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Preharvest application of salicylic acid, ethephon and allantoin enhances ripening and fruit quality of grape *cv.* Flame Seedless in subtropical condition of North India

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ABSTRACT

The present study investigated the effect of pre-harvest application of allantoin and salicylic acid (SA) on ripening behaviour, berry colour development, and fruit quality of grape (*Vitis vinifera* L.) cv. Flame Seedless under subtropical conditions. The experiment was conducted over two consecutive seasons (2023-2024) using single and double applications of allantoin (750, 1000 and 1250 ppm) and Salicylic acid (1, 2 and 3 mM), with ethephon (400 ppm) as a reference treatment and an untreated control. Ethephon significantly advanced ripening by 6-7 days, improved berry redness (a* value), and resulted in the highest total soluble solids (TSS), TSS:acidity ratio, total sugars, and anthocyanin content. Among the alternative treatments, double application of allantoin at 1250 ppm markedly enhanced berry colour, anthocyanin accumulation, and total phenolic content, producing colour development comparable to ethephon. Single applications of allantoin significantly increased tartaric acid concentration and antioxidant activity, indicating its positive influence on biochemical quality attributes. Salicylic acid treatments improved TSS and total sugar content but consistently delayed ripening by 2-3 days and showed limited effectiveness in enhancing berry colour. The untreated control recorded the lowest values for most quality parameters. Overall, the findings suggest that pre-harvest application of allantoin, particularly at higher concentrations with double sprays, is a promising alternative to conventional ripening agents for improving colour development and biochemical quality of Flame Seedless grapes under subtropical conditions.

Key words: Grape, allantoin, salicylic acid, ethephon, berry colour, anthocyanins.

INTRODUCTION

Grapes, among the earliest cultivated fruits, hold significant value in the food and health industries due to their rich nutritional profile, including antioxidants, minerals, soluble sugars, anthocyanins, flavonoids, organic acids, vitamins, and aromatic compounds. In India, grape cultivation spans approximately 180,000 hectares (Anonymous, 1), covering temperate, subtropical, and tropical regions, with key commercial varieties such as Thompson Seedless and Sharad Seedless. In Punjab, grapes are grown in arid irrigated region on an area 224 hectares (Anonymous, 2), predominantly the Perlette variety, which accounts for over 90% of the cultivated area.

To reduce dependence on monoculture, the Punjab Agricultural University recommended Flame Seedless, a crimson red, seedless variety known for its higher yield, superior total soluble solids (TSS) to acidity ratio, and improved bunch characteristics. However, Flame Seedless faces challenges such as inconsistent berry colouration due to suboptimal anthocyanin accumulation under prevailing sub-tropical climatic conditions (Arora *et al*., 3). Inconsistent colour development is related to anthocyanin accumulation which impairs the quality and reduces the economic returns of the grape growers (Ferrara *et al*., 6).

For achieving desired berry colour and quality under such conditions, application of plant growth regulators is adopted (Kadlag *et al*., 9). Under Indian conditions, PGRs such as ethephon (ethrel) and ABA are commonly applied to enhance anthocyanin development and uniform ripening in cultivars like Flame Seedless. While ethephon improves coloration, high doses may soften berries, and exogenous ABA is costly and often unstable in warm field environments.

Allantoin (N-(2,5-Dioxo-4-imidazolidinyl) urea) offers a promising alternative by promoting berry coloration through the release of bioactive ABA via β-glucosidase-mediated hydrolysis of ABA-glucose conjugates, effectively bypassing the NCED-dependent biosynthetic pathway (Moriyama *et al*., 17; Lu *et al*., 12). This enables a faster and more stable colour response under heat stress, making allantoin superior to direct ABA application. It is also cost-effective and does not induce excessive softening.

Salicylic acid (SA), a phenolic growth regulator, further enhances fruit size, firmness, ripening, and stress tolerance (Hayat *et al*., 7; Marzouk

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and Kassem 14). It has been shown to accelerate ripening in both climacteric and non-climacteric fruits, including strawberries and tomatoes (Karlidag *et al*., 11; Mady 13).Thus, this study aims to improve colour, quality, and marketability of Flame Seedless grapes through preharvest applications of allantoin and SA, offering an alternate approach over conventional PGRs used in Indian viticulture.

