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Bradyrhizobium Inoculation and/or Chicken Manure and Sulphur Fertilization of Soybean (*Glycine max* 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 and sulphur fertilization on physical characteristics and chemical composition of soybean (*Glycine max* L.) seeds. The results showed that chicken manure and sulphur fertilization in the presence or absence of *Bradyrhizobium* inoculation and their interactions significantly ($P \geq 0.05$) improved hydration coefficient and cookability of the seeds. Moisture and ash contents were not 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. However, inoculation 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 ($P \geq 0.05$) improved especially when chicken manure or sulphur was applied to inoculated seeds. The results also 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.

Key words: Bradyrhizobium, inoculation, chicken manure, soybean, proximate composition

INTRODUCTION

The importance of legumes as food lied primarily in their high protein content that averages 20 – 25%. Soybean (*Glycine max* L.) is a unique crop, containing 32 – 45% proteins extracted substances. Soybean seeds are used as a raw material for the production of top-quality dry oil, varnishes, soaps, plastics, candies, shampoos, pesticides, paints, disinfectants, strong glues and adhesives. The leading producer of soybean is the USA which accounted for 49%, Latin America and Caribbean produced 34%, Asia 14% and Africa less than 1%. The average yield in 2000 was 2210 kg/ha, ranging from about 3520 kg/ha in Western Europe and 2650 kg/ha in the USA, to 990 kg/ha in Africa[1]. Recently and due to increasing demand for soybean as a cash crop in Sudan the interest has been increased and the research work has also been reactivated[2]. Grain legumes such as cowpea and soybean are good nitrogen fixers, and they usually meet all of their nitrogen needs other than that absorbed from the soil[3]. Inoculation of soybean by *Bradyrhizobium japonicum* significantly increased nodulation, yield and seed quality[4]. 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[5,6]. Chicken manure is considered to have fertilizing properties intermediate between mineral fertilizers and farmyard manure and it has an appreciable residual effect[7]. 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 to reduce the pH, supply $SO^{=}_4$ to plants, makes phosphorus and micronutrients more available and reclaim soils[8]. The effectiveness of ES depends upon the soil type, pH, organic matter content, clay minerals, depth of soil profile and drainage status. However,

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Ghani *et al*.[9] reported that microbial population in soil is not a limiting in ES oxidation. *Rhizobium* inoculation significantly increased tannin content of faba bean[10] and groundnut[11] seeds. *Rhizobium* inoculation significantly increased the *in vitro* protein digestibility of groundnut[11] and faba bean[5,12] 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[10,13]. 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[14]. 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 and chicken manure fertilization on the physical characteristics and chemical composition of soybean seeds.

MATERIALS AND METHODS

Materials: Soybean (*Glycine max* L.) cultivar Jupiter used in this study was supplied kindly by the Arab Corporation for Agricultural Investment and Development, Khartoum, Sudan. *Bradyrhizobium* (TAL 109) 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[15] 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°C. Seeds were inoculated by mixing with a thick suspension of characoal based *Bradyrhizobium* inoculum, with average count of 1 x 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, Shambat, University of Khartoum, Sudan (Latitude 15° 40′ N and Longitude 32° 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:

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

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).

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

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 soybean seeds were processed in 200 ml of tap water in a conical flask at 110 °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[16].

Tannin Content Determination: Quantitative estimation of tannins was carried out using the modified vanillin–HCl method[17]. 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 °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)
- 20 = Sample weight (mg)

***In vitro* Protein Digestibility (IVPD) Determination:** IVPD was determined by the method of Saunder *et al.*[18]. 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°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[16]. Digestibility was calculated using the formula:

$$\text{IVPD \%} = \frac{\text{N in supernatant - enzyme N}}{\text{N in sample}} \times 100$$

Statistical Analysis: Data were analyzed statistically using analysis of variance and general linear model procedure of SAS Statistical Software[19]. The differences of means were identified by Duncan's test and data were considered significantly different when $p \geq 0.05$.

