimental plot consisted of 4 rows, 6 m long and 0.6 m between rows, giving a plot area of 14.4 m^2. Each row contained 36 plants after thinning to one plant per hill. Phosphorus fertilizer in form of mono-superphosphate (15.5% P_2O_5) was applied at planting at the rates of zero and 71.43 kg ha^{-1} and zero and 107.14 kg ha^{-1} to the calcareous and sandy soils, respectively. Nitrogen fertilizer in the form of ammonium nitrate (33.5%) was added at two equal doses; at planting and 21 days after planting at the rates of zero and 35.71 kg N ha^{-1} and zero and 71.4 kg N ha^{-1} to the calcareous and sandy soils, respectively. Potassium fertilizer was added in the form of potassium sulphate (48% K_2O) at the rate of 114.24 kg K_2O ha^{-1} with the second dose of N fertilizer for the two types of soil.

Wheat experiments: Inoculated and uninoculated wheat seeds (*Triticum vulgaris* L.) Sakha 69 variety were planted at the rate of 154.7 kg ha^{-1} during the last week of November 2000 and 2001. The experimental plot area was

16.8 m^2 (2.8 m in width and 6 m in length). Phosphorus fertilizer in the form of mono-superphosphate (15.5% P_2O_5) at the rates of zero and 107.14 kg ha^{-1} for the two soil types was added at planting. Nitrogen fertilizer in the form of ammonium nitrate (33.5% N) was added at three equal doses; at planting, tillering and heading stages at the rates of zero and 238.1 and zero and 285.71 kg N ha^{-1} to the calcareous and sandy soils, respectively. Potassium fertilizer was added in the form of potassium sulphate (48% K_2O) at the rate of 114.24 kg K_2O ha^{-1} with the second dose of N fertilizer for the two soil types.

All other agricultural practices for faba bean and wheat production were followed as common at the two experimental sites.

Sampling procedure

Number and weight of nodules per plant for faba bean were determined for each treatment 45 days after planting. At harvest, faba bean and wheat plant samples were collected from all treatment replications. Fresh plant samples (grains and straw) were washed by tap water followed by distilled water, dried at 65°C for 48 hrs and weighed, ground in stainless steel mill and stored for chemical analysis. Total yield for each plot was weighed and extrapolated to tons ha^{-1}. Also, 100 faba bean seeds and 1000 wheat grains were weighed.

Analytical procedure

Plant material (grain and straw) were wet digested with concentrated sulphuric acid and H_2O_2 (FAO, 1975). Total N (Chapman and Pratt, 1961) and P by the vanadomolybdate yellow method (Jackson, 1958) were determined.

The collected data were statistically analyzed according to procedures outlined by Snedecor and Cochran (1981) using SAS Software (1990).

RESULTS AND DISCUSSION

Initial state analyses of the two selected experimental soil sites (Table 1) indicated that the surface soil layer (0-30 cm) for the first site (El-Amal village) has a light texture of sandy loam with high content of total $CaCO_3$ (more than 22%). El-Ashrat Allaf village soil has a light texture of sandy with low content of total $CaCO_3$ (less than 10%). The two soil sites are of basic reaction (soil pH values ranged between 7.98 and 8.34) and non-saline soils (total soluble salts less than 1.97 dS m^{-1}) with low available NPK and organic matter content indicating their deficient fertility status.

