

Full Length Research Paper

Expression of bioactive compounds in different pepper cultivars (*Capsicum annum* L.) in response to different fertilizer treatments

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This research aimed at evaluating four cultivars of *Capsicum annum* L. with different nutrient sources to ascertain the nutrient source that would improve the soil characteristic and promote the accumulation of carotenoids and flavonoids in peppers. Four nutrient sources: poultry manure, pig manure, goat manure, and nitrogen: phosphorus: potassium (NPK) were used for the study. The experiment was conducted as a 4 × 5 Factorial in a Complete Randomized Design (CRD) at the Botanical Garden of the University of Nigeria, Nsukka. The soils characteristic features were analyzed by standard methods. The fruits biochemical content was quantified with the aid of a High-Performance Liquid Chromatography at the National Research Institute for Chemical Technology, Zaria, Nigeria. The α -carotene level (333.48 ± 0.27 mg/L) was the highest in "Nsukka yellow pepper" fruits grown on soil mixed with goat manure while β -carotene (45.56 ± 0.29 mg/L) was the highest when grown on soil mixed with poultry manure. "Tatase" cv. planted on soil mixed with poultry manure expressed the highest fruit capsanthin level while lutein was highly expressed when grown on soil mixed with goat manure. In conclusion, growing "Nsukka yellow pepper" with goat manure could increase the production of α -carotene, while poultry manure will increase the production of β -carotene.

Key words: Biochemical, inorganic fertilizer, micronutrient deficiency, organic manure, pepper.

INTRODUCTION

Deficiencies in nutrition and their resultant diseases remain prevalent in both the developed and developing world (Popkin et al., 2012). There are reports that about one-third of the world's population suffer deficiencies of vitamins mainly vitamins A and C and essential minerals (such as iodine, iron, and zinc) (Global Nutrition Report, 2014; Abu et al., 2019a) which results in health effects that range from mild to severe. More often than not, these deficiencies often go unnoticed and not tackled until its associated medical condition manifests itself. Because of their invisibility, such deficiencies are widely referred to

as "hidden hunger". Hidden hunger poses serious effects because people often do not realize that they are suffering from hidden hunger (Abu et al., 2019a). Pregnant women and young children who show rapid growth and development are the most susceptible to deficiencies of micronutrient and thus, suffer the maximum effects which are usually unpleasant (Meshram et al., 2012; Abu et al., 2019a). However, the production of pepper fruits of high nutritional value will contribute immensely in meeting up with the daily allowances of micronutrients as pepper fruits are consumed by every household either fresh or

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dried but the fresh form is mostly used in preparing delicacy (Abu et al., 2019a).

Pepper (*Capsicum annuum*) fruit is valued for its antioxidant capacity and high composition of bioactive molecules. However, it is ranked worldwide among the most popular fresh vegetables due to its combination of colour, flavour and nutritional value (Blanco-Ríos et al., 2013; Abu et al., 2019b). Pepper fruits are excellent sources of health-promoting molecules, such as ascorbic acid (vitamin C), antioxidants, provitamin A, sugars, carotenoids, and polyphenols (Jadczak et al., 2010). They are also repositories of various phenolics, flavonoids, and carotenoids (Materska and Perucka, 2005). Kelly and Boyhan, (2009) reported that one medium green pepper can provide up to 180% of vitamin C, 2% of iron, 8% of the Recommended Daily Allowance of vitamin A, and 2% of calcium. Lycopene, which protects against cancer is also present in red peppers (Perez-Lopez et al., 2007).

Genes responsible for the expression of nutritional traits are inherent in the DNA of pepper cultivars. However, for optimal expression of these genes, the perfect environment, and agronomic practice must be maintained. Liaven et al. (2008) found that amounts and characteristics of pepper fruits from plants cultivated in soil supplemented with organic manure were generally better than those from plants grown in soil only. Organic manure application has been one of the agronomic practices adopted by farmers to ensure optimal production. Organic fertilizers are more environmentally friendly as compared to chemical fertilizers. For a material to be eligible as an organic fertilizer, the material ought to occur in nature naturally. Generally, organic fertilizer is normally derived from single ingredients, thus, the types of organic fertilizers are derived from either plant, animal, or mineral sources. The organic fertilizers may supply nutrients to the soil but, a different type of source of fertilizer can give some different effects on the plant (Khandaker et al., 2017). Most often, landraces cultivated by farmers are poor in micronutrients due to poor agronomic practices such as nutrients application, planting date, postharvest handling among others. Understanding the best organic treatment that can help boost micronutrients composition in crops (pepper) is indispensable. Therefore, this study seeks to evaluate the biochemical composition of four cultivars of *C. annuum* fruits ("Shombo", "Tatase", "Ataragu" and "Nsukka yellow pepper") grown with different nutrient sources.

