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Assessing the impact of exogenous application of plant growth regulators on quality of watermelon (*Citrullus lanatus* Thunb.) with mulching

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Abstract
An experiment was conducted at the University Farm, Postgraduate Institute for Horticultural Sciences, SKLTSHU, Mulugu, Siddipet, during the *rabi* season from January to April 2024, to assess the impact of various plant growth regulators (PGRs) on the quality parameters of watermelon (*Citrullus lanatus* Thunb.). The study was laid out in a Randomized Block Design (RBD) with thirteen treatments, each replicated three times. The treatments included Naphthalene acetic acid (NAA) at 100 ppm (T_1) and 150 ppm (T_2); Gibberellic acid (GA_3) at 30 ppm (T_3) and 40 ppm (T_4); Ethrel at 250 ppm (T_5) and 500 ppm (T_6); Triiodobenzoic acid (TIBA) at 20 ppm (T_7) and 30 ppm (T_8); Maleic hydrazide (MH) at 100 ppm (T_9) and 150 ppm (T_{10}); Cycocel at 250 ppm (T_{11}) and 500 ppm (T_{12}); and a control (distilled water spray). Data were collected from five randomly selected plants per treatment. The results indicated a significant improvement in the quality parameters of watermelon, particularly with the application of TIBA at 30 ppm (T8), which produced the highest total soluble solids (TSS) of 11.80 °Brix, reducing sugars (3.45%), non-reducing sugars (4.80%) and total sugars (8.25%). This suggests that TIBA at 30 ppm is highly effective in enhancing the sweetness and overall quality of watermelon fruits, offering a viable approach to improving yield quality in commercial watermelon cultivation. These findings underline the potential of targeted PGR application in optimizing watermelon production.

Keywords: Flowering, GA_3, NAA, TIBA, watermelon, quality

Introduction
Watermelon (*Citrullus lanatus*), a significant fruit in tropical and subtropical regions, is valued for its refreshing nature and nutritional benefits. It contributes about 7% to global vegetable production, ranking just below tomatoes in output (Zheng, 2024) [25]. As a member of the Cucurbitaceae family, watermelon is an annual vine known for its lobed leaves, yellow flowers, and fruits classified botanically as pepos, which are berries with a hard rind and juicy flesh containing seeds (Meshram *et al*., 2022) [17]. Over 1,200 cultivars exhibit considerable genetic variation in fruit size, shape, flesh color, and seed traits (Freeman *et al*., 2021) [3].

Originally from Africa, watermelon thrives in warm, dry climates with temperatures between 22 °C and 30 °C. It is well-suited to areas with minimal rainfall, making it ideal for arid and semi-arid regions (Chomicki *et al*., 2020; Dahal *et al*., 2024) [5, 8]. In India, the crop covers approximately 124 million hectares, with an annual production of 3,505 metric tonnes (Agri Stats, 2022). It is known by various regional names, such as tarbuj, kalingada, and paniphal (Mahala, 2014) [15].

Nutritionally, watermelon is composed of about 93% water, offering hydration along with essential nutrients. Each 100-gram serving provides 30 kcal, 7.55 grams of carbohydrates, 6.2 grams of sugars, and 0.4 grams of dietary fiber, along with vitamins A (569 IU) and C (8.1 mg), calcium (7 mg), and iron (0.24 mg) (Kumar *et al*., 2022; Choo and Sin, 2012). Rich in lycopene, a powerful antioxidant, it has been linked to a reduced risk of certain cancers, with dark red-fleshed varieties containing higher levels than tomatoes, grapefruit, or guava (Cooperstone, 2019) [7]. China leads global production with over 60.6 million metric tonnes in 2019, while Asia accounts for nearly 79.5% of the world's output (Jordana *et al*., 2023) [13].

The crop's rapid growth and adaptability make it a suitable option for high-yield, short-duration cultivation (Singh *et al*., 2019) [21].

Watermelon exhibits diverse flowering behaviors, including monoecious, gynoecious, and andromonoecious types, with male flowers outnumbering female flowers. Since a lower number of female flowers can restrict fruit production, manipulating the male-to-female flower ratio through plant growth regulators (PGRs) has been employed to enhance yields (Manzano *et al*., 2014; Ji *et al*., 2015) [16, 24]. PGRs, which are substances that influence plant growth processes like flowering and fruit development, can modify hormone levels (Thakar *et al*., 2021) [23].

