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Improving Phytonutrient Constituents in the Leaf of *Cnidoscolus aconitifolius* (Tree Spinach) Through Soil Amendment with *Craseonycteris thonglongyai* Dung

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ABSTRACT: Generally, leafy vegetables form an integral part of our diets as they are among the major sources of phytonutrients and mineral elements required for development, maintenance of good health and prevention of degenerative diseases. The concentrations of these nutrients in vegetables depend on the physical and chemical properties of the soil where they were grown. It is for this reason that pot experiment was conducted to investigate the impact of graded levels of *Craseonycteris thonglongyai* dung on the concentrations of some phytotonutrients in the leaves of *Cnidoscolus aconitifolius*. The different levels of *C. thonglongyai* dung used were control (0 g), 25, 50, 75, 100 and 125 g per 20 kg soil. The leaves of *C. aconitifolius* were harvested at vegetative phase and subjected to standard analytical methods. The concentrations of ascorbic acid, was evaluated with titrimetric method, while concentrations of β-carotene, carotenoid, lycopene, chlorophyll, tocopherol were determined using spectrophotometric method. The concentration of tocopherol was not detected in the leaves of *C. aconitifolius* treated with different levels of *C. thonglongyai* dung. While moderate application of the dung significantly ($p < 0.05$) elevated the concentrations of carotenoid, β-carotene, ascorbic acid, chlorophyll and lycopene in the vegetable, the application of higher concentration of the dung particularly from 75 to 125 g per 20 kg soil decreased the concentrations of these phytonutrients significantly ($p < 0.05$). The study concludes that moderate application of *C. thonglongyai* dung particularly that of 50 g per 20 kg soil improves the nutritional quality of *C. aconitifolius*, as this quantity of the dung increased the bioaccumulations of the phytonutrients evaluated.

Keywords: Soil amendment, *Cnidoscolus aconitifolius*, Phytonutrients, *Craseonycteris thonglongyai* dung.

Introduction

Leafy vegetables are among the major sources of phytonutrients required for the maintenance of good health and prevention of degenerative and other age-related diseases. Some of these phytonutrients, which are also antioxidants and are abundant in leafy vegetables, include carotenoid, β-carotene, ascorbic acid, tocopherol, chlorophyll, lycopene, total phenol and flavonoid (Aliyu and Morufu, 2006: Musa, 2016). These substances when present at low concentrations, compared to those of oxidizable substrates, significantly delay or prevent the oxidation substrates (Ogbiko *et al*., 2017). Decrease in intake of nutritionally, and antioxidant food such as vegetables may increase the chances of oxidative stress which may lead to cell damage and diseases (Hossain *et al*., 2017).

There is a growing concern for a better and an improved value of health system that will translate good life due to an observable upsurge of diseases. Adequate intake of leafy vegetables and fruits has been linked to decrease in the risk of developing chronic diseases such as neurodegenerative diseases and cardiovascular diseases (Hossain *et al*., 2017; Liu *et al*., 2018; Musa *et al*., 2020; Musa, 2021). This is due to their phytonutrients

content with antioxidant potentials. The intake of natural antioxidants through diet, may provide protective effect against free radical induced diseases. These compounds are capable of preventing or attenuating tissue damages such as lipid peroxidation, oxidative damage to membranes, and inactivation of enzymes caused by free radicals. Nutrients and dietary components with antioxidant properties are important for protection against oxidative stress injury to the body by helping endogenous antioxidants to neutralize excess free radicals (Liu *et al*., 2018). Hence, there is increase in demand for healthy foods by the populace. Therefore, provision of healthy and nutrient rich foods that can improve overall health of the society are very critical, which has further increased the demand for plant-based foods. Therefore, the purpose of this research was to evaluate the influence of different levels *C. thonglongyai* dung on the improvement of the following phytonutrients; carotenoid, β-carotene, ascorbic acid, tocopherol, chlorophyll, lycopene, total phenol and flavonoid in the leaves of *Cnidoscolus aconitifolius*. These phytonutrients which are also known as dietary free radical scavengers play critical roles in human nutrition that they are important in sustenance of life and the help to prevent of both infectious and degenerative diseases (Musa *et al*., 2020; Musa, 2021).