MATERIALS AND METHODS

The experiment was carried out on 23-year-old own-rooted grapevines of *cv.*Flame Seedless at the Fruit Research Farm, Department of Fruit Science, Punjab Agricultural University, Ludhiana, Punjab (latitude 30.90° N and longitude 75.80° E). These vines were trained on a bower system and planted with the spacing of 3 m X 3 m. The study was carried out over two years, 2023 and 2024. Each vine received approximately 4-5 litres of spray solution using a Knapsack sprayer. To enhance the effectiveness of the growth regulators, Tween-20 was added at a concentration of 1 ml per litre. Salicylic acid was first dissolved in 100 ml ethanol before diluting to the desired volume with water, whereas allantoin, being readily water-soluble, was directly dissolved in water.

The treatments were designated as T_1 to T_{11}. Treatments T_1–T_6 involved the application of allantoin, viz., T_1 = Allantonin @ 750ppm, T_2 = Allantonin @ 1000ppm, T_3 = Allantonin @ 1250 ppm as single application at colour break stage; T_4 = Allantonin @ 750ppm as double application, T_5 = Allantonin @ 1000ppm as double application and T_6 = Allantonin @ 1250 ppm double application at both the colour break stage and 7 days later. Treatments T_7–T_9 consisted of salicylic acid, viz., T_7= Salicylic acid @ 1 mM, T_8= Salicylic acid 2 mM, and T_9 = Salicylic acid @ 3 mM during the pea stage and colour break stage. Treatment T_{10}, which involved ethephon application @ 400 ppm at the colour break stage, was included as a reference for comparison. T_{11} served as the control, with no treatment applied. Fully ripened bunches were harvested in the second week of June i.e. 146 days after pruning. Analysis was performed in the laboratory to assess the impact of treatments on ripening and fruit quality.

Date of ripening time was determined when the total soluble solids (TSS) of the fruit reached 16°Brix or more, and over 75% of the vine bunches exhibited uniform colouration. Unevenness (%) colouration was expressed as the percentage of berries in a bunch where less than 50% of their skin displayed red colour. TSS and titratable acidity were measured by crushing fifty randomly selected berries to extract juice. The TSS was recorded using a hand refractometer, titratable acidity was assessed using 0.1N NaOH, and the TSS-to-acid ratio was calculated. Berry peel colour (L*, a*, b*) was evaluated using a Hunter Lab ColourFlex® EZ meter, with measurements for L*, a*, and b* values recorded.

Total sugars were determined by diluting a 10 ml sample of grape juice to 100 ml with distilled water, followed by treatment with lead acetate (1g) and potassium oxalate (1g) to remove impurities. After overnight acid hydrolysis with HCl, the solution was neutralized with NaOH and titrated using Fehling's solutions and methylene blue as an indicator. The total sugar content was calculated based on the volume of filtrate used. Anthocyanin content (mg/100g) was measured by macerating a 5 g sample of berries in ethanolic HCl solution, filtering, and adjusting the volume to 100 ml. After dilution, the absorbance of the sample was measured at 535 nm using a spectrophotometer. Total phenolic content (mg GAE/100g) was determined using the Folin–Ciocalteu method. The extract was mixed with the reagent and Na_2CO_3 solution, incubated in the dark, and absorbance was measured at 725 nm. Results were compared to a gallic acid calibration curve and expressed as mg gallic acid equivalents per gram. Antioxidant capacity (% RSA) was analyzed by preparing berry extracts with 0.1% HCl acidified with methanol and centrifuging. The DPPH radical scavenging activity (% RSA) was measured spectrophotometrically at 515 nm, and the inhibition percentage was calculated. Sugar and organic acid profile (g/100g) was determined by homogenizing samples with 80% ethanol, centrifuging, and analyzing the supernatant using high-performance liquid chromatography (HPLC) at the DST-FIST laboratory, Department of Fruit Science, PAU, Ludhiana.

