**Bio-efficacy studies of biostimulant in relation to growth, yield and shelf-life of Thompson Seedless grape under multilocation**

**Abstract**

Grape cultivation is increasingly challenged by a range of abiotic stresses, including drought, salinity, excessive rainfall, high temperatures, intense solar radiation, and rising atmospheric CO2 levels. These factors, largely affected by global warming, are posing significant threats to sustainable grape production. Abiotic factors influence the synthesis and breakdown of primary metabolites (such as sugars, amino acids, and organic acids) and secondary metabolites (including phenolic compounds, volatile aroma compounds and their precursors). Application biostimulants is an innovative method to improve vine growth, quality and ultimately the final yield. This study aims to evaluate the effects of Budmaker, a biostimulant, on the growth, yield, and shelf-life of Thompson Seedless grapes under multilocation trials. The present research was conducted in two locations (at the farm of ICAR-National Research Centre for Grapes, Pune and at farmers field at Rahata in Ahmednagar of Maharashtra) during the year 2023-24 with the objective to assess the effects of bio-efficacy studies on growth, yield and quality of Thompson Seedless grapes. Budmaker were sprayed at three different stages (first at 1st leaf after the sub-cane, second at 3rd and 4th leaf after the sub-cane and third at 6th and 7th leaf after the sub-cane) with different concentrations (400,500 and 750 ml/acre). Among the different treatments, the application of 500 ml/acre exhibited a significant increase in vegetative growth parameters such as pruned biomass (kg/vine), % fruitfulness, early cane maturity, yield parameters such as average bunch weight, 50 berry weight, yield/vine, berry length and diameter and chlorophyll content. Biochemical and nutrient content such as phenol (mg/g), protein (mg/g), reducing sugar (mg/g), calcium (%), and phosphorus (%) were also estimated. All the treatments of Budmaker significantly increased fruit bud differentiation, early cane maturity, grape yield, and berry quality parameters as well as shelf life as compared to untreated control. The result revealed that the application of biostimulant *i.e*., Budmaker found suitable to improve the yield and quality parameter of grapes cv. Thompson Seedless under multilocation trial. Hence, the foliar spray of Budmaker with its higher concentration at all three different stages could be suggested to improve the quality and yield of grapevine.

**Keywords:** Budmaker, grapevine, yield, quality, shelf life , ICAR

**Introduction**

 Grape (*Vitis vinifera* L.) is one of the most widely cultivated fruit crops, prized for their versatility and nutritional value. In India, grape cultivation plays a crucial role in the agricultural sector, supporting the production of table grapes (78%), raisins (17-20%), wine and juice (2%) as reported by Somkuwar *et al*., (2024). However, grapes cultivation is increasingly challenged by a range of abiotic stresses, including drought, salinity, excessive rainfall, high temperatures, intense solar radiation, and rising atmospheric CO2 levels (Rafique et al. 2021;2023b). These factors, largely affected by global warming, are posing significant threats to sustainable grape production (Rafique et al. 2023ac). Abiotic factors influence the synthesis and breakdown of primary metabolites (such as sugars, amino acids, and organic acids) and secondary metabolites (including phenolic compounds, volatile aroma compounds and their precursors) (Bulgari *et al*. 2019). These factors also impact grapevine physiology, phenology and grape composition, ultimately affecting grape yield and quality directly or indirectly (Rao *et al*. 2016; Rafique et al 2024ab). This situation has led researchers to investigate different plant activators in the field of viticulture. Affecting plant growth, nutrition, product quality and yield positively; in order to increase the resistance of plants to abiotic stress (Rouphael, 2018; Bulgari *et al*. 2019: Yilmaz and Gazioglu 2021). Biostimulants are materials that are applied to plants from the leaves, soil or seeds (Bulgari *et al*. 2019). Grapes are amongst the main crops on which biostimulants are being used (Sharma *et al*., 2023). For instance, the effects of biostimulants on grapevines under high temperatures have been studied. The results revealed that biostimulants have a positive effect on promoting the growth of grapevine seedlings under high-temperature stress conditions. They also positively affect the accumulation of chlorophyll components in grapevine leaves, inhibiting chlorophyll degradation and maintaining photosynthesis. However, the effects of different biostimulants were inconsistent (Wu et al., 2024). Biostimulants have been classified by some researchers as humic substances, amino acids and other nitrogenous compounds, seaweed and plant extracts, chitin and chitosan-like polymers, inorganic compounds, beneficial fungi and beneficial bacteria, waste, exudates and extracts of seeds, leaves and roots (Yilmaz and Gazioglu, 2021). Protein hydrolysates (PHs) are important plant biostimulants based on mixtures of peptides and amino acids mainly produced by enzymatic and/or chemical hydrolysis of proteins from animal or plant-derived raw materials (Colla *et al*., 2015). Glycine betaine (GB) is an N-trimethyl glycine derivative compound that belongs to the quaternary amines. It is found in many bacteria, plant and animal species (Monterio *et al*., 2022). It plays an adaptive role in osmoregulation and protecting the sub-cellular structures in stressed plants (Hayes *et al*., 2020). Seaweed are macroscopic multicellular algae that can be brown, red and green. They are an important source of organic matter and fertilizer nutrients (Bulgari *et al*., 2019). They are applied as foliar spray and are able to enhance plant growth, abiotic stress tolerance, photosynthetic activity and resistance to fungi, bacteria and viruses, improving the yield and productivity of several crops (Norrie *et al*., 2006; Sharma *et al*., 2014). Seaweeds used for biostimulant production contain cytokinins and auxins or other hormone-like substances (Hamza, 2001). Numerous studies have demonstrated that both microbial and non-microbial plant biostimulants have the ability to induce various morpho-anatomical, biochemical, physiological, and molecular responses in plants. These responses encompass enhancements in crop productivity, nitrogen use efficiency, and increased resilience to abiotic stresses (Thomas et al., 2024). For a plant activator to be called a biostimulant, the product must also be effective against abiotic stress conditions on the plant (Bulgari *et al*., 2019). A study found that grapevine fungal disease control has been a great challenge to winegrowers worldwide. The use of chemicals in viticulture is high, which can result in the development of pathogen resistance, increasingly raising concerns regarding residues in wine and its effects on human and environmental health. Therefore, the use of biostimulants is emerging in order to reduce the consequences of biotic and abiotic stresses in the grapevine, namely preventing grape fungal diseases, improving grapevine resistance to water stress, and increasing yield and berry quality (Monteiro et al., 2022). This study aims to evaluate the effects of Budmaker, a biostimulant, on the growth, yield, and shelf-life of Thompson Seedless grapes under multilocation trials.

