

## Research Article

# Salicylic Acid Treatment Reduces Lipid Peroxidation and Chlorophyll Degradation and Preserves Quality Attributes of Pointed Gourd Fruit

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The marketability of pointed gourd fruit is drastically reduced after harvest due to moisture loss, chlorophyll degradation, yellowing of the skin, and shriveling. The present investigation studied the effect of exogenous salicylic acid (SA) treatment on senescence and fruit quality attributes of pointed gourd during storage under ambient conditions. Fruits were treated by immersing them in different concentrations of SA (1.0 mM, 2.0 mM, and 3.0 mM) and distilled water (control) for 5 minutes. The investigation showed beneficial effects of 3.0 mM SA treatment in lowering weight loss (16.8%), maintaining higher chlorophyll (32.8%) in the skin, and reducing lipid peroxidation (20.2%) compared to the control. SA (3.0 mM)-treated fruits retained 15.3% higher ascorbic acid and about 18% higher total phenol, flavonoids, and radical scavenging activity over pointed gourd fruits in the control group. However, significant difference in the total antioxidant capacity after 6 days of storage was not noted between SA-treated and control fruit. Thus, postharvest salicylic acid treatment can beneficially be used to extend marketability and delay quality deterioration of pointed gourd fruits stored under ambient conditions.

## 1. Introduction

Pointed gourd (*Trichosanthes dioica* Roxb.) is a perennial herbaceous plant belonging to the largest vegetable family Cucurbitaceae. It is originated in India and well known as “King of Gourds” because of high dietary fiber and rich nutritional and medicinal values. Pointed gourd is cultivated in tropical and subtropical regions of the world for its highly nutritious fruits. In India, the fruit is commercially cultivated in states like Bihar, Uttar Pradesh, Odisha, Assam, and West Bengal [1]. The pointed gourd fruit is also an abundant source of several phytochemical compounds like phenols, flavonoids, glycosides, sterol, saponin, and alkaloids [2].

Importantly, *Ayurveda*, a traditional Indian system of medicine makes use of innumerable botanicals to treat a wide range of disorders [3, 4]. Pointed gourd is used in the *Ayurvedic* medicine system for cholesterol-lowering activity, gastrointestinal complications, and diuretic, anthelmintic, and expectorant effects [1]. Moreover, traditional use of this fruit has also been reported for curing chicken pox scar, skin diseases, and swelling liver and spleen by some tribal communities of India in West Bengal and Odisha [5]. Due to these reasons, the demand of pointed gourd has increased considerably in domestic as well as export market. However, high perishability and rapid loss of market value after harvest is a major challenge in extending storability of pointed gourd

fruits beyond 2-3 days under ambient conditions [6]. The major reasons for reducing marketability of the fruit are rapid moisture loss, shriveling, loss of turgidity, yellowing of skin, and seed hardening [7]. Due to rapid degradation of appearance and sensory quality, sometimes growers fetch low market price for pointed gourd. Also, to maintain green colour and make the fruit attractive, some traders use synthetic colours that cause health hazards.

Salicylic acid (SA), as well as its natural analog acetyl salicylic acid, is a plant phenolic compound. It acts as a signaling molecule. It is involved in various physiological and developmental processes in plants [8]. SA has been found to be highly effective in delaying ripening and quality preservation of several fruits and vegetables due to its antiripening and antisenesescence properties. It is also reported to maintain sensory and nutritional quality and elicit natural resistance against postharvest biotic and abiotic stresses, eventually increasing postharvest shelf-life of the produce [9]. Studies have revealed that postharvest treatment with SA delayed weight loss, ripening-associated changes, and the incidence of diseases in fruits [10]. However, to the best of our knowledge, the response of pointed gourd fruits to exogenous SA treatment on different physicochemical and functional quality attributes has not been reported so far. The present investigation aimed to study the effect of postharvest salicylic acid treatment on the storage behavior of pointed gourd fruit.

