INTEGRATED NITROGEN, MULCH AND GIBBERELLIC ACID SIGNIFICANTLY ENHANCE PHOTOSYNTHATES IN MULTIPURPOSE PUMPKIN LEAVES

Isutsa, Dorcas Khasungu *and Mwaura, Margaret Mahiga

Department of Plant Sciences, Chuka University, P. O. Box 109-60400, Chuka

Email: profdkisutsa@gmail.com, disutsa@chuka.ac.ke, margretmwaura@gmail.com

*Telephone: +254-0721252293; + 254-0720571981. * Corresponding author.*

ABSTRACT

Pumpkin (*Cucurbita moschata* Duch.) edible leaves, fruits and seeds contribute to household food and nutrition enhancement. However, cultivation using limitedinputslead to poor growth. This study assessed effectsof nitrogen, mulch and gibberellic acid (GA_3) on photo synthates in pumpkin leaves. Nitrogen at 0, 50, 100 and 150 kgN/ha, mulchas none, unpainted and black-painted rice straws, and three GA_3at 0, 40 and 80mg/L were appliedin randomized complete block design, replicated three times in two seasons,with2mx2mplant-spacing. Data values were subjected to analysis of variance using SAS and means separated using the least significant difference test (α=0.05).Nitrogen significantly (P<0.05) increased moisture and proteins, while it negatively reduced fat, ash and carbohydrates. Mulch significantly reduced moisture, fat, ash and proteins, but increased total carbohydrates. GA_3significantly reduced moisture,fat and proteins only. Combined N, mulch and GA_3effect consistently increased moisture and proteins (highest 21% for $N_3M_1GA_1$), but reduced fat, ash and carbohydrates (lowest 46% for $N_3M_1GA_0$). This study recommends use of N, mulch and GA_3 that elicitthe desired response.

Keywords: Moisture content, Nutrient content, Proximate analysis, Neglected species.

1. INTRODUCTION

The oldest evidence on pumpkin-related seeds dating back to 7000 and 5500 BC are in Mexico (Milne, 2005).Although pumpkin is a native of Central America, it is now domesticated in many 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. Grubben and Chigumira-Ngwerume (2004) stated that pumpkin can grow in almost any part of East Africa and storage after harvesting can last over eight months provided the fruit stalk is retained, making it an appropriate food security crop (Horticultural Crops Development Authority, 2012). In Kenya, pumpkin production increased from 599 ha in 2015 to 681 ha in 2016, and volume rose from 3580 tonnes to 4017 tonnes (Horticulture Validated Report, 2016-17), although it is still regarded as a traditional vegetable. Karanja *et al.* (2014) stated that there is desertion of pumpkin production in Kenya. Nonetheless, FAO (2005) reported that pumpkin has immense economic potential as a food and industrial crop.It is famous for edible seeds, fruits and green parts (Matsui *et al*., 1998). Boiling, baking or soup thickening with fruits and dry-roasting of salted seeds produce delicious meals, while young leaves and flowers are perfect alternatives to kales (Oluoch, 2012).

Pumpkin production and consumption have risen due to several reasons including medicinal properties through the antioxidant beta-carotene that helps improve immune function and reduce cancer and heart disease risks (Ghanbari *et al*., 2007). Pumpkin contains mineral nutrients such as Ca, Fe, Mg, K, Zn, Se, niacin, foliate, and vitamins A, C, and E (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). Pumpkin fruits are good sources of carotenoids and g-aminobutyric acid (Murkovic *et al*., 2002). Pumpkin seeds are valued for their essential fatty acids like linoleic acid, amino acids, elements such as K, Cr, Na, Mg, Zn, Cu, Mo, Se (Glew *et al*., 2006), oils (50% w/w) and proteins (35%) that vary depending on cultivar (Fruhwirth and Hermetter, 2007). Several phytochemicals such as polysaccharides, phenolic glycosides, Non-Essential Fatty Acids (NEFA) and proteins have been isolated from pumpkin leaves and germinated seeds (Koike *et al*., 2005). Pumpkin fruit pulp has various hypoglycaemic polysaccharides. D-chiro-Inositol in pumpkin is an insulin secretor and sensitizer (Jun *et al*., 2006). Antibiotic components including anti-fungal ones have been characterized from various parts of pumpkin (Glew *et al*., 2006).

Despite of these documented immensebenefits, limited pumpkin is produced, commercialized and consumed in Africa (Ondigi *et al*., 2008). There are no documented pumpkin value chain preferences and consumption trends that exist in Kenya (Ondigi *et al*., 2008).Nevertheless,Hewett (2006) reported that temperature, relative humidity, water potential, light, cultural practices and pest management techniques are key pre-harvest factors that determine the inherent quality of horticultural produce. Nitrogen is by far the most critical plant growth element, yet soil testing is usually not practical due to nitrogen's mobility in the soil(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 a supplemental source of nutrients has been on the increase in pumpkin production but they are not applied in balanced proportions by most farmers. Furthermore, NPK fertilizer has been found to increase leaf area, stem diameter, leaves and nutrient contents (N, P, K, Ca, Na and Mg) in the soil under pumpkin production (Okonwu and Mensah, 2012). Mulch has been reported to enhance

germination of directly seeded pumpkin since it increases soil temperature. The high soil temperatures associated with mulch also accelerate establishment of transplants and promote subsequent crop development, thereby increasing yields and promoting crop maturity (Waterer, 2000). According to Yamaguch and Kamiya (2000)gibberellin (GA_3) plays an essential role in many aspects of pumpkin growth and development such as seed germination, stem elongation and flower development.The overall objective of the present study was to evaluate the interactive effects of nitrogen, mulch and GA_3on photosynthetic components of field-grown multi-purpose pumpkin.

