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EFFECT OF HARVEST MATURITY AND VARIETY ON YIELD AND NUTRITIONAL QUALITY OF SQUASH IN BANGLADESH

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Abstract

Squash (*Cucurbita pepo* L.) yield can vary significantly depending on harvesting date. To overcome this issue, a field experiment was conducted at the Vegetable Research field of Horticulture Research Centre, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh, during October 2019 to March 2020 to find out the effect of variety and harvesting date on yield and yield attributes of squash. The experiment comprised two sets of treatments viz., (A) Variety: (i) BARI squash1 (V_1) and (ii) Kajol-F_1 (V_2) and (B) Harvesting date: (i) 5 days after fruit setting (H_1); (ii) 10 days after fruit setting (H_2); (iii) 15 days after fruit setting (H_3); (iv) 20 days after fruit setting (H_4) and (v) 25 days after fruit setting (H_5) in a Randomized Complete Block Design (RCBD) with three replications. The results showed that the variety BARI squash1 showed significantly the highest number of fruit plant^{-1}, fruit length, yield and vitamin C content, five days after fruit setting showed significantly the highest fruit plant^{-1} and vitamin C content but the highest yield plant^{-1} and yield ha^{-1} were found from 20 days after fruit setting. The interaction between variety and harvesting date was significant for yield and quality parameters except moisture (%). Based on the study results it is concluded that maximum yield plant^{-1} (4350 g) and yield (43.30 t ha^{-1}) were recorded from BARI squash1 with 20 days after fruit setting, but higher TSS and vitamin C was found in BARI squash1 where crop harvested 5 days after fruit setting. Variety BARI squash1 harvesting at 20 days after fruit setting (V_1H_4) is sutable for higher yield and Variety BARI squash1 harvesting at 5 days after fruit setting (V_1H_1) is sutable for higher nutritional quality of squash in Bangladesh.

Keywords: Harvesting date, Nutritional value, Squash, Variety, Yield.

Introduction

Squash (*Cucurbita pepo* L.) is a type of vegetable in the cucurbit family, known as Cucurbitaceae. It is commonly known as Zucchini. It is also known as marrow, courgette, baby marrow, summer squash, bush squash etc. It is widely cultivated in the world. Short

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days, low temperature, high relative humidity and bright sunshine are ideal for squash cultivation. This crop is relatively new in Bangladesh, but it is becoming popularity day by day. The health benefits of summer squash, including its bioactive compounds and nutritional composition are widely recognized (Tadros *et al.*, 2023; Díaz *et al*., 2020). Additionally, the fruit of the summer squash is rich in fiber and vitamins and contains moderate amounts of mineral salts (Abdein, 2016).

Harvesting time of any vegetable crop is one of the most important factors which affect nutritional quality as well as total yield. Generally, squash is harvested during the growing season, while the skin is still tender and the fruit relatively small, mostly consumed at immature stage for culinary purposes before seeds begin to enlarge and harden. The whole tender fruit is edible, without discarding seeds and seed cavity tissues. It has soft seeds and thin, edible skin, and tender flesh with high water content (Herbst, 2001). Squash can be consumed at different stages of fruit development. Those picked up in advanced phases of development or in full maturity, when receive the diameter of about 20 cm, and a weight of 1.5-2.0 kg, can be directly supplied to the fresh market or after cold storage (Gajewski and Grzeszczuk, 2005). Small size fruits with diameter 3-6 cm, harvested at the time before the skin begin to harden and do not need to remove it (Gajewski and Grzeszczuk, 2005). However, such immature fruit has limited storage life. At later development stages of squash, there is an increase in yield, but squash at early stages of maturity contain high content of nutrients and health promoting compounds, such as protein, ash, crude fiber, phenolics and flavonoids (Magda *et al.*, 2015). Squash cultivation has a great opportunity and a promising vegetable in Bangladesh. Keeping in view, of growing importance of this crop in Bangladesh, the present studies have been undertaken to find out the optimum harvesting date and variety on yield and quality of squash for Bangladesh.

