EFFECT OF ORGANIC AND INORGANIC SOURCES OF NUTRIENTS ON THE BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF TOMATO

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Abstract. The study was conducted to compare the levels of bioactive compounds and antioxidant activities of (*Solanum lycopersicum* var. cerasiforme) fruits cultivated on different soil amendments (cow dung, chicken droppings and ([40 g/kg N: 60 g/kg N: 40 g/kg N] Nitrogen-Phosphorus-Potassium) in a shaded house. Tomato seedlings were transplanted into pre-treated soils and watered daily in the afternoon for a period of five months. The general growth and yield performances were in the order of NPK > Chicken droppings > Cow dung. The fruits were harvested at maturity (red-ripened stage) and subjected to bioactive compounds analysis using High Performance Liquid Chromatography (HPLC). The results of the study showed strong relationship between the level of macronutrients like nitrogen, phosphorus and potassium in the soil and the level phenolics, flavonoids, beta-carotene and lycopene contents in tomato. The level of beta-carotene was significantly higher ($p < 0.05$) in the fruits cultivated in the NPK amended soil which was totally different to the result of other bioactive compounds like phenolics, flavonoids, and lycopene which recorded their highest concentrations in the fruits harvested from control and cow dung amended soil. Antioxidant activity was determined by adding 2,2-diphenyl-1-picrylhydrazyl to an aliquot of methanolic extract. The ability to scavenge radical was measured by reading the decrease in purple coloration of the solution using Ultraviolet-Visible (UV) Spectrophotometer. The tomato fruit from cow dung showed highest percentage of radical scavenging activities.
Keywords: *abiotic and oxidative stress, acetate shikimate pathway, cow dung, physiological changes, reactive oxygen species*

Introduction

The use of chemical fertilizer has been a popular and widespread practice in the effort to address global food security challenge resulting from low fertile agricultural soil. The dependence on these chemical fertilizers, particularly nitrogen fertilizer has become necessary to replenish soil nutrients and invariably improve the quantity and quality of agricultural produce. High crop yield and high biomass of plant from N-fertilizer application are responsible for increasing dependent on N-fertilizer (Camara et al., 2003; Guo et al., 2010). However, intensive usage of N-fertilizer has been reported to cause accumulation of nitrogen residues, nutrient toxicity, metal pollution, greenhouse gas emission, groundwater contamination and soil acidification (Han and Zhao, 2009; Sierra et al., 2015). Furthermore, research has shown that increase in applied nitrogen leads to increase in the soil electrical conductivity, available nitrogen, distortion of C/N ratio, distorting soil microbial community and reduction in nutritional quality of agricultural produce (Stuart et al., 2014; Sierra et al., 2015; Norman and Dazzo, 2016; Wei et al., 2018). It has been established that crops only take up 30-50%

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of chemical fertilizers and the remaining percentage are lost to the environment (Norse, 2005; Mózner et al., 2012).

Conversely, the use of organic manure, has been reported to improve biological, chemical and physical properties of the soil and invariably increase plant growth and yield because of its high organic matter content due to high microbial activity (Stephen et al., 2014; Mitran et al., 2017). Organic manure, as opposed to inorganic fertilizer has also been reported to increase the level of secondary metabolites like phenolic, flavonoid, and antioxidant activity in plant (Zeinab et al., 2013; Fließbach et al., 2007). For example, Wiebel et al. (2000) reported a 19% higher phenolic content for organically produced apples compared to the inorganically produced ones. Higher level of phenolic content was also recorded for organically grown strawberries compared to inorganically cultivated ones (Hakkinen and Tomonen, 2000). All these reported cases have led to growing preference by consumers for organic agricultural produce (Hughner et al., 2007; Mie, et al., 2016). All over the world, attention has now shifted towards the use of organic manures because of the perceived health benefits and higher nutritional contents believed to be derived from products from these types of farming (Worthington, 2001; Magkos et al., 2001; Tarozzi et al., 2006). This submission is supported by the studies which reported that the positive attitude of the consumers towards organic food is based on their perception of higher nutritional contents and better tasting of organic food compared to the conventional or inorganic foods (Hunter et al., 2011; Smith-Springer et al., 2012). Organic manures are said to enhance antioxidant activity and bioactive compounds like phenol, flavonoid, beta-carotene and lycopene contents of fruits and vegetables (Dumas et al., 2003; Mohd et al., 2013). The beneficial effects of these plant bioactive compounds range from giving fruits their quality tastes and distinct colors in the case of phenolic and lycopene compounds, to their nutraceutical properties such as prevention of degenerative and carcinogenic diseases (Johnson et al., 2014; Canene-Adams et al., 2005). These reported reasons and benefits of organic farming have prompted scientists and farmers to embark on the use of organic manures in the cultivation of agricultural crops, fruits and vegetables.

