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INTERCROPPING SORGHUM (*SORGHUM BICOLOR* L.) AND COWPEA (*VIGNA UNGUICULATA* L.): EFFECT OF *BRADYRHIZOBIUM* INOCULATION AND FERTILIZATION ON MINERALS COMPOSITION OF COWPEA SEEDS

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Abstract: This investigation was carried out in order to gain a better understanding of the effect of *Bradyrhizobium* inoculation and fertilization on the nutritional values of macro- and micronutrients of cowpea intercropped with sorghum in the field under rainfed conditions. The results of the present study revealed various differences in the minerals content of cowpea seeds as affected by different treatments. Intercropping showed fluctuated results on both macro- and micronutrients of cowpea seeds. Although, intercropping significantly ($p \leq 0.05$) increased the concentrations of Mg, Mn and Cu it is also significantly ($p \leq 0.05$) decreased the concentrations of Ca and P as well as it has no effect on Na, K, Fe, and Zn in cowpea seeds. Despite *Bradyrhizobium* inoculation treatment alone showed variable effects on the concentration of all nutrients in cowpea seeds in both cropping system. The results of *Bradyrhizobium* inoculation together with P and N fertilization significantly ($p \leq 0.05$) improved both major and trace minerals composition of the seeds in intercropped plant.

Key words: *Bradyrhizobium* inoculation; Cowpea**;** Intercropping**;** Fertilization; Mineral content**.**

INTRODUCTION

Intercropping legumes and non-legumes is an agricultural practice of cultivating two or more crops in the same place of land at the same time which is commonly practiced in many parts of the world in order to increase the productivity per unit area of the land (Bhupinder et al., 2003). The crops are not necessarily sown at the same time and their harvest time may be quite different, but they are usually simultaneously grown for significant growing periods (Willey, 1990). Moreover, intercropping allows efficient use of both space and time to optimize beneficial effects (Potts, 1990). According to Campbel (1990) intercropping promotes diversification and allow greater flexibility in adjusting to short and long terms changes in the production and marketing situations. Intercropping provides better weed control and reduces pest and disease incidence (Finney, 1990). Furthermore intercropping is a popular cropping system among small scale farmers in the tropics (Vandermeer, 1989). Cereal/legume intercropping increased dry matter production and grain yield more than their monocultures. Nitrogen transfer from legume to cereal increased the cropping system's yield and efficiency of nitrogen use. The taller cereals reduce biological nitrogen fixation and yield of the associated legume (Fujita et al., 1992). According to Sangakkara (1994) the competitive relationships between the non- legume and the legume was affected the growth and yield of the leguminous crops in close proximity.

Cowpea (*Vigna unguiculata* L. Walp) is an important grain legume throughout the tropics and subtropics, covering Africa, Asia and Central and South America. The cultivated cowpea were domesticated in West Africa, from there, West African immigrants (Barno and Fellata) introduced the crop to western Sudan. In Sudan cowpea is grown under different environmental conditions using different agricultural practices which would certainly affect its growth, nodulation and nitrogen fixation. Cowpea is grown mainly in the rainfed land, but it is also grown in the irrigated schemes and in river banks after the flooding season. In rainfed areas, cowpea is grown alone or intercropped with other field crops such as cereals, or grown on field borders as in Nuba Mountains, and the average yield is about 475 Kg/ ha (Mohamed, 1984). Under irrigation cowpea is mainly grown accompanying vegetables, sorghum or cotton. It is grown almost all around the year, but it depends upon the availability of water, and the average of yield is about 650 Kg/ ha (Mohamed, 1984). In addition to the value of cowpea, as a leguminous plant, that can fix atmospheric nitrogen and improve soil nitrogen, cowpea is also drought and shade tolerant and, therefore, is compatible as an intercrop with many crops (IITA, 1997). Cowpea could be harvested at three different stages of maturity: green snaps, green mature seeds or dry seeds. Cowpea can be used at all stages of growth as a vegetable crop.

