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Composite effects of gibberellic acid and kinetin on the postharvest-life of *Solanum melongena* L.

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ABSTRACT

To reduce the qualitative loss and prolong the shelf-life of brinjal (*Solanum melongena* L), the application of an exogenous coating of gibberellic acid and kinetin separately and also multiple combinations of GA and KN (10 µM plus 10 µM, 20 µM plus 20 µM, and 30 µM plus 30 µM) were tested for a storage period of 14 days. The uncoated brinjal could sustain about 7 days and deteriorate early whereas the coated brinjal now has a successful storage period of 14 days. During the storage, it was observed a gradual decrement in titratable acidity, protein content, catalase, and peroxidase activity; whereas a gradual increment in proline content and DPPH radical scavenging activity. Among these three combinations, the combination of 10 µM GA plus 10 µM KN preserved the qualitative attributes and was effective in delaying the senescence process. The mathematical model and principal component analysis also justified the results of the present study. This study influences a better method to meet market and customer expectations for volume, quality, and other product and transaction attributes like nutrition, food security, and product safety.

Keywords: Brinjal, Gibberellic acid, Kinetin, Postharvest, Principal component analysis

INTRODUCTION

India acquires almost 70% of cultivable land, which produces numerous fruits and vegetables. Among these, brinjal is familiar and highly consumed in the subtropical and tropical regions (India, China, Japan, and Southern Europe) (Nothmann, 1986). Brinjal (Solanum melongena L) in the Solanaceae family is commonly known as eggplant; garden egg (French); baigan (Hindi); aubergine (Old English); vangi (Marathi). It has lots of health and nutritive benefits. The alkaloid “solanine” found in the roots and leaves of brinjal strengthens the bones. As the calorie content in brinjal is very low, it helps humans in weight loss (Aubert and Pochard, 1981). Alongside, it is rich in fiber, potassium (which balances the electrolytes in the body), vitamin B6, and phytonutrients (Aubert and Pochard, 1981). Its medicinal aspects can heal many diseases such as diabetes, asthma, bronchitis, cholera, and diarrhea. In addition to it, the tissue extracts are reported to lower the blood cholesterol level (Choudhury, 1976). However, the postharvest loss of brinjal is a great concern. This loss may be due to different insects attacking, rotting, cleaning, transportation, etc. (Kaysar *et al.,* 2016), and above all the accelerated senescence of the calyx (McColloch *et al.,* 1982). Also, brinjal cannot be preserved below 10 °C as it is chilling sensitive (Nothmann, 1986). To avoid postharvest loss, several edible coatings are available. However, as per our knowledge till now, these coatings have not been applied in brinjal. The plant growth regulators, directly associated with regulating the growth and development of plants may extend their help in delaying the senescence process. The use of GA and Naphthalene acetic acid (NAA) in brinjal, helped in extending the freshness of calyx of brinjal for a period of 3 days with and without fungicide (Temkin-Gorodeiski *et al.,* 1993). In this context, the present study emphasized the usage of two plant hormones (gibberellic acid and kinetin) as coatings to extend the postharvest period of the coated brinjal. Gibberellic acid (GA) was originally identified by the Japanese in 1935, as a metabolic compound produced by a fungus named Gibberella fujikuroi. When GA is purified, it becomes white to pale-yellowish solids (Brain *et al.,* 1954). GA was later identified in plants as a plant hormone and is produced in less amount, however, has a tremendous capacity to induce growth and elongation of plant cells. Generally, the postharvest shelf-life of fruits and vegetables is at best a week. But, if GA coatings are applied, then the postharvest period can be extended (Panigrahi *et al.,* 2017). Another report also suggested that if GA is coated on peach, it maintains firmness, and the ethylene emission rate can be reduced for a longer period (Martínez-Romero *et al.,* 2000).

Kinetin (KN) is a cytokinin class of plant hormone that reciprocates cell division as isolated by Miller *et al.,* (1956). Mostly cytokinin occurs as free purine bases, nucleosides or nucleotides, and t-RNA constituents (Horgan, 1998). It has been noted that kinetin minimizes necrosis by acting as an antioxidant and scavenges free radicals. Hence, it protects the outer layer of the skin, reduces wrinkles, improves skin texture, and decreases signs of mottled hyperpigmentation in humans. KN promotes cell division in meristematic centers directly reflects in the development of shoot length (Peeters *et al.,* 1991). Moreover, kinetin was also used for clonal propagation in many ornamental plants (Jain and Ochatt, 2010). Looking at the overpopulation of human beings and postharvest loss of fruits, it is very much a need for research to minimize the postharvest

loss and extend the longevity and freshness of the fruits. To eliminate the problem of degradation of fruits and vegetables, which are regarded as very perishable commodities, the preservation procedure must be enhanced (Trivedi *et al.,* 2022). The present study deals with the postharvest delaying in the ripening process, and enhancing the shelf-life of brinjal by treatment of various combinations of GA and KN. Each fruit was subjected to nine different combinations after which they were checked for weight loss, titratable acidity, protein estimation, proline estimation, catalase activity, peroxidase activity, and 2,2-diphenyl-1-picrylhydrazyl (DPPH). Also, mathematical modeling on weight loss was carried out to support the observations and principal component analysis was applied to find the variations among other parameters.

