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Bradyrhizobium Inoculation and Chicken Manure or Sulphur Fertilization of Hyacinth Bean (*Dolichos hyacinth* L.): Changes in Physical Characteristics and Chemical Composition of the Seeds

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Abstract: A field experiment was carried out at Shambat, Sudan (Latitude 15° 40´ N and Longitude 32° 32´ E) in three consecutive seasons (2000/03) to investigate the effect of *Bradyrhizobium* inoculation, chicken manure or sulphur fertilization on physical characteristics and chemical composition of hyacinth bean (*Dolichos hyacinth* L.) seeds. The results showed that chicken manure or sulphur fertilization in the presence or absence of *Bradyrhizobium* inoculation and their interactions significantly ($P \leq 0.05$) improved hydration coefficient and cookability of the seeds. Moisture and ash contents were not greatly affected while fat, fiber, protein and carbohydrates were greatly affected especially when 10 t/fed chicken manure or 100 kg/fed sulphur were applied with or without inoculation. Moreover, inoculation with or without chicken manure or sulphur fertilization greatly affected the chemical composition of the seeds. Tannin content of the seeds was increased by application of fertilizers especially when accompanied by inoculation. However, the *in vitro* protein digestibility was significantly ($P \leq 0.05$) improved especially when chicken manure or sulphur was applied to inoculated seeds. The results indicated that all measured parameters increased with increasing level of amendments (manure or sulphur) and the highest value of each parameter was observed with either 10 t/fed chicken manure or 100 kg/fed sulfur.

Keywords: *Bradyrhizobium*, inoculation, chicken manure, hyacinth bean, sulphur, proximate composition

INTRODUCTION

The importance of legumes as food lied primarily in their high protein content that averages 20–25%. Hyacinth bean (*Dolichos hyacinth* (L.) is an Asia origin crop, and was probably domesticated in India as a cultivated grain legume. The crop is widespread in India, Egypt and Sudan as well as in Madagascar and Ethiopia. Hyacinth seed contains an average of 17% protein with *in vitro* protein digestibility of 80% (Murphy and Colucci, 1999). These nutritional characteristics coupled with the other environmental benefits make hyacinth bean a suitable food and fodder crop for the tropics. Hyacinth is an old established irrigated crop in Sudan. The crop was introduced in the Gezira Scheme in 1926 as apart of rotation and main fodder for cattle. Also hyacinth is used for shifting cultivation or shorter rest periods of one or a few years for the usual method of maintaining soil fertility and organic matter (English *et al.,* 1999). Grain legumes fix about 15–210 kg/ha/y (Dakora and Keya, 1997). Inoculation of soybean by *Bradyrhizobium japonicum* significantly increased nodulation, yield and seed quality (Okereke and Onochie, 1996). Chicken manure amendments significantly improved the physical properties of the soil, such as water infiltration rate, water holding capacity, texture, reducing bulk density and hence increasing porosity. Moreover, chicken manure is readily available source of plant nutrients as well as a source of energy for soil biota and thus influences many of biological processes of the soil which was found to affect the seed quality of faba bean (Elsheikh and Alzidany, 1997a&b). Chicken manure is considered to have fertilizing properties intermediate between mineral fertilizers and farmyard manure and it has an appreciable residual effect (Abdelgani *et al*., 2003). Elemental sulphur (ES) has a variety of uses as soil amendment. The oxidation of ES to H_2SO_4 is particularly beneficial in alkaline soils in reducing the pH, supplying $SO^{=}_4$ to plants, making phosphorus and micronutrients more available and reclaiming soils (Lindemann *et al*., 1991).

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The effectiveness of ES depends upon the soil type, pH, organic matter content, clay minerals, depth of soil profile and drainage status. However, Ghani *et al.* (1997) reported that microbial population in soil is not a limiting in ES oxidation. *Rhizobium* inoculation significantly increased tannin content of faba bean (Babiker *et al.,* 1995) and groundnut (Elsheikh and Mohamedzein, 1998) seeds. *Rhizobium* inoculation significantly increased the *in vitro* protein digestibility of groundnut (Elsheikh and Mohamedzein, 1998) and faba bean (Elsheikh and Ahmed, 2000 and Elsheikh and Alzidany, 1997a) seeds. Efforts throughout the world are directed towards increasing the protein content and *in vitro* protein digestibility, and decreasing the tannin content, of beans and grains. Breeding, fertilization programmes and genetic engineering are directed towards improving seed quality. *Rhizobium* inoculation of faba beans was reported to increase yield and protein content (Babiker *et al.,* 1995; Elsheikh & Osman, 1995). Addition of sulphur to faba bean plants increased seed yield by 33% and significantly increased the amount of protein and the concentration of methionine and cystine in seeds (Farrag *et al.,* 1992). No trials were conducted to study the effect of chicken manure or elemental sulphur in combination with *Bradyrhizobium* inoculation on chemical composition of plant seeds. Therefore, in this study we would like to investigate the effect of *Bradyrhizobium* inoculation, sulphur or chicken manure fertilization on the physical characteristics and chemical composition of hyacinth bean seeds.

