

1 **Growth, nodulation and nutrients content of cowpea**
2 **(*Vigna unguiculata* (L.) Walp) following different Zinc**
fertilizer rates in the semi-deciduous forest zone of Ghana

ABSTRACT

4 Cowpea can fix atmospheric nitrogen through symbiotic association with indigenous rhizobia
5 but unfortunately, the amount of N-fixed is usually not enough due to the presence of
6 ineffective or low numbers of indigenous rhizobia in the soil. The effect of Zinc rates on
7 growth, nodulation and nutrient content of cowpea was investigated at the Plantation Section
8 of the Department of Crop and Soil Sciences, Faculty of Agriculture, KNUST/Kumasi
9 (Ghana) during the major and minor cropping seasons (2016). Cowpea seed varieties
10 (Asontem, Agyenkwa and Zamzam) were treated to three levels of Zinc fertilizer rates (0, 5
11 and 10 kg Zn/ha). The Zn fertilizer was applied as foliar application in both experiments. The
12 split plot design was used for both studies. All recommended cultural practices were timely
13 done. The results indicated that all yield components increased significantly following Zn
14 fertilizer application. Application of Zn fertilizer improved the N and K contents of cowpea
15 seeds. This implies the Zinc rates used can be applied to any of the varieties used. The
16 application of the Zn fertilizer did not affect nodulation, and the nodule number was nearly
17 successively decreased over time at all treatments and is not correlated with the Zinc fertilizer
18 applied. Percentage nodule effectiveness and nodule dry weight were not significantly
19 affected by Zinc rates at both sampling times. The amount of nodule dry biomass was
20 drastically reduced with the mineral Zinc fertilizer, whereas the amount of nodule biomass
21 was not affected in the control group, probably because the soil had satisfactory levels of

22 available N and P. The results suggest that cowpea responds differently to Zinc sulphate
23 application depending on its rates.

24 Keywords: N-fixed, Nodulation, Zinc fertilizer, NPK uptake and Yield

25 INTRODUCTION

26 Proper nutrition of plants with micronutrients depends on various factors, such as the rate of
27 absorption of nutrients by the plants, distribution of nutrients to functional sites and nutrient
28 mobility within the plant. Interactions occur between the micronutrients and some nutrients
29 [1, 2, 3]. The amount of nitrogen fixed is usually high in soils with low mineral N but with
30 sufficient water and enough of other nutrients capable of supporting plant growth [4].

31 Another factor is the differential response of plants to one nutrient in combination with
32 varying levels of a second element applied simultaneously i.e. the two elements combine to
33 produce an added effect not due to each of them acting alone [1, 2]. Such interactions may
34 take place in the soil and within the plant [3]. However, the amount of nutrients uptake is
35 strongly dependent on nutritional and environmental factors.

36 Cowpea is especially important for dry savannah of West Africa between latitudes 7 and
37 14°N [5] and second after groundnut as the most important legume of Ghana in terms of
38 space under cultivation (156,000 ha) and quantity produced and consumed annually (143,000
39 Mg) making Ghana among the largest cowpea producer in Africa [6]. Cowpea is a protein-
40 rich component of an otherwise protein-poor diet [7]. Many researchers have observed that
41 Zn have a positive relationship with the nitrogen metabolism pathway of plants, its deficiency
42 cause a reduction in protein synthesis into the plants. [8] identified the positive relationship
43 between the flowering and fruiting process and Zn. As micronutrient, Zinc has received much
44 recent attention [9] because it is present in all body tissues and fluids [10].

45 The native rhizobia are often low in numbers or ineffective and are therefore not able to fix
46 enough nitrogen to meet the nitrogen demand of plants. The study was undertaken to examine
47 the dynamics mineral contents in grain and haulm tissues and nutritional benefits following
48 by zinc fertilizer application. The nodule parameter was also under investigation.

49 **MATERIALS AND METHODS**

50 The study was conducted at the Plantation Section of the Department of Crop and Soil
51 Sciences, Faculty of Agriculture, KNUST, in the cropping seasons of 2016. The site is
52 located at 06° 45' N and 01° 31' W in the rainforest belt of Ghana. The site was located at
53 06° 45' N and 01° 31' W in the rainforest belt of Ghana. The total nitrogen content was low
54 with a mean value of 0.06%, available P content was low with value of 6.4 mg/kg, soil Zn
55 content was moderately low, found to be 1.290 mg/kg. Three early maturing cowpea
56 varieties (Asontem, Agyenkwa and Zamzam) were grown in both experiments and Zn
57 fertilizer rates of 0, 5 and 10 kg/ha were applied into the varieties. The Zn fertilizer was
58 applied as foliar application in both experiments. The split plot design, arranged in RCBD
59 **(The Randomized Complete Block Design)** was used for both cropping seasons. All
60 recommended cultural practices were done in
61 schedule. Cowpea varieties were obtained from the Crops Research (CSIR) at Fumesua,
62 Kumasi/Ghana. Zinc sulfate heptahydrate was applied at 3rd weeks (40%) and 5th weeks
63 (60%)
64 after sowing. The application was done early morning before 9:00 am, using a sprayer. The
65 plots were demarcated three days after harrowing and seeds were sown by hand using manual
66 labour. Seeds were sown at a spacing of 60 cm x 20 cm with a rate of two seeds per hill at the
67 depth of 3-5 cm. Urea and triple superphosphate (TSP) fertilizers were applied as band
68 placement by making a furrow of 5-7 cm deep and covering with 2 cm of soil. As starter

69 nitrogen, Urea was applied at the rate of 20 kg N/ha uniformly to all plots at two weeks after
70 sowing (WAS). Triple super phosphate (TSP) was also applied two weeks after??? (before)
71 sowing (WBS) to the cowpea plant at the rate of 40 kg P₂O₅/ha. Standard agronomic
72 and plant
73 protection treatments were used uniformly across the plots for the duration of the experiment.
74 Grass hoppers (*Empoasca kerri Pruth*), Thrips (*Caliothrips indicus Bagnall*) and Aphids
75 (*Aphis craccivora Koch*) were pests, respectively at vegetative stage and flowering to the end
76 of pod filling. Lambda master 2.5 % E.C. [Active ingredients (Lambda-Cyhalothrin, 9.8 %)]
77 was the pesticides used for pests' control.