However, there are a lot of reports which contradicts the suggestion of superior nutritional quality of organic produce over conventional ones. This study was therefore setup to investigate the effects of these different nutrient sources on the nutritional quality of *Solanum lycopersicum* var. Cerasiforme commonly known as tomato.

Tomato is a fruit bearing herbaceous plant grown annually for its many nutritional contents. It is an important fruit with a wide range of reported health benefits and cultivated in all continents except Antarctica (Ilahy et al., 2011; Pinela et al., 2012). It is one of the cheapest and most readily available sources of proteins, minerals, vitamins and essential amino acids (Stephen et al., 2014), and is said to be rich in antioxidants and bioactive compounds such as phenolics, flavonoids, beta-carotene and lycopene contents which are reported to serve as endogenous defense mechanisms produced in response to pathogens (Pinela et al., 2012; Bhowong et al., 2009; Simova-Stoilova et al., 2008). The concentration level of these bioactive compounds has been linked with the system of production. The effort to reduce the negative impacts of mineral fertilizers on the environment and the increasing demands of consumers for tomato fruit due to its nutritional and health benefits have caused scientists and growers to device means of meeting the demand for quality produce. This study was therefore conducted to compare the effect of organic manures and chemical fertilizer on the growth rate and the levels of bioactive compounds in tomato.

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Methodology

A farming experiment was set up at the production unit of Sefako Makgatho Health Sciences University located in the northern part of Pretoria (Tshwane metropolitan) South Africa, on a coordinate (25°37’8” and 28°1’22” E). The study area experiences a climate of long hot, rainy summer and a short cool and dry winter with an average annual temperature of 18 °C (65.7 °F).

Experimental design and planting

The farming experiment was carried out in the summer period of September 2015 – March 2016. The study was conducted on red beefsteak tomato with a globe shape fruit, smooth and glabrous skin and weighs between 0.12 – 0.18 kg. The design comprised three different soil amendments (cow dung, chicken droppings and Nitrogen-Phosphorus-Potassium (NPK) fertilizer with a control experiment of soil with without any amendment. The experimental design consisted of comparable amounts of 0.15 kg of inorganic amendment (NPK fertilizer) manufactured by Omina Fertilizers Johannesburg, South Africa was purchased from a registered marketer, Plantland Kwikery Akasia Pretoria North, South Africa. The dry organic amendments (cow dung and chicken droppings) were collected from a registered livestock farm De-Wildt Brits Road North west South Africa. The 0.15 kg weight of the $N_{40}P_{60}K_{40}$, i.e. N_{40} is 40% Nitrogen, P_{60} is 60% phosphorus and K_{40} is 40% potassium used as the basis for the comparison of the organic amendments falls within the recommended doses of NPK fertilizer for the cultivation of tomato (Hebbar et al., 2004). The experiment was a randomized block design which contained 32 pots, each with a 22 cm diameter (Alain et al., 2013) divided into four groups which were each filled with 5 kg of sandy-loam soil mixed with 0.15 kg of the amendment. The first group were each filled with an equal quantity of soil mixed with dry 0.15 kg of cow dung; the second group contained equal quantity of soil thoroughly mixed with dry 0.15 kg of chicken droppings; the third group were filled with the same quantity of soil thoroughly mixed with 0.15 kg of pellet $N_{40}P_{60}K_{40}$ fertilizer and the last group contained pot plants each filled with equal quantity of soil without any treatment which served as the control. The soils were continuously watered and turned over at two weeks interval with garden forks and left for over a month after the addition of the treatments for proper mineralization of the amendments to take place (Ayeni, 2014). The Pot plant saucers/ trays were placed under the perforated bottom pots to prevent nutrient loss by runoff during irrigation. The seedlings of tomato at two weeks old purchased from a registered nursery, Plantland Kwikery Nursery Akasia Pretoria North were transplanted into each pot plant and watered daily using a measuring cylinder with each plant receiving the same volume of water enough to moist the soil and the plants and preventing runoff.

