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Micronutrient uptake in common bean (*Phaseolus vulgaris* L.) as affected by Rhizobium inoculation, and the supply of molybdenum and lime

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Abstract

Pot and field experiments were done to establish the effect of *Rhizobium* inoculation, Molybdenum and lime supply on the availability of micronutrients (Cu, Zn, Mn, Fe, B and Mo) in *Phaseolus vulgaris* L. In both field and pot experiments, *Rhizobium* inoculation significantly improved uptake of Mn, Fe, Cu, Zn, B and Mo in all organs (roots, shoots, pods and whole plants) except the Mo uptake in roots. Supplying Mo at 6 and 12 $g.kg^{-1}$ of seeds significantly increased Mo uptake but reduced that of Mn, Fe, Cu, Zn and B in the roots of *P vulgaris* compared with the zero control. In the pods harvested from the glasshouse and field, and whole plant from glasshouse experiments, the uptake of Mn and Fe were significantly reduced in treatments supplied with Mo at 6 and 12 $g.kg^{-1}$ of seeds relative to the zero control. Compared with zero-lime control, the application of 2 and 3 $t.ha^{-1}$ in the glasshouse significantly reduced the uptake of Mn, Fe, Cu, Zn and B in the root, shoot and whole plant and increased that of Mo. In the field conditions lime significantly reduced the uptake of Fe, Zn and B in the roots, Fe and Cu in the shoot and whole plant, and Mn in pods and whole plants. Lime supply between 2 - 3 $t.ha^{-1}$ increased the uptake of Mo in all organs both in the greenhouse and field experiment. There was a significant interactive effect between *Rhizobium* inoculation and Mo, *Rhizobium* x lime and lime and Mo supply. Interactively, the best uptake of the micronutrients was recorded in treatments involving highest rate of Mo (12 $g.kg^{-1}$ of seeds) and that of lime (3 $t.ha^{-1}$).

Keywords: Nutrient uptake, Boron, Copper, Iron, Manganese, Zinc

Introduction

Phaseolus vulgaris L. is an important leguminous crop of greater nutritional status to poor communities in African countries. It is nicknamed as a poor man's protein due its potential role in the daily diet settings of the poor where expensive animal protein cannot be afforded. From this background, any technological or agronomical attempt to improve the nutritional quality such as the micronutrient content will be a positive move in sustaining health of poor communities relying on this crop. The productivity of *P. vulgaris* in many parts of developing world is constrained by soil related factors. To mention the few, they include: acidic soils with low pH (Whelan and Alexander, 1986; Richardson et al., 1988; Peoples et al., 1995); inadequate nitrogen levels in the soil (Woomer et al., 1997 and 1999) and low levels of molybdenum in acidic soils, an important nutrient in the N_2 fixation process in legumes (Hafner et al., 1992; Cardoso et al., 1998; Vieira et al., 1998). Its application in most legume nutritional programs is mostly neglected although positive results on the availability of nutrients and yield of legumes is widely acknowledged (López et al., 2007; Bambara and Ndakidemi 2010). Being a leguminous crop, *P. vulgaris* has a unique property of symbiotically associating with *Rhizobium leguminosarum* and convert atmospheric nitrogen into a usable form to the plants and some may be leaked to the soil and therefore influencing other biological process in the soil. Research evidence suggests that *Rhizobium* inoculation of legumes and the subsequent N_2 fixation process could have positive effects on plant growth and finally the micronutrient availability and hence improving the nutritional quality of different plant components (Rodelas et al., 1999; Rengel et al., 1999). It is established that certain groups of *Rhizobium* may produce siderophores (Wittenberg et al., 1996; Berraho et al., 1997; Duhan et al., 1998; Arora et al., 2001; Sridevi et al., 2008) which may facilitate the availability of nutrients such as Fe (Fabiano et al., 1994), Zn (Wani et al., 2008), P (Abd-Alla 1994; Sridevi et al., 2007) and render them more available to plants. Nevertheless, microorganisms such as *Rhizobium* inoculants may significantly affect the chemistry of micronutrients in soils by enhancing micronutrient uptake by plants. Based on these facts, it is therefore important to establish the possible role which could be played by bean *Rhizobium* inoculants on the availability of micronutrients in *P. vulgaris*. Soil acidity is an important abiotic factor affecting the availability of micronutrients in the soil (Rengel, 1999; Marschner, 1991). This constrain is alleviated by liming (Bambara and Ndakidemi 2010a). Research evidence suggest that the liming practice is antagonistic on the availability of micronutrients such as: Mn, Fe, Cu, Zn and B (Gupta, 1972a,b; Gupta, 1979,1992). Studies by (Prasad and Sinha, 1982) showed that liming the soil increased the soil pH and resulted in Mn, Fe, Cu, Zn and B deficiency in legumes. However, other research reports suggests improved uptake of certain

micronutrients such as Mo with liming (Fleming, 1980; Gupta, 1997 López, 2007). To-date, most *Rhizobium* inoculants have been developed and are primarily used for supplying N to plants. Little is known about their effect on supplying micronutrients in legumes such as *P.vulgaris*. Furthermore, limited studies in South Africa have reported the influence of lime and molybdenum on the availability of micronutrients in different plant organs of *P.vulgaris* including the edible parts such as pods and hence this study.

Materials and methods

Plant growth and growth conditions

Glasshouse and field experiments were conducted at the Cape Peninsula University of Technology, and the Agricultural Research Council Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa respectively, during the 2008 and 2009 summer seasons. The field experiment was conducted under irrigation. The experimental site for field experiment lies in the winter rainfall region of South Africa at an elevation of 146 m above sea level, with the mean annual rainfall of 713.4 mm and mean annual temperatures of 22.6 °C at day and 11.6 °C at night. The soil type of the experimental site was sandy loam (Glenrosa, Hutton form), which according to the Soil Classification Working Group (SCWG) is equivalent to skeletic leptosol (SCWG, 1991).

Experimental design

The experimental design followed a randomized complete block design in a factorial arrangement with 4 replications per treatment. The experimental treatments consisted of 2 levels of *Rhizobium* inoculation (with and without *Rhizobium*), 3 levels of dolomitic agricultural lime (0, 2 and 3 $t.ha^{-1}$) and 3 levels of Mo (ammonium molybdate [$(NH_4)_6Mo_7O_{24}2H_2O$]) (0, 6 and 12 $g.kg^{-1}$ of seeds). The field plots measured 2.5 m x 4 m with 5 rows 0.5 m apart from one another. *P. vulgaris* was sown with inter-row planting distance of 20 cm. The plots were interspaced by small terraces of 1 m to prevent contamination. The plant population density was 200,000 plants per hectare. Planting was done after ploughing and harrowing. Dolomtic agricultural lime application (Dolomite: $CaCO_3$ and $MgCO_3$) was done 2 weeks before planting. Twelve hours before planting, *P. vulgaris* seeds were soaked into Mo solution (ammonium molybdate [$(NH_4)_6Mo_7O_{24}2H_2O$]. The zero Mo control was also soaked in a water solution containing zero Mo. To avoid contamination, all *Rhizobium* uninoculated treatments were sown first. *Rhizobium* inoculation was done manually by putting the inoculant (*Rhizobium leguminosarum* biovar phaseoli-bakteriee registrasienr. L1795 wet 36/1947) in the planting hole. The inoculants used were obtained from University of Pretoria, South Africa. Weeding was done manually with a hoe at 3 and 8 weeks after planting.

Plant harvest and sample preparation

At 60 d after planting, *P. vulgaris* plants were sampled for nutrient analysis. About 10 plants were sampled respectively from the middle rows of each plot. The border plants within each row were excluded. The plants were carefully dug out with their entire root system, washed and divided into roots, shoots, pods. The plant organs were oven-dried at 60°C for 48 h weighed and ground into a fine powder (0.85 mm) for the analysis of micro nutrients.