Research shows that the application of PGRs such as gibberellic acid (GA_3) and naphthalene acetic acid (NAA) can significantly improve yield and fruit quality by promoting flowering, fruit set, and maturation (Zhumanova *et al*., 2024; Dhar & Awal, 2021) [26, 10]. GA_3 enhances growth by stimulating cell division, shoot elongation, and photosynthetic activity, while NAA aids in nutrient translocation and cell wall flexibility (Deepthi *et al*., 2008; Raj *et al*., 2022) [9, 19]. In cucurbits, hormones like auxins, gibberellins, and ethylene are crucial for sex expression. PGRs can adjust the male-to-female flower ratio to favor more female flowers, thus improving fruit yield (Hossain *et al*., 2006; Garg *et al*., 2020) [12, 11].

Mulching supports PGR use by conserving soil moisture, reducing weed competition, and stabilizing soil temperature, which is beneficial for watermelon grown in arid environments (Bellad and Hiremath, 2018) [2]. The synergy of mulching and PGRs enhances plant growth, fruit quality, and economic returns by creating favorable microclimate conditions (Singh *et al*., 2019) [21].

Traditionally, it is valued for its cooling effects and diuretic properties, while the seeds are believed to support cognitive function and are a good source of magnesium, which promotes heart health and helps regulate blood pressure (Singh *et al*., 2023; Rajappa *et al*., 2024) [22, 20]. With its economic and nutritional value, PGRs and mulching are crucial for improving watermelon yield and quality.

2. Materials and Methods

The experiment was conducted at the University Farm, Postgraduate Institute for Horticultural Sciences, SKLTSHU, Mulugu, Siddipet, during the *rabi* season, from January to April 2024. The study employed a Randomized Block Design (RBD) with three replications. The watermelon cultivar 'Shakkar Plus' was subjected to thirteen treatments: Naphthalene acetic acid (NAA) at 100 ppm (T_1) and 150 ppm (T_2); Gibberellic acid (GA_3) at 30 ppm (T_3) and 40 ppm (T_4); Ethrel at 250 ppm (T_5) and 500 ppm (T_6); Triiodobenzoic acid (TIBA) at 20 ppm (T_7) and 30 ppm (T_8); Maleic hydrazide (MH) at 100 ppm (T_9) and 150 ppm (T_{10}); Cycocel at 250 ppm (T_{11}) and 500 ppm (T_{12}); and a control (distilled water spray). Seeds were sown on January 8th at a spacing of 2.5 × 0.5 m². A uniform basal application of well-decomposed farmyard manure at a rate of 20 t/ha was applied to all experimental plots. Nitrogen, at a rate of 100 kg/ha in the form of urea, was administered in two equal split doses: one as a basal dose and the other 30 days after sowing. Phosphorus, at a rate of 50 kg/ha in the form of single super phosphate (SSP), and Potassium, at a rate of 50 kg/ha in the form of muriate of potash, were applied as basal doses. Standard cultural practices were maintained throughout the crop period across all experimental plots. The seedlings were treated with each PGR once at the 2-true leaf stage and again at the 4th leaf stage. Data was collected on five randomly selected and tagged plants from each treatment plot. TSS (Total soluble solids) was measured by digital hand refractometer and Total sugars were estimated with the procedure given. The sample absorbance was measured at 630 nm in a (HALO DB-20S UV VIS double beam) spectrophotometer, using petroleum ether as blank.

The collected data were analyzed using SPSS software to perform statistical tests and determine the significance of differences among treatments. Analysis of variance (ANOVA) was employed to compare the means, and significant differences were further analyzed using appropriate post-hoc tests.

3. Results and Discussion

3.1 Quality parameters

3.1.1 Total soluble solids (ºBrix)

The data pertaining to Total Soluble Solids (TSS) as influenced by different treatment concentrations of plant growth regulators are presented in Table 1 and Figure 1.

Significant differences were observed among the treatments in total soluble solids. The maximum total soluble solids (11.80 ºBrix) were recorded in treatment T_8: Triiodobenzoic acid (TIBA) @ 30 ppm. which was statistically on par with treatment T_7: Triiodobenzoic acid (TIBA) @ 20 ppm (11.54 ºBrix) and T_4: Gibberellic acid (GA_3) @ 40 ppm (11.35 ºBrix).