## 2. Materials and Methods

**2.1. Fruit Material and Salicylic Acid Treatment.** Commercially mature pointed gourd fruits of cultivar "Navdhari" were harvested in the early morning and immediately transported to the laboratory of horticulture department at Banaras Hindu University, Varanasi (India). After discarding the bruised or damaged fruit, a total of 400 healthy fruits having uniform size, shape, colour, and maturity were selected for the experiment. The selected fruits were then randomly divided into four groups. Treatments were performed by immersing the pointed gourd fruit in different concentrations (1.0 mM, 2.0 mM, and 3.0 mM) of salicylic acid solution for 5 minutes. For control, fruits were treated with distilled water for the same duration. The excess moisture on the fruit surface was dried and stored under ambient conditions (mean temperature 29°C and relative humidity 82%) in corrugated fibreboard boxes. At an interval of 2 days, fruits were selected at random from each treatment and analyzed for different quality attributes for a period up to 6 days.

**2.2. Fresh Weight Loss.** Reduction in fresh weight of the pointed gourd during storage was determined by recording the initial and final weight on the sampling day by using a digital weighing balance and calculated as percent (%).

**2.3. Total Chlorophyll and Total Carotenoid Content.** The level of total chlorophyll in the fruit skin was estimated from the acetone extract (80% v/v) by a spectrophotometric

method at 645 and 663 nm and calculated as mg/g fresh weight [11]. The level of total carotenoids in the skin was analyzed by using the spectrophotometer (Labtronics LT-2201 UV-Vis Double Beam Spectrophotometer) at 480 nm following the method of Roy [12] and calculated as mg/g fresh weight (FW).

**2.4. Lipid Peroxidation.** The level of malondialdehyde (nmol/g-FW) in the fruit was estimated to determine membrane lipid peroxidation. It was determined by the spectrophotometric method at 450, 532, and 600 nm following the method of Zheng and Tian [13] using 5% w/v trichloroacetic acid and 0.6% w/v thiobarbituric acid.

**2.5. Total Phenol and Total Flavonoid Content.** Analysis of total phenol was performed by using the Folin-Ciocalteu reagent (1.0 N) according to Singleton et al. [14]. The ethanol extract (80%) of the fruit and gallic acid standard were used for recording absorbance (760 nm). Gallic acid equivalent (GAE) ( $y = 0.0202x - 0.0225$ ;  $r^2 = 0.9942$ ) was used for determination of the total phenol content ( $\mu\text{g-GAE}/100 \text{ g-FW}$ ). The level of total flavonoids in the edible part of the pointed gourd was estimated by an aluminum chloride (10%) method [15]. Rutin equivalent (RE) ( $y = 0.2782x + 0.0628$ ;  $r^2 = 0.9982$ ) was used for calculation of flavonoid content ( $\mu\text{g-RE}/100 \text{ g-FW}$ ).

**2.6. Total Antioxidant Capacity.** The procedure of Apak et al. was followed for the estimation of total antioxidant capacity [16]. For this, the ethanolic sample extract (80%) was mixed with neocuproine ( $7.5 \times 10^{-3} \text{ M}$ ), an ammonium acetate buffer (1.0 M, pH 7.0), and copper(II) chloride ( $10^{-2} \text{ M}$ ) solution and absorbance was recorded at 450 nm. Trolox equivalent (TE) was used for calculation and expression of total antioxidant capacity ( $\mu\text{mol-TE}/\text{g-FW}$ ).

**2.7. DPPH Radical Scavenging Activity.** The scavenging activity of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was determined following the method of Brand-Williams et al. [17]. We analyzed the capacity of antioxidants present in the pointed gourd fruit to scavenge the stable DPPH radical (0.0634 mM), and results were presented in percent (%).

**2.8. Statistical Analyses.** The present investigation was laid out in a completely randomized design with four replications. Results obtained in respect of various parameters during this study were presented as the mean  $\pm$  standard error in tables and figures. The analysis for the study was carried out using SAS statistical system 9.2 (SAS Institute, Cary, NC, USA).

## 3. Results and Discussion

**3.1. Effect on Fresh Weight Loss.** It was observed that irrespective of treatments, fresh weight of pointed gourd fruits declined rapidly with the storage period (Figure 1(a)).

