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Research Article

Responses to foliar sprays of strawberry variety 'Portola' to biostimulants on growth, yield, quality, and bioactive compounds

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Abstract

The acceptance of strawberries in international trade has increased due to their organoleptic qualities, high nutritional value, and richness in antioxidants. This research evaluated the effect of foliar spray with chitosan, brassinosteroids, and thidiazuron as metabolic enhancers on growth, productivity, quality parameters, and bioactive compounds in 'Portola' strawberry cultivar in the first year of production. This experiment included six treatments and control with doses of CTS of 100, 300, and 400 mg L^{-1}, of Brassinosteroids (Vitazyme™) 0.5, 1-, and 5-mL L^{-1} and 0.15 mL L^{-1} of TDZ (Charger plex™) in applications of these biostimulants alone and combination CTS and BRs. The findings indicated that CTS treatments increased plant length, leaf count, leaf area, and fruit firmness. The joint application of CTS and BRs significantly promotes crown diameter, photosynthetic pigments, carotenoids, fresh and dry weight of roots and aerial parts, and ripening. Applications of CTS 300 mg L^{-1} and 1 mL L^{-1} of Vitazyme have shown the highest fruit length, diameter, and weight values. Regarding TDZ, this work showed an increase in crown diameter and in the fresh and dry weight of both the root and the aerial part without significant differences in the content of the chlorophyll and titratable acidity compared to the control. Therefore, it could be safely recommended that foliar application with CTS, BRs, and TDZ can be used as biostimulants to improve the growth, quality and bioactive compounds of strawberry cv 'Portola'.

Keywords: biostimulants; fruit yield; photosynthetic pigments; quality; strawberry; vegetative growth
Abbreviations: Brassinosteroids (BRs); chitosan (CTS); thidiazuron (TDZ)

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) represents a crop of relevant socioeconomic importance. The fruit is highly appreciated due to its nutritional, antioxidant, and organoleptic properties (González-Jiménez *et al.*, 2020; Hernández *et al.*, 2022; Mancini *et al.*, 2023). China, the United States of America, and Mexico are the top producers worldwide; they contribute about 59% of world production. Spain, the United States of America, and Mexico are the main dealers, contributing approximately 61% of global exports (Terrones *et al.*, 2022). Therefore, Mexico occupies a third place as a producer and exporter of strawberries worldwide.

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Agrochemicals are a worldwide practice common in horticulture to increase production (Albíter-Pineda *et al*., 2020). However, these synthetic products cause environmental pollution, soil degradation (Mashamaite *et al*., 2022), and biodiversity loss (Castro *et al*., 2019). Therefore, the strawberry industry is looking for research focused on new alternatives in the fertilization process and respect for the environment (Valle-Romero *et al*., 2023). A viable option is to apply foliar spray, organic compounds, and growth stimulants to enhance productivity and yield quality (Shams *et al*., 2014). In Mexico, efforts have been made to increase organic production by 20% per year in the last decade, making the state of Chihuahua one of the biggest producers of this type of agriculture (SIAP and SADER, 2019). In this change from traditional agriculture to an organic one, using biostimulants CTS, BRs, and TDZ have been studied.

CTS is a biodegradable and biocompatible obtained from chitin polymer (Dahmane *et al*., 2022), such as shrimp shells (Knidri *et al*., 2019) and offal red Scorpionfish (Laribi-Habchi *et al*., 2015), through a chemical process of N chemical deacetylation, deproteinization, and demineralization (Knidri *et al*., 2019; Lárez *et al*., 2019). CTS has demonstrated some biological properties (Dahmane *et al*., 2022), for example, to promote nutrition and crop yield; that is why it has been considered a biostimulant (Reyes-Pérez *et al*., 2020), increase the leaf area, the photosynthetic rate (Chen *et al*., 2023), leaf number (Gohari *et al*., 2023) and improve the quality and commercial life of the fruit (Kessel, 2018). The application of chitosan allows an increase in the content of indole-3-acetic acid (IAA), which stimulates cell division, and the overexpression of the PIN1 gene to regulate auxin translocation (López-Moya *et al*., 2017).

BRs have been defined as natural polyhydroxy steroids plant hormones that interact with the already studied plant hormones auxin, gibberellins, cytokinin, abscisic acid, and ethylene and resemble animal steroid hormones in structure (Clouse *et al*., 1996; Bajguz, 2009; Hansen *et al*., 2009; Clouse, 2011; Unterholzner *et al*., 2015; Nolan *et al*., 2020; Qureshi *et al*., 2023). They are known to generate remarkable growth responses (Mandava, 1988). BRs act in various physiological and biochemical processes in plants, for example, rhizogenesis in blueberries (Chávez-González *et al*., 2023), root growth, plant development (Wei and Li, 2016), cell division, elongation and proliferation (Ali, 2017), by control meristem size in roots (González-García *et al*., 2011), differentiation (Lee *et al*., 2019), reproduction phases (Sasse, 2003) the time of flowering (Divi and Krishna, 2009), morphology, photosynthetic characteristics (Liu *et al*., 2022), fruit ripening processes (He *et al*., 2018), reasons why they are worthy natural substances for their safety use to increase the agricultural production (Hernández and García-Martínez, 2016).

Another recently evaluated product is TDZ to achieve defoliation in cotton, *Gossypium hirsutum*, TDZ was made by the German Schering Corporation (Arndt *et al*., 1976). It is an unnaturally altered phenyl urea (*N*-phenyl-*N'*-1, 2, 3-thidiazol-5-yl-urea) or non-purine cytokinin (Ahmad *et al*., 2021). At low concentrations, 100 mg L^{-1}, it can be used as a stimulant for flower buds to stimulate sprouting, to advance the start of flowering and the time of full flowering in 'Shiro' plums (Alvarado *et al*., 2000). It has also been applied in foliar sprays combined with gibberellins to increase their sprouting and advance and concentrate the start of flowering in blueberries (Loera-Alvarado *et al*., 2017). Choi *et al*. (2023) reported that TDZ 5 mg L^{-1} treatment at full bloom showed the heaviest cluster weight, berry weight, and diameter of the 'Shine Muscat' grape.

However, plant responses to chitosan application may vary depending on their degree of deacetylation, molecular weight, viscosity or pKa, the concentration, solvents of chitosan, the mode of application (No *et al*., 2003; El Amerany *et al*., 2020). In addition, studies on the adequate doses of CTS in horticulture are still lacking (Martínez-González *et al*., 2017). Besides, the influence of the foliar applications of CTS and BRs on the yield and growth of strawberry cultivation is still unclear. On the other hand, while Talamini *et al*. (2002) did not find any effect of TDZ on the chlorophyll content in apple trees 'Fuji' and 'Gala' cultivars, Ferrante *et al*. (2003) in cut tulips registered an effect of TDZ in an increase of total chlorophyll. Besides, no reports were found on the regulation of the yield or growth of the strawberry crop by the use of TDZ. Therefore, it is necessary to continue research in this field of study.