Materials and Methods

Research Location

The research work was conducted at Vegetable Research field of Horticulture Research Centre, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh during the period of October 2019 to March 2020. The experimental site is located at 24.0° N latitude and 95.25° E longitude, respectively at an elevation of 8.4 meters from the sea level (Anon, 1995). Topsoil was sandy clay loam in texture having pH around 6.0. The selected plot was medium high land. Plenty of sunshine and moderately low temperature prevails during experimental period. The weather data during the study period are presented in Table 1.

Experimental design

The experiment consisted of two factors. Factor A: Variety (2) *viz*. (i) BARI squash1 and (ii) Kajol-F_1 and Factor B: Harvesting date (5): (i) 5 days after fruit setting (H_1); (ii) 10 days after fruit setting (H_2); (iii) 15 days after fruit setting (H_3); (iv) 20 days after fruit setting (H_4) and (v) 25 days after fruit setting (H_5). The experiment was conducted in a randomized complete block design (RCBD) with three replications. The layout of the experiment was prepared for distributing the combination of variety and

different harvesting dates. The 10 treatment combinations of the trail were assigned at arbitrary into 30 plots. The size of each unit plot 3m × 2m). The distance between replication to replication 1.0m and plot to plot distance was 0.5m.

Table 1. Monthly mean weather data during the crop growing periods at BARI, Gazipur

Year	Month	Temperature (°C)			Relative humidity (%)		Sunshine (hr./day)	Total rainfall (mm)
		Maximum	Minimum	Average	9 am	2 pm		
2019	July	32.95	26.76	29.86	84.94	78.52	3.85	360.2
	August	33.78	27.22	30.50	83.23	74.32	7.07	169.8
	September	33.29	26.16	29.73	85.53	76.43	4.68	210.2
	October	32.24	24.36	28.30	82.42	69.65	5.83	231.6
	November	31.13	20.26	25.70	81.97	61.93	6.47	22
	December	24.84	14.62	19.73	84.71	64.81	4.69	3.6
2020	January	24.36	13.61	18.985	88.68	64.16	4.25	28.2
	February	28.00	14.52	21.26	77.31	47.97	6.15	0.8
	March	32.70	19.63	26.165	66.94	45.55	8.06	16.6

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Raising of seedlings and crop management

The seeds of BARI squash1 were collected from Olericulture Division, Horticulture Research Centre (HRC), BARI, Gazipur and another squash variety Kajol-F_1 were collected from "Mollika Seed Company" 145, Siddique Bazar, Dhaka. Squash seeds were sown on 14 October 2019 in polybags at net house of Olericulture Division of BARI. Seeds were sown in polybags which were filled with loose friable, dead roots free, sandy loam soil previously mixed with well rotten cowdung. Eighteen days old seedlings were transplanted in the experimental plots. One fourth of cowdung (20 t ha^{-1}) and all of S (18.6 kg ha^{-1}), Zn (10.3 kg ha^{-1}) and B (1.2 kg ha^{-1}), ½ P (84 kg ha^{-1}) and 1/3rd K (90 kg ha^{-1}) were to be applied, respectively during final land preparation. Cowdung @ 10 kg, P @ 30 g, K @ 30 g and Mg @ 5 g were to be applied each pit in 7-10 days before planting. N @ 14 g was to be top dressed each pit at 4 split applications and K @ 15g to be applied 10-15 days after planting according to Projukti Hatboi (BARI, 2019). Healthy and uniform sized 18 days old seedlings were taken from the net house and were transplanted in the main field on 1 November, 2019. Plants were spaced at 1m × 1m spacing. The seedlings were watered after transplanting. The insects were controlled successfully by spraying Malathion 57 EC @ 2 ml/L water. The insecticide was sprayed fortnightly from a week after transplanting to a week before first harvesting. Squash fruits were harvested according to treatment. The harvested squashes of each plot collected separately, tagged and taken to laboratory for data collection.

Data collection and analysis method

The following data were collected from the experiment.

Fruit plant^{-1}

Total number of fruit was counted from each plant of each plot from first harvest to last harvest and average number of fruit was calculated and termed as number of fruits per plant.

Individual fruit weight

From first harvest to last harvest total fruit number was counted and total fruit weight was measured from each plant of each plot to determine single fruit weight.

