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

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Authors' contributions

Author DSW designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors DSW, PAS, GWN, and BDK reviewed the study design and all drafts of the manuscript. Author DSW managed the analyses of the study. Author DSW managed the literature searches. All authors read and approved the final manuscript.

ORIGINAL RESEARCH ARTICLE

ABSTRACT

Acidic soils with high exchangeable aluminium ions occur in most parts of Kenya, western Kenya inclusive. Aluminium toxicity 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 the 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 unfavorable 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, hence prompting the study. The objective of this study was to investigate the effect of NPK blended fertilizer application on chlorophyll content index and plant tissue mineral analysis. Randomized Complete Block Design with 0,25,50,75,100 kg application rates per acre of NPK blended fertilizer as the treatments were 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. Motsara and Roy Procedures were used to determine plant tissue analysis for nitrogen, phosphorus, potassium, calcium, and magnesium at physiological maturity from the leaves. Data were 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. 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, 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 regarding 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. A significant response to physiology (chlorophyll and plant leaf tissue mineral) might have been due to increased uptake of mineral nutrients

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present in the NPK blended fertilizer and increased soil pH caused by the liming action of the NPK blended fertilizer.

Keywords: chlorophyll content index; nitrogen; phosphorus; potassium; magnesium; calcium.

1. INTRODUCTION

Finger millet (*Eleusine coracana* (L.) Gaertn. ssp. coracana) originated from the highlands of Ethiopia and presently it is grown in eastern and southern Africa on small scale and low input farming systems [1]. The crop has food security, nutritional, cultural, medicinal, economic value and high industrial potential. The crop adapts better to poor soils, erratic weather conditions and droughts than main food grains like maize and wheat [2]. Though its production has been declining, the crop still has a significant demand, and its price has been much higher than other cereals in the past few years [16]. Finger millet is extensively cultivated in the tropical and sub-tropical regions of Africa, Kenya inclusive and is known to save the lives of poor farmers from starvation at times of extreme drought [17]. The crop also contains high nutritional value especially to pregnant women and children for weaning and its seeds can be stored for more than five years due to low vulnerability to insect damage, it provides food security for poor farmers [3].

The farmland soils of Western Kenya are acidic with widespread Ca, Mg, N and P deficiencies. Most soils found in the highlands of East of Rift valley and Western Kenya regions with a pH of 4.5 to 5.0 and high exchangeable Aluminium (III) ions which limits the availability and uptake of Ca, Mg and P in the soils through formation of insoluble aluminium complexes [15]. Acidic soils develop as a result of excessive leaching of basic cations, mainly Ca, Mg and K characterized by excessive rainfall and continuous use of acidifying fertilizer [14]. Several approaches have been made to ameliorate soil acidity such as liming or application of FYM, [14]. Calcium and Mg can be sourced from dolomitic limestones while P can be sourced from readily soluble sources (including superphosphates) or slowly soluble such as rock phosphates [15]. However, NPK blended fertilizer is known to contain both liming and nutrient components, hence it can provide good results and more agronomic potential. However, scientific information available with regard to improving crop physiology i.e. Chlorophyll content and plant tissue mineral uptake through soil nutrient supply by NPK blended fertilizer to finger millet varieties for the potential yield is limited. The productivity of the crop is negatively affected by the increased soil acidity.

The potential production of finger millet in Kenya remains largely low. For instance, in Western Kenya, millets were grown on 65,000 hectares in 2010 with an average yield of 1.3 tons/hectare [4]. The former Western province is known to be the largest producer of finger millet in Kenya with production rates of 0.5 ton/ha per year [5]. These low yields are primarily explained concerning depleted nutrients in soils such as calcium and magnesium among other factors. Acidity in soils causes nutrient immobilization through the formation of aluminium complexes that are insoluble. These make the nutrients unavailable to the plant hence limiting the physiology of such plants including finger millet. [6] concluded that Western Kenya continues to experience food insecurity due to increasing soil acidity and consequent phosphorus deficiencies with 0.9 million hectares of land having pH < 5.5. NPK blended fertilizer (10%N, 26%P_2O_5, 10%K_2O, 4%S, 8%CaO, 4%MgO and traces of B, Zn, Mo, Cu and Mn) is one of the P-based fertilizers currently gaining popularity in the region and can offset nutrient deficiency and improve crop yield. NPK blended fertilizer is known to contain liming materials that contribute to liming effects and their application in soils improves availability of nutrients such as phosphorus to plants resulting in high yields and improved soil properties [7].

