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Impact of β-sitosterol and stigmasterol 0.05% DF (Wilbond) on growth, yield and shelf life of Thompson seedless grapes

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Abstract

In this study, the bio-efficacy of the biostimulant Wilbond, containing β-sitosterol and stigmasterol at a 0.05% concentration was assessed on Thompson Seedless grapevines The grapevines were treated with various concentrations of Wilbond (56.25-150 g/ha) showed substantial improvements, particularly in treatment T_4 (150 g/ha) which increased shoot length (110.27 cm), diameter (7.51 mm) and leaf area (160.12 cm^2) as well as bunch weight (450.80 g) and yield per vine (18.33 kg/vine). Berry quality including berry diameter (18.90 mm), total soluble solids (18.70) and skin thickness (0.25 mm). In addition, T_4 treatment reduced physiological weight loss during storage, highlighting Wilbond positive impact on grape shelf-life. Overall, Wilbond application significantly boosted yield, quality and marketability of Thompson Seedless grapes, offering a sustainable alternative to traditional growth regulators for enhanced grape production.

Keywords: Grapevine, bio stimulant, vegetative growth, yield, shelf life

Introduction

In response to growing global demand for high-quality, increased shelf life and sustainably produced grapes, the grape industry has made significant steps in enhancing cultivation techniques. These improvements aim to boost not only grape quality and yield but also shelf life, factors essential for economic and environmental sustainability in modern agriculture. A significant catalyst in this evolution has been the development and application of bio stimulants and plant growth regulators, which serve to optimize vine health and productivity within eco-friendly frameworks (Deshmukh *et al*., 2023; Sharma *et al*., 2023) [3, 15]. Among these advancements, the bioactive compounds β-sitosterol and stigmasterol have shown substantial promise. Known for their ability to enhance plant growth, yield and resilience to stress, these compounds are particularly effective when formulated into products like β-Sitosterol and Stigmasterol 0.05% DF (Wilbond). As phytosterols, β-sitosterol and stigmasterol closely resemble plant sterols in structure, providing critical support for membrane stability, stress tolerance and metabolic efficiency (Moreau *et al*., 2018) [12]. Studies indicated that these compounds promote root and shoot growth, improve nutrient uptake and modulate stress response functions that are vital for grapevines, which are sensitive to both biotic and abiotic stress factors (Wu *et al*., 2024) [20]. Wilbond, a specially formulated product containing β-Sitosterol and Stigmasterol at 0.05%, aims to improve bioavailability and uptake, enhancing its impact on grape health and productivity. This formulation has demonstrated potential not only to improve yield but also to enhance quality attributes like berry size, sugar content and phenolic composition, while also boosting resilience to environmental stressors exacerbated by climate variability (Ruggiero *et al*., 2013) [14]. Furthermore, β-sitosterol and stigmasterol are associated with improved shelf life, attributed to their ability to regulate ethylene production and delay ripening, critical factors for the marketability of export-oriented grape cultivars (Hu *et al*., 2019; Ebrahimi *et al*., 2022) [10, 6]. The present study was aimed to evaluate the bio-efficacy and phytotoxicity of Wilbond in grape production, focusing on its effects on growth, yield and shelf life.

Materials and Methods

Experimental conditions

The experimental trial was conducted at the farm of ICAR-National Research Centre for Grapes, Pune (latitude 18°32′N and longitude 73°51′E) during the year 2023-24. 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 and three replications. 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). The treatments involved application of β-Sitosterol and Stigmasterol in varying doses (56.25 g/ha, 75 g/ha, 93.75 g/ha, and 150 g/ha) at two growth stages: full bloom and fruit set, alongside a standard check (gibberellic acid) and a control (water spray).

Growth parameter

Shoot length was measured 90 days after fruit pruning and expressed in cm. Shoot diameter and internodal length was recorded at fifth and sixth nodes using a Vernier caliper, on five canes per vine from in each treatment and was expressed in mm. 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, calculating the average number of bunches per vine 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 were 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 millimeters 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 micrometer 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, and PLW was 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 analysed using Randomized Block Design (RBD) and subjected to analysis of variance (ANOVA) as described by Panse and Sukhatme (1985) [13].

