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Soil Amendment with Graded Levels of *Craseonycteris thonglongyai* compost on the Concentrations of some Phytotoxins in the Leaf of *Cnidoscolus aconitifolius* (Tree Spinach)

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ABSTRACT

Generally, the nutrients and phytotoxins content in plants are influenced by physicochemical properties of the soil among other environmental factors. It is for this reason that pot experiment was conducted to investigate the influence of different levels of *Craseonycteris thonglongyai compost* on the concentrations of cyanide, nitrate, saponin, tannin, phytate and oxalate in the leaves of *Cnidoscolus aconitifolius*. The different levels of *C. thonglongyai* compost 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 oxalate and phytate were evaluated using titrimetric method while concentrations of nitrate, saponin and tannin were determined using spectrophotometric method. The results showed that application of *C. thonglongyai* dung significantly ($p < 0.05$) decreased the concentrations of the phytotoxins. Cyanide and phytate contents in the vegetable decreased with the application of various levels of the compost. Whereas moderate application of the compost had no significant effect on the concentrations of nitrate, saponin, tannin and oxalate, the concentrations of these phytotoxins increased significantly with increase in the application of the *C. thonglongyai* compost, particularly from 75 to 125 g per 20 kg soil. 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 does not increase the concentrations of the plant toxins evaluated in the vegetable except the nitrate content.

Keywords: Soil Amendment, *Cnidoscolus aconitifolius*, Phytotoxins, *Craseonycteris thonglongyai* compost.

Introduction

Leafy vegetables are extremely patchy group of crop plants that can be defined as crops cultivated for their edible leaves. They are prospective sources of mineral elements and phytonutrients with strong antioxidant properties. Adequate intake of vegetable has been linked with a lowered prevalence of degenerative diseases in humans.[1,2]In spite of these huge nutritional benefits, leafy vegetables also accumulate some toxic substances (such as oxalate, phytate, cyanide and nitrate, tannins, lectins) in them which can cause serious nutritional and health problems at high concentrations. For example, consumption of vegetable with high concentrations of phytate and oxalate reduce bioavailability of essential minerals, electrolyte imbalance and kidney stone in humans (calcium oxalate is the most common). High ingestion of cyanide on the other hand could lead to inhibition of ATP synthesis, respiratory poison, dizziness, cellular hypoxia, heart block and even death while high intake of nitrate results in the formation of alkylating agent ($^+CH_3$) that methylates the DNA leading to the formation of cancer. Other effects of nitrate are methaemoglobineamia, cyanosis, anoxia and death.[2-5]

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The concentrations of these nutrients and phytotoxins in vegetables are influenced by the nature and chemical contents of soil among other environmental and genetic factors.[5, 6]Therefore vegetable growers amend the soil with various form of organic fertilizer, with the realization that chemical or synthetic fertilizers are not environmental and health friendly. *Craseonycteris thonglongyai* dung is one of the organic fertilizers used by some farmers for cultivation of crops in some part of Niger State. However, the effect of the droppings on the concentration of plant toxins in the crops has not been evaluated. It is for this reason; this study is designed to evaluate the influence of different levels of *C. thonglongyai* droppings on the concentrations of nitrate, cyanide, phytate and oxalate, saponin and tannin in the leaf of *Cnidoscolus aconitifolius*.

Materials and Methods

Study area

Pot experiment was carried out in the Department of Biochemistry, Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria. The area 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 frequently occur in August and September, while the highest average monthly temperature of the study area (30-40^{0}C) is usually in March and the minimum (22.3^{o}C) in August.

Soil sampling and analysis

The surface (0 – 20 cm depth) soil sample was collected from 3 different locations on 8th June, 2020 at the Main Campus of Ibrahim

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Badamasi Babangida University, Lapai, Nigeria. The soil was mixed and sieved through 2 mm sieve to remove any wreckage. The physical and chemical properties of the soil and *C. thonglongyai* dung were analyzed according to the method of Juo.[7] 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.[7] Exchange acidity (E.A H^+ and Al^{3+}) was evaluated by titration method.[7] Total nitrogen was estimated by Macrokjedal procedure and available phosphorus by Bray No 1 method.[7]

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 the colony in a cave where the animals live 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, weighed and applied to grow *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 at angle 45° 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 10 pots replicated 3 times making total of 180 pots for the experiment. The plants were irrigated 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.[6]

Plant tissues analysis

The fresh leaves of *C. aconitifolius* grown in the pot experiment at different *C. thonglongyai* dung levels were harvested at market maturity and were subjected to standard analytical procedure to determine the phytotoxins content in the leaves of *C. aconitifolius*.