Measurement of mineral nutrients in organs

Measurements of micronutrients (Mn, Fe, Cu, Zn, B and Mo) were determined by ashing 1 g ground sample in a porcelain crucible at 500 °C overnight. This was followed by dissolving the ash in 5 mL of 6 M HCl and placing it in an oven at 50 °C for 30 min; 35 mL of deionised water were added and extract filtered through Whatman no. 1 filter paper. Nutrient concentrations in plant extracts were determined using an inductively-coupled plasma (ICP) emission spectrophotometer (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusettes, USA) (Giron, 1973).

Statistical analysis

A 3-factorial design (3-way ANOVA) was used to statistically analyse for miconutrients in plant organs. The analysis was done using the software of STATISTICA program 2010. Fisher's least significant difference was used to compare treatment means at $P \leq 0.05$ (Steel and Torrie, 1980).

Results

Effects of Rhizobium inoculation on micronutrient uptake in roots, shoots, pods and whole plants of P. vulgaris

Rhizobium inoculation in *P. vulgaris* plants significantly increased shoot uptake of Mn, Fe, Cu Zn B and Mo in both the glasshouse and field experiment relative to the control treatments in which inoculants were not supplied (Table 1). With roots, the provision of *Rhizobium* inoculants also significantly increased root uptake of all micronutrients listed above except Mo in the glasshouse and the field (Table 2) compared with the zero *Rhizobium* treatments. As shown in Table 3, with pods, the effect of *Rhizobium* inoculation in *P. vulgaris* also showed significant increases in the uptake of Mn, Fe, Cu Zn B and Mo in both the glasshouse and field. The micronutrient uptakes in pods were significantly elevated in the inoculated treatments relative to the uninoculated controls plots. At the whole-plant level, all nutrient uptakes (Mn, Fe, Cu Zn B and Mo) in bean plants were significantly increased with *Rhizobium* inoculation relative to the control treatments (Table 4).

Effects of Molybdenum application on micronutrient uptake in roots, shoots, pods and whole plants of P. vulgaris

The uptake of micronutrients Mn, Fe, Cu, Zn and B were significantly reduced by supplying Mo in the glasshouse and field experiment (Table 2). Generally, significantly lower uptake was found by supplying Mo at 6 and 12 $g.kg^{-1}$ of seeds relative to the zero control (Table 2). Contrary to the above results, supplying Mo at 6 and 12 $g.kg^{-1}$ of seeds significantly increased Mo uptake in the roots of *P vulgaris* compared with the zero control. Best Mo uptake was recorded in treatments which received the highest Mo level of 12 $g.kg^{-1}$ of seeds.

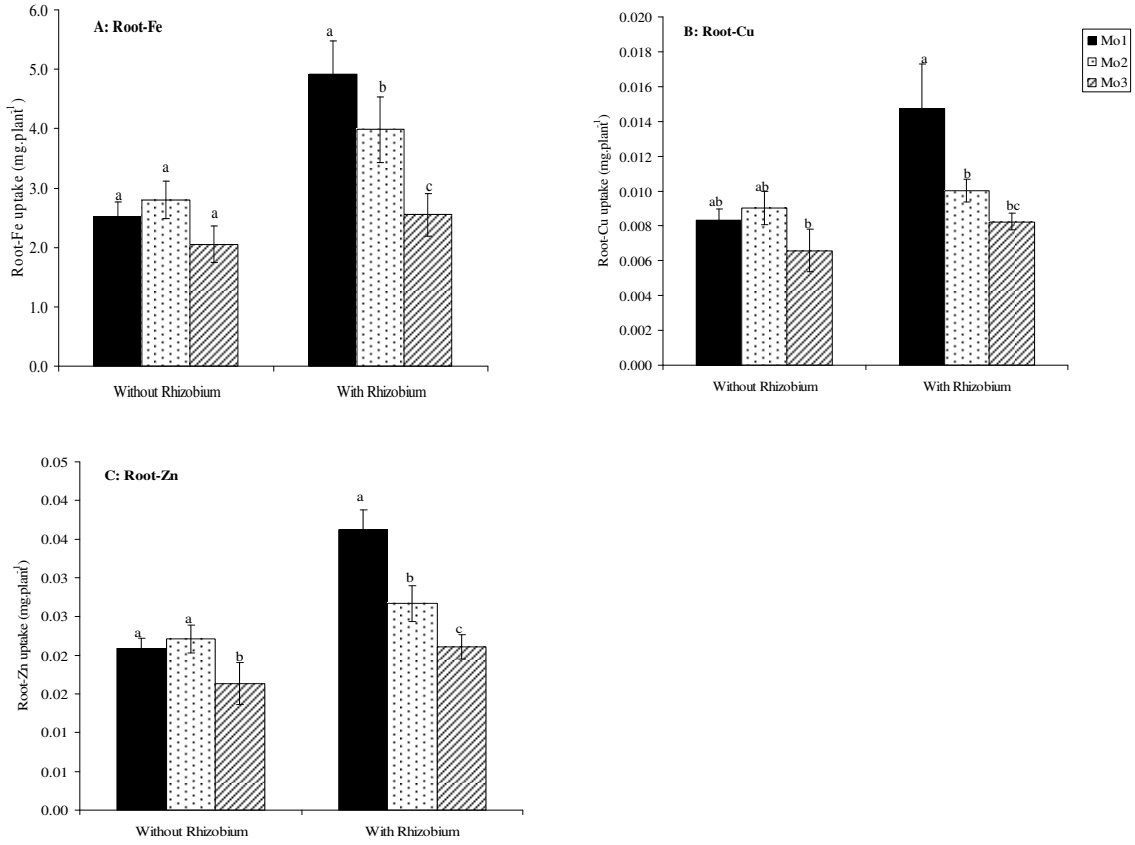

Fig.1: Interactive effects of *Rhizobium* and Molybdenum on the uptake of A) Root-Fe; B) Root-Cu, and C) Root-Zn of *P. vulgaris* grown in the glasshouse. Mo1 = No Molybdenum applied, Mo2 = Molybdenum applied at 6 $g.kg^{-1}$, Mo3 = Molybdenum applied at 12 $g.kg^{-1}$. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P = 0.05$

Applying Mo to *P. vulgaris* plants only significantly increased the shoot uptake of Mo in the glasshouse and field experiments. The shoot content of other micronutrients (Mn, Fe, Cu Zn and B) were not significantly altered by supplying Mo at 0, 6 and 12 $g.kg^{-1}$ kg of seeds (Table 1). As shown in Table 3, the pod uptake of Mn, Fe and Mo in the glasshouse and field were significantly affected by Mo application. The uptake of Mn and Fe were significantly reduced in treatments supplied with Mo at 6 and 12 $g.kg^{-1}$ of seeds relative to the zero control. However, as expected, applying Mo at 6 and 12 $g.kg^{-1}$ of seeds significantly increased the Mo uptake in the pods. At the whole-plant level, the tissue uptake of Fe and Mo were the only micronutrients that were significantly influenced by Mo application in the glasshouse (Table 4). In this experiment, the uptake of Fe in the glasshouse was significantly decreased with increasing Mo levels. But the uptake of Mo in both the glasshouse and field were significantly increased by increasing the Mo rates from 6 and 12 $g.kg^{-1}$ of seeds.

Effects of lime on micronutrient uptake in roots, shoots, pods and whole plants of P. vulgaris

Results from the glasshouse experiment on the uptake of micronutrients in the roots of *P vulgaris* showed that Mn, Fe, Cu, Zn were all significantly lowered by supplying lime (Table 2). The uptakes of these micronutrients were lowest in the highest lime treatment of 3 $t.ha^{-1}$ (Table 2). However, Mo application increased the root Mo uptake both in the glasshouse and in the field. Micronutrient uptake in roots followed the similar trend in the field experiment, but significant reductions in their uptake were only observed in Fe, Zn and B.

There was a significant response in the shoot micronutrient uptake of *P. vulgaris* to exogenous supply of lime. In the glasshouse experiment, the addition of lime significantly reduced the shoot uptake of Mn, Fe, Cu, Zn and B, and increased that of Mo. Compared with zero-lime control, the application of 2 and 3 $t.ha^{-1}$ significantly reduced the shoot uptake of Mn, Fe, Cu, Zn and B and increased that of Mo

Table 1. Effect of with and without *Rhizobium*, Mo and lime supply on the microelements uptake in roots of *P. vulgaris* as measured in the glasshouse and field.

