

Effect of intercropping, *Bradyrhizobium* inoculation and N, P fertilizers on yields, physical and chemical quality of cowpea seeds

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Abstract The present study was aimed to determine the effects of inoculation with *Bradyrhizobium* strain, intercropping, nitrogen and phosphorus fertilization and their interaction on the yield, physical and chemical properties of cowpea seeds. The results showed that the seed yield of cowpea was significantly ($P \leq 0.05$) increased by *Bradyrhizobium* inoculation, nitrogen and phosphorus fertilizers, but not by intercropping. All treatments of intercropping, P, *Bradyrhizobium* plus N and *Bradyrhizobium* plus P treatments significantly ($P \leq 0.05$) increased the hydration coefficient and cookability of cowpea seeds compared to untreated plants in both seasons. For chemical composition, all treatments significantly ($P \leq 0.05$) increased the dry matter, ash, protein and fiber content of the seeds compared to the untreated plants for the two systems and in both seasons, whereas it significantly ($P \leq 0.05$) decreased carbohydrate content of the seeds. Fat content of the seeds was not increased by *Bradyrhizobium* inoculation and intercropping, but it was significantly increased by nitrogen and phosphorus fertilization. Intercropping, *Bradyrhizobium* inoculation and nitrogen and phosphorus fertilization significantly ($P \leq 0.05$) increased tannin content and *in vitro* protein digestibility of the seeds compared to untreated plants for both systems and in the two seasons.

Keywords *Bradyrhizobium* inoculation, chemical composition, cowpea, intercropping, fertilization

Introduction

The rise of agriculture in the past 50 years has contributed to the appearance of problems such as soil erosion, environmental contamination by fertilizers and pesticides and also selection of diseases, pests, and weeds resistant to chemical treatments (Vandermeer et al., 1998). Consequently, the efficiency of agricultural systems needs to be improved and diversification of agro-systems has been proposed as one of several solutions for future agriculture (Altieri, 1999). Intercropping, the growing of two or more crops simultaneously on the same field represents one option of diversifying agro-ecosystems (Vandermeer et al., 1998; Malézieux et al., 2009). Intercropping is frequently practiced

in low-input farming of tropical regions of the world and by various indigenous peoples to stabilize and improve yield, this cropping system is so far of little interest to farmers in countries with temperate climates and highly mechanized agriculture (Zegada-Lizarazu et al., 2006). Intercropping has several advantages such as higher yield, higher use efficiency of natural resources and improved yield stability compared to monoculture cropping (Fukai and Trenbath, 1993). For these reasons there has been a renewed interest in intercropping (Malézieux et al., 2009) and particularly grain legume-cereal intercrops (Fukai and Trenbath, 1993). Legumes rely more on symbiotic nitrogen (N) fixation and cereals take up more N from soil when they are planted together than when they are sole cropped, which is considered complementary usage of N resources (Corre-Hellou et al., 2006; Fan et al., 2006; Neumann et al., 2007).

Cowpea can produce abundant biomass and fix substantial amounts of atmospheric nitrogen as well as it can suppress weeds (Creamer and Baldwin, 2000). In Sudan, cowpea is

Received March 7, 2011; accepted August 16, 2011

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grown widely as a subsistence crop in Kordofan, Darfur and Central and Southern regions as a rainfed crop. In 2004, the total area cultivated with cowpea was 367 thousand feddans with an average yield of 38 kg/ fed., and the total production was 8000 ton (Anon, 2006). In spite of its importance, very limited information is available on the extent of N₂-fixation by on farm grown cowpea under low soil fertility conditions, intercropping and as influenced by P application. The level of combined nitrogen in most Sudanese soils is very low (Mustafa and Gamar, 1971). Therefore, addition of nitrogen fertilizers is inevitable.

