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GROWTH AND NUTRITIONAL QUALITIES OF THREE OCIMUM SPECIES AS AFFECTED BY METHODS OF PROPAGATION

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ABSTRACT: *Ocimum species are well known for their medicinal values and this have caused increase in their demand by individual and many pharmaceutical industries. In order to meet the demand for this plant species three different Ocimum species (Ocimum basilicum, Ocimum gratissimum and Ocimum americanum) were propagated by different methods (stem cutting and seed) and were evaluated for their morphometric characters after 6 six and eight weeks. The chlorophyll content, proximate composition and phytochemicals content of the plants were also determined. The result obtained showed that Ocimum species propagated through stem performed better than the seeds. The results also revealed that there were strong correlation between the plants height and other morphometric characters. Also, the Ocimum species propagated by seed contained more phytochemicals than those propagated by stem. However, O. gratissimum propagated by stem cutting appeared to perform better than the other two Ocimum species in term of plant height, leaf number, stem girth and number of branches.*

KEYWORDS: Growth, Nutrition, Propagation, Ocimum

INTRODUCTION

The wellbeing of human is directly proportional to the wellbeing of botanicals around them, including agricultural, horticultural and ornamental plants as well as forest products. Despite the arrays of benefits derivable from plants, urbanization alongside different human's activities have contributed to the extinction of many important plants and has been the major factor enfeebling the growth and large production of botanicals in the world Gbadamosi *et al*., (2009). In Nigeria for example, these vital natural resources have suffered setback in growth and production because of the negligence by individuals and government. However, in recent years, the effluxion of many diseases which have developed resistant to many synthetic drugs and the effects of many organic wastes (including crude oil) on climate have called for planting and large production of botanicals. It is believed that plants that are medicinal contained numerous allelochemicals (Orafidiya *et al.*, 2005; Okunlola, 2010; Ade-Ademilua and Obi, 2013). Hence, researches have concentrated more on plant species with medicinal value as new thoroughfare of disease control as public awareness of the values associated with them is increasing swiftly. Hence, the need for means of multiplying them in large quantites in order to meet the public demands (Low and Hackett, 1981; Dag *et al.,* 2012).

Ocimum is a genus of about 35 species of aromatic annual and perennial herbs and shrubs in the family Lamiaceae found through the tropics and subtropics, both wild and cultivated (Ojeifo and Denton, 1993). *Ocimum americanum*, *Ocimum basilicum* and *Ocimum gratissimum* are important and the most popular member of this species in many African countries (Ehiagbonare, 2007). The fact that their foliage adds a distinctive flavor to many foods makes these species have a long history as culinary herbs (Deshpande *et al.*, 1997; Eze *et al*, 2006). The antioxidant activity present in high concentrations in the plants makes them important botanicals in the prevention of various diseases including upper respiratory tract infections, diarrhea, headache, diseases of the eye and skin, pneumonia, cough, fever and conjunctivitis (Lasisi and Ajuwon, 2002; Adebola and Salau, 2005; Sidhu*et al*, 2007). The oil from the plants clearly show antimicrobial, insect repellant and antihelminthic activities (Simon *et al*., 1999; Oboh, 2008).The flowers and leaves of the plants are rich in essential oils, and so it is used in preparation of teas and infusion (Rabelo *et al.*,2003). These important medicinal plants and culinary herbs *O. basilicum, O. gratissimum* and *O. americanum* are said to also display important effects at cellular level, including platelet anti-aggregate properties and inhibitory activities against HIV-1reverse transcriptase (Tomar*et al*., 2010).

In spite of the medicinal values associated with these species of Ocimum their propagation has not been given adequate attention yet the demand for them is on the increase. Although some plant scientists have suggested the micro-propagation of the three species but this has not been easily adaptable and accessible to the peasant farmers (Sofowora, 1992). In Nigeria, *Ocimum* species are propagated using both seeds and stem cutting, the seed however is a major means of propagation of *Ocimum* species (Faluyi, 2009) though, farmers have problems with cultivating the plants especially through seeds, due to its low viability (Sulistiarini, 1999), the use of stem cuttings on the other hand has not been promoted because of the difficulty of propagation. Therefore, the need to conduct studies on the method of propagation that will promote growth of the three species and its effect on the chemical composition of the herbs to ensure the plant can be conserved. The study therefore investigated the growth of *O. basilicum, O. gratissimum* and *O. americanum* propagated by two different methods.