The present study aims to investigate the effect of CTS, BRs, and TDZ foliar sprays on vegetative growth, quality parameters, and bioactive compounds of the 'Portola' strawberry cultivar under optimal light and temperature.

Materials and Methods

Research location

The treatments were carried out at the Faculty of Agrotechnological Sciences of the Autonomous University of Chihuahua at location coordinates: 28° 38' 20" North, 106° 05' 20" West inside a greenhouse protected with fiberglass from June 2022 to September 2023.

Plant materials

140 plants of the 'Portola' variety were established from June to September 2022 in the greenhouse from mother plants from Irapuato, Guanajuato. Portola is a day-neutral variety from California and its fruits are light in color and have good flavor (Hancock, 2020). For this activity, runners of at least 45 cm in length were selected whose mother plant had a crown of 2 cm minimum of radial diameter, which was measured with a Vernier Caliper 5", Aneng, made in China. The basal part of the terminal seedlings of uncut runners were placed in 6-inch pots containing a substrate prepared with agricultural soil, sand, and walnut leaf composted in a 2.5:1:2 ratio and disinfected with 4% NaClO, CAS number 7681-52-9 from Sigma-Aldrich. Sahu *et al*. (2022) mentioned that sodium hypochlorite (2%-10%) is used for material sterilization. The substrate was rinsed every third day, and 21 days were left in this medium. Then the rooting and anchoring of the new seedlings was verified. Subsequently, the runners were cut and transplanting was done on the 5th of December 2022. *Trichoderma harzianum,* from BIO THORK, México 100 mg L^{-1} was sprayed around the crown to promote preventive protection against pathogens the same day.

Experiment layout

Plants with a crown diameter of 1 cm were transplanted on December 5th, 2022 using the same substrate in a polyethylene sample bag 30 cm high, 28 cm in diameter, 82 cm in circumference, and 14 kg in capacity with perforations in the base for drainage. 200 g of solid earthworm humus was added to the substrate on both sides of the bag in an east-west direction, alternating with a north-south direction monthly.

Plants including control transplanted in bags, were placed in blocks for experimental divided plots within 10 cm distance between plants and 60 cm between treatments. The total area of the experimental plot was 24.372 m^2. Five plants were grouped which formed an experimental unit and four repetitions were carried out with twenty plants per treatment, 140 total plants, and six combinations of treatments between biostimulants of CTS, BRs, and TDZ in the split-plot were conducted with them. A group of untreated plants was used as a control, and each one of the treatments was applied to the experimental units randomly selected. The design of treatments is presented in Table 1, in which it is specified the doses of each treatment, from 1 to 6, and its combinations of biostimulants as well as the control. Regarding the weather, the daytime temperature conditions ranged between 12 °C and 32 °C, and the night ones fluctuated between 8 °C and 12 °C. An illumination of 900 to 1,000 lux was recorded in the warmest hours of the day.

Experiment treatments:

The foliage was sprayed five times during the growth period at 15, 40, 85, 110, and 135 days after transplanting from December 20th, 2022 through April 19th, 2023.

In this study, the following products and solutions were used:

CTS is a low molecular weight product of Sigma-Aldrich, CAS number: 9012-76-4, made in Iceland a synonym of Deacetylated chitin, poly (D-glucosamine) dissolved at 4 g L^{-1} H_2O, 0.5% acetic acid, and 0.07% potassium (Reyes-Pérez *et al*., 2020), used at a concentration of 300 mg L^{-1} H_2O in treatments 1 and 3, 100 mg L^{-1} H_2O in treatment 4, and 400 mg L^{-1} H_2O in treatment 2, in foliar spray application of 5 L to the plants.

Vitazyme® of SYNGENTA, made in the USA contains BRs: homobrasinolide, dolicolid, homodololicolid, and brassinone 0.00220 % in a solution of 0.5 mL L^{-1} H_2O in treatment 2, 1 mL L^{-1} H_2O in treatments 3 and 5-, and 5-mL L^{-1} H_2O in treatment 4.

CHARGER – PLEX This product of AgroScience, made in Mexico contains 42.5% TDZ synthetic cytokinin in a solution of 0.15 mL L^{-1} H_2O in treatment 6.

Control treatment:

The plants were sprayed with purified water.

Table 1. The design of treatments where the six treatments, biostimulant doses, and their combinations with CTS, BRs, and TDZ as well as the control are described

No Treatment	Applied biostimulant	Doses
7	Control	No apps
1	CTS	300 mg L^{-1}
5	BRs 0.00220% P/V	1 mL L^{-1}
6	TDZ	0.15 mL L^{-1} AgroScience Labs.
4	CTS + BRs 0.00220% P/V	100 mg L^{-1}+ 5 mL L^{-1}
3	CTS + BRs 0.00220% P/V	300 mg L^{-1} + 1 mL L^{-1} (Reyes-Pérez et al., 2020)/ SYNGENTA
2	CTS + BRs 0.00220% P/V	400 mg L^{-1} + 0.5 mL L^{-1}

Data recorded

As a representative sample, the twenty plants were taken from each experimental plot, and the following data were recorded:

Vegetable growth

The vegetative stage, in which the buds sprouted and the development of leaves unfolded without the presence of stolons, decreased on June, 2023. On the 27th, the formation of stolons began. Then, the number of leaves, plant height and diameter were measured, 204 days after transplant, with a digital tape measure, Stanley FatMAX classic, made in USA. Leaf area, petiole height, number of crowns, and crown diameter were measured with a Vernier used in plant materials.

Early fruit yield and dimensions

It was determined as a unit of the number of ripe, and unripe fruits (Mitcham, 2004), individual weight expressed in grams, with an analytical balance ADAM-PW-254, made in the United Kingdom, the equatorial and polar diameter of harvested fruits, measuring the largest part of the fruits from both poles for which a graduated Vernier used in plant materials was used at the ripe stage, in maturity index 6 (Figure 1), as it is described at Official Journal of the Federation (2012).

Maturity index

The color of the skin of the strawberry changes according to the mature fruit. For this reason, the strawberry maturity index is based on the red color of the strawberry surface (Mitcham, 2004). This is by the quality standards that apply. Strawberries usually take 30 days to reach full size and maturity. The parameters observed in this study were a color classification from 0 to 6. The NMX-FF-062-SCFI-2002 standard for human consumption of strawberry fresh fruit establishes that strawberry fruits must be harvested when they present a faint red or pink color on a maximum of 50% of their surface or consider the requirements of the destination market (Figure 1) (Official journal of the federation, 2012).

