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Effect of NPK fertilizer rates on secondary metabolites of pepino melon (*Solanum muricatum* Aiton)

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Secondary metabolites are bioactive compounds which are synthesized naturally in all plant parts. The quality and quantity of secondary metabolites produced by plants differ depending on the plant and environmental conditions under which they are produced. The purpose of the study was to investigate the effects of nitrogen, phosphorous and potassium (NPK) fertilizer (17:17:17) rates (0, 100, 200, 300 and 400 kg ha^{-1}) on the production of secondary metabolites in field and greenhouse grown pepino melons (*Solanum muricatum* Aiton). The experimental design was randomized complete block design with five NPK fertilizer treatments replicated three times. Results indicated that an increase in NPK fertilizer rate led to an increase of carotenoids (lutein, lycopene and β-carotene) up to a maximum at 200 kg NPK ha^{-1} after which the contents decreased in both growing environments and trials. The control (no fertilizer application) favored the accumulation of total phenolic content (TPC) in both growing environments and trials. Greenhouse grown pepino melon fruits which were not supplied with fertilizer (control) had a TPC content of 174.3 and 145.5 mg GAE 100g^{-1} fresh weight (FW) in trial one and two, respectively. Fertilizers could not enhance production of TPC in pepino melon fruits and application of 200 kg NPK ha^{-1} is recommended for maximum accumulation of carotenoids (lycopene, lutein and β-carotene).

Key words: Secondary metabolites, NPK fertilizer, greenhouse, field, pepino.

INTRODUCTION

Plants produce a wide variety of organic compounds which can be grouped as primary and secondary metabolites. Primary metabolites include organic acids, amino acids and phytosterols and they play vital roles in respiration, photosynthesis, growth and development in plants. In contrast, secondary metabolism is a pathway through which small molecule products are produced and they are not involved in growth and development of

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plants (Yang et al., 2018a). Secondary metabolites empower plants to adjust to biotic and abiotic stresses and furthermore as a method of correspondence with symbiotic microorganisms as well as to attract pollinators and seed dispersal agents (Wink, 2003). Plant secondary metabolites are classified based on their chemical structure and they include flavonoids, terpenoids, steroids and alkaloids (Yang et al., 2018a). Secondary metabolites have been used as conventional medicine, perfumery and as raw materials for industries (Balandrin et al., 1985).

Currently, carotenoids and phenolic compounds which are associated with secondary metabolites are of commercial importance because of their wide application in pharmaceutical, nutraceutical and cosmetic industries (Zheng et al., 2014). Carotenoids are a broad group of lipophilic yellow-orange pigments which are derivatives of tetraterpenes (Becerra et al., 2020). They are the most abundant pigments in nature and are needed by photosynthetic organisms (Sandmann et al., 2015). Carotenoids are classified as xanthophylls (lutein and zeaxanthin) and carotenes (β-carotene, lycopene) (Becerra et al., 2020). Carotenoids are found in leaves, roots, flowers and fruits and they possess an antioxidant activity which can protect humans against cardiovascular diseases, arthritis and cancer (Maiani et al., 2009). Additionally, β-carotene acts as pro-vitamin A while lutein protects the eye from UV radiation and is vital for brain development (Becerra et al., 2020).

Phenolic compounds are formed through the shikimate-phenylpropanoids-flavonoid pathway and are required by plants for growth, reproduction, pigmentation and protection from biotic and abiotic stresses (Ferrari, 2010). Phenolics are generally grouped into non-soluble phenolics like tannins, lignin and hydroxycinammic acids and soluble phenolics like phenolic acids, flavonoids and quinones (Krzyzanowska et al., 2010). Phenolics form an important part of human diet and they possess enormous natural antioxidant activity and other health benefits (Kumar and Goel, 2019). Phenolics have many biological and pharmacological properties such as antiviral, anticancer, anti-inflammatory, antimicrobial, antiallergic, antithrombotic, antidiabetic, hepatoprotective and food additive (Kumar and Goel, 2019).

Plant mineral nutrition not only promotes growth but also influences secondary metabolite content (Yang et al., 2018a). Nutrient deficiency can increase flavonoid accumulation specifically anthocyanins. Production of secondary metabolites in plants is also affected by soil nutrient availability (Wei et al., 2019). Suitable nutrient supply is required for the accumulation of secondary metabolites in plants (Gaude et al., 2007). The type and quantity of secondary metabolites produced by plants depends on the nutrients available in the soil (Wei et al., 2019). For instance, nitrogen deficiency in the soil favors accumulation of non-nitrogen secondary metabolites such as terpenoids and phenols whereas nitrogen sufficiency favors accumulation of nitrogenous secondary metabolites such as alkaloids and cyanogenic glycosides (Gershanzon, 1984). Anthocyanins, proanthocyanaidin and phenols accumulation was enhanced by application of 0, 50 and 100 kg nitrogen, phosphorous and potassium (NPK) ha^{-1} while application on 0 and 50 kg NPK ha^{-1} favored accumulation of flavonoids in pumpkin seeds (Oloyede et al., 2012). In another study, Ibrahim et al. (2013) found that application of NPK fertilizer above 90 kg NPK ha^{-1} resulted in reduction in TPC and flavonoids in *Labisia pumila* herb. β-carotene content in tomatoes was high in NPK treated plots compared to plots treated with organic fertilizers (Aina et al., 2019).

The synthesis and accumulation of secondary metabolites in plants depends on environmental conditions like light, temperature, soil water, soil fertility and salinity (Yang et al., 2018a). Plant secondary metabolites can be produced under environmental stresses and hence they play a role in adaptation and survival of plants in response to stimuli (Berini et al., 2018). Temperature is among the major environmental factors that significantly affect the composition of plant secondary metabolites, increasing temperature generally enhances the concentration of all secondary metabolites (Yang et al., 2018a). Increase in temperature increased phenolic compounds in three *Ribes nigrum* cultivars (Zheng et al., 2012). High temperatures were also reported to induce the biosynthesis of alkaloids (Yang et al., 2018a). Most of the studies have only majored on nitrogen and its effect on the accumulation of secondary metabolites in other vegetables but there is inadequate information on the effect of NPK fertilizer on accumulation of secondary metabolites in pepino melon. The present study therefore, aimed at investigating the effect of NPK fertilizer rates on accumulation of carotenoids and total phenolic content of field and greenhouse grown pepino melons.

