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Effects of Graded Levels of *Craseonycteris thonglongyai* Dung on the Concentration of Amino Acids in the Leaf of *Cnidoscolus aconitifolius* (Tree Spinach)

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

Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria

*Corresponding author Email: jelilibch@gmail.com Tel: +234 (0) 703 300 2211

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ABSTRACT: This research work determines the levels of amino acids in *Cnidoscolus aconitifolius* as influenced by graded levels of *Craseonycteris thonglongyai* dung. The effect of applied *C. thonglongyai* dung on the levels of amino acids was investigated with a view to determine the appropriateness of the application of *C. thonglongyai* dung in growing the vegetables. Complete randomized pot experiments were conducted to determine the effect of *C. thonglongyai* dung levels on amino acids (essential and non-essential) in *C. aconitifolius*. The leaves of the vegetable were harvested and analyzed at market maturity state (vegetative phase) and fruiting (reproductive phase) of the plant development. The results obtained showed that the applied *C. thonglongyai* dung significantly ($p<0.05$) elevate the essential amino acids from 50 to 100g of the dung; leucine (7.23±0.58 to 8.39±0.05 g/100g), lysine (3.58±0.39 to 4.43±0.02 g/100g), isoleucine (3.83±0.32 to 4.27±0.08 g/100g), phenylalanine (3.72±0.37 – 4.51±0.02 g/100g), methionine (0.83±0.05 – 1.46±0.23 g/100g), histidine (1.87±0.37 – 2.38±0.03 g/100g) and threonine concentration (3.05±0.37 – 3.82±0.03 g/100g). Similar results were also obtained for non-essential amino acids when the plants were treated from 50 to 100g of the dung; serine 2.87±0.32 – 3.48±0.04 g/100g, aspartic acids 6.83±0.43 – 7.79±0.06 g/100g, proline 2.63±0.24 – 3.24±0.06 g/100g, arginine 4.23±0.33 – 5.06±0.07 g/100g, tyrosine 2.51±0.23 – 3.14±0.15 g/100g, cysteine 0.91±0.06 – 1.11±0.02 g/100g, alanine 3.80±0.27 – 4.43±0.06 g/100g and glutamic acids concentration range from 11.38±0.62 – 12.68±0.04 g/100g. Application of *C. thonglongyai*, particularly at 50 g per 20 kg of the dung increased the synthesis of amino acids in the tree spinach.

Keywords: *Cnidoscolus aconitifolius, Craseonyteris thonglongyai,* Amino acids, Market maturity.

Introduction

Green leafy vegetables are important constituents of human diet all over the world and they are in diverse different region depending on purpose being used for. Leafy vegetables are good protective food, source of nutrients, beneficial for maintenance of health and prevention of diseases as they contain valuable food ingredient which can be utilized to build and repair the body (Welbaum, 2015). Leafy vegetable contain dietary fibres, mineral elements, vitamin and amino acids which are valuable in maintaining alkaline reserve in the body (Hanif *et al.,* 2006). Dark green leaves have high content of carotene, microelements and ascorbic acid, which are vital for slowing down degenerative diseases and metabolism of nutrients (Yi-Fang *et al.,* 2002). It is important to propagate the awareness of vegetable as a nutrient for health.
C. aconitifolius is a perennial shrub belonging to the family Euphorbiaceae. The plant is commonly grown in the tropic and sub-tropical region. People around the world, eat it as vegetable soup (Ganiyu, 2005). It has a succulent stem with milky sap when cut. It is usually pruned to about 2 meter for easier leaf harvest but usually

it can grow to 6 meters. It is called *'Iyana Ipaja'* in the Southwestern Nigeria. The raw leaves contain high contents of toxic hydrocyanic acid; therefore it should be cooked before eating (Mordi and Akanji, 2012).

However, amino acids play important roles in plants which are building blocks of protein and cannot be overlooked. With the exception of importance as major protein constituents, they also serve as plethora of cellular reactions and influence a number of physiological processes such as plants resistance to both abiotic and biotic stress, control intracellular pH, plant development and growth as well as, general metabolic energy (Moe, 2013, Watanabe *et al.,* 2013, Zeier, 2013, Fagard *et al.,* 2014, Galili *et al.,* 2014, Häusler *et al.,* 2014, Pratelli and Pilot, 2014).

