***Original Research Article***

# The Effect of *Croton macrostachyus*, *Plectranthus barbatus* leaf Aqueous Extracts, and inorganic fertilizers on the growth and nutrients concentration of kales in a greenhouse system.

Comment [E1]: effect


## ABSTRACT

Kales are popular vegetables grown in African society with short production cycle. They are used as ingredients for soup, sauces and accompaniment for carbohydrate. Over 75% of Kenyans consume popular kales among other vegetables. The consumption is expected to rise due to the increased demand on international markets and unfavorable soils condition. The growth parameters, quality and yields of vegetables differ based on soil type, available soil nutrients, and fertilizer management. From plant leaves decomposition studies, decomposed leaf aqueous extracts contributes to increased soil pH changes ,organic matter and soil nutrients through direct reaction with surface soil. The reaction involves direct exchange of hydroxyl groups of soil cations with those of organic groups of added leaf water extracts. A knowledge gap is presented on the use of *Plectranthus barbatus* and *Croton macrostachyus* leaf water extracts to improve soil nutrients, increase kale production and improve nutrients availability in kales. The leaves aqueous extracts of *Plectranthus barbatus* and *Croton macrostachyus* grown in Meru, Nyeri and Nyandarua Kenya were studied for their mineralization and their effect on growth rates, yield, and nutritive values of kales. The level of mineralization, nutritive values, and β-carotene, were determined by ICPE, HPLC, and UV-VIS.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*. There was significant difference levels of micro nutrients in leaf extracts. The mean growth rate: 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. 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) 261.82-294.31mg/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 results indicate *Plectranthus barbatus* and *Croton macrostachyus* leaf water extracts have plant growth-promoting substances and considerable amounts of micronutrients that might be responsible for better growth and production of kales. This study suggests that *croton* macrostachyus and *Plectranthus barbatus* leaves may be potential source of plant nutrients for crop production and higher nutritive values.


Comment [E2]: The abstract should be reconstructed following this order: Title>Methodology>Results>Discussion and Recommendation. Not more than 250 words.

## 1.0 **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 plant in Poland as early as 16$^{th}$ century (Sikora and Bodziarczyk, 2012). It's among the oldest specie of the cabbage family which originated from eastern Mediterranean sea. Kale was popularized round the world by immigrants from Mediterranean (Balkaya and Yanmaz, 2005). Kale growers use the tender leaves for human consumption and the older ones are used as forage for livestock (Balkaya and Yanmaz, 2005; Tıraşoğlu *et al.*, 2005). 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 (Chen and Aviad, 1990; Gosslau and Chen, 2004; Gundgaard *et al.*, 2003; Podsędek, 2007). In 2014, the area under production of Kales was 24,422 Ha which produced 348,637MT with a value of Kshs 4.8 billion (ERA, 2015). The productivity of kales is significantly affected by availability of K, Na, Mg, Ca, Fe, pH, P, Al and N nutrients in soils (Ohshiro *et al.*, 2016; Orr and Nelson, 2018). Plant leaves and other organic materials are great source of soil nutrients. Plants leaves contain humic acids existing as humate with cation exchange sites for Ca, Mg, Na, K ions (Tisdale *et al.*, 1993) .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 (Peiris *et al.*, 2002). Studies on *croton macrostachyus* and *Plectrathus bartatus* leaves shows abundant supply of element Ca, Mg , Fe, Na, P, and K (Ajasa *et al.*, 2004; Ganash and Qanash, 2018; Piotr Kalny *et al.*, 2007) which are essential for

