



## Differential Biomass Accumulation among African Leafy Vegetables as Affected by Wastewater Irrigation in Kitui County, Kenya

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author JMW designed the experiment, corrected data and developed the first manuscript draft. Authors SN and WN analyzed the data and read the manuscript. Author NKK reviewed the experimental design and read the manuscript while author JPGO conceptualized the idea, guided on collection of the study and read the final manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Water scarcity of fresh water in Sub-Saharan has led to utilization of the wastewater in home gardening and also in commercial production of vegetables. Wastewater is associated with various substances including nutrients and heavy metals hence it is pertinent to evaluate its effects on growth and yield of vegetables. An experiment was conducted to evaluate the effect of waste water released from the municipal council on the biomass accumulation in African leafy vegetables. Field experiments were carried out in two seasons and one greenhouse experiment. The field trial was laid out in a Randomized Complete Block Design (RCBD) and in the greenhouse the treatments were arranged in Complete Randomized Design (RCD) replicated three times. Four leafy

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vegetables were the treatments replicated three times. The vegetables were irrigated with waste water. Plant samples were collected at 6 WAP and 12 WAP, partitioned and dried in an oven and later weighed using electronic weighing balance. The findings revealed differences in biomass accumulation into various organs. Black nightshade depicted the highest leaf dry matter in the greenhouse at both 6 weeks after plant (WAP) and 12 WAP (24.62 g and 81.12 g respectively). Cowpea showed the highest increment (7 folds) in leaf weight between 6 to 12 WAP as compared to was paltry 3.6 folds. The highest stem dry weight was obtained in the amaranth species at 6 WAP and 12 WAP both in the greenhouse; recording 32.59 g and 90.12 g respectively. A similar trend was noted in root dry weight and root: shoot ratio. Cowpea had the least biomass accumulation potential across all the parameters in both seasons and in the greenhouse. The increased biomass growth is an indication sufficient availability of nutrient that promoted vibrant plant growth and also less toxicity from the heavy metals. Therefore, waste water can be put into use to enhance improved productivity of African leafy vegetables.

*Keywords: Nutrient benefits; plant growth; heavy metals.*

## 1. INTRODUCTION

African leafy vegetables are essential in meeting the constraints on malnourished people, particularly children and expectant mothers since many of these vegetables contain high nutritional value, and they also do well in range of soil types and thrive well on a number of environments [1,2]. With the current rising human population coupled with the climate change that has hit agricultural farming system, there is need to focus on diverse ways of growing African leafy vegetables as way of mitigating the dire consequences that come along with these current scenarios. Due to water scarcity in most of the regions in the country, most of the vegetable farmers have opted to use waste water from the municipal council as source of irrigation for African leafy vegetables [3]. Specifically, this takes place in urban area as way of reducing transport, handling and production costs, making the food products readily available to the urban poor [4]. This is based on the fact that the shorter the time of vegetables between harvest and consumption, the greater the nutritional quality.

In many developing countries, as a result of rapid urbanisation and the absence of wastewater treatment facilities, urban farmers often use wastewater either directly from sewage drains or indirectly through wastewater-polluted irrigation water. Wastewater uses in agriculture is common practice and is on an increasing trend as a result of the rising water scarcity worldwide [5]. The volume of wastewater has been increasing to the increasing population, improved living standards, urbanization and economic development [6]. The continued disposal of waste-water into land and water courses reduces the quality of water available for crop growth. Domestic sewage and

refuse find their way of the water from settlements, municipal wastes or institution drainages through leaching, direct discharge and runoff [7]. Human faeces contains high concentrations of toxic elements from the normal dietary matter presenting principal input of toxic metals to domestic waste water and sludge of domestic origin [8]. Due to nutrient accumulation in waste water it is considered beneficial to plant growth hence its extensive use for irrigation of crops.