RESULTS AND DISCUSSION

Effect of Treatments on Seed Physical Properties: As shown in Table 1, *Bradyrhizobium* inoculation showed a significant ($P \geq 0.05$) effect on the hydration coefficient of soybean seeds in all seasons. Chicken manure and sulphur fertilization were significantly ($P \geq 0.05$) 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 (153.73%, 152.93 and 153.20% for the first, second and residual seasons, respectively) or 100kg/fed of sulphur was applied (154.86%, 152.20 and 156.52% for the first, second and residual seasons, respectively). The results obtained for both fertilizers indicated that fertilization of soybean 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 [6]. It has been reported that a positive effect for hydration coefficient have been observed after application of chicken manure or sulphur to faba bean [6]. According to Abdelgani *et al.*[20] the hydration coefficient of fenugreek seeds were not affected by inoculation. However, other studies indicated that, inoculation significantly increased the hydration coefficient of groundnut [11] and faba bean[12] seeds. The difference in response to such treatments could be attributed to the difference in cultivars as well as the growing environment. Chicken manure and

Table 1: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on hydration coefficient of soybean 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	124.53	127.37	125.95	122.90	124.70	123.80	127.17	128.91	128.04
2.5 t/fed	127.70	134.98	131.34	125.83	130.61	128.22	131.11	133.37	132.24
5.0 t/fed	129.20	135.42	132.31	13.27	138.73	135.00	134.05	139.35	136.70
7.5 t/fed	137.77	145.93	141.85	139.00	143.36	141.18	139.23	141.95	140.59
10 t/fed	139.83	153.73	146.78	145.87	152.93	149.40	145.02	153.20	149.11
Means	131.80	139.48		132.97	138.07		135.32	139.36	
Overall	135.65			135.52			137.34		
				Sulphur					
Control	127.22	129.28	128.25	128.22	129.66	128.94	128.71	129.53	129.12
25 kg/fed	132.04	140.16	136.10	13.09	134.31	132.70	132.13	137.27	134.70
50 kg/fed	139.12	143.68	141.40	137.52	140.14	138.83	138.28	142.74	140.51
75 kg/fed	144.67	150.13	147.40	139.14	143.64	141.39	142.40	148.96	145.68
100 kg/fed	148.40	154.86	151.63	146.24	152.20	149.22	152.04	156.52	154.28
Means	138.28	143.6		136.44	140.00		138.71	143.01	
Overall	140.95			138.22			140.8		

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

sulphur application significantly ($P \geq 0.05$) 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 (22.36%, 23.97 and 23.39% for the first, second and residual seasons, respectively) or 100kg/fed of sulphur was applied (22.40%, 30.19 and 24.94% for the first, second and residual seasons, respectively). The results obtained for both fertilizers indicated that fertilization of soybean 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[21]. It has been found that chicken manure significantly increased the cookability of faba bean seeds in the presence or absence of *Bradyrhizobium* inoculation[6].

Effect of Treatments on Proximate Composition: The moisture (Table 3) and ash (Table 4) contents of soybean 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[5] 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[22]. Inoculation was reported to increase the ash content of guar[23], faba bean[11] and fenugreek[20] seeds. Chicken manure and sulphur significantly increased the fat

Table 2: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on cookability of soybean 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	10.69	14.35	12.52	11.26	15.74	13.50	12.43	13.77	13.10
2.5 t/fed	13.35	17.33	15.34	16.07	18.09	17.08	16.70	19.24	17.97
5.0 t/fed	15.93	19.27	17.60	19.35	22.27	20.81	18.96	22.54	21.13
7.5 t/fed	18.70	20.90	19.80	19.42	22.94	21.18	19.28	22.98	22.90
10 t/fed	19.24	22.36	20.80	22.71	23.97	23.34	22.41	23.39	23.34
Means	15.58	18.84		17.76	20.60		17.96	20.38	
Overall	17.21			19.18			19.17		
				Sulphur					
Control	11.13	13.97	12.55	13.24	18.98	16.11	11.77	13.29	12.53
25 kg/fed	14.26	17.32	15.79	15.81	20.19	18.00	15.24	17.18	16.21
50 kg/fed	16.21	19.49	17.85	18.37	23.35	20.86	17.98	20.26	19.12
75 kg/fed	19.30	21.50	20.40	21.80	26.08	23.94	20.29	23.31	21.80
100 kg/fed	20.04	22.40	21.22	25.91	30.19	28.05	23.24	24.94	24.09
Means	16.18	18.94		19.03	23.75		17.70	19.78	
Overall	17.56			21.39			18.74		