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 Mustafa, 2005). The latter effect is of special importance for countries, like Sudan, with predominantly low-input agricultural systems of production. Fertilizer programmes were also established in the Sudan to improve seed quality of legumes and cereals. (Babiker et al., 1995; Elsheikh and Elzidany, 1997). Inoculation of soybean by *Bradyrhizobium japonicum* significantly increased nodulation, yield and seed quality (Okereke and Onochie, 1996). 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 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. Intercropping, breeding, fertilization and genetic engineering programmes are directed towards improving grain yield and seed quality. The main objective of this study was to determine the effect of inoculation with *Bradyrhizobium* strain, intercropping, nitrogen and phosphorus fertilization and their interaction on minerals composition 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. 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 few minutes in shade. Seeds of cowpea (*Vigna unguiculate* L. Walp) were obtained from the local market of Abu Naama, whereas seeds of sorghum (*Sorghum biocolor* L. Monech) (Tabat variety) were obtained from the Sudanese Arabian Company. The germination test of both crops indicated that germination percentage was above 90% of the seeds were viable. Unless otherwise stated 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) to study the effect of intercropping, inoculation, and nitrogen and phosphorus fertilization on physical and chemical properties of sorghum seeds. The experimental site lies at the Demonstration Farm of the Faculty of Agriculture, University of Sennar, Abu Naama, 400 Kms South-east of Khartoum. It lies in the semi tropical savanna, at latitude 12 º 44´ N and longitude 34 º 7´ E. The area is dominated by rainfed agriculture where sorghum, sesame and groundnut are the main crops grown.

Experimental design and treatments

The experimental layout was arranged in split- split plot design with six replicates.
The following treatments were assigned to the main plots:
1- Uninoculated (Control)
2- Inoculated with *Bradyrhizobium* strain
The following cropping systems were assigned to the subplots:
1- Cowpea (monocropping system)
2- Cowpea /sorghum (intercropping system)
3- Sorghum (monocropping system)
The following fertilizers were assigned to the sub sub plots:
1- No fertilizers (Control)
2- 20 Kg N /ha
3- 50 Kg P_2O_5 /ha as 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, 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 20Kg N/ha and 50 Kg P_2O_5 /ha as TSP. Controls with no inoculation and/or no fertilizers were set throughout the experiments.

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 1.0 g 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 tests (1999 version; SAS Software Inst. Inc., Cary, N.C., U.S.A.). Least significant differences were computed at $P \leq 0.05$. Data were also analyzed using the correlation procedure (Pearson's correlation coefficients) in SAS.

RESULTS AND DISCUSSION

Food legumes in general contain appreciable quantities of iron and other minerals. Although legumes are often cited as a complement to cereals in terms of amino acid content, they also make a particularly important contribution to macro- and micro-nutrient nutrition and hence they play an important role in human nutrition mainly in developing countries. The determination of minerals and trace elements in foodstuffs is an important part of nutritional and toxicological analyses. Thus, the present study was aimed at understanding the status of minerals concentration in the seeds of cowpea growing as crop components in sole or mixed culture as affected by inoculation with *Bradyrhizobium* strain, nitrogen and phosphorus fertilization. The results of the present study revealed various differences in the minerals content of cowpea seeds as affected by different treatments. Intercropping significantly ($p \leq 0.05$) decreased the phosphorus (P) content of cowpea seeds in both seasons (Fig. 1top). In agreement with our results, growing cowpea in mixed culture with sorghum has been found to decrease the concentrations of P in the rhizosphere of cowpea plants, leading to markedly decreased content in tissues (Makoi, 2009). In contrast, it has been reported that intercropping increased the P concentrations of intercropped peanut and maize plants (Inal et al., 2007). As expected the highest values were observed in treatments when inoculation with *Bradyrhizobium* plus 50 Kg P/ha and/or 50 Kg P/ha were added, but in most instances were not significantly different compared to other treatments in both cropping system and both seasons. Similarly, it has been reported that inoculation with the locally isolated strains (ENRRI 16A and ENRRI 16C) as well as the introduced strains (TAL 169 and TAL 1371) of *Bradyrhizobium* failed to make significant increase in the mean phosphorus content of guar seeds (Ibrahim et al., 2010). In intercropped plant all the treatments of inoculation with *Bradyrhizobium*, and N and P fertilization enhanced the P content of cowpea seeds in both seasons, whereas in sole cropped plant P content was fluctuated in the two seasons. It is clear from untreated control (Fig. 1bottom) that intercropping significantly ($p \leq 0.05$) decreased the calcium (Ca) content of cowpea seeds in both seasons. In agreement with our results, significantly reduction of Ca concentrations of intercropped peanut and maize were reported (Inal et al. 2007). Moreover, growing cowpea in mixed culture with sorghum significantly decreased the concentrations of Ca content in the rhizosphere and tissues of cowpea plants (Makoi, 2009). In monocropping system, *Bradyrhizobium* inoculation and N fertilization slightly enhanced the Ca content while other treatments had no effect of Ca content in both seasons. Whereas, in intercropping system all the treatments increased Ca content with the highest value when 20 Kg N /ha was applied. Similarly, it has been reported that *Bradyrhizobium* inoculation significantly increased calcium content of guar genotypes (Elshiekh and Ibrahim 1999; Ibrahim et al., 2010), hyacinth bean seeds (Ibrahim et al., 2008) and soybean (Elsheikh et al., 2009). On the other hand, Elsheikh and Mohamedzein (1998) reported that groundnut seed Ca content did not show any pattern in 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 in response to such treatments could be attributed to the difference in cultivars as well as the growing environment. In the present study, the effect of all treatments on P and Ca content are more noticeable in intercropping system than in monocropping system.

