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Preharvest application of calcium and biostimulant elicitors mitigates postharvest decay and enhances quality in grapes (*Vitis vinifera* L.) cv. thompson seedless

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Abstract
The present study evaluated the effectiveness of preharvest interventions in mitigating postharvest losses in table grapes (*Vitis vinifera* L. cv. Thompson Seedless) through foliar application of four treatments: T_0 (Control), T_1 (biostimulant elicitor-based formulation), T_2 (calcium salt), and T_3 (combination of biostimulant and calcium-based formulation). Treatments were applied at three spray schedules: 2 days before harvest (2 DBH), 10 DBH, and a combination of 10 + 2 DBH. Among the treatments, T_1 significantly reduced berry rotting compared to the control at both 2 DBH and 10 DBH schedules. T_1 also effectively minimized rachis browning, maintaining rachis greenness up to 8 days after harvest (DAH). Application of T_2 at 10 DBH significantly decreased berry shattering, while T_2 at 2 DBH resulted in the greatest reduction in berry cracking. Furthermore, T_1 consistently enhanced total soluble solids, with a notable increase observed under the 2 DBH treatment. T_1 also exhibited the greatest efficacy in minimizing postharvest weight loss at 2 DBH. These findings highlight the potential of Velabs's biostimulant and calcium-based formulations as effective preharvest tools to reduce postharvest physiological disorders and quality deterioration in Thompson Seedless grapes, particularly when applied closer to harvest.

Keywords: Berry rotting, elicitors, foliar application**,** preharvest treatment and shelf life extension

Introduction

Grape (*Vitis vinifera* L.) is a commercially important fruit crop with substantial economic value worldwide. India, with its rich viticultural heritage, ranks among the leading grape-producing nations globally. The crop is cultivated over an area of approximately 171 thousand hectares, yielding around 3,781 thousand metric tonnes annually (1st advance estimates, 2024) [1]. Despite this strong production base, postharvest losses remain a major concern, primarily due to the decay of berries or entire bunches during handling, transportation, and storage (Blanckenberg *et al.,* 2021) [3]. Grapes, like many other perishable fruits, are particularly vulnerable to postharvest deterioration due to a complex interplay of factors such as climatic conditions, mechanical damage during handling, and suboptimal storage conditions (Ranjani *et al.,* 2023) [27]. Although table grapes are non-climacteric fruits with relatively low postharvest metabolic activity (Crisosto *et al.,* 2001) [8], they are prone to substantial physiological and microbial deterioration during prolonged storage and long-distance transport (Li *et al.,* 2015). Quality issues such as rapid moisture loss, rachis browning (Crisosto *et al.,* 2002) [7], weight loss (Jiang *et al.,* 2015) [14], berry shattering, wilting, and shrivelling (Ngcobo *et al.,* 2012) [26] contribute to significant quantitative and qualitative losses. Improper postharvest handling is a major contributor to the breakdown of natural defense mechanisms in grapes, thereby increasing their susceptibility to microbial decay (Sabir and Sabir, 2013) [31].

Postharvest berry and bunch decay is primarily caused by fungal pathogens, including *Botrytis cinerea*, *Aspergillus* spp., and *Penicillium* spp. (Sabir and Sabir, 2017; Wang *et al.,* 2024; Xie *et al.,* 2022) [30, 37, 38]. These opportunistic pathogens often exploit wounded or physiologically weakened tissues during harvest and transit. The widespread prevalence of such pathogens underscores the urgent need for innovative, sustainable approaches to enhance fruit resilience and extend shelf life. In recent years, elicitor-based strategies have gained attention as viable alternatives to synthetic postharvest fungicides, which are increasingly restricted due to health and environmental concerns (Alimadadi *et al.,* 2023) [2]. Elicitors are compounds that activate the plant's innate defense mechanisms, thus offering a proactive strategy to enhance resistance against pathogens and environmental stress. Several signalling molecules, including salicylic acid (Retamal-Salgado *et al.,* 2023) [28], jasmonic acid (Kanwal *et al.,* 2021) [15], and L-phenylalanine (Saidi *et al.,* 2021; Dey *et al.,* 2023) [9, 32], have been reported to induce acquired resistance by upregulating defense-related gene expression, thereby enhancing disease resistance in fruits (Mishra *et al.,* 2024) [24].