MATERIALS AND METHODS

Study location

The experiment was conducted in the screen house of the Department of Crop, Soil, and Pest Management, the Federal University of Technology, Akure Ondo State, Nigeria from April to July, 2013. The state lies between 4^1 30^o and 6^1 40^o east of the Greenwich meridian and latitudes 50^1 45^o and 8^o north of the equator located in the rainforest zone with two distinct seasons. The temperature ranges from 21^o- 29^o with relative humidity of 75±5% at 9.a.m.

Collection of plant materials

The seeds of *O. basilicum, O. gratissimum* and *O. americanum* used for the study were collected from parent stands. The viability of the seeds were tested by steeping them into suitable water containers. The floated seeds were discarded as non-viable while the sunken seeds were regarded as viable seeds. The seeds were removed from the water and expose to air for drying in the laboratory at temperature of 32±2^{o}C for three days. After drying, the seeds were kept in separate containers until further use. Also, the stem cuttings (with at least 3 nodes) of the Ocimum spp. were obtained from healthy parent stock. The stems were cut early in the morning with the aid of secateurs in order to avoid injury and ensure a clean cut. The seeds and stem cuttings of the plants were identified and authenticated in the Department of Crop, Soil and Pest management based on their vegetative (including shape and colour of leaves and stem) and reproductive (inflorescence) characteristics.

Preparation of growth medium

The type of growth medium that was used is top soil. The soil is rich in humus and was collected from the crop type museum of the Department of Crop, Soil and Pest Management. The soil was air dried, sieved and sterilized by heating in a drum before use. The physico-chemical properties of the soil was determined following routine procedure described by the Department of Crop, Soil and Pest Mangement, the Federal University of technology Akure (Laboratory Manual, 1998). The soil was equally distributed into poly pots of 11.4cm diameter and 20cm length with drainage holes.

Experimental procedure

The viable seeds of each plant were planted in seed trays before transplanting to poly pots. The stems were also planted in different poly pots. The experiment was laid out in a 2 by 3 factorial in complete randomized design and replicated five times. The plants were watered throughout the period of observation. The plant height, number of leaves, stem girth and total number of branches were observed and recorded.

Phytochemical analysis of the samples

The matured leaves of each of the Ocimum species were harvested, air-dried in the laboratory, grounded into fine powder and used to prepare crude aqueous extracts. Phytochemical analysis of the extracts were determined as described by Sofowora (1993), Trease and Evans (1989) as well as Harborne (1973). The extracts were evaluated for the presence of alkaloids, tannins, glycosides, saponins and flavonoids.

Proximate analysis of the plant samples

Proximate analysis of each of the plant samples was investigated to determine the difference in the concentration of nutritional components of each species cultivated through seeds and stem. Some of the proximate components tested for are moisture content, ash content, crude fibre, protein and carbohydrates content. Method of AOAC (1990) was used to determine the proximate composition of the samples.

Chlorophyll content analysis

To assess the chlorophyll contents of leaves amongst species, the uppermost leaves from each treatment were harvested, 1g of each were weighed and crushed in a small mortar with pistil. Each sample was collected in a test tube and its chlorophyll content repeatedly extracted with successive volume (20-30ml) of 80% acetone until no traces of green colour were noticed and the residue turned white. Three (3ml) of the extract from each sample were taken and the absorbance read with a Spectrophotometer at two wavelengths of 645nm and 663nm that correspond to absorption maximum of chlorophyll "a" and "b" respectively. Total chlorophyll content was calculated based on the equation described by McKinney (1941).

Total chlorophyll content (mg/100g tissue)= $[20.2\ A645 + 8.02\ A663][{}^{V}/_{10w}]$

Where;

A645 = absorbance at 645nm wavelength

A663 = absorbance at 663nm wavelength

V = final volume (cm^3) of chlorophyll extract in 80% acetone

W = fresh weight (g) of tissue extracted.