In contrast, the minimum total soluble solids (7.89 ºBrix) were recorded in treatment T_{13}: Absolute control (distilled water spray), which was statistically on par with treatments T_1: Naphthalene acetic acid (NAA) @ 100 ppm (8.43 ºBrix), T_{11}: Cycocel @ 250 ppm (8.60 ºBrix), T_{12}: Cycocel @ 500 ppm (8.72 ºBrix), and T_2: Naphthalene acetic acid (NAA) @ 150 ppm (8.85 ºBrix).

The application of TIBA (Triiodobenzoic Acid), has been shown to enhance photosynthetic efficiency and accelerate the ripening process, leading to a higher accumulation of sugars and other soluble solids in the fruit. This is consistent with the findings of Singh *et al*. (2019) [21], who reported that TIBA's role in promoting cell elongation and regulating growth contributes to more efficient resource allocation towards fruit development. The enhanced conversion of complex polysaccharides into simple sugars, as suggested by Rafeekher *et al*. (2005) [18], further supports this increase in TSS. This diversion of more solids towards the developing fruits can be attributed to the action of plant growth regulators, which ultimately results in improved fruit quality and higher TSS levels. The results support the earlier findings of Chaudhary *et al*. (2016) [27] in watermelon; and Meshram *et al*. (2022) [17] in watermelon.

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3.1.2 Reducing sugars (%)

The data pertaining to reducing sugars as influenced by different treatment concentrations of plant growth regulators are presented in Table 1 and Figure 2.

Significant differences were observed among the treatments in reducing sugars. The maximum reducing sugar content (3.45 %) was recorded in treatment T_8: Triiodobenzoic acid (TIBA) @ 30 ppm.

In contrast, the minimum reducing sugar content (2.11 %) was recorded in treatment T_{13}: Absolute control (distilled water spray).
TIBA (Triiodobenzoic Acid) is a plant growth regulator that affects various physiological processes in plants. When applied to watermelon plants, TIBA can increase the concentration of reducing sugars in the fruit. This effect is attributed to TIBA's role in altering carbohydrate metabolism within the plant. TIBA influences the activity of enzymes involved in carbohydrate synthesis and breakdown, thereby promoting the accumulation of sugars in the fruit. Specifically, TIBA can inhibit the activity of auxin, which in turn affects the balance of other growth regulators and metabolic pathways involved in sugar production and storage. This leads to higher levels of reducing sugars as the plant reallocates resources to enhance fruit development. The results support the earlier findings of Meshram *et al*. (2022) [17] in watermelon; and Chaudhary *et al*. (2016) [27] in watermelon.
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3.1.3 Non-reducing sugars (%)
The data pertaining to non-reducing sugars as influenced by different treatment concentrations of plant growth regulators are presented in Table 1 and Figure 2.
Significant differences were observed among the treatments in the non-reducing sugar content. The maximum non-reducing sugar content was recorded in treatments T_8: Triiodobenzoic acid (TIBA) @ 30 ppm (4.80%). which were statistically on par with T_7: Triiodobenzoic acid (TIBA) @ 20 ppm (4.79%), T_4: Gibberellic acid (GA_3) @ 40 ppm (4.63%) and T_3: Gibberellic acid (GA_3) @ 30 ppm (4.60%). In contrast, the minimum non-reducing sugar content was recorded in T_{13}: Absolute Control (Distilled water spray) (3.38%).
The application of TIBA (Triiodobenzoic Acid) in watermelon cultivation has been associated with increased levels of non-reducing sugars. TIBA influences plant hormonal balance, particularly by modulating auxin levels. This hormonal adjustment enhances carbohydrate metabolism and storage, leading to a higher accumulation of non-reducing sugars such as sucrose. Improved plant health and stress tolerance under TIBA treatment further contribute to better photosynthesis and carbohydrate production, thereby elevating non-reducing sugar content in watermelon fruits.
The results support the earlier findings of Meshram *et al*. (2022) [17] in watermelon; and Chaudhary *et al*. (2016) [27] in watermelon.

