

# Impact of Specific Leaf Extracts on Soil pH and Beta-Carotene in Amaranthus in Kiambu County, Kenya

## Abstract

*Amaranthus* (*Amaranthus spp.*) leaves are a good source of nutrients including vitamins, antioxidants and dietary minerals including calcium, iron, and potassium. Soil pH is a very important chemical property of the soil, as it dictates the availability of plant nutrients. Acidic soils reduce availability of plant nutrients and hinder growth. This is corrected by adding lime which is expensive, does not add nutrients and requires re-application. The objective of this study was to evaluate the effects of leaf extracts from selected tree leaves on soil acidity and beta-carotene levels of amaranthus. Plant leaves that were tested were Turril (*Vitex keniensis*), Mexican sunflower (*Tithonia diversifolia*) and Indian nettle (*Plectranthus barbatus*). The experiment setup was in a 2 x 7 factorial arrangement in a randomized complete block design (RCBD) replicated three times. The treatments were; the three leaves extracts, lime, farm yard manure, inorganic fertilizer and control. Leaves were dried, ground and soaked in distilled water for sixty days as pH was monitored and the resulting extract was added to soil for amaranthus grown with plant extracts. Data on plant height, leaf area, number of leaves, fresh weight and dry weight and levels of beta carotene levels were collected. AOAC method was used for analysis of beta carotene. All data was subjected to analysis of variance (ANOVA) at 5% level of significance using SAS and least significant difference (LSD) for means separation. The results showed that leaf extracts significantly reduced soil acidity to above 6 while inorganic fertilizer increased the acidity (pH 5.9) to (5.3) on all growing conditions while humus increased acidity during rainy and irrigation conditions. On beta carotene levels, Farmyard Manure had the highest levels (51.1 µg) followed by the leaf extracts with extract 3-*Plectras barbatus* having higher levels. Control had the lowest levels of beta carotene followed by inorganic fertilizer and lime. These results suggest that farmers should be encouraged to grow vegetables using farmyard manure and leaf extracts to increase antioxidant levels and reduce soil acidity. They should also be encouraged to grow more of trees and shrubs studied to increase forest cover.

**Keywords::** Plant extract, Beta-carotene, Soil pH

## 1. Introduction

There has been a rising demands of African Leafy Vegetables (ALVs) in the recent past in Kenya. The priority species marketed include leafy amaranth (*Amaranthus spp.*), African Black nightshade species (*Solanum spp.*), cowpeas (*Vigna unguiculata*), Ethiopian kale (*Brassica carinata*), kahuhura (*Cucurbita ficifolia*), jute plant (*Corchorus olitorius*) and pumpkin leaves (*Cucurbita maxima* and *C. moschata*), (Irungu, et al, 2011)

African Leafy Vegetables (ALVs) have gained commercial importance over the past 15 years as a result of the enormous growth in market (Irungu *et al.*, 2007). ALVs production has its advantages because of the uniqueness of such as short production cycles thrives in poor soil, are resistant to pests and diseases, require a few purchased inputs, and are quite acceptable to local tastes (Ekesa, *et al.*, 2009)

Continuous cultivation of diminishing farms to feed the growing population has resulted in soil degradation and consequently rise in use of inorganic fertilizers to increase crop yield. Increasing and continuous use of inorganic fertilizers lead to lowered soil pH, poor soil texture (Glinsiki and Witold, 1985), poor quality crop yields and finally pollution of the rivers and lakes resulting in eutrophication. The inorganic fertilizers are expensive and hence out of reach to most rural farmers, majority of whom are women. In addition they are not always available, especially the subsidized ones which is being blamed for late planting and thus poor yields. Simulation studies of plant leaves using leaf extracts showed increase in soil pH and crop biomass possibly due to availability of nutrients (Mwangi, 2012; Lawrence, 1990). Also certain plants have litter that can decompose easily releasing plant nutrient and increasing buffering capacity of soil (Murungi, 1990; Njagi 2008; Njagi *et al.*, 2012; Kikuchi, 2004, Mwangi, 2012).

However, these studies did not look at soil texture or actual crop yield and most were under controlled environment (Mwangi 2012). In addition, only a few non-leguminous plants were studied using only a mixture of leaves and leaf extracts. Therefore, there is need to optimize productivity using natural environment and individual as well as combined leaves and leaf extracts to optimize plant nutrients and soil pH. The present study used leaves extract of plants shown to have high buffering capacity and high rate of mineralisation individually and for being able to raise pH of the soil as well as adding nutrients to the soil and improving soil texture. Various leaves extracts were tested for their effect on soil pH and nutrients levels. They were used to improve the acidic soils where the amaranthus was grown on improved soils and their growth rate, biomass and yield monitored. Soil pH was also monitored at intervals.

