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Effects of microbial and chemical fertilization on yield and seed quality of faba bean (*Vicia faba*)

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Abstract: A field experiment was conducted at EL–Hudeiba Research Station farm in north Sudan for two consecutive seasons to examine the effect of inoculation by different *Rhizobium* and phosphate solubilizing bacteria strains and their interaction on yield and seed quality of faba bean, and to compare between the effects of chemical fertilizers and biofertilizers. *Rhizobium* inoculation individually increased significantly yield, seed moisture, ash, crude fiber and crude protein. Phosphate solubilizing bacteria individually significantly increased yield, seed moisture, ash and fat in faba bean. *Rhizobium* and phosphate solubilizing bacteria significantly increased yield and seed quality (moisture, crude protein, fat, crude fiber and ash content) and decreased seeds carbohydrate content of faba bean plants. A synergetic effect was observed when the two types of microorganisms were combined. Comparable increments in yield were obtained resulting from either microbial fertilizers (nitrogen fixing and/or phosphate solubilizing bacteria) or chemical fertilizers (nitrogen and phosphorus fertilizers). The study revealed the need for biological fertilizers with compatible effective strains to compensate the chemical fertilizers, to decrease the expenses of chemical fertilizers and to protect the environment from pollution hazards.

Keywords: ***Bacillus megatherium*, phosphate solubilizing bacteria, *Rhizobium*, seed quality, *Vicia faba***

Introduction

Legumes are second only to cereals as a source of human food and animal feed. Their importance as food lies primarily in their high protein content. Legumes' grain protein is the natural supplement to cereal grain protein. They also provide fat and carbohydrates. Moreover, legumes are high in bone building minerals and vitamins essential for good health (Porres *et al*., 2003). Faba bean is the most important food legume in the Sudan. It constitutes the main dish in two daily meals of the lower and middle income groups (Ali *et al*., 1982). Chemical properties of faba bean seeds are one of the important indicators of quality and nutritional values.

Many efforts were directed to improve yield and protein content of legumes through breeding, fertilization and genetic engineering in Sudan. Biofertilizers are inputs containing microorganisms which are capable of mobilizing nutritive elements from non-usable form to usable form through biological processes, they include mainly the nitrogen fixing, phosphate solubilizing and plant growth–promoting microorganisms (Goel *et al*., 1999). The symbiotic nitrogen fixing bacteria are gram-negative non spore formers soil bacteria, collectively called rhizobia (Young and Haukka, 1996), which can fix nitrogen in a symbiotic relationship with legume plants. Phosphate solubilizing microorganisms play a key role in the plant metabolism and crop productivity. They have been reported to increase the availability and uptake of native soil phosphorus by converting insoluble phosphates to soluble forms by producing various organic acids (Raja *et al*., 2002). Phosphate solubilizing microorganisms inoculants include species of *Aspergillus*, *Bacillus*, *Escherichia*, *Arthrobacter* and *Pseudomonas* and they add 30 – 35 kg P_2O_5 ha^{-1} $year^{-1}$ (Rodríguez and Fraga, 1999; Gaur *et al*., 2004). In this field, many experiments were conducted to study the effect of biofertilizers alone or in combination with other chemical fertilizers (Seema *et al*., 2000; Fisinin *et al*., 1999). The objective of this study was to assess the efficiency of microbial biofertilizers in improving production of faba bean, variety "Hudeiba 72".

Materials and Methods

Seeds, nitrogen-fixing and phosphate-solubilizing bacteria and fertilizers

Seeds of the faba bean variety "Hudeiba 72" were supplied by the Agricultural Research Corporation, Sudan. *Rhizobium* strain ENRRI 9 was locally isolated, whereas, strain TAL 1339 was supplied by NifTAL Project, Paia, HI. Two strains of Phosphate Solubilizing Bacteria, PSB (PSB1 and PSB2) of

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Bacillus megatherium var. *phosphaticum* were supplied by the National Centre for Research, Sudan. The bacterial strains were maintained on Yeast Extract Mannitol Agar (YEMA) stored at 4°C. Nitrogen was applied in the form of urea together with phosphorus which was added in the form of triple super phosphate. Three doses of chemical fertilizers were tested: D1 (0 N+0 P), D2 (25 kg N + 21 kg P ha^{-1}) and D3 (50 kg N + 42 Kg P ha^{-1}).