**Comment [E3]:** The plant

**Comment [E4]:** species

**Comment [E5]:** Sea

**Comment [E6]:** Close gap

**Comment [E7]:** Close gap

**Comment [E8]:**

crop production. Research finding points towards anaerobic decomposition of leaves aqueous extracts for faster release of nutrients. Strynchuk 2000, reported increased nutrient release in grass through decomposition. Similar results were observed by (Li *et al.*, 2011; Lin *et al.*, 2007) on decomposition of leaf solution. Mahari *et al* 2015, has reported 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* 2013 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 (Abdel-Monaim *et al.*, 2011). Ampofu (2009) has reported increased germination, growth and maize yields using aqueous teak leaf extracts. Talukder *et al.*, (2015) has 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 (Fischer and Richter, 1986; Kolbe *et al.*, 1995), red tomato (Caris-Veyrat *et al.*, 2004; Pither and Hall, 1990), kales, carrots (Leclerc *et al.*, 1991) and celeriac showed higher vitamin C, and β-carotene levels in organically-grown products. Weibel *et al.*, (1998) reported, no difference in nutritive values in leek, carrot or beetroot grown using organic fertilizers and mineral fertilizers. Altintas *et al.*,( 2012) reported no significant difference in mineral composition of bell pepper grown using mineral fertilizer and organic fertilizer. He noted increased ascorbic acid (110.07 mg 100 $g^{-1}$), Ca (0.83 g $kg^{-1}$), Mg (0.98 g $kg^{-1}$) and K (18.5 g kg-1) in bell pepper grown using mineral fertilizers and increased iron (57 mg, $kg^{-1}$) and zinc (11.8 mg $kg^{-1}$) in bell pepper grown

**Comment [E9]:** Comma before the year

**Comment [E10]:** Mahari et al. (2015) reported that

**Comment [E11]:** remove

**Comment [E12]:** Altintas *et al* ( 2012)

using organic liquid fertilizers. 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.

**Comment [E13]:** The Last paragraph of the introduction should state the purpose of and the objectives of study.

## 2.0 MATERIALS AND METHODS

**Comment [E14]:** remove

### 2.1 Study Area

The trials experiments were conducted at United States International University-Africa (USIU-A) greenhouse garden (1.214$^{o}$ S, 36.880$^{o}$ E) in Nairobi Kenya. The leaves of *Croton macrostachyus* Del (Mutundu) and *Plectranthus barbatus* Del (Maigoya) were collected from Mount Kenya region Meru (0.05$^{o}$ N, 37.6500$^{o}$ E), Nyeri (0.4167$^{o}$ S, 36.9500$^{o}$ E) and Nyandarua (.5500$^{o}$S, 36.6167$^{o}$ 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. This regions are home to large number of small scale vegetable farmers. Acidic Soil were sampled from areas near USIU-A neighborhood.

**Comment [E15]:** These

**Comment [E16]:** was

### 2.2 Materials, chemicals, seeds, tools and apparatus

The equipment's 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

**Comment [E17]:** equipments

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 leave 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, 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 (Adani *et al.*, 1995).

**Comment [E18]:** remove comma

**Comment [E19]:** ;

**Comment [E20]:** and then

### 2.4 Green House Experiment

5kg of Acidic Soil 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 (Odongo *et al.*, 2007; Van Der Heijden *et al.*, 2008) with small modification. *Brassica oleraceae* (kales) seeds were planted in a nursery bed 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 (Smoleń and Sady, 2009). 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.

Table 1.

| Treatment | Pots for Kales | Rate of application |
|---|---|---|
| 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 |
| US | 3 | Distilled water daily |
| 1F | 3 | fertilizer N-P-K by 25-50-50% **(Lee *et al.*, 2009)** |

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

**2.5 Sample digestion for analysis of macronutrients in the dried leaves, soils and crops**





**Comment [E21]:** beds

**Comment [E22]:** The table needs to be titlted

**Comment [E23]:** Remove grid

Exactly 0.1 ± 0.0001 dry sample was weighed into digestion specimen tubes. The samples were then placed in a muffle furnace for $4^1/_2$ hours at 450$^o$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 2.20g of pure potassium orthophosphate 500ml volumetric flask and distilled water added to mark. The working standards of 0, 2ppm, 4ppm, 6ppm, 8ppm, 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 (Okalebo *et al.*, 2002). 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.

**Comment [E24]:** Space unit from number

**Comment [E25]:** Space number from unit

**2.7 Determination of nitrogen as a nitrate**

The standard solution for nitrates (2500ppm) stock was prepared by diluting 1.179g 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. 30mg/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 650nm using Rayleigh 2000 UV/VIS spectrophotometer (Okalebo *et al.*, 2002). From the calibration curve of the standards the concentration of total nitrogen was obtained in mg/100g sample.

