**Bio-efficacy and phytotoxicity of Stigmasterol and Campesterol 0.01% w/w SL (Brand) on growth, yield and shelf life of Thompson Seedless**

**Abstract**

The study investigates the bio-efficacy and phytotoxicity of a phytosterol-based solution containing stigmasterol and campesterol (0.01% w/w SL, branded as Brand) on Thompson Seedless grapevines. The key parameters assessed includes shoot length, leaf area, bunch weight, berry size and physiological loss in weight (PLW) to determine post-harvest shelf life. Results indicated higher concentrations of Brand @ 2 ml/L significantly enhanced growth and yield. Treatment T4 recorded increase in shoot length (115.80 cm), leaf area (165.00 cm2), bunch weight (460.30 g), 50 berry weight (185.60 g) and yield (19.95 kg). Additionally, T4 demonstrated the highest skin and pedicel thickness (0.27 and 0.52 mm respectively), directly contributing to a reduction in PLW (5.20 % at 5th day), thereby enhancing post-harvest shelf life and resilience during storage. The potential of Stigmasterol and Campesterol as sustainable growth enhancers in viticulture.

**Keywords: Biostimulant, PGR, Grape, Yield, shelf life**

**Introduction**

Grapes (*Vitis vinifera* L.) are a major commercial fruit crop with significant economic importance, cultivated across tropical and subtropical regions. While native to temperate climates, grapevines have adapted well to tropical environments, particularly in areas where they do not undergo dormancy. In India, leading grape-producing states are Maharashtra, Karnataka, Andhra Pradesh, Madhya Pradesh and Mizoram. In 2024, India’s grape production reached approximately 3,896 thousand metric tons from 176.91 thousand hectares of cultivated land (Anonymous, 2024). In the fiscal year 2023-24, The demand for high-quality, visually appealing, seedless grape varieties is increasing, particularly for table grapes. Notable varieties such as Thompson Seedless and its clones (Tas-A-Ganesh, Manik Chaman, and Sonaka) are popular in both domestic and international markets. Consumers’ preferences for table grapes include attributes like bunch size, berry shape, skin colour, skin thickness, flesh texture, internal quality, flavor, aroma and a balanced sugar-to-acid ratio (Deshmukh et al., 2023). However, grape cultivation faces numerous challenges. Seasonal fluctuations and environmental stressors, both biotic and abiotic, impact grape yield and quality. Additionally, unseasonal rains, soil salinity, drought and suboptimal irrigation water quality make it difficult to achieve desired grape size and quality (Upadhyay et al., 2021). In response to these challenges, the application of biostimulants has gained attention as a promising strategy to boost metabolic activity and regulate growth during the vegetative and reproductive stages of grapevine development. Biostimulants can enhance plant resilience to environmental stresses, improve yield and maintain quality, making them integral to sustainable grape cultivation (Sharma et al., 2023; Deshmukh et al., 2023). Recently, the bio-efficacy and phytotoxicity of phytosterols have been key research areas for their potential to improve plant growth, yield and resistance to environmental stresses. These naturally occurring plant compounds have shown promise in modulating physiological responses under stress and enhancing resilience, as observed in many studies (Valitova et al., 2024). In grape production, stigmasterol and campesterol offer dual advantages: promoting growth and yield while potentially extending post-harvest shelf life through enhanced resistance to pathogens and environmental stresses. Considering this, the bio-efficacy study of Stigmasterol and Campesterol was conducted on Thompson Seedless grapes.

**Material and Methods**

**Experimental conditions**

The experimental trial was conducted at the farm of ICAR-National Research Centre for Grapes, Pune during the year 2023-24. The place is in mid-western Maharashtra (latitude 18°32ʹN and longitude 73°51ʹE). Four years old vines of Thompson Seedless grafted on Dogridge rootstocks were selected for the study. The vines were trained to extended Y trellis system of training spaced at 2.74 m between rows and 1.52 m between vines thus accommodating 968 vines per acre. The experiment was laid out in RBD design with six treatments replicated three times. Under the tropical conditions, the vines are pruned twice in a year. First pruning was done on 15th April 2023 (foundation pruning) while the second pruning on 20th October 2023 (forward pruning). Six treatments were implemented such as T1 (Brand SL at 0.75 ml/L of water), T2 (Brand SL at 1 ml/L of water), T3 (Brand SL at 1.25 ml/L of water), T4 (Brand SL at 2 ml/L of water), T5 (a standard check using gibberellic acid 0.001% L at 100 ppm), and T6 (control with water spray only). Each treatment, except the control, was applied twice (at the full bloom and fruit set stages) to observe responses across the growing period.