Field experiments

The field experiments were conducted at EL Hudeiba Research Station farm (latitude 17°33'N, longitude 33°56'E), River Nile State for two consecutive seasons in a multi factorial design with 3 replicates. The land was prepared by deep ploughing, harrowing and leveling. Then the land was ridged and divided into 3 x 6 m plots. Seeds were sown by hand on both sides of the ridge in holes with 20 cm between holes and 3 seeds per hole. Plots were irrigated immediately after sowing and then, subsequently, irrigated every 10 days. Hand–weeding of the field was carried out once after 3 weeks form sowing. Plots were harvested at 12 weeks after sowing. Treatments used were: Control (uninoculated unfertilized), inoculated with *Rhizobium* (two strains), inoculated with *Bacillus megatherium* var. *phosphaticum* (BMP) (two strains), chemical fertilizer (three dose levels) and the treatments combinations were: 3 *Rhizobium* strains x 3 BMP strains x 3 chemical fertilizers x 3 replicates.

Seeds inoculation was carried out by seed coating with the desired bacterial strain impregnated in charcoal powder carrier. Gum Arabic solution was used as an adhesive. The parameters measured were average yield per plot and harvested seeds chemical composition (moisture, crude protein, fat, crude fiber, ash and carbohydrate content).

Chemical analysis

Faba bean seeds (whole seeds) were cleaned then ground by a small mill to pass through a 0.4 mm screen and the powder obtained was used for proximate analysis, on a dry weight basis. This was conducted according to the methods of AOAC (1984).

Statistical analysis

Multifactor analysis of variance (ANOVA) was performed to determine the effect of each treatment, and interaction between treatments, on the measured parameters. Comparisons between means of treatments for various parameters measured were made by standard error calculation (Gomez and Gomez, 1984). The objective of the statistical analysis was also to separate the variation due to experimental treatments from the variation due to soil, climate, season, site and other intrinsic factors. Significance was accepted at $p \leq 0.05$.

Results and Discussion

Yield

Rhizobium inoculation with strain ENRRI 9 significantly ($P \leq 0.05$) increased faba bean seed yield (Table I). Similarly, Phosphate solubilizing bacteria (PSB) inoculation significantly ($P \leq 0.05$) increased faba bean seed yield. This result is in accord with several studies showing that using microorganisms in solubilizing phosphorus resulted in high crop yield (Sundara *et al*., 2002; Gull *et al*., 2004). Application of chemical fertilizers significantly ($P \leq 0.05$) increased yield by using both doses in the second season, and by using 50 kg N + 42 Kg P ha^{-1} (D3) in the first season.

Co-inoculation with *Rhizobium* and PSB increased yield significantly ($P \leq 0.05$) compared to uninoculated control and the best result was obtained when ENRRI 9 and PSB2 were combined. This result is in accord with results observed by Rudresh *et al*. (2004) in chickpea. Interaction between inoculation with *Rhizobium* and/or PSB and fertilizers application increased yield significantly ($P \leq 0.05$) compared to uninoculated control, and the highest yield was obtained by application of D3 and ENRRI 9 with PSB1 in the first season and with PSB2 in the second season.

