Effect of Nitrogen Source on Growth, Yield, Quality, and Nitrogen Use Efficiency of African Nightshade Varieties (*Solanum spp.*) Grown in Kenya

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ABSTRACT

African Nightshade (*Solanum spp*) is an important indigenous vegetable grown in Kenya and is ranked among the top ten priority indigenous vegetables in terms of production and consumption in the country. Production of this vegetable is affected by many factors, with the source of nitrogen fertilizer being among the most important. Currently, the production of this vegetable is 1 to 3 tons ha^{-1} versus a biological yield potential of 30 tons ha^{-1}. This disparity in yield could be rectified by appropriate fertilizer application and utilization. Recent studies showed that inorganic fertilizers have the potential to leach and cause environmental contamination, ultimately increasing the cost of production and with environmental ramifications. In comparison, organic fertilizers have the potential to improve the soil structure and water holding capacity reducing leaching of nutrients. Our study aimed at investigating the effect of nitrogen sources on the growth, yield, quality, and nitrogen use efficiency on African Nightshade. An experiment was conducted at Jomo Kenyatta University of Agriculture and Technology in 2017 having inorganic and organic nitrogen sources. The organic fertilizer consisted of composted plant material, minerals and treated bio-solids. The experiment was laid out in a randomized complete block design with four treatments and four replications. The nitrogen treatments included a control 0 g, 3.6 g (calcium ammonium nitrate), 2.11 g (urea) and 32.4 g (organic fertilizer) per plant. Data was collected on plant height (cm), number of leaves, fresh leaf weight (g), protein content, nitrate content and nitrogen use efficiency. The sources of nitrogen were shown to significantly ($P \leq 0.05$) affect plant growth with respect to plant height and leaf number. Plants applied with 2.11 g (Urea) per plant were the most productive.

INTRODUCTION

African nightshade (*Solanum spp*) is a leafy indigenous vegetable in the family Solanaceae that is widely produced and consumed in Kenya and the larger East African region. Commonly grown varieties in the country are *Solanum scabrum* and *Solanum villosum* with the former being preferred due to its proclaimed medicinal benefits such as treatment of stomach aches and diarrhea (Odongo et al., 2018). This vegetable plays an important role in poverty alleviation and malnutrition reduction in the region. Its production can be accomplished in small plots and utilizes limited resources which provides an opportunity for subsistence production and income generation among the rural poor without huge capital investments (DFID and R4D, 2010) .

Over the years African nightshade was mainly consumed in rural areas of the country, but it has recently gained popularity with urban communities due to its perceived nutritional and medicinal attributes. The leaves are high in proteins, iron, ascorbic acid and riboflavin (Onyango and Onyango, 2005). Recently, a variety of health promoting bioactive phytochemicals such as carotenoids and phenolic compounds have been identified in *Solanum scabrum* which could be resourceful in explaining its use as traditional medicine (Jiménez-Aguilar et al., 2015). Due to the changing trends in the consumption of this vegetable, there has been a need to increase production to meet the current demand. Low productivity of this vegetable is attributed to several factors including poor soils, small production plots, and drought (Tuwei et al., 2013). The general belief that indigenous vegetables are hardy and can grow under any environmental conditions has led to poor crop management by farmers, reducing yield quantity and quality significantly.

Nitrogen is an essential element for plant growth and development due to its role in cell division and expansion. While nitrogen plays a major role in the production, application techniques and sources can lead to reduced vegetable quality, economic losses and environmental contamination (Liu et al., 2014). The lack of information about variety specific application rates of nitrogen when utilizing different sources and its effects on plant yield, quality and the environment has created challenges in production. Most farmers incorrectly apply excessive amounts of fertilizers as insurance against yield losses without being aware of the cost and environmental implications (Umar et al., 2007). When applied to soils, inorganic fertilizers are rapidly released and absorbed by plants in comparison to organic fertilizers, which are slowly released, hence plant response to the fertilizer may be delayed. The rate of mineralization in organic fertilizer is affected by several factors including soil properties, temperature, available water and type of organic fertilizer (Nardi et al., 2018).

