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Influence of PGRs and $ZnSO_4$ on Biochemical Efficacy of Ber (*Ziziphus mauritiana* L.)

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ABSTRACT

Background: Ber is a significant fruit crop in desert regions. The fully ripe ber fruit is rich in calcium, vitamin C, vitamin B and vitamin A, among other nutrients. Spraying plants in mid-October and in last week of December with various treatments, NAA (20 ppm and 30 ppm), GA_3 (30 ppm and 40 ppm), salicylic acid (300 ppm), $ZnSO_4$ (0.5%) alone and in combination, along with a control group, each repeated three times.

Methods: This study investigated the impact of plant growth regulators (PGRs) and zinc sulfate ($ZnSO_4$) on the biochemical parameters of ber. Field trials were conducted involving foliar applications of PGRs and $ZnSO_4$, both individually and in combination. Spraying was done in two stages, the first in mid-October during the flowering stage and the second in the last week of November during the pealet stage. The experiment employed randomized block design (RBD). Ber fruits were assessed for total soluble solids (TSS), titratable acidity (TA), TSS acid ratio, sugars, vitamin C, total ash content, total protein content, total fat content, total fiber content and total moisture content.

Result: Results showed that T_{10}- GA_3+$ZnSO_4$+Salicylic acid (40 ppm+0.5%+300 ppm) has given best result followed by T_9 (30 ppm+ 0.5%+300 ppm) as compared to control (T_0). T_{10} exhibited highest TSS (14.50^a ^{o}Brix), minimum acidity (0.32^a), TSS acid ratio (45.37^b), highest total sugars (7.91^a%), reducing sugar (5.10^a %), non-reducing sugar (2.81^d %), ascorbic acid (101.56^{ab} mg/100 gm), highest ash content (2.38^b g/100gm), total protein (3.50^a g/100 gm), total fat (0.45^a g/100 gm) and total fiber content (5.37^a g/100 gm) and total moisture content (4.89^a%).

Key words: Ber, Biochemical parameters, Plant growth regulators, $ZnSO_4$.

INTRODUCTION

The fruit crop called Indian jujube, scientifically known as (*Ziziphus mauritiana* Lamk) is indigenous to India and belongs to the Rhamnaceae family Krishna *et al*. (2014). Other names for this kind of tropical fruit tree are Indian plum, jujube, ber (Hindi) and desert apple Karuppaiah *et al*. (2015) and Sapkota *et al*. (2020). Due to its great adaptability, it is grown almost everywhere in India, particularly in dry and semi-arid areas for its fresh fruit. As a result, it is commonly known as "the apple of arid zone fruits".

It is a common fruit crop in dry and semi-arid regions of India; the majority of plantations are found in the states of Uttar Pradesh, Maharashtra, Gujarat, Rajasthan and Haryana. Although it is mostly grown in India, Indian jujube is found all over the world in warm climates. According to National Horticulture Board (NHB) 2021-2022 data Madhya Pradesh is the hightest producer of ber which produce 121.76 lakh tonnes. Although ber is grown across various regions in India, it is predominantly found in dry and semi-arid areas. According to the National Horticulture Board (NHB) 2023 data, the area under ber cultivation in India is approximately 81,000 hectares, with a production estimate of around 8.6 lakh tonnes Jujube's late start-up and high nutritional content make it a reliable and feasible substitute crop Huang *et al*. (2017).

Ber is one of the many fruit crops that is becoming more and more popular with producers due to its high production, favourable returns and capacity to thrive in wasteland and drought circumstances. Among the many benefits of the jujube tree are its flexibility and food.

Plant growth regulators play a significant role in fruit development. They serve as a metabolic sink to redirect metabolites from one area of the plant to another, particularly in the direction of producing fruits. The use of plant growth regulators is a key component of contemporary agricultural practices aimed at enhancing the yield of high-quality fruits (Kumari *et al*., 2018).

