

PRODUCTION OF MINERAL NUTRIENT-RICH MULTIPURPOSE PUMPKIN FRUITS USING COMBINED NITROGEN, MULCH AND GIBBERELIC ACID

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ABSTRACT

The present study assessed effects of nitrogen, mulch and gibberellic acid (GA₃) on minerals and metabolites in pumpkin fruits. Nitrogen (0, 50, 100 and 150 kg N/ha), three mulches (none, unpainted, black-painted rice straws) and GA₃ (0, 40 and 80 mg/L) were used in randomized complete block design, replicated three times in two seasons, and each plant occupied 2m x 2m space. Post-harvest data values were subjected to analysis of variance using SAS and means were separated using the least significant difference test ($\alpha=0.05$). Nitrogen positively and significantly affected N, P and Mg. It negatively and significantly affected K, beta-carotene and TSS. Mulch positively and significantly influenced P, Mg and beta-carotene, but not TSS. GA₃ positively and significantly affected Mg and beta-carotene, while it negatively and significantly affected N, P, and K. There was consistent significant effect of combined N, mulch and GA₃ on N, P, K, Mg and beta-carotene (highest 29 ppm was for N₃M₀GA₀). Thus nitrogen, mulch, GA₃, alone or combined consistently enhance mineral nutrients and metabolites in pumpkin fruits.

Keywords: African leafy vegetables, Fruit quality, Mineral nutrients, Nutritional value

1. INTRODUCTION

Varieties of the multipurpose pumpkin fruit-vegetable grown widely in Kenya belong to *Cucurbita moschata* and *Cucurbita maxima*. Pumpkin belongs to the Cucurbitaceae family together with gourds, melons and squashes. It originated from Central America, but is now domesticated in other tropical and subtropical countries (Fedha, 2008). Globally, China is the major producer followed by India. In Africa, Egypt and South Africa are the leading producers (FAO, 2009). In Kenya, pumpkin production has been on the rise from 599 ha in 2015 to 681 ha in 2016, corresponding to 3580 to 4017 metric tonnes, respectively (Horticulture Validated Report, 2015-16). Regardless, pumpkin is still regarded as a traditional vegetable that can grow in all agro-ecological zones in Kenya (Karanja *et al.*, 2014). Multipurpose pumpkin is famous for its edible seeds, fruits and green parts that are edible (Matsui *et al.*, 1998).

The FAO (2005) report indicated that pumpkin has immense economic potential for use both as a food and industrial crop. Pumpkin production and consumption worldwide have risen due to several reasons, including medicinal through the antioxidant beta-carotene that helps improve immune function and reduce cancer and heart disease risks (Ghanbari *et al.*, 2007). Pumpkin also contains mineral nutrients such as Ca, Fe, Mg, K, Zn, Se, niacin, foliate, and vitamins A, C, and E (Ondigi *et al.*, 2008). Limited pumpkin is produced, commercialized and consumed in Africa (Ondigi *et al.*, 2008). Pumpkin is beneficial to human health because it contains various biologically active components such as polysaccharides, para-aminobenzoic acid, fixed oils, sterols, proteins and peptides (Caili *et al.*, 2006). The fruits are good sources of carotenoids and γ -aminobutyric acid (Murkovic *et al.*, 2002). Pumpkin seeds are valued for their high protein content and useful amounts of essential fatty acids like linoleic acid. Pumpkin seeds contain remarkably high proportions of essential amino acids. Additionally, the seeds contain relatively large amounts of various essential elements such as K, Cr and Na. The seeds are also good sources of Mg, Zn, Cu, Mo and Se (Glew *et al.*, 2006). The seeds are considered a nutritious food with high oil (50% w/w) and protein (35%) contents that vary depending on cultivar (Fruhworth and Hermetter, 2007). From pumpkin leaves and germinated seeds, several phytochemicals such as polysaccharides, phenolic glycosides, non-essential fatty acids and proteins have been isolated (Koike *et al.*, 2005). Various hypoglycaemic polysaccharides have been characterized from pumpkin fruit pulp. In this regard, D-chiro-inositol has been identified as an insulin secretor and sensitizer (Jun *et al.*, 2006). Various antibiotic components including anti-fungal ones have been characterized from various parts of pumpkin (Glew *et al.*, 2006).

These numerous benefits notwithstanding, there are no documented pumpkin value chain preferences and consumption trends that exist in Kenya (Ondigi *et al.*, 2008). These shortcomings can be overcome by the fact that pumpkin can grow in diverse climatic conditions and storage after harvesting can last for over 8 months provided the fruit stalk is retained (Grubben and Chigumira-Ngwerume, 2004; Horticultural Crops Development Authority, 2012). According to Hewett (2006), temperature, relative humidity, water potential, light, cultural practices and pest management techniques are key pre-harvest factors that determine the inherent quality of the produce that give rise to human pleasure. Colour, nutrition and flavour are some factors considered in determining good quality by consumers (Kitinoja *et al.*, 2011). Characteristics that are genetic and physiological establish post-harvest behaviour that is typical and that produces quality, which form the interaction basis (Kitinoja, 2010). The final indicator of quality is the inter-relationship between the produce and its environment.