MATERIALS AND METHODS

Materials: Hyacinth bean (*Dolichos lablab* L.) cultivar used in this study was supplied kindly by Dr. Abd El Wahab, H. Abdalla, Department of Agronomy, Faculty of Agriculture, University of Khartoum, Shambat, Sudan. *Bradyrhizobium* (TAL 102) was obtained from the Biofertilization Department, Environment and Natural Resources Institute, National Centre for Research, Khartoum, Sudan. The Yeast Extract Manitol (YEM) medium was prepared according to Cleyet-Marel (1993) method. Chicken manure was obtained from the Top Farm of Faculty of Agriculture, University of Khartoum, Shambat, Sudan. Elemental sulphur was obtained from El Geneed Sugar Industry, Sudan. Strains of *Bradyrhizobium* were preserved by streaking on YEM agar, mixed with 3.0 g of calcium carbonate per litre, in slants in screw-caps test tubes and kept in the refrigerator at 4^{o}C. Seeds were inoculated by mixing with a thick suspension of charcoal based *Bradyrhizobium* inoculum, with average count of 1×10^{9} cfu/g. Arabic gum solution (40%) was added for good adhesion. Seed inoculation was carried out in the farm and seeds were immediately sown in the soil and irrigated. Unless otherwise stated all chemicals and reagents used in this study are of reagent grade.

Field experiments: Three field experiments were conducted at the Demonstration Farm of the Faculty of Agriculture, University of Khartoum, Shambat, Sudan (Latitude 15^{o} 40´ N and Longitude 32^{o} 32´ E). The experiments were conducted during the seasons 2000/2001, 2001/2002 and 2002/2003.

Chicken manure and sulphur application: Two separate experiments were conducted, for one experiment chicken manure was applied at different levels (0, 2.5, 5.0, 7.5 and 10.0 t/fed). The fertilizer was distributed along the ridges and mixed with the soil and then the soil divided into plots. Thereafter, the plots were irrigated twice for two weeks before sowing. For the other experiment elemental sulphur was applied at different levels (0, 25, 50, 75 and 100 kg/fed), distributed along the ridges and mixed with the soil. The soil was divided into plots and the plots were irrigated twice before sowing for two weeks.

Treatments: The treatments were replicated three times in a split-split plot design. The treatments used during the first and second seasons were divided into groups as follows:

1. Uninoculated: Only chicken manure was applied at different levels (0, 2.5, 5.0, 7.5 and 10.0 t/fed).

2. Inoculated: The seeds were inoculated and the soil was amended with chicken manure at different levels (0, 2.5, 5.0, 7.5 and 10.0 t/fed).

3. Uninoculated: Only elemental sulphur was applied at different levels (0, 25, 50, 75 and 100 kg/fed).

4. Inoculated: The seeds were inoculated and the soil was amended with elemental sulphur at different levels (0, 25, 50, 75 and 100 kg/fed).

In the third season the residual effect of either chicken manure or sulphur in the presence or absence of *Bradyrhizobium* inoculation was investigated.

Sample preparation: Three samples from each plot were taken randomly after seeds matured. The seeds were dried by direct sun drying. The seeds were cleaned manually to remove husks, damage seeds and other extraneous materials. To determine the chemical composition, tannin and *in vitro* protein digestibility the cleaned seeds were ground to pass a 0.4 mm screen.

Physicals characteristics of the seeds

Soakabillity: From each plot 100 seeds were selected randomly, weighed and soaked in tap water at a ratio of 1: 4 for 16 hours. The percentage of non-soakers in each sample was calculated as follows:

$$\text{Non-soaker \%} = \frac{\text{Weight of non-soakers}}{\text{Initial weight}} \times 100$$

$$\text{Soakabillity\%} = 100 - \text{Non-soaker\%}$$

Hydration coefficient: The hydration coefficient percentage was calculated for each sample as follows using the data obtained above as follows:

$$\text{Hydration coefficient \%} = \frac{\text{Weight of soaked seeds}}{\text{Initial weight}} \times 100$$

Cookability: Twenty grams of hyacinth bean seeds were processed in 200 ml of tap water in a conical flask at 110 ^{o}C for 30 min. The sample was reweighed after processing. Cookability was calculated as follows:

$$\text{Cookability \%} = \frac{\text{Weight after processing} - \text{initial weight (20g)}}{\text{Initial weight (20g)}} \times 100$$

Chemical composition determination: Chemical composition of the seeds was determined according to the methods of AOAC (1995).

Tannin content determination: Quantitative estimation of tannins was carried out using the modified vanillin–HCl method (Price *et al*., 1978). A 200 mg sample was extracted using 10 mL 1% (v/v) concentrated HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 mL) was added to the extract (1 ml) and the absorbance of the colour developed after 20 min at 30 ^{o}C was read at 500 nm. A standard curve was prepared expressing the results as catechin equivalents, i.e. amount of catechin (mg/ml) which gives a colour intensity equivalent to that given by tannins after correcting for blank. Then tannin content (%) was calculated according to the equation:

$$\text{Catechin equivalent (CE)\%} = \frac{C \times 10 \times 100 \times 100}{200}$$

Where:

C = concentration obtained from the standard curve (mg/ ml).

