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Evaluation of Soil Amended with Graded Levels of *Craseonycteris thonglongyai* Dung on the Improvement of Mineral Element Constituents in the Leaf of *Cnidoscolus aconitifolius* (Tree Spinach)

Mary L. Abu, Abdulwasiu B. Lawal, Abduljelili Uthman, Amanabo Musa*

Department of Biochemistry, Faculty of Natural Sciences, Ibrahim Badamasi Babangida University, P. M. B. 11, Lapai, Niger State, Nigeria

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ABSTRACT

Mineral elements are among the micronutrients required for maintenance of good health and general well-being of individual as they play critical roles in enhancing immune system against infectious diseases. The concentrations of minerals in plant among other factors depend on the nutrient contents and compositions of soil. It is for this reason pot experiment was conducted to investigate the influence of graded levels of *Craseonycteris thonglongyai* dung on the concentrations of Fe, Mg, Zn, Ca, Mn, Cu, Na and K) in *Cnidoscolus aconitifolius*. The different levels of *C. thonglongyai* dung used were control (0 g), 25, 75, 100 and 125 per 20 kg soil. The leaves of *C. aconitifolius* were analysed at market maturirty for its mineral contents using Atomic Absorption Spectrophotometer (AAS) except that K and Na were analysed with Flame Photometer. The application of *C. thonglongyai* dung significantly ($p < 0.05$) increased the concentrations of Fe, Mg, Cu, Mn, Ca, Zn and K in the leaf of *C. aconitifolius* with increase application of the dung. Whereas treatment with the dung above 50 g significantly ($p<0.05$) decreased Fe, Cu and Zn contents, the concentrations of Mg, Ca and K did not differ significantly ($P>0.05$) with increasing concentration of the dung to 125 g. Similarly, application of different levels of the dung had no significant ($P > 0.05$) effect on Na in *C. aconitifolius*. The study concludes that moderate application of *C. thonglongyai* dung (particularly 50 g per 20 kg soil) enhance bioaccumulation of mineral contents in *Cnidoscolus aconitifolius*.

Keywords: Soil, *Craseonycteris thonglongyai* dung, *Cnidoscolus aconitifolius*, Dung analysis, Mineral elements.

Introduction

Cnidoscolus aconitifolius, commonly known as tree spinach, is a large, fast-growing leafy perennial shrub that is believed to have originated in the Yucatan Peninsula of Mexico. The vegetable is a hardy plant that bears numerous deep green leaves and resembles spinach. But it has far more nutritional value compared to spinach. The young shoots and tender leaves of tree spinach are cooked and eaten like spinach. The leaves can be added to salads, soups, or used as a tea. The leaves of the tree spinach can be only consumed cooked or boiled but never consume it raw because of the high content of toxic hydrocyanic acid.[1, 2]

C. aconitifolius comprise part of the staple diet and are the main dietary source of leafy vegetable for the indigenous people of Yucatan peninsula of Mexico.[3] It has succulent stems which exude a milky sap when cut. It can grow to be 6 meters tall, but is usually pruned to about 2 m for easier leaf harvest. With current renewal of interest in household gardens, attention is being focused on promoting *C. aconitifolius*, as leafy green vegetables among populations in the developing countries.[4] The edible parts of *C. aconitifolius*, which taste like spinach when cooked, provide important nutritional sources for protein, vitamins and mineral elements.[5] The leaves of the vegetables are broad and may consist of 3 or more lobes with fleshy petioles.[2] They contain high content of toxic hydrocyanic acid and therefore, should be cooked for at least one minute to destroy the acid before consumption of the vegetable.[1, 6]

C. aconitifolius is easy to cultivate and can tolerates heavy rain and has some drought tolerance. The stem cuttings about 6-12 inches long is usually used for the cultivation of the vegetable. The cutting is planted in 4-5" of soil, either in pots, or directly in the ground. The plant does well in compost or green manure soil. Growth of the plant is rapid and edible leaves and shoots could be produced within a short period (8 to 10 weeks). The leaves of *C. aconitifolius* can be harvested endlessly provided adequate care taken to guarantees healthy new plant growth.[6]

The leaves of *C. aconitifolius* are consumed for its nutritional and health enhancing potential due to the presence of vitamins, antioxidants and mineral elements which are crucial in normal metabolic activity in the body. Generally, the nutrient contents of vegetables such as *C. aconitifolius* besides other environmental factors, depend on the soil nutrients, physicochemical and biological activities of the soil.[7]

The dung of *Craseonycteris thonglongyai* is one of animal manures used for amendment of soil to increase the growth and improvement of plant. It is extremely effective manure as it has high concentrations of nitrogen, phosphorus and potassium which are the major vital nutrients required for plant growth and development. The dung also harbours some microorganisms which are actively involved in reconditioning the soil for appropriate nutrients absorption by plant 8. In spite of the use of *C. thonglongyai* dung for the production of vegetables such as *C. aconitifolius*, there is no information on the

*Corresponding author. E mail: musaamanabo@gmail.com
Tel: +234 81 45500676; 08186892655

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influence of the dung on the nutrient contents in the vegetable. It is in this context the present study was designed to evaluate the influence of graded levels of *C. thonglongyai* dung on the concentrations of some mineral elements (Fe, Mg, Zn, Ca, Mn, Cu, Na and K) in the leaves of *C. aconitifolius*.

