

Minerals Composition of Soybean (*Glycine max* L.) Seeds as Influenced by *Bradyrhizobium* Inoculation and Chicken Manure or Sulphur Fertilization

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Abstract: A field experiments were 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 and chicken manure or sulphur fertilization on minerals composition of soybean (*Glycine max* L.). The results obtained showed that inoculation, chicken manure, sulphur and their interactions significantly ($P = 0.05$) improved both major and trace minerals composition of the seeds. The results also indicated that all measured parameters increased with increasing level of chicken manure or sulphur and the highest value of each mineral was observed with either 10 tone/fed chicken manure or 100 kg/fed sulfur with or without *Bradyrhizobium* inoculation. The study also showed that the residual effect of chicken manure or sulphur significantly ($P = 0.05$) improved minerals content particularly at 10 tone/fed chicken manure or 100 kg/fed sulfur with or without *Bradyrhizobium* inoculation.

Key words: *Bradyrhizobium*, inoculation, chicken manure, soybean, mineral composition

Introduction

Soybean [*Glycine max* (L.)] is a legume that grows in tropical, subtropical and temperate climate. Approximately half of the world's soybeans are produced in the developing world and the other half in the developed world (IITA, 2002). Soybean is an important source of inexpensive and high quality content of protein and oil. With an average protein content of 40% and oil content of 20%, soybean has the highest protein content of all food crops and is second only to groundnut in terms of oil content among food legumes. Compared to other protein-rich foods such as meat, fish and eggs, soybean is by far the cheapest. Hamad (1986) reported that Ca content of soybean ranged from 268.75 to 293.0, Cu from 1.2 to 1.37, Fe from 9.04 to 13.32, Mg from 261.0 to 296.0, Mn from 3.38 to 4.94, K from 1500 to 1935, Na from 11.9 to 15.11 and Zn from 3.75 to 4.02 mg/100 g. Increasing population and the consequent increased demand for food production and food quality in the world, require that proposed agronomic strategies for improvement should, in general, avoid high input costs. Biofertilizers such as rhizobia and mycorrhiza, are steadily receiving increased attention and recognition from scientists. This could be attributed to the fact that they pose no ecological threats, usually have a longer-lasting effect and if properly managed can out-yield recommended doses of chemical fertilizers (Mahdi and Atabani, 1992). The latter effect is of special importance for countries, like Sudan, with predominantly low-input agricultural systems of production. Breeding programmes were started all over the world to improve

seed quality of legumes. Fertilizer programmes were also established in the Sudan to serve the same purpose (Babiker *et al.*, 1995; Elsheikh and Alzidany, 1997a). 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 improving the plant growth. 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). Elemental sulphur has a variety of uses as soil amendment. The oxidation of elemental sulphur to H_2SO_4 is particularly beneficial in alkaline soils to reduce the pH, supply SO_4^{2-} to plants, makes phosphorus and micronutrients more available and reclaim soils (Lindemann *et al.*, 1991). The effectiveness of elemental sulphur 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 elemental sulphur oxidation. Efforts throughout the world are directed towards improving the nutritional quality of crops by decreasing the level of antinutrients and improving the nutritional quality 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 and Osman, 1995). No trials were conducted to study the effect of chicken manure or elemental sulphur in combination with *Bradyrhizobium* inoculation on minerals composition of plant seeds. Therefore, the objective of this study was to assess the possibility of increasing minerals content of soybean by *Bradyrhizobium* inoculation and/or sulphur or chicken manure fertilization

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 Mannitol (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 3.0g 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 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, 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:

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.

Total mineral determination: Minerals were extracted from the samples by the dry ashing method described by Walsh (1980). About 1.0g sample was acid-digested with diacid mixture (HNO_3 : HClO_4 , 5: 1, v/v) in a digestion chamber. The digested samples were dissolved in double-distilled water and filtered (Whatman No. 42). The filtrate was made to 50 ml with double-distilled water and was used for the determination of total minerals. The amount of iron, zinc, manganese and copper were determined using atomic absorption spectroscopy (Perkin-Elmer 2380, USA). Calcium and magnesium were determined by the titration method described by Chapman and Pratt (1961). Sodium and potassium were determined using a flame photometer (CORNIG EEL, London, UK) according to the AOAC (1995) method.