10 = Volume of extract (ml)

200 = Sample weight (mg)

In vitro protein digestibility (IVPD) determination: IVPD was determined by the method of Saunder *et al.* (1973). A sample (0.2 g) was placed in a 50 ml centrifuge tube, 15 ml of 0.1M HCl containing 1.5 mg pepsin were added, and the tube was incubated at 37^{o}C for 3 h. The suspension was then neutralized with 0.5M NaOH and treated with pancreatin (4.0 mg) in 7.5 ml of 0.2M

phosphate buffer, pH 8.0, containing 0.05% sodium azide; the mixture was then gently shaken and incubated at 37°C for 24 h. After incubation, the sample was treated with 10% trichloroacetic acid (10 ml) and centrifuged at 5000x g for 20 min at room temperature. Nitrogen in the supernatant was determined by Kjeldahl method (AOAC, 1995). Digestibility was calculated using the formula:

$$\text{IVPD \%} = \frac{\text{N in supernatant - enzyme N}}{\text{N in sample}} \text{ X100}$$

Statistical analysis: Experimental data were analyzed by using the general linear models procedure, the ANOVA procedure, and Duncan's multiple range test (1999 version; SAS Software Inst. Inc., Cary, N.C., U.S.A.). Least significant differences were computed at $P \leq 0.05$. Data were also analyzed using the correlation procedure (Pearson's correlation coefficients) in SAS.

RESULTS AND DISCUSSION

Effect of treatments on seed physical properties: As shown in Table 1, *Bradyrhizobium* inoculation showed a significant ($P \leq 0.05$) effect on the hydration coefficient of hyacinth bean seeds in all seasons. Chicken manure and sulphur fertilization were significantly ($P \leq 0.05$) improved the hydration coefficient of uninoculated seeds with increasing level of each. However, after inoculation the rate of improvement greatly increased in all seasons with maximum values obtained when 10t/fed of chicken manure was used (255.60, 256.90 and 245.60% for the first, second and residual season, respectively) or 100kg/fed of sulphur was applied (243.64, 252.96 and 257.10% for the first, second and residual season, respectively). The results obtained for both fertilizers indicated that fertilization of hyacinth bean by chicken manure or sulphur greatly improved the hydration coefficient of the crop seeds. Moreover, fertilization of inoculated seeds caused further improvement in hydration coefficient of the seeds. Generally, hydration coefficient is a valuable factor for both consumers and processors. Low hydration coefficient indicates that the seeds are not capable of absorbing water efficiently. Legumes, in general have more than double of the initial weight after soaking in water (Elsheikh and Alzidany, 1997b).

Table 1. Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on hydration coefficient of hyacinth bean grown for three consecutive seasons.

Treatment	Hydration coefficient (%)								
	1st season			2nd season			Residual (3rd season)		
	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken M.									
Control	233.43	233.83	233.63	225.83	232.47	229.15	226.70	229.50	228.20
2.5 t/fed	235.70	236.96	236.33	231.27	238.70	234.98	232.40	233.00	232.70
5.0 t/fed	242.20	244.06	243.13	239.80	242.90	241.35	233.80	237.40	235.60
7.5 t/fed	247.80	250.40	249.10	245.40	246.90	246.15	236.00	238.20	237.10
10 t/fed	25126	255.60	253.43	250.60	256.90	253.30	239.00	245.60	242.30
Means	242.07	244.17		238.58	243.58		233.58	236.74	
Overall			243.12			241.08			235.16
Sulphur									
Control	215.24	222.30	218.77	218.40	227.30	222.85	221.70	227.50	224.60
25 kg/fed	219.36	232.18	225.77	222.17	233.61	227.89	226.80	232.40	229.60
50 kg/fed	227.09	241.53	234.31	229.67	240.93	235.30	230.30	236.10	233.20
75 kg/fed	233.84	240.72	237.28	237.07	244.21	240.64	239.00	247.40	243.20
100 kg/fed	237.90	243.64	240.77	239.10	252.96	246.04	245.70	257.10	251.40
Means	226.69	236.07		229.28	239.80		232.70	240.10	
Overall			231.38			234.54			236.4

Treatment(s)	LSD (5%)		
	1st season	2nd season	Residual (3rd season)
Amendments	10.53	12.65	12.55
Inoculated	6.27	7.54	8.25
Amendments X Inoculated	16.13	13.73	13.92
Amendment X level	26.22	15.91	15.83
Amendment X Inoculated X level	37.79	17.96	19.21

Table 2. Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on cookability of hyacinth bean grown for three consecutive seasons.