2. MATERIALS AND METHODS

2.1. Experimental Site

The field experiment was conducted in Chuka University'sHorticultural Research Farmbetween January 2019 and August 2020. The farm lies at0^o 19' S, 37^{o}38' E and 1535 m above sea level. The average annual temperature is 19.5^{o}C (12.2ºC to 23.2ºC). The area experiences two rainy seasons with the long rains occurring from March to June and short rains from October to December (Jaetzold *et al.,* 2006). The average annual rainfall is 1200 mm (http://en.climate-data.org). The soils are humic nitisols, deep, strongly weathered, well drained with a clayey subsurface horizon and high cation exchange capacity (Koskey *et al.,* 2017).

2.2. Experimental Design and Treatments

Three-factorplots embedded in a randomized complete block design with three replications were used. Each experimental plot measured 2m x 2m separated from others by 1 m space. The three factors tested were nitrogen fertiliserapplied as CAN, mulch and gibberellic acid (GA_3). Nitrogen fertiliser was assigned to main-plots, mulch to sub-plots and $GA_3$to split-plots. The four nitrogen rates were 0, 50, 100 and 150 kg N/ha appliedin two equal doses for each rate, at three weeks post-emergence and beginning of flowering. Amount of CAN fertilizer used per experimental unit was calculated as: a) 50 kg N/ha = 76.9 g CAN/4 m^2; b) 100 kg N/ha = 153.8 g CAN/4 m^2; c) 150 kg N/ha = 230.7 g CAN/4 m^2.

The mulch used wasnone, unpainted and black-painted rice straws obtained easilyfrom farms nearthe experimental site inrequired quantities. The black-painted and unpainted dry rice strawswere placed on the respective plots after land preparation. Painting of the rice straws was done by dipping them in a 200-L drum withblack paint solution, followed by spreading out on the soil to air-dry. The rice straws were uniformly spread on plots to achieve 20 cm thickness. Planting holes were marked and opened in rice straw mulch during pumpkin seed sowing.

The GA_3rates used were 0 mg/L, 40 mg/L and 80 mg/L. The $GA_3$was dissolved in 50ml alcohol and then the volume was topped to one litre stock solution by adding distilled water. The required concentration of spray solution was then prepared from the stock solution by diluting using distilled water. A few drops of commercial sticker were added to eachsolution to facilitate uptake of the GA_3 into leaves. The GA_3solution was applied to plants using a 1-L hand-held sprayer. The$GA_3$of lowconcentration was sprayed first followed by next higher rate. Spraying was done once during the fourth week after emergence. To avoid chemical drift, spraying was done during a calm morningwhile facing away from wind direction.

2.3. Pumpkin Plant Establishment and Maintenance

Three multipurpose pumpkin 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 sowed immediately after extraction following themethod recommended for handling pumpkin seeds for planting (AOAC, 1995).The field was prepared to appropriate tilth required for pumpkin growth. 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 was uprooted two weeks after emergence. All plots were kept weed-free through rogueing and manual weeding. Irrigation was done using drip tubes to supplement rain during drought. Control of insect pest and diseases was done using recommended pesticides and rates. The vines were coiled as necessary,while leaving them in contact with the soil. Data was taken from all the experimental plants except those in guard rows.

2.4. Data Collection and Analysis

Data was collected for the two experimental seasons with Season 1 running from March 2019 to July, 2019 with 1,004.3 mm rainfall, and Season 2 running from October 2019 to February 2020 with 1,259.6 mm rainfall.Moisture content determination was doneusing oven-drying method. The Mitamula MRK oven was calibrated to keep a steady temperature of 105^{o}C for three hours. Aluminum dishes were cleaned and dried in the oven for one hour and then cooled in a desiccator with dry silica gel for 15 minutes and weighed. The same process was repeated until their weights were constant and then the average weight for the dry empty dish was recorded as w_o. About 2 g of sample was placed onto each dry dish and the weight recorded as w_i. The sample dishes were placed in an oven in triplicates and dried for 1 hour, three times, until a constant weight was obtained for cooled dry samples. The final weight was noted for dry dish and sample as w_i. Moisturewas obtained using the formula: $\%\ moisture\ content = 100 - [(w_i - w_o/w_i - w_o) \times 100]$.

Determination of total ash was done using dry-ashing method (Ali*et al.,* 1988). Muffle furnace was set at steady temperature of 550^{o}C and clean dry porcelain crucibles were heated in the furnace for five hours. The crucibles were then cooled in a desiccator containing dry silica gel for 15 minutes and then weighed to get weight zero (w_o). About 2g of sample in the dry crucibles were weighed in triplicates to get weight one (w_i). The sample crucibles were burnt until they produced no smoke and placed in the furnace where they were incinerated for 5 hours. They were then removed from the furnace, cooled in a desiccator for 15 minutes and weighed to get weight two (w_i). Ash was calculated using the formula (Jones, 2001): $\%\ ash = (w_i - w_o/w_i - w_o) \times 100$.

