

Effects of Biological and Mineral Fertilization on Yield, Chemical Composition and Physical Characteristics of Faba Bean (*Vicia faba* L.) Cultivar Seleim

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Abstract: A field experiment was conducted at Dongola University Farm at Al-Seleim - Dongola University Farm, Northern State to study the effects of *Rhizobium leguminosarum biovar viceae* and *Bacillus megatherium* var. *Phosphaticum* (BMP) inoculation and phosphorus and nitrogen fertilization on the yield and seed quality of faba bean (*Vicia faba* L.) cultivar seleim. *Rhizobium* inoculation significantly ($p \leq 0.05$) increased yield, seed ash, fat, crude protein and 100-seed weight. BMP inoculation significantly ($p \leq 0.05$) increased seed moisture, fat, crude fiber and crude protein content. On the other hand, *Rhizobium* and BMP Co-inoculation significantly ($p \leq 0.05$) increased yield, seed ash, protein content and 100-seed weight. Application of chemical fertilizers increased yield, seed ash, fat, crude protein content and 100-seed weight. In addition to that, nitrogen fertilization significantly ($p \leq 0.05$) increased the hydration coefficient. Nitrogen fertilization in the presence of *Rhizobium* significantly ($p \leq 0.05$) increased the cookability.

Key words: *Rhizobium*, *Bacillus megatherium* var. *Phosphaticum*, inoculation, faba bean

INTRODUCTION

Legumes are the major direct source of proteins for both man and livestock, especially in poor countries, where animal protein is expensive (Hubbell and Gerald, 2003). Grain legumes play an essential role in human nutrition balancing the deficiencies of cereal-based diet (Dart and Krantz, 1977). The importance of legumes is that they can fix nitrogen in symbiotic association with rhizobia, and so they increase the soil nitrogen content (Poth *et al.*, 1986). This association enables legumes to benefit from an augmented nitrogen supply and can grow well on relatively poor soils (Heywood *et al.*, 1985).

Faba bean (*Vicia faba* L.) is one of the major legume crops cultivated in the Northern and the River Nile States of Sudan, produced in an average area of 69720 ha, with an average yield of 1896 kg/ha (AOAD, 2007). It is the main food for millions of people and the source of protein for the middle and low income groups (Salih, 1981). Faba bean is an important cash crop for farmers (Watson, 1981).

Crop productivity can be increased by the application of chemical, organic and biological fertilizers (Elsheikh *et al.*, 2009). Efforts throughout the world are directed towards improving the quality of food crops by increasing the nutritional value of the grains and decreasing the antinutrients level. The objectives of this study is to determine the effects of biological and mineral fertilization on yield, chemical composition and physical characteristics of faba Bean.

MATERIALS AND METHODS

A field experiment was conducted at Dongola University Farm at Al-Seleim, Northern State, Sudan for two consecutive winter seasons 2004/2005 and 2006/2007 in a factorial design with four replicates.

In these experiments, plants were fertilized with either nitrogen or phosphorus or with both of them. Plants were either inoculated with the introduced *Rhizobium* strain (TAL 1399) which was obtained from NifTAL project, University of Hawaii USA or with *Bacillus megatherium* var *phosphaticum* strain (BMP) which was locally isolated, in addition to combination of each (TAL 1399 + BMP). Control plants were kept for comparison. The land was prepared by deep ploughing, harrowing then leveling and ridging. The land was then divided into plots 6 x 3.8 m each. In treatments with nitrogen, 43 kg N/ha as urea was broadcasted immediately after sowing. Phosphorus was applied at a rate of 43 kg P/ha as triple super phosphate broadcasted before sowing.

Certified seeds of faba bean (*Vicia faba* L.) cultivar (Seleim-SM-L) were obtained from Dongola Research Station, Agricultural Research Corporation, Sudan.

Two to three seeds were placed in a hole on the top of the ridge with 20 cm spacing (between holes) and 70 cm (between ridges). Plots were immediately irrigated after sowing and then subsequently irrigated at 10 days intervals. Harvest was done at 13 weeks after sowing. Each plot was harvested separately by cutting the plants just above soil level. Plants were then threshed on

a large mat, then collected and weighed to determine yield of each plot. 100 seeds from the collected samples from each plot were counted in 4 replicates randomly then weighted.