MATERIALS AND METHODS

Plant

The fruits of the four cultivars of *C. annuum*: "Shombo", "Tatase", "Ataragu" and "Nsukka yellow pepper" were obtained from the germplasm of Dr. Mrs. N. E. Abu from the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The seeds were extracted by excising the fruits and the extracted seeds were dried under the sunlight.

Preparation of planting medium

Polypots were prepared by filling polythene bags which were perforated at the base to avoid water logging with 12 kg of the different nutrient medium. The different nutrient medium for the experiment was prepared by mixing topsoil with runoff sandy soil and different organic sources (poultry droppings, pig dung, and goat dung) separately at a ratio 3:2:1, respectively and allowed to cure for a period of seven days. While the polypots for nitrogen, phosphorus and potassium (NPK) fertilizer and the control were filled with topsoil and run off sandy soil (3:1) without mixing the organic source. Nevertheless, 30 g of NPK 20:10:10 was applied two weeks after transplanting using the ring method of application (Olatunji and Agele, 2015).

Planting

The seeds of the different cultivars were nursed separately in nursery baskets filled with topsoil mixed with poultry manure and run off sandy soil (3:2:1) and watered daily (Ojua et al., 2019; Abu et al., 2019a). After a period of 6 weeks, the seedlings were then transplanted into different poly pots filled with soil mixed with different organic manure. The plants were grown in the Botanical Garden of the University of Nigeria, Nsukka using a 4 × 5 factorial experimental laid out in a completely randomized design (CRD) with 15 replications for every treatment. The factors were the cultivars and the different nutrient medium.

Soil (planting medium) analysis

Following standard procedures, the five nutrient medium (soil) were analyzed for both the chemical {pH in water (H₂O) and potassium chloride (KCl) (McLean, 1982), organic matter (Nelson and Sommers, 1996), exchangeable cations (calcium, magnesium, sodium, potassium) (Chapman, 1965), exchangeable acidity (H⁺), available phosphorus (Olsen and Sommers, 1982), total nitrogen (Bremner and Mulvaney, 1982), cation exchange capacity (CEC) (Hendershot and Duquette, 1986), and base saturation (McLean, 1982)} and physical {Percentage particle size of clay, silt, fine sand, and coarse sand were determined by the Bouyoucos hydrometer method after destroying organic matter using hydrogen peroxide (H₂O₂) and dispersing the soil with sodium hydroxides (NaOH) in place of Calgon {sodium hexametaphosphate (NaPO₃)} (Bouyoucos, 1951; AOAC, 2005)} properties at the Laboratory of the Department of Soil Science, University of Nigeria, Nsukka.

Carotenoids and flavonoids quantification

At maturity, fully ripened fruits were harvested and bulked into separate groups according to the cultivars grown on the different medium for biochemical analysis. The bulked groups were further divided into 3 sets, deseeded and analyzed for carotenoids and flavonoids composition. The characterization and quantification were performed with the aid of a High-Performance Liquid Chromatography (HPLC) at the National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State, Nigeria.

Carotenoids extraction from fresh fruit samples was done according to the methodology of Bureau and Bushway (1986). Pepper fruit samples were chopped into small pieces, and a 10 g subsample was immediately combined with 1 g of magnesium carbonate (MgCO₃), 5 g of sodium sulphate (Na₂SO₄), and 125 mL tetrahydrofuran (THF) stabilized with 0.01% butylated hydroxytoluene (BHT). Each sample was homogenized with a Tekmar tissumizer (Cincinnati) for 5 min on medium speed. Samples were vacuum filtered (Whatman #42), and the residue was

Table 1. Physical and chemical characteristics of soil and nutrient mixtures used in this research.