In addition to these signalling molecules, structural elicitors such as chitosan (Romanazzi *et al.,* 2017) [29], methyl jasmonate (Lata *et al.,* 2021) [20], and oligosaccharides (Bose *et al.,* 2021) [4] have demonstrated potential in improving postharvest quality by reinforcing cell wall integrity, modulating oxidative stress-related enzyme activities, and inducing the synthesis of antimicrobial compounds (Khoshru *et al.,* 2023) [17]. However, the effectiveness of these elicitors largely depends on factors such as application timing, frequency, concentration, cultivar, and prevailing abiotic conditions (Gong *et al.,* 2022) [12]. Given this context, the present study aims to evaluate the efficacy of preharvest applications of defense response activation elicitors individually and in combination with calcium-based formulations in reducing postharvest rotting and maintaining the overall quality of *Vitis vinifera* L. cv. Thompson Seedless grapes.

Materials and Methods

The field experiment was conducted during the March 2024 grape season in a commercial vineyard located in Ambevani village, Nashik district, Maharashtra, India (20.245457°N, 73.898775°E). Four-year-old vines of *Vitis vinifera* L. cv.

Thompson Seedless grafted onto *Vitis champinii* L. cv. Dogridge rootstock was selected for uniformity. The experiment was laid out in a factorial completely randomized design (CRD) with three preharvest spray schedules: 2 days before harvest (2 DBH), 10 days before harvest (10 DBH), and a combined application at 10 + 2 DBH. Each schedule consisted of four treatments: T_0 (Control – no spray), T_1 (biostimulant-based formulation), T_2 (calcium salt), and T_3 (combination of biostimulant and calcium salt). The biostimulant and calcium-based formulations were developed by Velabs (Vegrow, Bengaluru, India) and comprised a proprietary blend of organic acids, amino acids, and calcium salts. All foliar treatments were applied at a volume of 500 mL per vine using a hand-held sprayer. Following treatment application, grape bunches were harvested as per the scheduled dates, packed in perforated liner bags, and transported under ambient conditions to Vegrow's Packhouse in Sakore, Nashik (20.143380°N, 73.942673°E). The bunches were stored at room temperature in ventilated plastic crates, and physiological and quality parameters were recorded at 2-day intervals from 0 to 8 days after harvest (DAH). The following parameters were assessed:

Berry Rotting

The incidence of berry rotting was evaluated based on the number and weight of decayed berries in each bunch. At each observation interval (0, 2, 4, 6, and 8 DAH), bunches were visually examined, and berries showing symptoms of rotting, such as fungal growth, tissue maceration, or water-soaked lesions, were manually separated. The rotten berries were counted and weighed, and the total number and weight of berries in the bunch were also recorded. Berry rotting was calculated and expressed as a percentage using the following formula:

$$Berry\ rotting\ (\%) = \left(\frac{Weight\ of\ rotten\ berries}{Total\ weight\ of\ berries\ per\ bunch}\right) \times 100$$

Rachis Browning

Rachis Browning was assessed to monitor the visual deterioration of the bunch stem, which affects market appeal and shelf life. The severity of browning was scored visually using a 4-point scale as follows: 1 = Fresh and green rachis; 2 = Browning initiation; 3 = Significant browning; 4 = Severe browning and desiccation (Ngcobo *et al.*, 2013) [25]. Observations were recorded at 0, 2, 4, 6, and 8 DAH for three bunches per treatment per replication.

Berry Shattering

Berry shattering, defined as the detachment of berries from the pedicle without mechanical force, was evaluated at each observation interval. Each bunch was gently lifted and shaken to collect naturally loosened berries. The loose berries were weighed, and the total bunch weight was recorded. The extent of berry shattering was calculated as a percentage using the formula

$$Berry\ Shattering\ (\%) = \left(\frac{Weight\ of\ shattered\ berries}{Total\ \ bunch\ weight}\right) \times 100$$

Berry Cracking

Berry cracking, often induced by internal pressure or weakened skin integrity, was measured as the proportion of cracked berries in each bunch. Cracked berries (exhibiting visible skin rupture or splits) were visually identified, manually separated, and weighed. The total bunch weight was recorded, and the percentage of cracked berries was calculated using the following formula

$$Berry\ cracking\ (\%) = \left(\frac{Weight\ of\ cracked\ berries}{Total\ \ bunch\ weight}\right) \times 100$$

Total Soluble Solids

Total soluble solids (TSS), representing the sugar content of the berries, were measured using a digital refractometer (Parisa Technologies, Mumbai, India). At each time point, five representative berries were randomly selected from each bunch, gently crushed, and the juice was filtered through a muslin cloth. A few drops of juice were placed on the refractometer prism, and the reading was recorded in °Brix. Three readings per replicate were averaged to obtain the final TSS value.