In rainfed agriculture, fertilizers are not routinely applied due to the following reasons: (I) It is not possible to determine when to fertilize, as it may rain at any time before or after the application, (II) how to minimize the production cost in risky agricultural systems due to rain fluctuation, (III) high cost of chemical fertilizers and (IV) low prices of the crop (cowpea). Therefore, it is important to adopt a cheap system to increase the nitrogen level in the soil, for example through intercropping with legumes. This is because intercropping not only avails the fixed nitrogen, but also ameliorates some conditions for the non-leguminous crops (Bloem and Barnard, 2000). The objectives of this study were to determine the effect of inoculation with *Bradyrhizobium* strain, intercropping, nitrogen and phosphorus fertilization and their interaction on physical and chemical properties of cowpea seeds.

Materials and methods

Materials

Bradyrhizobium strain TAL 169 as a charcoal inoculant was obtained from the National Center for Research, Environment and Natural Resources Research Institute, Biofertilization Department, Sudan. Seeds of cowpea (*Vigna unguiculata* L. Walp) were obtained from Abu Naama Research Station, whereas seeds of sorghum (*Sorghum bicolor* L. Monech) (Tabat variety) were obtained from the Sudanese Arabian Company. The germination test of both crops indicated that germination percentage was more than 90%. Cowpea seeds were wetted using 40% gum arabic solution and then mixed thoroughly with the charcoal based inoculum of *Bradyrhizobium*. Inoculated seeds were left to dry for a few minutes in shade. All chemicals used in this study are of reagent grade.

Field experiments and experimental site

A field experiment was conducted for two consecutive seasons (2004 and 2005) at the experimental site lies at the Demonstration Farm of the Faculty of Agriculture, University of Sennar, Abu Naama, 400 km South-east of Khartoum. It is located in the semi tropical savanna, at latitude 12°44'N and longitude 34°7'E. The area is dominated by rainfed agriculture where sorghum, sesame and groundnut are the

main crops growing. The soils of Abu Naama area are commonly known as Vertisol with high clay content (> 67% clay) in the top 30 cm depth. Cracks of 5–10 cm wide and 60–90 cm deep are distinct during summer. The physiochemical characteristics of the soil of the study area are shown in Table 1.

Table 1 Physiochemical characteristic of the soil in the experimental site

Parameters	Value
Depth (cm)	0–30
Sand (%)	11.5
Silt (%)	19.6
Clay (%)	68.9
pH (paste)	8.1
EC (ds/m)	0.4
Total N (%)	0.056
Organic carbon (%)	0.52
Available P (mg/kg soil)	4.3
CaCO ₃ (%)	2.0
SO ₄ ²⁻ (meq/L)	0–7
NO ₃ ⁻ (meq/L)	0–5

Experimental design and treatments

The experimental layout was arranged in split-split plot design with six replicates. The land was divided into plots of 4 m × 6 m, 70 cm between ridges, and six north-south ridges each.

The following treatments were assigned to the main plots:

Treatment 1—uninoculated (control),

Treatment 2—inoculated with *Bradyrhizobium* strain.

The following cropping systems were assigned to the subplots:

Cowpea plantation (monocropping system),

Cowpea/sorghum plantation (intercropping system),

Sorghum plantation (monocropping system).

The following fertilizers were assigned to the sub plots:

Plot 1—No fertilizers (control),

Plot 2—20 kg N/hm²,

Plot 3—50 kg P₂O₅ /hm² as triple superphosphate (TSP).

Five seeds of inoculated or uninoculated cowpea in conjunction with sorghum were sown by hand on the eastern side of the ridge in holes 30 cm apart, which were later thinned to three plants per hole for both crops. The crops were grown in alternate and single rows (Raw intercropping). To avoid rhizobial cross contamination, plots of uninoculated seeds were sown first. To the inoculated and uninoculated seeds, the soil was amended with 20 kg N/hm² and 50 kg P₂O₅/hm² as TSP. Controls with no inoculation and/or no fertilizers were set throughout 108 plots of the experiments.

Sample preparation

Three samples from each plot (4 m × 6 m) were taken randomly after seeds matured. The seeds were air-dried under sunlight. The seeds were cleaned manually to remove husks, damaged 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 sieves.