**Growth parameter**

Shoot length was measured at 90 days after fruit pruning and expressed in cm. Shoot diameter was recorded at the fifth and sixth nodes using a Vernier caliper, measuring five canes per vine from five vines in each treatment, with the average diameter expressed in mm. The internodal length between the fifth and sixth nodes was measured 90 days after forward pruning, with five randomly selected canes from each vine and averaged in cm. Leaf area was calculated using the LBK linear method, expressed in cm², based on the formula: Leaf area (A) = L x B x K (0.810), where L is leaf length, B is breadth, and K is a constant.

**Bunch and yield parameter**

To assess yield, the total number of bunches per vine was counted from five selected vines in each treatment after berry set. Average bunch weight was determined by weighing 10 randomly selected bunches from the five vines at harvest, expressed in grams. Berry weight was averaged from 50 berries collected from five vines at harvest. Yield per vine was recorded at harvest. Additionally, the number of berries per bunch was counted in five selected bunches per treatment to determine the average.

**Berry and quality parameters**

Berries were sampled randomly for juice extraction to measure total soluble solids (°Brix) using a hand refractometer. Titratable acidity was determined by titrating berry juice with 0.1 N NaOH and expressed as a percentage. Berry length and diameter were measured in mm using a Vernier caliper, with 10 berries selected per replication.

**Physical properties of treated grapes**

Pedicel thickness was measured with a Vernier caliper, expressed in mm, and skin thickness was determined using a micro meter gauge on skin samples peeled from ten randomly selected berries. Physiological loss in weight (PLW) was monitored daily for five days to assess shelf life, with PLW calculated as the percentage of mass loss from the beginning to the end of the storage period using the formula: PLW (%) = (Initial weight - Final weight) / Initial weight × 100.

**Statistical analysis**

 Data from the field experiment were analyzed using Randomized Block Design (RBD) and subjected to analysis of variance (ANOVA) as described by Panse and Sukhatme (1985).

**Result and discussion**

The data recorded on various growth parameters is presented in Table 1. Statistically non-significant variation was found for shoot diameter and internodal length with different concentrations of Stigmasterol and Campesterol 0.01% w/w SL (Brand). However, the differences among the different concentrations were significant. Treatment T4 recorded highest shoot length (115.80 cm) as compared with the other treatments. Higher shoot length was recorded in T3 treatment (106.20 cm) as compared with minimum in T6 (90.10 cm). Among the different treatments, T4 recorded higher leaf area of 165.00 cm2 as compared to the lowest in T6 (140.52 cm2). Stigmasterol and campesterol are key phytosterols that enhance grapevine growth by improving shoot length and leaf area. Stigmasterol promotes essential growth hormones (IAA and GA₃) and nutrient uptake (K⁺, Ca²⁺ and P³⁺), while also reducing stress impacts like salinity, supporting healthier growth (Bassuony et al., 2011; Hashem et al., 2011). Campesterol stabilizes cell membranes, aiding nutrient absorption and enhancing leaf area, which boosts photosynthesis and biomass production (Bhat et al., 2011). Together, these phytosterols strengthen grapevine development, optimizing growth and resilience.

**Table 1. Effect of Stigmasterol and Campesterol 0.01% w/w SL (Brand) on growth parameters**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatments** | **Shoot length (cm)** | **Shoot diameter (mm)** | **Internodal length (mm)** | **Leaf area (cm2)** |
| **T1**- Brand SL @ 0.75 ml/lit  | 93.60 | 7.60 | 5.53  | 157.10 |
| **T2**- Brand SL @ 1 ml/lit | 100.10 | 7.85 | 5.66 | 160.20 |
| **T3**- Brand SL @ 1.25 ml/lit | 106.20 | 7.90  | 5.80 | 162.40 |
| **T4**- Brand SL @ 2 ml/lit | 115.80  | 8.00 | 5.90  | 165.00 |
| **T5**- Standard Check Gibberellic Acid 0.001% L @ 100 ppm solution | 96.30 | 7.70 | 5.60 | 158.30 |
| **T6-** Control (Water Spray) | 90.10 | 7.20 | 5.24 | 140.52 |
| **SEm±** | **0.92** | **0.47** | **0.61** | **1.31** |
| **CD at 5%** | **2.91** | **1.48** | **1.93** | **4.13** |
| **Sig** | **\*\*** | **NS** | **NS** | **\*\*** |