Physiological Loss in Weight

PLW was assessed to monitor water loss and respiration-related weight decline during storage. The initial fresh weight of each bunch was recorded immediately after harvest (0 DAH), and the weight was measured again at each observation interval. The loss in weight was expressed as a percentage of the initial weight using the formula

$$PLW\ (\%) = \left(\frac{\text{Initial weight} - \text{Weight at observation interval}}{Initial\ weight}\right) \times 100$$

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using OPSTAT statistical software (Sheoran *et al.*, 1998) [34] to assess the effects of treatments, spray schedules, and their interactions. Mean comparisons were performed at a significance level of 5%. Graphs and data visualisations were generated using GraphPad Prism version 10.0.0 for Windows (GraphPad Software, Boston, Massachusetts, USA).

Results

Berry Rotting

The percentage of berry rotting increased progressively with advancing storage duration across all spray schedules and treatment groups. The highest incidence of rotting was consistently recorded in the untreated control (T_0) under all spray timings. In contrast, all the treated groups (T_1, T_2, and T_3) were effective in reducing berry rotting to varying extents. Among them, treatment T_1 resulted in the lowest rotting percentage in the 2 DBH spray schedule, whereas treatment T_3 was most effective in the 10 DBH group. Notably, in the combined spray schedule (10 + 2 DBH), both T_1 and T_3 treatments demonstrated similarly low levels of berry rotting, indicating a synergistic or additive effect of the dual application. Overall, treatment T_1 significantly reduced berry rotting under both 2 DBH and 10 DBH spray schedules when compared with their respective controls (Fig. 1).

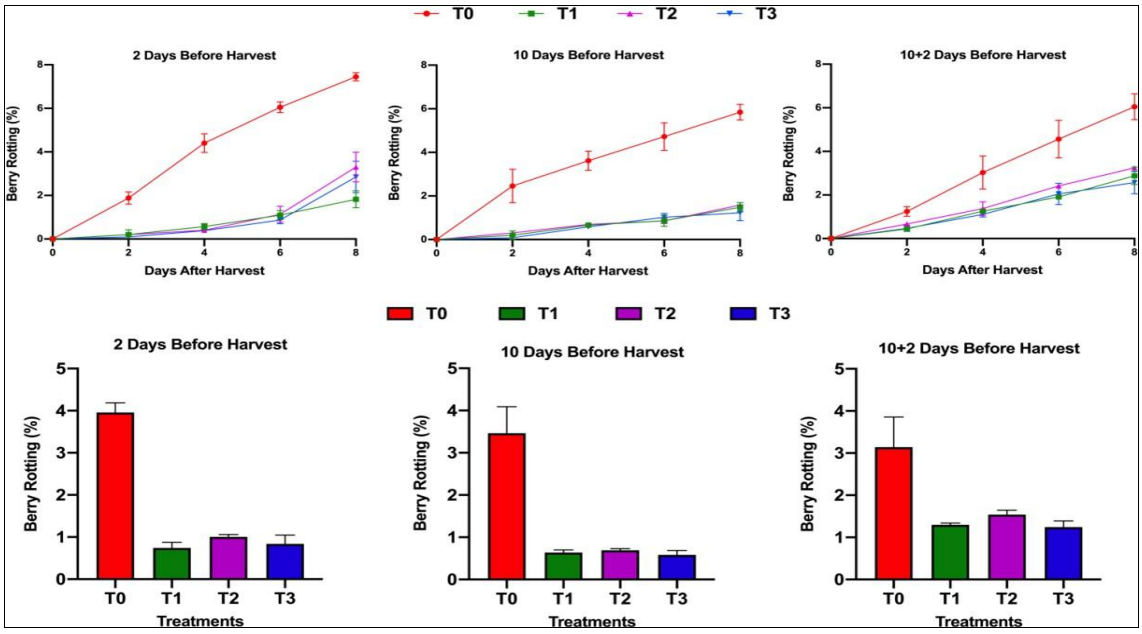

Fig 1: Berry rotting (%) in Thompson Seedless grapes under different preharvest treatments, T_0 (Control), T_1 (Biostimulant), T_2 (Calcium salt), and T_3 (Biostimulant + Calcium) and spray schedules (2 DBH, 10 DBH, and 10 + 2 DBH) during storage. Error bars represent standard error (n = 3).

