

# Effect of *Rhizobium* inoculation, organic and chemical fertilizers on proximate composition, *in vitro* protein digestibility, tannin and sulphur content of faba beans

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A field experiment was carried out to investigate the effect of *Rhizobium* inoculation, sulphur, nitrogen and chicken manure on proximate analysis, *in vitro* protein digestibility (IVPD) tannin and sulphur content of faba beans. The results showed that *Rhizobium* inoculation, sulphur, nitrogen and chicken manure treatments significantly ( $P \leq 0.05$ ) increased protein, IVPD and tannin content. Ash, moisture, fat and crude fibre content varied in their response to different fertilizers. The inoculation results were as good as the addition of 40 kg N ha<sup>-1</sup> and the efficiency of inoculation can be improved by the addition of fertilizers and/or amendments. Fertilization of faba beans with nitrogen, sulphur or chicken manure not only increased yield, but also improved seed nutritional value. Chicken manure is a promising fertilizer, not only because it increases yield but because it has a significant effect in improving seed protein and IVPD. Sulphur treatments significantly ( $P \leq 0.05$ ) increased the protein IVPD, tannin and sulphur content in seeds. © 1997 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

Leguminous crops are unique in the high protein content of their seeds and their ability to fix atmospheric nitrogen. In Sudan, faba bean is mainly grown for human consumption. Faba beans, like other beans, are good sources of calories, protein, carbohydrates and fibre and the main characteristic of faba bean is the high protein content although it is low in sulphur-containing amino acids (El Tinay *et al.*, 1993). The presence of tannin in legume seeds decreases digestive enzyme activity, energy, availability of protein and amino acids, and uptake of minerals, and causes other toxic effects (Price, 1980).

Efforts throughout the world are directed towards increasing the protein content and *in vitro* protein digestibility (IVPD), 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). Higher levels of nitrogen fertilization were found to enhance grain protein content and digestibility of ryegrass (Goh & Kee,

1978). 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).

The objective of this investigation was to study the effect of three groups of fertilizers—organic (chicken manure), chemical (nitrogen and sulphur) and bio-fertilizers (*Rhizobium*)—on seed protein, proximate composition, tannin content, IVPD and sulphur content of seeds.

## MATERIALS AND METHODS

### Seeds and *Rhizobium* strain

Seeds of faba bean cultivar Shambat 616 were kindly supplied by the Agricultural Research Corporation, Sudan; *Rhizobium leguminosarum viceae* strain TAL 1400 was kindly supplied by the Niftal Project USA. The strain was maintained at 4°C on yeast extract–mannitol–agar (YEMA) slopes.

### Field experiment

The experiment was carried out at the Demonstration Farm of the Faculty of Agriculture, Shambat, University of Khartoum (latitude 15°40' N, longitude 32°32' E) during the 1993/1994 cropping season, in a factorial design with four replicates. The land was prepared by deep ploughing, harrowing and levelling; the area was then ridged and divided into 5 m×4 m plots. Treatments used were:

1. 25 kg S ha<sup>-1</sup> (elemental sulphur).
2. 50 kg S ha<sup>-1</sup> (elemental sulphur).
3. 100 kg S ha<sup>-1</sup> (elemental sulphur).
4. 40 kg N ha<sup>-1</sup> (urea).
5. 80 kg N ha<sup>-1</sup> (urea).
6. 3 ton chicken manure ha<sup>-1</sup> (M ha<sup>-1</sup>).
7. 9 ton chicken manure ha<sup>-1</sup> (M ha<sup>-1</sup>).
8. 15 ton chicken manure ha<sup>-1</sup> (M ha<sup>-1</sup>).

Each of these treatments was either inoculated or uninoculated with *Rhizobium* strain. Elemental sulphur and chicken manure treatments were applied 3 weeks before sowing to minimize the harmful effect of chicken manure and to give enough time for the sulphur to react in the soil. Nitrogen was applied where required at sowing. Seeds were placed three to a hole on the top of the ridge with 20 cm spacing between holes and 70 cm between ridges. Plots were irrigated immediately after sowing and subsequently at 10 day intervals.