The study was carried out over two years, 2023 and 2024, to evaluate parameters related to quality enhancement. Data from both the years were pooled for analysis and examined using a Randomized Block Design (RBD). Variance analysis was performed using R (Version 4.4.1), and treatment means were compared using the Least Significant Difference (LSD) test at a significance threshold of $p \leq 0.05$.

RESULTS AND DISCUSSION

Ethephon notably advanced the ripening of grapes, with 7 and 6 days early in the year 2023 and 2024, respectively (Table 1). Allantoin treatments also accelerated ripening, with double applications slightly greater effects in terms of yield. Conversely, salicylic acid consistently delayed ripening by 2-3 days. Ethephon @ 400 ppm was the most effective

Table 1: Effect of pre-harvest application of allantoin and salicylic acid on date of ripening and unevenness and percentage of grape *cv*. Flame Seedless.

Treatment	2023	2024	Unevenness (%)
T1 -Allantoin@750ppm	06/06/2023	13/06/2024	26.86[cde]
T2-Allantoin@1000ppm	05/06/2023	12/06/2024	25.27[def]
T3-Allantoin@1250ppm	05/06/2023	12/06/2024	28.71[bcd]
T4-Allantoin@750ppm applied twice	05/06/2023	12/06/2024	31.54[b]
T5-Allantoin@1000ppm applied twice	06/06/2023	13/06/2024	19.76[g]
T6 Allantoin@1250ppm applied twice	05/06/2023	13/06/2024	29.82[bc]
T7-SA@1mM	11/06/2023	18/06/2024	21.89[fg]
T8-SA@2mM	12/06/2023	18/06/2024	22.95[efg]
T9-SA@3mM	12/06/2023	18/06/2024	23.71[efg]
T10-Ethephon@400ppm	02/06/2023	09/06/2024	13.02[h]
T11-Control	09/06/2023	15/06/2024	48.36[a]
LSD (p<0.05)			4.54

in reducing uneven colour development, showing significantly superior results compared to other treatments. Among allantoin applications, allantoin @ 1000 ppm, applied twice markedly reduced unevenness, while salicylic acid treatments exhibited limited efficacy, comparable to allantoin @ 1000 ppm. The ripening delay with salicylic acid may stem from its role in reducing respiration and ethylene biosynthesis as reported in banana (Srivastava and Dwivedi, 19). The advancements observed with allantoin and ethephon align with the positive correlation between ABA accumulation and berry ripening reported by Coombe and Hale (5).

The variation in TSS, TA (titratable acidity) and TSS: TA is given in Table 2. Ethephon@ 400 ppm resulted in maximum TSS content (18.95°B) and TSS:acidity ratio (43.60) in grape berries, significantly surpassing the control (T_{11}). Salicylic acid treatments (T_7–T_9) also improved TSS (up to 18.43°B) and TSS:acidity ratio (37.50–37.55) with reduced acidity. Among allantoin treatments, better performance was recorded in allantoin @ 1250 ppm applied twice. Control recorded with minimum TSS (15.33°B) and TSS:acidity ratio (21.89). These results align with Moriyama *et al.* (15) who reported comparable sugar content and acidity across allantoin treatments during berry maturation. The suppression of respiration and ethylene biosynthesis by salicylic acid (Zhang *et al.*, 21) likely contributed to the higher acidity observed. Findings by Omran *et al.* (17) and Champa *et al.* (4) also support the association of high ABA levels with enhanced TSS and reduced acidity during fruit maturation, consistent with the observed improvements in TSS:acidity ratios with allantoin application.