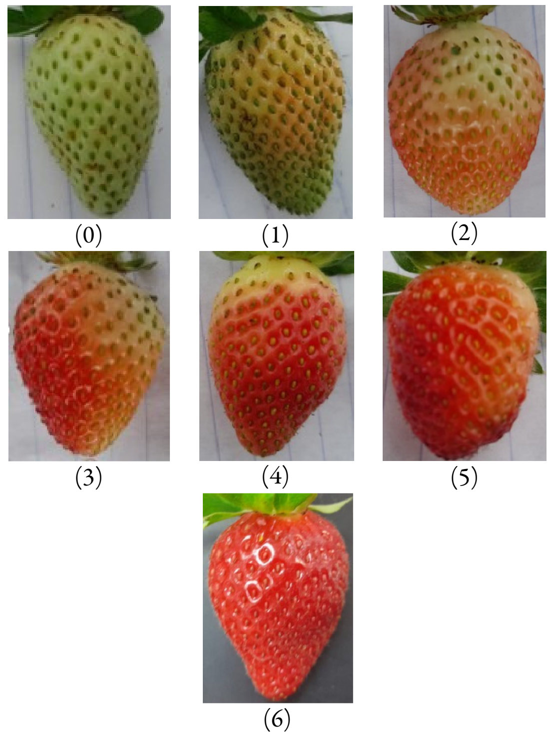

Figure 1. Color-based fruit maturity index according to the NMX- FF- 062- SCFI- 2002 standard.Where (0) is a well-developed greenish-white fruit, this state is known as physiological maturity; (1) The fruit is still greenish-white, with some pink areas in the apical zone; (2) The area of intense red color increases in the apical zone; (3) The pure red color covers up to the middle area of the fruit and the calyx area has pink hues; (4) Increase the deep red area towards the calyx; (5) The intense red color increases and begins to cover the area of the chalice; (6) The intense color covers the entire fruit.

Sugar content or total soluble solids content (TSS)

They were measured in the pulp and skin of the fruit. The fruits were macerated in a mortar until a homogeneous and smooth mixture of approximately 0.5 mL was obtained, which then was placed on the surface of a Red Rooster 90681 refractometer, made in USA scale from 0 to 32 °Brix. The values of the readings were expressed in °Brix. (Zhang and Whiting, 2011; Chicaiza, 2015).

Fruit firmness

It was evaluated with a penetrometer® Fruit Pressure Tested Wagner FT 327, made in the USA with a 2.3 mm thick tip at the height of the equatorial diameter. The results were expressed in Newton.

The titratable acidity

The titratable acidity was determined in triplicate with the method of Horwitz and Latimer (2000). 10 mL of strawberry juice per sample were taken. The sample was transferred to an Erlenmeyer flask and 4 drops of phenolphthalein 1% solution were added (0.5 g of phenolphthalein plus 70 mL of ethyl alcohol, calibrated to 100 mL with distilled water). The sample was titrated with a 0.1 N NaOH solution until the purplish color change was maintained for one minute. The titratable acidity was expressed as a percentage of citric acid and was calculated using the following formula:

$$\% \text{ acid} = \frac{\text{V NaOH} * \text{N NaOH} * \text{meq ácido} * 100}{\text{V}} \quad (1)$$

Sugar/Acidity ratio (TSS/TA)

It was expressed as a part of sugar for one part of acid (Flores *et al*., 2018), and it was determined from the total soluble solids for each part of the acid content.

$$Ratio\ \frac{\text{Sugar}}{\text{Acidity}} = \frac{\text{TSS}}{\text{TA}} \quad (2)$$

Fresh and dry weight of leaves and roots

The vegetative growth of fresh leaves and roots of each treatment was collected between 7:00 and 8:00 a.m. and they were placed into Kraft paper bags measuring 31 cm high × 8 cm long and 15.5 cm wide and were deposited inside a 25.4 cm × 27.94 cm closed plastic bag which was placed in a tray with ice. The fresh weight were measured at 8:15 a.m. using a TORREY LEQ, USA 10/20 high-precision digital scale Subsequently, the plant parts were dried at 70 °C in a drying oven LUZEREN® made in China until obtaining the constant weight. This temperature was demonstrated to be effective in strawberry leaf drying by El-Miniawy *et al*. (2013).

Photosynthetic pigments

The Wellburn (1994) method was used to determine photosynthetic pigments. Talolaes of 7 mm diameter with a weight of 0.2 to 0.3 g were extracted from fresh leaves. To these were added 10 mL of pure methanol (CH_3OH) and incubated in the dark for 24 hours at room temperature. For carotenoids, the absorbance was measured at 470 nm, for chlorophyll b, chl b, at 653 nm, and for chlorophyll a, chl a, at 666 nm.

The calculation of the pigment concentration was carried out with the following formulas (Wellburn, 1994):

$$\text{Chl a: } [15.65 \cdot (A_{666}) - 7.34\ (A_{653})]\ \frac{chl\ a\ \cdot\ V_1 \cdot p_1}{p_2 \cdot\ 2^2\ \cdot n} \quad (3)$$

$$\text{Chl b: } [27.05 \cdot (A_{653}) - 11.21\ (A_{666})]\ \frac{chl\ b \cdot\ V_1 \cdot p_1}{p_2 \cdot\ 2^2\ \cdot n} \quad (4)$$

$$Carotenoides\text{: } [1000 \cdot (A_{470}) - 2.86 \cdot (\text{chl a}) - 129.2(chl\ b)]/221 \frac{Carotenoides \cdot V_1 \cdot p_1}{(p_2 \cdot 2^2 \cdot \text{n})} \quad (5)$$

Where: V1: Volume of the extraction, p1: weight in g per talolae, p2: total weight in g, n: number of talolae. Chlorophyll a, chlorophyll b, and carotenoid concentrations were expressed as µg cm^{-2}.

Statistical analysis

The treatment arrangements were completely randomized with a classic analysis of variance based on a multiple comparison of means. The design was completely divided into plots. The variance homogeneity test was performed and when heterogeneity was detected, the Mann-Whitney test of medians was performed. For petiole length, the Mann-Whitney non-parametric test was used. To obtain the statistical results of foliar pigments, the analysis of variance for random blocks with the comparison of means by Tukey (alpha <= 0.05) was used. IBM, SPSS Statistics Version 25 (I.B.M. Corporation, 2017).