### Chemical analysis

Seeds were carefully cleaned and freed from dirt, stones, chips and other extraneous material, then ground to pass through a 0.4 mm screen for proximate analysis. AOAC (1975) methods were followed in the determination of moisture (7.003), petroleum ether extracts (7.048), crude fibre (7.057), ash (14.006) and crude protein (7.016). Carbohydrate was calculated by difference. IVPD was determined according to Saunder *et al.* (1973). Tannin was determined according to AOAC

(1975). The sulphur content of seeds was determined by the method outlined by Tabatabai (1982).

### Statistical analysis

Each sample was analysed in triplicate and the figures were then averaged. Data were assessed by analysis of variance (ANOVA) (Snedecor & Cochran, 1987) and by the Duncan multiple range test with a probability of  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

### Moisture content

Inoculation significantly ( $P \leq 0.05$ ) increased seed moisture content (Table 1). However, the highest value was obtained when manure at 15 ton M ha<sup>-1</sup> was added together with *Rhizobium* inoculation. There is a gradual increase in moisture content with increase in fertilizer dose (Table 1) in both the presence and the absence of *Rhizobium* inoculation.

### Crude fibre

With the exception of *Rhizobium* inoculation, all treatments significantly ( $P \leq 0.05$ ) increased the crude fibre percentage of faba bean seeds (Table 1). There was no significant difference in crude fibre content between different doses of sulphur and nitrogen; however, the effect of 15 ton M ha<sup>-1</sup> was significantly different from that of the other doses of manure (3 and 9 ton M ha<sup>-1</sup>). Chicken manure has the ability to provide a comparatively large amount of nutrients for a long period (El Tilib *et al.*, 1994). The crude fibre content, in general, is influenced by the environmental conditions and the varietal characteristics. Faba bean cultivars vary in their crude fibre content according to cultivar, location and time of harvest (El Tinay *et al.*, 1989). The fibre content

**Table 1.** Effect of nitrogen (N), sulphur (S) and chicken manure (M) on crude fibre and moisture content of seeds of faba bean cultivar Shambat 616, either uninoculated or inoculated with *Rhizobium* strain TAL 1400

Treatment	Crude fibre (%)		Moisture (%)	
	No <i>Rhizobium</i>	<i>Rhizobium</i>	No <i>Rhizobium</i>	<i>Rhizobium</i>
Control	5.75(±0.31) <sup>a</sup>	5.84(±0.20) <sup>a</sup>	6.60(±0.16) <sup>a</sup>	6.73(±0.08) <sup>a</sup>
40 kg N ha <sup>-1</sup>	6.07(±0.32) <sup>b</sup>	6.18(±0.12) <sup>b</sup>	7.40(±0.15) <sup>f</sup>	7.55(±0.11) <sup>de</sup>
80 kg N ha <sup>-1</sup>	6.05(±0.33) <sup>b</sup>	6.21(±0.21) <sup>b</sup>	7.50(±0.07) <sup>fg</sup>	7.85(±0.11) <sup>f</sup>
25 kg S ha <sup>-1</sup>	6.10(±0.19) <sup>b</sup>	6.24(±0.26) <sup>b</sup>	6.78(±0.08) <sup>b</sup>	6.85(±0.05) <sup>b</sup>
50 kg S ha <sup>-1</sup>	6.18(±0.21) <sup>b</sup>	6.30(±0.33) <sup>b</sup>	7.00(±0.11) <sup>c</sup>	7.28(±0.08) <sup>c</sup>
100 kg S ha <sup>-1</sup>	6.13(±0.17) <sup>b</sup>	6.27(±0.28) <sup>b</sup>	7.15(±0.11) <sup>d</sup>	7.49(±0.11) <sup>d</sup>
3 ton M ha <sup>-1</sup>	6.15(±0.12) <sup>b</sup>	6.34(±0.12) <sup>b</sup>	7.28(±0.16) <sup>e</sup>	7.43(±0.15) <sup>d</sup>
9 ton M ha <sup>-1</sup>	6.17(±0.09) <sup>b</sup>	6.26(±0.45) <sup>b</sup>	7.40(±0.07) <sup>f</sup>	7.55(±0.11) <sup>de</sup>
15 ton M ha <sup>-1</sup>	6.64(±0.46) <sup>c</sup>	6.78(±0.30) <sup>c</sup>	7.55(±0.11) <sup>h</sup>	7.80(±0.07) <sup>f</sup>

