

EFFECTS OF PHOSPHORUS AND *BRADYRHIZOBIUM JAPONICUM* ON GROWTH AND CHLOROPHYLL CONTENT OF COWPEA (*VIGNA UNGUICULATA* (L) WALP).

ABSTRACT:

Aims: To assess the effects of *Rhizobium* and phosphorus on growth and total leaf chlorophyll content of cowpea.

Study design: The experiment was laid down in split-plot design whereby the main plots comprised of two inoculation treatments (with and without *Rhizobium*), sub-plots included phosphorus (TSP) application at four different levels (0KgP.ha^{-1} , 20KgP.ha^{-1} , 40KgP.ha^{-1} , 80KgP.ha^{-1}). Both experiments were replicated four times.

Place and Duration of Study: The field and screen house experiment was conducted at Tanzania Coffee Research Institute and Seliani Agricultural Research Institute respectively in Tanzania, between March-July 2013.

Methodology: We measured plant growth parameters such as plant height; number of leaves per plant at different stages of plant growth, the stem girth was measured by vernier caliper at physiological maturity. The chlorophyll content was determined at 3, 5, and 7 weeks after planting (WAP). The chlorophyll was extracted by using dimethylsulphoxide (DMSO) and absorbance values were determined at 645 and 663nm, using UV/Visible spectrophotometer.

Results: *Rhizobium* inoculation significantly increased the plant height, number of leaves per plants, and stem girth relative to control. The height was increased by 11.23, 10.43 and 8.99% (screen-house) and by 8.11, 24.05, 9.29% (field) in the measurements taken at 4, 6, and 8 WAP respectively. Number of leaves per plant counted at 6 and 8 WAP, increased by 14 and 10.8% (screen-house) and 14 and 11.6% (field) respectively. *Rhizobium* also significantly increased chlorophyll content of cowpea by 26% (3 WAP) in screen-house and increased by 52, 37.9 and 13.3% (3, 5 and 7 WAP) in the field. Phosphorus also significantly increased different plant growth parameters and leaf chlorophyll content relative to control.

Conclusion: *Rhizobium* inoculation can function the same as inorganic N fertilizers. Therefore, their combined use with phosphorus boosts plant growth and chlorophyll content of the legumes and eventually legume production is increased.

Keywords: Legumes, Nitrogen, Photosynthesis, Plant Height, *Rhizobium*, Stem Girth

1. INTRODUCTION

Low level of soil P and N are the major constraints to crop growth and production in nutrients depleted sandy soil of Sub-Saharan Africa [1,2]. Phosphorus and Nitrogen are essential elements playing major role in plant growth, development and finally they determine the final yield of the crops. Phosphorus (P) is found in every living plant cell and is involved in several

key plant functions, including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant [3,4] nucleic acids, phospholipids, ATP, and it makes about 0.2% of a plant's dry weight [5]. It stimulates root development and growth of young plants, giving them a good and vigorous start [6,7] and control key enzyme reactions in the regulation of metabolic pathways [8]. Successful production systems based on legumes therefore requires P inputs [9] either from soil reserves or from added fertilizer [10]. Other workers [11,12,10,4,13] reported that plant height, leaf area, shoot, grain yields, and root dry weights of the legume plants increased with P application. Supplementing legumes with P nutrients enhances symbiotic establishment for increased N_2 fixation [10,14]. Plant cell functions such as photosynthesis, respiration, biosynthesis and the transfer of organic solutes across membranes, requires inorganic phosphate for its formation from ADP. Phosphorus have been reported to increase chlorophyll content and it plays an important role in both energy storage and transfer as ADP and ATP (adenosine di- and tri-phosphate) and DPN and TPN (di- and tri- phosphopyridine nucleotide) and a shortage of inorganic phosphate in the chloroplast reduces photosynthesis [15]. *Rhizobium* inoculation in legumes is an alternative to the expensive inorganic nitrogen fertilizers and is accredited for stimulating plant growth [16]. *Rhizobium* provides N through symbiotic fixation of atmospheric nitrogen [17,18]. Nitrogen is a building blocks of proteins and the amino acids [19] required for cellular synthesis of enzymes, chlorophyll, DNA and RNA, and is therefore important in plant growth [18,15]. The element is involved in many processes of plant growth and development which finally lead to yield and the quality of harvested organs (seeds or shoot biomass) [20]. Several research reports have indicated significant achievements in legume growth and yield in many parts of the world due to inoculation with the appropriate *Rhizobium* inoculants [21,22,23,24,25,26,11,27,28,29,30,12,10,16,17]. Inoculation of legumes with appropriate *Rhizobium* has been reported to increase the leaf Chlorophyll content of legume crops [16]. The increased chlorophyll content results in the increased photosynthesis and as a result plant gets enough carbohydrate for its growth and production. However, there is little information on the effects of phosphorus and *Rhizobium* on growth and chlorophyll content of cowpea in northern Tanzania. Therefore, it is important to study and establish the role that could be played by phosphorus and *Rhizobium* on growth and total leaf chlorophyll content of legumes grown under glasshouse and field conditions.