Rachis Browning Visual Score

Rachis Browning exhibited a progressive increase with storage duration across all treatments and spray schedules. By 8 days after harvest (DAH), the rachis in all spray groups reached a visual score of 4, indicating severe browning. In the 2 DBH spray group, both T_1 and T_3 effectively delayed the onset of browning, maintaining a fully green rachis (score 1) until 4 DAH. Among these, T_1 recorded a significantly lower browning score than the control (T_0) at 8 DAH. In the 10 DBH spray group, T_1 and T_3 showed a gradual transition from green rachis to early browning stages, with T_1 maintaining the lowest score by the end of the storage period. In the 10 + 2 DBH group, rachis browning increased progressively in all treatments, with T_1 and T_3 reaching a score of 3.5 at 8 DAH, comparable to the control. However, when considering the overall mean across all time points, treatment T_1 applied at 2 DBH demonstrated superior performance in preserving rachis greenness and delaying browning symptoms until the end of the storage period (Fig. 2).

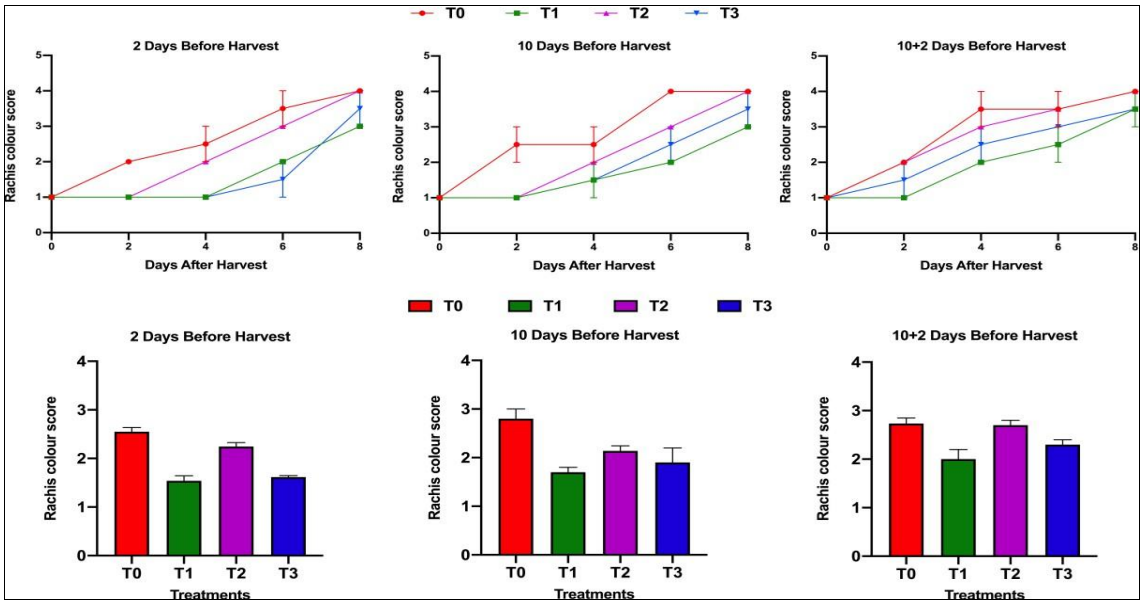

Fig 2: Rachis colour score in Thompson Seedless grapes under different preharvest treatments, T_0 (Control), T_1 (Biostimulant), T_2 (Calcium salt), and T_3 (Biostimulant + Calcium) and spray schedules (2 DBH, 10 DBH, and 10 + 2 DBH) during storage. Error bars represent standard error (n = 3).

Berry Shattering

Among all three spray schedules, 2 DBH, 10 DBH, and 10 + 2 DBH, the untreated control (T_0) consistently recorded the highest levels of berry shattering throughout the storage period. In contrast, treatment T_2 (calcium salt) showed a consistent and significant reduction in berry shattering across all spray schedules. Notably, the most pronounced reduction was observed in the 10 DBH group, where T_2

achieved the lowest shattering percentage among all treatments and schedules. This represented a 67.16% decrease in berry shattering compared to the corresponding control group (Fig. 3).

