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Effects of *Rhizobium* inoculation and cropping systems on micronutrients uptake and partitioning in common bean (*Phaseolus vulgaris*) and Lablab (*Lablab purpureus*)

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Key words: Soil fertility, N-fixation, Essential elements, Cropping systems, Nutrient uptake

Abstract

The current study was conducted to assess the effects of *Rhizobium* inoculation and cropping systems on the availability and uptake of Cu, Zn, Fe and Mn in common bean (*P. vulgaris*) and lablab (*L. purpureus*). To achieve this aim, field experiments were conducted at Selian Agricultural Research Institute (SARI) for two cropping seasons in a randomized complete block design with 3-factorial arrangement. The treatments included two levels of *Rhizobium* (with and without rhizobia), 2 legumes (*P. vulgaris* and *L. purpureus*) and 5 cropping systems (sole maize or sole legumes, 1 row maize to 1 row legumes (1:1) i.e. 0m or 0.45m of legume from maize row, 1 row maize to 2 rows of legumes (1:2) i.e. 0.1m or 0.2m of legumes from maize rows). The results indicated that, *Rhizobium* inoculation and cropping systems significantly improved the uptake of Cu, Zn, Fe and Mn in roots, shoots and whole plant relative to non-inoculated plots. Lablab was superior on the shoots and whole plant uptake of Cu in both cropping seasons, Zn, Fe and Mn in cropping season 1 but inferior on Zn, Fe and Mn uptake in cropping season 2. With roots micronutrients uptake, lablab was superior to common bean in micronutrients uptake in both cropping seasons. Sole legume had higher micronutrients uptake in shoots, roots and whole plant than other intercropping systems except Cu in shoots for both seasons. Therefore, the best uptake of the micronutrients was recorded in inoculated sole legumes indicating improvement of plant nutritional status and soil fertility through N fixation.

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Introduction

Soils fertility in different parts of the world is rapidly declining due to nutrient harnessing without replenishment (Omotayo and Chukwuka, 2009; Tairo and Ndakidemi, 2014). Crop plants require adequate amounts of all essential plant nutrients for optimum growth but only a few nutrients have been documented (Makoi *et al.,* 2013). These nutrients include phosphorus (P), nitrogen (N), zinc (Zn), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and to a much less extent boron (B), cupper (Cu), manganese (Mn) and iron (Fe) (Ndakidemi *et al.,* 2011). Plants often face nutrient shortages in their environment but they utilize sophisticated mechanisms in an attempt to acquire sufficient amounts of the macro- and micronutrients required for proper growth and reproduction (López-Arredondo, 2013), which include changes in the developmental program and root structure to better "mine" the soil for limiting nutrients, induction of high affinity transport systems (Li *et al.,* 2015), and the establishment of symbioses and associations that facilitate nutrient uptake (Ndakidemi *et al.,* 2011). Together, these mechanisms allow plants to maximize their nutrient acquisition abilities while protecting against the accumulation of excess nutrients. The micronutrients in the soil and their availability to plants are determined by the minerals contained in the original parent material and by the weathering processes that have taken place over the years (Regis, 1998) of the micronutrient deficiencies, Zn by far is the most common (Alloway, 2008). A study by Murata (2003) reported that all micronutrients other than Zn were found adequate for high yielding bean crops.

Availability of micronutrient in soils is governed by soil pH, cation and anion exchange capacity, nutrient interactions, soil physical and chemical properties (Bambara and Ndakidemi, 2010). Regis (1998) reported that as soil pH increases the availability of Fe, Mn, Cu and Zn decreases. For example, at low soil pH the availability of Mn and Zn may increase to toxic levels, and liming very acidic soils to pH 5.5 decreases the solubility and uptake of Mn sufficiently to eliminate the toxicity (Bambara and Ndakidemi, 2010).

In plants, Cu is an activator of many enzymes involved in nitrogen metabolism and an essential constituent of plastocyanin, protein which is a component of the electron transport chain of photosystem (Losak *et al.,* 2011). Mn in plants activates several enzymes involved in the metabolism of proteins, carbohydrates, lipids and more than 60% of the Mn contained in higher plants leaves is found in the chloroplast (Mousavi *et al.,* 2011). Fe is a component of many redox enzymes (Rout and Sahoo, 2015) and function as an activator in the synthesis of chlorophyll and some proteins. Zn is involved in the metabolism of carbohydrates, proteins, and phosphorus compounds and together with Cu and Mn, has a significant effect on carbohydrate metabolism (Kabata-Pendias, 2010)

Intercropping systems occupy an important ratio of land for food production in the world. In the intercropping system involving cereal and legumes, roots interaction could increase the root activity and microbial quantity in the rhizosphere (Zhang *et al.,* 2013). Interspecific interaction between species in the rhizosphere can also affect nutrient availability and uptake in intercropping (Głowacka, 2013). When plants are grown in mixture they have potentials of modifying nutrient availability in the soil by releasing exudates from their roots (Raynaud *et al.,* 2008; Ndakidemi, 2006). These exudates may contain various chemical compounds like organic anion, amino acids, protons, sugars and enzymes which are believed to modify nutrient availability for the plants.

Studies by Wani *et al.* (2008) and Ndakidemi *et al.* (2011) indicated the availability of certain micronutrients such as Fe and Zn in plants and perhaps other macronutrients may be influenced by certain rhizobial bacteria which enabled them to become more available to plants. Tairo and Ndakidemi (2014) reported that, *Bradyrhizobium japonicum* inoculation of legumes such as soybean and consequent N_2 fixation process might have constructive effects on legume growth and micronutrients availability in different plant components. Beneficial soil bacteria such as *B. japonicum* may significantly influence the chemistry of micronutrients in soils by enhancing or reducing micronutrient uptake by plants (Ndakidemi *et al.,* 2011). *Rhizobium* inoculants have been developed and are primarily used for supplying N_2 to plants. However, little information is known about their effect on influencing the availability of micronutrients such as Mn, Fe, Cu, and Zn in leguminous plants such as common bean and lablab. Therefore, this study aimed at assessing the effect of *Rhizobium* inoculation and intercropping systems on Cu, Zn, Fe and Mn content and uptake by legumes.

Materials and methods

Description of the research experimental site

Two field experiments were conducted at Selian Agricultural Research Institute (SARI) farm in northern part of Tanzania (April 2015 to September 2015 and October 2015 to February 2016). SARI lies at Latitude 3°21'50.08" and Longitude 36°38'06.29"E at an elevation of 1390masl with mean annual rainfall of 870mm. The mean maximum temperature ranges from 22°C to 28°C whiles the mean minimum temperature ranges from 12°C to 15°C respectively. Prior to planting, soil samples at 0–20cm depth were collected from the experimental plots in a zigzag mode, pooled, and sub-samples taken for physical and chemical analysis. The soil type was clay loam according to (USDA, 1975), which was slightly acidic ($pH_{(H2O)}$ 6.45), with low organic matter content (4.21%) and high abundance of available forms of copper (Cu) (7.65mg kg^{-1}), zinc (Zn) (1.15mg kg^{-1}), manganese (Mn) (127mg kg^{-1}) and iron (Fe) (137mg kg^{-1}).

Experimental design and treatments application

Land preparation involved clearing, ploughing, layout and finally planting. The experimental design followed a randomized complete block design (RCBD) in a 3-factorial arrangement with 4 replications per treatment. The experimental treatments consisted of 2 levels of *Rhizobium* inoculation (with and without *Rhizobium*), 2 legumes (legume 1 being *P. Vulgaris* and legume 2 being *L. purpureus*) and 5 cropping systems (sole maize or sole legumes, 1 row maize to 1 row legumes (1:1) i.e. 0m or 0.45m of legume from maize row, 1 row maize to 2 rows of legumes (1:2) i.e. 0.1m or 0.2m of legumes from maize rows).

The field plots measured 4m × 4m with 5 rows of maize spaced at (0.9m x 0.5m) apart and 8 rows of legumes spaced at (0.5m × 0.2m). The plots were interspaced by 1m to allow management of crops. The crops were planted upon rain onset. Prior to planting phosphate fertilizer as triple superphosphate was applied to all treatment plots at the rates of 20kg P/ha. The fertilizer was uniformly applied in to the holes and covered with little soil before planting maize or legume seeds to avoid seeds burning. The BIOFIX legume inoculants were obtained from *MEA* Company Nairobi-Kenya, sold under license from the University of Nairobi. Maize variety (SEEDCO 503) was obtained from SEEDCO seed company in Arusha and Common bean seeds variety (Lyamungo 90) and Lablab variety (Rongai) were obtained from Selian Agricultural Research Institute-Arusha-Tanzania. Before sowing, the specific legume seeds were thoroughly mixed with specific *Rhizobium* inoculants to supply (10^9cells/g seed), following procedures stipulated by products manufacturer. To avoid contamination, the non-inoculated seeds were planted first followed with the inoculated seeds. Three seeds were planted and thinned to two plants after full plant establishment. Interplant spacing was maintained at 0.5m throughout for maize and 0.2m for legumes. The plant density was kept constant on a total plot area basis set at the optimum for sole crops and kept the same in intercrops. The plant population density of maize and legumes were maintained at 44,000 and 200,000 plants per hectare respectively. Weeding and other agronomic practices were done manually using hand hoe at different growth stages of the crop plant.