Fruit output and quality were both improved by the use of NAA spray. They reroute metabolic activities from one area of the plant to another, particularly in the direction of fruit development, acting as a metabolic sink. Pre-harvest

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sprays are used by plant growth regulators (PGRs) to reduce fruit loss and increase fruit retention rates. Additionally thought to be essential for fruit development and growth is zinc. It is one among the components needed to synthesise chlorophyll, which makes it helpful for photosynthetic activity. Plants use certain enzymes, such as zinc, to synthesise indole acetic acid. A crucial gibberellin group growth regulator, gibberellic acid is utilized to increase the TSS and juice percentage of fruit in order to improve its quality and size. GA_3 is the cause of improved sugar content, flowering induction, maturity delay and seedlessness. When compared to the control, the application of urea, NAA and GA_3 significantly enhanced the number of leaves per shoot, the length of the shoots and the leaf area Salicylic acid (SA), a plant hormone found in nature, serves as a crucial signaling molecule and improves the ability of treated plants to withstand biotic stresses (Khan *et al*., 2012). Salicylic acid has been proposed to play an important role in plant water management, photosynthesis and overall growth Arfan *et al*. (2007). SA can increase the concentrations of phenolic compounds, flavonoids, anthocyanins and other biologically active substances, while also enhancing antioxidant activities in various fruits, such as apricots (Wang *et al*., 2015).

MATERIALS AND METHODS

Experimental site and plant material

This research experiment was conducted using ten-year-old ber plants from an established orchard at Lovely Professional University. The field trials took place at Lovely Professional University in Phagwara, Punjab, India (located between 30°57′ to 32° 7′N latitude and 75° 4′ to 76° 30′E longitude) during the cropping years 2022-23 and 2023-24. Situated between 270 and 300 meters above Mean Sea Level (MSL), the location boasts a humid subtropical environment with an average yearly precipitation of around 2029 millimetres.

Experimental design and treatment details

The experimental design used in the study was a randomized block design (RBD), with a total of eleven treatments and three duplicates. The treatment combinations *i.e*. T_0- Control (Water spray), T_1- NAA (20 ppm), T_2- NAA (30 ppm), T_3- GA_3 (30 ppm), T_4- GA_3(40 ppm), T_5- Salicylic acid (300 ppm), T_6- $ZnSO_4$ (0.5%), T_7- NAA+$ZnSO_4$+Salicylic acid (20 ppm+0.5%+300 ppm), T_8- NAA+$ZnSO_4$+Salicylic acid (30 ppm+0.5%+300 ppm), T_9- GA_3+$ZnSO_4$+Salicylic acid (30 ppm+0.5%+300 ppm), T_{10}- GA_3+$ZnSO_4$+Salicylic acid (40 ppm+ 0.5%+ 300 ppm), were tested in randomized blocks design with three replication.

Sample preparation

Fresh ber were harvested at their peak ripeness in April and transported to the laboratory for analysis. After thorough cleaning, the berries were blended into a smooth puree, which was then preserved at -80°C for later examination of various biochemical characteristics.The measurements taken included sugar content (total, reducing and non-reducing sugars), titratable acidity (TA), total soluble solids (TSS), TSS-acid ratio and total bioactive compounds. These bioactive compounds encompassed vitamin C, total ash, total protein, total fat, total fiber content and total moisture content.

Total soluble solids (°Brix), titratable acidity (%) and TSS/TA ratio

The total soluble solids (TSS) and titratable acidity (TA) were estimated using the modified methods of Roussos *et al.* (2022). In the first step, 2 grams of frozen fruit pulp were added to a centrifuge tube and centrifuged at 5000 ×g for 15 minutes with a Remi R-8C plus centrifuge. Hanna Woonsocket RI digital refractometer, with results expressed in °Brix. For the determination of TA, the juice sample was diluted with distilled water at a ratio of 1:50 and aliquots of the diluted solution were titrated with 0.1 N NaOH until a pink endpoint was achieved. The TA value was then calculated and expressed as a percentage (%). By dividing the TSS value by the TA value, the TSS/TA ratio was determined.

Estimation of sugars (%) and vitamin-C (mg/100 g)

The total and reducing sugar content was estimated using the method outlined by A.O.A.C. (1980), which involved the use of Fehling A and Fehling B solutions, with results reported as a percentage (%).