2. MATERIAL AND METHODS

2.1. Description of Location

Field and pot experiments were conducted at two different locations from mid March to late July 2013. A screen house experiments was conducted at Seliani Agricultural Research Institute (SARI) which is situated at an altitude of (1300) meter above sea level in Arusha, Tanzania at a latitude of (3°21'50.08"S) and longitude of (36°38'06.29"E). The field experiment was conducted at the Tanzania Coffee Research Institute which is situated at an altitude of (1268) meter above sea level in Kilimanjaro region, Tanzania at a latitude of (3°14'44")S and longitude of (37°14'48"E). The area where field experiment was conducted receives a mean annual rainfall of (1200) mm, bimodal rainfall pattern, where short rains begin late October through December and long rains commences in early April and ends in late June.

2.2. Experimental design

The experiment was laid down in split plot design. The main plots comprised of two inoculation treatments viz i) with inoculation of *Rhizobium* and ii) without inoculation of *Rhizobium*. Sub plots included application of Phosphorus at four different levels ie, 0, 20, 40, and 80 KgP.ha⁻¹. The *Rhizobium* inoculants were purchased from MEA Fertilizer Company in Nairobi Kenya.

2.3 Field and Screen house experiment:

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. The field was ploughed and harrowed by using tractor before planting. The crop was seeded at a spacing of 50cm by 20cm, where the plot size was 4m by 3m. 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 crop plant used for this experiment was Cowpea (*Vigna unguiculata* (L) Walp) supplied by the breeder from Sokoine University of Agriculture, Morogoro, Tanzania. Both screen house and field experiment was 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. Both experiments were replicated four times.

2.4 Growth data collection.

The growth parameters measured from 14 days after planting (DAP) to pod formation in both screen house and field experiment were: plant height measured using meter ruler from ground surface to the tip, number of leaves per plant, stem girth measured by using a vernier caliper and leaf area measured according to [31] shown below,

LA= 11.98 + 0.06LW. Where; L= leaf length and W= leaf width.

Leaf length (L) (cm) and leaf width (W) (cm) was measured by direct leaflet area measurements using a meter rule. Length was measured as the distance between the base and the apex of the leaflet; width was measured perpendicular to the length axis at the position on the leaflet yielding the greatest width. Ten randomly selected representative plants from the four central rows in each plot for field experiment were used while for screen house experiment, one plant from each pot was used to collect data. The number of days to 50% flowering and number of days to 50% pod formation was also counted as part of the growth data.

2.5 Chlorophyll content determination.

Extraction of chlorophyll concentrations by dimethylsulphoxide (DMSO) was done as described in [32]. A third of the plant leaves, from the tip was collected from each pot for screen house and five samples of leaves were taken from five randomly selected plants for field experiment. A hundred (100) mg of the middle portion of fresh leaf slices was placed in a 15 mL vial containing 7 mL DMSO and incubated at 4°C for 72 h. After the incubation, the extract was diluted to 10 mL with DMSO. The DMSO technique extracts chlorophyll from shoot tissue without grinding or maceration [32]. A 2 mL sample of chlorophyll extract was then transferred into curvets for absorbance determination. A spectrophotometer (UV/Visible Spectrophotometer) was used to determine absorbance values at 645 and 663 nm, which was then used in the equation proposed by [33] to determine total leaf chlorophyll contents against DMSO blank, expressed as mg L⁻¹ as shown below:

120 Chlorophyll total ($\text{Chl}t = 20.2D_{645} + 8.02D_{663}$).

121 Where “D” is the density values at the respective wavelength which was obtained on the
122 Beckman spectrophotometer.

123

124 2.6 Statistical Analysis

125 The statistical analysis was performed using the 2-way analysis of variance (ANOVA) in
126 factorial arrangement, with the computations being performed with the software program
127 STATISTICA. The fisher least significance difference (L.S.D.) was used to compare
128 treatment means at $P=0.05$ level of significance [34].

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130

131 3. RESULTS

132 3.1 Effects of *Rhizobium* and phosphorus on the height of the cowpea.