Data collection and laboratory analysis

Plant samples (common bean and lablab) collection involved uprooting of the ten plants which were randomly selected at flowering stage from each plot for the determination of shoots, roots and whole plant nutrient contents namely, Cu, Zn, Fe and Mn. With an aid of a sharpened peg the plants were uprooted and carefully washed by soaking in a half filled bucket. Then the roots and shoots were carefully cut at the ground level. Prior to analysis, the fresh plant samples were washed using distilled water and drip dried.

Thereafter, the samples were oven dried at 70°C to constant weights and ground to a fine powder (0.5mm sieve) for plant tissue analysis.

Micronutrients (Cu, Zn, Fe and Mn) were extracted by diethylenetriamine penta acetic acid (DTPA) according to Lindsay and Norvell (1978), and determined by an atomic absorption spectrophotometer. The dry matter yield determination involved ten whole legumes plant selected randomly at harvesting and sun dried for three days and then oven dried to constant weights at 70°C. After oven drying, samples were weighed and recorded as dry matter yield in kg/ha. The results were converted to dry weight and uptakes of each element by the legumes were calculated per hectare. Then nutrient concentration and uptake was calculated using the formula described by Amur (2003). Uptake (Kg/ha) = Concentration of nutrient (%) x Dry matter yield (Kg/ha).

Data analysis

A 3-way ANOVA was used to analyze the data collected. The analysis was done using STATISTICA software program 2010. Fisher's least significant difference was used to compare treatment means (Steel and Torrie, 1980), at 5% level of probability.

Results

Effects of Rhizobium inoculation on micronutrient uptake in shoots, roots and whole plant of P. vulgaris and L. purpureus

Rhizobium inoculation in *P. vulgaris* and *L. purpureus* plants significantly increased shoots and whole plant uptake of Cu, Zn, Fe and Mn in both cropping seasons relative to the plots in which inoculants were not supplied (Table 1 and 3). The increased shoots micronutrients uptake were by 17.6% and 29.9% for Cu, 29.1% and 41.1% for Zn, 18.4% and 31.7% for Fe, 24.3% and 36.7% for Mn in cropping season 1 and 2 respectively. The increase in whole plant micronutrients uptake were by 18.18% and 23.86% for Cu, 31.25% and 42.36% for Zn, 19.86% and 32.83 for Fe, 26.28% and 37.85 for Mn in cropping season 1 and 2 respectively. With roots, the provision of *Rhizobium* inoculants also significantly increased roots uptake of all micronutrients listed above in the two cropping seasons (Table 2) compared with the uninocuated plots. The increased roots micronutrients uptake were by 22.2% and 41.7% for Cu, 42.4% and 48.6% for Zn, 26.3% and 37.8% for Fe, 38.7% and 48.6% for Mn in cropping season 1 and 2 respectively. *P. vulgaris* had the less shoots and whole plant uptake of Cu, Zn, Fe and Mn in cropping season 1 and only Zn in cropping season 2 but more uptake of Cu, Fe and Mn in cropping season 2 than *L. purpureus*). Moreover, *P. vulgaris* had more root-uptake of Cu and Mn but less uptake of Zn and Fe in cropping season 1 and 2, respectively compared to *L. purpureus* (Table 2).

Effects of cropping systems on micronutrient uptake in shoots, roots and whole plant of P. vulgaris and L. purpureus

Results from the two cropping seasons on the uptake of micronutrients in the shoots and whole plant of *P vulgaris* and *L. purpureus* showed that Zn, Fe and Mn were all significantly higher in sole legumes (cropping system 1) than other cropping systems while Cu was higher in other cropping systems than in sole legumes (cropping system 1) in cropping season 1 and 2, respectively (Table 1 and 3). The increased shoots micronutrients uptake were by 18.2% and 16.9% for Zn, 21.5% and 20.8% for Fe, 21.2% and 18.2% for Mn in cropping season 1 and 2 respectively while Cu in shoots uptake had significant increase in other cropping systems by 14.7% and 15.3% than sole legumes (cropping system 1) in cropping season 1 and 2, respectively. The increased whole plant micronutrients uptake were by 22.3% and 22.7% for Zn, 21.7% and 21.5% for Fe, 20.5% and 17.9% for Mn in cropping season 1 and 2 respectively while Cu in whole plant uptake had significant increase in other cropping systems by 11.8% and 11.1% than sole legumes (cropping system 1) in cropping season 1 and 2, respectively. The uptake of micronutrients in the roots of *P vulgaris* and *L. purpureus* showed that Cu, Zn, Fe and Mn were all significantly higher in sole legumes (cropping system 1) than other cropping systems in cropping season 1 and 2, respectively (Table 2).

The increased roots micronutrients uptake were by 22.2% and 27.3% for Cu, 38.9% and 43.6% for Zn, 22.1% and 24.6% for Fe, 27.9% and 27.3% for Mn in season 1 and 2 respectively.

Interactive effects of Rhizobium inoculation, legumes and cropping systems on micronutrient uptake in shoots, roots and whole plant of P. vulgaris and L. purpureus.

There was a significant interactive effect between *Rhizobium* inoculation and legumes (*P. vulgaris* and *L. purpureus)* to the uptake of micronutrients in shoots, roots and whole plant in the two cropping seasons (Tables 1, 2 and 3). Generally, *Rhizobium* inoculation resulted into significantly more shoots and whole plant uptake of Cu in cropping season 1 but Zn, Fe and Mn in cropping season 2. Interaction between *Rhizobium* and cropping systems increased the uptake of Zn and Mn in cropping season 1 and 2 respectively. Interaction between legumes and cropping systems increased the uptake of Mn in both cropping seasons while the interaction between *Rhizobium*, legumes and cropping systems increased. the shoots and whole plant uptake of Zn and Mn in both cropping seasons. With roots, interaction between *Rhizobium* and legumes increased the uptake of Cu, Zn, Fe and Mn in cropping season 1 but Zn and Mn in cropping season 2. Interaction between *Rhizobium* and cropping systems increased the roots uptake of Zn in cropping season 1 but Cu, Zn, Fe and Mn in cropping season 2. Interaction between legumes and cropping systems increased the uptake of Mn in cropping season 1 but Cu, Zn, Fe and Mn in cropping season 2 while the interaction between *Rhizobium*, legumes and cropping systems increased the uptake of Zn and Fe in cropping season 1 but Zn, Fe and Mn in cropping season 2. Interestingly, in all treatments with no *Rhizobium* inoculation, the uptake of all the above mentioned micronutrients was lowest in the cropping systems 2, 3, 4 and 5 compared with cropping systems 1 (sole legumes).

The Rhizobium, legumes and cropping systems interaction was significant for Zn uptake in the shoots, roots and whole plant (Fig. 1-12) for both cropping seasons.

Table 1. Effect of *Rhizobium* inoculation and intercropping systems on shoots micronutrients uptake by two legumes (*P. vulgaris* and *L. purpureus*) in two cropping seasons.

