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Scientific Research

Influence of *Bradyrhizobium japonicum* and Phosphorus on Micronutrient Uptake in Cowpea. A Case Study of Zinc (Zn), Iron (Fe), Copper (Cu) and Manganese (Mn)

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ABSTRACT

The field and screen house experiments were carried out in the 2013 cropping season to assess the effects of *B. japonicum* inoculation and phosphorus supplementation on the uptake of micronutrients in the cowpea. The experiment was laid out in a split plot design where the main plots comprised two inoculation treatments (with and without *B. japonicum* inoculation) and sub plots included four different levels of phosphorus (0, 20, 40, and 80 kg P/ha). The results showed a significant improvement in the uptake of micronutrients in the *B. japonicum* inoculated treatments over the control. Phosphorus supplementation (40 kg P/ha) also showed a significant increase in the uptake of some micronutrients while decreasing the uptake of Zn in some plant organs. There was also a significant interaction between *B. japonicum* inoculation and phosphorus in the root uptake of Zn for the field experiment.

KEYWORDS

Bioavailability; Legume; P-Micronutrient Interactions; Triple Super Phosphate

1. Introduction

Cowpea is an important legume crop being the source of protein in the diet of many Tanzanians and the rest of sub Saharan Africa who cannot afford to incorporate animal proteins in their daily diet [1]. Cowpea production is constrained by various factors including pest and diseases and unavailability of mineral elements which are important for plant growth and production [2-4]. Like all animals, sustainability of all plant lives depends on the16 essential mineral elements [5]. Mineral elements are classified into two groups (macro elements and micro elements) depending on their importance in the plant. Macro elements (nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S)) are those which are required by plants in large amount while microelements (iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), chlorine (Cl), boron (B), nickel (Ni) and molybdenum (Mo)) are those which are required by plants in small amount [2,6]. Being required in small amount by plants doesn't mean they are not important to plant and human nutrition [7].

Shortage in any one of these elements restricts plant growth and reduces crop yields [2,8,9]. Bioavailability of mineral elements such as zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) and their uptake by plants is essential for crop production [2,10,11].

However, there is low supply and bioavailability of these mineral elements in the rhizosphere solution eventually limiting the accumulation of mineral elements in crops tissues [10]. Since there is low uptake of these mineral elements due to various factors, there is a growing gap between micronutrient concentration in plants and those required in the human diet [2,7,9]. Usually, these elements are taken up by plants either from soil

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solution in their cationic forms [10] or applied on the leaves as foliar application.

Phosphorus is widely known element playing many roles in the plants especially root and shoot growth [12], nodulation in legumes, and influences the efficiency of the rhizobium-legume symbiosis through facilitation of energy transfer reactions involving ATP in nitrogenase activity [13]. Not only can phosphorus enhance rhizobium-legumes symbiosis, but also Vesicular Arbuscular Mycorrhizal fungus enhance nodulation and ultimately biological nitrogen fixation [14]. The low availability of phosphorus nutrition in soils has become the limiting factor for plant and root growth [12,15,16]. The recent studies indicated that phosphorus enhanced root system which provides greater root-soil contact and eventually higher uptake of phosphorus and other important and low mobility nutrients and absorption of higher concentration of mineral nutrients [15]. However, there is little literature on the effects of phosphorus on the uptake of micronutrients in the tissues of cowpea under Tanzanian conditions.

Apart from phosphorus, nitrogen is another limiting macro element in the production of crops. However, legumes such as cowpea have a potential of fixing their own nitrogen through the process called biological nitrogen fixation which converts atmospheric nitrogen into a form that can be used by plants [17]. Various studies have reported that nitrogen accrued from rhizobia inoculants is essential for cellular synthesis of enzymes, proteins, chlorophyll, DNA and RNA, and as a result, it is important in plant growth [18]. Soil microorganisms such as *B. japonicum* inoculants and other plant growth promoting rhizobacteria (PGPR) can positively improve plant growth through different mechanisms such as nitrogen fixation and increasing plant water and nutrient uptake [19]. It is also reported that they can influence the chemistry of soil nutrients in many ways and enhance nutrients uptake by plants [20]. However, there is relatively little information in the literature about the effects of rhizobia (*B. japonicum*) inoculation on the uptake micronutrients in cowpea. Therefore, the currents study was conducted to assess the effects of phosphorus supplementation and *B. japonicum* inoculation on the uptake of microelements such as zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) in different tissues of cowpea plant.