The effect of pre-harvest treatments on berry colour (L*, a*, b*) in grape *cv.* Flame Seedless showed Lightness (L*) ranged from 39.93 with allantoin @ 1000 ppm to 50.27 in control (Table 2). Ethephon @ 400 ppm significantly enhanced redness (a*), achieving the highest value (18.82), a 185.5% increase over the control (6.59). Yellowness (b*) was highest in the control (17.17) and lowest in allantoin @ 1250 ppm applied twice (6.37). Ethephon @ 400 ppm was most effective in increasing redness, while allantoin @ 1250 ppm sprayed twice improved both redness and darkness (lower L* and b* values). Salicylic acid treatments (T_7-T_9) moderately affected L* and b* but had limited impact on a*. The control consistently exhibited the highest L* and b* values but the lowest a*, underscoring the treatments' effectiveness in enhancing berry colour. These findings align with Champa *et al.* (4), who reported that salicylic acid treatments reduced L* and b* values while increasing a* values, resulting in darker and redder fruits. Similarly, salicylic acid applications in this study produced comparable effects, regardless of concentration. Berry colour development was most pronounced under ethephon treatment, as evidenced by the highest a* value and reduced uneven colouration. Ethylene released from ethephon is known to stimulate anthocyanin biosynthesis by upregulating key enzymes of the phenylpropanoid pathway and by interacting synergistically with abscisic acid (ABA), which plays a central role in berry ripening. Consequently, ethephon-treated berries recorded the highest anthocyanin content, showing an almost 90% increase over the control. Enhanced anthocyanin accumulation following ethephon application has also been documented in Flame Seedless and other red

Table 2: Effect of pre-harvest application of allantoin and salicylic acid on TSS, acidity, TSS:acidity, L*, a* and b* of grape *cv.* Flame Seedless.

Treatment	TSS (°B)	Titratable acidity (%)	TSS:Acidity	L*	a*	b*
T1 -Allantoin@750ppm	17.10[c]	0.593[b]	28.91[d]	44.84[bc]	9.81[f]	12.77[b]
T2-Allantoin@1000ppm	17.48[bc]	0.595[b]	30.87[cd]	39.93[d]	10.52[e]	7.72[de]
T3-Allantoin@1250ppm	17.38[bc]	0.558[bc]	30.58[cd]	43.72[cd]	13.88[c]	9.81[bcde]
T4-Allantoin@750ppm applied twice	17.66[bc]	0.605[b]	29.20[d]	43.24[cd]	9.72[f]	10.88[bcd]
T5-Allantoin@1000ppm applied twice	1791[abc]	0.558[bc]	32.16[cd]	43.30[cd]	10.11[ef]	12.88[b]
T6 Allantoin@1250ppm applied twice	18.16[abc]	0.559[bc]	32.55[c]	40.32[d]	14.94[b]	6.37[e]
T7-SA@1mM	18.11[abc]	0.483[de]	37.55[b]	45.34[bc]	10.11[ef]	13.49[ab]
T8-SA@2mM	18.43[ab]	0.510[cd]	36.35[b]	47.87[ab]	10.44[e]	13.17[ab]
T9-SA@3mM	18.08[abc]	0.482[de]	37.50[b]	46.93[abc]	11.61[d]	8.06[cde]
T10-Ethephon@400ppm	18.95[a]	0.437[e]	43.60[a]	45.36[bc]	18.82[a]	12.01[bc]
T11-Control	15.33[d]	0.701[a]	21.89[e].	50.27[a]	6.59[g]	17.17[a]
LSD (p<0.05)	1.08	0.054	3.32	3.83	0.58	4.06

grape cultivars (Coombe and Hale, 5; Ferrara *et al*., 6). However, it leads to berry softening.

The effects of pre-harvest application of allantoin and salicylic acid on total sugars, phenols, and anthocyanin content in grape cv. Flame Seedless given in Table 3. Total sugar content ranged from 13.37% in control to 16.83% in Ethephon @ 400 ppm), representing a 25.9% increase. SA@2 mM also significantly improved sugar levels, recording 15.42%. Total phenols varied from 50.52 mg GAE/100 g in control to 79.29 mg in Allantoin@750 ppm applied twice a 56.9% increase. All allantoin treatments enhanced phenolic content significantly. Anthocyanin content was highest in Ethephon @ 400 ppm (41.55 mg), an 89.5% increase over the control, with Allantoin @ 1250 ppm sprayed twice (37.91 mg) also demonstrating strong results. These findings align with Hazarika and Marak (8), who reported significantly higher total sugar content (21.18%) in berries treated with 2 mM salicylic acid (SA) compared to other treatments, supporting our observation of increased total sugars. Similarly, Oraei *et al*. (18) found higher total phenolic content in SA-treated grapes, consistent with our results. The delayed ripening caused by SA, applied

Table 3: Effect of pre-harvest application of allantoin and salicylic acid on total sugars, phenols, anthocyanin, antioxidant activity and tartaric acid content of grape *cv.* Flame Seedless.