Soil nutrients

The soil is a sandy loamy with 71% sand, 15% silt and 14% clay. The control and cow dung amended soils were low in total N, available P and exchangeable K compared to NPK and chicken droppings treated soils (*Table 1*).

Data collection

Parameters such as plant height, stem girth and leaf area index were recorded for the determination of growth performance of tomato plants from different amendments and the

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control following the procedure of (Stephen et al., 2014). The Plant height was measured using a meter rule. Stem girth was recorded by using a vernier caliper and the leaf area index was measured by determining the average of the distances between the two longest and the two shortest branches using a meter rule (Witness et al., 2016).

Table 1. *Composition of soil nutrients*

Treatments	Total N (%)	Available P (µg/g)	Exchangeable K (µg/g)
Cow dung	0.8	552	535
Chicken droppings	1.2	647	443
NPK	1.8	670	456
Control	0.6	518	376

Preparation of tomato fruit for phytochemical and antioxidant activity analyses

Tomato fruits from soil treated with different amendments and that of the control were harvested after ripening. Visually selected injury free, ripened fruits were picked and immediately transferred to the laboratory where they were cut into small pieces and homogenized. The homogenates were then frozen in a -80 °C refrigerator and freeze-dried for the phytochemical and antioxidant activity analyses.

Extraction and determination of free radical scavenging activity

The methanolic extraction was carried out by sonicating the mixture of 100 ml of 50% methanol into an Erlenmeyer flask containing 6 g of freeze-dried powdery sample for 60 min. The resulting solution was thereafter filtered through a Whatman No. 1 filter paper and the filtrate (extract) placed in fume cupboard for 24 h for a complete evaporation of methanol. The determination of free radical scavenging activity was done by following the procedure described by (Biehler et al., 2010). An aliquot (10 mg) of methanolic extract was dissolved in 1 ml of 50% methanol from which 30 µl of the solution was pipetted into an Eppendorf tube. This was made up to 750 µl volume to which 750 µl of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was added. The solution was incubated for 40 min in the dark at room temperature. The decrease in the purple coloration of the reaction mixtures was read at 517 nm using an Ultraviolet-Visible Spectrophotometer (SPECORD 210 PLUS). Ascorbic acid was used as the standard and methanol used for extraction served as a negative control. The assay was performed in triplicate and the radical scavenging activity (RSA) was calculated using *Equation 1:*

$$RSA(\%) = A_0 - \frac{A_1}{A_0} \cdot 100 \quad \text{(Eq.1)}$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Beta-carotene and lycopene contents

Extraction and quantification of β-carotene were done using the method described by (Moyo et al., 2017). Samples were extracted with ice-cold hexane: acetone (1:1, v/v). The mixture was vortexed before centrifuging at 2000 rpm for 2 min and the organic phase decanted into another test tube containing 5 ml sodium chloride solution. The

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residue was similarly re-extracted and was combined in the saturated sodium chloride solution each time until the extract was colorless. The separated organic phase was injected into a High-Performance Liquid Chromatography (HPLC) equipped with a Photodiode Array Detector (PDA) and a mobile phase consisted of (7:2:1) acetonitrile-dichloromethane-methanol with an injection volume of 20 µl and detection at 450 nm. Peak identification and quantification were achieved with comparison to authentic β-carotene standard which was used for the plotting of the calibration curve (Moyo, et al., 2017).

Total phenolic

The method described by Singleton et al. (1999) was used for the extraction and the determination of the total phenolic content. An aliquot (10 ml) of 50% methanol was added into an Erlenmeyer flask containing 0.2 g of the freeze-dried tomato sample and the mixture sonicated for 30 min. The resulting solution was subsequently centrifuged in a HERMLE Z513 at 2000 rpm for 2 min. About 50 µl of the supernatant was pipetted into a new test tube and 450 µl of distilled water, 450 µl of 1N folin ciocalteu reagent, 125 µl of sodium carbonate solution were added. The solution was vortexed and incubated for 40 min at room temperature. Different aliquots of gallic acid (0.1 mg/ml) was used as the standard for plotting the calibration curve and total phenolic content was expressed in mg gallic acid equivalents (GAE) per g dry weight.