Results and Discussion

Vegetative growth of treated strawberry plants

All tested treatments of CTS and BRs applied together significantly increased plant length and diameter, number of leaves per plant, leaf area, and petiole length compared with the control (Table 2).

Table 2. Response of foliar application of CTS, BRs, and TDZ on the vegetative development of the plant

Treatment	Applied biostimulants	Plant length (cm)	Plant diameter (cm)	Number of leaves per plant	Leaf area (cm^2)	Petiole length (cm)	Crown diameter (cm)
7	Control No apps	18.0 b	32.9 c	28 d	71.7 d	8.3 c	1.6 d
1	CTS 300 mg L^{-1}	22.4 a	48.0 a	36 c	74.4 c	10.6 a	2.5 b
5	BRs 1 mL L^{-1}	18.1 b	38.3 b	28 d	70.3 d	9.4 b	2.2 bc
6	TDZ 0.15 mL L^{-1}	17.2 b	34.3 c	36 c	55.1 e	9.4 b	2.0 c
4	CTS 100 mg L^{-1} + BRs 5 mL L^{-1}	21.8 a	48.1 a	42 b	80.8 b	9.9 b	2.4 bc
3	CTS 300 mg L^{-1}+ BRs 1 mL L^{-1}	22.1 a	47.0 a	43 b	79.1 b	10.6 a	3.0 a
2	CTS 400 mg L^{-1} + BRs 0.5 mL L^{-1}	22.5 a	47.5 a	50 a	85.4 a	10.4 a	2.7 b

Means within a column followed by the same letter are not significantly different

The foliar applications at concentrations of CTS 400 mg L^{-1}, and BRs 0.5 mL L^{-1} highlighted by promoting plants with greater vigor in all the parameters evaluated. The results of this work revealed that the CTS improved vegetative development in plant length, diameter, and petiole length, and they are in agreement with published research that reported an increase in plant height and number of leaves in tomato (El-Tantawy, 2009; Islam *et al*., 2018), in strawberry (Abdel-Mawgoud *et al*., 2010; El-Minawy *et al*., 2013), in chilli (Islam *et al*., 2018). At the same time, we agree with authors who described an increment in leaf area as it was in strawberry (El-Miniawy *et al*., 2013; Metwaly *et al*., 2023), in summer squash plants (Ibraheim and Mohsen, 2015), in crow dipper (Chen *et al*., 2023). They also coincide with other works that divulged an enhancement in the total dry weight per plants as it was reported in summer squash plants (Ibraheim and Mohsen, 2015). In this part of the work the results also match with the study by Shams *et al*. (2014) in which, with the use of CTS, they improved vegetative growth in 'Sweet Charlie', 'Festival' and 'Florida' strawberry cultivars. The authors agree with Liu *et al*. (2022) who applied chitosan soaking and enhanced the leaf growth, height, stem diameter, and overground part dry weight of *Platicodon grandiflorus*. The beneficial effect of CTS as a growth promoter in plants may be attributed to an increase in the availability and uptake of water and essential nutrients due to the adjustment of cellular osmotic pressure and reducing the overexpression of free radicals by increasing antioxidants (Guan *et al*., 2009) or to an increment in the key enzyme activities of nitrogen metabolism such as nitrate reductase, glutamine synthetase and protease, and also to an improve of the transportation of nitrogen (N) in the functional leaves (Mondal *et al*., 2012). CTS can contribute to cell division and elongation, enzymatic activation, protein synthesis (Chakraborty *et al*., 2020), and to increment of endogenous phytohormones auxins (Román-Doval *et al.,* 2023), and therefore the vegetative growth. Besides, the polysaccharides contained in CTS intervene in the metabolism and phytoprotection of plants and thus develop plant growth (Shams *et al*., 2014).

BRs effect of their signalling is to promote cell division (Nakaya *et al*., 2002), and cell elongation (Yang *et al*., 2023). They are more actively synthesized in young and developing organs and are consistent with the notion that BRs function as growth vegetative promoting hormones in plants (Divi and Krishna, 2009; Sharma *et al*., 2015; Baghel *et al*., 2019). In this study, the foliar application of BRs increased the plant diameter, and the length of the petiole compared to the control, but in combination with chitosan in treatments, 4 (CTS 100 mg L^{-1} + BRs 5 mL L^{-1}), 3 (CTS 300 mg L^{-1}+ BRs 1 mL L^{-1}), and 2 (CTS 400 mg L^{-1} + BRs 0.5 mL L^{-1}) allowed further development of all the parameters studied. Pippatanawong *et al*. (1996) with the application of brassinosteroids, reported an increase in total leaf areas, the number of leaves, and petiole length over the untreated control plants for cv 'Miyoshi' and cv 'Enrai', two-day neutral strawberries which coincides with our registered results. The results of this work also coincide with Zheng *et al*. (2018) who got an increase of primary shoot lengths, and higher plant height for apple plants treated with exogenous brassinolide. The authors agree with Zheng *et al*. (2019) who worked with BRs and obtained a development of apple tree growth by regulating cell growth. The results of this work also concur with Que *et al*. (2017) who got higher plant height for carrot plants treated with 24-EBL compared with the control, and with Hayat *et al*. (2001) who worked with wheat grown from seed treated with 28-homobrassinolide and obtained enhanced leaf number per plant. This work also matches with the research of Lalarukh *et al*. (2022) who obtained a significant increase in the growth of wheat plants with the foliar application of 24-epibrassinolide.

The reason why BRs can promote vegetative growth was provided by Zurek *et al*. (1994) who suggested that BRs may elicit elongation by altering the mechanical properties of the cell wall through an increase of their plastic extensibility and they concluded an increase in BRU1 (BRs upregulated) transcript levels correlates with increases in stem elongation in response to brassinosteroids in soybean epicotyls. In addition, the elongation of young tissues may occur because BRs biosynthetic gene expression is under organ and developmental regulation, for example, the BR6ox genes are most expressed in apical shoots (Shimada *et al*., 2003). Yadav *et al*. (2023) demonstrated that BRs application improved N assimilation through the essential role of tomato Brassinazole resistant (BES1/BZR1) homolog 4 (BEH4) protein in regulating N metabolic response and in consequence growth physiology.

Besides, various studies have shown that the applications of BRs and CTS are growth enhancers: Martínez *et al*. (2016) used these bioactives and determined their positive effect on the growth of bean plants, and El Khateeb *et al*. (2017) recorded an improvement in the growth of marjoram plants.