Materials and Methods

Study area

Pot experiment was carried out in the Department of Biochemistry, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria. Lapai is positioned in the Southern Guinea Savanna region, on latitude 8^0 49'N and longitude 6^0 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 is frequently occurred in August and September, while the highest average monthly temperature (30-400C) is usually in March and the minimum (22.3^{0}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, Nigeria. 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.[9] The particle sizes were analyzed using hydrometer method; pH was determined potentiometrically in the water and 0.01M $CaCl_2$ solution in a 1: 2 soil/ liquid using a glass electrode pH meter and organic carbon by Walkey-Black method.[9] Exchange acidity (E.A H^+ and Al^{3+}) was evaluated by titration method. The exchangeable Ca, Mg, K and Na were leached from the soil sample with neutral 1N NH_4OAc solution before they were analysed. Sodium and potassium were determined by flame emission spectrophotometry while Mg and Ca were determined by EDTA versenate titration method.[9] Total nitrogen was estimated by Macrokjedal procedure and available phosphorus by Bray No 1 method.[9]

Source of Cnidoscolus aconitifolius cuttings and Craseonycteris thonglongyai dung

The Dung of *C. thonglongyai* which the local farmers used as organic fertilizer was collected into a polythene sack from a cave where the animals live in colony in Faso village of Edati Local Government Area of Niger State. The cuttings of *C. aconitifolius* were acquired from Teaching and Research Farm of Faculty of Agriculture, Ibrahim Badamasi Babangida University Lapai, Niger State.

Manure treatment and application

Dried dung of *Craseonycteris thonglongyai* were pulverized into powder 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 has ten (10) pots replicated 3 times making a 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.

Estimation of mineral elements

The determination of mineral elements (Fe, Mg, Zn, Ca, Mn, Cu, Na and K) in the leaves of *C. aconitifolius* was done according to the method described by.[10] Briefly, fresh leaves of the vegetable were harvested separately from the experimental plot and dried in an oven maintained at 110^{o}C for 24 hours. Precisely 0.50 g of each of the dry powdered leaves sample was weighed into different boiling tubes and 5.00 cm^3 of the digestion mixture, which comprises of concentrated perchloric and nitric acid in a ratio of 1:2 was added to each of the tubes. The resulting mixtures were swirled and left in a fume cupboard overnight. The mixtures were then digested on a hot plate at 150^{0}C for 2 hours. The resulting solutions were cooled for 10 minutes, after which 3.0 cm^3 of 6.0 M HCl was added and the samples were further digested for another $1^1/_2$ hour, and allowed to cool. The solution of each tube was then made up to 50 cm^3 with distilled deionized water in a volumetric flask and then transferred into cleaned sample bottles. The samples were analyzed using Atomic Absorption Spectrophotometer (Agilent 280FS AA) for Fe, Mg, Zn, Ca, Mn and Cu whereas Flame Photometer (Jenway PFP7) was used for Na and K.

Statistical analysis

Analysis of variance (ANOVA) was done using SPSS statistical package to determine the effect of graded levels of *C. thonglongyai* dung on the concentrations of the mineral elements in the leaves of *C. aconitifolius*. Duncan's Multiple Range Test (DMRT) was used for comparison of the means at $p < 0.05$.

Results and Discussion

Physical and chemical properties of soil.

Table 1 shows the result of soil analysis used for the pot experiment. The concentrations of total nitrogen, magnesium and calcium of the soil are very low whereas organic carbon and sodium are low. However, the concentrations of potassium and available phosphorus are high. The soil texture was sand soil which deduces that the soil has poor nutrient- and water-holding capacity while its water-infiltration capacity, aeration and workability are good [11]. The soil pH showed that the soil is strongly acidic in water and slightly basic in calcium chloride. Cation exchange capacity, which measures the nutrient holding capacity was very low while base saturation was very high[11]. The low concentrations of some essential soil nutrients required to support plant growth and development in the soil used for the experiment give credence for the amendment of soil by farmers in the vicinity with various manures to improve the physical and chemical properties of the soil for nutrients uptake and development of plant for optimal yield.[12, 13]