Treatments	Season 1 (Kg/ha)				Season 2 (Kg/ha)			
	Cu	Zn	Fe	Mn	Cu	Zn	Fe	Mn
Rhizobium								
R-	0.56±0.03b	1.24±0.04b	18.31±0.55b	1.84±0.06b	0.54±0.02b	1.13±0.03b	16.93±0.43b	1.78±0.05b
R+	0.68±0.03a	1.75±0.04a	22.44±0.68a	2.43±0.07a	0.77±0.03a	1.92±0.04a	24.80±0.76a	2.80±0.08a
Legumes								
1	0.46±0.02b	1.36±0.05b	19.03±0.77b	2.01±0.07b	0.55±0.02b	1.55±0.08a	21.64±1.08a	2.38±0.11b
2	0.78±0.02a	1.63±0.06a	21.72±0.54a	2.26±0.08a	0.76±0.03a	1.50±0.06a	20.10±0.61a	2.20±0.09a
Intercropping systems								
1	0.58±0.04a	1.65±0.08a	23.25±1.09a	2.45±0.15a	0.61±0.03a	1.66±0.09a	23.66±1.21a	2.58±0.16a
2	0.60±0.05a	1.53±0.12a	20.55±1.15b	2.31±0.15a	0.63±0.05a	1.56±0.14ab	20.97±1.55b	2.45±0.19a
3	0.63±0.06a	1.47±0.08b	20.05±0.97b	1.96±0.10b	0.66±0.06a	1.51±0.11b	20.57±1.38b	2.14±0.18b
4	0.68±0.06a	1.46±0.08b	19.78±1.14b	1.93±0.08b	0.72±0.06a	1.51±0.12b	20.39±1.49b	2.11±0.15b
5	0.62±0.05a	1.35±0.08c	18.25±0.89bc	2.03±0.08b	0.65±0.05a	1.38±0.10c	18.73±1.15c	2.16±0.10b
3-Way ANOVA (F-statistic)								
Rhiz	17.60***	168.59***	26.02***	116.97***	73.49***	454.9***	92.35***	360.48***
Leg	137.48***	44.34***	10.99***	19.03***	59.70***	1.61ns	3.53ns	10.94***
Cr syst	1.66ns	6.24***	4.04**	13.86***	1.79ns	6.91***	3.79**	12.36***
Rhiz*Leg	4.031**	0.000ns	1.669ns	1.077ns	2.812ns	7.410**	6.670**	12.586***
Rhiz*Cr syst	0.278ns	3.200**	0.082ns	6.462***	0.944ns	4.260**	0.192ns	7.642***
Leg* Cr syst	0.837ns	1.447ns	0.043ns	4.296**	0.619ns	2.113ns	0.108ns	5.419***
Rhiz* Leg*Cr Syst	0.019ns	4.271**	0.465ns	8.206***	0.045ns	3.766**	0.690ns	8.518***

R-: Without *Rhizobium*, R+; With *Rhizobium*, Legume 1: Common bean; Legume 2: Lablab; intercropping System 1, 2, 3, 4 and 5 are sole legumes, 0.1m, 0.2m, 0.45m and 0m of legumes from maize row respectively; Rhiz; Rhizobium, Leg; Legume, Cr Syst; Intercropping Systems. Values presented are means ± SE, n=4. **; *** = significant at P≤0.01, P≤0.001 respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter in a column are significantly different from each other at P=0.05 according to Fischer least significance difference (LSD).

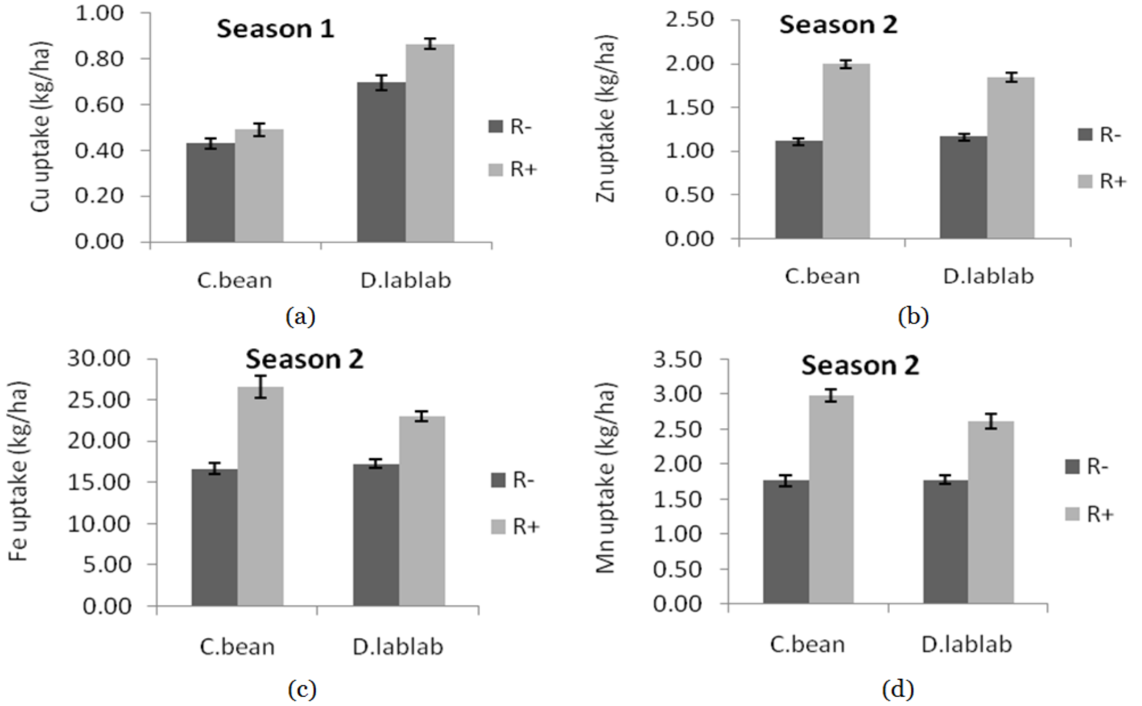


Fig. 1 (a-d): Interactive effects of *Rhizobium* and legumes on shoots Cu uptake in cropping season 1; Zn, Fe and Mn uptake in cropping season 2: (R-: Without *Rhizobium*, R+: With *Rhizobium*, C. bean: *P. vulgaris*, D. lablab: *L. purpureus*).

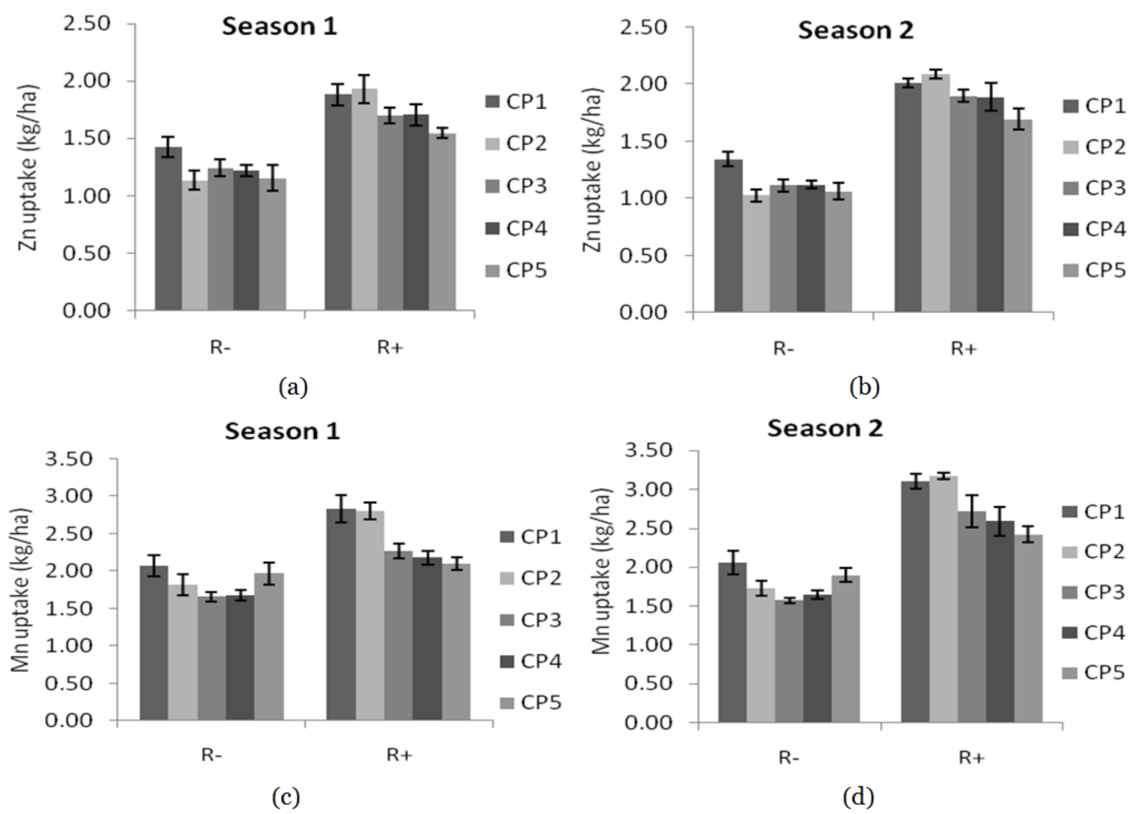


Fig. 2 (a-d): Interactive effects of *Rhizobium* and cropping systems on shoots Zn and Mn uptake in cropping season 1 and 2: (R-: Without *Rhizobium*, R+: With *Rhizobium*, CP1: Cropping system 1, CP2: Cropping system 2, CP3: Cropping system 3, CP4: Cropping system 4, CP5: Cropping system 5)

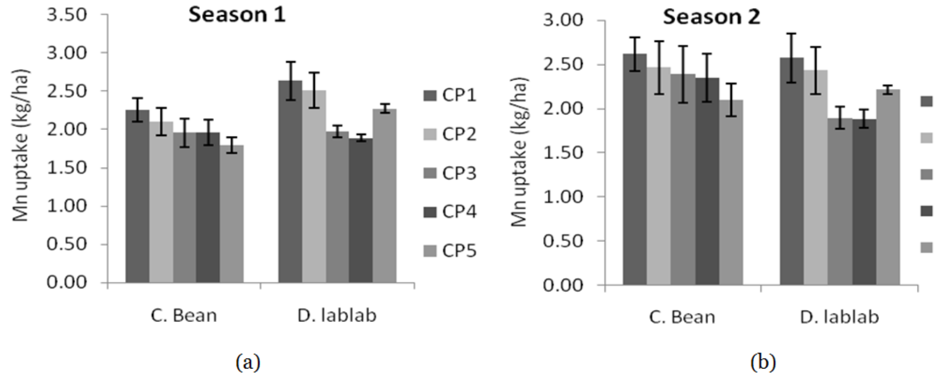


Fig. 3 (a-b): Interactive effects of legumes and cropping systems on shoots Mn uptake in cropping season 1 and 2: (C. Bean: *P. vulgaris*, D. lablab: *L. purpureus*, CP1: Cropping system 1, CP2: Cropping system 2, CP3: Cropping system 3, CP4: Cropping system 4, CP5: Cropping system 5).

