

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

3 **Abstract**

4 Cowpea can fix atmospheric nitrogen through symbiotic association with indigenous rhizobia
5 but unfortunately, the amount of N₂-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 applications
7 on growth, nodulation and nutrient uptakes of cowpea was investigated during the major and
8 minor cropping seasons (2016). Cowpea seed varieties were treated to foliar spray with three
9 different rates of Zinc sulfate (0, 5 and 10 kg Zn ha⁻¹) at 3 and 5 weeks after sowing. The split
10 plot design was used for both cropping seasons. This study shows that the supply of Zn
11 fertilizer applications did not affect growth and nodulation in 2016 major and minor cropping
12 seasons. The application of the 5 kg Zn ha⁻¹ led to better cowpea production and greatly
13 improve the quantity (haulm and grain yield) and quality (NPK content and crude protein) of
14 cowpea in both haulm and grain. The Zinc fertilizer significantly enhanced N₂-Fixed in both
15 cropping season's trial investigating. The results suggest that cowpea responds differently to
16 Zinc Sulphate application depending on its rates and the application of the 5 kg Zn ha⁻¹ is the
17 optimum rate that will enhance the yield and nutrient quality of cowpea in the Semi-
18 Deciduous Forest Zone of Ghana.

19 Keywords: N₂-fixed, Nodulation, Zn fertilizer, NPK uptake and yield

20 **INTRODUCTION**

21 Proper nutrition of plants with micronutrients depends on various factors, such as the rate of
22 absorption of nutrients by the plants, distribution of nutrients to functional sites and nutrient
23 mobility within the plant. Interactions occur between the micronutrients and some nutrients

24 [1, 2, 3]. The amount of nitrogen fixed is usually high in soils with low mineral N but with
25 sufficient water and enough of other nutrients capable of supporting plant growth [4].
26 Another factor is the differential response of plants to one nutrient in combination with
27 varying levels of a second element applied simultaneously i.e. the two elements combine to
28 produce an added effect not due to each of them acting alone [1, 2]. Such interactions may
29 take place in the soil and within the plant [3]. However, the amount of nutrients uptake is
30 strongly dependent on nutritional and environmental factors.

31 Cowpea is especially important for dry savannah of West Africa between latitudes 7 and
32 14°N [5] and second after groundnut as the most important legume of Ghana in terms of
33 space under cultivation (156,000 ha) and quantity produced and consumed annually (143,000
34 Mg) making Ghana among the largest cowpea producer in Africa [6]. Cowpea is a protein-
35 rich component of an otherwise protein-poor diet [7]. Many researchers have observed that
36 Zn have a positive relationship with the nitrogen metabolism pathway of plants, its deficiency
37 cause a reduction in protein synthesis into the plants. Epstein and Bloom [8] identified the
38 positive relationship between the flowering and fruiting process and Zn. As micronutrient,
39 Zinc has received much recent attention [9] because it is present in all body tissues and fluids
40 [10].

41 The native rhizobia are often low in numbers or ineffective and are therefore not able to fix
42 enough nitrogen to meet the nitrogen demand of plants. Including the Zn effect in this study
43 will help to determine the optimal rate that can maximize the dual mineral contents and
44 nodulation for better cowpea production.

45 **MATERIALS AND METHODS**

46 The study was conducted at the Plantation Section of the Department of Crop and Soil
47 Sciences, Faculty of Agriculture, KNUST, in the cropping seasons of 2016. The site is
48 located at 06° 45' N and 01° 31' W in the rainforest belt of Ghana. The total nitrogen content
49 was low with a mean value of 0.06%, available P content was low with value of 6.4 mg kg⁻¹,
50 soil Zn content was moderately low, found to be 1.290 mg kg⁻¹. The experiment design was
51 split plot, with treatments arranged in Randomized Completely Block Design (RCBD). The
52 factors assessed were cowpea varieties (main-plot factor) and Zn fertilizer (sub-plot factor).
53 The treatment combinations were replicated four times in 2016 major and minor cropping
54 seasons. Cowpea varieties were obtained from the Council for Scientific and Industrial
55 Research (CSIR) at Fumesua, Kumasi, Ghana. Three early maturing cowpea varieties
56 (Asontem, Agyenkwa and Zamzam) were grown in 2016 major and minor experiments and
57 selected according to their yield, number of days to physiological maturity (62-70 days) and
58 availability in the study area. The Zn fertilizer was obtained from “Chinese woman
59 company”, one of fertilizer shops in Kumasi, Ghana. Zinc sulfate heptahydrate
60 (ZnSO₄.7H₂O) was applied at a rate of 44.86 kg ZnSO₄ ha⁻¹ (equivalent to 10 kg Zn ha⁻¹) and
61 22.43 kg ZnSO₄ ha⁻¹ (equivalent to 5 kg Zn ha⁻¹). Foliar application to cowpea was done by
62 dissolving 1.0 kg of the zinc sulphate salt into 278 litres of distilled water [11] and was
63 sprayed on plant leaves at 3 weeks (40%) and 5 weeks (60%) after sowing when
64 canopy/leaves had established. The application was done early morning before 9:00 am,
65 using a sprayer. Urea and Triple Super Phosphate (TSP) fertilizers were applied as band
66 placement by making a furrow of 5-7 cm deep and covering with 2 cm of soil. Urea and
67 Triple Super Phosphate (TSP) fertilizers were applied as band placement by making a furrow
68 of 5-7 cm deep and covering with 2 cm of soil. As starter nitrogen, Urea at the rate of 20 kg
69 N ha⁻¹ and Triple Super Phosphate (TSP) at the rate of 40 kg P₂O₅ ha⁻¹ were applied uniformly
70 to all plots at two weeks after sowing (WAS). The plot (3 x 2 cm) was demarcated three days