3.1.4 Total sugars (%)
The data pertaining to total sugars, as influenced by different treatment concentrations of plant growth regulators, are presented in Table 1 and Figure 2.
Significant differences were observed among the treatments in total sugars. The maximum total sugars (8.25%) were recorded in treatment T_8: Triiodobenzoic acid (TIBA) @ 30 ppm, which was statistically on par with T_7: Triiodobenzoic acid (TIBA) @ 20 ppm (8.09 %) and T_4: Gibberellic acid (GA_3) @ 40 ppm (7.90%). In contrast, the minimum total sugars were recorded in T_{13}: Absolute control (distilled water spray) (5.49%).
The application of TIBA (Triiodobenzoic Acid) at 30 ppm resulted in a noticeable increase in the total sugar content in watermelon, which can be attributed to several physiological effects induced by TIBA. Firstly, TIBA enhances photosynthetic efficiency, leading to an increased production of carbohydrates that are ultimately converted into sugars. This boost in sugar synthesis is reflected in the higher total sugar content observed in the fruit.
Additionally, TIBA accelerates the ripening process by influencing the plant's hormonal balance. During ripening, the breakdown of complex carbohydrates into simpler sugars occurs, contributing further to the increase in total sugars. Moreover, TIBA's impact on cell elongation and growth regulation leads to more efficient resource allocation within the plant. This efficiency ensures that more nutrients and energy are directed towards fruit development, thereby increasing sugar concentration.
Lastly, TIBA's ability to regulate growth hormones, particularly by inhibiting auxin activity, can lead to favorable changes in growth patterns. These changes support the accumulation of sugars in the developing fruit, resulting in the higher total sugar content observed when TIBA is applied at this concentration. The results support the earlier findings of Meshram *et al*. (2022) [17] in watermelon and Chaudhary *et al*. (2016) [27] in watermelon.

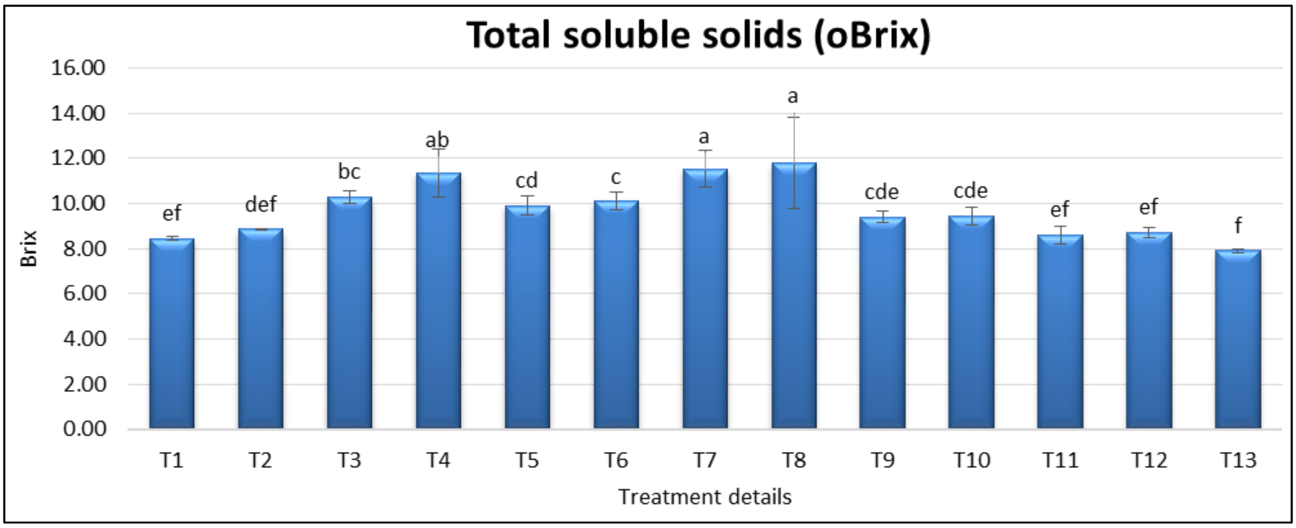

Fig 1: Assessing the impact of exogenous application of plant growth regulators on Total soluble solids (^{0}Brix) in watermelon