Values are means (±SD). Means not sharing a common superscript(s) in a column are significantly different at  $P \leq 0.05$  as assessed by Duncan's multiple range test.

of large seeds is important for food and animal feed and it is needed in a reasonable proportion as it gives bulk to the diet and helps in the movement of food through the digestive tract; also the crude fibre can contribute as a source of energy to the animal body (Salih & El Hardallou, 1986).

#### Ash content

*Rhizobium* inoculation did not significantly affect ash content (Table 2). The range of ash content under different treatments was 3.30–3.81%. Chicken manure treatments and 100 kg S ha<sup>-1</sup> significantly ( $P \leq 0.05$ ) increased the ash content both in the presence and in the absence of *Rhizobium* inoculation. Both nitrogen treatments did not affect ash content, with or without inoculation. The highest ash content was observed with 15 ton M ha<sup>-1</sup> combined with *Rhizobium* inoculation. High treatment doses resulted in high ash content; this could be attributed to the increase in seed weight and size as a result of improved physical, chemical and nutritional properties of the soil due to fertilizer application.

#### Fat content

With the exception of *Rhizobium* inoculation, 25 kg S ha<sup>-1</sup> and 50 kg S ha<sup>-1</sup>, all other treatments significantly ( $P \leq 0.05$ ) increased the fat content of faba bean seeds (Table 2). The fat content of different levels of sulphur, nitrogen and chicken manure were not significantly different in the presence or absence of *Rhizobium* inoculation. The highest values for the fat content were found in seeds treated with chicken manure, alone or combined with *Rhizobium* treatment; this could be attributed to the improved nutritional status of the plant and the role of manure in enhancing nutrient uptake (El Tilib *et al.*, 1994). El Tinay *et al.* (1989) found that the fat content of faba beans ranged from 1.1% to 2.2%.

#### Carbohydrate content

The carbohydrate content of faba bean seeds varied greatly with the different treatments in the range 49.9–57.1% (Table 3). The lowest carbohydrate contents occurred with the chicken manure treatments. The carbohydrate values resulting from the sulphur and

**Table 2.** Effect of nitrogen (N), sulphur (S) and chicken manure (M) on ash and fat content of seeds of faba bean cultivar Shambat 616, either uninoculated or inoculated with *Rhizobium* strain TAL 1400

Treatment	Ash (%)		Fat (%)	
	No <i>Rhizobium</i>	<i>Rhizobium</i>	No <i>Rhizobium</i>	<i>Rhizobium</i>
Control	3.30(±0.01) <sup>a</sup>	3.35(±0.01) <sup>a</sup>	0.70(±0.01) <sup>a</sup>	0.74(±0.01) <sup>a</sup>
40 kg N ha <sup>-1</sup>	3.47(±0.02) <sup>ab</sup>	3.52(±0.01) <sup>ab</sup>	0.83(±0.01) <sup>cd</sup>	0.87(±0.02) <sup>b</sup>
80 kg N ha <sup>-1</sup>	3.35(±0.01) <sup>a</sup>	3.42(±0.02) <sup>ab</sup>	0.85(±0.01) <sup>cd</sup>	0.86(±0.01) <sup>b</sup>
25 kg S ha <sup>-1</sup>	3.43(±0.02) <sup>ab</sup>	3.45(±0.01) <sup>ab</sup>	0.73(±0.01) <sup>a</sup>	0.75(±0.02) <sup>a</sup>
50 kg S ha <sup>-1</sup>	3.48(±0.01) <sup>ac</sup>	3.48(±0.01) <sup>ab</sup>	0.75(±0.02) <sup>ab</sup>	0.78(±0.01) <sup>a</sup>
100 kg S ha <sup>-1</sup>	3.55(±0.01) <sup>c</sup>	3.60(±0.01) <sup>b</sup>	0.80(±0.01) <sup>bc</sup>	0.87(±0.01) <sup>b</sup>
3 ton M ha <sup>-1</sup>	3.65(±0.01) <sup>cd</sup>	3.72(±0.01) <sup>bc</sup>	0.84(±0.01) <sup>cd</sup>	0.86(±0.01) <sup>b</sup>
9 ton M ha <sup>-1</sup>	3.71(±0.04) <sup>d</sup>	3.76(±0.01) <sup>bc</sup>	0.87(±0.01) <sup>d</sup>	0.89(±0.04) <sup>b</sup>
15 ton M ha <sup>-1</sup>	3.79(±0.02) <sup>d</sup>	3.81(±0.01) <sup>c</sup>	0.88(±0.01) <sup>d</sup>	0.92(±0.01) <sup>b</sup>