Treatments	GLASSHOUSE						FIELD					
	Mn	Fe	Cu	Zn	B	Mo	Mn	Fe	Cu	Zn	B mg.plant^{-1}	Mo
Rhizobium												
R-	0.019±0.002b	2.456±0.174b	0.008±0.001b	0.020±0.001b	0.016±0.001b	0.026±0.004a	0.264±0.036b	35.086±2.773b	0.115±0.009b	0.287±0.023b	0.233±0.018b	0.431±0.076a
R+	0.045±0.008a	3.818±0.324a	0.011±0.001a	0.028±0.002a	0.023±0.001a	0.033±0.004a	0.745±0.150a	60.733±6.019a	0.180±0.024a	0.446±0.034a	0.362±0.030a	0.506±0.067a
Molybdenum (g.kg^{-1})												
0	0.048±0.010a	3.718±0.393a	0.012±0.001a	0.028±0.002a	0.023±0.002a	0.015±0.003b	0.831±0.214a	60.942±7.950a	0.195±0.035a	0.469±0.048a	0.387±0.040a	0.250±0.055b
6	0.029±0.005b	3.392±0.333a	0.010±0.001ab	0.024±0.002b	0.020±0.001b	0.032±0.005a	0.419±0.079b	49.485±5.461a	0.138±0.010b	0.358±0.029b	0.291±0.025b	0.511±0.087a
12	0.020±0.004b	2.301±0.237b	0.007±0.001b	0.019±0.002c	0.015±0.001c	0.042±0.005a	0.263±0.051b	33.302±3.451b	0.108±0.011b	0.273±0.027b	0.214±0.019c	0.644±0.097a
Lime (t.ha^{-1})												
0	0.046±0.010a	4.188±0.452a	0.012±0.001a	0.029±0.002a	0.023±0.002a	0.013±0.002c	0.711±0.200a	60.900±8.097a	0.179±0.035a	0.412±0.038a	0.339±0.037a	0.195±0.036c
2	0.031±0.006b	3.050±0.201b	0.009±0.001b	0.024±0.002b	0.020±0.001b	0.027±0.003b	0.514±0.112a	49.481±5.323a	0.152±0.015a	0.385±0.043a	0.318±0.034a	0.419±0.050b
3	0.019±0.005b	2.173±0.189c	0.007±0.001b	0.019±0.002c	0.015±0.001c	0.049±0.006a	0.287±0.073a	33.348±3.337b	0.111±0.010a	0.303±0.033b	0.236±0.023b	0.791±0.109a
3 - Way ANOVA (F-Statistic)												
R	13.46***	32.38***	10.14**	27.22***	29.89***	3.02ns	11.4**	25.3***	7.1**	20.6***	19.6***	0.9ns
Mo	5.27**	12.83***	6.18**	12.99***	17.59***	16.46***	5.6**	9.9***	4.4*	10.5***	11.7***	8.7***
L	4.68*	23.75***	7.48**	11.26***	15.44***	29.76***	3.0ns	9.8***	2.6ns	3.5*	4.7*	19.8***
R*Mo	2.8ns	5.5**	3.2*	5.1**	3.0ns	0.1ns	3.1ns	4.7*	2.4ns	4.3*	2.8ns	0.3ns
R*L	1.8ns	9.9***	1.2ns	0.9ns	0.3ns	0.2ns	1.4ns	6.1**	1.0ns	0.7ns	0.5ns	0.2ns
Mo*L	0.1ns	0.3ns	0.5ns	0.3ns	0.7ns	1.4ns	0.2ns	0.4ns	0.5ns	0.6ns	0.5ns	0.8ns

-R: Without *Rhizobium*; +R; With *Rhizobium,* R; *Rhizobium,* Mo; Molybdenum, L; Lime. Values presented are means ± SE, n = 4. *; **; *** = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

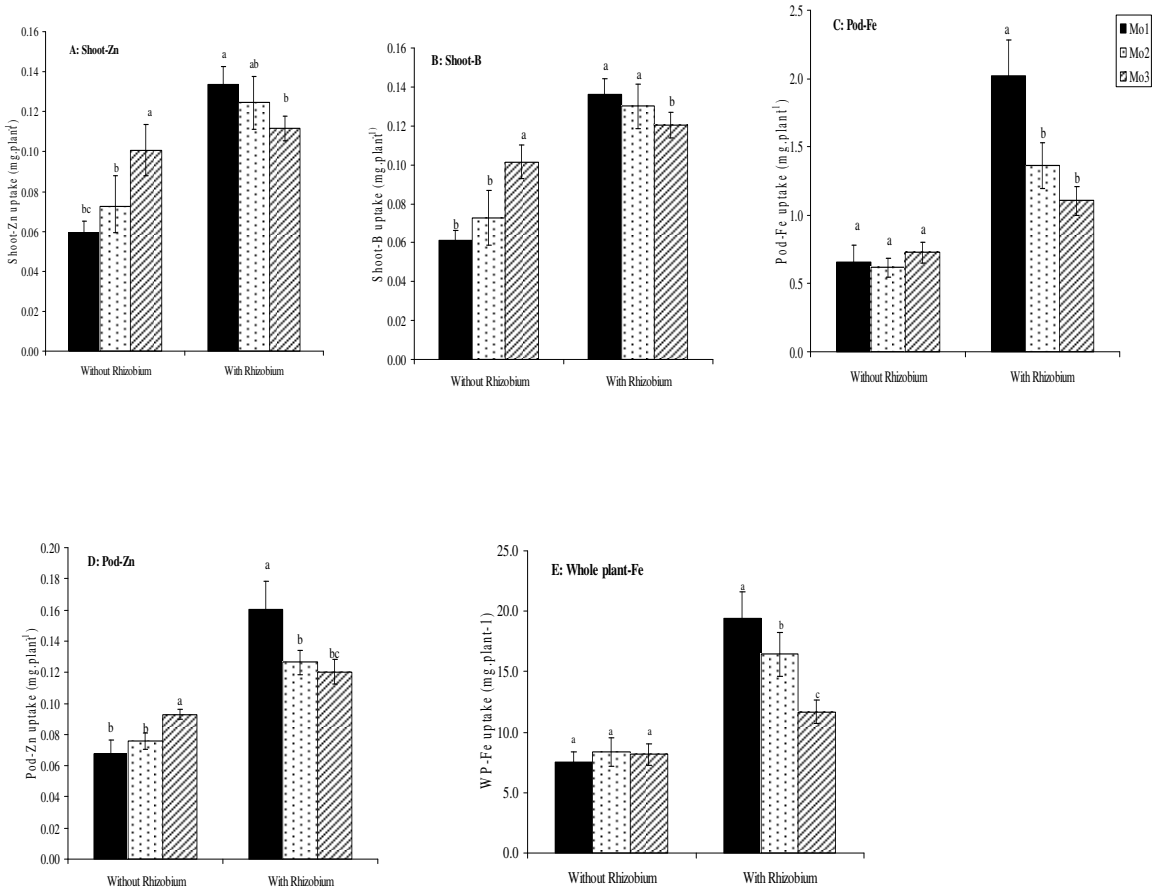

Fig.2: Interactive effects of *Rhizobium* and Molybdenum on the uptake of A) Shoot-Zn, B) Shoot-B, C) Pod-Fe, D) Pod-Zn and E) Whole plant-Fe of *P. vulgaris* grown in the glasshouse. Mo1 = No Molybdenum applied, Mo2 = Molybdenum applied at 6 $g.kg^{-1}$, Mo3 = Molybdenum applied at 12 $g.kg^{-1}$. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P = 0.05$

(Table 1). In the field conditions, supplying lime at 2 and 3 $t.ha^{-1}$ significantly reduced the shoot uptake of Fe and Cu and increased that of Mo relative to control (Table 1).