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

Table 3: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on moisture content of soybean 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	6.91	6.95	6.23	6.97	7.05	7.01	7.00	7.10	7.05
2.5 t/fed	7.03	7.17	6.40	7.03	7.15	7.09	7.05	7.15	7.10
5.0 t/fed	7.08	7.26	6.47	7.12	7.20	7.16	7.05	7.17	7.11
7.5 t/fed	7.40	7.50	6.75	7.18	7.22	7.20	7.17	7.25	7.21
10 t/fed	7.52	7.60	6.86	7.32	7.38	7.35	7.21	7.33	7.27
Means	7.18	7.30		7.12	7.20		7.10	7.20	
Overall	7.24			7.16			7.15		
				Sulphur					
Control	6.10	6.12	6.11	6.25	6.33	6.29	6.14	6.58	6.36

Table 3: Continued

25 kg/fed	6.17	6.21	6.19	6.33	6.39	6.36	6.33	6.73	6.53
50 kg/fed	6.23	6.25	6.24	6.52	6.62	6.57	6.36	6.84	6.60
75 kg/fed	6.29	6.35	6.32	6.75	6.77	6.76	6.44	6.86	6.65
100 kg/fed	6.32	6.38	6.35	6.86	6.98	6.92	6.49	6.91	6.70
Means	6.22	6.26		6.54	6.62		6.35	6.79	
Overall	624			6.58			6.57		

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 soybean 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	4.02	4.12	4.07	4.15	4.17	4.16	4.15	4.21	4.18
2.5 t/fed	4.14	4.20	4.17	4.18	4.20	4.19	4.17	4.21	4.19
5.0 t/fed	4.17	4.23	4.20	4.20	4.24	4.22	4.23	4.27	4.25
7.5 t/fed	4.21	4.29	4.25	4.21	4.29	4.25	4.25	4.33	4.29
10 t/fed	4.19	4.33	4.26	4.22	4.32	4.27	4.30	4.40	4.35
Means	4.15	4.23		4.19	4.25		4.22	4.28	
Overall	4.19			4.22			4.25		
Sulphur									
Control	4.10	4.12	4.11	4.32	4.34	4.33	4.17	4.23	4.20
25 kg/fed	4.13	4.17	4.15	4.32	4.46	4.39	4.27	4.33	4.30
50 kg/fed	4.19	4.21	4.20	4.33	4.57	4.45	4.39	4.47	4.43
75 kg/fed	4.24	4.26	4.25	4.40	4.64	4.52	4.51	4.53	4.52
100 kg/fed	4.27	4.31	4.29	4.46	4.88	4.67	4.60	4.58	4.59
Means	4.17	4.21		4.36	4.58		4.38	4.42	
Overall	4.19			4.47			4.40		

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 soybean 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	17.50	18.16	17.83	18.50	18.50	18.50	19.00	20.00	19.50
2.5 t/fed	18.16	19.50	18.83	19.50	19.50	19.50	21.00	20.66	20.83
5.0 t/fed	19.84	20.50	20.17	20.50	20.66	20.58	22.33	22.91	22.62
7.5 t/fed	20.34	21.00	20.67	20.16	21.00	20.58	22.33	23.67	23.00
10 t/fed	20.67	21.17	26.92	20.34	20.50	20.42	23.67	23.67	24.67
Means	19.30	20.08		19.80	20.04		21.66	22.18	
Overall	19.68			19.92			22.42		
Sulphur									
Control	17.32	17.50	17.42	18.67	19.33	19.00	19.33	21.01	20.17
25 kg/fed	17.33	18.83	18.08	19.66	20.00	19.83	21.01	21.67	20.84
50 kg/fed	18.18	20.00	19.08	19.84	21.00	20.42	21.33	22.01	22.17
75 kg/fed	19.50	20.50	20.00	20.33	20.67	20.50	22.67	23.33	23.00
100 kg/fed	20.16	21.50	20.83	19.83	21.33	20.58	23.00	23.66	23.33
Means	18.50	19.68		19.66	20.46		21.46	22.54	
Overall	19.08			20.06			22.00		