MATERIAL AND METHODS

Sample selection:

Solanum melongena L. fruits (Gujarat Anand Brinjal 6- Anand Doli) were collected from the local firm of Anand, Gujarat, India. The fruits were of uniform size (13±2 cm with a width of 6±2 cm). The postharvest brinjals were pricked and packaged in large-size polythene bags and transported to the local market on the same day of collection as confirmed by the vendors. The shape of these brinjals is club-shaped. For individual treatments and parameters, triplicates of fruits were used. All the fruits were surface washed under running tap water and allowed to dry over muslin cloth before the start of the experiment. The dipping solution was prepared in 9 different concentrations of gibberellic acid and kinetin emulsion in water (GA= 10 µM, 20 µM, 30 µM) (Kinetin= 10 µM, 20 µM, 30 µM) (GA + Kinetin= 10 µM + 10 µM, 20 µM + 20 µM, 30 µM + 30 µM). Fruits were dipped in these different hormone solutions for a period of 30 s at 25 ± 2° C temperature and were allowed to dry again before storing at 4 ± 1° C temperature in a top diameter of 5/8 inches, bottom diameter of 3/8 inches, and with a height of 3-inch storage container. This container was covered with a blotting sheet. This day was considered the zeroth day of the experiment.

Weight loss:

A separate lot of brinjals were considered for weight loss measurement. On a zeroth day, the weight of each brinjal was taken. After seven days, again the weight of each brinjal was measured. Now, the weight loss in grams was calculated by taking the difference of weight i.e., weight on day 7 minus weight on day zero. All these weight procedures were followed with triplicates. Likewise, it was calculated after 14 d. For the first time, a mathematical model was used to find out significant results in weight loss during the postharvest period of coated and uncoated brinjal.

Titratable acidity (TTA):

Since the fruits get matured, their organic acid level increases, and hence TTA was measured to know about its acidity level. 5 g of brinjal fruit was taken from the middle part (to ensure the properly grown part of the fruit) in 50 ml distilled water and a homogenate was made with a mortar and pestle. Then, it was centrifuged at 1956 g for 10 min. In 5 ml of supernatant 1-2 drops of phenolphthalein indicator were added (Yaman & Bayoindirli, 2002). Finally, it was titrated with 0.1N NaOH. The TTA in the sample was calculated using the formula given below

% Acid=(ml of NaOH× mili equivalent factor ×100)per g of fruit extract sample

Protein estimation:

5 g of fruit sample was taken in 10 ml of 80% acetone and macerated in mortar and pestle followed by centrifugation at 8000 rpm for 20 min at 40 C. Discarding the pellet and taking supernatant for further protein estimation in diverse aliquots of 0.5, 1.0, and 1.5 ml. Then the volume was made up to 2 ml in each tube by adding distilled water to continue adding 4.5 ml of reagent (2% Na_2CO_3 in 0.1N NaOH). Mixed it well and incubated for 10 min at room temperature and to it, 5 ml of sodium tartrate (reagent Ⅱ), 0.5 ml of Folin Ciocalteu Reagent (FCR) were poured and mixed properly. It was incubated in a dark condition for 30 min. A blank tube was served as 2 ml of distilled water along with the reagent I and II with FCR except for standard (Bovine serum albumin with 95 % purity purchased from Sigma-Aldrich and the fruit extract sample) (Lowry *et al.,* 1951). Lastly, the absorbance was measured at 660 nm in UV visible spectrophotometer (Model number 169; Systronic India) against blank. Later, the amount of protein was presumed after plotting the standard graph. The reagents were purchased from Himedia, Mumbai, India.

Proline estimation:

2 g of sample brinjal was combined with 10 ml of 80% acetone and clarified through Whatman No.2 filter paper. The extraction was repetitive and the filtrates were collected. 2 ml of this filtrate was mixed with 2 ml each of glacial acetic acid and ninhydrin (Bates *et al.,* 1973). The solution mix was boiled in a boiling water bath for 20 min. Once, cooled at room temperature, 4 ml of toluene was added which resulted in a red-colored solution that was measured at 520 nm against a blank. Proline quantity in the sample can be calculated by the formula:

[µmoles of proline /g tissue = (µg proline per ml × ml toluene)/115.5 × 5 g of sample]

Catalase Activity:
A smooth pulp of 1 g of brinjal with 0.1M phosphate buffer (pH 7.0) was made by using pre-chilled mortar and pestle and centrifuged at 5009 g for 20 min at 4º C. Collected the supernatant and later was used for enzyme activity. In a test tube, 2 ml of H_2O_2, 3 ml of phosphate buffer, and 1 ml of enzyme extract were mixed and incubated at 200 C for 1 min. Now, to stop the reaction 10 ml of 0.7N H_2SO_4 was added. Lastly, it was titrated with 0.01 N $KMnO_4$ till a faint purple color lasts for at least 15 sec (Sinha, 1972). The solution of the enzyme extract with the reaction mixture at zero time is considered blank.

Peroxidase (POD) activity:
For the initialization of the method, 3 g of sample fruit was crushed in mortar and pestle along with, 10 ml pre-chilled phosphate buffer (0.1 mM), pH 6.0. Muslin cloth (2 fold) was used to sieve the crushed mixture. Centrifuged the homogenate at 5009 g for 20 min at 4º C and took the supernatant and treated it as an enzyme source. 2.4 ml of distilled water, 0.5 ml H_2O_2, 1 ml of O-dianisidine, and 1 ml of phosphate buffer were added to the test tube. For blank, H_2O_2 was omitted, so far, the extra volume of water was added. To start the reaction, 0.2 ml of an enzyme was added and incubated at 30º C for 5 min in an incubator, and to stop the reaction 1 ml of 2N H_2SO_4 was poured, ending with taking the absorbance at 430 nm (Wang *et al.,* 2005).