Statistical analysis: Experimental data were analyzed by using the general linear models procedure, the ANOVA procedure and Duncan's multiple range test (Duncan, 1955). Least significant differences were computed at $P = 0.05$. Data were also analyzed using the correlation procedure (Pearson's correlation coefficients) in SAS (1995).

Results and Discussion

Effect of treatments on major minerals content: As shown in Table 1, *Bradyrhizobium* inoculation showed a significant ($P = 0.05$) effect on calcium (Ca) content of soybean seeds in all seasons. Chicken manure and sulphur fertilization were significantly ($P = 0.05$) increased Ca content of uninoculated seeds with increasing level of each. However, after inoculation it

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Table 1: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on calcium (Ca) content of soybean grown for three consecutive seasons

| Treatment | Ca content (mg/100g) | | | | | | | | |
|--------------------------------|------------------------|------------|----------|------------------------|------------|-------|-----------------------------------|------------|-------|
| | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means |
| Chicken M. | | | | | | | | | |
| Control | 225.0 | 229.0 | 227.0 | 230.0 | 240.0 | 235.0 | 244.0 | 266.0 | 255.0 |
| 2.5 t/fed | 230.0 | 236.0 | 233.0 | 234.0 | 244.0 | 239.0 | 250.0 | 260.0 | 255.0 |
| 5.0 t/fed | 250.0 | 260.0 | 255.0 | 236.0 | 248.0 | 242.0 | 270.0 | 294.0 | 282.0 |
| 7.5 t/fed | 280.0 | 300.0 | 290.0 | 254.0 | 274.0 | 264.0 | 310.0 | 330.0 | 320.0 |
| 10 t/fed | 320.0 | 330.0 | 325.0 | 290.0 | 310.0 | 300.0 | 350.0 | 380.0 | 365.0 |
| Means | 261.0 | 271.0 | | 248.8 | 263.2 | | 284.8 | 306.0 | |
| Overall | | | 266.0 | | | 256.0 | | | 295.4 |
| Sulphur | | | | | | | | | |
| Control | 230.0 | 242.0 | 236.0 | 226.0 | 230.0 | 228.0 | 200.0 | 240.0 | 220.0 |
| 25 kg/fed | 252.0 | 264.0 | 258.0 | 236.0 | 246.0 | 241.0 | 222.0 | 244.0 | 233.0 |
| 50 kg/fed | 294.0 | 310.0 | 302.0 | 245.0 | 259.0 | 252.0 | 250.0 | 278.0 | 264.0 |
| 75 kg/fed | 326.0 | 340.0 | 333.0 | 271.0 | 287.0 | 279.0 | 296.0 | 320.0 | 308.0 |
| 100 kg/fed | 352.0 | 368.0 | 360.0 | 305.0 | 307.0 | 306.0 | 330.0 | 370.0 | 350.0 |
| Means | 290.8 | 304.8 | | 256.6 | 265.8 | | 259.6 | 290.4 | |
| Overall | | | 297.8 | | | 261.2 | | | 275.0 |
| | | | LSD (5%) | | | | | | |
| Treatment(s) | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| Amendments | 13.72 | | | 12.62 | | | 17.12 | | |
| Inoculated | 8.68 | | | 7.80 | | | 10.15 | | |
| Amendments X Inoculated | 52.06 | | | 20.10 | | | 24.84 | | |
| Amendment X level | 36.63 | | | 33.30 | | | 38.21 | | |
| Amendment X Inoculated X level | 53.04 | | | 49.02 | | | 54.73 | | |