Leafy vegetables are bio accumulators and can accumulate nitrates to potentially harmful levels if the absorption of nitrates is higher than its conversion to ammonia (Qiu et al., 2014). The nitrate content varies in different parts of the plant and the physiological age of the crop (Umar et al., 2007). The toxic effects of nitrate are due to its endogenous conversion of nitrate to nitrite, which is implicated in the occurrence of methemoglobinemia, gastric cancer and several other diseases (Rao, 2000). The aim of this study was to investigate the effect of nitrogen source on plant growth, yield and nutritional quality of five different varieties of African nightshade.

MATERIALS AND METHODS

The study was carried out in a greenhouse between January 2017 and April 2017 in a pot experiment at the Jomo Kenyatta University of Agriculture and Technology. The university is in Juja, Thika constituency under the geographical co-ordinates -1.0870^0 S,37.0126^{0}E and 1524 meters above sea level. The five important varieties of African nightshade (two *S. villosum* and three *S. scabrum*) were used in the experiment. Three of the varieties were developed by Prof Abukutsa and named Abuku 1, Abuku 2 and Abuku 3. Two standard *S. villosum* and three *S. scabrum* varieties were sourced from the Wakulima market. The experiment was a completely randomized design with four treatments and four replications. Treatments included 0g control, 3.6g (Calcium ammonium nitrate (CAN)), 2.11g (Urea) and 32.4g (processed organic fertilizer) per plant (Table 1). Triple superphosphate (46% P 2O5) and Muriate of potash (60% K_2O) were applied as a basic dressing at the rate of 100kg ha^{-1} approximately 0.9g plant^{-1} of inorganic fertilizer. The amount of P and K in the processed organic fertilizer was above 10% respectively. Other crop management practices including watering and weeding was carried out after three days and two weeks respectively.

Data collection for plant height (cm) and number of leaves was initiated three weeks after treatment applications and were collected weekly while data on leaf yield and leaf area were collected three and a half months after planting. Data on vegetable quality which included the protein and nitrate content in the plant shoots was collected during the vegetative (35 days after treatment application) and reproductive growth stages (89 days after treatment application). Plant height was measured using a meter ruler and the leaf number counted manually. Leaf yield was measured in grams per plant using an electric balance (LIBROR EB-3200D, Shimadzu, Japan) and leaf area was measured in cm^2 using a leaf area meter (LI-COR LI-3000, Lincoln, NE, USA).

Proteins. Protein content in shoot tissue was determined using the semi-micro Kjeldahl method, specification 950.46 methods 20.87-37.1.22 (AOAC, 1995). Approximately 2 g of sample was weighed into a digestion flask together with a combined catalyst of 5 g potassium sulfate, 0.5 g of copper sulfate and 15 mL of H_2SO_4. The mixture was heated in a fume hood till the digest color turned blue. The digest was cooled, transferred to 100 mL volumetric flask and topped up to the mark with distilled water. Blank digestion with the catalyst was made. Exactly 10 mL of the diluted digest was transferred into the distilling flask and washed with distilled water. 15 mL of 40% NaOH was added and washed with distilled water. Distillation was done to a volume of approximately 60 mL distillate. The distillate was titrated using 0.02 N HCl to the orange color of the mixed indicator. The nitrogen in the sample was calculated and multiplied with the protein factor (0.65) to determine the percent protein content. Analysis of variance was performed to indicate whether fertilizer sources had a significant effect on protein content using the F-test at the 5% level for all variables. After significant effects on treatments were determined, separation of means was done using the Least Significant Difference (LSD 5%) approach. Post hoc tests were conducted where applicable.

Nitrates. The nitrate content in the shoot tissue was determined by the colorimetric method using salicylic acid (Cataldo et al.,1975). About 500 mg of fresh sample was weighed and put in a test tube, and 10 mL of hot (90-95°C) distilled water was added. The closed tubes were placed in a water bath at 80^0C for 30 minutes and shaken. The samples were cooled and centrifuged at 4500 rpm. The supernatant was decanted and weighed to determine the exact volume of the extract. Chlorophyll in the leaf extract was removed by adding 0.5 g $MgCO_3$ to the supernatant and centrifuged again. The supernatant containing the nitrate extract was treated with 2 N NaOH and a combination of Salicylic acid and H_2SO_4 in a ratio of 1:20 w/v. Standards were prepared using sodium nitrate. The absorption was measured at 410 nm with a UV-Vis spectrophotometer (PD-3000UV, SA). Analysis of variance was performed to indicate whether fertilizer source had a significant effect on nitrate content using F-test at 5%, levels on all variables. After significant effects on treatment were determined, separation of means was done using the Least Significant Difference (LSD5%) approach. Post hoc tests were conducted where applicable.