133 The results in table 1 clearly demonstrate that *Rhizobium* inoculation had significant effect
134 on the plant height assessed in this study. *Rhizobium* inoculation significantly improved the
135 plant height measured at four, six and eight weeks after planting (WAP) in both screen
136 house and field experiments relative to control treatment (Table 1). For example, in this
137 study, *Rhizobium* inoculation increased the plant height by 11.23, 10.43 and 8.99% in the
138 measurements taken at four, six and eight weeks after planting (WAP) respectively in the
139 screen house experiment where for the field experiment the *Rhizobium* inoculation increased
140 the plant height by 8.11, 24.05, 9.29% in the measurements taken at four, six and eight
141 weeks after planting respectively, compared with un-inoculated control.

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143 From this study, phosphorus application significantly increased the plant height measured at
144 four, six and eight weeks after planting in both screen house and field experiment as
145 compared with control (Table 1). In this study, phosphorus application in the screen house at
146 the rate of 20, 40 and 80 KgP.ha⁻¹ significantly increased the plant height by 19, 24 and 30%
147 (4WAP) respectively over the control. The measurements taken in the screen house at 6 and
148 8WAP shows that the application of phosphorus at the rate of 20, 40 and 80 KgP.ha⁻¹
149 significantly increased the plant height by 15.18, 16.8, 21.7% (6WAP) and 18, 23, 21.9%
150 (8WAP) respectively over control treatment (Table 1). Table 1 also indicated that the plant
151 height measured at 4, 6 and 8WAP in the field experiment had positive response to
152 phosphorus application at all levels. For example application of phosphorus at the rate of 20,
153 40 and 80 KgP.ha⁻¹ significantly increased plant height by 12.3, 11.8, 15.8% (4WAP); 9.8,
154 18.4, 27.2% (6WAP); and 14.9, 21.1, 28.8% (8WAP) respectively over the control.

155

156 3.2 Interactive effect of *Rhizobium* and phosphorus on plant height of cowpea.

157 This study shows significant ($P \leq .01$) interactive effect between *Bradyrhizobium japonicum*
158 and phosphorus on plant height of the cowpeas measured at six weeks after planting in the
159 field experiment. Plant height was increasing with the increase in phosphorus where the
160 greatest height was observed at the rate of 80 KgP.ha⁻¹ and the smallest height was seen in
161 the control treatment. *Rhizobium* supplied with P at highest level gave significantly higher
162 values of height compared with other treatments (Figure 1- A).

163 3.3 Effects of *Rhizobium* inoculation and phosphorus on number of cowpea 164 leaves per plant

165 In this study, *Rhizobium* inoculation significantly increased the number of leaves of cowpeas
166 measured at 6 and 8WAP in both the screen house and the field experiment (Table 2) as
167 compared with un-inoculated control treatments. *Rhizobium* did not show significant different
168 in the number of leaves measured from 2 and 4 WAP. The results in Table 2 shows that in
169 the screen house experiment, supplying P at 40 and 80 KgP.ha⁻¹ resulted into significant
170 number of leaves measured at 6 and 8 WAP compared with other treatments. In field

experiment, measurements taken at 6 WAP showed that P supplementation at 20, 40 and 80 KgP.ha⁻¹ resulted into significantly higher number of leaves per plant as compared with control treatment. However, at 8 WAP, supplying P at 40 KgP.ha⁻¹ and 80 KgP.ha⁻¹ resulted into significantly higher number of leaves relative to other treatments.

3.4 Interactive effect of *Rhizobium* and phosphorus on number of leaves per plant.

There was significant ($P = .05$) interactive effect between *Bradyrhizobium japonicum* and phosphorus on number of leaves per plant of the cowpeas measured at six weeks after planting in the field experiment (Figure 1-B). *Rhizobium* inoculation supplied with P at any level significantly gave the higher number of leaves per plant compared with the control.

3.5 Effects of *Rhizobium* inoculation and phosphorus application on the stem girth, leaf area, number of days to 50% flowering and number of days to 50% pod formation.

Table 3 clearly indicates that *Rhizobium* inoculation significantly affected almost all growth parameters measured in this study as compared with un-inoculated control. For example plant growth parameter measured and presented in table 3 were stem girth, leaf area, number of days to 50% flowering, and number of days to 50% pod formation. All parameter were significantly affected by inoculation of *Rhizobium*, compared with un-inoculated control in both screen house and field experiment, except leaf area in the screen house did not show significant difference with or without *Rhizobium* inoculation (Table 3). Inoculation of cowpeas with *Rhizobium* lowered the number of days to 50% flowering and pod formation in the screen house by 4.5 and 4% respectively, while it was vice versa in the field experiment where inoculation of cowpea seeds with *Rhizobium* increased the number of days to 50% flowering and pod formation by 2.32 and 2.4% respectively. Table 3 also shows that phosphorus supplementation at any level significantly affected the stem girth and leaf area of cowpea in both screen house and field experiment relative to control treatment. In addition, phosphorus supplementation at 20 Kg.ha⁻¹ showed significant effects on number of days to 50% flowering and 50% pod formation in the screen house experiment compared with all other treatments. Supplying P at 20 Kg.ha⁻¹ resulted in early flowering and pod formation in screen house experiment. However, in the field study, the results shows that supplying P at any level significantly affected the number of days to 50% flowering and pod formation compared with control.