78 A random sample of five plants from each plot and a random sample of five pods from each
79 of the five plants were selected to measure. Plant height, stem girth and number of leaves
80 were measured at 30, 45 and 60 days after sowing (DAS) and means for each plot were
81 calculated. Nodules were sampled at 30 and 45 days after sowing. Plants samples were
82 uprooted gently washed with water and the total nodules counted and the mean calculated for
83 each plot. The ground was sufficiently soaked with water 48 hours before sampling to each
84 uprooting of plants. To determine nodule effectiveness, nodules were cut open using a razor
85 blade and hand lens. Nodules with pink or reddish colour were considered effective and
86 fixing nitrogen, while those with green or colourless appearance were recorded as ineffective
87 nodules. Nodules per plot were kept in labelled envelopes and sent to the laboratory to oven-
88 dry at 70°C for 48 hours. Average dry weight of nodules per plant was computed and
89 expressed in grams. For mineral content analysis, random samples of five plants were
90 uprooted gently from each plot at harvest and the root system was removed. The above
91 ground parts were put in labelled envelopes and oven dry at 70° C for 72 hours and milled and
92 one hundred gram samples of each of the plant part (seeds and haulms) were taken to
93 determine nitrogen, phosphorus and potassium content. The nitrogen content was determined
94 using the Kjeldahl method [11]. The protein content of seed was determined on the basis of

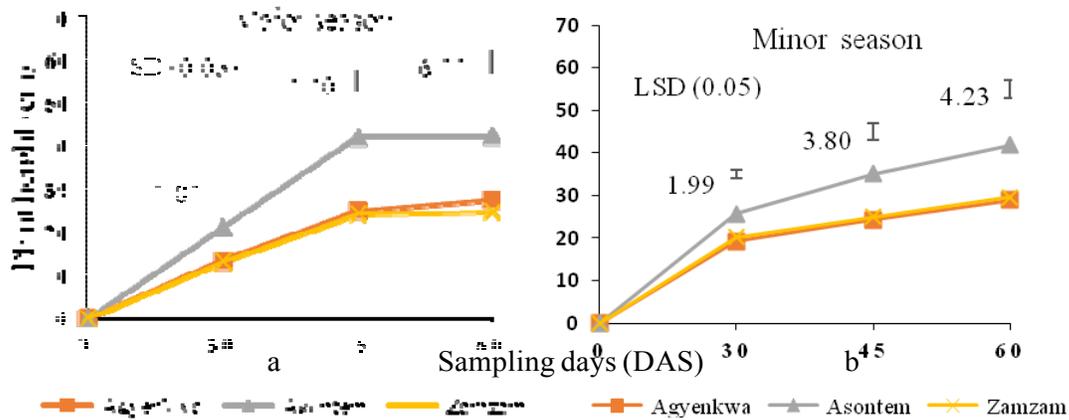
95 total nitrogen content [12]. N-fixed was obtained using the N-difference method. The total
96 nitrogen content of the maize (reference crop) was subtracted from that of the cowpea [13]. In
97 this study Omankwa maize variety was the reference crop. Phosphorus (P) content was
98 measured on the Spectronic 20 spectrophotometer to give absorbance measurements at a
99 wavelength of 420 nm. The observed absorbance was used to determine the P content from
100 the standard curve [14, 15] and Potassium (K) was obtained using the flame photometer.
101 From the standard curve, the concentration of K was calculated using the particular
102 absorbance observed for the sample. NPK uptake were done by multiplying the grain and
103 haulm yield in kilograms per hectare by each analysed parameters separately, nitrogen,
104 phosphorus and potassium, in the grain and haulm then divided by 100 percent. Zn content
105 was determined using Perkins model 403 atomic absorption spectrophotometer after
106 digestion. The file for the type of analysis and hollow cathode lamps were selected with
107 appropriate wavelengths of 213.9 nm [16]. The grain and straw yields were recorded
108 separately. Total Zn uptakes by grain and tissue were computed by multiplying Zn content
109 and their respective dry weights/ha. Data collected were subjected to analysis of variance
110 (ANOVA) using GenStat statistical package version 15th. The LSD test was used to compare
111 treatment means at 5% probability.

112 RESULTS

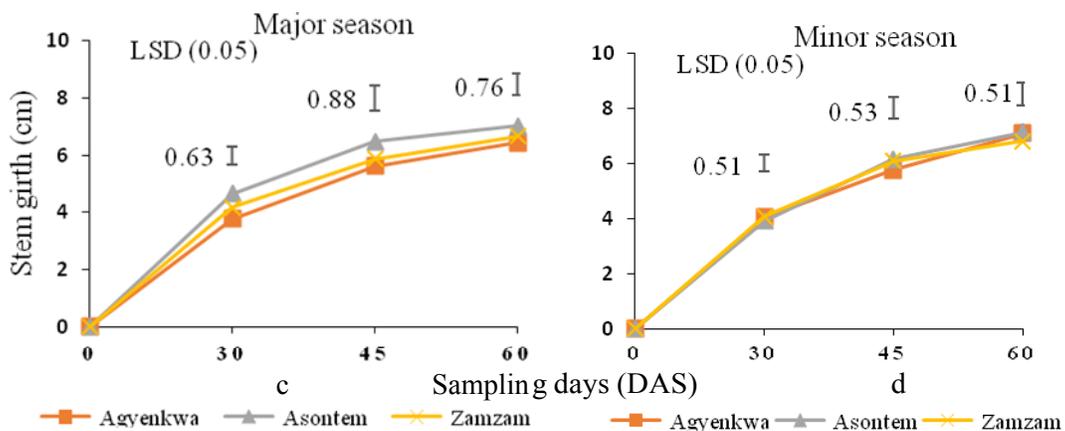
1. Effects of cowpea varieties on growth

113 **Figure 1a should be prepared again.**

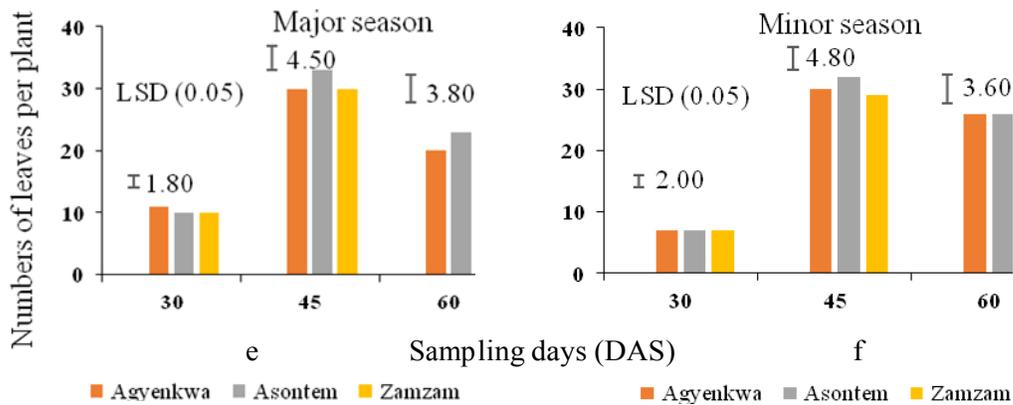
114 Fig 1 illustrates the effect of different cowpea varieties on plant height, stem girth and
115 number of leaves over the period of the experiment. The significant effect at 5% level of
116 probability of cowpea varieties used was recorded over all sampling period of the study. The
117 tallest plant was obtained by Asontem variety and the lowest by Zamzam. However, cowpea
118 varieties did not show any significant ($P > 0.05$) effect on stem girth and number of leaves.
119 Additionally, variety by Zinc rates was not significantly different on all days of sampling.



