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Research Article

Foliar application of organic liquid nutrient affects selected vitamins and antioxidants in two basils (*Ocimum gratissimum* L. and *Ocimum basilicum* L.)

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ABSTRACT

Study was conducted from December 2021 to June 2022 at the screen house of Osun State University, College of Agriculture to evaluate the influence of foliar application of organic liquid nutrient concentrations on the selected vitamins and antioxidant in two species of *Ocimum*. The experiment was laid out in a Complete Randomized Design (CRD) in ten replications with four treatments of organic liquid nutrient, with varied concentrations (T1: 5 ml/10 liters of water, T2: 10 ml/10 liters of water, T3: 15 ml/10 liters of water and Control: 0 ml). The two species of *Ocimum* leaf seedlings were nursed for five weeks and were transplanted into each pot at the nursery house. Results showed that for the antioxidant components, alkaloid was higher in *O. basilicum* with a value of 3.74% compared to *O. gratissimum* with a value of 3.60% under plants that did not receive organic liquid nutrient. For saponin, both species of *Ocimum* did not differ significantly (P<_0.05). For plants that received 15 ml/ litre of organic nitrogen, *O. basilicum* had 8.81% of flavonoid while *O. gratissimum* had 8.73%. Phenol was also higher in *O. basilicum* than *O. gratissimum*. For vitamins and carotene contents of *Ocimum gratissimum* and *Ocimum basilicum,* 10ml of liquid organic nutrient had a considerable effect on increasing parameters of both basils. Results showed in *Ocimum gratisimum*, carotene (mg/100g) was significantly highest at 0ml of liquid organic nutrient (16.37) and lowest at 5ml of liquid organic nutrient (13.33) compared to *Ocimum basilicum* at 0ml of liquid organic nutrient (13.77). Vitamin C and E was significantly higher in *Ocimum basilicum* at 0ml nutrient concentration (0.07-27.37) compared to *Ocimum gratissimum* at 5ml nutrient concentration (0.027) and 15ml nutrient concentration (20.23) respectively.

Keywords: Antioxidants; *Lamiaceae*; Organic Nutrient; Phytochemical

Introduction

Vegetables are the edible sections of herbaceous plants that are consumed whole or in pieces, raw or cooked, as a side dish or salad; they can be fragrant, bitter, or insipid [1]. Leaves, stalks, roots, flowers, seeds, fruits, and bulbs are among them. Leafy vegetables, on the other hand, are high in macro and micro nutrients, which are important for sustaining a healthy lifestyle. Leafy vegetables are common elements in the ordinary Nigerian's diet and supply significant amounts of nutritious nutrients [2]. Even though they are mostly water, green vegetables are a real natural pharmacy of minerals, vitamins, and phytochemicals [3]. *Ocimum basilicum* is used as spice in local dishes and even earned the name sweet basil. When added to boiling meat, it gives it a very nice smell and adds flavor to the cooked meat [4]. Extract from this plant is useful as pesticides, this plant when planted around human habitation is used to drive snakes away from the neighborhood [5]. *Ocimum gratissimum* is an herbaceous plant of the *Lamiaceae* family [6]. It is widespread in tropical and warm-weather locations [7].

Ocimum gratissimum is cultivated in gardens and the leaf is used for relieving fever [8].

It is germicidal and is widely used in toothpaste, mouthwashes, and topical ointments [9]. It works well as a gargle for sore throats and tonsillitis. It's also utilized as a cough suppressant and expectorant. The plant extract is used to treat animal and human gastrointestinal helminths [10]. According to phytochemical investigations, both aqueous and methanolic extracts of *Ocimum gratissimum* are high in tannins, steroids, terpenoids, flavonoids, and hydrogencyanide, and have a high antioxidant activity [11].

Ocimum gratissimum antioxidant action makes its phytochemicals useful in preventing illness [12]. Phytochemical ingredients define the therapeutic value and diverse biological functions of many plants [13]. The phytochemical and other chemical elements of leafy plants determine their medicinal efficacy [14]. It has anti-inflammatory, anti-bacterial, erythropoietic, anti-cholesterolemic and anti-diabetic effects [15]. However, much like many other polygenic characteristics, these chemical components, including phytochemicals, are altered by environmental influences.