Nitrogen use efficiency. Nitrogen use efficiency was calculated at the end of the pot experiment using data collected during the experiment (Moll et al.,1982). The data utilized in these calculations was total plant nitrogen at harvest, nitrogen source (treatments) and leaf yield (harvest index).

Nitrogen Use Efficiency (NUE)= *(uptake efficiency x utilization efficiency)*

Uptake efficiency = (Nt/Ns);

Utilization efficiency = (Gw/Nt)

Where:

Nt = total N in the plant at harvest,

Ns = Nitrogen supply rate,

Gw = Leaf yield of the produce.

RESULTS

A significant ($P \leq 0.05$) difference in plant height was observed among plants at 14 weeks after the application of nitrogen, with plants applied with urea being the tallest (Table 2). Plants that were applied with CAN and urea had significantly ($P \leq 0.05$) more leaves than the control and plants supplied with organic fertilizer (Table 3). There was a significant ($P \leq 0.05$) difference in leaf area, with plants supplied with CAN and urea having more leaf area than the plants in the control group and plants supplied with organic fertilizer (Figs. 1 and

2). However, there was no significant difference in leaf area between plants supplied with CAN and Urea. The *S. villosum* varieties were also observed to have smaller leaf area than *S. scabrum* varieties. There was a significant ($P \leq 0.05$) difference in nitrogen use efficiency among the different nitrogen sources, with plants in the control group being the most nutrient efficient (Figs. 3 and 4). A significant ($P \leq 0.05$) response in terms of leaf yield was observed, with plants supplied with CAN and urea having higher yields than plants in the control treatment and plants supplied with organic fertilizer (Figs. 5 and 6). The nitrogen source affected plant quality at both vegetative and reproductive growth stages of the African nightshade varieties. The nitrate content in the plants was higher during the reproductive stage compared to the vegetative stage (Tables 4 and 5). *Solanum villosum* varieties were observed to have a higher nitrate content at both vegetative and reproductive growth stages. The protein in the plants was significantly ($P \leq 0.05$) affected by the source of nitrogen (Tables 6 and 7) in both *S. scabrum* and *S. villosum* varieties. The protein content dropped over time, with plants at the vegetative stage having a higher protein content than plants at the reproductive stages.

DISCUSSION

The crop responses that were measured depended on the plant variety and the source of nitrogen applied. *Solanum scabrum* is known for its tall erect stems and broad leaves, while *S. villosum* is narrow-leaved and has short stems (Shippers, 2000). According to Overcash et al. (2005), plant growth characteristics such as plant height, number of leaves and leaf area strongly respond to nitrogen applications. The results of this experiment are in agreement with studies conducted on the physiological effect of organic and inorganic fertilizer on the growth of *S. nigrum* (Bvenura and Afolayan, 2014). Nitrogen is more responsible for plant growth than any other element. It stimulates vegetative growth resulting in large stems and leaves due to its effect on cell division and elongation (Abukutsa-Onyango, 2002). In the studies mentioned above inorganic fertilizers were shown to be effective in increasing the growth and yield of the five nightshade varieties compared to organic fertilizer. These results are similar to those of Akinrinde (2006), who reported a significant response of various crop species to the application of inorganic fertilizers.

In our study, African nightshade plants subjected to organic fertilizers showed signs of nitrogen deficiency during early growth stages due to the slow release nature of the fertilizer. Compared to inorganic fertilizers, organic fertilizers are not readily available for uptake because mineralization has to occur for nutrients to be available. Inorganic fertilizers are refined to a state that make it easy for plants to take up the nutrients. Wheeler (2008) identified a number of challenges for farmers using organic fertilizers. The challenges include slow availability of organic fertilizer, sourcing of manure, and the typically higher cost of organic fertilizer. Our results show that the source of nitrogen affected the quality of African nightshade by affecting the nitrate and protein content. During the vegetative growth stage, all plants supplied with additional nitrogen, except for the control treatment, accumulated nitrate levels higher than the recommended daily intake for nitrate, which is 3.7 mg/kg according to EFSA (2008). The nitrate concentrations increased with physiological age, with the highest levels recorded at the reproductive growth stage. This observation was in line with a review conducted by Anjana et al. (2009) that identified physiological age as a factor for nitrate accumulation in plants.