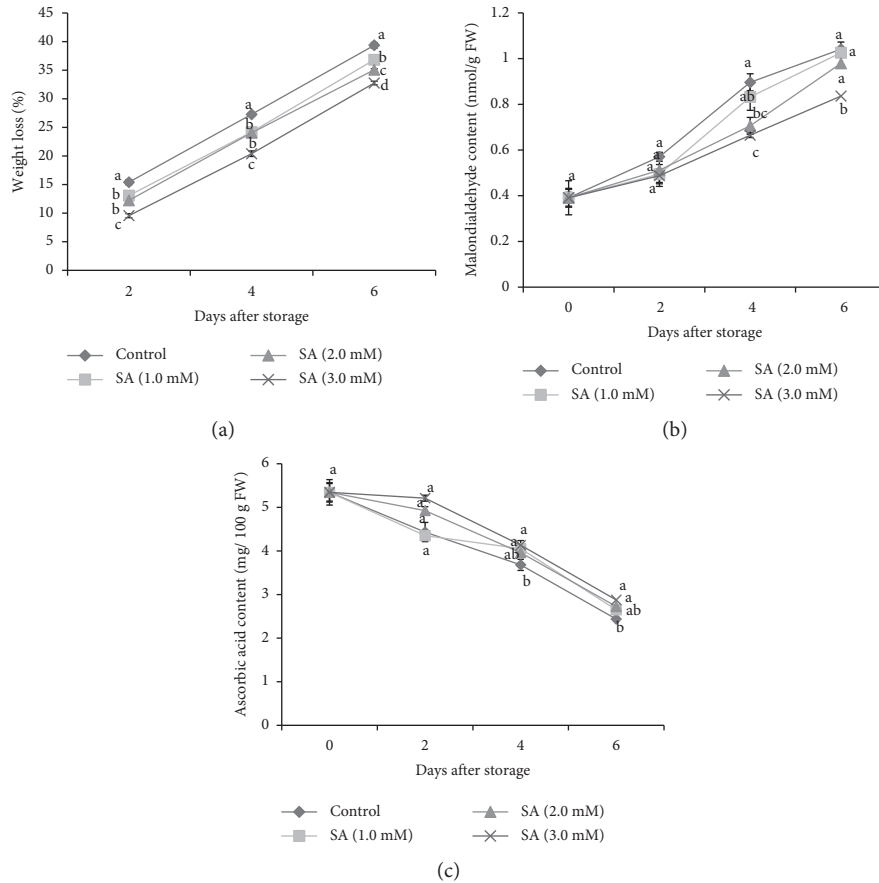


FIGURE 1: The effect of salicylic acid treatments on weight loss (a), malondialdehyde content (b), and ascorbic acid content (c) of the pointed gourd fruit during storage under ambient conditions. On each day, data with the same letters are not significantly different ( $p < 0.05$ ).

TABLE 1: The effect of salicylic acid treatments on total chlorophyll and carotenoid content of pointed gourd fruits during storage under ambient conditions.

Treatments	Total chlorophyll content (mg/g-FW)			Total carotenoid content (mg/g-FW)		
	2 DAS	4 DAS	6 DAS	2 DAS	4 DAS	6 DAS
Control	$0.73 \pm 0.02$ b	$0.60 \pm 0.03$ c	$0.43 \pm 0.03$ c	$1.19 \pm 0.05$ a	$1.56 \pm 0.04$ a	$1.87 \pm 0.05$ a
SA (1.0 mM)	$0.88 \pm 0.02$ a	$0.74 \pm 0.01$ b	$0.47 \pm 0.02$ c	$0.99 \pm 0.03$ a	$1.32 \pm 0.03$ b	$1.79 \pm 0.03$ b
SA (2.0 mM)	$0.88 \pm 0.03$ a	$0.84 \pm 0.02$ a	$0.56 \pm 0.02$ b	$1.05 \pm 0.05$ a	$1.29 \pm 0.07$ b	$1.59 \pm 0.02$ b
SA (3.0 mM)	$0.91 \pm 0.03$ a	$0.87 \pm 0.03$ a	$0.64 \pm 0.01$ a	$1.10 \pm 0.07$ a	$1.21 \pm 0.02$ b	$1.51 \pm 0.009$ b
	Initial value: $0.98 \pm 0.01$ mg/g-FW			Initial value: $0.98 \pm 0.03$ mg/g-FW		

Values are the mean  $\pm$  standard error of four replicate determinations ( $n = 4$ ). Treatment values followed by the same letters on each day are not significantly different ( $p < 0.05$ ).