About TDZ, it increased the number of leaves per plant and petiole length in contrast to the control. The quality promotion in plants is because it is a highly effective synthetic-based phenyl urea plant growth regulator (Mok *et al*., 1982; Ali *et al*., 2022). This work also coincides with Talamini *et al*. (2002) who worked with different doses of TDZ, 0, 5, 10, and 20 g ha^{-}, in 'Gala' and 'Fuji' apples and reported an increase in shoot growth in both cultivars, and with Zhang *et al*. (2019) who reported that TDZ in low concentrations, 30 or 60 mg L^{-1} promoted the emergence of new shoots in a study carried out on *Dendrobium* 'Sunya Sunshine Portola' orchids, and except for stem diameter in the TDZ_{30} treatment, there were no significant differences between the TDZ treatments and the control in leaf length and width. In this sense, their results also coincide with ours because we do not find differences concerning the control in plant length and diameter.

Regarding the diameter of the crown, treatment 3 (CTS 300 mg L^{-1} + BRs 1 mL L^{-1}) stood out by obtaining the most vigorous diameter (3 cm), 1.4 cm wider than the control, followed by treatment 2 (2.7 cm), and 1 (2.5 cm) which did not obtain a significant difference. At least, treatment 6 had a diameter 0.4 cm wider than the control. The results coincide with Rizk and Mohamed (2012) who, by applying chitosan, obtained an increase in the diameter of the crown in the 'Festival' variety strawberry crop compared to the control. They also agree with Furio *et al*. (2022) who studied the application of two BRs, BB16 and EP24, in cv Festival strawberries and recorded an increase in crown diameter. This study matches with Li *et al*. (2021) in their work, the crowns of strawberry plants treated with TDZ and TDZ combined with auxins were larger than those of the other treatments with different cytokinins: 6-benzyladenine, kinetin, and zeatin.

The number of fruits produced with the treatments sprayed with chitosan or chitosan plus BR increased considerably. While considerably enhanced immature and mature fruits and the weight, length, and diameter of fruits by the same biostimulants (Table 3).

Table 3. Response of foliar application of CTS, BRs, and TDZ on the average number of immature and ripe fruits, fruits weight and length, and fruit diameter at 111 days after transplant because fruit ripening began

Treat ment	Applied biostimulants	Number of unripe fruits/plants	Number of ripe fruits/plant	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)
7	Control No apps	4.3 b	2 c	14.9 f	3.3 c	2.4 c
1	CTS 300 mg L^{-1}	8.5 a	2 c	18.8 c	3.3 c	2.4 c
5	BRs 1 mL L^{-1}	5.3 b	2 c	17.2 e	3.5 c	2.7 b
6	TDZ 0.15 mL L^{-1}	5.3 b	2 c	18.0 d	3.4 c	2.7 bc
4	CTS 100 mg L^{-1} + BRs 5 mL L^{-1}	9.3 a	3 b	18.2 cd	4.1 b	2.7 bc
3	CTS 300 mg L^{-1} + BRs 1 mL L^{-1}	8.4 a	4 a	21.2 a	4.7 a	2.9 b
2	CTS 400 mg L^{-1} + BRs 0.5 mL L^{-1}	9.2 a	2 c	19.9 b	4.3 b	3.5 a

Means within a column followed by the same letter are not significantly different

The weight and length of the fruit showed a significant increase with treatment 3, 6.3 g heavier, and 1.4 cm more length with regard to the control. Besides, treatments 3 and 4 stood out by presenting more ripe fruits and increased the yield due to the rise in the number of fruits. There are similar results from several studies: Sultana *et al*. (2017) reported that treatments with 60 and 100 ppm chitosan had a positive effect in increasing total yield $plot^{-1}$ of tomato, 41.67 and 38.30 kg, respectively, then the control 22.79 kg. Hernández *et al*. (2022) applied 1 g L^{-1} of chitosan to the aerial part and obtained an increase of 27% in tomato production compared to the control. Kowalski *et al*. (2007) with the application of soluble chitosan obtained an increase in the yield of potato minitubers, and Saad *et al*. (2022) applied chitosan NPs as a nano-nitrogen fertilizer combined with mineral nitrogen fertilization and enhanced the productivity of wheat plants with 120 kg Mn-N and 14 L Nan-N/ha obtained the heaviest 1000-grain weight, 55.8 and 57.4 g, compared to the control, 47.6 and 45.5 g.

Regarding the BRs, in strawberry plants, it is confirmed that this hormone applied consecutively achieved an increase in the mass, length, and diameter of the fruits. Therefore, the results of this work also coincide with those of some authors who reported an increase in fruit yield (Assis *et al*., 2006; Isci and Gökbayrak, 2015). These responses are because BRs are involved in several important processes, for example, in cell division (Nakaya *et al*., 2002). They are involved in the reproduction and photomorphogenesis phases (Sasse, 2003), the flowering season (Divi and Krishna, 2009), and fruit ripening (Harpreet *et al*., 2015). However, we found even better results in the variables studied when the CTS and the BR act together.

About TDZ foliar applications in these results, it was obtained that an increase in fruit weight, on average 3.1 per fruit, and a small difference, 0.3 cm, was recorded in the diameter of the fruit compared to the control. TDZ has been shown to have the same results as auxins and cytokinins; nevertheless, each of these regulators has a different chemical structure (Guo *et al*., 2011). In this study, the authors agreed with the work of Famiani *et al*. (1999) in which they determined that the treatments with TDZ stimulate kiwi fruit growth, and promote fruit enlargement to improve fruit quality. At the same time, results coincided with Reynolds *et al*. (1992) who obtained through the use of TDZ an increase of berry and cluster weight in seedless table grapes, and with Attia (2022) whose results with TDZ treatment at 5 mg L^{-1} increased fruit set and fruit diameter of 'LeConte' pear trees. This work is consistent with previous results of Greene (1995) who described a fruit weight increase in 'McIntosh' using a dose of 50 mg L^{-1} TDZ and a lower dose of 15 mg L^{-1} for 'Empire' apples sprayed at full Bloom. Therefore, the authors agreed with the work of Famiani *et al*. (1999) in which they determined that the treatments with TDZ stimulate kiwi fruit growth, and promote fruit enlargement to improve fruit quality.

The quality of the fruit and the leaf was appreciated as well as the vigor of the plants in contrast to the control (Figure 2). The images show the plants treated in the fruit productive stage at 111 days after the transplant where (A) is treatment 1, (B) treatment 2, (C) treatment 3, which presented more fruit load, (D) is treatment 4, (E) 5, (F) 6 and (G) the control.

Figure 2. Productive stage of the treated plants and the control

Data presented show clearly that chemical and physical fruit quality characteristics such as fruit firmness, expressed in Newton, Brix, pH, and titratable acidity concentration expressed in percentage of citric acid (CA), were significantly different among the tested treatments (Table 4).

