

1 **Influence of NPK blended fertilizer application on**
2 **chlorophyll content and tissue mineral contents of two**
3 **finger millet varieties grown in acid soils of Kakamega,**
4 **Western Kenya.**

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7
8 **ABSTRACT**
9

Acidic soils with high exchangeable aluminium ions occur in most parts of Kenya, western Kenya inclusive. Aluminium ions is a serious environmental problem that affects crop productivity in Western Kenya region. The county governments of Kakamega, Bungoma, Vihiga, Busia and Trans-Nzoia are promoting application of NPK blended fertilizer to ameliorate the soil acidity to increase maize production. Finger millet (*Eleusine coracana* L) is one of the important cereal crops in Kenya and has the ability to grow under unfavourable environmental conditions much better than other cereal crops. It is for this reason that it is currently being popularized in efforts to address food security in the region, however the effects of NPK blended fertilizer application on the selected physiological parameters of the crop is little known. The objective of this study to investigate the effect of NPK blended fertilizer application on chlorophyll content index and plant tissue mineral analysis. The experiment was conducted at the Kenya Agricultural and Livestock research Organization (KALRO) Kakamega station to determine how the NPK blended fertilizer application affects the physiology of finger millet varieties grown on acidic soils. Randomized Complete Block Design with 0,25,50,75,100 kg application rates per acre of NPK blended fertilizer as the treatments was applied in two equal split applications. The measured parameters were chlorophyll content index using CCM-200 spectrophotometer, (Opti-Sciences Inc., Hudson, USA) from the plant leaves at 50% plot maturity. [12], [13] Procedures were used to determine plant tissue analysis for nitrogen, phosphorus, potassium, calcium and magnesium at physiological maturity from the leaves. Data was subjected to analysis of variance (ANOVA) using GenStat statistical package version 15.1. Means were separated by Least Significant Difference (LSD) test at 0.05 probability level where significant differences were observed. Regression analysis was used to estimate the relationship between variables. At the 75 kg/acre rate the leaves showed the significant $P < 0.05$ chlorophyll content. Check that it is correct, calcium and potassium in both varieties for the two seasons, short rain and long rain respectively. Control had the lowest physiological activities for both seasons in terms of chlorophyll content, tissue calcium, magnesium, nitrogen, phosphorus and potassium. Significant nitrogen content was observed on Gulu-E variety for both seasons on the highest rate whereas the same trend was observed on P-224 variety, and during the short rainy season, a linear increase was observed with increasing NPK blended fertilizer rates. No conclusive pattern was observed during the short rainy season but with the control exhibiting the lowest phosphorus content for both varieties. Significant response to physiology (chlorophyll and plant leaf tissue mineral) might have been due to increased uptake of mineral nutrients present in the NPK blended fertilizer, and increased soil pH caused by the liming action of the NPK blended fertilizer.

10
11 **Keywords:** Soil acidity; finger millet; NPK blended; chlorophyll content index; nitrogen; phosphorus;
12 potassium; magnesium; calcium.

13
14 **1. INTRODUCTION**

15 Finger millet (*Eleusine coracana* (L.) Gaertn. ssp. coracana) originated from the highlands of Ethiopia
16 and presently it is grown in eastern and southern Africa [1]. In eastern and southern Africa small-scale
17 farmers grow it in low input farming systems. The crop has food security, nutritional, cultural,
18 medicinal, economic value and high industrial potential. The crop adapts better to poor soils, erratic
19 weather conditions and droughts than main food grains like maize and wheat [2]. Though its
20 production has been declining, the crop still has a significant demand. Finger millet price has been
21 much higher than other cereals in the past few years. Finger millet is extensively cultivated in the
22 tropical and sub-tropical regions of Africa, Kenya inclusive and is known to save the lives of poor
23 farmers from starvation at times of extreme drought. The crop also contains high nutritional value