Flavonoid content

The flavonoid content was quantified using aluminum chloride colorimetric method as described by (Zhisten et al., 1999). An aliquot (0.2 g) of the freeze-dried tomato sample was weighed into an Erlenmeyer flask into which 10 ml of 50% methanol was added and the mixture sonicated for 30 min. The solution was then vortexed and centrifuged at 2000 rpm for 2 min and the supernatant pipetted into a new test tube. About 250 µl of the supernatant was pipetted into a new test tube and 1 ml of distilled water, 75 µl of 5% $NaNO_2$ were added. After 5 min, 75 µl of 10% $AlCl_3$, 500 µl of 1 M NaOH and 600 µl of distilled water were added and the solution vortexed. Different aliquots of gallic acid (1 mg/ml) catechin was used as standard for the calibration curve and flavonoid content was expressed in mg catechin equivalents (CE) per g DW.

Statistical analysis

All statistical analyses were performed using SPSS 24.0 by carrying out analysis of variance to determine the statistical significance and post hoc test using Tukey for the separation of the means of growth performance, bioactive compounds and scavenging activity from the different treatments.

Results

Effect of different soil amendments on the growth and yield performances

The results of the growth and yield performances were presented in *Tables 1* and *2* (Aina et al., 2018). Although, plants from control soil and cow dung amended soil recorded higher plant height and canopy size at the early stage after transplanting. This observation according to literature, may be due to the physiological adjustment of plant

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to the new environment (Ayeni et al., 2014). Overall, the tomato cultivated in inorganic fertilizer (NPK) recorded the best growth rate which was determined by recording the height, canopy size and stem girth of the plant. Similarly, the yield results showed that NPK produced the highest number of tomato fruits, followed by chicken droppings and with a yield pattern of NPK > chicken droppings > cow dung > control. This yield result is also consistent with other study which suggested that it may be a result of deficiency and or slow mineralization of nutrient of organic manure during the plant growing and developmental stages (de Ponti et al., 2012).

Effect of different soil amendments on the level of bioactive compound concentrations

The varied level of bioactive compounds of the tomato fruits as influenced by different soil amendments are presented in *Table 2*. The results showed that the level of phenolics, flavonoids, and lycopene were lower in the fruits harvested from inorganic NPK fertilizer compared to their counterparts from the control and cow dung amended soil. The difference in the level of phenolics flavonoids and lycopene in the fruits cow dung manures, NPK and control soil were statistically significant, the difference in the lycopene across different treatments was statistically significant. However, in reverse pattern, beta-carotene (*Fig. 1*) was significantly higher in fruit harvested from the soil treated with NPK fertilizer compared to the ones from the rest of the amendments and control. Finally, although the tomato fruit from cow dung amended soil recorded the highest concentration of potential radical scavenging activity, there was however no significant difference in the radical scavenging activity of the fruits across the group (*Fig. 2*).

Discussion

It has been suggested that nitrogen stimulates more roots production which in turn enhance plant growth and yield performance (Sultana et al., 2012; Rai et al., 2013). The study done by Mani et al. (2002) reported higher plant height and yield in plant cultivated with NPK fertilizer and attributed this observation to higher N and P contents. Chemical fertilizer like NPK has been reported to increase plant biomass and crop yield due to its richness in N and P contents which are reported to promote growth in plant (Ekbic et al., 2010; Zeng et al., 2011; Stephen et al., 2014; Mishra and Singh, 2006). The growth result of the current study is consistent with all these previous observations. In this study, application of NPK fertilizer enhances plant height, stem girth and leaf area index considered to be key factors in determining growth vigor. The higher growth response of tomato may be due to the higher nitrogen (N) and phosphorus (P) contents in the NPK compared to other treatments (*Table 1*).

This observation corroborates previous findings that NPK and poultry manure recorded better yield compared to control and cow dung (Znidarcic et al., 2007; Hague, 2012). The better growth rate and yield performance of NPK and chicken droppings compared to the cow dung and control could be due to the high content of essential nutrients like N and P in poultry manure which have been reported to enhance photosynthetic activity capable of promoting root development, vegetative growth and crop yield (John et al., 2004; Lima et al., 2012; Isah et al., 2014; Nafiu et al., 2011; Meysam et al., 2017).

