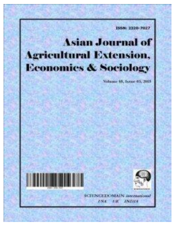

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The Effect of *Croton macrostachyus*, *Plectranthus barbatus* Leaf Aqueous Extracts and Inorganic Fertilizers on Growth and Nutrients Concentration of *Brassica oleracea* L. in a Greenhouse at Nairobi

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Authors' contributions

This work was carried out in collaboration between all authors. Author KO designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors JM and RW managed supervised the analysis of the study. Author NN managed the literature searches and editing of first draft

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ABSTRACT

The study was undertaken in Sustainable Development Initiatives Centre greenhouse at United States international university -Africa. The greenhouse experiment was laid out in potted containers in a randomised complete Block design (RCBD), with 3 replications. A total of four treatments composed of decomposed leaves extracts of *Plectranthus barbatus* (PB) and *Croton macrostachyus* (CM), inorganic fertilizer (IF) and Untreated soils (US) as control were used. The level of mineral and nutritive elements in leaf extracts and kales were determined by ICPE, β-carotene was determined by HPLC while Phosphorus Nitrogen were determined by UV-VIS. Data was analyzed using (ANOVA) and significant treatment means separated using the Turkey's Honestly Significant

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Difference Test. The highest mean levels macro nutrients in leaves (μg/g) K^+; 228.31±1.76; Mg^{2+}, 188.35 ± 1.24; $PO4^{3-}$, 16.21 ±3.36 and NO_3^-, 95.35 ± 2.36 for croton macrostachyus and K^+: 412.71 ± 2.55. Mg^{2+}: 369.72± 3.25, $PO4^{3-}$: 29.59 ± 2.04 and NO_3^-, 63.24± 1.47 for *Plectranthus barbatus*. Application of croton macrostachyus, and *Plectranthus barbatus* leaf water extracts resulted in increased mean shoot length, leaf length, and number of leaves of kales as compared to the control. The mean growth rate for kales (shoot length 8.69 ± 3.68-12.64cm, Mean leaf length 8.34 ± 4.17-12.82 ± 5.53, Mean number of leaves 8.38 ± 2.94-12.53± 4.73 and Yield (t/ha) ranged from 2.44 - 5.89. Kales grown using leaf extracts had higher nutritive values as compared to the control. The mean nutritive values in kales were Iron (Fe) 3.87 – 5.24 mg/100g; Magnesium (Mg) 250.96-323.67 mg/100g; Sodium (Na) 216.21-320.81mg/100g; Phosphorus (P) 261.82-294.31 mg/100g; zinc (Zn) 1.17 -1.36 mg/100g; and β-carotene 4.73±0.15 - 3.38± 0.09 mg/100g.There was significant difference in nutritive values between leaf extracts and control. The growth of kales using leaf extracts of *Croton macrostachyus* responded better and gave a yield that was comparably higher to those of inorganic fertilizer and control. This study suggests that *Croton macrostachyus* and *Plectranthus barbatus* leaves may be potential source of plant nutrients for crop production and higher nutritive values.

Keywords: Plectranthus barbatus; Croton macrostachyus; soil improvement; growth of Brassica oleracea L; improved nutritive values.