Waste water is associated to have high accumulations of plant growth-promoting and metal-tolerant bacteria increase plant growth and reduce metal uptake or translocation to above-ground tissues of plants by reducing the availability of soil heavy metal [9]. This makes it quite reliable for the above ground growth which is a good measure of yield in vegetables. Wastewater thus serves as source of fertilizer, especially in low income regions where farmers have the inability of acquiring farm inputs [9]. However, over-supply of these nutrients to the vegetables, particularly leafy ones, may cause detrimental effects and also could negatively affect the yield returns. There is need for new and alternative approaches to ensuring food and nutrition security in these developing countries. Such solutions should be sustainable, resilient and of practical solutions to challenges due to climate change and variability. This need has led to renewed focus on identifying and improving underutilized indigenous and traditional crops. The waste-water use is such alternative to improving the productivity of indigenous vegetables [10]. Having much being known on the benefits of waste water, this study focused at evaluating the influence of the wastewater on the accumulation of biomass in the selected African leafy vegetables.

## 2. MATERIALS AND METHODS

### 2.1 Site Description

The field experiment was carried out in Kitui County which is located in Eastern part of Kenya and lies within latitudes 0°10' and 3°0' South and longitudes 37°50' and 39°0' East and covers an area of about 30,570 km<sup>2</sup>. 6,369 km<sup>2</sup> of the total area is under Tsavo National Park, 14,137.2 km<sup>2</sup> is arable agricultural land and 6,364.4 km<sup>2</sup> non arable land. The experiment was evaluated for two seasons from September to November 2017 and January to March 2018.

### 2.2 Experimental Design, Treatments and Data Collection

The field experiment was laid out in a Randomized Complete Block Design (RCBD). The treatments were four vegetable species replicated three times and irrigated with waste water. The waste water was obtained from the municipal sewage of Kitui County. In the greenhouse the treatments were arranged in complete Randomized Design (CRD).

The land was cultivated using hand hoe tools and the plot later harrowed using rakes into finer tilth. The seeds of different crop species were drilled and later thinned to recommended spacing two weeks after emergence. The plots size was 3 by 2 m<sup>2</sup>. All agronomic management practices such as weeding, watering and spraying against pests and diseases were performed uniformly when necessary using recommended fungicides and pesticides respectively.

Ten (10) plants were sampled from each plots, stored in a cool box for subsequent measurements of dry weight and laboratory analysis. This was done in week 6 and week 12 of the vegetables growth cycles. The samples were washed in tap water, and were separated into root, stem and leaves and dried in a forced air oven. At 72°C for 48 hours, the samples were fully dried such that no significant changes occurred before the tests were done. The partitioned plant parts, shoot, leaves, and the roots were weighed using the electronic balance model 6354.

### 2.3 Data Analysis

The collected data was managed in the excel spreadsheet and subjected to analysis of variance using GenStat statistical software version 15. Significance differences between

means was performed using Fischer's Protected Least Significance Difference (L.S.D) test at 5% level of significance.

## 3. RESULTS AND DISCUSSION

### 3.1 Leaf Dry Weight

Vegetable species exhibited significant differences ( $P \leq 0.05$ ) in leaf biomass accumulation under the effect of waste water in both seasons and greenhouse experiment. Black nightshade was the most superior in biomass accumulation in both week 6 and week 12 for both field experiments. In week 6 and 12 the highest shoot biomass for black nightshade was recorded for the greenhouse experiment, recording 24.62 g and 81.12 g respectively. The cowpea vegetable species showed the least biomass accumulation across the experiment recording 6.43 in season 1 and 24.42 g in the greenhouse (Table 1). All the species showed an increase in leaf biomass between week 6 and 12, an implication that waste water did not have negative impact (due to heavy metals) on their growth. However, cowpeas showed a greater increment of 7 folds between 6 and 12 WAP in season 1 from 6.43 g (6WAP) to 46.46 g (12 WAP). On the other hand, biomass accumulation between this period was 3.7 folds for black nightshade. This could be as result proper utilization of the available plant nutrients that enhance growth of cowpea or it implies that cowpea is more tolerant of heavy metals that is mostly associated with waste water.