Fertilizer application is a cheap and fast way of replacing the nutrients content of the soil that have been depleted through continuous use of the land for cultivation of crops in order to improve plant growth and development (Nidhi *et al.,* 2014). Due to the constant use of land for cultivation of crops, soils becomes impoverished of essential nutrients required for plant growth and development and hence the application of fertilizers to enrich the soil become necessary (Musa *et al.*, 2017). Manures on the other hand, are the major sources of plant nutrients (Abdel-Aziz, 2007; Musa *et al.,* 2016) and are the most important and controllable factor affecting the nutritional value of vegetables (Heaton, 2001).

Craseonycteris thonglogyai dung is a waste from animal, which farmers used as manure for amendment of the soil to improve their farm produce. This animal compost is highly effective as manure for farmer due to its high concentration of phosphorous, potassium and nitrogen which are major nutrients required for plant growth and development. This study therefore determines the effects of graded levels of *C. thonglongyai* dung on the concentrations of both essential and non-essential amino acids present in the leaves of *C. aconitifolius.*

Material and methods

Study area: The study was conducted in the Department of Biochemistry, Faculty of Natural Science, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria. Niger State has a savannah climate located within the latitude of 8°49'N and longitude 6°41'E. The mean monthly temperature of Lapai in March is 30-40 °C highest and lowest in August at 22.3 °C. Highest rainfall in Lapai occurred in August and September and is between 300 and 330 mm. (Shittu *et al*., 2008).

Soil sampling and analysis: The soil samples used in the study was collected at depth of 0-20 cm from three different sites. The samples were bulked, dried, ground and sieved through 2 mm sieve to remove debris. The soil samples were then analyzed for particle size, pH, organic carbon, exchangeable acidity, exchangeable Ca, Mg, K and Na and total nitrogen according to the method described by Jou (1979).

Source of Cnidoscolus aconitifolius stem and Craseonycteris thonglongyai droppings: The stem of *Cnidoscolus aconitifolius* was collected in a garden in the Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Niger State while *Craseonycteris thonglongyai* dropping was collected in a polythene bags from a cave in Faso village of Edati Local Government Area of Niger State, Nigeria.

Planting and experimental design: Two cutting stems of *Cnidoscolus aconitifolius* were planted in a polythene bag filled with 20.00 kg of top soil and were thinned to one plant per pot after sprouting. Complete randomized design (CRD) was adopted, using six (6) different treatment levels; 0 g (as control), 25, 50, 75, 100, and 125 g of the *C. thonglonyai* dropping. Each treatment had 10 pots replicated three times and this gave a total of 180 pots. The stems cutting were planted at angle of 45° and were watered twice daily (mornings and evenings) using watering can except on the raining days. The experimental area and the surroundings were weeded regularly to prevent harboring of pests. The pots were lifted from time to time to prevent the roots of the plants from growing out of the container.

Harvesting of *Cnidoscolus aconitifolius*: Leaves of the vegetable grown with *C. thonglonyai* droppings were harvested at vegetative phase of plant development after three months of planting and were used for the analysis.

Determination of amino acid profile: The amino acids profile in the leaves of *Cnidoscolus aconitifolius* was determined using methods described by Benitez (1989). Leaves of *C. aconitifolius* was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

Defatting sample: The sample was defatted using chloroform/methanol mixture of ratio 2:1. Exactly 1.00 g of the sample was put in extraction thimble and extracted for 5 hours in Soxhlet extraction apparatus (AOAC, 2006).

Nitrogen determination: Nitrogen content was determined using Kjeldhal digestion procedure, according to the method described by AOAC (2006).

Hydrolysis of the sample: A known weight (mentioned in the calculation sheet) of the defatted sample was weighed into glass ampoule. Exactly 7 ml of 6 N HCl was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g. methionine and cystine). It should be noted that tryptophan is destroyed by 6 N HCl during hydrolysis. The tryptophan in the known sample was hydrolyzed with 4.2 M Sodium hydroxide (Maria *et al.*, 2004).
Statistical analysis: Data was analyzed using statistical package for social science (SPSS) version 16 and presented as mean±SEM. Data were subjected to Analysis of Variance (ANOVA) and means were compared by Duncan's Multiple Range Test (DMRT). $p<0.05$ was considered statistically significant.