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

content of soybean 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 21.17, 20.50 and 23.67% for the first, second and residual seasons, respectively. Application of 100kg/fed sulphur to inoculated seeds gave maximum values of 21.50, 21.33 and 23.66% fat during the first, second and residual seasons, respectively. The results obtained are similar to those reported by Abdelgani *et al*. [20] for fenugreek and Elsheikh and Alzidany[5] 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[24]. Chicken manure, sulphur fertilization before and after inoculation slightly increased the fiber content of soybean in all seasons (Table 6). The maximum values obtained for fiber after application of 10t/fed chicken manure to inoculated seeds were 5.95, 5.94 and 6.45% for the first, second and residual seasons, respectively. Application of 100kg/fed sulphur to inoculated seeds gave maximum values of 7.10, 5.47 and 6.83% fiber during the first, second and residual seasons, respectively. Fiber content of legume seeds was reported to be affected by chemical and organic fertilizers[5], environmental condition[11] and plant variety[20]. 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 ($P \geq 0.05$) increased the protein content of soybean seeds during

Table 6: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on fiber content of soybean 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	3.73	5.03	4.38	3.80	4.86	4.33	4.00	5.32	4.66
2.5 t/fed	4.00	5.50	4.75	3.84	5.30	4.57	4.57	6.01	5.29
5.0 t/fed	4.07	5.77	4.92	4.00	5.04	4.52	5.63	6.13	5.88
7.5 t/fed	4.13	5.93	5.03	4.00	5.70	4.85	6.00	6.30	6.14
10 t/fed	4.30	5.96	5.13	4.10	5.94	5.02	6.03	6.45	6.24
Means	4.04	5.64		3.95	5.37		5.24	6.04	
Overall	4.84			4.66			6.14		
Sulphur									
Control	3.77	4.03	3.90	3.70	3.84	3.77	4.00	4.26	4.13
25 kg/fed	4.50	4.96	4.73	4.17	4.23	4.20	5.53	6.09	5.81
50 kg/fed	4.90	5.54	5.22	4.40	4.50	4.45	5.73	6.29	6.01
75 kg/fed	5.27	6.51	5.88	5.13	5.17	5.15	5.80	0.60	6.20
100 kg/fed	5.88	7.10	6.49	4.83	5.47	5.15	5.97	6.83	6.40
Means	4.86	5.62		4.44	4.64		5.41	6.01	
Overall	5.24			4.54			5.71		

Treatment (s)	LSD (5%)		
	1st season	2nd season	Residual (3rd season)
Amendments	0.09	0.07	0.13
Inoculated	0.06	0.04	0.09
Amendments X Inoculated	0.13	0.11	0.18
Amendment X level	0.15	0.17	0.29
Amendment X Inoculated X level	0.09	0.07	0.41

all seasons (Table 7). The maximum values obtained for protein after application of 10t/fed chicken manure to inoculated seeds were 39.84, 40.25 and 36.84% for the first, second and residual seasons, respectively. Application of 100kg/fed sulphur to inoculated seeds gave maximum values of 40.15, 40.46 and 37.10% protein during the first, second and residual seasons, respectively. The results obtained are in a good agreement with those reported by Mendoza *et al*.[25] and Mohamed and Mustafa,[26]. Manure fertilization was found to increase the protein content significantly for faba bean[5] and fenugreek[20] seeds. The increase in the protein content of soybean 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 soybean plant and better growth and production were obtained by biofertilizers application. Inoculation with *Bradyrhizobium* strain significantly ($P \geq 0.05$) decreased the carbohydrate content of soybean in all seasons (Table 8). Generally, the carbohydrate content of leguminous crops seeds was found to decrease with *Rhizobium* inoculation[22]. Chicken manure and sulphur fertilization also significantly decreased the carbohydrate content, in all seasons. The results obtained confirmed the findings of Elsheikh and Alzidany[5] for faba bean and Abdelgani *et al*.[20] for fenugreek seeds. The reduction in carbohydrates content is likely to be due to increase of other constituents of the seeds after chicken manure and sulphur fertilization in the presence or absence of *Bradyrhizobium* inoculation.