2. Material and Methods

2.1. Study Location

The field and pot experiments were conducted at two different locations in the long rain season from mid March to late July 2013. The field experiment was conducted at the Tanzania Coffee Research Institute situated in an area which is 1390 m above the sea level in Kilimanjaro region, Tanzania of latitude (3˚14'44"S) and longitude (37˚14'48"E). The field experiment was conducted in an area with bimodal rainfall pattern and mean annual rainfall of 1200 mm. A screen house experiment was conducted at Seliani Agricultural Research Institute (SARI), situated in an area which is 1390 m above the sea level in Arusha, Tanzania of latitude 3˚21'50.08"S and longitude 36˚38'06.29"E.

2.2. Experimental Design

The experiment was laid out in a split plot design, where the main plots comprised two inoculation treatments (with and without *B. japonicum* inoculation) and sub plots included four levels of phosphorus (0, 20, 40, and 80 kg P/ha). Both experiments were replicated four times. The *B. japonicum* used were Biofix legume inoculants for cowpea, Batch Number 08021302P, purchased from MEA Fertilizer Company in Nairobi, Kenya. The inoculants packets were supplied with gum Arabic for sticking as many cells as possible into the seeds.

2.3. Inoculation Procedure

The crop plant used for this experiment was Cowpea (*V. unguiculata* (L) Walp) supplied by the breeder from Sokoine University of Agriculture, Morogoro, Tanzania. The *B. japonicum* inoculants were applied following manufacturers' instructions as follows: three (3) gram of gum Arabic was added to two tablespoonful of water and mixed to form a solution. 1 kg of cowpea seeds was weighed and 2 tablespoonful of gum Arabic solution was added and mixed well. 10 gm of legume inoculants was added and mixed well so that all seeds are coated. The inoculated seeds were put under shade and the seeds were then sown immediately in a wet moist soil.

2.4. Field and Screen House Preparation and Management

Before set up of experiments soil was sampled and analyzed to assess the physical and chemical characteristics of study area soil. The field was ploughed and harrowed by using tractor before planting. The crop was seeded at a spacing of 50 cm by 20 cm, where the plot size was 4 m by 3 m. In the field trial, three seeds were seeded per hill and then thinned to two plants. The plots were weeded twice where the first weeding was done two weeks after emergence and the second weeding was done just before flowering. Each plot comprised of six rows. Data were collected from the four middle rows.

The soil for screen house experiment was collected from the site where field experiment was conducted.

The soil was packed into 4 kg pots where four seeds were germinated in each pot, and later thinned to two after germination and uniform established. Both experiments were planted at the mid of March 2013, and closely monitored from this point until physiological maturity for field, and pod formation for screen house experiment.

2.5. Plant Harvest and Sample Preparation

At 50% pod formation, the cowpea (*V. unguiculata* (L) Walp) was sampled for dry matter and nutrient determination. Plants were excavated carefully from the soil with their entire root system, washed, and separated into roots, shoots and pods. The plant organs were oven-dried at 60˚C for 48 hrs, weighed and ground into a fine powder for nutrient analysis.

2.6. Measurement of Micronutrients in Plant Tissues

Micronutrients (Cu, Zn, Fe and Mn) were extracted by diethylenetriaminepentaacetic acid (DTPA) [21] and determined by an atomic absorption spectrophotometer.

2.7. Statistical Analysis

The statistical analysis was performed using the 2-way analysis of variance (ANOVA) in factorial arrangement, with the computations being performed with the software program STATISTICA. The fisher's least significance difference (L.S.D.) was used to compare treatment means at $p = 0.05$ level of significance [22].