1. INTRODUCTION

1.1 Kales (*Brassica oleracea* L. var. *acephala*)

The kale (*Brassica oleracea L. var. acephala*), also called (*sukuma wiki*) is classified as a family of *Brassicaceae*, known as cultivatory plan [1]. The plant is among the oldest species of the cabbage family which originated from eastern Mediterranean Sea. Kale was popularized round the world by immigrants from Mediterranean [2]. Kale growers use the tender leaves for human consumption and the older ones are used as forage for livestock [2,3]. Due to the important functions that kales play in the diet in human, it has attracted increasing attention in recent years. Several studies reveal numerous health benefits of kale ranging from reduced cases of chronic diseases, precursors for cancer and cardiovascular diseases [4,5,6,7]. The area under production of Kales has continued to decrease to 24,422 Ha by 2014 with production of 348,637 MT with a value of Kshs 4.8 billion [8]. The productivity of kales is significantly affected by availability of K, Na, Mg, Ca, Fe, pH, P, Al and N nutrients in soils [9,10]. Plant leaves and other organic materials are great source of soil nutrient [11]. Plants leaves contain humic acids existing as humate with cation exchange sites for Ca, Mg, Na, K ions [12]. The humate cations exchanges with NO_3^-, and H^+ ions in soil thus raising the soil pH. The humates also modify soil cation exchange capacity, increases the nutrients availability which in turn increases crop production [13]. Studies on *croton macrostachyus* and *Plectrathus bartatus* leaves shows abundant supply of element Ca, Mg , Fe, Na, P, and K [14,15,16] which are essential for crop production. Research finding points towards anaerobic decomposition of leaves aqueous extracts for faster release of nutrients. Strynchuk et al. [17] reported increased nutrient release in grass through decomposition. Similar results were observed by [18,19] on decomposition of leaf solution. Mahari et al. [20], reported that there was increased nutrient release by *croton macrostachyus* and *Cordia africanas* leaves in solution form. The use of plant aqueous extracts for crop production is well documented. Rady et al. [21] reported increased growth rates and yields of beans using Moringa leaf extracts, the leaf extracts of *Nerium oleander, Eugenia jambolana, and Citrullus colocynthis* have been found to improve the growth of lupine plants. [22] has reported increased germination, growth and maize yields using aqueous teak leaf extracts. Talukder et al. [22] reported increased spinach production using *Terminalia belerica* herbal plant extracts, while the neem extract decreased the germination and growth of turnip.

The nutritional quality of kales grown using organic techniques is largely unknown. Studies done on potatoes [23,24], red tomato [25,26], kales, carrots [27] and celeriac showed higher vitamin C, and β-carotene levels in organically-grown products. Weibel et al. [28] reported, no difference in nutritive values in leek, carrot or beetroot grown using organic fertilizers and mineral fertilizers. Altintas et al. [29] reported no significant difference in mineral composition of bell pepper grown using mineral fertilizer and organic fertilizer. The potential of *croton macrostachyus* and *Plectrathus bartatus* leaf extracts as a source of plants nutrients, for growth, yields as well as increasing nutritive values of kales in a green house is presented in the current study.

2. MATERIALS AND METHODS

2.1 Study Area

The trials experiments were conducted at United States International University-Africa (USIU-A) greenhouse garden (1.214° S, 36.880° E) in Nairobi Kenya. The study area has subtropical highland climate at an altitude of above 1895m above sea level with rainfall averaging to above 800mm. The leaves of *Croton macrostachyus* Del (Mutundu) and *Plectranthus barbatus* Del (Maigoya) were collected from Mount Kenya region Meru (0.05° N, 37.6500° E), Nyeri (0.4167° S, 36.9500° E) and Nyandarua (.5500°S, 36.6167° E). The three areas were chosen since they are home to many species of *Croton macrostachyus* and *Plectranthus barbatus* which are either widely found naturally as fences or intercropped together with food crops. These regions are home to large number of small scale vegetable farmers. Acidic Soil was sampled from areas near USIU-A neighborhood.

2.2 Materials, Chemicals, Seeds, Tools and Apparatus

The equipments used in this study included; pH meter (Sanxin MP521), ultra violet spectrophotometer (Rayleigh UV-9200), inductively coupled plasma emission spectroscopy (Shimadzu ICPE-9000 Multitype), blender (BRUHM M012), analytical balance (OHAUS PA214), grinder (Basant) and muffle furnace (carbolite). All the reagent used were analytical grade; Sulphuric acid, hydrogen peroxide, multi element standard for ICPE, Ethylene diamine tetra acetic acid, (EDTA), Ammonium fluoride, hydrochloric acid, nitric acid, ammonium acetate, ammonium molybdate, sodium nitro prusside, sodium citrate, sodium tartrate, sodium hypochlorite and antimony potassium tartrate were all purchased from were sourced from Sigma-Aldrich Chemical Co., USA and supplied by Kobian Kenya limited. All the apparatus used in this study were rinsed thoroughly with distilled water and oven dried. High quality Kale seeds were obtain from Amiran Kenya.