Response of faba bean plants to inoculation treatments

1. Faba bean biomass and nodulation

Faba bean dry weight per plant (Tables 3 and 4) significantly varied with either biofertilizer inoculation or mineral fertilization in the two investigated soil types. Dry weight per plant was about 53% higher at F_2 as compared to F_1 in case of calcareous soil, while it was higher by 9% for sandy soil. Statistical analysis also showed that the higher dry weight per plant was obtained by inoculation with the mixture of the three inocula AM + R + PDB (191.6 and 111.8 g $plant^{-1}$ for calcareous and sandy soils, respectively) and with AM + R (183.7 and 107.5 g plant^{-1} for the same two soil types, respectively) with insignificant differences between them. The lowest dry weight per plant was obtained by uninoculated plants followed by inoculated plants with PDB, AM and AM + PDB with insignificant differences between them for the two soil types. In general, faba bean dry weight was increased by about 25% with inoculation as compared to uninoculated plants in both soils. Moreover, the response of faba bean dry weight to the inoculum mixture was about 13% and 8% higher than the single inoculation for calcareous and sandy soils, respectively. This indicated that there was a positive interaction between the two and/or three inocula for the benefit of the faba bean plants, a response that was observed by Koreish *et al.*(1998), Attia and Badr El-Din (1999) and Shalaby and Hanna (2000). Johansen (1999) reported that plant dry matter accumulation was unaffected or slightly affected by mycorrhizal colonization, where shoot dry weight was slightly increased, while root dry weight was slightly decreased compared to uninoculated control plants. Shalaby and Hanna (2000) concluded that the plants inoculated with mycorrhizal fungi and *Bradyrhizobium japonicum* showed a highly significant increase in growth of soybean plants compared to plants treated with either organisms separately.

Table (3): Effects of single and combined inoculation of biofertilizers on yield and some yield components of faba bean in calcareous soil (combined analysis).

Factors		Dry weight (g plant^{-1})	No. of nodules plant^{-1}	Weight of nodules (g plant^{-1})	100-seed weight (g)	Seed yield (Ton ha^{-1})	Relative yield (%)	Nutrients uptake (kg ha^{-1})	
								N	P
Inoculation (In)	Un	133.2 e	55 c	2.58 b	89.9 d	2.97 b	-	26.77 d	24.57 c
	In_1	152.9 cd	69 b	3.23 b	93.2 bc	2.99 b	0.67	26.89 d	25.73 b
	In_2	163.2 cd	118 a	5.15 a	100.2 a	3.28 a	9.76	27.92 b	24.93 c
	In_3	149.6 de	66 b	3.20 b	92 5 cd	2.98 b	0 37	26.83 d	25.08 c
	In_4	155.1 cd	74 b	3.32 b	95.7 b	2.99 b	0.67	27.17 c	28.24 ab
	In_5	189.5 bc	119 a	5.19 a	100.1 a	3.21 a	8.08	27.91 b	25.13 c
	In_6	183.7 ab	117 a	5.26 a	100.2 a	3.36 a	13.13	27.99 ab	25.96 ab
	In_7	191.6 a	121 a	5.73 a	101.8 a	3.39 a	14.14	28.13 a	26.43 a
Mineral fertilizer (F)	F_1	128.5 b	97 a	3.96 b	93.8 b	2.17 b	-	16.59 b	13.43 b
	F_2	196.2 a	69 a	4.42 a	99.8 a	4.12 a	89.86	38.31 a	37.74 a
$L.S.D_{0.05}$	In	17.62	12	1.03	3.14	0.193	-	0.196	0.587
	F	29.78	8	0.37	1 77	0.631	-	7.611	8.264
In		*	*	*	**	*	-	**	*
F		**	N.S	**	**	**	-	**	**
In x F		*	*	*	*	*	-	*	*

Un: Uninoculated. In: Inoculated.
F_1: Zero mineral fertilizer.
F_2: 35.71 kg N ha^{-1} + 71.43 kg P_2O_5 ha^{-1} + 114.24 kg K_2O ha^{-1}.