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Table 2. *The mean concentration of bioactive active compounds in response to different soil treatments with different letter(s) indicating significant difference at 95% confidence interval*

Treatments	Phenolics (mg GAE/g DW)	Flavonoids (mg CE/g DW)	Lycopene (mg/100 g DW)
Cow dung	3.90[a]	0.40[b]	129.76[b]
Chicken droppings	3.20[b]	0.27[c]	114.50[d]
NPK	3.81[a]	0.34[b]	123.21[c]
Control	3.91[a]	0.56[a]	132.66[a]

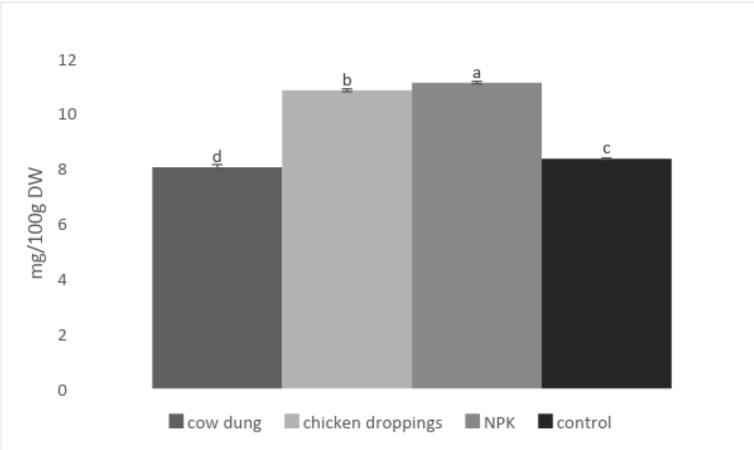

Figure 1. *The level of beta-carotene in tomato fruits with different letters indicating significant differences ($p < 0.05$)*

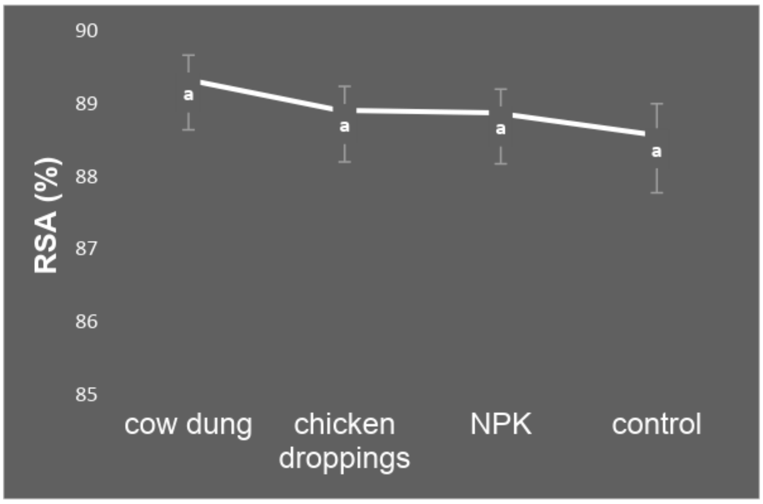

Figure 2. *Free radical scavenging activity of tomato fruits same letter indicating no significant difference ($p > 0.05$)*

On the other hand, organic manures are reportedly less concentrated nutrient sources compared to mineral or chemical fertilizers and are noted to be very slow in mineralization rates, leading to lower nutrient bioavailability during growth and development nutrients demanding stages (Zhao, et al., 2009; Lester and Saftner, 2011;

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Seufert et al., 2012). This condition causes organically cultivated tomato to experience; 1. oxidative stress from superoxide dismutase which is a key enzyme in plant defense and development (Chang et al., 2008), and 2. abiotic stress which causes accumulation of Reactive Oxygen Species (ROS) that inhibits enzymatic activity, disturbs cellular homeostasis and ruptures membrane with deleterious effects on plant growth (Luis, 2015; Baxter et al., 2014). Consequently, organically grown vegetables react to this condition by activating their own defense mechanisms which leads to the synthesizing of more bioactive and antioxidant compounds (Vallverdu-Queralt et al., 2011). They activate signaling pathway for the detoxification of ROS by synthesizing antioxidant that scavenges on ROS (Sharma et al., 2012).