Fruit length

The length of the fruit was measured with a meter scale in centimeter from the neck of the fruit to the bottom of the fruit. It was measured from each plot and their average was calculated in centimeters.

Fruit diameter

The diameter of individual fruit was measured in several directions from five selected fruits with slide calipers and the average of all directions was finally recorded and expressed in centimeter.

Yield plant^{-1}

Weight of matured fruits harvested from each picking in the tagged plants in each replication was recorded till final harvest and total yield of fruits per plant computed in kilogram.

Yield ha^{-1}

After collection of fruit per plot, it was converted to ton per hectare by the following formula:

$$\text{Yield (ton ha}^{-1}) = \frac{\text{Fruit yield plot}^{-1}\text{ (kg)} \times 10000\ \text{m}^2}{\text{Plot size (m}^2) \times 1000\ \text{kg}}$$

Moisture (%)

Squash slice (10g) was taken in porcelain crucible and placed in an oven and heat at 80 ºC for 72 hours and until constant weight was obtained. The crucible with the sample was then transferred to a desiccator containing anhydrous calcium chloride and kept there for about 10 minutes for cooling are final weight was taken. Percent moisture content was calculated using following formula-

$$\%\ \text{moisture content} = \frac{I - F}{I} \times 100$$

Where,

I = Initial weight of slice,

F= Final weight of dry matter

TSS

Total Soluble Solids (TSS) content was determined by a refractometer by placing of drop of pulp on its prism. TSS obtained from direct reading of the refractometer.

Vitamin C content

The reagent used for the estimation vitamin C were as follows-

a) Metaphoporic acid solution (3%)

b) Standard ascorbic solution

c) Dye solution

For estimation of vitamin C were as follows

Five ml of Standard ascorbic solution was taken in conical flask and 5 ml metaphoporic acid (HPO_3) was added to it and shaken.

A micro burette was filled with dye solution then the mixed solution was titrated with dye using phenolphthalein as indicator solution to a pinked coloured end point, which persisted at least for 15 seconds. Dye factor was calculated using the following formula-

$$\textit{Dye factor} = \frac{0.5}{\textit{Titre}}$$

Preparation of sample

10 g of sample was taken and transferred to 250 ml volumetric flask and the volume was made up to the mark with metaphosphoric acid.

Titration

Five ml of metaphosphoric acid extracted sample was taken in an aliquot and titrated with standard dye solution, using phenolphthalein as indicator to a pink coloured end point which persisted for at least for 15 seconds.

Vitamin C content was calculated using the following formula-

$$\text{Vitamin C content (mg/100g sample)} = \frac{T \times D \times V_1}{V_2 \times W} \times 100$$

Where,

T= Titre

D= Dye factor

V_1= Volume made up

V_2= Volume of extract taken for estimation

W= Weight of sample taken for estimation

Firmness

Fruit firmness of squash fruit was measured (Rahman *et al.*, 2014) using a Digital Firmness Tester (DFT 14, Agro-Technologie, France) equipped with 5 mm diameter stainless probe. The tester was checked before use. The plunger of the tester was moved in and out about 10 times to ensure that it was running smoothly. Firmness was reported in kilogram-force cm^{-2} (kg-f-cm^{-2}). Measurements were taken at three different places of each fruit and mean was calculated.

Statistical analysis of data

The recorded quantitative data were analyzed statistically by using MSTAT-C a computer-based program to find out the variation among different treatments, treatment combinations and their interactions. Treatment means were compared by Duncan Multiple Range Test (DMRT).

Results and Discussion

Effect of variety

The result presented in Table 2 showed that significant effect of variety on fruit $plant^{-1}$, individual fruit weight, fruit length, fruit diameter, yield $plant^{-1}$ and yield ha^{-1}. The maximum number of fruits $plant^{-1}$ (5.07), longest fruit (43.87 cm), yield $plant^{-1}$ (3328 g) and yield (33.05 t ha^{-1}) was obtained from V_1 (BARI squash1). The highest individual fruit weight (969.7 g) and fruit diameter (7.63 cm) was obtained from V_2 (Kajol-F_1).