3. Results

3.1. Soil Physical and Chemical Characteristics

The physical and chemical characteristics of soils from study area are presented in Table 1.

The proposed deficiency levels for Zn (DTPA) in the soil 0.4 - 0.6 mg·kg^{-1} and the values above 10 - 20 mg·kg^{-1} were considered as excess. For Cu, 0.2 mg·kg^{-1} is considered as below critical level. The proposed critical levels for Fe (DTPA) in the soil ranged from 0.3 - 10 mg·kg^{-1} and from this study the level of Fe was 31 mg·kg^{-1} (normal). The level of Mn in the soil was 25 mg·kg^{-1} and considered normal for plant growth. The critical values of Mn were 2.0 - 5.0 mg·kg^{-1} and the values greater than 140 - 200 mg·kg^{-1} regarded as excess. The critical levels are based on the recommendation work by Ndakidemi and Semoka [23].

3.2. Micronutrients Uptake in Roots of Cowpea

The result presented in Table 2 indicated that there was a significant difference in the uptake of most micronutrients tested in the roots as results of inoculation with *B. japonicum* in the field experiments compared with the control. For the screen house experiment, there was a significant increase in the uptake of Zn and Fe following inoculation of *B. japonicum* while uptake of Cu and Mn was not affected by inoculation. For example, inoculation significantly increased the roots uptake of Zn, Cu, Fe and Mn by 54.9%, 26.3%, 63.7%, and 34.8% respectively, in field experiment relative to the un-inoculated treatments. Over the control, inoculation significantly increased the uptake of micronutrients in the roots of cowpea in the screen house experiments by 33.8% (Zn) and = 32.2% (Fe) (Table 2).

In the screen house experiment, application of phosphorus at 40 kg P/ha significantly increased the uptake of Fe and Mn by 67.6% and 225.2% respectively, while in the field experiment, P application significantly increased the uptake of Fe and Mn by 186.8% (40 kg P/ha) and 90.3% (80 kg P/ha) respectively compared with the control. The results on root Zn uptake in the field experiment indicated that application of phosphorus at more than 20 kg P/ha significantly decreased the uptake of Zn (Table 2).

Table 1. **Physical and chemical characteristics soil.**

Particle size analysis	<2	µm	29
	2 - 20	µm	20
	20 - 50	µm	11.4
	50 - 2000	µm	39.6
pH 1:2.5		H_2O	6.4
		KCl	6.1
ORG C KCl		%	1.96
TOTAL N		%	0.197
AVAIL. P BRA-I		mg/Kg	5.1
Exchangeable bases	Ca	meq/100g	2.69
	Mg	meq/100g	1.42
	K	meq/100g	0.94
	Na	meq/100g	0.11
	Zn	mg·kg^{-1}	5
	Cu	mg·kg^{-1}	11
	Fe	mg·kg^{-1}	31
	Mn	mg·kg^{-1}	25
EC		mS/cm	0.12

Table 2. **Effects of *B. japonicum* and phosphorus on nutrient uptake in roots of cowpea.**

