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Biomass yields and crude protein content of two African indigenous leafy vegetables in response to kraal manure application and leaf cutting management

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African indigenous leafy vegetables have the important role of providing essential minerals, vitamins and amino acids in diets of poor smallholder farmer households in sub-Saharan Africa. A study was conducted to quantify the influence of applying cattle and goat manure on the biomass yields and crude protein content of two commonly used African leafy vegetables (*Cleome gynandra* and *Amaranthus hybridus*) when subjected to varying leaf cutting management. Treatments for each vegetable consisted of a combination of three manures (control, cattle and goat) and three cutting regimes (cutting edible leaves, all harvestable leaves and cut only at the end) utilised in randomised block design. The results showed significant increase in leaf biomass yields and crude protein content of both vegetables due to manure application. Goat manure was superior to cattle manure due to its higher quality in terms of nutrient content and lower C:N ratio. Cutting the edible leaves more frequently, every fortnight, was associated with the highest leaf biomass yield and crude protein in treatments where manure was applied. It was concluded that potential exist for smallholder farmers to benefit from adopting appropriate manure and leaf cutting regime. A combination of goat manure and frequent cutting of the tender edible leaf tips is recommended. The results point to the potential of maximizing biomass yield and quality of the vegetable leaves by adopting appropriate nutrient supply and leaf cutting regime.

Key words: Animal kraal manure, nutritional security, smallholder farmers, indigenous vegetables, leaf defoliation.

INTRODUCTION

African indigenous leafy vegetables, also referred to as traditional leafy vegetables, are crops that grow wild or are cultivated and are gathered or harvested for food within a particular African ecosystem (Alleman et al., 1996; Aphane et al., 2003). Van Rensburg et al. (2007) and Schippers (2000) have described leafy vegetables as plant species of which the leafy parts, which may include young succulent stems, flowers and very young fruits are used as vegetables. Oniang'o et al. (2004) and Flyman and Afolayan (2006a) have suggested that the food and nutritional insecurity that most African countries face today could potentially be mitigated and sustainably reversed if a manifest change can be realised through the appreciation and domestication of African indigenous foods including leafy vegetables. This is because indigenous leafy vegetables constitute important sources

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of both micronutrients and non-nutrient bio-active phytochemicals that have been linked to protection against cardiovascular and other degenerative diseases (Akhtar et al., 2012; Aphane et al., 2003; Flyman and Afolayan, 2006a; Modi et al., 2006, Uusiku et al., 2010; Smith and Eyzaguirre, 2007). In South Africa, however, African indigenous vegetables are mostly underexploited and have received insufficient attention within the mainstream research on food security and management interventions (Van Rensburg et al., 2004). The consumption of African indigenous vegetables has however increased over the years in South Africa (Venter et al., 2007; Van Rensburg et al., 2004). The reawakening of demands for superior nutrition and health by modern communities has gradually created an increase in consumer demand for traditional food crops and African indigenous leafy vegetables are on the spotlight again due to their superior nutritive and medicinal value (Flyman and Afolayan, 2006a; Mwangi and Kimathi, 2006; Smith and Eyzaguirre, 2007; FAO, 1988).

The yield of leafy vegetables has been shown to be affected by the leaf cutting regime and sequence (Diz et al., 1995). In the context of African tradition, there are many techniques used to harvest African indigenous leafy vegetables (Chweya and Mnzava, 1997). In many African communities, the harvesting of the leaves is done sequentially at different times during the growing period to enable the plant to grow and produce more leaves (Schippers, 2000). In some cases, African indigenous leafy vegetables can be harvested by uprooting the whole plants or by cutting the top part, cutting back to ground level or picking individual leaves or leafy branches at frequent intervals (Mnzava, 1997). Defoliation is an important leaf management factor, especially in the productivity of C_4 crops such as leaf amaranth (Abou-khalefa et al., 2008). In leaf amaranth, and other vegetables, the type and frequency and stage of development of leaves that are removed can have a significant effect not only on the development (Baloyi et al., 2013; Odeleye and Olufolayi, 2010; Lestiene et al., 2006), but also on the yield and quality of leaves (Diz et al., 1995). The harvesting of consumable parts of indigenous leafy vegetables among rural households generally involves different practices, which include uprooting the entire young plants, cutting back established plants to encourage growth and picking of the top part of stem and branches close to the growing point (Schippers, 2000).

