

Combined Effects of Nitrogen, Mulch and Gibberellic Acid on Postharvest Physiology of Multi-Purpose Pumpkin Leaves and Fruits

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Abstract— The leaves, fruits and seeds of multi-purpose pumpkin (*Cucurbita moschata* Duch.) species are consumed as vegetables, snacks and blended dishes to boost household health, food and nutritional security. However, cultivation without using inputs leads to poor postharvest physiological attributes. Consequently, a study was conducted to assess the effects of combined nitrogen, mulch and gibberellic acid (GA_3) on postharvest physiology of pumpkin. The treatments comprised four N rates (0, 50, 100 and 150 kg N/ha) supplied as CAN, three mulch types (none, unpainted, and black-painted rice straws) and three GA_3 rates (0, 40 and 80 mg/L). Experimentation was done in two seasons using split-split plots arranged in randomized complete block design with three replications, and 2 m x 2 m plant spacing. Nitrogen occupied main plots, mulch sub-plots, and GA_3 split plots. Post-harvest measures reported in this paper were harvested edible leaf weight, leaf and fruit moisture losses. Data values were subjected to analysis of variance using SAS Version 9.3. Separation of significant means was done using the least significant difference test at $\alpha=0.05$. Nitrogen fertilizer did not significantly ($P>0.05$) affect leaf weight and physiological weight loss, but it significantly ($P<0.05$) increased fruit physiological weight loss. Mulch had no significant effect on leaf weight and physiological weight loss, but it significantly increased fruit physiological weight loss. The effect of GA_3 on leaf weight and physiological weight losses was not significant, although the trend was positive in fruits. Similarly, combined nitrogen, mulch and GA_3 did not significantly affect leaf weight and physiological weight losses in both seasons. Thus, the increase of weight loss in pumpkin fruits produced using high nitrogen, mulch or GA_3 application should be counteracted by taking appropriate postharvest deterrent measures. On the other hand, the influence of combined nitrogen, mulch and GA_3 on multi-purpose pumpkin performance cannot be entirely depicted by analysing postharvest physiology.

Keywords— *Cucurbita moschata*, Leaf weight, Physiological weight loss, Shrinkage, Shriveling.

I. INTRODUCTION

Pumpkin is an important, healthy food crop, which is rich in vitamins, minerals and antioxidants, but low in calorific content, making it weight-loss-friendly (Kiharason *et al.*, 2017). Its nutrients and antioxidants are good boosters of the immune system, protectors of eyesight, reducers of certain cancer risks, and promoters of heart and skin health (Ghanbari *et al.*, 2007). Proper pumpkin growth,

production, development and physiology require integration of inputs. However, growers often concentrate on application of sole inputs at unverified rates, leading to poor nourishment that does not benefit post harvest physiology. Poor postharvest physiology leads to lessor wastage of produce before it is utilised as food or for income generation. This is despite the fact that the increased demand of pumpkin produce can only be

fulfilled by using integrated inputs that enhance post-harvest physiology.

Nitrogen is an essential element in plant growth. Although NH_4^+ , NO_2^- and NO_3^- account for less than 5% of the total N in the soil, Liu *et al.* (2014) indicated that N is a critical element that most plants absorb. Nitrogen is the most important element for proper plant growth and development, which substantially increases and enhances yields and quality, as it plays a critical role in biochemical and physiological processes (Ullah *et al.*, 2010). Nitrogen enhances total leaf biomass which is a determinant of pumpkin leaf vegetable yield (Nasim *et al.*, 2012). Mulch prevents soil leaching and runoff of fertilizer, conserves soil moisture, increases soil temperature, suppresses weeds and prevents pathogen splash, thereby enhancing growth, yield, quality and shelf-life of pumpkin leaves, fruits and seeds (Cerniauskiene *et al.*, 2015). Endogenous gibberellins help transport water and nutrients through the xylem and influence many biochemical and physiological processes like photosynthesis, respiration, protein synthesis, cell extension, wall thickness and stability (Abbas *et al.*, 2011), which are important in productivity and quality enhancement. Gibberellins strengthen parthenocarpic flowers and fruits to prevent abortion, which is common in pumpkins particularly when pollination is inadequate (Mwaura *et al.*, 2014; Isutsa and Mwaura, 2017; Kiramana and Isutsa, 2019).