Table 2: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on magnesium (Mg) content of soybean grown for three consecutive seasons

| Treatment | Mg content (mg/100g) | | | | | | | | |
|--------------------------------|------------------------|------------|----------|------------------------|------------|-------|-----------------------------------|------------|-------|
| | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means |
| Chicken M. | | | | | | | | | |
| Control | 200.0 | 220.0 | 210.0 | 190.0 | 198.0 | 194.0 | 196.0 | 204.0 | 200.0 |
| 2.5 t/fed | 210.0 | 228.0 | 219.0 | 206.0 | 212.0 | 209.0 | 200.0 | 220.0 | 210.0 |
| 5.0 t/fed | 232.0 | 244.0 | 236.0 | 230.0 | 232.0 | 231.0 | 236.0 | 248.0 | 242.0 |
| 7.5 t/fed | 256.0 | 272.0 | 264.0 | 239.0 | 255.0 | 247.0 | 260.0 | 286.0 | 273.0 |
| 10 t/fed | 260.0 | 284.0 | 272.0 | 256.0 | 278.0 | 267.0 | 272.0 | 290.0 | 281.0 |
| Means | 231.6 | 249.6 | | 224.2 | 235.0 | | 232.8 | 249.6 | |
| Overall | | | 240.6 | | | 229.6 | | | 241.2 |
| Sulphur | | | | | | | | | |
| Control | 196.0 | 208.0 | 201.0 | 201.0 | 205.0 | 203.0 | 201.0 | 205.0 | 203.0 |
| 25 kg/fed | 216.0 | 226.0 | 221.0 | 214.0 | 224.0 | 219.0 | 215.0 | 225.0 | 220.0 |
| 50 kg/fed | 234.0 | 246.0 | 240.0 | 226.0 | 240.0 | 233.0 | 219.0 | 229.0 | 224.0 |
| 75 kg/fed | 248.0 | 258.0 | 253.0 | 236.0 | 242.0 | 239.0 | 241.0 | 257.0 | 249.0 |
| 100 kg/fed | 270.0 | 296.0 | 283.0 | 254.0 | 264.0 | 259.0 | 270.0 | 282.0 | 276.0 |
| Means | 232.8 | 246.8 | | 226.2 | 235.0 | | 229.6 | 239.6 | |
| Overall | | | 239.8 | | | 230.6 | | | 234.4 |
| | | | LSD (5%) | | | | | | |
| Treatment(s) | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| Amendments | 10.36 | | | 10.47 | | | 11.71 | | |
| Inoculated | 5.64 | | | 4.48 | | | 7.31 | | |
| Amendments X Inoculated | 15.07 | | | 15.12 | | | 16.23 | | |
| Amendment X level | 24.41 | | | 24.20 | | | 24.72 | | |
| Amendment X Inoculated X level | 33.93 | | | 32.81 | | | 33.35 | | |

was greatly increased in all seasons with maximum values obtained when 10t/fed of chicken manure was applied (330.0, 310.0 and 380.0 mg/100g) for the first,

second and residual season, respectively) or 100kg/fed of sulphur (368.0, 307.0 and 370.0 mg/100g for the first, second and residual seasons, respectively). The results

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Table 3: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on potassium (K) content of soybean grown for three consecutive seasons

| Treatment | K content (mg/100g) | | | | | | | | |
|------------|------------------------|------------|--------|------------------------|------------|--------|-----------------------------------|------------|--------|
| | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means |
| Chicken M. | | | | | | | | | |
| Control | 1190.0 | 1204.0 | 1197.0 | 1174.0 | 1188.0 | 1181.0 | 1194.0 | 1210.0 | 1202.0 |
| 2.5 t/fed | 1185.0 | 1215.0 | 1198.0 | 1198.0 | 1218.0 | 1208.0 | 1205.0 | 1235.0 | 1220.0 |
| 5.0 t/fed | 1224.0 | 1246.0 | 1235.0 | 1242.0 | 1270.0 | 1256.0 | 1256.0 | 1278.0 | 1267.0 |
| 7.5 t/fed | 1250.0 | 1276.0 | 1263.0 | 1296.0 | 1302.0 | 1299.0 | 1310.0 | 1330.0 | 1320.0 |
| 10 t/fed | 1292.0 | 1316.0 | 1304.0 | 1324.0 | 1356.0 | 1340.0 | 1356.0 | 1374.0 | 1365.0 |
| Means | 1228.2 | 1251.4 | | 1246.8 | 1266.8 | | 1264.2 | 1285.4 | |
| Overall | | | 1239.8 | | | 1256.8 | | | 1274.8 |
| Sulphur | | | | | | | | | |
| Control | 1184.0 | 1196.0 | 1190.0 | 1168.0 | 1182.0 | 1175.0 | 1191.0 | 1199.0 | 1195.0 |
| 25 kg/fed | 1198.0 | 1216.0 | 1207.0 | 1187.0 | 1199.0 | 1193.0 | 1209.0 | 1237.0 | 1223.0 |
| 50 kg/fed | 1220.0 | 1244.0 | 1232.0 | 1230.0 | 1250.0 | 1240.0 | 1247.0 | 1267.0 | 1257.0 |
| 75 kg/fed | 1268.0 | 1290.0 | 1279.0 | 1276.0 | 1294.0 | 1285.0 | 1287.0 | 1303.0 | 1295.0 |
| 100 kg/fed | 1316.0 | 1328.0 | 1322.0 | 1320.0 | 1336.0 | 1328.0 | 1325.0 | 1345.0 | 1335.0 |
| Means | 1237.2 | 1254.8 | 229.2 | 1236.2 | 1252.2 | | 1251.8 | 1270.2 | |
| Overall | | | 1246.0 | | | 1244.2 | | | 1261.0 |