The essential element nitrogen is by far the most critical plant growth nutrient with far reaching benefits, although testing for it in soil is usually not practical due to its rapid mobility (Cameron *et al.*, 2013). Cucurbits require from 22.5 to 45 kg of actual nitrogen per acre per season (Bratsch, 2009). Use of chemical fertilizers as supplemental sources of nutrients has been on the increase in pumpkin production, but they are not applied in balanced proportions by most farmers. Connectedly, NPK fertilizer has been found to increase leaf area, stem diameter, number of leaves and mineral nutrient contents (N, P, K, Ca, Na and Mg) in the soil under pumpkin production (Okonwu and Mensah, 2012). Mulch enhances germination of pumpkin seeds by increasing soil temperature. The high soil temperatures also accelerate transplant establishment and promote

subsequent crop development and maturity, thereby increasing yields (Waterer, 2000). Gibberellins play an essential role in many aspects of pumpkin growth and development such as seed germination, stem elongation and flower development (Yamaguchi and Kamiya, 2000). The present study determined the interactive effects of nitrogen, mulch and GA₃ on mineral nutrients and metabolites in multi-purpose pumpkin fruit pulp.

2. MATERIALS AND METHODS

2.1 Research Site

The Research Farm used lies at 0° 19' S, 37° 38' E and 1535 m above sea level. The prevailing annual temperature ranges from 12.2°C to 23.2°C. The area experiences average annual rainfall of 1200 mm and two rainy seasons, with the long rains occurring in March through June and short rains in October through December (Jaetzold *et al.*, 2006; <http://en.climate-data.org>). The soils at the research site are humic nitisols, deep, strongly weathered, well drained with a clayey subsurface horizon made of angular, blocky structural elements with shiny faces and high cation exchange capacity (Koskey *et al.*, 2017).

2.2. Experiment Layout

A three-factor experiment was set in a randomized complete block design (RCBD) with three replications in two seasons. Each experimental plant occupied 2m x 2m space, separated from other plants by 1 m. The three factors tested were nitrogen, mulch and GA₃, assigned to main-plots, sub-plots and split-plots, respectively. The nitrogen rates were 0, 50, 100 and 150 kg N/ha, applied as CAN in two equal doses at three-weeks post-emergence and at beginning of flowering. Amount of CAN fertilizer used per experimental unit was calculated as: a) 50 kg N/ha = 76.9 g CAN/4 m²; b) 100 kg N/ha = 153.8 g CAN/4 m²; c) 150 kg N/ha = 230.7 g CAN/4 m².

Mulch used was none, unpainted and black-painted rice straw that were easily available near the experimental site in required quantities. The black-painted dry rice straws and unpainted dry rice straws were placed on respective split plots after land preparation. Painting of the rice straws was done by dipping in a 200-L drum containing black paint solution and spreading out on the soil to air-dry. The rice straws were uniformly spread on the plots to achieve 20 cm thickness. Planting holes were marked and opened in rice straws during pumpkin seed sowing.

The GA₃ rates used were 0, 40 and 80 mg/L prepared by dissolving in 50ml alcohol and then topping the volume to one litre stock solution using distilled water. The required concentration of spray solution was prepared from the stock solution by diluting with distilled water. A few drops of commercial sticker were added to the solutions to facilitate leaf-uptake of the GA₃. Spraying the GA₃ to pumpkin plants using a 1-L hand-held sprayer. Spray solution of lower rate was applied first followed by the next with higher rate. Spraying was done once during the fourth week after emergence on a calm morning while observing wind direction.

2.3. Pumpkin Seed Sowing and Maintenance

Three fruits of uniform size, free from diseases and pests and from one mother plant were used. The fruits were sourced from farmers near the present research site. Seeds were used immediately after extraction following recommendations for handling pumpkin seeds for planting (AOAC, 1995). The field was prepared to appropriate tillage. Mulch was laid in line with treatments just before sowing seeds. All recommended phosphorus and potassium straight fertilizers were applied just before seed sowing. Two seeds were placed at the centre of each split plot and one seedling was uprooted two weeks after emergence. All plots were kept weed-free through manual roguing and cultivation. Irrigation was done using drip tubes to supplement rain during drought. Pest control was done when appropriate using recommended pesticides. Pumpkin vines were coiled once beyond their allocated area, while leaving them in contact with the soil. Data values were collected from all experimental plants, except guard row ones.