MATERIALS AND METHODS

Experimental Site description

The experiment was conducted at the Horticulture Research and Teaching Field, Egerton University, Kenya. The field lies at a latitude of 0° 23' South, longitudes 35° 35' East in the Lower Highland III Agro Ecological Zone (LH3) at an altitude of approximately 2,238 m above sea level. Average maximum and minimum temperatures range from 19 to 22°C and 5 to 8°C, respectively, with a total annual rainfall ranging from 1200 to 1400 mm. The soils are predominantly mollic andosols (Jaetzold and Schmidt, 2006). The greenhouse used was 8 m by 60 m and the covering material was UV stabilized polythene sheet with a thickness of 12×150 microns purchased from Amiran Kenya Ltd, Nairobi, Kenya. The mean monthly temperatures in the greenhouse and field during the experiment are presented in Table 1.

Pepino grows well within a temperature range of 10-30°C and the optimum temperature for growth is between 15 and 25°C (Lim,

Table 1. Average monthly field and greenhouse temperature (°C) in trial one and two.

		2018				2019			
		Nov	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**
Trial one	Field	20.9	19.7	20.9	21.7	22.8	22.6	21.2	18.9
	Greenhouse	30.3	21.0	33.4	30.2	29.4	34.0	35.8	28.0
		2019						**2020**	
		July	Aug	Sept	Oct	Nov	Dec	Jan	Feb
Trial two	Field	19.1	19.2	20.5	19.3	19.3	18.9	19.1	22.6
	Greenhouse	18.5	29.4	30.0	28.0	32.0	28.0	35.3	36.7

Table 2. Pre-planting soil analytical results in trial one and two.

Soil properties	Field		Greenhouse	
	Trial 1	**Trial 2**	**Trial 1**	**Trial 2**
Total nitrogen (%)	0.28-0.45	0.21-0.40	0.21-0.78	0.20-0.72
Potassium (mg kg^{-1})	12.6-22.13	10.2-20.45	19.4-48.6	17.5-38.7
Available P (mg kg^{-1})	1.31-1.72	1.23-1.63	1.96-3.06	1.88-2.99
pH	4.8-5.7	4.6-5.2	4.38-6.03	4.25-6.00

2015). If the temperatures are below 10°C or above 30°C, fruit set is reduced (Prohens et al., 2000). High temperatures interfere with pollination and fruit set (Burge, 1989).

Experimental design and treatment application

The experimental design was randomized complete block design (RCBD) with five treatments and three replications. The five treatments included (0, 100, 200, 300 and 400 NPK (17:17:17) kg ha^{-1}). Pepino seedlings (Ecuadorian Gold variety) were obtained from Garlic and Pepino Farm, Nakuru. For the field experiment, each experimental plot was 3.2 m × 3.2 m and the seedlings were planted in rows 80 cm apart and 50 cm (FAO, 1994) within the plants to give a total of 24 plants per plot. In the greenhouse experiment, each experimental plot was 2 m × 5 m at the same spacing as in the field experiment to give a total of 25 plants per plot. Soil samples were collected from the experimental plots in the field and greenhouse and analyzed for total N, P, K and pH before the experiment was carried out. Soil sampling was done at a depth of 0-40 cm with a soil auger, bulked to form a composite sample and taken for selected analysis. Subsoil samples were air dried and crushed to pass through a less than 1mm sieve. Analysis was carried out using the method described by Okalebo et al. (2002) and the results are presented in Table 2. The NPK fertilizer was applied and thoroughly mixed with the soil before placing the seedlings in the transplanting holes. Weeding was done uniformly to all experimental units. Field capacity was determined as described by Cong et al. (2014), thereafter tensiometers were placed in two experimental units in each block. Irrigation was done when the field capacity fell below 60% since pepino requires a field capacity of 60-65% (Lim, 2015). Drip irrigation was used in the greenhouse experiment. Trial one was carried out from November 2018 to June 2019 and trial two from July 2019 to February 2020. Ripe pepino fruits collected from selected plants in the field and greenhouse were used for analysis of secondary metabolites.

Determination of carotenoids (β-carotene, lycopene, lutein)

Carotenoids were extracted as described by Fish et al. (2002). β-carotene determination was done by spectrophotometric analysis at 453 nm (β-carotene) and lutein at 445 nm. Results were calculated using the formula as described by Nagata and Yamashita (1992) and expressed as mg g^{-1}: β-carotene and lutein (E_x x V)/FW where E_x is absorbance depending on the carotenoid, V is volume of the solution (25 ml) and FW is the fresh weight of the sample. Lycopene was extracted from pepino fruits of the different treatments using acetone and absorbance measured at 503 nm using a UV/Vis spectrophotometer SP-756PC Spectrum Instruments Shanghai China in the plant molecular biology and biotechnology laboratory, Egerton University, Kenya. Lycopene content (mg 100 g^{-1} FW) was then calculated using the formula by Ranganna (1997) where:

Lycopene Content mg $100g^{-1}$ FW = 3.1206×A×V×D×100/W×1000

Where: A=Absorption, V=Volume made up, D= Dilution, W= Weight of Sample
Lycopene content was then expressed in mg g^{-1}.

Determination of total phenolic content

Sample extractions were done according to the method of Ndhalala et al. (2008). Total phenolic content was analyzed by Folin Ciocalteau method as described by Singleton et al. (1999) and absorbance was measured at 760 nm using a UV/Vis spectrophotometer SP-756PC Spectrum Instruments Shanghai China in the plant molecular biology and biotechnology laboratory, Egerton University, Kenya. TPC was quantified by a calibration curve obtained by measuring the absorbance of gallic acid standard. The concentrations were expressed as mg of gallic acid equivalents (GAE) 100 g^{-1} FW.