Given the importance of this vegetable in people's lives, it has become vital to research on methods of raising its production to fulfill the rising demand for its products. Soil fertility is one of the key issues limiting agricultural productivity [16]. So far, this has been checked by the administration of plant nutrients to the soil, either organic or inorganic. Organic fertilizers, in addition to releasing nutrients into the soil, improve its physical features, promoting plant growth and development. However, the release of nutrients in organic fertilizers is slower, but longer lasting, as compared to the speeder release of nutrients in inorganic fertilizers, which are frequently lost quickly through leaching in porous soil and high rainfall locations. Since soil boosters will not leave the phytochemicals in the treated plants the same, this study evaluated the influence of different concentration of liquid organic nutrients on selected vitamins and phytochemicals in two basils.

Materials and Methods

Plant Propagation and Experimental Design

Study Area

The experiment was carried out at the screen house of College of Agriculture (Latitude 7°, °N and Longitude 4°, 18'13, 76°E) Osun State University, Nigeria. The pot experiment was set up under temperature range of 25oC to 35oC. The soil type was a well-drained sandy-loam. The experiment was laid out in a two-factorial Complete Randomized Design (CRD) in ten replications with four concentrations (T1: 5ml/10 liters of water, T2: 10ml/10 liters of water, T3: 15ml/10 liters of water, and Control T4: 0ml) of liquid organic nutrient. According to GNLD, 2019, a proven test was done on this product and the table below reflects the minerals present in it.

Table 1. Minerals present in liquid organic nutrient done by GNLD (2019)

Minerals	Quality	Type
Nitrogen	72g per liter	Macro minerals
Phosphorus	45g per liter	Macro minerals
Sulphur	15g per liter	Macro minerals
Calcium	9g per liter	Macro minerals
Magnesium	7g per liter	Macro minerals
Iron	5mg per liter	Micro minerals
Iodine	3mg per liter	Micro minerals
Marine	1mg per liter	Micro minerals
Zinc	1mg per liter	Micro minerals

Collection of Planting Materials

The seeds of the two species of *Ocimum* were sourced from the department of Agronomy, Osun State University, Osogbo, Nigeria, nursed for five weeks and were transplanted into pots at the nursery house. Irrigation was done regularly at the same rate. Liquid organic nutrient at different rates was applied every two weeks.

Laboratory Analysis

The phytochemicals including flavonoids, saponins, alkaloids, tannins and phenols were analyzed. Flavonoid was determined according to the method of [17].

Determination of Flavonoids: This was determined according to the method of [8]. 300gram of the sample was boiled in 150ml of 2M HCL solution for 30mins under reflux. It was allowed to cool and then filtered through Whatman No 42-filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The flavonoids precipitated was recovered by filtration using weighed filter paper.

Determination of Alkaloids: This was also determined according to the method of [8]. 0.5g of the sample was weighed in 96% ethanol and 20% H_2SO_4 (1:1). 1 ml of the filtrate was added to 5 ml of 60% prepared sulphuric acid and allowed to stand for 5 min. Then; 5 ml of 0.5% Formaldehyde of 5 ml was added and allowed to stand for three hours. The absorbance of 565 nm was taken.

Determination of Oxalate: The concentration of Oxalate was carried out using the method reported by [18]; and [19]. Exactly 20 ml of 0.3 M HCl in the sample (2.50 g) was extracted three (3) times by warming at a temperature of 50°C for 1 hour with constant stirring using a magnetic stirrer. For oxalate estimation, 1ml of 5 M ammonium hydroxide was added to 5ml of extract to ensure alkalinity. The addition of 2 drops of phenolphthalein indicator, three drops of glacial acetic acid, and 1ml of 5% calcium chloride to make the mixture acidic before standing for 3 hours was followed by centrifugation at 3000 rpm for 15 minutes. Then 2 ml of 3 M sulphuric acid was added and the precipitate dissolved by warming in a water bath at 70°C. Freshly prepared 0.01 M potassium permanganate ($KMNO_4$) was titrated until a pink color appeared and persisted for at least 30 seconds. The concentration of Oxalate was calculated as：

Oxalate= (Titre value×0.9004) mg/g.

