



RESEARCH PAPER

## The Effect of *Craseonycteris thonglongyai* (Hog-nosed Bat) Droppings on the Antioxidant Constituents in *Ocimum gratissimum* (Basil) Leaf

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### ABSTRACT

Leafy vegetables are among the major sources of antioxidants that play critical roles in human nutrition and wellbeing of individuals, as they are important in the maintenance and strengthening of immune systems. The concentrations of these health-protecting and maintaining compounds in vegetables, among other environmental factors are influenced by the physicochemical properties of the soils. It is for this reason, the research is designed to investigate the influence of *Craseonycteris thonglongyai* droppings on the improvement of some antioxidants ( $\beta$ -carotene, ascorbic acid, lycopene, chlorophyll, tocopherol, carotenoid, flavonoid and phenol) in the leaf of *Ocimum gratissimum*. The leaves of *O. gratissimum* cultivated with *Craseonycteris thonglongyai* droppings and chemical fertilizer (commercial reference) were harvested at the vegetative phase and subjected to standard analytical methods. The concentrations of tocopherol, lycopene, chlorophyll,  $\beta$ -carotene, total carotenoid, flavonoid and phenol were determined by spectrophotometric method while ascorbic acid content was analysed by titrimetric method. The results showed that the application of *C. thonglongyai* droppings and chemical fertilizer significantly increased ( $p < 0.05$ ) the concentration of  $\beta$ -carotene in *O. gratissimum*, though; the concentration of this parameter was significantly higher ( $p < 0.05$ ) in the vegetable treated with the organic fertilizer. While the concentration of vitamin C in *O. gratissimum* fertilized with *C. thonglongyai* droppings and Chemical fertilizer did not differ significantly from each other, the concentration of the parameter in the vegetable treated with *C. thonglongyai* droppings was significantly higher than the control. Similarly, whereas the application of chemical fertilizer has no significant effect on the concentrations of lycopene, chlorophyll, vitamin E, carotenoid, flavonoid and phenol in *O. gratissimum*, the concentrations of these antioxidants increased significantly with the application of *C. thonglongyai* droppings. The study concludes that the application of *C. thonglongyai* droppings elevated the concentrations of the antioxidants evaluated in the leaves of *Ocimum gratissimum* than conventional chemical fertilizer (commercial reference) and should be the prefer fertilizer in growing the vegetable for enhancement of the antioxidant constituents.

**Keywords:** Soil amendment, *Craseonycteris thonglongyai*, chemical fertilizer *Ocimum gratissimum*,

### INTRODUCTION

Green leafy vegetables are an essential part of our diets as they are the major sources of micronutrients such as vitamins, minerals elements, dietary fibres, antioxidants and some essential amino acids required for the maintenance of good health and prevention of degenerative diseases (Musa and Ogbadoyi, 2014; Musa *et al.*, 2015; Musa *et al.*, 2016 a). Leafy vegetables are succulent edible plant parts that can be eaten as accompanying food or side dishes in the raw state or the cooked form together with staple food (Musa *et al.*, 2016 a). Adequate intake of vegetable has been linked with a lowered prevalence of degenerative diseases in humans (Musa *et al.*, 2015).

*Ocimum gratissimum* (basil) is one of the leafy vegetables consumed as a healthy vegetable in West Africa, as it contains some micronutrients such as carotenoids, vitamins B<sub>1</sub>, B<sub>2</sub>, C and E, and minerals. The dark-green leaves of the vegetable bioaccumulates different proportions of calcium, iron,  $\beta$ -carotene, vitamin C, dietary fibres and protein required for good health (Schippers *et al.*, 2000). The concentrations of these nutrients in *Ocimum gratissimum* like other vegetables depend on the nutrient contents and composition of the soil. *Craseonycteris thonglongyai* droppings are one of the organic fertilizers used to amend the soil in order to

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improve plant growth and development (Emerson and Roark, 2007; Musa, 2016 a). The dung is a highly effective fertilizer due to exceptionally high concentrations of nitrogen, phosphorus and potassium which are essential nutrients for plant growth. *C. thonglongyai* droppings also contain some microorganisms whose activities in the soil help to recondition the soil for proper nutrient uptake by plants (Emerson and Roark, 2007; Musa *et al.*, 2016 a; Musa, 2016 a). Despite the use of the dung for growing vegetables such as *O. gratissimum*, there is a scarcity of information on the upshot of the dung on the concentrations of micronutrients in the vegetable. It is in this context the present research is designed to evaluate the influence of *C. thonglongyai* droppings on the bioaccumulation of some antioxidants (ascorbic acid, tocopherol,  $\beta$ -carotene, carotenoid, lycopene, chlorophyll, flavonoid and phenol) in the leaf of *Ocimum gratissimum*.