| Physio-chemical properties | Poultry mixture | Pig mixture | Goat mixture | NPK mixture | Control |
|--|-------------------------|-------------|--------------|-------------|---------|
| Clay (%) | 7 | 7 | 7 | 7 | 7 |
| Silt (%) | 4 | 4 | 4 | 4 | 4 |
| Fine sand (%) | 30 | 34 | 36 | 48 | 45 |
| Coarse sand (%) | 59 | 55 | 53 | 41 | 44 |
| Textured class | Sandy, clay, loam (SCL) | SC L | SCL | SCL | SCL |
| Soil pH (in H ₂ O) | 9.1 | 8.1 | 7.3 | 7.6 | 7.5 |
| Soil pH (in KCl) | 8.2 | 7.2 | 6.5 | 6.5 | 6.8 |
| Total carbon (%) | 0.890 | 0.890 | 1.122 | 0.697 | 0.658 |
| Total organic matter (%) | 1.535 | 1.535 | 1.935 | 1.201 | 1.134 |
| Total nitrogen (%) | 0.154 | 0.126 | 0.154 | 0.154 | 0.070 |
| Exchangeable sodium (meq/100 g of soil) | 0.03 | 0.03 | 0.05 | 0.03 | 0.03 |
| Exchangeable potassium (meq/100 g of soil) | 0.06 | 0.06 | 0.08 | 0.06 | 0.06 |
| Exchangeable calcium (meq/100 g of soil) | 2.00 | 2.20 | 2.80 | 1.80 | 2.20 |
| Exchangeable magnesium (meq/100 g of soil) | 1.40 | 1.20 | 1.20 | 0.60 | 0.60 |
| Exchangeable aluminum (meq/100 g of soil) | - | - | - | - | - |
| Exchangeable hydrogen (meq/100 g of soil) | 1.20 | 1.00 | 1.40 | 2.00 | 1.20 |
| Cation exchange capacity (meq/100 g of soil) | 8.40 | 6.40 | 15.20 | 14.80 | 4.80 |
| Base saturation (%) | 41.55 | 54.53 | 27.17 | 16.82 | 60.21 |
| Available phosphorus (ppm) | 40.02 | 53.16 | 72.75 | 60.62 | 51.30 |

Source: Authors

re-extracted with an additional 125 mL THF. A 20 mL portion of the sample was concentrated under nitrogen gas and stored at -20°C until HPLC analysis where the samples were resuspended in 0.4 mL THF and vortexed.

All samples were filtered through a nylon Magna 0.22 µm filter into HPLC vials for injection. The HPLC (Waters C 18 column, Shimadzu, Japan) analysis was done to confirm the presence and quantity of specific carotenoids and flavonoids. The column conditions were HPLC column of waters C18 symmetry, column (4.6 × 250 mm), and waters 600 pump 7725 rheodyne 7725 injector, waters 2487 dual-wavelength UV absorbance Detector 230n m. The mobile phase components (acetonitrile: water) was used in a gradient form, which varied with a change in time and a flow rate of 1.2 mL/min.

The column temperature was set at 40°C. The range on the photodiode array detector was also set at 380 to 550 nm, with maximum detection at 454 nm. The carotenoids were identified by their absorption spectra captured by the photodiode array detector, and HPLC retention times in comparison with authentic standards. Also, samples were spiked with standards (Sigma-Aldrich, European reference standard) to verify the identity of sample peaks with similar retention times.

Total carotenoids were quantified as capsanthin equivalents and β-carotene by using an authentic standard. All standards were handled under low light on the ice. Standard solutions of β-carotene were prepared in 20 mL THF, and capsanthin standards in methanol: acetonitrile (1:1). Aliquots were diluted in methanol: acetonitrile (1:1) to provide standard concentrations ranging from 2 to 10 µg/L with a detection limit of 0.1 µg/L.

Statistical analysis

Data collected from the research work were analyzed using Genstat Discovery Edition 4 to get the Analysis of Variance (ANOVA) and

Least Significant Difference (LSD) was used to separate the means at $P \leq 0.05$ level of significance.

RESULTS AND DISCUSSION

The percentage clay and silt composition of the nutrient medium were similar while soil mixed with poultry manure and NPK had a higher percentage of fine and coarse sand. Similarly, the soil pH in both water and potassium chloride, exchangeable magnesium was higher in poultry manure mixed soil, while the goat manure had the highest percentage concentration of total carbon, organic matter, exchangeable sodium, potassium, calcium, available phosphorus and cation exchange capacity (Table 1). On the whole, the organic nutrient sources were better in the available organic carbon, organic matter, exchangeable calcium, magnesium, and pH when compared with the NPK and the control. This is an indication of a sufficient supply of essential nutrient elements by the organic nutrient sources to bridge the deficiency gap in the soil used (Baiyeri et al., 2016). Following the recommendation of Baiyeri et al. (2016) that higher soil pH can help bridge the putative nutrient deficiency gap of the soil, poultry, and pig manure would be more useful in bridging the nutrient deficiency gap in the soil than the commercial NPK fertilizer. The total nitrogen recorded in poultry and goat manure was comparable to NPK fertilizer. Olatunji and Agele (2015) had earlier linked the increase in plant agro-

Table 2. Effect of *Capsicum annuum* L. cultivars and nutrient sources on mean value of fruits carotenoids.