Treatments	Screen house				Field			
	Zn	Cu	Fe	Mn	Zn	Cu	Fe	Mn
	mg/plant				mg/plant			
Rhizobium								
−R	18.74 ± 0.89b	5.71 ± 0.45a	49.78 ± 4.84b	6.04 ± 0.74a	36.47 ± 1.88b	17.91 ± 1.01b	154.74 ± 17.51b	130.75 ± 11.18b
+R	25.08 ± 1.04a	5.74 ± 0.49a	65.81 ± 5.74a	6.25 ± 0.76a	56.49 ± 2.96a	22.62 ± 0.88a	253.26 ± 25.15a	176.28 ± 12.29a
P Levels Kg·ha^{-1}								
0	20.68 ± 1.49ab	4.84 ± 0.92a	40.38 ± 3.36b	2.62 ± 0.23c	53.49 ± 5.79a	17.58 ± 1.36b	97.09 ± 19.14c	99.60 ± 9.31b
20	24.08 ± 2.55a	5.02 ± 0.42a	59.52 ± 6.39ab	5.63 ± 0.84b	53.99 ± 4.22a	22.76 ± 1.63a	186.07 ± 28.49b	161.29 ± 15.46a
40	22.70 ± 1.72ab	6.40 ± 0.53a	67.68 ± 8.76a	8.52 ± 0.63a	41.02 ± 4.29b	20.56 ± 1.53ab	278.43 ± 30.99a	163.60 ± 13.59a
80	20.18 ± 0.89b	5.63 ± 0.48a	63.60 ± 9.31b	7.82 ± 0.78a	37.41 ± 3.03b	20.16 ± 1.44ab	254.39 ± 20.96a	189.57 ± 18.56a
2-way ANOVA (F-statistics)								
R	24.87***	0.001 ns	5.42*	0.09ns	88.24***	14.21***	29.94***	13.05**
P	2.03 ns	1.98 ns	3.08*	16.02***	15.98***	2.89 ns	20.39***	9.16***
R*P	1.60 ns	0.36 ns	0.81 ns	1.35 ns	3.14*	0.58 ns	0.57 ns	0.20 ns

+R: With *Rhizobium*; −R: Without *Rhizobium*; R: *Rhizobium*; P: Phosphorus; Values presented are means ± SE; *, **, ***: significant at $p \le 0.05$, $p \le 0.01$, $p \le 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at $p = 0.05$ according to Fischer least significance difference (LSD).

3.3. Micronutrients Uptake in Shoots of Cowpea

There was a significant difference in the uptake of Zn, Cu, Fe, and Mn in the shoots following inoculation of *B. japonicum* and phosphorus application in both the screen house and field experiments. Over the control, inoculation significantly increased the uptake of micronutrients in the shoots from both the screen house and field experiments by; Zn = 33.8%, Cu = 49.6%, Fe = 46.6% and Mn = 50.03% in the screen house and for the field experiment micronutrients were increased by Zn = 47.6%, Cu = 46.8%, Fe = 24.3% and Mn = 56.3% (**Table 3**).

Phosphorus supplementation in this study resulted in a significant increase in shoot uptake of Cu, Fe and Mn in the screen house and the shoot uptake of Fe and Mn in the field experiment over the control. For example, the shoot uptake of Cu was increased by 123.1% at 40 kg P/ha, Fe was increased by 170.4% at 80 kg P/ha, and Mn was increased by 175.1% at 80 kg P/ha in the screen house experiment relative to the control. In the field experiment, there was a significant decrease in the shoot uptake of Fe by 18.2% and the shoots uptake of Mn was increased by 162.2% at 80 kg P/ha relative to control (**Table 3**).

3.4. Micronutrients Uptake in Pods of Cowpea

B. japonicum inoculation significantly improved the pods uptake of micronutrients in the screen house and field experiment. For example, inoculation significantly increased the pods uptake of micronutrients by: Zn = 105.7%, Cu = 88.9%, Fe = 77.8% and Mn = 62.5% in the screen house and in the field experiment micronutrients was increased by; Zn = 36.5%, Cu = 16.1%, Fe = 72.5% and Mn = 26.3% over the control (**Table 4**). The results also showed that phosphorus supplementation significantly affected the uptake of micronutrients in both the screen house and field experiment relative to the control. In the screen house, P supplementation at any level significantly increased the uptake of Cu, Fe, and Mn over the control while the uptake of Zn was reduced by P supplementation at the level of 40 kg P/ha and 80 kg P/ha (**Table 4**). In the field experiment the pods uptake of Zn was significantly reduced by phosphorus application. Pods uptake of Fe was significantly improved by 20 kg of phosphorus and then decreased with supplying phosphorus at 40 and 80 kg P/ha. The pods uptake of Cu, and Mn was increased by application of P at the level of 40 kg P/ha which was statistically the same as supplying 80 kg P/ha (**Table 4**).