Treatment	Cookability (%)								
	1st season			2nd season			Residual (3rd season)		
	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken M.									
Control	85.36	90.30	87.83	85.26	91.42	88.34	71.20	89.20	80.40
2.5 t/fed	87.11	97.57	92.34	89.32	97.96	93.64	78.59	87.97	83.28
5.0 t/fed	9.324	101.72	97.48	95.18	104.46	99.82	79.50	90.30	84.90
7.5 t/fed	99.81	102.43	101.12	103.74	115.60	109.67	82.58	96.12	89.35
10 t/fed	104.48	110.72	107.60	117.94	131.00	124.47	92.13	100.07	96.10
Means	94.00	100.54		98.28	108.08		80.80	92.74	
Overall			97.27			97.27			86.77
Sulphur									
Control	82.36	88.44	85.40	73.24	78.98	76.11	75.23	84.17	79.70
25 kg/fed	87.04	92.98	90.01	75.82	94.08	89.95	87.52	90.66	89.09
50 kg/fed	89.43	96.19	92.81	78.37	100.03	94.20	88.48	98.50	93.49
75 kg/fed	90.64	104.08	97.36	91.80	100.08	95.94	93.52	101.10	97.31
100 kg/fed	95.12	111.56	103.34	95.91	105.19	100.55	98.54	102.72	100.63
Means	88.91	98.65		83.03	95.67		88.65	95.43	
Overall			93.78			93.78			92.04

Treatment(s)	LSD (5%)		
	1st season	2nd season	Residual (3rd season)
Amendments	2.09	2.73	1.43
Inoculated	1.84	1.22	0.94
Amendments X Inoculated	3.52	3.82	2.16
Amendment X level	5.23	5.94	3.82
Amendment X Inoculated X level	6.82	8.40	5.24

It has been reported that a positive effect for hydration coefficient have been observed after application of chicken manure or sulphur to faba bean (Elsheikh and Alzidany, 1997b). Contradictory results were reported by Abdelgani *et al.,* (1999) who stated that hydration coefficient of fenugreek seeds were not affected by inoculation. However, other studies indicated that, inoculation significantly increased the hydration coefficient of groundnut (Elsheikh and Mohamedzein, 1998) and faba bean (Elsheikh and Ahmed, 2000) seeds. The difference in response to such treatments could be attributed to the difference in cultivars as well as the growing environment. Chicken manure or sulphur application significantly ($P \leq 0.05$) improved cookability of uninoculated seeds with increasing level of each (Table 2). However, after inoculation of the seeds the rate of improvement greatly increased in all seasons with maximum values obtained when 10t/fed of chicken manure was used (110.72, 131.00 and 100.07% for the first, second and residual season, respectively) or 100kg/fed of sulphur was applied (111.56, 105.19 and 102.72% for the first, second and residual season, respectively). The results obtained for both fertilizers indicated that fertilization of hyacinth bean by chicken manure or sulphur greatly improved cookability of the crop seeds. Moreover, fertilization of inoculated seeds caused further improvement in cookability of the seeds. It was also observed that the hydration coefficient greatly affected cookability of the seeds. Cookability is known to be affected by soaking time, type of water, environmental factors, location and time of harvesting (El Mubarak *et al*., 1988). It has been found that chicken manure significantly increased the cookability of faba bean seeds in the presence or absence of *Bradyrhizobium* inoculation (Elsheikh and Alzidany, 1997b).

Effect of treatments on proximate composition: The moisture (Table 3) and ash (Table 4) contents of hyacinth bean seeds were not significantly affected neither by chicken manure nor by sulphur application in the presence or absence of *Bradyrhizobium* inoculation in all seasons. Elsheikh and Alzidany (1997a) reported that inoculation of faba bean seeds with *Bradyrhizobium* was found to affect the moisture content of the seeds. Generally moisture content of the seeds was found to be affected by factors other than treatments such as the relative humidity of the surrounding atmosphere at the time of harvest, during inoculation and storage (Elsheikh, 2001). Inoculation was reported to increase the ash content of guar (Elsheikh and Ibrahim, 1999), faba bean (Elsheikh