Statistical analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) and means were separated with Tukey's Test. Also, the linear regression analysis was carried out to ascertain the correlations between the morphometric characters of the plants. SPSS version 17 was used for the analysis.

RESULTS

Chemical composition of the growth medium used and its soil particles

The chemical composition and the percentage soil particles of the growth medium are presented in Figure 1 and 2 respectively. Among all the nutrients analyzed, phosphorus had the highest quantity (28.78mg/kg) while nitrogen had the lowest (0.15%). The pH of the growth medium is 5.52, percentage sand 52.57%, clay, 28.4% and silt 18.99%.

Morphometric characteristics of three Ocimum species grown from two different plant parts

Table 1 and 2 showed the morphometric characters of the three Ocimum species. Regardless of the vegetative part used and the species of the Ocimum, height, stem girth, leaf number and number of branches increased with increase in period of planting. At six weeks after planting (WAP) *O. gratissimum* propagated by stem cutting recorded the highest height (31.82cm) significantly different from others ($F_{5, 24}$=385.983, $p<0.0005$); *O. gratissimum* by stem cutting also recorded the highest leaf number (12.61), stem girth (0.57cm) and number of branches (8.67) significantly different (leaf number: $F_{5, 24}$=1448.265, $p<0.0005$;stem girth: $F_{5, 24}$= 678.368, $p<0.0005$; number of branches: $F_{5, 24}$= 18152.744, $p<0.0005$) from the other species grown by stem cutting and seed.

At 8WAP, *O. gratissimum* grown by stem cutting recorded the highest height, leaf number, stem girth and number of branches of 40.37cm, 16.32, 0.77cm and 12.00 respectively. The growth of *O. gratissimum* was significantly different (height: $F_{5, 24}$=12302.222; leaf number: $F_{5, 24}$=62.598, $p<0.0005$; stem girth: $F_{5, 24}$= 168.348, $p<0.0005$; number of branches: $F_{5, 24}$= 6125.402, $p<0.0005$) from others that were grown by stem cutting as well as by seed. Tables 1 and 2 showed that irrespective of the period of planting Ocimum species grown by stem cutting performed better than those grown by seed. Also, the tables showed that *O. basilicum* grown by seed recorded the highest values for the parameters observed than other Ocimum species propagated by seed.

Correlation between height of the plants and their other morphometric characters

Table 3 showed the correlation between the plant height and other morphometric characters of Ocimum species at 8WAP. The correlation between the heights of the plants and the other morphometric characters is evident as reflected by their R value which is tending towards 1. The R^2 value showed that only 72.3, 35.7 and 66.3% of the leaf number, stem girth and number of branches can be explained by the plant height value respectively. The R^2 reflected high correlation as the values are large. However, the correlation between the plants heights and leaf number, plant height and stem girth as well as plant height and number of branches appeared to be high and statistically significant (plant height/leaf number: $F_{1,28}$= 79.955, $p < 0.0005$; plant height/stem girth: $F_{1, 28}$= 15.567, $p < 0.0005$; and plant height/number of branches: $F_{1, 28}$=55.143, $p < 0.0005$ respectively).

Proximate composition and chlorophyll content of three Ocimum spp grown from two plant parts

The proximate composition of the three Ocimum species as well as their chlorophyll contents are presented in Figures 3 and 4 respectively. Irrespective of the vegetative part used for the propagation of the plants, the three Ocimum species recorded high moisture content above 68% which were not significantly different from each other at $p<0.05$. Although, the three species of Ocimum had low carbohydrate content irrespective of the vegetative part used for propagation. *Ocimum basilicum* grown by stem cutting recorded the highest moisture and crude fibre content as well as the lowest carbohydrate content (0.33%). However, there were no significant differences between the 3 Ocimum species with respect to their proximate composition.

Figure 4 presented the chlorophyll content of the three Ocimum species. *Ocimum gratissimum* propagated by stem cutting recorded the highest chlorophyll content (963.66) while *O. americanum* propagated by stem cutting recorded the lowest chlorophyll content (699.99). Except for *O. gratissimum* all the Ocimum species propagated by seed recorded high chlorophyll content than those propagated by stem cutting.