Table 3. Effect of NPK fertilizer rates and growing environment on lutein (mg g^{-1} FW) content of pepino melon.

Environment	Fertilizer(kg ha^{-1})	Lutein (mg g^{-1} FW)	
		Trial 1	Trial 2
Greenhouse	0	34.78^{cd*}	30.39^{f}
	100	41.62^{b}	35.92^{ef}
	200	54.16^{a}	47.88^{bc}
	300	42.04^{b}	38.34^{de}
	400	39.64^{bc}	34.90^{ef}
Field	0	23.23^{e}	36.97^{de}
	100	30.86^{d}	42.45^{cd}
	200	42.53^{b}	67.25^{a}
	300	32.16^{d}	52.35^{b}
	400	31.43^{d}	44.05^{c}

*Means followed by the same letter (s) within a column are not significantly different according to Tukey's test ($p \leq 0.05$).

Data analysis

Data collected was subjected to Analysis of Variance (ANOVA) and significant means separated using Tukey's honestly significant difference (Tukey's HSD) test at $p \leq 0.05$. The SAS (Version 9.1; SAS Institute, Cary, NC) statistical package was used for data analysis.

RESULTS AND DISCUSSION

Lutein

NPK fertilizer rates and growing environment had a significant effect on lutein content of pepino melon fruits in both trials. In trial one, fruits from greenhouse grown plants which were supplied with 200 kg NPK ha^{-1} had the highest lutein content of 56.16 mg g^{-1} FW, while the control fruits from field grown plants had the lowest lutein content 23.23 mg g^{-1} FW (Table 3). In trial two, fruits from field grown plants supplied with 200 kg NPK ha^{-1} had the highest lutein content. Generally, it was observed that as the fertilizer rate increased lutein content also increased and reached its peak at 200 kg NPK ha^{-1} after which the content dropped in both growing environments and trials.

The current study revealed that an increase in NPK fertilizer led to an increase in lutein content of pepino melon fruits. Similar results indicated that increasing nitrogen fertilizer led to an increase in the lutein content of kales and tomatoes (Kopsell et al., 2007; Neugart et al., 2018; Zhang et al., 2016). Furthermore, the findings are in agreement with those of Chenard et al. (2005) who reported that lutein content of parsley increased with an increase in nitrogen fertilizer rates. Barickman et al. (2009) also reported that a positive correlation existed between nitrogen fertilizer rates and the concentration of antioxidant carotenoids like lutein in watercress (*Nasturtium officinal* R. Br.). Lutein is a lipid soluble tetraterpenoid and is found in the plastids (Baslam et al., 2013). Lutein is a xanthophyll pigment of the light harvesting photosystem II and light harvesting antenna and it plays the function of dissipating excess heat from the photosystem and is a reactive oxygen species (ROS) scavenger (Jahns and Holzwarth, 2012). Based on these functions, the concentration of lutein and other xanthophylls may decrease due to nitrogen deficiency because the photosystem will lack nitrogen for chlorophyll synthesis and this explains the low content of lutein in the control (no fertilizer). The low lutein content in fruits from the control could also be due to the fact that plants which are grown in areas with low resources have reduced growth, low production and decreased production of secondary metabolites (Fanciullino et al., 2014). The soil in this study had low nutrient resources according to Horneck et al. (2011) and hence the low lutein content in the control. On the other hand, plants growing in areas with intermediate resources including fertilizers will have the highest allocation of secondary metabolites and this could explain why lutein content was high and reached its peak in fruits which were supplied with 200 kg NPK ha^{-1} compared to plants supplied with higher fertilizer rates. Under intermediate nutrient resources, high production of secondary metabolites occurs due to the availability of an excess pool of carbon to synthesize carbon-based secondary metabolites like carotenoids and lutein being one of them (Fanciullino et al., 2014). The low lutein content in plants supplied with 400 kg NPK ha^{-1} could be due to high fertilizer rate resulting to the allocation of most of the photosynthates to growth and development and thus low accumulation of secondary metabolites.

Phytoene synthase enzyme catalyzes the first-rate limiting step in carotenoid biosynthesis which involves the condensation of two geranylgeranyl diphosphate (GGPP)

Table 4. Effect of NPK fertilizer rates and growing environment on β-carotene (mg g^{-1} FW) content of pepino melon.

Environment	Fertilizer(kg ha^{-1})	β-carotene (mg g^{-1} FW) content	
		Trial 1	Trial 2
Greenhouse	0	2.18^{fg*}	1.18^{g}
	100	2.74^{ef}	1.63^{fg}
	200	17.31^{a}	13.23^{b}
	300	9.49^{c}	6.49^{d}
	400	6.86^{d}	4.51^{e}
Field	0	0.86^{g}	1.74^{fg}
	100	1.25^{fg}	3.39^{ef}
	200	14.28^{b}	21.59^{a}
	300	5.71^{d}	9.85^{c}
	400	3.97^{e}	6.97^{d}

*Means followed by the same letter (s) within a column are not significantly different according to Tukey's test at ($p \leq 0.05$).

molecules into one phytoene molecule (Fanciullino et al., 2014). Phytoene synthase (PSY) enzyme is sensitive to temperature and this means that the carotenoid biosynthetic pathway may be involved in temperature stress response (Stanley and Yuan, 2019). High temperature leads to the production of ROS and this increases the activity of PSY enzyme and hence increases in lutein content (Yang et al., 2018a). The increase in ROS leads to increase in biosynthesis of carotenoids through redox signaling by increasing the expression of genes and enzymes involved in carotenogenesis (Fanciullino et al., 2014). This might explain why lutein content was high for greenhouse grown pepino melons in trial one because of the high temperature in the greenhouse (Table 1). In trial two, field grown pepino melon supplied with 200 kg NPK ha^{-1} had the highest lutein content and the temperatures in the field ranged from 19.1 to 22.6°C. This contrasts a previous study which reported that temperatures of 18.5°C led to a decrease in carotenoids in tobacco leaves because of decrease in PSY enzyme (Yang et al., 2018b).