3.5. Micronutrients Uptake in the Whole Plant

The results in **Table 5** showed that there was a significantly increase in the uptake of micronutrients in the whole cowpea plant following inoculation of *B. japoni-*

Table 3. Effects of ***B. japonicum*** **and phosphorus on nutrient uptake in shoots of cowpea.**

Treatments	Screen House				Field			
	Zn	Cu	Fe	Mn	Zn	Cu	Fe	Mn
	mg/plant				mg/plant			
Rhizobium								
−R	47.36 ± 2.79b	19.15 ± 2.17b	63.84 ± 8.01b	15.61 ± 1.98b	160.53 ± 7.92b	42.30 ± 2.65b	728.46 ± 23.86b	44.81 ± 4.57b
+R	63.36 ± 2.19a	28.64 ± 2.55a	93.62 ± 9.02a	23.42 ± 1.93a	236.99 ± 8.33a	62.11 ± 3.67a	905.22 ± 24.52a	70.03 ± 6.33a
P levels $Kg{\cdot}ha^{-1}$								
0	55.27 ± 5.89ab	13.44 ± 2.69b	38.19 ± 6.52c	9.69 ± 1.98c	203.61 ± 20.38a	45.51 ± 6.09a	867.89 ± 41.84a	30.20 ± 4.02c
20	59.89 ± 4.32a	22.84 ± 2.35a	76.63 ± 6.68b	17.16 ± 1.73b	212.42 ± 16.67a	54.31 ± 4.30a	892.005 ± 46.57a	54.39 ± 6.35b
40	58.05 ± 4.15ab	29.99 ± 2.91a	96.84 ± 11.51ab	24.54 ± 1.96a	193.35 ± 18.39a	54.71 ± 7.13a	773.39 ± 46.78b	65.90 ± 5.25b
80	48.24 ± 2.95b	29.30 ± 3.79a	103.26 ± 11.49a	26.66 ± 2.29a	185.66 ± 17.65a	54.30 ± 5.37a	734.08 ± 30.74b	79.19 ± 9.11a
2-way ANOVA (F-statistics)								
R	22.41***	13.63**	13.39**	29.23***	39.85***	17.58***	44.19***	33.73***
P	2.29 ns	8.93***	12.98***	28.51***	0.93 ns	0.89 ns	8.01***	22.92***
R*P	0. 73 ns	0.02 ns	1.02 ns	0.18 ns	0.07 ns	0.29 ns	0.55 ns	1.43 ns

+R: With *Rhizobium*; −R: Without *Rhizobium*; R: *Rhizobium*; P: Phosphorus; Values presented are means ± SE; **, ***: significant at $p \leq 0.01$, $p \leq 0.001$ respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at $p = 0.05$ according to Fischer least significance difference (LSD).

Table 4. Effects of ***B. japonicum*** **and phosphorus on nutrient uptake in pods of cowpea.**