Physical characteristics of the seeds

100-seed weight

From the collected samples of each plot, 100 seeds were counted randomly in triplicate and weighed.

Hydration coefficient

Hydration coefficient was determined as described previously (Elsheikh et al., 2009), and its percentage was calculated for each sample using the data obtained above and equation as follows:

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

Cookability

The cookability of treated cowpea seeds was estimated as described previously (Elsheikh et al., 2009). Twenty grams of cowpea 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:

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

Chemical composition determination

The chemical composition of each sample was determined according to the Standard Official Methods of Analysis (AOAC, 1995). Total carbohydrate of the samples was calculated by subtracting the value of protein, oil, fiber, ash and moisture content from 100.

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 color 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 color intensity equivalent to that given by tannins after correcting for blank. Then tannin content (%) was calculated according to the equation:

$$\text{Catechin equivalent(CE) \%} = \frac{C \times \text{Volume extracted(10 mL)}}{\text{Sample weight(g)}} \times 100,$$

where C is the concentration obtained from the standard curve (mg/mL).

In vitro protein digestibility (IVPD) determination

The IVPD was determined by the method of Saunders et al. (1973). A sample (0.2 g) was placed in a 50 mL centrifuge tube, 15 mL of 0.1 mol/L HCl containing 1.5 mg pepsin was added, and the tube was incubated at 37°C for 3 h. The suspension was then neutralized with 0.5 mol/L NaOH and treated with pancreatin (4.0 mg) in 7.5 mL of 0.2 mol/L potassium phosphate buffer, at 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 5000×g for 20 min at room temperature. Nitrogen in the supernatant was determined by Kjeldahl method (AOAC, 1995). Digestibility was calculated using the formula:

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

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., USA). 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 the yields of cowpea seeds

Bradyrhizobium inoculation significantly ($P \leq 0.05$) increased the yields of cowpea compared to the control in the monocropping and intercropping systems in the two seasons (Table 2). Application of nitrogen or phosphorus in the presence of inoculation showed significant results compared to *Bradyrhizobium* alone. Similar results were observed when 50 kg/hm² of P was applied. However,

Table 2 Effect of inoculation, N, P and intercropping (sorghum/cowpea) on yields of rain fed cowpea (kg/hm²) growing for two consecutive seasons

Treatments	Yield (kg/hm ²)	
	1st season	2nd season
Monocropping system		
Control	334.89 ^b	344.26 ^d
20 kg/hm ² of N	367.93 ^{ab}	400.28 ^{bc}
50 kg/hm ² of P	406.17 ^a	426.70 ^{ab}
TAL 169	383.31 ^a	384.06 ^c
TAL 169 + 20 kg/hm ² of N	402.23 ^a	407.91 ^b
TAL 169 + 50 kg/hm ² of P	418.17 ^a	435.82 ^a
Intercropping system		
Control	302.37 ^c	310.49 ^c
20 kg/hm ² of N	337.00 ^{bc}	397.39 ^a
50 kg/hm ² of P	383.97 ^{ab}	406.33 ^a
TAL 169	371.27 ^b	353.39 ^b
TAL 169 + 20 kg/hm ² of N	389.50 ^{ab}	401.52 ^a
TAL 169 + 50 kg/hm ² of P	415.11 ^a	417.57 ^a

The same letter(s) within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test (DMRT) between tow treatments.

application of 20 kg/hm² of N showed inconsistent results in the two seasons under both systems. The increase in yield of cowpea due to inoculation indicated the efficiency of the *Bradyrhizobium* strain in fixing the atmospheric nitrogen. In agreement with our results, Kishan et al. (2001) found that the yield of cowpea was increased by *Rhizobium* inoculation and nitrogen and phosphorus fertilizers. Moreover, it was also reported that the addition of nitrogen fertilizer to inoculated cowpea increased the yield of cowpea (Desai et al., 2001). The results of the current study showed that intercropping reduced the yield of cowpea compared to monocropping

system in both seasons. The lower grain yield of the intercrop might be due to the reduced cowpea plant population per unit area under the intercropping systems compare to their monocrops. Similarly, Khan et al. (2002) found that grain yield of cowpea was reduced under intercropping systems. Furthermore, the intercropping reduced all the yield components of sunflower and sesame compared to the solo crops (Olowe and Adeyemo, 2009).

