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Effect of Foliar Application of Chitosan, Triacontanol, and Benzyladenine on the Concentration of Some Phytohormones and Yield of Strawberry

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ABSTRACT

This study aims to assess the effect of foliar application with chitosan, triacontanol and benzyl adenine on the growth and Yield of Strawberry plants. A factorial experiment (3 × 3 × 2) was carried out in Randomized Complete Block design (RCBD) within split-plot arrangement with three replication, at the University of Baghdad from July the, 1st, 2023 to July the, 1st, 2024, The main plots of the experiment consisted of 2 levels of chitosan (0, 500 mg L^{-1}), whereas the sub-plots were 3 levels of triacontanol (0, 1 and 2 mg L^{-1}) and benzyladenine (0, 250 and 500 mg L^{-1}). Application of 500 mg L^{-1} chitosan exerted significant improvement in all traits studied. Triacontanol at 2 mg L^{-1} improved plant height, crown number, chlorophyll content, auxin and cytokinin levels, and yield. Benzyl adenine at 500 mg L^{-1} increased hormone levels, Flowers number, and fruit yield.. Notably, the interaction among the three factors had a synergistic effect on most traits. These findings suggest that combined application of these substances can effectively promote growth and productivity in strawberry cultivation

Key words: : auxins , cytokinins , gibberellins , small fruits

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INTRODUCTION

Strawberry (*Fragaria ananassa* Duch.), belonging to the Family of Rosaceae (Lateef et al., 2021; Saleem et al., 2021), is one of the most popular small fruits cultivated in the world. Being perennial, it is also known to adapt itself to various elements of climates, mostly the temperature. As of 2022, the world production of strawberries was estimated by the Food and Agriculture Organization (FAO) to be 9,569,865 tonnes, representing a 2.18% increase compared to 2021. In Iraq, strawberry production increased from 6 tonnes in 2021 to 25 tonnes in 2022, reflecting an absolute increase of 19 tonnes and a relative growth rate of approximately 317%. Despite this notable improvement, Iraq remains among the countries with limited global strawberry production. Besides consumption of fresh strawberries, the fruits are widely utilized. The fruit is also known for its health and medicinal properties because of its high antioxidants, vitamins, minerals, proteins, carbohydrates, fats, and dietary fiber content. And strawberries are also famous for their high content of organic acids and various essential mineral elements (Khodaei and Hamidi-Esfahani, 2019). Over the past fifteen years, there has been increased interest in growing strawberry cultivation in Iraq in particular and globally, following increased consumer demand.This trend has been followed by an increase in research for improving vegetative growth and yield . Biostimulants, in particular, have attracted much attention as they play vital roles in enhancing plant growth, yield, and stress tolerance through the scavenging of reactive oxygen species (ROS) (Salloom and Al-Hachami, 2020). Chitosan is identified as an effective biostimulant which enhances the growth, productivity of plant and useful in promoting the stress tolerance. The antioxidant defense function is due to its special chemical structure and the lots of available amine groups as well as the hydroxyl groups that can

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bind to ROS. Furthermore, chitosan itself provides a natural source of nitrogen and amino acids (Katel et al., 2022). Several recent studies focused on the role of chitosan in growth promotion and subsequently in yield improvement (Metwaly et al., 2023; Rahman et al., 2018). On the other hand, a recent investigations pointed at the importance of Plant Growth Regulators (PGRs) . Those organic compounds have a great influence on the vegetative growth by inducing the resistance of plant against environmental stresses and in turns this appears in the yield. This is achieved through their role in regulating most of the important biochemical activities of horticultural crops in the plant life cycle. Triacontanol (TRIA) is recognized as a non-nutritive organic/chemical compound with effective biological impacts. It increases the rate of photosynthesis, enhances water and nutrient uptake from the soil, increases the number of leaves and leaf area, promotes flower number, elevates the content of photosynthetic pigments in leaves, reduces the damage from environmental stresses, and increases secondary metabolite compounds such as alkaloids and phenols , Studies have shown that foliar application of TRIA significantly enhances plant height, leaf area, dry weight of shoots and roots, and yield, as well as improves physical and chemical traits of the plant (Hussein and Al-Doori, 2021; Katel et al., 2022) . Cytokinins, such as the naturally occurring zeatin, are key regulators of cell division (Hamza and Al-Taey, 2020). Benzyl adenine, a synthetic cytokinin, plays a vital role in regulating plant organ growth, plastid differentiation, suppression of apical dominance, stimulation of lateral buds, and control of stomatal movement , Hussein and Al-Doori (2021) highlighted its positive effects on vegetative and floral growth and yield. Therefore, this study aims to examine the impact of chitosan, triacontanol, and benzyl adenine on plant hormone concetration (auxin, gibberellic acid, and zeatin) and yield.