Determination of Saponins: Weighed 0.5g of the samples, 20 ml of 1NHCl, and was added and boiled for four hours. After cooling, it was filtered, and 50 ml of petroleum ether was added and evaporated to dryness. 5 ml of acetone ethanol was added to the residue. 0.4 ml of the sample was taken into three different test tubes. 6 ml of Ferrous sulfate reagent was added, followed by 2 ml of con H2SO4. The absorbance was taken after 10 mins at 490 nm [20].

Determination of Phytate: To 4 g of grounded sample was added 100 ml of 2% HCl. The mixture was constantly shaken for 3 h and filtered. To 25 ml of the filtrate was added 5 ml of 0.3% ammonium thiocyanate (NH_4SCN). Fifty milliliters of distilled water was added to afford the desired acidity. This solution was titrated against a 0.00195 g/ml ferric chloride solution. The end point was characterized by a persistent brownish yellow coloration. Phytate content was estimated from Equation (4) below;

$$\%\text{Phytic acid} = \frac{8.24t \times 100}{1000 \times \text{sample mass}}$$

Where t= titer value.

Determination of Phenols: Defatting of 2g sample was carried out for 2 hours in 100 ml of ether using a soxhlet apparatus. The defatted sample (0.50 g) was boiled for 15 minutes with 50 ml of ether for the extraction of the phenolic components. Exactly 10 ml distilled water, 2 ml of 0.1 N ammonium hydroxide solution, and 5 ml of concentrated amyl alcohol were all added to 5 ml of the extract for 30 minutes for color development. The absorbance was then measured at 505 nm. Standard curve was prepared for the phenol standard and left for 30 minutes to develop color. [21]

Determination of Carotenoids: A measure weight of each sample was homogenized in ethanol using a laboratory blender. A 1:10 (1%) mixture was used. The homogenate was filtered to obtain the initial crude extracts. 20ml of ether was added to the filtrate to take up the carotenoids mixed well and then treated with 20ml of distilled water in a separating funnel. The outer layer was recovered and evaporated to dryness at low temperature (35-50^{0}C) in a vacuum dessicator. The dry extract was then saponified with 20ml of ethanolic potassium hydroxide and left over night in a dark cupboard. The next day, the carotenoid was taken up in 20ml of ether and then washed with two portions of 20ml-distilled water. The carotenoid extract (ether layer) was dried in a dessicator and then treated with a light petroleum (petroleum spirit) and allowed to stand overnight in a freezer (-10^{0}C). The next day the precipitation steroid was removed by centrifugation and the carotenoid extracts was evaporated to dryness in a weighed evaporation dish, cooled in a desiccator and weighed. The weight of carotenoid was determined and expressed as a percentage of the sample weight [22]

Statistical Analysis

Data collected were subjected to one-way analysis of variance (ANOVA) using SPSS, where significant differences were found, the means were separated using Duncan Multiple Range Test (DMRT).

Results

Carotene and Vitamin contents of *Ocimum gratissimum* L. and *Ocimum basilicum* L.

Figure 1, 2 and 3 present vitamins and carotene contents of *Ocimum gratissimum* and *Ocimum basilicum,* means with different superscript on the same row are significantly different except vitamin E. Results showed in *Ocimum gratisimum*, carotene (mg/100g) was significantly highest at 0ml of liquid organic nutrient (16.37) and lowest at 5ml of liquid organic nutrient (13.33) compared to *Ocimum basilicum* at 0ml of liquid organic nutrient (13.77). Vitamin C and E was significantly higher in *Ocimum basilicum* at 0ml nutrient concentration (0.07-27.37) compared to *Ocimum gratissimum* at 5ml nutrient concentration (0.027) and 15ml nutrient concentration (20.23) respectively.