**Material and Methods**

**Experimental conditions**

 The experimental trials were carried out at two different locations (at ICAR-National Research Centre for Grapes in Pune (18°32ʹN and 73°51ʹE) and farmer’s plots at Rahata (19°42ʹN and 74°28ʹE) in Ahmednagar of Maharashtra) during the year 2023-24. The grape variety Thompson Seedless was selected for the study in both locations. The experiment was laid out in RBD design with four treatments of five replications, each replication comprised of five vines. The vines were pruned twice a year in both locations. The first pruning was done during the mid-last week of April 2023 (foundation pruning) while the second pruning was done during the mid-last week of October 2023 (forward pruning). The treatments imposed during the experiment are T1: control, T2: foliar application of Budmaker @400 ml/acre, T3: foliar application of Budmaker @500 ml/acre and T4: foliar application of Budmaker @750 ml/acre. The Budmaker was applied at three different stages (at 1st leaf after sub-cane, second at 3rd and 4th leaf after sub-cane and third at 6th and 7th leaf after sub-cane). Budmaker was applied as a foliar spray, water volume used was based on the canopy size (250 to 400 L/acre).

**Growth parameters**

The length of the shoots was measured from 1st node during 120 days after fruit pruning and expressed in centimetres. The shoot diameter of the matured cane was measured between the fifth and sixth node with Vernier calliper for five cane per vine at 120 days after pruning (foundation pruning) from five vines and their mean was expressed in mm. Leaf area was measured by linear method (LBK method) expressed in cm2 (Ghule *et al*., 2019). The mathematical relationship for calculation was given as follows: Leaf area (A) = L x B x K (0.810). Pruned biomass was collected from each vine immediately after pruning and the weight of biomass was recorded using weighing balance and the mean was calculated and expressed in kg/vine. The percentage of fruitful canes was computed from the number of canes and the number of fruitful canes. Days taken for cane maturity were calculated from the date of foundation pruning to the cane maturity for individual vines and the mean was calculated.

**Bunch and yield parameters**

 The total number of bunches was counted from five vines in each treatment and the mean number of bunches per vine was calculated after the berry set. The total number of berries was counted from five bunches in each treatment and a mean number of berries per bunch was calculated. The mean weight of the bunch was recorded by averaging the weight of 10 bunches from five vines selectedrandomly at harvest. This was expressed in grams. The berries from five vines were collected randomly during harvesting. The mean weight of the berry was derived by averaging the weight of 50 berries and was expressed in grams. The grapes were harvested after attaining maturity (Total Soluble Solids (TSS) and acidity). The yield was recorded at the time of harvest and expressed in kg.

**Berry quality parameters**

 Ten berries were randomly selected from each replication and berry length and berry diameter (mm) were measured using Vernier Caliper. Randomly selected berries were taken for juice extraction and total soluble solids in the juice were determined using a hand refractometer. The TSS was measured in degree brix (°Brix). Total titratable acidity was determined by titrating the berry juice with 0.1 N NaOH and was expressed in %.

**Biochemical parameter**

 Chlorophyll content in leaves was estimated using the Dimethyl sulfoxide (DMSO) method. Phenol was estimated by Folin-Ciocalteu as suggested by Singleton and Rossi, (1965) and expressed in mg/g. Fruit soluble protein content at harvest was estimated as per the method suggested by Lowry *et al.* (1951) and expressed as milligrams per gram of fresh weight (mg/g).The percentage of reducing sugars in the grape berries was determined by the Dinitro-Salicylic acid (DNSA) method as suggested by Miller (1972). A known volume of alcohol extract was taken and allowed to evaporate the alcohol completely. The clear solution was taken for estimation of reducing sugar-using DNSA-reagent by following the above method and results were expressed in percentage. The diacid extract was used for the calcium (ppm) determination. It was determined by using the neutral normal ammonium acetate method. The digest prepared with a diacid mixture was used for the determination of phosphorous content (%) from petiole samples. The phosphorus was estimated by Venadomolybdo phosphoric acid yellow color method with a Spectrophotometer as given by Jackson (1973). The intensity of the yellow color was measured on a Double Beam Spectrophotometer using wavelength 470 nm.

**Physical properties of treated grapes**

The thickness of the pedicel was measured using a vernier caliper and expressed in millimeters. The skin of ten randomly selected berries was peeled off using a Lazar blade and skin thickness was measured by a mini portable digital caliper micrometer thickness gauge and expressed in mm. To study the change in physical properties of treated grapes with advancement in storage time, physiological loss in weight (PLW) was studied as described by Sharma *et al*., (2023). Shelf-life in terms of physiological loss in weight (%) was calculated as the percentage of mass lost by the bunch from the beginning to the end of the shelf-life period. The mass of each treatment was taken on a daily basis for 5 days. The Physical Loss in Weight (PLW) (%) at each interval was calculated as:

$$Physiological loss in weight (\%)=\frac{Initial weight-Final weight}{Initial weight }×100$$

**Statistical analysis**

The data recorded from field experiment was statistically analyzed by using Randomized Block Design (RBD) as described by Panse and Sukhatme (1985).