The data recorded on number of bunches/vines, average bunch weight (g), 50-berry weight, yield/vine and number of berries/bunches is presented in Table 2. The differences for number of bunches/vine and number of berries/bunches were non-significant. This was mainly because the fruit bud differentiation was already been completed during the period of 40 to 70 days after the foundation pruning. In addition, considering the quality yield for export purpose, bunch thinning is also done after berry set. With the increase in Brand SL concentration, there was increase in average bunch weight. The average bunch weight varied from 428.17 g in T1 to 460.30 g in T4 as compared with the local check T5 (445.80 g) while the control treatment T6 recorded the lowest average bunch weight (417.27 g). Significant differences were also recorded for 50-berry weight. Higher 50-berry weight was recorded in T4 treatment (185.60 g) while the lowest in T1 (176.05 g) as compared to standard check T5 (181.81 g) and 172.85 g in T6 (control). With the increase in 50-berry weight and average bunch weight, the yield per vine also increased in T4 treatment (19.95 kg) as compared with the standard check T5 (19.11 kg) and control (16.69 kg). Stigmasterol and campesterol, two key phytosterols, play crucial roles in improving grapevine traits like bunch weight, berry size and overall yield by supporting membrane integrity and nutrient uptake (Cabianca et al., 2021; Griebel & Zeier, 2010). These sterols may work with plant growth regulators (PGRs) to optimize growth, enhancing berry set and weight (Flouda, 2022). Manipulating sterol levels has shown promising results in increasing yield per vine (Berli et al., 2021) and genetic research indicates that targeting sterol composition could aid breeding programs aimed at better grapevine resilience and productivity (Flutre et al., 2019). Additionally, the balance of sterols may affect stress responses, indirectly supporting yield by enhancing resilience to pathogens (Griebel & Zeier, 2010). These results are in line with the reports of Khatoon et al., (2021); Ghorbani et al., (2017); Deshmukh et al., (2023) and Sharma et al., (2023).

**Table 2. Effect of Stigmasterol and Campesterol 0.01% w/w SL (Brand) on yield parameters**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment** | **Number of bunches/vine** | **Average bunch weight (gm)** | **50 berry weight (gm)** | **Yield (kg/ vine)** | **Number of berries/bunch** |
| **T1**- Brand SL @ 0.75 ml/lit  | 41.15 | 428.17 | 176.05 | 17.62 | 121.60 |
| **T2**- Brand SL @ 1 ml/lit | 43.17  | 447.93  | 181.94 | 19.34 | 123.00 |
| **T3**- Brand SL @ 1.25 ml/lit | 43.27 | 451.30 | 183.45  | 19.53  | 123.10 |
| **T4**- Brand SL @ 2 ml/lit | 43.34 | 460.30 | 185.60 | 19.95 | 124.00 |
| **T5**- Standard Check Gibberellic Acid 0.001% L @ 100 ppm solution | 42.87 | 445.80 | 181.81 | 19.11 | 122.60 |
| **T6-** Control (Water Spray) | 40.00 | 417.27 | 172.85 | 16.69 | 120.70 |
| **S Em±** | **3.08** | **3.79** | **1.53** | **0.16** | **0.98** |
| **CD at 5%** | **9.72** | **11.93** | **4.81** | **0.51** | **3.09** |
| **Sig** | **NS** | **\*\*** | **\*\*** | **\*\*** | **NS** |

The grape quality mainly consists of berry length, berry diameter, TSS, acidity and juice pH. The data recorded on grape berry quality is presented in Table 3. Acidity, berry length and berry diameter varied significantly among the different treatments. The treatment T4 recorded highest berry length (22.88 mm) and berry diameter (18.90 mm) which was at par with treatment T3 (22.65 and 18.60 mm respectively) over untreated control in T6 (20.45 mm and 17.40 mm respectively). The differences for TSS and pH of grape berry were non-significant. However, TSS ranged from 18.20°Brix (T1) to 18.90°Brix (T4). The acidity ranged from 0.50 to 0.58 %. Maximum acidity was recorded in T6 (0.58 %) which was at par with treatment T1 (0.57 %) and T5 (0.55 %). The acidity in grape berries was within the acceptable limit in all the treatments. Though the differences for juice pH was non-significant, the pH values were within grange of 3.26 to 3.36. Similar to our findings, the researchers viz., Asghari and Reazei-Rad (2018); Isci and Gokbayrak (2015); Salvi et al. (2016) and Symons et al. (2006) reported the non-significant effect of brassinosteroids on TSS and titratable acidity. Stigmasterol and campesterol, two essential phytosterols, enhance berry size in grapevines by interacting with growth regulators like gibberellic acid (GA₃) and brassinosteroids (BRs). These compounds support cell wall integrity and elasticity, promoting cell expansion and resulting in larger berries. GA₃ for instance is known to increase berry size and weight significantly by enlarging cell size (Li et al., 2024; Ferrara et al., 2014; Bedrech, 2022), while BRs, when applied optimally, further boost berry weight and dimeter (Champa et al., 2015). Together, these phytosterols and PGRs offer a promising approach to improving grape yield and quality. The increase in berry size might be due to enhanced cell division and cell elongation in response to the application of biostimulants (Deshmukh et al., 2023; Sharma et al., 2023)