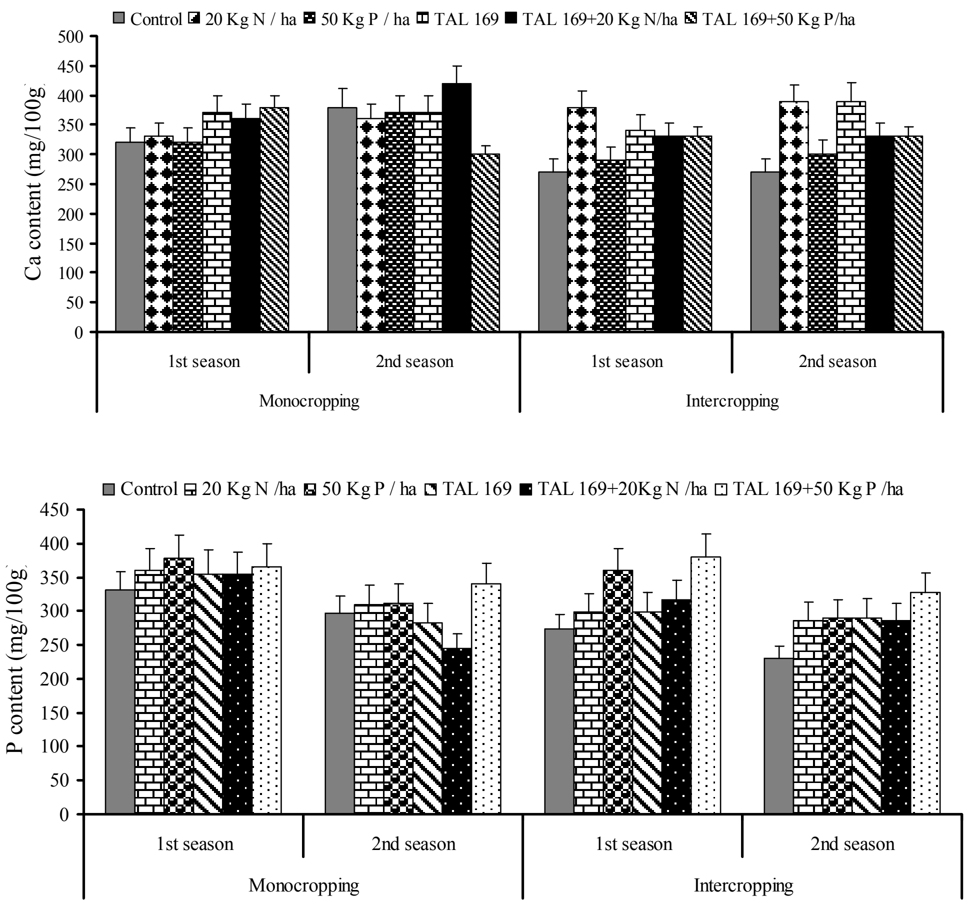

Figure 1. Effect of inoculation, N, P and intercropping (sorghum/cowpea) on calcium (top) and phosphorus (bottom) contents of cowpea seeds grown for two consecutive seasons.