Moisture content

The average moisture content of faba bean seeds was found to be in the range of 6.35–8.95%, in the control treatments. It was previously reported to vary between 6.6 – 7.8% (Elsheikh and Elzidany, 1997) and 3.2 – 5.7% (Mohamed Ahmed, 2000). *Rhizobium* inoculations significantly ($P \leq 0.05$) increase the moisture content of faba bean seeds (Table II). The increment of faba bean seeds moisture due to *Rhizobium* inoculation was reported by Mohamed Ahmed (2000). PSB inoculation with PSB2 significantly ($P \leq 0.05$) increased moisture content of faba bean seeds in the second season. Application of 25 kg N + 21 kg P ha^{-1} (D2) chemical fertilizer significantly ($P \leq 0.05$) increased moisture content in the second season. Co-inoculation with ENRRI 9 and PSB2 resulted in the highest significant ($P \leq 0.05$) increase in moisture content compared to uninoculated control in the second season.

Crude protein

Faba bean contains a high protein content compared to other legumes amounting to 33%

Table 1. Strains and chemical fertilizer om faba bean seed yield (Kg/Ha)

Treatments		First season			Second season		
PSB inoculation	***fertilizer dose***	***No Rhizobium***	***ENRRI 9***	***TAL 1399***	***No Rhizobium***	***ENRRI 9***	***TAL 1399***
No PSB	D1	2670.00	3080.56	3183.33	1656.67	1556.11	1980.56
	D2	2389.44	2896.11	3173.33	2022.78	2066.67	2209.44
	D3	3463.33	3131.11	2716.11	1658.89	1992.22	1797.78
PSB 1	D1	2913.89	3045.00	3078.89	1610.56	1736.67	1676.11
	D2	3436.11	3213.33	2541.67	2078.89	1741.11	1857.22
	D3	3599.44	3357.78	3519.44	2012.22	1968.33	1912.22
PSB2	D1	2711.11	2928.89	2907.22	2100.56	1903.89	1681.67
	D2	2863.33	2396.67	3306.67	1878.33	2410.00	1776.11
	D3	2855.00	3266.11	2850.56	1675.00	2321.11	2320.56

		First season	*Second season*
LSD for *Rhizobium*	= +	68.19	51.56
LSD for PSB	= +	68.19	51.56
LSD for Fertilizer	= +	68.19	51.56
LSD for *Rhizobium* x PSB Interaction	= +	204.59	154.69
LSD for *Rhizobium* x Fertilizer Interaction	= +	204.59	154.69
LSD for PSB x Fertilizer Interaction	= +	204.59	154.69
LSD for *Rhizobium* x PSB x Fertilizer Interaction	= +	613.77	464.08

Table 2. Effects of *rhizobium*, PSB strains and chemical fertilizer on faba bean seeds moisture content (%)

Treatments		First season			Second season		
PSB inoculation	***fertilizer dose***	***No Rhizobium***	***ENRRI 9***	***TAL 1399***	***No Rhizobium***	***ENRRI 9***	***TAL 1399***
No PSB	D1	8.30	8.30	8.23	6.65	8.95	6.35
	D2	8.05	8.05	8.28	5.98	9.23	8.35
	D3	7.93	8.28	7.85	7.38	6.23	6.25
PSB 1	D1	8.18	8.20	8.37	5.88	5.65	7.73
	D2	8.12	7.37	8.23	7.03	7.75	5.40
	D3	8.30	8.35	8.03	7.55	5.78	6.63
PSB2	D1	8.02	7.88	8.25	8.50	7.00	6.13
	D2	7.70	8.10	8.32	7.18	6.93	7.95
	D3	7.65	8.08	8.07	8.65	7.75	6.08

		First season	*Second season*
LSD for *Rhizobium*	= +	0.06	0.003
LSD for PSB	= +	0.06	0.003
LSD for Fertilizer	= +	0.06	0.003
LSD for *Rhizobium* x PSB Interaction	= +	0.19	0.010
LSD for *Rhizobium* x Fertilizer Interaction	= +	0.19	0.010
LSD for PSB x Fertilizer Interaction	= +	0.19	0.010
LSD for *Rhizobium* x PSB x Fertilizer Interaction	= +	0.57	0.030