24 especially to pregnant women and children for weaning and its seeds can be stored for more than five
25 years due to low vulnerability to insect damage, it provides food security for poor farmers [3].
26 The farmland soils of Western Kenya are acidic with widespread Ca, Mg, N and P deficiencies. Most
27 soils found in the highlands of East of Rift valley and Western Kenya regions with a pH of 4.5 to 5.0
28 and high exchangeable Aluminium (III) ions which limits the availability and uptake of Ca, Mg and P in
29 the soils through formation of insoluble aluminium complexes [15]. Acidic soils develop as a result of
30 excessive leaching of basic cations, mainly Ca, Mg and K characterised by excessive rainfall and
31 continuous use of acidifying fertilizer [14]. Several approaches have been made to ameliorate soil
32 acidity such as liming or application of FYM [you should write its clear name.](#), [14]. Calcium and Mg
33 can be sourced from dolomitic
34 limestones while P can be sourced from readily soluble sources (including superphosphates) or slowly
35 soluble such as rock phosphates [15]. However, NPK blended fertilizer is known to contain both liming
36 and nutrient components, hence it can provide good results and more agronomic potential. However,
37 scientific information available with regard to improving crop physiology i.e. Chlorophyll content and
38 plant tissue mineral uptake through soil nutrient supply by NPK blended fertilizer to finger millet
39 varieties for the potential yield is limited. The productivity of the crop is negatively affected by the
40 increased soil acidity.

41 The potential production of finger millet in Kenya remains largely low. For instance, in Western Kenya,
42 millets were grown on 65,000 hectares in 2010 with an average yield of 1.3 tons/hectare [4]. Former
43 Western province is known to be the largest producer of finger millet in Kenya with production rates of
44 0.5 ton/ha per year [5]. These low yields are largely explained in terms of depleted nutrients in soils
45 such as calcium and magnesium among other factors. Acidity in soils causes nutrient immobilization
46 through formation of aluminium complexes that are insoluble. These make the nutrients unavailable to
47 the plant hence limiting the physiology of such plants including finger millet. [6] concluded that
48 Western Kenya continues to experience food insecurity due to increasing soil acidity and consequent
49 phosphorus deficiencies with 0.9 million hectares of land having pH < 5.5. NPK blended fertilizer
50 (10%N, 26%P₂O₅, 10%K₂O, 4%S, 8%CaO, 4%MgO and traces of B, Zn, Mo, Cu and Mn) is one of
51 the P-based fertilizers currently gaining popularity in the region and can offset nutrient deficiency and
52 improve crop yield. NPK blended fertilizer is known to contain liming materials that contribute to liming
53 effects and their application in soils improves availability of nutrients such as phosphorus to plants
54 resulting in high yields and improved soil properties [7].

55 [8] Found that chlorophyll content significantly increased in soya bean planted under Diammonium
56 phosphate (DAP) fertilizer treatment and lowest in control in Tharaka Nithi and Meru whose soils are
57 acidic. The result was due increased P uptake which is key in the chlorophyll synthesis. Phosphorus
58 uptake relies on the soil pH and since DAP lacks liming materials in it, there is need to adopt a
59 fertilizer that can offer liming as well as growth nutrients such as NPK blended fertilizer.

60 [9] Reported increased Calcium and decreased aluminium (Al) toxicity on application of liming
61 materials; calcium hydroxide (Ca(OH)₂), Calcium Oxide (CaO), Calcium Carbonate (CaCO₃) grown
62 under sugarcane crop in acidic soils of Kisumu. Increased Ca was attributed to increased pH due to
63 reduced soil acidity which in turn reduced the leaching of nutrients such as Ca. However, liming
64 materials alone may not be enough for the provision of other nutrients necessary for the plant growth,
65 hence need for a fertilizer with such qualities. [10] Found that NPK blended fertilizer in combination
66 with manure application on maize, led to increased uptake of plant nutrients including Ca, Mg, and
67 NPK. Finger millet is also grown in similar agro-ecological environment like maize. Therefore,
68 application of NPK blended fertilizer would also improve the productivity of finger millet. However, the
69 effect of NPK blended fertilizer on the physiology i.e chlorophyll content, tissue mineral content of
70 nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) of the finger millet is
71 not clearly known, hence prompting the current study.