According to Anwar et al. [11], irrigation with waste water leads to an increase in biomass formation as a result of increased growth rate which agrees with the findings of this study. In another study by Zu et al. [12], waste water has the potential for stimulating vibrant growth and consequent biomass accumulation on the above ground as result of adequate concentration for nitrogen and phosphorus that have promoted an increase in the growth. An increase in leaf biomass and leaf area was also noted in the green grams grown in waste water as compared to the control which agrees with the current findings [13]. The results of this study also agree with those of previous reported work [14], which reported improved fruit fresh weight and yield as a result of waste water applications as source of irrigation.

### 3.2 Stem Dry Weight

Significant differences ( $P \leq 0.05$ ) were observed in stem dry weights in all vegetables specie in two

seasons and greenhouse experiments. In week 6 and 12 amaranthus species was superior in biomass accumulation in both two seasons and greenhouse with the highest dry weight being recorded in the greenhouse with 32.59 g and 90.12 g in 6 and 12 WAP respectively (Table 2). This could imply that amaranthus may be a phytoaccumulators, hence not significantly affected by the availability of heavy metals in waste water. The lowest biomass was revealed in the cowpea species in all the experiments with season 1 in week 6 recording the least figure of 8.28 g (Table 2). However, in 6 WAP and 12 WAP there was a noticeable increase in the biomass accumulation. Similar to leaf dry weight (Table 1), cowpeas consistently showed the greatest increment; with stem dry weight being four in season 1 from 8.28 g at 6 WAP to 33.96 g (12 WAP). This could be as result of improved growth rate as result of nutrients available in the waste water.

As reported by Hussein, [15] an increase in vegetable species stem dry weight as a result of treated sewage water irrigation may be due to the increase in organic matter, macro-and micronutrients in the sewage water where

beneficial nutrients improved the metabolic activities and hence the vegetative growth which is confirmed from this study. Bedbabis et al. [16] found that waste water irrigation of olive trees resulted in significant yield increase when compared to yields from plot using well water. Availability of nutrients in waste water enables the plant to develop strong organs such as the stem to enhance proper growth and support maximization of yield [17]. An increase in plant biomass is also an indication that the vegetable species were not affected by the heavy metals such as lead and cadmium that are evident in waste water have the potential of limiting crop growth [18].

### 3.3 Root Dry Weight

Root dry weight showed significance differences ( $P \leq 0.05$ ) in vegetable species in 6 and 12 WAP during the two seasons and greenhouse experiment. Amaranthus recorded the highest root dry weights with the greenhouse being more superior with 43.26 g and 124.34 g in 6 WAP and 12 WAP respectively. Cowpea also was less superior in accumulation of the root biomass recording (8.92 g) in season one as revealed in Table 3. An increase in the root

**Table 1. Influence of waste water on leaf biomass accumulation in in two seasons and green house**

Species	Leaves dry weight (g)					
	Week 6			Week 12		
	S1	S2	GH	S1	S2	GH
Cowpea	6.43 <sup>c</sup>	8.43 <sup>c</sup>	6.80 <sup>d</sup>	46.42 <sup>b</sup>	51.62 <sup>b</sup>	24.42 <sup>c</sup>
Amaranthus	10.41 <sup>b</sup>	12.57 <sup>b</sup>	15.31 <sup>c</sup>	62.47 <sup>a</sup>	68.63 <sup>a</sup>	63.48 <sup>b</sup>
Kale	11.38 <sup>b</sup>	12.58 <sup>b</sup>	20.00 <sup>b</sup>	47.53 <sup>b</sup>	51.08 <sup>b</sup>	64.08 <sup>b</sup>
Black nightshade	16.93 <sup>a</sup>	19.68 <sup>a</sup>	24.62 <sup>a</sup>	62.63 <sup>a</sup>	68.42 <sup>a</sup>	81.12 <sup>a</sup>
<b>LSD</b>	<b>1.16</b>	<b>2.01</b>	<b>3.18</b>	<b>7.37</b>	<b>7.46</b>	<b>5.48</b>