120



121

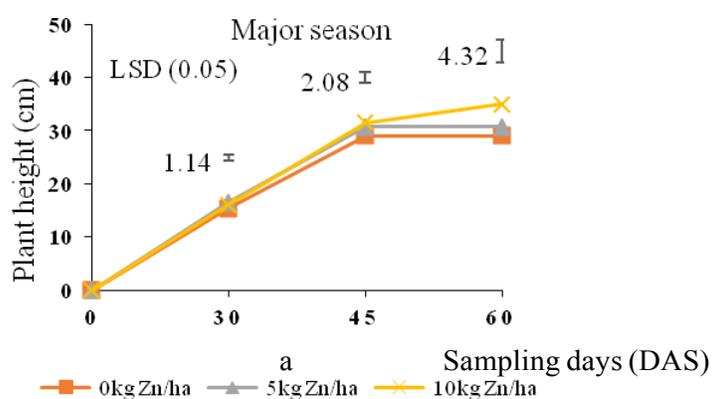


122

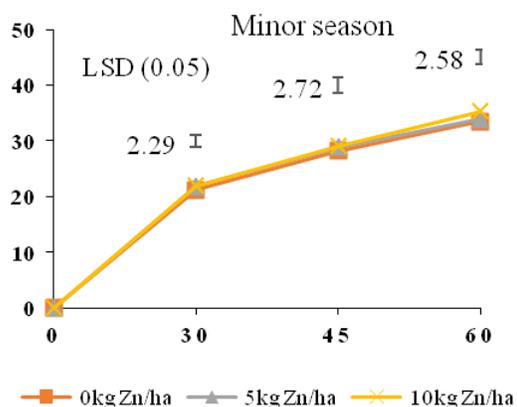
123 **Figure 1. Effects of cowpea varieties on plant height (a and b), stem girth (c and d) and**
 124 **number of leaves (e and f) under Zinc foliar application in 2016 cropping seasons**

2. Effects of Zinc rates on growth

125 Fig 2 illustrates the effects of Zn fertilizer application on plant height, stem girth and number
 126 of leaves over the period of the experiment. Analysis of variance showed no significant effect
 127 of Zn fertilizer on plant height and leaf production. However, plots with Zn application had
 128 the tallest plants compare to the control. Branch production was significantly affected by Zn
 129 rates in both seasons. At 30 DAS, the 5 kg/ha treatment effect was significantly higher than
 130 other treatment effects. Treatment effect at 45 DAS was similar. At 60 DAS, the control
 131 treatment effect was significantly lower ($P < 0.05$) than all Zinc treatments. Variety by zinc
 132 rates interaction was not significant at 5% level of probability on all sampling days.

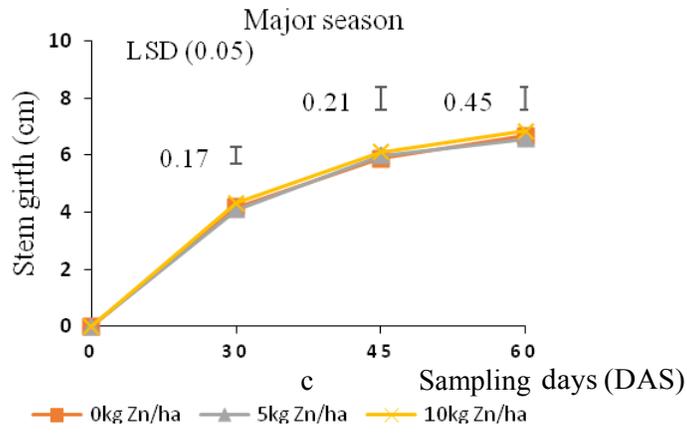


b



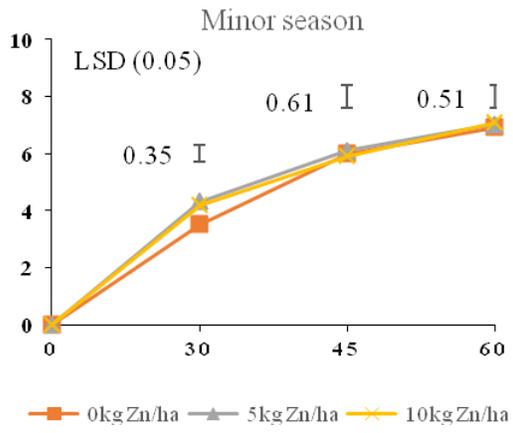
133

134

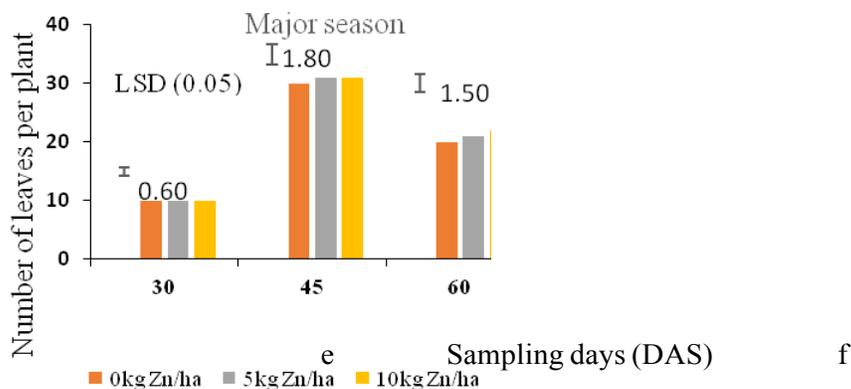


135

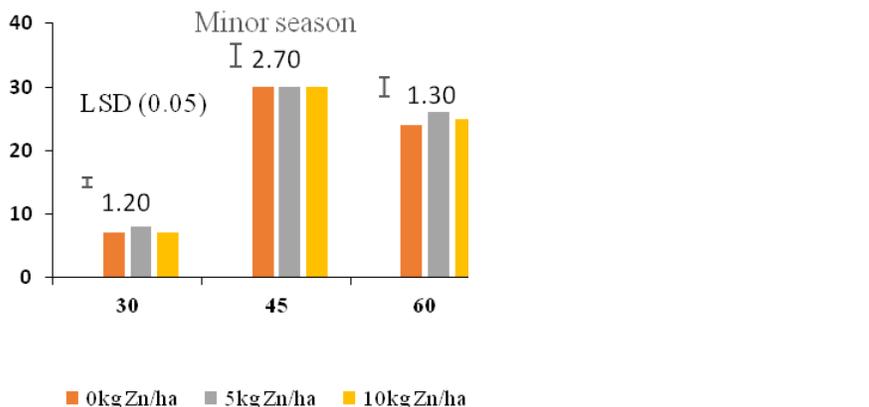
d



136



137



138

139 **Figure 2. Effects of Zinc rates on plant height (a and b), stem girth (c and d) and**
 140 **number of leaves (e and f) in 2016 cropping seasons**

3. Nodulation parameters

141 Results on number of nodules per plant, effective nodules per plant and nodule dry weight per
 142 plant as influenced by cowpea varieties and Zinc fertilizer application in the two sampling
 143 periods in both experiments are presented in Tables 1 and 2. Treatment differences for all
 144 parameters on all days at both seasons were not significant ($P > 0.05$). The interaction effect
 145 was also not significant at 5% probability. Nodule number was nearly successively decreased
 146 over time at all treatments and is not correlated with the Zinc fertilizer applied. No interaction
 147 effect was significant for all parameters at all sampling periods.