2.4. Data Collection and Analysis

Data values were collected, as described below for the two experimental seasons with Season 1 spanning March 2019 to July, 2019 with 1,004.3 mm rainfall, and Season 2 spanning October 2019 to February 2020 with 1,259.6 mm rainfall. Sampled fruits were cleaned using water and rinsed with distilled water to remove surface contaminants. Fruit flesh and seeds were separated using a metallic spoon, after dissecting the fruit into two pieces. The fruit flesh was cut into thin slices of 5 mm and oven-dried at 60°C to a constant weight. The samples were then crushed to a fine powder using a mortar and a pestle, sieved through 20 mm mesh and stored in an air-tight plastic container to wait for respective analysis.

A sample of 1 g was transferred into 300 ml Kjeldahl digestion flask to which 5 g of potassium sulphate, 0.5 g of copper (II) sulphate and 15 ml of concentrated sulphuric acid were added. Digestion was done on a heater in a fume hood until the final digest was clear blue. The digest was then diluted into 100-volume with distilled water. Diluted digest measuring 10 ml was transferred to Pannas and Wagner distillation apparatus and 15 ml of 40% sodium hydroxide solution was added. Steam distillation was done and about 70 ml of distillate was received in 4% boric acid solution containing several drops of mixed indicator. A blank was set by using the same procedure but with no sample. The received aliquot was titrated with standard 0.02 N hydrochloric acid and total nitrogen content was calculated using the Kjeldahl method formula: % Total nitrogen = $(V-B) \times 0.02 \times 0.014 \times 100/v \times 100/s$, Where V= Titre volume of 0.02 N HCl, B= Blank titre volume of 0.02 N HCl (Kirk, 1950).

Each sample was wet-digested using a mixture of concentrated nitric, perchloric and sulphuric acids in the ratio of 9:2:1. Colorimetric method was used to determine phosphorus (AOAC, 1990). Diluted ash solution, measuring 1 ml, was pipetted into a test-tube to which 3 drops of 0.5% p-nitro phenol indicator solution was added. The content was made yellow in colour by adding 6 N ammonia solution drop-wise, followed by 1 N nitric acid drop-wise until the solution turned colourless (about 1.2 ml was used for decolourizing). A 1 ml of ammonium molybdate/vanadate was added and allowed to stand for 15 minutes for colour development. Absorbance was then read at a wavelength of 420 nm in UV-VIS Spectrophotometer. Standard solutions of concentration between 0 ppm and 20 ppm were prepared and run under the same

condition in the Spectrophotometer. A standard curve was constructed and phosphorus content in the sample was determined using the formula: $P(ppm) = \frac{Conc \times DF \times 0.063}{Sample\ Weight}$.

Potassium was determined using Atomic Absorption Spectrometer, while magnesium was determined using Fourier Transform Infrared Spectrophotometry. Muffle furnace was set at a steady temperature of 550°C to heat clean dry porcelain crucibles for five hours. About 2 g of sample in dry crucible was weighed in triplicates. The crucibles with samples were charred until they produced no smoke and placed inside a furnace where they were incinerated for five hours. They were then removed from the furnace and cooled in a desiccator for 15 minutes. The ash was dissolved in 6 N nitric acid and diluted up to 100 ml volume. A small volume of the diluted sample was aspirated into Shimadzu Atomic Absorption Spectrometer. The emission for potassium and the absorbance for magnesium were then read. Standards for each element were run under the same conditions and standard curves used to calculate the content of each mineral.

Beta-carotene standards were prepared using pure beta-carotene powder (from Sigma) in analytical grade 40-60 petroleum ether at concentration from 1 ppm to 10 ppm. The absorbance was read at 440nm wavelength in APEL-PD3000UV-VIS spectrophotometer and a graph of concentration against absorbance was constructed (beta-carotene standard curve). About 5g of sample was weighed, finely chopped and transferred into a mortar with about 10ml of acetone. A pinch of acid-washed sand was added and the sample was thoroughly ground using a pestle until it produced no more colour. The acetone extract was transferred into a 25ml volumetric flask and topped up with more acetone. The extract was evaporated to dryness and 1ml of analytical grade 40-60 petroleum ether was added. The solution was introduced into a 3cm open chromatography column pre-packed with 60 mesh silica gel, a pinch of anhydrous sodium sulphate, and conditioned with 40-60 petroleum ether. The sample was eluted through the column and 25ml of beta-carotene (yellow pigment) was received. The absorbance was read at 440 nm wavelength in a spectrophotometer and the beta-carotene concentration was determined from the standard curve. Total soluble solids in fruit pulp for each treatment were assessed using a hand-held refractometer with 0-30 brix range and expressed as percent sugar. Data values for mineral nutrients (N, P, K, Mg), beta-carotene content and total soluble solids in fruit samples were subjected to analysis of variance, using the SAS software version 9.3. Mean separation was performed using the least significant difference test at $\alpha = 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of Nitrogen on Minerals and Metabolites in Fruit Pulp