Results

Physical and chemical properties of soil: The result of analysis of the soil sample used in the pot experiment is presented in Table 1. The mean pH of the soil was measured to be 5.7 which indicated that the soil is strongly acidic. The organic carbon (4.57 g kg^{-1}) and calcium (3.68 cmol kg^{-1}) were determined to be low, while available phosphorous (16.30 mg kg^{-1}) and magnesium (1.28 cmol kg^{-1}) are rated moderate. The concentration of sodium (3.70 cmol kg^{-1}) and potassium (1.13 cmol kg^{-1}) were very high whereas the total nitrogen content of 1.82 g kg^{-1} was rated medium. The soil textural class was loamy sand indicating that the water holding capacity is moderate. The Cation Exchange Capacity (CEC) of 9.77 cmol kg^{-1} was low while the base saturation (93.9%) was rated very high.

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

Parameter	**Value (Mean ±SD)**
Sand (%)	84.5±0.23
Silt (%)	6.9±0.03
Clay (%)	8.6±0.15
Textural class	Loamy sand
pH (H_2O)	5.7±0.08
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})	1.13±0.01
Ca^{2+} (cmol kg^{-1})	3.68±0.02
Mg^{2+} (cmol kg^{-1})	1.28±0.11
Acidity (cmol kg^{-1})	0.60±0.01
EC(cmol kg^{-1})	9.17±0.42
CEC (cmol kg^{-1})	9.77±0.21
Base saturation (%)	93.86±3.27

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

Chemical properties of C. thonglongyai dung: The chemical property of *C. thonglongyai* dung is shown in Table 2. The total nitrogen, organic carbon, available phosphorous, sodium and potassium of the dung are very high as indicated in Table 2. The magnesium content (3.79 cmol kg^{-1}) of the dung was found to be high while the calcium content of 2.88 cmol kg^{-1} was rated low. The mean pH (7.64) of the dung showed that it was slightly basic.

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

Parameter	**Value (Mean ±SD)**
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±45.2
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 mean values of triplicate determinations

Effect of graded levels of Craseonycteris thonglonyai dung on the concentration of essential amino acids in Cnidoscolus aconitifolius: The result of the graded levels of *Craseonycteris thonglonyai* dung on the concentration of essential amino acids in *Cnidoscolus aconitifolius* is shown in Table 3. The application of different levels of the dung had no significant (p>0.05) effects on the concentrations of leucine, lysine, isoleucine, phenylalanine, tryptophan, valine, and histidine. The application of the dropping at 100 g had significant (p<0.05) effect on the concentration of methionine in the leaves of *C. aconitifolius*. The concentration of this amino acid increased with the application of 25 g to 100 g of the dung. More so, treatment at 75 and 125 g *C. thonglongyai* dung had significant (p<0.05) effect on the concentration of threonine in the leaves of *C. aconitifolius.* In addition, application of the dung increases the concentration of almost or all the amino acids from 50 to 100 g treatment.

Table 3: Effect of graded level of *Craseonycteris thonglongyai* dung on the concentration of essential amino acid in *Cnidoscolus aconitifolius*