71 after harrowing and seeds were sown by hand using manual labour. Seeds were sown at a
72 spacing of 60 cm x 20 cm with a rate of two seeds per hill at the depth of 3-5 cm. The first (3
73 weeks after sowing) and the second (7 weeks after sowing) weeding were done manually
74 using hand hoe. Standard agronomic and plant protection treatments were used uniformly
75 across the plots for the duration of the experiment. Grass hoppers (*Empoasca kerri Pruth*),
76 Thrips (*Caliothrips indicus Bagnall*) and Aphids (*Aphis craccivora Koch*) were pests,
77 respectively at vegetative stage and flowering to the end of pod filling. Lambda master 2.5 %
78 E.C. [Active ingredients (Lambda-Cyhalothrin, 9.8 %)] was the pesticides used for pests'
79 control.

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

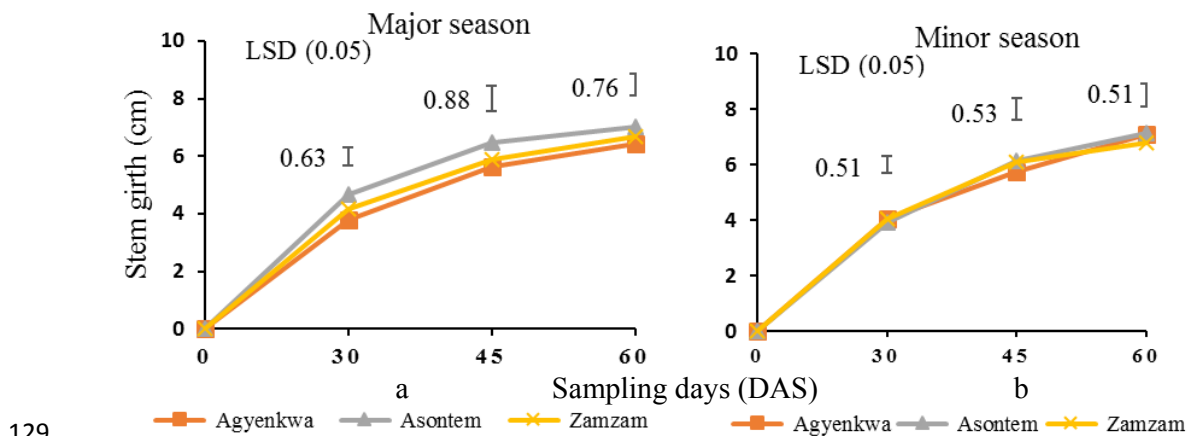
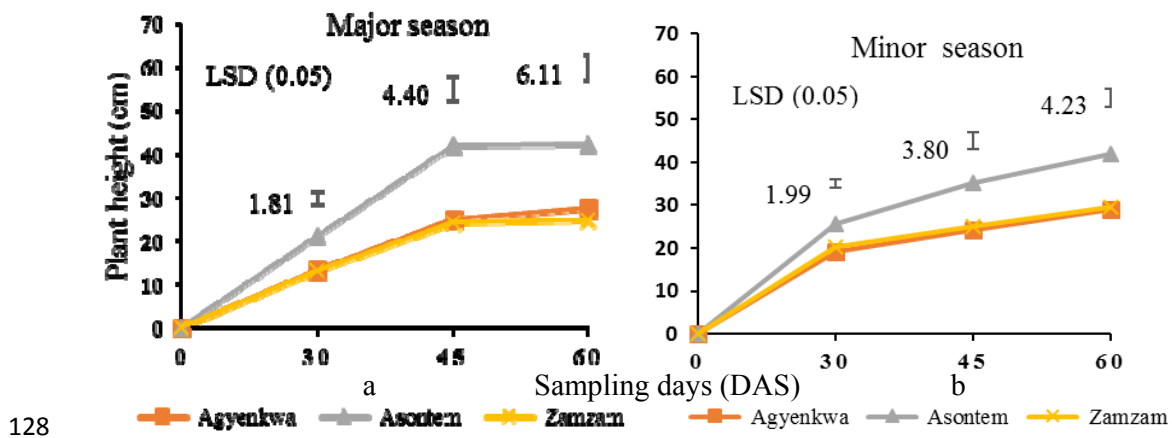
95 nitrogen content was determined using the Kjeldahl method [12]. The protein content of seed
96 was determined on the basis of total nitrogen content [13]. Phosphorus (P), the content was
97 measured on the Spectronic 20 spectrophotometer to give absorbance measurements at a
98 wavelength of 420 nm. The observed absorbance was used to determine the P content from
99 the standard curve [15, 16] and Potassium (K) was obtained using the flame photometer.
100 From the standard curve, the concentration of K was calculated using the particular
101 absorbance observed for the sample. NPK uptake were done by multiplying the grain and
102 haulm yield in kilograms per hectare by each analysed parameter separately, nitrogen,
103 phosphorus and potassium, in the grain and haulm then divided by 100 percent. This was
104 done by multiplying the haulm yield in kilograms per hectare by concentrations of Nitrogen,
105 Phosphorus and Potassium. From N uptake in both grain and haulm of cowpea and reference
106 crop (Omankwa maize variety), N-fixed was obtained using the N-difference method [14].
107 The reference crop was planted at the same time with cowpea varieties during the major and
108 minor seasons (2016). The total nitrogen content of the maize was 1.27 % in the grain and
109 0.62 % in the haulm. The yield of the reference crop was 1949 kg ha⁻¹ (grain yield) and 2285
110 kg ha⁻¹ (haulm yield). Zn content was determined using Perkins model 403 atomic absorption
111 spectrophotometer after digestion. The file for the type of analysis and hollow cathode lamps
112 were selected with appropriate wavelengths of 213.9 nm [17]. The grain and straw yields
113 were recorded separately. Total Zn uptakes by grain and tissue were computed by multiplying
114 Zn content and their respective dry weights ha⁻¹. Data collected were subjected to analysis of
115 variance (ANOVA) according to [18] using GenStat statistical software [19]. The Least
116 Significant Difference (LSD's test) was used to compare mean data when the probability level
117 was significant.