Effect of treatments on hydration coefficient, 100 seeds weight and cookability of cowpea seeds

Table 3 shows the effect of inoculation, N, P and intercropping (sorghum/cowpea) on hydration coefficient and cookability of rainfed cowpea seeds grown for two consecutive seasons. Phosphorus, *Bradyrhizobium* plus N and *Bradyrhizobium* plus P treatments significantly ($P \leq 0.05$) increased the hydration coefficient of cowpea compared to untreated samples for the two cropping systems in both seasons. Intercropping significantly ($P \leq 0.05$) increased hydration coefficient of cowpea in both seasons. In this study, the hydration coefficient of cowpea seeds was not affected by inoculation, but significantly increased by nitrogen and phosphorus fertilization as well as intercropping. The reports showed variable responses of hydration coefficient to different treatments of different plants. Obied (2003) found that the inoculation did not increase the hydration coefficient of hyacinth bean. By contrast, inoculation with Isolate-2 significantly increased the hydration coefficient of soybean seeds under both cropping systems (Elsheikh et al., 2009). A significant increase in hydration coefficient was reported for soybean (Salih, 2002), faba bean (Elsheikh and Ahmed, 2000) and groundnut seeds (Elsheikh and Mohamedzein, 1998b). According to Abdelgani et al. (1999), nitrogen

Table 3 Effect of inoculation, N, P and intercropping (sorghum/cowpea) on hydration coefficient and cookability (%) of rain fed cowpea seeds growing for two consecutive seasons

Treatments	Hydration coefficient		Cookability		100-seed weight	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Monocropping system						
Control	205.43 ^c	207.00 ^b	10.09 ^c	12.08 ^a	18.22 ^d	16.88 ^c
20 kg/hm ² of N	208.38 ^b	210.54 ^{ab}	11.64 ^{bc}	12.30 ^a	20.27 ^c	18.74 ^{ab}
50 kg/hm ² of P	208.97 ^{ab}	212.97 ^a	11.29 ^{bc}	12.22 ^a	20.88 ^{bc}	18.88 ^{ab}
TAL 169	208.06 ^{bc}	208.39 ^{ab}	10.99 ^c	12.25 ^a	21.54 ^{ab}	18.54 ^{ab}
TAL 169 + 20 kg/hm ² of N	209.53 ^{ab}	213.63 ^a	12.64 ^{ab}	14.33 ^a	22.56 ^a	19.56 ^a
TAL 169 + 50 kg/hm ² of P	210.07 ^{ab}	213.73 ^a	12.27 ^b	14.18 ^a	22.81 ^a	19.81 ^a
Intercropping system						
Control	210.43 ^{ab}	211.93 ^{ab}	12.33 ^{ab}	13.65 ^a	20.06 ^d	18.23 ^b
20 kg/hm ² of N	213.16 ^{ab}	214.49 ^a	13.64 ^{abc}	13.92 ^a	20.78 ^{bc}	18.88 ^{bc}
50 kg/hm ² of P	212.54 ^{ab}	214.21 ^a	13.70 ^{abc}	14.13 ^a	21.14 ^{bc}	18.97 ^{bc}
TAL 169	212.65 ^{ab}	212.83 ^{ab}	14.95 ^a	13.88 ^a	20.81 ^{bc}	18.88 ^{bc}
TAL 169 + 20 kg/hm ² of N	214.82 ^a	215.15 ^a	14.86 ^a	14.32 ^a	21.51 ^b	19.23 ^c
TAL 169 + 50 kg/hm ² of P	215.03 ^a	216.28 ^a	14.74 ^{ab}	14.40 ^a	21.52 ^{ab}	19.20 ^a

The same letter(s) within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test (DMRT) between tow treatments.