**Result and Discussion:**

       The data recorded on the growth parameters of Thompson Seedless grapes is presented in Table 1. Statistically significant variation was recorded in shoot length, shoot diameter, leaf area, pruned biomass, percent fruitful canes and days took to cane maturity with different concentrations of Budmaker across locations. Treatment T1 showed the highest shoot length (100.00 cm), and shoot diameter (7.44 mm). This variation could be attributed to environmental conditions and cultivation practices (Somkuwar *et al*., 2024). The maximum leaf area) was recorded in T3 (163.83 cm2) treatment whereas the lowest shoot length (82.25 cm) and minimum shoot diameter (7.05mm) was recorded in T3 which was followed by T2 and minimum leaf area in T2 (152.46 cm2) at ICAR-NRCG. The Rahata site exhibited a somewhat similar trend with distinct values. Vegetative parameters like shoot length and diameter indirectly influenced grape yield and quality. As shoot length increases, more photosynthetic products are utilized, reducing the resources available for cane development and sink growth (Somkuwar *et al*., 2024).  During October pruning, the treatment T3 recorded a higher % of fruitful canes (92.52%) and pruned biomass (0.62 kg) over the control treatment (73.21 % and 0.53 kg respectively). The early days to cane maturity were also achieved in treatment T3 (118.4 days) which was followed by T4 (123.0 days) whereas late cane maturity was achieved in T1 (Control) with123.0 days at ICAR-NRCG. Similar trends with different values were recorded for pruned biomass and fruitfulness but non-significant result was obtained in the case of days taken to cane maturity at the Rahata location. The increase in pruned biomass in Budmaker treatment over control is due to biostimulants helping plants to uptake more Nitrogen by promoting Carbon and Nitrogen metabolism in plants (Yilmaz and Gazioglu, 2021).

**Table 1: Effect of Budmaker on growth parameters of Thompson Seedless grape**

|  |  |  |  |
| --- | --- | --- | --- |
| Treatments | Foundation pruning (90 Days) | October pruning |  |
| Shoot length(cm) | Shoot diameter (mm) | Leaf area (cm2) | Pruned biomass (kg/vine) | Fruitful canes(%) | Days taken to cane maturity |
| Pune location |
| T1- Control | 100.00 | 7.44 | 155.05 | 0.53 | 73.21 | 123 |
| T2- Budmaker @ 400 ml/L | 90.01 | 7.28 | 152.46 | 0.55 | 79.67 | 121 |
| T3- Budmaker @ 500 ml/L | 82.25 | 7.05 | 163.83 | 0.62 | 92.52 | 118.4 |
| T4- Budmaker @ 750 ml/L | 85.50 | 7.2 | 160.5 | 0.54 | 82.85 | 119.8 |
| CD at 5% | 2.00 | 0.17 | 3.95 | 0.016 | 2.47 | 1.7 |
| Sig | \*\* | \*\* | \*\* | \*\* | \*\* | \*\* |
| Rahata location |
| T1- Control | 97.50 | 7.60 | 152.00 | 0.56 | 80 | 120.99 |
| T2- Budmaker @ 400 ml/L | 88.00 | 7.45 | 150.10 | 0.59 | 85 | 120.5 |
| T3- Budmaker @ 500 ml/L | 80.10 | 7.00 | 165.10 | 0.64 | 92 | 117.61 |
| T4- Budmaker @ 750 ml/L | 83.40 | 7.10 | 158.60 | 0.54 | 85.4 | 118.9 |
| CD at 5% | **1.96** | **0.18** | **4.06** | **0.02** | **2.34** | **2.74** |
| Sig | **\*\*** | **\*\*** | **\*\*** | **\*\*** | **\*\*** | **ns** |

**Bunch and yield parameters**

The data recorded on number of bunches/vines, number of berries/bunch, average bunch weight (g), 50-berry weight and yield/vine are presented in Table 2. It was observed that the application of Budmaker had no significant effect on a number of bunches per vine across locations while the number of berries/bunches had a non-significant effect at the Rahata location. This was mainly due to the fact that the fruit bud differentiation had already been completed during the period of 40 to 70 days after foundation pruning. In addition, considering the quality yield for export purposes, bunch thinning is also done after the berry set. Similarly, no significant difference in a number of bunches per vine was reported by Sharma *et al*., (2023). The treatment T3 significantly showed the highest average bunch weight (446.56 g), 50-berry weight (204.88 g) and yield/vine (15.10 kg) followed by T4 (415.60 g, 185.14 g, 13.79 kg respectively) over the control treatment T1 (351.26 g, 144.80 g, 12.00 kg respectively). The increase in yield was primarily attributed to the larger size and heavier weight of the bunches and berries, likely enhancing the efficiency of carbon assimilation through photosynthesis and protein synthesis due to the application of biostimulants Deshmukh *et al*., (2023). The greatest increase in berry and bunch weight was also reported by Secco *et al*. (2016). Use of Biostimulant significantly increased yield over control in Thompson Seedless and Sharad Seedless as reported by Sharma *et al*., (2023) and Deshmukh *et al*. (2023). The treatment T4 recorded a maximum number of berries per bunch (119.00) while the minimum number of berries was recorded in T3 (114.60) at ICAR-NRCG. A more or less similar trend with different values was recorded at the Rahata location. The increase in bunch and yield parameters in Budmaker might be due to the stimulator's ability to modify some molecular processes that allow for improvement in water and nutrient use efficiency of crops, stimulate plant development and counteract abiotic stresses (Van *et al*., 2017) by enhancing primary and secondary metabolism (Rao *et al*. 2016).