and Mohamedzein, 1998) and fenugreek (Abdelgani *et al*., 1999) seeds. Chicken manure or sulphur fertilization significantly increased the fat content of hyacinth bean in the presence or absence of *Bradyrhizobium* inoculation in all seasons (Table 5). The maximum values obtained for fat after application of 10t/fed chicken manure to inoculated seeds were 2.71, 2.68 and 3.03% for the first, second and residual season, respectively. Application of 100kg/fed sulphur to inoculated seeds gave maximum values of 3.70, 3.13 and 3.93% fat during the first, second and residual season, respectively. The improvement trend obtained in this study is similar to that reported by Abdelgani *et al*. (1999) for fenugreek and Elsheikh and Alzidany (1997a) for faba bean. Fats are important dietary constituents because of their high energy value, vitamins and essential fatty acids which are associated with fat of natural food (Egan *et al*., 1989). Chicken manure or sulphur fertilization before and after inoculation slightly increased the fiber content of hyacinth bean in all seasons (Table 6). The maximum values obtained for fiber after application of 10t/fed chicken manure to inoculated seeds were 6.97, 6.06 and 8.43% for the first, second and residual season, respectively. Application of 100kg/fed sulphur to inoculated seeds gave maximum values of 8.20, 7.53 and 8.25% fiber during the first, second and residual season, respectively. Fiber content of legume seeds was reported to be affected by chemical and organic fertilizers (Elsheikh and Alzidany, 1997a), environmental condition (Elsheikh and Mohamedzein, 1998) and plant variety (Abdelgani *et al*., 1999). Treatments applied to the seeds provide fiber to be likely consisting of high level of cellulose together with proportion of lignin and hemicellulose. The fiber content is an important constituent of human and animal food and it is needed in a reasonable proportion as it gives the bulk to the diet and helps in movement of food through the digestive tract. Chicken manure or sulphur fertilization with or without *Bradyrhizobium* inoculation significantly ($P \leq 0.05$) increased the protein content of hyacinth bean seeds during all seasons (Table 7). The maximum values obtained for protein after application of 10t/fed chicken manure to inoculated seeds were 28.45, 27.66 and 27.89% for the first, second and residual season, respectively. Application of 100kg/fed sulphur to inoculated seeds gave maximum values of 28.45, 29.49 and 27.99% protein during the first, second and residual seasons, respectively. The results obtained are in a good agreement with those reported by Mendoza *et al*. (1994) and Mohamed and Mustafa, (1994).

Table 3. Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on moisture content of hyacinth bean grown for three consecutive seasons.

Treatment	Moisture content (%)								
	1st season			2nd season			Residual (3rd season)		
	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken M.									
Control	4.90	4.92	4.91	5.35	5.39	5.37	5.38	5.40	5.39
2.5 t/fed	5.00	5.04	5.02	5.48	5.52	5.50	5.40	5.52	5.46
5.0 t/fed	5.12	5.18	5.15	5.58	5.84	5.71	5.45	5.65	5.55
7.5 t/fed	5.19	5.31	5.25	5.78	5.94	5.86	5.68	5.94	5.81
10 t/fed	5.23	5.43	5.33	6.13	6.15	6.14	5.90	6.00	5.95
Means	5.08	5.18		5.66	5.76		5.56	5.70	
Overall			5.13			5.71			5.63
Sulphur									
Control	5.70	6.00	5.86	5.87	6.03	5.95	5.80	5.90	5.85
25 kg/fed	5.85	6.13	5.98	5.97	6.13	6.05	5.85	6.07	5.96
50 kg/fed	5.93	6.19	5.99	6.07	6.11	6.08	5.89	6.11	6.00
75 kg/fed	6.03	6.23	6.13	5.92	6.22	6.07	6.03	6.15	6.09
100 kg/fed	6.12	6.26	6.19	5.92	6.18	6.05	6.08	6.24	6.16
Means	5.92	6.16		5.95	6.13		5.93	6.09	
Overall			6.04			6.04			6.01

Treatment(s)	LSD (5%)		
	1st season	2nd season	Residual(3rd season)
Amendments	0.5	0.7	0.7
Inoculated	0.3	0.5	0.4
Amendments X Inoculated	0.9	1.1	1.0
Amendment X level	1.2	1.7	1.5
Amendment X Inoculated X level	1.9	2.5	2.1

Table 4. Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on ash content of hyacinth grown for three consecutive seasons.

Treatment	Ash content (%)								
	1st season			2nd season			Residual (3rd season)		
	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken M.									
Control	5.49	5.73	5.61	5.76	5.96	5.86	5.50	5.58	5.54
2.5 t/fed	5.80	6.14	5.97	5.85	6.07	5.96	5.66	5.82	5.74
5.0 t/fed	5.80	6.38	6.09	6.14	6.18	6.16	5.75	5.93	5.84
7.5 t/fed	6.03	6.67	6.35	5.87	6.27	6.07	5.79	6.03	5.91
10 t/fed	6.24	6.88	6.56	6.12	6.32	6.22	5.82	6.16	5.99
Means	5.87	6.35		5.94	6.16		5.70	5.90	
Overall			6.11			6.05			5.80
Sulphur									
Control	5.11	5.49	5.30	5.02	5.54	5.28	4.98	5.16	5.07
25 kg/fed	5.15	5.57	5.36	5.05	5.59	5.32	5.04	5.22	5.13
50 kg/fed	5.29	5.69	5.49	5.25	5.65	5.45	5.20	5.38	5.29
75 kg/fed	5.37	5.75	5.56	5.36	5.70	5.53	5.49	5.71	5.60
100 kg/fed	5.43	5.97	5.70	5.42	5.78	5.60	5.54	5.80	5.67
Means	5.27	5.69		5.23	5.65		5.85	5.45	
Overall			5.48			5.44			5.65

Treatment(s)	LSD (5%)		
	1st season	2nd season	Residual(3rd season)
Amendments	0.12	0.16	0.12
Inoculated	0.08	0.11	0.06
Amendments X Inoculated	0.18	0.22	0.18
Amendment X level	0.24	0.35	0.28
Amendment X Inoculated X level	0.42	0.50	0.40

Table 5. Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on fat content of hyacinth bean grown for three consecutive seasons.