3.6 Effects of *Rhizobium* inoculation and phosphorus on total leaf chlorophyll content of cowpea.

The results in table 4 indicated that the *Rhizobium* inoculation had positive effects on the total leaf chlorophyll content of cowpeas measured from three weeks after planting and taken three times at two weeks interval. The total leaf chlorophyll content measured at 3 WAP significantly increased with *Rhizobium* inoculation in both screen house and field experiment compared with un-inoculated pots and plots (Table 4). The total leaf chlorophyll content of cowpea measured at five and seven weeks after planting were also significantly increased with inoculation of *Rhizobium* in the field experiment. However, the total leaf chlorophyll content measured at the same time from the screen house experiment did not show significant different from *Rhizobia* inoculated and un-inoculated control (Table 4). In the screen house, P supplementation at all levels did not show significant effect on the chlorophyll content of the cowpeas measured at 3, 5 and 7 WAP. Table 4 also shows that supplementation of phosphorus at all levels significantly increased the chlorophyll content of cowpea measured at 5 and 7 WAP compared to control. Supplementation of P at 80 Kg.ha⁻¹ (5 WAP) and 40 Kg.ha⁻¹ (7 WAP) resulted in greater values of chlorophyll content compared to other treatments

3.7 Interactive effect of *Rhizobium* and phosphorus on the total leaf chlorophyll content of cowpea.

There was significant ($P = .05$) interactive effect between *Rhizobium* and phosphorus on the total leaf chlorophyll content of cowpeas measured at three weeks after planting in the screen house and seven weeks after planting in the field experiment. (Figure 2 A and B).

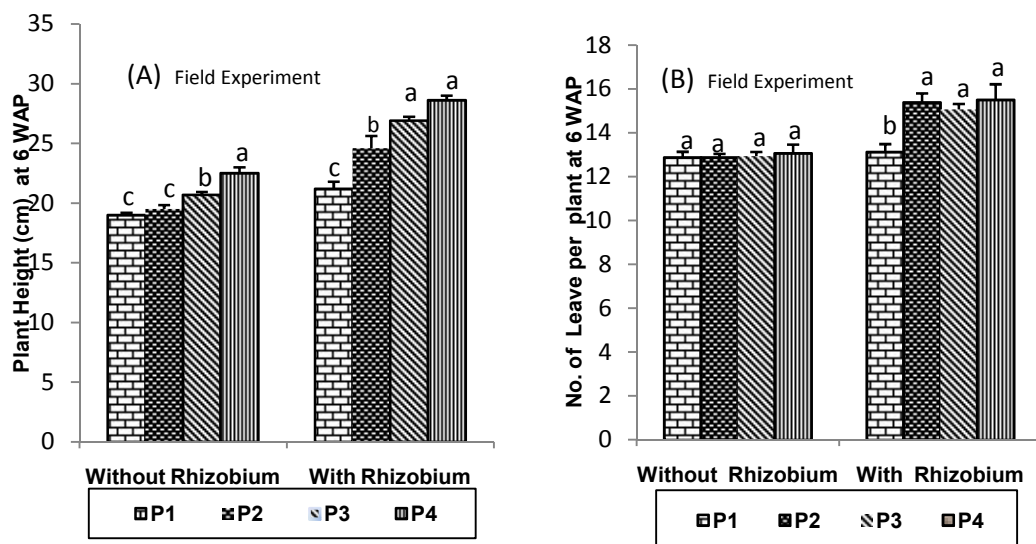
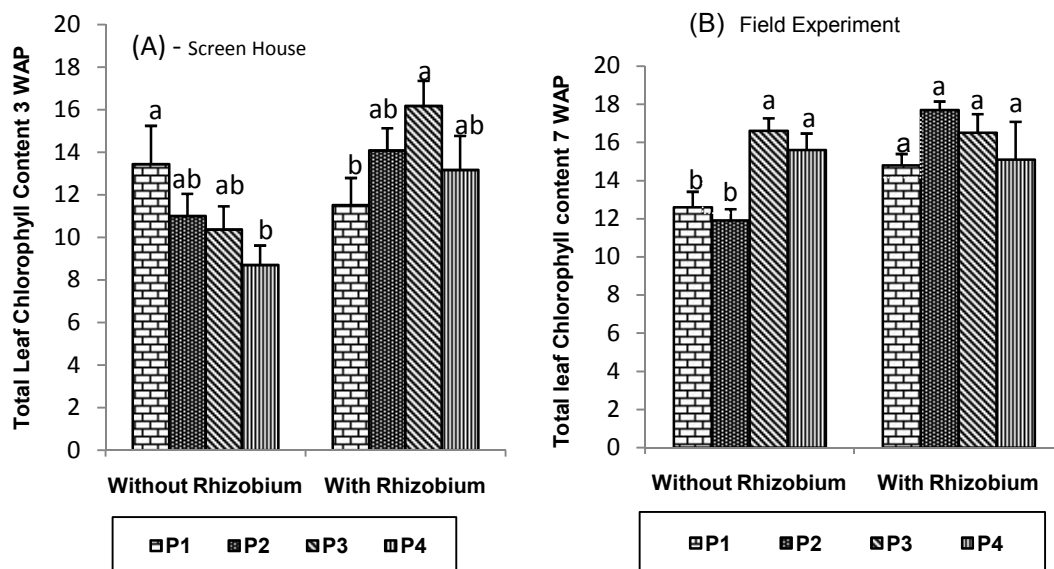