**Table 2: Effect of Budmaker on bunch and yield parameters of Thompson Seedless grapes**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatments | No. of bunches/ vine | No. of berries/bunch | Average bunch weight (g) | 50 berry weight (g) | Yield/vinekg) |
| Pune location |
| T1- Control | 34.20 | 117.60 | 351.26 | 144.80 | 12.00 |
| T2- Budmaker @ 400 ml/L | 33.04 | 116.00 | 372.98 | 164.46 | 12.33 |
| T3- Budmaker @ 500 ml/L | 33.84 | 114.60 | 446.56 | 204.88 | 15.10 |
| T4- Budmaker @ 750 ml/L | 33.16 | 119.00 | 415.60 | 185.14 | 13.79 |
| CD at 5% | 1.17 | 2.65 | 45.14 | 31.57 | 1.6 |
| Sig | NS | \* | \*\* | \*\* | \*\* |
| Rahata location |
| T1- Control | 33.10 | 100.00 | 450.00 | 225.02 | 14.92 |
| T2- Budmaker @ 400 ml/L | 32.00 | 105.00 | 500.10 | 238.16 | 17.31 |
| T3- Budmaker @ 500 ml/L | 34.50 | 106.29 | 540.00 | 254.40 | 18.50 |
| T4- Budmaker @ 750 ml/L | 35.00 | 108.00 | 535.20 | 249.41 | 17.51 |
| CD at 5% | 2.38 | 7.85 | 13.57 | 18.66 | 1.11 |
| Sig | NS | NS | \*\* | \* | \*\* |

**Berry quality parameters**

 The grape quality mainly consists of berry length, berry diameter, TSS and acidity (Anjum et al. 2020). The data recorded on grape berry quality is presented in Table 3. The use of Budmaker significantly increased berry length and berry diameter. The treatment T3 recorded the highest berry length (21.12 mm) and berry diameter (18.40 mm) followed by T4 (20.05 and 18.00 mm respectively) as compared to the untreated control T1 (18.51 and 16.80 mm respectively) at ICAR-NRCG. A comparable pattern with varying values was observed at the Rahata location. In the present study, the treatment with 500 ml/L concentration proved better in terms of berry diameter. The increase in berry size could be attributed to the stimulation of cell division and elongation, likely triggered by the application of biostimulants (Warusavitharana *et al*., 2008; Deshmukh *et al*. 2023). Berry length and berry diameter together contribute to the shape of the berry. Our result confirms the finding of Sharma *et al*. (2023) who also reported biostimulants contribute to increasing berry length and diameter significantly over control.

The use of different concentrations of Budmaker showed non-significant variation for the TSS of the grape berry. However, the TSS ranged between 18.08°Brix to 18.22°Brix where control (T1) showed maximum TSS (18.22°Brix) while least in treatment T2 (18.08°Brix). Lower TSS in treated berries was reported by Norrie and Keathley (2006). The acidity ranged from 0.52 % in T1 to 0.64 % in T3 treatment at ICAR-NRCG. The acidity in grape berries was within the acceptable limit in all the treatments. Similar trends, though with different figures were noted at Rahata. At harvest non-significant effect on total soluble solids was also reported by Frioni *et al*. (2019; Sharma *et al*. (2023) and Deshmukh *et al*., (2023).

**Table 3: Effect of Budmaker on berry quality parameters of Thompson Seedless grapes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatments** |  **Berry length (mm)** |  **Berry diameter (mm)** | **TSS (°Brix)** | **Acidity (%)** |
| **Pune location** |
| **T1- Control** | 18.51 | 16.80 | 18.22 | 0.52 |
| **T2- Budmaker @ 400 ml/L** | 19.16 | 17.50 | 18.08 | 0.62 |
| **T3- Budmaker @ 500 ml/L** | 21.12 | 18.40 | 18.12 | 0.64 |
| **T4- Budmaker @ 750 ml/L** | 20.05 | 18.00 | 18.20 | 0.58 |
| **CD at 5%** | **0.68** | **0.45** | **0.47** | **0.04** |
| **Sig.** | **\*\*** | **\*\*** | **NS** | **\*\*** |
| **Rahta location** |
| **T1- Control** | 20.00 | 18.00 | 18.00 | 0.55 |
| **T2- Budmaker @ 400 ml/L** | 21.50 | 18.50 | 18.40 | 0.60 |
| **T3- Budmaker @ 500 ml/L** | 23.60 | 20.00 | 18.80 | 0.62 |
| **T4- Budmaker @ 750 ml/L** | 22.80 | 19.40 | 18.50 | 0.56 |
| **CD at 5%** | **0.60** | **0.49** | **1.40** | **0.04** |
| **Sig.** | **\*\*** | **\*\*** | **NS** | **\*\*** |