MATERIALS AND METHODS

This study was conducted in one of the plastic houses at Research Station A, affiliated with the College of Agricultural Engineering Sciences, University of Baghdad, Al-Jadriyah, during the period from July 1, 2023, to July 1, 2024. Strawberry plants of the cultivar *Albion* were obtained from nurseries operated by the Ministry of Agriculture – Agricultural Research Directorate – Najaf Al-Ashraf Branch. The soil of the plastic house (30 × 9 m) was prepared by first removing weeds, followed by plowing and levelling using a tractor specialized for greenhouse operations. Thermal soil sterilization was carried out from July 1, 2023, to September 10, 2023. Pesticides were applied to protect against insect infestations as well as fungal and bacterial diseases. The soil was then shaped into raised terraces with a width of 0.80 m, height of 0.30 m, and length of 30 m. A 0.50 m space was left at both the front and rear ends of the plastic house. *Albion* saplings were planted according to the experimental design layout. To evaluate soil properties, random samples were collected from various locations in the plastic house at a 0–30 cm depth to form a representative composite sample. Table (1) summarizes the soil's physical and chemical characteristics, analyzed at the Soil and Water Sciences Laboratory, College of Agricultural Engineering Sciences, University of Baghdad.

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Table 1. The results of the soil sample analysis inside the plastic house

Measured Parameter	Unit	Analysis Result
Electrical conductivity EC	dS m^{-1}	3.22
pH 1:1	—	7.15
Available nitrogen (N)	mg kg^{-1} soil	27
Available phosphorus (P)	mg kg^{-1} soil	3.20
Available potassium (K)	mg kg^{-1} soil	122.1
Organic matter (OM)	%	0.66
Calcium carbonate	%	31.34
Soluble calcium (Ca^{2+})	meq L^{-1}	17.5
Soluble magnesium	meq L^{-1}	10.20
Soluble sodium (Na^{+})	meq L^{-1}	5.79
Soluble bicarbonates	meq L^{-1}	1.4
Soluble chloride (Cl^{-})	meq L^{-1}	20.9
Soluble potassium (K^{+})	meq L^{-1}	1.23
Moisture content at field	cm^3 cm^{-3}	0.339
Moisture content at	cm^3 cm^{-3}	0.159
Sand	g kg^{-1} soil	275
Silt	g kg^{-1} soil	313
Clay	g kg^{-1} soil	412
Soil texture class	—	Clay

The strawberry saplings were planted on October 1, 2023, two weeks after applying preventive pesticides against insect, fungal, and bacterial infections. A double-row terraces system was used , with a spacing of 0.20 m between the two rows on each terrace, 0.30 m between plants within the same row, and 1.40 m between the centers of adjacent terraces. The study included three factors:

First factor (C): Foliar application of the biostimulant chitosan, applied at two concentrations: 0 and 500 mg L^{-1}, designated as C0 and C1, respectively (Rahman et al., 2018).

Second factor (T): Foliar application of the growth regulator triacontanol at three concentrations: 0, 1, and 2 mg L^{-1}, designated as T0, T1, and T2, respectively (El-Beltagi et al., 2022).

Third factor (B): Foliar application of the growth regulator benzyladenine at three concentrations: 0, 250, and 500 mg L^{-1}, designated as B0, B1, and B2, respectively (Ahmed and Al-Atrakchii, 2023). The first spray of the biostimulant Chitosan was applied after the plants had developed five true leaves, on November 15, 2023. Triacontanol was sprayed three days after the Chitosan application, and benzyl adenine was sprayed three days after the Triacontanol application. Plants were treated until July 1, 2024, at a rate of one spray per month. The last spraying date was June 15. The experiment was conducted as Randomized Complete Block design (RCBD) with split-plot arrangement with three replication. The experiment consist of three factors, the first factor (Chitosan) was randomly distributed in the main plot. The combinations between the second and third factors (3*3=9) (Triacontanol at three concentrations and Benzyladenine at three concentrations) were randomly distributed and represented the subplots. This resulted in 54 experimental units. The results were analyzed using the GenStat statistical program, and the means were compared according to the least significant differences (LSD) at a 5% probability level.

Estimation of (Auxins, Gibberellins, and Cytokinins) Using HPLC Technique

Extraction Method: Extraction was carried out following the method described by Unyayar *et al.* (1996), with modifications to suit the Fast Isocratic Chromatography (FIC) column used in the High-Performance Liquid Chromatography (HPLC) system.

Quantitative and qualitative estimation of growth regulators: Standard solutions of PGRs were injected at a concentration of 25 µg/ml to determine the retention time and peak

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area. Sample solutions to be quantified for all parameters were then prepared and injected into an HPLC device in a volume of 20 µL under the same conditions as the standard sample injections. Hormone concentrations were determined by comparing the quantitative estimation results in the sample samples for retention time and band area with the retention time and band area of the standard samples according to the following equation:

Sample concentration = (Sample area / Standard area) * Standard concentration * Number of dilutions