118

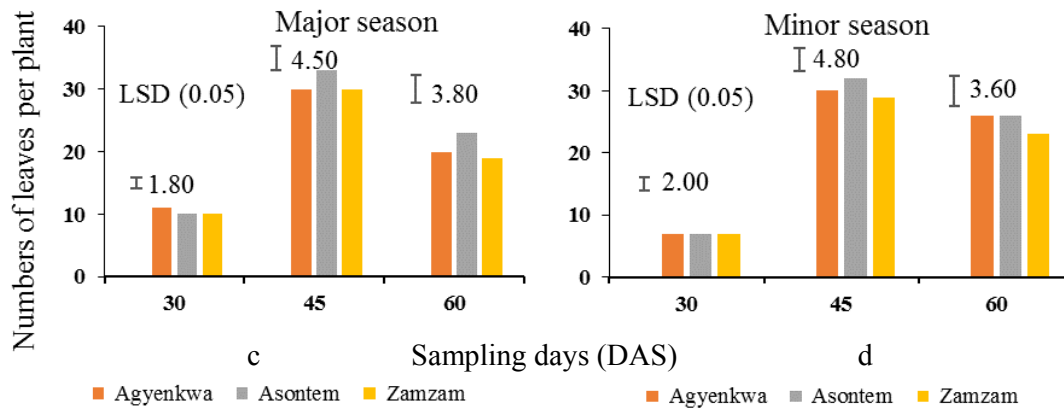
119 **RESULTS**

120 **1. Effects of cowpea varieties on growth**

121 Fig. 1 illustrates the effect of different cowpea varieties on plant height (cm), stem girth (cm)
 122 and number of leaves/plant over the period of the experiment. The significant effect at 5%
 123 level of probability of cowpea varieties used was recorded over all sampling period of the
 124 study. The tallest plant was obtained by Asontem variety and the lowest by Zamzam.
 125 However, cowpea varieties did not show any significant ($P > 0.05$) effect on stem girth and
 126 number of leaves. Additionally, variety by Zinc rates was not significantly different on all
 127 days of sampling.