2.3 Collection of Leaves and Preparation of Leaf Extract

Croton macrostachyus and *Plectranthus barbatus* leaves were collected from small scale farmers in Meru, Nyeri, and Nyandarua in Kenya. The leaves were washed to remove dirt; dried under shade for 2 to 4 weeks in separate groups based on sampling site. The dry leaves were then ground into fine powder using a hammer mill grinder and given codes (CM) for *Croton macrostachyus* and (PB) for *Plectranthus bartatus*. Part of the ground powder was analyzed for macronutrients, and then kept aside to be mixed with selected soils at a rate of 5t/Ha during planting. The rest were subdivided into different codes CM1, CM2 and CM3 for *Croton macrostachyus* and, PB1, PB2 and PB3 for *Plectranthus bartabus*. The leaves were soaked in distilled water into 20L containers at rate of 100g/L in laboratory under room temperature for a period of 90 days to gain compost stability [30].

2.4 Green House Experiment

5kg of Acidic Soil sourced from USIU–A neighbourhood were sampled, sieved to break down big particles and filled in 5kg pots. Prior to planting the upper layer of 15cm of soils were mixed with powdered leave at a rate of (5t/Ha biomass) as recommended by [31 and 32] with small modification. *Brassica oleraceae* (kales) seeds were planted in a nursery beds adjacent to the greenhouse and Seedlings were transplanted two weeks after sowing. The healthy and vigorously growing seedlings were selected and transplanted in 24 pots with different treatments (Table 1). Prior to transplanting, the seedling bed were watered to allow lifting with soil clods attached to the roots. The application of aqueous extracts were at a rate of 600L/Ha foliar nutrition measurements [33]. A complete randomize design was applied for the greenhouse trials, with application of aqueous extracts twice in a month after transplanting and distilled water daily. Inorganic fertilizer (IF) and untreated soils (US) were the control in these experiment with inorganic fertilizer being applied once during transplanting period.

2.5 Sample Digestion for Analysis of Macronutrients in the Dried Leaves, Soils and Crops

Exactly 0.1 ± 0.0001g dry sample was weighed into digestion specimen tubes. The samples were then placed in a muffle furnace for $4^1/_2$

hours at 450°C for ashing. The samples were allowed to cool down then digested with 10 ml of 1:1 HCl and HNO_3. After digestion, 20% H_2O_2 was added to complete digestion. The mixture was then subjected to heat on the hot plate till the sample mixture completely evaporated. The sample were Re-dissolved using 10 ml 0.5N of HCl, the specimen tube cocked and allowed to stand for at least 5 hours to re – extract the elements.

2.6 Determination of Total Phosphorus

The standard solution for phosphorus analysis was prepared by dissolving 2.20 g of pure potassium orthophosphate 500 ml volumetric flask and distilled water added to mark. The working standards of 0, 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm were prepared from these stock solutions. A 5 ml of wet digested sample was pipetted into 50 ml volumetric flask. 2 ml of ammonium molybdate/antimony potassium tartrate and 10 ml of ascorbic acid reducing agent were added, and made to 50 v/v ml with distilled water. The solution was allowed to stand for 1 hour to permit full color development and absorbance was measured at 880 nm using UV/visible spectrophotometer [34]. Blanks and standards were also treated the same way. From the calibration curve of the standards the concentration of total phosphorus was obtained in mg/100g sample.

2.7 Determination of Nitrogen as a Nitrate

The standard solution for nitrates (2500 ppm) stock was prepared by diluting 1.179 g of ammonium sulphate in distilling water. Working standard of 0, 4, 8, 12, 16, 20 and 24 (ppm) were prepared from the stock solution. A 5 ml of powdered sample were diluted to ratio of 1:9 (v/v) with distilled water in a digestion tube and shaken on a shaker for 30 minutes. 5 ml of a mixture of (34.00 g of sodium salicylate, 25.00 g of sodium citrate, 25.00 g of sodium tartrate and 0.12 of sodium nitroprusside in one liter of distilled water) was added vortexed for 10 minutes. 30 mg/l of sodium hydroxide was then added and the mixture vortexed for another ten minutes. The solution was allowed to stand for 2 hours. The procedure was repeated for standard and absorbance for standards and samples measured at 650 nm using Rayleigh 2000 UV/VIS spectrophotometer [34]. From the calibration curve of the standards the concentration of total nitrogen was obtained in mg /100 g sample.