148 **Table 1. Effect of cowpea varieties in changes of nodule number (nodules per plant),**
 149 **nodule dry weight (g per plant) and effective nodules (%)**

| Varieties | Time (Days after sowing) | | | |
|--------------------------|--|-------|--------------|-------|
| | Major season | | Minor season | |
| | 30 | 45 | 30 | 45 |
| | Nodule number (nodules per plant) | | | |
| Agyenkwa | 5 | 3 | 7 | 6 |
| Asontem | 8 | 5 | 10 | 6 |
| Zamzam | 5 | 4 | 8 | 6 |
| LSD (0.05) | NS | NS | NS | NS |
| CV (%) | 21.8 | 31.8 | 14.2 | 22.4 |
| | Nodule dry weight (g per plant) | | | |
| Agyenkwa | 0.06 | 0.09 | 0.22 | 0.16 |
| Asontem | 0.04 | 0.08 | 0.19 | 0.14 |
| Zamzam | 0.05 | 0.12 | 0.18 | 0.13 |
| LSD (0.05) | NS | NS | NS | NS |
| CV (%) | 18.8 | 21.0 | 10.5 | 20.3 |
| | Effective nodules (%) | | | |
| Agyenkwa | 82.54 | 37.82 | 84.48 | 47.55 |
| Asontem | 74.44 | 32.64 | 76.56 | 42.11 |
| Zamzam | 76.94 | 65.13 | 79.72 | 68.27 |
| LSD (0.05) | NS | NS | NS | NS |
| CV (%) | 4.9 | 5.5 | 2.4 | 13.3 |
| Variety x Zn rate | NS | NS | NS | NS |

150

151 **Analysis of variance and LSD evaluations should be re-established. Because the**
mean values in the ‘effective nodules (%)’ section are very different (32.64% and
65.13%), but they are shown as NS.

152

153

154

155

156

157

158

159

160 Table 2. Changes in nodule number (nodules per plant), nodule dry weight (g per plant)
161 and effective nodules (%) of cowpea growing under Zinc foliar application

| Rates | Time (Days after sowing) | | | |
|------------|--|------|--------------|------|
| | Major season | | Minor season | |
| | 30 | 45 | 30 | 45 |
| | Nodule number (nodules per plant) | | | |
| 0 | 6 | 4 | 8 | 6 |
| 5 | 5 | 4 | 8 | 5 |
| 10 | 7 | 4 | 9 | 6 |
| LSD (0.05) | NS | NS | NS | NS |
| CV (%) | 11.3 | 13.8 | 5.0 | 11.9 |

| Nodule dry weight (g per plant) | | | | |
|--|-------|-------|-------|-------|
| 0 | 0.05 | 0.11 | 0.18 | 0.15 |
| 5 | 0.05 | 0.09 | 0.20 | 0.15 |
| 10 | 0.06 | 0.08 | 0.21 | 0.14 |
| LSD (0.05) | NS | NS | NS | NS |
| CV (%) | 9.8 | 22.8 | 7.1 | 28.5 |
| Effective nodules (%) | | | | |
| 0 | 78.56 | 43.33 | 81.97 | 49.25 |
| 5 | 74.26 | 53.11 | 76.48 | 48.88 |
| 10 | 81.11 | 39.16 | 82.31 | 59.80 |
| LSD (0.05) | NS | NS | NS | NS |
| CV (%) | 3.6 | 8.8 | 4.4 | 8.4 |
| Variety x Zn rate | NS | NS | NS | NS |

4. Yield and harvest index

162 The cowpea grain yield was significant ($P < 0.05$) under Zinc fertilizer application in all the
 163 two seasons (Table 3). Cowpea grain yield recorded on the application of Zinc fertilizer at 5
 164 kg/ha increased at 28 % for Agyenkwa > Zamzam (20 %) > Asontem (19%) compare to the
 165 control in major season and the minor season the results followed the same trend. The Zinc
 166 levels are increased the cowpea grain yield in the order: 5 kg Zn/ha > 10 kg Zn/ha > 0 kg
 167 Zn/ha during all cropping seasons (2016 major and minor seasons). The cowpea grain yield
 168 decline over increasing the Zinc rate beyond 5 kg/ha. There was about 6 % and 10 % yield
 169 reduction in the main season obtained with Agyenkwa and Asontem respectively. 100 seed
 170 weights were different at 5% level of probability. One hundred seeds weight was higher with
 171 Zamzam following by Agyenkwa and at the end Asontem with the lowest one. The shoot dry
 172 weight was significant ($p < 0.05$) affect by Zinc fertilizer application. Similarly, cowpea
 173 varieties did significant ($P < 0.05$) affect the cowpea biomass yield in all sampling periods
 174 and the interaction follows the same trend.

175

170 Table 3. Effects of Zn rates on harvest index, haulm and grain yield of cowpea

176 **If there is a statistically significant difference between the averages, the averages should be grouped with the letters.**

| Treatments | Major season | | | Minor season | | |
|------------|------------------|-------|-------------|------------------|-------|-------------|
| | 100 seeds weight | Haulm | Grain yield | 100 seeds weight | Haulm | Grain yield |

| | ← g → | ← Kg/ha → | ← g → | ← Kg/ha → | | |
|---------------------------------------|----------|-----------|---------|-----------|---------|-------|
| <u>Varieties</u> | | | | | | |
| Agyenkwa | 16.25 ab | 1382.74 | 1142.23 | 16.20 | 1311.58 | 1620 |
| Asontem | 13.63 b | 1596.68 | 1082.15 | 13.26 | 1650.58 | 1326 |
| Zamzam | 17.18 a | 1470.64 | 1423.62 | 17.07 | 1630.33 | 1707 |
| LSD (0.05) | 1.29 | 342.94 | 268.69 | 1.25 | 317.90 | 125.2 |
| CV (%) | 4.7 | 13.40 | 12.80 | 4.7 | 6.40 | 6.1 |
| <u>Zn levels (kg.ha⁻¹)</u> | | | | | | |
| 0 | 15.34 | 1451.48 | 1087.45 | 14.93 | 1440.58 | 1493 |
| 5 | 15.58 | 1440.24 | 1283.94 | 15.61 | 1587.67 | 1600 |
| 10 | 16.14 | 1558.34 | 1276.60 | 16.00 | 1564.25 | 1560 |
| LSD (0.05) | NS | 225.13 | 120.87 | 0.79 | 218.492 | 79.4 |
| CV (%) | 5.5 | 17.20 | 16.00 | 4.7 | 10.40 | 4.7 |