**2.8 Measurement of macro and micro nutrients using ICPE**

Extractable macronutrients (potassium, calcium, magnesium, sodium iron zinc and aluminium) were determined using Inductively 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, 0.1ppm, 0.2ppm, 0.5ppm, 1ppm, 2ppm, 5ppm. 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/100g sample obtained.

**Comment [E26]:** Close gap

**Comment [E27]:** Check this again

**Comment [E28]:** Check previous comment

**Comment [E29]:** Check previous comment

**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) |

Comment [E30]: Remove grid



**2.9 Measurements 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 0.8g butylated 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).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. 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 $D_f$ = dilution factor

**2.10 Measurement of growth parameters**

I. **Plant height (cm)**

Kales from each pot were measured at 20th, 40th, 60th day, and at harvest time. The average Plant height was measured in cm from the pot ground level to the tip of fully open leaves by use of ruler.

II. **Number of leaves per plant**

The number of leaves of each pot was counted on 20th, 40th, 60th day, and at harvest stage. The average expressed as number of leaves per plant.

III. **Length of leaves (cm)**

The length of leaves of kales were randomly selected from each pot. The leaves were measured by ruler on 20th, 40th, 60th day, and at harvesting stage. The average was expressed in centimeters.

IV. **Yield for kales and carrots**

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.

**Comment [E31]:** Too many details. Summarize into 4 lines for all the growth parameters

**2.11 Analysis 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.

**Comment [E32]:** Close gap

**3.0 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 were: pH = 4.79, total N = 0.21%, available P = 0.004% and exchangeable K= 0.11%. aluminium 0.0273% exchangeable calcium 0.0168 % and magnesium 0.0119%. The chemical composition of decomposed croton macrostachyus were: pH = 8.02, total N= 0.87%,

**Comment [E33]:** Is as follows:

**Comment [E34]:** Separate unit from number and reconcile others.

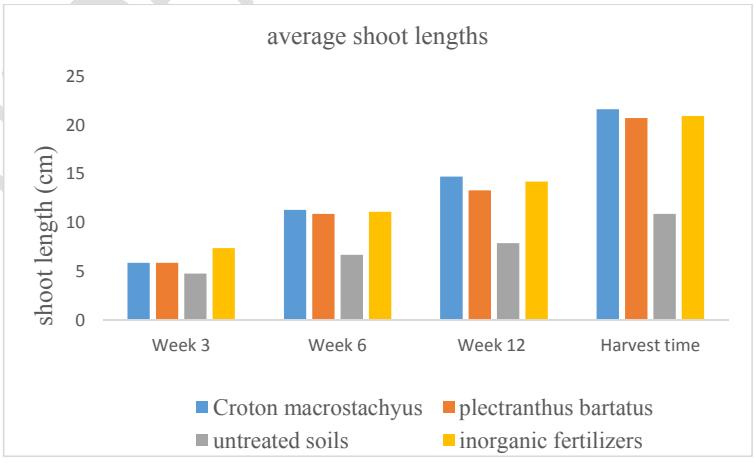
available phosphorus P= 0.61%, exchangeable k = 0.29% and available Magnesium Mg= 0.59%. The chemical composition of decomposed *Plectranthus barbatus* were: pH = 7.99, total N= 0.604%, available phosphorus P= 0.32%, exchangeable k = 3.49% and available Magnesium Mg= 0.37%.

**Table 3. Growth parameters**

| treatment | Mean shoot length(cm) | Mean leaf length (cm) | Mean number of leaves | Yield (t/Ha) |
|---|---|---|---|---|
| CM | 12.24 ± 5.06a | 12.16 ± 5.17a | 11.93 ± 4.52a | 5.89a |
| PB | 11.29 ± 4.91b | 11.77 ± 5.21b | 11.19 ± 4.05a | 4.79b |
| 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 | 2.44d |
| p value | 0.047 | 0.0145 | 0.025 | 0.031 |