RESULTS AND DISCUSSION

The results in Table(2) indicate that the Chitosan treatment (C_1) significantly outperformed the control treatment (C_0) in terms of plant height, recording a value of 4.143 cm, compared to only 3.545 cm under C_0. Regarding the effect of Triacontanol (T) treatments, significant differences were also observed. The T2 treatment led to the highest plant height of 4.127 cm, significantly greater than T0 (3.512 cm). Statistical analysis indicated that Benzyl adenine (B) exhibited a relatively limited influence on plant height. At the B_2 treatment level, the mean plant height was reduced to 3.678 cm, compared to 3.936 cm in the control treatment (B_0). Similarly, the B_1 treatment resulted in a height of 3.919 cm, which did not differ significantly from the control. As for the effect of the three-way interaction between Chitosan, Triacontanol, and Benzyladenine, the statistical analysis of Table (2) showed a significant superiority in the C1*T2*B0 treatment, which gave the highest height of 4.733 cm, compared to the C0*T0*B0 treatment, which recorded the lowest height of 2.553 cm. The results of the statistical analysis of Table (2) show that spraying plants with Chitosan, The treatment C_1 (Chitosan at 500 mg L^{-1}) recorded an average of 3.765 crowns. $Plant^{-1}$, which was significantly higher than the control treatment C_0 (no Chitosan), which gave only 3.084 crowns. $Plant^{-1}$. Regarding the effect of Triacontanol (T) , the data also revealed significant differences among treatments. The highest number of crowns (3.910 crowns. $plant^{-1}$,) was observed under treatment T_2, followed by treatment T_1, which recorded 3.667 crowns per plant. Both T_1 and T_2 were significantly better than the control treatment T_0, which had the lowest value at 2.697 crowns. Plant-1. The B_2 treatment (500 mg L^{-1}) gave the highest value among Benzyladenine treatments at 3.562 crowns $Plant^{-1}$, which was significantly greater than the control treatment B_0 (0 mg L^{-1}), which recorded 3.165 crowns $Plant^{-1}$. However, B_2 did not differ significantly from B_1 (250 mg L^{-1}), which recorded 3.546 crowns $Plant^{-1.}$ Furthermore, the three-way interaction between Chitosan, Triacontanol, and Benzyladenine showed a significant effect on this trait. The combination $C_1 \times T_2 \times B_1$ significantly outperformed all other combinations, producing an average of 4.733 crowns $Plant^{-1}$, compared to the lowest value of only 1.100 crowns $Plant^{-1}$ under the control combination $C_0 \times T_0 \times B_0$ The results in Table 2 shows that the bio- stimulant Chitosan had an influence on chlorophyll concetration. The control treatment C_0 (no Chitosan) obtained the lowest chlorophyll concetration of 242.73 mg/100 g fresh weight and was significantly lower than treatment C_1 which had a value of 257.78 mg/100 g fresh weight. Regarding Triacontanol, the highest chlorophyll concetration was found under T2 at 261.58 mg per 100 g fresh weight and was significantly higher than T0, which had 232.66 per 100 g fresh wt. Regarding the effect of Benzyladenine (B), the results revealed significant differences. The lowest chlorophyll content (240.94 mg per 100 g fresh weight) was found in the control treatment B_0, while treatment B_1 showed the highest value at 257.95 mg per 100 g fresh weight, which was significantly higher than both B_0 and B_2, the latter recording 251.88 mg per 100 g fresh weight.The interaction among Chitosan, Triacontanol, and Benzyladenine also had a clear influence on this trait. The combination $C_1 \times T_1 \times B_2$ produced the highest chlorophyll concetration of 286.19 mg per 100 g fresh weight, whereas the lowest value was recorded for the control combination $C_0 \times T_0 \times B_0$, at 231.00 mg per 100 g fresh weight .

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Table 2. Effect of spraying with the biostimulant Chitosan and the growth regulators Triacontanol and Benzyladenine and their interaction on some vegetative growth traits

Treatment	Plant height (cm)	Number of crowns (crowns plant^{-1})	Leaf chlorophyll content (mg 100 g^{-1} fresh weight)
C0	3.545	3.0844	242.73
C1	4.143	3.7652	257.78
LSD 0.05	0.1022	0.03065	0.678
T0	3.512	2.6972	232.66
T1	3.894	3.6672	256.52
T2	4.127	3.9100	261.58
LSD 0.5	0.122	0.03753	0.831
B0	3.936	3.1656	240.94
B1	3.919	3.5461	257.95
B2	3.678	3.5628	251.88
LSD 0.5	0.125	0.03753	0.831
C0T0B0	2.553	1.1000	231.00
C0T0B1	3.399	2.3667	243.66
C0T0B2	3.208	2.6667	236.59
C0T1B0	3.599	3.9167	236.78
C0T1B1	3.615	3.2167	256.94
C0T1B2	3.745	3.7333	240.91
C0T2B0	3.982	3.1167	250.31
C0T2B1	4.549	3.5333	251.99
C0T2B2	3.256	4.1100	236.39
C1T0B0	4.256	3.1167	219.02
C1T0B1	3.715	3.7167	228.51
C1T0B2	3.941	3.2167	237.18
C1T1B0	4.491	4.2100	234.69
C1T1B1	4.350	3.7100	283.60
C1T1B2	3.567	3.2167	286.19
C1T2B0	4.733	3.5333	273.82
C1T2B1	3.889	4.7333	283.00
C1T2B2	4.350	4.4333	274.00
LSD 0.05	0.306	0.09194	2.035