Results and Discussion

Among the different parameters studied, significant variations were recorded for shoot length (cm), shoot diameter (mm), internodal length (mm) and leaf area (cm^2). Among the different treatments of Wilbond, lowest shoot length was recorded in T_6 treatment (95.00 cm) whereas the highest shoot length was recorded in T_4 (110.27 cm). The same trend was observed for internodal length. The treatment T_6 recorded controlled internodal length of 5.30 cm as compared to the higher internodal length in T_4 treatment (5.93 mm). However, T_4 recorded maximum shoot diameter and leaf area (7.51 mm and 160.12 cm^2 respectively) as compared to the minimum shoot diameter and leaf area in control treatment T_6 (7.00 mm and 130.00 cm^2 respectively). The increase in shoot length and diameter can be attributed to the role of both β-sitosterol and stigmasterol in maintaining membrane fluidity and permeability, which are essential for cell elongation and division (Du *et al*., 2022; Valitova *et al*., 2024) [5, 19]. These sterols facilitate the synthesis of brassinosteroids a class of plant hormones known to promote cell expansion and elongation thereby contributing to increased internodal length (Aboobucker & Suza, 2019) [1]. Additionally, by enhancing stress tolerance, β-sitosterol and stigmasterol help sustain optimal photosynthetic activity and nutrient uptake, leading to larger leaf areas (Du *et al*., 2022; Valitova *et al*., 2024) [5, 19]. Overall, β-sitosterol and stigmasterol contribute to plant growth by improving membrane properties, supporting hormone synthesis and boosting stress resilience, ultimately resulting in improved shoot and leaf development.

Table 1: Effect of β-Sitosterol and Stigmasterol 0.05% DF (Wilbond) on growth parameters

Treatments	Shoot length (cm)	Shoot diameter (mm)	Internodal length (mm)	Leaf area (cm^2)
T_1-β-Sitosterol & Stigmasterol 0.05% DF (Wilbond) @ 56.25 g/ha	97.00	7.00	5.35	150.00
T_2-β-Sitosterol & Stigmasterol 0.05% DF (Wilbond) @ 75 g/ha	105.40	7.14	5.50	155.42
T_3-β-Sitosterol & Stigmasterol 0.05% DF (Wilbond) @ 93.75 g/ha	106.37	7.43	5.71	158.20
T_4-β-Sitosterol & Stigmasterol 0.05% DF (Wilbond) @ 150 g/ha	110.27	7.51	5.93	160.12
T_5-Standard Check Gibberellic Acid Technical @ 50 ppm solution	102.10	7.01	5.43	150.50
T6-Control (Water Spray)	95.00	7.00	5.30	130.00
SEm±	1.03	0.06	0.05	1.37
CD at 5%	3.25	0.19	0.17	4.31
Sig	**	**	**	**

The data recorded on various bunch parameters i.e., number of bunches/vines, average bunch weight (g), 50-berry weight, yield per vine and number of berries/bunches is presented in Table 2. It was observed that with the application of β-Sitosterol and Stigmasterol 0.05% DF (Wilbond), there was non-significant effect on number of bunches per vine and number of berries per bunch. 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 the berry set. Among the different treatments studied, treatment T_4 significantly showed maximum average bunch weight (450.80 g), 50 berry weight (187.83 g) and yield/vine (18.33 kg/vine) which was at par with treatment T_3 (440.93 g, 183.70 g, 17.90 kg/vine, respectively) over the control treatment T_6 (376.50 g, 154.30 g, 14.64 kg/vine respectively). By enhancing stress tolerance, β-sitosterol and stigmasterol enable plants to maintain optimal photosynthetic activity and nutrient uptake, leading to larger leaf areas (Du *et al*., 2022; Valitova *et al*., 2024) [5, 19]. A greater leaf area increases the surface available for photosynthesis, the process that converts sunlight into energy, thus supporting overall plant growth and fruit development (Somkuwar *et al*., 2024a; 2024b; 2024c; Kakade *et al*., 2024) [16, 17, 18, 11]. With more leaf surface, the plant can absorb a higher volume of nutrients from the soil, which are essential for the growth and development of grape bunches, resulting in heavier, more abundant grape bunch and increased yield (Diago *et al*., 2012) [4]. Additionally, an expanded leaf area enhances the plant's ability to regulate water through transpiration, helping maintain optimal hydration levels a critical factor for developing healthy, robust grape bunches (Howell, 2001). Similar to our result, significant yield improvements by the use of biostimulant in grape varieties such as Thompson Seedless and Sharad Seedless were also reported by Sharma *et al*. (2023) [15] and Deshmukh *et al*. (2023) [3].