Means followed by the same letter within the same column are not significantly different ( $P \leq 0.05$ ). S1- season 1, S2- season 2, GH-greenhouse

**Table 2. Influence of waste water on stem biomass accumulation in in two seasons and green house**

Species	Stem dry weight (g)					
	Week 6			Week 12		
	S1	S2	GH	S1	S2	GH
Cowpeas	8.28 <sup>d</sup>	10.65 <sup>c</sup>	9.17 <sup>d</sup>	33.96 <sup>c</sup>	37.72 <sup>c</sup>	28.48 <sup>c</sup>
Black nightshade	12.77 <sup>b</sup>	15.03 <sup>c</sup>	10.03 <sup>c</sup>	49.42 <sup>b</sup>	53.72 <sup>c</sup>	61.47 <sup>b</sup>
Kale	18.85 <sup>b</sup>	21.35 <sup>b</sup>	29.33 <sup>b</sup>	56.06 <sup>b</sup>	50.67 <sup>b</sup>	66.64 <sup>ab</sup>
Amaranthus	26.27 <sup>a</sup>	31.07 <sup>a</sup>	32.59 <sup>a</sup>	82.05 <sup>a</sup>	89.21 <sup>a</sup>	90.12 <sup>a</sup>
<b>LSD</b>	<b>1.8</b>	<b>3.36</b>	<b>2.21</b>	<b>7.63</b>	<b>6.63</b>	<b>18.55</b>

Means followed by the same letter within the same column are not significantly different ( $P \leq 0.05$ ). S1- season 1, S2- season 2, GH-greenhouse

**Table 3. Influence of waste water on vegetable species root biomass accumulation in in two seasons and green house**

Species	Root dry weight (g)					
	Week 6			Week 12		
	S1	S2	GH	S1	S2	GH
Cowpeas	8.92 <sup>b</sup>	11.49 <sup>c</sup>	9.48 <sup>c</sup>	85.05 <sup>b</sup>	93.49 <sup>ab</sup>	41.23 <sup>d</sup>
Black nightshade	12.77 <sup>b</sup>	15.15 <sup>bc</sup>	19.05 <sup>bc</sup>	79.70 <sup>b</sup>	86.83 <sup>b</sup>	99.20 <sup>b</sup>
Kale	17.00 <sup>b</sup>	20.88 <sup>b</sup>	26.72 <sup>b</sup>	54.13 <sup>c</sup>	59.81 <sup>c</sup>	69.90 <sup>c</sup>
Amaranthus	28.91 <sup>a</sup>	34.44 <sup>a</sup>	43.26 <sup>a</sup>	100.48 <sup>a</sup>	109.82 <sup>a</sup>	124.24 <sup>a</sup>
<b>LSD</b>	<b>6.93</b>	<b>4.65</b>	<b>8.12</b>	<b>8.82</b>	<b>13.53</b>	<b>12.75</b>

Means followed by the same letter within the same column are not significantly different ( $P \leq 0.05$ ). S1- season 1, S2- season 2, GH-greenhouse

biomass was noted across all the experiments by the vegetable species which is an indication of a vibrant root growth as a result of available nutrient such as phosphorus and potassium in the sewage water that enhance growth. Although cowpea had less root biomass accumulation compared to other species, there was a noticeable increase from week 6 to week 12 during season by 10 folds increasing from 8.92 g to 85.05 g (Table 3).

The results of this study does not agree with the result of Uzma et al. [19] who reported that waste water used in irrigation of vegetables resulted in a decrease of the root biomass. According to the authors, this was as a result of high levels of heavy metals that are thought to inhibit further growth of the root, limiting supply and access of nutrient required for plant growth. Also, in another study carried out in Pakistan, waste water reduced root biomass root growth, leading to inhibition of nutrient uptake and consequently stunted root systems in vegetables [20]. The finding which does not conform with the current study. Therefore, based on the current findings it could be the heavy metals in the waste water had not reached toxic limit of interfering with the growth of these particular vegetable species.