β-Carotene

NPK fertilizer rates and growing environment had a significant effect on β-carotene content of pepino melon in both trials. In trial one, fruits from greenhouse grown plants supplied with 200 kg NPK ha^{-1} were significantly different at $p \leq 0.05$ compared to the other fertilizer rates and field grown plants. Field grown plants supplied with 200 kg NPK ha^{-1} had a β-carotene content of 14.28 mg g^{-1} FW in trial one (Table 4). In trial two, fruits from field grown plants supplied with 200 kg NPK ha^{-1} had a β-carotene content which was significantly different $p \leq 0.05$ from other fertilizer rates in both growing environments. Greenhouse grown pepino plants supplied with 200 kg NPK ha^{-1} were not significantly different at $p \leq 0.05$ from field grown fruits supplied with 300 kg NPK ha^{-1} in trial two. It was observed that as the fertilizer rate increased the β-carotene content also increased and reached its peak at 200 kg NPK ha^{-1} after which the content dropped in both growing environments and trials.

Results of this study revealed that an increase in NPK fertilizer application led to an increase in β-carotene content of pepino melon fruits. This is in harmony with the discovery of Boskovic-Rakocevik et al. (2012) who noted that an increase in nitrogen fertilizer application prompted an increase in β-carotene content of carrot roots. Similarly, Chenard et al. (2005) found that β-carotene content of parsley leaves was influenced by increasing nitrogen rates. On the contrary, Musa et al. (2010) reported that the applied nitrogen did not significantly affect β-carotene of *Corchorus olitorius* at fruiting. Comparative outcomes were accounted for by Sorensen (1999) who revealed that a decrease of nitrogenous fertilizer from 240 to 60 kg N ha^{-1} resulted to a 12% decrease in β-carotene content of carrots. Increment in temperature from 15 to 30°C prompted an increase in the β-carotene content of kales and spinach (Lefsrud et al., 2005). Vitamin A carotenoids particularly β-carotene are fundamentally affected by NPK nutrition but the results differ between different vegetables and fruits (Jones et al., 2015). On the contrary, Neugart et al. (2018) found that increase in nitrogen fertilizer did not have a significant effect in β-carotene content of kales. In trial two, field grown plants supplied with 200 kg NPK ha^{-1} had the highest β-carotene content of 21.59 mg g^{-1} FW. This could be due to the fact that β-carotene content decreases with increasing temperature because the activity of enzymes PSY and phytoene desaturase

Table 5. Effect of NPK fertilizer rates and growing environment on lycopene (mg g^{-1} FW) content in pepino melon.

Environment	Fertilizer(kg ha^{-1})	Lycopene (mg g^{-1}) content	
		Trial 1	Trial 2
Greenhouse	0	4.05d[ef*]	2.30[d]
	100	4.47[cde]	2.67[d]
	200	14.25[a]	11.16[a]
	300	7.71[bc]	4.65[cd]
	400	6.19[bcd]	3.43[d]
Field	0	1.39[f]	2.39[d]
	100	2.37[ef]	2.81[d]
	200	8.98[b]	12.87[a]
	300	5.76[bcde]	7.96[b]
	400	4.39[cdef]	6.49[bc]

*Means followed by the same letter (s) within a column are not significantly different according to Tukey's test ($p \leq 0.05$).

catalyzing the synthesis of β-carotene is influenced by temperature above 30°C (Lurie et al., 1996). At temperatures above 30°C PSY levels are reduced and hence the reduced levels of β- carotene in the greenhouse in trial two. In trial one, the average monthly temperature in the field was 18.9 to 22.8°C (Table 1) and in trial two, the temperature was 19.1-22.6°C; while in the greenhouse the temperature was 21-35.8 and 18.5-36.7°C in trial one and two respectively (Table 1). On the contrary, Menegol et al. (2017) detailed that carotenes (β-carotene and lycopene) are not influenced by temperature.

Lycopene

NPK fertilizer rates and growing environment had a significant effect on lycopene content of pepino melon plants in both trials. Greenhouse grown plants supplied with 200 kg NPK ha^{-1} had the highest lycopene content of 14.25 mg g^{-1} FW compared to the other treatments in trial one (Table 5). In trial two, greenhouse and field grown plants supplied with 200 kg NPK ha^{-1} had the highest lycopene content compared to the other fertilizer rates. It was observed that as the fertilizer rate increased the lycopene content also increased and reached its peak at 200 kg NPK ha^{-1} after which lycopene content decreased. Lycopene content was higher in trial one compared to trial two.

The present study indicated an increase in lycopene content as the NPK fertilizer rates increased with a peak at 200 kg NPK ha^{-1} in both growing environments. Similar trend of results was obtained by Dorais (2007) who reported that increased nitrogen application led to a decrease in lycopene content of tomatoes. This explains the decrease in lycopene content when NPK fertilizer rates exceeded 200 kg NPK ha^{-1}. In trial one, lycopene content was high for greenhouse grown pepino fruits and the temperature in the greenhouse ranged from 21 to 35.8°C (Table 2). Helyes et al. (2003) also found out that greenhouse grown indeterminate tomatoes had a high lycopene content compared to field grown tomatoes. Brandt et al. (2006) stated that maximum lycopene content occurs at temperatures of 25 to 30°C and is completely inhibited at temperatures above 32°C. Fruits grown at high temperatures have a low lycopene content although temperature regulation of carotenoids is crop specific. This could be due to the fact that fruits which are exposed to high temperatures as in the case of greenhouse grown pepino fruits had low lycopene content. When the air temperature is 30°C, the surface temperature of the fruit may range between 40-50°C and this decreases lycopene synthesis (Adegoroye and Joliffe, 1983). In the greenhouse the temperature ranged from 18.5-36.7°C and therefore when the temperature was above 30°C the lycopene content was low due to conversion to β-carotene. In the field the fruits were exposed to direct sunlight and this led to an increase in the surface temperature of the fruit and hence low lycopene content. In trial two, temperature in the field ranged from 19.1 to 22.6°C (Table 1) and the lycopene content was higher than that for the greenhouse grown fruits. The results are in agreement with Abushita et al. (2000) who also reported that field grown tomatoes have higher lycopene content than greenhouse grown tomatoes. Lycopene content increases as temperature increases from low to medium then drastically declines from medium to high temperatures. Hamauzu et al. (1998) stated that high temperatures above 35°C inhibit the accumulation of lycopene by converting it into β-carotene. This further explains the high lycopene content in the field grown pepino fruits in the studies in trial two