Phytochemicals of three Ocimum species grown from two different plant parts

The phytochemical composition of the three Ocimum species is presented in Table 4. The propagation method used notwithstanding, there were significant differences between the three Ocimum species as regard their phytochemicals. *O. americanum* propagated by seed recorded the highest oxalate, phytate and phenol content which differed significantly from others (oxalate: $F_{5, 12}$ = 14.616, $p < 0.0005$; phytate: $F_{5, 12}$ = 7.771, $p < 0.01$; phenol: $F_{5, 12}$ = 54.305, $p < 0.0005$). *O. basilicum* propagated by seed recorded the highest flavonoid content also significantly different from others at $F_{5, 12}$ = 42.352, $p < 0.0005$. *O. gratissimum* propagated through seed had the highest saponins and tannin content significantly different from others (sapon ins: $F_{5, 12}$ = 31.666, $p < 0.0005$; tannin: $F_{5, 12}$ = 46.397, $p < 0.0005$). However, regardless of the species of Ocimum, those propagated through seed had higher phytochemical content than those propagated by stem cutting.

DISCUSSION

Multiplying the population of plant species that are of high medicinal value within short period of time as well as maintaining the allechemicals contained in them are of great importance among plant scientist in a world of increasing demand for plant and plant products. Saglamet al. (2004), Capecka (2012) as well as Eed and Burgoyne (2015) reported that propagation method affects the growth of plants. Different plant species have different propagation methods that can promote high yield. Therefore, propagation methods are directly or inversely proportional to the development of different morphometric characters of botanicals.

The result obtained in this research showed that the three Ocimum species evaluated for their growth differed significantly with the type of vegetative part used for their growth. Also, regardless of the propagation method used, there were increases in height, leaf number, stem girth and number of branches of the three species as time of planting increased. However, those that were propagated by stem cutting performed significantly better than those that were propagated by seed. This could be attributed to the fact that plants propagated by seed took longer time to get to maturity. In addition, to the fact that plants propagated through stem develop root system within short period after planting (REF). The result obtained showed that percentage of sand in the media used is higher than the clay and silt content this could have contributed to the performance of the stem cutting species of the three Ocimum species because plants propagated through stem are believed to perform better on sandy soils. The result also acquiesced with the study by Capecka (2012) that stem cutting propagated *Salvia officinalis* and *Melissa officinalis* performed better than those propagated by seed in term of their morphometric characters. The findings from this research however, disagree with the result obtained by Saglam et al.(2004) in which seed propagated *M. officinalis* performed better than those propagated using stem cutting. The differences could be attributed to the different study location because it is believed that environmental factors have significant effect on plant growth. Furthermore, the results revealed that *O. gratissimum* propagated by stem cutting had the highest performance in term of plant height, leaf number, stem girth and number of branches while seed propagated *O. gratissimum* recorded the lowest performance in term of morphometric characters as earlier mentioned. This result also agree with the findings of Orwa et al. (2009) that seed propagated *O. basilicum* performed better than the seed propagated *O. gratissimum.* The study showed that there was high significant correlation between the height of the plants and other morphometric characters. The findings from the work established the report of Ardakani et al. (2012) that increase in height of plants is directly proportional to other morphometric characters. Furthermore, the findings of Yan et al. (2012) agreed with the result of this work that increase in plant height strongly correlate with increased leaf number, leaf area and other morphometric characters. The high value recorded in height of the plants may be related to the high phosphorus, potassium and sodium contained in the growth medium used in this research because the elements are believed to increase plant height (Moyin-Jesu &Adekayode, 2010). All the three Ocimum species recorded high chlorophyll content reflecting fact that the propagation method has no significant effect on their chlorophyll content. In the same vein, the proximate composition of the three Ocimum species indicated that the propagation method used had no effect on their proximate content. However, this work indicated that the stem cutting propagated *O. basilicum, O. gratissimum* and *O. americanum* had low phytochemicals content compared to the seeds.