The response of faba bean to inoculation under fertilizer levels (Tables 3 and 4) revealed that the number of nodules per plant was insignificantly decreased by increasing fertilization. Number of nodules was insignificantly reduced by about 9 and 2% at F_2 fertilizer level as compared to F_1 for calcareous and sandy soils, respectively. Inversely, weight of nodules was significantly increased with fertilization, it was higher by about 12% and the double of it at F_2 level as compared to F_1 level for the same soil types, respectively. Moreover, nodulation of faba bean plants was similarly affected by inoculation with *Rhizobium* and/or any other inocula mixed with *Rhizobium*

(Tables 3 and 4). The number and dry weight of nodules were significantly higher in the presence of the dual inocula (R + AM) or (R + PDB) and the mixture of (R + AM + PDB). This indicated that there was a positive interaction effects between the *Rhizobium* and the other inocula. The higher number of nodules per plant was obtained by inoculation with the mixtures of AM + R + PDB (121 and 102 nodules per plant for calcareous and sandy soils, respectively) and followed by (R + AM), (R + PDB) and (R) for the two soil types. The same trend was observed for nodules weight, it was about 64 and 61% higher in the presence of R inoculation alone and/or its mixtures with any other inocula as compared to the absence of R and the other inoculum treatments for both soil types.

Table (4): Effects of single and combined inoculation of biofertilizers on yield and some yield components of faba bean in sandy soil (combined analysis).

Factors		Dry weight (g $plant^{-1}$)	No. of nodules $plant^{-1}$	Weight of nodules (g $plant^{-1}$)	100-seed weight (g)	Seed yield (Ton ha^{-1})	Relative yield (%)	nutrients uptake (kg ha^{-1})	
								N	P
Inoculation (In)	Un	81.6 f	51 c	2.36 b	83.76 c	2.57 b	-	22.31 c	23.14 d
	In_1	95.8 e	61 b	2.74 b	89.12 b	2.59 b	0.78	22.74 c	24.19 b
	In_2	101.4 cd	101 a	4.22 a	92.86 a	2.98 a	15.95	24 46 b	23.18 d
	In_3	93.2 e	58 b	2.42 b	89.11 b	2.58 b	0.39	22.52 c	23.97 c
	In_4	96.7 de	81 b	2 77 b	89.26 b	2.59 b	0.78	22.73 c	24.68 a
	In_5	103.8 bc	101 a	4.23 a	93.19 a	2.97 a	15.56	24.60 ab	24 19 b
	In_6	107 5 ab	102 a	4.29 a	93.52 a	2.99 a	16.34	24.62 ab	24.34 b
	In_7	111.8 a	102 a	4.25 a	94.71 a	3.04 a	17.83	24.91 a	24.87 a
Mineral fertilizer (F)	F_1	94.5 b	81 a	3.04 b	82.41 b	1.94 b	-	11.86 b	8.82 b
	F_2	103.4 a	79 a	3.76 a	98.97 a	3.84 a	97.94	35.36 a	39.32 a
$L.S.D_{0.05}$	In	4.87	9	0.917	1.939	0.213	-	0.376	0.213
	F	3.92	2	0.096	10.114	0.512	-	9.865	9.975
In		**	**	*	*	*	-	*	**
F		**	*	**	**	**	-	**	**
In x F		*	**	*	*	*	-	*	*

Un: Uninoculated. In: Inoculated.
F_1: Zero mineral fertilizer.
F_2: 71.40 kg N ha^{-1} + 107.14 kg P_2O_5 ha^{-1} + 114.24 kg K_2O ha^{-1}.

2. Faba bean yield and yield components

The response of faba bean plants to inoculation and/or fertilization (Tables 3 and 4) revealed that seed yield and 100-seed weight were significantly increased by either inoculation or fertilization in both calcareous and sandy soils. 100-seed weight was about 7 and 20% higher at F_2 as compared to F_1 for calcareous and sandy soils, respectively. On the other hand, seed yield was 90 and 98% higher with increasing fertilizer level from F_1 to F_2 for the same soil types, respectively. Such effect might be related to the better nutritional status for the growing plants upon fertilizer applications and inoculation, especially under sandy soil conditions. Moreover, statistical analysis showed that, in the two soil types, the higher 100-seed weight and seed yield were obtained by inoculation with the mixture of AM + R + PDB, followed by AM + R, R and R + PDR with insignificant differences between them. Conversely, the lowest values of 100-seed weight or seed yield were observed with uninoculated plants followed by single inocula (AM and PDB) and dual inocula (AM + PDB) for two soil types. The maximum relative increase in yield (14.14 and 17.83%) was recorded with inoculation by the mixture of (AM + R + PDB), while it was (13.13 and 16.34%), (9.76 and