It is therefore conceivable that the result of this study which showed the tomato fruits cultivated in control and organic cow dung amended soils producing higher phenolic, flavonoid and lycopene contents in comparison to their counterparts from chicken droppings and especially inorganic NPK fertilizer amended soil corroborates these reports. The cow dung amended soil as well as the control were low in essential nutrients like N and P in comparison to the soil treated with chemical (NPK) fertilizer (*Table 1*). Several literatures have linked the accumulation of phenolics, flavonoids and lycopene to the level of available N in the soil. For instance, Oliveira et al. (2013) and Mitchell et al. (2007) in their separate studies which investigated the effects of organic and inorganic fertilizers on plant secondary metabolites, reported that organically produced tomatoes accumulated higher concentrations of bioactive compounds like total phenolics and flavonoids compared to inorganically grown ones. These compounds have been reported to accumulate more in plants cultivated on organic manures due to low level of N (Mittler, 2002; Abd El-Moniem et al., 2012; Mohd et al., 2013). Numerous studies have explained that plants tend to produce more or higher level of bioactive compounds and antioxidant as a preventive or protective measure against oxidative and abiotic stress which may result from low level or slow release of macronutrient like N (Caris-Veyrat et al., 2004; Toor et al., 2006; Wang et al., 2008; Vallverdu-Queralt et al., 2012; Oliveira et al., 2013). This conclusion is backed up by several other studies on the nutritional quality of fruits and vegetables, which reported that organically produce have approximately three times less nitrate in comparison to conventional crops due to lower availability of nitrogen in organic farming systems (Worthington, 2001; Williams, 2002; Tuomisto et al., 2012).

The result of the current study is consistent with the above submissions as evident in the concentrations of phenolics, flavonoids and lycopene in the tomato fruits from control and cow dung amended soils, both growing media with lower level of available N compared to NPK fertilizer and chicken droppings. Whereas lower concentrations of total phenolic, flavonoid and lycopene were detected in the fruits harvested from soils amended with chicken droppings and NPK fertilizer, both growing media with higher level of available N. It was therefore assumed that there is an inverse relationship between the concentration of bioactive compounds like phenolics, flavonoids, lycopene and the level of available N in the soil.

Other studies concluded that organic manures have stimulatory effect on the accumulation of bioactive compounds by inducing acetate shikimate pathway in biosynthesis, resulting in higher production of secondary metabolites like flavonoids and phenolics (Sousa et al., 2008; Yoldas et al., 2008). It must be noted that although the results of this study showed a significantly higher lycopene content in both the control and the organic cow dung amended soils, some studies have argued that, types

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of soil fertilization have no effect on lycopene content of fruits. It was explained that lycopene content is accumulated more in the deep ripen stage (Toor et al., 2006; Ilahy et al., 2011). The current study therefore identified this area for further studies in future research.

This study however showed a result of opposite pattern for beta-carotene which was significantly higher in the fruit cultivated in the soil treated with NPK fertilizer and closely followed by that from chicken droppings. This could be due to the higher content of available P in the NPK fertilizer and chicken droppings manure which has been reported to increase beta-carotene contents in fruits and vegetables (George and Zhou, 2002; Abd El-Baky, 2010). Furthermore, the action of phosphorus on enzymes like phosphofructokinase, pyruvate kinase and precursors of pyruvate has been linked with the biosynthesis of carotenoid (Bramely, 2002; Black et al., 2008). The study by Meysam et al. (2017) also reported that increased level of P application led to increase level of carotenoid production.

Although, there was no statistical significance in the potential ability to inhibit and retard oxidation processes, also known as radical scavenging activity of the fruits, it was however slightly higher in the fruits from the soil treated with cow dung. There is no conclusive report on what is responsible for higher production or stimulation antioxidant activity in plant. While some reports have suggested that organic production increases plant antioxidant activity (Janzantti et al., 2012; Vinha et al., 2014), other studies with similar observation to the current study have reported no significant difference in the antioxidant activity of plants from organic and conventional system of production (Fischer et al., 2007; Reche et al., 2019).

Conclusion

In general, this study demonstrated the responsiveness of bioactive compounds in tomato to the level of soil macronutrients which are relatively low or slowly released in organic amendments. It was observed that nitrogen content in the soil has a significant effect on the level of certain bioactive compound synthesis. Although, inorganic NPK fertilizer produced highest growth, yield and beta-carotene, control and organic cow dung amended soils had a higher stimulatory effect on the bioactive compounds like phenolic, flavonoid, lycopene and antioxidant activity. This study therefore concludes that in the effort to reduce environmental degradation through minimum dependence on chemical fertilizer, more usage of free and readily available organic manures as a source of soil fertility management should be recommended. The study also identifies that the link between method of farming system and other secondary metabolites as well as radical scavenging activity requires extensive and detailed study in future research.

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