169

171

5. N-fixed and crude protein

172 The results of cowpea N-fixed and crude protein at all sampling periods are presented in
 173 Table 4. In this study Zinc fertilizer application interacted to significantly ($p < 0.05$) affect
 174 cowpea N-fixed and crude protein. Zinc levels increased the cowpea N-fixed and crude
 175 protein in the order: 5 kg Zn/ha > 10 kg Zn/ha > 0 kg Zn/ha. N-fixed and crude protein
 176 interaction differed significantly ($p < 0.05$) among some the treatment interactions. Zamzam
 177 variety interacted markedly to produce the highest value of N-fixed in cowpea haulm and
 178 grain and Agyenkwa presented the lowest one. For the cowpea crude protein, the result is
 179 presented in the following order: Asontem > Agyenkwa > Zamzam.

180 **Table 4. Effects of Zn rates on cowpea N-fixed and crude protein**

| Treatment | Major season | | | Minor season | | |
|---------------------------------------|---|--|-------------------------|---|--|-------------------------|
| | Grain N-fixed ← Kg.ha ⁻¹ → | Tissue N-fixed ← Kg.ha ⁻¹ → | Crude protein (%) | Grain N-fixed ← Kg.ha ⁻¹ → | Tissue N-fixed ← Kg.ha ⁻¹ → | Crude protein (%) |
| <u>Varieties</u> | | | | | | |
| Agyenkwa | 22.40 | 26.72 | 25.80 | 39.61 | 15.70 | 24.84 |
| Asontem | 26.40 | 27.64 | 29.44 | 32.23 | 16.59 | 26.86 |
| Zamzam | 31.80 | 30.82 | 24.87 | 39.14 | 20.95 | 23.44 |
| LSD (0.05) | NS | 12.32 | 0.91 | 4.81 | NS | 1.46 |
| CV (%) | 16.60 | 13.50 | 2.00 | 10.40 | 3.20 | 3.40 |
| <u>Zn levels (kg.ha⁻¹)</u> | | | | | | |

| | | | | | | |
|-------------------------|-------|-------|-------|-------|-------|-------|
| 0 | 21.80 | 26.12 | 26.88 | 33.59 | 14.12 | 24.57 |
| 5 | 30.90 | 27.26 | 27.28 | 38.39 | 20.09 | 25.56 |
| 10 | 27.90 | 31.79 | 25.95 | 39.00 | 19.03 | 25.01 |
| LSD (0.05) | 5.26 | 7.11 | 0.59 | 4.81 | 5.58 | 1.15 |
| CV (%) | 17.30 | 14.30 | 1.40 | 4.00 | 2.30 | 4.50 |
| Variety x Znrate | * | * | * | * | * | * |

183

6. Effects of Zinc rates on NPK content

184 The results of grain nutrients analysis showed no significant varietal effects for content of
 185 nitrogen and Phosphorus (Table 5). Additionally, Potassium content in the haulms was not
 186 different among varieties. However, for Potassium content in seed, the Zn treatments effects
 187 were similar, but greater either effect was greater than the control treatment effect in both
 188 seasons.

189 Table 5. NPK content of cowpea as affected by varieties

| Rates | Nutrient uptakes (kg/ha) | | | |
|----------|--------------------------|-------|--------------|-------|
| | Major season | | Minor season | |
| | Haulm | Grain | Haulm | Grain |
| | N | | | |
| Agyenkwa | 40.92 | 47.21 | 29.90 | 64.41 |
| Asontem | 41.84 | 51.18 | 30.80 | 57.03 |
| Zamzam | 45.46 | 56.61 | 35.20 | 63.94 |

| | | | | |
|------------|-----|-----|------|-----|
| LSD (0.05) | NS | NS | NS | NS |
| CV (%) | 8.4 | 6.9 | 9.40 | 6.2 |

P

| | | | | |
|------------|------|------|------|-------|
| Agyenkwa | 3.23 | 5.46 | 3.53 | 12.19 |
| Asontem | 3.39 | 5.09 | 3.80 | 14.33 |
| Zamzam | 2.75 | 5.68 | 3.77 | 12.33 |
| LSD (0.05) | NS | NS | NS | NS |
| CV (%) | 7.5 | 6.5 | 7.1 | 7.70 |

K

| | | | | |
|------------|-------|-------|-------|-------|
| Agyenkwa | 14.32 | 12.01 | 13.95 | 20.92 |
| Asontem | 13.17 | 13.61 | 13.58 | 16.84 |
| Zamzam | 10.79 | 13.83 | 11.72 | 20.43 |
| LSD (0.05) | NS | 1.27 | NS | 2.89 |
| CV (%) | 12.1 | 7.20 | 7.70 | 4.30 |

191

192 For the Zn treatments, N content of grain was affected by Zn application in both seasons.
 193 Haulm N content was also significantly affected by Zn fertilizer application. In all these
 194 cases, treatments differences between the Zinc treatments were similar, but either effect was
 195 greater than the control treatment (Table 6). Haulm P content were significantly affected by
 196 Zn fertilizer application (Table 6), with the exception haulm Zinc content in the major season,
 197 where the control treatment effect was similar to the 5 kg/ha Zn treatment. In all cases, the Zn
 198 treatment effects were similar, and either effect was significantly higher than the control
 199 treatment effect. Haulm K content was not affected by Zn fertilizer in both seasons (Table 6).
 200 However, grain K content significantly affected by the Zn fertilizer in both seasons. In both
 201 seasons, grain K content in the control treatment was lower than the Zn treatment effects.

202 Table 6. NPK content of cowpea as affected by Zinc fertilizer

| Rates | Nutrient uptakes (kg/ha) | | | |
|-------|--------------------------|-------|--------------|-------|
| | Major season | | Minor season | |
| | Haulm | Grain | Haulm | Grain |
| 0 | 40.32 | 46.63 | 28.30 | 58.39 |

| | | | | |
|------------|-------|-------|-------|-------|
| 5 | 41.46 | 55.69 | 34.30 | 63.19 |
| 10 | 45.99 | 52.68 | 33.20 | 63.80 |
| LSD (0.05) | NS | 5.26 | 5.59 | 2.29 |
| CV (%) | 8.90 | 8.20 | 9.40 | 2.40 |