The results in Table (3) indicate that leaf auxin concetration (IAA) was significantly influenced by the experimental treatments. Foliar application of Chitosan increased this trait, particularly under treatment C_1, which recorded the highest IAA concetration at 61.039 µg g^{-1} fresh weight, significantly higher than treatment C_0, where the lowest value of 42.550 µg g^{-1} fresh weight was observed. Regarding the effect of Triacontanol (T), the data show that treatment T_1 gave the highest leaf IAA concetration at 56.631 µg g^{-1} fresh weight, followed by treatment T_2 with 51.164 µg g^{-1} fresh weight, both of which were significantly higher than the control treatment T_0, which recorded 47.588 µg g^{-1} fresh weight. In contrast, foliar application of Benzyladenine (B) led to a significant decrease in leaf auxin concetration. The lowest IAA level was observed under treatment B_1, with a value of 47.940 µg g^{-1} fresh weight, followed by treatment B_2 at 53.489 µg g^{-1} fresh weight. The highest auxin concetration among Benzyladenine treatments was recorded under B_0 at 53.954 µg g^{-1} fresh weight, which was significantly higher than both B_1 and B_2 .The interaction between the bio-stimulant Chitosan and the plant growth regulators Triacontanol and Benzyladenine had a significant effect on leaf auxin concetration. The highest IAA concentration was observed in the combination $C_1 \times T_0 \times B_1$, reaching 72.626 µg g^{-1} fresh weight, while the lowest level was recorded for the combination $C_0 \times T_2 \times B_1$, at only 24.092 µg g^{-1} fresh weight. The results in Table (3) indicate significant differences among the bio-stimulant treatments in terms of leaf gibberellin concetration (GA). The C_1 treatment (Chitosan at 500 mg L^{-1}) significantly increased GA levels, reaching 84.700 µg g^{-1} fresh weight, compared to the control treatment C_0, which recorded the

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lowest value at 83.060 µg g^{-1} fresh weight.The result shows that the T_2 treatment (Triacontanol at 2 mg L^{-1}) significantly outperformed T_1, with a leaf GA concetration of 84.830 µg g^{-1} fresh weight, while T_1 recorded 83.038 µg g^{-1} fresh weight. The control treatment T_0 had a GA concetration of 83.919 µg g^{-1} fresh weight.Regarding the effect of Benzyladenine (B) , the data show that increasing its concentration led to a decrease in leaf gibberellin concetration. The highest GA level was observed under B_0 (no Benzyladenine), at 84.607 µg g^{-1} fresh weight, followed by treatment B_1 at 83.590 µg g^{-1} fresh weight, which was significantly lower. Treatments B_1 and B_2 (500 mg L^{-1}) showed no significant difference, with B_2 recording 83.589 µg g^{-1} fresh weight. The three-way interaction between the bio-stimulant Chitosan and the growth regulators Triacontanol and Benzyladenine had a significant effect on this trait. The combination $C_1 \times T_2 \times B_0$ produced the highest leaf gibberellin concetration at 96.219 µg g^{-1} fresh weight. the combination $C_0 \times T_2 \times B_0$ recorded the lowest value at 70.297 µg g^{-1} fresh weight, which did not differ significantly from the combination $C_1 \times T_1 \times B_1$, which gave a value of 70.960 µg g^{-1} fresh weight. The control treatment (unspecified but likely $C_0 \times T_0 \times B_0$) recorded a GA concetration of 86.738 µg g^{-1} fresh weight. The results shows that foliar application of the bio-stimulant Chitosan significantly affected leaf cytokinin concetration CK (Table 3).

Table 3. Effect of spraying with the biostimulant Chitosan and the growth regulators Triacontanol and Benzyladenine and their interaction on leaf hormone concentration

Treatment	Leaf IAA content (µg g^{-1} fresh weight)	Leaf GA content (µg g^{-1} fresh weight)	Leaf CK content (µg g^{-1} fresh weight)
C0	42.550	83.060	50.137
C1	61.039	84.798	73.060
LSD 0.05	0.0984	0.3314	0.1412
T0	47.588	83.919	56.113
T1	56.631	83.038	66.730
T2	51.164	84.830	61.953
LSD 0.5	0.1205	0.4058	0.1729
B0	53.954	84.607	63.614
B1	47.940	83.590	56.488
B2	53.489	83.589	64.694
LSD 0.5	0.1205	0.405	0.1729
C0T0B0	40.153	86.738	47.316
C0T0B1	31.763	88.546	37.426
C0T0B2	25.094	84.103	29.571
C0T1B0	63.650	81.206	74.999
C0T1B1	34.232	87.364	40.336
C0T1B2	58.630	85.886	69.084
C0T2B0	41.160	70.297	48.499
C0T2B1	24.092	85.955	28.387
C0T2B2	64.172	77.446	75.614
C1T0B0	60.197	78.869	71.166
C1T0B1	72.626	77.955	85.569
C1T0B2	55.698	87.302	65.629
C1T1B0	55.638	94.313	65.558
C1T1B1	66.618	70.960	78.501
C1T1B2	61.020	78.497	71.901
C1T2B0	62.927	96.219	74.147
C1T2B1	58.309	90.763	68.706
C1T2B2	56.321	88.300	76.363
LSD 0.05	0.2951	0.994	0.4236

Treatment C_1 (Chitosan at 500 mg L^{-1}) significantly increased CK levels, recording a value of 73.060 µg g^{-1} fresh weight, which was notably higher than treatment C_0 (no Chitosan), where the concetration was only 50.137 µg g^{-1} fresh weight. Regarding the effect of the growth regulator Triacontanol (T), the result indicate that treatment T_1