By deducting the reducing sugars from the overall sugar amount, the non-reducing sugar content was determined. The technique outlined by Ranganna (2003) was used to determine the vitamin C content of strawberries. This involved titrating filtered strawberry extracts, diluted with 3% metaphosphoric acid, against a standardized solution of 2,6-dichlorophenol indophenol dye until a pink endpoint was achieved.

Total ash content (g/100 gm), total protein (g/100 gm), total fat (g/100 gm) and total fiber content (g/100 gm) and total moisture content (%)

Ash content was determined by drying the samples at 105°C for 1 day in an oven and then transferred the crucibles to a muffle furnace. The temperature was gradually raised to 600! and samples were ashed for 10-12 hours until they were white in colour Paquat and Houtfenne method, (1987). A micro-Kjeldahl flask was filled with a fruit sample of known weight (0.25 g). Each sample received an addition of 15 ml conc. H_2SO_4 (36 N), a small quantity of glass beads was added to minimize sample bumping, along with a catalyst combination of 0.42 g $CuSO_4$ and 9.0 g K, SO_4. The sample was broken down at 410°C for 45 minutes, or until a clear green solution was formed, in order to guarantee that all organic components had completely oxidised. The distillation apparatus was fitted with a micro-Kjeldahl flask after the digest had been diluted

with 50 millilitres of distilled water. After adding 45 ml of 15 N NaOH, the sample was distilled to capture the released ammonia in a boric acid solution that contained the indicators methyl red and methylene blue. Standardised 0.1 N H, SO_4 was used to titrate the borate anion, representing the quantity of nitrogen present. H_2SO_4 was standardized using 0.1 N Na_2CO_3 as the primary standard. A blank for reagent was ran concurrently.

The following formula was used to compute the sample nitrogen content:

$$N\ (\%) = \frac{(\text{ml } H_2SO_4 \text{for sample - ml of } H_2SO_4 \text{ for blank}) \times \text{Normality of } H_2SO_4 \times 1.4007}{\text{Weight of sample (g)}}$$

$$\text{Protein } (\%) = N\ (\%) \times 5.32$$

Soxhlet apparatus was utilized in this study to determine the FC of fresh and dried pulp sample. Petroleum Ether used as the solvent to extract the FC of sample, solvent was removed by evaporation and residue of fat was weighed. Petroleum ether of 25 ml was mixed with the known amount of sample was mixed with 25 ml and the mixture was stored for overnight. The sample was dried after the petroleum ether was decanted. The drying process was carried out until the sample attains constant weight. 5 ml of 1.25% H_2SO_4 as used to treat the sample and it was processed in the boiling water bath for about 30 minutes. The distilled water was used to wash the residue for three times. 5 ml of 1.25% NaOH was used to treat the washed residue and it was processed in the boiling water bath for about 30 minutes. Washed with distilled water for three times and oven dried until attain the constant weight. 5 ml of 0.06 M potassium dichromate reagent was used to oxidize the residue and was processed in the boiling water bath for about 30 minutes. Then it was 52 brought back to room temperature. The absorbance was measured at 590 nm after the volume was adjusted to 50 ml with the addition of distilled water. The fruit pulp sample's CFC (g/100 g) was reported as a percentage. For this measurement, a standard of 5 to 25 mg of pure cellulose was utilised. By drying the kernel samples in a hot air oven set to 105°C for the whole night, the moisture content was determined. Next, the moisture was ascertained and computed using the AOAC (1990) technique.

Statistical analysis

Statistical analysis of the data obtained in the different set of experiments. Opstat software was used for analysis of the parameters.