Sub-optimal inputs contribute to poor returns through high post-harvest loss (Nakazibwe *et al.*, 2019). According to Kader (2013), food security can be enhanced through reduction of post-harvest loss and increase of produce shelf-life. Some procedures that can be carried out include using cultivars with long shelf life, integrated crop management systems that promote yield, quality and postharvest stability, as well as appropriate pre-harvest and post-harvest handling procedures that sustain physiology, quality and safety of crops and their products (Kitinoja *et al.*, 2011).

Promoting postharvest physiology through integrated input management practices will guarantee pumpkin yields and income increment for producers, as well as food and nutrition security boost for households and consumers (Gomez *et al.*, 2020). Owing to the increasing need of pumpkin produce in Kenya, coupled with the challenge of ensuring that it is plentiful and remains wholesome, determining optimal inputs for enhancing postharvest physiology is very imperative. The present paper determined the interactive effects of nitrogen fertiliser, mulch and GA_3 in enhancing preharvest physiology of pumpkin leaves and fruits.

II. MATERIALS AND METHODS

2.1. Research Site

The present experiment was conducted from January 2019 to August 2020 in two seasonal trials. Season 1 ran from March 2019 to July, 2019 with 1,004.3 mm rainfall, and Season 2 ran from October 2019 to February 2020 with 1,259.6 mm rainfall. The research site lies at $0^\circ 19' \text{ S}$, $37^\circ 38' \text{ E}$ and 1535 m above sea level. The average annual temperature is 19.5°C derived from 12.2°C to 23.2°C . The research 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

The experiment used three-factor plots embedded in randomized complete block design with three replications. Each experimental plot measured 2m x 2m and was separated from others by 1 m space. The three factors tested were nitrogen, mulch and GA_3 assigned to main-plots, sub-plots and split-plots, respectively. Nitrogen was applied as CAN to provide 0, 50, 100 and 150 kg N/ha. The 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. Nitrogen fertilizer was applied as two equal doses at four weeks from seedling emergence and at the beginning of flowering.

Mulch applied was none, unpainted and black-painted rice straws easily available in a close proximity to the experimental site and quantities required. The black-painted dry rice straws and unpainted dry rice straws were placed on their respective plots after land preparation. Painting of the rice straws was done by dipping them 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 soil to achieve 20 cm thickness. Planting holes were marked and opened in rice straw mulch during pumpkin seed sowing.

The GA_3 rates were 0 mg/L, 40 mg/L and 80 mg/L. The GA_3 granules were dissolved in 50ml alcohol and then the volume made up to one litre stock solution by adding distilled water. The required concentration of spray solution was then prepared from the stock solution by diluting with distilled water. A few drops of commercial sticker were added to the solutions to facilitate uptake of the GA_3 into leaves. The GA_3 was sprayed onto plants using a 1-L hand-held sprayer. Spray solution with low GA_3 rate was applied first followed by next high rate.

Spraying was done once during the fourth week after emergence. To avoid chemical drift, spraying was done during a calm morning while observing wind direction.

2.3. Pumpkin Establishment and Management

Three multipurpose pumpkin fruits of uniform size, free from disease and insect pests, and from one mother plant were used. The fruits were sourced from farmers endorsed by Extension Officer near the research site. Seeds were prepared as recommended to handle pumpkin seeds for planting and used immediately after extraction (AOAC, 1995).

The field was prepared to appropriate tillage 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 planting hole and one seedling was uprooted two weeks after emergence. All plots were kept weed-free through roguing and manual cultivation. Irrigation was done using drip tubes to supplement rain during drought. Insect pest and disease control was done when appropriate using recommended pesticides and rates. The vines were coiled when they became long, while leaving them in contact with the soil. Data values were taken from all plants for experimentation, except those in guard rows.