CONCLUSION

The result of this research showed that the growth of the three Ocimum species evaluated was directly proportional to the method of propagation used. Also, it was found that the stem girth, leaf number and the number of branches of the plants were dependent on the plant height as reflected by the regression analysis. The performance of the stem cutting propagated Ocimum species can be arranged as follow *O. gratissimum*> *O. basilicum*> *O. americanum* while the performance of the three plant species propagated by seed could be arranged as *O. basilicum*> *O. americanum*> *O. gratissimum.* Since stem cutting propagation method has yielded high growth of the three Ocimum species, it is therefore recommended as method for propagating the plants. However, the pharmaceutical industries may be advised to use seed propagation method for growing the three species of Ocimum since they contain more phytochemical (major materials in production of drugs) than those propagated by stem cutting.

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APPENDIX

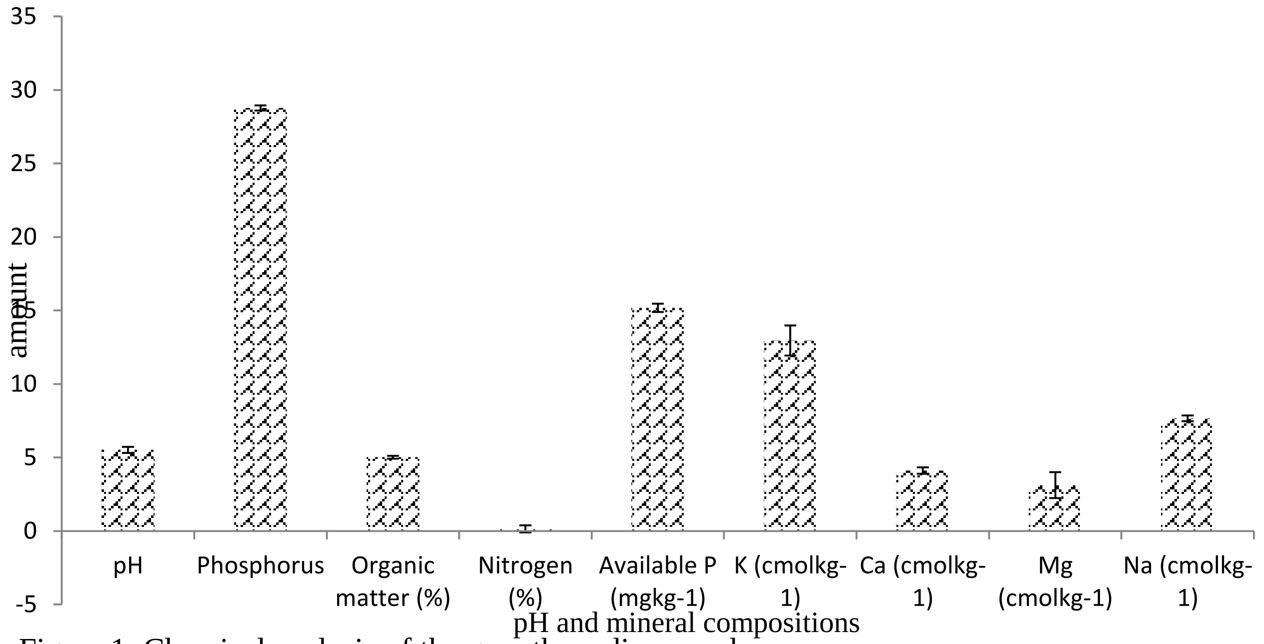

Figure 1: Chemical analysis of the growth medium used

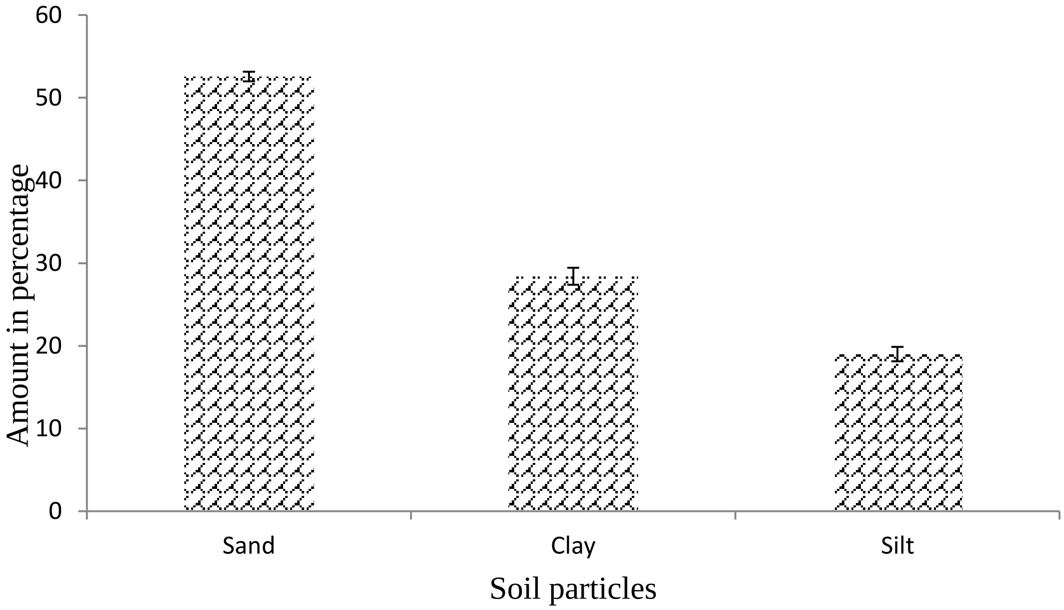

Figure 2: Particles of the growth medium

Table 1: Morphometric characteristics of three Ocimum species grown from two different plant parts after six weeks

plant parts	Plants	Height (cm)	Leaf number	Stem girth (cm)	Number of branches