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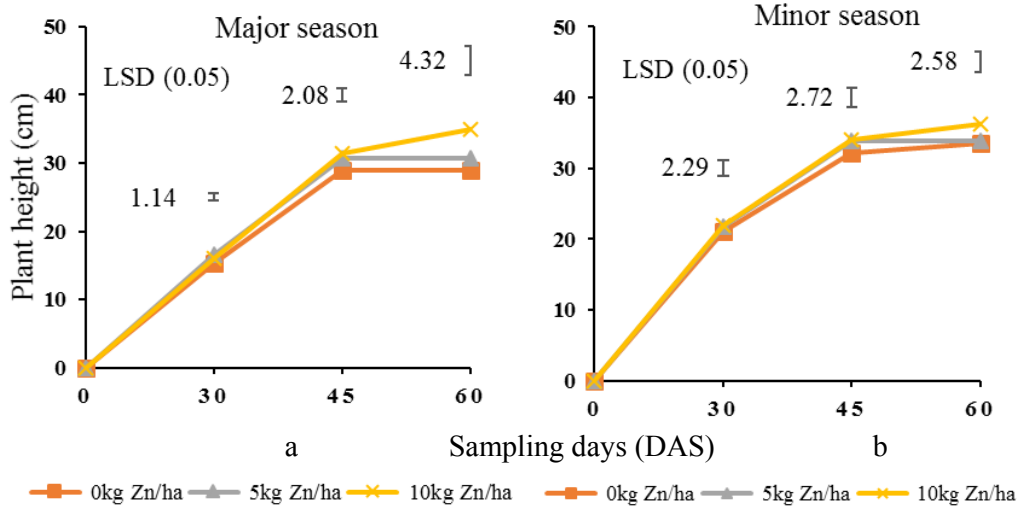


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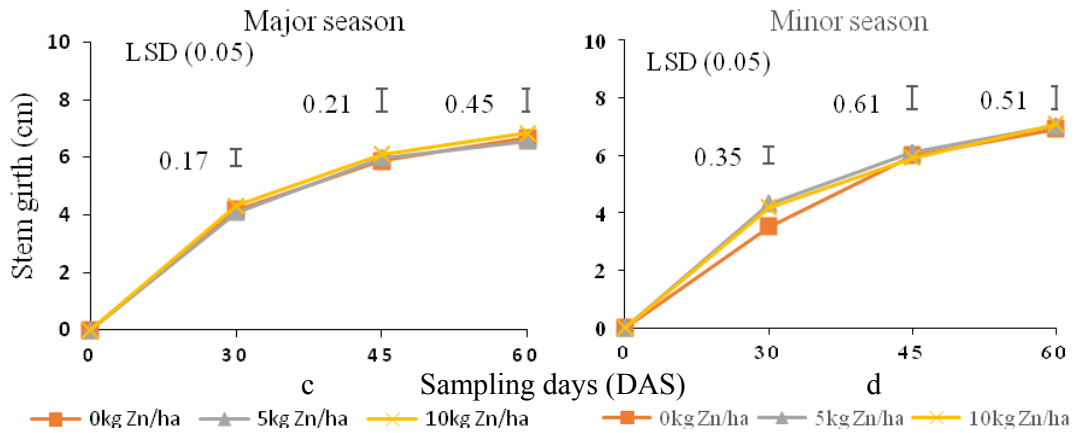
131 Figure 1. Effects of cowpea varieties on Plant height (a and b), stem girth (c and d) and
 132 number of leaves (e and f) under Zinc foliar application in 2016 cropping seasons

133 **2. Effects of Zinc rates on growth**

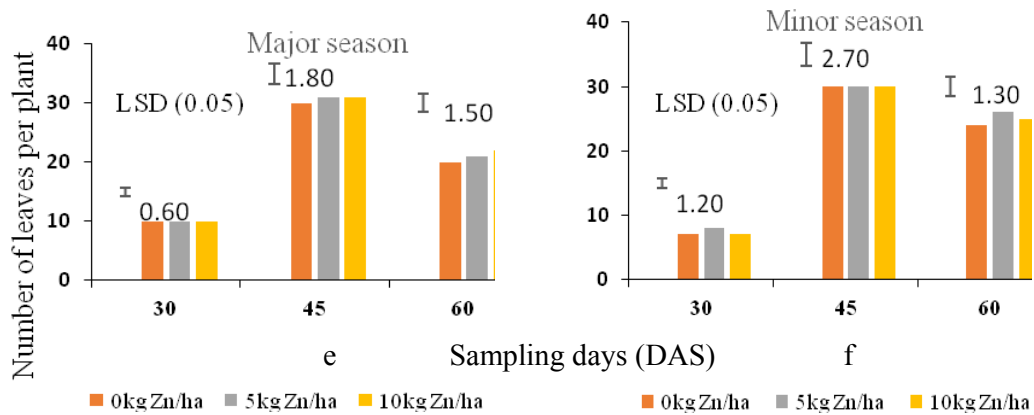
134 Fig. 2 illustrates the effect of different rates of Zn fertilizer application on plant height (cm),
 135 stem girth (cm) and number of leaves/plant over the period of the experiment. Analysis of
 136 variance showed no significant effect of Zn fertilizer on plant height and leaf production.
 137 Branch production was significantly affected by Zn rates in both seasons. At 30 DAS and 45
 138 DAS, the 5 kg/ha treatment effect was significantly higher than other treatment effects. At 60
 139 DAS, the control treatment effect was significantly lower ($P < 0.05$) than all Zinc treatments.
 140 Variety by zinc rates interaction was not significant at 5% level of probability on all sampling
 141 days.