Treatment	Fat content (%)								
	1st season			2nd season			Residual (3rd season)		
	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken M.									
Control	1.73	1.81	1.77	1.70	1.78	1.74	1.87	1.95	1.91
2.5 t/fed	1.90	2.30	2.10	1.83	2.33	2.08	2.30	2.66	2.48
5.0 t/fed	2.20	2.70	2.45	2.63	2.47	2.25	2.77	2.85	2.81
7.5 t/fed	2.63	2.87	2.75	2.33	2.87	2.60	2.53	2.89	2.71
10 t/fed	2.87	2.71	2.78	2.70	2.68	2.68	3.37	3.03	3.20
Means	2.27	2.47		2.12	2.42		2.56	2.68	
Overall			2.37			2.27			2.62
Sulphur									
Control	2,33	2.93	4.80	2.37	2.83	2.60	2.29	3.43	2.86
25 kg/fed	2.50	3.00	5.37	2.60	2.88	2.73	2.65	3.77	3.31
50 kg/fed	2.70	3.40	5.67	2.80	2.90	2.85	2.76	3.70	3.23
75 kg/fed	2.73	3.47	6.17	2.82	3.20	3.02	3.01	3.97	3.48
100 kg/fed	2.90	3.70	6.70	2.97	3.13	3.05	2.89	3.93	3.41
Means	2.63	3.30		2.71	2.99		2.72	3.76	
Overall			2.97			2.85			3.24

Treatment(s)	LSD (5%)		
	1st season	2nd season	Residual(3rd season)
Amendments	0.27	0.30	0.54
Inoculated	0.21	0.23	0.41
Amendments X Inoculated	0.38	0.42	0.76
Amendment X level	0.59	0.67	1.21
Amendment X Inoculated X level	0.88	0.95	1.72

Table 6. Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on fiber content of hyacinth bean grown for three consecutive seasons.

Treatment	Fiber content (%)								
	1st season			2nd season			Residual (3rd season)		
	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken M.									
Control	4.27	5.33	4.80	3.90	4.72	4.32	5.07	5.65	5.36
2.5 t/fed	4.90	5.82	5.36	4.13	5.01	4.57	6.90	6.82	6.86
5.0 t/fed	5.27	6.07	5.67	4.40	5.48	4.92	7.50	7.40	7.45
7.5 t/fed	5.83	6.51	6.17	4.73	6.13	5.43	7.47	8.53	8.00
10 t/fed	6.43	6.97	6.70	5.20	6.06	5.63	8.23	8.43	8.33
Means	5.34	6.14		4.47	5.48		7.03	7.37	
Overall			5.74			4.98			7.20
Sulphur									
Control	5.50	581	5.68	5.13	5.49	5.32	5.97	6.15	6.06
25 kg/fed	6.03	6.31`	6.17	5.50	5.76	5.63	7.20	8.26	7.73
50 kg/fed	6.40	7.04	6.72	5.97	6.33	6.15	8.23	7.83	8.03
75 kg/fed	7.00	7.58	7.28	6.30	6.86	6.58	6.63	7.73	7.18
100 kg/fed	7.40	8.20	7.80	6.97	7.53	7.25	8.53	8.25	3.40
Means	6.47	6.99		5.97	6.40		7.31	7.65	
Overall			6.73			5.58			7.48

Treatment(s)	LSD (5%)		
	1st season	2nd season	Residual(3rd season)
Amendments	0.09	0.07	0.13
Inoculated	0.05	0.04	0.0
Amendments X Inoculated	0.13	0.11	0.18
Amendment X level	0.15	0.17	0.29
Amendment X Inoculated X level	0.19	0.24	0.41

Table 7. Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on protein content of hyacinth bean grown for three consecutive seasons.

Treatment	Protein content (%)								
	1st season			2nd season			Residual (3rd season)		
	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken M.									
Control	22.89	23.58	23.23	22.16	23.64	22.90	22.14	23.36	22.75
2.5 t/fed	24.18	24.26	24.22	24.21	24.37	24.29	23.40	27.14	25.27
5.0 t/fed	24.81	25.61	25.21	25.40	26.40	25.90	23.57	27.01	25.29
7.5 t/fed	25.67	26.14	25.91	25.81	26.91	26.36	25.59	28.41	27.00
10 t/fed	27.08	28.45	27.77	26.16	27.66	26.91	25.33	27.89	26.61
Means	24.92	25.60		24.74	25.80		24.00	26.76	
Overall			25.26			25.27			25.38
Sulphur									
Control	21.65	21.91	21.78	21.68	23.28	22.48	22.47	25.51	23.99
25 kg/fed	22.35	21.53	23.94	24.96	25.12	25.04	22.85	26.85	24.85
50 kg/fed	24.80	26.58	21.69	25.34	27.48	26.41	23.35	27.15	24.25
75 kg/fed	25.14	27.30	26.22	25.96	30.62	28.29	25.20	27.72	26.46
100 kg/fed	25.67	28.45	27.07	29.49	29.49	29.49	25.25	27.99	26.62
Means	23.93	25.15		25.48	27.20		23.82	27.04	
Overall			24.54			26.34			25.48