2.4. Data Collection and Analysis

Data was collected as described below for the two experimental seasons. Dry matter accumulation was measured on two vegetable leaf samples per experimental unit. The two leaves were oven-dried at 60°C until a constant weight was achieved. Picking of edible leaves was done at the 3rd and 4th internode on three randomly selected branches per plant. Harvesting was done by cutting the leaf stalk with a knife ensuring that each leaf had a 15 cm stalk. The first, second, third and fourth leaf harvesting was done three, four, five and six weeks after leaf production, respectively (Mwaura *et al.*, 2014). Close monitoring was done to enable marking of the identified leaves at production. Special markers with different colours to represent each treatment were used. The harvested leaves were weighed on a balance scale and assessed for dry matter using the method described by Windham *et al.* (1987). Three leaves were left at the tip of each branch to allow growth to continue. The measured fresh weight of the vegetable leaves was recorded in grams.

Weight loss was determined by weighing and recording leaves and fruits. Three leaves per treatment harvested at the 6th node of two previously marked branches were used. The first weight measurement was taken at harvesting. Leaves were then weighed after every two (2) days until a constant weight was achieved and the weight recorded in

grams. Three (3) fruits per treatment were weighed at harvesting and the weight recorded as initial weight in kg. Fruits were weighed once per week for 6 weeks. Subsequent weekly measurements for fruits and day measurements for leaves were treated as the weight in the tested week/day. Physiological weight loss was calculated as a percentage of the initial weight using the equation: $WL(\%) = ((W_o - W_i)/W_o) \times 100$; Where W_o is weight on the first day of storage and W_i is the weight in the tested day (Moalemiyan and Ramaswamy, 2012). Data values on leaf weight, leaf and fruit physiological weight losses were subjected to analysis of variance, using the SAS software version 9.3. Mean separation was performed using the least significant difference test at $\alpha = 0.05$.

III. RESULTS AND DISCUSSION

3.1. Effect of Nitrogen on Leaf Weight and Physiological Weight Loss

Nitrogen had no significant ($P > 0.05$) effect on leaf weight (Figure 1). The 150 kg N/ha had highest leaf weight of 17.21 g and 16.59 g in S1 and S2, respectively. Leaf weight increased with increase in nitrogen up to 150 kg N/ha. Nonnitrogen produced the lowest leaf weight of 15.40 g and 15.08 g in S1 and S2, respectively. Nitrogen had a significant ($P > 0.05$) effect on leaf physiological weight loss in both seasons. The 150 kg N/ha had the highest leaf physiological weight loss of 6.90 g and 6.73 g in S1 and S2, respectively. Physiological weight loss increased with increase in nitrogen up to 150 kg N/ha in both seasons. No nitrogen produced physiological weight loss of 5.50 g and 5.60 g in S1 and S2, respectively. Nitrogen had a significant ($P < 0.05$) effect on fruit physiological weight loss in S2 only. The 150 kg N/ha produced the highest physiological weight loss of 1.05 g and 0.82 g in S1 and S2, respectively. Fruit physiological weight loss increased with increase in nitrogen up to 150 kg N/ha. The control had the lowest fruit physiological weight loss of 0.89 g and 0.62 g in S1 and S2, respectively.

The findings of the present study were similar to those of Yildirim *et al.* (2007), who found that head and leaf dry matter of broccoli were negatively affected by nitrogen and foliar urea. In their study, urea statistically and significantly decreased the head dry matter content. Sorensen (1999) and Balik *et al.* (2003) reported that increasing nitrogen amounts in growth of broccoli, cabbage and maize resulted in lower dry matter percentages in leaves, stems and heads. These responses could be attributed to high succulent growth, as opposed to dense growth.

3.2. Effect of Mulch on Leaf Weight and Physiological Weight Loss

Mulch had no significant ($P>0.05$) effect on leaf weight of multi-purpose pumpkin fruits during both seasons as shown in Figure 2. Nevertheless, application of black-painted rice straw mulch produced the highest leaf

weight of 17.39 g and 16.03 g during S1 and S2, respectively. In both seasons, lowest leaf weight of 15.29 g and 15.78 g for S1 and S2, respectively, was obtained when unpainted rice straw mulch was applied.