142



143



144

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

147 **3. Nodulation parameters**

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

155 **Table 1. Effect of cowpea varieties in changes of nodule number (nodules/ plant), nodule**
 156 **dry weight (g/ plant) and effective nodules (%)**

Varieties	Time (Days after sowing)			
	Major season		Minor season	
	30	45	30	45
	Nodule number (nodules/ 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/ 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	45.13	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
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157

158 **Table 2. Changes in nodule number (nodules per plant), nodule dry weight (g per plant)**
 159 **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/ 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/ 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

160

4. Yield

161 The cowpea grain yield was significant ($P < 0.05$) under Zinc fertilizer application in all the
 162 two seasons (Table 3). Cowpea grain yield recorded on the application of Zinc fertilizer at 5
 163 kg/ha increased at 28 % for Agyenkwa > Zamzam (20 %) > Asontem (19%) compare to the
 164 control in major and the minor seasons. The Zinc levels are increased the cowpea grain yield
 165 in the order: 5 kg Zn ha⁻¹ > 10 kg Zn ha⁻¹ > 0 kg Zn ha⁻¹ during all cropping seasons (2016

166 major and minor seasons). The cowpea grain yield decline over increasing the Zinc rate
 167 beyond 5 kg ha⁻¹. There was about 6 % and 10 % yield reduction in the main season obtained
 168 with Agyenkwa and Asontem respectively. For one hundred seed weights, there were
 169 different results at 5% level of probability. One hundred seeds weight was higher with
 170 Zamzam following by Agyenkwa and at the end Asontem with the lowest one. The shoot dry
 171 weight was significant (p < 0.05) affect by Zinc fertilizer application. Similarly, cowpea
 172 varieties did significant (P < 0.05) affect the cowpea biomass yield in all sampling periods
 173 and the interaction follows the same trend.

174

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

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 ^a	1352.74 ^b	1142.23 ^{ab}	16.20 ^a	1311.58 ^b	1620.10 ^a
Asontem	13.63 ^b	1596.68 ^a	1082.15 ^b	13.26 ^b	1650.58 ^{ab}	1326.17 ^b
Zamzam	17.18 ^a	1470.64 ^{ab}	1423.62 ^a	17.07 ^a	1630.33 ^a	1707.63 ^a
LSD (0.05)	1.29	242.94	268.69	1.25	317.90	125.21
CV (%)	4.7	13.40	12.80	4.71	6.40	6.10
<u>Zn levels (kg ha⁻¹)</u>						
0	15.34	1351.48 ^b	1087.45 ^b	14.93 ^b	1340.58 ^b	1493.22 ^b
5	15.58	1440.24 ^{ab}	1283.94 ^a	15.61 ^a	1587.67 ^a	1600.13 ^a
10	16.14	1558.34 ^a	1276.60 ^a	16.00 ^a	1564.25 ^a	1560.27 ^a
LSD (0.05)	NS	205.13	120.87	0.79	218.49	79.41
CV (%)	5.5	17.20	16.00	4.72	10.40	4.70
Variety x Zn rate	*	NS	*	*	NS	*

176

177 **5. N-fixed and crude protein**

178 The results of cowpea N-fixed and crude protein at all sampling periods are presented in
 179 Table 4. In this study, Zinc fertilizer application interacted to significantly (p < 0.05) affect

180 cowpea N-fixed and crude protein. Zinc levels increased the cowpea N₂-fixed and crude
 181 protein in the order: 5 kg Zn ha⁻¹ > 10 kg Zn ha⁻¹ > 0 kg Zn ha⁻¹. N-fixed and crude protein
 182 interaction differed significantly (p < 0.05) among some the treatment interactions. Zamzam
 183 variety interacted markedly to produce the highest value of N-fixed in cowpea haulm and
 184 grain and Agyenkwa presented the lowest one. For the cowpea crude protein, the result is
 185 presented in the following order: Asontem > Agyenkwa > Zamzam.

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187

188

189 **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 →	Crude protein (%)	Grain N-fixed ← kg ha ⁻¹ →	Tissue N-fixed →	Crude protein (%)
<u>Varieties</u>						
Agyenkwa	22.40	16.72 ^b	25.80 ^b	39.61 ^a	15.70	24.84 ^b
Asontem	26.40	27.64 ^a	29.44 ^a	32.23 ^b	16.59	26.86 ^a
Zamzam	31.80	30.82 ^a	24.87 ^c	39.14 ^a	20.95	23.44 ^c
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 ^b	24.12 ^b	25.88 ^b	33.59 ^b	14.12 ^b	24.27 ^b
5	30.90 ^a	27.26 ^{ab}	27.28 ^a	38.39 ^a	20.09 ^a	25.56 ^a
10	27.90 ^a	31.79 ^a	26.95 ^a	39.00 ^a	19.03 ^a	25.01 ^{ab}
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 Zn rate	*	NS	*	*	NS	*

190

191 **6. Effects of Zinc rates on NPK content**

192 The results of grain nutrients analysis showed no significant varietal effects for content of
 193 nitrogen and phosphorus (Table 5). Additionally, potassium content in the haulms was not
 194 different among varieties. However, for potassium content in seed, the Zn treatments effects
 195 were similar, but greater either effect was greater than the control treatment effect in both
 196 cropping seasons (2016 major and minor seasons).