2.8 Determination of Macro and Micro Nutrients Using ICPE

Extractable macronutrients (potassium, calcium, magnesium, sodium iron zinc and aluminium) were determined usingInductively Coupled Plasma Emission (ICPE) spectroscopy (Kalra 1998).1000ppm of commercial premixed metal standard (for 23 metals), was used to prepare different working standards. One set of standards for macro element determination ranged from 10ppm to 50ppm while the other set for trace elements ranged from 0ppm, 0.1 ppm, 0.2 ppm, 0.5 ppm, 1 ppm, 2 ppm, 5 ppm. All the standard solutions were prepared in 10% (v/v) in nitric acid. All Samples analyses were performed on a Shimadzu ICPE 9000 system with CCD that allows measurements of all elements at all wavelength. The ICPE conditions were set as in the Table 2. The Measurements were determined in comparison to premix all metal standard calibration curves and concentration in mg /100 g sample obtained.

2.9 Determination of Beta Carotene in Crops

Fresh clean samples of Kales were homogenized using an electric blender. 10g ± (0.05) sample, were mixed with 0.6 grams of $MgCO_3$ (acid neutralizing agents) and 0.8gbutylated hydroxytoluene (BHT) sonicated for 2 hours and then extracted with hexane/acetone/ethanol, 2:1:1 v/v). 50ml solution of sodium hydroxide was added and sonicated to enhance separation of aqueous and organic phase containing carotenoids [36]. The organic phase was evaporated to near dryness under nitrogen and then reconstituted by 5 ml methanol. The reconstituted extract were analyzed using Agilent high performance liquid chromatography with column C_{18} (Gemini-NX 5uc 18110A, 250 × 4.6 mm id × 5 µm particle size), mobile phase (Acetone: Methanol: Dichloromethane) in the ratio (70:10:20 v/v) respectively. The flow rate was set at 1.6ml/min on and elution time 10 minutes. Beta carotene was detected at 450 nm, using UV-visible detector [36]. The peak for beta carotenoid was identified by comparing the retention time to that of the standard. The measurement was done in triplicate and concentration determined in mg/100g sample.

$$C_X \ (mg/100g) = \left[\frac{P_X - B}{S}\right] \times D_f \times 100g$$

C_X = concentration of analyte in mg/100g of fresh sample, P_X = Peak area of analyte, B = intercept, S = slope and D_f = 5

Table 1. Design and treatment application

Treatment	No. of pots	Application rate
CM1	3	5t DM /ha before planting +100ml extract twice a month
CM2	3	5t DM /ha before planting +100ml extract twice a month
CM3	3	5t DM /ha before planting +100ml extract twice a month
PB1	3	5t DM /ha before planting +100ml extract twice a month
PB2	3	5t DM /ha before planting +100ml extract twice a month
PB3	3	5t DM /ha before planting +100ml extract twice a month
IF	3	fertilizer N-P-K by 25-50-50% [35]
US	3	Distilled water daily

DM= dry Matter, ha = hector, CM1, CM2 and CM3 are Croton macrostachyus. PB1, PB2, PB3 Plectranthus barbatus; US- Untreated soils; IF- inorganic fertilizers

Table 2. Conditions of ICPE equipment

Pressure of gas (pure argon)	450±10KPa
Radio frequency power	1.20KW
Plasma gas flow rate	10.0L/min
Auxillary gas	0.60L/min
Carrier gas flow rate	0.70L/min
Exposure time	30sec
Vacuum pressure	>10Pa
Couple Charged Device (CCD) detector temp	15°C
External coolant (water)	20±2°C
Detector direction view	axial
Plasma torch	standard mini torch
Nebulizer	pneumatic (coaxial nebulizer)

2.10 Determination of Growth Parameters

The average growth parameters plant height (cm), number of leaves, leaf length (cm) were all determined by measuring using a ruler and physical counting on 20^{th}, 40^{th}, 60^{th} day, and at harvest stage. The Plant height was measured in cm from the pot ground level to the tip of fully open leaves. The edible mature leaves were harvested twice from each pot for entire period of planting. The leaves were weighed per treatment at harvest stage and their average mean expressed as fresh weight in t/ha.

2.11 Determination of Levels of Mineral Elements and β-carotene Values in Harvested Kales

The levels of mineral elements (calcium, iron, magnesium, sodium, phosphorus and zinc) were determined in dry matter of kales while and β-carotene was determined in fresh kales. Kales from the same extracts were mixed and samples analyzed in triplicate.

2.12 Data Analysis

The results obtained for the macronutrients, growth rate of kales and carrots were calculated as means of three replicate measurements ± standard deviation, the values were also compared by One –Way ANOVA and Student-Newman-Keuls (SNK) test at α = 0.05 to indicate differences among means.