Treatment(s)	LSD (5%)		
	1st season	2nd season	Residual(3rd season)
Amendments	0.62	0.73	0.67
Inoculated	0.13	0.16	0.49
Amendments X Inoculated	0.94	1.04	0.95
Amendment X level	1.51	1.64	1.50
Amendment X Inoculated X level	2.16	2.33	2.13

Manure fertilization was found to increase the protein content significantly for faba bean (Elsheikh and Alzidany, 1997a) and fenugreek (Abdelgani *et al*., 1999) seeds. The increase in the protein content of hyacinth bean due to inoculation is an expected result as *Bradyrhizobium* inoculation increased N-fixing efficiency where more nitrogen was fixed in the nodules and translocated to the seeds. Moreover, inoculation enhanced the symbiotic properties of hyacinth bean plant and better growth and production were obtained by biofertilizers application. Inoculation with *Bradyrhizobium* strain significantly ($P \leq 0.05$) decreased the carbohydrate content of hyacinth bean in all seasons (Table 8). Generally, the carbohydrate content of leguminous crops seeds was found to decrease with *Rhizobium* inoculation (Elsheikh, 2001). Chicken manure or sulphur fertilization also significantly decreased the carbohydrate content, in all seasons. The results obtained confirmed the findings of Elsheikh and Alzidany (1997a) for faba bean and Abdelgani *et al*. (1999) for fenugreek seeds. The reduction in carbohydrates content is likely to be due to increase of other constituents of the seeds after chicken manure or sulphur fertilization in the presence or absence of *Bradyrhizobium* inoculation.

Effect of treatments on tannin content and *in vitro* protein digestibility: Tannin content of hyacinth bean seeds was found to be ranged from 0.03 to 0.04 mg/ml depend on the growing conditions (Table 9). Application of chicken manure or sulphur with or without *Bradyrhizobium* inoculation increased tannin content of the seeds. The maximum value obtained for tannin after application of 10t/fed chicken manure to inoculated seeds was 0.11 mg/ml for all seasons. Application of 100kg/fed sulphur to

Table 8. Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on carbohydrate content of hyacinth bean grown for three consecutive seasons.

Treatment	Carbohydrate content (%)								
	1st season			2nd season			Residual (3rd season)		
	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken M.									
Control	60.72	58.63	59.68	61.13	58.51	59.82	60.00	58.06	59.03
2.5 t/fed	58.22	56.43	57.32	58.50	56.70	57.60	56.34	52.04	54.19
5.0 t/fed	57.00	54.66	55.83	56.45	53.63	55.04	55.06	51.16	63.11
7.5 t/fed	54.80	52.71	53.76	54.64	52.14	53.39	53.14	48.20	50.67
10 t/fed	52.35	49.96	51.14	53.69	51.97	52.83	51.35	48.83	50.09
Means	56.61	54.47		56.88	54.60		55.17	51.65	
Overall			55.54			55.74			53.41
Sulphur									
Control	59.71	57.86	58.78	59.93	56.83	58.38	58.49	53.85	56.17
25 kg/fed	58.12	57.16	57.69	55.92	54.52	55.22	56.31	49.83	53.07
50 kg/fed	55.16	51.34	53.25	54.57	51.53	53.05	54.57	49.83	52.20
75 kg/fed	53.73	49.77	51.75	53.64	47.40	50.52	53.64	48.72	51.18
100 kg/fed	52.78	47.82	50.30	49.23	47.89	48.56	51.71	47.79	49.75
Means	55.91	52.79		54.65	51.63		54.94	50.00	
Overall			54.35			53.14			52.47

Treatment(s)	LSD (5%)		
	1st season	2nd season	Residual(3rd season)
Amendments	0.90	0.87	1.22
Inoculated	0.33	0.29	0.83
Amendments X Inoculated	1.29	1.23	1.74
Amendment X level	2.01	1.95	2.72
Amendment X Inoculated X level	2.81	2.76	3.85

inoculated seeds gave maximum values of 0.12, 0.12 and 0.09 mg/ml tannin during the first, second and residual season, respectively. Tannin content have been found to lower the nutritive value of food and feeds by lowering palatability due to a stringency and bitter taste, complexing with protein and carbohydrates and lower the digestibility of both (Babiker and El Tinay, 1993) likely by inhibition of the digestive and microbial enzymes, toxicity to rumen microorganisms (Hahn *et al*., 1981). Chicken

manure or sulphur, application with or without *Bradyrhizobium* inoculation significantly (P ≤ 0.05) increased the *in vitro* protein digestibility of hyacinth bean in all seasons (Table 10).