Table 6. Effect of NPK fertilizer rates and growing environment on Total Phenolic Content (mg GAE 100g^{-1} FW) content of pepino melon.

Environment	Fertilizer(kg ha^{-1})	TPC (mg GAE 100 g^{-1} FW)	
		Trial 1	Trial 2
Greenhouse	0	174.3^{a*}	148.5^{a}
	100	128.3^{b}	104.7^{b}
	200	104.2^{bc}	92.0^{bc}
	300	93.8^{bc}	87.0^{bc}
	400	83.6^{cd}	85.1^{bc}
Field	0	129.1^{b}	105.4^{b}
	100	72.6^{cde}	70.5^{cd}
	200	50.1^{de}	61.7^{cd}
	300	39.6^{ef}	46.5^{de}
	400	14.0^{f}	19.4^{e}

*Means followed by the same letter (s) within a column are not significantly different according to Tukey's test ($p \leq 0.05$).

because the temperatures were lower in the field compared to the greenhouse. Fruits which were exposed to high temperatures had low lycopene content. Lycopene content is high in fruits which are exposed to light compared to those which are shaded (Dumas et al., 2003). It should be noted that the carotenoid content varies depending on growing seasons, locations and cultivars and this depends on the regulation of genes, particularly zeoxanthin epoxidase (ZEP) and violaxanthin de-epoxidase (VDE) and other genes involved in biosynthesis of carotenoids (Othman et al., 2014).

Total phenolic content (TPC)

NPK fertilizer rates and growing environment had a significant effect on TPC of pepino melon in both trials. Greenhouse grown pepino plants which were not supplied with any fertilizer (control) recorded the highest TPC compared to the other fertilizer rates (Table 6). In trial one greenhouse grown pepino plants which were not fertilized had a TPC of 174.3 mg GAE 100 g^{-1} FW while in trial two they recorded 148.5 mg GAE 100 g^{-1} FW. Field grown plants not supplied with fertilizer also had a TPC which was not significantly different from greenhouse grown plants supplied with 100, 200 and 300 kg NPK ha^{-1} in both trials. Field grown plants supplied with 400 kg NPK ha^{-1} had the lowest TPC though not significantly different from plants that received 300 kg NPK ha^{-1} in both trials. Generally, greenhouse grown plants had a higher TPC compared to field grown pepino plants. It was also observed that as the NPK fertilizer rate increased from 0 kg ha^{-1} to 400 kg ha^{-1} the TPC also decreased in both growing environments and trials.

The current study revealed up regulated buildup of TPC where no NPK fertilizer (control) was supplied to pepino plants. The results are in harmony with the findings of Ibrahim et al. (2011) which showed that accumulation phenolic content in plant tissues was increased under conditions of low nitrogen. Similar trend of results was obtained by Munene et al. (2017) who reported high TPC in amaranth plants which were not supplied with any fertilizer (control). Argyropoulou et al. (2015) also reported that the synthesis of secondary metabolites was stimulated by nitrogen deficiency and this enhanced the accumulation of TPC of sweet basil (*Ocimum basilicum* L.). These results are also in agreement with Vanitha and Mehalai (2016) who reported the total phenolic content of ripe pepino fruits to be 93.02 mg GAE 100 g^{-1} FW. On the contrary, Kola (2010) reported that the TPC of pepino melon was 480-540 mg GAE 100 g^{-1} FW which is quite high compared to the results obtained in the current study. This could be due to differences in environmental conditions in Turkey and Kenya. It has been reported that environmental conditions play a key role in the quality of pepino melon fruits (Kola, 2010). Phenylalanine is a precursor in the biosynthesis of phenolics and is also an amino acid used in protein synthesis. Therefore, there might be a competition for phenylalanine between protein synthesis and secondary metabolite synthesis and therefore biosynthesis of secondary metabolites might be inhibited due to incorporation of phenylalanine into protein synthesis (Margna, 1977). A positive correlation exists between activity of phenylalanine lyase (PAL) an enzyme of the phenylpropanoid pathway and accumulation of carbon-based secondary metabolites in plants (Jeyaramraja et al., 2003). Plants grown in nitrogen deficient soils increase the supply of ammonia by enhancing PAL activity hence a rise in the accumulation of polyphenolic compounds (Margna,1977).

Nitrogen is efficient for protein synthesis and thus phenolic content decreased for a given amount of phenylalanine. In addition, the expression of phenylalanine genes increases under nitrogen depletion (Larbat et al., 2012). Greenhouse grown plants had a higher TPC due to high temperature in the greenhouse. Temperature might have different responses depending on the species and their tolerance or sensitivity to high temperatures. Exposure of tomato plants to a temperature of 35°C led to a significant increase of total phenols, while a decrease in TPC was observed in watermelon (Toscano et al., 2019). The increase or decrease of TPC could be due to high or low PAL enzyme activity, suggesting a vital role for this enzyme in regulating plant stress response (Toscano et al., 2019).