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74 2. MATERIAL AND METHODS

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76

76 2.1 Study site

77 The study was conducted as an on-station experiment at the Kenya Agricultural and Livestock
78 Research Organization (KALRO) field station located in the upper medium (UM) ecological zone in
79 Kakamega County in Western Kenya which borders Vihiga County to the South, Siaya County to the
80 west, Bungoma County to the North and Nandi County to the east. The station lies on the latitude of
81 (00° 16' N; 34° 45' E; 1585 m asl) in the Western part of Kenya during short and long rains season of

82 2015 and 2016, the short rains (SR) season which starts in October to February and the long rains
83 (LR) season which starts in March to August.

84 2.2 Experimental design and treatments

85 The study adopted ~~Randomized Complete Block-split parcel trial~~ Design (RCBD), replicated thrice with five
86 treatments

87 of five levels of NPK blended fertilizer. The treatments were: 0, 25, 50, 75 and 100 kg per acre applied
88 in two equal split applications, at planting and at four weeks after planting. The experimental unit
89 measured 2 m x 1.7 with a 2 m pathway between blocks and a 1 m pathway between plots for easier
90 plot management. **Blocks measured 18 m x 1.7 m translating to an experimental field of 18m x
13.1m**~~This sentence is not understood. explain.~~

91 The finger millet varieties P-224 and Gulu-E were obtained from Kenya Agricultural and Livestock
92 Research Organization (KALRO)-Kakamega station. These are the varieties commonly grown within
93 the Kakamega area.

93 2.3 Cultural operations

94 Soil samples were taken on the plots at a depth of 0 – 30 cm before planting then after harvesting to
95 monitor the change in soil chemical properties. The seeds were planted with 30 cm spacing between
96 rows and later thinned after four weeks. In each plot three rows of each of the two varieties (P-224
97 and Gulu-E) were planted. The seeds were drilled in each line. The first weeding was done 14 days
98 after germination (DAG) and the second weeding 14 days after the first weeding. To ensure enough
99 space for the individual plants thinning of the rows was done during the first weeding [11] to have
100 plants with 10 cm gap between each individual plant.
101

102 2.4 Data collection.

103 2.4.1. Chlorophyll content index.

104 The chlorophyll content index (CCI) was measured from the second leaf from the apex of five plants
105 from each variety per treatment at random points along the 5 cm section using an Opti- Sciences
106 CCM-200 spectrophotometer, (Opti- Sciences Inc., Hudson, USA) at 50% plot maturity following
107 procedures by [12].

108 2.4.2. Plant tissue mineral analysis

109 [13] Procedures were used to determine nitrogen, phosphorus, potassium, calcium and magnesium
110 at ~~physiological maturity~~ from the leaves of the plant at physiological maturity.

111 2.4.2.1. Tissue Nitrogen, N. (Motsara & Roy, 2008).

112 0.5g of leaf sample were wet digested by di-acid in Kjeldahl flask. Then 0.7g CuSO₄, 1.5 g K₂SO₄ and
113 30ml 0.05 M H₂SO₄ in that order and boiled for 10 minutes. 50 ml water was added to cool &
114 transferred to distilling flask. 3 drops of Methyl red were added, followed by 30ml of 30% NaOH and
115 heated for 15 minutes and excess acid titrated with 0.1 M NaOH. A blank was made from 0.1M HCl in
116 conical flask.

117
$$N\% = (\text{Sample reading} - \text{Blank reading} * \text{df}) / \text{wt.}$$

118

119 2.4.2.2. Tissue Phosphorus, P. (Motsara & Roy, 2008).

120 0.5 of leaf sample were wet digested in a di-acid and volume topped to 100 ml. 5ml of 100ml solution
121 were put in 50 ml flask and KH₂PO₄ then added. The solution was read on AAS to measure
122 concentration of Phosphorus. Absorbance range of 0.1 was read & used to determine P from
123 standard curve and calculated from;

124
$$P = C * \text{df}$$

125 2.4.2.3. Tissue K (Motsara & Roy, 2008).

126 0.5g of leaf sample were wet digested in di-acid to 100ml, 5ml of it put in 50 ml volumetric flask. 10 ml
127 KCl was added and water added to volume and shaken for 10 minutes. Absorbance was read on AAS
128 and K determined from standard curve.