Phytotoxin Contents

The nitrate content in the fresh leaves of *C. aconitifolius* was determined by the colourimetric method of Sjoberg and Alanko, [8] while the alkaline picrate method of Ikediobi et al. [9]was used to evaluate the cyanide concentration in the samples. Titrimetric method by AOAC [10]and Maga[11] were used to determine the oxalate and phytate contents, respectively in the leaves of the vegetable. Similarly, the concentrations of tannin and saponin in the samples were evaluated quantitatively using colourimetric method as reported in the manual of food quality control.[10]

Statistical Analysis

Analysis of variance (ANOVA) was done using SPSS statistical package (version 26.0 of 2016) to determine the effect of graded levels of *C. thonglongyai* dung on the concentrations of phytotoxins 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

The analysis of the soil used in the pot experiment is presented in Table 1. The pH of the soil which is 5.7 indicates that the soil is strongly acidic. The organic carbon and calcium are low, while available phosphorus and magnesium are moderate. Whereas the concentrations of sodium and potassium are very high, the total nitrogen content is medium. The soil textural class is sand indicating that the soil has poor water- and nutrient- holding capacity while its aeration, water-infiltration capacity and workability are good quality. The cation exchange capacity (CEC) is low while the base saturation is very high.[12]The low concentrations of some essential nutrient in the soil used for the study, particularly the mineral elements could be as a result of low organic matter content of the soil as it is well documented that organic matter decrease with land use resulting in low content of mineral element in the soil. The low concentrations of these essential plant nutrients needed for plant growth and development justify the necessities to amend the soil with adequate manure to improve nutrient content of the soil to support growth and yield.[6]

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. The pH of the dung showed that it is slightly basic. [12]Similarly, the high concentrations of soil nutrients, particularly, nitrogen, phosphorus and potassium in the droppings of *C. thonglongyai* which are among the essential nutrients of the soils required by plants for maximum performance validate the use of the dung by farmers for amending the soil to improve the nutrient contents of the soil that will support the growth and development of plants that will lead to improved yields.[6]

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

Parameters	Values
Sand (%)	84.50 ± 0.23
Silt (%)	6.90 ± 0.03
Clay (%)	8.60 ± 0.15
Textural class	Sand
pH (H_2O)	5.70 ± 0.08
pH ($CaCl_2$)	7.35 ± 0.20
Organic carbon (g kg^{-1})	4.57 ± 0.04
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})	0.01 ± 0.01
Mg^{2+} (cmol kg^{-1})	1.28 ±0.02
Ca^{2+} (cmol kg^{-1})	3.68 ± 0.11
Acidity (cmol kg^{-1})	0.60 ± 0.01
CEC (cmol kg^{-1})	9.77 ± 0.21
EC(cmol kg^{-1})	9.17 ± 0.42
Base saturation (%)	93.86 ± 3.27

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

Effect of graded levels of C .thonglongyai dung on the phytotoxin contents

The determination of effect of graded levels of *C. thonglongyai* dung on the concentrations of phytotoxins in *C. aconitifolius* plant showed that application of different levels of. *thonglongyai* dung significantly ($p < 0.05$) decreased the concentration of cyanide in the leaf of the vegetable. The mean values of cyanide recorded for control, 25, 50, 75, 100 and 125 g of the dung per kg soil were 352.84±11.54, 268.57 ± 45.29, 248.57 ± 14.55, 230.62 ± 43.33, 221.18 ± 71.51 and 220.64 ± 54.63 mg/kg, respectively (Table 3).

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The application of 25 g of *C. thonglongyai* dung had no significant ($p > 0.05$) effect on the concentration of nitrate in *C. aconitifolius*, however, the nitrate content increased significantly with increasing application of the dung from 50 to 125 g per 20 kg of the soil. Nitrate concentration in the leaves of the vegetable was highest (1,758.33±16.67 mg/kg) at the highest application level of 125 g of the dung while the lowest concentration (658.33±88.19 mg/kg) was recorded at 25 g application (Table 3).

While treatment with 25 and 50 g per 20 kg of the dung had no significant effect on the concentration of saponin in the leaves of *C. aconitifolius,* increased application of the dung from 75 to 125 g significantly elevated the saponin content in the vegetable. The mean values of 39.43 ± 28.27, 35.77± 7.76,34.62 ± 10.59, 69.28 ± 13.13, 79.78 ± 6.94 and 84.16 ± 6.16 mg/g of saponin were recorded for control, 25, 50, 75, 100 and 125 g, respectively (Table 3).