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significantly improved leaf cytokinin concetration, reaching 66.730 µg g^{-1} fresh weight , followed by treatment T_2 at 61.953 µg g^{-1} fresh weight , with a statistically significant difference between them. The control treatment T_0 recorded the lowest level at 56.113 µg g^{-1} fresh weight. As for the effect of Benzyladenine (B), treatment B_2 showed a significant increase in leaf cytokinin concetration, reaching 64.694 µg g^{-1} fresh weight, compared to the control treatment B_0, which recorded 63.614 µg g^{-1} fresh weight. Treatment B_1, however, gave the lowest cytokinin concetration at 56.488 µg g^{-1} fresh weight. In terms of the three-way interaction among the experimental factors (the bio-stimulant and growth regulators), the combination $C_1 \times T_0 \times B_1$ achieved the highest leaf cytokinin concetration at 85.569 µg g^{-1} fresh weight . In contrast, the combination $C_0 \times T_2 \times B_1$ recorded the lowest level at 28.387 µg g^{-1} fresh weight, while the distilled water control (likely representing untreated plants) recorded 47.316 µg g^{-1} fresh weight. Upon reviewing the results of the study on vegetative growth traits, which included the biostimulant *Chitosan* and the plant growth regulators *Triacontanol* and *Benzyladenine*, it was evident that the treatments induced significant improvements in most measured indicators. Specifically, foliar application of *Chitosan* at 500 mg L^{-1} significantly enhanced both plant height and crown number (Table 2). This enhancement may be attributed to *Chitosan's* role in activating signaling pathways associated with the biosynthesis of plant hormones (Safikhan et al., 2018), which, in turn, led to increased gibberellin levels (Table 2). Gibberellins are known for their indirect effect in enlarging plant cell size by enhancing cell wall extensibility and promoting cell expansion. After leading to the induction of auxin and increasing the concetration (Table 2), which plays a role in cell growth and its importance in the process of stimulating and modifying the process of gene replication and then the process of translation and then stimulating the construction of RNA and protein (Uozu et al., 2005) , as the auxins induced by gibberellin play an important role in stimulating the loosening of cell walls (Wall Loosening) by breaking the bonds of the cell walls and rearranging them in new locations under the influence of turgor pressure, which contributes to increasing the size and expansion of cells. In addition, auxins may affect the enzymes that compose them, especially the enzyme Cellulase, which in turn weakens the fiber systems and the construction and decomposition of cell wall components, which may come about through activating the pumping of hydrogen ions and reducing the cell pH, which causes an increase in the acidity of the cell wall and a change in the bonds and then an increase in the flexibility of the cell wall (Du et al., 2020), as it leads to a change in the water relations of the plant, especially the turgor pressure and osmosis of the cell, which causes the flow of water into the cell and an increase in its expansion. On the other hand, auxin stimulates the division of cells in the apical meristematic tissues, thereby promoting an increase in plant height. Alternatively, the significant improvement observed in vegetative growth traits particularly plant height and number of crowns (Table 2) may be attributed to an increase mineral content in the leaves, Auxin promotes root growth and branching, thereby increasing the uptake of mineral nutrients. It also regulates the gene expression of mineral transporter proteins and enhances the development of vascular tissues, which improves the distribution of nutrients within the plant. These mechanisms collectively contribute to increasing the mineral content in plant tissues (De Rybel et al., 2016; Giehl and von Wirén, 2014; Krouk et al., 2016) . As for the observed increase in total photosynthetic pigments—chlorophyll content—in leaves (Table 2), this could be due to a synergistic or combined effect among plant hormones. It may also be attributed to the significant increase in cytokinin levels in plant tissues under Chitosan application (Table 3). Cytokinins promote chlorophyll accumulation in leaves by stimulating chloroplast formation. In fact, cytokinins are known to encourage the development of plastids, thylakoid membranes, and overall chlorophyll synthesis (Zwack and Rashotte,