Results on sodium (Na), potassium (K), and Magnesium (Mg) contents are presented in Figure. 2. As could be seen from untreated control, intercropping did not affect Na content of cowpea seeds in both seasons (Fig. 2 top). In monocropping system, *Bradyrhizobium* inoculation and N fertilization slightly increased Na content in both season but the enhancement was not significant, whereas, in intercropped seeds both treatments significantly ($p \leq 0.05$) reduced Na content in both seasons. It has recently been reported that inoculation with *Bradyrhizobium* strain TAL 377 significantly increased Na content of monocropped soybean (Elskeikh et al., 2009) and hyacinth bean (Ibrahim et al., 2008). However, previously, Elsheikh and Ibrahim (1999) reported that inoculation insignificantly affected Na contents of guar clutivars. The difference in response to such treatments could also be attributed to the difference in cultivars as well as the growing environment. All treatments did not significantly affect the K content of cowpea seeds compared to the untreated control in the two cropping systems in both seasons (Fig. 2 middle). Intercropping also did not affect the K content in both seasons. Similarly, Makoi (2009) reported that growing cowpea in mixed culture with sorghum significantly decreased the concentrations of K content in the rhizosphere and tissues of cowpea plants. Moreover, it has been reported that K concentration of wheat was decreased significantly by intercropping with chickpea or lentil (Gunes et al., 2007). Inconsistent with our results, Inal et al. (2007) reported that intercropping significantly enhanced K concentrations of the plants when compared to the monocropping system. Moreover, *Bradyrhizobium* inoculation and fertilization by chicken manure were reported to increase K content of the soybean seeds in both cropping systems (Elsheikh et al., 2009).