LSD (5%)

| Treatment(s) | 1 st season | 2 nd season | Residual (3 rd season) |
|--------------------------------|------------------------|------------------------|-----------------------------------|
| Amendments | 8.26 | 9.87 | 9.87 |
| Inoculated | 3.03 | 5.42 | 3.89 |
| Amendments X Inoculated | 10.65 | 13.96 | 13.96 |
| Amendment X level | 18.30 | 22.07 | 22.07 |
| Amendment X Inoculated X level | 27.06 | 31.22 | 31.22 |

Table 4: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on sodium (Na) content of soybean grown for three consecutive seasons

| Treatment | Na content (mg/100g) | | | | | | | | |
|------------|------------------------|------------|-------|------------------------|------------|-------|-----------------------------------|------------|-------|
| | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means |
| Chicken M. | | | | | | | | | |
| Control | 9.42 | 9.84 | 9.63 | 9.24 | 9.56 | 9.35 | 9.36 | 9.66 | 9.51 |
| 2.5 t/fed | 10.24 | 10.38 | 10.31 | 9.78 | 9.90 | 9.84 | 9.86 | 10.12 | 9.99 |
| 5.0 t/fed | 10.39 | 10.71 | 10.55 | 10.06 | 10.38 | 10.22 | 10.28 | 10.54 | 10.36 |
| 7.5 t/fed | 11.23 | 11.67 | 11.45 | 10.56 | 10.82 | 10.69 | 10.76 | 10.94 | 10.85 |
| 10. t/fed | 11.82 | 12.16 | 11.99 | 11.12 | 11.20 | 11.16 | 11.17 | 11.31 | 11.24 |
| Means | 10.65 | 10.95 | | 10.15 | 10.37 | | 10.29 | 10.51 | |
| Overall | | | 10.80 | | | 10.26 | | | 10.40 |
| Sulphur | | | | | | | | | |
| Control | 8.52 | 8.78 | 8.65 | 7.96 | 8.18 | 8.07 | 8.12 | 8.28 | 2.20 |
| 25 kg/fed | 9.04 | 9.24 | 9.14 | 8.32 | 8.64 | 8.48 | 8.54 | 8.78 | 8.66 |
| 50 kg/fed | 9.58 | 9.84 | 9.71 | 8.98 | 9.16 | 9.07 | 9.10 | 9.36 | 9.23 |
| 75 kg/fed | 10.24 | 10.42 | 10.33 | 9.47 | 9.75 | 9.61 | 9.64 | 9.88 | 9.76 |
| 100 kg/fed | 10.64 | 11.04 | 10.84 | 10.15 | 10.35 | 10.25 | 10.12 | 10.38 | 10.25 |
| Means | 9.60 | 9.86 | | 8.97 | 9.21 | | 9.10 | 9.34 | |
| Overall | | | 9.73 | | | 9.09 | | | 9.22 |