Values are means (±SD). Means not sharing a common superscript(s) in a column are significantly different at  $P \leq 0.05$  as assessed by Duncan's multiple range test.

**Table 3.** Effect of nitrogen (N), sulphur (S) and chicken manure (M) on protein and carbohydrate content of seeds of faba bean cultivar Shambat 616, either uninoculated or inoculated with *Rhizobium* strain TAL 1400

Treatment	Protein (%)		Carbohydrate (%)	
	No <i>Rhizobium</i>	<i>Rhizobium</i>	No <i>Rhizobium</i>	<i>Rhizobium</i>
Control	26.6(±0.58) <sup>a</sup>	27.0(±0.19) <sup>a</sup>	57.1(±0.01) <sup>a</sup>	56.3(±0.01) <sup>a</sup>
40 kg N ha <sup>-1</sup>	27.2(±0.57) <sup>b</sup>	28.1(±0.44) <sup>bc</sup>	55.1(±0.02) <sup>bc</sup>	53.4(±0.01) <sup>b</sup>
80 kg N ha <sup>-1</sup>	28.4(±0.70) <sup>c</sup>	29.2(±0.19) <sup>de</sup>	53.9(±0.01) <sup>cd</sup>	52.8(±0.02) <sup>b</sup>
25 kg S ha <sup>-1</sup>	27.0(±0.26) <sup>b</sup>	27.7(±0.41) <sup>b</sup>	55.9(±0.02) <sup>ab</sup>	55.2(±0.01) <sup>a</sup>
50 kg S ha <sup>-1</sup>	27.5(±0.93) <sup>b</sup>	28.2(±0.40) <sup>c</sup>	55.1(±0.01) <sup>bc</sup>	52.1(±0.01) <sup>bc</sup>
100 kg S ha <sup>-1</sup>	28.3(±0.35) <sup>c</sup>	28.9(±0.60) <sup>d</sup>	54.1(±0.01) <sup>c</sup>	52.9(±0.01) <sup>b</sup>
3 ton M ha <sup>-1</sup>	28.4(±0.35) <sup>c</sup>	29.5(±0.46) <sup>e</sup>	53.2(±0.01) <sup>d</sup>	52.1(±0.01) <sup>bc</sup>
9 ton M ha <sup>-1</sup>	29.4(±0.64) <sup>d</sup>	30.3(±0.48) <sup>f</sup>	52.5(±0.04) <sup>de</sup>	51.2(±0.01) <sup>c</sup>
15 ton M ha <sup>-1</sup>	30.3(±0.43) <sup>e</sup>	31.3(±0.40) <sup>g</sup>	51.3(±0.02) <sup>e</sup>	49.9(±0.01) <sup>c</sup>

Values are means (±SD). Means not sharing a common superscript(s) in a column are significantly different at  $P \leq 0.05$  as assessed by Duncan's multiple range test.

nitrogen treatments were comparable. *Rhizobium* inoculation significantly ( $P \leq 0.05$ ) decreased the carbohydrate content of faba bean seeds. This could be attributed to the high concentration of nitrogen available to the plant which increased the protein content in treated plants. The low carbohydrate content in all fertilization treatments is directly related to the high protein content of faba bean seeds compared to the uninoculated control.