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

[9] Reported increased Calcium and decreased aluminium (Al) toxicity on application of liming materials; calcium hydroxide ($Ca(OH)_2$), Calcium Oxide (CaO), Calcium Carbonate ($CaCO_3$) grown under sugarcane crop in acidic soils of Kisumu. Increased Ca was attributed to increased pH due to reduced soil acidity which in turn reduced the leaching of nutrients such as Ca. However, liming materials alone may not be enough for the provision of other nutrients necessary for the plant growth, hence need for a fertilizer with such qualities. [10] Found that NPK blended fertilizer in combination with manure application on maize, led to increased uptake of plant nutrients including Ca, Mg, and NPK. Finger millet is also grown in similar agro-ecological environment like maize. Therefore, application of NPK blended fertilizer would also improve the productivity of finger millet. However, the effect of NPK blended fertilizer on the physiology i.e chlorophyll content, tissue mineral content of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) of the finger millet is not clearly known, hence prompting the current study.

2. MATERIAL AND METHODS

2.1 Study site

The study was conducted as an on-station experiment at the Kenya Agricultural and Livestock Research Organization (KALRO) field station located in the upper medium (UM) ecological zone in Kakamega County in Western Kenya which borders Vihiga County to the South, Siaya County to the west, Bungoma County to the North and Nandi County to the east. The station lies on the latitude of (00^o 16' N; 34^o 45' E; 1585 m asl) in the Western part of Kenya during short and long rains season of 2015 and 2016, the short rains (SR) season which starts in October to February and the long rains (LR) season which starts in March to August.

2.2 Experimental design and treatments

The study adopted Randomized Complete Block Design (RCBD), replicated thrice with five treatments of five levels of NPK blended fertilizer. The treatments were: 0, 25, 50, 75 and 100 kg per acre applied in two equal split applications, at planting and at four weeks after planting. The experimental unit measured 2 m x 1.7 with a 2 m pathway between blocks and a 1 m pathway between plots for easier plot management. Blocks measured 18 m x 1.7 m translating to an experimental field of 18m x 13.1m. The finger millet varieties P-224 and Gulu-E were obtained from Kenya Agricultural and Livestock Research Organization (KALRO)-Kakamega station. These are the varieties commonly grown within the Kakamega area.

2.3 Cultural operations

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

2.4 Data collection.

2.4.1. Chlorophyll content index.

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

2.4.2. Plant tissue mineral analysis

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

2.4.2.1. Tissue Nitrogen, N. (Motsara & Roy, 2008)
0.5g of leaf sample were wet digested by di-acid in Kjeldahl flask. Then 0.7g $CuSO_4$, 1.5 g K_2SO_4 and 30ml 0.05 M H_2SO_4 in that order and boiled for 10 minutes. 50 ml water was added to cool & transferred to distilling flask. 3 drops of Methyl red were added, followed by 30ml of 30% NaOH and heated for 15 minutes and excess acid titrated with 0.1 M NaOH. A blank was made from 0.1M HCl in conical flask.

N% = (Sample reading ---Blank reading *df) /wt.

2.4.2.2. Tissue Phosphorus, P. (Motsara & Roy, 2008)
0.5g of leaf sample were wet digested in a di-acid and volume topped to 100 ml. 5ml of 100ml solution were put in 50 ml flask and KH_2PO_4 then added. The solution was read on AAS to measure concentration of Phosphorus. Absorbance range of 0.1 was read & used to determine P from standard curve and calculated from;

P = C*df

2.4.2.3. Tissue K (Motsara & Roy, 2008)
0.5g of leaf sample were wet digested in di-acid to 100ml, 5ml of it put in 50 ml volumetric flask. 10 ml KCl was added and water added to volume and shaken for 10 minutes. Absorbance was read on AAS and K determined from standard curve.

K = C*df.

2.4.2.4. Tissue Mg. (Motsara & Roy, 2008)
0.5g of leaf sample were wet digested by di-acid and topped to 100ml by water. To 5ml in 50ml volumetric flask, 10ml of Magnesium standard reagent was added. Absorbance of final solution was measured on AAS.

Mg = C*df.