Table 1: Treatment details for figure 1

T_1 - Naphthalene acetic acid (NAA) @ 100 ppm	T_6 - Ethrel @ 500 ppm	T_{11} - Cycocel @ 250 ppm
T_2 - Naphthalene acetic acid (NAA) @ 150 ppm	T_7 - Triiodobenzoic acid (TIBA) @ 20 ppm	T_{12} - Cycocel @ 500 ppm
T_3 - Gibberellic acid (GA_3) @ 30 ppm	T_8 - Triiodobenzoic acid (TIBA) @ 30 ppm	T_{13} - Control (Distilled Water Spray)
T_4 - Gibberellic acid (GA_3) @ 40 ppm	T_9 - Malic hydrazide (MH) @ 100 ppm	
T_5 - Ethrel @ 250 ppm	T_{10} - Malic hydrazide (MH) @ 150 ppm	

Note: Error bars indicate standard deviation. Columns with different letters are statistically different ($p \leq 0.05$).

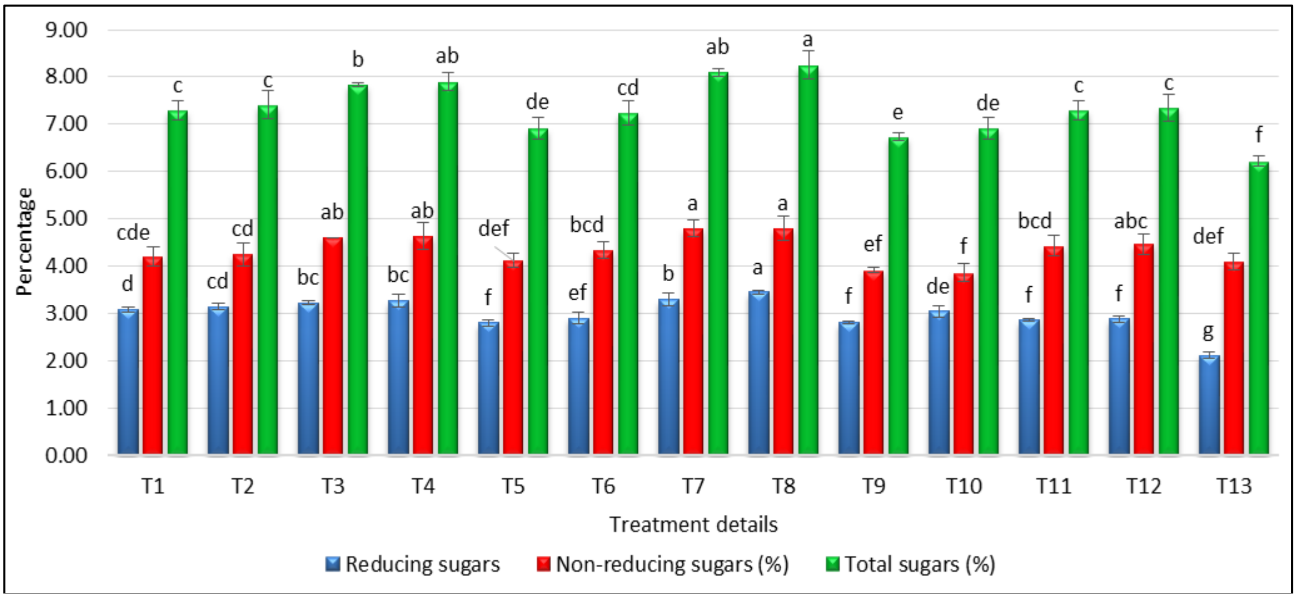

Fig 2: Assessing the impact of exogenous application of plant growth regulators on Reducing sugars (%), non-reducing sugars (%), and Total sugars (%) in watermelon

Table 2: Treatment details for figure 2

T_1 - Naphthalene acetic acid (NAA) @ 100 ppm	T_6 - Ethrel @ 500 ppm	T_{11} - Cycocel @ 250 ppm
T_2 - Naphthalene acetic acid (NAA) @ 150 ppm	T_7 - Triiodobenzoic acid (TIBA) @ 20 ppm	T_{12} - Cycocel @ 500 ppm
T_3 - Gibberellic acid (GA_3) @ 30 ppm	T_8 - Triiodobenzoic acid (TIBA) @ 30 ppm	T_{13} - Control (Distilled Water Spray)
T_4 - Gibberellic acid (GA_3) @ 40 ppm	T_9 - Malic hydrazide (MH) @ 100 ppm	
T_5 - Ethrel @ 250 ppm	T_{10} - Malic hydrazide (MH) @ 150 ppm	

Note: Error bars indicate standard deviation. Columns with different letters are statistically different ($p \leq 0.05$).