Chemical properties of C. thonglongyai dung

The chemical properties of *C. thonglongyai* dung is shown in Table 2. The organic carbon, total nitrogen, phosphorus, sodium and potassium contents of the dung are very high. Nevertheless, the magnesium content is high whereas the concentration of calcium is low. The pH of the dung is slightly basic[11]. The high concentrations of essential nutrients particularly, N, P and K in the *C. thonglongyai* dung justify the use of the organic fertilizer by plant growers for amending the soil to enrich the nutrient contents of the soil to support plants growth, development and increase their yields.[13-17]

Effect of graded levels of C. thonglongyai dung on the mineral elements content

The determination of effect different levels of *C. thonglongyai* dung on mineral elements concentration in *C. aconitifolius* showed that the Fe content increased significantly ($p < 0.05$) with the application of the dung. However, the concentration of Fe in the vegetable treated with 75, 100 and 125 g was significantly ($p < 0.05$) lower when compared with the vegetable grown with 50 g. The mean values of Fe in the control, 25, 50, 75, 100 and 125 g were 2.01± 0.16, 3.02 ± 0.37, 4.27 ± 0.34, 2.28 ± 0.27, 2.34 ± 0.06 and 2.34 ± 0.06 mg/kg, respectively (Table 3).

Similarly, treatment with graded level of *C. thonglongyai* dung significantly ($p < 0.05$) elevated the concentration of Mg in the vegetable with increased quantity of the dung. Nevertheless, the concentration of the mineral in the vegetable treated with 75, 100 and 125 g were not significantly different from the vegetable treated with 50 mg (Table 3).

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

Parameters	Values
Sand (%)	92.29 ± 3.20
Silt (%)	5.94 ± 0.31
Clay (%)	1.77 ± 0.31
Textural class	Sand
pH (H_2O)	5.97 ± 0.69
pH ($CaCl_2$)	7.35 ± 0.30
Organic carbon (g kg^{-1})	4.61 ± 0.11
Total nitrogen (g kg^{-1})	0.18 ± 0.01
Available phosphorus (mg kg^{-1})	51.90 ± 2.10
Na^+ (cmol kg^{-1})	0.23 ± 0.01
K^+ (cmol kg-^{1})	1.13 ± 0.05
Mg^{2+} (cmol kg^{-1})	0.09 ± 0.01
Ca^{2+} (cmol kg^{-1})	1.39 ± 0.03
Acidity (cmol kg^{-1})	0.16 ± 0.02
CEC (cmol kg^{-1})	3.00 ± 0.16
EC(cmol kg^{-1})	2.86 ± 0.08
Base saturation (%)	94.66 ± 2.73

CEC = Cation exchange capacity, EC = Exchangeable cations. Values represent mean values of triplicate determinations.

Application of the dung had no significant effect on the concentrations of Cu, Zn and Na in *C. aconitifolius*, except that the Cu and Zn contents in the vegetable treated with 50 g of the compost significantly ($p < 0.05$) increased in the vegetable (Table 3). Whereas treatment with 25 g of the dung had no significant ($p > 0.05$) on the Mn content in the leaf of *C. aconitifolius*, fertilization with 50 g and above significantly ($p < 0.05$) elevated the mineral content in the vegetable. The mean concentrations of 1.28 ± 0.08, 1.33 ± 0.50, 1.45 ± 0.43, 1.60 ± 0.26, 2.08 ± 0.25 and 2.45 ± 0.04 mg/kg were recorded for control, 25, 50, 75, 100 and 125 g of the dung, respectively (Table 3). Similarly, application of *C. thonglongyai* dung significantly ($p < 0.05$) increased the concentrations of Ca and K in the leaf of *C. acanitifolius*, while the concentrations of these mineral elements in the vegetable treated with 50 g of the dung did not differ significantly from the vegetable treated with 25 g, those treated with 75 and above were significantly higher in Ca and K compared with vegetable treated with 25 g of the dung (Table 3).

The higher contents of Fe, Mg, Cu, Mn, Ca, Zn and K in the leaf of *C. aconitifolius* grown on the soil treated with *C. thonlongyai* dung when compared with the control is in consonance with the submission of the following researchers[8,18-21,23] to the effect that organic manures increase the concentrations of some mineral elements in vegetables. [18]Assertion that organic manures activate species of microbes in the soil, and their microbial actions help to trigger nutrients assimilation that enhance plant growth and development. Similarly,[19]suggested that the increase in the levels of Ca, Fe and Zn in *Lactuca sativa* cultivated with compost of bounce back can be attributed to the balanced nutrient contents in the compost which facilitates absorption of nutrients by the plant. The dung of *C. thonglongyai* used in this study is an organic fertilizer with high concentrations of carbon, nitrogen, essential mineral elements and also contain beneficial microorganism. When applied to soil, the dung improved the physicochemical properties of the soil and microbial population. It thus follows that the elevation in the concentrations of the minerals (Fe, Mg, Cu, Mn, Ca, Zn and K) in *C. aconitifolius* following application of the dung may firstly suggest, a direct nutrients interaction and assimilation from the soil by the plant, since the dung has high concentrations of essential nutrients including some mineral elements required by plants for normal metabolic activity.[13, 21, 23] Secondly, the high nutrient contents and the activities of the microbial population in the dung help breakdown organic materials in the soil and convert them to nutrients for plants' root. This in turn helped to improve the density of soil, creating good texture and recondition soil for optimal nutrients assimilation by plant.