Nitrates are relatively non–toxic, however they may be transformed endogenously to nitrite which can react with amines and amides to produce N-nitroso compounds which are associated with increased disease susceptibility (Santamaria, 2006). Nitrogen was observed to influence the protein content, with plants supplied with inorganic fertilizer accumulating more proteins during the vegetative stage. Protein content across all plants reduced during the reproductive stage, a change attributed to senescence. According to Watanabe et al. (2013), during senescence there are metabolic changes that include hydrolysis and remobilization of macromolecules like proteins, nucleic acids, and pigments that accumulate during growth. There are several factors affecting the nitrogen use efficiency of a crop, including type of crop, type of fertilizer, timing of fertilizer application and climatic conditions (Lakesh and Bali, 2018). Inorganic fertilizers have a lower nitrogen use efficiency compared to organic fertilizers because they can be easily lost through leaching, runoff, or volatilization.

Results of this study show that nitrogen supply plays a dominant although not exclusive role in the yield and quality of African nightshade. The use of organic fertilizer can be more economical and useful in the development of long-term sustainable food production systems. Due to the slow release nature of organic fertilizers the cumulative economic and agronomic value of some of the organic materials applied can be greater in the post application phase. Short season crops like African nightshade can be at a disadvantage in terms of growth and yield when using organic fertilizer due to N unavailability. More research needs to be conducted on the mineralization rate of different organic fertilizers that is affected by factors such as source of organic matter and physical state of the organic fertilizer. This will provide the foundational information on organic fertilizers and which would be most suitable for the production of short season African indigenous vegetables.

CONCLUSIONS

The source of nitrogen fertilizer affected plant growth, yield, quality, and nitrogen use efficiency in African nightshade varieties. There is a need for farmers to be equipped with information on various ways in which to improve fertilizer efficacy. Due to the effect of physiological age of crop on nutritive quality of African nightshade harvesting should be done at the vegetative stage so as to maintain its nutritional benefits.

Table 1. Nitrogen treatments applied during the experiment.

Treatment (g per plant)	Nitrogen source	Comments
T_1	No nitrogen	Control
T_2 (36.4 g)	Processed organic fertilizer	Organic
T_3 (3.6 g)	Calcium ammonium nitrate (CAN; 27% N, half in nitrate and half in ammonium)	Commonly used by farmers as nitrogen source
T_4 (2.11 g)	Urea	Inorganic

Table 2. Average Plant height of African nightshade *Solanum villosum* and *S. scabrum* varieties at fourteen weeks after treatment applications of different nitrogen sources.

Nitrogen source	*S. scabrum* varieties Plant Height (cm)	*S. villosum* varieties Plant Height (cm)
Control	56.55 ^{a}	61.76 ^{a}
Organic	66.80 ^{b}	69.58 ^{b}
CAN	67.50 ^{b}	71.87 ^{b}
Urea	68.77 ^{b}	72.11 ^{b}
	LSD 4.17 CV 6.9%	LSD 5.51 CV 9.7%

Mean separation is within columns same letters indicated there was no significant difference at (P≤0.05), different letters indicate significant difference at (P≤0.05). CV indicates the ratio of standard deviation to the mean; LSD indicates least significant difference between the means at the required level of probability (P≤0.05).

Table 3. Average number of leaves of African Nightshade *Solanum villosum* and *S. scabrum* varieties at fourteen weeks after treatment applications of different nitrogen sources.