Stem cuttings	*O. basilicum*	26.38±0.19[d]	11.41±0.02[e]	0.51±0.01[d]	8.33±0.01[e]
	O. gratissimum	31.82±0.05[e]	12.61±0.01[f]	0.57±0.01[e]	8.67±0.01[f]
	O. americanum	25.00±0.98[cd]	7.65±0.21[d]	0.52±0.01[d]	8.00±0.02[d]
Seed	*O. basilicum*	24.33±0.01[c]	6.33±0.09[c]	0.28±0.01[c]	2.25±0.03[c]
	O. gratissimum	8.98±0.01[a]	3.32±0.01[a]	0.18±0.01[a]	1.00±0.01[a]
	O. americanum	17.15±0.01[b]	5.55±0.01[b]	0.20±0.01[b]	2.00±0.06[b]

Each value is mean± standard error of five replicates. Values followed by the same letters are not significantly ($p > 0.05$) different from each other using Tukey's Test.

Table 2: Morphometric characteristics of three Ocimum species grown from two different plant parts after eight weeks

plant parts	Plants	Height	Leaf number	Stem girth	Number of branches
Stem cuttings	*O. basilicum*	34.33±0.01[e]	14.65±0.01[f]	0.73±0.01[d]	10.00±0.01[e]
	O. gratissimum	40.37±0.01[d]	16.32±0.01[e]	0.77±0.01[f]	12.00±0.01[f]
	O. americanum	28.68±0.02[cd]	8.98±1.46[d]	0.68±0.01[e]	8.25±0.01[d]
Seed	*O. basilicum*	27.00±0.01[c]	9.80±0.05[c]	0.63±0.01[c]	5.50±0.11[c]
	O. gratissimum	12.98±0.02[a]	4.03±0.01[a]	0.53±0.01[a]	3.00±0.01[a]
	O. americanum	21.45±0.00[b]	7.66±0.01[b]	0.57±0.01[b]	4.50±0.02[b]

Each value is mean± standard error of five replicates. Values followed by the same letters are not significantly ($p > 0.05$) different from each other using Tukey's Test.