DPPH radical scavenging activity:
5 g of brinjal fruit from its middle part was macerated meticulously with 50 ml of distilled water in a mortar and pestle. It was centrifuged at 5009 g for 20 min at 4°C. The supernatant was taken and treated as an enzyme source. In 10 µl of an enzyme extract, dimethyl sulfoxide (DMSO) was added to make it to 40 µl. 2.96 ml of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was poured into it and incubated in dark conditions for 20 min at room temperature. The absorbance of the mixture was observed at 517 nm. As a control, 3 ml of DPPH was taken. The % radical scavenging activity of the sample extracts was calculated by using the following formula: [% Radical Scavenging Activity = (Abs. control – Abs. of sample× 100)/Abs. control] (Molyneux, 2004).

Statistical Analysis:
Each coated and uncoated brinjal was tested and repeated thrice with three replicates for various parameters. Duncan's multiple range test (Duncan, 1955) and two-way Analysis of variance (ANOVA) at $P < 0.05$ statistical analysis were conducted in SPSS version 20.0, IBM Inc. Further, principal component analysis (PCA) was applied to detect the variations in the parameters except for weight loss (MetaboAnalystR 3.0 software) (Pang *et al.,* 2020).

RESULTS

Internal fruit section:
As shown in **Fig. 1**, it is easy to see that the internal section of control brinjal rotten early at just 7 days whereas coated brinjal with GA, KN and GK emerged far better than control even on the 14th day of storage. Among them, GK10 was suitable in all aspects of morphology and without any hindrance, it can be prescribed for consumption. As observed in **Fig. 1**, GK10 treated brinjal appearance was better followed by GK20, KN20, GK30, GA10 and the rest could not be referred for consumption.

Weight loss:
The effect of coating at different concentrations on weight loss of brinjal under each interval of 7 days is shown in **Table 1**. There was a significant decrement observed in the weight of brinjal during the storage period of 14 days. The coating might reduce the loss of water, hormones, moisture, nutrient, and energy. Therefore, coated brinjal with GA and KN had a lower amount of weight loss compared to control ones (**Table 1**). The uncoated brinjal couldn't last more than 7 days, but it could last 14 days when coated with 10µ M gibberellic acid and (10+10) µM coating of gibberellic acid and kinetin, even though it had less intact water content. Fruits initially contain more water after being harvested. There is no doubt that water loss happens throughout the day, although it will gradually be less than on days 0 and 7.

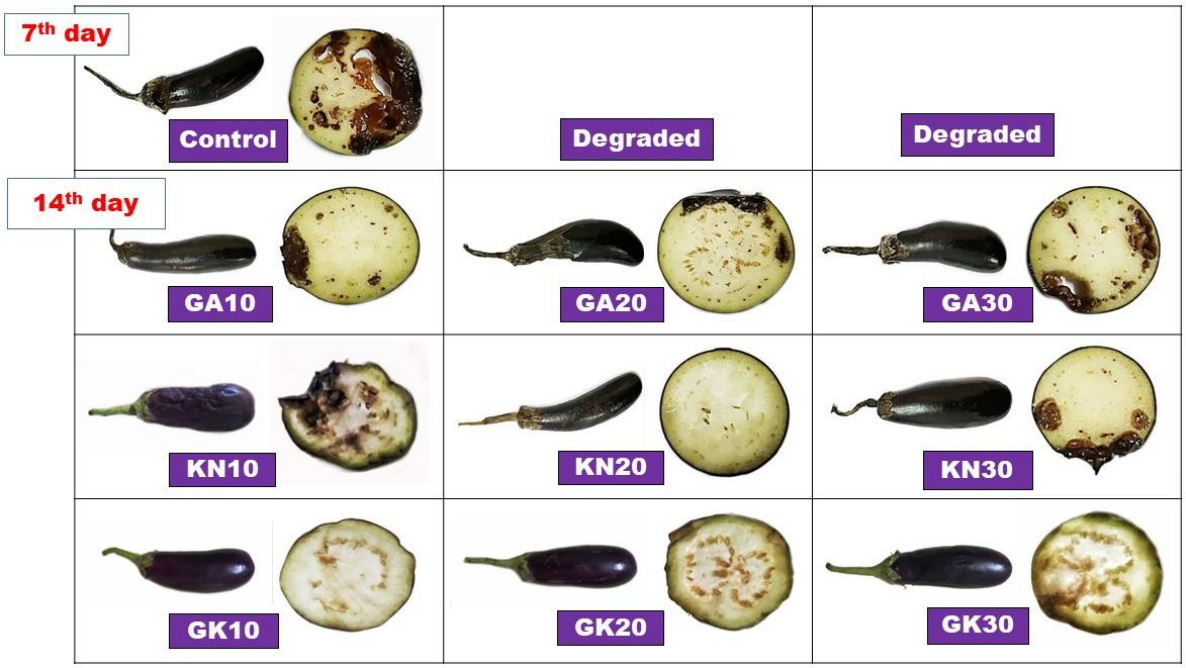

Fig. 1. Internal brinjal fruit section at the final postharvest stage i.e., in control (7th day) and in treated (14th day). GA10- 10 µM Gibberellic acid; GA20- 20 µM Gibberellic acid; GA30- 30 µM Gibberellic acid; KN10- 10 µM kinetin; KN20- 20 µM kinetin; KN30- 30 µM kinetin; GK10- 10 µM Gibberellic acid + 10 µM kinetin; GK20-20 µM Gibberellic acid + 20 µM kinetin; GK30- 30 µM Gibberellic acid + 30 µM kinetin