**Chlorophyll content in leaf**

 The application of Budmaker significantly increased chlorophyll content in leaves in the present study. The data recorded on chlorophyll content in the leaf at 90 days after foundation and fruit pruning of grapes is presented in Table 4. Chlorophyll b content in the leaf at 90 days after the foundation pruning and also at 90 days after the fruit pruning was non-significant among treatments. Treatment T3 showed the highest chlorophyll a and total chlorophyll content (12.07 ug/ml and 16.18 ug/ml) while treatment T1 had the least chlorophyll a content (9.82 ug/ml and 13.06 ug/ml) with the application of foliar spray of Budmaker at ICAR-NRCG. slightly different set of values but a similar trend was recorded for foundation pruning at Rahata. Except, after 90 days of fruit pruning, with the application of Budmaker the treatment T2 showed maximum chlorophyll-a (15.50 ug/ml) followed by T4 (14.10 ug/ml) compared to the lowest in control T1 (12.80 ug/ml). The chlorophyll b was higher in T3 (4.10 ug/ml) compared to the lowest in control T1 (2.70 ug/ml). Total chlorophyll content in grape leaf was higher in T2 (19.30 ug/ml) followed by T3 (17.60 ug/ml) while lowest in control T1 (15.50 ug/ml). The increase in chlorophyll content in Budmaker treatments might be due to an increase in photosynthesis, nutrient uptake, iron and magnesium which are essential elements for chlorophyll biosynthesis. The rise in chlorophyll content resulted from a decrease in its degradation and an enhancement in chloroplast biogenesis. One of the roles of biostimulant treatment is an increase in chlorophyll content in the treated plant has been recorded by Battacharyya, *et al*. (2015) and Sharma *et al*. (2023).

**Table 4. Effect of Budmaker on chlorophyll content in the leaf of Thompson Seedless grapes**

|  |  |  |
| --- | --- | --- |
| **Treatments** | **90 Days Foundation Pruning** | **90 Days Fruit Pruning** |
| **Chlorophyll a (ug/ml)** | **Chlorophyll b (ug/ml)** | **Total Chlorophyll (ug/ml)** | **Chlorophyll a (ug/ml)** | **Chlorophyll b (ug/ml)** | **Total Chlorophyll (ug/ml)** |
| **Pune location** |
| **T1- Control** | 9.82 | 3.24 | 13.06 | 11.44 | 2.76 | 14.20 |
| **T2- Budmaker @ 400 ml/L** | 10.77 | 3.71 | 14.48 | 12.55 | 2.56 | 15.12 |
| **T3- Budmaker @ 500 ml/L** | 12.07 | 4.12 | 16.18 | 11.69 | 3.04 | 14.73 |
| **T4- Budmaker @ 750 ml/L** | 11.96 | 3.91 | 15.87 | 12.21 | 2.92 | 15.13 |
| **CD @ 5%** | 1.20 | 0.75 | 1.90 | 1.09 | 0.52 | 1.36 |
| **Sig** | \*\* | **NS** | **\*** | **NS** | **NS** | **NS** |
| **Rahta location** |
| **T1- Control** | 9.00 | 3.80 | 12.80 | 12.80 |  2.70 |  15.50 |
| **T2- Budmaker @ 400 ml/L** | 11.50 | 4.60 | 16.10 | 15.50 |  3.80 | 19.30 |
| **T3- Budmaker @ 500 ml/L** | 13.20 | 5.10 | 18.30 | 13.50 | 4.10 | 17.60 |
| **T4- Budmaker @ 750 ml/L** | 12.00 | 4.00 | 16.00 | 14.10 | 3.00 | 17.10 |
| **CD @ 5%** | 0.37 | 1.00 | 1.17 | 0.33 | 0.12 | 0.74 |
| **Sig** | \*\* | **NS** | **\*\*** | **\*\*** | **\*\*** | **\*\*** |

**Biochemical contents in grape berries**

 The data recorded on biochemical contents (phenol, protein, reducing sugar, calcium and phosphorus) is presented in Table 5. Statistically significant variation was found in phenol, protein, reducing sugar, calcium and phosphorous % at full bloom and veraison stage of berry development except at ICAR-NRCG non-significant difference was recorded in phosphorous content. Phenolic compounds constitute one of the most important groups of plant metabolites, as they participate in a multitude of physiological processes (Martínez-Lorente *et al*. 2024). Phenol was relatively higher in T3 (0.52 mg/g) while it was lowest in T1 (0.36 mg/g) treatment. The application of biostimulant has been found to increase phenolic compounds in different plant parts such as fruits, leaves and roots of multiple crops (Martínez-Lorente *et al*. 2024). Similarly, treatment T3 recorded the highest protein and reducing sugar (23.50 and 245.20 mg/g respectively) followed by T4 (22.18 and 240.94 mg/g respectively) whereas T1 showed the lowest protein content (18.20 mg/g) while reducing sugar was less in T2 (175.10 mg/g). The application of biostimulants provides a balance during maturity, preserves the sugar content of fruits and increases the anthocyanin and polyphenol contents (Salvi *et al*., 2015).

 The maximum calcium content in grape berries was recorded in treatment T4 (33.48 ppm) followed by T2 and T3 (33.08 and 31.74 ppm) while the minimum in T1 (23.72 ppm) at ICAR-NRCG. Rahata's location showed a comparable trend but with variations in the recorded values for phenol, protein and reducing sugar. However, the maximum calcium content was recorded in treatment T3 (34.00 ppm) which was followed by T4 (33.80 ppm) and T2 (32.20 ppm) while minimum calcium in T1 (24.00 ppm). The maximum phosphorous content in leaf petiole at full bloom and veraison stage was recorded in T3 (0.520 %) and T4 (0.251 %), whereas minimum phosphorous content in leaf petiole at full bloom and veraison stage was recorded in T1 (0.410 and 0.225 %). Phosphorus (%) content in leaf petiole was positively correlated with fruitful canes percent (0.880). Phosphorus is essential for plant energy transfer through the formation of ATP and other nucleotide triphosphates. It supports the synthesis of key molecules like sucrose, phospholipids, cellulose, and nucleic acids (DNA and RNA) which are crucial for cell structure and function, including protoplasm, the nucleus and cell walls. Its mobility within plants allows efficient translocation, ensuring it reaches all parts to sustain vital cellular processes (El-Boray *et al*. 2007). Nutrient absorption and assimilation from the soil are crucial for healthy plant growth as they are required for the production of essential metabolites and enzymes, as well as serving as cofactors in various physiological processes. Many researchers reported that different biostimulants can significantly improve the uptake of phosphorus (P) and calcium (Ca) in different fruit crops (Martínez-Lorente *et al*. 2024).