Whereas application of 25 and 50 g of the dung per 20 kg soil did not have significant ($p > 0.05$) effect on the tannin and oxalate contents in the leaves of *C. aconitifolius,* the concentrations of these parameters increased significantly ($p < 0.05$) when the vegetable was treated with 75, 100 and 125 g of the dung per 20 kg soil (Table 3).

The concentration of phytate in *C. aconitifolius* in the control was highest (3.73±0.60 g/100g) while treatment with 125 g of the dung per 20 kg recorded the lowest content of phytate (2.68±0.12 g/100g). However, the phytate content in the vegetable treated with 25, 50, 75 and 100g of *C. thonglongyai* had the mean values of 3.14±0.34, 3.17±0.45, and 3.20±0.24 and 3.49±0.33 g/100 g, respectively. These values did not differ significantly from each other ($p>0.05$). However, the results showed that application of the dung decrease the phytate content in the vegetable (Table 3).

Phytotoxins which some of them are also antinutritional factors are known to interfere with nutrients uptake and bioavailability of nutrients for metabolic process.[13,14]Linamarin is the pre-dominant cyanogenic glycosides in the tissues of *Cnidoscolus* species; this compound has a β-linkage that can only be released under high pressure and temperature, use of mineral acids, and enzymes including β-glycosidase and linamarase which is the endogenous enzyme that hydrolyzed the β-linkage of the glycoside.[15] The linamarin is hydrolyzed by the enzyme β-glycosidase, which removes the glucose portion. Significant decrease in cyanide content in the leaves of *C. aconitifolius* treated with *C. thonglogyai* dung could be as a result of enzyme inhibition of cyanogenic glycoside. Cyanogenic glycosides are compounds present in foods that release hydrogen cyanide. Similar report in cassava leaves by[16] revealed that the leaves of the plant treated with organic fertilizers had the lowest concentration of cyanide. Likewise, [5] reported that organic manures overhaul the soil with required nutrients and promotes plant's health. However, the application of the *C. thonglongyai* dropping is likely to reduce the enzymatic conversion of tyrosine to hydroxymandelonitrile, an intermediate compound for production of cyanide and thereby reduce the concentration of respiratory poison.[2, 17]

Naturally, nitrate concentration in leafy vegetables has been reported to be higher than other tissues of vegetable such as roots, stems, flowers, fruits and seeds.[17,18]The significant increase in the concentration of nitrate with increasing application of the dung could be as a result of high concentration of nitrogen in the dung. This finding corroborates with the submission of Musa [17, 19] that when plants are treated with high concentration of nitrogen in excess of what the plant require to perform it function for growth and biosynthesis of protein, the excess nitrogen will be converted to nitrate and store principally in the leaves. The higher nitrate content recorded in the leaves of the vegetable with increase application of the dung could suggest that appropriate caution should be taken when applying *C. thonglongyai* dung for the cultivation of *C. aconitifolius* to avoid nitrate toxicity that could result to health problems such cancers, methaemoglobinemia, cyanosis, anoxia and even death.[2]

The concentration of saponin in the leaves of *C. aconitifolius* treated with different levels of *C. thonglongyai* dung is within the safe limit, since saponins at levels <10 % in a diet is said to be harmless to the body.[20] However, in humans and animals high saponin content has been connected with gastroenteritis, manifested by diarrhoea, dysentery and haemolysis of red blood cells of.[21] This secondary metabolite protects plant from fungal and insect attacks [22, 23] and serves to protect the plants that secreted them against pathogens and other environmental threat.

Vegetable tannins are water soluble phenolic secondary metabolites compounds that are widely distributed in the plant kingdom. Tannins can produce undesirable effects in food when present in considerably high amounts. They form a complex with proteins through hydrogen bonding and covalent linkages and reduce the bioavailability of the proteins. The increase in the tannin content upon the application of *C. thonglongyai* droppings suggests that the droppings have potentials to elevate the plant protective effects against array of disease causing pathogens due to their free radical scavenging activity. However, high concentration of tannins in food samples could have deleterious effects in the body.[13]

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

Parameters	**Values**
pH (H_2O)	7.64 ± 0.14
Organic carbon (g kg-^{1})	35.20 ± 2.13
Total nitrogen (g kg-^{1})	7.80 ± 0.12
Available phosphorus (mg kg-^{1})	8742.0 ± 45.20
Na^+ (cmol kg-^{1})	3.20 ± 0.06
K^+ (cmol kg-^{1})	12.00 ± 0.30
Mg^{2+} (cmol kg-^{1})	3.79 ± 0.13
Ca^{2+} (cmol kg-^{1})	2.83 ± 0.21

Values represent means of triplicate determinations.