To summarize, increase in temperature may enhance the accumulation of most secondary metabolites in plants (Yang et al., 2018a). This explains why greenhouse grown fruits which were not supplied with NPK fertilizer had the highest TPC compared to field grown pepino melon fruits because in the greenhouse, the temperatures were higher than in the field. In trial one, the average monthly temperature in the greenhouse temperature was 21-35.8°C and in trial two, the temperature was 18.5-36.7°C; while in the field the temperature was 18.9-22.8°C and 19.1-22.6°C in trial one and two respectively (Table 1). There is a negative correlation between proteins and phenols because phenylalanine is used in protein synthesis and not phenolics under conditions of excess nitrogen supply (Li et al., 2008). This might be the reason for the low TPC recorded in this study when high NPK fertilizer rates were used. NPK fertilizer rates have an effect on the accumulation of secondary metabolites in pepino melon fruits grown in the field and greenhouse.

CONCLUSION AND RECOMMENDATION

Accumulation of carotenoids (lutein, β-carotene and lycopene) was favored by application of 200 kg NPK ha^{-1} with high content being recorded in greenhouse grown pepino fruits. On the other hand, accumulation of TPC was high in the control (no fertilizer) in both field and greenhouse grown pepino melons. It is therefore recommended that application of 200 kg NPK ha^{-1} for both field and greenhouse grown pepino melons will help to enhance the accumulation of carotenoids which have good antioxidant capacity. No fertilizer application is recommended for maximum accumulation of TPC of pepino melon fruits in the location where this study was carried.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abushita AA, Daood HG, Biacs PA (2000). Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. Journal of Agriculture and Food Chemistry 48(6):2075-2081.

Adegoroye AS, Joliffe PA (1983). Initiation and control of sunscald injury in tomato fruit. Journal of American Society of Horticultural Science 108:23-28.

Aina OE, Amoo SO, Mugivhisa LL, Olowoyo JO (2019). Effect of organic and inorganic sources of nutrients on the bioactive compounds and antioxidant activity of tomato. Applied Ecology and Environmental Research 17(2):3681-3694. http://dx.doi.org/10.15666/aeer/1702_36813694

Argyropoulou K, Salahas G, Hela D, Papasavvas A (2015). Impact of nitrogen deficiency on biomass production, morphological and biochemical characteristics of sweet basil (*Ocimum basilicum* L.) plants cultivated aeroponically. Agriculture and Food 3:32-42.

Balandrin MF, Klocke, JA, Wurtele ES, Bollinger WH (1985). Natural plant chemicals: sources of industrial and medicinal materials. Science 228:1154-1159. http://dx.doi.org/10.1126/science.3890182

Barickman TC, Kopsell DA, Sams CE (2009). Impact of nitrogen and Sulphur fertilization on the phtochemical concentration in watercress, *Nasturtium officinal* R. BR. Acta Horticulturae 841:479-482. https://doi.org/10.17660/ActaHortic.2009.841.63

Baslam M, Morales F, Garmendia I, Goicoechea N (2013). Nutritional quality of outer and inner leaves of green and red pigmented lettuces (*Lactuca sativa* L.) consumed as salads. Scientia Horticulturae 151:103-111.https://dx.doi.org/10.1016/j.scienta.2012.12.023

Becerra M, Contreras LM, Lo MH, Diaz JM (2020). Lutein as a functional food ingredient: stability and bioavailability. Journal of Functional Foods 66:1-2. https://doi.org/10.1016/j.jff.2019.103771

Berini JL, Brockman SA, Hegeman AD, Reich PB, Muthukrishnan R, Montgomery RA, Forester JD (2018). Combinations of abiotic factors differentially alter production of plant secondary metabolites in five woody plant species in the boreal-temperate transition zone. Frontiers in Plant Science 9:1257-1273. https://doi.org/10.3389/fpls.2018.01257

Boskovic-Rakocevik L, Pavlovic R, Zdravkovic J, Zdravkovic M, Pavlovic N, Djuric M (2012). Effect of nitrogen fertilization on carrot quality. African Journal of Agricultural Research 7(18):2884-2900. https://doi.org/10.5897/AJAR11.1652

Brandt S, Pek Z, Barna E, Lugasi A, Helyes L (2006). Lycopene content and colour of ripening tomatoes as affected by environmental conditions. Journal of Science, Food and Agriculture 86:568-572. https://doi.org/10.1002/jsfa.2390

Burge GK (1989). Fruit set in pepino (*Solanum muricatum* Ait.). Scientia Horticulturae 41:63-68. https://doi.org/10.1016/0304-4238(89)90050-2

Chenard CH, Kopsell DA, Kopsell DE (2005). Nitrogen concentration affects nutrient and carotenoid accumulation in parsley. Journal of Plant Nutrition 28(2):285-297.https://doi.org/10.1081/PLN-200047616

Cong Z, Lu H, Ni HG (2014). A simplified dynamic method for field capacity estimation and its parameter analysis. Water Science and Engineering 7(4):351-362.https://doi.org/10.3882/j.issn.1674-2370.2014.04.001

Dorais M (2007). Effect of cultural management on tomato fruit health qualities. Acta Horticulturae 744:279-294. https://doi.org/10.17660/ActaHortic.2007.744.29

DumasY, Dadomo M, Di Lucca G, Grolier P (2003). Review: Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. Journal of Science, Food and Agriculture 83:369-382. https://doi.org/10.1002/jsfa.1370

Fanciullino AL, Bidel LPR, Urban L (2014). Carotenoid responses to environmental stimuli: integrating redox and carbon controls into a fruit model. Plant Cell Environment 37:273-289. https://doi.org/10.1111/pce.12153

Food and Agriculture Organization (FAO) (1994). Neglected crops: 1492 from a different perspective. FAO plant protection series, No. 26