Materials and methods

Study area: Pot experiment was conducted in the Department of Biochemistry, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria. Lapai is positioned in the Southern Guinea Savanna region, on latitude 8° 49'N and longitude 6° 41'E. The wet season of Lapai happens between April and October with mean annual rainfall of 1334 mm. The highest rainfall of between 300 and 330 mm frequently occurred in August and September, while the highest average monthly temperature of the study areas (30-40 °C) is usually in March and the minimum (22.3 °C) in August.
Soil sampling and analysis: The superficial (0 – 20 cm depth) soil sample was obtained from 3 different locations at the main campus of Ibrahim Badamasi Babangida University, Lapai, Nigeia. The soil was mixed and sieved to remove any wreckage. The physical and chemical properties of the soil and *C. thonglongyai* dung were analyzed according to the method of Bieganowski *et al.* (2015).
Source of Cnidoscolus aconitifolius cuttings and Craseonycteris thonglongyai dung: The dungs of *C. thonglongyai*, which the local farmers used as one of the organic fertilizers, were collected into a polythene sack from the colony of the animal in a cave in Faso village of Edati Local Government Area of Niger State. The cuttings of *C. aconitifolius* were obtained from Teaching and Research Farm of Faculty of Agriculture, Ibrahim Badamasi Babangida University Lapai, Niger State.
Manure treatment and application: The droppings of *C. thonglongyai* were air-dried, pulverized into fine particles and applied to cultivate *C. aconitifolius* in pot experiment at six different levels, which were control (No application), 25, 50, 75, 100 and 125 g per 20 kg soil.
Planting, experimental design and nursery management: Two cuttings of *C. aconitifolius* were planted in 20 kg bag of soil containing different levels of *C. thonglongyai* dung and thinned to one plant per pot after sprouting. Completely Randomized Design (CRD) was used for the six treatments. Each treatment had 10 pots replicated 3 times making total of 180 pots for the experiment. The plants were watered twice daily (morning and evening) using watering can except on rainy days in which the pots were not irrigated. The surrounding was kept clean regularly to avoid pest and the pots were lifted from time to time to avoid the roots of the plants from growing out of the pot.
Analysis of plant samples: The freshly harvested leaves of *C. aconitifolius* grown in the pot experiment were subjected to standard analytical procedure at market maturity to evaluate the phytonutrients content in the leaves of the vegetable amended with different levels of *C. thonglongyai* dung.
The concentration of ascorbic acid in the sample was determined by 2, 6- dichlorophenol indophenols titrimetric method of Jones and Hughes (1983). B-carotene content was evaluated by ethanol and petroleum ether method as described by Musa *et al*. (2010). Similarly, α-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 were evaluated by Zakaria *et al*. (1979). Likewise, the chlorophyll content in the leaves of the vegetable was determined according to the method reported by Branisa *et al*. (2014).
Statistical analysis: Analysis of variance (ANOVA) was done using SPSS statistical package (version 26.0 of 2016) to evaluate the influence of graded levels of *C. thonglongyai* manure on the bioaccumulation of phytonutrients in the leaves of *C. aconitifolius*. Duncan's Multiple Range Test (DMRT) was used for comparison of the means at $p < 0.05$.

Results

Physicochemical properties of soil: Result of the analysis of the soil used for pot experiment is presented in Table 1. The pH of the soil which is 5.6 signifies that the soil is strongly acidic. The calcium and organic carbon are low, whereas available phosphorus and magnesium are moderate. While the concentrations of sodium and potassium are very high, the total nitrogen content is medium.