Total carbohydrates (simple sugars, crude fibre, starch and all other polysaccharides) were determined by the difference, after proximate analysis results were obtained, using McCready*et al.,* (1950) formula:$\%\ total\ carbohydrates = 100 - (\%\ moisture + \%\ ash + \%\ fat + \%\ protein)$.

Crude nitrogen was determined using the semi micro-Kjeldhal procedure. Crude protein value was obtained by multiplying the nitrogen value by a factor of 6.25, which was taken as the general protein factor. A sample of 1g was transferred into 300ml Kjeldahl digestion flask of which5 g of potassium sulphate and 0.5g of copper (II) sulphate were added. To the sample, 15ml of concentrated sulphuric acid was added and digested on a heater in a fume hood until it turned clear blue. The digest was diluted to 100 ml volume using distilled water. An aliquot of10 ml of the diluted digest was transferred to Pannas and Wagner distillation apparatus and 15 ml of 40% sodium hydroxide added. Steam distillation was then done. About 70 ml of distillate was received in 4% boric acid with several drops of mixed indicator. A blank was set up using the same procedure. The received aliquot was titrated with 0.02 N standard hydrochloric acid and crude protein content was calculated using the formula:*% crude protein = [(V-B) × 0.02 × 0.014 × 100/v× 100/s] × 6.25,* where: V= Tire volume of 0.02N HCl, B= Blank titre volume of 0.02N HCl, V= volume of the diluted digest taken for distillation, and S= sample weight taken.

Fat content in samples was determinedusingSoxhlet continuous extraction method, whereby 300ml fat receiver flasks (ground joint flat-bottomed flasks) were cleaned, dried at 105^{o}C, cooled in a desiccator, weighed and recorded as w_0. A 5g of sample was added into cellulose thimble, flagged with defatted cotton wool and inserted in the Soxhlet extraction apparatus. The receiver flask was half-filled with analytical grade 40 – 60 petroleum ether and connected to the Soxhlet extraction apparatus.The apparatus was then connected to a condenser and the whole assembly was heated at the flask base on a water bath. Extraction was carried out for 16 hours. The ether was then evaporated from the receiver flasks using Rotary evaporator and the flasks were dried for one hour at 105^{o}C in a hot air circulation oven.The flasks were cooled in a desiccator and then weighed.The final weight was obtained and recorded as w_1. The percent fat content was determined using the formula suggested by AOAC (1990) as: *%fat = [(w_1-w_0)/sample weight] ×100.*Data values 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 Moisture Content and Photosynthates in Leaves

Nitrogen had a significant (P<0.05) effect on moisture content in both seasons (Table 1). Application of 150kg N/ha produced the highest moisture content of 12.99% and 12.75%in S1 and S2, respectively. Control pumpkin leaves had the lowest moisture content of 12.46% and 12.26% for S1 and S2, respectively.

Nitrogen had a significant (P<0.05) effect on the fat content in both seasons. Application of 150kg N/ha produced the lowest fat content of0.90% and 0.85% for S1 and S2, respectively (Table 1). Fat content decreased with increase in nitrogen up to 150 kg N/ha in both seasons. In the control treatment, pumpkins had 1.22% and 1.56% fat content in S1 and S2, respectively.

Nitrogen had a significant (P<0.05) effect on protein content in both seasons. Application of 150 kg N/ha produced the highest protein content of 17.98% and 18.48% during season 1 and 2, respectively (Table 1). Protein content increased with increase in nitrogen up to 150 kg N/ha in both seasons. The protein content for zero nitrogen was 4.53% and 4.15% in S1 and S2, respectively. There was a 126.9% and 154.7% increase in protein content of the pumpkin leaf vegetables when 50 kg N/ha was applied compared to zero nitrogen.

Nitrogen fertiliser had a significant (P<0.05) effect on ash content in leavesin both seasons (Table 1). The control treatment produced the highest ash contentof 21.98% and 22.84%in S1 and S2, respectively. When 150 kg N/ha was applied, the ash content was lowest of18.42% and 20% in S1 and S2, respectively. Ash content reduced with increase in nitrogen rate.

Nitrogen had a significant (P<0.05) effect on carbohydrates in both seasons. The control treatment produced the highest carbohydrates of 59.69% and 59.42% in S1 and S2, respectively. Total carbohydrates decreased with increase in nitrogen up to 150 kg N/ha in both seasons.

Nitrogen had a significant effect on moisture, fat, proteins, ash and total carbohydrates in pumpkin leaves. Additionally, moisture and proteins were enhanced as N increased. The fat, ash and total carbohydrate contents in leaves were negatively affected by nitrogen since there was reduction as the nitrogen fertilizer was increased. The results are agreed with those of Litke*et al.* (2018) and Weber *et al.* (2008), who reported a significant effect of N on the moisture, protein and carbohydrate contents in wheat straws and grains. Nitrogen is thus a significant factor in enhancing the moisture and protein contents in leaves of multi-purpose pumpkin probably through enhancement of biosynthetic and resource accumulation processes.