Treatments	Screen House				Field			
	Zn	Cu	Fe	Mn	Zn	Cu	Fe	Mn
	mg/plant				mg/plant			
Rhizobium								
−R	1.05 ± 0.14b	2.89 ± 0.27b	42.77 ± 4.48b	2.75 ± 0.31b	122.32 ± 2.84b	24.33 ± 2.51a	153.07 ± 7.53b	165.53 ± 12.75b
+R	2.16 ± 0.20a	5.46 ± 0.51a	76.03 ± 7.16a	4.47 ± 0.34a	166.96 ± 4.85a	28.24 ± 2.37a	263.99 ± 7.73a	209.13 ± 13.81a
P levels $Kg{\cdot}ha^{-1}$								
0	2.08 ± 0.27a	2.70 ± 0.48b	31.41 ± 4.82c	2.25 ± 0.41c	158.82 ± 10.62ab	16.19 ± 1.73b	216.50 ± 22.06ab	109.19 ± 7.94c
20	1.93 ± 0.32ab	4.33 ± 0.92a	56.31 ± 9.91b	3.49 ± 0.64b	156.83 ± 9.96a	24.96 ± 2.16a	237.96 ± 22.61a	189.64 ± 12.36b
40	1.40 ± 0.35bc	4.88 ± 0.65a	73.62 ± 5.94a	4.01 ± 0.27ab	139.76 ± 10.23bc	32.52 ± 2.93a	201.97 ± 22.82b	232.99 ± 13.68a
80	1.01 ± 0.15c	4.81 ± 0.63a	76.26 ± 11.16a	4.68 ± 0.45a	131.14 ± 10.59c	31.45 ± 3.59a	177.68 ± 21.52c	217.48 ± 9.04a
2-way ANOVA (F-statistics)								
R	33.12***	26.09**	39.62**	30.38***	107.02***	1.95ns	188.64***	33.84***
P	6.52**	4.09*	15.28*	10.89***	7.03**	7.22**	9.83***	54.02**
R*P	1.32 ns	0.93 ns	2.31 ns	2.52 ns	144 ns	0.05 ns	0.01 ns	0.86 ns

+R: With *Rhizobium*; −R: Without *Rhizobium*; R: *Rhizobium*; P: Phosphorus; Values presented are means ± SE; *, **, ***: 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 letter(s) in a column are significantly different from each other at $p = 0.05$ according to Fischer least significance difference (LSD).

Table 5. Effects of *B.japonicum* and phosphorus on nutrient uptake in the whole cowpea plant.

Treatments	Screen House				Field			
	Zn	Cu	Fe	Mn	Zn	Cu	Fe	Mn
	mg/plant				mg/plant			
Rhizobium								
−R	67.15 ± 2.91b	27.79 ± 2.48b	156.39 ± 13.65b	24.39 ± 2.76b	319.31 ± 11.01b	84.54 ± 4.97b	1036.27 ± 24.36b	341.09 ± 25.06b
+R	90.60 ± 2.81a	39.82 ± 3.21a	235.46 ± 18.81a	34.14 ± 2.79a	460.44 ± 12.73a	112.97 ± 4.34a	1422.47 ± 33.89a	455.44 ± 29.73a
P Levels Kg·ha^{-1}								
0	78.02 ± 6.82ab	20.99 ± 3.27c	109.98 ± 10.33c	14.55 ± 2.29c	407.93 ± 33.72ab	79.28 ± 7.95b	1181.49 ± 77.57b	239.001 ± 16.48c
20	85.91 ± 6.60a	32.19 ± 3.27b	192.47 ± 15.03b	26.29 ± 2.43b	423.25 ± 30.14a	102.03 ± 7.43a	1316.04 ± 90.41a	405.32 ± 29.74b
40	82.15 ± 4.96a	41.27 ± 3.42a	238.14 ± 21.93a	37.07 ± 2.25a	374.12 ± 30.77bc	107.79 ± 8.62a	1253.79 ± 91.54ab	462.49 ± 27.86a
80	69.43 ± 3.54b	40.74 ± 3.40ab	233.12 ± 20.65a	36.17 ± 2.89a	354.21 ± 25.40c	105.92 ± 5.91a	1166.15 ± 64.41b	486.25 ± 32.98a
2-way ANOVA (F-statistics)								
R	45.07***	15.64***	38.66***	30.34***	92.95***	25.82***	100.10***	41.79***
P	4.09*	9.76***	32.52***	40.98***	4.60**	5.57**	3.22*	39.71***
R*P	1.29 ns	0.01 ns	1.89 ns	0.26 ns	0.62 ns	0.37 ns	0.47 ns	0.59 ns

+R: With *Rhizobium*; −R: Without *Rhizobium*; R: *Rhizobium*; P: Phosphorus; Values presented are means ± SE; *, **, ***: 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 letter(s) in a column are significantly different from each other at $p = 0.05$ according to Fischer least significance difference (LSD).

cum and phosphorus application in both the screen house and field experiments. For example, inoculation of cowpea with *B. japonicum* significantly increased the uptake of Zn Cu, Fe and Mn in the whole plant by 34.9%, 43.3%, 50.6%, and 39.9% respectively in the screen house experiment relative to the un-inoculated treatments. Inoculation also significantly increased the uptake of Zn, Cu, Fe and Mn in the field experiment by 44.2%, 33.6%, 37.3% and 35.5% respectively over the control (**Table 5**).