Treatment	Total sugars (%)	Total phenols (mgGAE/ 100g)	Anthocyanin content (mg/100g)	Antioxidant activity (%RSA)	Tartaric acid content (g/100g)
T1 -Allantoin@750ppm	14.28[de]	62.87[de]	33.46[c]	74.78[a]	0.289[b]
T2-Allantoin@1000ppm	14.37[d]	61.57[e]	33.88[c]	66.33[bc]	0.435[a]
T3-Allantoin@1250ppm	14.34[d]	60.07[e]	31.95[cd]	75.72[a]	0.364[ab]
T4-Allantoin@750ppm applied twice	14.47[d]	79.29[a]	33.30[c]	69.69[ab]	0.199[c]
T5-Allantoin@1000ppm applied twice	14.46[d]	76.47[ab]	34.03[c]	63.22[bcd]	0.133[c]
T6 Allantoin@1250ppm applied twice	14.01[e]	75.07[b]	37.91[b]	62.64[bcde]	0.183[c]
T7-SA@1mM	14.89[c]	68.06[c]	29.73[de]	54.74[f]	0.165[c]
T8-SA@2mM	15.42[b]	69.71[c]	27.01[e]	58.48[cdef]	0.177[c]
T9-SA@3mM	15.05[c]	66.19[cd]	26.46[e]	54.97[ef]	0.150[c]
T10-Ethephon@400ppm	16.83[a]	73.76[b]	41.55[a]	56.06[def]	0.307[b]
T11-Control	13.37[f]	50.52[f]	21.93[f]	43.04[g]	0.125[c]
LSD (p<0.05)	0.30	3.98	3.47	7.87	0.077

at veraison, likely resulted in treated grapes maturing slightly later than controls at harvest. Enhanced phenolic content with allantoin aligns with Neto *et al*. (16), who observed increased phenolic compounds with multiple S-ABA applications. Moriyama *et al*, (15) reported greater anthocyanin accumulation in allantoin-treated berries, particularly with* additional applications, supporting our finding of a 72% increase in anthocyanin content. Oraei *et al.* (18) further confirmed that salicylic acid treatments significantly boosted anthocyanin content, validating our results. The data presented in Table 3 highlights the significant effects of pre-harvest applications of allantoin and salicylic acid on antioxidant activity and tartaric acid content in grape *cv.* Flame Seedless ($p < 0.05$). Antioxidant activity ranged from 43.04% in control to 75.72% in allantoin @ 1250 ppm, with latter showing a 75.9% increase over the control, followed by Allantoin @ 750 ppm (74.78%). Allantoin treatments generally exhibited higher antioxidant activity than salicylic acid or ethephon. Tartaric acid content varied from 0.125 g in control to 0.435 g with Allantoin @ 1000 ppm. While allantoin and ethephon significantly enhanced tartaric acid accumulation, salicylic acid treatments showed minimal impact, similar to the control. The higher tartaric acid levels observed in the single allantoin treatments may help explain the enhanced antioxidant activity. This can be attributed to the work of Kakan *et al.* (10) and Tabata *et al.* (20), who concluded that ABA, via the transcription factor ABI4, negatively regulates the expression of the VTC2 gene, which is crucial for ascorbic acid biosynthesis. Ascorbic acid is an essential precursor in the biosynthetic pathway of tartaric acid.

As ABA promotes ripening, it can be concluded that allantoin application enhances ABA content, which further promotes ripening and the accumulation of glucose and fructose. Also its role in enhancing tartaric acid needs to be exploited in varieties suitable for wine making. Salicylic acid enhance physical characteristics of bunches and yield but delays ripening which is not desirable under Punjab conditions. Thus it can be applied in combination with ripening promotor like ethephon.

AUTHORS' CONTRIBUTION

Conceptualization and designing of experiment (NKA, GDK and GS); field/lab experiments and data collection (GS); data interpretation and preparation of manuscript (GS and NKA), statistical analysis (GS and RA).

DECLARATION

The authors declare that they don't have any conflict of interest.

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