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129 $K = C \cdot df.$
130 **2.4.2.4. Tissue Mg. (Motsara & Roy, 2008).**
131 0.5g of leaf sample were wet digested by di-acid and topped to 100ml by water. To 5ml in 50ml
132 volumetric flask, 10ml of Magnesium standard reagent was added. Absorbance of final solution was
133 measured on AAS.

134 $Mg = C \cdot df.$
135 **2.4.2.5. Tissue Ca. (Motsara & Roy, 2008).**
136 0.5g of the leaf sample were wet digested by di-acid and topped to 100ml by water. 5ml in 50ml
137 volumetric flask, 10ml of Calcium standard reagent was added. Absorbance of final solution was
138 measured on AAS.

139 $Mg = C \cdot df.$

140 **KEY.**
141 **C.....:Concentration**
142 **df.....:dilution**
143 **factor**
144 **wt.....:weight of leaf sample used.**
AAS.....:Atomic Absorption Spectrophotometer.

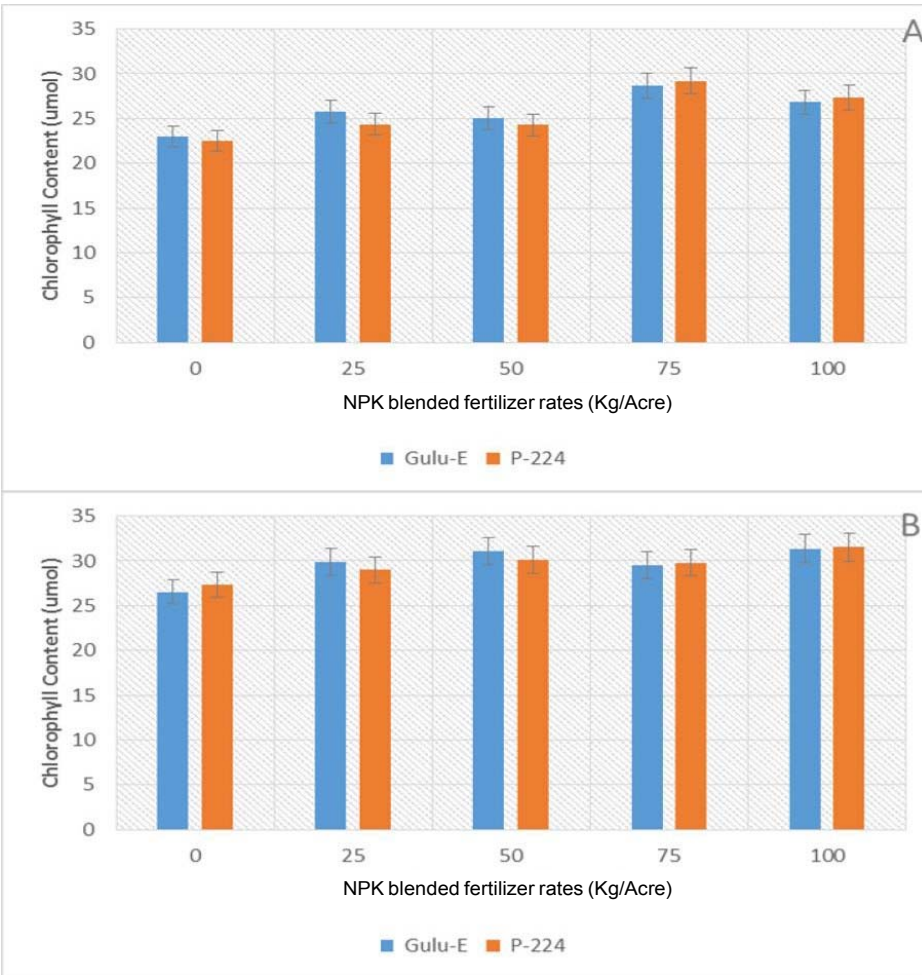
145 **2.5 Statistical analysis**

146 Analysis of variance (ANOVA) was performed on the collected data using GenStat statistical software
147 Version 15.1 to test treatment effect at 0.05 level of significance. The means were separated using
148 the Fischer's Protected LSD test where significant differences between treatments were observed.