3.2. Effect of Mulch on Moisture Content and Photosynthates in Leaves

Mulch had a significant (P<0.05) effect on moisture content in both seasons (Table 2). Application of unpainted mulch produced the highest moisture content of 12.79% and 12.59%in S1 and S2, respectively. In both seasons, lowest moisture content of 12.54% and 12.37% for S1 and S2, respectively, was obtained when black-painted rice straw mulch was applied.

Mulch had a significant (P<0.05) effect on the fat content in both seasons. No mulch produced the highest fat content of 1.19% and 1.14% in S1 and S2, respectively, while the lowest fat content was produced when unpainted rice straw mulch was applied on the soil.

Mulch type had a significant (P<0.05) effect on protein content in both seasons. No mulch produced highest protein content of 11.43% and 12.11% in S1 and S2, respectively (Table 2). Protein content was lowest 10.51% and 10.99% in S1 and S2, respectively, when unpainted mulch was applied. When black-painted mulch was applied, protein content was 11.29% and 11.20% in S1 and S2, which showed that pumpkin performed slightly better than with no mulch.Mulch had no significant (P>0.05) effect on ash content in both seasons (Table 2). No much produced the highest ash contentof 20.84% and 21.88% in S1 and S2, respectively. In S1 and S2, the ash content was lowest 19.85% and 21.06%, respectively, when black-painted mulch was applied. Mulch had a significant (P<0.05) effect on total carbohydrates during both seasons. Unpainted rice straw mulch treatment produced the highest total carbohydrates of 55.55% in S1, while black-painted rice straw mulch had the highest total carbohydrates of 54.36% in S2.

Mulch significantly affected moisture, fat, proteins and total carbohydrates in leaves (Table 2). Unpainted rice straw had the highest moisture content. Mulched plants had the highest fat, proteins and ash contents. Mulched plants have been confirmed to have better growth, owing to stable soil temperatures and moisture, leading to increase in fat, protein and ash contents. Mulch positively affected total carbohydrates by conserving moisture that leads to better physiological processes for manufacture and production. Mulches also function by absorbing and maintaining high soil temperatures around plants, which in turn promote total carbohydrate manufacture and assimilation into plants including cucurbits (Iqbal *et al.,* 2020; Kosterna *et al.*, 2010). These effects of mulch might explain the trends observed in the present study.

3.3. Effect of GA_3 on Moisture Content and Photosynthates in Leaves

Gibberellic acid had a significant ($P<0.05$) effect on moisture content in S1 and no significant ($P>0.05$) effect in S2 (Table 3). In both seasons, moisture content was highest 12.74% and 12.52% in S1 and S2, respectively, when no GA_3 was applied. Lowest moisture content of 12.60% and 12.40% in S1 and S2, respectively, was obtained when 40 mg/L GA_3 was applied. There was a significant ($P<0.05$) effect of GA_3 on fat content in S1 only. The 0 mg/L GA_3 had the lowest fat content of 1.06% and 1.02% in S1 and S2, respectively, while highest fat content wasfor 80 mg/L GA_3. Fat content increased as the GA_3 was increased.

The effect of GA_3 on protein content was significant ($P<0.05$) in both seasons. In the treatment where 40 mg/L GA_3 was applied, protein content was highest of 11.68% and 12.18% in S1 and S2, respectively (Table 3). Protein content was lowest of 10.67% and 10.84% during S1 and S2, respectively, under the control treatment. The applied GA_3 had no significant ($P>0.05$) effect on ash content in both seasons. The 0 mg/L GA_3 had the highest ash contentof 20.37% and 21.73%in S1 and S2, respectively. The ash content was lowest of 20.19% and 21.03% in S1 and S2 when 80 mg/L GA_3 was used. The ash content declined as GA_3 increased.

The GA_3 trend on total carbohydrates showed 80 mg/L GA_3 yield the highest total carbohydrates of 55.33% and 54.01% in S1 and S2, respectively. Lowest total carbohydrates of 54.27% and 52.96% in S1 and S2 were obtained when 40 mg/L GA_3 was applied. The effect of GA_3 was significant in S1 for moisture, fat and protein contents only. Moisture and ash contents were highest under the control treatment, implying that GA_3 had a negative effect on them.

Table 1: Effect of nitrogen on moisture content and photosynthates in leaves

Nitrogen	Moisture content (%)		Fat (%)		Protein content (%)		Ash (%)		Total carbohydrates (%)	
(kg/ha)	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
0 (Control)	12.46d	12.26c	1.22a	1.56a	4.53d	4.15d	21.98a	22.84a	59.69a	59.42a
50	12.71b	12.45b	1.22a	1.09ab	10.28c	10.57c	21.28a	22.07ab	56.37b	55.10b
100	12.57c	12.43b	1.11b	1.07b	11.51b	12.56b	19.42b	20.79bc	53.63c	52.04c
150	12.99a	12.75a	0.90c	0.85c	17.98a	18.46a	18.42c	20.00c	49.71d	47.93d
P-value	*0.001**	*0.001**	*0.001**	*0.001**	*0.001**	*0.001**	*0.001**	*0.034**	*0.001**	*0.001**
LSD 5%	0.005	0.145	0.002	0.077	1.187	0.555	1.158	1.844	1.727	2.009