Essential Amino Acid (g/100g)	Graded levels of *Craseonycteris thonglongyai* dung (g)					
	0	**25**	**50**	**75**	**100**	**125**
Leucine	7.53 ± 0.24[a]	7.29 ± 0.40[a]	7.23 ± 0.58[a]	7.88 ± 0.07[a]	8.39 ± 0.05[a]	8.36 ± 0.26[a]
Lysine	3.60 ± 0.21[a]	3.59 ± 0.25[a]	3.58 ± 0.39[a]	4.06 ± 0.11[a]	4.43 ± 0.02[a]	4.20 ± 0.09[a]
Isoleucine	3.89 ± 0.17[a]	3.85 ± 0.18[a]	3.83 ± 0.32[a]	4.22 ± 0.12[a]	4.27 ± 0.08[a]	4.18 ± 0.09[a]
Phenylalanine	3.82 ± 0.22[a]	3.74 ± 0.27[a]	3.72 ± 0.37[a]	4.25 ± 0.09[a]	4.51 ± 0.02[a]	4.41 ± 0.10[a]
Tryptophan	0.84 ± 0.09[a]	0.82 ± 0.06[a]	0.86 ± 0.11[a]	0.98 ± 0.05[a]	1.14 ± 0.01[a]	2.00 ± 0.95[a]
Valine	3.42 ± 0.20[a]	3.38 ± 0.22[a]	3.45 ± 0.32[a]	3.79 ± 0.09[a]	3.98 ± 0.06[a]	3.90 ± 0.06[a]
Methionine	0.85 ± 0.07[b]	0.83 ± 0.05[b]	0.87 ± 0.14[b]	0.92 ± 0.03[b]	1.46 ± 0.23[a]	1.04 ± 0.09[b]
Histidine	2.12 ± 0.08[a]	2.09 ± 0.11[a]	1.87 ± 0.37[a]	2.28 ± 0.03[a]	2.38 ± 0.03[a]	2.35 ± 0.03[a]
Threonine	3.19 ± 0.17[b]	3.11 ± 0.17[b]	3.05 ± 0.37[b]	3.52 ± 0.06[ab]	3.82 ± 0.03[a]	3.66 ± 0.08[ab]

Mean ±SEM on the same row with different superscripts are significantly different (p ≤ 0.05)

Effect of graded levels of Craseonycteris thonglonyai dung on the concentration of nonessential amino acids in Cnidoscolus aconitifolius: The effect of graded levels of *Craseonycteris thonglongyai* dung on the concentration of non-essential amino acids in *Cnidoscolus aconitifolius* revealed that the application of different levels of the dropping had no significant effect (p>0.05) on serine, aspartic acid, glutamic acid and glycine content in the vegetable.

Application of the dung at 75 g had significant effect (p<0.05) on the concentrations of proline, arginine, alanine and cysteine in *C. aconitifolius* leaves. The result also shows that the concentrations of all the non-essential amino acids decreased from 100 to 125 g of the dung application except in glycine.

Furthermore, application of the dung at 0, 25, 50 and 75 g had no significant effect (p>0.05) on the concentration of tyrosine in the leaves of *C. aconitifolius*. But the tyrosine level in the vegetable had significant effect when the vegetable was fertilized with 100 and 125 g of the dung. The mean value recorded in the control, 25, 50, 75, 100 and 125 g of the dung for tyrosine were 2.60 ± 0.14, 2.52 ± 0.18, 2.51 ± 0.23, 2.78 ± 0.09, 3.14 ± 0.15 and 2.95 ± 0.11 respectively. Application of the dung at the different levels had no significant effect (p>0.05) on the concentration of glycine. Meanwhile, glycine concentration increased from the application of 100 to 125 g of the dung (Table 4).

Table 4: Effect of graded level of *Craseonycteris thonglongyai* dung on the concentration of some non-essential amino acid in *Cnidoscolus Aconitifolius*