Table 1: Physicochemical properties of the soil (0 – 20 cm depth) used for pot experiment

Parameters	Values
Sand (%)	85.40 ±0.23
Silt (%)	6.70 ±0.03
Clay (%)	7.90 ± 0.15
Textural class	Sand
pH (H_2O)	5.60 ±0.07
pH ($CaCl_2$)	7.35 ± 0.20
Organic carbon (g kg^{-1})	4.57 ± 0.03
Total nitrogen (g kg^{-1})	1.82 ± 0.01
Available phosphorus (mg kg^{-1})	16.30 ± 1.00
Na^+ (cmol kg^{-1})	3.70 ± 0.09
K^+ (cmol kg^{-1})	0.02 ± 0.01
Mg^{2+} (cmol kg^{-1})	1.29 ±0.02
Ca^{2+} (cmol kg^{-1})	3.72 ± 0.21
Acidity (cmol kg^{-1})	0.60 ± 0.01
CEC (cmol kg^{-1})	9.33 ± 0.21
EC (cmol kg^{-1})	8.73 ± 0.32
Base saturation (%)	93.56 ± 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 compost: The chemical properties of *C. thonglongyai* dung are shown in Table 2. The total nitrogen, organic carbon, available phosphorus, sodium and potassium of the dung are high. While the magnesium content of the dung is high, the calcium content is low.

Table 2: Chemical properties of the *Craseonycteris thonglongyai* dung

Parameters	Values
pH (H_2O)	7.54 ± 0.15
Organic carbon (g kg^{-1})	36.20 ± 2.03
Total nitrogen (g kg^{-1})	7.90 ± 0.13
Available phosphorus (mg kg^{-1})	8741.0 ± 45.10
Na^+ (cmol kg^{-1})	3.20 ± 0.05
K^+ (cmol kg^{-1})	12.00 ± 0.40
Mg^{2+} (cmol kg^{-1})	3.79 ± 0.23
Ca^{2+} (cmol kg^{-1})	2.84 ±0.22

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

Effect of graded levels of C. thonglongyai dung on the phytonutrient contents: The determination of effect of graded levels of *C. thonglongyai* dung on the concentrations of phytonutrients in the leaves of *C. aconitifolius* showed that the chlorophyll content increased significantly ($p < 0.05$) with application of the dung. However, the concentrations of chlorophyll in the vegetable treated with 50, 75 and 125 g were significantly ($p < 0.05$) higher when compared with vegetable treated with 25 g. The mean value of chlorophyll in control, 25, 50, 75, 100 and 125 g per 20 kg soil were 0.17 ± 0.02, 0.18 ± 0.04, 0.31 ± 0.01, 0.30 ± 0.04, 0.30 ± 0.01 and 0.34 ± 0.02 mg/g, respectively. While treatment with 25 g of *C. thonglongyai* dung had no significant effect on the β-carotene content in the vegetable, the concentration of this parameter increased significantly ($p < 0.05$) with 50 g of the dung and decreased significantly when the vegetable was fertilized with 75 to 125 g of the dung. The mean values of 25602.92 ± 21.49, 30331.39 ±40.86, 60045.22 ± 59.04, 17627.78 ± 10 .86, 17004.90 ± 20.67 and 16109.90 ± 60.67 µg/100 g were recorded for control, 25, 50, 75, 100 and 125 g, respectively. The

concentration of tocopherol in the leaves of *C. aconitifolius* treated with different levels of *C. thonglongyai* dung was not detected with the analytical method used for the evaluation.
Whereas application of 25 g of *C. thonglongyai* dung had no significant effect on the concentration of ascorbic acid in *C. aconitifolius*, the application of 50 to 125 g of the dung significantly ($p < 0.05$) decreased the ascorbic acid content in the vegetable. Although, the concentration of ascorbic in the vegetable treated with 50, 75,100 and 125 g of the dung were not significantly different from each other. Similarly, while treatment with 50 g of the dung significantly ($p < 0.05$) elevated the concentration of carotenoid in *C. aconitifolius*, fertilization with 75, 100 and 125 g of the dung decreased its content whereas treatment 25 g of *C. thonglongyai* dung had no significant effect on the carotenoid content of the vegetable. Lycopene content increased significantly ($p < 0.05$) with increase in application of *C. thonglogyai* dung in *C. aconitifolius* except that the concentration of the parameter decreases with application of 125 g of the dung (Table 3).