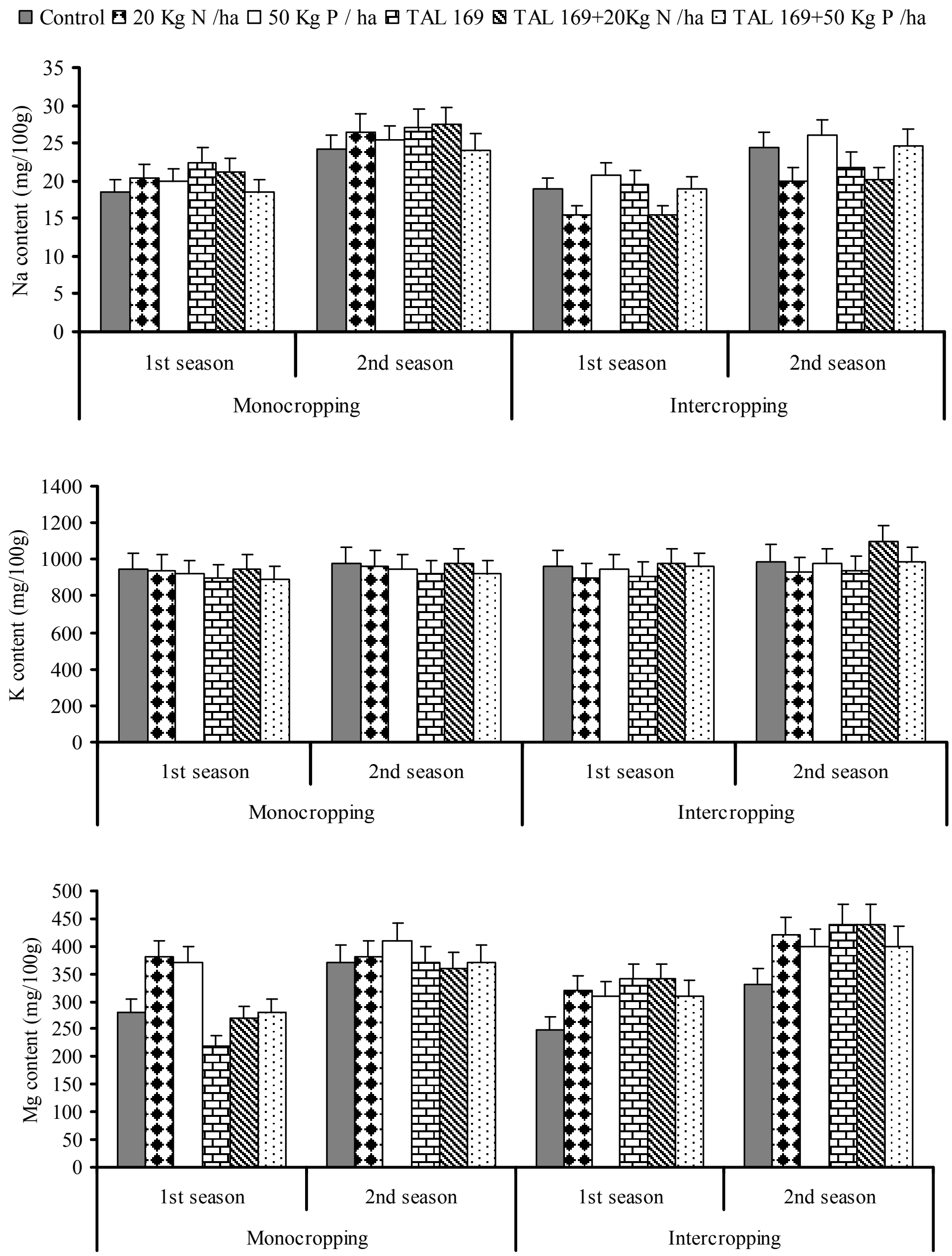

Figure 2. Effect of inoculation, N, P and intercropping (sorghum/cowpea) on Na (Top), K (Middle) and Mg (Bottom) contents of rain fed cowpea seeds grown for two consecutive seasons.

In the present study, high potassium content was found in cowpea seeds of both cropping systems compared to other macronutrients. The amount of K content in cowpea seeds are slightly lower than the daily recommended value (1200 mg/100g) but is higher than the values reported in many cereals and legumes. Intercropping significantly increased the Mg content in cowpea seeds in both seasons (Fig. 2 bottom). However, the treatments of *Bradyrhizobium* inoculation alone and/or in combination with N and P fertilization significantly ($p \leq 0.05$) reduced the amount of Mg in the seeds of sole cropped cowpea plant in both seasons. These treatments enhanced the content of Mg in the seeds of intercropped cowpea plant in the two seasons. Fertilization with both N and P significantly ($p \leq 0.05$) increased Mg content in both cropping systems in the two seasons. Recently, Elsheikh et al. (2009) reported that inoculation with *Bradyrhizobium* strain TAL 377 and addition of chicken manure significantly increased Mg in intercropping system. Previously, Elsheikh and Ibrahim (1999) reported that inoculation with *Bradyrhizobium* strains significantly increased Mg content of five cultivars of guar seeds. Moreover, Ibrahim et al. (2008) indicated that fertilization of hyacinth bean by chicken manure or sulphur greatly increased Mg content of the crop seeds. Elsheikh and Mohamedzein (1998) reported that inoculation with *Bradyrhizobium* and/or VA mycorrhiza significantly increased the seed content of Mg. By contrast, Makoi (2009) reported that growing cowpea in mixed culture with sorghum significantly decreased the concentrations of Mg in the rhizosphere of cowpea plants, leading to markedly decreased content in tissues. This discrepancy of result suggests that different genotypes under different agronomic practices may lead to significant variation in the concentration of mineral element in the plant tissues and seeds.