Samples were taken from seeds of each plot, ground and used for proximate analysis which were conducted according to the methods of AOAC (1984), except for *In vitro* Protein Digestibility (IVPD) which was determined using the method of Maliwal (1983) as modified by Manjula and John (1991), Tannin content which was estimated using the modified vanillin HCl method (Price *et al.*, 1978) and Hydration coefficient and Cookability which were determined using the methods described by Elsheikh *et al.*, 2009.

Multifactor Analysis of Variance (ANOVA) was used to determine the effect of different treatments on the measured parameters.

Least significance difference was used to compare between means (Gomez and Gomez, 1984). Significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effects of treatments on faba bean yield: Inoculation of faba bean plants with *Rhizobium* strain TAL 1399 significantly ($p \leq 0.05$) increased seed yield in both

seasons (Table 1). In previous studies similar results were reported by Osman and Mohamed (1994); Rugheim and Abdelgani, (2009). However, Co-inoculation with *Rhizobium* and BMP significantly ($p \leq 0.05$) increased faba bean seed yield in both seasons as was previously found by Rugheim and Abdelgani (2009).

Effects of treatments on the proximate analysis of faba bean seeds moisture content: The average moisture content of faba bean seeds was found to range from 2.26-5.65%. None of the treatments had a significant effect on the moisture content of faba bean seeds except inoculation with BMP as phosphobacterin (Table 2).

Ahmed (1998) reported that there is no effect on moisture content for faba bean following *Rhizobium* inoculation. However, BMP inoculation with phosphobacterin significantly increased moisture content of faba bean seeds (Rugheim and Abdelgani, 2009).

Generally, the moisture content of legume seeds was found to be affected by the relative humidity of the surrounding atmosphere at the time of harvest and during storage (Elsayed, 1994).

Table 1: Effects of treatments on faba bean yield (kg/f)*

Treatments	Yield	
	First season	Second season
No inoculation		
Control	283.40	112.80
Nitrogen (43 kg N/h)	346.50	167.08
Phosphorous (43 kg P ₂ O ₅ /h)	298.45	122.68
Nitrogen + Phosphorous	341.40	197.50
Mean	317.43	150.02
Inoculation with <i>Rhizobium</i> (TAL 1399)		
Control	348.00	139.39
Nitrogen	358.50	143.32
Phosphorous	323.60	131.67
Nitrogen + Phosphorous	386.10	176.98
Mean	354.05	147.84
Inoculation with phosphobacterin (BMP)		
Control	294.90	134.47
Nitrogen	372.70	110.00
Phosphorous	300.90	204.59
Nitrogen + Phosphorous	332.60	130.86
Mean	325.27	144.98
Inoculation with <i>Rhizobium</i> + phosphobacterin		
Control	377.50	167.41
Nitrogen	397.50	113.00
Phosphorous	340.90	141.09
Nitrogen + Phosphorous	228.00	126.73
0 Mean	335.97	137.06
LSD for <i>Rhizobium</i>	46.24	25.6685
LSD for phosphobacterin	8.1746	4.5376
LSD for <i>Rhizobium</i> x phosphobacterin	92.49	51.3371
LSD for nitrogen	8.1746	4.5376
LSD for phosphorous	8.1746	4.5376
LSD for nitrogen x phosphorous	16.3492	9.0753
LSD for <i>Rhizobium</i> x phosphobacterin x nitrogen x phosphorous	184.97	102.6742

*f(feddan) = 0, 42 ha

Table 2: Effects of treatments on moisture, ash and fat and crude fiber content of faba bean seeds