**Table 3. Effect of Stigmasterol and Campesterol 0.01% w/w SL (Brand) on quality parameters**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment** | **TSS (0Brix** | **Acidity (%)** | **Berry length (mm)** | **Berry diameter (mm)** | **Juice pH** |
| **T1**- Brand SL @ 0.75 ml/lit | 18.32 | 0.57 | 21.53 | 18.20 | 3.26 |
| **T2**- Brand SL @ 1 ml/lit | 18.47 | 0.51 | 22.27 | 18.40 | 3.31 |
| **T3**- Brand SL @ 1.25 ml/lit | 18.73  | 0.50  | 22.65 | 18.60 | 3.35  |
| **T4**- Brand SL @ 2 ml/lit | 18.80 | 0.50 | 22.88 | 18.90 | 3.36 |
| **T5-** Standard Check Gibberellic Acid 0.001% L @ 100 ppm solution | 18.45 | 0.55 | 22.08 | 18.35 | 3.30 |
| **T6-** Control (Water Spray) | 18.02 | 0.58 | 20.45 | 17.40 | 3.26 |
| **SEm±** | **0.16** | **0.02** | **0.19** | **0.15** | **0.04** |
| **CD at 5%** | **0.50** | **0.07** | **0.58** | **0.48** | **0.12** |
| **Sig** | **NS** | **\*** | **\*\*** | **\*\*** | **NS** |

 The data presented in Figure 1 revealed significant differences for skin thickness and pedicel thickness of fresh berries. Treatment T4 recorded the highest skin thickness (0.270 mm) and pedicel thickness (0.520 mm), while T6 showed the lowest values, with a skin thickness of 0.190 mm and pedicel thickness of 0.440 mm. Across all treatments, PLW% increased as storage duration progressed (Fig. 2). Treatment T4 improved shelf life which might be due to its greater skin and pedicel thickness, highlighting the effectiveness of T4 in prolonging storage quality. On day 1, treatment T4 recorded a PLW% of 1.03%, which gradually increased to 5.20% by day 5. In contrast, the control treatment experienced a more rapid increase in PLW%, starting at 1.85% on day 1 and reaching 6.40% by day 5. This suggests that the T4 treatment contributed to better preservation and reduced weight loss over time. Stigmasterol and campesterol are key phytosterols that enhance grapevine skin and pedicel thickness while reducing post-harvest weight loss (PLW). These sterols stabilize plant cell membranes and act as precursors to brassinosteroids, which promote cell growth and tissue strength, crucial for grape durability (Valitova et al., 2016). When combined with growth regulators, they extend grape shelf-life by slowing decay and increasing resistance to pathogens, thus minimizing PLW and reducing berry rot (Ramteke et al., 2002; Hmidene et al., 2023). This makes them valuable in improving grape quality and resilience in viticulture.

**Phytotoxicity:**

Phytotoxicity data indicated no observed phytotoxic effects.

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| Fig. 1. **Effect of Stigmasterol and Campesterol 0.01% w/w SL (Brand) on pedicel thickness and skin thickness (mm) of grapes** |

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| **Fig. 2. Effect of Stigmasterol and Campesterol 0.01% w/w SL (Brand) on Shelf life of Thompson Seedless** |

**Conclusion:**

 All treatments with Stigmasterol and Campesterol 0.01% w/w SL (Brand) notably enhanced grape yield, berry quality and shelf life compared to the untreated control. Among these treatments, T4 @ 2 ml/L Stigmasterol and Campesterol 0.01% w/w SL Brand was particularly effective, showing superior results in bunch size, berry quality and shelf life, leading to an overall increase in vine yield. Consequently, applying a foliar spray of Stigmasterol and Campesterol 0.01% w/w SL (Brand) at this higher concentration across two growth stages is recommended for optimizing the yield and quality of Thompson Seedless grapevines.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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