application had no effect on hydration coefficient of fenugreek seeds, while Elsheikh and Elzidany (1997a) found that nitrogen application significantly increased hydration coefficient of faba bean. Moreover, Mohamedzein (1996) reported that phosphorus fertilization decreased the hydration coefficient of groundnut seeds. Intercropping system together with the other treatments applied significantly ($P \leq 0.05$) improved cookability of cowpea compared to the untreated samples especially during the first season. However, cookability of cowpea seeds was not affected by *Bradyrhizobium* inoculation, nitrogen and phosphorus fertilization and intercropping in the second season. Similarly, in previous studies, inoculation did not affect the cookability of soybean and hyacinth bean (Ibrahim et al., 2008), soybean (Elsheikh et al., 2009) and groundnut (Elsheikh and Mohamedzein, 1998b). All treatments of *Bradyrhizobium* inoculation, nitrogen and phosphorus fertilization and intercropping significantly ($P \leq 0.05$) increased the 100-seed weight of cowpea compared to untreated samples for the two systems and in both seasons. The increment of 100-seed weight may be due to high content of nitrogen and phosphorus during the reproductive period. Similarly, in previous studies, inoculation increased the 100-seed weight of soybean (Elsheikh et al., 2009) and chickpea (El Hadi and Elsheikh, 1999). The improvement of 100-seed weight of chickpea due to nitrogen fertilization was also reported by El Hadi and Elsheikh (1999).

Effect of treatments on chemical composition of cowpea seeds

Table 4 shows the effects of inoculation, N, P and intercropping (sorghum/cowpea) on the contents of dry matter and ash of rain fed cowpea seeds grown for two

consecutive seasons. The dry matter of cowpea seeds was significantly increased by *Bradyrhizobium* inoculation and nitrogen and phosphorus application, and was not affected by intercropping. Intercropping significantly ($P \leq 0.05$) increased the ash content of cowpea seeds for the two systems in both seasons. With the exception of those treated with 20 kg /hm² of N, all other treatments of *Bradyrhizobium* inoculation, nitrogen and phosphorus fertilization significantly ($P \leq 0.05$) increased ash content of cowpea seeds for the two systems and in both seasons. In agreement with our results, Elsheikh and Ahmed (2000) reported that the dry matter content of faba bean was significantly increased by inoculation as well, but not affected by intercropping. It was also reported that inoculation increased the dry matter and ash contents of various legumes (Ahmed, 1998; Elsheikh and Ibrahim, 1999; Ibrahim et al., 2008). Furthermore, intercropping did not affect the dry matter and ash content of several legumes (Ahmed, 1998; Salih, 2002). Generally, the dry matter content of the seeds was found to be affected by factors other than the treatments applied such as the relative humidity of the surrounding atmosphere at the time of harvest, during inoculation and storage (Elsheikh and Ibrahim, 1999; Elsheikh, 2001).

Table 5 shows the effects of inoculation, N, P, and intercropping (sorghum/cowpea) on fat and fiber contents of rainfed cowpea seeds growing for two consecutive seasons, although intercropping had no significant effect on fat content of cowpea seeds during the two seasons. *Bradyrhizobium* significantly ($P \leq 0.05$) decreased the fat content compared to the untreated samples in the first season and had no effect in the second season. All other treatments were not significantly different compared to untreated samples in the first season and slight differences were observed for the second season harvest. Whereas, fiber content of cowpea seeds was

Table 4 Effect of inoculation, N, P and intercropping (sorghum/cowpea) on dry matter and ash contents (%) of rain fed cowpea seeds growing for two consecutive seasons