Table 3: Effect of graded levels of *Craseonycteris thonglongyai* dung on the concentrations of phytonutrients in *Cnidoscolus aconitifolius*

Phytonutrients	**Graded levels of *Craseonycteris thonglongyai* dung (g)**					
	0 (Control)	**25**	**50**	**75**	**100**	**125**
Chlorophyll (mg/g)	0.17 ± 0.02^{a}	0.18 ± 0.04^{a}	0.31 ± 0.01^{b}	0.30 ± 0.04^{b}	0.30 ± 0.01^{b}	0.34 ± 0.02^{b}
β-Carotene (µg/100g)	25602.92 ± 21.49^{b}	30331.39 ± 40.86^{b}	60045.22 ± 59.04^{c}	17627.78 ± 10.86^{a}	17004.90 ± 20.67^{a}	16109.90 ± 60.67^{a}
Tocopherol (µg/100 g)	ND	ND	ND	ND	ND	ND
Vitamin C (mg/100g)	13.05 ± 0.97^{b}	12.00 ± 1.48^{b}	9.83 ± 01.39^{a}	8.95 ± 0.98^{a}	7.53 ± 0.21^{a}	7.15 ± 0.27^{a}
Carotenoid (mg/g)	5413.52 ± 56.34^{b}	5336.32 ± 16.46^{b}	8492.59 ± 25.13^{c}	3477.28 ± 69.95^{a}	3247.95 ± 48.85^{a}	2897.87 ± 47.01^{a}
Lycopene (mg/g)	275.04 ± 59.73^{a}	405.20 ± 79.13^{b}	1072.13 ± 64.82^{c}	854.75 ± 40.63^{c}	393.95 ± 12.30^{b}	348.40 ± 12.30^{ab}

ND = Not detectable. Row mean values carrying the same superscript are not significantly different from each other ($p > 0.05$)

Discussion

The soil textural class used for the pot experiment is sand indicating that it has poor-water holding capacity and poor nutrients while water-infiltration capacity, aeration and workability is good. The cation exchange capacity (CEC) is low whereas the base saturation is very high (FAO, 2016). The observed low contents of some vital soil nutrient in the soil utilized for the research, mainly the minerals may be attributed to low organic matter constituents of the soil since it is well known that organic matter diminish with continuous land use giving rise to low concentration of mineral element in the soil. The low levels of these essential plant nutrients required for growth and development give credence why it is imperative to amend the soil with sufficient manure to improve soil nutrient content and composition that will enhance plant growth and yield (Abu *et al*., 2020; Musa *et al*., 2022). The pH of the dung used for the soil amendment is slightly basic (FAO, 2016). Correspondingly, the high contents of nitrogen, phosphorus and potassium in the dung 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 dung 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).
Ascorbic acid (vitamin C) supports synthesis of hormone, iron absorption and collagen, which is extensively used in cosmetics and pharmaceuticals. It acts as water soluble antioxidant, which plays a major role in free radical scavenging activity and activates immune system (Locato *et al*., 2013). The significant decrease in ascorbic acid content in the leaves of *C. aconitifolius* treated with different levels of *C. thonglongyai* dung in the present study is in agreement with the submission of Musa *et al.* (2010) and Virginia (2001) who reported similar observation in their study of effect of nitrogen fertilizer on the vitamin C content in the vegetables. They observed that application of nitrogen fertilizer increases the proteins and decrease carbohydrates content and that since vitamin C synthesis is derived from carbohydrates, its synthesis is also decreased. This observation could be correct, since the dung of *C. thonglongyai* is very rich is nitrogen and increase application of the dung will overload the soil with excessive nitrogen that will enhance the synthesis of protein over carbohydrate and

thus decrease the formation of ascorbic acid. Therefore, in order to optimize the nutritional benefit from the use of the dung in the cultivation of vegetable, moderate application of the dung is required (Musa, 2021).