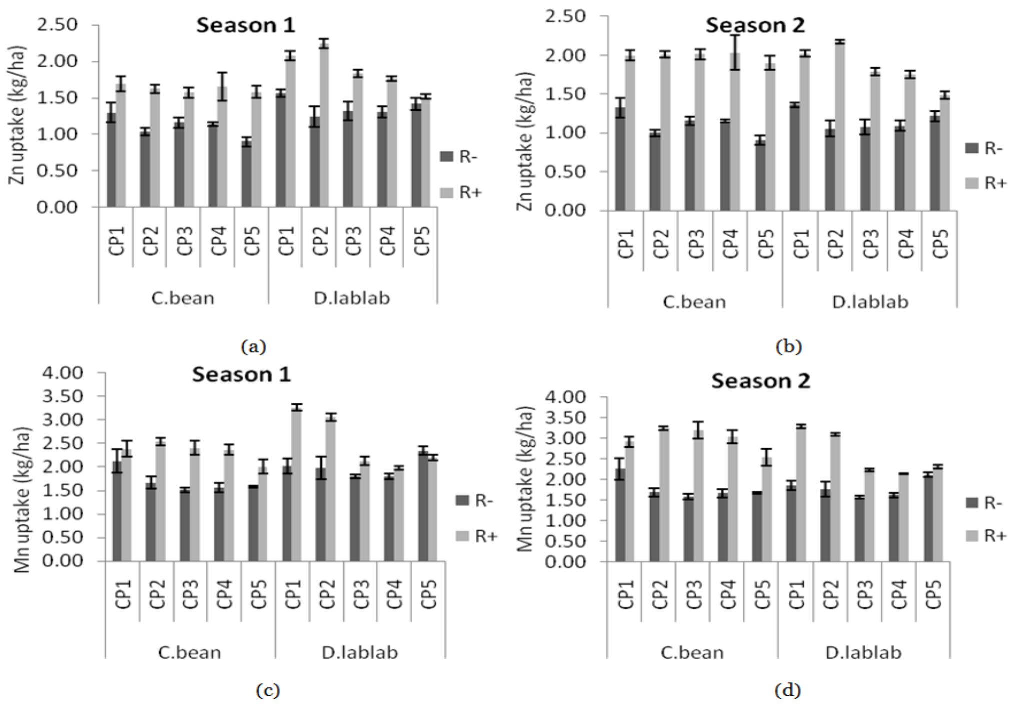


Fig. 4 (a-d): Interactive effects of *Rhizobium*, Legumes and cropping systems on shoots Zn and Mn uptake in season 1 and 2: (-R: Without *Rhizobium*, +R: With *Rhizobium*, C. bean: *P. vulgaris*, D. lablab: *L. purpureus*, CP1: Cropping system 1, CP2: Cropping system 2, CP3: Cropping system 3, CP4: Cropping system 4, CP5: Cropping system 5).

Table 2. Effect of *Rhizobium* inoculation and intercropping systems on roots micronutrients uptake by two legumes (*P. vulgaris* and *L. purpureus*) in two cropping seasons.

	Season 1 (Kg/ha)				Season 2 (Kg/ha)			
Treatments	Cu	Zn	Fe	Mn	Cu	Zn	Fe	Mn
Rhizobium								
R-	0.07±0.002b	0.19±0.006b	3.72±0.10b	0.19±0.00b	0.07±0.003b	0.19±0.01b	3.52±0.08b	0.19±0.01b
R+	0.09±0.002a	0.33±0.018a	5.05±0.13a	0.31±0.01a	0.12±0.004a	0.37±0.02a	5.66±0.08a	0.37±0.01a
Legumes								
1	0.09±0.003a	0.22±0.011b	3.89±0.12b	0.27±0.01a	0.11±0.005a	0.26±0.02b	4.58±0.19a	0.34±0.02a
2	0.07±0.003b	0.31±0.019a	4.89±0.15a	0.22±0.01b	0.08±0.003b	0.29±0.02a	4.60±0.18a	0.22±0.01b
Intercropping systems								
1	0.09±0.005a	0.36±0.038a	4.97±0.26a	0.29±0.02a	0.11±0.009a	0.39±0.04a	5.25±0.31a	0.33±0.03a
2	0.08±0.004a	0.26±0.017b	4.57±0.21ab	0.25±0.02a	0.09±0.008b	0.27±0.02b	4.81±0.31a	0.29±0.03a

Treatments	Season 1 (Kg/ha)				Season 2 (Kg/ha)			
	Cu	Zn	Fe	Mn	Cu	Zn	Fe	Mn
Rhizobium								
3	0.08±0.004a	0.25±0.018b	4.17±0.24b	0.24±0.02a	0.09±0.007b	0.26±0.02b	4.48±0.28ab	0.28±0.03a
4	0.08±0.004a	0.23±0.016b	4.33±0.19b	0.25±0.02a	0.08±0.005b	0.24±0.02b	4.46±0.21ab	0.27±0.03ab
5	0.07±0.004b	0.22±0.021b	3.87±0.24c	0.21±0.02b	0.08±0.007b	0.22±0.02b	3.96±0.30b	0.24±0.03b
3-Way ANOVA (F-statistic)								
Rhiz	228.00***	526.03***	418.42***	1215.52***	548.07***	1165.14***	1983.62***	3606.99***
Leg	109.85***	223.47***	248.66***	161.90***	393.48***	31.35***	0.30ns	1447.19***
Cr syst	25.42***	77.56***	32.91***	47.84***	31.03***	120.95***	79.59***	104.14***
Rhiz*Leg	4.97**	86.215***	8.43**	42.36***	2.19ns	58.48***	0.12ns	257.73***
Rhiz*Cr syst	0.42ns	24.921***	0.81ns	0.94ns	3.52**	40.17***	9.69***	8.37***
Leg* Cr syst	0.80ns	2.058ns	2.50ns	3.37**	5.08***	5.12***	2.67**	17.33***
Rhiz* Leg*Cr Syst	0.26ns	7.086***	5.19***	0.60ns	2.13ns	7.00***	6.88***	9.44***

R-: Without *Rhizobium*, R+; With *Rhizobium*, Legume 1: Common bean; Legume 2: Lablab; intercropping System 1, 2, 3, 4 and 5 are sole legumes, 0.1m, 0.2m, 0.45m and 0m of legumes from maize row respectively; Rhiz; Rhizobium, Leg; Legume, Cr Syst; Intercropping Systems. Values presented are means ± SE, n=4. **; *** = significant at P≤0.05, P≤0.01, P≤0.001 respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter in a column are significantly different from each other at P=0.05 according to Fischer least significance difference (LSD).