RESULTS AND DISCUSSION

Total sugars, reducing sugar, non-reducing sugar

From Table 1 we can see that the application GA_3+$ZnSO_4$ + Salicylic acid (40 ppm + 0.5% + 300 ppm- T_{10}) resulted in the highest total sugars (9.62%), followed by GA_3+$ZnSO_4$ + Salicylic acid (30 ppm + 0.5% + 300 ppm- T_9) that was (9.44%) and control treatment (T_0) produced the lowest total sugars (5.85%). Application GA_3+$ZnSO_4$ + Salicylic acid (40 ppm + 0.5% + 300 ppm- T_{10}) produced the highest reducing sugar (6.36%), followed by application GA_3+$ZnSO_4$ + Salicylic acid (30 ppm + 0.5% + 300 ppm- T_9), which was (6.30%) and the application control (T_0), which produced the lowest reducing sugar (3.50%). With GA_3+$ZnSO_4$ + Salicylic acid applied at (40 ppm + 0.5% + 300 ppm - T_9), the maximum non-reducing sugar (3.23 %) was obtained, which was followed by GA_3+$ZnSO_4$ + Salicylic acid (30 ppm + 0.5% + 300 ppm- T_9), which produced (3.13 %) and the T_0 control yielded (2.34%). The highest vitamin C (103.92 g/100 g) was produced by applying GA_3+$ZnSO_4$ + Salicylic acid (40 ppm + 0.5% + 300 ppm). This was followed by T_9 GA_3+$ZnSO_4$ + Salicylic acid (30 ppm + 0.5% + 300 ppm), which produced (101.56 g/100 g) of vitamin C, while the lowest vitamin C (85.20 g/100 g) was obtained in the T_0 control.

Proximate composition

Table 2 showed that the application of GA_3 + $ZnSO_4$ + Salicylic acid (40 ppm + 0.5% + 300 ppm- T_{10}) resulted in significantly higher in all proximate compositions. GA_3+$ZnSO_4$ + Salicylic acid (40 ppm + 0.5% + 300 ppm- T_{10}), yielded in highest ash content (2.38 g/100g), followed by GA_3+$ZnSO_4$ + Salicylic acid (30 ppm + 0.5% + 300 ppm- T_9), which was (2.36 g/100 g) and the lowest ash content (2.19 g/100 g) founded in control (T_0). The application of GA_3+$ZnSO_4$ + Salicylic acid (40 ppm + 0.5% + 300 ppm- T_{10}) resulted in the greatest protein content (3.50 g/100 g), followed by GA_3+$ZnSO_4$ + Salicylic acid (30 ppm + 0.5% + 300 ppm- T_9) and control (T_0) generated the lowest protein content (2.98 g/100 g). GA_3+ $ZnSO_4$ + Salicylic acid (40 ppm + 0.5% + 300 ppm- T_{10}) resulted in the greatest fat content (0.45 g/100 g), which was followed by GA_3+$ZnSO_4$ + Salicylic acid (30 ppm + 0.5% + 300 ppm - T_9) that was (0.43 g/100 g) and the control (T_0) had the least amount of fat (0.19 g/100 g). Application GA_3+$ZnSO_4$ + Salicylic acid (40 ppm + 0.5% + 300 ppm- T_{10}) produced the highest fiber content (5.37 g/100 g), followed by the application of GA_3+$ZnSO_4$ + Salicylic acid (30 ppm + 0.5% + 300 ppm - T_9) that was (5.31 g/100 g) whereas, treatment control (T_0) produced the lowest fiber content (2.59 g/100 g). Application GA_3+$ZnSO_4$ + Salicylic acid (40 ppm + 0.5% + 300 ppm- T_{10}) produced the highest moisture content (4.89 g/100 g), followed by application GA_3 + $ZnSO_4$ + Salicylic acid (30 ppm + 0.5% + 300 ppm - T_9) that was (4.84 g/100 g), whereas treatment control (T_0) produced the lowest moisture content that was (3.10 g/100 g).

