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Effects of Biofertilization on Yield, Physical Characteristics and Chemical Composition of Pigeon Pea (*Cajanus cajan* L.)

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Abstract: A field experiment was conducted at Elbagair area, Al Jazirah state, central Sudan for two successive seasons 2009/2010-2010/2011. In these experiments pigeon pea plants were either inoculated with five introduced or locally isolated *Rhizobium* or *bradyrhizobium* strains each alone or with *Bacillus Megatherium* var. *Phosphaticum* strain (BMP) alone and combination of each rhizobial strain with *Bacillus Megatherium* var. *Phosphaticum* strain (BMP). In addition to control for comparison. Inoculation with *Rhizobium* significantly ($p \leq 0.05$) increased pigeon pea seed yield, 100-seed weight, cookability, moisture content, ash, fat, fiber and protein. BMP inoculation significantly ($p \leq 0.05$) increased seed yield, 100-seed weight, cookability, fat, fiber and protein content. *Rhizobium* and BMP co-inoculation significantly ($p \leq 0.05$) increased seed yield, 100-seed weight, cookability, moisture, fat, fiber and protein content. Inoculation with *Rhizobium* strain TAL 1132 and co-inoculation with *Rhizobium* strain USDA 3472 and BMP as phosphobacterin was efficient to give significant yield, with good quality.

Key words: *Rhizobium*, *Bacillus megatherium* var. *Phosphaticum*, inoculation, pigeon pea

INTRODUCTION

Worldwide legumes (including pigeon pea) occupy 2.4 million km^2 or 18% of the global harvested area (FAOStat, 2008). The legumes and grains families are by far the world's most important sources of food; grains supply starch while legumes which include bean, peas supply protein and fats (John, 2005). Legumes are very important not only as food crops but possess high propensity to grow in depleted soils thereby serving as a medium of fertilizing succeeding crops through their unique symbiotic capability with nitrogen-fixing *Rhizobium* bacteria which are inhabited in root nodules of the legumes and the nitrogen balance in the soil is thereby preserved (Okaka *et al.*, 2002).

Pigeon pea is an important food in developing tropical countries. An excellent source of protein, the seeds (and sometimes the pods) are eaten as a vegetable, as a flour additive to other foods, in soups and with rice (Center for New Crops and Plants Products, 2002). Although pigeon pea makes excellent forage, because of the brittleness of its stems (Department of Primary Industries, 2002). The species is planted as a green manure crop, nurse crop, cover crop, a windbreak hedge. The stalks are used for fuel, thatch and basketry (Center for New Crops and Plants Products, 2002). In spite of the importance and benefits of this crop it does not enjoy global popularity, hence there is limited information available on this plant. Most of the rhizobia inoculation studies done in Sudan have concentrated on faba bean, groundnut, alfalfa, guar, chickpea and common bean with little focus on pigeon pea (Elhassan *et al.*, 2010).

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 aim of this study is to determine the effects of biological fertilization on Yield, physical characteristics and chemical composition of pigeon pea.

MATERIALS AND METHODS

A field experiment was conducted at Elbagair area (Latitude 15° 38´ N and longitude 32° 76´ E), Al Jazirah state, central Sudan for two consecutive seasons 2009/2010 and 2010/2011 in a factorial design with four replicates.

In these experiments, plants were either inoculated with five introduced or locally isolated *Rhizobium* or *Bradyrhizobium* strains (ENRRI 63, ENRRI 4, TAL 209, TAL 1132 and USDA 3472) each alone or with *Bacillus Megatherium* var *Phosphaticum* strain (BMP) alone in addition to combination of each rhizobial strain with *Bacillus Megatherium* var *Phosphaticum* strain (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 5 x 4 m each.

Local variety of pigeon pea (*Cajanus cajan*) was obtained from Central Market, Khartoum, Sudan.

Three to four seeds were placed in a hole on the top of the ridge with 25 cm spacing (between holes) and 60 cm (between ridges). Plots were immediately irrigated

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after sowing and then subsequently irrigated at 12-15 days intervals. Harvest was done at 20 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 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 pigeon pea yield: All *Rhizobium* strains used in this study significantly ($p \leq 0.05$) increased pigeon pea seed yield in both seasons (Table 1), except ENRRI 63 and ENRRI 4 in the first season and TAL 209 in the second season. The highest yield in both seasons was obtained by TAL 1132. Pulses seeds treated with specific strains of *Rhizobium* increases the yield through better nodulation (Saxena and Tilak, 1999). BMP inoculation significantly ($p \leq 0.05$) increased seed yield in both seasons. Rugheim and Abdelgani (2011) found that phosphate solubilizing bacteria individually significantly increased faba bean yield. Generally, *Rhizobium* strains and phosphobacterin co-inoculation significantly ($p \leq 0.05$) increased seed yield in both seasons. Combined inoculation with *Rhizobium* and *Bacillus megatherium* var. *phosphaticum* and full dose of N and P increased pigeon pea yield by 34.70% compared to un-inoculated control, dual inoculation with half dose of fertilizer gave 20.22% yield higher than control (Arunachalam *et al*., 1995).