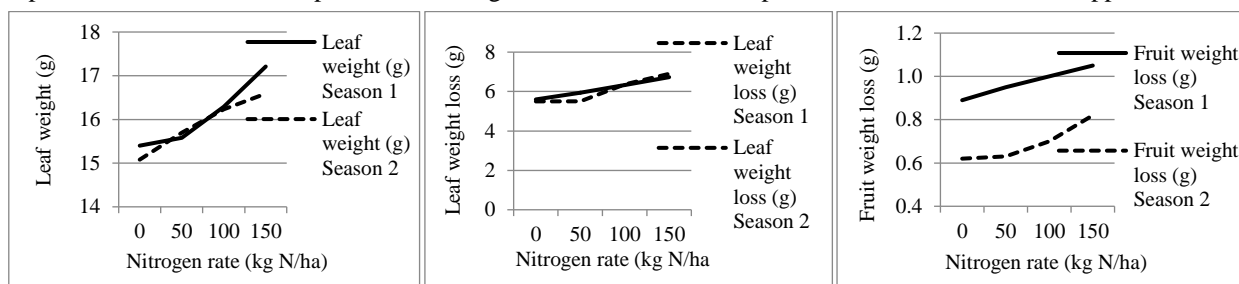


Fig.1: Effect of nitrogen on leaf weight and physiological weight loss. Season 2 fruit weight loss $P = 0.031$, $LSD_{0.05} = 0.13$

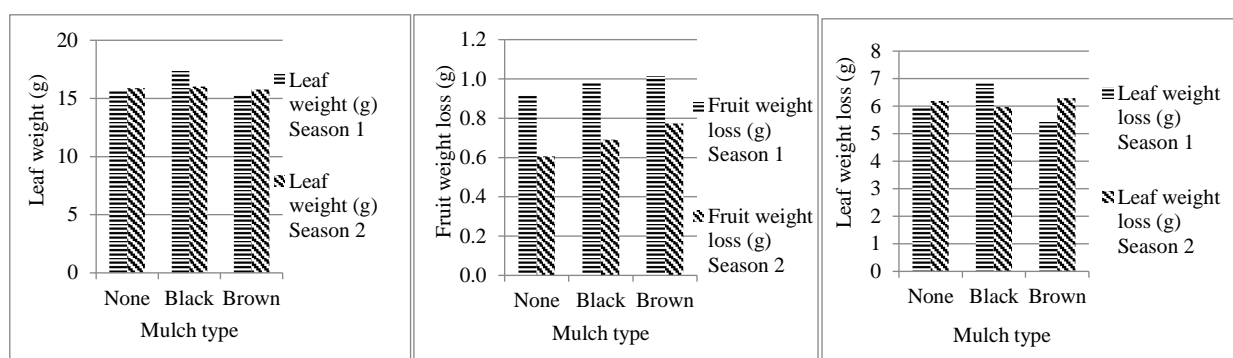


Fig.2: Effect of mulch on leaf weight and physiological weight loss. Season 2 fruit weight loss $P = 0.001$, $LSD_{0.05} = 0.047$

Mulch had no significant ($P>0.05$) effect on physiological weight loss in both seasons. Black-painted rice straws had the highest leaf physiological weight loss of 6.83 g in S1, while no mulch had the highest leaf physiological weight loss of 6.19 g in S2. Mulch had a significant ($P<0.05$) effect on leaf physiological weight loss of fruits in S2, but the effect was not significant ($P>0.05$) in S1. Fruit physiological weight loss was lowest 0.915 g and 0.606 g in S1 and S2, respectively, while use of unpainted rice straw mulch produced the highest leaf physiological weight loss of 1.015 g and 0.774 g during S1 and S2, respectively (Figure 2).