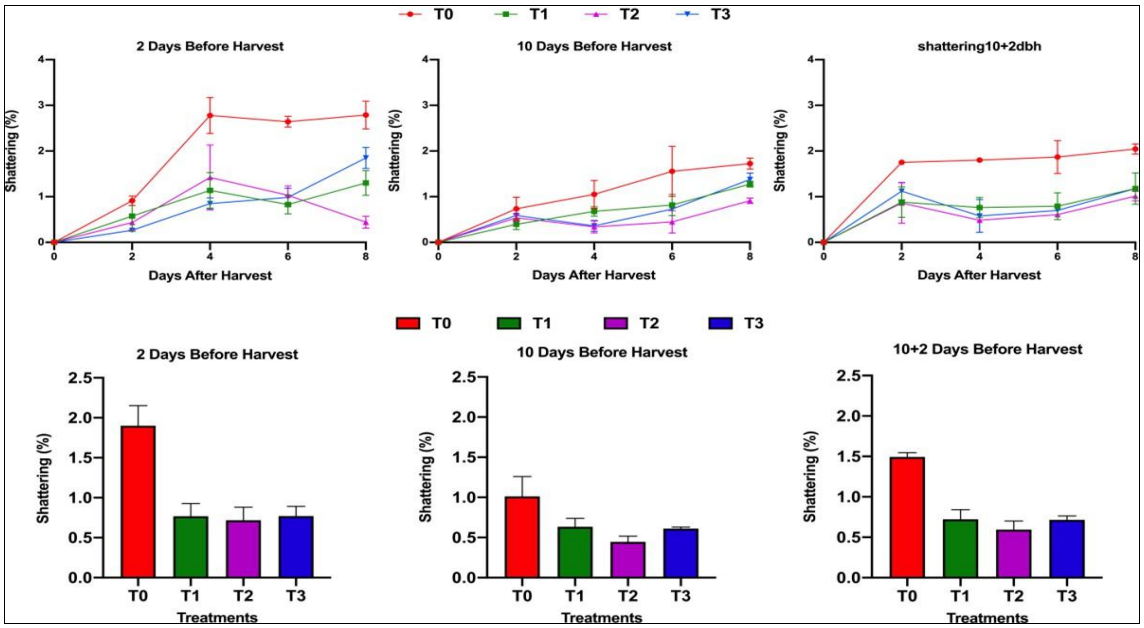

Fig 3: Berry shattering (%) in Thompson Seedless grapes under different preharvest treatments, T_0 (Control), T_1 (Biostimulant), T_2 (Calcium salt), and T_3 (Biostimulant + Calcium) and spray schedules (2 DBH, 10 DBH, and 10 + 2 DBH) during storage. Error bars represent standard error (n = 3).

Berry Cracking

Berry cracking was consistently highest in the untreated control (T_0) across all spray schedules. In contrast, treatments T_1 and T_2 effectively reduced the incidence of cracking, with the lowest percentage observed in T_2 under the 10 DBH spray schedule. Within the 2 DBH group, both T_1 and T_2 significantly reduced berry cracking compared to the control. In the 10 + 2 DBH group, T_2 and T_3 exhibited similar trends in reducing cracking severity. The overall pooled data indicate that T_1 and T_2 were particularly effective in minimizing cracking when applied at 2 DBH, while T_2 alone showed consistent reductions under 10 DBH and 10 + 2 DBH applications relative to their respective controls (Fig. 4).