| Cultivar | Nutrient sources | α -carotene (mg/L) | β -carotene (mg/L) | Capsanthin (mg/L) | Lutein (mg/L) | Total carotenoids (mg/L) |
|---------------|------------------|----------------------------|----------------------------|-----------------------------|----------------------------|------------------------------|
| Atarugu | Poultry | 0.91 ^g ± 0.03 | 0.97 ^e ± 0.01 | 0.46 ^f ± 0.01 | 0.00 ^f ± 0.00 | 11.14 ^l ± 0.12 |
| | Pig | 0.42 ^{ghi} ± 0.02 | 0.48 ^{fg} ± 0.02 | 0.15 ^f ± 0.08 | 0.00 ^f ± 0.00 | 5.10 ^l ± 0.03 |
| | Goat | 0.63 ^{gh} ± 0.01 | 0.86 ^{ef} ± 0.02 | 0.39 ^f ± 0.01 | 0.00 ^f ± 0.00 | 13.78 ^l ± 0.01 |
| | NPK | 0.00 ⁱ ± 0.00 | 0.00 ⁱ ± 0.00 | 0.00 ^f ± 0.00 | 0.00 ^f ± 0.00 | 2.28 ^l ± 0.05 |
| | Control | 0.00 ⁱ ± 0.00 | 0.00 ⁱ ± 0.00 | 0.00 ^f ± 0.00 | 0.00 ^f ± 0.00 | 190.13 ^j ± 3.95 |
| Shombo | Poultry | 26.89 ^d ± 0.02 | 0.16 ^{ghi} ± 0.09 | 2.32 ^f ± 0.02 | 0.09 ^{ef} ± 0.05 | 70.36 ^k ± 1.00 |
| | Pig | 0.12 ^{hi} ± 0.01 | 0.02 ^{hi} ± 0.003 | 0.13 ^f ± 0.003 | 0.07 ^{ef} ± 0.01 | 0.67 ^l ± 0.01 |
| | Goat | 0.10 ^{hi} ± 0.003 | 0.4 ^{gh} ± 0.29 | 0.16 ^f ± 0.01 | 0.09 ^{ef} ± 0.003 | 1.47 ^l ± 0.02 |
| | NPK | 137.88 ^b ± 0.78 | 0.23 ^{ghi} ± 0.03 | 164.19 ^b ± 0.54 | 0.23 ^{def} ± 0.13 | 933.08 ^g ± 7.07 |
| | Control | 0.04 ^{hi} ± 0.03 | 0.22 ^{ghi} ± 0.06 | 0.20 ^f ± 0.12 | 0.08 ^{ef} ± 0.04 | 915.72 ^g ± 0.76 |
| Tatase | Poultry | 76.06 ^c ± 0.38 | 0.41 ^{gh} ± 0.25 | 213.13 ^a ± 0.66 | 0.08 ^{ef} ± 0.03 | 571.6 ^l ± 0.39 |
| | Pig | 0.18 ^{hi} ± 0.03 | 0.04 ^{hi} ± 0.02 | 82.55 ^{cd} ± 28.45 | 0.35 ^{de} ± 0.19 | 839.29 ^h ± 3.59 |
| | Goat | 0.07 ^{hi} ± 0.03 | 25.09 ^c ± 0.09 | 168.07 ^b ± 0.42 | 255.4 ^a ± 0.26 | 928.94 ^g ± 0.47 |
| | NPK | 0.00 ^l ± 0.00 | 0.01 ⁱ ± 0.01 | 100.72 ^c ± 0.79 | 0.06 ^{ef} ± 0.03 | 2184.21 ^a ± 41.7 |
| | Control | 0.13 ^{hi} ± 0.06 | 2.55 ^d ± 0.05 | 67.74 ^d ± 0.81 | 0.08 ^{ef} ± 0.04 | 2053.28 ^b ± 10.74 |
| Nsukka yellow | Poultry | 7.53 ^f ± 0.17 | 45.56 ^a ± 0.29 | 93.74 ^c ± 0.16 | 45.20 ^c ± 0.14 | 1247.78 ^c ± 11.26 |
| | Pig | 9.08 ^e ± 0.04 | 29.56 ^b ± 0.29 | 46.32 ^e ± 0.18 | 0.49 ^d ± 0.09 | 1081.32 ^d ± 4.11 |
| | Goat | 333.48 ^a ± 0.27 | 0.26 ^{ghi} ± 0.13 | 5.60 ^f ± 0.16 | 185.16 ^b ± 0.26 | 955.51 ^f ± 0.71 |
| | NPK | 0.06 ^{hi} ± 0.03 | 0.09 ^{ghi} ± 0.07 | 9.67 ^f ± 0.17 | 0.06 ^{ef} ± 0.03 | 1000.53 ^e ± 1.13 |
| | Control | 0 ^l ± 0 | 0.05 ^{hi} ± 0.03 | 0.03 ^f ± 0.01 | 0.03 ^f ± 0.03 | 923.77 ^g ± 0.46 |
| LSD | | 0.59 | 0.39 | 18.21 | 0.30 | 19.15 |

Values are presented as mean ± standard error and significant means are separated with different alphabets on the same column using Least Significant Difference Test (F-LSD) at P ≤ 0.05.