Soil fertility depletion in small scale farming areas has been cited as a fundamental biophysical cause of the declining per-capita food production in sub-Saharan Africa (Bationo and Mokwunye, 1991). Soil fertility problems under small scale farming in South Africa are no exception. Chemical fertilisers are expensive for the poor farmers who often utilize African leafy vegetables (Van Rensburg et al., 2003). In the case of the South African smallholder sector, which is largely responsible for the production of most African indigenous leafy vegetables, animal manures have long been the primary way in which plant nutrients are returned to cultivated soils (Van Averbeke and Yoganathan, 2003; Edmeades, 2003). Kraal manure is an important resource for the supply of plant nutrients especially nitrogen and phosphorus under most crop production systems (Mhlontlo et al., 2007).

The production of African indigenous leafy vegetables has been advocated as part of a food security strategy aimed at combating micronutrient deficiencies among many rural communities in South Africa (Faber et al., 2010; Van Rensburg et al., 2004). Consequently, many smallholder farmers, who are presently largely responsible for the production of African indigenous leafy vegetables, have taken to the cultivation of indigenous leafy vegetables on their farms (Cunning et al., 1992; Odhav et al., 2007). However, there is generally limited information on the agronomic practices related to the cultivation of African indigenous vegetables including the effects of cutting on the leaf yields of African indigenous vegetables and how this would interact with availability of soil nutrients. Harvesting of leaves of leafy vegetables for human consumption is becoming an important management practice among African communities (Baloyi et al., 2013; Materechera and Medupe, 2006). This is because leaf harvesting practices and procedures have the potential to reduce or improve the yield of essential components of the crop (Rahman et al., 2008). Saidi et al. (2010) and Baloyi et al. (2013) have shown that the intensity or extent, frequency and timing of foliage removal from leafy vegetables can affect the performance, in terms of biomass yield and nutritional quality of the crop. Furthermore, information on the influence of kraal manure on growth of African indigenous leafy vegetables especially under smallholder farming management is still rudimentary. This information is important as it underpins the agronomic practices necessary for the production of African indigenous leafy vegetables.

The aim of this study was therefore to investigate the response of two commonly utilized African indigenous leafy vegetables, amaranth (*Amaranthus hybridus*) and cleome (*Cleome gynandra*), to additions of different kraal manures (cattle and goat) and leaf cutting management. It was hypothesized that the biomass yield and quality of the two African indigenous leafy vegetables will improve with manure and appropriate leaf cutting management regime.

MATERIALS AND METHODS

Soil sampling and analysis

The soil used was collected at Molelwane University farm located 8 km from the city of Mafikeng (25°48' S 25°38' E) on the road to Gaborone, Botswana. The soil is a dark reddish brown sandy loam classified as Hutton form according to the South African Soil Classification System (Soil Classification Working System, 1991). It

Table 1. Dates of cutting the leaves of *A. hybridus* and *C. gynandra* and mean daily temperature during the growing period.

Activities	*A. hybridus*		*C. gynandra*		Mean daily temperature (°C)
	Date	WAT	Date	WAT	
Transplanting	13th Oct 2010	0	15th Oct 2010	0	n/a
Thinning	25th Oct 2010	2	28th Oct 2010	2	25 ±3.5
1st cutting	7th Nov 2010	4	12th Nov 2010	4	24 ±3.8
2nd cutting	16th Nov 2010	5	21th Nov 2010	5	27 ±2.3
3rd cutting	22th Nov 2010	6	28th Nov 2010	6	22 ±3.6
4th cutting	29th Nov 2010	7	5th Dec 2010	7	24 ±2.6
5th cutting	6th Dec 2010	8	12th Dec 2010	8	27 ±3.2
Harvest	15th Dec 2010	9	18th Dec 2010	9	24 ±2.5

WAT = Weeks after transplanting. n/a=not applicable.

was collected randomly at a depth of 0-20 cm from a 0.5 ha area of uncultivated land within the farm whose natural vegetation is composed of grasses with scattered shrubs and bushes of mostly *Acacia* species.

The soil was air dried, passed through a 2 mm sieve and analysed. Organic carbon was determined using the procedure of Walkley-Black method (Okalebo et al., 1993). Soil pH and EC were determined as outlined by The Non-Affiliated Soil Analysis Work Committee (1990). Available P was measured using the Bray 1 method (Bray and Kurtz, 1945). Extractable cations were extracted with 1.0 N ammonium acetate (Anderson and Ingram, 1993) and analysed for the elements (Ca, K, Mg) using atomic absorption spectroscopy.