Figure 1. Interactive effects of *Rhizobia* and Phosphorus (P) on: (A) Plant height (cm) measured at 6 WAP, (B) Number of leaves per plant counted at 6 WAP.

P1 = Control, P2 = 20 KgP.ha⁻¹, P3 = 40 KgP.ha⁻¹, P4 = 80 KgP.ha⁻¹, WAP = Weeks After Planting. Bars followed by similar letter are not significantly different.



237 **Figure 2. Interactive effects of *Rhizobia* and Phosphorus (P) on: (A) Total Leaf**
 238 **Chlorophyll Content at 3 WAP, (B) Total leaf chlorophyll content at 7 WAP.**
 239 P1 = Control, P2 = 20 KgP.ha⁻¹, P3 = 40 KgP.ha⁻¹, P4 = 80 KgP.ha⁻¹, WAP = Weeks After
 240 Planting. Bars followed by similar letter(s) are not significantly different

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245 **Table 1: Effects of *Rhizobium* and phosphorus on plant height of cowpea grown under screen house and field condition.**

Treatment s	Screen House				Field			
	Plant Height (cm) 2 weeks after planting	4 weeks after planting	6 weeks after planting	8 weeks after planting	Plant Height (cm) 2 weeks after planting	4 weeks after planting	6 weeks after planting	8 weeks after planting
<i>Rhizobium</i>								
-R	16.78±0.42a	22.08±0.58b	32.88±0.83b	120.88±3.59b	6.41±0.12a	10.23±0.14b	20.42±0.39b	49.94±1.71b
+R	17.18±0.36a	24.56±0.74a	36.31±0.73a	131.75±2.52a	6.77±0.19a	11.06±0.32a	25.33±0.77a	54.58±2.05a
P Levels (Kg.ha⁻¹)								
0	15.13±0.45b	19.69±0.43c	30.50±0.91c	109.13±3.80c	6.33±0.15a	9.67±0.15b	20.09±0.50d	44.98±2.12c
20	17.56±0.38a	23.50±0.82b	35.13±1.26b	128.88±3.22b	6.71±0.25a	10.86±0.36a	22.06±1.09c	51.66±2.35b
40	17.39±0.42a	24.50±0.73ab	35.63±0.91ab	134.25±2.82a	6.59±0.26a	10.81±0.31a	23.78±1.18b	54.48±2.20ab
80	17.84±0.34a	25.60±0.66a	37.13±0.48a	133.0±2.87ab	6.72±0.27a	11.23±0.41a	25.56±1.19a	57.93±2.39a
2 – Way ANOVA (F-Statistics)								
R	1.04ns	23.80***	27.58***	17.90***	2.21 ns	8.76**	181.91***	4.24*
P	10.43***	25.54***	19.07***	20.67***	0.58 ns	5.76**	41.41***	85.96***
R*P	1.53ns	0.6 ns	1.64ns	0.5 ns	0.35 ns	1.42 ns	6.65**	0.09 ns

246 **+R:** With *Rhizobium*; **-R:** Without *Rhizobium*; **R:** *Rhizobium*; **P:** Phosphorus; Values presented are means ± SE; *, **, ***; significant
 247 at P= .05, P≤ .01, P≤ .001 respectively, **ns** = not significant, **SE** = standard error. Means followed by dissimilar letter(s) in a column
 248 are significantly different from each other at P= .05 according to Fischer least significance difference (LSD).