In pods, except for Zn and B in the glasshouse, other uptakes of micronutrients (Mn, Fe Cu) were significantly reduced in the pods supplied with lime at 2 and 3 $t.ha^{-1}$ (Table 3). Similar results were reported in the field experiment, but in addition to Zn and B, Cu uptake in the pods was also not significantly affected by lime treatments. Whether measured in the pods collected from the glasshouse or in the field, Mo uptake was significantly increased with lime supply at 2 and 3 $t.ha^{-1}$ compared with the control. At whole plant level, lime application significantly reduced the uptake of Mn, Fe, Cu, Zn and B in the glasshouse, and that of Fe, Cu and Zn in the field (Table 4). The uptake of these micronutrients was lowest as lime rates were increased from 2 to 3 $t.ha^{-1}$ relative to the lime zero control.

Interactive effects of Rhizobium inoculation, Mo and lime supply on micronutrient uptake in roots, shoots, pods and whole plants of P. vulgaris.

There was a significant interactive effect between *Rhizobium* inoculation and Mo supply to the *P vulgaris* organs grown in the glasshouse and in the field (Figure 1 - 2). Generally, *Rhizobium* inoculation resulted into significantly more uptake of Zn and B in shoots, Fe, Cu and Zn in the roots and pods and Fe in the whole plant. Interestingly, in all treatments with no *Rhizobium* inoculation, the uptake of all the above mentioned nutrients was lowest in the zero Mo treatment. However, supplying inoculants and with no Mo resulted into greater

Table 2. Effect of with and without *Rhizobium*, Mo and lime supply on the microelements uptake in shoots of *P. vulgaris* as measured in the glasshouse and field.

Treatments	GLASSHOUSE						FIELD					
	Mn	Fe	Cu	Zn	B	Mo	Mn	Fe	Cu	Zn	B mg.plant^{-1}	Mo
Rhizobium												
R-	0.08±0.01b	3.23±0.31b	0.02±0.00b	0.08±0.01b	0.08±0.01b	0.05±0.01b	2.0±0.4b	68.1±6.3b	0.4±0.0b	1.6±0.1b	1.6±0.1b	0.9±0.2b
R+	0.18±0.01a	5.63±0.38a	0.03±0.00a	0.12±0.01a	0.13±0.01a	0.08±0.01a	5.6±0.7a	175.7±18.9a	0.8±0.1a	3.7±0.3a	3.9±0.3a	2.3±0.3a
Molybdenum (g.kg^{-1})												
0	0.14±0.02a	4.57±0.53a	0.02±0.00a	0.10±0.01a	0.10±0.01a	0.03±0.01c	3.5±0.8a	115.1±21.1a	0.5±0.1a	2.4±0.4a	2.4±0.3a	0.6±0.2c
6	0.12±0.02a	4.64±0.58a	0.02±0.00a	0.10±0.01a	0.10±0.01a	0.06±0.01b	4.0±0.9a	138.2±22.6a	0.7±0.1a	2.8±0.4a	2.9±0.4a	1.7±0.3b
12	0.12±0.02a	4.07±0.32a	0.03±0.00a	0.11±0.01a	0.11±0.01a	0.11±0.02a	3.8±0.9a	112.3±17.6a	0.6±0.1a	2.7±0.3a	2.9±0.4a	2.6±0.4a
Lime (t.ha^{-1})												
0	0.16±0.02a	5.66±0.57a	0.03±0.00a	0.11±0.01a	0.12±0.01a	0.03±0.01c	4.9±1.0a	159.2±24.2a	0.7±0.1a	3.1±0.4a	3.2±0.4a	0.8±0.2c
2	0.12±0.02ab	4.48±0.40b	0.02±0.00b	0.10±0.01ab	0.11±0.01ab	0.06±0.01b	3.5±0.7a	115.6±17.8ab	0.6±0.1ab	2.6±0.3a	2.7±0.3a	1.5±0.3b
3	0.10±0.02b	3.15±0.32c	0.02±0.00b	0.08±0.01b	0.09±0.01b	0.11±0.02a	2.9±0.6a	90.8±16.7b	0.5±0.1b	2.2±0.3a	2.4±0.3a	2.6±0.4a
3 - Way ANOVA (F-Statistic)												
R	32.00***	30.88***	2.18ns	24.70***	45.24***	10.77**	15.9***	28.1***	22.0***	36.1***	37.7***	22.2***
Mo	0.29ns	0.69ns	1.11ns	0.41ns	1.00ns	22.07***	0.1ns	0.7ns	1.7ns	0.4ns	0.9ns	14.7***
L	3.39*	11.27***	3.51*	3.69*	5.11**	19.61***	1.8ns	3.9*	3.3*	2.6ns	2.0ns	12.4***
R*Mo	1.9ns	3.0ns	3.2*	4.1*	4.8*	0.0ns	0.2ns	0.2ns	0.0ns	0.1ns	0.1ns	1.3ns
R*L	0.6ns	1.1ns	0.3ns	0.2ns	0.6ns	3.6ns	0.6ns	1.7ns	2.0ns	1.8ns	1.4ns	1.7ns
Mo*L	0.1ns	0.2ns	0.1ns	0.1ns	0.2ns	1.6ns	0.0ns	0.1ns	0.2ns	0.2ns	0.2ns	0.4ns

-R: Without *Rhizobium*; +R; With *Rhizobium,* R; *Rhizobium,* Mo; Molybdenum, L; Lime. Values presented are means ± SE, n = 4. *; **; *** = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference.

Table 3. Effect of with and without *Rhizobium*, Mo and lime supply on the microelements uptake in pods of *P. vulgaris* as measured in the glasshouse and field.

Treatments	GLASSHOUSE						FIELD					
	Mn	Fe	Cu	Zn	B	Mo	Mn	Fe	Cu	Zn	B	Mo
						mg.plant^{-1}						
Rhizobium												
R-	0.03±0.00b	0.67±0.05b	0.02±0.00b	0.08±0.00b	0.06±0.00b	0.03±0.00b	0.02±0.00b	0.53±0.04b	0.01±0.00b	0.06±0.00b	0.05±0.00b	0.02±0.00b
R+	0.08±0.01a	1.50±0.12a	0.03±0.00a	0.14±0.01a	0.10±0.00a	0.05±0.01a	0.08±0.01a	1.60±0.15a	0.03±0.00a	0.14±0.01a	0.10±0.00a	0.06±0.01a
Molybdenum (g.kg^{-1})												
0	0.06±0.01a	1.34±0.20a	0.02±0.00a	0.11±0.01a	0.07±0.00a	0.02±0.01c	0.06±0.01a	1.26±0.21a	0.02±0.00a	0.11±0.02a	0.07±0.01a	0.02±0.01c
6	0.05±0.01ab	0.99±0.12b	0.02±0.00a	0.10±0.01a	0.07±0.00a	0.04±0.01b	0.05±0.01ab	1.05±0.19ab	0.02±0.00a	0.10±0.01a	0.07±0.01a	0.04±0.01b
12	0.04±0.01b	0.92±0.07b	0.02±0.00a	0.11±0.01a	0.08±0.00a	0.06±0.01a	0.04±0.01b	0.88±0.10b	0.02±0.00a	0.10±0.01a	0.08±0.00a	0.06±0.01a
Lime (t.ha^{-1})												
0	0.06±0.01a	1.50±0.17a	0.03±0.00a	0.11±0.01a	0.08±0.00a	0.02±0.00c	0.06±0.01a	1.51±0.23a	0.02±0.00a	0.11±0.01a	0.07±0.01a	0.02±0.00c
2	0.05±0.01ab	1.06±0.12b	0.03±0.00a	0.11±0.01a	0.08±0.00a	0.05±0.01b	0.05±0.01ab	1.01±0.13b	0.02±0.00a	0.11±0.01a	0.07±0.01a	0.04±0.01b
3	0.04±0.01b	0.68±0.08c	0.02±0.00b	0.10±0.01a	0.07±0.00a	0.06±0.01a	0.04±0.01b	0.68±0.08c	0.02±0.00a	0.09±0.01a	0.07±0.01a	0.06±0.01a
3 - Way ANOVA (F-Statistic)												
R	78.13***	107.46***	124.98***	49.19***	89.01***	36.68***	113.4***	84.9***	183.6***	74.1***	190.3***	55.6***
Mo	3.45*	10.68***	0.26ns	0.85ns	2.31ns	33.83***	3.2*	3.7*	0.5ns	0.3ns	2.0ns	25.1***
L	4.79*	34.71***	3.17*	1.81ns	0.19ns	37.87***	4.9*	17.7***	1.7ns	0.9ns	0.0ns	27.2***
R*Mo	3.2*	12.8***	7.0**	5.5**	4.1*	0.1ns	4.5*	6.6**	7.9**	5.1**	5.8**	0.3ns
R*L	1.8ns	7.8**	1.3ns	1.3ns	0.5ns	5.1**	2.7ns	8.2***	1.7ns	1.7ns	1.0ns	5.5**
Mo*L	0.2ns	1.8	0.9ns	0.3ns	0.6ns	4.3**	0.1ns	0.3ns	0.3ns	0.0ns	0.5ns	2.1ns