Supplementation of phosphorus significantly increased uptake of Cu, Fe, and Mn in both experiments, while supplementation of phosphorus at 80 kg P/ha significantly induced a reduction in the uptake of Zn in both experiments (**Table 5**). Phosphorus application (40 kg P/ha) in the screen house experiment increased the uptake of Cu, Fe, and Mn by 96.6%, 116.5%, and 154.8% respectively over the control. For the field experiment, the results indicated the significant increase in the uptake of Cu, Fe, and Mn by 35.9% (40 kg P/ha), 11.4% (20 kg P/ha) and 103.5% (80 kg P/ha) respectively over the control (**Table 5**).

3.6. Interactive Effect of *B. japonicum* and Phosphorus Supplementation on Roots Zn Uptake

There was a significant interaction between *B. japonicum* and phosphorus on the uptake of Zn in the roots of cowpea grown in the field experiment. Whether inoculated or not inoculated phosphorus supplementation at 40 and 80 kg per hectare significantly reduced the root uptake of Zn (**Figure 1**).

4. Discussion

B. japonicum inoculation significantly improved the uptake of Zn, Fe, Cu and Mn in all plant organs (roots, shoots, pods and whole plant) tested from screen house and field experiment relative to the control (**Tables 2-5**). Improvements in the uptake of micronutrient in the *B. japonicum* inoculated pots and the plots in all plant organs over the control are beneficial as apart from improving plant growth and production through atmospheric nitrogen fixation; these minerals can now be available for human taking cowpea in their diet. Similar to our findings, several researchers [11,17,24,25] have reported on the improvements in the nutrient uptake in plants as influenced by rhizobial inoculation. The mechanism by which these minerals are made available for uptake by plant is not well known but several studies have reported on the possible mechanism of increasing availability of these minerals. For example, [17,26] reported on the improved uptake of micronutrients in *Phaseolus vulgaris* and associated it to the improved soil pH by microorganism to the level which favored the uptake of micronutrients. It was similarly reported that soil microorganisms

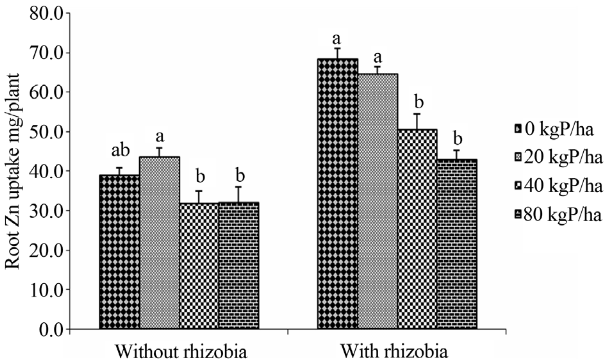

Figure 1. Interactive effects of *B. japonicum* and phosphorus (P) on zinc (Zn) uptake in the roots of cowpea grown in the field experiment. Bars followed by similar letter(s) are not significantly different from each other.

such as plant growth promoting rhizobacteria (PGPR) can positively improve plant growth [18] through different mechanisms such as nitrogen fixation and increasing plant water and nutrient uptake [19]. Other work reported by [20,24] showed that soil microorganisms can influence the chemistry of soil by producing iron carrier compound called siderophores which facilitate formation of soluble Fe^{3+} complexes which is then reduced by ferric reductases to F^{2+} [10] that can be taken by plants. *Rhizobium* inoculation was also found to increase the concentration of nutrients in the rhizosphere soil, which is consequently associated to the increased uptake of these minerals by plant [26]. Several research findings [11,20,25,27] have revealed that rhizobium inoculation can increase availability of plant nutrients by releasing dead cells which may contain plant nutrients or chemical molecules that can solubilize unavailable nutrients such as Fe to a form that can be taken up by plants.