Table 2: Effects of *Rhizobium* and phosphorus on number of leaves of cowpea grown under screen house and field condition.

Treatments	Screen House				Field			
	2 weeks after planting	4 weeks after planting	6 weeks after planting	8 weeks after planting	2 weeks after planting	4 weeks after planting	6 weeks after planting	8 weeks after planting
<i>Rhizobium</i>								
-R	3±0.00a	4.75±0.14a	9.56±0.38b	19.69±0.41b	3.49±0.05a	6.13±0.21a	12.94±0.12b	26.85±0.89b
+R	2.94±0.06a	4.56±0.13a	10.94±0.35a	21.81±0.49a	3.48±0.05a	6.58±1.58a	14.77±0.33a	29.96±1.16a
P Levels (Kg.ha⁻¹)								
0	3±0.00a	4.63±0.18a	8.75±0.37c	18.88±0.35b	3.61±0.07a	6.10±0.13a	13.00±0.21b	25.29±0.70c
20	3±0.00a	4.75±0.25a	10.00±0.42b	20.00±0.60b	3.49±0.06ab	6.21±0.31a	14.13±0.52a	27.14±1.36bc
40	3±0.00a	4.50±0.19a	10.75±0.56ab	21.63±0.63a	3.48±0.08ab	6.95±2.21a	14.00±0.43a	29.53±1.58ab
80	2.9±0.13a	4.75±0.16a	11.5±0.42a	22.50±0.63a	3.36±0.08b	6.15±0.33a	14.28±0.60a	31.68±1.54a
2 – Way ANOVA (F-Statistics)								
R	ns	0.82 ns	13.2**	26.68***	0.0 ns	1.28 ns	45.38***	5.89*
P	ns	0.33 ns	9.6***	15.57***	1.8 ns	0.99 ns	4.56*	4.72*
R*P	ns	0.33 ns	0.69 ns	0.52 ns	0.3 ns	0.76 ns	3.85*	0.26 ns

+R: With *Rhizobium*; **-R:** Without *Rhizobium*; **R:** *Rhizobium*; **P:** Phosphorus; Values presented are means ± SE; *, **, ***; significant at $P = .05$, $P \leq .01$, $P \leq .001$ respectively, **ns** = not significant, **SE** = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at $P = .05$ according to Fischer least significance difference (LSD).

272 **Table 3: Effects of *Rhizobium* and Phosphorus on growth parameters of cowpea grown under screen house and field condition.**

Treatments	Screen House				Field			
	Stem Girth (mm)	Leaf Area (cm ²)	Days to 50% flowering	Days to 50% Pod formation	Stem Girth (mm)	Leaf Area (cm ²)	Days to 50% Flowering	Days to 50% Pod formation
<i>Rhizobium</i>								
-R	0.47±0.01b	28.46±0.71a	65.31±0.28a	68.81±0.29a	7.64±0.12b	29.17±0.39b	70.25±0.64a	75.63±0.51b
+R	0.53±0.02a	29.90±0.81a	62.50±0.70b	66.19±0.66b	8.31±0.14a	30.49±0.55a	71.88±0.49b	77.44±0.48a
P Levels K.gha⁻¹								
0	0.45±0.02b	26.83±0.66b	64.88±0.77a	68.63±0.63a	7.48±0.18b	28.21±0.42b	69.63±0.73b	75.25±0.62b
20	0.52±0.02a	30.31±1.00a	62.13±1.14b	65.75±1.01b	7.95±0.26a	29.88±0.64ab	71.75±0.56a	76.63±0.42ab
40	0.50±0.02a	29.79±1.18a	64.38±0.56a	67.75±0.56a	8.22±0.20a	30.36±0.53a	72.00±1.10a	77.5±1.00a
80	0.54±0.02a	29.79±1.18a	64.25±0.84a	67.88±0.91a	8.25±0.10a	30.88±0.87a	70.88±0.77ab	76.75±0.80ab
2 – Way ANOVA (F-Statistics)								
R	9.97**	1.84 ns	20.18***	18.77***	20.44***	4.58*	4.47*	7.40*
P	4.66*	2.22 ns	3.78*	4.11*	5.67**	3.52*	1.95 ns	1.98 ns
R*P	0.08 ns	0.07 ns	2.61 ns	2.06 ns	1.46 ns	0.42 ns	0.96 ns	1.06 ns

273 **+R:** With *Rhizobium*; **-R:** Without *Rhizobium*; **R:** *Rhizobium*; **P:** Phosphorus; Values presented are means ± SE; *, **, ***; significant
 274 at P= .05, P≤ .01, P≤ .001 respectively, **ns** = not significant, **SE** = standard error. Means followed by dissimilar letter(s) in a column
 275 are significantly different from each other at P= .05 according to Fischer least significance difference (LSD).