2.4.2.5. Tissue Ca. (Motsara & Roy, 2008)
0.5g of the leaf sample were wet digested by di-acid and topped to 100 ml by water. 5 ml in 50ml volumetric flask, 10 ml of Calcium standard reagent was added. Absorbance of final solution was measured on AAS.

Mg = C*df.

KEY.
C……. Concentration
df……… dilution factor
wt ………weight of leaf sample used.
AAS…….Atomic Absorption Spectrophotometer.

2.5 Statistical analysis

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

3. RESULTS AND DISCUSSION

3.1 Chlorophyll content index

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

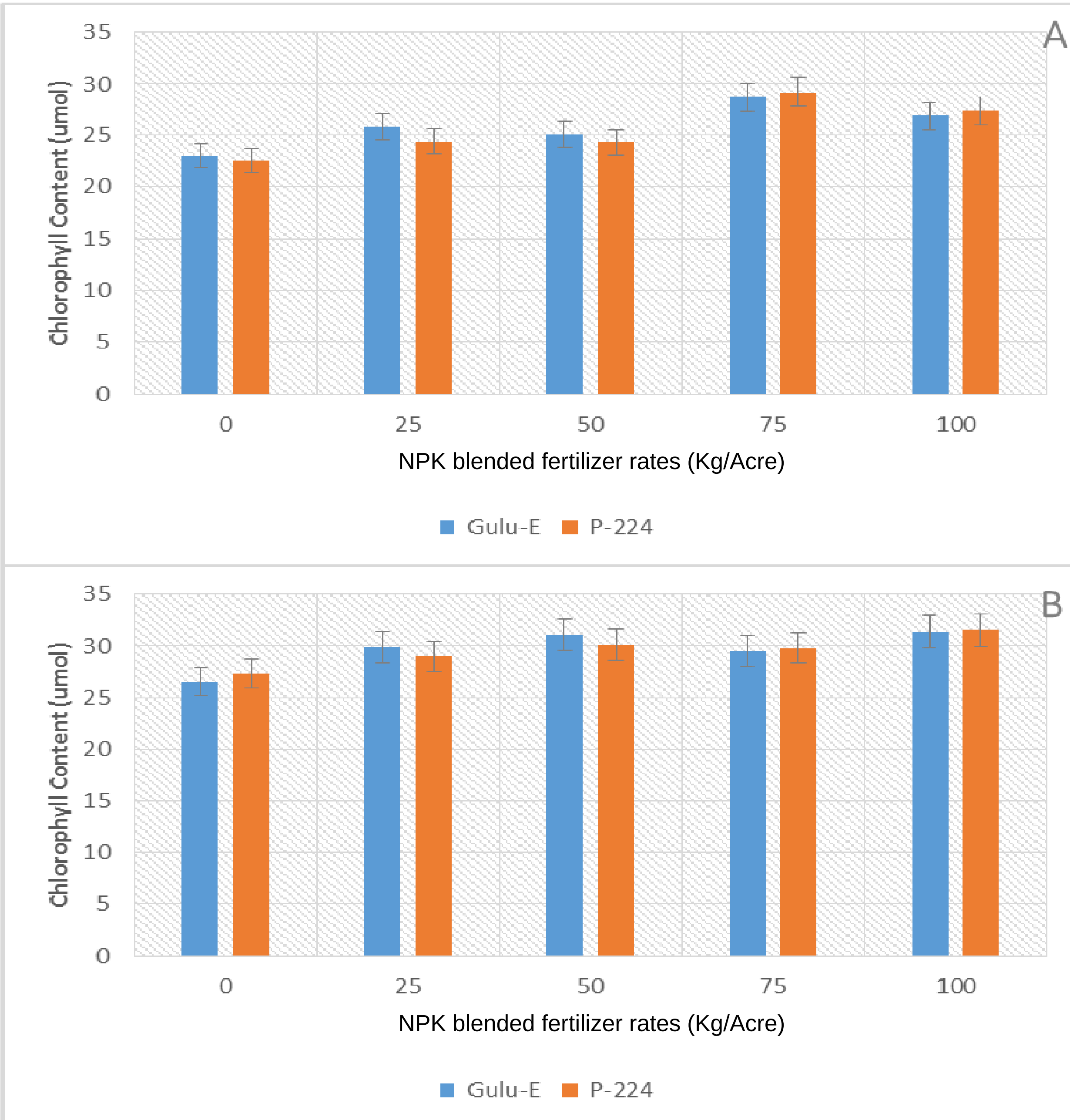

Fig 1. The influence of NPK blended fertilizer on the chlorophyll content of Gulu-E and P-224 finger millet varieties at Kakamega during the Short rain (A) and long rain (B) seasons. Error bars indicate the SE at P≤0.05