**Comment [E35]:** Remove grid

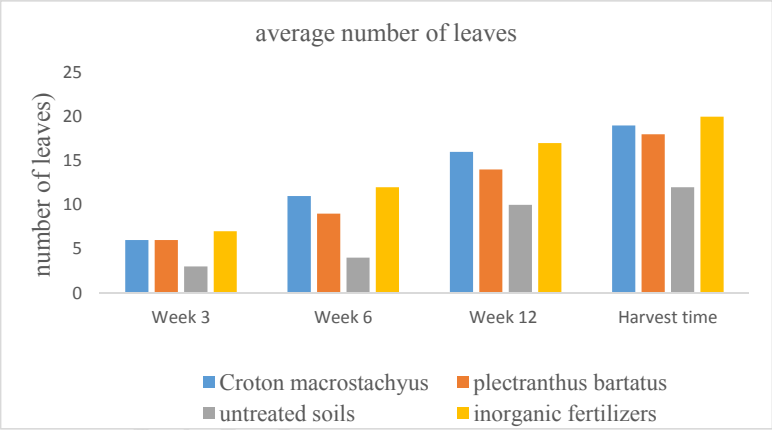
The mean growth parameters followed by different small letters (a, b,c and d) in the same column are significantly different ($P<0.05$, n=3, SNK-test). *Croton macrostachyus* (CM,), *plectranthus barbatus* (PB), inorganic fertilizer (IF) and Untreated Soil (US)



**a.) Shoot length**

The mean shoot length (cm) showed a very signficant difference ($p<0.047$)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 .

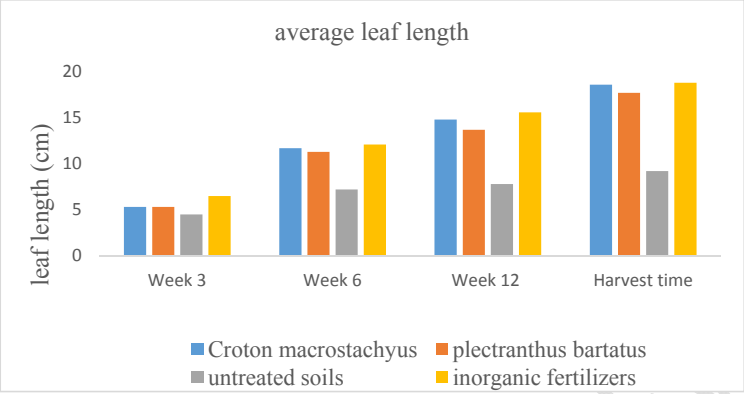
Comment [E36]: space




**b.) Leaf number**

Mean leaf number per plant, as expected, increased with time over the growing period and was almost equal in the IF,CM and PB treatments. Differences in mean number were however very significant ($p<0.025$) in all the treatment 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.

Comment [E37]: Not right to use the word "expected" in scientific writing. Reconstruct statement everywhere it appeared.




**c) Leaf length**

Average leaf length per plant, as expected, 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 the treatment and control .The mean leaf length per plant ranged from 8.13 -18.38cm. There was significant difference in leaf length between IF,CM and PB ($p<0.015$).

**Comment [E38]:** expunge

**d) 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* (5.89t/Ha) leaf extracts while the least was recorded in the control (2.44t/Ha). The inorganic fertilizer treatment gave a (5.65t/Ha) which was not significantly different from those of the croton extract while the *Plectranthus barbatus* gave a yield of (4.79t/Ha). The leaf extracts contains high humus content that retain more water in the leaves leading to high yields and high strength of leaves.

**Comment [E39]:** Change to "ha"

### 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 below.

**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).

Comment [E40]: Remove grid

The level of mineral elements in Kales are shown in table 4 above. 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) 216.21-320.81mg/100g; Phosphorus (P) 261.82-294.31mg/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.*, 2006).

### 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 (Britton and Khachik, 2009) (Ismail and Cheah, 2003). Study by Ismail (Ismail and Cheah, 2003) reported high β- carotene level in organically grown vegetables than the conventionally grown ones.

### 3.4 Discussion and conclusion

**Comment [E41]:** Separate the result discussion from conclusion of the article.

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 (Ayaz *et al.*, 2006).

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.

These study has concluded 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.

**Comment [E42]:** Replace with……The study therefore showed that

## References


Abdel-Monaim, M., Abo-Elyousr, K., and Morsy, K. (2011). Effectiveness of plant extracts on suppression of damping-off and wilt diseases of lupine (Lupinus termis Forsik). *Crop protection,* 30(2), 185-191.