### Protein content

All treatments significantly ( $P \leq 0.05$ ) increased the protein content of faba bean seeds (Table 3); the range varied from 26.6% to 31.3% according to treatment. Sulphur and nitrogen treatments showed a similar effect on protein content. *Rhizobium* inoculation increased the protein content and it is comparable to that of 40 kg N ha<sup>-1</sup>. Chicken manure treatments significantly ( $P \leq 0.001$ ) increased the protein content in both the presence and the absence of *Rhizobium* inoculation. The highest protein content was obtained when both *Rhizobium* and 15 ton M ha<sup>-1</sup> were added, the increment being 17.7% compared to the uninoculated control (Table 3). The significant effect of chicken manure may be due to the fact that this manure consists of different nutritive elements, which is why it is considered to be a balanced fertilizer that encourages the photosynthetic process and other physiological factors that increase protein synthesis. El Tilib *et al.* (1994) reported that protein content increases with improved plant nutrition and that the application of manure results in a high exchangeable capacity, hence a considerable quantity of phosphorus is diverted to available form and thus increased protein. Nitrogen fertilization was found to enhance grain protein content and the digestibility of ryegrass (Goh & Kee, 1978). Inoculation and nitrogen fertilization were found to increase the protein content of faba beans (Babiker *et al.*, 1995). Faba beans can be used as a protein supplement to other staple foods in Sudan, such as sorghum, millet and cassava which all

have a low protein content, or can provide a good supplement to produce weaning foods of high nutritive value. This is because faba bean protein is characterized by its high lysine content and low methionine content (El Tinay *et al.*, 1993), whereas sorghum and millet are deficient in lysine and contain moderate quantities of methionine (Salih & El Hardallou, 1986).

### *In vitro* protein digestibility (IVPD)

The results for IVPD in this study ranged from 92.6% (for the control) to 95.1% (for 15 ton M ha<sup>-1</sup> with *Rhizobium* inoculation). All treatments significantly ( $P \leq 0.05$ ) increased IVPD content in seeds (Table 4). Chicken manure treatment, 100 kg S ha<sup>-1</sup> and 80 kg N ha<sup>-1</sup> significantly increased the IVPD in both the presence and absence of *Rhizobium* inoculation. It was reported that higher levels of fertilization significantly increased grain protein and IVPD (Babiker *et al.*, 1995). The IVPD varies greatly in faba beans according to genotype, method of extraction, tannin value, time of soaking and time of swelling. Not all proteins are equally absorbed and some proteins are more readily digestible than others.

### Tannin content

All treatments significantly ( $P \leq 0.05$ ) increased the tannin content of faba beans in both the presence and absence of *Rhizobium* inoculation. The tannin content ranged from 0.024% to 0.038% (Table 4). Although all treatments improved seed composition and quality, the only drawback was the increased tannin content. Babiker *et al.* (1995) reported similar results. There are many factors that affect tannin content, such as genotype, time of harvest and temperature. Price (1980) reported that the deleterious effects of various tannins include depression of food/feed intake, formation of tannin complexes with dietary protein and other food components, inhibition of digestive enzymes, and increased excretion of endogenous protein.

**Table 4.** Effect of nitrogen (N), sulphur (S) and chicken manure (M) on *in vitro* protein digestibility and tannin content of faba bean cultivar Shambat 616, either uninoculated or inoculated with *Rhizobium* strain TAL 1400