The lack of significant effect of mulch on leaf weight and physiological weight loss in both seasons was similar to that reported by Helaly *et al.* (2017) in *Physalis pubescens*. On the contrary, Israt (2018) and Ibarra-Jimenez *et al.* (2008) found a significant effect of mulch on dry matter in squash and physiological weight of cucumber. The variation may be due to the different crop species and environments assessed.

3.3. Effects of GA₃ on Leaf Weight and Physiological Weight Loss

There was no significant ($P>0.05$) effect of GA₃ on leaf weight in both seasons (Figure 3). Leaf weight of 17.16 g and 16.35 g in S1 and S2, respectively, was highest when no GA₃ was applied. Leaf weight was lowest 15.54 g when 80 mg/L GA₃ was applied in S1 and 15.13 g when 40 mg/L GA₃ was applied in S2. GA₃ had no significant ($P>0.05$) effect on leaf physiological weight loss in both seasons. No GA₃ produced the highest physiological weight loss of 7.03 g in S1, while highest physiological weight loss of 6.58 g was obtained when 40 mg/L GA₃ was applied in S2. Physiological weight loss decreased as the GA₃ was increased in S1.

The GA₃ had no significant effect on physiological weight loss in both seasons. The 40 mg/L GA₃ fruit physiological weight loss was lowest 0.960 g and 0.667 g in S1 and S2, respectively (Figure 3). Fruit physiological weight loss of 0.989 g and 0.710 g in S1 and S2, respectively, was highest when 40 mg/L GA₃ was applied.

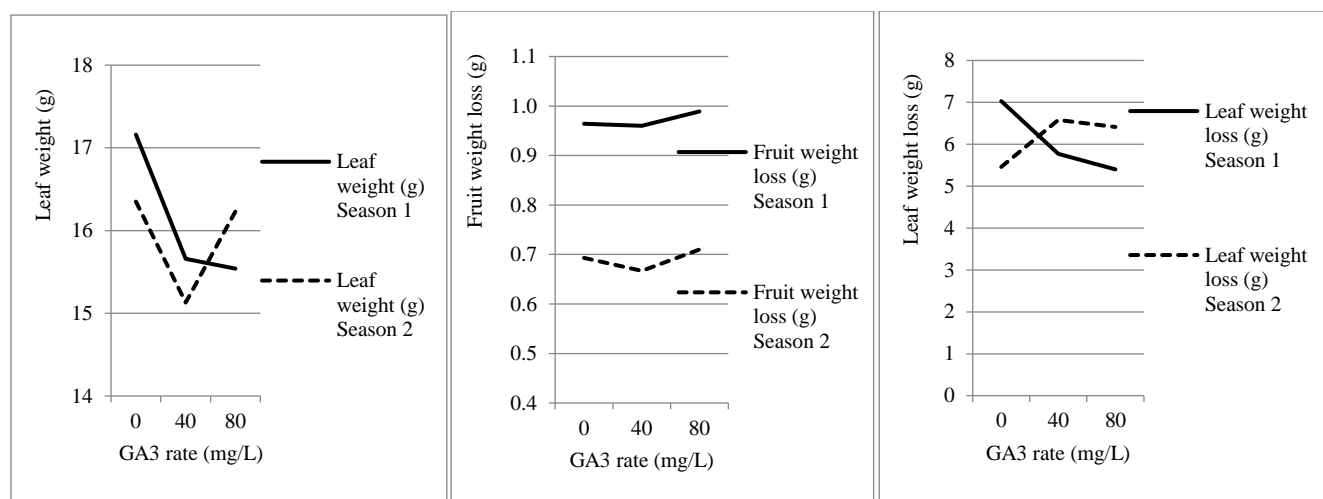


Fig.3: Effect of GA₃ on leaf weight and physiological weight loss

Application of GA₃ reduced the leaf weight in this study which contradicts the results of Shafeek *et al.* (2016), who reported increased leaf weight and significant effect in squash plants. Physiological weight loss in both the leaves and fruits was low when GA₃ was applied during season 1, but during season 2, application of GA₃ increased physiological weight loss in both the leaves and fruits. Growth regulators were found to improve the physiological performance of sweet cherry (Correia *et al.*, 2020). The lack of consistency in seasonal responses is attributed to climatic variations that may be beyond the control of researchers. Regardless, the low leaf weight and high postharvest physiological weight loss could be attributed to promotion of more succulent growth in pumpkin by the applied GA₃ (Abbas *et al.*, 2011).