Table 1: Effect of GA and KN coating and storage period on weight loss, titratable acidity (TTA), protein content, proline content, catalase activity, peroxidase activity, DPPH radical scavenging activity (DPPH RSA), in brinjal (*Solanum melanogena*). The data represents mean ± standard deviation. Data for each column followed by the different alphabets are significantly different according to Duncan's multiple range test (Duncan, 1955) at P<0.05. The absence of data represents a lack of information due to the postharvest deterioration of fruit.

(µM)	Storage (days)	Weight loss (g)	TTA (mg g^{-1} extract)	Protein content (µg g^{-1} extract)	Proline content (µmol g^{-1} extract)	Catalase activity (nmol H_2O_2 min^{-1} g^{-1} extract)	Peroxidase activity (U g^{-1} extract)	DPPH RSA (%)
0	0	22.41±2.24ab	0.828±0.034a	5.42±0.49a	0.669±0.013h	4.168±0.097	0.541±0.04d	27.5±1.88e
(Control)	07	21.43±2.52ab	0.035±0.021abcd	3.62±0.83ef	2.602±0.78a	1.733±0.075fg	0.557±0.04cd	48.64±1.5cd
	14	0±0	0.0±0.0	0.0±0.0	0±0	0±0	0±0	0±0
GA 10	07	18.96±1.76 ab	0.064±0.046ab	4.98±0.62ab	0.829±0.05gh	3.78±0.088	0.772±0.006a	44.7±3.14d
	14	17.88±1.69 ab	0.059±0.039abc	3.20±1.24bcde	1.015±0.19fgh	2.643±0.075b	0.639±0.002abcd	60.79±1.39abc
GA 20	07	20.25±3.63 ab	0.034±0.019abcd	3.56±0.62ef	1.238±0.07efgh	1.683±0.011gh	0.626±0.001abcd	51.25±0.935bcd
	14	17.31±4.66 ab	0.017±0.007bcd	2.51±0.13g	1.887±0.04bc	1.543±0.105h	0.039±0.003e	70.21±1.56a
GA 30	07	19,27±3.28 ab	0.046±0.026abcd	4.58±0.08bcd	0.968±0.031fgh	2.946±0.198a	0.749±0.002ab	50.88±0.2bcd
	14	16.34±1.8 ab	0.017±0.007bcd	3.06±0.36fg	1.288±0.264efg	1.776±0.075efg	0.587±0.004bcd	64.79±0.347ab
KN 10	07	19.0±3.41 ab	0.055±0.019abc	3.76±0.16def	1.321±0.03defg	1.733±0.075fg	0.63±0.005abcd	55.99±8.61abcd
	14	16.04±3.65 ab	0.029±0.007ef	3.12±0.43fg	1.996±0.29b	1.603±0.075gh	0.039±0.039e	70.43±1.23a
KN 20	07	20.7±5.92 ab	0.042±0.026abcd	4.74±0.22abc	1.477±0.35bcdef	1.993±0.15cd	0.748±0.005ab	49.0±1.41cd
	14	18.07±4.48b	0.012±0.002lcd	3.66±0.19ef	1.869±0.16bcd	1.093±0.02c	0.603±0.002abcd	65.45±0.802ab
KN 30	07	20.51±4.41 ab	0.082±0.034bc	4.4±0.5bcde	1.682±0.033bcde	2.166±0.075c	0.72±0.005abc	47.71±2.33cd
	14	16.59±3.46b	0.012±0.003cd	3.59±0.47ef	1.706±0.061bcde	1.906±0.075de	0.588±0.013bcd	66.11±0.486a
GK 10	07	18.43±3.18 ab	0.051±0.038abc	4.97±0.04cde	0.885±0.02gh	2.99±0.13a	0.747±0.031ab	47.38±0.516cd
	14	15.24±2.69ab	0.038±0.025abcd	3.76±0.16def	1.181±0.08efgh	1.993±0.15cd	0.624±0.001abcd	59.45±1.294abcd
GK 20	07	18.47±5.09 ab	0.042±0.026hi	4.38±0.13bcde	0.942±0.10fgh	1.686±0.05gh	0.62±0.001abcd	46.57±1.92cd
	14	17.51±9,68 ab	0.025±0.012bcd	2.69±0.18g	1.207±0.17efgh	1.656±0.05gh	0.605±0.002abcd	64.44±0.751ab
GK 30	07	20.13±3.36 ab	0.034±0.026jk	4.36±0.15bcde	1.08±0.92fgh	2.253±0.075c	0.737±0.002ab	47.85±1.98cd
	14	17.88±1.69 ab	0.029±0.007bcd	2.69±0.18g	1.365±0.01cdefg	1.863±0.075de	0.619±0.002abcd	64.3±0.161ab