However, compared to control, SA-treated fruits showed lower weight loss. The beneficial effect of SA in reducing weight loss was more prominent with the increase in concentration. Up to 4 days of storage, significant difference in weight loss was not recorded between 1.0 mM and 2.0 mM SA-treated fruit, whereas the best result was observed in fruits treated with 3.0 mM-SA. Six days after storage (DAS), 3.0 mM-SA-treated fruits registered minimum weight loss (32.75%), while it was the maximum (39.35%) in control fruit. The high transpiration rate and rapid water flow between the internal and external atmospheres caused weight loss in fruits [18]. Moreover, catabolic processes associated with postharvest senescence cause cellular breakdown

leading to accelerated respiration and transpiration rates [19]. It leads to shriveling, turgidity loss, and reduction in the market value of pointed gourd fruit. SA treatment probably regulated stomata closure [20, 21] and altered composition and content of wax [22] in fruit, leading to reduced transpiration and respiration rates in pointed gourd, thereby reducing weight loss in treated fruits.

**3.2. Effects on Total Chlorophyll and Total Carotenoid Content.** Gradual loss of total chlorophyll with storage period was noted in the skin of pointed gourd fruits during the study (Table 1). Control fruits showed a maximum loss of

chlorophyll of about 2.3-fold, while the loss was about 1.8 fold on the final day of storage in 3.0 mM SA-treated fruit. The latter treatment showed maximum chlorophyll retention in comparison to other treatments. The level of total carotenoids in the skin of pointed gourd fruits increased gradually up to the end of the storage period (Table 1). SA treatments showed beneficial effects in reducing accumulation of carotenoid pigments compared to control. Nevertheless, the total carotenoid content in pointed gourd fruits treated with different concentrations of SA was statistically at par irrespective of storage duration. In the overall experiment, 3.0 mM-SA-treated fruits exhibited their superior performance in delaying synthesis of carotenoids (1.51 mg/g-FW) compared to control (1.87 mg/g-FW).

The skin colour of pointed gourd fruits remains green when it is harvested; however, with the onset of ripening, colour changes to yellow or orange due to synthesis of carotenoid pigments. The retention of chlorophyll or green colour is critical to maintain visual appeal of pointed gourd fruit. Colour change drastically reduces consumer acceptance and marketability. Therefore, delaying degradation of chlorophyll and suppressing accumulation of carotenoids are desirable for pointed gourd fruit. The decrease in chlorophyll content in fruits with storage was most likely due to generation of chromoplasts from chloroplasts [23, 24]. SA treatment might have reduced chlorophyll degradation by maintaining a higher number of chloroplasts and suppressing the activity of chlorophyllase, Mg-dechelataase, and pheophytinase, which play a major role in chlorophyll degradation; as a result, the rate of carotenoid synthesis was also decreased [24].

**3.3. Effect on Lipid Peroxidation.** In the present study, progressive increase in the level of malondialdehyde in the fruit indicated an increase in membrane lipid peroxidation with the storage period (Figure 1(b)). About a 2.7-fold increase in the MDA content was observed in control fruit. On the final day of the experiment (day 6), only 3.0 mM SA treatment showed a superior effect (2.1-fold) in reducing accumulation of MDA (0.83 nmol/g-FW) compared to lower doses of SA. The MDA content on day 6 was statistically at par between control and SA-treated (1.0 mM and 2.0 mM) fruits. Salicylic acid initiates accumulation of a higher amount of proline, thus protecting plants by working as a cellular osmotic regulator in the vacuole and cytoplasm [25]. Salicylic acid has also been reported to increase ascorbate peroxidase, catalase, and superoxide dismutase activity and stabilize antioxidant systems, which might have reduced accumulation of MDA in fruits [7]. Salicylic acid treatment also showed similar results in different crops like cucumber [26], sponge gourd [27], and plum [28].