197 **Table 5. NPK content of cowpea as affected by varieties**

Rates	Nutrient uptakes (kg ha ⁻¹)			
	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	12.19 ^b	13.95	20.92 ^a
Asontem	13.17	14.33 ^a	13.58	16.84 ^b
Zamzam	10.79	13.33 ^a	11.72	20.43 ^a
LSD (0.05)	NS	1.27	NS	2.89
CV (%)	12.1	7.70	7.70	4.30

198
 199 For the Zn treatments, N content of grain was affected by Zn application in both seasons.
 200 Haulm N content was also significantly affected by Zn fertilizer application. In all these

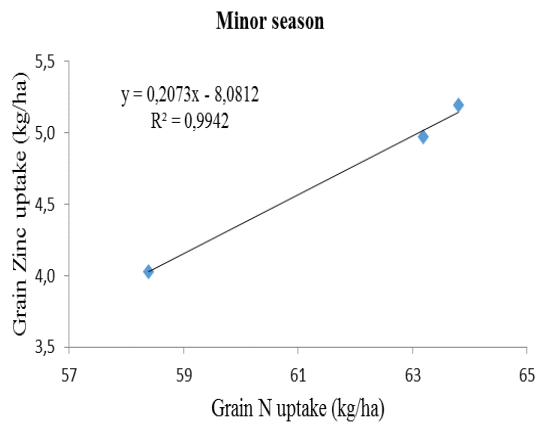
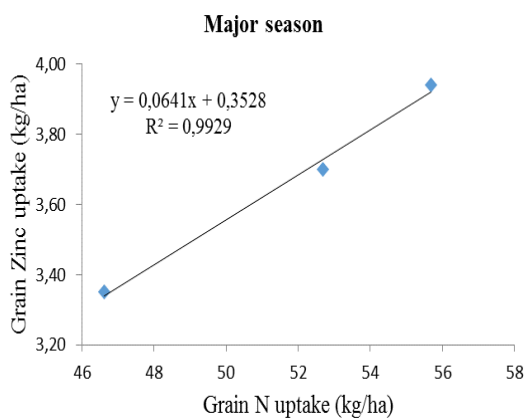
201 cases, treatments differences between the Zinc treatments were similar, but either effect was
 202 greater than the control treatment (Table 6). Haulm P content were significantly affected by
 203 Zn fertilizer application (Table 6), with the exception haulm Zinc content in the major season,
 204 where the control treatment effect was similar to the 5 kg ha⁻¹ Zn treatment. In all cases, the
 205 Zn treatment effects were similar, and either effect was significantly higher than the control
 206 treatment effect. Haulm K content was not affected by Zn fertilizer in both seasons (Table 6).
 207 However, grain K content significantly affected by the Zn fertilizer in both seasons. In both
 208 seasons, grain K content in the control treatment was lower than the Zn treatment effects.

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

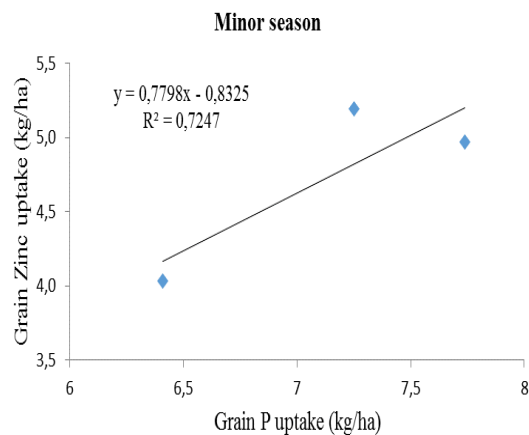
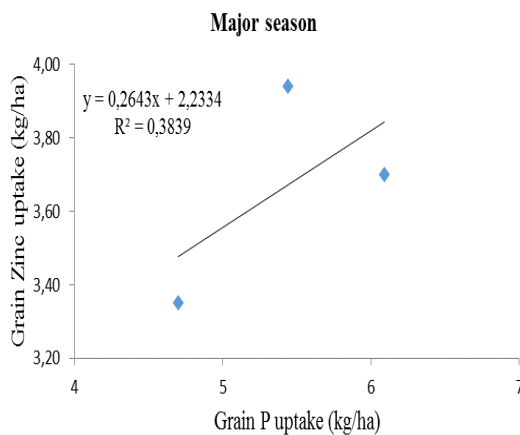
	Nutrient uptakes (kg ha ⁻¹)			
	Major season		Minor season	
	Haulm	Grain	Haulm	Grain
N				
Rates				
0	40.32	46.63 ^b	28.30 ^b	58.39 ^b
5	41.46	55.69 ^a	34.30 ^a	63.19 ^a
10	45.99	52.68 ^a	33.20 ^{ab}	63.80 ^a
LSD (0.05)	NS	5.26	5.59	2.29
CV (%)	8.90	8.20	9.40	2.40
P				
0	2.94 ^b	4.70 ^c	3.22 ^b	12.01 ^b
5	2.89 ^b	6.09 ^b	3.88 ^{ab}	13.61 ^a
10	3.54 ^a	5.44 ^a	4.01 ^a	13.83 ^a
LSD (0.05)	0.55	0.70	0.65	1.27
CV (%)	9.20	13.50	9.60	7.20
K				
0	12.45	12.11 ^b	12.42	17.84 ^b
5	13.38	13.81 ^a	14.38	20.27 ^a
10	12.45	13.93 ^a	12.45	20.08 ^a
LSD (0.05)	NS	1.26	NS	1.67
CV (%)	14.30	8.30	17.30	8.10