15.95%) and (8.08 and 15.56%) with inoculation by (AM + R), (R) and (R + PDB) in both calcareous and sandy soils, respectively. The positive interaction between two organisms or more was observed by Pacovsky *et al.*(1986) and Shalaby and Hanna (2000).

3. Nutrients uptake by faba bean plants

Results in Tables (3 and 4) show considerable increase in N and P uptake by faba bean plants with increasing fertilizer levels from F_1 to F_2 in both soil types. Nitrogen and phosphorus uptake values at harvesting stage were about (131% & 180%) and (198% & 345%) higher at F_2 as compared to F_1 for calcareous and sandy soils, respectively. On the other hand, data revealed that, in both soil types, inoculation with *Rhizobium* and/or with its mixture with AM and PDB induced significant increase in N and P uptake by faba bean plants as compared to uninoculated or other inoculated treatments without *Rhizobium*. Shalaby and Hanna (2000) found that soybean plants inoculated with mycorrhizal fungi and *Br. japonicum* showed a highly significant increase in growth and phosphorus and nitrogen contents compared to plants treated with either organisms separately. Sainz *et al.*(1998) reported that the AM fungi increased crop productivity and P uptake in P deficient soils or when low to moderate P doses are applied. Faber *et al.*(1990) reported that the fungal hyphae absorb phosphate and other elements in soils and translocate them into the plant. Growth increase due to dual inoculation of soybean plants with mycorrhizal fungi and *Br. japonicum* was reported by Pacovsky *et al.*(1986), similar response was also observed by Fitter and Stickland (1991) and Trotta *et al.*(1996). Shalaby and Hanna (2000) concluded that VA mycorrhizal fungi stimulated soybean plants to produce greater plant mass and higher nitrogen and phosphorus content as well as more nodules.

Response of wheat plants to inoculation treatments

1. Wheat plant dry matter

Data in Tables (5 and 6) show that the dry weight per plant significantly varied with either biofertilizer inoculation or mineral fertilization for both investigated soil types, calcareous and sandy soils. Dry weight per plant was about 25 and 19% higher at F_2 as compared to F_1 in calcareous and sandy soils, respectively. Statistical analysis, also, showed that the higher dry weight per plant was obtained by inoculation with the mixtures of AM + A + PDB (51.32 and 47.16 g/10 plants for calcareous and sandy soils, respectively) and the dual inoculation AM + A (51.26 and 47.13 g/10 plants) and A + PDB (51.30 and 47.06 g/10 plants) and with single inoculation with *Azotobacter* (51.21 and 47.10 g/10 plants) with insignificant differences between them. Uninoculated treatment and inoculated treatments by AM, PDB and AM + PDB gave the lowest plant dry weight for the two soil types. In general, wheat dry weight was increased by about 3% with inoculation as compared to uninoculated plants for the two soil types. Moreover, the response of wheat dry weight to the mixture inocula was about 1 and 2% higher than the single inoculation for calcareous and sandy soils, respectively.

Table (5): Effects of single and combined inoculation of biofertilizers on yield and some yield components of wheat in calcareous soil (combined analysis).

