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BIO-EFFICACY STUDIES OF BIO-STIMULANT (CALSIB^{++}) FOR IMPROVING BERRY QUALITY IN THOMPSON SEEDLESS GRAPES

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ABSTRACT

In the present study, Thompson Seedless grapes grafted on Dogridge rootstock were treated with CalSiB^{++} (Biostimulant), a foliar nutrient to enhance yield, quality and shelf-life. Four different dosages of CalSiB^{++} were used at three critical growth stages (berry setting, 8–10 mm berry size and 12–14 mm berry size) and was compared with untreated control. The treatment T_2 (CalSiB^{++} @ 2.0 g/L) significantly improved bunch weight (451.25 g), 50-berry weight (189.88 g), total yield (34.65 t/ha) and reduced PLW to 5.19%. However, T_2 recorded higher nutrient levels in the grape berries, with increased level of phosphorus, calcium and magnesium. The additional benefits included enhanced berry firmness (74.50%), skin thickness (0.30 mm) which were the critical factors for preserving fruit quality during storage and transport. These findings indicated that foliar application of CalSiB^{++} @ 2.0 g/L, applied at three growth stages has optimally improved both yield and quality, supporting its recommendation as an effective practice for grape production.

Key words : Bio-Stimulant, Foliar Spray, Nutrient, Calcium, Boron, Yield.

Introduction

Grape (*Vitis vinifera* L.) is a temperate fruit crop well adapted to the tropical and subtropical regions of India. Grapes has a varied usage as table grapes, raisins, juice and wine, purpose depends on the crop variety and place of cultivation also (Shabeer *et al*., 2023). Comparing with other countries, India has fastened production of grapes in the recent years. In India, 78% of total grape consumed as fresh grapes, 17-20% used as raisins and only 2% is used in wine making. The grapes are mainly consumed as fresh grapes in India. The total production of grapes in India is 38.96 lakh MT, grown over an area of 1.76 lakh ha with average productivity of 22.15 MT/ha (Anonymous, 2024). Maharashtra has major share with about 70% contribution of the total grape production followed by Karnataka, Tamil Nadu, Mizoram and some parts of North India. In Maharashtra, Ahmednagar, Nasik, Pune, Solapur and Sangli districts are the major grape growing regions (Bhosale *et al*., 2016). Good quality yield results from the influence of climate, irrigation, nutrients and soil. Pruning at regular interval has influence in growth and yield. Manures and Fertilizers has a role in increasing yield and quality. Nutrition plays an important role in managing the growth, crop yield and berry composition. Regular analysis of soil and petiole nutrients is being performed for formulation of fertilizer schedule and to avoid excess application of fertilizers. Nutrient as soil application, fertigation and as a foliar application is generally being followed after each pruning to supplement the growth. Foliar application avoids the leaching losses compared to the soil application. Each stage of growth demands specific nutrients. After berry set, supply of nitrogen, potassium, calcium, magnesium, boron and zinc becomes essential. Deficiency of nutrients may result into physiological disorders and affects growth. Foliar application has positive impact in vines even in stress condition unless like soil application. Foliar application of urea has notable increase in aromatic and phenolic compounds of wine (Gutiérrez *et al.*, 2022). It increases the yield as well as the quality. Each nutrient has irreplaceable role in vines physiology and function. The lack of nutrient in certain stage cause deficiency which affects crops productivity. Among the nutrients, calcium and boron are well known for development and maintenance of structural properties (Swathi *et al.*, 2019). Ca deficiency causes blossom end rot results low bunch yield and quality. Grapes require adequate and regular supply of boron especially in flowering and berry set which also has indispensable role in pollen formation. Foliar application of boron increases berry set and finally the yield. In case of boron deficiency, grape affects with hen and chicken disorder. Calcium and boron are applied as a foliar spray rather than as general spray applied to avoid deficiency while development. The nutrients are being applied as single fertilizer or mixed fertilizer. Micro encapsulated nutrients are needed in lesser quantity, but has a sustained nutrient releasing behaviour (Timilsena *et al.*, 2015). Encapsulated nutrients have release rate and release pattern in water soluble fertilizers, by which nutrient absorption of crop is increased (Bons *et al.*, 2023). Also, calcium application helps to reduce physiological disorders and increases berry firmness and gives resistance to the diseases (Colapietra *et al.*, 2005). Considering this, the research was conducted to study the effect of CalSiB^{++} especially nutrient on berry development in Thomson Seedless grapes grafted on Dogridge rootstock.