Source: Authors

morphological traits to the availability of nitrogen that helps in plant growth and development. Therefore, poultry and goat manure would be a reliable replacement of the inorganic fertilizer for the supply of nitrogen required for plant growth and development.

Significant (P ≤ 0.05) variations were observed across the nutrient sources and cultivars for the different carotenoids concentration (Table 2). While α -carotene was the highest in Nsukka yellow planted on soil mixed with goat manure, β -carotene was the highest in Nsukka yellow planted on soil mixed with poultry manure. Tatase cultivar planted on soil mixed poultry manure expressed the highest Capsanthin level while Lutein was highly expressed in Tatase cultivar planted on soil mixed with goat manure. This could be an indication that the availability of some nutrient sources supported the synthesis of some of the carotenoids in some of the cultivars. These observations were in harmony with the works of Antonius et al. (2014), which reported that concentration of carotenoids and antioxidant content in the fruits of *C. annuum* varied and were significantly dependent on soil treatment. Ha et al. (2007) and Guzman et al. (2010) also indicated that concentrations

of carotenoids in pepper fruits are highly dependent on soil nutritional factors, growth stage of fruit and also the colour of fruits, while Sarafi et al. (2018) asserted that, the concentration of carotenoid for a given cultivar depends mainly on the morphological and physiological characteristics of that cultivar in addition to certain growth factors. From the results of this research, the organic nutrient sources especially the poultry and goat nutrient source was generally better than the inorganic and control in enhancing the production of different carotenoids. This observation conforms with the work of Wu et al. (2013), who reported antioxidant activity under organic fertilization were higher than under mineral fertilization. Therefore, the presence of various major and minor elements in organic fertilizers may have contributed to the increase in secondary metabolites and antioxidant potentials as compared to the case of mineral fertilizers that contain only three basic minerals which include; Nitrogen, Potassium, and Phosphorous (Ibrahim et al., 2013).

Variations in the concentration of different flavonoids such as myricetin, quercetin, kaempferol, and luteolin, including the total flavonoids across the cultivars and

Table 3. Effect of *Capsicum annuum* L. cultivars and nutrient sources on mean value of fruits flavonoids.

| Cultivar | Treatment | Myricetin (mg/L) | Quercetin (mg/L) | Kaempferol (mg/L) | Luteolin (mg/L) | Total flavonoids (mg/L) |
|---------------|-----------|-----------------------------|------------------------------|---------------------------|----------------------------|----------------------------|
| Atarugu | Poultry | 721.10 ^a ± 4.55 | 0.11 ^{de} ± 0.06 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 727.12 ^b ± 0.11 |
| | Pig | 460.86 ^b ± 0.34 | 285.97 ^a ± 0.95 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 724.80 ^b ± 0.46 |
| | Goat | 165.30 ^c ± 0.21 | 0.19 ^{de} ± 0.10 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 179.35 ^h ± 0.39 |
| | NPK | 0.05 ^f ± 0.04 | 0.14 ^{de} ± 0.01 | 24.13 ^c ± 0.16 | 3.13 ^b ± 0.06 | 54.38 ⁱ ± 0.29 |
| | Control | 0.00 ^f ± 0.00 | 0.00 ^e ± 0.00 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 91.84 ⁱ ± 0.20 |
| Shombo | Poultry | 0.08 ^f ± 0.04 | 0.03 ^e ± 0.02 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 527.89 ^e ± 2.66 |
| | Pig | 0.002 ^f ± 0.0003 | 0.004 ^e ± 0.0003 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 285.49 ^g ± 1.16 |
| | Goat | 0.00 ^f ± 0.00 | 0.00 ^e ± 0.00 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 54.77 ^l ± 0.24 |
| | NPK | 0.00 ^f ± 0.00 | 0.00 ^e ± 0.00 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 415.09 ^f ± 0.13 |
| | Control | 0.001 ^f ± 0.001 | 0.29 ^{de} ± 0.02 | 0.00 ^d ± 0.00 | 6.95 ^a ± 0.39 | 895.55 ^a ± 2.11 |
| Tatase | Poultry | 6.34 ^e ± 0.17 | 0.0003 ^e ± 0.0003 | 42.31 ^a ± 0.34 | 0.00 ^d ± 0.00 | 693.97 ^c ± 0.53 |
| | Pig | 72.20 ^d ± 0.21 | 181.73 ^b ± 0.53 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 599.42 ^d ± 1.59 |
| | Goat | 0.00 ^f ± 0.00 | 0.002 ^e ± 0.0001 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 81.46 ^k ± 0.63 |
| | NPK | 0.002 ^f ± 0.001 | 0.59 ^{de} ± 0.02 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 0.61 ^o ± 0.01 |
| | Control | 0.00 ^f ± 0.00 | 0.82 ^d ± 0.02 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 3.69 ⁿ ± 0.07 |
| Nsukka yellow | Poultry | 5.19 ^e ± 0.04 | 0.002 ^e ± 0.001 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 6.15 ⁿ ± 0.09 |
| | Pig | 0.003 ^f ± 0.0003 | 0.14 ^{de} ± 0.03 | 25.03 ^b ± 0.04 | 0.28 ^c ± 0.01 | 84.96 ⁱ ± 0.41 |
| | Goat | 5.5 ^e ± 0.25 | 0.31 ^{de} ± 0.04 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 10.08 ^m ± 0.14 |
| | NPK | 0.002 ^f ± 0.001 | 9.22 ^c ± 0.26 | 0.00 ^d ± 0.00 | 0.00 ^d ± 0.00 | 9.51 ^m ± 0.12 |
| | Control | 0 ^f ± 0 | 0.001 ^e ± 0.001 | 0.03 ^d ± 0.006 | 0.03 ^{cd} ± 0.003 | 5.29 ⁿ ± 0.09 |
| LSD | | 2.93 | 0.72 | 0.24 | 0.26 | 2.63 |