Mathematical application of weight loss:

The exponential decay model has been extensively studied in various scientific experiments. Exponential growth or exponential decay is used in everyday life. For example, the half-life of a radioactive substance, in environmental forensic finding the contamination age (Domoradzki, *et al.,* 2003) The experimental decay model can be best applicable in this study i.e., described by the following equation (1).

$$y(t)=Ae^{-bt}\ldots(1)$$

Here A represents the initial quantity present and b represents the rate at which the decay or deterioration takes place. If the value of b is small then the decay is slow, and if the value of b is large then the decay is faster. The effect of the control group, GA10, GA20, GA30, KN10, KN20, KN30, GK10, GK20, and GK30 on weight-loss when modeled by the equation $y(t)=Ae^{-bt}$, then the corresponding equations and the values of b were found and tabulated in **Table 2**. Then, the equations obtained were utilized to extrapolate the data of weight loss for a period of 14 days keeping a 2-d gap. This was plotted in Figure 2. Here, it was observed that the b value of the control fruits (uncoated brinjal) was 0.1. Whereas the b value for the coated brinjal was greater than 0.1. So, this shows that the weight loss in the coated brinjal fruits was reduced. The yellow curve in **Fig. 2** corresponds to the control group lying above all the remaining curves.

Table 2: Mathematical application on weight loss. The model is following the equation: $y(t) = Ae^{-bt}$

		Weight loss			b value
0	(Control)	0	7	14	0.01
		22.41	21.43	19.46	
		$y=22.604\ e^{(-0.01t)}$			
1	**GA 10**	0	7	14	0.016
		22.41	18.96	17.88	
		$y=22.008\ e^{(-0.016t)}$			
2	**GA 20**	0	7	14	0.018
		22.41	20.25	17.31	
		$y=22.618\ e^{(-0.018t)}$			
3	**GA 30**	0	7	14	0.023
		22.41	19.27	16.34	
		$y=22.462\ e^{(-0.023t)}$			
4	**KN 10**	0	7	14	0.024
		22.41	19	16.04	
		$y=22.426\ e^{(-0.024t)}$			
5	**KN 20**	0	7	14	0.015
		22.41	20.7	18.07	
		$y=22.622\ e^{(-0.015t)}$			
6	**KN 30**	0	7	14	0.021
		22.41	20.51	16.59	
		$y=22.876\ e^{(-0.021t)}$			
7	**GK 10**	0	7	14	0.028
		22.41	18.43	15.24	
		$y=21.99\ e^{(-0.028t)}$			
8	**GK 20**	0	7	14	0.018
		22.41	18.47	17.51	
		$y=21.235\ e^{(-0.018t)}$			
9	**GK 30**	0	7	14	0.016
		22.41	20.13	17.88	
		$y=22.452\ e^{(-0.016t)}$			

Titratable acidity:

In this study, the value of TTA was obtained in intervals of 7 days and the results suggested that TTA values in all the concentrated groups were gradually and considerably decreased with increasing storage period. Decrement of TA occurred in both uncoated and coated groups. But, comparatively different concentrations of GA and kinetin showed better firm quality than uncoated. On the 7th day of the storage period, the titratable acidity value of the uncoated control sample resulted in immense fallout while all coated samples showed a better result (Table 1). Here the outcomes also proved that after the 14th day of the storage period, the sample of the uncoated control group was completely vitiated and the coated sample showed better maintenance, especially in 10 µM concentration of gibberellic acid and (10+10) µ M concentration of gibberellic acid and kinetin (**Fig. 1**). These outcomes were also significant at $P < 0.05$ (**Table 3**).

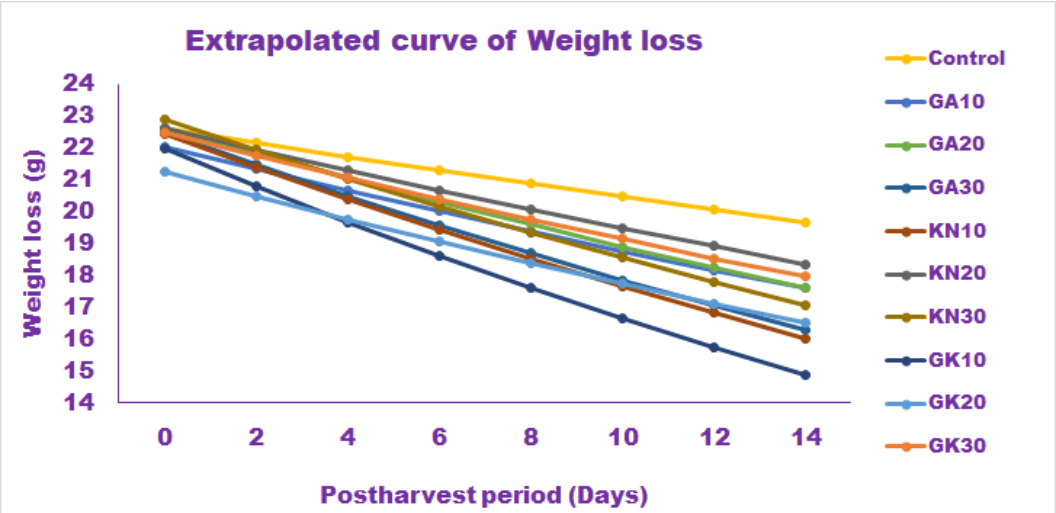

Fig. 2: Weight loss after 7 days of harvest in different treatments of Gibberellic acid and kinetin. GA10- 10 µM Gibberellic acid; GA20- 20 µM Gibberellic acid; GA30- 30 µM Gibberellic acid; KN10- 10 µM kinetin; KN20- 20 µM kinetin; KN30- 30 µM kinetin; GK10- 10 µM Gibberellic acid + 10 µM kinetin; GK20-20 µM Gibberellic acid + 20 µM kinetin; GK30- 30 µM Gibberellic acid + 30 µM kinetin