Nitrogen fertiliser had a significant ($P < 0.05$) effect on nitrogen content in both seasons (Table 1). Application of 150kg N/ha produced the highest nitrogen content of 0.35 ppm and 0.44 ppm in S1 and S2, respectively. The 0 kg N/ha had the lowest nitrogen content of 0.05 ppm and 0.06 ppm in S1 and S2, respectively. Nitrogen had a significant ($P < 0.05$) effect on phosphorus content in both seasons. Use of 150kg N/ha produced the highest phosphorus content of 149.56 ppm and 148.82 ppm in S1 and S2, respectively. Phosphorus content increased with increase in nitrogen rate up to 150kg N/ha in both seasons. The 0 kg N/ha produced 134.79 ppm and 138.87 ppm P content in S1 and S2, respectively.

There was a significant ($P<0.05$) effect of nitrogen fertiliser on potassium content in both seasons. Application of 50 kg N/ha produced the highest potassium of 823.6 ppm and 866.8 ppm in S1 and S2, respectively (Table 1). The 0 kg N/ha had 574 ppm and 565 ppm potassium in S1 and S2, respectively. Nitrogen had a significant ($P<0.05$) effect on magnesium content in S1 only. Application of 150 kg N/ha produced the highest magnesium content of 60.48 ppm and 61.44 ppm in S1 and S2, respectively. Application of 50 kg N/ha produced the lowest magnesium content of 55.87 ppm and 58.64 ppm during S1 and S2, respectively. Nitrogen had a significant ($P<0.05$) effect on beta-carotene content in pumpkin fruits in both seasons, where beta-carotene content was highest when 50 kg N/ha of fertilizer was applied.

The effect of nitrogen on TSS content in pumpkin fruits was significant ($P<0.05$) in S1 only (Table 1). The TSS content was highest when 50 kg N/ha of fertilizer was applied, yielding 3.15 ppm and 2.62 ppm during S1 and S2, respectively. The TSS content was lowest 1.10 ppm and 1.93 ppm when 150 kg N/ha was applied during S1 and S2, respectively.

Interactive effect of nitrogen fertiliser was significant in both seasons, when application of 100 and 150 kg N/ha produced the highest N, P and Mg contents. These results were attributed to the capacity of plants supplied with high amounts of nitrogen to build metabolites which in turn contributed the high levels and increases of N, P and Mg. Similar responses were reported by Ibrahim and Selim (2007). Das *et al.* (2015) concluded that TSS in bottle gourd fruits were enhanced positively by application of nitrogen, but this was not the case in the present study where TSS increased and then decreased. Acar *et al.* (2008) found that nitrogen rate had no significant effect on TSS of the leafy lettuce in Turkey.

3.2. Effect of mulch on Minerals and Metabolites in Fruit Pulp

Mulch had a significant ($P<0.05$) effect on nitrogen content in S1 only (Table 2). Use of black-painted rice straw mulch produced the highest nitrogen contents of 0.18 ppm in S1. The unpainted rice straw mulch produced the lowest nitrogen content of 0.17 ppm in S1.

The effect of mulch on phosphorus was significant ($P<0.05$) in both seasons. Unpainted rice straw mulch produced the highest phosphorus of 139.28 ppm and 140.80 ppm in S1 and S2, respectively, whereas black-painted rice straws produced the lowest in both seasons (Table 2).

Potassium content in both seasons was significantly ($P<0.05$) affected by mulch. No mulch produced fruits with the highest potassium content of 712.4 ppm and 745.4 ppm in S1 and S2, respectively (Table 2). The 640.2 ppm and 680.0 ppm potassium content in S1 and S2, respectively, was lowest under black-painted rice straw mulch.

Mulch had a significant ($P<0.05$) effect on magnesium content in S1 only. Application of black-painted rice straw mulch produced the highest magnesium content of 58.94 ppm and 63.59 ppm in S1 and S2, respectively. Use of no mulch produced the lowest magnesium content of 55.96 ppm and 58.00 ppm during S1 and S2, respectively (Table 2).

There was a significant ($P < 0.05$) effect of mulch on beta-carotene in both seasons (Table 2). The beta-carotene content of 17.78 ppm and 21.31 ppm in S1 and S2, respectively, was highest when unpainted rice straw mulch was applied. Lowest beta-carotene content was obtained under no mulch. Mulch had no significant ($P > 0.05$) effect on TSS in both seasons. The TSS of 2.39 ppm and 2.25 ppm in S1 and S2, respectively, were the highest when unpainted rice straw mulch was applied. The TSS content of 1.50 ppm and 2.21 ppm in S1 and S2, respectively, was the lowest produced when black-painted rice straw mulch was applied (Table 2).