Nitrogen Source	Scabrum varieties No. of leaves	Villosum varieties No. of leaves
Control	64.08 ^{a}	58.00 ^{a}
Organic	69.92 ^{a}	68.33 ^{a}
CAN	74.00 ^{b}	73.67 ^{b}
Urea	77.08 ^{b}	79.17 ^{b}
	Lsd 5.81	Lsd 12.38
	CV 14.7%	CV 14.3%

Mean separation is within columns same letters indicated there was no significant difference at (P≤0.05), different letters indicate significant difference at (P≤0.05). CV indicates the ratio of standard deviation to the mean; LSD indicates least significant difference between the means at the required level of probability (P≤0.05).

Table 4. Nitrate content of African nightshade *Solanum villosum* and *S. scabrum* varieties at vegetative and reproductive growth stages after treatment applications of different nitrogen sources.

Nitrogen Sources	Vegetative stage Nitrate content (mg/kg)	Reproductive stage Nitrate content (mg/kg)
Control	3.872 ^{a}	7.236 ^{a}
Organic	4.483 ^{b}	8.171 ^{ab}
CAN	4.808 ^{bc}	8.621 ^{b}
Urea	5.312 ^{c}	9.889 ^{c}
	Lsd 0.556	Lsd 1.078
	CV 14.6%	CV15.4%

Mean separation is within columns same letters indicated there was no significant difference at (P≤0.05), different letters indicate significant difference at (P≤0.05). CV indicates the ratio of standard deviation to the mean; LSD indicates least significant difference between the means at the required level of probability (P≤0.05).

Table 5. Nitrate content of African nightshade Solanum villosum and S. scabrum varieties at vegetative and reproductive growth stages after treatment applications of different nitrogen sources.

Nitrogen Sources	Vegetative stage Nitrate content (mg/kg)	Reproductive stage Nitrate content (mg/kg)
Control	5.008 ^{a}	9.13 ^{a}
Organic	6.459 ^{b}	9.89 ^{a}
CAN	8.535 ^{c}	10.81 ^{ab}
Urea	5.558 ^{ab}	12.68 ^{b}
	Lsd 1.391	Lsd 2.43
	CV 21.1%	CV 22.1%

Mean separation is within columns same letters indicated there was no significant difference at (P≤0.05), different letters indicate significant difference at (P≤0.05). CV indicates the ratio of standard deviation to the mean; Lsd indicates least significant difference between the means at the required level of probability (P≤0.05).

Table 6. Protein content of African nightshade *Solanum villosum* and *S. scabrum* varieties at vegetative and reproductive growth stages after treatment applications of different nitrogen sources.

Nitrogen Sources	Vegetative stage Protein content (%)	Reproductive stage Protein content (%)
Control	3.386 ^{a}	1.90^{a}
Organic	4.349 ^{a}	2.26^{a}
CAN	5.883 ^{b}	3.34^{b}
Urea	8.199 ^{c}	4.41^{c}
	Lsd 1.157 CV 25.6%	Lsd 1.23 CV 28.9%

Mean separation is within columns same letters indicated there was no significant difference at ($P \leq 0.05$), different letters indicate significant difference at ($P \leq 0.05$). CV indicates the ratio of standard deviation to the mean; Lsd indicates least significant difference between the means at the required level of probability ($P \leq 0.05$).

Table 7. Protein content of African nightshade *Solanum villosum* and *S. scabrum* varieties at vegetative and reproductive growth stages after treatment applications of different nitrogen sources.

Nitrogen Sources	Vegetative stage Protein content (%)	Reproductive stage Protein content (%)
Control	3.27 ^{a}	1.641 ^{a}
Organic	4.337 ^{ab}	2.516 ^{ab}
CAN	6.232 ^{b}	3.447 ^{b}
Urea	8.596 ^{c}	4.52 ^{c}
	Lsd 1.921 CV 33.2%	Lsd 1.001 CV 32%

Mean separation is within columns same letters indicated there was no significant difference at ($P \leq 0.05$), different letters indicate significant difference at ($P \leq 0.05$). CV indicates the ratio of standard deviation to the mean; Lsd indicates least significant difference between the means at the required level of probability ($P \leq 0.05$).