Treatment	Moisture (%)	Ash (%)	Fat content (%)	Crude fiber (%)
No inoculation				
Control	5.07	2.90	1.17	7.52
Nitrogen (43 kg N/h)	4.28	7.45	1.12	7.43
Phosphorous (43 kg P ₂ O ₅ /h)	4.39	7.23	1.28	7.03
Nitrogen + Phosphorous	5.19	2.48	1.40	7.08
Mean	4.73	5.02	1.24	7.26
Inoculation with <i>Rhizobium</i> (TAL 1399)				
Control	2.26	5.28	1.39	7.04
Nitrogen	3.64	4.63	1.31	8.12
Phosphorous	5.52	5.39	1.39	7.73
Nitrogen + Phosphorous	3.67	16.95	1.20	7.93
Mean	3.77	8.69	1.32	7.55
Inoculation with phosphobacterin (BMP)				
Control	5.65	2.73	1.30	7.99
Nitrogen	5.42	3.50	1.27	7.87
Phosphorous	4.75	6.07	1.32	7.17
Nitrogen + Phosphorous	4.57	2.49	1.21	7.19
Mean	5.09	3.96	1.27	7.73
Inoculation with <i>Rhizobium</i> + phosphobacterin				
Control	5.03	4.37	1.26	7.16
Nitrogen	4.79	2.74	1.31	7.12
Phosphorous	3.98	5.34	1.20	7.28
Nitrogen + Phosphorous	4.40	2.68	1.19	8.33
Mean	4.55	3.78	1.24	7.47
LSD for <i>Rhizobium</i>	0.132	0.186	0.083	0.201
LSD for phosphobacterin	0.132	0.186	0.083	0.201
LSD for <i>Rhizobium</i> x phosphobacterin	0.260	0.372	0.167	0.402
LSD for nitrogen	0.132	0.186	0.083	0.201
LSD for phosphorous	0.132	0.186	0.083	0.201
LSD for nitrogen x phosphorous	0.260	0.372	0.167	0.402
LSD for <i>Rhizobium</i> x phosphobacterin x nitrogen x phosphorous	2.08	1.488	0.664	1.608

Ash content: *Rhizobium* inoculation and co-inoculation with *Rhizobium* and BMP significantly ($p \leq 0.05$) increased ash content of faba bean seeds (Table 2). Rugheim and Abdelgani (2009) reported that inoculation and co-inoculation significantly increased the ash content of faba bean seeds. BMP inoculation didn't affect the ash content of faba bean seeds. However Rugheim and Abdelgani (2009) found that BMP inoculation significantly increased ash content of faba bean seeds. Application of nitrogen and phosphorus chemical fertilizers separately significantly increased ash content of faba bean seeds. This result is in accord with the observations of Rugheim and Abdelgani (2009).

Fat content: *Rhizobium* and BMP inoculation and application of chemical fertilizers separately significantly ($p \leq 0.05$) increased fat content of faba bean seeds (Table 2). The increase in fat content of faba bean due to biological, chemical and organic fertilization was reported by Elsheikh (1998), Elsheikh and Ahmed (2000) and Elsheikh (2001).

Fiber content: None of the treatments had significant effect on the crude fiber content of faba bean seeds except, inoculation with BMP as phosphobacterin (Table 2). *Rhizobium* inoculation didn't affect the crude fiber

content of faba bean seeds. This result is in accord with the findings of Elsheikh (1998) and Elsheikh and Ahmed (2000) and contradictory to the findings of Rugheim and Abdelgani (2009). BMP inoculation significantly ($p \leq 0.05$) increased crude fiber content of faba bean seeds. This result is in accord with observations of Rugheim and Abdelgani (2009). Co-inoculation, chemical fertilization and the 4-way interaction didn't affect crude fiber content of faba bean seeds.

Protein content: Faba bean contains a high protein content compared to other legumes amounting to 33% (Elsheikh *et al.*, 2000). The individual or combined inoculation of *Rhizobium* and BMP significantly ($p \leq 0.05$) increased crude protein content in faba bean seeds compared to uninoculated control plants (Table 3). This finding prove the results of Lucas-Garcia *et al.* (2004) and Rugheim and Abdelgani (2009). Application of nitrogen and phosphorus separately and their combination significantly ($p \leq 0.05$) increased crude protein in faba bean seeds, as was previously found by Rugheim and Abdelgani (2009). The 4-way interaction reduced crude protein content in faba bean seeds.