**Table 5: Effect of Budmaker on biochemical parameters of Thompson Seedless grapes**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | **Phenol mg/g** | **Protein mg/g** | **Reducing sugar mg/g** | **Calcium (ppm)** | **Phosphorus (%) full bloom** | **Phosphorus (%)****at veraison**  |
| **Pune location** |
| **T1- Control** | 0.36 | 18.20 | 222.00 | 23.72 | 0.419 | 0.219 |
| **T2- Budmaker @ 400 ml/L** | 0.49 | 22.02 | 175.10 | 33.08 | 0.503 | 0.225 |
| **T3- Budmaker @ 500 ml/L** | 0.52 | 23.50 | 245.20 | 31.74 | 0.531 | 0.238 |
| **T4- Budmaker @ 750 ml/L** | 0.46 | 22.18 | 240.94 | 33.48 | 0.497 | 0.243 |
| **CD at 5%** | **0.07** | **0.62** | **6.22** | **3.05** | **0.09** | **0.04** |
| **Sig** | **\*\*** | **\*\*** | **\*\*** | **\*\*** | **NS** | **NS** |
| **Rahta location** |
| **T1- Control** | 0.40 | 19.00 | 230.00 | 24.00 | 0.410 | 0.225 |
| **T2- Budmaker @ 400 ml/L** | 0.54 | 22.60 | 180.00 | 32.20 | 0.500 | 0.230 |
| **T3- Budmaker @ 500 ml/L** | 0.56 | 24.00 | 248.00 | 34.00 | 0.520 | 0.245 |
| **T4- Budmaker @ 750 ml/L** | 0.51 | 23.10 | 244.10 | 33.80 | 0.495 | 0.251 |
| **CD at 5%** | **0.02** | **0.62** | **6.33** | **1.41** | **0.013** | **0.005** |
| **Sig** | **\*\*** | **\*\*** | **\*\*** | **\*\*** | **\*\*** | **\*\*** |

**Shelf life**

              The data on the shelf life of grapes, in terms of PLW (%) during storage at room temperature is presented in Table 6. In all the treatments, the PLW (%) increased with the advancement in storage duration. The minimum physiological loss in weight (%) was recorded in treatment T3 from 1st day (1.23 %), 2nd day (2.39 %), 3rd day (3.03 %), 4th day (3.26 %) and 5th day (5.00 %). The physiological loss in weight (%) in grape berries of control treatment increased rapidly from 1st day (1.54 %), 2nd day (2.82 %), 3rd day (3.70 %), 4th day (4.19 %) and 5th day (6.12 %) at ICAR-NRCG. At Rahata, the trends were similar, but the values varied. The data recorded on pedicel thickness and skin thickness of fresh grape berries is presented in Table 7. Pedicel thickness was relatively higher in T3 (0.544 mm) while it was lowest in T1 (0.420 mm) treatment. The treatment T4 recorded maximum skin thickness (0.247 mm) while it was minimum in T1 (0.183 mm) treatment at the Pune location. A trend of a similar nature, though with fluctuating values was seen at Rahata. However, the maximum pedicel and skin thickness contribute in increasing the storability of grape bunch. Similarly, Deshmukh *et al*. (2023) also reported maximum skin thickness in biostimulant-treated vines leads to an increase in the storage life of grapes compared to untreated ones. The application of biostimulants may activate various lipid peroxidation processes and defense-related enzymes, which contribute to preserving the firmness of grape berries. This also helps reduce fruit drop, minimize physiological weight loss, and prevent berry decay during storage (Liu *et al*., 2016; Zaharah *et al*., 2012; Deshmukh *et al*., 2023; Sharma *et al*., 2023).

**Table 6: Effect of Budmaker on physiological loss in weight (%) of Thompson Seedless grapes**

|  |  |
| --- | --- |
| **Treatments** | **Physiological loss in weight (%)** |
| **1 day** | **2 day** | **3 day** | **4 day** | **5 day** |
| **Pune location** |
| **T1- Control** | 1.54 | 2.82 | 3.70 | 4.19 | 6.12 |
| **T2- Budmaker @ 400 ml/L** | 1.46 | 2.55 | 3.59 | 4.00 | 5.67 |
| **T3- Budmaker @ 500 ml/L** | 1.23 | 2.39 | 3.03 | 3.26 | 5.00 |
| **T4- Budmaker @ 750 ml/L** | 1.36 | 2.50 | 3.34 | 3.85 | 5.12 |
| **Rahta location** |
| **T1- Control** | 1.85 | 3.13 | 3.86 | 4.51 | 6.29 |
| **T2- Budmaker @ 400 ml/L** | 1.59 | 2.96 | 3.83 | 4.14 | 5.97 |
| **T3- Budmaker @ 500 ml/L** | 1.28 | 2.73 | 3.26 | 3.49 | 5.22 |
| **T4- Budmaker @ 750 ml/L** | 1.48 | 2.78 | 3.59 | 4.11 | 5.28 |

**Table 7: Effect of Budmaker on pedicel thickness (mm) and skin thickness (mm) of Thompson Seedless grapes**

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Pune location** | **Rahta location** |
| **Pedicel thickness (mm)** | **Skin thickness (mm)** | **Pedicel thickness (mm)** | **Skin thickness (mm)** |
| **T1- Control** | 0.420 | 0.183 |  0.410 | 0.195 |
| **T2- Budmaker @ 400 ml/L** | 0.506 | 0.199 |  0.450 | 0.200 |
| **T3- Budmaker @ 500 ml/L** | 0.544 | 0.223 |  0.535 | 0.250 |
| **T4- Budmaker @ 750 ml/L** | 0.456 | 0.247 |  0.500 | 0.235 |
| **CD at 5%** | 0.09 | 0.04 | 0.011 | 0.006 |
| **Sig** | **\*** | **\*** | **\*\*** | **\*\*** |