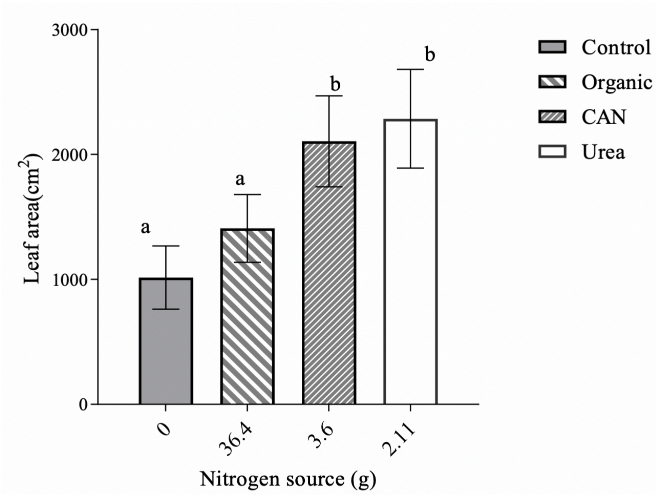

Figure 1. Leaf area of *Solanum scabrum* variety treated with different sources of nitrogen. Vertical bars with same letter are not significantly different ($P \leq 0.05$); vertical bars with different letters are significantly different ($P \leq 0.05$).

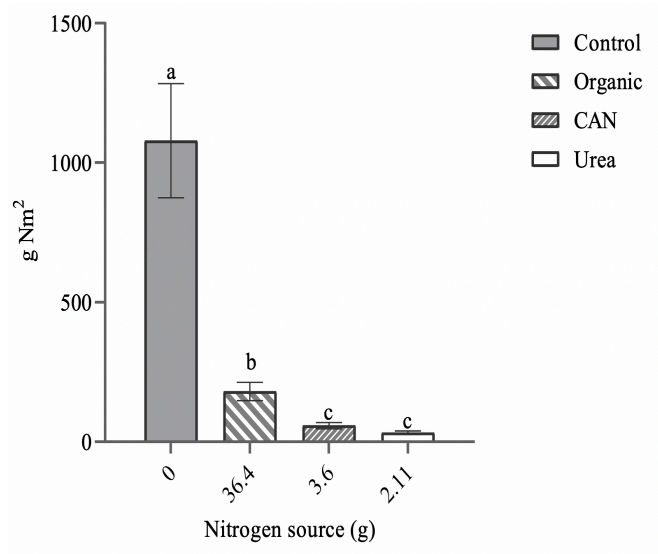

Figure 3. Nitrogen use efficiency of *Solanum scabrum* variety treated with different sources of nitrogen. Vertical bars with same letter are not significantly different ($P \leq 0.05$); vertical bars with different letters are significantly different ($P \leq 0.05$).

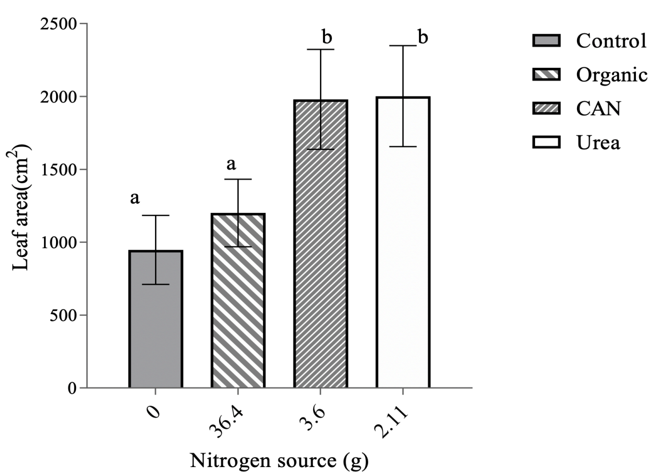

Figure 2. Leaf area of *Solanum villosum* variety treated with different sources of nitrogen. Vertical bars with same letter are not significantly different ($P \leq 0.05$); vertical bars with different letters are significantly different ($P \leq 0.05$).