149 **3. RESULTS AND DISCUSSION**

151 **3.1 Chlorophyll content index**

152 NPK blended fertilizer rate of 75 kg/acre significantly ($P < 0.05$) showed the highest total chlorophyll
153 content in the leaves. The varieties showed very minimal differences in the chlorophyll content index
154 and was the lowest under the control (Figure 1). Though insignificant, the highest rate on the P-224
155 variety showed the highest chlorophyll content (31.53 μmol) per unit fresh weight of leaves. There
156 were no significant differences between the NPK blended fertilizer treatments during the long rainy
157 season but there was a distinct trend where the control had the lowest chlorophyll content and 100
158 kg/acre fertilizer rate having the highest chlorophyll content in the finger millet varieties. The increase
159 might have been attributed to the applied NPK blended fertilizer which increased soil pH and availed
160 the important nutrients such as phosphorus, magnesium and calcium that are important for the
161 synthesis of chlorophyll in the chloroplasts. Since the fertilizer had magnesium, iron, calcium and
162 nitrogen which are components in chlorophyll molecule structure, hence the significant relationship
163 between the NPK blended and chlorophyll content. Nitrogen is a component of the enzymes
164 associated with chlorophyll synthesis and hence the chlorophyll concentration reflects relative N
165 status in the soil which was greatly enhanced through the NPK blended fertilizer. The results agree
166 with those of [8] which indicated that chlorophyll content was significantly higher in soya bean planted
167 under DAP fertilizer treatment and lowest in control treatment in acidic soils of Tharaka Nithi and
168 Meru.
169



170 Figure 1. The influence of NPK blended fertilizer on the chlorophyll content of Gulu-E and P-224
 171 finger millet varieties at Kakamega during the Short rain (A) and long rain (B) seasons. Error bars
 172 indicate the SE at $P \leq 0.05$
 173
 174

175 **3. 2 Tissue calcium content**

176 The application of NPK blended fertilizer led to an increase in the tissue calcium content of the plant.
 177 The 75 kg/acre NPK blended fertilizer rate elicited significantly the highest calcium content in the
 178 tissues of finger millet varieties (Table 1a and 1b). The control had the lowest plant tissue calcium
 179 content in both varieties with a decrease observed in the highest NPK blended fertilizer rate from the
 180 75 kg/acre rate onwards. In the long rainy season, a conclusive trend could not be determined but the
 181 25 kg/acre treatment exhibited the highest calcium content while the control had the lowest on variety
 182 Gulu-E with 81 mg/100 g and 64.3 mg/100 g respectively. Variety P-224 had the highest calcium
 183 content of 81.3 mg/100 g and lowest content of 60.3 mg/100 g. Variety P-224 had the highest calcium
 184 content of 81.3 mg/100 g and lowest content of 60.3 mg/100 g. The results are in agreement with
 185 those of [10] who found that NPK blended fertilizer in combination with manure application in maize
 186 led to increased calcium uptake. Results also agree with those of [9]. This could be attributed to the
 187 increased levels of potassium uptake which antagonistically affects calcium uptake at the highest rate.

188 **3.3 Tissue potassium content**

189 Application of NPK blended fertilizer led to increase in the tissue potassium uptake in both seasons.
 190 Significant differences at $P < 0.05$ were observed between the NPK blended fertilizer treatments during
 191 the short rainy season on the plant tissue potassium content (Table 1a and 1b). There were no
 192 significant differences between the treatments observed during the long rainy season. The 75 kg/acre
 193 rate showed the highest K content in the plant tissues of finger millet where P-224 had 1057 mg/100 g
 194 and Gulu-E had 997 mg/100 g during the short rainy season. Though not significantly different,
 195 Please check the values. There is an error in your analysis. The difference between the values
 196 obtained is high. This should be important in this case. This difference can not be significant, he
 197 control showed the lowest amount of tissue K in the long rainy season with 758 mg/100 g and 757
 198 mg/100 g for Gulu-E and P-224 varieties respectively, the decrease in long rain season could have
 199 been due to nutrient leaching and surface run off. These results are in agreement with those of [10]. The results on the increased potassium tissue content could have been due to optimal levels of calcium and nitrogen that enhanced potassium uptake.