-R: Without *Rhizobium*; +R; With *Rhizobium,* R; *Rhizobium,* Mo; Molybdenum, L; Lime. Values presented are means ± SE, n = 4. *; **; *** = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P = 0.05$ according to Fischer least significance difference.

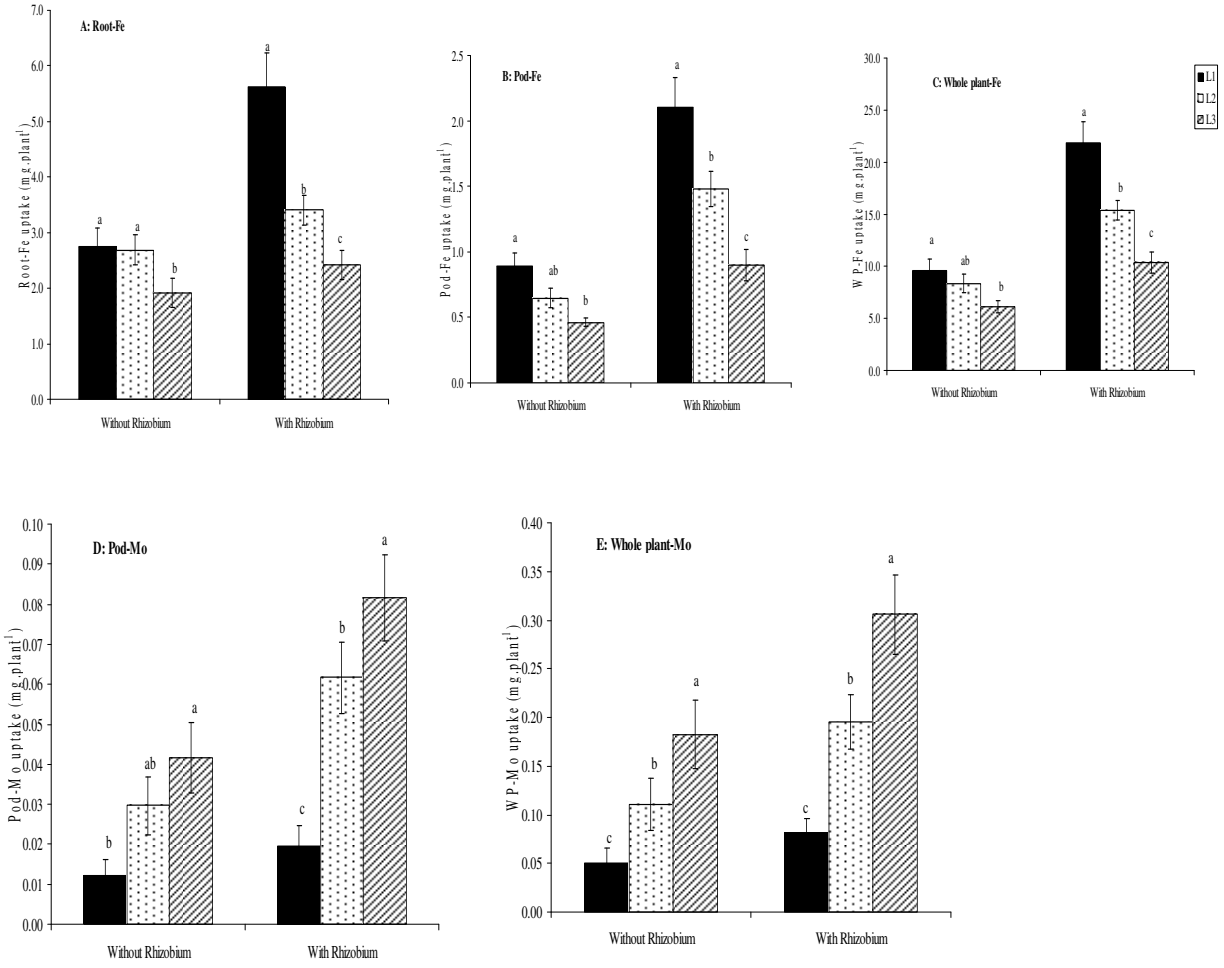

Fig.3: Interactive effects of *Rhizobium* and Lime on the uptake of A) Root-Fe, B) Pod-Fe, C) whole plant-Fe D) Pod-Mo, and E) Whole plant-Mo of *P. vulgaris* grown in the glasshouse. L1 = No lime applied, L2 = Lime applied at 2 t.ha^{-1}, L3 = Lime applied at 3 t.ha^{-1}. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P = 0.05$.

uptake of all nutrients in all organs (Figure 1 - 2). In most cases, increasing the supply of Mo from 6 and 12 g.kg^{-1} of seeds in combination with *Rhizobium* inoculation significantly reduced the uptake of micronutrients (Figure 1 - 2). The *Rhizobium* x lime interaction was significant for Fe uptake in the roots, pod and whole plant (Figure 3A - C). In general, the uptakes of these micronutrients were better in the plots supplied with *Rhizobium*. However, the best uptake of Fe was recorded in the zero lime control treatments supplied with *Rhizobium* and the uptake of this element was significantly reduced as lime rates were increased from 2 - 3 t.ha^{-1}. The *Rhizobium* x lime interaction was also significant for Mo uptake in the pods and whole plant (Figure 3D - E). Generally, *Rhizobium* inoculated treatments exhibited the greatest uptake of Mo. Applying lime at 2 - 3 t.ha^{-1}, significantly elevated the uptake of Mo relative to the zero lime treatment. In this study, liming and Mo application interacted positively and influenced the uptake of Mo and B in pods and Mo in the whole plants grown in the glasshouse (Figure 4). Generally, the uptake of these elements in their organs increased steadily and significantly by increasing the application rates of Mo from 0 - 12 g.kg^{-1} of seeds and that of lime from 0 - 3 t.ha^{-1} respectively. The best uptake of the micronutrients was recorded in treatments involving highest rate of (12 g.kg^{-1} of seeds) and that of lime (3 t.ha^{-1}).

Table 4. Effect of with and without *Rhizobium*, Mo and lime supply on the microelements uptake in whole plant in *P. vulgaris* as measured in the glasshouse and field.