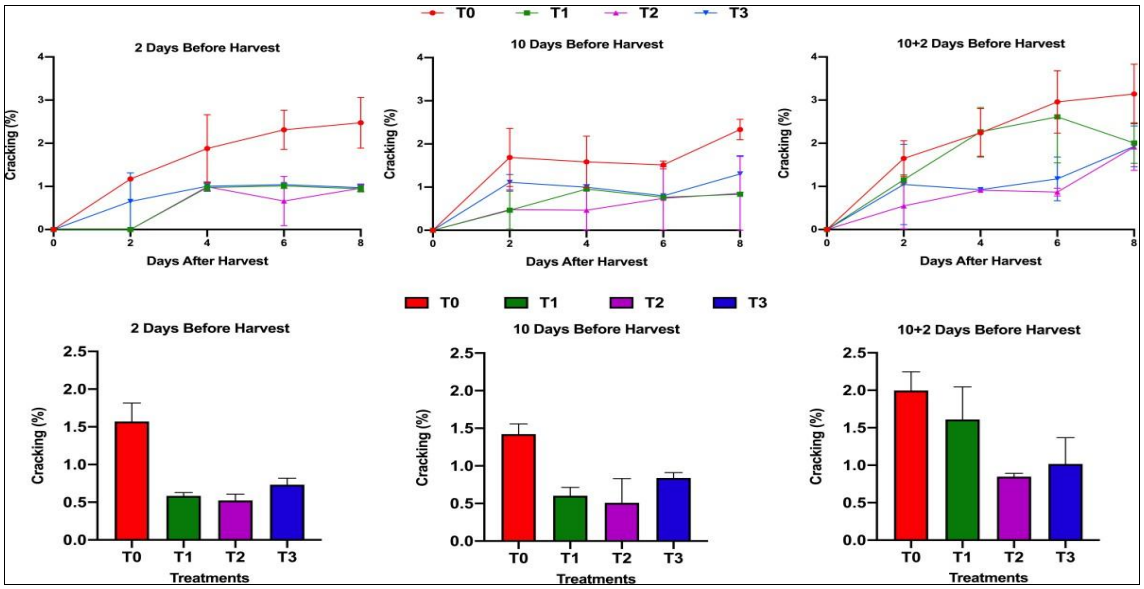

Fig 4: Berry cracking (%) in Thompson Seedless grapes under different preharvest treatments, T_0 (Control), T_1 (Biostimulant), T_2 (Calcium salt), and T_3 (Biostimulant + Calcium) and spray schedules (2 DBH, 10 DBH, and 10 + 2 DBH) during storage. Error bars represent standard error (n = 3).

Total Soluble Sugars

Total soluble sugar (TSS) content exhibited slight fluctuations across treatments during the storage period in all spray schedules. The untreated control (T_0) consistently recorded the lowest °Brix values, indicating lower sugar accumulation. In contrast, treatment T_1 resulted in significantly higher TSS values across all spray groups, with the most pronounced increase observed in the 2 DBH schedule. Specifically, T_1-treated vines under the 2 DBH spray recorded a 20.90% higher TSS compared to the corresponding control. This enhancement suggests an improved carbohydrate partitioning or metabolic activation

in response to the biostimulant application. While T_1 also maintained elevated sugar levels in the 10 DBH and 10 + 2 DBH groups, the effect was most substantial in the 2 DBH schedule (Fig. 5).

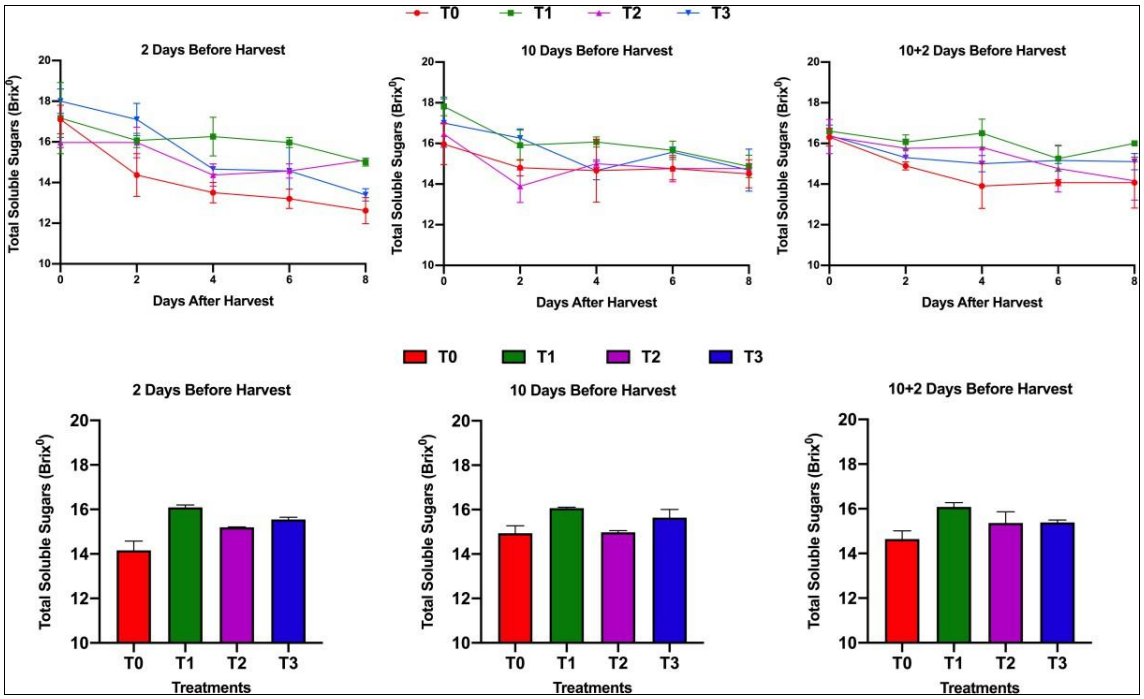

Fig 5: TSS in Thompson Seedless grapes under different preharvest treatments, T_0 (Control), T_1 (Biostimulant), T_2 (Calcium salt), and T_3 (Biostimulant + Calcium) and spray schedules (2 DBH, 10 DBH, and 10 + 2 DBH) during storage. Error bars represent standard error (n = 3).