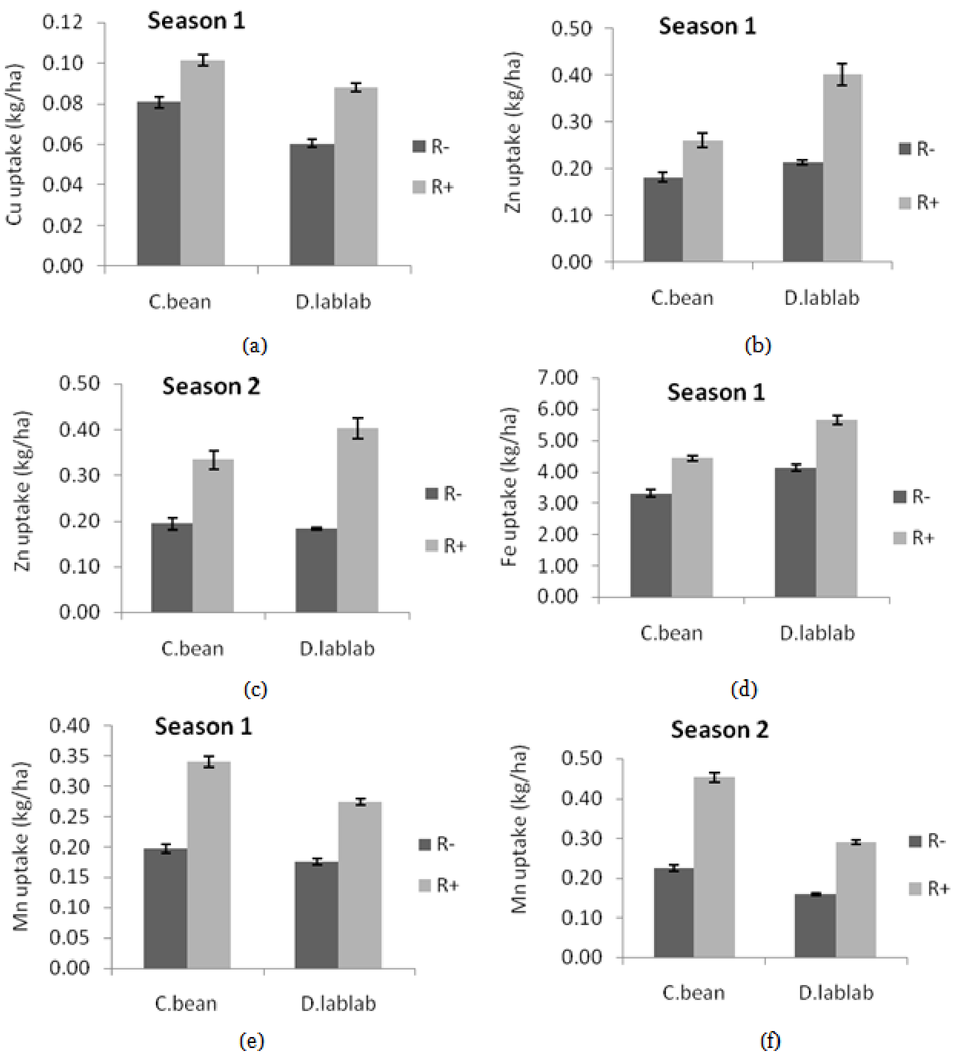


Fig. 5 (a-f): Interactive effects of *Rhizobium* and legumes on roots Cu and Fe uptake in season 1 and Zn and Mn uptake in cropping season 1 and 2: (R-: Without *Rhizobium*, R+: With *Rhizobium*, C.bean: *P. vulgaris*, D. lablab: *L. purpureus*).

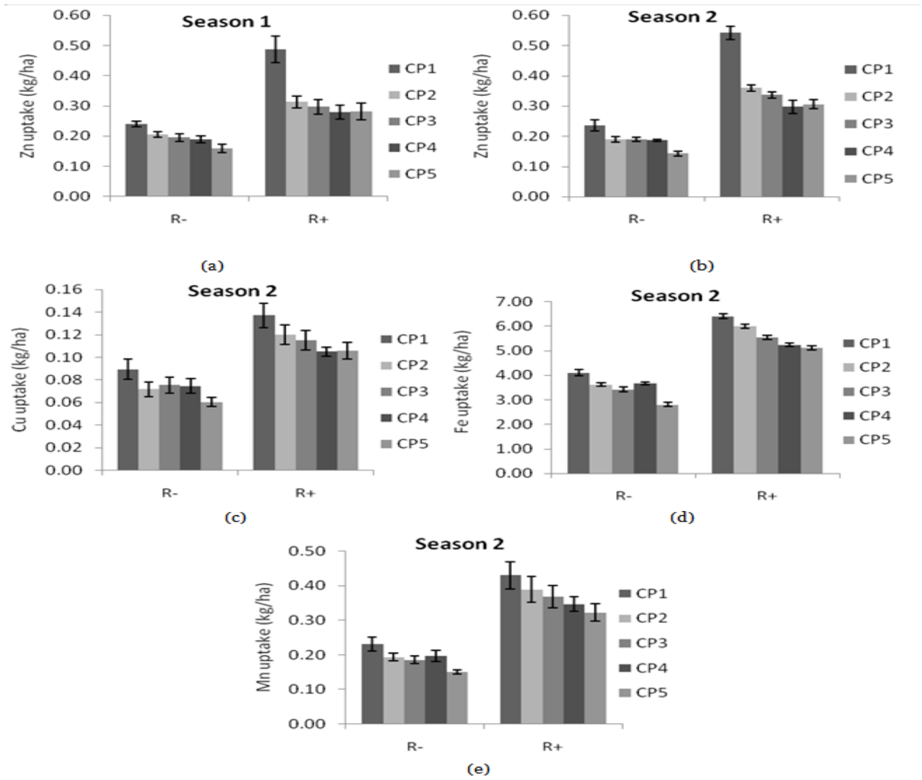


Fig. 6 (a-e). Interactive effects of *Rhizobium* and cropping systems on roots Zn uptake in cropping season 1 and 2; Cu, Fe and Mn uptake in cropping season 2: (R-: Without *Rhizobium*, R+: With *Rhizobium*, CP1: Cropping system 1, CP2: Cropping system 2, CP3: Cropping system 3, CP4: Cropping system 4, CP5: Cropping system 5).

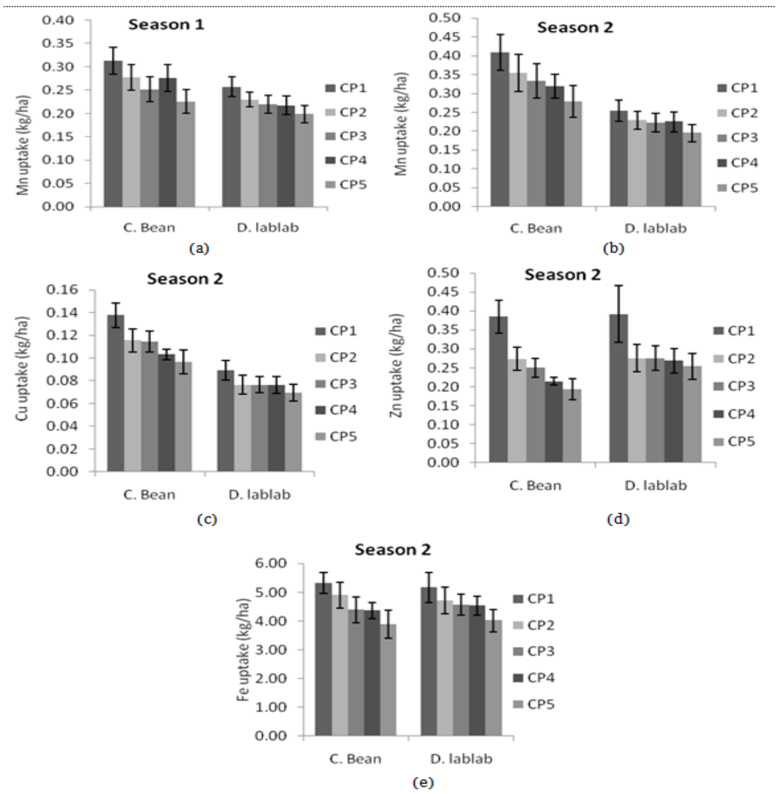


Fig. 7 (a-e). Interactive effects of legumes and cropping systems on roots Mn uptake in cropping season 1 and 2; Cu, Zn and Fe uptake in cropping season 2: (C. Bean: *P. vulgaris*, D. Lablab: *L. purpureus*, CP1: Cropping system 1, CP2: Cropping system 2, CP3: Cropping system 3, CP4: Cropping system 4, CP5: Cropping system 5).