Table 2: Effect of mulch on moisture content and photosynthates in leaves

Mulch type	Moisture content (%)		Fat (%)		Proteins (%)		Ash (%)		Total carbohydrates (%)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Control	12.72b	12.47ab	1.19a	1.14a	11.43a	12.11a	20.84	21.88	53.82b	52.40b
BL	12.54c	12.37b	1.14b	1.07b	11.29a	11.20b	19.85	21.06	55.19ab	54.36a
BR	12.79a	12.59a	1.01c	0.97b	10.51b	10.99b	20.14	21.34	55.55a	54.11a
P-value	*0.001**	*0.005**	*0.001**	*0.001**	*0.001**	*0.018**	*0.346*	*0.311*	*0.044**	*0.004**
LSD 5%	0.001	0.121	0.002	0.064	1.057	0.776	1.432	1.123	1.396	1.130

Table 3: Effectof GA_3on moisture content and photosynthates in leaves

GA_3 (mg/L)	Moisture content (%)		Fat (%)		Proteins (%)		Ash (%)		Total carbohydrates (%)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
0 (Control)	12.74a	12.52	1.06c	1.02	10.67b	10.84b	20.37	21.73	54.95	53.89
40	12.60c	12.40	1.12a	1.04	11.68a	12.18a	20.27	21.52	54.27	52.96
80	12.71b	12.50	1.17b	1.06	10.88b	11.39b	20.19	21.03	55.33	54.01
P-value	*0.001**	*0.186*	*0.001**	*0.49*	*0.001**	*0.001**	*0.915*	*0.388*	*0.090*	*0.130*
LSD 5%	0.003	0.138	0.002	0.072	2.084	0.581	0.884	1.031	0.961	1.121

BL= black-painted rice straw mulch; BR= unpainted rice straw mulch

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

The fat and protein contents being lowest under the control implied that GA_3 positively affected them. Previously, Olaiya *et al.* (2010) reported that ash content was higher when auxins were applied in tomato. Nandi *et al.* (1995) got an increase in protein content in tea shoots and oak tissues treated with plant growth regulators. These results contrasted with those of the present study probably because they concerned auxins and woody plants.

3.4. Effect of Nitrogen, Mulch and GA_3 on Leaf Moisture Content and Photosynthates

A significant(P<0.05)interactiveeffect of N, mulch and GA_3on moisture content was observed in S1 and S2 (Table 4). Highest moisture content was 14.23% for $N_3M_1GA_0$, whilelowest was 11.37%for $N_1M_1GA_0$in S1. In S2, highest moisture content of 14.14% wasfor $N_3M_1GA_0$, while lowest of 11.00% was for $N_1M_2GA_0$. The $N_3M_1GA_0$ (150kg N/ha, black-painted mulch and 0 mg/LGA_3) had the highest combined effect of nitrogen, mulch and GA_3on moisture content.

A significant (P<0.05) interactive effect was observed on fat content in S1 and S2. Fat content was highest 1.88% for $N_1M_1GA_1$, while the lowest fat content of 0.53% was for $N_3M_2GA_2$ during S1 (Table 4). In S2, highest fat content of 1.75% was recorded for $N_3M_0GA_0$, while the lowest fat content of 0.48% was recorded for $N_3M_2GA_2$. The $N_1M_1GA_1$ (50 kg N/ha, black-painted rice straw mulch and 40 mg/L GA_3) and $N_3M_0GA_0$ (150 kg N/ha, no mulch and 0 mg/L GA_3) had the highest combined effect on fat content in S1 and S2, respectively.

Protein content was highest 20.40% for $N_3M_1GA_0$, while the lowest of 2.43% was for $N_0M_0GA_0$ during S1. The 20.75% was the highest protein content obtained for $N_3M_1GA_1$, while $N_0M_0GA_0$ had the lowest of 1.80% in S2.The $N_3M_1GA_0$ (150 kg N/ha, black painted rice straw mulch and 0 mg/L GA_3) and $N_3M_1GA_1$ (150 kg N/ha, black painted rice straw mulch and 40 mg/L GA_3) had the highest interactive effect on protein content in S1 and S2 respectively. There was a significant (P<0.05) effect due to interaction on protein content produced in S1 and S2.

Table 4: Effect of nitrogen, mulch and GA_3 on leaf moisture content and photosynthates