Physiological Loss in Weight

Physiological loss in weight (PLW) increased significantly with storage duration in all treatment groups and spray schedules. The highest PLW was observed in the control (T_0), highlighting its vulnerability to moisture loss and respiration-induced shrinkage during storage. Among the treatments, T_2 showed the greatest efficacy in reducing PLW in the 10 DBH spray schedule, while T_1 effectively minimized weight loss in both the 2 DBH and 10 + 2 DBH groups. Notably, T_1 applied at 2 DBH resulted in the lowest overall weight loss by the end of storage, indicating its superior performance in maintaining postharvest freshness and water retention (Fig. 6).

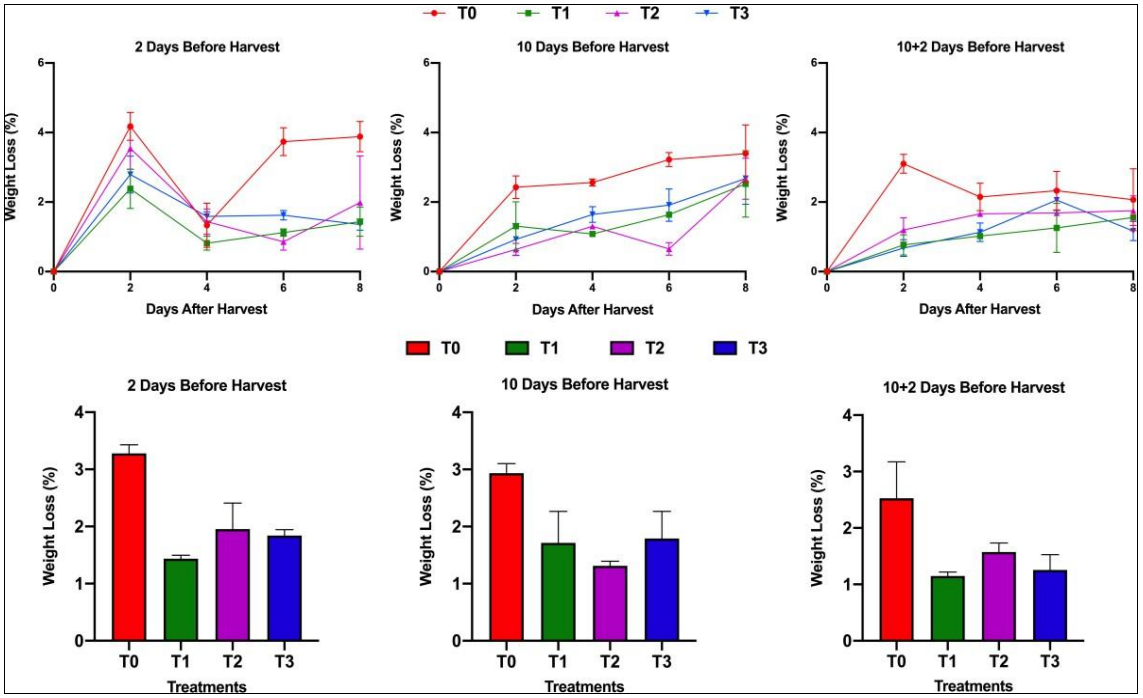

Fig 6: Weight loss (%) in Thompson Seedless grapes under different preharvest treatments, T_0 (Control), T_1 (Biostimulant), T_2 (Calcium salt), and T_3 (Biostimulant + Calcium) and spray schedules (2 DBH, 10 DBH, and 10 + 2 DBH) during storage. Error bars represent standard error (n = 3).

Discussion

Fresh produce, including grapes, possesses limited shelf life and is highly susceptible to postharvest losses, primarily due to fungal decay and various physiological processes such as high respiration rates, hormonal interactions, and suboptimal storage conditions. Although the use of postharvest fungicides has traditionally been effective in mitigating fungal decay in grapes, growing concerns regarding pathogen resistance, human health hazards, and environmental safety have led to increasing restrictions on their application in fresh fruits. In the present study, the preharvest foliar application of Velabs's biostimulant formulation significantly reduced berry rotting, particularly when applied 2 days before harvest (2 DBH). The formulation, composed of organic acids and amino acids, likely functions as an elicitor, activating the plant's innate defence mechanisms such as the phenylpropanoid pathway, thereby enhancing resistance against postharvest pathogens. Comparable outcomes have been reported with L-phenylalanine preharvest sprays in strawberry, mandarin, and mango, where fungal decay was notably reduced (Kumar *et al.*, 2020) [19]. Similarly, preharvest applications of chitosan, fulvic acid, and salicylic acid were shown to be effective in controlling rotting in grapes cv. Thompson Seedless (El-kenawy, 2017) [10]. In addition, preharvest bunch dipping in 1% calcium propionate at 3 DBH has been documented to significantly reduce berry rotting in grapes (Sun *et al.*, 2021) [35].