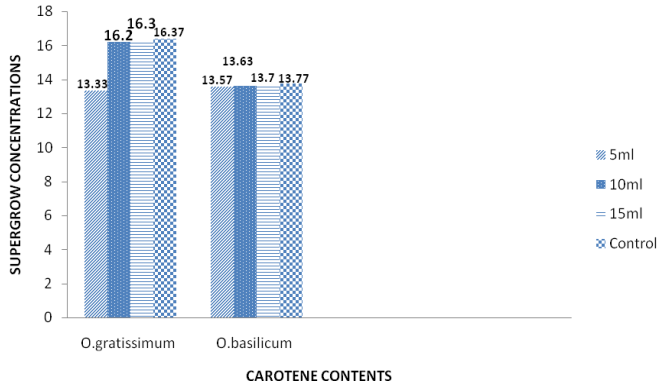


Figure 1. Carotene contents of *Ocimum gratissimum* and *Ocimum basilicum* as influenced by different level of liquid organic nutrient

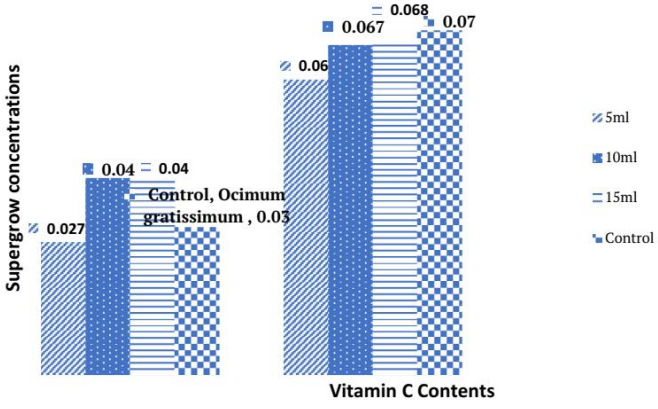


Figure 2. Vitamin C contents of *Ocimum gratissimum* and *Ocimum basilicum* as influenced by different level of liquid organic nutrient

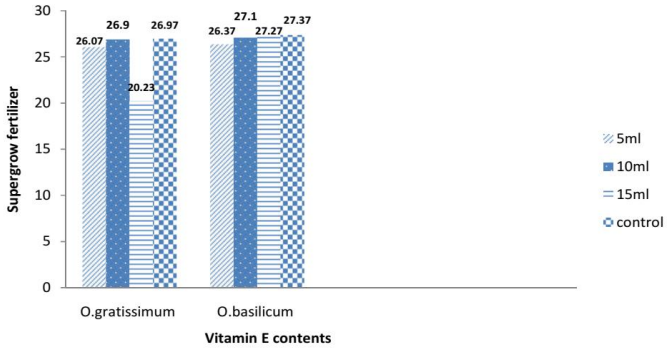


Figure 3. Vitamin E contents of *Ocimum gratissimum* and *Ocimum basilicum* as influenced by different level of liquid organic nutrient

Antioxidant components of Ocimum gratissimum and Ocimum basilicum

Table 2 presents antioxidant components of *Ocimum gratissimum* and *Ocimum basilicum* with means on the same row with different superscript which are significantly different (P<0.05).

Ocimum basilicum showed higher alkaloids content at 0ml of liquid organic nutrient (3.74) and lowest at *Ocimum gratissimum* at 5ml of liquid organic nutrient (3.13). *Ocimum basilicum* at 0ml has the highest saponin content (4.67) compared to *Ocimum gratissimum* at 15 ml of nutrient concentration and lowest at *Ocimum basilicum* with 5ml of liquid organic nutrient concentration (4.20). Tannin was significantly highest at *Ocimum basilicum* at 0ml of liquid organic nutrient with (3.90) and lowest at *Ocimum gratissimum* at 5ml of liquid organic nutrient (3.63).

Flavonoid was significantly highest at *Ocimum basilicum* at 15ml of liquid organic nutrient (8.81) and lowest at *Ocimum gratissimum* at 5ml of liquid organic nutrient with a (8.61). Ocimum basilicum exhibited higher phenol content at 15ml of liquid organic nitrogen (0.77) and lowest at *Ocimum gratissimum* at 5 ml of liquid organic nutrient (0.59).