Factors		Dry weight (g/10 plants)	1000-seed weight (g)	Yield (Ton ha^{-1})		Relative yield (%)		Nutrients uptake (kg ha^{-1})	
				Grain	Straw	Grain	Straw	N	P
Inoculation (In)	Un	49.13 b	52.91 c	5.512 c	10.97 d	-	-	49.97 b	26.18 d
	In_1	49.83 b	58.33 b	5.540 c	11.07 cd	0.508	0.912	50.47 b	28.21 b
	In_2	51.21 a	62.73 a	5.682 b	11.57 ab	3.084	5.469	53.37 a	26.36 d
	In_3	49.71 b	57.91 b	5.563 c	11.18 c	0.925	1.914	50.82 b	27.74 c
	In_4	49.92 b	58.73 b	5.571 c	11.19 c	1.070	2.005	50.26 b	28.92 ab
	In_5	51.30 a	62.79 a	5.681 b	11.49 b	3.066	4.740	53.45 a	27.96 c
	In_6	51.26 a	63.25 a	5.712 ab	11.68 a	3.628	6.472	53.48 a	28.63 b
	In_7	51.32 a	63.41 a	5.793 a	11.86 a	5.098	8.113	53.74 a	29.32 a
Mineral fertilizer (F)	F_1	44.79 b	55.10 b	4.049 b	8.699 b	-	-	18.59 b	14.90 b
	F_2	56.13 a	64.16 a	7.211 a	14.061 a	78.093	61.639	85.23 a	40.93 a
$L.S.D_{0.05}$	In	0.964	3.814	0.098	0.179	-	-	1.072	0.613
	F	4.721	1.932	0.814	2.124	-	-	16.851	11.352
In		*	*	**	*	-	-	*	**
F		**	**	**	**	-	-	**	**
In x F		*	*	**	*	-	-	*	*

Un: Uninoculated. In: Inoculated.
F_1: Zero mineral fertilizer.
F_2: 238.1 kg N ha^{-1} + 107.14 kg P_2O_5 ha^{-1} + 114.24 kg K_2O ha^{-1}.

Table (6): Effects of single and combined inoculation of biofertilizers on yield and some yield components of wheat in sandy soil (combined analysis).

Factors		Dry weight (g/10 plants)	1000-seed weight (g)	Yield (Ton ha^{-1})		Relative yield (%)		Nutrients uptake (kg ha^{-1})	
				Grain	Straw	Grain	Straw	N	P
Inoculation (In)	Un	44.92 b	50.85 c	4.674 b	9.162 b	-	-	43.93 b	26.28 d
	In_1	45.34 b	56.30 b	4.693 b	9.222 b	0.407	0.655	43.95 b	27.38 bc
	In_2	47.10 a	61.20 a	4.979 a	9.681 a	6.525	5.665	46.68 a	26.46 d
	In_3	45.31 b	56.29 b	4.689 b	9.218 b	0.321	0.611	43.95 b	27.18 c
	In_4	45.36 b	56.43 b	4.694 b	9.215 b	0.428	0.578	44.17 b	27.76 a
	In_5	47.06 a	61.18 a	4.988 a	9.679 a	6.718	5.643	46.72 a	27.24 bc
	In_6	47.13 a	61.21 a	4.996 a	9.688 a	6.889	5.741	46.77 a	27.59 ab
	In_7	47.16 a	61.26 a	5.013 a	9.694 a	7.253	5.807	46.82 a	27.87 a
Mineral fertilizer (F)	F_1	42.12 b	52.56 b	3.355 b	7.025 b	-	-	9.58 b	11.85 b
	F_2	50.23 a	63.62 a	6.319 a	11.873 a	88.346	69.011	81.17 a	42.59 a
$L.S.D_{0.05}$	In	0.915	2.733	0.039	0.206	-	-	0.958	0.376
	F	3.611	1.176	0.748	1.942	-	-	20.350	13.461
In		*	*	*	*	-	-	*	**
F		**	**	*	**	-	-	**	**
In x F		*	*	*	*	-	-	*	*

Un: Uninoculated. In: Inoculated.
F_1: Zero mineral fertilizer. F_2: 285.71 kg N ha^{-1} + 107.14 kg P_2O_5 ha^{-1} + 114.24 kg K_2O ha^{-1}.