Table 2: Effect of β-Sitosterol and Stigmasterol 0.05% DF (Wilbond) on yield parameters

Treatments	Number of bunches/vines	Average bunch weight (gm)	50 berry weight (gm)	Yield (kg/vine)	Number of berries/ bunch
T_1-β-Sitosterol & Stigmasterol 0.05% DF (Wilbond) @ 56.25 g/ha	39.48	420.01	175.00	16.58	118.10
T_2-β-Sitosterol & Stigmasterol 0.05% DF (Wilbond) @ 75 g/ha	40.20	425.10	180.08	17.21	120.00
T_3-β-Sitosterol & Stigmasterol 0.05% DF (Wilbond) @ 93.75 g/ha	40.50	440.93	183.70	17.90	120.01
T_4-β-Sitosterol & Stigmasterol 0.05% DF (Wilbond) @ 150 g/ha	40.67	450.80	187.83	18.33	120.00
T_5-Standard Check Gibberellic Acid Technical @ 50 ppm solution	40.60	420.45	177.81	16.90	122.00
T6-Control (Water Spray)	38.90	376.50	154.30	14.64	118.00
SEm±	0.40	3.46	1.88	0.14	1.02
CD at 5%	1.26	10.91	5.94	0.44	3.20
Sig	NS	**	**	**	NS

The grape quality mainly consists of TSS, acidity, berry length, berry diameter and juice pH. The data recorded on various grape berry quality parameters is presented in Table 3. Acidity, berry length, berry diameter and juice pH varied significantly among the different treatments. The treatment T_4 recorded highest berry length (22.52 mm) and berry diameter (18.90 mm) which was at par with treatment T_3 and T_2 over untreated control T_6 (20.10 mm and 17.40 mm). Application of β-Sitosterol and Stigmasterol 0.05% DF showed non-significant variation for TSS in grape berry. However, the TSS ranged between 18.00°Brix to 18.70°Brix in which T_4 showed maximum TSS (18.70°Brix) while least in T_6 (18.00°Brix). The acidity in juice ranged from 0.47 to 0.58 %. Maximum acidity was recorded in T_6 which was at par with treatment T_1 and T_2. However, the acidity in grape berries was within the acceptable limit in all the treatments studied. The juice pH was also within grange of 3.29 to 3.39. Sterols play a critical role in regulating plant architecture by influencing the synthesis and transport of auxin, a key hormone for growth and structural development. In addition to auxin regulation, sterols also affect cellulose content, which directly shapes plant architecture. Both β-sitosterol and stigmasterol contribute to maintaining membrane fluidity and permeability, essential for cell elongation and division (Du *et al*., 2022) [5]. This enhanced cellular environment results in increased berry length and diameter in treated plants compared to controls. Similar findings were also reported by Deshmukh *et al*., (2023) [3]; Sharma *et al*., (2023) [15].

Table 3: Effect of β-Sitosterol and Stigmasterol 0.05% DF (Wilbond) on quality parameters

Treatments	TSS (⁰Brix)	Acidity (%)	Berry length (mm)	Berry diameter (mm)	Juice pH
T_1-β-Sitosterol & Stigmasterol 0.05% (Wilbond) @ 56.25 g/ha	18.20	0.57	22.18	18.20	3.32
T_2-β-Sitosterol & Stigmasterol 0.05% (Wilbond) @ 75 g/ha	18.53	0.55	21.62	18.40	3.38
T_3-β-Sitosterol & Stigmasterol 0.05% (Wilbond) @ 93.75 g/ha	18.60	0.51	21.70	18.60	3.38
T_4-β-Sitosterol & Stigmasterol 0.05% (Wilbond) @ 150 g/ha	18.70	0.47	22.52	18.90	3.39
T_5-Standard Check Gibberellic Acid Technical @ 50 ppm solution	18.47	0.50	20.30	18.35	3.36
T6-Control (Water Spray)	18.00	0.58	20.10	17.40	3.29
SEm±	0.22	0.02	0.37	0.15	0.02
CD at 5%	0.70	0.07	1.17	0.48	0.05
Sig	NS	*	**	**	*