Collection and analysis of manure samples

Both cattle and goat kraal manures were collected with a spade from kraal floors at Molelwane University research farm. Both manures were air dried and passed through a 4 mm sieve to homogenize and achieve proper mixing with soil. The manure was thoroughly mixed with soil (1:2 ratio of manure: water, v/v) in 7 L capacity plastic garden pots with bottom holes to allow drainage of excess water. The soil:manure mixture was kept moist for 3 weeks to allow decomposition to commence before planting. The mixture was kept moist to near field capacity to speed up the decomposition and mixing of manure and soil. The manure was analysed for moisture, ash, organic carbon, total N and P, pH and EC using by the procedure described by Okalebo et al. (1993). Four replicate sub-samples were analysed for each manure type.

Treatments and experimental design

Two separate experiments were conducted at the same time which involved two African indigenous leafy vegetables species, viz amaranth (*A. hybridus*) and cleome (*C. gynandra*). For each vegetable species, there were nine treatments resulting from a combination of three types of manure (M) and three cutting management (C) The treatments were utilized in a 3 × 3 factorial. The factors were three types of kraal manure (cattle, goat and control) and three leaf cutting techniques. The three cutting techniques involved (i) cutting all leaves throughout: this involved cutting by hand all the fully extended young and mature leaves, (ii) cutting only edible tips which involved cutting all young but fully extended leaves, usually pale green in colour, smoother and tender than mature leaves, and (iii) cutting once at final cut. The treatments were laid in a Randomized Complete Block Design with four replicates.

Raising and transplanting of seedlings

Seeds of the two indigenous vegetables (*A. hybridus* and *C. Gynandra*) were sown in seedling trays filled with growing media hygromix. The seedling trays were kept moist by watering regularly using a watering can whenever the growing medium looked to be dry. The seedlings were transplanted after they had grown 5 to 6 leaves. Three seedlings were transplanted into large 7 L PVC pots with perforations at the bottom to allow drainage. They were later thinned to leave two plants per pot. Seedlings were watered regularly to ensure that the soil was moist throughout the growing period. The plants grew for two months in a screen net house where temperature was controlled by a fun. Weeds were uprooted by hand whenever they appeared and Aphids were controlled by applying Malathion (50% EC) while locusts were picked by hand.

Leaf cutting management

In both vegetable species, cutting of leaves began three weeks after transplanting when the plants showed an extended flower stalk of at least 10 cm. The leaves were cut by hand once every week over a two month period as shown in Table 1. After cutting, the fresh leaves were weighed to obtain biomass yields. The height of each plant was measured at the final cut using a ruler. The harvested materials were placed in envelopes and dried in the oven set at 60°C for 48 h and weighed to obtain dry biomass yields. The total biomass of leaves for each treatment was obtained by adding the yields for all the cuttings during the experiment.

Determination of crude protein in leaves

The harvested leaves from both vegetable species were analysed for crude protein content following the procedure described by the AOAC (1990). The leaves from all cuttings in each treatment were bulked and a sample was collected and ground in a Wiley mill with 0.5 mm sieve. About 0.5 g of the ground plant material was placed digested in Kjeldahl tube and crude protein was calculated by first determining the percent N and multiplying it by 6.25 to obtain crude protein (AOAC, 1990).

Table 2. Properties of manure used in the study.

Property	Unit	Cattle	Goat
pH		7.9	7.6
Organic carbon	%	42	47
Total nitrogen	%	1.81	2.53
Total phosphorus	%	1.04	0.94
Electrical conductivity	mS cm^{-1}	4.45	5.9
Ash	%	27.07	19.99
Moisture content at sampling	%	3.38	3.57
C:N ratio	-	23:1	18:1
C:P ratio	-	40:1	50:1

Values are means of four replicates.

Table 3. Properties of soil used in the study

Property	Unit value
Sand (%)	68.7
Clay (%)	16.9
Silt (%)	14.4
Textural class	Sandy loam
Soil pH (KCl)	5.38
Organic carbon (%)	0.43
Available phosphorus (%)	1.2
Total nitrogen (%)	0.49
Electrical conductivity (ms cm^{-1})	0.07
K^{+} (mg kg^{-1})	418
Ca^{2+} (mg kg^{-1})	614.19
Mg^{2+} (mg kg^{-1})	240.28

Values are means of four replicates.