Treatments	GLASSHOUSE						FIELD					
	Mn	Fe	Cu	Zn	B	Mo	Mn	Fe	Cu	Zn	B	Mo
											mg.plant^{-1}	
Rhizobium												
R-	0.12±0.02b	6.35±0.42b	0.05±0.00b	0.18±0.01b	0.15±0.01b	0.10±0.02b	2.3±0.4b	103.7±7.2b	0.6±0.0b	1.9±0.1b	1.9±0.1b	1.4±0.2b
R+	0.30±0.02a	10.94±0.73a	0.07±0.00a	0.29±0.01a	0.25±0.01a	0.17±0.02a	6.4±0.8a	238.0±21.1a	1.0±0.1a	4.3±0.3a	4.3±0.3a	2.9±0.4a
Molybdenum (g.kg^{-1})												
0	0.25±0.04a	9.63±1.06a	0.06±0.00a	0.24±0.02a	0.19±0.02a	0.06±0.02c	4.4±0.9a	177.3±26.1a	0.7±0.1a	3.0±0.4a	2.9±0.3a	0.9±0.3c
6	0.20±0.03a	9.02±0.90a	0.06±0.00a	0.22±0.02a	0.19±0.02a	0.13±0.02b	4.5±0.9a	188.7±25.0a	0.8±0.1a	3.2±0.4a	3.3±0.4a	2.2±0.4b
12	0.19±0.02a	7.29±0.50b	0.06±0.00a	0.23±0.01a	0.21±0.01a	0.22±0.03a	4.1±0.9a	146.5±19.4a	0.8±0.1a	3.1±0.4a	3.2±0.4a	3.3±0.4a
Lime (t.ha^{-1})												
0	0.27±0.03a	11.34±1.04a	0.06±0.00a	0.25±0.02a	0.22±0.02a	0.06±0.01c	5.7±1.1a	221.6±29.0a	0.9±0.1a	3.6±0.5a	3.6±0.4a	1.0±0.2c
2	0.21±0.03ab	8.59±0.61b	0.06±0.00a	0.24±0.02a	0.20±0.01ab	0.14±0.02b	4.1±0.8a	166.1±19.7ab	0.8±0.1ab	3.1±0.3ab	3.0±0.4a	2.0±0.3b
3	0.16±0.02b	6.00±0.49c	0.05±0.00b	0.20±0.01b	0.18±0.01b	0.22±0.03a	3.2±0.7a	124.8±17.0b	0.6±0.1b	2.6±0.3b	2.7±0.3a	3.4±0.5a
3 - Way ANOVA (F-Statistic)												
R	42.0***	73.1***	45.2***	63.8***	86.4***	24.7***	18.8*	41.6***	36.6***	45.0***	45.9*	22.6***
Mo	1.8ns	6.8**	0.0ns	0.4ns	0.8ns	43.6***	0.1ns	1.5ns	0.5ns	0.2ns	0.6ns	19.2***
L	4.9*	33.0***	8.3***	5.4**	4.6*	47.5***	2.4ns	7.3**	6.1**	3.3*	2.6ns	20.1***
R*Mo	2.8ns	8.5***	8.1***	8.4***	6.5	0.0ns	0.3ns	0.5 ns	0.6 ns	0.3 ns	0.1ns	0.8ns
R*L	0.9ns	6.5**	0.5ns	1.0ns	0.7ns	4.9*	0.8 ns	3.6*	3.2*	2.2 ns	1.6ns	1.3ns
Mo*L	0.1ns	0.4ns	0.3ns	0.1ns	0.2ns	3.2*	0.0 ns	0.0 ns	0.1 ns	0.2 ns	0.2ns	0.5ns

-R: Without *Rhizobium*; +R; With *Rhizobium,* R; *Rhizobium,* Mo; Molybdenum, L; Lime. Values presented are means ± SE, n = 4. *; **; *** = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letters in a column are significantly different from each other at $P=0.05$ according to Fischer least significance difference

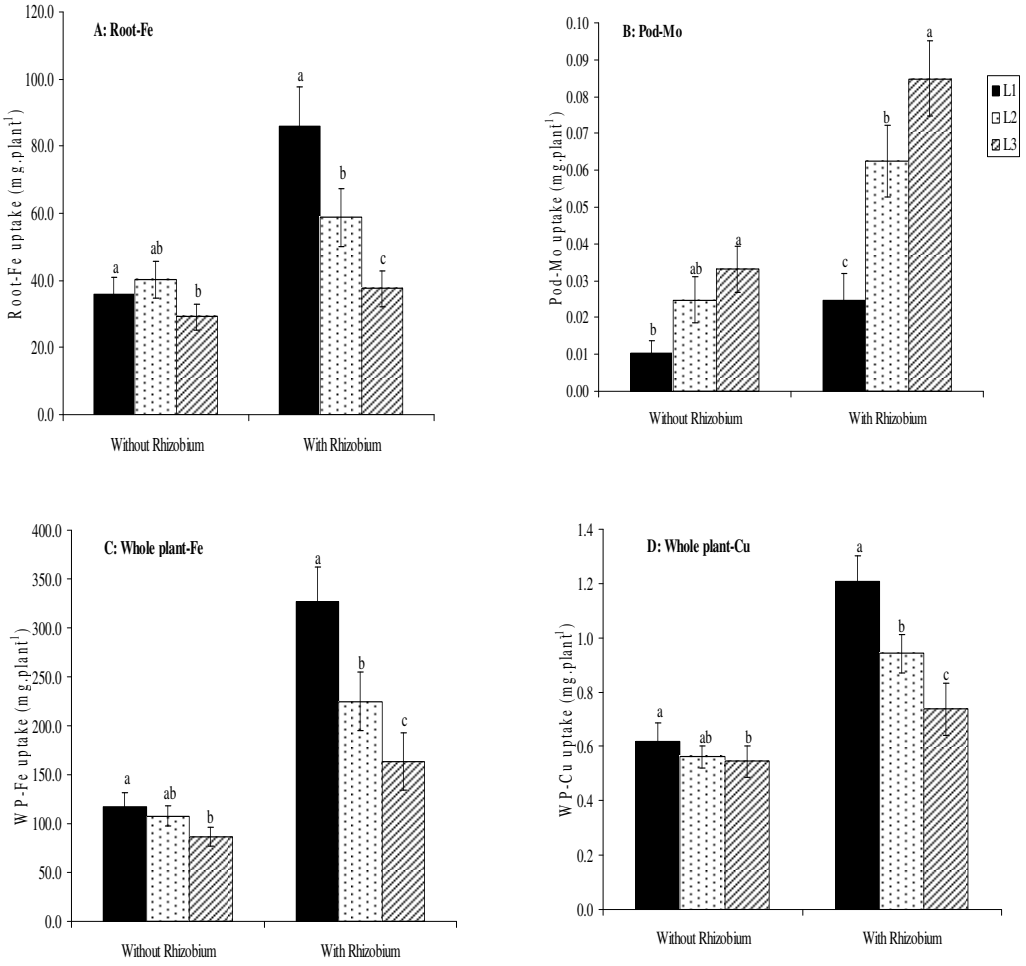

Fig.4: Interactive effects of *Rhizobium* and Lime on the uptake of A) Root-Fe, B) Pod-Mo, C) whole plant-Fe and D) Whole plant-Cu of *P. vulgaris* grown in the field experiment. L1 = No lime applied, L2 = Lime applied at 2 $t.ha^{-1}$, L3 = Lime applied at 3 $t.ha^{-1}$. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P = 0.05$.

Discussion

Supplying plants of *P vulgaris* with *Rhizobium* inoculants significantly altered the uptake of Mn, Fe, Cu Zn, B and Mo in organs of *P. vulgaris* (shoot, root, pod and whole plant) grown in the glasshouse and verified in the field (Table 1 - 4). There was a significant increase in micronutrient content in all organs for the *Rhizobium* inoculated treatments. Therefore, the greater levels of Mn, Fe, Cu Zn, B and Mo in shoots, roots, pods, and whole plants of *P vulgaris* 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 (Wittenberg et al., 1996; Berraho et al., 1997; Duhan et al., 1998; Arora et al., 2001; Sridevi et al. 2008) which may facilitate the solubility of nutrients such as Fe (Fabiano et al., 1994) Zn (Wani et al., 2008) P (Young et al., 1990; Abd-Alla 1994; Chabot et al., 1996; Sridevi et al., 2007) 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, Howell (1987) reported that superior rhizobial strains enhanced the uptake of other minerals and balanced the nutritional requirements of peanut plants.