**Conclusion:**

 A multilocation trial was conducted in Thompson Seedless for bio efficiency studies of Budmaker during 2023-24. Different doses of Budmaker were applied through sprays and compared with untreated control. All the treatments of Budmaker significantly increased fruit bud differentiation, early cane maturity, grape yield, and berry quality parameters as well as shelf life as compared to untreated control. In both locations, among the different treatments of Budmaker, the treatment T3 i.e., application of 500 ml/L Budmaker through foliar spray (after sub-cane-emergence of 1st leaf, after emergence of 3rd and 4th leaf and after 6th and 7th leaf emergence) showed better performance for fruitfulness, 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 Budmaker with its higher concentration at all three different stages could be suggested to improve the quality and yield of grapevine.

**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.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

**References**

Anjum, N., Feroze, M.A., Rafique, R., Shah, M.H., 2020. Effect of gibberellic acid on berry yield and quality attributes of grapes cv. sultanina. PAB 9 (2), 1319–1324. https://doi.org/10.19045/bspab.2020.90137.

Battacharyya D, Babgohari M, Rathor P and Prithiviraj B (2015) Seaweed extracts as biostimulants in horticulture. Sci. Hortic 196: 39-48.

Bulgari, R., Franzoni, G., & Ferrante, A. (2019). Biostimulants application in horticultural crops under abiotic stress conditions. *Agronomy*, *9*(6), 306.

Colla, G.; Nardi, S.; Cardarelli, M.; Ertani, A.; Lucini, L.; Canaguier, R.; Rouphael, Y. Protein hydrolysates as biostimulants in horticulture. Sci. Hortic. (Amst.) 2015, 196, 28–38.

Deshmukh, N. A., Saste, H., Gat, S., & Gather, S. K. (2023). Influence of a Biostimulant on Yield and Quality of Sharad Seedless Grape. *Grape Insight*, 89-95.

El-Boray, M. S., Mostafa, M. F., & Hamed, A. A. (2007). Effect of some biostimulants on yield and berry qualities of grapevines. *Journal of Plant Production*, *32*(6), 4729-4744.

El-Boray, M. S., Mostafa, M. F., & Hamed, A. A. (2007). Effect of some biostimulants on yield and berry qualities of grapevines. *Journal of Plant Production*, *32*(6), 4729-4744.

Frioni, T, Tombesi S, Quaglia M, Calderini O, Moretti C, Poni S and Palliotti A (2019) Metabolic and transcriptional changes associated with the use of Ascophyllum nodosum extracts as tools to improve the quality of wine grapes (Vitis vinifera cv. Sangiovese) and their tolerance to biotic stress. J. Sci. Food Agric 99(14): 6350-6363.

Frioni, T.; Tombesi, S.; Quaglia, M.; Calderini, O.; Moretti, C.; Poni, S.; Gatti, M.; Moncalvo, A.; Sabbatini, P.; Berrìos, J.G.; *et al*. Metabolic and transcriptional changes associated with the use of Ascophyllum nodosum extracts as tools to improve the quality of wine grapes (Vitis vinifera cv. Sangiovese) and their tolerance to biotic stress. J. Sci. Food Agric. 2019, 99, 6350–6363.

Ghule V, Zagade P, Bhor V and Somkuwar R (2019) Rootstock affects graft success, growth and physiological parameters of grape varieties (Vitis vinifera L.). Int. J. Curr. Microbiol. Appl. Sci. 8(1): 799-805.

Hamza,B.B.; Suggars, A. Biostimulants: Myths and Realities. TurfGrass Trends 2001, 8, 6–10.

Hayes, M. A., Shor, A. C., Jesse, A., Miller, C., Kennedy, J. P., Feller, I. (2020). The role of glycine betaine in range expansions; protecting mangroves against extreme freeze events. Journal of Ecology 108(1): 61-69.

Jackson, M. L. (1973). Vanadomolybdo phosphoric yellow colour method for determination of phosphorus. *Soil Chemical Analysis*, 151-154.

Khatoon F, Kundu M, Mir H and Nahakpam S (2021) Efficacy of foliar feeding of brassinosteroid to improve growth, yield and fruit quality of strawberry (Fragaria × Ananassa Duch.) grown under subtropical plain. Communications in Soil Science and Plant Analysis 52(8):803-814 https:// doi.org/10.1080/00103624.2020.1869765.

Liu Q, Xi Z, Gao J, Meng Y, Lin S and Zhang Z (2016) Effects ofexogenous 24-epibrassinolide to control grey mould and maintain post-harvest quality of table grapes. International Journal of Food Science and Technology 51:236-1243 <https://doi.org/10.1111/ijfs.13066>.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J biol Chem*, *193*(1), 265-275.

Martínez-Lorente, S. E., Martí-Guillén, J. M., Pedreño, M. Á., Almagro, L., & Sabater-Jara, A. B. (2024). Higher Plant-Derived Biostimulants: Mechanisms of Action and Their Role in Mitigating Plant Abiotic Stress. *Antioxidants*, *13*(3), 318.

Miller G. L. (1972). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry, 31, 426 – 428.

Monteiro, E., Gonçalves, B., Cortez, I., & Castro, I. (2022). The role of biostimulants as alleviators of biotic and abiotic stresses in grapevine: A review. *Plants*, *11*(3), 396.

Norrie J and Keathley J (2006) Benefits of Ascophyllum nodosum marine-plant extract applications to ‘Thompson Seedless’ grape production. Acta Hort 727: 243–248.