Adani, F., Genevini, P., and Tambone, F. (1995). A new index of organic matter stability. *Compost Science and Utilization,* 3(2), 25-37.

Ajasa, A. M. O., Bello, M. O., Ibrahim, A. O., Ogunwande, I. A., and Olawore, N. O. (2004). Heavy trace metals and macronutrients status in herbal plants of Nigeria. *Food Chemistry, 85*(1), 67-71. Altintas, Sureyya, Eryilmaz Acikgoz, and Funda. (2012). The effects of mineral and liquid organic fertilizers on some nutritional characteristics of bell pepper (Vol. 11).

Ampofo, K. G. (2009). *Effect of tectona grandis leaf extract, mulch and woodlot soil on germination and growth of maize.*

Ayaz, F. A., Glew, R. H., Millson, M., Huang, H., Chuang, L., *et al.* (2006). Nutrient contents of kale (Brassica oleraceae L. var. acephala DC.). *Food Chemistry, 96*(4), 572-579.

Balkaya, A., and Yanmaz, R. (2005). Promising kale (Brassica oleracea var. acephala) populations from Black Sea region, Turkey. *New Zealand Journal of Crop and Horticultural Science, 33*(1), 1-7.

Britton, G., and Khachik, F. (2009). Carotenoids in food. *Carotenoids*, 45-66.

Caris-Veyrat, C., Amiot, M.-J., Tyssandier, V., Grasselly, D., Buret, M., *et al.* (2004). Influence of organic versus conventional agricultural practice on the antioxidant microconstituent content of tomatoes and derived purees; consequences on antioxidant plasma status in humans. *Journal of agricultural and food chemistry, 52*(21), 6503-6509.

Chen, Y., and Aviad, T. (1990). Effects of humic substances on plant growth. *Humic substances in soil and crop sciences: Selected readings*(humicsubstances), 161-186.

ERA. ( 2015). Economic review of agriculture. In m. o. a. Government of Kenya, livestock and fisheries (Ed.).

Fischer, A., and Richter, C. (1986). *Influence of organic and mineral fertilizers on yield and quality of potatoes.* Paper presented at the Importance of biological agriculture in a world of diminishing resources: proc of the 5th IFOAM Int Scientific Conference at the Univ of Kassel (Germany), Aug 27-30, 1984/H. Vogtmann, E. Boehncke, I. Fricke, eds.

Ganash, M., and Qanash, S. (2018). Phenolic Acids and Biological Activities of Coleus forskohlii and Plectranthus barbatus as Traditional Medicinal Plants. *International Journal of Pharmacology, 14*(6), 856-865.

Gosslau, A., and Chen, K. Y. (2004). Nutraceuticals, apoptosis, and disease prevention. *Nutrition, 20*(1), 95.

Gundgaard, J., Nielsen, J. N., Olsen, J., and Sørensen, J. (2003). Increased intake of fruit and vegetables: estimation of impact in terms of life expectancy and healthcare costs. *Public health nutrition, 6*(01), 25-30.

Ismail, A., and Cheah, S. F. (2003). Determination of vitamin C, β-carotene and riboflavin contents in five green vegetables organically and conventionally grown. *Malaysian journal of nutrition, 9*(1), 31-39.

Kolbe, H., Meineke, S., and Zhang, W.-L. (1995). Differences in organic and mineral fertilization on potato tuber yield and chemical composition compared to model calculations. *Agribiological research (Germany).*

Leclerc, J., Miller, M., Joliet, E., and Rocquelin, G. (1991). Vitamin and mineral contents of carrot and celeriac grown under mineral or organic fertilization. *Biological Agriculture & Horticulture, 7*(4), 339-348.

Lee, G.-J., Lee, J.-T., Zhang, Y.-S., Hwang, S.-W., Park, C.-S., *et al.* (2009). Recommendations of NPK Fertilizers based on Soil Testing and Yied Response for Carrot in Highland. *Korean Journal of Soil Science and Fertilizer, 42*(6), 467-471.

Li, L.-J., Zeng, D.-H., Yu, Z.-Y., Fan, Z.-P., Yang, D., *et al.* (2011). Impact of litter quality and soil nutrient availability on leaf decomposition rate in a semi-arid grassland of Northeast China. *Journal of Arid Environments, 75*(9), 787-792.