In our study, supplying Mo at fro 0 - 12 $g.kg^{-1}$ of seeds significantly increased the uptake of this element in all organs at the glasshouse and in the field (Table 1 - 4). Molybdenum is

known to be a relatively mobile nutrient in the plant system and readily concentrated by plants (Clarkson and Hanson, 1980). However, Mo supply lead to the significantly reduced uptake of Mn, Fe, Cu Zn and B in roots (Table 1), Mn and Fe in pods (Table 3) and Fe in the whole plant (Table 4). The decrease in mineral content of plant parts was more dramatic with increasing the supply rates of Mo. A separate report from this study (Bambara and Ndakidemi, 2010b) showed significant increases in soil pH from 6.1 to 6.3 and 6.5 by supplying Mo at 0, 6 and 12 $g.kg^{-1}$ of seeds respectively. It is widely documented that, Mo is the only trace mineral whose availability to plants increases with an increase in soil pH under normal soil conditions (Fleming, 1980; Gupta, 1997) a situation similar to the observations reported in this study. Furthermore, the reduction in the uptake of Mn, Fe, Cu Zn and B 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 (Brady and Weil, 2008). Similar to our observation, Fleming (1980) showed that high Mo concentrations in biological systems can induce Fe-deficiency. The interactions between Mo and the bioavailability of Fe, Cu Zn and B and are well documented in animals (Dowdy and Matrone, 1968; Smith and Wright, 1975; Bremner, 1979; Suttle et al., 1984; Gengelbach et al., 1994), but little information is available for plants. Studies involving different animal types have shown that high Mo intakes may interfere with the bioavailability of these micronutrients, a scenario which was also confirmed in *P. vulgaris* in our study. These changes in micronutrient content of *P. vulgaris* shoots with Mo supply could have implications in the dietary use of the leaf component as vegetables in Africa. Therefore, this calls for detailed studies to establish other possible mechanism of Mo decreasing the uptake and bioavailability of Mn, Fe, Cu Zn and B in *P. vulgaris* organs. Results from our study showed that liming had antagonistic effect on the bioavailability and the uptake of Mn, Fe, Cu, Zn and B. Although in some organs the availability was not significantly reduced (Tables 1 - 4), but in all instances, the amounts of Mn, Fe, Cu, Zn and B were significantly reduced with the addition of lime. The levels of these micronutrients were consistently lower by increasing lime rates from 2 - 3 $t.ha^{-1}$ relative to the zero lime treatment. The results are consistent with reports by Chen et al. (1982); Rengel et al. (1999); Basu et al. (2008). As expected, lime in this study significantly increased the soil pH (Bambara and Ndakidemi 2010b) and thus increasing the uptake and bioavailability of the Mo in organs of *P vulgaris*. Similar to our report, López et al. (2007) also reported an increase in Mo in a leguminous plant *Trifolium Pratense* L. with the addition of lime. The interactive effects between *Rhizobium*, and Mo application were observed in the glasshouse for the uptake of Fe, Cu and Zn in roots (Fig. 1A - C), Zn and B in shoots, Fe and Zn in pods and Fe in the whole plant (Fig 2A - E). Generally, more micronutrient uptake occurred in the treatments involving *Rhizobium* inoculation. Interestingly, the best significant micronutrients uptake was found in treatments involving *Rhizobium* inoculation with zero Mo (Figs. 1A-C and 2A - E). Supplying inoculants and applying Mo at 6 and 12 $g.kg^{-1}$ of seeds had significant negative effects on the uptake of micronutrients in different organs and the whole plant of *P. vulgaris*. The mechanism involved is not well established, thus, warranting further investigation. The interactive effect between *Rhizobium* and lime were also recorded in the glasshouse study on the uptake of Fe in roots and pods, and whole plant (Fig. 3A, B and C and 5C), and Mo in pods and whole plant (Figs 3C and D and 5C). Plants receiving lime and rhizobial inoculants had significantly higher uptake of Fe and Mo relative to the uninoculated treatments. Significant reduction in the uptake of Fe in root, pods and whole plant were mostly recorded in inoculated treatments and were aggravated more by supplying lime at 2 - 3 $t.ha^{-1}$ relative to the zero lime treatment. It is well established that lime has a positive effects on increasing the soil pH (Bambara and Ndakidemi, 2010b) and ultimately decreasing the uptake of Fe (Patra and Mohanty, 1994). In our study, *Rhizobium* inoculation in combination with lime increased the soil pH (Bambara and Ndakidemi, 2010b), thus, reducing the solubility of Fe in the soil solution (Römheld, 1987; Patra and Mohanty, 1994), and then, reduced the availability of Fe to plants and hence its uptake and translocation into roots, pods and whole plant as observed in our study. Significant increases in Mo levels were observed in pods and whole plants of *P. vulgaris* supplied with a combination of lime and *Rhizobium* inoculation. The treatments supplied with *Rhizobium* and lime at 2 - 3 $t.ha^{-1}$ had significantly more Mo than the uninoculated treatments (Fig. 3C and E). In this study it is evident that *Rhizobium* inoculation in combination with lime increased the Mo in pods, whole plant and roots supporting the fact that the microorganism had a mechanism which facilitated the uptake (Figs 3C & E 5A & B). This is due to the fact that Mo levels in the uninoculated treatments supplied with lime at 2 - 3 $t.ha^{-1}$ were lower, and by introducing the inoculants they were significantly doubled (Figs. 3C & E 5A & B). Looking at the means of two control treatments (0 $t.ha^{-1}$ of lime) in the inoculated and uninoculated groups (Figs. 3C & E 5A & B), the response in Mo uptake in inoculated treatment was induced by *Rhizobium* inoculation. Therefore, apart from lime, increased Mo uptake in pods and whole plants of *P vulgaris* reported in this study was due to *Rhizobium* inoculation. In their studies, other workers have also reported that lime application increased the soil pH; promoting the molybdate desorption and hence making it more available to a leguminous plant *T. Pratense* (López, 2007). Increased uptake of Mo in pods observed in this study (Fig. 4A) with the supply of lime and Molybdenum in plants is similar to results reported by other researchers (During, 1984; Wheeler, 1998). The increased uptake of Mo with Mo application was expected. However, the improved uptake of Mo attributed to lime could be associated with increased pH (Bambara and Ndakidemi, 2010b) which made Mo more available into the pods (López et al., 2007). The uptake of B in the pods followed the similar trend to that of Mo (Figure 4B).

In conclusion, the provision *Rhizobium* inoculants to *P. vulgaris* appear to promote greater nutrient uptake (Mn, Fe, Cu, Zn B and Mo) and accumulation in their tissues. The supply of external nutrients such as Mo and lime also had some antagonistic influences by reducing the availability of Mn, Fe, Cu, Zn and B. The findings obtained in this study clearly suggest the need for further experimentation on the effects of microorganisms and other fertilizer inputs on the nutrient quality and quantity of different legumes used in the food industry.

Acknowledgements

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References

Abd-Alla MH (1994) Solubilization of rock phosphates by *Rhizobium* and B*radyrhizobium*. Folia Microbiol 39:53-56

Arora NK, Kang SC, Maheshwari DK (2001) Isolation of siderophore producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. Curr Sci 81:673–677

Bambara S, Ndakidemi PA (2010a) *Phaseolus vulgaris*response to *Rhizobium* inoculation, lime and molybdenum in selected low pH soil in Western Cape, South Africa. Afr J Agric Res 5(14):1804-1811

Bambara S, Ndakidemi PA (2010b) Changes in selected soil chemical properties in the

rhizosphere of *Phaseolus vulgaris* L. supplied with *Rhizobium* inoculants, molybdenum and lime. Sci Res Essays 5(7):679-684

Basu M, Bhadoria PBS, Mahapatra SC (2008) Growth, nitrogen fixation, yield and kernel quality of peanut in response to lime, organic and inorganic fertilizer levels. Biores Technol 99(11): 4675-4683

Berraho EL, Lesueur D, Diem HG, Sasson A (1997) Iron requirement and siderophore production in *Rhizobium ciceri* during growth on an iron-deficient medium. World J Microbiol Biotechnol 13:501-510

Brady NC, Weil RR (2008) *The Nature and Properties of Soil*. Prentice-Hall Press, New York