210 **7. Interrelationship between Zinc and NPK uptake in plant grain**

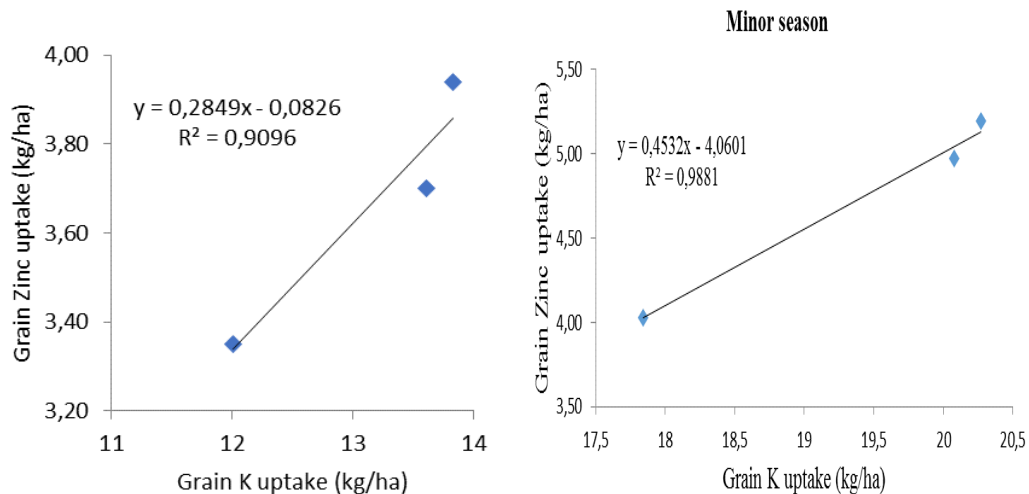
211 The linear regression showed the positive relationship between grain Zn uptake and NPK
212 content for three sampling periods during the experiment in the major and minor seasons
213 (Fig. 3). The argument on the enhanced NPK uptake y Zn content was ably supported by the
214 significant positive relationship observed in the present study between NK and Zn uptake
215 (0.9929*** with N and 0.9096** with K) in the major cropping season. The minor cropping
216 season also follows the same trend with 0.9942** and 0.9389** with N and K respectively.
217 And with P the relationship was weak but positive (0.3839 in major season and 0.7289 in
218 minor season).



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220



221

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

224 **DISCUSSION**

225 Plant height was affected by both variety and Zn rates. Among the Zn treatments, Plant
 226 height was greatest in the Zn plots applied in both cropping seasons. Malakooti *et al.* [20]
 227 reported that added Zn significantly increased plant height by increasing internodes distances.
 228 Kaya *et al.* [21] stated that grain yield was positively correlated with leaf weight, stem
 229 weight, plant height and number of branching per plant. Zinc fertilizer application did not,
 230 however, have any significant effect on the number of leaves and stem girth in all the days
 231 examined. It was reported that application of zinc had positive effects on growth parameters
 232 [22]. Contrarily, foliar application of micronutrients increased the diameter of plant over the
 233 control treatment [23]. So, these findings conclude that the entire cowpea varieties gave equal
 234 stem diameter at all treatments of zinc application.