Lin, Y. M., Liu, J. W., Xiang, P., Lin, P., Ding, Z. H., *et al.* (2007). Tannins and nitrogen dynamics in mangrove leaves at different age and decay stages (Jiulong River Estuary, China). *Hydrobiologia, 583*(1), 285-295.

Mahari, A., Giday, K., and Gashaw, T. (2015). Soil nutrient status under different vegetation cover classes of Desa'aa dry Afromontane forest in Northern Ethiopia. *Research Journal of Agriculture and Environmental Management. Vol, 4*(3), 131-133.

Odongo, N. E., Hyoung-Ho, K., Choi, H.-C., Van Straaten, P., McBride, B. W., *et al.* (2007). Improving rock phosphate availability through feeding, mixing and processing with composting manure. *Bioresource Technology, 98*(15), 2911-2918.

Ohshiro, M., Hossain, M. A., Nakamura, I., Akamine, H., Tamaki, M., *et al.* (2016). Effects of soil types and fertilizers on growth, yield, and quality of edible Amaranthus tricolor lines in Okinawa, Japan. *Plant production science, 19*(1), 61-72.

Orr, R., and Nelson, P. N. (2018). Impacts of soil abiotic attributes on Fusarium wilt, focusing on bananas. *Applied Soil Ecology*.

Peiris, D., Patti, A. F., Jackson, W. R., Marshall, M., and Smith, C. J. (2002). The use of Ca-modified, brown-coal-derived humates and fulvates for treatment of soil acidity. *Soil Research, 40*(7), 1171-1186.

Piotr Kalny, Fijałek, Z., Daszczuk, A., and Ostapczuk, P. (2007). Determination of selected microelements in polish herbs and their infusions. *Science of the Total Environment, 381*(1), 99-104.

Pither, R., and Hall, M. (1990). *Analytical Survey of the Nutritional Composition of Organically Grown Fruit and Vegetables: MAFF Project*: Campden Food and Drink Research Association.

Podsędek, A. (2007). Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *LWT-Food Science and Technology, 40*(1), 1-11.

Rady, M. M., Varma, B., and Howladar, S. M. (2013). Common bean (Phaseolus vulgaris L.) seedlings overcome NaCl stress as a result of presoaking in Moringa oleifera leaf extract. *Scientia Horticulturae, 162*, 63-70.

Sikora, E., and Bodziarczyk, I. (2012). Composition and antioxidant activity of kale (Brassica oleracea L. var. acephala) raw and cooked. *Acta scientiarum polonorum. Technologia alimentaria, 11*(3).

Smoleń, S., and Sady, W. (2009). The effect of various nitrogen fertilization and foliar nutrition regimes on the concentrations of sugars, carotenoids and phenolic compounds in carrot (Daucus carota L.). *Scientia Horticulturae, 120*(3), 315-324.

Strynchuk, J., Royal, J., and England, G. (2000). Grass and leaf decomposition and nutrient release study under wet conditions *Watershed Management and Operations Management 2000* (pp. 1-10).

Talukder, M. A., M. Rahaman, B. R., and Saha, K. C. (2015). Effects of on germination and seedling growth of some vegetables. *International Journal for Science and Nature, 6*(3), 421-425.

Tıraşoğlu, E., Cevik, U., Ertuğral, B., Apaydın, G., Baltaş, H., *et al.* (2005). Determination of trace elements in cole (Brassica oleraceae var. acephale) at

Trabzon region in Turkey. *Journal of Quantitative Spectroscopy and Radiative Transfer, 94*(2), 181-187.

Tisdale, S., Nelson, W., Beaton, J., and Havlin, J. (1993). Soil fertility and fertilizers. Macmillan Publ. Co., New York. *Soil fertility and fertilizers. 5th ed. Macmillan Publ. Co., New York.*

Van Der Heijden, M. G., Bardgett, R. D., and Van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters, 11*(3), 296-310.

Weibel, F., Bickel, R., Leuthold, S., and Alföldi, T. (1998). *Are organically grown apples tastier and healthier? A comparative field study using conventional and alternative methods to measure fruit quality.* Paper presented at the XXV International Horticultural Congress, Part 7: Quality of Horticultural Products 517.