The effect of mulch on N, P, K, Mg and beta-carotene was significant (Table 2). The high amounts of mulched plants could be explained by the fact that there was high absorption of nutrients due to high activity of roots in response to the accumulation of heat in mulched soil. The non-significant effect on TSS agreed with findings of Ekinici and Dursun (2009) in watermelon fruits. Eissa (2002) showed that strawberry plants grown with plastic mulch had significant increase in fruit TSS. Mulch significantly affected TSS in summer squash (Kumar and Sharma, 2018), watermelon (Ansary and Roy, 2005), and tomatoes (Sharma and Narendra, 2004; Aruna *et al.*, 2007). Perhaps other variables caused disparities in the various studies.

3.3. Effect of GA₃ on Minerals and Metabolites in Fruit Pulp

The effect of GA₃ was significant ($P < 0.05$) on nitrogen content in both seasons (Table 3). No GA₃ produced the highest nitrogen content of 0.19 ppm and 0.23 ppm in S1 and S2, respectively. In both seasons, nitrogen content was lowest of 0.16 ppm and 0.20 ppm in S1 and S2, respectively, when 80 mg/L GA₃ was applied.

Table 1: Effect of nitrogen on minerals and metabolites in fruit pulp

Nitrogen (kg/ha)	Nitrogen (ppm)		Phosphorous (ppm)		Potassium (ppm)		Magnesium (ppm)		Beta-carotene (ppm)		TSS (ppm)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
0 (Control)	0.05d	0.06d	134.79b	138.87b	574.0d	564.8d	56.80b	60.24	15.30c	15.49d	1.38b	2.39
50	0.17b	0.21b	115.96d	122.26d	823.6a	866.8a	55.87c	58.64	19.02a	22.19a	3.15a	2.62
100	0.12c	0.15c	127.49c	131.77c	614.5c	664.8c	56.03c	59.54	13.81d	21.60b	1.84b	1.99
150	0.35a	0.44a	149.56a	148.82a	702.4b	770.5b	60.48a	61.44	16.19b	17.88c	1.10b	1.93
<i>P-value</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.155</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.032*</i>	<i>0.351</i>
LSD 5%	0.011	0.016	0.813	4.027	1.006	55.12	0.507	2.578	2.578	0.199	1.297	0.994

Table 2: Effect of mulch on minerals and metabolites in fruit pulp

Mulch type	Nitrogen (ppm)		Phosphorous (ppm)		Potassium (ppm)		Magnesium (ppm)		Beta-carotene (ppm)		TSS (ppm)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Control	0.17b	0.22	134.10b	140.14a	712.4a	745.4a	55.96c	58.00b	13.62c	16.89c	1.72	2.23
Black	0.18a	0.22	122.48c	125.35b	640.2c	680.0b	58.94a	63.59a	16.85a	19.68b	1.50	2.21
Brown	0.17b	0.21	139.28a	140.80a	683.2b	724.7a	56.99b	58.31b	17.78b	21.31a	2.39	2.25
<i>P-value</i>	<i>0.013*</i>	<i>0.21</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.018*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.161</i>	<i>0.900</i>
LSD 5%	0.009	0.014	0.742	2.836	0.769	41.91	0.183	1.071	1.071	0.112	0.965	0.205

Table 3: Effect of GA₃ on minerals and metabolites in fruit pulp

GA ₃ (mg/L)	Nitrogen (ppm)		Phosphorous (ppm)		Potassium (ppm)		Magnesium (ppm)		Beta-carotene (ppm)		TSS (ppm)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
0(Control)	0.19a	0.23a	145.24a	147.97a	724.3a	782.0a	53.12c	57.49c	17.18b	19.54b	1.52	2.22
40	0.17b	0.21b	128.54b	131.57b	663.9b	692.8b	58.08b	58.69b	12.51c	18.28c	1.61	2.23
80	0.16c	0.20b	122.08c	126.76c	647.7c	675.4b	60.75a	63.72a	18.55a	20.06a	2.47	2.24
<i>P-value</i>	<i>0.001*</i>	<i>0.003*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.001*</i>	<i>0.077</i>	<i>0.988</i>
LSD 5%	0.009	0.014	0.959	3.368	0.697	41.73	0.240	0.955	0.150	0.092	0.909	0.228

S1= Season 1 (March 2019-July 2019), S2= Season 2 (October 2019-February 2020)

*Means followed by the same letter or no letter within a column are not significantly different according to the LSD Test at $P=0.05$

Gibberellic acid had a significant ($P<0.05$) effect on phosphorus in both seasons. The 0 mg/L GA₃ treatment yielded the highest phosphorus of 145.24 ppm and 147.97 ppm in S1 and S2, respectively. Lowest phosphorus of 122.08 ppm and 126.76 ppm in S1 and S2, respectively, was obtained when 80 mg/L GA₃ was applied (Table 3).