ISBN 92-5-103217-3.
Ferrari S (2010). Biological elicitors of plant secondary metabolites: Mode of action and use in the production of neutraceutic. Advances in Experimental Medicine and Biology 698:152-166. https://doi.org/10.1007/978-1-4419-7347-4_12
Fish WW, Perkins-Veazie P, Collins JK (2002). A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. Journal of Food Composition and Analysis 15:309-317. https://doi.org/10.1006/jfca.2002.1069
Gaude N, Brehelin C, Tischendorf G, Kessler F, Dormann P (2007). Nitrogen deficiency in Arabidopsis affects galactolipid composition and gene expression and results in accumulation of fatty acid phylesters. Plant Journal 49(7):29-39. https://doi.org/10.1111/j.1365-313X.2006.02992.x
Gershanzon J (1984). Changes in the levels of plant secondary metabolites under water and nutrient stress. In: Timmerman BN, Steelink C, Loewus FA (eds). Phytochemical adaptations to stress. Recent advances in phytochemistry (proceedings of the phytochemical society of North America) vol. 18, Springer, Boston, MA. Springer pp. 273-320. https://doi.org/1007/978-1-4684-1206-2_10
Hamauzu Y, Chachin K, Ueda Y (1998). Effects of postharvest temperature on the conversion of 14C-mevalonic acid to carotene in tomato fruit. Journal of Japanese Society of Horticultural Science 67:549-555. https://doi.org/10.2503/jjshs.67.549
Helyes L, Brandt S, Reti K, Barna E, A. Lugasi A (2003). Appreciation and analysis of lycopene content of tomato. Acta Horticulturae 604:531-537. https://doi.org/10.17660/ActaHortic.2003.604.61.
Horneck DA, Sullivan DM, Owen JS, Hart JM (2011). Soil test interpretation guide. EC 1478. Corvallis, OR: Oregon State University Extension Service. https://doi.org/10.1016/S0031-9422(00)994321-8
Ibrahim MH, Jaafar HZ, Rahmat A, Rahman ZAM (2011). Effects of nitrogen fertilization on synthesis of primary and secondary metabolites in three varieties of Kacip Fatimah (*Labisia pumila* Blume). International Journal of Molecular Science 12:5238-5254. https://doi.org/10.3390/ijms12085238
Ibrahim MH, Jaafar HZE, Karimi E, Ghasemzade A (2013). Impact of organic and inorganic fertilizer application on the phytochemical and antioxidant activity of Kacip Fatimah (*Labisia pumila* Benth). Molecules18:10973-10988. https://doi.org/10.3390/molecules180910973
Jaetzold R, Schmidt H (2006). Farm management handbook of Kenya. Natural conditions and farm management information, Ministry of Agriculture, Kenya.
Jahns P, Holzwarth AR (2012). The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. Biochimica Biophysica Acta Bioenergetics 1817:182-193. https://doi.org/10.1016/j.bbabio.2011.04.012
Jeyaramraja PR, Pius PK, Kumar RR, Jayakumar D (2003). Soil moisture stress-induced alterartions in bioconstituents determining tea quality. Journal of Science, Food and Agriculture 83:1187-1191. https://doi.org/10.1023/B:PHOT.0000027523.51145.a0
Jones RB, Stefanelli D, Tomkins RB (2015). Pre-harvest and post-harvest factors affecting ascorbic acid and carotenoid content in fruits and vegetables. Acta Horticulturae 1106:30-41. https://doi.org/10.17660/ActaHortic.2015.1106.6
Kola O (2010). Physical and chemical properties of the ripe pepino (*Solanum muricatum)* fruit grown in Turkey. Journal of Food, Agriculture and Environment 8(2):168-171. https://doi.org/10.1234/4.2010.1595
Kopsell DA, Kopsell DE, Curran-Celentaro J (2007). Carotenoid pigments in kale are influenced by nitrogen concentration and form. Journal of Science, Food and Agriculture 87(5):900-907. https://doi.org/10.1002/jsfa.2807
Krzyzanowska JA, Czubacka A, Oleszek W (2010). Dietary phytochemicals and human health. In: Bio-Farms for nutraceuticals: Functional food and safety control by biosensors. (eds. Giardi, M. T., Rea, G., Berra, B.), Springer US.698(7):74-99. https://doi.org/10.1007/978-1-4419-7347-4_7.
Kumar N, Goel N (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnology Reports 24:1-10.https://doi.org/10.1016/j.btre.2019.e00370
Larbat R, Olsen KM, Slimestad R, Lovdal T, Benard C, Verheul M (2012). Influence of repeated short-term nitrogen limitations on leaf phenolics metabolism in tomato. Phytochemistry 77:119-128. https://doi.org/10.1016/j.phytochem.2012.02.004
Lefsrud MG, Kopsell DA, Kopsell DE, Curran-Celentano J (2005). Air temperature affects biomass and carotenoid pigment accumulation in kale and spinach grown in a controlled environment. HortScience 40:2026-2030. https://doi.org/10.21273/HORTSCI.40.7.2026
Li J, Zhu Z, Gerendas J, (2008). Effects of nitrogen and sulfur on total phenolic and antioxidant activity in two genotypes of leaf mustard. Journal of Plant Nutrition 31(9):1642-1655. https://doi.org/10.1080/01904160802244860
Lim TK (2015). Fruits. In: T.K. Lim (Ed.), Edible Medicinal and Non-Medicinal Plants. Retrieved from http://www.springer.com>book pp. 390-394 (Accessed on 5th April 2020). https://doi.org/10.1007/978-94-017-9511-1
Lurie S, Handros A, Fullik E, Shapira R (1996). Reversible inhibition of tomato gene expression at high temperature. Plant Physiology 110:1207-1214. https://doi.org/10.1104/pp.110.4.1207
Maiani G, Caston MJP, Catasta G, Toti E, Cambrodon IG, Bysted A, Schlemmer U (2009). Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. Molecular Nutrition and Food Research 53(2):194-218. https://doi.org/10.1002/mnfr.200800053
Margna U (1977). Control at the level of substrate supply-An alternative in the regulation of phenylpropanoid accumulation in plant cells. Phytochemistry16:419-426.https://doi.org/10.1016/S0031-9422(00)94321-8
Menegol T, Diprat AB, Rodrigues E, Rech R (2017). Effects of temperature and nitrogen concentration on biomass composition of *Heterochlorella luteoviridis*. Food Science and Technology 37:28-35. https://doi.org/10.1590/1678-457x.13417
Munene R, Changamu E, Korir N, Gweyi-Onyango, J (2017). Effects of different nitrogen forms on growth, phenolics, flavonoids and antioxidant activity in amaranth species. Tropical Plant Research 4(1):81-89.https://doi.org/10.10.22271/trp.2017.v4.il.012
Musa A, Ezenwa IS, Oladiran JA, Akanya, HO, Ogbadoyi EO (2010). Effect of soil nitrogen levels on some micronutrients, antinutrients and toxic substances in *Corchorous olitorius* grown in Minna, Nigeria. African Journal of Agricultural Research 5(22):3075-3081.
Nagata M, Yashimita I (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Journal of Japanese Society of Food Science and Technology 39:925-928. https://doi.org/10.3136/nskkk1962.39.925
Neugart S, Baldermann, S, Hanschen FS, Klopsch R, Weisner-Reinhold M, Schreiner M (2018). The intrinsic quality of brassicaceous vegetables: How secondary metabolites are affected by genetic, environmental and agronomic factors. Scientia Horticulturae 233:460-478. https://doi.org/10.1016/j.scienta.2017.12.038.
Ndhalala ARM, Muchuweti C, Mupure K, Chitindingu T, Murenje AK., Benhura M (2008). Phenolic content and profiles of selected wild fruits of Zimbabwe: *Ximenia caffra, Arbotrys brachpetalus* and *Syzygium cordatum*. International Journal of Food Science and Technology 43:1333-1337.https://doi.org/10.1111/j.1365-2621.2007.01611.x
Okalebo JR, Gathua KW, Woomer PL (2002). Laboratory methods of soil and water analysis. A working manual. Second edition, p. 128.
Oloyede FM, Obisesan IO, Agbaje GO, Obuotor EM (2012). Effect of NPK fertilizer on chemical composition of pumpkin (*Cucurbita pepo* Linn.) seeds. The Scientific World Journal pp. 1-6. https://doi.org/10.1100/2012/808196
Othman R, Zaifuddin FAM, Hassan NM (2014). Carotenoid biosynthesis regulatory mechanism in plants. Journal of Oleo Science 63(8):753-760. https://doi.org/10.5650/jos.ess13183
Prohens J, Ruiz JJ, Nuez F (2000). Growing cycles for a new crop, the pepino in the Spanish Mediterranean. Acta Horticulturae 29:53-60. https://doi.org/10.17660/ActaHortic.2000.523.6
Ranganna S (1997). Manual of Analysis of Fruit and Vegetable