Micronutrient deficiency in plant foods is becoming an increasingly important global problem. Proper metal transport and improvement in micronutrient content in the edible portions of the plant will be helpful for alleviating human nutritional disorders. Results on zinc (Zn) and copper (Cu) content in the seeds of cowpea under different cropping systems combined with various treatments are presented in Figure 3. Intercropping did not affect the content of Zn in the seeds cowpea plant of both growing seasons (Fig 3 top). In the literature different effects of intercropping on the content of Zn have been reported. Reduction of Zn content in the tissues of cowpea plants growing mixed culture with sorghum has been reported (Makoi, 2009). Whereas, intercropping enhanced of Zn contents of intercropped wheat/chickpea under field condition (Gunes et al., 2007), peanut/graminous species (wheat, maize, parley, oats) under greenhouse condition (Zuo and Zhang, 2008; Inal et al., 2007).

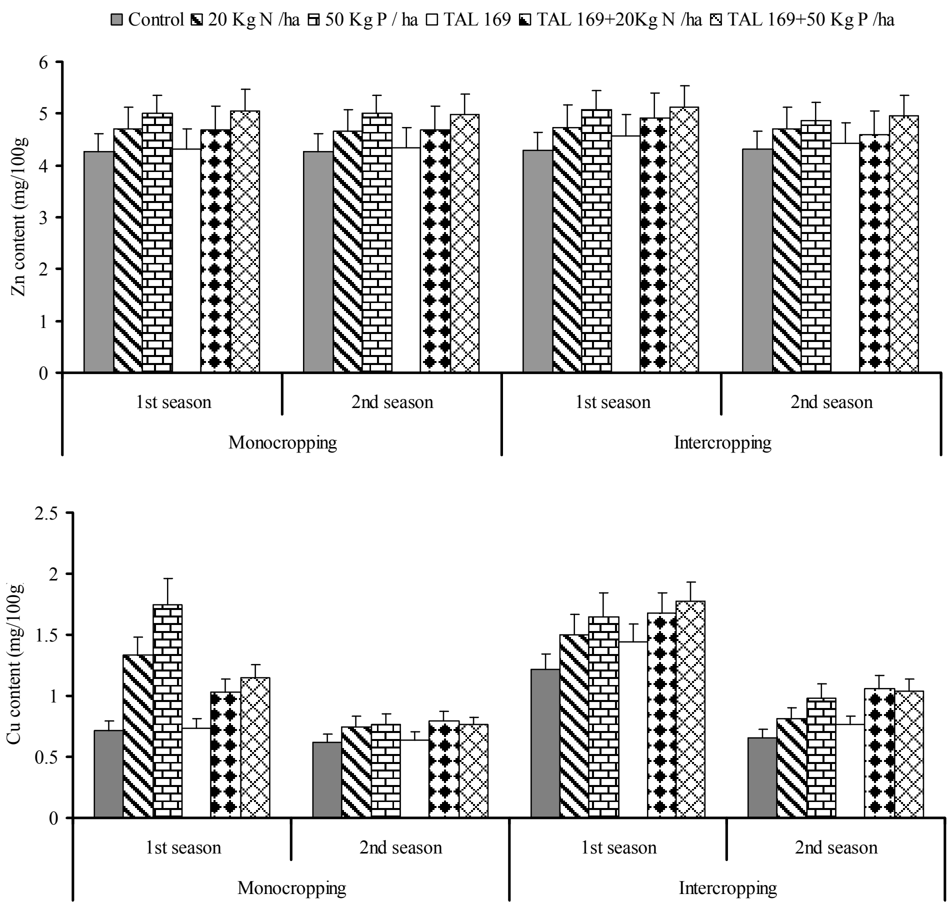

Figure 3. Effect of inoculation, N, P and intercropping (sorghum/cowpea) on Zinc (Top) Copper (Bottom) contents of rain fed cowpea seeds grown for two consecutive seasons.