3. RESULTS AND DISCUSSION

3.1 Soil and Leaves Analysis

Soil conditions at the top 15 cm in pots done before the start of the experiment is as follows: pH = 4.79, total N = 0.21 %, available P = 0.004 % and exchangeable K= 0.11%.aluminium 0.0273 % exchangeable calcium0.0168 % and magnesium 0.0119 %. The chemical composition of decomposed *Croton macrostachyus* were: pH = 8.02, total N= 0.87%, available phosphorus P= 0.61%, exchangeable k = 0.29 % and available Magnesium Mg= 0.59 %. The chemical composition of decomposed *Plectranthus barbatus* were: pH = 7.99, total N= 0.604 %, available phosphorus P= 0.32 %, exchangeable k = 3.49 % and available Magnesium Mg= 0.37 %.

3.1.1 Shoot length

The mean shoot length (cm) showed avery signficantdifference (p<0.047) between the IF,CM, PB treatments and the control throughout the growing period. Shoot length ranged from a mean of 8.69 - 12.64 cm.There was also no signficant difference in shootlength between CM, PB and IF treatment.

3.1.2 Leaf number

Mean leaf number per plant increased with time over the growing period and wasalmost equal in the IF,CM and PB treatments. Differences in mean number were however very significant (p<0.025) in all thetreatment and control .The mean number of leaves per plant ranged from 8.38 - 12.5. There was no significant difference in number of leaves between IF,CM and PB.

3.1.3 Leaf length

Average leaf length per plant, increased with time over the growing period for all the treatments. Differences in mean leaf length were however very significant (p<0.015) in the all thetreatment and control .The mean leaf length per plant ranged from 8.13 -18.38 m. There was significant difference in leaf length between IF,CM and PB (p<0.015).

3.1.4 Yields per pot

The results in Table 3 represents the effects of different treatments on yields of kales. The highest vegetable yields were recorded in *Croton macrostachyus* (5.89 t/ha) leaf extracts while the least was recorded in the control (2.44 t/ha). The inorganic fertilizer treatment gave a (5.65 t/ha) which was not significantly different from those of the croton extract while the *Plectranthus barbatus* gave a yield of (4.79 t/ha). The leaf extracts contains high humus content that retain more water in the leaves leading to high yields and high strength of leaves.

3.2 Mineral Elements in Vegetables

The levels of mineral elements (calcium, iron, magnesium, sodium, phosphorus and zinc) and β-carotene were determined in dry matter of kales. Kales from the same treatment were mixed and analyzed in triplicate. The results were recorded in the Table 4.

The level of mineral elements in Kales are shown in Table 4. The mineral element were significantly different per treatment (p< 0.005). Iron (Fe) ranged between 3.87 – 5.24 mg/100g; Magnesium (Mg) 250.96 - 323.67 mg/100g; Sodium (Na) 216.21 - 320.81 mg/100g; Phosphorus (P) 261.82 - 294.31 mg/100g; and zinc (Zn) 1.17 - 1.36 mg/100g. Kales grown using extracts of *Croton macrostachyus* had higher amounts of iron, phosphorus and zinc. This could be attributed to higher rate of decomposition and nutrients release to soils .The observation further supports the fact that the type, quality and amount of organic input added to kales, will affect the overall nutrients availability in the crop. Similar result were obtained in a research by Ayaz et al. [37].

3.3 Beta Carotene

There was a significant difference in levels of β-carotene in kales grown using different treatments (p<0.05). The levels ranged between 4.73 ± 0.15 - 3.38 ± 0.09 mg/100g. Kales grown using *Croton macrostachyus* leaf extracts and inorganic fertilizers generally showed higher levels of β-carotene as compared to the control and those of *Plectranthus barbatus*. The levels were quite within the ranges reported in other studies [38,39]. Study by Ismail [39] reported high β- carotene level in organically grown vegetables than the conventionally grown ones.