Bremner I (1979) The toxicity of cadmium, zinc and molybdenum and their effects on copper metabolism. Proc Nutr Soc 38:235-242

Cardoso EJB, Vieira C, Cassini STA (1998) Foliar application of molybdenum in common beans. I. Nitrogenase and reductase activities in a soil of high fertility. J Plant Nutr 21(1): 169-180

Chabot R, Antoun H, Cescas, MP (1996) Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar. *Phaseoli* Plant Soil 184(2):311-321

Chen Y, Navrot J, Barak P (1982) Remedy of lime-induced chlorosis with iron-enriched muck. J Plant Nutr 5:927-940

Clarkson DT, Hanson JB (1980) The mineral nutrition of higher plants Ann Rev Plant Physiol 31:239-298

Dowdy RP, Matrone G (1968) A copper-molybdenum complex: its effects and movement in the piglet and sheep. J Nutr 95(2):197-201

Duhan JS, Dudeja SS, Khurana AL (1998) Siderophore production in relation to N_2 fixation and iron uptake in pigeon pea–*Rhizobium* symbiosis. Folia Microbiol 43:421-426

During C (1984) Fertiliser and soils in New Zealand farming. Wellington, Government Printer

Fabiano E, Gualtieri G, Pritsch C, Polla G, Arias A (1994) Extent of high-affinity iron transport systems in field isolates of rhizobia. Plant Soil 164:177-185

Fleming GA (1980) Essential micronutrients 1. Boron and molybdenum. In: Davies BE (ed) Applied Soil Trace Elements, Wiley-Interscience, New York pp155-197

Gengelbach GP, Ward JD, Spears JW (1994) Effect of dietary copper, iron, and molybdenum on growth and copper status of beef cows and calves. J Animal Sci 72(10):2722-2727.

Giron HC (1973) Comparison between dry ashing and wet digestion in the preparation of plant materials for atomic absorption analysis. Atomic Absorption Newsletters 12:28-29

Gupta UC (1972a) Effects of boron and lime on boron concentration and growth of forage legumes under greenhouse conditions, Commun Soil Sci Plant Anal 3:355-365

Gupta UC (1972b) Effects of manganese and lime on yield and on the concentration of manganese, molybdenum, boron, copper and iron in the boot stage tissue of barley. Soil Sci 114:131-136

Gupta UC (1979) Boron nutrition of crops. Adv Agron.31:273-307

Gupta UC (1992) Characterization of the iron status in plant parts and its relation to soil pH on acid soils. J Plant Nutr 15:1531-1540

Gupta UC (1997) Soil and plant factors affecting molybdenum uptake by plants. In: Gupta UC (ed) Molybdenum *in Agriculture*, Cambridge University Press, Cambridge, UK, pp. 71-91

Hafner H, Ndunguru BJ, Bationo A, Marschner H (1992) Effect of nitrogen, phosphorus and molybdenum application on growth and symbiotic N_2-fixation of groundnut in an acid sandy soil in Niger. Fert Res 31(1):69-77

Howell RK (1987) *Rhizobium* induced mineral uptake in peanut tissues. J Plant Nutr 10:(9-16) 1297-1305

López RS, Marysol, A, Liliana G, de la Luz Mora, M (2007) Molybdenum availability in andisols and its effect on biological parameters of soil and red clover (*Trifolium Pratense* L.). Soil Sci 172(11):913-924

Marschner H (1991) Mechanisms of adaptation of plants to acid soils. Plant Soil 134:1-20.

Patra BN, Mohanty SK (1994) Effect of nutrients and liming on changes in pH, redox potential, and uptake of iron and manganese by wetland rice in iron-toxic soil. Biol Fert Soils 17(4):285-288

Peoples MB, Lilley DM, Burnett VF, Ridley AM, Garden DL (1995) The effects of surface applications of lime and superphosphate to acid soils on the growth and N_2 fixation by subterranean clover in mixed pasture swards. Soil Biol Biochem 27: 663-671

Prasad B, Sinha NP (1982) Changes in the status of micronutrients in soil with long term applications of chemical fertilizers, lime and manure. Plant Soil 64(3): 437-441

Rengel Z (1999) Mineral Nutrition of Crops Fundamental Mechanisms and Implications CRS Press. pp 399

Rengel Z, Batten GD, Crowley DE (1999) Agronomic approaches for improving the micronutrient density in edible portions of field crops. Field Crops Res 60(1-2): 27-40

Richardson AE, Henderson AP, James GS, Simpson RJ (1988) Consequences of soil acidity and the effect of lime on nodulation of *Trifolium subterraneum* L. growing in an acid soil. Soil Biol Biochem 20:439-445

Rodelas B, González–López J, Martínez-Toledo MV, Pozo C, Salmerón V (1999) Influence of *Rhizobium/Azotobacter* and *Rhizobium/Azospirillum* combined inoculation on mineral composition of faba bean (*Vicia faba* L.) Biol Fert Soils 29(2):165-169

Römheld V (1987) Different strategies for iron acquisition in higher plants. Physiol Plantar 70(2):231-234

Smith BSW, Wright H (1975) Copper: molybdenum interaction, effect of dietary molybdenum on the binding of copper to plasma proteins in sheep. J Comp Pathol 85:299-305.

Soil Classification Working Group (SCWG) (1991) Soil classification: A Taxonomic System for South Africa. Mem Natural Agric Resources for SA p. 15

Sridevi M, Kumar KG, Mallaiah KV (2008b) Production of catechol-type of siderophores by *Rhizobium* sp. isolated from stem nodules of *Sesbania procumbens* (Roxb.) W and A. Res J Microbiol 3:282-287

Sridevi M, Mallaiah KV, Yadav NCS (2007) Phosphate solubilization by *Rhizobium* isolates from *Crotalaria* species. J Plant Sci 2:635-639

Steel RGD, Torrie JH (1980) Principles and procedures of statistics: A biometrical approach. 2nd ed. mcGraw-Hill, New York

Suttle NF, Abrahams P, Thornton I (1984) The role of a soil × dietary sulphur interaction in the impairment of copper absorption by ingested soil in sheep. J Agric Sci 103:81-86

Vieira RF, Vieira C, Cardoso EJBN, Mosquim PR (1998) Foliar application of molybdenum in common bean. II. Nitrogenase and nitrate reductase activities in a soil of low fertility. J Plant Nutr 21(10):2141-2151

Wani PA, Khan MS, Zaidi A (2008) Impact of zinc-tolerant plant growth promoting rhizobacteria on lentil grown in zinc-amended soil. Agron Sustain Dev 28:449-455

Wheeler DM (1998) Investigation into the mechanisms causing lime responses in a grass/clover pasture on a clay loam soil'. New Zealand J Agric Res 41:(4): 497-515

Whelan AM, Alexander M (1986) Effects of low pH and high Al, Mn and Fe on the survival of *Rhizobium trifolii* and the nodulation of subterranean clover. Plant Soil 92:363-371

Wittenberg JB, Wittenberg BA, Day DA, Udvardi MK, Appleby CA (1996) Siderophore bound iron in the peribacteroid space of soybean root nodules. Plant Soil 178:161-169

Woomer PL, Karanja NK, Mekki EI, Mwakalobe B, Tembo H, Nyika M, Nkwiine C, Ndakidemi PA, Msumali G (1997) Indigenous populations of Rhizobia, legume response to inoculation and farmers awareness of inoculants in East and Southern Africa. Afr Crop Sci Con Proc 3(1): 297-308

Woomer PL, Savala CN, Ndakidemi PA, Kizza C, Nyika M (1999) Evaluation of liquid formulation legume inoculants in East and Southern Africa. Afr Crop Sci Con Proc 4:77-82

Young CC, Chen CL, Chao CC (1990) Effect of *Rhizobium*, vesicular-arbuscular mycorrhiza and phosphate solubilizing bacteria on yield and mineral phosphorus uptake of crops in subtropical-tropical. In: Transactions of the 14th International Congress of Soil Science. Kyoto, Japan, ICSS. III. p. 55-60.