Materials and Methods

Experimental condition

The experimental trial was conducted at Farmers field in Rahata Taluka, Dist. Ahmednagar during the year 2020-21. The place is in Maharashtra (latitude 19°71′N and longitude 74°48′E). Four years old of Thompson Seedless grapes grafted on Dogridge rootstocks were selected for the study. The vines were trained to Y trellises spaced at 2.743 m between rows and 1.524 m between vines thus accommodating 968 vines per acre. The experiment was laid out in RBD design with five treatments and four replications. Under the tropical conditions the vines are pruned twice in a year. First pruning was done at 14th April, 2020 (foundation pruning) while the second pruning in the month of 21th October, 2020 (forward pruning). The experiment consisted of five treatments of CalSiB^{++} (a speciality nutrient with calcium, silicon and boron fortified with other micronutrients; calcium is sourced from organic source, making it suitable for foliar spray): T_1 (1 g/L of CalSiB^{++}), T_2 (2 g/L of CalSiB^{++}), T_3 (3 g/L of CalSiB^{++}), T_4 (1 g/L, Chelated Calcium) and T_5 (control, no application). These treatments were applied at three distinct stages of berry development: berry set, 8-10 mm berry diameter and 10-12 mm berry diameter.

Table 1 : Treatment details.

Stage	Berry Setting	8-10 mm (Berry stage)	12-14 mm (Berry stage)
T_1	1.0 g/L	2.0 g/L	1.0 g/L
T_2	2.0 g/L	2.0 g/L	2.0 g/L
T_3	3.0 g/L	3.0 g/L	3.0 g/L
T_4 (Recommended)	1.0 g/L	1.0 g/L	1.0 g/L
T_5 (Control)	Untreated	Untreated	Untreated

Total number of bunches were counted from selected vines in each treatment. The mean weight of a bunch was recorded by averaging the weight of 10 bunches collected from five vines selected randomly. The berries from five vines were collected randomly and the mean berry weight was derived by averaging the weight of 50 berries. The individual vine was harvested and weight of all harvested bunches was recorded. The mean berry diameter and berry length was derived by averaging the length of 10 berries using Vernier calliper. Berry firmness was recorded by Agrostat instrument (model no Agrostat 100 field version 6), while berry skin thickness was measured by a mini portable digital micro-meter thickness gauge. Randomly selected berries were taken for juice extraction and total soluble solids in the juice was recorded using hand refractometer and was expressed in degree brix. Titratable acidity of fresh juice was determined using 0.1N NaOH with phenolphthalein indicator (Paul *et al.*, 2010). Grape berries were stored and weight loss of berries were studied.

To study content of minerals in petiole, physiologically matured grape berries free from damage or defects and petiole at veraison stage were collected. The separated petioles were washed carefully to removed surface contamination with liquid soap solution followed by tap water and then distilled water. For nutrient analysis, the samples were placed in paper bags and dried in a hot air oven at 70°C for 72 hours. Dried petiole samples were crushed using sample mill grinder (Labtech). The digested sample was taken for further analysis of macro and micro nutrients. All other nutrients were simultaneously analysed by Inductively Coupled Plasma Mass Spectrometer (Thermo Scientific).

Statistical analysis

The recorded data was analysed using SAS Version 9.3. Tukey's test was used for comparing treatment means.