200 **3.4 Tissue magnesium content**

201 Application of NPK blended fertilizer led to increase in the tissue magnesium uptake in both seasons
 202 Significant differences ($P < 0.05$) were observed between the treatments for both seasons ($P < 0.05$) as
 203 shown on (Table 1a and 1b). Gulu-E and P-224 elicited the lowest magnesium content in the control
 204 during the long and short rainy seasons. The highest ~~K-Mg~~ tissue content in Gulu-E was observed on
 205 the 50 kg/acre NPK blended fertilizer rate for both seasons while P-224 showed a different trend
 206 where above 50 kg/acre rate had the highest Mg content during the short rainy season, while the 25
 207 kg/acre and 100 kg/acre rates had the highest during the long rainy season. The highest K tissue
 208 content (160 mg/100 g) in Gulu-E was observed on the 50 kg/acre NPK blended fertilizer rate while
 209 P-224 showed a different trend where above 50 kg/acre rate had the highest Mg content while the
 210 25 kg/acre (163 mg/100 g) and 100 kg/acre rates had the highest in the first trial. This results are in
 211 agreement with those of [10]. The results might have been due to increased amounts of
 212 Magnesium that was released and made available to plants due to increased soil pH and
 213 absorbed by the plant in exchangeable form. More magnesium was present in the soil solution
 214 and thereby making it conducive for uptake hence higher uptake. Higher tissue magnesium levels
 215 might have also been due to optimum levels of nitrogen which is synergistic to magnesium uptake.

216
 217 Table 1a. The influence of NPK blended fertilizer on the calcium, magnesium, nitrogen, phosphorus
 218 and potassium contents in Gulu-E and P-224 finger millet varieties at Kakamega during
 219 the short rain season.

Variety	Fertilizer Rate	Calcium mg/100 g	Potassium mg/100 g	Magnesium mg/100 g	Nitrogen mg/100 g	Phosphorus mg/100 g
Gulu-E	0	113.3b	670b	55b	1778b	335a
	25	119a	847ab	63ab	1920ab	340a
	50	120a	797ab	65a	1877ab	337a
	75	126.7a	997a	63ab	1940ab	353a
	100	121.7a	823ab	70a	2200a	358a
P-224	0	110b	713ab	53b	1758b	322a
	25	120a	860ab	60ab	1885ab	330a
	50	120a	757ab	67a	1830ab	340a
	75	126.7a	1057a	67a	2065a	347a
	100	123.3a	793ab	67a	2193a	357a
P-Value		0.044	0.008	0.033	0.036	0.555
LSD		13.3	167.8	10.62	270.7	39.68

220 Values in columns followed by the same letter do not differ significantly at $P \leq 0.05$.

221
 222
 223
 224
 225

226 Table 1b. The influence of NPK blended fertilizer on the calcium, magnesium, nitrogen, phosphorus
 227 and potassium contents in Gulu-E and P-224 finger millet varieties at Kakamega during the long rains
 228 season

Variety	Fertilizer Rate	Calcium mg/100 g	Potassium mg/100 g	Magnesium mg/100 g	Nitrogen mg/100 g	Phosphorus mg/100 g
Gulu-E	0	64.3b	758a	142b	2361b	358b
	25	81a	1012a	152ab	2514a	413ab
	50	71.7ab	855a	160a	2595a	459a
	75	71.7ab	983a	150ab	2493ab	393ab
	100	75.3ab	950a	142ab	2650a	430a
P-224	0	60.3b	757a	133b	2463ab	353b
	25	81.3a	1027a	163a	2467ab	402ab
	50	73.3ab	857a	148ab	2572a	447a
	75	72ab	1003a	142ab	2468ab	400ab
	100	72.7ab	1017a	157a	2598a	389ab
P-Value		0.139	0.223	0.045	0.042	0.003
LSD		14.16	263.6	23.6	254.6	62.59

229 Values in columns followed by the same letter do not differ significantly at $P \leq 0.05$

230 3. 5 Tissue nitrogen content

231 Application of NPK blended fertilizer significantly ($P < 0.05$) influenced the nitrogen content in the finger
 232 millet tissues for both seasons (Table 1a and 1b). The highest content was observed on Gulu-E
 233 variety for both seasons on the highest rate whereas the same trend was observed on P-224 variety.
 234 During the short rainy season, a linear increase was observed with increasing NPK blended fertilizer
 235 rates peaking at 100 kg/acre with 2650 mg/100 g for Gulu-E variety and 259 mg/100 g for P-224
 236 variety. The increase in tissue nitrogen might have been due to optimal levels of copper and boron
 237 nutrients present in the NPK blended fertilizer that could have promoted nitrogen uptake by the finger
 238 millet varieties. These results are in agreement with those of [10].