Table 2. Antioxidant components of *Ocimum gratissimum* and *Ocimum basilicum*

	Ocimum gratissimum L.				*Ocimum basilicum* L.				
Parameters (%)	**5 ml/**	**10 ml**	**15 ml**	**Control**	**5 ml**	**10 ml**	**15 ml**	**Control**	**SEM**
Alkaloids	3.13^{f}	3.47^{d}	3.57^{c}	3.60^{c}	3.30^{e}	3.63^{abc}	3.70^{ab}	3.74^{a}	0.003
Saponins	4.22^{c}	4.47^{b}	4.60^{a}	4.60^{a}	4.20^{c}	4.47^{b}	4.60^{a}	4.67^{a}	0.001
Tannins	3.63^{e}	3.77^{d}	3.80^{cd}	3.84^{bc}	3.80^{bc}	3.87^{a}	3.87^{a}	3.90^{a}	0.001
Flavonoids	8.61^{d}	8.67^{cd}	8.73^{bc}	8.73^{bc}	8.69^{b}	8.70^{b}	8.81^{a}	8.80^{a}	0.001
Phenols	0.59^{e}	0.66^{d}	0.67^{d}	0.67^{d}	0.72^{c}	0.75^{b}	0.77^{a}	0.76^{ab}	0.000

abc Means on the same row with different superscript are significantly different (P<0.05)

Discussion

Vitamin contents of *Ocimum gratissimum* and *Ocimum basilicum*

Vitamin C, also known as ascorbic acid, promotes wound healing; strengthens resistance to infections by boosting the immune system and improves absorption of iron [24]. Vitamin C was significantly highest at *Ocimum basilicum* at 0ml of liquid organic nutrient. Vitamin E was also highest at *Ocimum basilicum* at 0 ml of liquid organic nutrient.

Antioxidant components of *Ocimum gratissimum* and *Ocimum basilicum*

Alkaloids, phenols, tannins, flavonoids, and saponins were detected in both *O. gratissimum* and *O. basilicum*. On the contrast, [12] reported absence of saponin in O. basilicum in their study area. The variation may have been attributed to nutrient source and level as used in this study; saponin was highest at control and 15 ml liquid organic nutrient of *O. basilicum* and *O. gratissimum*. This thus negates the claim by [12]. This result is like that of [16] and [13] who also reported presence of these phytochemicals.

In *Ocimum gratissimum*, alkaloid concentration was highest at control and 15 ml of liquid organic nutrient. Alkaloid concentration in *Ocimum basilicum* was also highest at control. Tannin concentration was highest in *O. gratissimum* and *O. basilicum* at control. Flavonoid was highest at 15 ml of liquid organic nutrient in *O. gratissimum* and *O. basilicum*. Phenols were also highest in *O. gratissimum* and *O. basilicum*.

Observably, O. basilicum had the highest values for all antioxidant components parameters evaluated. This validates study of [17] and [12] who reported that *O basilicum* which are traditionally used in folklore medicine were reported to contain higher to trace amounts of phytochemical. [12] posit that *O. basilicum* crude extracts validate the presence of a wide range of phytochemicals thus indicating the plant's ability to provide the anticipated promising biological response. However, in this study, *O. basilicum* and *O. gratissimum* shared high values for proximate (crude) contents. Hence, it is important to note that only few of these parameters may be liable for improvement of phytochemical in *O. basilicum*.

Conclusion

The leaves of *O. gratissimum* and *O. basilicum* contain an appreciable amount of carotene, vitamin and antioxidants. However, inclusion of liquid organic nutrient at different level had influence on phytochemicals except tanin; 10 ml of liquid organic nutrient had a considerable effect on increasing parameters of both basils. *O. basilicum* had the best antioxidants compared to *O. gratissimum*. Hence, *Ocimum basilicum* is highly recommended for pharmaceutical and nutraceutical industries.

Author Contributions

FMO conceived the research idea and managed all aspects of the manuscript. VMA collected field data and wrote the first draft of the manuscript. GGD did the laboratory analysis. All authors read and approved the final manuscript.

Declaration of Generative AI and AI-Assisted Technologies in Scientific Writing

Nothing to disclose.

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Data Availability Statement

All relevant data are available within the manuscript

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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