LSD (5%)

| Treatment(s) | 1 st season | 2 nd season | Residual (3 rd season) |
|--------------------------------|------------------------|------------------------|-----------------------------------|
| Amendments | 0.83 | 0.13 | 0.33 |
| Inoculated | 0.51 | 0.09 | 0.14 |
| Amendments X Inoculated | 1.17 | 0.09 | 0.47 |
| Amendment X level | 1.86 | 0.29 | 0.73 |
| Amendment X Inoculated X level | 2.62 | 0.41 | 0.97 |

obtained for both fertilizers indicated that fertilization of soybean by chicken manure or sulphur greatly increased Ca content of the crop seeds. Moreover, fertilization of

inoculated seeds caused further increase in Ca content. Elsheikh and Mohamedzein (1998) reported that groundnut seed Ca content did not show any pattern in

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Table 5: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on iron (Fe) content (of soybean grown for three consecutive seasons

| Treatment | Fe content (mg/100g) | | | | | | | | |
|--------------------------------|------------------------|------------|-------|------------------------|------------|-------|-----------------------------------|------------|-------|
| | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means |
| Chicken M. | | | | | | | | | |
| Control | 6.90 | 7.16 | 7.03 | 7.01 | 7.09 | 7.05 | 7.79 | 7.89 | 7.84 |
| 2.5 t/fed | 6.95 | 7.23 | 7.09 | 7.21 | 7.23 | 7.22 | 8.12 | 8.44 | 8.28 |
| 5.0 t/fed | 7.29 | 7.69 | 7.49 | 7.78 | 8.10 | 7.94 | 8.76 | 8.92 | 8.84 |
| 7.5 t/fed | 8.18 | 8.60 | 8.39 | 8.65 | 9.15 | 8.90 | 9.19 | 9.39 | 9.29 |
| 10 t/fed | 9.24 | 10.16 | 9.70 | 10.15 | 10.55 | 10.35 | 9.91 | 10.23 | 10.07 |
| Means | 7.71 | 8.17 | | 8.16 | 8.42 | | 8.75 | 8.97 | |
| Overall | | | 7.94 | | | 8.29 | | | 8.86 |
| Sulphur | | | | | | | | | |
| Control | 6.94 | 7.08 | 7.01 | 6.85 | 7.05 | 6.95 | 7.50 | 7.82 | 7.66 |
| 25 kg/fed | 7.02 | 7.34 | 7.18 | 7.25 | 7.37 | 7.31 | 8.11 | 8.27 | 8.19 |
| 50 kg/fed | 7.73 | 7.97 | 7.85 | 7.71 | 7.97 | 7.84 | 8.50 | 8.98 | 8.74 |
| 75 kg/fed | 8.24 | 8.78 | 8.51 | 8.46 | 8.76 | 8.61 | 9.24 | 9.84 | 9.54 |
| 100 kg/fed | 9.10 | 10.02 | 9.56 | 9.20 | 9.84 | 9.52 | 10.13 | 10.73 | 10.43 |
| Means | 7.81 | 8.23 | | 7.89 | 8.19 | | 8.70 | 9.12 | |
| Overall | | | 8.02 | | | 8.04 | | | 8.91 |
| LSD (5%) | | | | | | | | | |
| Treatment(s) | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| Amendments | 0.16 | | | 0.18 | | | 0.22 | | |
| Inoculated | 0.08 | | | 0.10 | | | 0.14 | | |
| Amendments X Inoculated | 0.23 | | | 0.26 | | | 0.31 | | |
| Amendment X level | 0.38 | | | 0.41 | | | 0.44 | | |
| Amendment X Inoculated X level | 0.55 | | | 0.58 | | | 0.57 | | |

Table 6: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on copper (Cu) content of soybean grown for three consecutive seasons