Statistical analysis of data

All the data was subjected to analysis of variance (ANOVA) using the General Linear Model procedure of the Statistical Analysis System (SAS) programme (SAS Institute Inc., 2006). Tukey's t test was used to compare treatment means at 5% probability.

RESULTS

The goat manure had a higher concentration of nutrients and lower C:N ratio than the cattle manure (Table 2). Both manures had not decomposed completely. Table 3 shows that the soil pH was optimum for most crops. The soil has low organic carbon, available phosphorus and total nitrogen (Table 3). Although the soil pH was ideal, the N and P concentrations were very low. The organic carbon concentration was high in both kraal manure types. Cattle manure had higher total phosphorus level than goat manure. There was a high electrical conductivity in both kraal manure types and goat manure had higher EC than cattle manure. The C:N ratio of cattle was higher than that of goat manure.

The ANOVA showed that in both vegetables, both manure type and leaf cutting management had significant influence on all the parameters measured (Table 4). The interaction of manure type and leaf cutting management were also significant except for total dry leaf mass. There was a significant influence of kraal manure and leaf cutting management on protein content of *C. Gynandra* but not of *A. hybridus*. In both vegetable species, plants that were grown in soil amended with goat kraal manure produced the highest fresh leaf mass across all the manure types followed by cattle kraal manure (Table 5). Dry leaf biomass followed a similar pattern as in that of fresh leaf biomass. Plants that were grown in soil amended with cattle kraal manure recorded highest leaf moisture content except in *A. hybridus* whereby highest leaf moisture content was obtained from plants that were added with goat manure. Stem and root yield followed the same trend as fresh leaf biomass and dry leaf biomass.

Table 4. Analysis of variance (F values) for total fresh leaf mass, total dry leaf mass, leaf moisture content, fresh stem mass, dry stem mass, fresh root mass and dry root mass of *C. gynandra* and *A. hybridus*.

Source of variation	df	Total fresh leaf mass (g/pot)	Total dry leaf mass (g/pot)	Leaf moisture content (%)	Fresh stem mass (g/pot)	Dry stem mass (g/pot)	Fresh root mass (g/pot)	Dry root mass (g/pot)	CP (%)
C. gynandra									
Block	3	0.47^{ns}	0.07^{ns}	1.07^{ns}	0.21^{ns}	0.17^{ns}	0.68^{ns}	2.82^{ns}	2.33^{ns}
Manure type (MT)	2	255.59***	160.1***	70.78**	160.49**	195.03***	34.62*	47.96*	25.3**
Cutting technique (CT)	2	81.38***	18.9***	31.68***	124.76***	353.20***	17.28***	26.63***	4.97*
MT × CT	4	17.91***	10.33***	3.95*	16.87***	53.49*	3.15***	3.33*	0.52ns
Error	24								
Total	35								
A. hybridus									
Block	3	1.92^{ns}	1.73^{ns}	2.01^{ns}	1.30^{ns}	1.92^{ns}	1.77^{ns}	1.63^{ns}	1.13^{ns}
Manure type (MT)	2	163.3***	8.16**	221.81***	21.99***	27.57***	25.11***	21.29***	14.51
Cutting technique	2	63.4***	1.52^{ns}	71.90***	31.26***	77.96***	41.13***	71.41***	2.66^{ns}
MT × CT	4	7.4**	2.65^{ns}	6.97**	4.61*	11.47**	6.27**	15.04***	0.83^{ns}
Error	24								
Total	35								

ns = Not significant; * = significant at (p<0.05); ** = significant at (p=<0.01); *** = significant at (p<0.0; CP = crude protein.

Table 5. Effect of kraal manure application on biomass yields, moisture content and crude protein of *C. gynandra* and *A. hybridus*.