Results and Discussion

Significant differences were recorded for bunches/ vine, number of berries per bunch, 50-berry weight and average bunch weight. It was observed that with the application of CalSiB^{++}, there was no effect on number of bunches per vine. This was mainly because the fruit bud differentiation was already completed during the period of 40 to 70 days after the foundation pruning (Table 2). Average bunch weight varied significantly among the different doses of CalSiB^{++} over the untreated control vines. At harvest, treatment T_3 recorded highest average bunch weight (460.88 g) followed by T_2 (451.258 g) as compared to the untreated control (311.25 g). The weight of individual berry contributes for average bunch weight via 50-berry weight. The treatment T3 recorded 195.50 g berry weight as compared to lowest in untreated control (149.88 g). Similar findings were reported in previous studies. Kumar *et al*. (2004) observed that the application of boron, as well as a combination of zinc and boron, increased cluster weight in Muscat grapes. Similarly, Bonomelli *et al*. (2010) found that calcium application enhanced crop yield by increasing berry weight. A significant increase in average bunch weight at harvest was noted with varying doses of CalSiB^{++}. Ekbic *et al*. (2018) reported that 0.2% boric acid application improved berry weight, a result also supported by Fawzi *et al*. (2014).

Table 2 : Effect of CalSiB^{++} on bunch parameter.

Treatments	Bunches /vine	Av. bunch wt. (g)	50 berry wt. (g)
T_1	31.00	402.00	184.38
T_2	31.75	451.25	189.88
T_3	31.00	460.88	195.50
T_4 (Recommended)	31.00	365.88	163.75
T_5 (Control)	30.25	311.25	149.88
S Em ±	0.435	16.74	9.932
CD at 5%	1.341	51.6	30.6
Significance	NS	**	*

Table 3 : Effect of CalSiB^{++} on yield parameters.

Treatments	Yield/vine (kg)	Yield/acre (Tons)	Yield/ha (Tons)
T_1	12.47	12.07	30.17
T_2	14.32	13.86	34.65
T_3	14.29	13.83	34.57
T_4 (Recommended)	11.35	10.99	27.47
T_5 (Control)	9.41	9.11	22.77
S Em ±	0.576	0.552	1.381
CD at 5%	1.775	1.702	4.256
Significance	**	**	**

Application of CalSiB^{++} resulted into increase in yield per vine and yield/ha (Table 3). The treatment T_2 recorded higher yield (34.65 t/ha) followed by T_3 (34.57 t/ha) while the untreated control T5 recorded lowest yield of 22.77 t/ha indicating the significance of CalSiB^{++} in improving the grape yield. Our results align with the previous findings of Wang *et al*. (2019) on enhancing grape yield through nutrient applications who reported that calcium chloride treatment significantly increased wine grape yields. Similarly, El-Katawy *et al*. (2024) found that combining calcium and magnesium fertigation with molybdenum spray yielded the highest grape production. Swathi *et al*. (2019) reported higher grape yield using combined application of boric acid (0.2%) and calcium nitrate (0.5%) compared to individual applications of calcium and boron which might be likely due to the slow release of encapsulated nutrients. Ekbic *et al*. (2018) and Fawzi *et al*. (2014) both demonstrated yield improvements with boron, and Fawzi highlighting further benefits when boron was used with urea. Hegazy *et al*. (2018) confirmed increased yield per hectare with a combination of Zn, B, and Mg. Most recently, Lateef *et al*. (2023) observed substantial yield gains from applying a calcium-boron complex. These studies collectively underscore the benefits of targeted nutrient combinations in optimizing grape production.

The grape quality consists mainly of berry diameter, berry length, skin thickness, berry firmness, TSS and acidity in the grape berries. The data recorded on grape berry quality is presented in Table 4. Berry diameter varied significantly among the different treatments. At harvest, the treatment T_2 recorded higher berry diameter (19.00 mm) followed by T_3 (18.75 mm) which was significantly superior over T_1 (18.25 mm) and untreated control T5 (17.50 mm). The quality of grapes considered for export are at 18.00 mm berry diameter. Berry length also varied significantly among the different treatments. At harvest, the treatment T_2 recorded higher berry length (24.00 mm) followed by T_3 (23.25 mm) and untreated control T_5 (21.38 mm). In the present study, all the treatments proved better in terms of berry diameter and berry length over the untreated control. Berry length and berry diameter together contributes for shape of the berry. Ekbic *et al*. (2018) observed that applying 0.2% boron resulted in the longest berry length, along with an increase in berry size and volume. Similarly, Hegazy *et al*. (2018) reported that the combined application of zinc, boron and magnesium

Table 4 : Effect of Calsib++ on berry quality.