| Treatment | Cu content (mg/100g) | | | | | | | | |
|--------------------------------|------------------------|------------|-------|------------------------|------------|-------|-----------------------------------|------------|-------|
| | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means |
| Chicken M. | | | | | | | | | |
| Control | 0.75 | 0.93 | 0.84 | 0.70 | 0.94 | 0.82 | 0.69 | 0.91 | 0.80 |
| 2.5 t/fed | 1.07 | 1.59 | 1.33 | 1.04 | 1.44 | 1.24 | 0.98 | 1.16 | 1.07 |
| 5.0 t/fed | 1.91 | 2.37 | 1.75 | 1.79 | 2.09 | 1.94 | 1.67 | 1.85 | 1.76 |
| 7.5 t/fed | 2.84 | 3.12 | 2.98 | 2.71 | 3.05 | 2.88 | 2.12 | 2.72 | 2.42 |
| 10 t/fed | 2.96 | 3.24 | 3.10 | 2.94 | 3.30 | 3.12 | 3.04 | 3.18 | 3.11 |
| Means | 1.91 | 2.25 | | 1.84 | 2.16 | | 1.70 | 1.96 | |
| Overall | | | 2.08 | | | 2.0 | | | 1.83 |
| Sulphur | | | | | | | | | |
| Control | 0.80 | 0.98 | 0.89 | 1.00 | 1.22 | 1.11 | 0.72 | 0.88 | 0.80 |
| 25 kg/fed | 1.15 | 1.63 | 1.39 | 1.29 | 1.47 | 1.38 | 1.10 | 1.36 | 1.23 |
| 50 kg/fed | 1.87 | 2.31 | 2.09 | 1.92 | 2.42 | 2.17 | 1.89 | 2.01 | 1.95 |
| 75 kg/fed | 2.76 | 2.98 | 2.87 | 2.86 | 3.10 | 2.98 | 2.54 | 2.90 | 2.72 |
| 100 kg/fed | 3.16 | 3.44 | 3.30 | 3.21 | 3.39 | 3.60 | 3.17 | 3.35 | 3.26 |
| Means | 1.95 | 2.27 | | 2.06 | 2.32 | | 1.88 | 2.10 | |
| Overall | | | 2.11 | | | 2.19 | | | 1.99 |
| LSD (5%) | | | | | | | | | |
| Treatment(s) | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| Amendments | 0.02 | | | 0.04 | | | 0.014 | | |
| Inoculated | 0.01 | | | 0.02 | | | 0.009 | | |
| Amendments X Inoculated | 0.05 | | | 0.06 | | | 0.020 | | |
| Amendment X level | 0.07 | | | 0.08 | | | 0.032 | | |
| Amendment X Inoculated X level | 0.20 | | | 0.13 | | | 0.045 | | |

response to *Bradyrhizobium* and/or VA mycorrhiza. However, Kawai and Yamamoto (1986) reported that inoculation with VAM increased plant development

through supply of some elements such as Ca. Moreover, Giri (1993) reported that application of 25 kg N/ha to groundnut increased crop uptake of Ca. The difference

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Table 7: Effect of *Bradyrhizobium* inoculation and chicken manure or sulphur fertilization on zinc (Zn) content of soybean grown for three consecutive seasons.

| Treatment | Zn content (mg/100g) | | | | | | | | |
|--------------------------------|------------------------|------------|-------|------------------------|------------|-------|-----------------------------------|------------|-------|
| | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means |
| Chicken M. | | | | | | | | | |
| Control | 1.65 | 1.79 | 1.72 | 1.62 | 1.84 | 1.73 | 1.73 | 1.87 | 1.80 |
| 2.5 t/fed | 1.85 | 1.99 | 1.92 | 1.94 | 1.98 | 1.96 | 2.01 | 2.43 | 2.22 |
| 5.0 t/fed | 2.17 | 2.35 | 2.26 | 2.14 | 2.36 | 2.25 | 2.57 | 2.85 | 2.71 |
| 7.5 t/fed | 2.75 | 3.05 | 2.90 | 2.65 | 2.97 | 2.81 | 3.17 | 3.53 | 3.35 |
| 10 t/fed | 3.17 | 3.41 | 3.29 | 3.10 | 3.42 | 3.26 | 3.91 | 3.42 | 4.13 |
| Means | 2.31 | 2.51 | | 2.29 | 2.51 | | 2.68 | 2.96 | |
| Overall | | | 2.41 | | | 2.40 | | | 2.82 |
| Sulphur | | | | | | | | | |
| Control | 7.70 | 1.82 | 1.76 | 1.72 | 1.90 | 1.81 | 1.81 | 1.93 | 1.87 |
| 25 kg/fed | 1.92 | 2.04 | 1.98 | 1.98 | 2.16 | 2.07 | 2.05 | 2.65 | 2.35 |
| 50 kg/fed | 2.35 | 2.73 | 2.54 | 2.41 | 2.79 | 2.60 | 2.94 | 3.14 | 3.04 |
| 75 kg/fed | 3.04 | 3.22 | 3.13 | 3.10 | 3.28 | 3.19 | 3.24 | 3.46 | 3.35 |
| 100 kg/fed | 3.42 | 3.56 | 3.49 | 3.56 | 3.74 | 3.65 | 3.79 | 3.93 | 3.86 |
| Means | 2.49 | 2.67 | | 2.55 | 2.77 | | 2.76 | 3.02 | |
| Overall | | | 2.58 | | | 2.66 | | | 2.89 |
| LSD (5%) | | | | | | | | | |
| Treatment(s) | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| Amendments | 0.06 | | | 0.07 | | | 0.21 | | |
| Inoculated | 0.04 | | | 0.05 | | | 0.13 | | |
| Amendments X Inoculated | 0.09 | | | 0.10 | | | 0.30 | | |
| Amendment X level | 0.15 | | | 0.17 | | | 0.48 | | |
| Amendment X Inoculated X level | 0.22 | | | 0.24 | | | 0.68 | | |