3.4. Effect of Nitrogen, Mulch and GA₃ on Leaf Weight and Physiological Weight Loss

The highest leaf weight of 21.23g for N₃M₁GA₀ and 20.23 g for N₂M₀GA₁, while the lowest of 10.98g for N₂M₀GA₂ and 11.10 g for N₀M₀GA₂ were recorded in S1 and S2, respectively (Table 1). The N₃M₁GA₀ (150 kg N/ha, black-painted rice straw mulch and 0 mg/L GA₃) and N₂M₀GA₀ (100 kg N/ha, no mulch and 0 mg/L GA₃) had the highest interactive effect on leaf weight in S1 and S2, respectively. No significant effect of interaction was observed in S1 and S2 on pumpkin leaf weight.

The highest leaf physiological weight loss of 10.27 g was for N₂M₀GA₀, while the lowest of 1.30 g was for N₂M₀GA₂ in S1. In S2, highest leaf physiological weight

loss of 10.27 g was for N₃M₂GA₀, while the lowest of 2.20 g was for N₃M₀GA₀. The N₂M₀GA₀ (100 kg N/ha, no mulch and 0 mg/L GA₃) and N₃M₂GA₀ (150 kg N/ha, unpainted rice straw mulch and 0 mg/L GA₃) had the highest interactive effect on leaf physiological weight loss in S1 and S2, respectively. No significant effect occurred due to interactive effect in both seasons (Table 1).

Fruit physiological weight loss was highest 1.38 g for N₂M₀GA₂, while the lowest of 0.52 g was for N₃M₀GA₂ in S1 (Table 1). Highest fruit physiological weight loss of 1.00 g was for N₃M₁GA₂, while N₁M₀GA₀ had the lowest of 0.50 g in S2 (Table 1). The N₂M₀GA₂ (100 kg N/ha, no mulch and 80 mg/L GA₃) and N₃M₁GA₂ (150kg N/ha, black-painted rice straw mulch and 80 mg/L GA₃) had the highest interactive effect in S1 and S2, respectively. No significant effect was observed due to interactive effect on physiological weight loss in both seasons.

The results of the interactive effect that showed no significant effect were similar to those reported by Tsiakaras *et al.* (2014) on leaf weight of lettuce. Nonetheless, significant interactive effect of GA₃ and N on leaf weight of brussel sprouts has been reported (Selman and Bora, 1999). The reported results contrasted probably because of different crop species and two factors tested. The increase in physiological weight loss may be attributed to increased accumulation of moisture in produce during production under high rates of nitrogen, mulch and GA₃, which is then available for loss after harvest of produce.

Table 1: Effect of nitrogen, mulch and GA₃ on leaf weight and physiological weight loss in pumpkin leaves and fruits