Panse, V.G. and Sukhatme, P.V. 1985. Statistical methods for Agricultural workers. ICAR Pub, New Delhi, pp 115-130.

Rafique, R., Ahmad, T., Ahmed, M., Abbasi, N.A., Hoogenboom, G., 2021. Seasonal variability in temperature affects key phenological stages of table grapes cultivars- a case study from Pothwar region of Pakistan [Abstract]. In: Proceedings of the ASA, CSSA, SSSA International Annual Meeting. Salt Lake City, UT. https://scisoc.confex. com/scisoc/2021am/meetingapp.cgi/Paper/134870. Accessed on 12 Feb 2023.

Rafique, R., Ahmad, T., Ahmed, M., Khan, M.A, Wilkerson, C.J., Hoogenboom, G., 2023b. Seasonal variability in the effect of temperature on key phenological stages of four table grapes cultivars. Int. J. Biometeorol. [https://doi.org/10.1007/s00484-023- 02452-0](https://doi.org/10.1007/s00484-023-%2002452-0).

 Rafique, R., Ahmad, T., Kalsoom, T., Khan, M.A., Ahmed, M., 2023a. Climatic challenge for global viticulture and adaptation strategies. Global Agricultural Production: Resilience to Climate Change. Springer International Publishing, Cham, pp. 611–634.

Rafique, R., Ahmad, T., Khan, M.A., Ahmed, M., 2023c. Exploring key physiological attributes of grapevine cultivars under the influence of seasonal environmental variability. Oeno One 57 (2), 381–397.

Rafique, R., Ahmad, T., Khan, M.A., Ahmed, M., Hoogenboom, G., 2024. Developing a simple and efficient modeling solution for predicting key phenological stages of table grapes in a non-traditional viticulture zone in south Asia. Int. J. Biometeorol. 1–15. <https://doi.org/10.1007/s00484-024-02686-6>.

Rafique, R., Ahmad, T., Ahmed, M., & Khan, M. A. (2024). Adapting the process based STICS model to simulate phenology and yield of table grapes-a high value fruit crop in a new emerging viticulture zone of South Asia. Scientia Horticulturae, 336, 113419.

Rao, N.K.S.; Laxman, R.H.; Shivashankara, K.S. Physiological and Morphological Responses of Horticultural Crops to Abiotic Stresses. In Abiotic Stress Physiology of Horticultural Crops; Rao, N.K.S., Shivashankara, K.S., Laxman, R.H., Eds.; Springer: New Delhi, India, 2016; pp. 3–7, ISBN 978-81-322-2723-6.

Rouphael, Y.; Colla, G. Synergistic Biostimulatory Action: Designing the Next Generation of Plant Biostimulants for Sustainable Agriculture. Front. Plant Sci. 2018, 9, 1655.

Salvi L, Cataldo E, Secco S, Mattii GB, 2015, November. Use of natural biostimulants to improve the quality of grapevine production: first results. In II World Congress on the Use of Biostimulants in Agriculture 1148 (pp. 77-84).

Secco, S., Mattii, G. B., Salvi, L., & Cataldo, E. (2015, November). Use of natural biostimulants to improve the quality of grapevine production: first results. In *II World Congress on the Use of Biostimulants in Agriculture 1148* (pp. 77-84).

Sharma, A. K., Somkuwar, R. G., Upadhyay, A. K., Kale, A. P., Palghadmal, R. M., & Shaikh, J. (2023). Effect of Bio-stimulant Application on Growth, Yield and Quality of Thompson Seedless. *Grape Insight*, 48-53.

Sharma, H.S.S.; Fleming, C.; Selby, C.; Rao, J.R.; Martin, T. Plant biostimulants: A review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses. J. Appl. Phycol. 2014, 26, 465–490.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

Somkuwar RG, Kakade PB, Ghule VS, Sharma AK. Performance of grape varieties for raisin recovery and raisin quality under semi-arid tropics. Plant Archives. 2024b;24(1):61-66.

Van Oosten, M.J.; Pepe, O.; De Pascale, S.; Silletti, S.; Maggio, A. The role of biostimulants and bio effectors as alleviators of abiotic stress in crop plants. Chem. Biol. Technol. Agric. 2017, 4.1, 5.

Warusavitharana AJ, Tambe TB and Kshirsagar DB (2008) Effect of cytokinin’s and brassinosteroid with gibberellic acid on yield and quality of Thompson Seedless.

Yılmaz, Y., & Şensoy, R. İ. G. (2021). The use of biostimulants in sustainable viticulture. *Journal of the Institute of Science and Technology*, *11*(2), 846-856.

Zaharah SS, Singh Z, Symons GM and Reid JB (2012) Role of brassinosteroids, ethylene, abscisic acid, and indole-3- acetic acid in mango fruit ripening. Journal of Plant Growth Regulation 31:363-372 https://doi.org/10.1007/ s00344-011-9245-5.

Wu, J., Zhong, H., Ma, Y., Bai, S., Yadav, V., Zhang, C., ... & Wang, X. (2024). Effects of different biostimulants on growth and development of grapevine seedlings under high-temperature stress. *Horticulturae*, *10*(3), 269.

Monteiro, E., Gonçalves, B., Cortez, I., & Castro, I. (2022). The role of biostimulants as alleviators of biotic and abiotic stresses in grapevine: A review. *Plants*, *11*(3), 396.

Thomas, J. M., Reshmi CR, Rafeekher M, Priyakumari I, & Aparna B. (2024). Biostimulants for Promoting Growth, Yield and Flower Quality in Anthurium andreanum Lind. *International Journal of Environment and Climate Change*, *14*(2), 330–339.