This result under the present study might be due to high genetic variability. Similar results were also observed by Wetzel and Stone (2019) and Esho and Saeed (2017) in squash, and they found significant variation on fruit yield per plant among different varieties of squash.

Table 2. The yield contributing parameters and yield of squash as influenced by different varieties

Variety	Yield contributing parameters and yield					
	Fruit $plant^{-1}$ (no.)	Individual fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Yield $plant^{-1}$ (g)	Yield (t ha^{-1})
V_1	5.07 a	907.3 b	43.87 a	6.85 b	3328 a	33.05 a
V_2	4.78 b	969.7 a	33.77 b	7.63 a	3240 b	32.79 b
LSD (0.05)	0.103	5.711	3.063	0.272	10.76	0.123
CV (%)	7.99	3.85	4.96	4.9	2.70	2.03

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per DMRT. V_1 = BARI squash1 and V_2 = Kajol-F_1

The effect of variety did not show significant variation on moisture content, total soluble solid (TSS) and firmness of squash but vitamin C content showed significant effect (Table 3). However, results indicated that the highest moisture content (95.64%) and firmness (2.05 kg-f-cm^{-2}) was recorded in V_2 (Kajol-F_1) variety whereas the highest (5.81%) total soluble solid (TSS) was recorded from V_1 (BARI squash1).

The highest vitamin C content (16.34 mg/100 g) was found in the V_1 (BARI squash1) whereas the lowest percentage of vitamin C content (12.71 mg/100 g) was observed in the variety V_2 (Kajol-F_1). The BARI squash1 and Kajol-F_1 cultivars differ significantly in their vitamin C content, which is probably due to inherent genetic variations. High genetic heterogeneity in the biosynthetic pathways and regulatory mechanisms influencing vitamin-C accumulation in the fruit is reflected in these variations.

Table 3. Quality parameters of squash as influenced by different varieties

Variety	Quality parameters and firmness			
	Moisture (%)	TSS (%)	Vitamin C Content (mg/100g)	Firmness (kg-f-cm^{-2})
V_1	94.82	5.81	16.34 a	1.93
V_2	95.64	4.57	12.71 b	2.05
LSD (0.05)	NS	NS	1.079	NS
CV (%)	0.75	1.50	4.49	5.37

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per DMRT. V_1 = BARI Squash-1 and V_2 = Kajol-F_1

Effect of harvesting date

The result presented in Table 4 showed that significant effect of harvesting date on fruit plant^{-1}, individual fruit weight, fruit length, fruit diameter, yield plant-^{1} and yield ha^{-1}. The maximum number of fruit plant^{-1} (10.49) was found from H_1 (5 days after fruit setting) whereas the minimum number of fruits plant^{-1} (1.88) was found from H_5 (25 days after fruit setting). The highest individual fruit weight (1710 g), longest fruit (53.0 cm) and highest fruit diameter (11.1 cm) were obtained from H_5 (25 days after fruit setting). The maximum yield plant^{-1} (4315 g) and yield ha^{-1} (42.97 t) were found from H_4 (20 days after fruit setting).

The lowest individual fruit weight (194.2 g), shortest fruit (21.5 cm), lowest fruit diameter (4.03 cm), minimum yield plant^{-1} (2010 g) and yield ha^{-1} (20.48 t) were found from H_1 (5 days after fruit setting).

Table 4. Yield contributing parameters and yield of squash as influenced by different harvesting dates

Harvesting date	Yield contributing parameters and yield					
	Fruit plant^{-1} (no.)	Individual fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Yield plant^{-1} (g)	Yield (t ha^{-1})
H_1	10.49 a	194.2 e	21.50 e	4.03 e	2010 e	20.48 e
H_2	5.01 b	595.0 d	32.33 d	5.68 d	3085 d	30.92 d
H_3	3.94 c	920.8 c	39.58 c	6.91 c	3700 b	37.23 b
H_4	3.33 d	1273.0 b	47.67 b	8.47 b	4315 a	42.97 a
H_5	1.88 e	1710.0 a	53.00 a	11.10 a	3310 c	32.98 c
LSD (0.05)	0.478	29.80	2.336	0.430	10.95	0.812
CV (%)	7.99	3.85	4.96	4.9	2.70	2.03