**3.4. Effect on Ascorbic Acid Content.** During storage, the level of ascorbic acid in pointed gourd fruits declined progressively with the storage period in both untreated and SA-treated fruits (Figure 1(c)). Control fruits demonstrated a maximum loss of ascorbic acid (2.2-fold) compared to fruits treated with SA. In the overall experiment, the maximum

ascorbic acid retention (2.87 mg/100 g) was estimated in fruits treated with 3.0 mM-SA. Nevertheless, significant variation in ascorbic acid was not observed among the different doses of SA-treated fruits. Ascorbic acid acts as a powerful antioxidant and scavenging agent against the free radicals produced in the fruit and subsequently inhibits its degradation. In this study, oxidation of ascorbic acid with the progress of storage duration might have caused its loss [29]. However, higher ascorbic acid retention in SA-treated fruits was probably due to its antisenescence property. Similar response of salicylic acid has also been observed in green asparagus [30], cucumber [26], and tomato [31, 32].

**3.5. Effect on Total Phenol Content.** Loss of phenolic compounds with the storage period was recorded in this study irrespective of treatments (Table 2). SA treatment was found effective in better retention of phenolic compounds compared to control. About 1.8-fold reduction in phenolic compounds was observed in the control fruit, while the 3.0 mM SA-treated fruit maintained 1.2-fold higher amount of phenolics compared to control. However, at the end of the storage duration, significant difference in the level of phenolic compounds was not observed among pointed gourd fruits treated with different doses of SA. Oxidation of phenolic compounds due to the activity of polyphenol oxidase (PPO), disruption of membrane integrity, and sub-cellular disintegration might have caused loss of phenolic compounds [33, 34]. The reason behind a higher content of phenolic compounds in SA-treated fruits might be increased phenylalanine ammonia lyase (PAL) activity, resulting in production of polyphenolic compounds and reduced activity of PPO, thereby reducing oxidation of phenolic compounds [35]. Salicylic acid treatment also showed similar results in green asparagus [30], tomato [32], and avocado [36].

**3.6. Effect on Total Flavonoid Content.** The flavonoid content in pointed gourd fruits declined irrespective of treatments, the maximum loss (3.2-fold) being noted in control fruits (Table 2). Although SA treatments showed a delay in loss of flavonoids, at the end of the storage, the flavonoid content in control and SA-treated fruits was statistically at par, except those treated with 3.0 mM SA. The latter treatment maintained the maximum flavonoid content (1.90  $\mu\text{g}\cdot\text{RE}/\text{g}\cdot\text{FW}$ ) after 6 days, which was 1.2-fold higher than of the control. The decline in flavonoids in pointed gourd fruits with the storage period is linked with the loss of phenolic compounds [37]. With the onset of fruit ripening, the level of flavonoids declined probably due to breakdown of enzyme activity into secondary phenolic compounds [38]. In this study, SA treatment reduced the loss of flavonoids which might have been due to slowdown of the ripening and senescence processes.

**3.7. Effects on Total Antioxidant Capacity and DPPH Radical Scavenging Activity.** The present investigation showed reduction in the total antioxidant capacity with storage duration irrespective of treatments (Figure 2(a)). Different SA



TABLE 2: The effect of salicylic acid treatments on total phenol and flavonoid content of pointed gourd fruits during storage under ambient conditions.

Treatments	Total phenol content ( $\mu\text{g}\cdot\text{GAE}/\text{g}\cdot\text{FW}$ )			Total flavonoids content ( $\mu\text{g}\cdot\text{RE}/\text{g}\cdot\text{FW}$ )		
	2 DAS	4 DAS	6 DAS	2 DAS	4 DAS	6 DAS
Control	102.43 $\pm$ 2.30 a	84.00 $\pm$ 1.62 c	63.68 $\pm$ 2.90 b	3.87 $\pm$ 0.11 b	2.80 $\pm$ 0.08 c	1.55 $\pm$ 0.09 b
SA (1.0 mM)	103.50 $\pm$ 3.67 a	92.75 $\pm$ 1.88 ab	72.56 $\pm$ 3.80 a	3.90 $\pm$ 0.12 b	3.10 $\pm$ 0.17 bc	1.65 $\pm$ 0.09 ab
SA (2.0 mM)	110.50 $\pm$ 2.98 a	88.81 $\pm$ 2.07 bc	74.31 $\pm$ 1.45 a	4.30 $\pm$ 0.12 a	3.30 $\pm$ 0.12 ab	1.55 $\pm$ 0.09 b
SA (3.0 mM)	112.25 $\pm$ 3.21 a	97.25 $\pm$ 1.03 a	78.25 $\pm$ 1.61 a	4.30 $\pm$ 0.12 a	3.65 $\pm$ 0.9 a	1.90 $\pm$ 0.05 a
	Initial value: 116.25 $\pm$ 5.06 $\mu\text{g}\cdot\text{GAE}/\text{g}\cdot\text{FW}$			Initial value: 4.92 $\pm$ 0.04 $\mu\text{g}\cdot\text{RE}/\text{g}\cdot\text{FW}$		