Table 4. Effect of foliar application of CTS, BRs, and TDZ on fruit quality of strawberry in 2022, median of fruit firmness, TSS (brix), fruit pH and titratable acidity, and SST/Titratable acidity ratio

Treatment	Applied biostimulants	Fruit firmness (N)	TSS Brix (%)	Fruit pH	Titratable acidity (% CA)	TSS/ Titratable acidity ratio
7	Control No apps	2.0 b	6.3 d	3.895 a	0.9 a	7.0
1	CTS 300 mg L^{-1}	2.3 a	12.0 a	3.87 ab	0.3 c	32.2
5	BRs 1 mL L^{-1}	2.0 b	10.0 c	3.76 c	0.8 a	11.1
6	TDZ 0.15 mL L^{-1}	2.1 b	10.4 c	3.84 bc	0.8 a	11.6
4	CTS 100 mg L^{-1}+ BRs 5 mL L^{-1}	2.1 b	12.8 a	3.81 c	0.7 b	16.1
3	CTS 300 mg L^{-1}+ BRs 1 mL L^{-1}	2.2 ab	11.8b	3.90 a	0.7 b	15.3
2	CTS 400 mg L^{-1}+ BRs 0.5 mL L^{-1}	2.0 b	12.7 a	3.89 ab	0.7 b	16.3

Titratable acidity HSD Tukey "Means for groups in homogeneous subsets are displayed. It is based on statistical means.

The fruits with greater firmness were those treated with only CTS in treatment 1. Meanwhile, brix degrees were increased with treatments 1, 4, and 2. The highest pH was obtained with treatment 3, and titratable acidity was obtained with the control, and treatments 5, 6.

Furthermore, CTS, BRs, and TDZ significantly increased the concentrations of soluble sugar compared with the control in this study. Soluble sugars act as cell osmoprotectants (Pilon-Smits *et al*., 1995) regulating osmotic adjustment (Bohnert *et al*., 1996; Khan *et al*., 2020) and decreasing reactive oxygen species (Bohnert *et al*., 1996). Treatments 1 showed an increase in firmness and a decrease in pH, which coincides with the results of Sree *et al*. (2020) who used CTS with different concentrations in tomato coating by dipping method. Moreover, Abdel-Mawgoud *et al*. (2010) and El- Miniawy *et al*. (2013) matched that the application of CTS allows to increase in the content of total soluble solids in strawberries (TSS), so their results agree with this study. However, in the last work, the authors reported that it depends on the dose of CTS applied concentration because total acidity and total sugars increased significantly compared to the control treatment as it increased up to 2 cm^3 L^{-1} and after the effect started to decrease. The results of this work are also similar to those registered by Ávila *et al*. (2023) who reported that the application of CTS under water-deficit conditions promoted increases in the accumulation of total soluble sugars in sorghum plants, and Metwaly *et al*. (2023) who applied foliar spraying of CTS, 100 mg L^{-1}, and phosphorus fertilizer based on Micro Carbon Technology™ and considerably improved in strawberry crop cv 'Fortuna' the fruit firmness, soluble solid content and total sugars compared with the untreated plants.

On the other hand, in tomato fruits coated by dipping method with different concentrations of chitosan, 0.5%, 1%, 2%, and 2.5%, Sree *et al*. (2020) results recorded the lowest TSS value (5.1% Brix) in 2.5% chitosan and the highest TSS value (6.8% Brix) in control on 20th day of storage. Meanwhile, Abd *et al.* (2012) results showed that an increment in CTS concentrations increased the pH range, and reduced Brix values in apple juice. In this work, results were recorded in Brix values of 12% with CTS, and from 11.8 to 12.8% with CTS and BRs foliar spray, and the lowest in the control (6.3%), and there were minor differences in pH between treatments and control. In this case, the acidic highest index was obtained in the control (0.9%) and the lowest (0.3%) with CTS in treatment 1. Therefore, the fact that the increase in total soluble solids depends on the application of very low concentrations of CTS is strengthened.

Regarding the responses of BRs, this work coincides with Symons *et al*. (2006) in which BRs stimulate ripening as demonstrated in 'Cabernet Sauvignon' grapes, and Assis *et al*. (2006) who applied 0.1 mg L^{-1} of BR analogue BB-16 three consecutive weeks after the appearance of the first flowers of yellow passion plants and obtained an increase of 1% Brix of soluble solid greater than the control. Also, in concordance with Zhu *et al*.

(2015) who worked applying brassinolide in tomato ripening and got an increase in soluble sugars. At the same time, agreed with the results of Isci and Gökbayrak (2015) who observed a decrease in TTS in table grape 'Alphonse Lavallee 'compared to the control using low doses of BRs during the first year of their investigation.

Concerning the effects of TDZ, there were no significant differences in the content of the titratable acidity compared to the control; Therefore, this result coincides with those of Attia (2022) who found an increase in acidity in pears, and Reynolds *et al*. (1992) who obtained high titratable acidity contents in Selection 535 and 'Simone' grapes. However, contrary to their research, the present study shows a slight increase in degrees Brix. In this aspect, this work agrees with the results of Greene (1995) because the application of TDZ on 'McIntosh' and 'Empire' apple cultivars increased soluble solids concentration.

On the other hand, significant differences on the fresh and dry weight of the root and vegetative aerial growth are shown between the treatments in response to the foliar application of the biostimulants studied (Table 5).

Table 5. Effect of foliar application of CTS, BRs, and TDZ median of root and vegetative growth, fresh and dry weights of strawberry plants at the end of growth vegetative cycle on October, 2022

Treatment	Applied biostimulants	Root fresh weight (g)	Root dry weight (g)	Fresh weight of vegetative growth (g)	Dry weight of vegetative growth (g)
7	Control No apps	16.2 d	3.8 d	80.7 d	15.3 d
1	CTS 300 mg L^{-1}	20.6 c	4.8 c	102.5 c	19.4 c
5	BRs 1 mL L^{-1}	16.5 d	3.9 d	81.3 d	15.4 d
6	TDZ 0.15 mL L^{-1}	20.6 c	4.8 c	102.6 c	19.5 c
4	CTS 100 mg L^{-1} + BRs 5 mL L^{-1}	24.4 b	5.8 b	123.8 b	23.6 b
3	CTS 300 mg L^{-1} + BRs 1 mL L^{-1}	25.1 b	5.9 b	124.6 b	23.6 b
2	CTS 400 mg L^{-1} + BRs 0.5 mL L^{-1}	29.1 a	6.8 a	144.7 a	27.4 a

Means within a column followed by the same letter are not significantly different