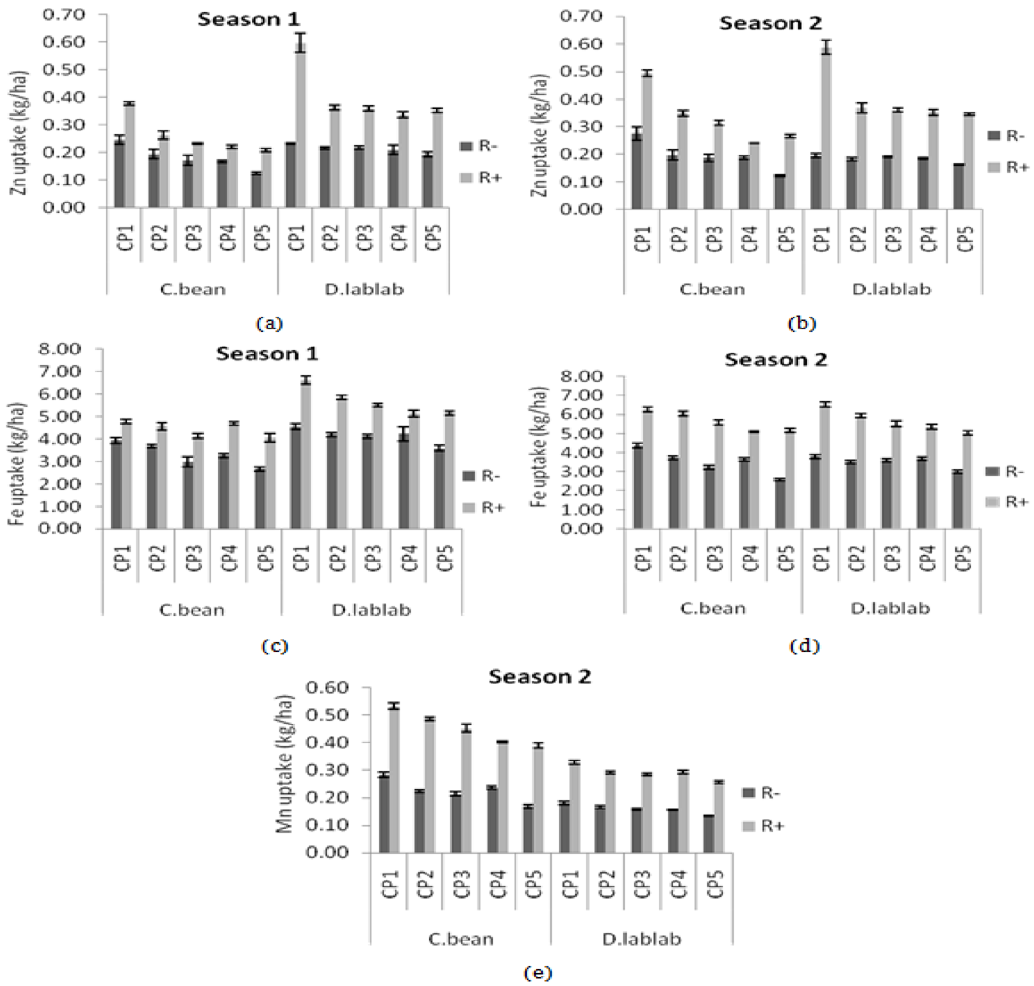


Fig. 8 (a-e). Interactive effects of *Rhizobium*, Legumes and cropping systems on roots Zn and Fe uptake in season 1and 2; Mn uptake in season 2: (R-: Without *Rhizobium*, R+: With *Rhizobium*, C. bean: *P. vulgaris*, D. lablab: *L. purpureus,* CP1: Cropping system 1, CP2: Cropping system 2, CP3: Cropping system 3, CP4: Cropping system 4, CP5: Cropping system 5).

Table 3. Effect of *Rhizobium* inoculation and intercropping systems on whole plant micronutrients uptake by two legumes (*P. vulgaris* and *L. purpureus*) in two cropping seasons.

Treatments	Season 1 (Kg/ha)				Season 2 (Kg/ha)			
	Cu	Zn	Fe	Mn	Cu	Zn	Fe	Mn
Rhizobium								
R-	0.63±0.02b	1.43±0.04b	22.03±0.62b	2.02±0.06b	0.88±0.02a	1.32±0.03b	20.46±0.48b	1.97±0.05b
R+	0.77±0.03a	2.08±0.05a	27.49±0.73a	2.74±0.07a	0.67±0.03b	2.29±0.04a	30.46±0.79a	3.17±0.08a
Legumes								
1	0.55±0.02b	1.58±0.06b	22.91±0.84b	2.28±0.08b	0.83±0.03a	1.81±0.09a	26.21±1.23a	2.72±0.13a
2	0.86±0.02a	1.93±0.07a	26.62±0.63a	2.48±0.09a	0.73±0.03b	1.79±0.08a	24.70±0.77a	2.42±0.10b
Intercropping systems								
1	0.67±0.04a	2.02±0.12a	28.22±1.25a	2.73±0.16a	0.73±0.03a	2.07±0.13a	28.91±1.44a	2.91±0.18a
2	0.68±0.05a	1.79±0.14b	25.12±1.23b	2.56±0.16b	0.72±0.05a	1.83±0.16b	25.78±1.77b	2.74±0.22a
3	0.71±0.06a	1.72±0.09b	24.23±1.14b	2.19±0.11c	0.76±0.06a	1.77±0.13b	25.05±1.61b	2.42±0.21b
4	0.76±0.06a	1.69±0.09bc	24.12±1.23b	2.17±0.10c	0.81±0.06a	1.75±0.13b	24.85±1.64b	2.39±0.17b
5	0.69±0.05a	1.57±0.09c	22.11±1.07c	2.25±0.09c	0.73±0.05a	1.60±0.12b	22.69±1.42c	2.39±0.11b
3-Way ANOVA (F-statistic)								
Rhiz	26.53***	268.68***	46.37***	168.83***	109.10***	694.12***	147.98***	513.54***
Leg	126.72***	78.73***	21.40***	12.70***	42.49***	0.22ns	3.38ns	30.31***
Cr syst	1.33ns	13.91***	6.16***	16.48***	1.42ns	17.13***	5.99***	16.51***

Treatments	Season 1 (Kg/ha)				Season 2 (Kg/ha)			
	Cu	Zn	Fe	Mn	Cu	Zn	Fe	Mn
Rhizobium								
Rhiz*Leg	4.69*	1.82ns	1.15ns	2.09ns	2.61ns	2.68ns	6.51*	20.31***
Rhiz*Cr syst	0.25ns	3.17**	0.09ns	6.52***	0.85ns	4.05**	0.17ns	8.36***
Leg* Cr syst	0.89ns	1.32ns	0.05ns	4.42**	0.91ns	1.58ns	0.15ns	5.41***
Rhiz* Leg*Cr Syst	0.02ns	4.79**	0.59ns	8.19***	0.10ns	4.38**	0.78ns	8.49***

R-: Without *Rhizobium*, R+; With *Rhizobium*, Legume 1: Common bean; Legume 2: Lablab; intercropping System 1, 2, 3, 4 and 5 are sole legumes, 0.1m, 0.2m, 0.45m and 0m of legumes from maize row respectively; Rhiz; Rhizobium, Leg; Legume, Cr Syst; Intercropping Systems. Values presented are means ± SE, n=4. *; **; *** = significant at P≤0.05, P≤0.01, P≤0.001 respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter in a column are significantly different from each other at P=0.05 according to Fischer least significance difference (LSD).

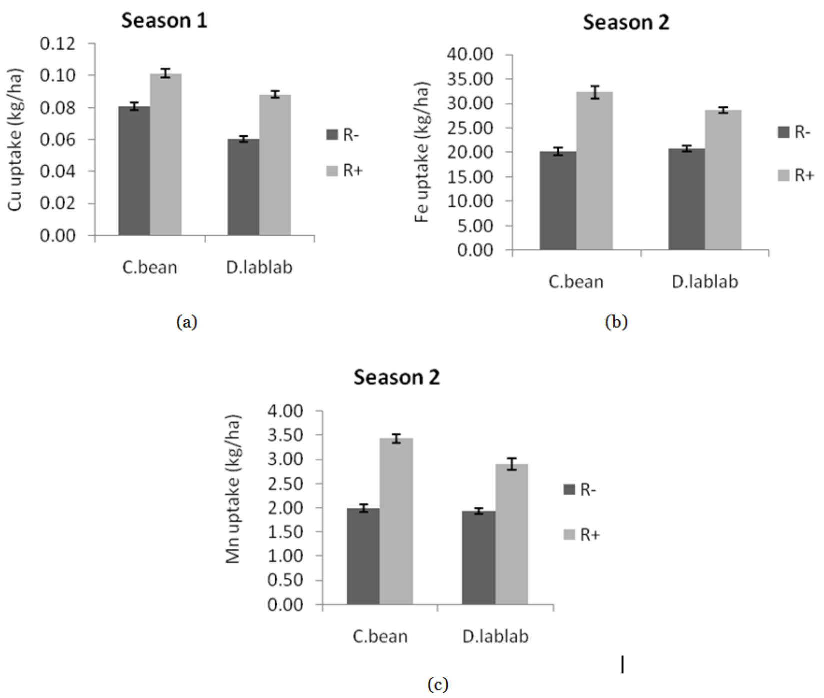
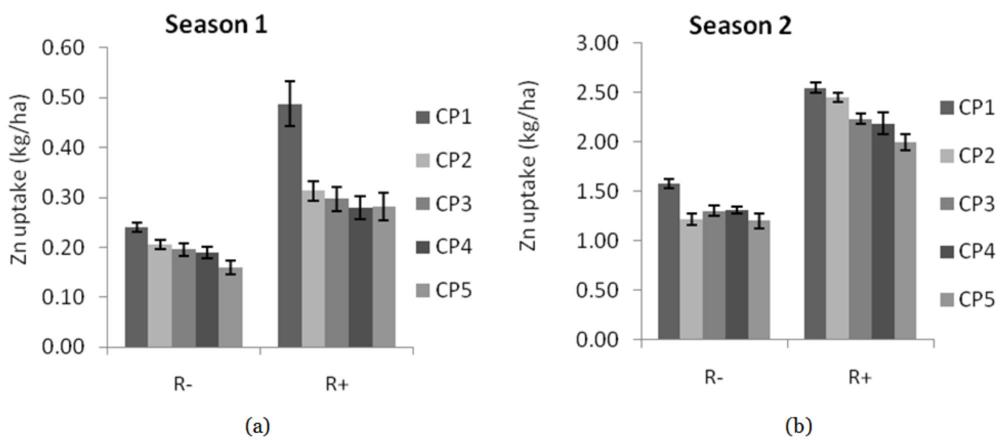


Fig. 9 (a-c). Interactive effects of *Rhizobium* and legumes on whole plant Cu uptake in cropping season 1; Fe and Mn uptake in cropping season 2: (R-: Without *Rhizobium*, R+: With *Rhizobium*, C. bean: *P. vulgaris*, D. lablab: *L. purpureus)*.