2. Wheat grain yield and yield components

The data in Tables (5 and 6) show significant increases in wheat grain and straw yield and 1000-seed weight due to fertilizers application and/or inoculation in both calcareous and sandy soils. 1000-seed weight was about 16 and 21% higher at F_2 as compared to F_1 for the two soil types, respectively. On the other hand, grain and straw yield were 78% and 62% higher by increasing mineral fertilizer level from F_1 to F_2 for calcareous soil, while they were 88% and 69% higher for sandy soil. Moreover, statistical analysis showed that, in the two soil types, the higher 1000-seed weight was obtained by inoculation with the mixture of AM + A + PDB, followed by AM + A, A and PDB + A with insignificant differences between them. The inoculation with *Azotobacter* singly or in combination with other inocula (AM + A + PDB), (AM + A) and (PDB + A) significantly increased grain and straw yield of wheat as compared with the uninoculated treatment and dual inoculation with (AM + PDB) or (AM) and (PDB) separately, in both soil types. The maximum relative increase in grain yield (5.098 and 7.253%) was recorded by inoculation with the mixture of (AM + A + PDB), while it was (3.628 and 6.889%), (3.066 and 6.718%) and (3.084 and 6.525%) by inoculation with (AM + A), (PDB + A) and (A) in calcareous and sandy soils, respectively. Similar trend was observed for the straw yield.

3. Nutrients uptake by wheat plants

The data presented in Tables (5 and 6) show a significant increase in N and P uptake by wheat plants with increasing fertilizer levels from F_1 to F_2 in both soil types. Nitrogen and phosphorus uptake values at harvesting stage were about (358% & 747%) and (175% & 259%) higher at F_2 as compared to F_1 for calcareous and sandy soils, respectively. Moreover, data revealed that, in both soil types, inoculation with *Azotobacter* and/or with its mixtures with AM and PDB induced significant increase in N and P uptake by wheat plants as compared to uninoculated or inoculated plants by single inoculation AM and PDB and dual inocula (AM + PDB). As mentioned above, results indicated that there was a positive interaction between the *Azotobacter* and the other inocula. Grain yield was about 3.72 and 6.85% higher in the presence of *Azotobacter* inoculum singly and/or its mixtures with any other inoculum as compared to absence of A and the other inoculum treatments for calcareous and sandy soils, respectively. On the other hand, they were 6.20 and 5.71% for straw yield, respectively.

In general, several workers reported that the application of phosphate solubilizing bacteria increased the efficiency of phosphorus fertilizers through solubilizing the fixed forms by acids produced from bacteria (Laheurte and Barthelin, 1988). Also, arbuscular mycorrhizal fungi may positively affect the acquisition of mineral nutrients by the host plant by functionally increasing the absorptive surface of the root system. This has most clearly been demonstrated in the case of phosphorus, with numerous combinations of plants and AM-fungi species (Smith *et al.*, 1986 and Smith and Read, 1997). It has also been shown that external AM hyphae are able to uptake nitrogen both as NH_4 and NO_3 and translocate N to the host plant in considerable amounts (Ames *et al.*, 1983; Johansen *et al.*, 1992, 1993, 1994; George *et* *al.*, 1992 and Tobar *et al.*, 1994). Beside the transport of N, it has also been shown that the external hyphae efficiently deplete the soil of inorganic N (George *et al.*, 1992 and Frey and Schuepp, 1993). Barea *et al.*(1987 and 1989) found that external AM hyphae may utilize N sources that are not available to the roots alone. The effect may be mediated partially by direct hyphal transport mechanism, but also by the changes in the root system architecture (Amijee *et al.*, 1989; Berta *et al.*, 1990; Kothari *et al.*, 1990 and Hooker *et al.*, 1992). Moreover, Azcon-Aguilar and Barea (1978) reported that the inoculation with PDB increased the total percentage of root colonization by native AM fungi, also plants inoculated with AM fungi produced significantly higher percentage of root infection than the uninoculated ones. Attia and Badr El-Din (1999) found that the inoculation with phosphate solubilizing bacteria increased the number of phosphate solubilizing bacteria to 5.8×10^8 cells g^{-1} soil in the rhizosphere after 6 weeks of growth. Also, the number of phosphate solubilizing bacteria in the maize rhizosphere was not affected by inoculation with AM fungi. They, also, reported that the inoculation with phosphate solubilizing bacteria and AM fungi singly or in combination significantly increased plant dry matter of maize as compared with the uninoculated treatment. Comparatively, mycorrhizal inoculation was more effective in increasing plant dry matter than inoculation with phosphate solubilizing bacteria. This indicates that AM fungi was most efficient in increasing P uptake and other nutrients from the soil than are phosphate solubilizing bacteria. However, dual inoculated plants with the two endophytes had significantly greater dry matter accumulation than uninoculated treatments or singly inoculated with phosphate solubilizing bacteria.