Carotenoids, have been credited with other beneficial effects on human health enhancement of the immune response and reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract, and muscular degeneration (Astrog 1997, Bendich 1994, Burri 1997, Gaziano and Hennekens 1993, Krinsky 1993, Mayne 1996, Olson 1999a, Olson and

Krinsky 1995). The action of carotenoids against diseases has been attributed to antioxidant property, specifically, their ability to quench singlet oxygen and interact with free radicals (Palozza and Krinsky 1992). Therefore, it was selected for analysis

It is hoped that the results of this study would be used to improve soil, increase vegetable production, with high levels of betacarotene and alleviate poverty amongst small-scale farmers, as well as encouraging planting of more plants and trees thus increase plant cover especially in Kenya.

### **General Objective**

To determine the effect of selected leaves extracts on soil pH, and betacarotene levels of amaranthus species.

### **Specific Objectives:**

- (i) To determine the effect of *Vitex keniensis*, *Tithonia diversifolia* and *Plectranthus barbatus* leaves extracts on pH of acidic soil.
- (ii). To determine levels of betacarotene in amaranthus grown after treatment with leaves extracts of *Vitex keniensis*, *Tithonia diversifolia* and *Plectranthus barbatus*, inorganic fertilizer, lime and with no treatment.

## **2.0 Materials and Methods**

### **3.1 Study Area and Experiment Procedure**

The leaves of the target species were collected from Juja Ward in Kiambu County, where also the experiment was carried out in the field. This was done under three regimes; under irrigation, on rainy season and in a greenhouse at Kenyatta University field demonstration site.

Analysis for soil pH, were done at Chemistry Department Laboratories at Kenyatta University, while for betacarotene analysis was done at JKUAT, Laboratories Food Science Department.

Plant leaves tested were Turrill (*Vitex keniensis*), Mexican sunflower (*Tithonia diversifolia*) and Indian nettle (*Plectranthus barbatus*). The concentration of the leaves extract was 500g diluted with two litres of distilled water.

The leaves were washed, sun dried subjected to milling/hand crushing, weighing packing and labeling. For leave extracts, both milled and hand crushed leaves were soaked in distilled water for 60 days and pH and buffering capacity monitored for individual plant leaves. Optimization of the leaf extracts for soil remediation and growth of the crops was done for open fields. For pH and buffering capacity controls, distilled water was kept under the same conditions and monitored for pH and buffering capacity (Miller and Kissel, 2010).

### **3.2 Study Design and Treatment Application**

Land preparation was done one month before sowing by clearing the weeds followed by ploughing. The field was harrowed to create a suitable tilth. The experiment setup was in a randomized complete block design (RCBD), in a two by seven factorial. Three blocks were made and spaced at 1 m apart while the plots within the blocks separated by 0.5m. There were 14 experimental plots per block and hence a total of 42. Individual plots measured 2 m by 1.2 m. The treatments were, the three leaves extracts, lime, Farm Yard Manure inorganic fertilizer and the control. All the seven treatments were replicated three times.

The seeds were planted directly and plots prepared to a fine tilth at a spacing of 30cm x 10cm at a depth of 2 mm in the raised plots and each had 40 plants excluding the border lines.

Plants were subjected to treatments after two weeks of germination in the plots. The concentration of each of the leaves extract was 2kg of ground leaves diluted with 20 litres of distilled water. To apply treatment on the amaranthus on weekly basis, 3.5 litres of this solution was diluted with 15 litres of water. This was then applied through drenching in the soil at the rate of 75 ml of each of the leaf extract per plant, two middle rows once per week for six weeks. Farm yard manure was applied once during planting at the rate of 200g per hole. Di-ammonium phosphate and Lime were applied once at planting at a recommended rate of 5g per plant and 130g respectively.

### **3.3 Management of the Experiment**

Routine field maintenance practices such as weeding, watering were done and also spraying against pests and fungal diseases when necessary using recommended pesticides and fungicides respectively.

### **3.4 Data Collection and Analysis**

The data collected per individual plot for the five sampled plants per plot included: fresh weight, plant height, root length, number of leaves, leaf area, and dry weight. This was done after six weeks from planting and continued for four weeks.

### **3.5 Sample preparation and analysis**

#### **Effects of Leaves Extract on Soil pH**

Soil samples for pH were taken from the experiment site and using 1:2 ratio, Soil to Water, from a depth of 0-20 and 20-40 cm. The samples were air-dried and ground to pass through a 2 mm sieve and analyzed for pH according to (Miller and Kissel, 2010).