Treatment	Moisture content (%)		Fat (%)		Proteins (%)		Ash (%)		Total carbohydrates (%)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
$N_0M_0GA_0$	13.3	13.1	0.69	0.64	2.43	1.80	21.6	22.8	62.0	61.7
$N_0M_1GA_0$	11.5	11.5	1.23	1.27	4.00	3.18	22.4	22.2	60.9	61.9
$N_0M_2GA_0$	12.3	12.0	1.56	1.52	4.51	3.55	19.8	21.7	61.8	61.2
$N_0M_0GA_1$	13.2	13.0	1.73	1.68	7.02	8.72	23.4	23.7	54.6	52.9
$N_0M_1GA_1$	12.4	12.2	1.49	1.15	4.12	3.29	19.5	22.2	62.6	61.2
$N_0M_2GA_1$	12.7	12.4	0.61	0.57	4.07	(3.08)	25.2	26.3	57.5	57.6
$N_0M_0GA_2$	12.7	12.4	1.30	1.25	4.17	3.83	22.7	21.7	59.2	60.8
$N_0M_1GA_2$	13.5	14.0	1.33	1.28	7.88	6.76	21.4	22.8	55.9	55.2
$N_0M_2GA_2$	11.6	11.3	1.07	1.03	(2.58)	3.12	21.9	22.3	**62.7**	**62.3**
$N_1M_0GA_0$	12.7	12.4	1.15	1.11	10.99	10.06	21.1	22.2	54.1	54.2
$N_1M_1GA_0$	(11.4)	(11.0)	1.21	1.17	10.58	8.13	18.5	20.5	58.3	59.2
$N_1M_2GA_0$	14.0	13.9	0.73	0.68	7.89	7.36	17.0	19.0	60.4	59.1
$N_1M_0GA_1$	12.6	12.4	0.73	0.68	10.17	11.20	18.2	18.9	58.2	56.8
$N_1M_1GA_1$	12.2	11.9	**1.88**	1.07	12.05	12.51	20.4	21.5	53.5	53.1
$N_1M_2GA_1$	12.5	12.2	1.43	1.38	10.22	11.64	19.3	21.2	56.6	53.7
$N_1M_0GA_2$	12.9	12.6	1.06	1.01	12.17	14.23	18.3	19.5	55.6	52.6
$N_1M_1GA_2$	12.5	12.2	1.52	1.47	11.24	11.59	21.8	22.6	52.9	52.1
$N_1M_2GA_2$	13.7	13.5	1.29	1.24	7.17	8.38	20.1	21.9	57.8	55.0
$N_2M_0GA_0$	12.7	12.5	1.40	1.36	11.66	11.17	19.3	20.6	54.9	54.4
$N_2M_1GA_0$	12.2	11.9	0.54	0.50	11.83	16.22	22.6	23.8	52.8	47.6
$N_2M_2GA_0$	12.7	12.4	1.19	1.15	12.19	13.52	22.7	24.0	51.3	48.9
$N_2M_0GA_1$	12.5	12.2	1.33	1.28	12.78	14.57	**26.6**	**27.0**	46.8	(44.9)
$N_2M_1GA_1$	12.2	11.9	0.75	0.71	12.07	11.18	16.8	17.7	58.2	58.5
$N_2M_2GA_1$	12.4	12.7	1.39	1.35	12.25	11.70	22.5	23.3	51.5	51.0
$N_2M_0GA_2$	12.2	11.9	1.06	1.01	13.13	13.78	21.0	22.4	52.6	50.9
$N_2M_1GA_2$	12.9	12.7	1.15	1.11	4.68	4.71	19.2	20.6	62.1	60.9
$N_2M_2GA_2$	12.5	12.2	1.22	1.18	13.02	16.20	20.9	19.2	52.4	51.2
$N_3M_0GA_0$	12.9	12.6	1.79	**1.75**	14.85	16.77	20.2	23.3	50.4	45.6
$N_3M_1GA_0$	**14.2**	**14.1**	0.60	0.55	**20.40**	19.07	18.7	19.0	(46.1)	47.2
$N_3M_2GA_0$	13.1	12.9	0.62	0.57	19.21	19.21	20.6	21.8	46.5	45.6
$N_3M_0GA_1$	13.0	12.7	1.04	0.99	18.80	19.79	19.4	21.3	47.8	45.2
$N_3M_1GA_1$	12.3	12.1	1.18	1.14	20.11	**20.75**	18.2	19.5	48.3	46.6
$N_3M_2GA_1$	13.4	13.2	0.54	0.49	16.48	16.49	(13.8)	(15.8)	55.7	54.0
$N_3M_0GA_2$	12.1	11.7	0.97	0.93	18.94	19.39	18.3	19.4	49.7	48.6
$N_3M_1GA_2$	13.2	12.9	0.82	0.77	16.46	16.99	18.8	20.5	50.8	48.8
$N_3M_2GA_2$	12.8	12.5	(0.53)	(0.48)	16.54	17.68	17.9	19.6	52.3	49.7
P-value	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*
LSD 5%	0.009	0.444	0.005	0.232	2.084	2.073	3.459	3.694	3.731	3.956

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

Bolded values = Highest; Bracketed values = Lowest.

The effect of interaction on ash in S1 and S2 was significant (P<0.05). Ash content of 26.61% was the highest for $N_2M_0GA_1$, while the least ash of 13.83% was for $N_3M_2GA_1$ in S1. The 27% ash content was the highest obtained for $N_2M_0GA_1$, while $N_3M_2GA_1$had the least fat content of 15.81% in S2. The $N_2M_0GA_1$ (100 kg N/ha, no mulch and 40 mg/L GA_3) had the highest interactive effect on ash content in both seasons.