P

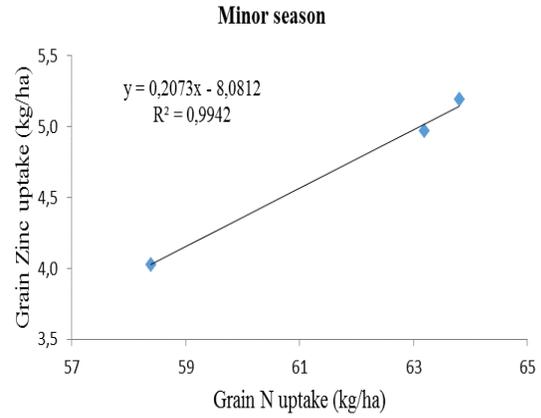
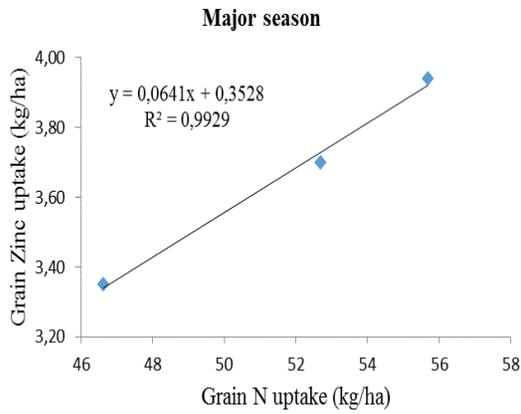
| | | | | |
|------------|------|-------|------|-------|
| 0 | 2.94 | 4.70 | 3.22 | 12.01 |
| 5 | 2.89 | 6.09 | 3.88 | 13.61 |
| 10 | 3.54 | 5.44 | 4.01 | 13.83 |
| LSD (0.05) | 0.55 | 0.70 | 0.65 | 1.27 |
| CV (%) | 9.20 | 13.50 | 9.60 | 7.20 |

K

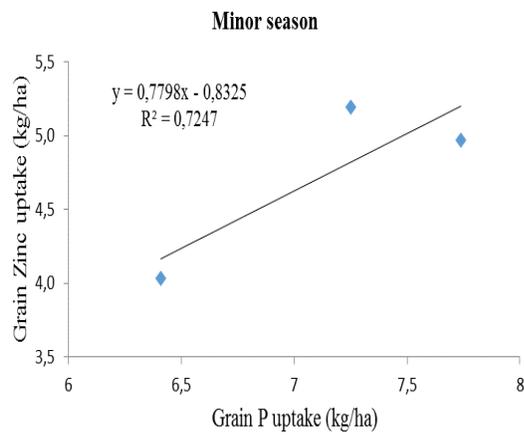
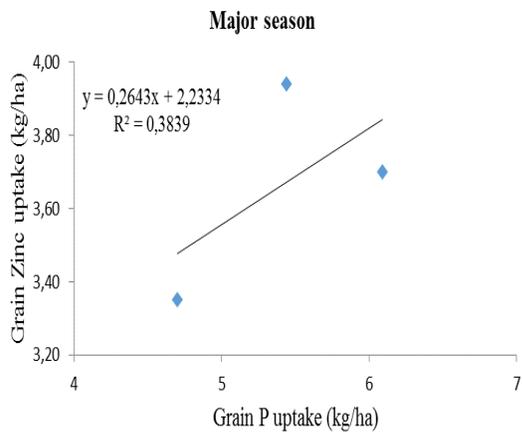
| | | | | |
|------------|-------|-------|-------|-------|
| 0 | 12.45 | 12.01 | 12.42 | 17.84 |
| 5 | 13.38 | 13.61 | 14.38 | 20.27 |
| 10 | 12.45 | 13.83 | 12.45 | 20.08 |
| LSD (0.05) | NS | 1.27 | NS | 1.67 |
| CV (%) | 14.30 | 7.20 | 17.30 | 8.10 |

7. Interrelationship between Zinc and NPK uptake in plant grain

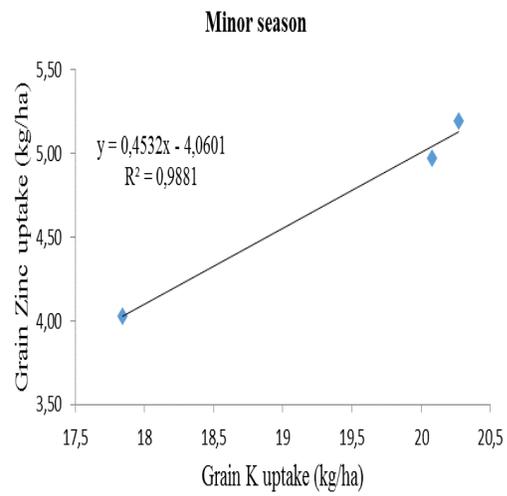
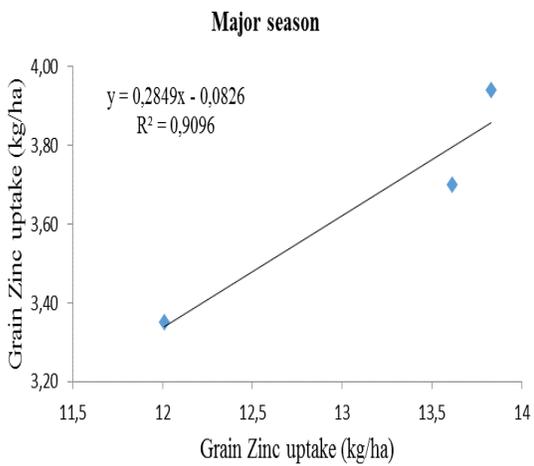
203 The linear regression showed the positive relationship between grain Zn uptake and NPK
 204 content for three sampling periods during the experiment in the major and minor seasons
 205 (Fig. 3). The argument on the enhanced NPK uptake y Zn content was ably supported by the
 206 significant positive relationship observed in the present study between NK and Zn uptake
 207 (0.9929*** with N and 0.9096** with K) in the major cropping season. The minor cropping
 208 season also follows the same trend with 0.9942** and 0.9389** with N and K respectively.
 209 And with P the relationship was weak but positive (0.3839 in major season and 0.7289 in
 210 minorseason).



211



212



213

214 **Figure 3. Relationship between Zn uptake and the macronutrients (NPK) in 2016**
215 **cropping seasons**

216 **DISCUSSION**

217 Plant height was affected by both variety and Zn rates. Among the Zn treatments, plant
218 height was greatest in the Zn plots applied in both cropping seasons. [17] reported that added
219 Zn significantly increased plant height by increasing internodes distances. [18] stated that
220 grain yield was positively correlated with leaf weight, stem weight, plant height and number
221 of branching per plant. Zinc fertilizer application did not, however, have any significant
222 effect on the number of leaves and stem girth in all the days examined. It was reported that
223 application of zinc had positive effects on growth parameters [19]. Contrarily, foliar
224 application of micronutrients increased the diameter of plant over the control treatment [20].
225 So, these findings conclude that the entire cowpea varieties gave equal stem diameter at all
226 treatments of zinc application.