The data recorded on skin thickness and pedicel thickness of fresh berries shown in Fig. 1. It was observed that skin thickness was relatively higher in T_4 (0.250 mm) while it was lowest in T_6 (0.170 mm). The treatment T_4 recorded maximum pedicel thickness (0.510 mm) while it was minimum in T_6 (0.450 mm). The data in Fig. 2 represents the shelf-life of grapes specifically the physiological loss in weight (PLW%) during room-temperature storage. Across all the treatments, PLW% increased progressively with storage duration. Notably, the minimum physiological loss in weight was recorded in treatment T_4 in all the days under shelf. The minimum in PLW was recorded from 1st day (1.04 %), 2nd day (1.96 %), 3rd day (2.54 %), 4th day (3.56 %) and 5th day (5.11 %) of storage. The PLW reached to a level of 5% on 5th day. The physiological loss in weight (%) in berries of control treatment increased rapidly from 1st day (1.72 %), 2nd day (2.70 %), 3rd day (3.74 %), 4th day (5.19 %) and 5th day (6.08 %). The enhanced retention of freshness in berries of T_4 treatment over the storage period compared to the control, reflecting the treatment's positive effect on grape shelf life. β-Sitosterol and stigmasterol are phytosterols that play a vital role in reducing postharvest physiological loss in weight (PLW) and enhancing the thickness of skin and pedicel in grapevine through various biochemical pathways. These compounds are particularly valuable in grape cultivation due to their ability to promote cellular health and structural integrity. By enhancing membrane fluidity and permeability, they improve cellular resilience against stress (Bacchetti *et al*., 2011) [2]. β-Sitosterol, for instance, has anti-inflammatory properties that reduce inflammation markers in skin tissues, supporting skin thickness and health (Han *et al*., 2014) [8]. It also promotes hyaluronic acid synthesis and boosts the expression of skin barrier proteins, enhancing moisture retention (Haiyuan *et al*., 2019) [7]. The combined anti-inflammatory and moisture-preserving properties of β-sitosterol and stigmasterol support structural integrity in plant tissues, thus reducing PLW during storage. This improved moisture retention and cellular health contribute to greater skin and pedicel thickness, crucial for prolonging the shelf life and quality of harvested crops (Han *et al*., 2014; Haiyuan *et al*., 2019) [8, 7].

Phytotoxicity

Phytotoxicity data indicated no observed phytotoxic effects.