Treatment	Leaf weight (g)		Leaf weight loss (g)		Fruit weight loss (g)	
	S1	S2	S1	S2	S1	S2
N ₀ M ₀ GA ₀	18.14	14.67	9.65	5.00	0.72	(0.50)
N ₀ M ₁ GA ₀	14.82	15.35	4.83	6.03	0.98	0.77
N ₀ M ₂ GA ₀	17.69	16.38	7.70	6.37	0.78	0.57
N ₀ M ₀ GA ₁	13.59	18.45	3.60	8.43	0.88	(0.50)
N ₀ M ₁ GA ₁	19.60	14.74	9.60	4.77	1.10	0.67
N ₀ M ₂ GA ₁	12.20	11.50	2.90	3.47	0.97	0.70
N ₀ M ₀ GA ₂	13.19	(11.10)	3.53	2.43	0.88	0.60
N ₀ M ₁ GA ₂	16.96	18.40	3.63	8.40	0.77	0.63
N ₀ M ₂ GA ₂	14.07	15.10	4.07	5.47	0.88	0.62
N ₁ M ₀ GA ₀	14.74	14.89	4.73	4.90	0.68	(0.50)
N ₁ M ₁ GA ₀	18.10	13.44	8.10	4.43	0.70	0.87
N ₁ M ₂ GA ₀	13.35	12.32	3.33	2.23	1.13	0.63
N ₁ M ₀ GA ₁	15.18	17.13	5.17	7.47	0.83	0.52
N ₁ M ₁ GA ₁	15.67	15.04	5.67	5.07	0.95	0.65
N ₁ M ₂ GA ₁	16.16	16.37	6.17	6.80	1.12	0.55
N ₁ M ₀ GA ₂	13.74	19.40	4.40	9.37	1.05	0.57
N ₁ M ₁ GA ₂	18.28	17.30	8.30	7.63	0.92	0.65
N ₁ M ₂ GA ₂	13.36	15.40	3.70	5.43	1.13	0.70
N ₂ M ₀ GA ₀	20.25	14.05	10.27	4.40	1.07	0.63
N ₂ M ₁ GA ₀	17.96	15.48	7.93	5.80	1.25	0.77
N ₂ M ₂ GA ₀	15.84	17.50	5.83	7.50	0.87	0.65
N ₂ M ₀ GA ₁	16.29	20.23	6.30	10.2	0.92	0.65
N ₂ M ₁ GA ₁	18.27	16.32	8.27	6.33	0.77	0.73
N ₂ M ₂ GA ₁	14.36	16.20	4.70	6.23	0.97	0.67
N ₂ M ₀ GA ₂	(10.98)	15.80	(1.30)	6.10	1.38	0.67
N ₂ M ₁ GA ₂	17.30	14.50	7.30	4.50	1.08	0.75
N ₂ M ₂ GA ₂	15.35	16.00	5.33	5.93	1.17	0.78
N ₃ M ₀ GA ₀	17.53	11.23	7.53	(2.20)	1.08	0.73
N ₃ M ₁ GA ₀	21.23	16.25	7.90	6.23	1.18	0.93
N ₃ M ₂ GA ₀	16.26	19.95	6.57	10.27	1.12	0.77
N ₃ M ₀ GA ₁	14.56	15.73	4.87	5.73	0.97	0.73
N ₃ M ₁ GA ₁	14.92	17.07	4.93	7.10	1.07	0.87
N ₃ M ₂ GA ₁	17.09	17.36	7.07	7.37	0.98	0.77
N ₃ M ₀ GA ₂	19.97	18.10	9.97	8.03	(0.52)	0.67
N ₃ M ₁ GA ₂	15.55	15.40	5.53	5.37	1.02	1.00
N ₃ M ₂ GA ₂	17.76	18.30	7.77	8.30	1.07	0.88
<i>P</i> -value	0.102	0.720	0.195	0.773	0.540	0.917
LSD 5%	6.440	7.606	6.098	6.931	0.472	0.241

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

Bolded values = Highest; Bracketed values = Lowest

IV. CONCLUSION AND RECOMMENDATION

Nitrogen fertilizer does not significantly affect leaf weight and physiological weight loss, but it significantly increases fruit physiological weight loss. Mulch has no significant effect on leaf weight and physiological weight loss, but it significantly increases fruit physiological weight loss. The effect of GA₃ on leaf weight and physiological weight loss is not significant, although the trend is positive on weight loss. Similarly, combined nitrogen, mulch and GA₃ consistently do not have a significant effect on leaf weight and physiological weight losses. The present study indicates that the increase of weight loss in pumpkin fruits produced using high nitrogen, mulch or GA₃ application should be counteracted by taking appropriate postharvest deterrent measures. On the other hand, the influence of combined nitrogen, mulch and GA₃ on multi-purpose pumpkin performance cannot be wholly depicted by analysing postharvest physiology.

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