General Conclusion

In light of the presented data and in both investigated soil types, it seems evident that inoculation with *Rhizobium* for faba bean and *Azotobacter* for wheat or by their combinations with mycorrhiza and/or phosphate dissolving bacteria, as compared to inoculation with mycorrhiza or phosphate dissolving bacteria singly or in dual, markedly enhanced number and weight of nodules for faba bean and nutrients uptake, seed yield and yield components for faba bean and wheat. The maximum seed yield was recorded by inoculation with the mixture of *Rhizobium* or *Azotobacter* + mycorrhiza + phosphate dissolving bacteria with higher relative increasing in yield in sandy soil than in calcareous soil. Data also revealed that seed yield was more enhanced by dual inocula (AM + PDB) and mycorrhiza alone than phosphate dissolving bacteria with insignificant difference between them. The maximum nutrient uptake (nitrogen and phosphorus) was recorded by inoculation with the mixture of (R or A + AM + PDB) with higher relative increase in yield, followed by (R or A + AM), (R or A + PDB) and (R or A) with insignificant difference between them. This indicated that there was a positive interaction between the dual and/or the inoculum mixture for the welfare of the faba bean or wheat plants.

Finally, under the experimental conditions, it is recommended that faba bean seed treatment with the mixture of (*Rhizobium* + mycorrhiza +

phosphate dissolving bacteria) under NPK mineral fertilizer rates of 35.71, 71.43 and 114.24 kg ha^{-1} for calcareous soil and the rates of 71.43, 107.14 and 114.24 kg ha^{-1} for sandy soil, markedly enhanced bio-availability and uptake of soil nutrients and consequently seed production. The same effects were observed for wheat seed treatments with the mixture of (*Azotobacter* + mycorrhiza + phosphate dissolving bacteria) under NPK mineral fertilizer rates of 238.1, 107.14 and 114.24 kg ha^{-1} for calcareous soil and the rates of 285.71, 107.14 and 114.24 kg ha^{-1} for sandy soil.

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دراسة مقارنة بين مخاليط الأسمدة الحيوية على محصولى الفول البلدى والقمح فى أراضى حديثة الإستصلاح

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يهدف هذا البحث إلى دراسة التأثير المتداخل لفطر الميكورهيزا والبكتيريا المذيبة للفوسفات تحت ظروف التلقيح أو عدم التلقيح بالريزوبيم أو الأزوتوباكتر لمحصولى الفول والقمح على الترتيب وكذلك دراسة العلاقات المزدوجة أو التأثيرات الفردية لكافة التداخلات المحتملة لهذه الملقحات الحيوية على نمو وإنتاجية محصولى الفول البلدى والقمح تحت ظروف الأراضى الجيرية والأراضى الرملية بمنطقة غرب النوبارية. وقد تم تنفيذ هذا البحث فى موقعين الأول يمثل الأراضى الجيرية (قرية الأمل) والثانى يمثل الأراضى الرملية (قرية العشرة آلاف) خلال الموسمين الشتويين لعامى ٢٠٠٠/٢٠٠١ و ٢٠٠١/٢٠٠٢. وقد أستخدم تصميم القطاعات المنشقة فى أربع مكررات فى الدراسة.