In these parameters evaluated, treatment 2 obtained the highest values: in the root fresh weight 12.9 g, in the root dry weight 3 g, in fresh weight of vegetative growth 64 g, and 12.1 g in dry weight of vegetative growth more than the control. In addition, the results clearly show the positive effects of CTS, and BRs in a combined action on dry and fresh weight of roots and aerial part while CTS, BRs, and TDZ independently applied obtained lower weight in root and aerial growth, but higher than the untreated plants. The authors of this work agree with other previous studies in the literature in which CTS was used in several crops, and they reported an increase in vegetative growth and therefore in fresh and dry mass, for example, in tomato (El-Tantawy, 2009), strawberry (Abdel-Mawgoud *et al*., 2010), sweet pepper (Ghoname *et al*., 2010), cowpea (Farouk and Amany, 2012), okra (Mondal *et al*., 2012). These results also match with Guan *et al*. (2009) who observed an increase in shoot height, root length, and shoot and root dry weights in maize lines compared with the control. While Badizadegan *et al*. (2023) in the propagation of zamioculcas (*Zamioculcas zamiifolia*) by leaf cuttings demonstrated that chitosan concentrations of 250 mg L^{-1} increased the number and proliferation of roots. For their part, Martin *et al*. (2023) studied the effect of chitosan hydrochloride 2.5% and recorded an increase in root development of 'Tempranillo' grape cultivar. Regarding strawberry, El-Miniawy *et al*. (2013) obtained an increase in the root and vegetative fresh and dry weight with respect to the control. We also agree with the results of Abdel-Mawgoud *et al.* (2010) in their work with CTS application to strawberry plants they improved the fresh and dry weights of the leaves. Chitosan leads to the accumulation of phytohormones like auxins in the apex of roots, thus promoting the elongation of the organ (Suárez-Fernández *et al*., 2020).

In addition, BRs, as growth hormones in plants, are highest produced in juvenile organs (Divi and Krishna, 2009). Particularly, in the roots they act in cell elongation and development, including maintaining meristematic size and root hair generation (Wei and Li, 2016), and to provoke lateral root genesis, they act with auxins (Bao *et al*., 2004). As biostimulants CTS and BRs were recommended to improve fresh and dry weights of herb plants in *Majorana hortensias* (El-Khateeb *et al*., 2017).

Regarding TDZ, this work showed an increase in fresh and dry weight of both the root and the aerial part compared to the control. This increase is comparable to that of CTS, but less than that of CTS and BRs. Our results contrast with those of Talamini *et al*. (2002) because they did not find any effect on foliar dry mass on 'Gala' and 'Fuji' apple cultivars. However, they go along with Sajid and Aftab's (2009) study in vitro where the maximum fresh and dry weight of potato plantlets cv. 'Cardinal' was obtained on MS medium containing 10^{-9} M TDZ, and with Ghorbanpour and Hatami (2015) because they found the highest increase of Fresh and dry weights of aerial parts of geranium plant with 75 µM TDZ contrasting to untreated plants.

Another important aspect of this work is the notable statistical differences in the studied parameters of chlorophyll and carotenoids (Table 6).

Table 6. Effects of foliar sprays of CTS, BRs, and TDZ concentrations on chlorophyll a, b, total, and carotenoids in Portola variety strawberry leaves

Treat ment	Applied biostimulants	Chlorophyll a µg cm^{-2}	Chlorophyll b µg cm^{-2}	Total chlorophyll µg cm^{-2}	Carotenoids µg cm^{-2}
7	Control No apps	145.0 c	72.7 c	217.7 c	54.2 b
1	CTS 300 mg L^{-1}	211.3 b	94.2 c	305.5 b	86.4 a
5	BRs 1 mL L^{-1}	203.2 b	93.5 c	296.7 b	73.8 ab
6	TDZ 0.15 mL L^{-1}	139.1 c	64.9 c	204.0 c	52.2 b
4	CTS 100 mg L^{-1} + BRs 5 mL L^{-1}	230.5 a	119.8 a	350.3 a	84.3 a
3	CTS 300 mg L^{-1} + BRs 1 mL L^{-1}	232.6 a	110.0 b	342.6 a	85.6 a
2	CTS 400 mg L^{-1} + BRs 0.5 mL L^{-1}	198.7 b	87.1 d	285.8 b	82.1 a

Means within a column followed by the same letter are not significantly different B. Alpha = .05.

In this part of the study, the highest chlorophyll a content was treatments 4, and 3, followed by treatments 1, 5, and 2. The control, and treatment 6 did not show significant differences. For chlorophyll b highlighted treatment 4, and for total chlorophyll treatments 4, and 3. Regarding carotenoids, treatments 1, 4, 3, and 2 stood out.

Regarding photosynthetic pigments, CTS improves the photosynthetic quality by enlarging chloroplasts and the stimulation of the biosynthesis of photosynthetic pigments (Chamnanmanoontham, 2015). The results of this work were similar to those shown by Bakhoum *et al*. (2020) who revealed an improvement of this kind of pigments by the application of CTS at a concentration of 50 mg L^{-1} to sunflower plants (*Helianthus annuus* L.) even under salinity stress conditions. Metwaly *et al*. (2023) registered their improvement in strawberry when applying CTS and phosphorus fertilizer. El Amerany *et al*. (2023) with arbuscular mycorrhizal fungi, green compost, and chitosan reported an increase in chlorophyll contents which promoted vegetative development in tomato plants. In coffee seedlings, Dzung *et al*. (2011) obtained in their work an increase in the chlorophyll content with the use of CTS. For their part, Santo *et al*. (2017) with the use of CTS nanoparticles revealed an increase in the levels of chlorophylls and carotenoids in *Phaseolus vulgaris*. Our study is in harmony also with Van *et al*. (2013) who found that CTS nanoparticles developed the content of chlorophyll in the coffee leaves of 'Robusta' and this was probably induced by plants' improvement in taking up the nutrients.

On the other hand, BRs enhanced the levels of chlorophyll a, b, and total in comparison with untreated plants. These results agree with several authors: Anuradha and Ram (2003) showed that the application of BRs to rice seeds increased the pigment level in plants. Besides, it was observed an increment in photosynthetic pigment accumulation in the green alga *Chlorella vulgaris* in comparison with the control as an effect of BRs (Bajguz *et al*., 2014). Hayat *et al*. (2001) noted a pronounced increment in Chlorophyll content in chestnut mustard with the foliar application of homobrassinolide. Fariduddin *et al*. (2003), who worked with mungbean, described in their results an increment of leaf chlorophyll content. It has also been reported that BRs work with exogenous auxins in increasing the content of chlorophyll in green alga, *Chlorella vulgaris* cultures (Bajguz *et al*., 2013).