Products, Tata McGraw Hill Publishing Co. Ltd., New Delhi.
Sandman G (2015). Carotenoids and biotechnological importance. Advances in Biochemical Engineering and Biotechnology 148:449-467. https://doi.org/10.1007/10_2014_277
Singleton VL, Orthofer RM, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substances and antioxidants by means of Folin-Ciacalteu reagent. Methods in Enzymology 299:152-178. https://doi.org/10.1016/S0076-6879(99)99017-1
Sorensen JN (1999). Nitrogen effects on vegetable crop production and chemical composition. Acta Horticulturae 506:41-50. https://doi.org/10.17660/ActaHortic.1999.506.4
Stanley L, Yuan Y, (2019). Transcriptional regulation of carotenoid biosynthesis in plants: So many regulators, so little consensus. Frontiers in Plant Science 10:1017. https://doi.org/10.3389/fpls.2019.01017
Toscano S, Trivellin A, Cocetta G, Bulgari R, Francini A, Romano D, Ferrante A (2019). Effect of preharvest abiotic stresses on the accumulation of bioactive compounds in horticultural produce. Frontiers in Plant Science 10:1212. https://doi.org/10.3389/fpls.2019.01212
Vanitha T, Mehalai V (2016). Studies on quality changes of pepino (*Solanum muricatum*) fruit during storage. International Journal of Science and Technolodge 4(3):138-143.
Wei W, Ye C, Huang H, Yang M, Mei X, Du F, He X, Zhu S, Liu Y (2019). Appropriate nitrogen application enhances saponin synthesis and growth mediated by optimizing root nutrient uptake ability. Journal of Ginseng Research 2019: 1-10. https://doi.org/10.1016/j.jgr.2019.04.003
Wink M (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64:3-19. https://doi.org/10.1016/S0031-9422(03)00300-5
Yang L, Wen K, Zhao K, Wei F, Wang Q (2018a). Response of plant secondary metabolites to environmental factors, Molecules 23:1-26. https://doi.org/10.3390/molecules23040762
Yang LY, Yang SL, Li JY, Ma JH, Pang T, Zou CM, He B (2018b). Effects of different growth temperatures on growth, development and plastid pigments metabolism of tobacco (*Nicotiana tabacum* L.) plants. Botanical Studies 59(5):1-13. https://doi.org/10.1186/s40529-018-0221-2
Zhang E, Duan Y, Zhang S (2016). Effects of long-term nitrogen and organic fertilization on antioxidants content of tomato fruits. Journal of Horticulture 3(1):172. https://doi.org/10.1016/j.scienta.2017.12.038
Zheng J, Yang B, Ruusunen V, Laaksonen O, Tahvonen R, Hellsten J, Kallio H (2012). Compositional differences of phenolic compounds between black currant (*Rubes nigrum* L.) cultivars and their response to latitude and weather conditions. Journal of Agriculture and Food Chemistry 60:6581-6593. https://doi.org/10.1021/jf3012739
Zheng Z, Fu YY, Li BH, Zhang ML, Xin CW, Huang P (2014). PSY-1, a *Taxus chinensis* var. *mairei* extract, inhibits cancer cell metastasis by interfering with MMPs. Natural product communications 9(2):241-245. https://doi.org/10.1177/2F1934578X1400900228