Effect of Treatments on Tannin Content and *in vitro* Protein Digestibility: Tannin content of soybean seeds was found to be ranged from 0.04 to 0.05 mg/ml depend on the growing conditions (Table 9).

Table 7: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on protein content of soybean 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	33.80	35.32	34.56	34.43	36.09	35.26	30.04	32.58	31.31
2.5 t/fed	34.48	36.72	35.60	35.49	37.59	36.54	30.36	31.70	31.03
5.0 t/fed	34.96	36.94	35.95	34.56	39.20	36.88	35.16	30.66	32.91
7.5 t/fed	37.05	38.97	38.02	37.05	38.55	37.80	35.43	35.87	35.65
10 t/fed	38.12	39.84	38.98	38.53	40.25	39.39	36.50	36.84	36.67
Means	35.68	37.56		36.01	38.33		33.09	33.53	
Overall	36.62			36.62			33.32		
				Sulphur					
Control	33.50	34.64	34.07	34.84	36.14	35.49	29.17	30.41	29.79
25 kg/fed	36.90	36.88	36.89	37.53	39.99	38.76	31.42	33.18	32.50
50 kg/fed	38.76	39.08	38.92	39.44	41.18	40.31	33.56	36.46	35.01
75 kg/fed	37.25	39.57	38.41	37.42	38.76	38.09	34.62	35.76	35.19
100 kg/fed	37.47	40.15	38.81	37.06	40.46	38.76	36.70	37.10	36.90
Means	36.78	38.06		37.26	39.32		33.09	34.57	
Overall	37.42			37.42			33.83		

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

Table 8: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on carbohydrate content of soybean 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	19.90	30.46	25.18	32.15	29.33	30.74	35.81	30.79	33.30
2.5 t/fed	17.22	27.05	22.13	29.96	26.26	28.11	32.85	30.15	31.50
5.0 t/fed	13.89	25.48	19.69	29.52	23.86	26.69	25.60	29.76	27.68
7.5 t/fed	26.87	22.31	24.55	27.29	23.56	25.43	25.82	25.18	25.50
10 t/fed	29.20	21.20	23.20	25.29	21.91	23.60	6.49	1.45	23.97
Means	20.62	25.30		28.84	24.98		29.31	27.47	
Overall	22.96			26.91			28.39		
Sulphur									
Control	35.21	33.87	34.54	32.22	29.52	30.87	37.19	33.51	35.35
25 kg/fed	31.03	28.95	29.99	28.09	24.53	26.31	31.44	27.80	29.62
50 kg/fed	28.05	25.05	26.55	25.47	22.23	23.85	29.13	22.93	26.03
75 kg/fed	27.65	22.93	25.29	25.89	24.39	25.14	27.36	23.76	25.56
100 kg/fed	27.08	20.72	23.90	27.26	21.40	24.33	26.44	22.12	24.28
Means	29.86	26.30		27.79	24.41		30.31	26.01	
Overall	28.05			26.10			28.16		

Table 8: Continued

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

Table 9: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on Tannin content of soybean 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	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.05
2.5 t/fed	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.07	0.06
5.0 t/fed	0.06	0.08	0.07	0.06	0.06	0.06	0.07	0.09	0.08
7.5 t/fed	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.`0	0.09
10 t/fed	0.10	0.12	0.11	0.09	0.09	0.09	0.09	0.13	0.11
Means	0.07	0.07		0.06	0.06		0.07	0.07	
Overall	0.07			0.06			0.08		
Sulphur									
Control	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06
25 kg/fed	0.06	0.06	0.06	0.06	0.08	0.07	0.07	0.07	0.07
50 kg/fed	0.07	0.09	0.08	0.07	0.09	0.08	0.08	0.10	0.09
75 kg/fed	0.09	0.09	0.09	0.08	0.10	0.10	0.10	0.10	0.10
100 kg/fed	0.11	0.11	0.11	0.10	0.10	0.10	0.10	0.12	0.11
Means	0.08	0.08		0.07	0.08		0.08	0.09	
Overall	0.08			0.075			0.085		