235 The application of the Zn fertilizer did not affect nodulation, indicating that some of
 236 inoculation factors were limiting such as soil pH, initial phosphorus and others

237 micronutrients. And also, Gourion *et al.* [24] reported that the nodule initiation may depend
238 on the relative concentrations of plant-specific signals and host species appears to be a
239 significant factor determining the maximum number of nodules generated. Effective nodule is
240 essential for a functioning Legumes-Rhizobium symbiosis and Zinc, chloride and cobalt have
241 no effect on nodulation but are required for the growth of the host legume [25]. Two hosts
242 may have the same sensitivity to bacterial signal molecules, but might differ in their ability to
243 elicit synthesis of required nodulation signals in the bacteria [24, 26]. Cowpea root exudates
244 have also been reported to contain substances that enhance nodule initiation [27, 28, 29].
245 However, lower efficiency of cowpea cannot be readily explained in terms of reduced
246 numbers of bacteria in contact with the root [24]. Varieties most susceptible to infection and
247 capable of producing effective nodules should have greater potential to fix more atmospheric
248 N. However, this assumption often depends on other factors such as the environment and
249 crop management [30]. Indeed Giller [31] reported that the ability to form nodules is not
250 enough to obtain an effective nitrogen fixation symbiosis. Nodule number was nearly
251 successively decreased over time at all treatments and is not correlated with the Zinc fertilizer
252 applied. The amount of nodule dry biomass was drastically reduced with the mineral Zinc
253 fertilizer, whereas the amount of nodule biomass was not affected in the control group,
254 probably because the soil had satisfactory levels of available N and P. Nodule number
255 correlated negatively with nodule dry weight [32]. The interaction effect was also not
256 significant at 5% probability.

257 The present results were supported by Arif *et al.* [33] who reported that foliar application of
258 micronutrients help in improving yield. In both seasons, foliar spray of Zn fertilizer had effect
259 on hundred grain weights. In all these parameters, the control treatment effect was lower than
260 Zn treatments, whereas among the Zn treatments. Pandey *et al.* [34] reported that following

261 Zn fertilization increased hundred seed weight. Also, Zeidan *et al.* [35] reported that yield
262 and its components in lentil are improved by foliar application of micronutrients. Crop yields
263 and quality are reduced by Zn inadequate in soil; therefore, Zn utilization is essential to obtain
264 high yield and quality in crops as showed the results (Table 3). These results are in close
265 conformity with those of [36, 37, 38]. This was because of the fact that better and higher
266 availability of Zinc, resulting better nutritional environment, higher dry matter accumulation
267 and its associated effect on growth attributes increased haulm and grain yield. It is also
268 evident from table 3 that all the Zinc treated plots increased the grain yield over the control,
269 as there was a consistent increase in cowpea grain yield up to 10 kg Zn/ha. This suggests that,
270 the application of Zn significantly affect cowpea yield. Similar results were reported as in
271 Moswatsi [39] and Oseni [40] studies. In this connection, Banks [41] reported that the foliar
272 application of Zn affected yield and its components of soybean. Also, Seifi *et al.* [42]
273 reported that the highest yield of common bean was obtained by Zinc foliar application.
274 Abdoli *et al.* [43] reported that more production of chlorophyll and IAA can cause delay in
275 plant oldness and prolong the period of photosynthesis. This incident improves the
276 production of carbohydrates and their transportation to the growing seeds.

277 The Zn deficiency symptoms can be prevented by the application of Zn fertilizers. The actual
278 causal relationship and mechanisms are still not fully understood [3]. As shown in Tables 4
279 and 5 the mean percentage total nitrogen, phosphorus and potassium uptakes in the harvested
280 leaves were quantitatively higher under zinc fertilizer application and increased with
281 incremental zinc rates. These results corroborate the findings of Fagaria [44] and Sunitha *et*
282 *al.* [45] who reported that zinc is an essential micronutrient for plant growth and plays an
283 important role in the catalytic part of several enzymes its deficiency will result in stunted
284 growth and nutrient uptakes. And also, Potarzycki and Grzebisz [46] reported that zinc exerts

285 a great influence on basic plant life processes, such as (i) nitrogen metabolism – uptake of
286 nitrogen and protein quality; (ii) photosynthesis - chlorophyll synthesis and carbon anhydrase
287 activity. Also, many researchers have observed that Zn is closely related to the nitrogen
288 metabolism pathway of plants, thus causing a reduction in protein synthesis for Zn deficient
289 plants. Zinc deficiency significantly affects the root system including root development [47].

290 **CONCLUSION**

291 Zn fertilizer significantly affected NPK content and grain yield of cowpea varieties used. The
292 increment of Zn content in the grain had a positive relationship with NK, which will
293 definitely enhance nutrition of both human and animals. At all sampling periods, nodule
294 number per plant was not affected by Zinc rates and nodule number was nearly successively
295 decreased over time at all treatments and is not correlated with the Zinc fertilizer applied. The
296 Zinc fertilizer significantly enhanced N-Fixed and Crude protein in both cropping season's
297 trial investigating effect of Zinc rates on growth, nodulation and mineral content of cowpea in
298 the semi-deciduous forest zone of Ghana. This implies the 5 kg Zn ha⁻¹ is the optimum rate
299 that will enhance the yield and nutrient quality of cowpea in the Semi-Deciduous Forest Zone
300 of Ghana.

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