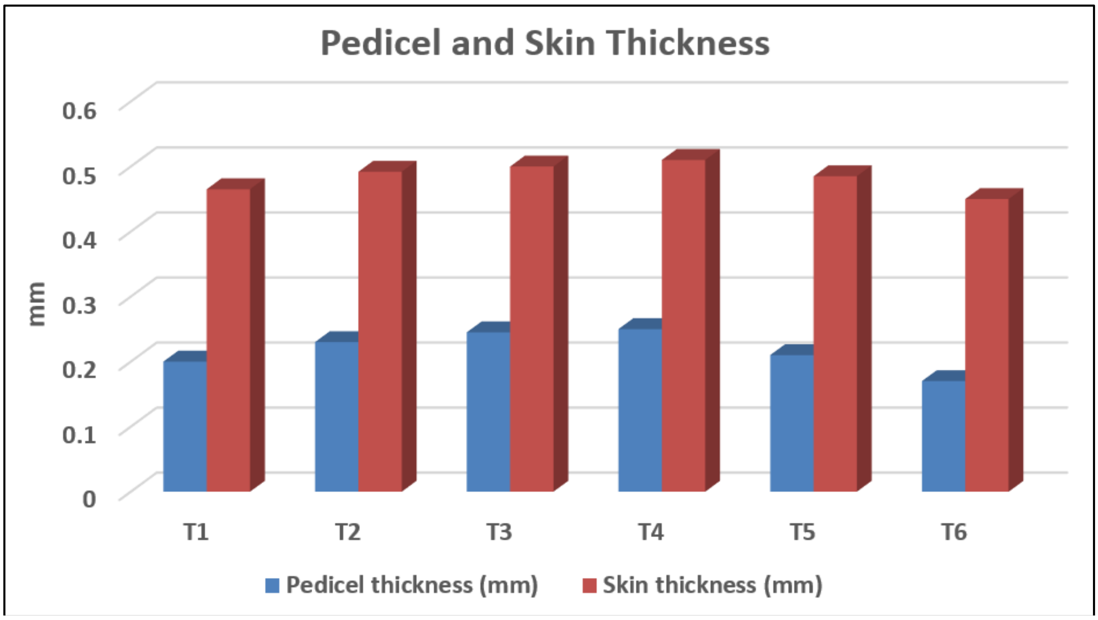

Fig 1: Effect of β-Sitosterol and Stigmasterol 0.05% DF (Wilbond) on pedicel thickness and skin thickness (mm)

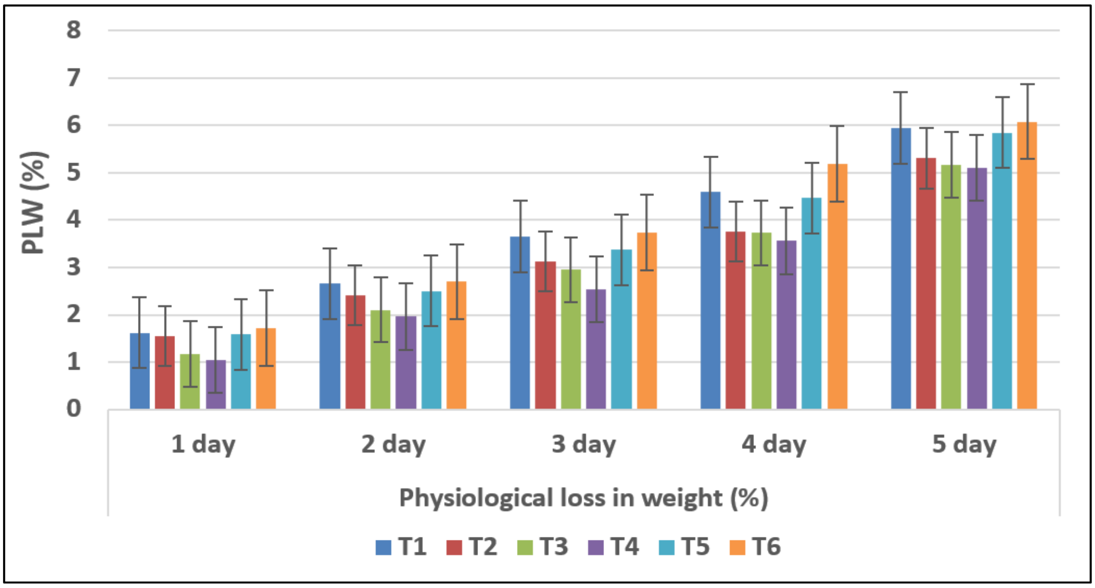

Fig 2: Effect of β-Sitosterol and Stigmasterol 0.05% DF (Wilbond) on Shelf life

Conclusion

All the treatments of β-Sitosterol and Stigmasterol 0.05% DF (Wilbond) significantly increased grape yield and berry quality as well as shelf-life as compared to untreated control. Among the different treatments of β-Sitosterol and Stigmasterol 0.05% DF (Wilbond), the treatment T_4 [(93.75 g/ha β-Sitosterol and Stigmasterol 0.05% DF (Wilbond)] showed better performance for bunch, berry quality parameters as well as shelf-life, however as a result it also improved the final yield of the vines. Therefore, the foliar spray of β-Sitosterol and Stigmasterol 0.05% DF (Wilbond) with its higher concentration at two different stages can be suggested to improve the quality and yield of Thompson Seedless grapevine.

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