GA_3 is a plant hormone that regulates various growth processes. It promotes cell elongation, division and differentiation, which can lead to larger fruit size and increased biomass. GA_3 enhances the photosynthetic rate by improving leaf expansion and chlorophyll content, leading to greater sugar production in the leaves. Zhang *et al.* (2022) reported that GA_3 treatment in tomatoes significantly increased TSS levels due to enhanced fruit size and sugar. GA_3 application in strawberries increased TSS by promoting

photosynthesis and fruit growth. The increased proportion of non-reducing sugar pectin may have resulted from GA_3 regulation facilitating the effective transfer of photosynthates to the fruits Marwaha *et al*. (2023). Maurya *et al.* (2018) reported similar findings in guava with the application of GA_3 alone, while Tripathi and Kumar (2022) observed increased sugar and ascorbic acid levels in mango, along with reduced fruit drop, when using a combination of GA_3 and $ZnSO_4$. Additionally, González-Villagra *et al*. (2024) found that applying salicylic acid increased TSS levels in sweet cherry in their study. Mohammad *et al.* (2022) found that GA_3 application significantly increased ascorbic acid content in ber fruits, likely due to enhanced metabolic activity and improved fruit quality. Patel *et al*. (2023) observed that

Table 1: Effect of different growth regulators and $ZnSO_4$ on fruit biochemical attributes of ber.

Treatments	TSS (°Brix)	Acidity (%)	TSS: acid	Total sugar (%)	Reducing sugar (%)	Non reducing sugar (%)	Ascorbic acid (mg/100 g)
T_0: Control (water spray)	10.26^{f}	0.46^{a}	22.31^{h}	5.85^{f}	3.50^{g}	2.34^{f}	85.20^{d}
T_1: (NAA 20 ppm)	12.60^{d}	0.42^{a}	29.32^{f}	7.38^{e}	3.53^{f}	2.83^{ef}	95.23^{c}
T_2: (NAA 30 ppm	13.23^{c}	0.40^{a}	32.32^{e}	7.42^{e}	3.55^{f}	2.85^{e}	96.34^{c}
T_3: (GA_3 30 ppm)	13.30^{c}	0.39^{a}	34.14^{e}	7.61^{cd}	4.59^{de}	2.99^{cd}	96.53^{bc}
T_4: (GA_3 40 ppm)	13.63^{bc}	0.37^{a}	36.91^{d}	7.66^{cd}	4.61^{cd}	3.02^{bcd}	97.55^{bc}
T_5: (Salicylic acid 300 ppm)	11.50^{e}	0.41^{a}	27.36^{fg}	7.77^{c}	4.80^{c}	2.96^{bc}	96.19^{c}
T_6: ($ZnSO_4$ 0.5%)	11.63^{e}	0.44^{a}	26.55^{g}	7.40^{dc}	4.98^{e}	2.41^{d}	95.62^{c}
T_7: (20 ppm NAA + 0.5% $ZnSO_4$ + 300 ppm Salicylic acid)	13.80^{b}	0.35^{a}	38.89^{cd}	8.12^{b}	5.10^{b}	3.00^{b}	97.68^{bc}
T_8: (30 ppm NAA + 0.5% $ZnSO_4$ + 300 ppm Salicylic acid)	13.93^{b}	0.34^{a}	41.09^{c}	8.24^{b}	5.12b	3.10^{b}	98.78^{bc}
T_9: (30 ppm GA_3+ 0.5% $ZnSO_4$ + 300 ppm Salicylic acid)	14.50^{a}	0.32^{a}	45.37^{b}	9.44^{a}	6.30^{a}	3.13^{a}	101.56^{ab}
T_{10}: (40 ppm GA_3 + 0.5% $ZnSO_4$ + 300 ppm Salicylic acid)	14.83^{a}	0.29^{a}	51.55^{a}	9.62^{a}	6.36^{a}	3.23^{a}	103.92^{a}
SE (m)	0.130	0.007	0.825	0.100	0.240	0.049	1.546
SE (d)	0.184	0.009	1.166	0.141	0.081	0.064	2.186
C.D. at 5% level	0.386	0.020	2.450	0.297	0.114	0.135	4.592

Table 2: Effect of different growth regulators and $ZnSO_4$ on fruit biochemical attributes of ber.