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

Parameters	Values
pH (H_2O)	7.66 ± 0.22
Organic carbon (g kg-^{1})	33.00 ± 1.33
Total nitrogen (g kg-^{1})	7.50 ± 0.13
Available phosphorus (mg kg-^{1})	8651.39 ± 49.95
Na^+ (cmol kg-^{1})	3.15 ± 0.07
K^+ (cmol kg-^{1})	11.90 ± 0.31
Mg^{2+} (cmol kg-^{1})	3.86 ± 0.15
Ca^{2+} (cmol kg-^{1})	2.78 ± 0.12

Values represent mean values of triplicate determinations

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

Minerals	Graded levels of *Craseonycteris thonglongyai* dung					
(mg/kg)	0 g (control)	25 g	50 g	75 g	100 g	125 g
Fe	2.01 ± 0.16^a	3.02 ± 0.37^b	4.27 ± 0.34^c	2.28 ± 0.27^{ab}	2.34 ± 0.06^{ab}	2.34 ± 0.06^{ab}
Mg	40.67 ± 6.36^a	45.13 ± 2.90^b	50.78 ± 3.80^c	51.60 ± 8.24^c	53.97 ± 4.51^c	53.45 ± 8.70^c
Cu	0.08 ± 0.01^a	0.09 ± 0.01^a	0.13 ± 0.06^b	0.09 ± 0.00^a	0.08 ± 0.01^a	0.07 ± 0.01^a
Mn	1.28 ± 0.08^a	1.33 ± 0.50^a	1.45 ± 0.43^b	1.60 ± 0.26^b	2.08 ± 0.25^c	2.45 ± 0.04^c
Ca	29.61 ± 2.03^a	33.43 ± 2.56^b	39.38 ± 0.99^{bc}	46.32 ± 3.10^c	53.45 ± 3.86^c	48.79 ± 8.93^c
Zn	1.41 ± 0.01^a	1.53 ± 0.16^a	1.75 ± 0.17^b	1.66 ± 0.15^a	1.52 ± 0.15^a	1.42 ± 0.30^a
Na	22.22 ± 1.40^a	19.83 ± 0.38^a	22.26 ± 1.11^a	23.49 ± 1.07^a	23.16 ± 0.42^a	21.29 ± 1.95^a
K	132.97 ± 8.81^a	157.93 ± 3.46^b	167.03 ± 17.93^{bc}	176.51 ± 12.67^c	181.88 ± 11.85^c	180.07 ± 6.32^c

Mean values on the same row with different superscript are significantly different ($p < 0.05$)

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Furthermore, the dung has bioremediation capacity, by acting as fungicide in the soil, putrefying fungi and control nematodes with harmful insects, thereby helps to keep soils and plants free from diseases.[22-24] It is established that plant grown on disease free soil with adequate nutrients will support maximum nutrients absorption from the soil for optimal metabolic and physiological activities resulting in bioaccumulation and synthesis of essential nutrients.[8, 22, 23]
Nonetheless, reduction in the concentrations of some mineral elements and the insignificant effect of application of *C. thonglongyai* dung above 50 g per 20 kg on the concentration of the studied minerals could suggest that, even though the dung enriched the nutrient contents of soil, enhanced growth and bioaccumulation of nutrients, excessive fertilization can alter the physical and chemical properties of the soil that could negatively affect absorption of nutrients.[8] Thus, in order to effectively optimise *C. thonglongyai* dung in the improvement of mineral contents in *C. aconitifolius*, moderate application of the dung is required. In this current study, better improvement in the mineral contents in the vegetable was obtained with fertilization of 50 g per 20 kg of *C. thonglongyai* dung.

Conclusion

The current study reveals that the application of *C. thonglongayai* dung improved the accumulation of mineral elements in *C. aconitifolius*. With moderate application, particularly that of 50 g per 20 kg soil being the preferred one, as this quantity of the dung increased the bioaccumulations of mineral elements evaluated in the vegetable. This finding therefore suggests that the public health problems associated with mineral element deficiency can be alleviated by consumption of the leaves of *C. aconitifolius* cultivated with moderate amount of *C. thonglogyai* dung.

Conflict of interest

The authors declare no conflict of interest regarding the manuscript

Authors' Declaration

We hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by us.

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