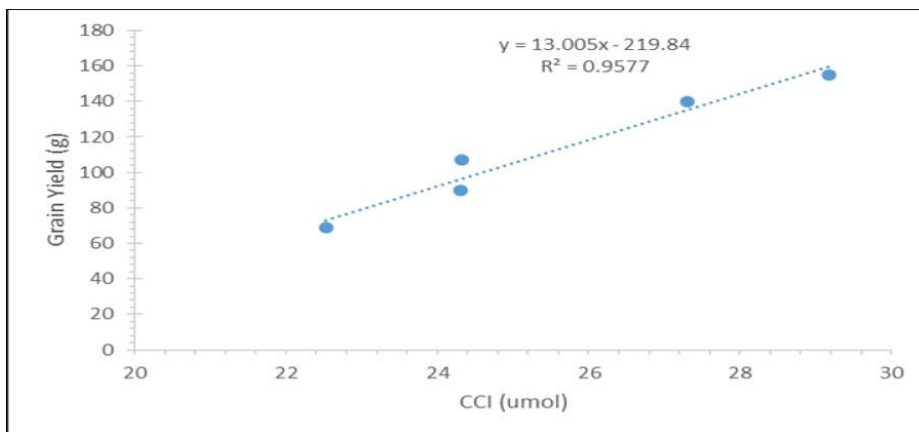
239 3. 6 Tissue phosphorus content

240 The phosphorus tissue content was significantly influenced by the NPK blended fertilizer treatment
 241 during the long rainy season (Table 1b). There was no conclusive pattern observed during the short
 242 rainy season but with the control exhibiting the lowest phosphorus content for both varieties. The 50
 243 kg/acre NPK blended fertilizer rate elicited the highest P tissue content under both varieties with Gulu-
 244 E having 459 mg/100 g and P-224 having 447 mg/100 g. These results are in agreement with those of
 245 [10] who found that NPK blended fertilizer in combination with manure application in maize, led to
 246 increased phosphorus uptake by the plant. This might be due the Phosphorus component in the
 247 fertilizer that could have led to increased uptake of the nutrient and also the increase of the soil pH
 248 with increasing NPK blended rates that might have led to the reduction of Fe and Al ion concentration
 249 in the soil thereby decreasing the adsorption/precipitation of P thus more uptake and accumulation in
 250 the plant tissues.

252 3.6 Relation between chlorophyll content index and finger millet grain yield.

253 There was a linear increase in the finger millet grain yield with increase in the amount of chlorophyll
 254 index in the leaves (Fig. 2). At 22.5 umol of chlorophyll, the grain yield was recorded to be 69 g per
 255 plant but the grain yield increased to 155 g per plant when the level of chlorophyll in the leaves was at
 256 29.17 umol. The linear increase in yield with chlorophyll content may have been due to enhanced
 257 synthesis of chlorophyll. This was due to availability of Mg, Ca, P etc supplied by NPK blended
 258 fertilizer. Increased chlorophyll content increased primary productivity of finger millet plant hence
 259 increased yield.

260



261 Figure 2: Linear relationship between the chlorophyll content index and the grain yield of finger
 262 millet at Kakamega.

263
 264 **4.0. CONCLUSION**

265 The physiological components (chlorophyll content, calcium, magnesium, potassium, nitrogen and
 266 phosphorus) were positively increased by application of NPK blended fertilizer compared to the
 267 absolute control due to increased absorption of mineral elements under fertilizer applied treatments. It
 268 was observed that with the increasing chlorophyll content index, there was a positive linear increase
 269 on the grain yield per plant of finger millet.

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