وقد أظهرت النتائج مايلى:

- التلقيح بالريزوبيم أو الأزوتوباكتر منفردا أو مزدوجاً مع الميكورهيزا أو البكتريا المذيبة للفوسفات أو فى خليط ثلاثى من الريزوبيم أو الأزوتوباكتر مع الميكورهيزا والبكتيريا المذيبة للفوسفات كان له تأثيرا معنويا على زيادة عدد ووزن العقد الجذرية بالفول البلدى بالإضافة إلى الوزن الجاف ووزن الحبوب لكل من الفول البلدى والقمح كما حدثت زيادة معنوية لمحصول البذور لكلا المحصولين مقارنة بالنباتات الغير ملقحة أو الملقحة بالميكورهيزا أو البكتيريا المذيبة للفوسفات منفردة أو مزدوجة معا.
- التلقيح بالخليط الثلاثى الريزوبيم + الميكورهيزا + البكتيريا المذيبة للفوسفات لنباتات الفول البلدى أو الأزوتوباكتر + الميكورهيزا + البكتيريا المذيبة للفوسفات لنباتات القمح أدت إلى زيادة المادة الجافة بنسبة ٢٥% لكل من المحصولين.
- حدث زيادة فى نسبة النتروجين الممتص بواسطة نباتات الفول والقمح الملقحة بالريزوبيم أو الأزوتوباكتر منفردة أو فى مخاليطها مع الميكورهيزا أو البكتيريا المذيبة للفوسفات وبلغت نسبة الزيادة ٣,٩٧% و ٦,٣٨% على الترتيب بالمقارنة بالنباتات الغير ملقحة أو الملقحة بالميكورهيزا أو البكتيريا المذيبة للفوسفات منفردة أو مزدوجة معا وذلك بالأراضى الجيرية بينما بلغت هذه النسبة ٨,٩٥% و ٤٤,١% بالأراضى الرملية.
- التلقيح بالميكورهيزا أو البكتيريا المذيبة للفوسفات منفردة أو فى مخاليطها أدى إلى زيادة صلاحية الفوسفور والذى إنعكس على نسبة الفوسفور الممتص بواسطة نباتات الفول البلدى والقمح بالمقارنة بالنباتات الغير ملقحة أو الملقحة بالريزوبيم أو الأزوتوباكتر منفردة وبلغت نسبة الزيادة فى الفوسفور الممتص ١,٩١% ، ٣,٥٧% بالأراضى الجيرية بينما بلغت ٢,٢٨% ، ١,٩٤% بالأراضى الرملية لكلا النباتين على الترتيب.
- حدث زيادة فى محصول الحبوب للفول البلدى والقمح قدرها ١١,٢٨% ، ٣,٧٢% على الترتيب بالأراضى الجيرية وذلك للنباتات الملقحة بالريزوبيم أو الأزوتوباكتر أو بأى من مخاليطها مع الميكورهيزا أو البكتيريا المذيبة للفوسفات مقارنة بالنباتات الغير ملقحة أو الملقحة بالميكورهيزا أو البكتيريا المذيبة للفوسفات منفردة أو مزدوجة معا وبلغت هذه النسبة ١٦.٤٢% و ٦,٨٥% بالأراضى الرملية لكلا المحصولين على الترتيب.

مما سبق وبصفة عامة تبين أن هناك إستجابة ملحوظة لنباتات الفول البلدى والقمح للتلقيح بمخاليط الملقحات سواء المزدوجة أو الثلاثية وذلك عن الملقحات المنفردة مما يشير إلى أن هناك تفاعل إيجابى بين الملقحات المستخدمة والذى إنعكس إيجابيا على نمو وإنتاجية المحصولين تحت ظروف كلا نوعى التربة وظروف التجربة.