All treatments of inoculation with *Bradyrhizobium* and N and P fertilization showed similar effect on Zn content in both cropping systems of the two seasons. Inoculation with *Bradyrhizobium* did not affect the content of Zn in cowpea seeds in both cropping systems and both seasons, whereas fertilization with N and P alone or in combination with *Bradyrhizobium* inoculation significantly increased Zn content in the cowpea seeds of both cropping systems in the two seasons. Recently, Elsheikh et al. (2009) reported that inoculation *Bradyrhizobium* strain TAL 377 significantly increased Zn of soybean seeds in monocropping system and insignificantly increased this mineral of intercropped soybean seeds. Previously, Elsheikh and Ibrahim (1999) reported that inoculation with *Bradyrhizobium* strains significantly increased Zn content of five cultivars of guar seeds. Intercropping significantly ($p \leq 0.05$) increased the Cu content of cowpea seeds in both seasons (Fig. 3 bottom). There was no uniform pattern in the effect of treatments on Cu content of cowpea seeds compared to the untreated control in the monocropping systems in the two seasons. It has been reported that growing cowpea in mixed culture with sorghum significantly decreased the concentrations of Cu in the tissues of cowpea plants (Makoi, 2009). This discrepancy of result might be due to differences of cowpea genotypes and growing location and agronomic practices, which assumed to lead to significant variation in the concentration of mineral element in the plant tissues and seeds. Inoculation with *Bradyrhizobium* did not affect the content of Cu in cowpea seeds in sole cropped plant, whereas it slightly enhanced Cu content in the seeds of intercropped cowpea plant. Fertilization with N (20 Kg/ha) and P (50 Kg/ha) alone or together with *Bradyrhizobium* inoculation significantly increased Cu content in the cowpea seeds of both cropping systems in the two seasons compared to the untreated control. Recently, Elsheikh et al. (2009) reported that inoculation with *Bradyrhizobium* strains and addition of chicken manure significantly increased Cu of soybean seeds in monocropping system. It has also been reported that inoculation with *Bradyrhizobium* strains had no significant effect on Cu content of guar cultivars (Ibrahim and Elsheikh, 1999).

Results of manganese (Mn) and iron (Fe) content of the cowpea seeds under different treatments and cropping systems are shown in Figure 4. Intercropping significantly ($p \leq 0.05$) increased the Mn content of cowpea seeds in both seasons (Fig. 4 top). Similarly, Inal et al. (2009) found that Mn concentration of peanut grown with maize increased significantly while the Mn concentrations of maize remained unchanged in response to intercropping. Moreover, under the field conditions, shoot concentration of Mn in wheat and chickpea were also reported to increase by intercropping (Gunes et al., 2007). With the exception of *Bradyrhizobium* treatment, all other treatments significantly ($p \leq 0.05$) increased the Mn content compared to the untreated control in the two cropping systems in both seasons. The highest content of Mn in the seeds of cowpea was noticed in intercropping system when 50 Kg P/ha together with *Bradyrhizobium* treatment were applied. Intercropping did not affect the content of Fe in both seasons compared to untreated control (Fig. 4 bottom). Similar to Mn, all treatments, except *Bradyrhizobium* treatment, significantly ($p \leq 0.05$) increased the Fe content compared to the untreated control in the two cropping systems in both seasons. In agreement with our results, it has been reported that growing cowpea in mixed culture with sorghum significantly decreased the concentrations of Fe in the rhizosphere of cowpea plants, leading to markedly decreased content in tissues (Makoi et al., 2009). On the other hand, Zuo and Zhang (2008) reported that in the intercropping systems of two maize genotypes, barley, oats and wheat not only acquired adequate Fe to meet their own demand, but also improved the Fe status of peanut. However, insignificant improvements of Fe nutrition of intercropped wheat and chickpea were also reported (Gunes et al., 2007). This discrepancy of the might be due to the variation in the plants genotypes as well as agronomical practices and locations. Generally, grain legumes contain all of the essential minerals required by man, although concentration may vary in response to both genetic and environmental factors (Trevor et al., 2003).

Several minerals such as Ca, Fe, K, Mo, Na, Ni and P are essential for human and animal health. The knowledge about their level in different raw foods provides information on the nutritional adequacy of diets. Other minerals such as Cu, Se and Zn though are essential; however, they have a limited range between required and toxic levels. Minerals content of leguminous seeds were to vary with different cropping, inoculation, and fertilization treatments.