227 The application of the Zn fertilizer did not affect nodulation, indicating that some of
228 inoculation factors were limiting such as soil pH, initial phosphorus and others
229 micronutrients. And also, [21] reported that the nodule initiation may depend on the relative
230 concentrations of plant-specific signals and host species appears to be a significant factor
231 determining the maximum number of nodules generated. Effective nodule is essential for a
232 functioning Legumes-Rhizobium symbiosis and Zinc, chloride and cobalt have no effect on
233 nodulation but are required for the growth of the host legume [22]. Two hosts may have the
234 same sensitivity to bacterial signal molecules, but might differ in their ability to elicit
235 synthesis of required nodulation signals in the bacteria [23]. Cowpea root exudates have also
236 been reported to contain substances that enhance nodule initiation [24, 25, 26]. However,

237 lower efficiency of cowpea cannot be readily explained in terms of reduced numbers of
238 bacteria in contact with the root [21]. Varieties most susceptible to infection and capable of
239 producing effective nodules should have greater potential to fix more atmospheric N.
240 However, this assumption often depends on other factors such as the environment and crop
241 management [27]. Indeed [28] reported that the ability to form nodules is not enough to
242 obtain an effective nitrogen fixation symbiosis. Nodule number was nearly successively
243 decreased over time at all treatments and is not correlated with the Zinc fertilizer applied. The
244 amount of nodule dry biomass was drastically reduced with the mineral Zinc fertilizer,
245 whereas the amount of nodule biomass was not affected in the control group, probably
246 because the soil had satisfactory levels of available N and P. Nodule number correlated
247 negatively with nodule dry weight [29]. The interaction effect was also not significant at 5%
248 probability.

249 The present results were supported by [30] who reported that foliar application of
250 micronutrients help in improving yield. In both seasons, foliar spray of Zn fertilizer had effect
251 on hundred grain weights. In all these parameters, the control treatment effect was lower than
252 Zn treatments, whereas among the Zn treatments. [31] reported that following Zn fertilization
253 increased hundred seed weight. Also, [32] reported that yield and its components in lentil are
254 improved by foliar application of micronutrients. Crop yields and quality are reduced by Zn
255 inadequate in soil; therefore, Zn utilization is essential to obtain high yield and quality in crops as
256 showed the results (Table 3). These results are in close conformity with those of [33, 34, 35].
257 This was because of the fact that better and higher availability of Zinc, resulting better
258 nutritional environment, higher dry matter accumulation and its associated effect on growth
259 attributes increased haulm and grain yield. It is also evident from table 3 that all the Zinc
260 treated plots increased the grain yield over the control, as there was a consistent increase in

261 cowpea grain yield up to 10 kg Zn/ha. This suggests that, the application of Zn significantly
262 affect cowpea yield. Similar results were reported as in [36] and [37]. In this connection, [38]
263 reported that the foliar application of Zn affected yield and its components of soybean. Also,
264 [39] reported that the highest yield of common bean was obtained by Zinc foliar application.
265 [40] believe that more production of chlorophyll and IAA can cause delay in plant oldness
266 and prolong the period of photosynthesis. This incident improves the production of
267 carbohydrates and their transportation to the growing seeds.

268 The Zn deficiency symptoms can be prevented by the application of Zn fertilizers. The actual
269 causal relationship and mechanisms are still not fully understood [3]. As shown in Tables 4
270 and 5 the mean percentage total nitrogen, phosphorus and potassium uptakes in the harvested
271 leaves were quantitatively higher under zinc fertilizer application and increased with
272 incremental zinc rates. These results corroborate the findings of [41] and [42] who reported
273 that zinc is an essential micronutrient for plant growth and plays an important role in the
274 catalytic part of several enzymes its deficiency will result in stunted growth and nutrient
275 uptakes. And also, [43] reported that zinc exerts a great influence on basic plant life
276 processes, such as (i) nitrogen metabolism – uptake of nitrogen and protein quality; (ii)
277 photosynthesis - chlorophyll synthesis and carbon anhydrase activity. Also many researchers
278 have observed that Zn is closely related to the nitrogen metabolism pathway of plants, thus
279 causing a reduction in protein synthesis for Zn deficient plants. Zinc deficiency significantly
280 affects the root system including root development [44].

281 **CONCLUSION**

282 Zn fertilizer significantly affected NPK content and grain yield of cowpea varieties used. The
283 increment of Zn content in the grain had a positive relationship with NK, which will

284 definitely enhance nutrition of both human and animals. At all sampling periods, nodule
285 number per plant was not affected by Zinc rates and nodule number was nearly successively
286 decreased over time at all treatments and is not correlated with the Zinc fertilizer applied. The
287 Zinc fertilizer significantly enhanced N-Fixed and Crude protein in both cropping season's
288 trial investigating effect of Zinc rates on growth, nodulation and mineral content of cowpea in
289 the semi-deciduous forest zone of Ghana. This implies the Zn rates used can be applied to
290 any of the varieties used.

291 REFERENCES

- 292 1. Olsen SR. Micronutrient Interactions. *JM Mortved, JJ Goirdano, WL Lindsay (eds)*
293 *Micronutr Agric Soil Sci Soc Am.* 1972;6(August):243-264.
- 294 2. Imtiaz M., Alloway BJ., Shah KH., Siddiqui SH., Memon MY., Alsam M. and Khan
295 P. Zinc Nutrition of Wheat: II: Interaction of Zinc with other Trace Elements. *Asian J*
297 *Plant Sci.* 2003;2:156-160.
- 298 3. Alloway BJ. Zinc in Soils and Crop Nutrition. Second edition, published by IZA and
299 IFA, Brussels, Belgium and Paris, France. 2008;139.
- 300 4. Unkovich, M. and Baldock, J. Measurement of asymbiotic N₂ fixation in Australian
301 agriculture. *Soil Biol Biochem.* 2008;40:2912 – 2921.
- 302 5. Craufurd PQ., Summerfield RJ., Ellis RH. and Roberts EH.. Photoperiod, temperature,
303 and the growth and development of cowpea. *Adv cowpea Res Int Inst Trop Agric*
304 *Ibadan, Niger Japan Int Res Cent Agric Sci Tsukuba, Ibaraki, Japan.* 1996;II:75-86.
- 305 6. Gates M. Bulletin of Tropical Legumes. *Area.* 2011;(October):5-8.
- 306 7. Hall AE. Breeding for adaptation to drought and heat in cowpea. *Eur J Agron.*