290 **Table 4: Effects of *Rhizobium* and phosphorus on total Leaf Chlorophyll content of cowpea grown under screen house and field**
 291 **condition**

Treatments	Screen House			Field		
	Total Leaf Chlorophyll Content (mgL ⁻¹)			Total Leaf ChlorophyllContent (mgL ⁻¹)		
	3 weeks after planting	5 weeks after planting	7 weeks after planting	3 weeks after planting	5 weeks after planting	7 weeks after planting
<i>Rhizobium</i>						
-R	10.88±0.72b	13.30±0.71a	13.54±0.51a	7.65±0.79b	11.61±0.76b	14.17±0.61b
+R	13.74±0.72a	12.49±0.81a	13.76±0.44a	11.17±0.42a	16.01±0.65a	16.05±0.60a
P Levels (Kg.ha⁻¹)						
0	12.48±1.08a	12.73±1.30a	14.59±0.71a	8.90±1.34a	12.39±1.58b	13.70±0.63b
20	12.54±0.90a	13.63±1.24a	13.93±0.48a	10.34±1.03a	13.92±1.22ab	14.81±1.16ab
40	13.27±1.33a	13.00±1.01a	13.01±0.66a	8.41±0.99a	13.55±0.82ab	16.55±0.55a
80	10.93±1.20a	12.23±0.83a	13.07±0.72a	10.00±0.98a	15.38±1.33a	15.37±0.99ab
2 – Way ANOVA (F-Statistics)						
R	10.03**	0.62 ns	0.10 ns	14.33***	21.20***	7.50*
P	1.19 ns	0.32 ns	1.23 ns	0.96 ns	1.67*	2.97*
R*P	3.50*	2.79 ns	0.56 ns	0.30 ns	1.38 ns	4.45*

292 **+R:** With *Rhizobium*; **-R:** Without *Rhizobium*; **R:***Rhizobium*; **P:** Phosphorus; Values presented are means ± SE; *, **, ***; significant
 293 at P= .05, P≤ .01, P≤ .001 respectively, **ns** = not significant, **SE** = standard error. Means followed by dissimilar letter(s) in a column
 294 are significantly different from each other at P= .05 according to Fischer least significance difference (LSD).

295 4. DISCUSSION

296 The field and Screen house experiment was carried out in 2013 at northern Tanzania to assess the effects of
 297 *Rhizobium* (*Bradyrhizobium japonium*) on growth and total leaf chlorophyll content of cowpeas (*Vigna*
 298 *unguiculata* (L) Walp). The results of this study revealed that *Rhizobium* inoculation had significant effect on
 299 different plant growth parameters. For example, *Rhizobium* inoculation significantly increased the plant height,
 300 number of leaves per plant, stem girth and leaf area of cowpea (Table 1, 2 and 3 respectively) in both screen
 301 house and field experiment over un-inoculated control. The positive plant height, stem girth, leaf area and
 302 maximum number of leaves per plant observed in the inoculated pots and plots might be due to symbiotic
 303 relationship between *Rhizobium* and nodulated roots of legumes which fixes atmospheric nitrogen into a
 304 usable form by plants [10,35,36] and ultimately results in the plant growth over the control. The fixed nitrogen
 305 is an important input in the plant growth [37,38,39]. Therefore, if there is reduced nitrogen fixation due to
 306 different factors in nutrient depleted soils, there will be reduced plant growth [17]. Similar to our study, other
 307 workers [36,40,4] have reported that *Rhizobia* inoculation significantly increased the plant height, number of
 308 leaves per plant and biomass of Groundnuts (*Arachis hypogaea*) and Soybean (*Glycine max*).
 309

310 This study also showed significant improvement on the total leaf chlorophyll content of cowpeas as a result of
 311 *Rhizobium* inoculation in both screen house and field experiment relative to un-inoculated treatments. These
 312 findings concur with the results found by [16] in which *Rhizobium* inoculation improved chlorophyll content in
 313 *Phaseolus vulgaris*. Furthermore, inoculation with *Rhizobium* significantly increased the number of days to
 314 50% flowering and pod formation in the field experiment. The late maturity of legumes in the field experiment
 315 may be attributed to biological nitrogen fixation by *Rhizobium* inoculation on cowpea seeds which produce
 316 adequate nitrogen and make the plant to grow luxuriously vegetative. These results differ from the study by
 317 [41] on the effect of *Rhizobium* inoculant on nodulation, yield and yield traits of chickpea (*Cicer arietinum* L)
 318 who found that *Rhizobium* inoculation resulted in the early maturity of chickpea.
 319 The improved plant growth parameters in the inoculated treatments over the control is an indication that
 320 *Rhizobium* were efficient in fixing nitrogen which is a building block of plant proteins many of which
 321 determines the size and structure of plant tissues and consequently plant growth is enhanced.
 322