Manure type	Fresh leaves (g/pot)	Dry leaves (g/pot)	Leaf moisture content (%)	Fresh stem (g/pot)	Dry stem (g/pot)	Fresh roots (g/pot)	Dry roots (g/pot)	Crude protein (%)
Cleome gynandra								
No manure	80.14^{c}	14.99^{c}	78.97^{c}	29.98^{c}	6.73^{c}	14.35^{b}	2.91^{c}	5.8^{c}
Cattle	328.42^{b}	41.26^{b}	86.96^{a}	98.49^{b}	15.56^{b}	27.74^{b}	5.19^{b}	12.2^{b}
Goat	470.86^{a}	65.49^{a}	84.89^{b}	169.79^{a}	29.77^{a}	41.37^{a}	8.57^{a}	14.2^{a}
Mean	293.14	40.58	83.61	99.42	17.35	27.82	5.55	10.7
Amaranthus hybridus								
No manure	116.45^{c}	22.38^{c}	80.59^{c}	52.06^{c}	8.66^{c}	39.82^{c}	6.78^{c}	6.7^{c}
Cattle	259.56^{b}	27.16^{b}	88.91^{b}	90.09^{b}	11.15^{b}	67.52^{b}	8.36^{b}	11.44^{b}
Goat	366.13^{a}	33.31^{a}	90.09^{a}	159.22^{a}	23.01^{a}	88.62^{a}	14.38^{a}	13.44^{a}
Mean	247.38	27.61	86.53	100.45	14.27	65.32	9.84	10.5

Values are means, n = 36; Means within a column with similar letter are not significantly different (p<0.05) by the LSD test.

Table 6. Effect of leaf cutting management on biomass yields and moisture content of *C. gynandra* and *A. hybridus* .

Leaf cutting techniques	Fresh leaves (g/pot)	Dry leaves (g/pot)	Leaf moisture content (%)	Fresh stem (g/pot)	Dry stem (g/pot)	Fresh roots (g/pot)	Dry roots (g/pot)	CP (%)
C. gynandra								
Only edible tips throughout	342[b]	45.38[a]	84.61[b]	84.01[b]	10.32[b]	23.01[b]	4.46[b]	8.6[c]
All leaves throughout	371.24[a]	45.79[a]	85.74[a]	46.95[c]	6.46[c]	21.64[b]	4.21[b]	12.33[a]
Cut once at harvest	165.35[c]	30.58[b]	80.47[c]	167.31[a]	35.27[a]	38.81[a]	7.99[a]	11.33[b]
Mean	292.86	40.58	83.61	99.42	17.35	27.82	5.55	10.75
A. hybridus								
Only edible tips throughout	294.52[a]	29.77[a]	87.95[b]	82.53[b]	8.99[b]	57.80[b]	6.70[b]	9.4[c]
All leaves throughout	290.34[a]	27.99[a]	88.49[a]	46.53[c]	4.85[c]	38.45[c]	4.58[c]	12.2[a]
Cut once at harvest	157.28[b]	25.09[b]	83.14[c]	172.31[a]	28.97[a]	99.72[a]	18.23[a]	9.9[b]
Mean	247.38	27.61	86.53	100.45	14.27	65.32	9.84	10.5

Values are means, n = 36; Means within a column with similar letter are not significantly different ($p<0.05$) by the LSD test.

The results indicated that *C. gynandra* produced highest leaf yield when compared to *A. hybridus.* For both vegetables, application of kraal manure increased the crude protein content of leaves and the increase was higher with goat than cattle kraal manure. In both vegetable species, the crude protein content was increased when plants were cut every fortnight than when they were cut once at the end (Table 6).

The interactive effects of kraal manure and leaf cutting regime showed that both the leaf biomass yields and crude protein content of leaves of both vegetables depended on a combination of manure and leaf cutting regime (Figure 1). The results showed that fresh leaf biomass of *C. gynandra* and *A. hybridus* were increased when kraal manure was added. Goat manure produced higher biomass than cattle manure. The yield was higher in goat manure when all the leaves were cut than in cattle manure for both vegetables. When edible tips were cut, better biomass from *C. gynandra* was obtained than in *A. hybridus*. There was no significant difference in fresh leaf biomass for both vegetables when leaves were cut once at the end of study. *C. gynandra* obtained highest fresh leaf yield than *A. hybridus*. *A. hybridus* responded better than *C. gynandra* where kraal manure was not added. Figure 2 shows that the application of kraal manure in both *C. gynandra* and *A. hybridus* was associated with improved biomass yields of loves for a longer time after transplanting than the control (without manure amendment). This may suggest that the manure provided nutrients to the plants longer. The yields for *C. gynandra* were generally higher than *A. hybridus* across the cutting regimes. The results in Figures 3 and 4 suggests that cutting only of the tender edible leaves at the tip of the plants produced slightly higher albeit not significant biomass yields. This implies that farmers could still gain by selectively cutting the tender edible leaves as long as nutrients were available to the plants.