(Elsheikh *et al*., 2000). *Rhizobium* inoculation increased crude protein content of faba bean seeds over the control plants, this increase was significant ($P \leq 0.05$) when plants were inoculated with strain ENRRI 9 and insignificant by using strain TAL 1399 (Table III). The crude protein increment in *Rhizobium* inoculated plants may be ascribed to the nitrogen fixed within the root nodules and translocated to the seeds. Increase in seeds protein content of other legumes as a response to *Rhizobium* inoculation has previously been reported for fenugreek (Abdelgani *et al*., 1998). PSB inoculation with both strains significantly ($P \leq 0.05$) increased crude protein content. This result may be explained by the effect of PSB on nodulation and nitrogen fixation in legume plants as reported by Zhang *et al*. (1996), Andrade *et al*. (1998) and Lucas–Garcia *et al*. (2004). Application of chemical fertilizers significantly ($P \leq 0.05$) increased crude protein content in the second season and insignificantly in the first season field experiments. Co–inoculation with TAL 1399 and PSB1 resulted in a significant ($P \leq 0.05$) increase in crude protein content compared to control treatment whereas the crude protein percentage was less than ENRRI 9 inoculation individually and PSB1 individually.

Fat content

Rhizobium and PSB inoculation and applying of chemical fertilizers separately increased fat content of faba bean seeds in both seasons (Table IV). This increase was significant ($P \leq 0.05$) by using strains TAL 1399 in the first season. PSB2 in the second season increased seed fat content, and D2 of the fertilizer in the second season. Fats are important in diet constituents because of their high energy value, vitamins and the essential fatty acids which are associated with the fat of natural food (Mohamed Ahmed, 2000). The increase in fat content of faba bean due to chemical, organic and biological fertilization was reported by Elsheikh and Elzidany (1997) and Mohamed Ahmed (2000).

Crude fiber

Rhizobium inoculation significantly ($P \leq 0.05$) increased crude fiber content of faba bean seeds compared to uninoculated control in both seasons (Table V), these results were in accord with the observations of Mohamed Ahmed (2000), and are contradictory to the findings of Elsheikh and Elzidany (1997) and Ahmed (1998). Application of chemical fertilizers significantly ($P \leq 0.05$) increased crude fiber of the seeds in both seasons. Inoculation with ENRRI 9 alone gave a higher result than co-inoculation with ENRRI 9 and PSB2 which gave the highest significant ($P \leq 0.05$) crude fiber content compared to the other interactions and control. Fiber content of legume seeds was reported to be affected by chemical and organic fertilizers (Elsheikh and Elzidany, 1997) and plant variety (Abdelgani *et al*., 1998). The fiber content is an important constituent

Table 3. Effects of *rhizobium*, PSB strains and chemical fertilizer on faba bean seeds crude protein content (%)

Treatments		First season			Second season		
PSB inoculation	*fertilizer dose*	*No Rhizobium*	*ENRRI 9*	*TAL 1399*	*No Rhizobium*	*ENRRI 9*	*TAL 1399*
No PSB	D1	31.67	31.31	32.26	30.20	31.29	27.28
	D2	31.50	31.80	30.94	27.28	29.68	29.06
	D3	30.76	32.73	32.10	29.81	31.03	30.24
PSB 1	D1	31.20	31.03	33.03	30.46	27.63	27.71
	D2	32.77	31.86	32.51	29.37	27.84	30.16
	D3	31.66	32.13	31.49	28.28	31.47	30.33
PSB2	D1	33.08	33.33	30.68	30.51	27.71	27.98
	D2	31.89	31.57	32.88	30.38	28.28	28.80
	D3	31.47	31.76	30.85	30.46	30.28	30.51

		First season	*Second season*
LSD for *Rhizobium*	= +	0.12	0.004
LSD for PSB	= +	0.12	0.004
LSD for Fertilizer	= +	0.12	0.004
LSD for *Rhizobium* x PSB Interaction	= +	0.38	0.010
LSD for *Rhizobium* x Fertilizer Interaction	= +	0.38	0.010
LSD for PSB x Fertilizer Interaction	= +	0.38	0.010
LSD for *Rhizobium* x PSB x Fertilizer Interaction	= +	1.16	0.040