There was a significant (P<0.05) interactive effect on total carbohydrates produced in S1 and S2. Total carbohydrates were highest 62.70% for $N_0M_2GA_2$, while the lowest total carbohydrates of 46.12% were recorded for $N_3M_1GA_0$ during S1. In S2, highest total carbohydrates of 62.26% were for $N_0M_2GA_2$, while the lowest total carbohydrates of 44.90% were for $N_2M_0GA_1$. The $N_0M_2GA_2$ (0 kg N/ha, unpainted mulch and 80 mg/L GA_3) had the highest interactive effect on total carbohydrates of pumpkin leaves in both seasons.

The combined effect on moisture and photosynthates was significant in both seasons (Table 4). Highest moisture content was for the combination with the highest amount of N, while lowest was observed when N was at 50 kg N/ha, implying that N enhances moisture content in pumpkin leaves. Total carbohydrates were highest where no N was applied and lowest where either 100 kg N/ha or 150 kg N/ha were applied. This indicates that N negatively affected total carbohydrates in leaves when combined with mulch and GA_3.

Treatments receiving low GA_3 had high moisture, fat, protein, ash and total carbohydrates. Lee and Rosa (2011) reported that GA_3 significantly reduced starch in green leaves of tobacco perhaps through dilution effect. When a plant receives growth regulators such as GA_3 that promote vegetative growth, it tends to be more succulent than starchy. When such plants are dried, they lose high amounts of water, leaving behind very little biomass. Results contrasted with those of Kibria*et al.* (2016), who reported no significant effect in protein content in tomatoes when nitrogen and plant residues were used. Elsewhere, application of fertilizers and GA_3 significantly affected total sugar, starch and protein contents in cucumbers (Pal *et al.,* 2018).

4. CONCLUSIONS AND RECOEMMENDATIONS

Nitrogen fertilizer positively and significantly increases moisture and protein contents, while it negatively and significantly reduces fat, ash and carbohydratecontents in leaves. The effect of mulchis negative and significant effect on moisture, fat, protein, but positive and significant on total carbohydrate in leaves. Mulch has no significant effect on ash content in leaves.Gibberellic acid has a negative effect on moisture content, but positive and significant on fat andprotein contents in leaves. In addition, the effect is positive,but not significant on total carbohydrates in leaves. Combined nitrogen, mulch and GA_3effect on moisture, fat, protein, ash, total carbohydrate contents in leaves is consistently significant, with the trend being positive for moisture and protein contents, but negative for fat, ash and carbohydrate contents. The present study recommends application of nitrogen, mulch and GA_3 that produce the desired response.

REFERENCES

Ali, M. W., Zoltai, S. C., and Radford, F. G. (1988). A comparison of dry and wet ashing methods for the elemental analysis of peat. *Canadian Journal of Soil Science, 68*, 443–447.

AOAC (1990). Official Methods of Analysis (15thed.). *Association of Official Analytical Chemists.* Washington DC USA. 400-2200Wilson Boulevard, Arlington Virginia USA,*2*, 910-928.

AOAC (1995). Official Methods of Analysis of the AOAC International, (16thed). Method 970.12. *Association of Official Analytical Chemists International*. Washington, DC.

Caili, F., Huan, S., and Quanhong, L. (2006). A review on pharmacological activities and utilization technologies of pumpkin. *Plant Foods Human Nutrition, 61*, 73-80.

Cameron, K. C., Di, H. J., and Moir, J. (2013). Nitrogen losses from the soil/plant system: A review. *Annals of Applied Biology, 162*(2), 10.1111/aab.12014.

Ding, Y., Luo, W., and Xu, G. (2006). Characterization of magnesium nutrition and interaction of magnesium and potassium in rice. *Annals of Applied Biology, 149*, 111–123.

FAO (2005). Improving nutrition through home gardening. a training package for preparing field workers in Africa, FAO of the United Nations, Nutrition Programme Service, Food and Nutrition Division. Viale delle Terme di Caracalla, 00100 Rome, Italy.

Fedha, M.S. (2008). Physicochemical characterization and food application potential of pumpkin (*Cucurbita* sp.) fruit and seed kernel flours. Unpublished Thesis. JJKUAT, Juja.

Fruhwirth, G.O., and Hermetter, A. (2007). Seeds and oil of the Styrian oil pumpkin: Components and biological activities. *European Journal of Lipid Science and Technology, 109,* 1128–1140.

Ghanbar, A., Nadjali, F., and Shabahang, J. (2007). Effects of irrigation regimes and row arrangement on yield, yield components and seed quality of pumpkin. *Asian Journal of Plant Sciences, 6*(7), 1072-1079.

Glew, R., Glew, R. and Chuang, L. (2006). Amino acid, mineral and fatty acid content of pumpkin seeds (*Cucurbita* spp) and *Cyperusesculentus* nuts in Niger. *Plant Foods Human Nutrition*, *61,* 51-56.

Grubben, G.J.H., and Chigumira-Ngwerume, F. (2004). *Cucurbita moschata* Duch. *In*: Grubben, G.J.H. and Denton, O.A. (Eds.). PROTA 2: Vegetables/Legumes. [CD-ROM]. PROTA, Wageningen, Netherlands.

Hewett, E.W. (2006). An overview of pre-harvest factors influencing post-harvest quality of horticultural products. *International Journal of Postharvest Technology and Innovation, 1*(1), 4–15.