Values are presented as mean ± standard error and significant means are separated with different alphabets on the same column using Least Significant Difference Test (F-LSD) at P ≤ 0.05.

Source: Authors

nutrient sources were observed (Table 3). Myricetin concentration was significantly the highest in Atarugu cultivar grown with poultry manure; quercetin was the highest in the same cultivar grown with pig manure, while kaempferol was the highest in Tatase grown with poultry manure (Table 3). Generally, the nutrient sources affected the flavonoids concentrations differently. More so, the organic nutrient sources favoured the synthesis of more flavonoids than the control and NPK fertilizer. This report corroborates the works of Mohd et al. (2013) that phenolics and flavonoids were enhanced by 12 and 22%, respectively in treatment with organic fertilizer as compared to inorganic fertilization. Zhang and Hamazu (2003), Marinova et al. (2005) and Navarro et al. (2006) also emphasized that the concentration of flavonoids and phenols depends greatly on cultivation, ripeness, storage, and soil salinity. The positive influence of organic fertilizers in producing crops with enhanced phytochemicals and bioactive compounds was also asserted in the reports of Mitchell et al. (2007). They observed that organic crop management practices led to the increase in flavonoids content in tomatoes and also an increase in the levels of antioxidant as was analyzed

in strawberry. The variations recorded among the cultivars in this research are an excellent indication of the variations abounds in the concentration of different flavonoids in different cultivars of pepper. This observation could be attributed to the genotypic variation inherent in the DNA of these pepper cultivars. Araceli et al. (2011) reported similar findings, that apart from the fruit shape and appearance, pepper fruits also vary in their content of vitamin C, phenols, flavonoids, beta-carotene, capsaicin, and dihydrocapsaicin depending on the ripeness stage. This report is also in agreement with the works of Sarafi et al. (2018), who opined that genetic and abiotic stress may be responsible for the overall phytochemical concentration in peppers. Therefore, genotypes respond differently to an organic treatment.

Within the scope of this research, it could be established that growing crops with organic nutrient sources would be more productive; they also cost less amount in acquisition when compared with the inorganic fertilizer and most importantly, they contribute to a safer environment with less pollution than the inorganic nutrients. Invariably, consumption of pepper fruits with high concentration of the micronutrients most especially

the carotenoids and flavonoids would help combat a lot of latent but dangerous diseases that could result to serious health challenges because pepper fruits with high concentration of these micronutrients will help provide the Recommended Daily Allowances (RDA) of these micronutrients when consumed in our diets.