Table 3: Assessing the impact of exogenous application of plant growth regulators on total soluble solids, reducing sugars, non-reducing sugars and total sugars in watermelon.

Treatments	Total soluble solids (°Brix)	Reducing sugars (%)	Non-reducing sugars (%)	Total sugars (%)
T_1 - Naphthalene acetic acid (NAA) @ 100 ppm	$8.43^{ef}\pm0.09$	$3.09^{d}\pm0.06$	$4.20^{cdef}\pm0.20$	$7.29^{c}\pm0.20$
T_2 - Naphthalene acetic acid (NAA) @ 150 ppm	$8.85^{def}\pm0.01$	$3.15^{cd}\pm0.07$	$4.25^{cde}\pm0.24$	$7.41^{c}\pm0.30$
T_3 - Gibberellic acid (GA3) @ 30 ppm	$10.27^{bc}\pm0.29$	$3.23^{bc}\pm0.04$	$4.60^{ab}\pm00$	$7.84^{b}\pm0.04$
T_4 - Gibberellic acid (GA3) @ 40 ppm	$11.35^{ab}\pm1.06$	$3.27^{bc}\pm0.13$	$4.63^{ab}\pm0.28$	$7.90^{ab}\pm0.20$
T_5 - Ethrel @ 250 ppm	$9.90^{cd}\pm0.42$	$2.8^{f}\pm0.08$	$4.13^{def}\pm0.16$	$6.93^{de}\pm0.23$
T_6 - Ethrel @ 500 ppm	$10.09^{c}\pm0.40$	$2.9^{ef}\pm0.12$	$4.33^{bcd}\pm0.18$	$7.24^{cd}\pm0.26$
T_7 - Triiodobenzoic acid (TIBA) @ 20 ppm	$11.54^{a}\pm0.80$	$3.3^{b}\pm0.14$	$4.79^{a}\pm0.18$	$8.09^{ab}\pm0.08$
T_8 - Triiodobenzoic acid (TIBA) @ 30 ppm	$11.80^{a}\pm2.01$	$3.45^{a}\pm0.05$	$4.80^{a}\pm0.26$	$8.25^{a}\pm0.31$
T_9 - Malic hydrazide (MH) @ 100 ppm	$9.40^{cde}\pm0.27$	$2.81^{f}\pm0.03$	$3.92^{ef}\pm0.05$	$6.73^{e}\pm0.08$
T_{10} - Malic hydrazide (MH) @ 150 ppm	$9.44^{cde}\pm0.38$	$3.05^{de}\pm0.12$	$3.87^{f}\pm0.18$	$6.91^{de}\pm0.22$
T_{11} - Cycocel @ 250 ppm	$8.61^{ef}\pm0.39$	$2.86^{f}\pm0.03$	$4.43^{bcd}\pm0.21$	$7.29^{c}\pm0.21$
T_{12} - Cycocel @ 500 ppm	$8.72^{ef}\pm0.23$	$2.88^{f}\pm0.06$	$4.47^{abc}\pm0.22$	$7.35^{c}\pm0.28$
T_{13} - Control (Distilled Water Spray)	$7.89^{f}\pm0.07$	$2.12^{g}\pm0.07$	$3.38^{g}\pm0.23$	$5.49^{f}\pm0.17$
Overall mean value	9.71	2.99	4.29	7.29
S.E (m)±	0.40	0.04	0.11	0.12
CD at 5 %	1.18	0.14	0.33	0.35
CV (%)	7.19	2.84	4.67	2.91

4. Conclusion

The application of Triiodobenzoic acid (TIBA) at 30 ppm significantly improved watermelon quality, with the highest total soluble solids (11.80 °Brix), reducing sugars (3.45%), non-reducing sugars (4.80%), and total sugars (8.25%) recorded. This suggests that TIBA, applied at the 2-true and 4-true leaf stages, enhances sweetness and overall fruit quality, offering watermelon growers a practical strategy to boost production and consumer satisfaction. The study highlights the role of PGRs in promoting sustainable and efficient watermelon cultivation.

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