DISCUSSION

The increase of biomass yield when kraal manures were applied is consistent with the results of the chemical properties of soil and manure used in the present study. This suggests that the observed increase in biomass yield of plants amended with kraal manure could be due to higher contents of N, P and organic carbon in kraal manure compared to soil. This was confirmed by Azeez et al. (2010) in their study. They found that manure at the tested levels contained highest levels of N, P and K and suggested that the positive effect of kraal manure was due to the release of plant nutrients contained in the manure. The increased biomass yields of vegetable species in the present study could be due to highest levels of N and P following kraal manure applications as suggested by Azeez et al. (2010).

Makinde et al. (2010) has shown that the protein content of amaranth leaves was improved with high NPK fertilisation in Nigeria and suggested that the increase in protein content might be because N is an important element in protein synthesis. In our case, the high nitrogen content that was available in the manure amended soils compared to the control provided the base for improved crude protein. Similar explanations were given by Mhlontlo et al. (2007) who found that the uptake of N and P in the leaves of vegetables was increased by increase in manure application and they suggested that because of close relationship between N and protein, crude protein was increased in the leaves with highest N and P uptake. The finding that the tender edible leaves had highest crude protein can be explained by the translocation of nitrogenous compounds are out of senescent leaves and flowers and relocation into younger areas of the plant (Baloyi et al., 2013). Flowers and Yeo(1992) have shown that if growing tips are removed from plants more frequently, senescence of other parts

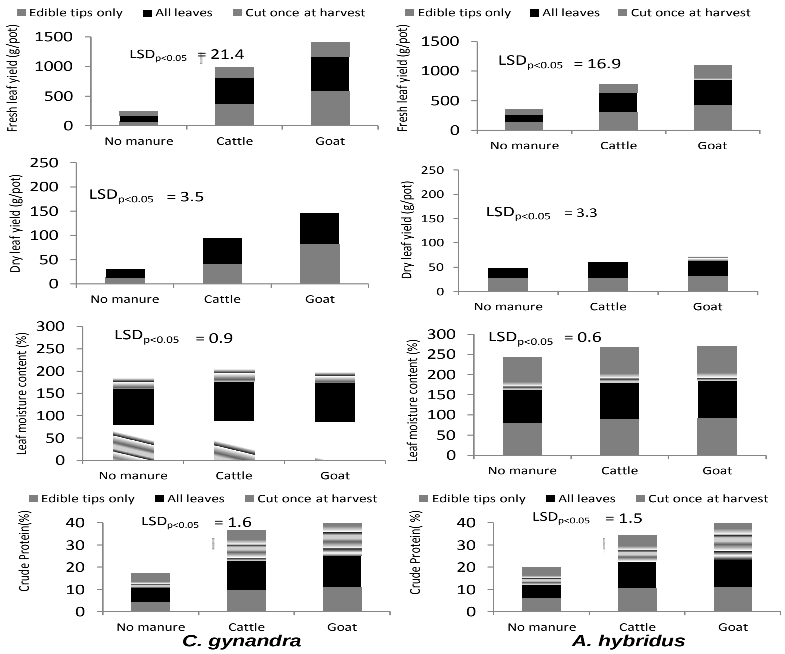

Figure 1. The interactive effects of manure type and cutting techniques on the biomass of fresh leaf , dry leaf, leaf moisture content and crude protein of *C. gynandra* and *A. hybridus*.