323 In this study, phosphorus supplementation significantly increased the plant height, number of leaves per plant
 324 and stem girth (Table 1, 2 and 3 respectively) of cowpea in both screen house and field experiment. These
 325 findings are supported by other workers [42] who concluded that supplying P irrespective of the rate
 326 significantly increase the plant height, the number of leaf per plant, stem girth and leaf area. These results
 327 suggest that P is an important element for plant growth and development. In relation to our findings, other
 328 workers [43,44] have reported that supplying P to legume and cereal crops significantly improved the plant
 329 height. The improvement in growth parameters and chlorophyll content observed in this experiment might
 330 have been caused by the activity of phosphorus which plays major role in structural and metabolic function in
 331 the plant leading to growth and development [45]. Therefore, an adequate supply of phosphorus is needed for
 332 the promotion of early root formation and plant growth because phosphorus is a component of numerous cell
 333 constituents and performs major roles in several key processes, such as photosynthesis, respiration, energy
 334 storage and transfer, cell division, and cell enlargement [46,47].
 335

336 This study also showed significant interactive effects between *Rhizobium* and phosphorus on plant height,
 337 number of leaves per plant (Figure 1-A & B) and chlorophyll content of cowpeas (Figure 2-A & B). For instance,
 338 whether supplied alone or with *Rhizobium*, P significantly gave the greater plant height at its highest level of
 339 80 kg P.ha⁻¹ (Figure 1-A). The maximum numbers of leaves per plant were also observed where *Rhizobium*
 340 was supplied with phosphorus relative to control (Figure 1-B). There was also significant interactive effect
 341 between *Rhizobium* and phosphorus on the chlorophyll content of cowpea where the greatest value of
 342 chlorophyll content was recorded in *Rhizobium* supplied with 40 KgP.ha⁻¹ relative to the control. P
 343 supplementation alone without *Rhizobium* decreased chlorophyll content in the screen house compared with
 344 control (Figure 2-A). The decreasing values of chlorophyll content with increasing P without *Rhizobium*
 345 inoculation suggests that increasing P alone does not increase chlorophyll concentration in the leaves, but
 346 rather nitrogen is a necessary building blocks of chlorophyll molecule in the plant and therefore, it influences
 347 the formation of green pigment in the plant leaves. These results also suggest that there was limited amount
 348 of nitrogen in the small volume of soil in the pots compared with the field experiment where the plant roots
 349 can move far searching nitrogen and consequently chlorophyll content of plant leaves was improved in both
 350 inoculated and un-inoculated treatments in the field trial. Figure 2- B shows interactive effects between
 351 *Rhizobium* and phosphorus on the total leaf chlorophyll content of cowpea in which P supplementation alone
 352 produced greater values of chlorophyll content at 40 and 80 Kg.ha⁻¹ relative to other treatments while both
 353 *Rhizobium* inoculation and P supplementation had no significant difference over the control. However,
 354 treatments which received *Rhizobium* inoculation and supplemented with P produced greater values of

chlorophyll content compared with the treatments received P alone (Figure 2-B). The increased plant height, number of leaves and chlorophyll content of cowpea might have been contributed by combination of *Rhizobium* and phosphorus which enhance root formation, nodulation, N-fixation and finally plant growth with dark green color for food production [28,30,11, 12].

5. CONCLUSION

In conclusion, our results showed the importance of *rhizobium* inoculation and phosphorus on the growth and total leaf chlorophyll content of legumes crops in the nutrient depleted soils. The growth parameters such as plant height, number of leaves per plant, leaf area, stem girth, were significantly improved with *Rhizobium* inoculation and application of P over the control treatments. Inoculation with *Rhizobium* also significantly improved the total leaf chlorophyll content of the cowpea. There was also significant interaction between *Rhizobium* and phosphorus on the plant height, number of leaves per plant, and total leaf chlorophyll content of cowpeas. Therefore, for improved plant growth, number of leaves and chlorophyll content in the legume production system, it is important to supplement the depleted soils of sub-Saharan Africa with phosphorus and *Rhizobium* inoculation.

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