The increase in β-carotene content in the leaves of the vegetable treated with *C. thonglogyai* dung could be as a result of elevation in the content and activity of chlorophyll and associated light absorbing pigments, including caroteniods (Havling *et al.*, 2006; Musa, 2010). Previous study revealed that increase in potassium content enhance the concentration of β-carotene in vegetable leaves since the mineral element speeds up the rate of acetic thiokinase activity for catalysis the condensation of acetyl CoA units which form the initial steps in the biosynthesis of carotenoids (Fanasca *et al.*, 2006). Therefore, the increase in the concentration of carotenoids with application of the dung may be attributed to the high content of potassium in the dung which helps in the activation of acetic thiokinase activity, one of the key enzymes in the biosynthetic pathway of carotenoids (Tjhia *et al*., 2018). Similarly, previous reports has shown that application of organic manure rich in phosphorus increase the concentrations of β-carotene and carotenoids in vegetables due to the action of phosphorus on enzymes such phosphofructokinase, pyruvate kinase and precursors of pyruvate which has been linked with the biosynthesis of carotenoid (Black *et al*., 2008; Meysam *et al.*, 2017; Aina *et al*., 2018).

The observed highest concentration of carotenoid and lycopene in the leaves of *C. acontififolius* fertilized with 50 g of *C. thonglogyai* dung further strengthening the submission by Musa (2021) that moderate application of the dung is required to improve the nutrient and antioxidants content in the vegetables. Musa *et al*. (2020) put forward that application of both *C. thonglongyai* compost increased the nutrient contents and compositions of the soil which ultimately improves nutrients availability and their uptake by plant. This leads to proper plant growth and development which in turn increases the formation of nutrients, antioxidants and other plant pigments including phytochemicals which are harnessed by plants to fulfilled ecological purposes and protect themselves from predators and pathogens (Musa *et al*., 2019).

Result of this study clearly demonstrated that increase in the quantity of the *C. thonglongyai* dung increases the concentration of chlorophyll content in the leaves of *C. aconitifolius*. Physiologically, the colours of *C. aconitifolius* leaves treated different levels of *C. thonglongyai* dung become more greenish compared with the control, as a result of increased in the content of the chlorophyll. This finding corroborates with the submission of by El-Arwy *et al*. (2016) who reported higher chlorophyll-a concentrations following the application of fertilizer which enhanced the formation of the pigments. This may be due to the fact that nitrogen is one of the structural components of chlorophyll and it is also an essential nutrient required by plants for proper growth and development (Bannari *et al.*, 2007; Marschner, 2012). It is worth to note that this essential nutrient for plant is very high in the droppings used in the cultivation of *Vernonia amygadalina* and would have been responsible for this observation. Similar report was made by El-Arwy *et al.* (2016) to the effect that organic fertilizer increases the concentration of plant pigments including chlorophyll content, which is a key indicator for crop growth and development, therefore accurately determining and assessing of chlorophyll concentration is essential (Bannari *et al.*, 2007).

Conclusion

The current study reveals that application of various levels of *C. thonglongayai* dung improve the bioaccumulation of phytonutrients in the leaves of *C. aconitifolius*. The study suggest that moderate application the dung, particularly that of 50 g per 20 kg soil being the preferred one in improving the nutritional quality of *C. aconitifolius*, as this quantity of the dung increased the bioaccumulations of phytonutrient constituents evaluated in the vegetable.

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