Treatments	Ash content (g/100 gm)	Protein content (g/100 gm)	Fat content (g/100 g)	Fiber content (g/100 gm)	Moisture content (%)
T_0: Control (water spray)	2.19^{de}	2.98^{f}	0.19^{a}	2.59^{d}	3.10^{d}
T_1: (NAA 20 ppm)	2.23^{cde}	3.16^{de}	0.23^{a}	3.24^{c}	3.16^{d}
T_2: (NAA 30 ppm	2.27^{bcde}	3.20^{cde}	0.26^{a}	3.29^{c}	3.19^{d}
T_3: (GA_3 30 ppm)	2.30^{bcd}	3.25^{cd}	0.29^{a}	4.21^{b}	4.10^{c}
T_4: (GA_3 40 ppm)	2.33^{bc}	3.29^{bc}	0.32^{a}	4.27^{b}	4.25^{c}
T_5: (Salicylic acid 300 ppm)	3.12^{a}	3.12^{e}	0.21^{a}	2.24^{e}	3.12^{d}
T_6: ($ZnSO_4$ 0.5%)	2.17^{e}	3.14^{de}	0.23^{a}	3.20^{c}	3.16^{d}
T_7: (20 ppm NAA + 0.5% $ZnSO_4$ + 300 ppm Salicylic acid)	2.31^{bcd}	3.38^{ab}	0.38^{a}	5.21^{a}	4.72^{b}
T_8: (30 ppm NAA + 0.5% $ZnSO_4$ + 300 ppm Salicylic acid)	2.33^{bc}	3.40^{ab}	0.41^{a}	5.25^{a}	4.78^{ab}
T_9: (30 ppm GA_3+ 0.5% $ZnSO_4$ + 300 ppm Salicylic acid)	2.36^{b}	3.47^{a}	0.43^{a}	5.31^{a}	4.84^{ab}
T_{10}: (40 ppm GA_3 + 0.5% $ZnSO_4$ + 300 ppm Salicylic acid)	2.38^{b}	3.50^{a}	0.45^{a}	5.37^{a}	4.89^{a}
SE (m)	0.035	0.041	0.006	0.050	0.053
SE (d)	0.050	0.058	0.009	0.071	0.075
C.D. at 5%	0.104	0.122	0.018	0.150	0.158

salicylic acid treatment in various fruit crops, including ber, led to higher vitamin C content, attributed to improved metabolic activity and fruit ripening processes.

GA_3 promoted cell division and biomass growth, $ZnSO_4$ enhanced enzyme activity for protein synthesis and salicylic acid improved stress tolerance and biochemical processes, increasing fat, fiber and moisture content. Similar to our findings, Opabode and Raji (2019) found that the ideal concentration of GA_3 was 80 mg•L^{-1}. Their analysis encompassed various components in the leaves, including proteins, crude fiber, ash, carbohydrates and minerals such as sodium, phosphorus, magnesium, calcium, iron and zinc. The application of GA_3 and salicylic acid is crucial for promoting the growth and development of "Zaghloul" fruit. This enhancement was associated with higher levels of dry matter, crude fiber, ash, total soluble solids, total sugars, carbohydrates and protein (Talaat *et al.,* 2023). Using $ZnSO_„$ fertilizer at a rate of 5 kg ha^{-1} improved various quality parameters, including moisture content, protein, fat, carbohydrates and ash (Adekiya *et al.* 2018).

CONCLUSION

The current study concludes that in terms of TSS, acidity, TSS-acid ratio, total sugars, reducing sugars, non-reducing sugars, ash content, protein content, fibre content and moisture content were seen best in the combination of GA_3, $ZnSO_4$ and salicylic acid, applied at concentrations of 40 ppm, 0.5% and 300 ppm (T_{10}), followed by (T_9) GA_3, $ZnSO_4$ and salicylic acid in combination of 30 ppm, 0.5% and 300 ppm. The results of my study, which I carried out alone, are remarkable and very helpful for Punjabi farmers, orchard owners and researchers. To those who are concerned, I would like to suggest that spraying growth regulators such GA_3, salicylic acid and micronutrient Zn is very beneficial in order to boost fruiting quality.

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Conflict of interest

The author declares no conflict of interest. The manuscript has not been submitted for publication in other journals.

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