## METHODOLOGY

### Study Area

The Pot experiment was carried out in the premise of Department of Biochemistry of Ibrahim Badamasi Babangida University, Lapai, Niger State. Lapai is located in the Southern Guinea Savanna region of Nigeria at latitude 6° 49' N and longitude 6° 41' E. The rainy season occurs between April to October with a mean annual rainfall of 1334 mm, with August and September having the highest rainfall of 300-330 mm. The mean monthly temperature of Lapai is highest in March (30 – 40 °C) and lowest in August (22.3°C).

### Soil Sampling

The soil samples used in the present study were obtained from Ibrahim Badamasi Babangida University, Lapai: Soil samples were collected at a depth (0-20 cm). The samples were bulked, dried, ground and sieved through a 2 mm sieve to remove debris.

### Source and Analysis of *Cratogeomys thonglongyai* Dung

The dried samples of *C. thonglongyai* dung were collected into polythene bags from a Cave in Faso, Edati Local Government Area of Niger State. The physical and chemical properties of the soil and *C. thonglongyai* dung were evaluated according to the method of Bieganowski *et al.* (2015).

### Source of Seeds

The seeds of *Ocimum gratissimum* (basil) were obtained from National Horticultural Research Institute (NIHORT), Ibadan, Oyo State, Nigeria.

### Manure Treatment and Nursery Management

The dried dung of *C. thonglongyai* was ground into powder form and the fine particles of the dung were applied for the cultivation of *O. gratissimum* in a pot experiment at 50 g of the dung per 10 kg soil. While the application of the synthetic fertilizer was done at the recommended dose of 30 mg N/kg soil, 30 mg P<sub>2</sub>O<sub>5</sub>/kg soil and 30 mg K<sub>2</sub>O/kg soil (NIHORT, 1983; Musa, 2010). The control pots were not treated with any manure. Complete Randomized Design (CRD) was adopted using three soil treatments, namely; control (no fertilizer application), synthetic fertilizer application at the recommended dose and 50 g of *C. thonglongyai* droppings per 10.00 kg soil. Each treatment has 10 pots repeated 3 times giving rise to a total of 90 pots for the trial. The plants were irrigated in the mornings and evenings daily except on rainy days in which the pots were not watered. The surrounding and inside the pots were weeded regularly to prevent harbouring of pests. The pots were lifted from time to time to prevent the roots of the plants from growing out of the container (Musa, 2016 b).

### Analysis of Plant Samples

The fresh leaves of *O. gratissimum* grown in the pot experiment were harvested at market maturity and subjected to standard analytical methods to evaluate the antioxidant content in the leaves of *O. gratissimum* amended with synthetic fertilizer and *C. thonglongyai* droppings.

### Antioxidant Contents

The concentration of ascorbic acid in the samples was determined by the 2, 6- dichlorophenol indophenols titrimetric method of Jones and Hughes (1983).  $\beta$ -carotene content was evaluated by ethanol and petroleum ether method as described by Musa *et al.* (2010). Similarly, aluminium chloride colourimetric method was used for the determination of flavonoid (Chang *et al.*, 2002) and total phenol content was assayed according to Singleton *et al.* (1999).  $\alpha$ -tocopherol content in the samples was determined by the Emmerie-Engel reaction as reported by Rosenberg (1992) while total carotenoids and lycopene in the samples was

analysed by Zakaria *et al.* (1979). Likewise, the chlorophyll content in the leaves of the vegetable was evaluated according to the method of Branisa *et al.* (2014).

### Statistical Analysis

Analysis of variance (ANOVA) was carried out using SAS statistical package to determine the variation between the application of *C. thonglongyai* dung and chemical fertilizer on concentrations of antioxidants in *O. gratissimum*. Mean values were separated using Duncan's Multiple Range Test (DMRT) and the significance was tested at  $P < 0.05$ .

## RESULTS

### Physical and Chemical Properties of Soil

The result of the investigation of the soil used for the pot experiment is shown in Table 1. The pH of the soil which is 5.8 signifies that the soil is strongly acidic. The calcium and organic carbon are low, whereas available phosphorus and magnesium are moderate. Whereas the concentrations of sodium and potassium are very high, the total nitrogen content is medium.