Protein estimation:

In this study, the protein content of coated and uncoated samples is measured at 660 nm. And it showed a significant downturn in both. According to this study, at the end of the 14th day, high content of protein was found in the 10µM concentration of gibberellic acid and (10+10) µM concentration of gibberellic acid and kinetin compared to others (**Fig. 1**). Notably, it can also be observed that the uncoated control sample doesn't outlast than 7 days and becomes vitiate. It clearly shows the shelf-life extended in the coated sample. The two-way ANOVA value is also in concurrence with the combination of gibberellic acid and kinetin coating and convincing with the storage state at $P < 0.05$ (**Table 3**).

Table 3: Two-way ANOVA showing individual and interaction effect of the GK coating and storage period on weight loss, titratable acidity (TTA), protein content, proline content, catalase activity, peroxidase activity, and DPPH radical scavenging activity (DPPH RSA) in brinjal (*Solanum melanogena*). *Significant at P<0.05.

Source	df	Weight loss		TTA		Protein content		Proline content	
		Mean square	F	Mean square	F	Mean square	F	Mean square	F
Corrected model	20	65.379	4.166	0.001	2.337	3.773	18.202	0.911	10.076
GK	9	45.89	2.924*	0.001	1.581*	3.578	17.261*	0.524*	5.794*
Storage duration	2	277.481	17.682	0.009	14.156	24.189	116.694	4.609	0.016
GK × Storage duration	9	61.746	3.935*	0.001	0.889*	1.317	6.354*	1.359	15.034
Error	42	15.693		0.001		0.207		0.090	
Total	63								
Source	df	Catalase activity		Peroxidase activity		DPPH RSA			
		Mean square	F	Mean square	F	Mean square	F		
Corrected model	12	2.240	254.514	0.164	20.623	769.150	13.315		
GK	3	2.333	265.095	0.179	22.490	701.902*	12.151*		
Storage duration	3	13.502	1534.445	0.559	70.209	701.825*	12.150*		
GK × Storage duration	6	0.577	65.545*	0.077*	9.641*	634.927*	10.991*		
Error	26	0.009		0.008		57.766			
Total	38								

Proline estimation:

As shown in **Table 1**, during the storage period, the proline content was augmented in all uncoated and coated samples. Measuring after the 7 days of the interval with the ninhydrin method, an exceedingly increment of proline content occurred in the uncoated sample compared to coated and it was prejudiced and vitiated before 14 days. Whereas coated samples, with GA 20µ M and kinetin 10µM, showed significantly higher proline content in a gradually increasing manner throughout the 14 days of extended shelf-life compared to other coated samples (**Table 1**). These results were also approved at $P < 0.05$ in two-way ANOVA valuation.

Catalase activity:
With an increasing storage period of up to 14 days, a gradual decrement of catalase activity was observed in this study. Moreover, a decline in catalase activity during chilling conditions leads to the aggregation of H_2O_2 and it terminates the antioxidant defense of the plant. The result of our study indicates that the sample which is coated with 10 µM GA3 showed higher and better catalase activity over control and other concentrations (**Table 1**).

Peroxidase Activity:
In the current study, both coated and uncoated samples showed a decrement in peroxidase activity throughout the extended shelf-life up to 14 days. But in comparison to the uncoated control sample which got spoiled and did not survive till 14 days, a coated sample with a 10 µM concentration of gibberellic acid and (10+10) µM concentration of Gibberellic acid and kinetin showed a salutary effect for extension of the shelf-life in Solanum melongena L (**Table 1**). The combination of the coating was significant at $P < 0.05$ (**Table 3**).

DPPH radical scavenging activity:
In the present work, antioxidant activity was assessed by applying the DPPH decolorization assay which measures total racial antioxidant abilities to scavenge the free radicals by spectrophotometer at 517 nm. Outcomes exhibited as both coated and uncoated samples show a significant difference ($P < 0.05$) in their response to antioxidants. As shown in Table 1, the DPPH radical scavenging activity was increased in both treated and untreated as well. But a kind of better result was observed in the 10µM concentration of GA3 and (10+10) µM concentration of GA3 and Kinetin compared to others. These both are the most effective scavenger with the lowest value at the end of the 14th day.

PCA analysis of various parameters:
A mathematical algorithm such as PCA shows the maximum variations in the dataset and reduces the dimensionality (Ringnér, 2008). By using it, similarities and differences between these samples based on various parameters such as TTA, protein, proline, catalase, and peroxidase activity compared with control were visually assessed as shown in **Fig. 3** A and B. Two principal components expressly PC1 and PC2 derived that enumerated for 70.5 % of the variance and 21.9% of the variance respectively in 30 variable systems of different coating concentration levels including control. Moreover, a petty extent of variance occurred in PC 3 (data not shown). As with most of all the variables obtained under these two components though maximum weightage was found in the case of PC1. Moreover, it was clearly found that the control values could not match the coating values as indicated by various spots and apart from it, the PCA score showed a similar variable cluster together as PC1 have it more.