Treatments	Berry diameter (mm)	Berry length (mm)	Skin thickness (mm)	Berry firmness (%)	TSS (^{0}Brix)	Acidity (g/L)
T_1	18.25	21.25	0.213	61.50	17.90	5.11
T_2	19.00	24.00	0.305	74.50	18.30	5.20
T_3	18.75	23.25	0.244	76.25	18.17	5.15
T_4 (Recommended)	18.00	21.50	0.275	62.00	18.13	5.19
T_5 (Control)	17.50	21.38	0.209	68.75	18.00	5.02
S Em ±	0.27	0.559	0.023	3.719	0.08	0.023
CD at 5%	0.832	1.725	0.072	11.45	0.247	0.073
Significance	*	*	NS	*	*	**

significantly increased grape yield by enhancing cluster size. More recently, El-Katawy *et al.* (2024) found that fertigation with calcium and magnesium maximized cluster length in grapes. The differences for berry skin thickness among the different concentrations of CalSiB^{++} were non-significant. However, at harvest the treatment T_2 recorded higher skin thickness (0.305 mm) followed by T_4 (0.275 mm) as compared to the untreated control (0.290 mm). The highest skin firmness was recorded in T_3 (76.25 %) followed by T_2 (74.50%) which was significantly superior over untreated control T_5 (68.75%). The variation in increase in skin thickness and skin firmness among the different treatments was recorded in the present study. For fresh grape consumption, mechanical properties such as grape firmness and skin thickness are essential for quality (Poposka *et al.*, 2023). Swathi *et al.* (2019) reported the highest berry firmness when grapes were treated with a calcium nitrate and boric acid spray, while untreated grapevines showed the lowest firmness. Additionally, Martin *et al.* (2021) confirmed that calcium spray applications can improve berry firmness, enhancing the consistency of the fruit. The differences for TSS among the different treatments were non-significant. However, minimum requirement of berry TSS (above 18°Brix) was achieved in the vine treated with CalSiB^{++}. Swathi *et al.* (2019) observed an increase in total soluble solids (TSS) in grape berries treated with a combination of calcium and boron. Similarly, El-Katawy *et al.* (2024) reported higher TSS levels when calcium and magnesium were applied together in equal doses. Qaoud *et al.* (2019) found that a combined application of magnesium, boron and amino acids also led to an increase in TSS in grapes. The acidity ranged from 5.20 g/L in T_2 to 5.02g/L in untreated control treatment. The acidity in grape berries was within the acceptable limit in all the treatments studied. Ekbic *et al.* (2018) reported that higher concentrations of boron in spray treatments increased the acidity of grape berries. Amiri *et al.* (2009) observed that greater calcium applications similarly resulted in higher acidity. In contrast, Qaoud *et al.* (2019) found that a combined application of magnesium and boron reduced the acidity percentage in grape berries.

Shelf life helps to understand the time duration that a commodity may be stored without becoming unfit for consumption. The grapes kept under shelf if remain fresh for longer time indicates the good quality. Improved shelf life of grapes can fetch better price in the market. The data recorded on shelf life is presented in Fig. 1. It was observed that on the 4th day under shelf, the PLW was less in T_2 treatment (5.19%) followed by T_1 (6.03%) and T_4 (6.26%) as compared to T_3 (6.34%) and control treatment (7.17%). The results of the present study indicated that the use of CalSiB^{++} @ 2.0 g/L at three stages (berry setting stage, 8-10 mm and 12-14 mm berry size stage) helped the grapes to remain fresh for longer time thus indicating the superiority of the product. Zhang *et al.* (2017) reported that applying silicon can extend the shelf life of grapes by reducing respiratory losses and decay incidence. Similarly, Parthiban *et al.* (2021) found that chelated calcium and boron applications can also increase grape shelf life by minimizing physiological water loss.