Table 1: Physical and chemical properties of the soil (0 – 20 cm depth) used for the experiment

Parameters	Values
Sand (%)	84.50 ± 0.31
Silt (%)	6.58 ± 0.02
Clay (%)	8.92 ± 0.24
Textural class	Sand
pH (H <sub>2</sub> O)	5.80 ± 0.05
pH (CaCl <sub>2</sub> )	7.33 ± 0.10
Organic carbon (g kg <sup>-1</sup> )	4.49 ± 0.04
Total nitrogen (g kg <sup>-1</sup> )	1.74 ± 0.01
Available phosphorus (mg kg <sup>-1</sup> )	16.30 ± 1.00
Na <sup>+</sup> (cmol kg <sup>-1</sup> )	3.73 ± 0.07
K <sup>+</sup> (cmol kg <sup>-1</sup> )	0.03 ± 0.01
Mg <sup>2+</sup> (cmol kg <sup>-1</sup> )	1.30 ± 0.02
Ca <sup>2+</sup> (cmol kg <sup>-1</sup> )	3.81 ± 0.20
Acidity (cmol kg <sup>-1</sup> )	0.63 ± 0.02
CEC (cmol kg <sup>-1</sup> )	9.50 ± 0.21
EC (cmol kg <sup>-1</sup> )	8.87 ± 0.32
Base saturation (%)	93.37 ± 4.27

CEC = Cation exchange capacity, EC = Exchangeable cations. Values represent Mean ± SEM of triplicate determinations. SEM = Standard error mean.

### Chemical Properties of *C. thonglongyai* Droppings

The chemical properties of *C. thonglongyai* droppings are presented in Table 2. The results showed that the available phosphorus, organic carbon, total nitrogen, potassium and sodium of the droppings are high. Whereas the concentration of magnesium in the dropping is high, the calcium content is low. The pH of the droppings which is 7.56 indicates that it is slightly basic.

Table 2: Chemical properties of the *C. thonglongyai* droppings

Parameters	Values
pH (H <sub>2</sub> O)	7.56 ± 0.14
Organic carbon (g kg <sup>-1</sup> )	37.10 ± 2.01
Total nitrogen (g kg <sup>-1</sup> )	7.95 ± 0.12
Available phosphorus (mg kg <sup>-1</sup> )	8752.0 ± 43.11
Na <sup>+</sup> (cmol kg <sup>-1</sup> )	3.30 ± 0.04
K <sup>+</sup> (cmol kg <sup>-1</sup> )	11.00 ± 0.50
Mg <sup>2+</sup> (cmol kg <sup>-1</sup> )	3.80 ± 0.21
Ca <sup>2+</sup> (cmol kg <sup>-1</sup> )	2.79 ± 0.32

Values represent Mean ± SEM of triplicate determinations. SEM = Standard error mean.

### Antioxidant Contents

Analysis of the influences of *C. thonglongyai* droppings and chemical fertilizer on the concentrations of some antioxidants in *O. gratissimum* is presented in Table 3. The results showed that the application of chemical fertilizer and *C. thonglongyai* droppings significantly increased ( $p < 0.05$ ) the concentration of  $\beta$ -carotene in *O. gratissimum*, though; the concentration of this parameter was significantly higher ( $p < 0.05$ ) in the vegetable treated with the organic fertilizer. The mean values of 779.78 ± 23.84, 1650.00 ± 377.75 and 1015.24 ± 127.50  $\mu\text{g}/100\text{ g}$  of  $\beta$ -carotene were recorded in the control, vegetable treated with *C. thonglongyai* droppings and Chemical fertilizer, respectively. While the concentration of vitamin C in *O. gratissimum* fertilized with *C. thonglongyai* droppings and Chemical fertilizer did not differ significantly from each other, the concentration of this parameter in the vegetable treated with *C. thonglongyai* droppings was significantly higher than the control. The mean values recorded for control, vegetable treated with *C. thonglongyai* droppings and chemical fertilizer were 50.60 ±

3.16,  $61.67 \pm 2.74$  and  $56.93 \pm 4.75$  mg/100 g, respectively.

Similarly, whereas the application of chemical fertilizer has no significant effect on the concentrations of lycopene, chlorophyll, vitamin

E, carotenoid, flavonoid and phenol in *O. gratissimum*, the concentration of these antioxidants increased significantly with the application of *C. thonglongyai* droppings (Table 3).

Table 3: Effect of *C. thonglongyai* droppings and Chemical fertilizer on the concentrations of antioxidants in the leaf of *O. gratissimum*