Horticulture Validated Report 2016-2017. Data and Resources.

Iqbal, R., Raza, M.A.S., Valipour, M. *et al.* (2020). Potential agricultural and environmental benefits of mulches: A review. *Bull National Resource Centre,*44,75.

Jaetzold R., Schmidt, H., Hornetz, B., and Shisanya C. (2006). Farm Management Handbook of Kenya. Vol. II Natural Conditions and Farm Management Information 2nd Ed. Part B Central Kenya. Central Province.

Jones, J. B. (2001). Laboratory guide for conducting soil tests and plant analysis. Boca Raton, Fl.: CRC Press.

Jun, H.I., Lee, C.H., and Song, G.S. (2006). Characterization of the pectic polysaccharides from pumpkin peel. *Food Sci Tech*, 39, 554-561.

Karanja, J.K., Mugendi, B.J., Khamis, F.M., and Muchugi, A.N. (2014).Nutritional evaluation of some Kenyan pumpkins (*Cucurbita* spp.). *International Journal of Agriculture and Forestry, 4*(3), 195-200.

Kibria, G., Mazharul, I., and Alamgir, D. (2016). Yield and nutritional quality of tomato as affected by chemical fertilizer and biogas plant residues. *International Journal of Plant and Soil Science, 13*(2), 1-10.

Koike, K., Li, W., and Liu, L. (2005). New phenolic glycosides from the seeds of *Cucurbita moschata*. Chem Pharm Bull *53*, 225-228.

Koskey, G., Mburu, S.W., Njeru, E.M., Kimiti, J.M., Ombori, O., and Maingi, J.M. (2017). Potential of native rhizobia in enhancing nitrogen fixation and yields of climbing beans (*Phaseolus vulgaris* L.) in contrasting environments of Eastern Kenya. Front. Plant Sci. 8:443.doi: 10.3389/fpls.2017.

Kosterna, E., Zaniewicz-Bajkowska, A., Rosa, R., Franczuk, J., Borysiak-Marciniak, I., and Chrominska, K. (2010).Effect of black synthetic mulch on fruit quality and selected component of nutritive value of melon. *Acta Sci. Pol. Hortorum, 9*(3), 27-36.

Litke, L., Gaile, Z., and Ruza, A. (2018). Effect of nitrogen fertilization on winter wheat yield and yield quality. *Agronomy Research, 16*(2), 500-509.

Lee, T., and Rosa, N. (2011). Regulation of starch and sugar levels in tobacco leaves by gibberellic acid. *Canadian Journal of Botany, 47*(10), 1595-1598.

McCready, R. M. *et al.* (1950). Determination of Starch and Amylose in Vegetables. *Analytical Chemistry, 22*(9), 1156-1158.

Matsui, T., Guth, H., and Grosch, W. (1998). A comparative study of potent odorants in peanut, hazelnut, and pumpkin seed oils on the basis of aroma extract dilution analysis and gas chromatography – olfactometry of headspace samples. *Lipid Fett,100,* 51 – 56.

Milne, G. W. A. (2005). Gardner's commercially important chemicals: Synonyms, trade names, and properties. New York: Wiley-Interscience.

Murkovic, M., Mulleder, U., and Neunteufl, H. (2002). Carotenoid content in different varieties of pumpkins. *J Food Comp Anal,* 15, 633-638.

Nandi, S. K., Palni, L. M., and Raslimi, S. (1995). Chemical manipulation of dormancy in tea shoots and associated biochemical changes. *Journal of Plantation Crops, 23,* 53-58.

Okonwu, K., and Mensah, S.I. (2012). Effects of NPK (15:15:15) fertilizer on some growth indices of pumpkin. *Asian Journal of Agricultural Research, 6,*137-143.

Olaiya, C. O., Soetan, K. O., and Ogunkolade, N. S. (2010).Evaluation of the biochemical effects of auxins on nutritional quality of tomato (*Solanumlycopersicon*), genotype JM 94/47.*African Journal of Food Science, 4*(2), 41-45.

Oluoch, M.O. (2012).Production practices of pumpkins for improved productivity. *Scripta Horticulturae, 15*, 181-190.

Ondigi, A.N., Toili, W.W., Ijani, S.M.A., and Omuterema, O.S. (2008). Comparative analysis of production practices and utilization of pumpkins (*Cucurbita pepo* and *Cucurbita maxima*) by smallholder farmers in the Lake Victoria Basin, East Africa. *African Journal Environmental Science and Technology, 2*(9), 296-304.

Pal, P., Yadav, K., Kumar, K., and Singh, N., (2018). Efficacy of foliar application of GA_3 and K on growth and biochemical parameters of two F1 hybrid parthenocarpic cultivars of cucumber. *Journal of Horticultural Research, 26*(2), 5-13.

Waterer, D. (2000). Influence of soil mulches and method of crop establishment on growth and yields of pumpkins. *Canadian Journal of Plant Sciences, 80*, 385–388.

Weber, E.A., Graeff, S., Koller, W.D., Hermann, W., Merkt, N., and Claupein, W. (2008). Impact of nitrogen amount and timing on the potential of acrylamide formation in winter wheat (Triticumaestivum L.). *Field Crops Research,106*, 4452.