Table 4. Effects of *rhizobium*, PSB strains and chemical fertilizer on faba bean seeds fat content (%)

Treatments		First season			Second season		
PSB inoculation	*fertilizer dose*	*No Rhizobium*	*ENRRI 9*	*TAL 1399*	*No Rhizobium*	*ENRRI 9*	*TAL 1399*
No PSB	D1	2.02	1.97	2.12	2.35	2.50	2.45
	D2	1.40	2.05	2.25	2.10	2.48	1.80
	D3	1.90	1.62	1.65	2.50	2.63	1.93
PSB 1	D1	1.68	1.88	2.18	2.53	2.45	2.20
	D2	2.10	1.83	1.62	3.03	1.85	2.70
	D3	1.88	1.53	1.67	2.30	1.60	2.05
PSB2	D1	1.53	1.38	1.80	2.40	1.70	2.23
	D2	1.95	1.80	1.48	2.80	2.90	2.08
	D3	1.60	1.83	1.97	2.88	2.35	1.80

		First season	Second season
LSD for *Rhizobium*	= +	0.04	0.005
LSD for PSB	= +	0.04	0.005
LSD for Fertilizer	= +	0.04	0.005
LSD for *Rhizobium* x PSB Interaction	= +	0.12	0.010
LSD for *Rhizobium* x Fertilizer Interaction	= +	0.12	0.010
LSD for PSB x Fertilizer Interaction	= +	0.12	0.010
LSD for *Rhizobium* x PSB x Fertilizer Interaction	= +	0.37	0.050

Table 5. Effects of *rhizobium*, PSB strains and chemical fertilizer on faba bean seeds crude fibre content (%)

Treatments		First season			Second season		
PSB inoculation	*fertilizer dose*	*No Rhizobium*	*ENRRI 9*	*TAL 1399*	*No Rhizobium*	*ENRRI 9*	*TAL 1399*
No PSB	D1	8.15	8.38	7.90	6.63	7.63	7.83
	D2	8.60	7.87	8.07	7.83	8.38	7.33
	D3	7.92	8.43	7.60	6.83	6.18	6.63
PSB 1	D1	7.85	7.72	7.78	6.50	6.38	7.40
	D2	7.55	7.68	8.12	7.03	8.00	6.55
	D3	7.95	8.03	8.33	7.95	6.10	6.55
PSB2	D1	7.90	7.63	8.15	6.55	7.48	7.33
	D2	7.60	8.42	7.80	6.60	7.93	7.50
	D3	8.27	8.25	8.13	6.50	6.53	6.38

		First season	*Second season*
LSD for *Rhizobium*	= +	0.09	0.030
LSD for PSB	= +	0.09	0.030
LSD for Fertilizer	= +	0.09	0.030
LSD for *Rhizobium* x PSB Interaction	= +	0.29	0.090
LSD for *Rhizobium* x Fertilizer Interaction	= +	0.29	0.090
LSD for PSB x Fertilizer Interaction	= +	0.29	0.090
LSD for *Rhizobium* x PSB x Fertilizer Interaction	= +	0.87	0.270

of human food and animal feed and is needed in a reasonable proportion as it gives the bulk to the diet and helps in movement of food through the digestive tract.

Ash content

Rhizobium Inoculation had no effect on ash content of faba bean seeds in the first season (Table VI), but in the second season *Rhizobium* inoculation with either strain significantly ($P \leq 0.05$) increased the seeds ash content. Mohamed Ahmed (2000) reported that inoculation with *Rhizobium* had no effect on ash content of faba bean seeds. PSB inoculation significantly ($P \leq 0.05$) increased ash content in all field experiment, and always PSB2 results were higher than PSB1. Application of chemical fertilizers significantly ($P \leq 0.05$) increased ash content in both seasons. Co-inoculations significantly ($P \leq 0.05$) increased ash content of the seeds in both seasons compared to uninoculated control. Generally, ash content represents the residue after burning the organic matter. The ash obtained is not necessarily of exactly the same composition as the mineral matter present in the original food as they may be lost due to volatilization.