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2015).The results also indicate that foliar application of the plant growth regulator Triacontanol at concentrations of 1 and 2 mg L^{-1} had a positive effect on vegetative growth traits. Specifically, Triacontanol treatment led to an increase in plant height (Table 2). This improvement may be attributed to a significant increase in endogenous plant hormone levels—particularly auxins, gibberellins , and cytokinins, as well as enhanced activity of antioxidant enzymes and increased production of secondary metabolites, especially phenolic compounds (Patel and Singh, 2024). As previously discussed, these hormonal changes contributed to the observed significant increase in plant height.The increase in number of crowns Table (2) could be due to the role of Triacontanol in enhancing internal hormone levels such as gibberellins (GA) Salloom and Al-Hachami (2020) and zeatin, while simultaneously reducing the level of abscisic acid (ABA) (Hayat and Ahmad, 2011) . The synergistic or complementary interaction among these hormones, as mentioned earlier, also likely played a role in this response. As for the observed increase in leaf chlorophyll concetration (Table 2), this may be explained by the significant rise in cytokinin levels in plant tissues under Triacontanol treatment. Chlorophyll can be retained by cytokinins through suppressing the activity of degradation enzymes, dehydrogenase and ribonuclease (Taiz and Zeiger, 2010). This enhances photosynthetic rate by increasing the activity of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the key enzyme involved in carbon fixation in the Calvin cycle. Then the (total) rate of sugar (carbohydrate) and energy (ATP) generation on the one hand and in soluble proteins and amino-acids on the other hand is increased and the relative growth rate of the vegetative parts, such as plant length (or culm length) and number of culms will increase. This may be due to the increased concetration of endogenous hormones such as GA (Salloom and Al-Hachami, 2020). Treating plants with Benzyladenine led to significant differences in the vegetative growth of the plants, exhibiting both positive and negative effects on vegetative growth characteristics. A shortening of plants treated with Benzyladenine was observed (Table 2), which may be attributed to the redirection of nutrients and phytohormones towards the growth of lateral buds at the expense of plant height. Cytokinin promotes the development of xylem vessels connecting the axillary buds and the main stem, leading to the breaking of apical dominance controlled by auxin, which consequently caused a significant increase in the number of crowns (Table 2). The results of the statistical analysis show an increase in leaf chlorophyll concentration when plants were treated with Benzyladenine. This may be primarily because cytokinin is essential for the differentiation of chloroplasts and secondarily because cytokinin inhibits chlorophyll degradation and delays leaf senescence. Additionally, it plays a role in the phenomenon of Phytogerontology in plants. In addition, The increase in the concetration of nutrients in the leaves, especially nitrogen and iron, plays a major role in its formation (Khalil et al., 2015), as nitrogen enters into the composition of porphyrins, which enter into the formation of chlorophyll molecules, while iron enters into the composition and construction of chloroplasts, as they contain 80% of the total iron in the plant through its role in building the proteins of these plastids, especially electron transport proteins. Statistical analysis of the triple-interaction treatment revealed that chitosan and triacontanol without benzyl adenine ($C_1 \times T_2 \times B_0$) yielded the highest mean plant height (Table 2). This improvement is attributed to the synergistic effect between chitosan—which stimulates indole-3-acetic acid (IAA) biosynthesis—and triacontanol, which enhances endogenous accumulation of key phytohormones, particularly gibberellin (GA). Concurrently, the exclusion of benzyl adenine likely preserved apical meristem activity by preventing apical dominance suppression, thereby facilitating cellular elongation and promoting longitudinal expansion of plant tissues (Rademacher, 2015). Significantly, treatment $C_1 \times T_2 \times B_1$ demonstrated statistically superior performance in crown number per plant (Table 2). This may be ascribed to hormonal stimulation from elevated auxin and cytokinin

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levels Table (3) induced by chitosan and triacontanol, combined with moderate benzyl adenine. These hormonal interactions contributed to apical dominance breakage, activating axillary meristems and stimulating lateral branching and crown proliferation (Werner et al., 2001) . Furthermore, treatment $C_1 \times T_1 \times B_2$ produced the highest chlorophyll concentration (Table 2). This increase may result from enhanced cytokinin activity Table (3) promoted jointly by chitosan and benzyl adenine, given cytokinin's established role in inhibiting chlorophyll degradation through suppression of catabolic enzymes, while simultaneously enhancing chloroplast differentiation and development. Consequently, this contributed to chlorophyll accumulation and improved photosynthetic efficiency (Zwack and Rashotte, 2015). Treatment $C_1 \times T_0 \times B_1$ recorded the peak auxin concentration (Table 3), attributed to chitosan's stimulatory effect on auxin production via priming of primary defense responses, supplemented by moderate benzyl adenine. The latter likely activates gene expression associated with (IAA) biosynthesis (Kowalczyk et al., 2016). Regarding gibberellins, the maximum concentration occurred under $C_1 \times T_2 \times B_0$ (Table 3). This reflects the concerted action of chitosan and triacontanol in stimulating GA biosynthesis, coupled with the absence of benzyl adenine—which may suppress GA at elevated concentrations (Sarkar and Srivastava, 2018). Conversely, treatment $C_1 \times T_0 \times B_1$ most effectively increased cytokinin concentration (Table 3), demonstrating the compound effect of chitosan and benzyl adenine in promoting cytokinin accumulation through either activation of biosynthetic pathways or inhibition of degradation mechanisms, resulting in elevated leaf-tissue concentrations (Davies, 2010) .

Flowers number: The results in Table (4) indicate that plants treated with Chitosan at 500 mg L^{-1} (C_1) showed a significant increase in the number of flowers, recording the highest value of 30.430 flowers $plant^{-1}$. In contrast, the control treatment (distilled water) gave the lowest flower count at 24.870 flowers $plant^{-1}$. Regarding the effect of Triacontanol (T), treatment T_2 significantly outperformed the others, with an average of 29.667 flowers $plant^{-1}$, compared to 25.433 flowers $plant^{-1}$ under the control treatment T_0. Treatment T_1 recorded an intermediate value of 27.850 flowers $plant^{-1}$, which was significantly higher than T_0 but lower than T_2. In terms of Benzyladenine (B) application, the data show that treatment B_2 significantly improved flower production, reaching 29.967 flowers $plant^{-1}$, which was significantly higher than treatment B_1 , where the number of flowers $plant^{-1}$ was 27.667 . The lowest flower count was observed in the control treatment B_0, with only 25.317 flowers $plant^{-1}$. Furthermore, the three-way interaction among the experimental factors (Chitosan, Triacontanol, and Benzyladenine) had a significant effect on flower number. The combination $C_1 \times T_2 \times B_1$ produced the highest number of flowers per plant (37.700 flowers $plant^{-1}$), significantly outperforming the control combination $C_0 \times T_0 \times B_0$, which recorded the lowest value at 16.700 flowers $plant^{-1}$.