Table 3: Effects of different levels of *Craseonycteris thonglongyai* dung on the concentrations of phytotoxins in the leaf of *Cnidoscolus aconitifolius*

	Graded levels *Craseonycteris thonglongyai* compost (g)					
Phytotoxins	**0 g (Control)**	**25 g**	**50 g**	**75 g**	**100 g**	**125 g**
Cyanide (mg/kg)	352.84 ± 11.54^b	268.57 ± 45.29^a	248.57 ± 14.55^a	230.62 ± 43.33^a	221.18 ± 71.51^a	220.64 ± 54.63^a
Nitrate (mg/kg)	708.33 ± 10.42^a	658.33 ± 88.19^a	975.00 ± 87.80^b	1008.33 ± 41.67^b	1316.67 ± 154.34^c	1758.33 ± 16.67^d
Saponin (mg/g)	39.43 ± 28.27^a	35.77± 7.76^a	34.62 ± 10.59^a	69.28 ± 13.13^b	79.78 ± 6.94^c	84.16 ± 6.16^c
Tannin (mg/g)	1.76 ± 0.29^a	1.69 ± 0.24^a	1.82 ± 0.75^{ab}	1.91 ± 0.13^b	2.04 ± 0.22^b	2.43 ± 0.05^b
Phytate (g/100g)	3.73 ± 0.60^c	3.14 ± 0.34^b	3.17 ± 0.45^b	3.20 ± 0.24^b	3.49 ± 0.33^b	2.68 ± 0.12^a
Oxalate (mg/g)	14.95 ± 0.00^a	12.65±1.33^a	14.75 ± 2.51^a	16.48 ± 2.68^b	16.87 ± 1.38^b	18.40 ± 1.99^b

Row mean values carrying the same superscripts are not significantly different from each other ($p > 0.05$)

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Application of different levels of *C. thonlongyai* droppings decreases the concentration of phytate in the leaves of *C. aconitifolius*. This decrease in phytate is more effective with increased concentration of *C. thonglogyai* dung. In most plants, phytate is a major storage form of phosphorus, an important mineral used in the production of energy as well as the formation of structural components like cell membrane.[2, 24]Although recent studies have shown that phytic acid has potential health benefits as it can act as antioxidant, exhibits anticancer properties, and have a positive impact in lowering the cholesterol and blood glucose. However, at high concentration, phytic acid is a potent antinutrient which forms complexes with numerous divalent and trivalent metal cations. This antinutrient has six reactive phosphate groups and meets the criterion of a chelating agent. The major concern about the presence of phytate in the diet is its negative effect on mineral uptake. Minerals of concern in this regard include Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mn^{2+} and Cu^{2+}. The chelating of the minerals by phytate in the gastrointestinal tract, leads to unavailability of the dietary minerals for absorption and utilization in the body. Phytate also interacts with proteins and reduce their bioavailability of the dietary proteins.[2,13-25] Therefore; the decrease in the concentration of this potent antinutrient with application of the dung is a good one as it will help to increase the bioavailability of minerals and proteins in the body which are essential for metabolic activity and general wellbeing of the body.

Consumption of leafy vegetables is of concern when there is a risk of high oxalate concentration in them due to the public health problems associated with the ingestion of the antinutrient such as reduction of bioavailability of mineral elements, formation of kidney stone (calcium oxalate is common) and electrolyte imbalance.[2] The significant increase in the concentration oxalate in the leaves of *C. aconitifolius* treated with 75, 100 and 125 g compared to 25 and 50 g per 20 kg soil could suggests that moderate application of the dung had no influence on the bioaccumulation of the antinutrients in the leaves of the vegetable. Therefore, moderate use of the dung should be encouraged over excessive application. Thus our current findings is a good one as it serves as good guide to vegetable growers in their bid to reduce the public health problems associated with high of oxalate and also optimized the use of the dung and thereby reduce the cost of production of the vegetable.

Conclusion

The current study reveals that application of various levels of *C. thonglongayai* dung decreases cyanide and phytate contents in the vegetable. However, while moderate application of the dung had no significant effect on the concentrations of nitrate, saponin, tannin and oxalate in the vegetable, the concentrations of these plant toxins increases significantly with increased in the application of the *C. thonglongyai* dung, particularly from 75 to 125 g per 20 kg soil. These findings therefore suggest that moderate application particularly that of 50 g per 20 kg soil being the preferred in improving the nutritional quality of *C. aconitifolius*, as this quantity of the dung do not increase the concentrations of the phytotoxins in vegetable except the nitrate content.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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