Regarding TDZ, in this work in the chlorophyll content, no significant differences were found with respect to the control. That is in accord with Talamini *et al*. (2002) because in their work they did not find any effect of TDZ on the chlorophyll content in apple trees cultivars 'Gala' and 'Fuji'. On the other hand, our results disagree with those of Ferrante *et al*. (2003) in cut tulips at the end of the experiment where it was noticed an effect of TDZ in 62% increase of total chlorophyll than the initial value measured at harvest time. However, the increase in photosynthetic pigments depends on the dose of TDZ applied (Ghorbanpour and Hatami, 2015).

Finally, the results in the harvest yield were highly enhanced with the use of biostimulants as can be observed in Table 7.

Table 7. Effects of foliar sprays of CTS, BRs, and TDZ concentrations on strawberry yield variety 'Portola', where the average weight of 20 fruits per treatment was obtained and then multiplied by two, due two harvests were carried out weekly showed in column 5

Treatment	Applied biostimulants	No. of ripe fruits/ plant	Average fruit weight/plant (g)	Average weight/20 plants/week (g)
7	Control No apps	2 c	14.9 f	596
1	CTS 300 mg L^{-1}	2 c	18.8 c	752
5	BRs 1 mL L^{-1}	2 c	17.2 e	688
6	TDZ 0.15 mL L^{-1}	2 c	18.0 d	720
4	CTS 100 mg L^{-1} + BRs 5 mL L^{-1}	3 b	18.2 cd	1.092
3	CTS 300 mg L^{-1} + BRs 1 mL L^{-1}	4 a	21.2 a	1.696
2	CTS 400 mg L^{-1} + BRs 0.5 mL L^{-1}	2 c	19.9 b	796

Data are means of 20 fruits and three replications per treatment.

In this part of the study, CTS foliar spray produced fruits 3.9 g greater than the control. Treatments 3 and 4 showed a greater number of ripe fruits compared to the control. Treatment 3, in a combination of CTS and BRs, stood out because it doubled the number of ripe fruits compared to the control and its fruit weighed 6.3 g more than the control which makes this treatment a promise in economic profitability. An interesting finding is that spray application of CTS, BRs, and TDZ in all the treatments significantly enhanced fruit weight and yield compared with the non-treated control.

Regarding the yield components, the results showed that the applications based on CTS influenced by increasing the number of fruits and fruit weight, improving fruit and yield attributes which confirms the results of Guan *et al*. (2009), El-Miniawy *et al*. (2013), Shams *et al*. (2014), Islam *et al*. (2018), Reyes-Pérez *et al*. (2020). Our results are also in harmony with different works: Metwaly *et al*. (2023) obtained an increment of the average fruit weight, early fruit yield, and total yield with foliar spraying of CTS. Abdel-Mawgoud *et al*. (2010) used a chitosan solution application with different doses: 1, 2, 3, and 4 cm^3 L^{-1} and improved in number and weight the fruits of the strawberry cultivar 'Sweet Charlie' obtained positive responses with the highest peak recorded with 2 cm^3 L^{-1}. For their part, Boonlertnirun *et al*. (2008) demonstrated a significant increase in rice yield over the other treatments by the application of CTS both to seeds and to the soil four times during the growing season, and Farouk and Amany (2012) used 50 mg/l of CTS in a foliar manner and increased yield in cowpea.

Concerning BRs, applied in isolation, the authors agree with several Works: Assis *et al*. (2006) reported in the passion fruit plants a 65% increase in yield by the application of BR analogue for three consecutive weeks after Bloom. Champa *et al*. (2015) treated clusters with BRs at 0.5 and 1.0 mg l^{-1} and found a significant increase in weight and berry weight. Cortés *et al*. (2003) applied two BRs analogues to cladodes of *Opuntia ficus-indica* L. and revealed an increase in the number and fresh weight of harvested cladodes. In addition, El-Khateeb *et al*. (2017) recommended CTS and brassinolide to improve oil yield in marjoram, *Majorana hortensis*.

Besides, in this work, the results are in harmony with others of them using TDZ: Pasa *et al*. (2017) applied it on pear trees varieties 'Hosui' and 'Packam´s Triumph' and reported an increase in fruit yield. Alves *et al*. (2015) used a dose of 0.9 g ha^{-1} of TDZ in tillering on rice crop cv. 'BRS Esmeralda' and the productivity increased by 23.5%. Taiz *et al*. (2017) clarified that Cytokinins stimulate nutrient mobilization through vigorous drains that are more advantaged in competition for nutrients, in this way, TDZ can increase crop productivity.

Conclusions

CTS, BRs, and TDZ as stimulants are highly promising for their use in increasing growth improvement and strawberry production. The present study strongly recommends the safe use of such treatments at the early growth stage to obtain fruit quality and quantity. Foliar application of CTS alone or combined with BRs effected positively on the growth of strawberry plants: plant height and diameter, number of leaves, foliar área, and petiole length under greenhouse conditions. The treatment that developed greater vigor by fresh and dry weight of roots and aerial part was number 2, CTS 400 mg L^{-1} + Vitazyme 0.5 mL L^{-1} and in the enhancing content of chlorophylls highlighted treatments 3, CTS 300 mg L^{-1} + Vitazyme 1 mL L^{-1}, and 4, CTS 100 mg L^{-1} + Vitazyme 5 mL L^{-1}. Regarding the weight, length, and diameter of the fruit, the most significant was treatment three which increased production by 113% compared to the control, and also increased the crown diameter of the plants. In this study, the joint application of BRs and CTS show to promote ripening significantly in a nonclimateric fruit as a strawberry. Our research indicates that exogenously applied CTS and BRs exert a positive influence on strawberry ripeness. Regarding the use of TDZ in foliar applications in low doses to strawberry plants, the results conclude an increase in the quality of the plants in crown diameter, the number of leaves, the length of the petiole, and the weight and diameter of the fruits. The concentration of soluble sugar also increased and no statistically significant differences were obtained in the content of titratable acidity and chlorophyll compared to the control. TDZ also showed an increase in the fresh and dry weight of both the root and the aerial part was obtained in comparison with the control and higher fruit yield. Further studies may lead to their application to the strawberry growers and other Rosaceae plants such as *Cydonia, Rubus, Rosa,* and *Prunus*.

Authors' Contributions

Conceptualization: MEMP and TJRA; Methodology: JLJC and GCZ; Validating: JLJC and GCZ; Strawberry cultivation, laboratory work, and data curation: MEMP; Project administration: MEMP and TJRA; Formal analysis: JLJC; Investigation: MEMP; Writing original draft: MEMP TJRA and JLJC; Writing review and editing: MEMP TJRA JLJC and GCZ; All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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