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per DMRTH_1 = 5 days after fruit setting, H_2 = 10 days after fruit setting, H_3 = 15 days after fruit setting, H_4 = 20 days after fruit setting, H_5 = 25 days after fruit setting

The effect of harvesting date did not show significant variation on moisture content and total soluble solid (TSS) but vitamin C content and firmness of squash showed significant effect (Table 5). However, results indicated that the moisture content ranges from 94.37-95.70% and TSS ranges from 4.47%-5.93% from all treatments. The highest vitamin C content (25.95 mg/100 g) was recorded in the H_1 (5 days after fruit setting) and the lowest vitamin C content (5.75 mg/100 g) was found in the H_5 (5 days after fruit setting). The highest firmness (2.95 kg-f-cm^{-2}) was found in H_5 (25 days after fruit setting) and the lowest firmness (1.2 kg-f-cm^{-2}) was found in H_1 (5 days after fruit setting). Vitamin C is heat-sensitive and easily oxidized. Vitamin C is degraded by late harvesting because it is exposed to high temperatures or oxygen.

Table 5. Quality parameters of squash as influenced by different harvesting date

Harvesting date	Quality parameters and firmness			
	Moisture (%)	TSS (%)	Vitamin C Content (mg/100g)	Firmness (kg-f-cm^{-2})
H_1	95.70	5.93	25.95 a	1.20 e
H_2	95.55	5.53	18.98 b	1.47 d
H_3	95.44	5.21	12.47 c	1.85 c
H_4	95.10	4.82	9.48 d	2.48 b
H_5	94.37	4.47	5.75 e	2.95 a
LSD (0.05)	NS	NS	0.791	0.127
CV (%)	0.75	1.50	4.49	5.37

NS = Non-significant.

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per DMRT. H_1 = 5 days after fruit setting, H_2 = 10 days after fruit setting, H_3 = 15 days after fruit setting, H_4 = 20 days after fruit setting, H_5 = 25 days after fruit setting

Interaction effect of variety and harvesting date

Interaction effect of variety and harvesting date showed significant effect on yield parameters and yield (Table 6). The maximum number of fruits plant^{-1} (10.97) was found from V_1H_1 whereas the minimum number of fruits plant^{-1} (1.78) was found from V_2H_5. The highest individual fruit weight (1827 g) was observed from V_2H_5 and the lowest individual fruit weight (191.7 g) and (196.7 g) were observed from V_2H_1 and V_1H_1 treatments. The longest fruit (59.67 cm) was produced from V_1H_5 and the shortest fruit (22.83 cm) and (20.17 cm) were produced from V_1H_1 and V_2H_1. The highest fruit diameter (11.57 cm) was found from V_2H_5 and the lowest fruit diameter (4.0 cm and 4.07 cm) were observed from V_1H_1 and V_2H_1. The highest yield plant^{-1} (4350 g) and yield ha^{-1} (43.3 t) were obtained found from V_1H_4 and the lowest yield plant^{-1} (1970 g) and yield ha^{-1} (19.93 t) were obtained from V_2H_1.

Variety BARI squash1 harvesting at 20 days after fruit setting (V_1H_4) is sutable for higher yield and Variety BARI squash1 harvesting at 5 days after fruit setting (V_1H_1) is sutable for higher nutritional quality of squash in Bangladesh.

Table 6. Yield contributing parameters and yield of squash as influenced by different variety and harvesting dates