3. 2 Tissue calcium content

The application of NPK blended fertilizer led to an increase in the tissue calcium content of the plant. The 75 kg/acre NPK blended fertilizer rate elicited significantly the highest calcium content in the tissues of finger millet varieties (Table 1 and 2). The control had the lowest plant tissue calcium content in both varieties with a decrease observed in the highest NPK blended fertilizer rate from the 75 kg/acre rate onwards. In the long rainy season, a conclusive trend could not be determined but the 25 kg/acre treatment exhibited the highest calcium content while the control had the lowest on variety Gulu-E with 81 mg/100 g and 64.3 mg/100 g respectively. Variety P-224 had the highest calcium content of 81.3 mg/100 g and lowest content of 60.3 mg/100 g. Variety P-224 had the highest calcium content of 81.3 mg/100 g and lowest content of 60.3 mg/100 g. The results are in agreement with those of [10] who found that NPK blended fertilizer in combination with manure application in maize led to increased calcium uptake. Results also agree with those of [9]. This could be attributed to the increased levels of potassium uptake which antagonistically affects calcium uptake at the highest rate.

3. 3 Tissue potassium content

Application of NPK blended fertilizer led to increase in the tissue potassium uptake in both seasons. Significant differences at P<0.05 were observed between the NPK blended fertilizer treatments during the short rainy season on the plant tissue potassium content (Table 1 and 2). There were no significant differences between the treatments observed during the long rainy season. The 75 kg/acre rate showed the highest K content in the plant tissues of finger millet where P-224 had 1057 mg/100 g and Gulu-E had 997 mg/100 g during the short rainy season. Though not significantly different, the control showed the lowest amount of tissue K in the long rainy season with 758 mg/100 g and 757 mg/100 g for Gulu-E and P-224 varieties respectively, the decrease in long rain season could have 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.

3. 4 Tissue magnesium content

Application of NPK blended fertilizer let to increase in the tissue magnesium uptake in both seasons Significant differences (P<0.05) were observed between the treatments for both seasons (P<0.05) as shown on (Table 1 and 2). Gulu-E and P-224 elicited the lowest magnesium content in the control during the long and short rainy seasons. The highest K tissue content in Gulu-E was observed on the 50 kg/acre NPK blended fertilizer rate for both seasons while P-224 showed a different trend where above 50 kg/acre rate had the highest Mg content during the short rainy season while the 25 kg/acre and 100 kg/acre rates had the highest during the long rainy season. The highest K tissue content (160 mg/100 g) in Gulu-E was observed on the 50 kg/acre NPK blended fertilizer rate while P-224 showed a different trend where above 50 kg/acre rate had the highest Mg content while the 25 kg/acre (163 mg/100 g) and 100 kg/acre rates had the highest in the first trial. This results are in agreement with those of [10]. The results might have been due to increased amounts of Magnesium that was released and made available to plants due to increased soil pH and absorbed by the plant in exchangeable form. More magnesium was present in the soil solution and thereby making it conducive for uptake hence higher uptake. Higher tissue magnesium levels might have also been due to optimum levels of nitrogen which is synergistic to magnesium uptake.

Table 1. The influence of NPK blended fertilizer on the calcium, magnesium, nitrogen, phosphorus and potassium contents in Gulu-E and P-224 finger millet varieties at Kakamega during 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**

Values in columns followed by the same letter do not differ significantly at P≤0.05.

Table 2. The influence of NPK blended fertilizer on the calcium, magnesium, nitrogen, phosphorus and potassium contents in Gulu-E and P-224 finger millet varieties at Kakamega during the long rains 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**

Values in columns followed by the same letter do not differ significantly at P≤0.05

3. 5 Tissue nitrogen content

Application of NPK blended fertilizer significantly ($P<0.05$) influenced the nitrogen content in the finger millet tissues for both seasons (Table 1 and 2). The highest content was observed on Gulu-E variety for both seasons on the highest rate whereas the same trend was observed on P-224 variety. During the short rainy season, a linear increase was observed with increasing NPK blended fertilizer rates peaking at 100 kg/acre with 2650 mg/100 g for Gulu-E variety and 259 mg/100 g for P-224 variety. The increase in tissue nitrogen might have been due to optimal levels of copper and boron nutrients present in the NPK blended fertilizer that could have promoted nitrogen uptake by the finger millet varieties. These results are in agreement with those of [10].