Antioxidants	Control (No application)	<i>C. thonglongyai</i> droppings	Chemical fertilizer
$\beta$ -carotene ( $\mu$ g/100 g)	$779.78 \pm 23.84^a$	$1650.00 \pm 377.75^c$	$1015.24 \pm 127.50^b$
Vitamin C (mg/100g)	$50.60 \pm 3.16^a$	$61.67 \pm 2.74^b$	$56.93 \pm 4.75^{ab}$
Lycopene (mg/g)	$1229.70 \pm 314.89^a$	$1445.80 \pm 195.20^b$	$1036.97 \pm 72.16^a$
Chlorophyll (mg/100g)	$19.15 \pm 3.83^a$	$46.78 \pm 4.78^b$	$21.08 \pm 3.33^a$
Vitamin E (mg/100g)	$39.93 \pm 26.94^a$	$58.60 \pm 0.35^b$	$43.42 \pm 14.57^a$
Carotenoid (mg/g)	$15798.40 \pm 44.17^a$	$24204.53 \pm 0.35^b$	$16016.53 \pm 66.87^a$
Flavonoid (mg/100g)	$3.30 \pm 0.17^a$	$7.70 \pm 0.81^b$	$4.43 \pm 0.38^a$
Phenol (mg/100g)	$1.07 \pm 0.62^a$	$6.55 \pm 0.03^b$	$1.60 \pm 0.92^a$

Mean  $\pm$  Standard errors on the same row with different superscripts are significantly different ( $p \leq 0.05$ )

## DISCUSSION

The soil textural class used for the study is sand signifying that it has poor-water holding capacity and poor nutrients while water-infiltration capacity, aeration and workability are good. The cation exchange capacity (CEC) is low whereas the base saturation is very high (FAO, 2006). The low concentrations of some essential soil nutrient in the soil used for the research, mainly the mineral elements may be attributed to low organic matter constituents of the soil since it is well known that organic matter diminish with continuous land use for crops production, giving rise to low concentration of mineral elements in the soil. The low concentrations of these essential plant nutrients required for growth and development justify why it is vital to modify the soil with sufficient manure to improve soil nutrient content and composition that will boost plant growth and yield (Abu *et al.*, 2020; Musa *et al.*, 2022 a, Musa *et al.*, 2022 b). The pH of the dung used for the soil amendment is slightly basic (FAO, 2006). Correspondingly, the high contents of nitrogen, phosphorus and potassium in the droppings of *C. thonglongyai*, which are among the crucial nutrients of the soils needed by plants for optimum performance give good reason for the use of the droppings by crop growers for modifying the soil to improve the nutrient constituents of the soil that would

support the growth and development of plants resulting in improved yields (Abu *et al.*, 2020; Musa *et al.*, 2022 a).

The significantly higher concentration of  $\beta$ -carotene in the leaves of *O. gratissimum* cultivated with both chemical fertilizer and *C. thonglongyai* droppings compared to control put forward that soil amendment with both fertilizers increased nutrient contents of the soil which ultimately enhances the synthesis of this powerful antioxidant that is required for vision, healthy skin and mucous membrane, bone and tooth growth and boosting of the immune system (George, 1999; Musa *et al.*, 2019; Musa, 2021). The higher concentrations of the  $\beta$ -carotene, vitamin C, lycopene, chlorophyll, vitamin E, carotenoid, flavonoid and phenol in the leaf *O. gratissimum* with the application *C. thonglongyai* droppings and the insignificant effect of chemical fertilizer on these antioxidants except,  $\beta$ -carotene in the vegetable corroborates with the finding of the previous researchers (Makinde *et al.*, 2010; Funda *et al.*, 2011; Musa, 2016 b; Musa *et al.*, 2019; Musa, 2021) who reported high nutrient contents in crops grown with organic manures compared to inorganic fertilizer. (Makinde *et al.*, 2010) further stressed that nutrients in organic material are released slowly and progressively, since the materials

have to be decaying and organic nutrients mineralized unlike nutrients in chemical fertilizers which are readily lost by leaching and this could be responsible for the lower antioxidant contents in the vegetable cultivated with chemical fertilizer compared with the organic manure. It is well documented that organic manures have microorganisms whose activities in the soil result in the mineralization of organic matter in the soil and improve nutrient availability for plant uptake (Musa, 2016 b; Musa et al., 2019). Organic fertilizers are also well-known to activate numerous species of living organisms in the soil to release some chemical messengers that may stimulate plant growth and nutrient formation and such organisms need nitrogen for multiplication (Adekayode, 2004; Ouda and Mahadeen, 2008). The higher concentrations of the antioxidants in the leaves of *O. gratissimum* treated with the *C. thonglongyai* droppings compared to chemical fertilizer further give credence to the preference of organic foods as health-promoting foods over conventional foods as the high concentrations of antioxidants in them help to prevent degenerative diseases linked with aging (Musa et al., 2019; Musa, 2021).

### Conclusion

The present study concludes that soil amendment with *C. thonglongyai* droppings significantly improves the accumulation of antioxidants in the leaf of *O. gratissimum* when compared with the chemical fertilizer (commercial reference). Thus suggesting that growing the vegetable with *C. thonglongyai* droppings will help in reducing the incidence of degenerative and age-related diseases orchestrated by the generation of free radicals.

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