Treatments	Yield contributing parameters and yield					
	Fruit plant^{-1} (no.)	Individual fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Yield plant^{-1} (g)	Yield (t ha^{-1})
V_1H_1	10.97 a	196.7 h	22.83 g	4.00 g	2050 i	21.03 f
V_1H_2	5.03 c	601.7 g	36.67 e	5.47 ef	3120 g	31.43 de
V_1H_3	3.98 d	898.3 f	45.50 c	6.47 e	3750 c	37.07 b
V_1H_4	3.42 de	1247.0 d	54.67 b	7.67 d	4350 a	43.30 a
V_1H_5	1.97 f	1593.0 b	59.67 a	10.63 b	3370 e	32.40 d
V_2H_1	10.02 b	191.7 h	20.17 g	4.07 g	1970 j	19.93 f
V_2H_2	4.98 c	588.3 g	28.00 f	5.90 e	3050 h	30.40 e
V_2H_3	3.90 de	943.3 e	33.67 e	7.37 d	3650 d	37.40 b
V_2H_4	3.23 e	1298.0 c	40.67 d	9.27 c	4280 b	42.63 a
V_2H_5	1.78 f	1827.0 a	46.33 c	11.57 a	3250 f	33.57 c
LSD (0.05)	0.675	42.14	3.304	0.609	15.33	1.148
CV (%)	7.99	3.85	4.96	4.9	2.70	2.03

In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per DMRT. V_1 = BARI squash1, V_2 = Kajol-F_1, H_1 = 5 days after fruit setting, H_2 = 10 days after fruit setting, H_3 = 15 days after fruit setting, H_4 = 20 days after fruit setting, H_5 = 25 days after fruit setting

Table 7 presented the interaction effect of variety and harvesting date had significant effect on total soluble solid (TSS), vitamin C content and firmness.

Table 7. Quality parameters of squash as influenced by different varieties and harvesting dates

Treatments	Quality parameters and firmness			
	Moisture (%)	TSS (%)	Vitamin C Content (mg/100g)	Firmness (kg-f-cm^{-2})
V_1H_1	95.20	6.37 a	29.33 a	1.20 f
V_1H_2	95.13	6.17 a	20.47 c	1.43 e
V_1H_3	95.07	5.92 b	14.13 e	1.80 d
V_1H_4	94.80	5.43 c	11.75 f	2.30 c
V_1H_5	93.90	5.17 d	6.03 h	2.90 a
V_2H_1	96.20	5.50 c	22.57 b	1.20 f
V_2H_2	95.97	4.90 e	17.50 d	1.50 e
V_2H_3	95.80	4.50 f	10.80 f	1.90 d
V_2H_4	95.40	4.20 g	7.20 g	2.67 b
V_2H_5	94.83	3.77 h	5.47 h	3.00 a
LSD (0.05)	NS	0.217	1.118	0.118
CV (%)	0.75	1.50	4.49	5.37

NS = Non-significant. In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per DMRT. V_1 = BARI squash1, V_2 = Kajol-F_1

H_1 = 5 days after fruit setting, H_2 = 10 days after fruit setting, H_3 = 15 days after fruit setting, H_4 = 20 days after fruit setting, H_5 = 25 days after fruit setting

Moisture (%) did not differ significantly. The highest total soluble solid (6.37% and 6.17%) were recorded from V_1H_1 and V_1H_2 and maximum vitamin C content (29.33 mg/100 g) were recorded from V_1H_1 and the lowest TSS (3.77%) and vitamin C content (5.47 mg/100 g) were recorded from V_2H_5. The highest firmness (3.0 kg-f-cm^{-2}) was found in V_2H_5 and the lowest firmness (1.2 kg-f-cm^{-2}) was found in V_1H_1 and V_2H_1.

Conclusion

Based on the findings, it can be concluded that yield, quality and yield contributing characters of squash can be effectively manipulated by different harvesting date and variety. Fruit harvesting 5 days after fruit setting produces the higher number of fruits plant^{-1} and vitamin C content. Among the tested varieties, BARI squash1 consistently outperformed Kajol-F_1 in terms of both yield and quality. Therefore, for optimal squash production, BARI Squash-1 is recommended, with harvesting schedules tailored according to the desired priority early harvest for nutrition, or later harvest for yield. However, further experimentation need to be executed in different agro-ecological zones with more varieties.

Author's contribution

The study conception, formulation of the research program, provision of materials, statistical data analysis, preparation of tables and graphs, manuscript editing, and research project funding were carried out by M A. Hossen and M. N. Islam. Fieldwork, chemical analyses, and data collection were conducted by M. A. Hosesn, S. Akther and H. M. Rashid. The initial draft of the manuscript was prepared by M. Al-Amin. A. A. Sabuz performed the postharvest test and analysis. All authors reviewed and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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