Treatments	Dry matter		Ash	
	1st season	2nd season	1st season	2nd season
Monocropping system				
Control	92.00 ^{ab}	7.87 d	3.08 ^b	3.08 ^c
20 kg/hm ² of N	91.63 ^{bc}	8.75 ^b	3.97 ^a	3.35 ^c
50 kg/hm ² of P	91.25 ^c	8.67 ^b	3.98 ^a	3.58 ^b
TAL 169	91.67 ^b	8.25 ^c	3.73 ^a	3.52 ^b
TAL 169 + 20 kg/hm ² of N	91.17 ^c	9.18 ^a	4.03 ^a	3.87 ^a
TAL 169 + 50 kg/hm ² of P	91.25 ^c	9.30 ^a	4.08 ^a	3.87 ^a
Intercropping system				
Control	92.17 ^a	8.47 ^b	4.07 ^b	3.57 ^c
20 kg/hm ² of N	91.25 ^c	9.00 ^a	4.40 ^b	4.10 ^b
50 kg/hm ² of P	91.17 ^c	9.08 ^a	4.47 ^a	4.07 ^b
TAL 169	91.17 ^c	8.53 ^b	4.55 ^a	4.12 ^b
TAL 169 + 20 kg N/hm ² of N	91.25 ^c	9.33 ^a	4.62 ^a	4.38 ^b
TAL 169 + 50 kg P/hm ² of P	91.17 ^c	9.33 ^a	4.68 ^a	4.42 ^a

The same letter(s) within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test (DMRT) between tow treatments.

Table 5 Effect of inoculation, N, P, Intercropping (sorghum/cowpea) on fat and fiber contents (%) of rain fed cowpea seeds growing for two consecutive seasons

Treatments	Fat		Fiber	
	1st season	2nd season	1st season	2nd season
Monocropping system				
Control	1.95 ^{ab}	1.67 ^d	4.18 ^{ab}	4.08 ^d
20 kg/hm ² of N	2.17 ^a	2.06 ^{ab}	4.43 ^{abc}	4.87 ^c
50 kg/hm ² of P	2.17 ^a	2.00 ^{abcd}	4.30 ^{ab}	5.02 ^{abcd}
TAL 169	1.56 ^c	1.67 ^d	4.12 ^d	4.98 ^{bcd}
TAL 169 + 20 kg/hm ² of N	2.11 ^a	2.00 ^{abcd}	4.50 ^{abc}	5.18 ^{abcd}
TAL 169 + 50 kg P/hm ² of P	2.06 ^a	2.22 ^a	4.67 ^{ab}	5.43 ^a
Intercropping system				
Control	1.72 ^{bc}	1.78 ^{cd}	4.32 ^{abc}	4.90 ^{cd}
20 kg/hm ² of N	1.89 ^{abc}	2.17 ^a	4.82 ^a	5.23 ^{abc}
50 kg/hm ² of P	2.11 ^a	2.22 ^a	4.88 ^a	5.30 ^{ab}
TAL 169	1.84 ^{abc}	1.72 ^{cd}	4.75 ^{ab}	4.33 ^c
TAL 169 + 20 kg/hm ² of N	2.11 ^a	2.11 ^{ab}	4.93 ^a	5.33 ^{ab}
TAL 169 + 50 kg/hm ² of P	2.17 ^a	2.22 ^a	4.98 ^a	5.30 ^{ab}

The same letter(s) within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test (DMRT) between two treatments.

significantly ($P \leq 0.05$) increased by *Bradyrhizobium* inoculation, nitrogen and phosphorus fertilization as well as intercropping. Fiber content is an important constituent in 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. In previous studies, fluctuated results were reported on the effects of inoculation, N, P and/or intercropping on fat and fiber contents of several plants. While, Ahmed (1998) found that intercropping increased the fat content of faba bean seeds, Salih (2002), on the other hand, reported that intercropping did not affect the fat content of soybean seeds. *Rhizobium* inoculation and N and P fertilization were found to increase

the fat and fiber contents of many legumes (Joshi et al., 1989; Elsheikh and Mohamedzein, 1998a; Elsheikh and Ibrahim, 1999; Ibrahim et al., 2008; Elsheikh et al., 2009). Recently, Elsheikh et al. (2009) reported that inoculation with *Bradyrhizobium* and application of chicken manure to inoculated or uninoculated soybean seeds had no significant effect on fiber content of both monocropped and intercropped soybean seeds. Whereas, Abdelgani et al. (1999) and Mohamedzein (1996) reported that nitrogen phosphorus fertilization had no effect on fiber content of fenugreek and groundnut seeds, respectively. The inconsistency of the results on the effect of inoculation, N, P, and intercropping on fat and fiber contents in the aforementioned studies might