Table 8: Effect of *Bradyrhizobium* inoculation and amendments on chicken manure or sulphur fertilization on manganese (Mn) content of soybean grown for three consecutive seasons

| Treatment | Mn content (mg/100g) | | | | | | | | |
|--------------------------------|------------------------|------------|-------|------------------------|------------|-------|-----------------------------------|------------|-------|
| | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means | Uninoculated | Inoculated | Means |
| Chicken M. | | | | | | | | | |
| Control | 2.20 | 2.48 | 2.34 | 2.00 | 2.10 | 2.05 | 2.25 | 2.43 | 2.34 |
| 2.5 t/fed | 2.74 | 2.92 | 2.83 | 2.50 | 2.84 | 2.67 | 2.84 | 3.14 | 2.99 |
| 5.0 t/fed | 3.02 | 3.24 | 3.13 | 3.10 | 3.24 | 3.17 | 3.25 | 3.51 | 3.28 |
| 7.5 t/fed | 3.50 | 3.70 | 3.60 | 3.39 | 3.81 | 3.60 | 3.76 | 4.04 | 3.90 |
| 10 t/fed | 3.84 | 4.04 | 3.94 | 3.94 | 4.08 | 4.01 | 4.02 | 4.21 | 4.30 |
| Means | 3.06 | 3.28 | | 2.99 | 3.21 | | 3.26 | 3.50 | |
| Overall | | | 3.17 | | | 3.10 | | | 3.38 |
| Sulphur | | | | | | | | | |
| Control | 2.04 | 2.18 | 2.11 | 1.97 | 2.17 | 2.07 | 2.21 | 2.33 | 2.27 |
| 25 kg/fed | 2.34 | 2.68 | 2.51 | 2.32 | 2.58 | 2.45 | 2.63 | 2.89 | 2.76 |
| 50 kg/fed | 2.90 | 3.12 | 3.01 | 2.84 | 3.04 | 2.94 | 3.01 | 2.21 | 2.11 |
| 75 kg/fed | 3.34 | 3.56 | 3.45 | 3.22 | 3.54 | 3.38 | 3.49 | 3.77 | 3.63 |
| 100 kg/fed | 3.71 | 3.87 | 3.79 | 3.86 | 4.02 | 3.94 | 3.90 | 4.10 | 4.00 |
| Means | 3.04 | 3.08 | | 3.04 | 3.08 | | 3.04 | 3.26 | |
| Overall | | | 3.06 | | | 3.06 | | | 3.15 |
| LSD (5%) | | | | | | | | | |
| Treatment(s) | 1 st season | | | 2 nd season | | | Residual (3 rd season) | | |
| Amendments | 0.10 | | | 0.06 | | | 0.22 | | |
| Inoculated | 0.06 | | | 0.04 | | | 0.13 | | |
| Amendments X Inoculated | 0.14 | | | 0.09 | | | 0.09 | | |
| Amendment X level | 0.22 | | | 0.15 | | | 0.50 | | |
| Amendment X Inoculated X level | 0.33 | | | 0.21 | | | 0.70 | | |

in response to such treatments could be attributed to the difference in cultivars as well as the growing environment. Chicken manure and sulphur application