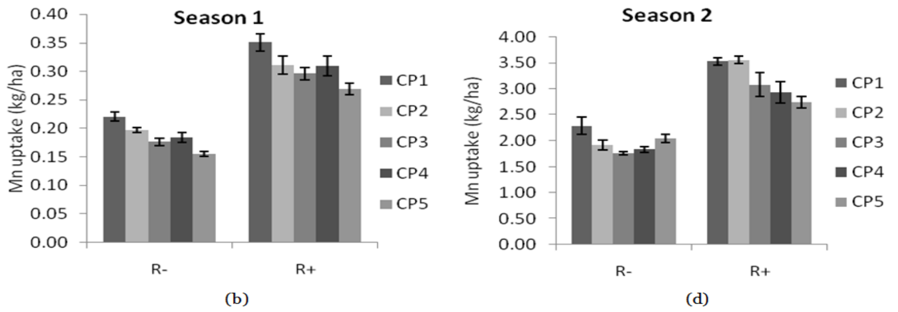


Fig. 10 (a-d). Interactive effects of *Rhizobium* and cropping systems on whole plants Zn and Mn uptake in cropping season 1 and 2: (R-: Without *Rhizobium*, R+: With *Rhizobium*, CP1: Cropping system 1, CP2: Cropping system 2, CP3: Cropping system 3, CP4: Cropping system 4, CP5: Cropping system 5).

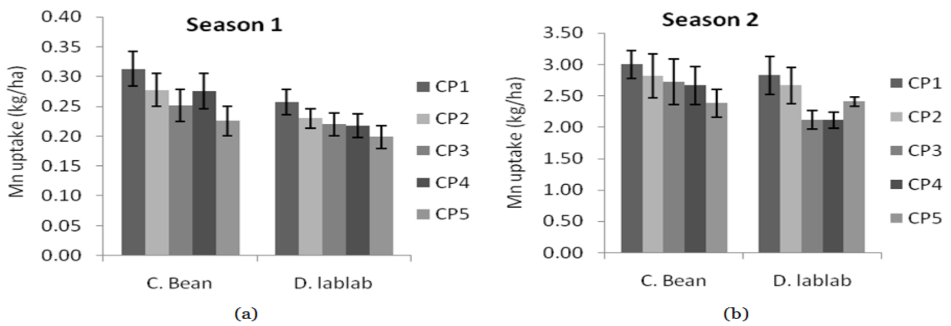


Fig. 11 (a-b). Interactive effects of legumes and cropping systems on shoots Mn uptake in cropping season 1 and 2: (C. Bean: *P. vulgaris*, D. Lablab: *L. purpureus*, CP1: Cropping system 1, CP2: Cropping system 2, CP3: Cropping system 3, CP4: Cropping system 4, CP5: Cropping system 5).

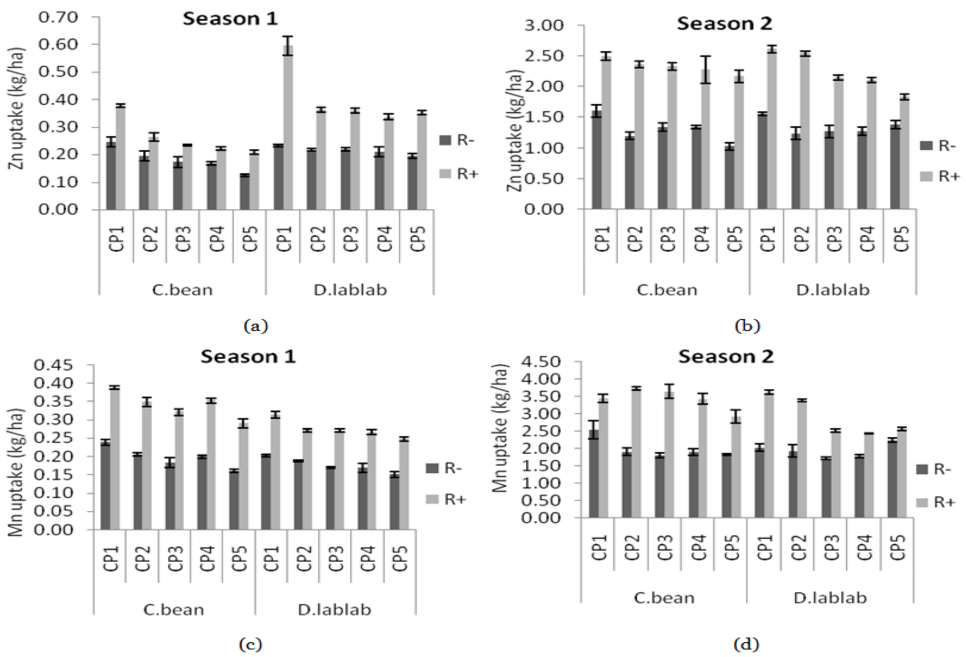


Fig. 12 (a-d). Interactive effects of *Rhizobium*, Legumes and cropping systems on whole plant Zn and Mn uptake in season 1and 2: (R-: Without *Rhizobium*, R+: With *Rhizobium*, C. bean: *P. vulgaris*, D. lablab: *L. purpureus,* CP1: Cropping system 1, CP2: Cropping system 2, CP3: Cropping system 3, CP4: Cropping system 4, CP5: Cropping system 5.

Discussion

Supplying plants of *P. vulgaris* and *L. purpureus* with *Rhizobium* inoculants significantly altered the uptake of Cu, Zn, Fe and Mn in shoot, root and whole plant grown in the two cropping seasons. There was a significant increase in micronutrient content in all organs for the *Rhizobium* inoculated plots. Therefore, the greater levels of Cu, Zn, Fe and Mn in shoots, roots and whole plant of *P. vulgaris* and *L. purpureus* with *Rhizobium* inoculation is an additional advantage apart from the basic function of fixing the atmospheric nitrogen into usable forms in legumes. The mechanisms involved are not well documented. However, research evidence suggests that different nitrogen fixing organisms may produce siderophores (Gamit and Tank, 2014) which may facilitate the solubility of nutrients such as Fe (Sandy and Butler, 2009) and Zn (Wani *et al.,* 2008) from different sources, a phenomenon similar to the observations manifested in our study. The simulative effect of *Rhizobium* on the uptake could be due to their activities on the solubilization of the micronutrients, a phenomenon which requires quantification. Similar to our study, a study by Howell (2008) as cited by Ndakidemi *et al.* (2011) reported that superior rhizobial strains enhanced the uptake of other minerals and balanced the nutritional requirements of peanut plants. Micronutrients are known to be a relatively mobile nutrient in the plant system and readily concentrated by plants (Ndakidemi *et al.,* 2011). However, in plots with no *Rhizobium* inoculants, there was reduction in micronutrients uptake. Furthermore, the reduction in the uptake of Cu, Zn, Fe and Mn may also be strongly related to similar changes in soil pH as it is widely accepted that the availability and uptake of these micronutrients is pH dependent. Bambara and Ndakidemi (2010) reported that when *Rhizobium* inoculation is applied in to the high soil pH, ultimately decrease the uptake of Fe thus, reducing the solubility of Fe in the soil solution then reduced the availability of Fe to plants and hence its uptake and translocation into shoots, roots and whole plant as observed in our study. Due to the limited solubility of Fe in many soils, a study by Connolly and Walker (2008) indicated that plants often must first mobilize iron in the rhizosphere before transporting it into the plant.