3. 6 Tissue phosphorus content

The phosphorus tissue content was significantly influenced by the NPK blended fertilizer treatment during the long rainy season (Table 2). There was no conclusive pattern observed during the short rainy season but with the control exhibiting the lowest phosphorus content for both varieties. The 50 kg/acre NPK blended fertilizer rate elicited the highest P tissue content under both varieties with Gulu-E having 459 mg/100 g and P-224 having 447 mg/100 g. These results are in agreement with those of [10] who found that NPK blended fertilizer in combination with manure application in maize, led to increased phosphorus uptake by the plant. This might be due the Phosphorus component in the fertilizer that could have led to increased uptake of the nutrient and also the increase of the soil pH with increasing NPK blended rates that might have led to the reduction of Fe and Al ion concentration in the soil thereby decreasing the adsorption/precipitation of P thus more uptake and accumulation in the plant tissues.

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

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

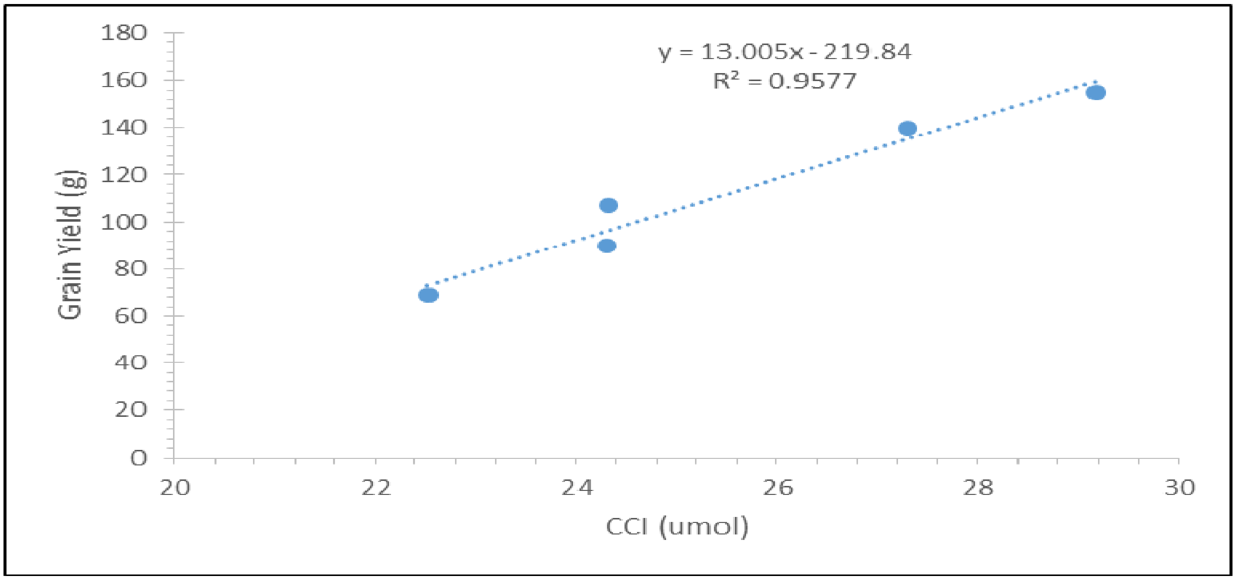

Fig.2: Linear relationship between the chlorophyll content index and the grain yield of finger millet at Kakamega.

4.0. CONCLUSION

The physiological components (chlorophyll content, calcium, magnesium, potassium, nitrogen, and phosphorus) positively increased by application of NPK blended fertilizer compared to the absolute control. This could have been due to increased absorption of mineral elements under fertilizer applied treatments. The increased uptake of the nutrients could be as a result of liming caused by the liming components present in the NPK blended fertilizer. The Nutrients taken up positively influenced the physiology of the two finger millet varieties such chlorophyll content of the plant. It was observed that with the increasing chlorophyll content index, there was a positive linear increase in the grain yield per plant of finger millet.

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