- 307 2004;21(4):447-454.
- 308 8. Epstein E. and Bloom AJ. Mineral Nutrition of Plants: Principles and Perspectives.
309 *Sinauer Assoc.*; 2005.
- 310 9. Latham MC. Human Nutrition in the Developing World. Food and N. Rome/Italy;
311 1997.
- 312 10. FAO and WHO. Vitamin and Mineral Requirements in Human Nutrition Second
313 Edition.; 1998.
- 314 11. AOAC. Official Methods of Analysis. 17th ed. Gaithersburg, Maryland, USA, AOAC
315 International; 2000.
- 316 12. Jones DB. Factors for Converting Percentages of Nitrogen in Foods and Feeds into
317 Percentages of Proteins. Circular No. 183. Washington, DC, United States Department
318 of Agriculture.; 1941.
- 319 13. Varvel GE. and Peterson TA. Nitrogen fertilizer recovery by corn in monoculture and
320 rotation systems. *Agron J.* 1990;82:935-938.
- 321 14. Motsa, M.R. and Roy RN. Guide to Laboratory Establishment for Plant Nutrient
322 Analysis. FAO. Rome, Italy; 2008.
- 323 15. Moss P. Limits of interference by Fe, Mn, Al and phosphate in the EDTA
324 determination of Calcium in the presence of Mg using calcon-red as indicator. *J Sci, F*
325 *Agric.* 1961;12:30-34.
- 326 16. Okalebo JR., Gathua KW. and Woomer PL. Laboratory Methods of Soil and Plant
327 Analysis: *A Working Manual*. Vol 1. Tropical S. Nairobi/Kenya: Soil Science Society
328 of East Africa; 1993.

- 329 17. Kaya C., Higgs D. and Burton A. Phosphorus acid phosphates enzyme activity in
330 leaves in leaves of tomato cultivars in relation to Zn supply. *Commun Soil Sci Plant*
331 *Anal.* 2000;31:3239-3248.
- 332 18. Jatasra, D.S. and Dahiya B. Relative importance of forage yield components in
333 cowpeas under dry land conditions. *Indian J Agric Resour.* 1988;22(1):1-5.
- 334 19. Malakouti MJ. The Effect of Micronutrients in Ensuring Efficient Use of
335 Macronutrients. *Turkish J Agric For.* 2008;32:215-220.
- 336 20. Shuman LM. Fractionation Method for Soil Micronutrients. *Soil Sci.* 1985;140:11-22.
- 337 21. Bhuvanewari, T.V., Lesniak, A.P., and Wolfgang D. Efficiency of Nodule Initiation
338 in Cowpea and Soybean. *Plant Physiol.* 1988;86:1210-1215.
- 339 22. FAO. Legume Inoculants and Their Use. Rome; 1984.
- 340 23. Peters, N.K, Frost, J.W and Long S. A plant flavone, luteolin induces expression of
341 *Rhizobium meliloti* nodulation genes. *Sci Agric.* 1986;234:977-980.
- 342 24. Bhagwat A.A and Thomas J. Legume-Rhizobium interactions: cowpea root exudate
343 elicits faster nodulation response by *Rhizobium* species. *Appl Environ Microbiol.*
344 1982;43:800-805.
- 345 25. Bhagwat, A.A and Thomas J. Legume-Rhizobium interactions: role of cowpea root
346 exudate in polysaccharide synthesis and infectivity of *Rhizobium* species. *Arch*
347 *Microbiol.* 1983;136:102-105.
- 348 26. Bhagwat, A.A and Thomas J. Legume-Rhizobium interactions: host induced
349 alterations in capsular polysaccharides and infectivity of cowpea rhizobia. *Arch*
350 *Microbiol Microbiol.* 1984;140:260-264.

- 351 27. Kellman AW. Rhizobium inoculation cultivar and management effects on the growth,
352 development and yield of common bean (*Phaseolus vulgaris* L.). 2008.
- 353 28. Giller KE. Nitrogen Fixation in Tropical Cropping System. Wallingfor. UK; 2001.
- 354 29. Sarkodie-Addo J. Evaluation of Bradyrhizobium japonicum isolates from Ontario 74
355 soybean fields. 1991.
- 356 30. Arif M., Chohan MA., Ali S., Gul R. and Khan S. Response of wheat to foliar
357 application of nutrients. *J Agric Biol Sci.* 2006;1:30-34.
- 358 31. Yilmaz A., Ekiz H., Torun B., Gulekin I., Karanlink S., Bagci S.A. and Cakmak I.
359 Effect of different zinc application methods on grain yield and zinc concentration in
360 wheat cultivars grown on zincdeficient calcareous soils. *J Plant Nutr.* 1997;20:461-
361 471.
- 362 32. Zeidan M.S., Hozayn M., and Abd El-Salam MEE. Yield and quality of lentil as
363 affected by micronutrient deficiencies in sandy soils. *J Appl Sci Res.* 2006;2:1342-
364 1345.
- 365 33. Sharma SK. and Jat ML. Effect of sulphur on growth and yield of cowpea [*Vigna*
366 *unguiculata* (L.) Walp]. *Ann Agric Res New Ser.* 2003;24:215-216.
- 367 34. Yadav SS. Growth and yield of cowpea [*Vigna unguiculata* (L.) Walp] as influenced
368 by phosphorus and sulphur fertilization. *Haryana J Agron.* 2004;20:10-12.
- 369 35. Tripathi H.C., Pathak R.K., Kumar A. and Dimsec S. Effect of sulphur and zinc on
370 yield attributes, yield and nutrient uptake in chickpea. *Ann Plant Soil Res.* 2011;13:134-
371 136.
- 372 36. Moswatsi MS. Response of cowpea to variable rates and methods of zinc. Thesis,

- 373 University of Limpopo. 2015; 77.
- 374 37. Oseni T. Growth and Zinc Uptake of Sorghum and Cowpea in Response to Phosphorus
375 and Zinc Fertilization. *World J Agric Sci.* 2009;5(6):670-674.
- 376 38. Banks L. Effect of timing of foliar zinc fertilizer on yield component of soybean. *Aust*
377 *J Exp Agric Anim Husb.* 2004;22:226-231.
- 378 39. Seifi NM., Yarnia M. and Rahimzade KF. Effect of zinc and manganese and their
379 application method on yield and yield components of common bean (*Phaseolus*
380 *vulgaris* L. cv. Khomein). *Middle-East J Sci Res.* 2011;8:859-865.
- 381 40. Hemantaranjan, A. and Grag OK. Iron and zinc fertilization with reference to the grain
382 quality of triticum Aestivum L. *J Plant Nutr.* 1988;11:1439-1450.
- 383 41. Fageria NK. Dry matter yield and nutrient uptake by lowland rice at different growth
384 stages. *J Plant Nutr.* 2004;27:947-958.
- 385 42. Sunitha, K Padma, S N Vasandha, S and Anitha S. Microbial Inoculants- A Boon to
386 Zinc Deficient Constraints in Plants : A Review. *Int J Sci Res Publ.* 2014;4(6):4-7.
- 387 43. Potarzycki, J. and Grzebisz W. Effect of zinc foliar application on grain yield of maize
388 and its yielding components. *Plant, Soil Environ.* 2009;55:519-527.
- 389 44. Fageria N. Influence of micronutrients on dry matter yield and interaction with other
390 nutrients in annual crops. *Pesq Agropec Bras.* 2002;37:1765-1772.