Results showed that *L. purpureus* had more effect on the uptake of Cu in both cropping seasons, Zn, Fe and Mn in cropping season 1, while *P. vulgaris* had more effect on the uptake of Zn, Fe and Mn for shoots and whole plant in cropping season 2. With roots, *P. vulgaris* had higher uptake of Cu and Mn but lower uptake of Zn and Fe in both cropping seasons. A study by Sarker *et al.* (2011) indicated that the different micronutrients uptakes are associated with different plant species potential as observed in our study.

Cropping systems significantly affect the micronutrients uptake in shoots and whole plant of *P. vulgaris* and *L. purpureus* where the sole legumes had higher shoots and whole plant uptake of Zn, Fe and Mn except Cu in both cropping seasons. However, sole legumes had higher uptake micronutrients (Cu, Zn, Fe and Mn) in roots than other cropping systems. The high nutrients uptake in shoots, roots and whole plant of sole legumes were attributed by lack of competition of the component crops in the system. A study by Ndakidemi (2006) showed that the decreased micronutrients uptake in legume shoots, roots and whole plant of the intercrop was probably due to high interspecific competition (negative interaction) by intercropped cereal and legumes. Although it might be interspecific facilitation (or positive interaction) in which one plant species enhances the survival, growth, or fitness of another, especially of nutrients uptake in intercropping systems (Li *et al.,* 2003). They also indicated that the decreased in micronutrients uptake might be due to roots intermingling resulted mainly from the enhancement of the volume of soil exploited by the roots rather than a rhizosphere effect.

The interactive effects between *Rhizobium* and legumes were observed in the shoots and whole plant uptake of Cu in season 1 but Zn, Fe and Mn in cropping season 2 (Fig. 1-4 and 9-12) while the roots micronutrients uptake were on Cu, Zn, Fe and Mn in season 1 but Zn and Mn in season 2 (Fig. 5-8).

The interactive effect between *Rhizobium*, cropping systems and intercrops were also recorded in the shoots, roots and whole plant on the micronutrients uptake. Generally, more micronutrient uptake occurred in the treatments involving *Rhizobium* inoculation.

In this study it is evident that *Rhizobium* inoculation in combination with legumes intercrops increased the micronutrients uptake supporting the fact that the microorganism had a mechanism which facilitated the uptake (Ndakidemi *et al.,* 2011). These interaction and mechanisms on the micronutrients uptake by legumes, *Rhizobium* and cropping systems are not well known and therefore, warrant further investigation.

Conclusion

The study suggested that the use of the *Rhizobium* inoculants in common bean or lablab intercropped with maize increased micronutrients uptake. These enhanced the micronutrients concentration in rhizosphere of the intercropped common bean and lablab, as consequence; increase for micronutrients uptake in shoot, roots and whole plant. Lablab had more positive effect on interspecific competition through micronutrients uptake and partitioning than common bean with the intercropped maize. This confirmed the advantage of intercropping maize-common bean or lablab over sole cropping system on micronutrients uptake.

Therefore, the provision *Rhizobium* inoculants to *P. vulgaris* and *L. purpureus* appear to promote greater nutrient uptake (Cu, Zn, Fe and Mn) in whole plant and accumulation in their plant tissues.

References

Alloway BJ. 2008. Zinc in Soils and crop nutrition. Second edition, published by IZA and IFA Brussels, Belgium and Paris France.

Amur N. 2003. Assessment of the fertility status of soils of rice growing areas of Same District, Kilimanjaro Region, Tanzania. Unpublished Dissertation for award of the MSc. Degree at Sokoine University of Agriculture, Morogoro, Tanzania 94pp.

Bambara S, Ndakidemi PA. 2010. *Phaseolus vulgaris* Response to *Rhizobium* inoculation, lime and molybdenum in selected low pH soil in Western Cape, South Africa. African Journal of Agricultural Research **5,** 1804-1811.

Connolly EL, Walker EL. 2008. Time to pump iron: iron-deficiency-signaling mechanisms of higher plants. Current Opinion in Plant Biology **11,** 530-535.

Gamit DA, Tank SK. 2014. Effect of siderophore producing microorganism on plant growth of *Cajanus cajan* (Pigeon pea). International Journal of Research in Pure and Applied Microbiology **4,** 20-27.

Głowacka A. 2013. Uptake of Cu, Zn, Fe and Mn by maize in the strip cropping system. Plant Soil Environment **7,** 322-328.

Howell RK. 2008. *Rhizobium* induced mineral uptake in peanut tissues. Journal of Plant Nutrition **10**, 1297-1305.

Kabata-Pendias A. 2010. Trace elements in soils and plants (4th Ed.).

Li LF, Zhang X, Li P, Christie J, Sun S, Yang, Tang C. 2003. Interspecific facilitation of nutrient uptake by intercropped maize and faba bean. Nutrient Cycling in Agro ecosystems **65,** 61-71.

Li X, Zeng R, Liao H. 2015. Improving crop nutrient efficiency through root architecture modifications. Journal of Integrative Plant Biology **58,**193-202.

Lindsay WL, Norvell WA. 1978. Development of DTPA soil test for zinc, iron, manganese and cupper. Soil Science Society of America Journal **42,** 421-428.

López-Arredondo SL, Leyva-González MA, Alatorre-Cobos L, Herrera-Estrella L. 2013. Biotechnology of nutrient uptake and assimilation in plants. International Journal of Developmental Biology **57,** 595-610.

Losak TJ, Hlusek J, Martinec J, Jandak M, Szostkowa R, Filipcik J, Manasek K, Prokes J, Peterka L, Varga L, Ducsay F, Orosz, Martensson A. 2011. Nitrogen fertilization does not affect micronutrient uptake in grain maize (*Zea mays* L.). Acta Agriculture Scandinavica, section B: Soil and Plant Science **61,** 543-550.

Makoi JHJ, Bambara RS, Ndakidemi PA. 2013. "Rhizobium Inoculation and the supply of molybdenum and lime affect the uptake of macroelements in common bean (*P. vulgaris* L.) plants." Australian Journal of Crop Science **7,** 784-794.

Mousavi SR, Shahsavari M, Rezaei M. 2011. A general overview on manganese (Mn) importance for crops production. Australian Journal of Basic and Applied Sciences **5,** 1799-1803.

Murata MR. 2003. The impact of soil acidity amelioration on groundnut productivity on sandy soils of Zimbabwe. PhD thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Plant Production and Soil Science, University of Pretoria etd.

Ndakidemi PA, Bambara RS, Makoi JHJ. 2011. Micronutrient uptake in common bean (*Phaseolus vulgaris* L.) as affected by *Rhizobium* inoculation, and the supply of molybdenum and lime. Plant Omics Journal **4,** 40-5.

Ndakidemi PA. 2006. Manipulating legume/cereal mixtures to optimize the above and below ground interactions in the traditional African cropping systems. African Journal of Biotechnolgy **5,** 2526-2533.

Omotayo OE, Chukwuka KS. 2009. Soil fertility restoration techniques in sub-Saharan Africa using organic resources. African Journal of Agricultural Research **4,** 144-150.

Raynaud XB, Jaillard, Leadley PW. 2008. Plants may alter competition by modifying nutrient bioavailability in rhizosphere: a modeling approach. The American Naturalist **171,** 44-58.

Regis V. 1998. Micronutrients. Department of Agronomy Iowa State University Ames, IA50011. Pp 69.

Rout GR, Sahoo S. 2015. Role of iron in plant growth and metabolism. Reviews in Agricultural Science **3,** 1-24.

Sandy M, Butler A. 2009. Microbial iron acquisition: Marine and terrestrial siderophores. Chemical Reviews **109,** 4580-4595.

Sarker MJU, Siddiky MA, Jahiruddin M, Mian MH, Islam MS, 2011. Uptake of different nutrient elements by legume crops in wheat -legume- T. Aman cropping pattern. Bangladesh Journal of Agricultural Research **36,** 247-253.

Steel RGD, Torrie JH. 1980. Principles and procedures of statistics: A biometrical approach, Second Edition. McGraw Hill, New York.

Tairo EV, Ndakidemi PA. 2014. Micronutrients uptake in soybean (*Glycine max* L.) as affected by *Bradyrhizobium japonicum* inoculation and phosphorus (p) supplements. World Journal of Soil and Crop Sciences Research **1,** 1-9.

United State Development of Agriculture (USDA) Soil Taxonomy. 1975. A basic system of soil classification for making and interpreting soil surveys. Soil survey Staff, Soil Conservation Service, Washington, D.C 754pp.

Wani PA, Khan MS, Zaidi A. 2008. Impact of zinc tolerant plant growth promoting rhizobacteria on lentil grown in zinc amended soil. Agronomy for Sustainable Development **28,** 449-455.

Zhang X, Huang G, Bian X, Zhao Q. 2013. Effects of root interaction and nitrogen fertilization on the chlorophyll content root activity, photosynthetic characteristics of intercropped soybean and microbial quantity in the rhizosphere. Plant, Soil and Environment **59,** 80-88.