#### **Effects of Leaves Extracts on Betacarotene Levels**

The amaranthus leaves were harvested at intervals of one week and dried under shade and later grounded and betacarotene extract was made. Determination of Beta-carotene was by carotene equivalent using acetone as solvent, by AOAC method (AOAC, 1995). The concentration of carotene was read directly from UV visible spectrophotometer at 440 nm after proper calibration of the instrument with standard solutions of pure beta-carotene (Sigma chemical Co., St. Louis, and Mo).

#### **Statistical Analysis**

All the plant biochemical data were subjected to analysis of variance (ANOVA) using the General Linear Model. Proc GLM code of SAS- computer software (SAS 2002; Version 16.0) where significant, mean separation were done with LSD.

### 3. Results and Discussions

#### 4.1 Effects of adding ground leaves extracts on distilled water pH from day 1 to 60 days

The pH of leaf extract in day 1 was not significantly different ( $p > 0.05$ ) from the initial pH. However, by day 15, there was a drop in pH in all the leaf extracts. The pH for *Plectranthus barbatus* ranged from 6.45 to 6.67, which implies that its leaf extracts would be best for treatment of acidic soil. The pH of leaf extract for the other species also ranged from 6.22 to 6.40 for *Vitex keniensis* and *Tithonia diversifolia* from 6.32 to 6.49. (Table 1) The trend for leaf extracts in this study agrees with those of (Murungi, 1990; Njagi, 2008) where pH dropped and then increased depending on the type of leaves and the length of soaking in distilled water.

#### 4.2 Effect of treatments on the soil pH across the three regimes

The soil pH under greenhouse condition ranged from 5.604 to 6.233. The leaf extract *Plectranthus barbatus* gave the highest pH while soil treated with inorganic fertilizer had the lowest pH. (Table 2) This concurs with (Mwaura and Woomer, 1999; Monica, 2003), that inorganic fertilizers increase soil acidity. This is because under greenhouse the soils are not leached, hence a lot of retention both for the leaf extracts and the inorganic fertilizer. Under irrigation, leaf extract *Plectranthus barbatus* and lime had the highest soil pH, 6.172 and 6.153 respectively. This agrees with (Nekesa 2007; Kisinyo et al., 2009; Kikuchi, 2004). Fukushima and Tokushi (2009) that liming curbs soil acidity, though reapplication is needed, hence cost implication, not sustainable and does not add nutrients.

The soil pH under the rainy season, lime and leaf extract *Plectranthus barbatus* had 6.153 and 6.148 means respectively. The control and the inorganic fertilizer had the lowest 5.956 and 5.555

respectively. These soils pH were slightly higher than in the other regimes as there is vertical movement of dissolved cations, and this concur (Olojugba M.R.2015).

The Least Significance Difference across the regimes ranged between 0.028 and 0.049 showing significance.

#### **4.3 Mean squares and standard error for soil pH across the three regimes**

The soil pH indicated significant ( $P < 0.05$ ) differences among the treatments, however there were no differences in the two varieties (Table 3) This agrees with (Murungi, 1990; Njagi 2008; Njagi *et al.*, 2012; Kikuchi, 2004, Mwangi, 2012) that certain plants have litter that can decompose easily increasing buffering capacity of the soil. In all the regimes, there were significant ( $P < 0.05$ ) differences on the interaction between treatments and time.

#### **4.4 Effect of Treatments on Betacarotene Levels across Regimes**

On betacarotene levels (Table 4), under green house conditions FYM had the highest levels (51.51  $\mu\text{g}$ ) that was significantly different ( $p < 0.05$ ) from the others. Control had the lowest (26.67  $\mu\text{g}$ ) that was significant different ( $p < 0.05$ ) from the others.

Under irrigation, FYM had the highest betacarotene levels (38.36  $\mu\text{g}$ ) that was significantly different ( $p < 0.05$ ) from the others. Control had the lowest (19.22  $\mu\text{g}$ ) levels. Those under the rainy conditions, FYM had the highest betacarotene levels (45.02  $\mu\text{g}$ ) that was significant different ( $p < 0.05$ ) from the others. Control had the lowest levels (21.89  $\mu\text{g}$ ).

There were significant differences ( $P < 0.05$ ) on the various treatments on betacarotene under the three regimes. There were no significant differences on the two varieties and also on the interaction between the variety, treatment and regime.