Table 3: Correlation between height of the plants and their other morphometric characters

Parameters	R	R^2	K ± S.E	R_c± S.E	R_E	Sig.
Leaf number	0.880	0.723	-2.02±1.47	0.43±0.05	-2.02+0.43(height)	0.000
Stem girth	0.598	0.357	0.75±0.05	-0.01±0.00	0.75-0.01(height)	0.000
Number of branches	0.814	0.663	-1.30±1.17	0.30±0.04	-1.30+0.30(height)	0.000

K=constant; R_c= regression coefficient; R_E= regression equation

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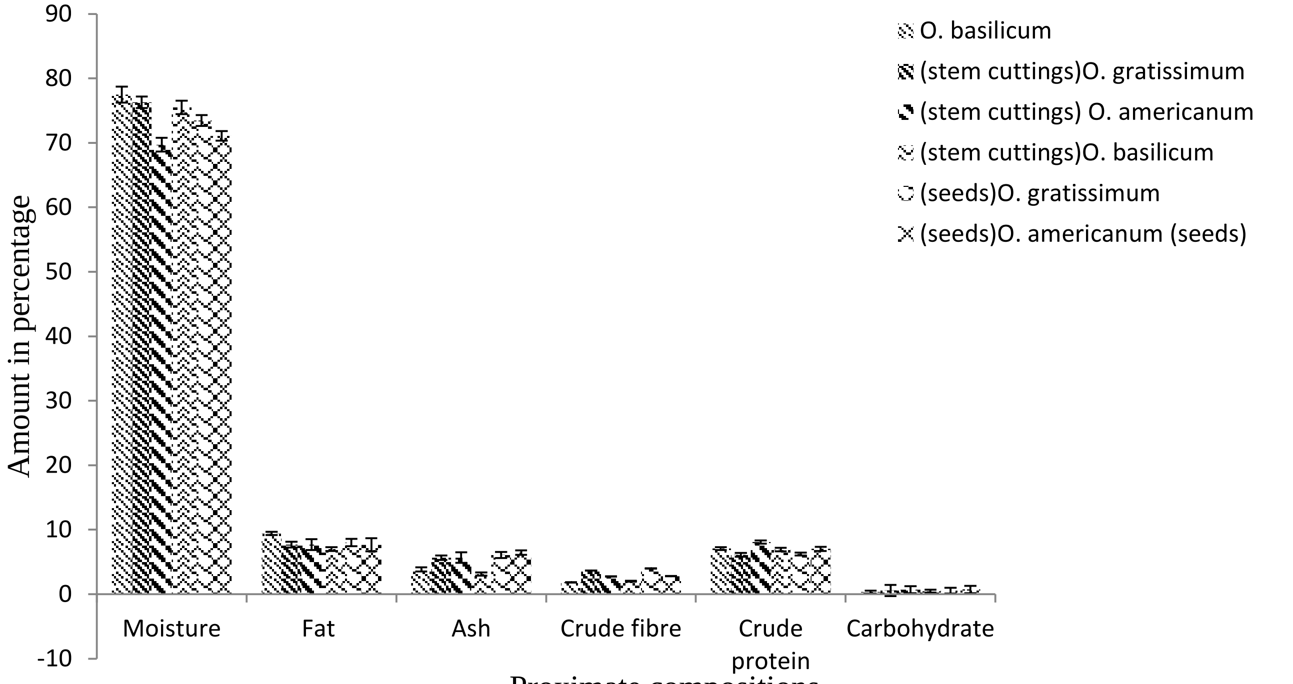