Carbohydrates content: *Rhizobium* and BMP inoculation, co-inoculation with *Rhizobium* and BMP and

Table 3: Effects of treatments on crude protein, carbohydrates, *in vitro* protein digestibility and tannin content of faba bean seeds

Treatments	Crude protein	Carbohydrates	IVPD	Tannin
	----- (%) -----			
No inoculation				
Control	29.16	54.18	59.74	0.188
Nitrogen (43 kg N/ha)	30.04	49.68	57.96	0.138
Phosphorous (43 kg P ₂ O ₅ /ha)	30.91	49.16	52.92	0.363
Nitrogen + Phosphorous	31.20	52.65	55.76	0.063
Mean	30.32	51.41	56.59	0.188
Inoculation with <i>Rhizobium</i> (TAL 1399)				
Control	32.44	51.59	49.22	0.124
Nitrogen	29.61	52.71	51.65	0.119
Phosphorous	32.13	47.84	58.51	0.033
Nitrogen + Phosphorous	32.95	37.3	49.59	0.266
Mean	31.78	47.36	52.24	0.136
Inoculation with phosphobacterin (BMP)				
Control	32.66	49.67	47.07	0.119
Nitrogen	33.25	48.69	52.56	0.041
Phosphorous	31.5	49.19	51.79	0.075
Nitrogen + Phosphorous	28.0	55.82	54.35	0.044
Mean	31.35	50.84	51.44	0.096
Inoculation with <i>Rhizobium</i> + phosphobacterin				
Control	31.58	50.60	56.97	0.099
Nitrogen	32.43	51.69	50.83	0.091
Phosphorous	30.91	51.29	57.42	0.053
Nitrogen + Phosphorous	28.79	54.61	52.96	0.049
Mean	30.92	52.04	54.54	0.073
LSD for <i>Rhizobium</i>	0.8573	0.9749	0.4972	0.0538
LSD for phosphobacterin	0.8573	0.9749	0.4972	0.0538
LSD for <i>Rhizobium</i> x phosphobacterin	1.7146	1.9498	0.9945	0.1077
LSD for nitrogen	0.8573	0.9749	0.4972	0.0538
LSD for phosphorous	0.8573	0.9749	0.4972	0.0538
LSD for nitrogen x phosphorous	1.7146	1.9498	0.9945	0.1077
LSD for <i>Rhizobium</i> x phosphobacterin x nitrogen x phosphorous	6.8584	7.7993	3.9780	0.086

application of chemical fertilizers decreased carbohydrates content in faba bean seeds (Table 3). This result is in accord with observation of Rugheim and Abdelgani (2009).

Generally the carbohydrates content in the seeds of leguminous crops was found to decrease with *Rhizobium* inoculation (Elsheikh, 2001).

***In vitro* protein digestibility:** None of the treatments had a significant effect on the *in vitro* protein digestibility of faba bean (Table 3). Similar results were reported by Elsheikh *et al.* (2009) and Rugheim and Abdelgani (2009). However Elsheikh and Ahmed (2000) found that *Rhizobium* inoculation gave significant increment in the *in vitro* protein digestibility of faba bean seeds (Elsheikh and Ahmed, 2000). The *in vitro* protein digestibility has been reported to be affected by many factors such as genotype and tannin content (Babiker *et al.*, 1995).

Tannin content: None of the treatments had a significant effect on the tannin content of faba bean seeds, except phosphorous chemical fertilization (Table 3).

Rhizobium inoculation and chicken manure fertilization had no significant effect on tannin content of soybean seeds Elsheikh *et al.* (2009). However, *Rhizobium* inoculation significantly increased the tannin content of

groundnut and faba bean seeds (Elsheikh and Mohamedzein, 1998; Babiker *et al.*, 1995).

100-Seed weight: *Rhizobium* inoculation and co-inoculation with *Rhizobium* and BMP significantly ($p \leq 0.05$) increased 100-seed weight compared to uninoculated control (Table 4). Application of 43 kg N/ha + 43 kg P₂O₅/ha and phosphorus chemical fertilizers significantly ($p \leq 0.05$) increased 100-seed weight.