Plant yield (g $plant^{-1}$): The results in Table (4) show that the bio-stimulant Chitosan significantly increased the yield per plant. Treatment C_1 (500 mg L^{-1} Chitosan) produced the highest yield at 1176.8 g $plant^{-1}$, which was significantly higher than treatment C_0 (no Chitosan), where the yield was only 790.9 g $plant^{-1}$. Regarding the effect of the plant growth regulator Triacontanol (T), the data indicate a positive response with increasing concentration. The highest yield was recorded under T_2 at 1075.8 g $plant^{-1}$, which was significantly greater than treatment T_1, yielding 994.5 g $plant^{-1}$. The control treatment (distilled water) gave the lowest yield at 881.3 $plant^{-1}$. In the case of Benzyladenine (B) application, statistical analysis revealed a significant increase in yield under treatment B_2 , which recorded the highest value at 1065.8 g $plant^{-1}$, significantly outperforming treatment B_1 , which yielded 1007.0 g $plant^{-1}$. The lowest yield among Benzyladenine treatments was observed in the control treatment B_0 at 878.8 g $plant^{-1}$ Furthermore, the three-way interaction among the experimental factors (Chitosan, Triacontanol, and Benzyladenine) had a significant impact on yield. The combination

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$C_1 \times T_2 \times B_1$ showed the best performance, producing the highest yield of 1523.8 g plant^{-1}, significantly surpassing the control combination $C_0 \times T_0 \times B_0$, which recorded the lowest yield at 445.9 g plant^{-1}.

Table 4. Effect of foliar application of the biostimulant Chitosan and the growth regulators Triacontanol and Benzyladenine, and their interaction, on the number of flowers and yield per plant

treatments	Number of flowers (flowers plant^{-1})	Yield per plant (g plant^{-1})
C0	24.870	790.9
C1	30.430	1176.8
LSD 0.05	0.0893	7.81
T0	25.433	881.3
T1	27.850	994.5
T2	29.667	1075.8
LSD 0.5	0.1094	9.57
B0	25.317	878.8
B1	27.667	1007.0
B2	29.967	1065.8
LSD 0.5	0.1094	9.57
C0T0B0	16.700	445.9
C0T0B1	23.567	752.1
C0T0B2	28.567	936.4
C0T1B0	24.200	778.3
C0T1B1	25.300	831.0
C0T1B2	29.100	921.3
C0T2B0	26.400	786.2
C0T2B1	25.300	882.7
C0T2B2	24.700	784.5
C1T0B0	22.700	749.0
C1T0B1	29.233	1123.9
C1T0B2	31.833	1280.6
C1T1B0	33.100	1418.1
C1T1B1	24.900	928.7
C1T1B2	30.500	1089.6
C1T2B0	28.800	1095.3
C1T2B1	37.700	1523.8
C1T2B2	35.100	1382.5
LSD 0.05	0.2680	23.43

It is evident from the above that foliar application of the biostimulant *Chitosan* positively influenced floral growth traits in strawberry plants, as reflected in the significant increase in flower number (Table 4). This increase may be attributed to the rise in crown number (Table 2), which represents lateral branching in strawberry plants branches that often develop terminal buds capable of flowering . Another possible explanation is the role of auxin (Table 2) in stimulating ethylene production, which promotes flowering (Perveen et al., 2021). Additionally, auxin contributes to hormonal and nutritional balance, ensuring the translocation of synthesized assimilates from leaves to floral buds. While the response of floral growth to spray treatments with the growth regulator Triacontanol differed after an increase in the number of flowers was recorded (Table 3), the reason for this may be attributed to a significant increase in the concetration of the plants of the plant hormones auxin, gibberellin and cytokinin (Table 2), and this increase may be due to the role of Triacontanol in raising the levels of internal plant hormones Perveen et al. (2021) by raising the activity of enzymes, especially antioxidant enzymes, and increasing the production of secondary metabolites, especially phenols (Khalil & Al-Rawi, 2015) , which had an effect, as we mentioned previously. The results also showed a significant increase in flower number in plants treated with the growth regulator Benzyladenine. This may be attributed to the essential role of cytokinins in various physiological processes during the reproductive stage, particularly cell division,