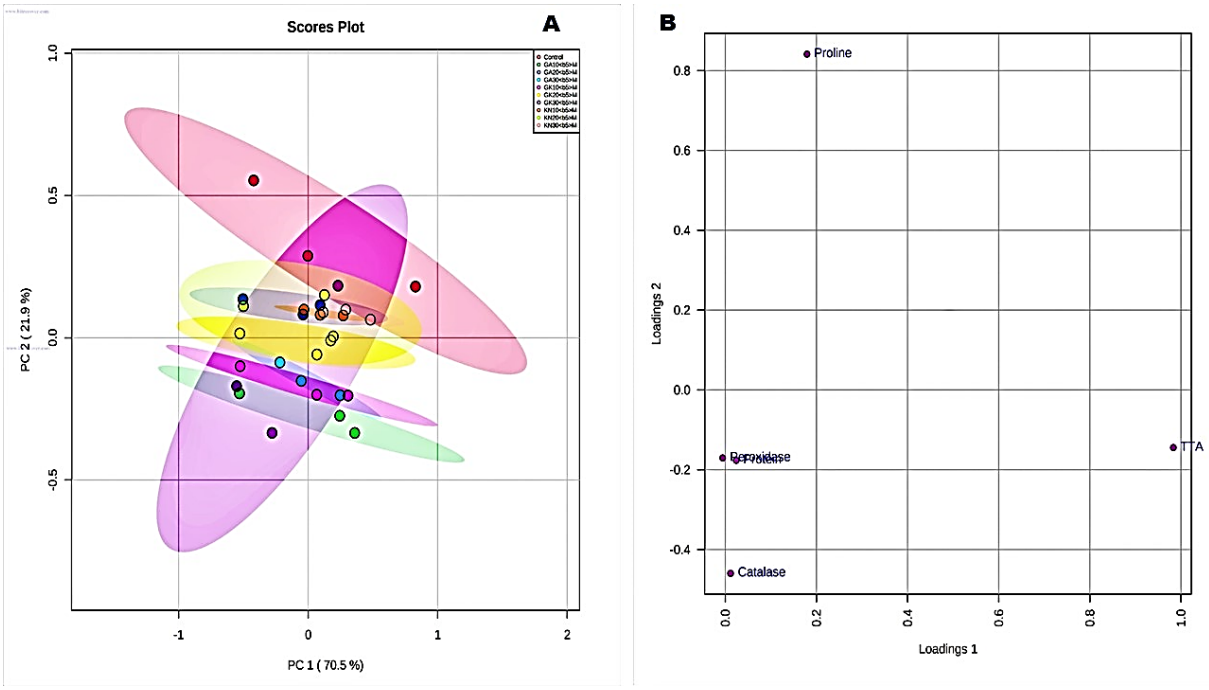

Fig. 3. Principal component analysis on different parameters. A- Scores plot, B- Loading plot

DISCUSSION

During the storage period, the quality of fruit can't be improved but can be maintained. And if it doesn't maintain properly, then fruit losses its optimal eating quality, nutritive quality, and caloric quality, and becomes shriveled. Also, the fruits get rotted and flavorless due to climacteric bursts of ethylene, physiological changes, pathological infection, and transportation. Thus, it leads to diminishing the shelf life of fruit. Moreover, its

internal morphology should be protected and maintained in all adverse circumstances for sellers' and consumers' goods.

Hence the experiment results of weight loss approved the same trend as the previous study on green chili (Panigrahi *et al.,* 2017), Capsicum annuum (Patel *et al.,* 2018), and cucumber (Patel and Panigrahi 2019). The obtained results were also affirmed by a two-way ANOVA assessment and found to be significant at $P < 0.05$ (Table 2.). In a previous study, the wrinkle of the brinjal was increased with the storage time for the control (Al- Juhaimi *et al.,* 2012). The color changes for the GA and KN coated brinjal compared to the uncoated ones were insignificant. Storage at room temperature expands the shelf life of brinjal and provides a better result in all respects as per days of survival. Excessive transpiration, respiration, and metabolic activity are the major cause of weight loss (Shafiee *et al.,* 2010). After harvesting, vegetables further undergo stressed conditions due to the loss of nutrients, energy, moisture, water, and hormones, and initiate senescence (King and Morris, 1994). A possible solution to this was to use the gibberellic acid and kinetin coating that fills the opening of the vegetable surface. The weight loss is related to the physiological and morphological characteristics as well as the type of crop assessed (Nunes *et al.,* 2007; Khatri *et al.,* 2020). Therefore, the weight loss of brinjal results in economic loss for farmers.

It establishes that the various coatings have reduced the weight loss in brinjal during the postharvest period and have extended to 14 d instead of a week. Among these coatings, GK10 was found to be the best i.e., the weight loss was minimal. Remarkably, titratable acidity (TTA) is the chief soothsayer of bitterness (Sadler and Murphy, 2010) and bitterness causes when protons are detected. Organic acids such as citric acid leading substrates for the enzymatic process involved in respiration. In due course of the storage period, decrement in acidity and augmentation of pH occurs consecutively (Yaman and Bayoindirli, 2002). As earlier scientists represent that coating diminishes the respiration rate and breaks the activities of organic acid (Yaman and Bayoindirli, 2002).