Carbohydrates content

The carbohydrates content is inversely related to

Table 6. Effects of *rhizobium,* PSB strains and chemical fertilizer on faba bean seeds ash content (%)

Treatments		First season			Second season		
PSB inoculation	***fertilizer dose***	***No Rhizobium***	***ENRRI 9***	***TAL 1399***	***No Rhizobium***	***ENRRI 9***	***TAL 1399***
No PSB	D1	3.28	3.33	3.18	3.85	3.58	3.70
	D2	3.93	3.27	3.05	4.03	3.60	4.30
	D3	3.40	3.68	3.65	3.60	3.68	4.20
PSB 1	D1	3.60	3.42	3.13	3.60	4.23	3.90
	D2	3.20	3.47	3.68	3.10	4.33	3.40
	D3	3.72	3.77	3.63	3.80	4.50	4.08
PSB2	D1	3.73	3.90	3.50	3.60	4.40	3.95
	D2	3.35	3.47	3.82	3.30	3.18	4.03
	D3	3.70	3.47	3.33	3.20	3.75	4.33

		First season	*Second season*
LSD for *Rhizobium*	= +	0.04	0.001
LSD for PSB	= +	0.04	0.001
LSD for Fertilizer	= +	0.04	0.001
LSD for *Rhizobium* x PSB Interaction	= +	0.12	0.004
LSD for *Rhizobium* x Fertilizer Interaction	= +	0.12	0.004
LSD for PSB x Fertilizer Interaction	= +	0.12	0.004
LSD for *Rhizobium* x PSB x Fertilizer Interaction	= +	0.38	0.010

Table 7. Effects of *rhizobium*, PSB strains and chemical fertilizer on faba bean seeds carbohydrate content (%)

Treatments		First season			Second season		
PSB inoculation	***fertilizer dose***	***No Rhizobium***	***ENRRI 9***	***TAL 1399***	***No Rhizobium***	***ENRRI 9***	***TAL 1399***
No PSB	D1	46.58	46.71	46.31	50.33	46.06	52.40
	D2	46.52	46.97	47.41	52.80	46.65	49.16
	D3	48.09	45.25	47.15	49.89	50.27	50.76
PSB 1	D1	47.49	47.76	45.51	51.04	53.68	51.06
	D2	46.26	47.79	45.84	50.46	50.23	51.79
	D3	49.49	46.18	46.85	50.13	50.56	50.37
PSB2	D1	45.73	45.89	47.62	48.44	51.71	52.40
	D2	47.51	46.65	45.71	49.75	50.80	49.65
	D3	47.31	46.60	47.65	48.32	49.34	50.92

		First season	*Second season*
LSD for *Rhizobium*	= +	0.17	0.030
LSD for PSB	= +	0.17	0.030
LSD for Fertilizer	= +	0.17	0.030
LSD for *Rhizobium* x PSB Interaction	= +	0.52	0.090
LSD for *Rhizobium* x Fertilizer Interaction	= +	0.52	0.090
LSD for PSB x Fertilizer Interaction	= +	0.52	0.090
LSD for *Rhizobium* x PSB x Fertilizer Interaction	= +	1.58	0.270

the protein content of faba bean seed. *Rhizobium* and PSB inoculation decreased carbohydrates content of faba bean seeds (Table VII). Application of chemical fertilizers significantly ($P \leq 0.05$) decreased carbohydrates content in the second season. This result reflects the effects of these factors on moisture, fat, fiber and protein contents, caused from the inverse relation between these parameters and carbohydrates content.

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