Table 6 Effect of inoculation, N, P and intercropping (sorghum/cowpea) on protein and carbohydrates contents (%) of rain fed cowpea seeds growing for two consecutive seasons

Treatments	Protein		Carbohydrates	
	1st season	2nd season	1st season	2nd season
Monocropping system				
Control	26.17 ^d	24.86 ^f	56.62 ^a	58.44 ^a
20 kg/hm ² of N	27.33 ^c	26.77 ^{cd}	53.69 ^b	54.21 ^{bc}
50 kg/hm ² of P	28.21 ^{abc}	26.66 ^{cd}	52.59 ^{bc}	54.02 ^{bc}
TAL 169	27.44 ^c	26.69 ^{cd}	52.99 ^{bc}	54.89 ^{bc}
TAL 169 + 20 kg/hm ² of N	28.16 ^{bc}	28.20 ^{ab}	52.36 ^c	51.57 ^d
TAL 169 + 50 kg/hm ² of P	29.26 ^{ab}	29.33 ^a	51.19 ^{cd}	49.85 ^{de}
Intercropping system				
Control	28.03 ^c	26.39 ^f	54.03 ^a	55.50 ^a
20 kg/hm ² of N	29.70 ^{ab}	27.90 ^{abc}	50.60 ^{bc}	51.60 ^{bc}
50 kg/hm ² of P	29.95 ^{ab}	29.87 ^a	49.46 ^c	49.46 ^d
TAL 169	29.66 ^{ab}	27.49 ^{bcd}	50.36 ^b	53.70 ^b
TAL 169 + 20 kg/hm ² of N	31.98 ^a	28.47 ^{ab}	47.61 ^c	50.87 ^{cd}
TAL 169 + 50 kg/hm ² of P	32.64 ^a	27.92 ^{abc}	46.70 ^c	50.81 ^{cd}

The same letter(s) within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test (DMRT) between two treatments.

be due to the variation in the experimental conditions and locations as well as the plant species.

Table 6 shows the effects of inoculation, N, P and intercropping (sorghum/cowpea) on protein and carbohydrates contents of rain fed cowpea seeds growing for two consecutive seasons. The protein content of cowpea seeds was significantly ($P \leq 0.05$) increased by intercropping, *Bradyrhizobium* inoculation, and nitrogen and phosphorus fertilization. Whereas, all the treatments of intercropping, *Bradyrhizobium* inoculation, and nitrogen and phosphorus fertilization significantly ($P \leq 0.05$) decreased the carbohydrate content of cowpea seeds compared to the untreated control for both cropping systems in both seasons. In agreement with our results, the treatments of either intercropping, inoculation, and/or nitrogen and phosphorus fertilization were found to increase the protein content of various cereals and legumes (Singh and Singh, 1990; Babiker et al., 1995; Mohamedzein, 1996; Abd El Lattif, 1997; Elsheikh and Elzidany, 1997b; Ahmed, 1998; Elsheikh and Mohamedzein, 1998a; Elsheikh and Ibrahim, 1999; Elsheikh and Ahmed, 2000; Mpairwe et al., 2002; Azraf-ul-Haq et al., 2007; Ibrahim et al., 2008; Dahmardeh et al., 2009; Elsheikh et al., 2009). On the other hand, reduction of the carbohydrate contents of various legumes following the aforesaid treatments was reported previously (Ahmed, 1998; Elsheikh and Mohamedzein, 1998a; Salih, 2002; Ibrahim et al., 2008). The reduction in carbohydrate content is likely to be due to the increase in other constituents of the treated seeds.