During the protein estimation, peptide bond presents in the protein react with copper and produce Cu^{+}. The copper-catalyzed oxidation of aromatic acid occurred as Cu^{+} reacts with FCR and resulting in the reduction of phosphomolybdotungstate to heteropolybdenum blue. This reaction gives a blue-colored compound because of tyrosine and tryptophan content in protein (Waterborg, 2009). This whole process was pH-sensitive besides the decrement of protein obtained with increasing storage period. It's because of the conversion of protein to amino acid residue (Patel *et al.,* 2019; Panigrahi *et al.,* 2018; Panigrahi *et al.,* 2007). Several published results confirm that under some precise conditions, such as in proline dehydrogenase mutants where impaired proline degrades and resulted in severe toxicity too. So, the exact influences of proline accumulation on particular regulatory pathways in complex stress responses are still not fully known (Ábrahám *et al.,* 2010; Patel *et al.,* 2018; Patel and Panigrahi, 2019). Accumulation of proline occurs when a plant faces some environmental and biotic stress. Such as high salinity, heavy metal, drought, hypoxia, water stress, and pathogen infection. In response to such conditions, proline plays a dual role as an osmoprotective compound and molecular chaperon and further protects the cellular structure, membranes, and protein during osmotic stress. It is also investigated as a scavenger of reactive oxygen species and competent to impoverish oxidative damage. So, it gives a good contribution to adaptation to stressed conditions. Data on proline accumulation gives an idea about the physiological status of plants. Moreover, the augmented level of proline enhances salt tolerance (Ábrahám *et al.,* 2010).

Though the shelf life, countenance, and flavour of brinjals declined with diminishing catalase activity. This result was significantly upheld by a two-way ANOVA assessment at $P < 0.05$ (Table 2). Uncoated brinjal actively survived in the first interval of 7 days but after then it was completely vitiated. Catalase has a versatile role in plants as it works as a protector against oxidative damage which occurs at high H_2O_2 concentrations and it also conduces with other antioxidant enzymes to detain the formation of reactive oxygen species (ROS). H_2O_2 detrimentally impacts the process such as plant pathogen response and catalase does the degradation of H_2O_2 to water and molecular oxygen (Heinze and Gerhardt, 2002; Suthar *et al.,* 2021). Therefore, this outcome showed the ameliorative effect of coating to extend the shelf-life of brinjal. H_2O_2 can induce intracellular reactive oxygen species and it causes oxidative damage in plant cells. Though it plays a duple role as emergently conducible at low concentrations and noxious at high concentrations (Petrov *et al.,* 2012). Always, the balance between the production and scavenging of H_2O_2 should be maintained. If it gets disturbed under any stressed condition, the excessive production of H_2O_2 occurs and it leads to damage to cell structure (Anjum *et al.,* 2015). To cope with this situation, aerobic organisms have developed some enzymes including 'Peroxidase'. Peroxidase activity abolishes the toxic level of H_2O_2 (Ozyigit, 2016). As a result, during stressful situations, catalase activity and the amount of H_2O_2 generated both rises (Vaishnani, 2022). Remarkably there is a factual relationship between peroxidase activity and the enhancement of odor and flavors in vegetables (Burnette, 1977).

Here also it proves that a combination of gibberellic acid and kinetin coating in a particular concentration leads to beneficial effects towards the extension of shelf-life and retention of the quality of brinjal. The DPPH (1,1-diphenyl-2-picryl-hydrazyl) is a smooth, prompt, accurate, non-enzymatic, and reasonable assay method for scaling the capability of different compounds to act as hydrogen donors or free radical scavengers and to assess the antioxidant activity of food (Marinova *et al.,* 2011; Panigrahi *et al.,* 2018). From this PCA plot, it can be concluded that these types of coatings are acceptable to prevent qualitative loss in

Brinjal. Furthermore, the loading plot presents in **Fig. 3** B showed the correlation between the variables as peroxidase placed as opposed to the other 4 variables with bit negative value, and the other parameters were well placed in loadings 1 plot. From these PCA loadings, it can be said that the GA and KN coating may show an impact on the postharvest storage of brinjal.

CONCLUSION

The results of this study demonstrated that the brinjal coated with gibberellic acid and kinetin laid out an emergent change in numerous parameters, including weight loss, increase DPPH radical scavenging activity, decrease titratable acidity, in addition to catalase and peroxidase activity, under stressed condition proline content was increased, and protein content was decreased, in comparison to uncoated control brinjal. Additionally, this work demonstrates the beneficial effects of gibberellic acid and kinetin as an exogenous coating for Solanum melongena L. shelf-life extension. However, the combination of gibberellic acid and kinetin at a concentration of 10+10 µM had a bigger impact than any other level of concentration on the lengthening of storage life. Additionally, the principal component analysis and mathematical decay model made a considerable contribution to justifying the hypothesis as had been expected before the experiment. Overall, it has been indicated that covering this GA3 + kinetin will slow down the ripening and senescence process and encourage the extension of brinjal's shelf life. As a result, it can be used to preserve fruits and vegetables. Better postharvest management of brinjal is therefore crucial at the national level, since it not only lowers postharvest losses but also improves nutrition, increases food and financial security for the populace, and generates jobs.

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