The differences for calcium and magnesium content in petiole at full bloom stage was non-significant. However, the treatment T_2 recorded higher phosphorous in petiole (0.274%) followed by T_3 (0.247%). T_1 recorded highest berry (0.123%) and petiole (3.062%) potassium content followed by T_5 (0.12% and 2.824%) and T_3 (0.12% and 2.746%). Calcium content in grape berries was significantly differed among the different treatments as compared to the control. The calcium content in grape berries of T_2 was higher (184.00ppm) followed by T_4 (180.25 ppm) as compared to the control treatment

Table 5 : Effect of $CalSiB^{++}$ on nutrient status of berry and petiole.

Treatments	Nutrient Status							
	Phosphorous (%)		Potassium (%)		Calcium (ppm)		Magnesium (ppm)	
	Berry	Petiole	Berry	Petiole	Berry	Petiole	Berry	Petiole
T_1	0.019	0.232	0.123	3.062	173.50	1.79	45.50	0.69
T_2	0.018	0.274	0.097	2.746	184.00	1.74	58.25	0.79
T_3	0.018	0.247	0.120	2.765	180.00	1.92	65.25	0.82
T_4 (Recommended)	0.017	0.153	0.103	3.003	180.25	1.69	63.75	0.78
T_5 (control)	0.018	0.138	0.120	2.824	166.00	1.81	45.25	0.81
CD at 5%	0.0004	0.075	0.0038	0.141	4.506	-	2.158	-
SEm ±	0.0001	0.023	0.0012	0.043	1.462	0.06	0.7	0.03
Significance	**	**	**	**	**	NS	**	NS

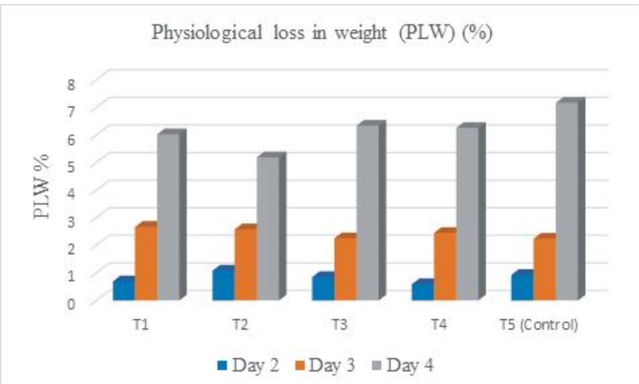

Fig. 1 : Effect of $CalSiB^{++}$ on shelf-life study in Thompson seedless grapes.

(166.00 ppm). Highest magnesium content was observed in T_3 in grape berries (65.25 ppm). The remaining berry magnesium was in the order of $T_4 > T_2 > T_1 > T_5$. Treated vines has higher nutrient content than the untreated vines in case of P, Ca and Mg, while untreated vine had higher potassium level compared to treated vines. Several studies highlight the positive effects of nutrient treatments on grapevine health. Fawzi *et al*. (2014) found higher potassium in boric acid-treated vines. Qaoud *et al*. (2019) reported that boron and magnesium increased micronutrients like P, Fe, Mn and Zn. Ekbic *et al*. (2018) observed increased Ca, Mn, Cu and Zn with higher boric acid concentrations, while Garde *et al*. (2023) found calcium and silicon applications improved grape flavor and aroma. Bonomelli *et al*. (2010) noted that soil-applied calcium was more effective than foliar application. Overall, foliar treatments not only increased targeted nutrients but also enhanced other essential nutrients.

Conclusion

The application of $CalSiB^{++}$ treatments significantly enhanced grape yield and berry quality compared to the untreated control. Among these treatments, T_2 ($CalSiB^{++}$ at 2.0 g/L applied at three growth stages (berry setting, 8-10 mm berry size and 12-14 mm berry size) demonstrated the best results for both bunch and berry quality. Specifically, T_2 increased berry diameter to 19.00 mm, achieved a yield of 14.32 kg per vine, and minimized postharvest loss with respect to PLW to 5.19%. Although T_3 treatment increased average bunch weight and the weight of 50 berries, it resulted in a lower overall yield. Thus, T_2 outperformed other treatments in terms of yield and berry quality. Based on these findings, foliar application of CalSiB++ at 2.0 g/L during the specified growth stages could be recommended to improve grape yield, quality and shelf life.

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