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which is crucial for flower development and growth. Cytokinins are considered important endogenous factors for flowering plants in transitioning from the vegetative to the floral growth phase. Statistical analysis further revealed that foliar application of Chitosan significantly increased fruit yield per plant (Table 4). This improvement can be attributed to the bio-stimulant's role in enhancing several yield components, including the number of flower clusters, flower count, fruit set percentage, fruit size, and individual fruit weight. The results also indicate that foliar application of the plant growth regulator Triacontanol had a positive effect on the physical or physiological characteristics of strawberry fruits, as reflected in the increased yield per plant (Table 3). Moreover, plant hormones induced by Triacontanol play a vital role in enhancing vegetative vigor, thereby boosting carbon assimilation and nutrient accumulation. These nutrients are then translocated from source tissues (leaves) to sink organs (fruits) (Mengel and Kirkby, 2001) , leading to increased fruit size and weight and, with more fruits, a higher total yield per plant. Reviewing the results also shows that Benzyladenine-treated plants achieved a significant increase in yield per plant (Table 4). This might be because of its beneficial effect on the shoot and root growth characteristics (by improving the N, P, Ca, and Fe concentration in the leaves). These improvements may in part have led to improved floral traits (Khalil and Hammooodi, 2020) , particularly flower number Table (4) which would have driven increased fruit number, size and weight—affecting enhanced plant yield. The triple interaction treatment $C_1 \times T_2 \times B_1$ demonstrated significant superiority in both flower number and plant yield (Table 4). This can likely be attributed to the multilevel synergistic interaction between chitosan, triacontanol, and benzyl adenine. Chitosan acts by enhancing auxin (IAA) synthesis, activating the expression of genes associated with hormonal signaling and floral bud formation. It also induces plant priming through the activation of systemic defense pathways, leading to enhanced cell division and expansion, particularly during the transition from the vegetative to the reproductive phase (El-Tanany et al., 2023; Kowalczyk and Treder, 2016).Triacontanol, at the high concentration (T_2), promotes the accumulation of plant hormones such as gibberellins and cytokinins, which play pivotal roles in flowering initiation and fruit set. This regulator also enhances photosynthetic efficiency and increases the activity of antioxidant enzymes, thereby improving the translocation of photosynthetic assimilates from leaves to floral and fruit organs (Perveen et al., 2021). Benzyl adenine, at the medium concentration (B_1), contributes to the breaking of apical dominance, activation of lateral buds, and formation of floral tissues. It regulates the process of cell division in floral buds and the formation of floral organs (Mok and Mok , 2001). Additionally, it delays senescence and enhances chloroplast functions, prolonging the efficiency of photosynthesis that supports reproductive growth (Zwack and Rashotte, 2015). The synergy of these three factors chitosan, triacontanol, and benzyl adenine results in a harmonized hormonal regulation that enhances both flowering initiation and fruit development. Elevated levels of auxin and gibberellin, as shown in Table (3), stimulate floral initiation and fruit enlargement, while cytokinins ensure the formation of floral organs and the distribution of photoassimilates. These interactions are reflected in increased flower number, fruit set ratio, fruit size and weight, and consequently, higher total plant yield (Rademacher , 2015) .

CONCLUSION

The foliar application of a biostimulant, chitosan, in combination with the growth regulators triacontanol and benzyladenine, was found to improve the vegetative and reproductive yield of strawberry (*Fragaria ananassa* Duch) plants. Foliar treatment of 500 mg L^{-1} chitosan led to significant enhancement in plant height, crown number, chlorophyll content and IAA, GA_3, CK (hormone) content and acted directly on flower production and fruit yield. Triacontanol (2 mg L^{-1}) and benzyladenine (500 mg L^{-1}) were also found to increase growth parameters, hormone content, and yield. The interaction between these compounds boosted yield-related traits

and physiological mechanisms. In general, the joint foliar application of chitosan, triacontanol, and benzyladenine was an effective approach for enhancing growth, hormonal status, and productivity of strawberry under controlled environment.

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CONFLICT OF INTEREST

We, the authors, hereby declare that we have no conflicts of interest regarding the publication of this manuscript

AUTHOR/S DECLARATION

We confirm that all Tables in the manuscript are original to us.

AUTHOR'S CONTRIBUTION STATEMENT

All authors made equal contributions to the study design, methodology, experimental work, data analysis, and manuscript writing. All authors reviewed and approved the final version of the manuscript.

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تأثير الرش بالكيتوسان والتراي كونتانول والبنزل أدنين في محتوى نبات الشليك من بعض الهرمونات النباتية والحاصل

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المستخلص

الهدف من البحث تقييم تأثير الرش الورقي بالكايتوسان، والترياكونتانول، والبنزيل أدينين في نمو وحاصل نبات الشليك. أُجريت تجربة عاملية (3 × 3 × 2) باستخدام تصميم القطاعات العشوائية الكاملة وبترتيب القطع المنشقة وبثلاث مكررات خلال المدة من 1 تموز 2023 إلى 1 تموز 2024 في جامعة بغداد، شملت المعاملات الرئيسة الكايتوسان بتركيزين (0 و500 ملغم لتر$^{-1}$)، في حين وُزعت التراكيز (0، 1، 2 ملغم لتر$^{-1}$) للترياكونتانول و(0، 250، 500 ملغم لتر$^{-1}$) للبنزيل أدينين على القطع الثانوية. أدى الكايتوسان بتركيز 500 ملغم لتر$^{-1}$ إلى زيادة معنوية في جميع الصفات المقاسة. كما زاد الترياكونتانول بتركيز 2 ملغم لتر$^{-1}$ من ارتفاع النبات وعدد التيجان وتركيز الكلوروفيل والأوكسين والسيتوكاينين والحاصل. وزاد البنزيل أدينين بتركيز 500 ملغم لتر$^{-1}$ من تركيز الهرمونات وعدد الأزهار وحاصل الثمار. ولوحظ أن التداخل بين العوامل الثلاثة كان له تأثير تآزري في معظم الصفات. وتشير النتائج إلى أن الاستخدام المشترك لهذه المواد يمكن أن يعزز النمو والإنتاجية بفاعلية في زراعة الشليك.

الكلمات المفتاحية: الأوكسينات, السيتوكاينينات, الجبريلينات, ثمار صغيرة.