Effect of treatments on tannin content and in vitro protein digestibility of cowpea seeds

Table 7 shows the effects of inoculation, N, P and intercropping (sorghum/cowpea) on percent tannin content

and protein digestibility of rain fed cowpea seeds for two consecutive seasons. Intercropping significantly ($P \leq 0.05$) increased tannin content and in vitro protein digestibility of cowpea seeds in the two seasons. The *Bradyrhizobium* when combined with 20 kg /hm² of N or 50 kg /hm² of P significantly ($P \leq 0.05$) increased the tannin content of monocropped cowpea seeds in the two seasons. All treatments of *Bradyrhizobium* inoculation and nitrogen and phosphorus fertilization significantly ($P \leq 0.05$) increased the tannin content and the in vitro protein digestibility of intercropped cowpea seeds in the two seasons. The enhancement of tannin content and in vitro protein digestibility due to inoculation, and N and P fertilization were reported for various legumes (Mohamedzein, 1996; Elsheikh and Elzidany, 1997a; Elsheikh and Mohamedzein, 1998a; Salih, 2002; Ibrahim et al., 2008; Elsheikh et al., 2009). Tannin content was found to lower the nutritive value of food and feeds by lowering the 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 (Babiker and El Tinay, 1993). It was reported that tannins adversely affected the protein digestibility (Babiker and El Tinay, 1993). However, in this study all treatments increased both tannin content and protein digestibility of cowpea seeds and this observation was 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 plants tannins.

Conclusion

Generally, all the treatments of intercropping, inoculation with

Table 7 Effect of inoculation, N, P and intercropping (sorghum/cowpea) on tannin content and protein digestibility (%) of rain fed cowpea seeds growing for two consecutive seasons

Treatments	Tannin		Protein digestibility	
	1st season	2nd season	1st season	2nd season
Monocropping system				
Control	1.28 ^c	1.37 ^c	66.67 ^f	67.84 ^d
20 kg/hm ² of N	1.38 ^{bcde}	1.45 ^{bc}	79.64 ^{ab}	80.64 ^{ab}
50 kg/hm ² of P	1.35 ^{de}	1.45 ^{bc}	76.66 ^{cd}	80.78 ^{ab}
TAL 169	1.32 ^c	1.40 ^b	79.47 ^a	78.78 ^b
TAL 169 + 20 kg/hm ² of N	1.47 ^{abc}	1.57 ^{ab}	73.13 ^{cd}	76.57 ^c
TAL 169 + 50 kg/hm ² of P	1.45 ^{abcd}	1.53 ^{ab}	72.01 ^c	81.73 ^a
Intercropping system				
Control	1.37 ^{cde}	1.42 ^{bc}	69.70 ^g	72.20 ^g
20 kg/hm ² of N	1.50 ^a	1.58 ^{ab}	77.31 ^c	84.16 ^b
50 kg/hm ² of P	1.48 ^{bc}	1.57 ^{ab}	75.18 ^d	87.92 ^a
TAL 169	1.47 ^{abc}	1.52 ^b	71.26 ^{fg}	81.82 ^{bc}
TAL 169 + 20 kg/hm ² of N	1.52 ^a	1.60 ^{ab}	79.61 ^{ab}	85.84 ^{ab}
TAL 169 + 50 kg/hm ² of P	1.53 ^a	1.62 ^a	81.48 ^a	86.25 ^{ab}

The same letter(s) within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test (DMRT) between two treatments.

Bradyrhizobium strain, and nitrogen and phosphorus fertilization significantly influenced the physical and chemical properties of cowpea seeds. Intercropping and application of different treatments significantly reduced the carbohydrate content of cowpea seeds with inconsistent results for fat content in the two seasons. Interestingly, *Bradyrhizobium* inoculation and intercropping of sorghum/cowpea improved the yield and quality of cowpea seeds. Thus, we assumed that the adoption of intercropping and/or *Bradyrhizobium* inoculation in the vast rainfed areas of Sudan might contribute to food security by increasing the yield and quality of cowpea, through the full exploitation of the land and the free rainwater potentials.

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