Values are mean  $\pm$  standard error of four replicate determinations ( $n=4$ ). Treatment values followed by the same letters on each day are not significantly different ( $p < 0.05$ ).

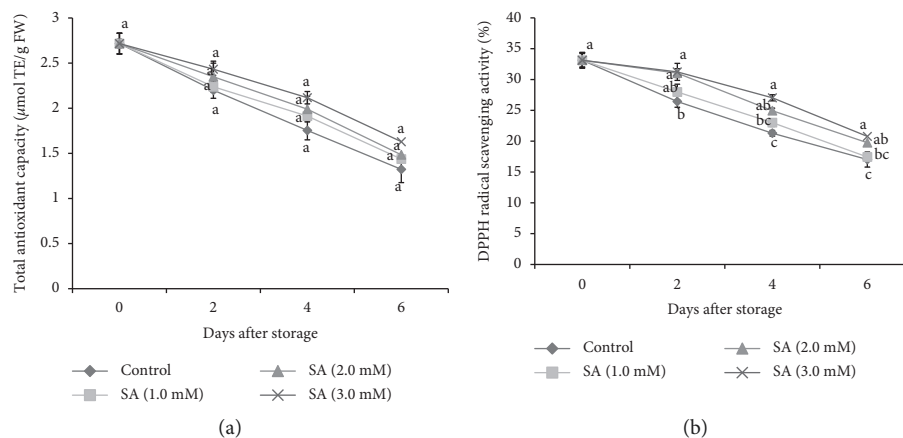


FIGURE 2: The effect of salicylic acid treatments on total antioxidant capacity (a) and DPPH radical scavenging activity (b) of the pointed gourd fruit during storage under ambient conditions. On each day, data with the same letters are not significantly different ( $p < 0.05$ ).

treatments of pointed gourd fruits minimized the loss of total antioxidant capacity. However, its level was statistically at par between control and SA-treated fruits in all doses and at all time of storage. Likewise, the DPPH radical scavenging activity of pointed gourd declined progressively with the storage period (Figure 2(b)). Throughout the storage period, the minimum DPPH scavenging activity was registered in untreated pointed gourd, while the 3.0 mM SA-treated fruit demonstrated the maximum radical scavenging activity (20.77%). On the termination day of experiment (day 6), the DPPH scavenging activity was not significantly different between 2.0 mM and 3.0 mM SA-treated fruit. Total antioxidant capacity and DPPH scavenging activity in pointed gourd are mainly contributed by phenols, ascorbic acid, and flavonoids. Thus, the decrease in antioxidant capacity with storage duration may be linked with the loss of the above compounds. During the entire period of storage, free radical scavenging activity decreases due to the continuous decline in antioxidant capacity in pointed gourd. Higher antioxidant capacity and DPPH radical scavenging ability in SA-treated fruits probably attributed to delayed ripening, senescence, and reduced PAL and PPO enzyme activity than the control [30].

#### 4. Conclusions

The findings of the present study revealed that exogenous application of salicylic acid to the pointed gourd fruit has

beneficial effects in delaying senescence and preserving quality during storage under ambient conditions. SA treatment at 3.0 mM proved to be the most effective in reducing weight loss, chlorophyll degradation, and lipid peroxidation as evidenced by less malondialdehyde accumulation in the fruit. This treatment also preserved higher total phenol, flavonoid, and radical scavenging activity over control. Nevertheless, the effect of combined application of salicylic acid with edible coating may be studied further as a future line of work.

#### Data Availability

The data used to support the findings of this study are included within the article.

#### Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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