The potassium content in both seasons was significantly ($P<0.05$) affected by gibberellic acid. The control 0 mg/L GA₃ treatment had the highest potassium content of 724.3 ppm and 782.0 ppm in S1 and S2, respectively (Table 3). The 80 mg/L GA₃ potassium content was lowest of 647.7 ppm and 675.4 ppm during S1 and S2, respectively.

Results showed that gibberellic acid had a significant ($P<0.05$) effect on magnesium content in pumpkin fruits in both seasons. The 80 mg/L GA₃ produced the highest magnesium content of 60.75 ppm and 63.72 ppm during S1 and S2, respectively. On the other hand, the magnesium content was lowest of 53.12 ppm and 57.49 ppm during S1 and S2, respectively, under the control 0 mg/L GA₃ treatment (Table 3).

There was a significant ($P<0.05$) effect of GA₃ on beta-carotene in both seasons (Table 3). The 80 mg/L GA₃ had the highest beta-carotene of 18.55 ppm and 20.06 ppm in S1 and S2, respectively. Beta-carotene was lowest in S1 and S2 when 40 mg/L GA₃ was applied.

The effect of GA₃ on TSS in both seasons was not significant ($P<0.05$) in both seasons (Table 3). Nevertheless, the trend showed that 80 mg/L GA₃ yielded the highest TSS of 2.47 ppm and 2.24 ppm in S1 and S2, respectively. The TSS content was lowest of 1.52 ppm and 2.22 ppm in S1 and S2, respectively, under no GA₃ application.

The effect of GA₃ was consistently significant on mineral nutrients N, P, K, Mg and beta-carotene, but not significant on TSS in multipurpose pumpkin fruit pulp (Table 3). The N, P and K contents were lowest when 80 mg/L GA₃ was applied, implying that GA₃ led to their decrease. These results occurred probably because GA₃ plays an important role in promoting cell elongation and cycling, thereby bringing about a significant increase in morphological growth, but chemical composition dilution in fruit pulp (Goldberg-Moeller *et al.*, 2013). Results showing lowest Mg, beta-carotene and TSS in control treatments with 0 mg/L GA₃ and increase with high rates of GA₃ implied that GA₃ positively affects Mg, beta-carotene and TSS contents in multipurpose pumpkin fruit pulp.

Contrasting results showing an increase of K⁺ content, and decrease of beta-carotene content with increasing GA₃ rate in wax apple fruit (Moneruzzaman *et al.* 2011). The contrast in the results observed was attributed to the difference in species that were tested in the two studies.

3.4. Effect of nitrogen, mulch and GA₃ on Minerals and Metabolites in Fruit Pulp

Highest nitrogen content of 0.42 ppm was for N₃M₀GA₀, while lowest of 0.02 ppm was for N₀M₂GA₂ in S1 (Table 4). In S2, highest nitrogen content of 0.57 ppm was for N₃M₀GA₀, while the lowest of 0.03 was for N₀M₂GA₂. The N₃M₀GA₀ (150 kg N/ha, no mulch and 0 mg/L GA₃) had the highest combined effect on nitrogen content in S1 and S2. A significant ($P < 0.05$) effect of interaction was observed on nitrogen content in both seasons.

Phosphorus was highest 180.56 ppm for N₃M₂GA₁, while the lowest of 11.43 ppm was for N₁M₂GA₂ in S1 (Table 4). In S2, highest phosphorus of 175.39 ppm was for N₁M₂GA₀, while the lowest of 13.58 ppm was recorded for N₁M₂GA₂. The N₃M₂GA₁ (150 kg N/ha, unpainted rice straw mulch and 40 mg/L GA₃) and N₁M₂GA₀ (50 kg N/ha, unpainted rice straw mulch and 0 mg/L GA₃) had the highest combined effect on phosphorus content in both seasons. A significant ($P < 0.05$) effect of interaction on phosphorus content resulted in both seasons.

Table 4: Effect of nitrogen, mulch and GA₃ on minerals and metabolites in fruit pulp