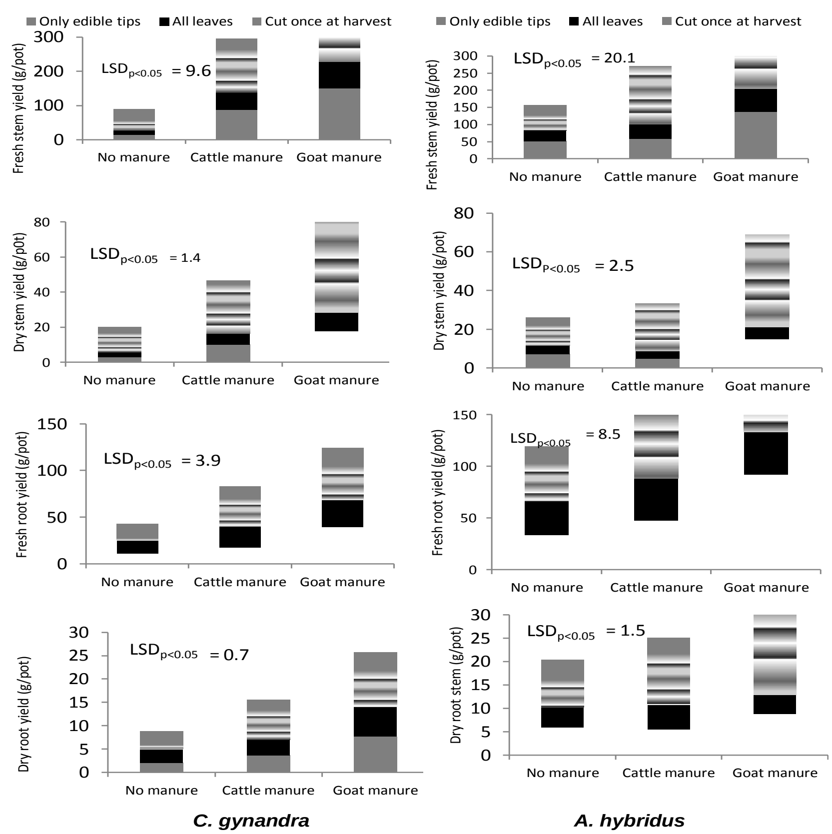

Figure 2. The interactive effects of manure type and cutting techniques on biomass yield of fresh stem , dry stem , fresh root and dry root of *C. gynandra* and *A. hybridus.*

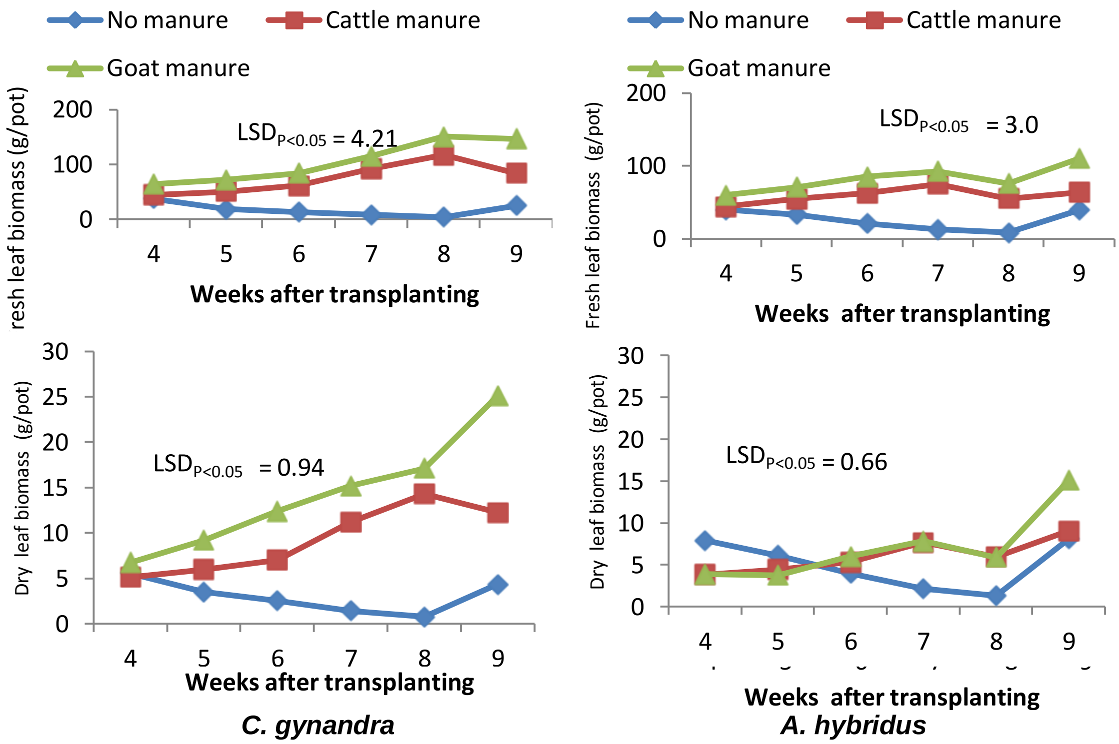

Figure 3. Trends in the leaf biomass yields of *C. gynandra* and *A. hybridus* during the experimental period as influenced by manure types.