Non-Essential amino acids (g/100g)	Graded levels of *Craseonycteris thonglongyai* dung (g)					
	0	**25**	**50**	**75**	**100**	**125**
Serine	3.01 ± 0.13[a]	2.87 ± 0.24[a]	2.87 ± 0.32[a]	3.38 ± 0.12[a]	3.48 ± 0.04[a]	3.38 ± 0.05[a]
Aspartic acid	6.90 ± 0.32[a]	6.93 ± 0.31[a]	6.83 ± 0.43[a]	7.50 ± 0.10[a]	7.79 ± 0.06[a]	7.68 ± 0.06[a]
Proline	2.76 ± 0.13[a]	2.74 ± 0.13[a]	2.63 ± 0.24[a]	2.93 ± 0.07[ab]	3.24 ± 0.06[b]	3.10 ± 0.05[b]
Arginine	4.26 ± 0.18[a]	4.27 ± 0.21[a]	4.23 ± 0.33[a]	4.64 ± 0.12[ab]	5.06 ± 0.07[b]	4.82 ± 0.09[b]
Tyrosine	2.60 ± 0.14[a]	2.52 ± 0.18[a]	2.51 ± 0.23[a]	2.78 ± 0.09[a]	3.14 ± 0.15[b]	2.95 ± 0.11[b]
Cysteine	0.93 ± 0.04[a]	0.88 ± 0.05[a]	0.91 ± 0.06[a]	1.00 ± 0.06[ab]	1.11 ± 0.02[b]	1.06 ± 0.03[b]
Alanine	3.85 ± 0.08[a]	3.80 ± 0.10[a]	3.80 ± 0.27[a]	4.10 ± 0.10[ab]	4.43 ± 0.06[b]	4.24 ± 0.09[b]
Glutamic acid	11.57 ± 0.36[a]	11.53 ± 0.45[a]	11.38 ± 0.62[a]	12.26 ± 0.14[a]	12.68 ± 0.04[a]	12.39 ± 0.05[a]
Glycine	4.22 ± 0.24[a]	4.21 ± 0.27[a]	4.15 ± 0.40[a]	4.69 ± 0.07[a]	3.33 ± 0.89[a]	4.90 ± 0.17[a]

Mean ± SEM on the same row with different superscript are significantly different (p ≤ 0.05)

Discussion

The medium concentration of nitrogen, phosphorus and magnesium in the analyzed soil samples could due to average amount of organic matter content available in the soil. This finding disagreed with the finding reported by Riezebos and Loerts (1998), Jaiyeoba, (2003) and Jimoh *et al.* (2017) that organic matter decrease with land use resulting in low mineral content in the soil. The low concentration of the essential nutrients required for plant growth and development justify the modification of soil with manures to improve the nutrient content of the soil (Agboola and Unamma, 1991).

The organic matter content in soil is the basic indicator of the soil quality and fertility and the key factor for the adequate growth, development and efficiency of plant (Hayes, 2005; Lal, 2011; Krasowicz *et al.*, 2011). The results showed that the *Craseonycteris thonglongyai* dung used in this study contained high total nitrogen, organic carbon, available phosphorous, sodium and potassium content. These are among the major nutrients required by plants for growth and development. The chemical composition of *C. thonglongyai* dung shows that they have a great ability to improve soil quality and crop yields. The decomposition of *C. thonglongyai* dung releases nutrients to plants and humus increases the nutrient content, which has a positive effect on the physical, chemical and biological properties of soil.

The fertility of a soil is determined by the presence and content of nutrients and organic matter (Kaho *et al.*, 2007). Organic matter is an important component of soil fertility (Rahman and Parkinson, 2007). This means that a soil with high organic matter has a good structure, high stability, good mobility and availability of nutrients (Ros *et al*., 2006). This justifies the increase in the amino acid yield of *C. aconitifolius* vegetables with application of *C. thonglongyai* dung. The soil nitrogen increased with treatment of *C. thonglongyai* dung, which increased the nitrogen uptake by *C. aconitifolious* resulting in increase in amino acid synthesis.

The results revealed an increase in amino acids content of *C. aconitfolious* treated with high *C. thonglongyai* dung against control. The variation in the levels of amino acids content in the leaves of *C. aconitifolious* may be attributed to the differential rate of organic nitrogen assimilation towards nitrogen transport. Amino acids are used to transfer nitrogen from source organs to sink tissues and to build up reserves during periods of nitrogen availability for subsequent use in growth, defense and reproductive processes. Methionine, a precursor of succinyl-CoA, homocysteine, cysteine, creatine, and carnitine, is an essential sulfur-containing amino acid. It is necessary for the metabolism of polyamines, creatine, and phosphatidylcholine. Methionine is the precursor for cellular methylation and the synthesis of cysteine, and can thus decrease the dietary cysteine requirement (Finkelstein *et al*. 1988; Mackay *et al.* 2012).