Treatment	Tannin content (%)		<i>In vitro</i> protein (%)	
	No <i>Rhizobium</i>	<i>Rhizobium</i>	No <i>Rhizobium</i>	<i>Rhizobium</i>
Control	0.024(±0.002) <sup>a</sup>	0.034(±0.008) <sup>a</sup>	92.6(±0.580) <sup>a</sup>	93.6(±3.540) <sup>b</sup>
40 kg N ha <sup>-1</sup>	0.033(±0.009) <sup>c</sup>	0.034(±0.008) <sup>a</sup>	93.1(±1.940) <sup>bc</sup>	93.4(±0.490) <sup>ab</sup>
80 kg N ha <sup>-1</sup>	0.034(±0.008) <sup>cd</sup>	0.036(±0.006) <sup>ab</sup>	93.4(±0.050) <sup>c</sup>	93.5(±2.900) <sup>b</sup>
25 kg S ha <sup>-1</sup>	0.028(±0.009) <sup>b</sup>	0.036(±0.007) <sup>ab</sup>	93.0(±1.320) <sup>b</sup>	93.2(±1.120) <sup>a</sup>
50 kg S ha <sup>-1</sup>	0.028(±0.004) <sup>b</sup>	0.038(±0.005) <sup>b</sup>	93.0(±0.480) <sup>b</sup>	93.3(±0.530) <sup>ab</sup>
100 kg S ha <sup>-1</sup>	0.031(±0.100) <sup>bc</sup>	0.038(±0.010) <sup>b</sup>	93.1(±0.860) <sup>bc</sup>	93.4(±1.860) <sup>ab</sup>
3 ton M ha <sup>-1</sup>	0.034(±0.009) <sup>cd</sup>	0.036(±0.008) <sup>ab</sup>	93.2(±1.650) <sup>bc</sup>	93.2(±1.230) <sup>a</sup>
9 ton M ha <sup>-1</sup>	0.037(±0.007) <sup>d</sup>	0.037(±0.007) <sup>ab</sup>	93.3(±0.940) <sup>c</sup>	94.5(±0.600) <sup>c</sup>
15 ton M ha <sup>-1</sup>	0.036(±0.003) <sup>d</sup>	0.038(±0.005) <sup>b</sup>	93.4(±2.900) <sup>c</sup>	95.1(±1.900) <sup>d</sup>

Values are means (±SD). Means not sharing a common superscript(s) in a column are significantly different at  $P \leq 0.05$  as assessed by Duncan's multiple range test.

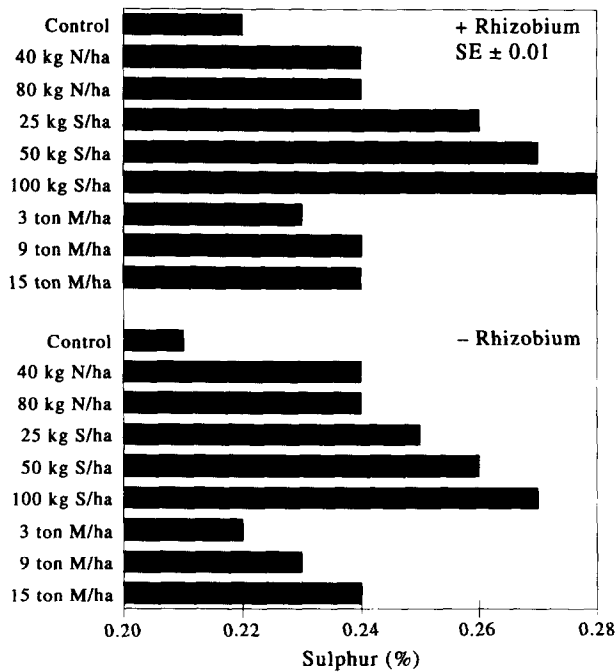


Fig. 1. Effect of nitrogen (N), sulphur (S) and chicken manure (M) on sulphur content of faba bean cultivar Shambat 616, either uninoculated or inoculated with *Rhizobium* strain TAL 1400.

#### Sulphur content of seeds

The sulphur content of seeds significantly ( $P \leq 0.05$ ) increased with all sulphur treatments; this is clearly due to the availability of sulphur ions to plants. All other treatments did not affect sulphur content either in the presence or absence of *Rhizobium* inoculation (Fig. 1). Farrag *et al.* (1992) found that sulphur fertilization of faba beans resulted in increased yield, and protein and total amino acid content. Hago and Salama (1987) found that addition of elemental sulphur at  $150 \text{ kg S ha}^{-1}$  increased the sulphur content of groundnut seeds by more than 93% and the protein content by more than 15%. The increased sulphur in faba bean seeds due to sulphur treatment may indicate an increase in sulphur-containing amino acids which are lacking in the legume proteins.

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