Table 9. Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on tannin content (mg/ml) of hyacinth bean grown for three consecutive seasons.

Treatment	Tannin content (mg/ml)								
	1st season			2nd season			Residual (3rd season)		
	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken M.									
Control	0.03	0.05	0.04	0.05	0.05	0.05	0.04	0.04	0.04
2.5 t/fed	0.04	0.06	0.05	0.06	0.06	0.06	0.04	0.06	0.05
5.0 t/fed	0.06	0.08	0.07	0.06	0.08	0.07	0.05	0.07	0.06
7.5 t/fed	0.08	0.10	0.09	0.08	0.10	0.09	0.07	0.09	0.08
10 t/fed	0.09	0.11	0.10	0.11	0.11	0.11	0.09	0.11	0.10
Means	0.06	0.08		0.07	0.08		0.06	0.07	
Overall			0.07			0.075			0.065
Sulphur									
Control	0.04	0.04	0.04	0.04	0.06	0.05	0.04	0.04	0.04
25 kg/fed	0.05	0.07	0.06	0.05	0.07	0.06	0.05	0.07	0.06
50 kg/fed	0.06	0.08	0.07	0.06	0.08	0.07	0.05	0.09	0.07
75 kg/fed	0.08	0.08	0.08	0.07	0.09	0.08	0.07	0.09	0.08
100 kg/fed	0.10	0.12	0.11	0.10	0.12	0.11	0.09	0.09	0.09
Means	0.06	0.08		0.06	0.08		0.10	0.12	0.11
Overall			0.07			0.07			0.09

Treatment(s)	LSD (5%)		
	1st season	2nd season	Residual(3rd season)
Amendments	0.011	0.05	0.03
Inoculated	0.008	0.03	0.01
Amendments X Inoculated	0.015	0.06	0.05
Amendment X level	0.024	0.10	0.07
Amendment X Inoculated X level	0.034	0.21	0.10

Table 10. Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on *in vitro* protein digestibility (IVPD) of hyacinth bean grown for three consecutive seasons.

Treatment	IVPD (%)								
	1st season			2nd season			Residual (3rd season)		
	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken M.									
Control	67.07	70.35	68.71	66.00	71.86	68.93	62.86	77.02	69.94
2.5 t/fed	75.82	79.08	77.45	76.65	84.51	80.58	77.39	81.33	79.37
5.0 t/fed	88.10	81.48	84.79	88.46	76.30	82.38	76.98	92.32	84.65
7.5 t/fed	89.46	83.72	86.58	80.85	85.27	83.06	79.17	94.65	86.91
10 t/fed	95.38	88.95	92.17	95.00	85.08	90.04	88.37	97.33	92.85
Means	83.17	80.71		81.39	80.61		76.95	88.53	
Overall			81.94			81.00			82.74
Sulphur									
Control	71.13	73.61	72.37	65.73	69.17	67.45	60.61	69.51	65.06
25 kg/fed	75.24	78.06	76.65	70.14	76.38	73.26	66.60	72.14	69.37
50 kg/fed	83.49	89.29	86.39	82.04	89.82	85.88	76.12	79.28	77.70
75 kg/fed	87.51	93.17	90.34	84.00	88.24	86.12	78.19	86.67	82.43
100 kg/fed	90.63	98.33	94.48	94.09	100.31	97.20	82.46	90.80	86.63
Means	81.61	86.49		79.20	84.78		72.80	79.68	
Overall			84.05			81.99			76.24

Treatment(s)	LSD (5%)		
	1st season	2nd season	Residual(3rd season)
Amendments	3.11	3.11	2.27
Inoculated	2.21	1.06	1.92
Amendments X Inoculated	4.40	4.40	3.21
Amendment X level	6.95	6.95	5.07
Amendment X Inoculated X level	9.83	9.83	7.17

The maximum values obtained for the protein digestibility after application of 10t/fed chicken manure to inoculated seeds were 88.95, 85.08 and 97.33% for the first, second and residual season, respectively. Application of 100kg/fed sulphur to inoculated seeds gave maximum values of 98.33, 100.31 and 90.80% protein digestibility during the first, second and residual season, respectively. It has been reported that inoculation increased the *in vitro* protein digestibility of groundnuts (Elsheikh and Mohamedzein, 1998) and faba bean (Elsheikh and alzidany, 1997a). It has been reported that tannins adversely affected the protein digestibility (Babiker and El Tinay, 1993). However, in this study inoculation of the seeds increased both tannin content and protein digestibility and this observation is a departure from an otherwise good correlation between tannin content and protein digestibility. The explanation for this difference is not clear, but may lie in chemical (as well as quantitative) differences between tannins of different plants.

CONCLUSION

Chicken manure or sulfur fertilization significantly improved the seed quality of hyacinth bean especially at a level of 10t/fed of chicken manure or 100kg/fed of sulphur. Further improvement was observed when the seeds were inoculated with *Bradyrhizobium*.

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