significantly ($P = 0.05$) increased Magnesium (Mg) content 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 applied (284.0, 278.0 and 290.0 mg/100g for the first, second and residual season, respectively) or 100kg/fed of sulphur (296.0, 264.0 and 282.0 mg/100g for the first, second and residual season, respectively). The results obtained for both fertilizers indicated that fertilization of soybean by chicken manure or sulphur greatly increased Mg content of the crop seeds. Moreover, fertilization of inoculated seeds caused further increase in Mg content. Elsheikh and Mohamedzein (1998) reported that inoculation with *Bradyrhizobium* and/or VA mycorrhiza significantly (~50.05) increased the seed content of Mg. Further they reported that the Mg percentage increased by N and P treatments and decreased when 0.01 mton manure were applied. The seed content of Mg was increased by inoculation. Kawai and Yamamoto (1986) reported that inoculation with VAM increased plant development through supply of some elements such as Mg. Giri (1993) reported that application of 25 kg N ha⁻¹ to groundnut increased crop uptake of Mg. Chicken manure and sulphur application significantly (P = 0.05) increased Potassium (K) content of uninoculated seeds with increasing level of each (Table 3). However, after inoculation of the seeds it was greatly increased in all seasons with maximum values obtained when 10t/fed of chicken manure was applied (1316.0, 1356.0 and 1374.0 mg/100g for the first, second and residual season, respectively) or 100kg/fed of sulphur (1328.0, 1336.0 and 1345.0 mg/100g for the first, second and residual season, respectively). The results obtained for both fertilizers indicated that fertilization of soybean by chicken manure or sulphur greatly increased K content of the crop seeds. Moreover, fertilization of inoculated seeds caused further increase in K content. Elsheikh and Mohamedzein (1998) reported that inoculation with *Bradyrhizobium* and/or VA mycorrhiza significantly (~50.05) increased the seed content of K. The seed content of K was increased by inoculation and fertilization of the plant seeds. Chicken manure and sulphur application significantly (P = 0.05) increased Sodium (Na) content of uninoculated seeds with increasing level of each (Table 4). Inoculation of the seeds significantly (P = 0.05) increased Na content in all seasons with maximum values obtained when 10t/fed of chicken manure was applied (12.16, 11.20 and 11.31 mg/100g for the first, second and residual season, respectively) or 100kg/fed of sulphur (11.04, 10.35 and 10.38 mg/100g for the first, second and residual season, respectively). The results obtained for both fertilizers indicated that fertilization of soybean by chicken manure or sulphur greatly increased Na content of the seeds. Fertilization of inoculated seeds caused further increase in Na content. Elsheikh and Mohamedzein (1998) reported that inoculation with *Bradyrhizobium* and/or VA mycorrhiza significantly (~50.05) increased the seed content of Na.

Effect of treatments on trace minerals content: As shown in Table 5, *Bradyrhizobium* inoculation showed a significant (P = 0.05) increase in iron (Fe) content of soybean seeds in all seasons. Chicken manure and sulphur fertilization were significantly (P = 0.05) increased Fe content of uninoculated seeds with increasing level of each. However, after inoculation it was greatly increased in all seasons with maximum values obtained when 10t/fed of chicken manure was applied (10.16, 10.55 and 10.23 mg/100g) for the first, second and residual season, respectively) or 100kg/fed of sulphur (10.02, 9.84 and 10.73 mg/100g 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 increased Fe content of the crop seeds. Moreover, fertilization of inoculated seeds caused further increase in Fe content. The results obtained (rate of change) for Cu (Table 6), Zn (Table 7) and Mn (Table 8) were similar to that obtained for Fe. In general proper fertilization and inoculation provides the plant with both major and trace elements which reflects the nutritional status of the plant. Several minerals such as Ca, Fe, K, Na and Mg are essential for human and animal health. Knowledge about their level in different raw foods will provide information on the nutritional adequacy of diets. Other minerals, such as Cu, Mn and Zn though essential, have a limited range between required and toxic levels.

Conclusion: Chicken manure or sulfur fertilization in the presence or absence *Bradyrhizobium* inoculation significantly increased mineral composition of soybean especially at a level of 10t/fed of chicken manure or 100kg/fed of sulphur. 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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