Table 3. Effects of different treatments on growth parameters of kales

Treatment	Mean shoot length(cm)	Mean leaf length (cm)	Mean no. of leaves	Yield (t/ha)
CM	12.24 ± 5.06a	12.16 ± 5.17a	11.93 ± 4.52a	5.89 a
PB	11.29 ± 4.91b	11.77± 5.21b	11.19 ± 4.05a	4.79 b
IF	12.64± 5.06a	12.82 ± 5.53a	12.53± 4.73a	5.65a
US	8.69 ± 3.68c	8.34 ± 4.17c	8.38± 2.94b	2.44 d
p value	0.047	0.0145	0.025	0.031

The mean growth parameters followed by different small letters (a, b,c and d) in the same column are significantly different (P<0.05, n=3, SNK-test). Croton macrostachyus (CM,),Plectranthus barbatus (PB), inorganic fertilizer (IF) and Untreated Soil (US)

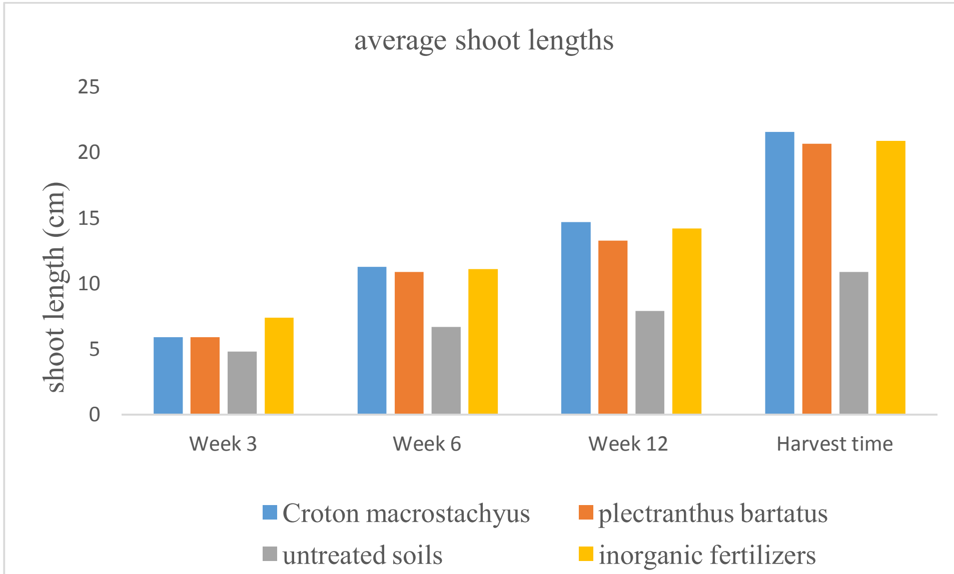

Fig. 1. Average shoot length of kales

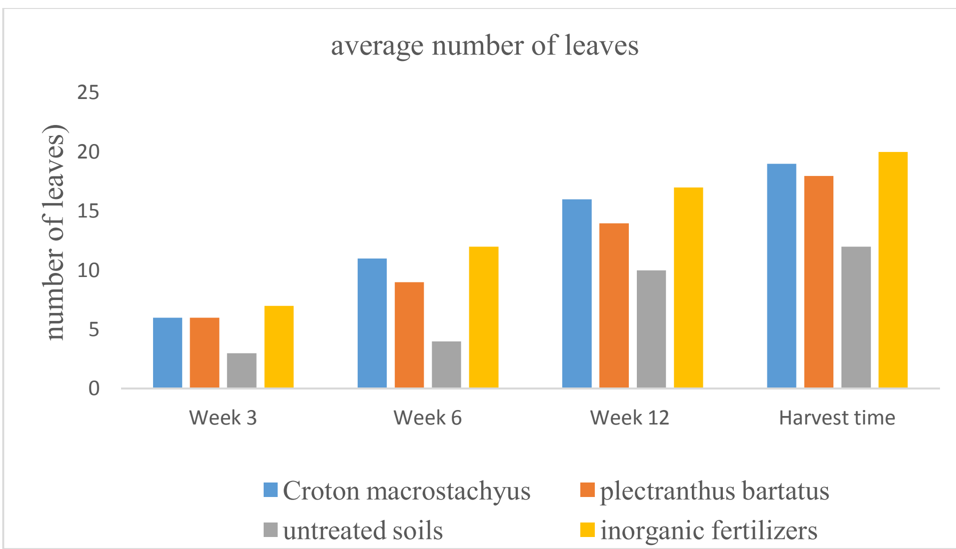

Fig. 2. Average number of leaves of kales

Table 4. The mean levels of mineral composition and β-carotene in kales

Elements (mg/100g)	CM	PB	IF	US	p
Fe	5.24± 0.61a	4.16± 0.53b	4.81 ± 0.14a	3.87 ± 0.55b	0.05
Mg	314.16 ±5.4a	284.33 ± 4.2b	323.67 ± 4c	250.96 ± 3.3d	0.02
Na	312.31 ± 1.63a	252.76± 6.23b	320.81 ± 2.51a	216.21 ± 3.55c	0.04
P	294.31 ± 4.58a	277.61± 2.72b	288.33 ± 2.08c	261.82± 2.51d	0.05
Zn	1.36 ± 0.31a	1.23 ± 1.01a	1.12 ± 0.31b	1.17 ± 0.32b	0.41
β-carotene	4.63 ± 0.24a	3.54 ± 0.15b	4.73± 0.15c	3.38 ± 0.09d	0.04

The mean of mineral elements followed by different small letters (a, b,c and d) in the same raw are significantly different (P<0.05, n=3, SNK-test). Croton macrostachyus (CM,), Plectranthus barbatus (PB), inorganic fertilizer (IF) and Untreated Soil (US).

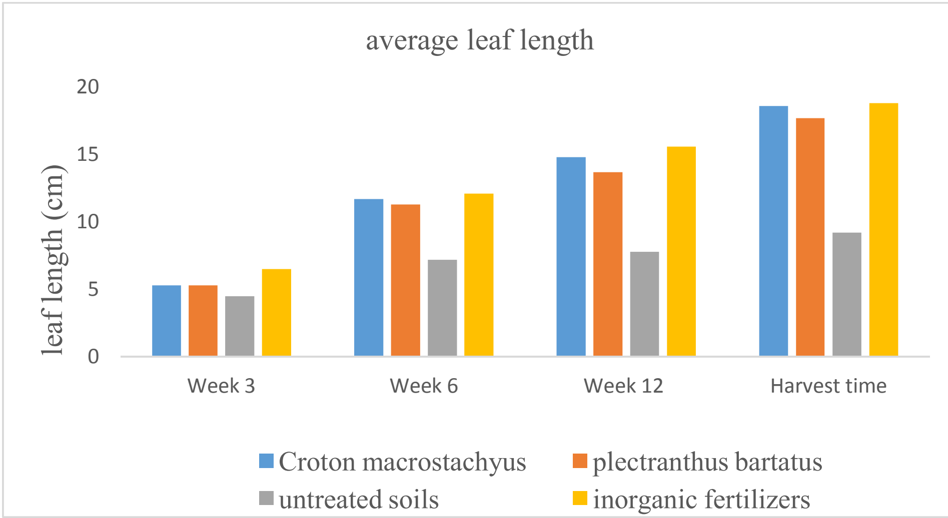

Fig. 3. Average leaf length of kales

3.4 Discussion

Shoot length, leaf length and yields were important indicators of the nutrient levels (especially of (N, P, K) in the soil. The pots with liquid aqueous extracts in the experiment had an increase in nitrogen, phosphorus and potassium levels three times the control. The greater quantities of available nitrogen