Treatment	Nitrogen (ppm)		Phosphorous (ppm)		Potassium (ppm)		Magnesium (ppm)		Beta-carotene (ppm)		TSS (ppm)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
N ₀ M ₀ GA ₀	0.04	0.04	143.3	145.2	515.4	590.8	48.9	50.8	12.7	13.8	1.18	2.30
N ₀ M ₁ GA ₀	0.06	0.06	142.6	141.2	775.8	774.9	69.5	73.6	8.6	13.4	0.83	2.63
N ₀ M ₂ GA ₀	0.07	0.08	150.4	152.7	884.0	944.7	52.8	63.5	25.8	18.4	1.62	2.13
N ₀ M ₀ GA ₁	0.06	0.09	107.7	109.3	587.4	624.9	60.3	62.5	12.1	15.5	1.32	2.07
N ₀ M ₁ GA ₁	0.05	0.04	141.2	143.5	353.5	402.9	47.4	51.6	26.2	17.5	2.07	2.63
N ₀ M ₂ GA ₁	0.06	0.06	145.8	149.3	444.9	505.3	60.8	63.1	7.3	13.0	2.45	2.43
N ₀ M ₀ GA ₂	0.06	0.07	144.6	160.1	739.7	644.9	61.4	62.1	8.2	(11.5)	1.10	2.33
N ₀ M ₁ GA ₂	0.07	0.07	101.3	107.8	534.6	609.8	60.8	64.3	24.7	18.4	1.03	2.43
N ₀ M ₂ GA ₂	(0.02)	(0.03)	136.1	140.8	(330.5)	(329)	49.4	50.8	12.2	18.0	0.83	2.50
N ₁ M ₀ GA ₀	0.18	0.24	149.5	150.7	539.1	601.7	49.0	48.5	17.4	13.6	2.28	1.50
N ₁ M ₁ GA ₀	0.17	0.18	128.5	136.9	717.8	955.2	57.3	62.5	25.9	21.6	0.82	2.23
N ₁ M ₂ GA ₀	0.13	0.14	170.3	175.4	998.3	1062.5	49.1	55.4	22.1	15.5	2.87	2.10
N ₁ M ₀ GA ₁	0.13	0.17	141.7	159.6	1293.1	1148.2	47.3	58.3	12.6	12.4	2.47	2.20
N ₁ M ₁ GA ₁	0.42	0.48	15.4	19.9	622.5	691.9	56.6	58.4	8.9	14.5	1.95	2.10
N ₁ M ₂ GA ₁	0.10	0.14	133.7	138.6	560.0	639.0	58.4	56.3	25.8	23.6	8.70	1.47
N ₁ M ₀ GA ₂	0.19	0.25	152.3	161.6	872.6	981.4	77.6	68.3	12.8	14.3	3.03	2.13
N ₁ M ₁ GA ₂	0.11	0.16	140.8	144.2	592.6	711.8	50.9	56.2	22.5	24.7	2.38	(1.43)
N ₁ M ₂ GA ₂	0.11	0.11	(11.4)	(13.6)	1016.6	1009.2	56.7	64.1	23.4	20.7	3.38	2.17
N ₂ M ₀ GA ₀	0.15	0.17	116.0	125.4	787.3	743.7	54.1	69.7	16.1	24.7	2.18	2.10
N ₂ M ₁ GA ₀	0.12	0.17	150.5	155.6	630.1	717.6	58.2	63.5	17.5	22.6	2.48	1.57
N ₂ M ₂ GA ₀	0.14	0.19	165.8	159.3	539.6	612.4	49.9	51.5	25.6	20.5	0.97	2.27
N ₂ M ₀ GA ₁	0.06	0.07	129.7	136.7	492.8	559.6	49.0	(37.0)	9.7	17.5	2.98	1.50
N ₂ M ₁ GA ₁	0.12	0.15	115.8	118.1	706.4	795.3	60.2	61.8	3.1	23.6	2.47	2.27
N ₂ M ₂ GA ₁	0.14	0.16	111.6	118.4	539.3	605.6	69.5	75.9	2.3	21.4	1.80	2.13
N ₂ M ₀ GA ₂	0.12	0.15	114.8	117.4	613.5	658.4	55.8	67.6	1.7	17.6	0.32	2.33
N ₂ M ₁ GA ₂	0.03	0.04	95.6	99.7	569.5	626.4	49.7	50.6	25.8	25.1	0.82	2.13
N ₂ M ₂ GA ₂	0.18	0.26	147.7	155.3	652.1	664.3	57.8	58.3	22.6	21.6	2.53	1.57
N ₃ M ₀ GA ₀	0.42	0.57	161.7	167.4	939.2	1054.1	47.6	47.4	28.8	24.5	0.23	2.87
N ₃ M ₁ GA ₀	0.36	0.46	117.5	117.5	622.2	707.8	57.9	63.6	(1.3)	21.3	0.48	2.20
N ₃ M ₂ GA ₀	0.39	0.44	146.7	148.5	543.1	618.3	(43.2)	40.4	21.0	24.5	2.32	2.80
N ₃ M ₀ GA ₁	0.30	0.38	154.1	155.2	613.2	702.2	54.3	40.9	16.9	18.5	0.55	2.77
N ₃ M ₁ GA ₁	0.32	0.38	165.2	165.7	612.6	682.1	56.8	84.9	21.6	25.3	2.52	2.40
N ₃ M ₂ GA ₁	0.34	0.44	180.6	164.6	1140.5	1092.1	55.7	53.9	3.8	16.6	0.42	2.70
N ₃ M ₀ GA ₂	0.30	0.43	93.8	93.2	555.8	616.8	78.6	83.1	14.4	18.8	2.97	2.90
N ₃ M ₁ GA ₂	0.35	0.41	155.3	154.0	745.4	846.7	62.1	72.7	16.2	27.8	(0.20)	2.43
N ₃ M ₂ GA ₂	0.36	0.46	171.2	173.4	549.4	614.5	68.1	66.6	21.7	22.4	0.25	2.50
<i>P-value</i>	<i>0.001</i>	<i>0.001</i>	<i>0.001</i>	<i>0.001</i>	<i>0.001</i>	<i>0.001</i>	<i>0.001</i>	<i>0.001</i>	<i>0.001</i>	<i>0.001</i>	<i>0.088</i>	<i>0.91</i>
LSD 5%	0.029	0.047	3.001	10.92	2.425	142.4	0.843	3.824	0.504	0.352	3.134	1.14