Moreover, amino acids are the raw materials for protein synthesis and products of protein decomposition. Application of *C. thonglongyai* dung to the plant was found to be stimulatory towards higher biosynthesis and accumulation of amino acid in leaves of *C. aconitifolius.* The enhanced level of amino acids in leaves of *C. aconitifolus* treated with *C. thonglongyai* dung might due to effective conversion of nitrogen to amino acids due to readily available mineral elements in the soil and their translocation in the plant system (Ram Rao *et al.*, 2007).

The increased synthesis of these amino acids in *C. aconitifolius* leave could be as a result of treatment with droppings which is tightly linked to compartmentalization, carbohydrate metabolism, absorbed nitrate and demand for protein synthesis and secondary metabolism. Amino acid synthesis takes place mainly in the chloroplasts, where reducing power as well as ATP can be provided directly (Widyarani *et al.,* 2016). This vegetable contains appreciable amount of essential amino acids which the body cannot synthesize directly and are needed for the maintenance and repair of tissue in the body. The presence of all the essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine) in these vegetables makes them important in human diet because they cannot be synthesized by humans body (Widyarani *et al.*, 2016).

Application of dung was found to be stimulatory towards biosynthesis and accumulation of non-essential amino acids in leaves of *C. aconitifolius.* The effect of *C. aconitifolius* at different graded levels of treatment (50 to 100 g) resulted in increase in almost all the non-essential amino acids content as the treatment increase and these may be related to environmental stress or different osmotic adjustment. According to Barnerjee *et al.* (2012), the accumulation of proline in the leaves can be stimulated by growth retardant and reflecting the osmotic adjustment of crop plants. The leaves of *C. aconitifolius* have high cyanide content and increase in synthesis of cysteine could lead to cyanide reduction. Meyers & Ahmad (1991); Ogunlabi & Agboola (2007), agrees that β-cyanoalanine synthase pathway is the principal route for cyanide detoxification in plants. Biochemically, the incorporation of cyanide into cysteine which is facilitated by β-cyanoalanine synthase (an enzyme in the synthesis of cyanide) and the nitrogen in cyanide are allocated to amino acid or for ammonia production (Machingura *et al.,* 2016). The cysteine produced can be used in protein translation and the synthesis of the antioxidant glutathione and the osmolyte taurine (Rezzi *et al.* 2007; Nicholson *et al.* 2008).

Chlorophyll content is approximately proportional to leaf nitrogen content and the biosynthesis of glutamic acid and aspartic acid, two amide amino acids, are tightly regulated by light (Oliveira *et al.*, 2001). Arginine continuous increase in the leaves of *C. aconitifolious* could be as a result of used for nitrogen storage and transport to various parts of plants. Thus, mobilization of nitrogen from source tissues requires arginine degradation, and the enzymes involved are induced during senescence and germination (Witte, 2011). Increased tyrosine level resulted in growth and yield of the plant which may come from increased use of *C. thonglongyai* dung and alanine increases from stress in the plants and in relation to intracellular pH regulation (Pavlík *et al.*, 2010). Increase in the concentration of glycine and serine may be attributed to increase in Na level in the leaf of *C. aconitifolius* when *C. thonglongyai* dung was applied. This suggested that the effects of increase in Na lead to an increase in the photorespiration rate and both glycine and serine are strongly involved in photo-respiratory cycle. Glycine is produced by the transaminase between glyoxylate and glutamate, and is the substrate for glycine decarboxylase while serine hydroxymethyltransferase complex, that, in turn, produces serine, ammonia, and carbon dioxide.

The decrease in mean values of amino acids observed at 125 g fertilizer treatment suggests that excessive supply of *C. thonglongyai* dung may be detrimental to vegetable quality which may arose from the inhibition of the nutrients present in the organic matters transported and possible protein degradation.

Conclusion

This study reveals that the effect of graded levels of *Craseonycteris thonglongyai* dung in *Cnidoscolus aconitifolius* leaves indicates an increase concentration in both essential and non-essential amino acids when applied from 50 to 100 g. The application of *C. thonglongayai* dung thus improved synthesis of amino acids particularly at 50 g per 20 kg soil in the leaves of the vegetable.

Recommendation

For its health benefits, it is recommended that *C. thonglogyai* dung should not to be limited to vegetable research only but rather it should be used for fertilization of other economic plants.

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