While minimizing fungal decay is a top priority in reducing postharvest losses, rachis browning remains a key quality determinant due to its influence on consumer preference (Lichter, 2016) [23]. In this study, the application of T_1 maintained rachis greenness up to 8 DAH, potentially due to improved antioxidant activity that delays chlorophyll degradation and alleviates osmotic stress. Previous studies have demonstrated similar effects, where salicylic acid and oxalic acid applications preserved rachis colour in grapes (Champa *et al.*, 2015) [6]. The calcium salt treatment (T_2), especially when applied at 10 DBH, effectively reduced berry cracking and shattering. This response can be attributed to the limited calcium uptake by berries after veraison, primarily due to dysfunctional xylem and reduced transpiration rates in the fruit (Knipfer *et al.*, 2015) [18]. The solubilization of pectin and depletion of calcium from cell wall complexes compromises cell wall integrity, reducing elasticity and increasing cracking susceptibility (Bruggenwirth and Knoche, 2017). Therefore, calcium supplementation through foliar application may strengthen the cell wall, enhance osmotic regulation, and reduce tissue swelling, thereby improving the mechanical properties of berry skin. Similar outcomes have been reported with calcium nitrate sprays during veraison, resulting in reduced berry cracking and shattering (Young-Sik *et al.*, 2022).

Berry shattering, another major cause of commercial loss in table grapes, is often triggered by abscission zone activation during postharvest storage and transport (Uzquiza *et al.*, 2014) [36]. The application of prohexadione calcium has been shown to reduce berry shattering by downregulating abscisic acid (ABA) levels and modifying hormonal balance (Li *et al.*, 2024). A similar mechanism may be at play in the present study, where calcium salt application potentially influenced ABA signalling pathways, thereby reducing abscission zone formation and preserving berry integrity. These results are consistent with previous findings where nano-calcium and calcium chloride sprays applied two weeks before harvest enhanced berry detachment force and reduced abscission in Thompson Seedless grapes (Ilie *et al.*, 2017) [13]. The biostimulant treatment (T_1) also enhanced total soluble sugar (TSS) content, particularly when applied at 2 DBH. This could be linked to the presence of organic and amino acids in the formulation, which may regulate sugar metabolism by promoting α- and β-amylase activity, facilitating starch-to-sugar conversion. Comparable findings have been reported in apples treated with amino acid sprays, which showed significant increases in TSS levels (Kazemi *et al.*, 2011) [16].

Weight loss increased progressively across all treatments during storage, consistent with postharvest metabolic activity, transpiration, and respiration as reported by Shafiee *et al.* (2010). Notably, T_1 application at 2 DBH significantly reduced PLW compared to other treatments and controls, likely due to enhanced stress tolerance and reduced moisture loss. This observation agrees with studies conducted in kiwi and apple, where elicitor-based treatments contributed to better retention of physiological quality and reduced postharvest weight loss (Fattahi *et al.*, 2010; Kazemi *et al.*, 2011) [11, 16].

Conclusion

The application of preharvest interventions, including Velabs's biostimulant elicitor-based formulation (T_1), derived from organic and amino acids, and foliar-applied calcium salt (T_2), demonstrates significant potential in alleviating key postharvest challenges in table grapes. Treatment T_1, particularly when applied 2 days before harvest (2 DBH), was effective in reducing berry decay, preserving rachis greenness, enhancing total soluble solids (TSS), and minimizing physiological weight loss. In parallel, T_2 applied at 10 DBH notably reduced berry cracking and shattering, likely through modulation of cell wall structure and inhibition of abscission processes. These findings suggest that a strategic preharvest application of T_1 at 2 DBH and T_2 at 10 DBH can serve as an effective, synergistic approach for improving the postharvest quality and marketability of Thompson Seedless grapes. Further investigations are warranted to elucidate the molecular and physiological mechanisms underlying elicitor-induced defence responses and calcium-mediated structural integrity, with emphasis on gene expression and metabolic regulation triggered by these novel formulations.

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Author Contributions

SS: Conceptualization, investigation, data curation, formal analysis, software, and writing original draft. KJH: Methodology, writing, review and editing. AAB: Resources, project administration.

Conflicts of Interest

The authors declare no conflict of interest.

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