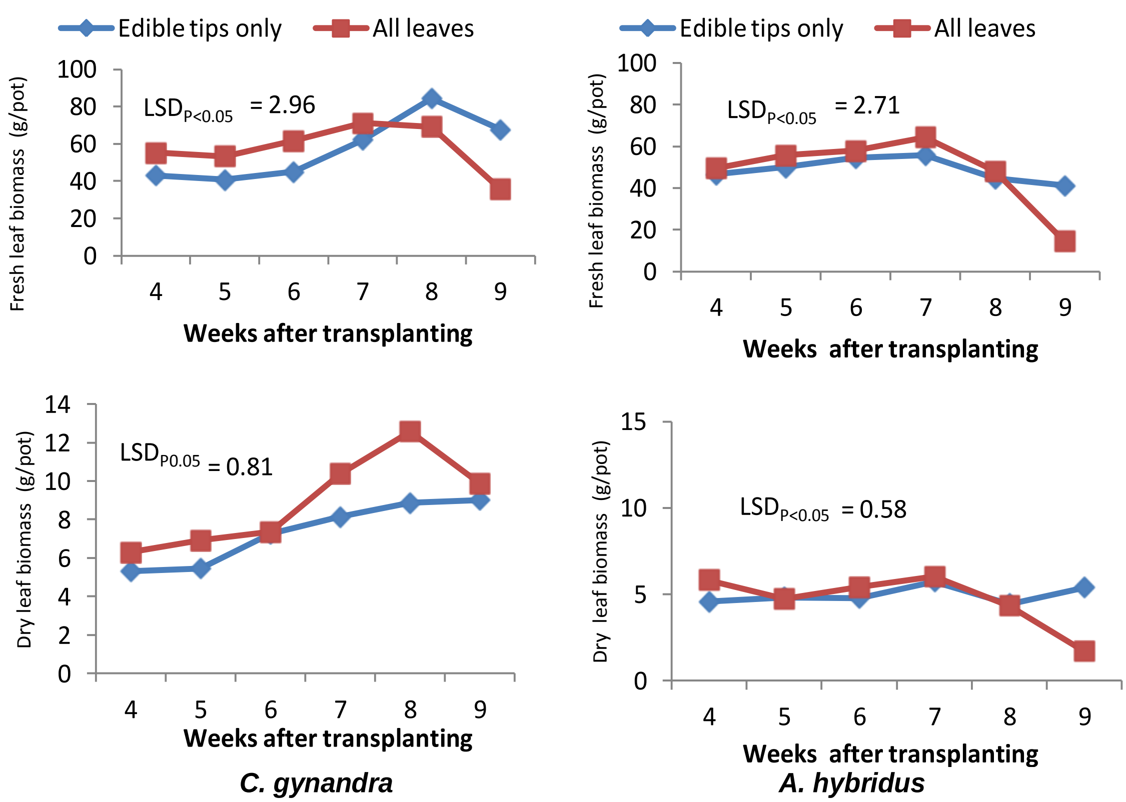

Figure 4. Trends in the leaf biomass yields of *C. gynandra* and *A. hybridus* during the experimental period as influenced by cutting techniques.

such as leaves is delayed but that more nutrients accumulate in the leaves. Alleman et al. (1996) have suggested that more nutrients might have been used for leaf proliferation and growth as leaves were cut frequently but to those plants which were cut once, some nutrients might have been translocated to the stems and roots. Barimavandi et al. (2010) reported that when vegetable leaves are not cut, there is least use of stored assimilates because of sufficient amount of nutrients from photosynthesis via leaves and this can increase in other parts of the plant such as stems and roots. Similar explanations have been suggested by other authors (Asiegbu, 2005; Belesky and fedders, 1994; Lestiene et al., 2006; Ogar and Asiegbu, 2005; Boogoard et al., 2001).

Conclusion

Both cattle and goat kraal manure improved the growth and biomass yields of African indigenous leafy vegetables but the effect of goat manure was superior to that of cattle manure. The biomass yields of leaves of African indigenous leafy vegetables can significantly be improved by cutting leaves frequently with enough soil nutrients and water. It must be mentioned that the experiments in this study were conducted in pots and cognisance must be taken of the limitation of extrapolating these results to field conditions. However, these results provide a useful indication of the nature of the responses that can be expected in the field and justifies further validation under field conditions.

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