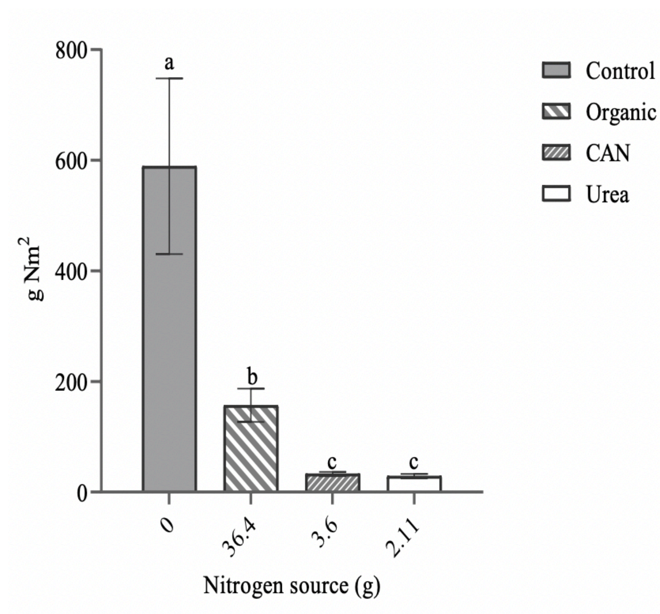

Figure 4. Nitrogen use efficiency of *Solanum villosum* variety treated with different sources of nitrogen. Vertical bars with same letter are not significantly different ($P \leq 0.05$); vertical bars with different letters are significantly different ($P \leq 0.05$).

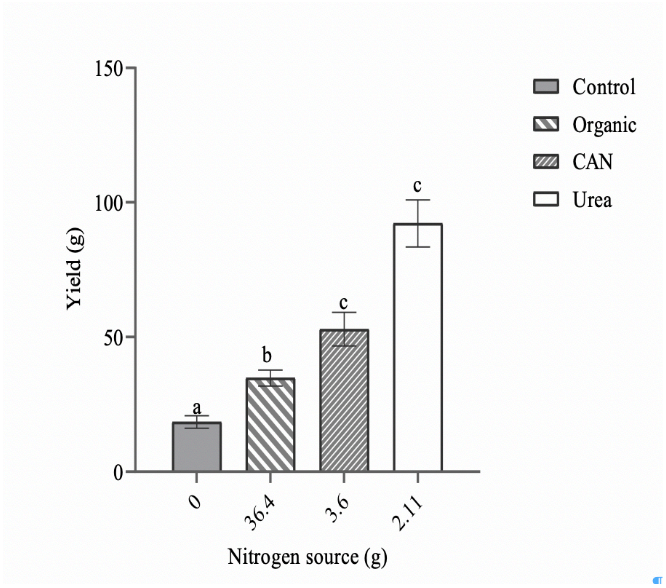

Figure 5. Yield (plant fresh weight) of *Solanum scabrum* variety treated with different sources of nitrogen. Vertical bars with same letter are not significantly different (P≤0.05); vertical bars with different letters are significantly different (P≤0.05).

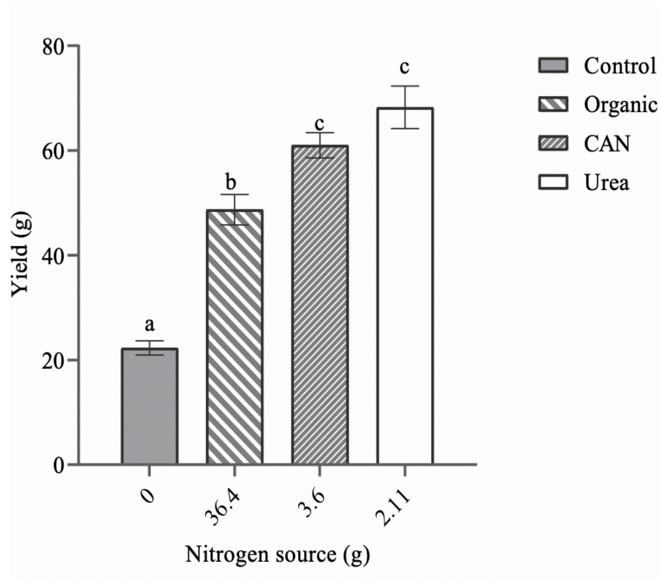

Figure 6. Yield (plant fresh weight) of *Solanum villosum* variety treated with different sources of nitrogen. Vertical bars with same letter are not significantly different (P≤0.05); vertical bars with different letters are significantly different (P≤0.05).

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