Table 1: Effects of treatments on pigeon pea seed yield (kg/ha)

Treatments		First season	Second season
Uninoculated	Control	988.51	863.69
Rhizobium	ENRRI 63	995.36	1117.13
	ENRRI 4	1081.55	1266.67
	TAL 209	1251.68	1092.26
	TAL 1132	1483.30	1335.71
	USDA 3472	1303.63	1109.25
Phosphobacterin	B	1356.01	1051.78
	ENRRI 63+B	1279.70	1142.86
	ENRRI 4+B	1336.19	1159.52
	TAL 209+B	847.63	1269.05
	TAL 1132+B	1428.27	1257.74
	USDA 3472+B	1515.44	1311.90
LSD for *Rhizobium*		93.494	229.416
LSD for phosphobacterin		53.989	132.454
LSD for *Rhizobium* x phosphobacterin		132.221	324.444

Table 2: Effects of treatments on 100 seed weight, cookability and hydration coefficient of pigeon pea

Treatments		100 SW (g)	C (%)	HC (%)
Uninoculated	Control	8.93	6.97	199.07
Rhizobium	ENRRI 63	10.13	8.73	191.11
	ENRRI 4	9.65	7.87	197.67
	TAL 209	9.04	10.23	196.57
	TAL 1132	9.43	9.60	207.50
	USDA 3472	9.13	7.08	202.85
Phosphobacterin	B	9.33	8.50	197.67
	ENRRI 63+B	9.56	8.73	189.63
	ENRRI 4+B	9.58	10.21	183.22
	TAL 209+B	9.51	10.41	186.77
	TAL 1132+B	9.53	10.86	195.67
	USDA 3472+B	9.15	9.83	191.62
LSD for *Rhizobium*		0.396	1.834	8.467
LSD for phosphobacterin		0.229	1.498	4.888
LSD for *Rhizobium* x phosphobacterin		0.560	2.594	11.974

100 SW = 100 Seed Weight (g); C = Cookability (%), HC = Hydration Coefficient (%)

Effects of treatments on the physical characteristics of faba bean seeds

100-Seed weight: Inoculation with *Rhizobium* strains ENRRI 63, ENRRI 4 and TAL 1132 significantly ($p \leq 0.05$) increased pigeon pea 100-seed weight, while the inoculation with other two strains insignificantly increased it (Table 2). The increment in 100-seed weight resulted from *Rhizobium* inoculation was observed for faba bean by Osman *et al*. (2010). BMP inoculation significantly ($p \leq 0.05$) increased 100-seed weight in both seasons. This increasing occurred by BMP inoculation may be ascribed to the effect of phosphorus on early plant maturity (Mullins *et al*., 1996). All co-inoculation treatments with *Rhizobium* strains and phosphobacterin significantly ($p \leq 0.05$) increased the 100-seed weight except USDA 3472 and phosphobacterin interaction. This increment may be taken as evidence on the effect of BMP on increasing nitrogen fixation, which was reported before by Barea *et al*. (2005).

Cookability: From the results shown in Table 2 it appears that inoculation with *Rhizobium* strains TAL 209, TAL 1132 and BMP significantly ($p \leq 0.05$) increased cookability of pigeon pea seeds. *Rhizobium* and phosphobacterin co-inoculation significantly ($p \leq 0.05$) increased seed cookability except ERNNI 63. It was previously reported that fertilization with nitrogen in the presence of *Rhizobium* improved cookability of faba bean seeds (Osman *et al*., 2010).

Hydration coefficient: Table 2 shows that none of the treatments had a significant effect on this parameter. Inoculation with *Rhizobium* strains TAL 1132 and USDA

Table 3: Effects of treatments on moisture, ash, fat and fiber content of pigeon pea seeds