Treatment (s)	LSD (5%)		
	1st season	2nd season	Residual (3rd season)
Amendments	0.02	0.011	0.03
Inoculated	0.009	0.008	0.01
Amendments X Inoculated	0.06	0.015	0.05
Amendment X level	0.09	0.024	0.07
Amendment X Inoculated X level	0.18	0.034	0.10

Application of chicken manure or sulphur with or without *Bradyrhizobium* inoculation increased tannin content of the seeds. The maximum values obtained for tannin after application of 10t/fed chicken manure to inoculated seeds were 0.12, 0.09 and 0.13 mg/ml for the first, second and residual seasons, respectively. Application of 100kg/fed sulphur to inoculated seeds gave maximum values of 0.11, 0.10 and 0.12 mg/ml tannin during the first, second and residual seasons, 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 likely by inhibition of the digestive and microbial enzymes, toxicity to rumen microorganisms[27]. Chicken manure or sulphur, application with or without *Bradyrhizobium* inoculation significantly ($P \geq 0.05$) increased the *in vitro* protein digestibility of soybean in all seasons (Table 10). The maximum values obtained for the protein digestibility after application of 10t/fed chicken manure to inoculated seeds were 94.10, 94.23 and 79.67% for

Table 10: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on *in vitro* protein digestibility (IVPD) of soybean 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	53.54	61.10	57.32	53.49	61.49	57.49	45.96	54.36	50.16
2.5 t/fed	58.79	67.61	63.20	59.64	67.07	63.36	47.51	60.39	53.95
5.0 t/fed	66.45	72.47	69.46	66.57	76.57	71.57	49.01	66.87	57.94
7.5 t/fed	83.78	87.04	85.41	83.10	79.04	81.07	57.17	76.97	67.07
10 t/fed	86.96	94.10	90.53	89.47	94.23	91.85	67.33	79.67	73.50
Means	69.90	76.46		70.46	75.68		53.39	67.65	
Overall	73.18			73.07			60.52		
				Sulphur					
Control	57.90	58.38	58.14	61.90	58.12	60.01	55.01	61.01	58.01
25 kg/fed	65.90	61.90	63.90	64.10	65.76	64.92	57.89	65.01	61.45
50 kg/fed	80.33	77.51	78.92	78.93	80.17	79.55	67.82	68.10	67.96
75 kg/fed	83.26	85.34	84.30	82.75	93.39	81.07	70.16	71.82	70.99
100 kg/fed	93.17	99.19	96.18	91.19	97.71	64.45	72.15	74.57	73.36
Means	76.11	76.47		75.77	79.03		64.6	68.10	
Overall	76.29			77.40			60.35		

Treatment (s)	LSD (5%)		
	1st season	2nd season	Residual (3rd season)
Amendments	3.11	2.15	2.27
Inoculated	2.21	1.06	1.92
Amendments X Inoculated	4.40	3.05	3.21
Amendment X level	6.95	4.82	5.07
Amendment X Inoculated X level	9.83	6.82	7.17

the first, second and residual seasons, respectively. Application of 100kg/fed sulphur to inoculated seeds gave maximum values of 99.19, 97.71 and 74.57% protein digestibility during the first, second and residual seasons, respectively. It has been reported that inoculation increased the *in vitro* protein digestibility of groundnuts[11] and faba bean[5]. It has been reported that tannins adversely affected the protein digestibility[27]. 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 soybean 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*. Proper fertilization programmes, focusing on biofertilization should be implemented to improve the productivity of food legumes and thereby increase total food production, improve the supply of good quality proteins as well as minerals in the diet of people who largely depend on food legume crops, and improve seed quality. The latter implies processing, consumer, nutritional value and export quality. This investigation also calls food scientists to allow for the previous agronomic treatments, the history of the seeds, their origin, and certification, before starting their experiments, analysis or interpreting their data.

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