According to Faizan et al. [21] waste water contains significant amount of nutrients that are known to accelerate root proliferation enhancing them to extract more nutrients near the root zone and consequently leading to a higher dry matter; which agrees with the findings of this study. Another study by Parveen et al. [22] agrees with this study that waste water increases root biomass as a result of proper mineral nutrition.

### 3.4 Root: Shoot Ratio

There were varied significance differences ( $P \leq 0.05$ ) observed in root: shoot ratios of vegetable species during the two seasons and greenhouse experiment. Amaranthus exhibited the highest root: shoot ratio as influenced by waste water, with the highest being in the greenhouse at 6 WAP recording 0.94 g (table 4). The least root: shoot ratio was noted in black nightshade in 6WAP recording 0.44 g in season 1, season 2 and greenhouse experiments. At 12 WAP kales had the least root: shoot ratio of 0.51, 0.58 and 0.53 in season 1, 2 and greenhouse respectively. Root to shoot ratio increased across the weeks, however at 6WAP and 12 WAP in season two cowpea recorded an increment by 77%. (Table 4).

**Table 4. Influence of waste water on vegetable species root biomass accumulation in in two seasons and green house**

Species	Root: Shoot ratio					
	Week 6			Week 12		
	S1	S2	GH	S1	S2	GH
Black nightshade	0.44 <sup>b</sup>	0.44 <sup>b</sup>	0.44 <sup>b</sup>	0.71 <sup>b</sup>	0.71 <sup>b</sup>	0.70 <sup>a</sup>
Kale	0.60 <sup>ab</sup>	0.64 <sup>ab</sup>	0.57 <sup>b</sup>	0.51 <sup>b</sup>	0.58 <sup>b</sup>	0.53 <sup>a</sup>
Cowpea	0.60 <sup>ab</sup>	0.60 <sup>ab</sup>	0.60 <sup>b</sup>	1.07 <sup>a</sup>	1.06 <sup>a</sup>	0.89 <sup>a</sup>
Amaranthus	0.78 <sup>a</sup>	0.78 <sup>a</sup>	0.94 <sup>a</sup>	0.71 <sup>b</sup>	0.71 <sup>b</sup>	0.83 <sup>a</sup>
<b>LSD</b>	<b>0.21</b>	<b>0.18</b>	<b>0.2</b>	<b>0.14</b>	<b>0.18</b>	<b>0.37</b>

Means followed by the same letter within the same column are not significantly different ( $P \leq 0.05$ ). S1- season 1, S2- season 2, GH-greenhouse

An increase in root to shoot ratio is an indication of plant response to available moisture and nutrition supplied by waste water during irrigation. The higher the root: shoot ratio the healthier the plants due to increased absorption of the nutrients by the roots and a higher absorption of energy transformed from yield making in the plants [23]. This is an indication that waste water supplied in this study provided the plants with sufficient requirements for maximum plant growth of the case the case of black nightshade, and cowpea. However, for kale and *Amaranthus*, there was a decrease in root to shoot ratio which could be as a result of toxicity of the heavy metal present in the soils hence limited the growth of plant. The findings of this study agrees with those of Singh and Agrawal, [24] who reported an increase in root to shoot ratios of crops grown in waste water. Similar findings were also reported by Heitholt and Sloan [25] working with vegetables in calcareous clay soils.

#### 4. CONCLUSION

Wastewater used in irrigation of African leafy vegetables increased biomass in all vegetable species. This was noted from the week 6 and week 12 of planting where an increase in root, stem leaf and root: shoot ratio was revealed in season 1, season 2 and the greenhouse experiments. Biomass accumulation being a measure of yield in vegetables implied that waste water is rich in mineral capable of nourishing and supporting faster growth rate. Also, it can be concluded that the water water did not contain toxic heavy metals such as lead and cadmium that would inhibit maximum plant growth. Therefore, use of this waste water from the municipal council can be recommended for improved vegetable farming.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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