S1= Season 1 (March 2019-July 2019); S2= Season 2 (October 2019-February 2020).

Bolded values = Highest; Bracketed values = Lowest.

Highest K of 1293.1 ppm resulted for $N_1M_0GA_1$, while the lowest 330.5 ppm was for $N_0M_2GA_2$ in S1 (Table 4). The highest K of 1148.2 ppm was for $N_1M_0GA_1$, while $N_0M_2GA_2$ had the lowest K of 329.0 ppm in S2. The $N_1M_0GA_1$ (50 kg N/ha, no mulch and 40 mg/L GA_3) had highest K in S1 and S2. A significant ($P<0.05$) interactive effect on K occurred in both seasons.

Magnesium was highest 78.60 ppm for $N_3M_0GA_2$, while the lowest 43.22 ppm was obtained for $N_3M_2GA_0$ in S1. The highest Mg of 84.85 ppm was for $N_3M_1GA_1$, while $N_2M_1GA_0$ had the lowest 36.95 ppm in S2. The $N_3M_0GA_2$ (150 kg N/ha, no mulch and 80 mg/L GA_3) and $N_3M_1GA_1$ (150 kg N/ha, black-painted rice straws and 40 mg/L GA_3) had the highest effect in both seasons. There was a significant ($P<0.05$) effect of interaction on Mg in both seasons.

The highest beta-carotene of 28.77 ppm was for $N_3M_0GA_0$, while the lowest 1.33 ppm was for $N_3M_1GA_0$ in season 1 (Table 4). In S2, highest beta-carotene of 27.81 ppm was for $N_3M_1GA_2$, while 11.45 ppm was for $N_0M_0GA_2$. The $N_3M_0GA_0$ (150 kg N/ha, no mulch and 0 mg/L GA_3) and $N_3M_1GA_2$ (150 kg N/ha, black painted rice straws and 80 mg/L GA_3) had the highest combined effect on beta-carotene in both seasons. There was a significant ($P<0.05$) effect of interaction on beta-carotene produced in both seasons.

The TSS content of 8.70 ppm were highest for $N_1M_2GA_1$, while the lowest of 0.20 ppm were for $N_3M_1GA_2$ during S1. The 2.90 ppm were highest TSS for $N_3M_0GA_2$, while $N_1M_1GA_2$ had the lowest TSS of 1.43 ppm. The $N_1M_2GA_1$ (50 kg N/ha, unpainted rice straws and 40 mg/L GA_3) and $N_3M_0GA_2$ (150 kg N/ha, no mulch and 80 mg/L GA_3) had the highest combined effect on TSS produced in both seasons. There was no significant ($P>0.05$) interactive effect on TSS in both seasons. Similarly, Leilah and Khan (2021) observed foliar application of GA_3 and N significantly decrease TSS in sugar beet.

CONCLUSIONS AND RECOMMENDATIONS

Fruit pulp results showed that nitrogen fertilizer positively and significantly affects N, P and Mg consistently. It negatively and significantly affects K, beta-carotene and TSS. The effect of mulch is positive and significant on P, Mg and beta-carotene, but not significant on TSS content. Gibberellic acid has a positive and significant effect on Mg and beta-carotene. The effect of GA_3 is negative and significant on N, P, and K contents, while it is positive and significant on Mg and beta-carotene. The GA_3 effect is consistently not significant on TSS. Combined nitrogen, mulch and GA_3 effect on N, P, K, Mg and beta-carotene is consistently significant. The interactive effect on TSS is consistently not significant. Sole nitrogen fertilizer, mulch, GA_3 , and combined rates that promote the desired mineral nutrient and metabolite contents in pumpkin fruit pulp should be adopted and applied, depending on the kind.

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