Phosphorus is one of the major fundamental macronutrients for biological growth and development of any plant [28]. Its concentration in the soil is usually very low for plant uptake [29]. Supplementation of phosphorus in known to increase its concentration in the soil [30], plant tissues [31] and availability of other nutrients in the rhizosphere [32,33]. From the current study, phosphorus supplementation at different levels significantly improved the uptake of micronutrients in different tissues of cowpea grown under screen house and field condition. For instance, phosphorus supplementation significantly increased the shoot uptake of Cu, Fe, and Mn in the screen house experiment and the shoot uptake of Mn in the field experiment, while the shoot uptake of Fe was decreased at 40 and 80 kg P/ha in the field experiment relative to the control. The results in **Table 4** also indicated the significant improvement in the pod uptake of Cu, Fe and Mn in the screen house, while for the field experiments phosphorus significantly improved the pod uptake of Cu and Mn. Furthermore, phosphorus supplementation improved the root uptake of Fe and Mn for plants collected from both screen house and field experiment relative to the control. At the whole plant level, there was a significant increase in the uptake of Cu, Fe and Mn in both the screen house and field experiment over the control treatments. Phosphorus is known for its functions on root formation in plants [13,34,35]. The improved uptake of different micronutrients in different plant tissues in this study, might have been caused by the possibility of increased root system which explored the bulk of soil for water and other mineral nutrients uptake which comes in contact with the roots [36]. However, the data from this study (**Tables 2-5**) showed that phosphorus supplementation either did not show significant difference (shoot) or significantly decreased the uptake of Zn and Fe in different plant organs tested. For example, root uptake of Zn in both the screen house and field experiment was significantly reduced with phosphorus supplementation. In **Table 3** the data showed that the shoot uptake of Fe was significantly decreased with P supplementation. There was also a significant reduction in the pod uptake of Zn in the screen house, while for the field experiment there was a significant decrease in the pod uptake of Zn and Fe following supplementation of phosphorus. Similar to our findings [36,37] observed that an increase in P supply lowered Zn concentration in *Phaseolus vulgaris*, and attributed this effects to a dilution effect of plant growth. Generally, this study showed that phosphorus supplementation significantly reduced the uptake of Zn in all plant parts tested for both screen house and field experiments. Despite the fact that the mechanism is not clear, but several studies [32,38] have shown that increasing P in the growth medium can induced Zn deficiency in plants by altering soil and plant factors. However, [38] pointed out some possible mechanisms such as: a P × Zn interaction in the soil; dilution effect on the concentration of Zn in plant tops due to growth response to P; a slower rate of translocation of Zn from the roots to tops; and a metabolic disorder within the plant cells related to an imbalance between P and Zn. The current study showed a significant interactive effect of *B. japonicum* inoculation and phosphorus supplementation on the root uptake of Zn (**Figure 1**). *B. japonicum* inoculation without P fertilization (0 kg P/ha) resulted in high value of Zn uptake than inoculation plus other levels of phosphorus (20 - 80 kg P/ha) which showed the declining trend over increasing P concentration.

In conclusion, this study indicated that *B. japonicum* significantly improved the uptake of micronutrients in different plant tissues in both the screen house and field experiments. Phosphorus supplementation also improved the uptake of some micronutrients while inducing the deficiency of other nutrients such as Zn and in some organs Fe deficiency was observed. The uptake of most nutrients was enhanced at 40 kg P/ha which was statistically the same as 80 kg P/ha. However, phosphorus at 80 kg P/ha, numerically decreased the uptake of most nutrients indicating that excessive P may limit the uptake of nutrients in plants.

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