Conclusion

Poultry and goat manure were found to increase soil nutrients and could be reliable replacement of the inorganic fertilizer for the supply of nitrogen required for plant growth and development. The manure sources influenced the production of bioactive compounds in peppers. On the average, poultry manure supported the synthesis of bioactive compounds more. Growing Nsukka yellow with goat manure increased the production of α -carotene, while poultry manure increased the production of β -carotene. Growing Tatase on poultry manure increased the production of capsanthin levels while goat manure increased the production of lutein. Poultry manure favoured the production of myricetin in Atarugu, while pig manure increased the production of quercetin and kaempferol was the highest in Tatase grown with poultry manure.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Abu NE, Ojua EO, Udensi OU (2019b). Induction of variability in three Nigerian pepper varieties using gamma irradiation. *Journal of Experimental Agriculture International* 42(4):111-119.
- Abu NE, Omeke JO, Ojua EO (2019a). Effect of different mutagens on some mineral, phytochemical and proximate composition of two red pepper varieties. *Annual Research and Review in Biology* 33(2):1-13. <https://doi.org/10.9734/ARRB/2019/v33i230116>
- Antonius GF, Turley ET, Hill RR (2014). Antioxidant contents of bell pepper and melon fruits grown in soil amended with recycled waste. *Journal of Environmental Science and Health, Part B* 49(5):361-365. <https://doi.org/10.1080/03601234.2014.882173>
- Araceli MV, Jose LC, Jose CC, Mercedes GL (2011). Phytochemical evaluation of wild and cultivated pepper (*Capsicum annuum* L. and *C. pubescens* Ruiz and Pav.) from OAXACA Mexico. *Chilean Journal of Agricultural Research* 71(4):578-585.
- Association of Official Analytical Chemist (AOAC) (2005). Official methods of analysis, 6th ed., Washington DC, USA.
- Baiyeri KP, Otitoju GT, Abu NE, Umeh S (2016). Poultry manure influenced growth, yield and nutritional quality of containerized aromatic pepper (*Capsicum annuum* L., var "Nsukka Yellow"). *African Journal of Agricultural Research* 11(23):2013-2023. <https://doi.org/10.5897/AJAR2015.10512>
- Blanco-Ríos AK, Medina-Juarez LA, González-Aguilar GA, Gamez-Meza N (2013). Antioxidant activity of the phenolic and oily fractions of different sweet bell peppers. *Journal of the Mexican Chemical Society* 57(2):137-143. <https://doi.org/10.29356/jmcs.v57i2.226>
- Bouyoucos GJ (1951). A Recalibration of the Hydrometer Method for Making Mechanical Analysis of Soils¹. *Agronomy Journal* 43(9):434-438. <https://doi.org/10.2134/agronj1951.00021962004300090005x>
- Bremner JM, Mulvaney CS (1982). Total nitrogen In: Page, A. L; Miller, R. D. and Keeney, D. R. (Eds.), *Methods of Soil Analysis*. Soil Science Society of America, Madison, WI, USA.
- Bureau JL, Bushway RJ (1986). HPLC determination of carotenoids in fruits and vegetables in the United States. *Journal of Food Science* 51(1):128-130. doi:10.1111/j.1365-26211986.tb10851.x
- Chapman HD (1965). Cation-exchange capacity. In: Black, C.A. (ed.). *Methods of soil analysis Part 2-Chemical and microbiological properties*, Agronomy Monograph 9.2. American Society of Agronomy, Soil Science Society of America. Madison, WI, USA pp. 891-901. <https://doi.org/10.2134/agronmonogr9.2.c6>
- Global Nutrition Report (2014). *Actions and Accountability to Accelerate the World's Progress on Nutrition*. Washington, D.C.: International Food Policy Research Institute. Available at <https://www.ifpri.org/publication/global-nutrition-report-2014-actions-and-accountability-accelerate-worlds-progress> Accessed 03/04/2020
- Guzman IS, Hamby S, Romero J, Bosland PW, O'Connell MA (2010). Variability of carotenoid biosynthesis in orange colored *Capsicum* spp. *Plant Science* 179(1-2):49-59. <https://doi.org/10.1016/j.plantsci.2010.04.014>
- Ha SH, Kim JB, Park JS, Lee SW, Cho KJ (2007). A comparison of the carotenoid accumulation in *Capsicum* varieties that show different ripening colours: deletion of the capsanthin-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. *Journal of Experimental Botany* 58(12):3135-3144.
- Hendershot WH, Duquette M (1986). A simple barium chloride method for determining cation exchange capacity and exchangeable cations. *Soil Science Society of America Journal* 50(3):605-608. <https://doi.org/10.2136/sssaj1986.03615995005000030013x>
- Ibrahim MH, Jaafar HZ, Karimi E, Ghasemzadeh A (2013). Impact of Organic and Inorganic Fertilizers Application on the Phytochemical and Antioxidant Activity of Kacip Fatimah (*Labisia pumila* Benth). *Molecules* 18(9):10973-10988. <https://doi.org/10.3390/molecules180910973>
- Jadczak D, Grzeszczuk M, Kosecka D (2010). Quality characteristics and content of mineral compounds in fruit of some cultivars of sweet pepper (*Capsicum annuum* L.). *Journal of Elementology* 15(3):509-515. <https://doi.org/10.5601/jelem.2010.15.3.509-515>
- Kelly WT, Boyhan G (2009). Cultural practices and varieties. *Commercial Pepper Production Handbook: Bulletin 1309*. Cooperative Extension, The University of Georgia College of Agriculture and Environmental Sciences, Cooperative Extension Bulletin. Athens, GA.
- Khandaker MM, Rohani F, Dalorima T, Mat M (2017). Effects of different organic fertilizers on growth, yield and quality of *Capsicum Annuum* L. Var. Kulai (Red Chilli Kulai). *Bioscience and Biotechnology Research of Asia* 14(1):185-192. <https://doi.org/10.13005/bbra/2434>
- Liaven MA, Jimenez JL, Coro BI, Rosales RR, Molina JM, Dendooven L, Miceli FA (2008). Fruit Characteristics of bell pepper cultivated in sheep manure vermicompost substituted Soil. *Journal of Plant Nutrition* 31(9):1585-1598. <https://doi.org/10.1080/01904160802244738>
- Marinova D, Ribarova F, Atanassova M (2005). Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy* 40(3):255-260.
- Materska M, Perucka I (2005). Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). *Journal of Agricultural and Food Chemistry* 53(5):1750-1756.
- Mclean EO (1982). Soil pH and Lime Requirement. In: Page, A. L; Miller, R. D. and Keeney, D. R. (Eds.), *Methods of Soil Analysis, Part 2*, American Society of Agronomy, Madison, WI, USA. <https://doi.org/10.1002/jpln.19851480319>
- Meshram II, Arlappa N, Balakrishna N, Laxmaiah A, Mallikarjun RK, Gal RC, Ravindranath M, Sharad KS, Brahmam GN (2012). Prevalence and determinants of undernutrition and its trends among pre-school tribal children of Maharashtra State, India. *Journal of Tropical Pediatrics* 58(2):125-132. <https://doi.org/10.1093/tropej/fmr035>
- Mitchell AE, Hong Y, Koh E, Barret DM, Bryam DE, Denison RF (2007). Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. *Journal of Agricultural and Food Chemistry* 55(15):6154-6159.