Figure 3: Proximate composition of three Ocimum spp grown with different methods

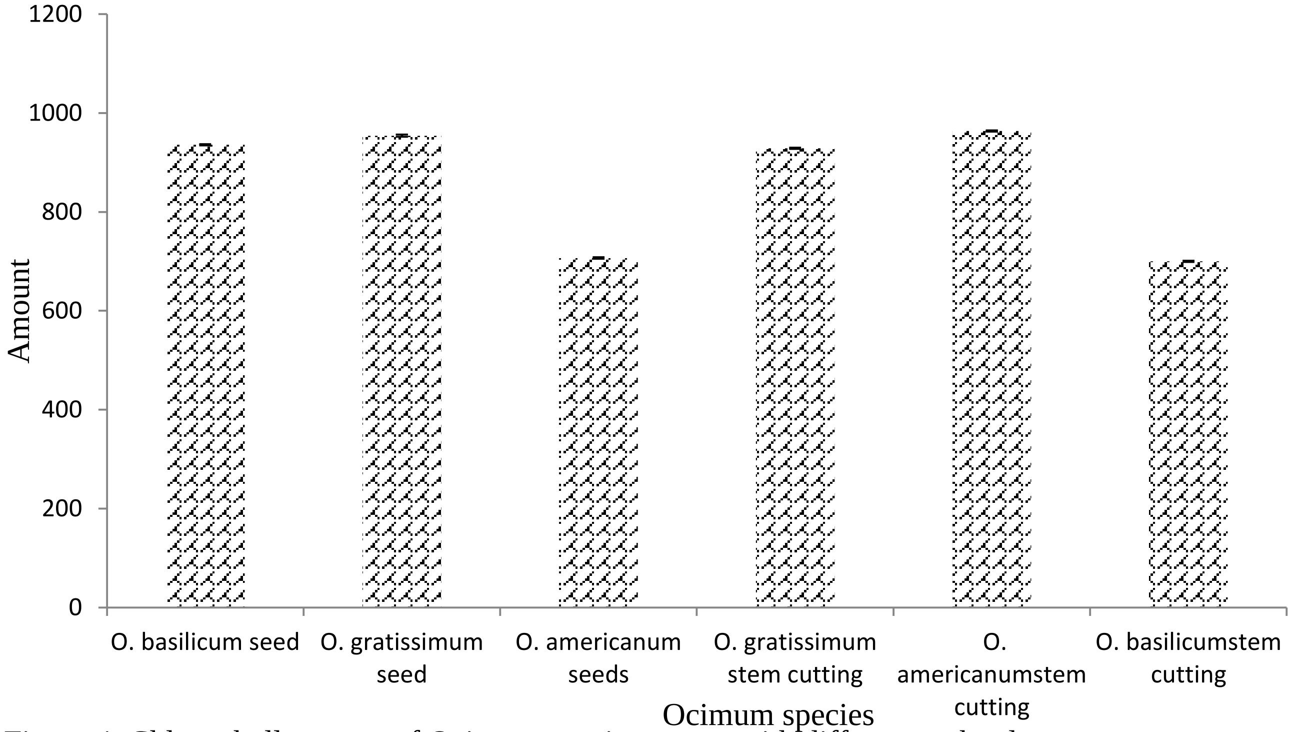

Figure 4: Chlorophyll content of Ocimum species grown with different mehtods

Table 4: Phytochemicals of three Ocimum species grown by two different plant parts

Plant parts	Plants	Oxalate	Phytate	Flavonoids	Saponin	Tannin	Phenols
Stem cuttings	*O. basilicum*	0.05±0.00[a]	0.58±0.04[a]	0.52±0.02[cd]	3.25±0.02[a]	2.70±0.03[bc]	0.02±0.00[a]
	O. gratissimum	0.06±0.01[abc]	0.65±0.00[ab]	0.28±0.02[ab]	4.50±0.01[b]	3.29±0.08[c]	0.07±0.04[a]
	O. americanum	0.07±0.00[c]	0.67±0.01[b]	0.46±0.01[bc]	3.01±0.22[b]	0.81±0.02[a]	0.56±0.10[b]
Seed	*O. basilicum*	0.06±0.00[abc]	0.62±0.01[ab]	0.78±0.06[f]	4.52±0.03[b]	3.30±0.01[c]	0.07±0.01[a]
	O. gratissimum	0.06±0.00[abc]	0.70±0.01[b]	0.36±0.01[b]	6.86±0.43[c]	4.60±0.01[d]	0.12±0.01[a]
	O. americanum	0.09±0.01[d]	0.88±0.01[c]	0.64±0.01[de]	4.13±0.35[a]	1.87±0.01[b]	0.79±0.02[c]

Each value is mean± standard error of three replicates. Values followed by the same letters are not significantly ($p > 0.05$) different from each other using Tukey's Test.