CONCLUSION

Combined intercropping and biofertilization could overcome potential nutrient deficiencies, particularly in harvested seeds, and the value of such agronomic arrangements very important for countries that cannot afford biotechnological research, and where farmers are often forced to rely on their own inexpensive seed resources. The results of our experiments strongly support that the cowpea intercropped with sorghum and treated with chemical and/or biofertilization could enhance critical macro- and micronutrients of cowpea seeds. Proper agronomical programmes, focusing on biofertilization and intercropping 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. Furthermore, increasing mineral nutrient concentration of the seed is a high priority research task, and will greatly contribute to alleviation of micronutrient deficiencies in human populations worldwide.

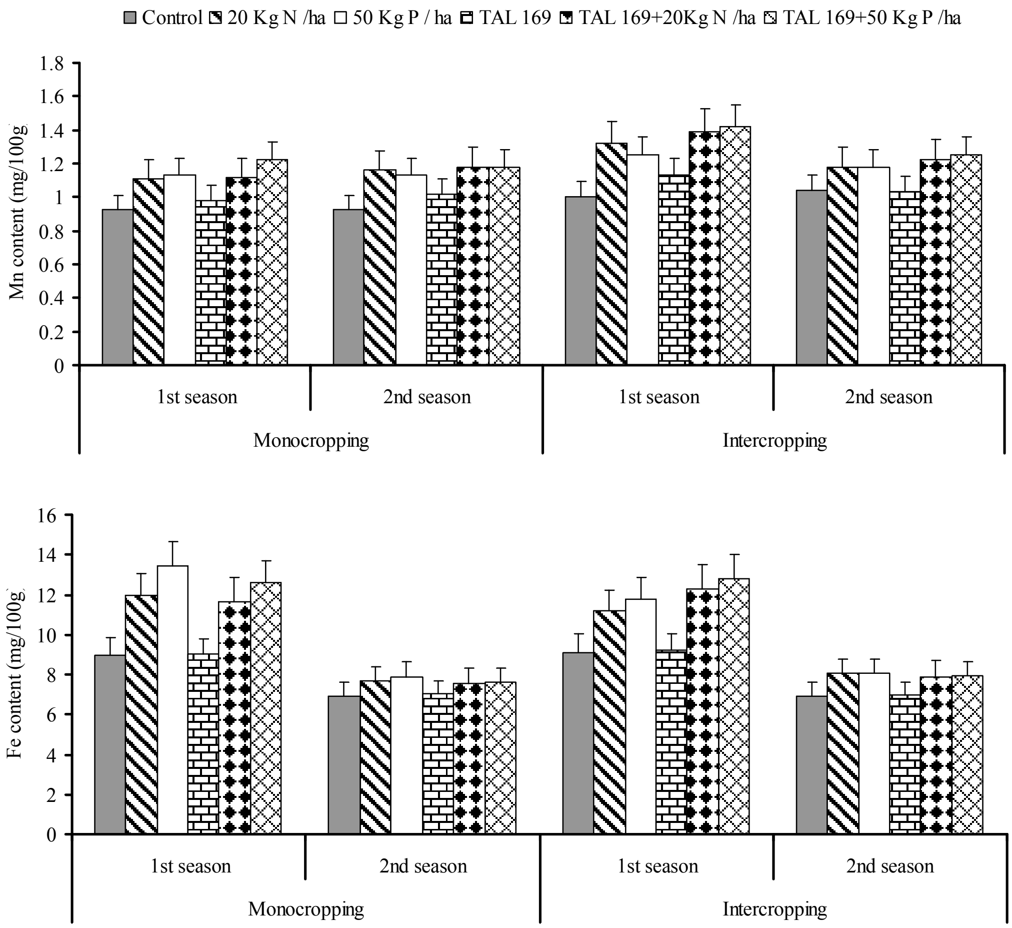

Figure 4. Effect of inoculation, N, P and intercropping (sorghum/cowpea) on Manganese (Top) Iron (Bottom) contents of rain fed cowpea seeds grown for two consecutive seasons.

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