- Mohd HI, Hawa ZEJ, Ehsan K, Ali G (2013). Impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of Kacip Fatimah (*Labisia pumila* Benth). *Molecules* 18(9):10973-10988.
- Navarro JM, Flores P, Garrido C, Martinez V (2006). Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chemistry* 96(1):66-73.
- Nelson DW, Sommers, LE (1996). Total carbon, organic carbon and organic matter. In: DL Sparks, AL Page, PA Helmke, RH Loeppert, PN Soltanpour, MA Tabatabai, CT Johnston, ME Sumner (Eds). *Methods of Soil Analysis, Part 3, 2nd edition*. American Society of Agronomy, Madison, WI, USA.
- Ojua EO, Abu NE, Ojua DN, Omeke JO, Eze NM, Okanwu JO, Chukwuma CK (2019). Effect of gamma irradiation on fruits of three pepper varieties. *International Journal of Science and Technology* 7(1):26-30. <https://doi.org/10.24940/theijst/2019/v7/i1/ST1901-020>
- Olatunji A, Agele OS (2015). Effects of nutrients sources and variety on the growth and yield of three cultivars of pepper (*Capsicum annuum* L.) in Southwestern Nigeria. *New York Science Journal* 8(10):21-29.
- Olsen SR, Sommers LE (1982). Phosphorus. In: Page AL, Keeney DR, Baker DE, Miller RH, Roscoe Ellis Jr, Rhoades JD (Eds.) *Methods of soil analysis, Part 2. Chemical and Microbiological Properties*. Agronomy Monograph No. 9. (Second edition), Madison, WI, USA.
- Perez-Lopez AJ, Del Amor FM, Serrano-Martinez A, Fortea MI, Nunez-Delicado E (2007). Influence of agricultural practices on the quality of sweet pepper fruits as affected by the maturity stage. *Journal of the Science of Food and Agriculture* 87(11):2075-2080. <https://doi.org/10.1002/jsfa.2966>
- Popkin BM, Adair LS, Ng SW (2012). Global nutrition transition and the pandemic of obesity in developing countries. *Nutrition Reviews* 70(1):3-21. <https://doi.org/10.1111/j.1753-4887.2011.00456.x>
- Sarafi E, Siomos A, Tsouvaltzis P, Chatzissavvidis C, Therios I (2018). Boron and maturity effects on biochemical parameters and antioxidant activity of pepper (*Capsicum annuum* L.) cultivars. *Turkish Journal of Agriculture and Forestry* 42(4):237-247. <https://doi.org/10.3906/tar-1708-31>
- Wu CS, Gao QH, Kjelgren RK, Guo XD, Wang M (2013). Yields, phenolic profiles and antioxidant activities of *Ziziphus jujube* Mill. in response to different fertilization treatments. *Molecules* 18(10):12029-12040. <https://doi.org/10.3390/molecules181012029>
- Zhang D, Hamauzu Y (2003). Phenolic compound, ascorbic acid, carotenoid and antioxidant properties of green, red and yellow peppers. *Journal of Food Agriculture and Environment* 1(2):22-27.