Nitrogen chemical fertilizer, BMP inoculation and the 4-way interaction didn't affect 100-seed weight. The increase in 100-seed weight resulted from *Rhizobium* inoculation was observed in faba bean earlier by Mohamed Ahmed (2000). The increase in 100-seed weight due to co-inoculation with *Rhizobium* and BMP or phosphorus chemical fertilization was previously observed by Barea *et al.* (2005). However, Rugheim and Abdelgani (2009) found that chemical fertilizers didn't affect 100-seed weight when interacted with *Rhizobium* and BMP.

Hydration coefficient: None of the treatments had a significant effect on the hydration coefficient of faba bean seeds except fertilization with nitrogen as urea in comparison with control (Table 4). Elsheikh *et al.* (2009) reported that hydration coefficient of soybean seeds was

Table 4: Effects of treatments on 100-seed weight, hydration coefficient and cookability of faba bean seeds

Treatments	100-seed weight (g)	Hydration coefficient (%)	Cookability (%)
No inoculation			
Control	52.73	207,74	15,48
Nitrogen (43 kg N/h)	52.82	223,39	14,21
Phosphorous (43 kg P ₂ O/h)	57.97	193,23	19,53
Nitrogen + Phosphorous	56.94	204,14	19,55
Mean	55.10	207.13	17.19
Inoculation with <i>Rhizobium</i> (TAL 1399)			
Control	57.91	209,53	12.46
Nitrogen	56.19	206,80	25.46
Phosphorous	52.85	201,39	18.72
Nitrogen + Phosphorous	50.20	204,14	12.30
Mean	54.29	205.47	17.24
Inoculation with phosphobacterin (BMP)			
Control	51.81	201,12	11.67
Nitrogen	50.62	206,58	17.79
Phosphorous	57.37	206,99	9.52
Nitrogen + Phosphorous	54.60	213,88	12.34
Mean	53.6	207.14	12.83
Inoculation with <i>Rhizobium</i> + phosphobacterin			
Control	58.91	199,74	19.11
Nitrogen	53.15	197,78	18.95
Phosphorous	56.39	207,81	20.90
Nitrogen + Phosphorous	50.39	197,34	22.01
Mean	54.71	200.72	20.24
LSD for <i>Rhizobium</i>	1.91	2,06	1.64
LSD for phosphobacterin	1.91	2,06	1.64
LSD for <i>Rhizobium</i> x phosphobacterin	3.82	4,12	3.28
LSD for nitrogen	1.91	2,06	1.64
LSD for phosphorous	1.91	2,06	1.64
LSD for nitrogen x phosphorous	3.82	4,12	3.28
LSD for <i>Rhizobium</i> x phosphobacterin x nitrogen x phosphorous	15.31	16,47	13.13

significantly increased by inoculation with *Rhizobium* isolate-2 and not significantly with *Rhizobium* strain TAL-377.

Cookability: None of the treatments had a significant effect on the cookability of faba bean seeds except fertilization with nitrogen in the presence of *Rhizobium* inoculation in comparison with control (Table 4). Elsheikh *et al.* (2009) found that inoculation and chicken manure fertilization insignificantly affected the cookability of soybean seeds. It was previously reported that chicken manure significantly increased the cookability in the presence or absence of *Rhizobium* inoculation (Elsheikh and Elzidany, 1997).

Conclusion: Inoculation with *Rhizobium* indicated an increase in faba bean seed yield, ash, fat, crude protein, and 100-seed weight. BMP inoculation increased moisture, fat, crude fiber and crude protein content. Co-inoculation with *Rhizobium* and BMP increased seed yield, ash, protein content and 100-seed weight. Application of chemical fertilizers increased seed yield, ash, fat, crude protein content and 100-seed weight. None of the treatments had a significant effect on the hydration coefficient of faba bean seeds except fertilization with nitrogen as urea in comparison with

control and also none of the treatments had a significant effect on the cookability of faba bean seeds except fertilization with nitrogen in the presence of *Rhizobium* inoculation in comparison with control.

From all these results we can conclude that inoculation with *Rhizobium* and co-inoculation with *Rhizobium* strain TAL 1399 and BMP as phosphobacterin was efficient to give significant yield, with good quality.

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