It is important to note that under various regimes, inorganic fertilizer had the lowest beta carotene compared to leaf extract and lime. Its levels were only higher than that of the control.

#### 4.5 Mean Squares and Standard Error for Betacarotene Levels across the Three Regimes

From (Table 5) on the betacarotene levels, there were significant differences ( $P < 0.05$ ) in all treatments under all the regimes. However, there were no significant differences in the varieties and also on the interaction between the varieties and the treatments under all the regimes.

#### 4. Conclusions and Recommendations

Significant differences ( $P \leq 0.05$ ) were observed between the treatments under all the three regimes. This was important as buffering of the soil made the soil pH rise and assisted in maintaining it enabling the growth of the amaranthus. This was observed in the greenhouse unlike in the rainy season as some of the soils were leached. Leaf extract<sup>3</sup>, *Plectranthus barbatus* had the highest rise in soil pH in the three regimes while control the lowest.

There was no significant difference in the two varieties. The inorganic fertilizer decreased the soil pH.

FYM and *Plectranthus barbatus* had the highest on betacarotene levels.

More research is needed to evaluate the effect of the leaf extracts over a longer period of time on the soil pH, and also the effect of combining the leaf extracts and Farm Yard Manure. Also growing other different vegetables especially those with betacarotene to check their levels.

5. Acknowledgements

6. Competing Interests

7. Authors' Contribution

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## Tables

Table 1 The pH of distilled water after soaking ground leaves extracts from day 1 to 60 days

Plant species	Days				
	1	15	30	45	60
<i>T.diversifolia</i>	6.32	6.21	6.30	6.41	6.49
<i>V.keniesis</i>	6.22	6.15	6.25	6.33	6.40
<i>P.barbatus</i>	6.45	6.32	6.41	6.50	6.67

Table 2: Effects of Treatments on the Soil pH Across the Regimes

Treatment	Greenhouse	Irrigation	Rainy
CONTROL	5.96e	5.94c	5.95d
FARM YARD MANURE	6.03d	5.96c	5.97cd
INORGANIC FERTILIZER	5.60f	5.53d	5.55e

LEAF EXTRACT 1-TITHONIA	6.06cd	6.02b	6.01bc
LEAF EXTRACT 2-VITEX KENIESIS	6.09c	6.02b	6.04b
LEAF EXTRACT 3-PECTRAS BARBATUS	6.23a	6.172a	6.14a
LIME	6.18b	6.15a	6.15a
LSD	0.03	0.02	0.04
CV(%)	0.80	0.60	1.00

Treatments with different letters in the same column and rows are significantly different at 5% probability

Table 3: Mean squares and standard error for soil pH across the three regimes

Regimes	Time	Treatment	Time*Treatment	Variety * Treatment
Green house conditions	4.72(0.011)s	0.50(0.021)s	0.42(0.030)s	0.01(0.030)ns
Irrigation conditions	2.696(0.005)s	0.548(0.010)s	0.437(0.014)s	0.0004(0.014)ns
Rainy conditions	2.838(0.009)s	0.490(0.017)s	0.384(0.024)s	0.003(0.024)ns

s = significant at  $\alpha$  0.05      ns = not significant at  $\alpha$  0.05

Table: 4 Effect Of Treatment On Betacarotene Levels Across The Three Regimes.

Treatment	Greenhouse	Irrigation	Rainy
CONTROL	26.67l	19.22o	21.89n
FARM YARD MANURE	51.51a	38.36e	45.02o
INORGANIC FERTILIZER	30.47j	23.64m	24.47m
<i>LEAF EXTRACT 1-TITHONIA</i>	41.82d	33.49h	37.82ef
<i>LEAF EXTRACT 2-VITEX</i>	37.17f	31.67i	34.5h
<i>LEAFEXTRACT 3-PLECTRAS BARBATUS</i>	46.14b	35.98g	41.98d
LIME	32i	26.13l	28.13k
LSD	1.08	0.98	1.15
CV(%)	2.80	2.81	2.9

Treatments with different letters in the same column and rows are significantly different at 5% probability

Table 5: Mean Squares and Standard Error for Betacarotene Levels across the Three Regimes

Regimes	Variety	Treatment	Variety * Treatment
Green house conditions	0.1249(0.201)ns	485.120(0.377) s	0.9702(0.533)ns
Irrigation conditions	1.6363(0.18) ns	291.829(0.377)s	0.4821(0.477)ns
Rainy conditions	0.942(0.211)ns	469.475(0.396)s	1.6634(0.559)ns

s = significant at  $\alpha$  0.05      ns =not significant at  $\alpha$  0.05