coupled with moisture retention by organic matter, and beneficial soil micro- organisms. The extracts may also have stabilized the soil to prevent leaching of nutrients as compared to soil in the inorganic treatment and control which may have had higher chances of leaching. Leaf number was also an important parameter for leafy vegetables as leaves are the main consumable plant parts over the growing seasons.Mean leaf number per plant was increasing and almost equal in all treatments except the control. The leaf nutritive levels show the range of nutrients availability for treatments. Kales grown using extracts of *Croton macrostachyus* had higher amounts of iron, phosphorus and zinc. This could be attributed to higher rate of decomposition and nutrients release to soils .The observation further supports the fact that the type, quality and amount of organic input added to kales, will affect the overall nutrients availability in the crop. Similar result were obtained in a research by [37].

4. CONCLUSION

The growth of kales in the acidic soil mixed with leaf extracts of *Croton macrostachyus* responded better and gave a yield that was comparably higher to those of inorganic fertilizer. In addition the leaves of kales that were watered with leaf extract were heavier and resisted wilting at high temperatures in the green house. The nutritive values of kales grown using fertilizer was similar to those grown using leaf extracts with no significant difference. The study revealed that vegetables planted using leaf extracts will not have compromised nutritive values. The study therefore showed that *Croton macrostachyus* and *Plectranthus barbatus* leaf extracts are source of cheap soil nutrients that can substitute use of inorganic fertilizers and still generate high nutritive values of food especially among the poor in Kenya.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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