Treatments		Moisture (%)	Ash (%)	Fat (%)	Fiber (%)
Uninoculated	Control	5.12	4.02	1.23	7.46
Rhizobium	ENRRI 63	5.00	4.14	1.39	7.68
	ENRRI 4	5.47	4.37	1.33	8.05
	TAL 209	5.17	4.31	1.38	8.33
	TAL 1132	5.58	4.01	1.12	8.01
	USDA 3472	5.37	4.16	1.25	8.24
Phosphobacterin	B	4.88	4.00	1.30	7.71
	ENRRI 63+B	5.59	3.89	1.31	8.09
	ENRRI 4+B	5.54	4.12	1.27	8.21
	TAL 209+B	4.97	4.10	1.50	7.73
	TAL 1132+B	5.41	3.98	1.38	7.68
	USDA 3472+B	4.94	4.20	1.30	8.06
LSD for *Rhizobium*		0.128	0.241	0.129	0.784
LSD for phosphobacterin		0.074	0.139	0.074	0.453
LSD for *Rhizobium* x phosphobacterin		0.180	0.361	0.182	1.109

Table 4: Effects of treatments on protein, carbohydrate and tannin content of pigeon pea seeds

Treatments		P (%)	C (%)	T (%)
Uninoculated	Control	17.06	62.71	0.04
Rhizobium	ENRRI 63	18.08	63.71	0.04
	ENRRI 4	19.83	60.95	0.05
	TAL 209	19.54	61.27	0.08
	TAL 1132	17.79	63.50	0.04
	USDA 3472	18.08	62.90	0.06
Phosphobacterin	B	19.83	62.27	0.03
	ENRRI 63+B	18.67	62.45	0.03
	ENRRI 4+B	19.54	61.32	0.05
	TAL 209+B	19.25	62.44	0.05
	TAL 1132+B	19.54	62.01	0.04
	USDA 3472+B	19.83	61.67	0.03
LSD for *Rhizobium*		2.074	2.079	0.027
LSD for phosphobacterin		1.197	1.200	0.022
LSD for *Rhizobium* x phosphobacterin		2.933	2.940	0.038

P (%) = Protein, C (%) = Carbohydrate; T (%) = Tannin

3472 insignificantly increased the hydration coefficient. Similar results were reported by Elsheikh *et al.* (2009) for soybean seeds and Osman *et al.* (2010) for faba bean seeds.

Effects of treatments on the proximate analysis of faba bean seeds

Moisture content: *Rhizobium* inoculation with strains ENRRI 4, TAL 209 and USDA 3472 significantly ($p \leq 0.05$) increased the seeds moisture content compared to uninoculated control (Table 3). BMP inoculation didn't affect the moisture content of pigeon pea seeds. Co-inoculation with phosphobacterin and *Rhizobium* strains ENRRI 63, ENRRI 4 and TAL 1132 significantly ($p \leq 0.05$) increased the seeds moisture content compared to un-inoculated control. Generally, the moisture content of legume seeds was found to be affected by the relative humidity of surrounding atmosphere at the time of harvest and during storage (Elsayed, 1994).

Ash content: *Rhizobium* strains ENRRI 4 and TAL 209 significantly ($p \leq 0.05$) increased ash content compared to uninoculated control. All other treatments had no effect on pigeon pea seeds ash content (Table 3). 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.

Fat content: Inoculation with *Rhizobium* strains ENRRI 63 and TAL 209 significantly ($p \leq 0.05$) increased pigeon pea seeds fat content compared to control (Table 3). Inoculation with BMP alone or with *Rhizobium* strains significantly ($p \leq 0.05$) increased seeds fat content except co-inoculation with phosphobacterin and TAL 209 compared to un inoculated control. Similar result was obtained by Osman *et al.* (2010) for faba bean.

Fiber content: All treatments increased pigeon pea fiber content compared to uninoculated control (Table 3). Inoculation with *Rhizobium* strains TAL 209 and USDA 3472 significantly ($p \leq 0.05$) increased fiber content of pigeon pea seeds compared to uninoculated control. This result is in accord with observations of Rugheim and Abdelgani (2011) for faba bean.

Protein content: Inoculation with *Rhizobium* and BMP strains each alone or in combination increased pigeon pea seeds protein content compared to uninoculated control (Table 4). *Rhizobium* strains ENRRI 4 and TAL 209 and phosphobacterin alone significantly ($p \leq 0.05$) increased protein content of pigeon pea seeds compared to uninoculated control. The increase in protein content due to biological fertilizers was reported by Elsheikh (1998 and 2001).

Carbohydrates content: *Rhizobium* and BMP inoculation and co-inoculation insignificantly improved pigeon pea Carbohydrates content compared to control (Table 4). This result is in contradictory to the findings of Osman *et al.* (2010) and Rugheim and Abdelgani (2011) for faba bean.

Tannin content: Only inoculation with *Rhizobium* strain TAL 209 significantly ($p \leq 0.05$) increased tannin content of pigeon pea seeds compared to control (Table 4). Osman *et al.* (2010) reported a similar result for faba bean. *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 faba bean and groundnut seeds (Elsheikh and Mohamedzein, 1998; Babiker *et al.*, 1995).

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