Colegio de Postgraduados

Zinc oxide nanoparticles *vs.* Zn-EDTA in the growth and production of strawberry crops (*Fragaria*×*ananassa* Duch)

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ABSTRACT

Objective: The objective of this study was to evaluate and to compare the effects on growth between zinc oxide nanoparticles (ZnO NPs) and Zn-EDTA, nutrient concentration of zinc in leaf and fruit, and strawberry production.

Design/methodology/approach: Strawberry plants were used, established in plastic bags with a mixture of substrate, peat and agrolite. Two factors were evaluated: first, the concentration of ZnO NPs (100, 200, 500, 1000 mg L^{-1}), plus treatments with Zn-EDTA and a control; and the second, form of application (foliar and to the substrate). A completely randomized increased factorial design was used. Growth was measured, leaf and fruit zinc concentration was determined, the number of flowers and weight of fruits per plant, and the variables of firmness and total soluble solids of fruits were quantified.

Results: The results obtained indicated that the foliar application of the 200 mg L^{-1} dose of ZnO NPs caused the greatest plant height, as well as the highest number of flowers, crowns and fruits per plant, and the greatest production. The highest leaf zinc concentrations within the sufficiency interval were observed with the leaf application of 200, 500 and 1000 mg L^{-1} of ZnO NPs.

Findings/conclusions: The study allows inferring that the use of zinc oxide nanoparticles is an alternative source of fertilizer with respect to conventional Zn sources to improve growth, leaf zinc concentration and fruit production in strawberry.

Keywords: Nanotechnology, plant nutrition, zinc, *Fragaria*×*ananassa* Duch.

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INTRODUCTION

Strawberry fruits (*Fragaria*×*ananassa* Duch) are among the most appreciated and consumed in the world due to their nutritional, organoleptic and nutraceutical properties (Liu *et al.*, 2018). Its cultivation is of great economic importance for Mexico, ranking fourth in worldwide production and as the main exporter of strawberry fruit to the United States (FAOSTAT, 2022).

As with any plant species, mineral nutrition through the application of agrochemicals is necessary to complement the natural fertility of the soil; however, in recent years, given the incorrect technical views or recommendations in a good number of production areas, there has been an indiscriminate use of chemical fertilizers, causing damage to production, the environment and human health (Davarpanah *et al.*, 2016). Therefore, it is necessary to look for alternatives to maintain productivity without affecting the quality of the fruit and, in turn, reduce environmental damage.

Nanotechnology applied to agriculture arises as an emerging technology focused on sustainable crop production, using various strategies (Lira Saldivar *et al.*, 2018). One of them is the production and use of nanometric-sized fertilizers, seeking to make the use of agrochemicals more efficient and sustainable (Singh *et al.*, 2018). Examples of these are carbon-based organic nanomaterials, nanostructured from zeolite as carriers for nitrogen, phosphorus and potassium, and nanofertilizers developed from metallic nanoparticles (NPs), such as zinc oxide, iron, copper, titanium dioxide and selenium (Singh *et al.*, 2018).

Specifically, zinc oxide nanoparticles (ZnO NPs) have gained great interest in the agricultural sector, since Zn plays a vital role in the physiological and anatomical responses of plants (Amezcua Romero and Lara Flores, 2017). The effects of the use of these nanoparticles on the growth and physiology of crops such as chickpea (*Cicer arietinum*), coffee (*Coffea arabica* L.) and chili pepper (*Capsicum annum*) have been documented (Pavani *et al.*, 2014; Rossi *et al.*, 2019; García-López *et al.*, 2019).

In order to verify the viability of ZnO NPs as a fertilization alternative, it is necessary to conduct comparative studies to determine the efficiency of nanofertilizers (ZnO NPs) versus that of conventional fertilizers (Zn-EDTA). Therefore, the objective of this study is to compare the effect of foliar and substrate application of ZnO NPs and Zn-EDTA on the growth, foliar zinc concentration, and production of strawberry.

MATERIALS AND METHODS

The study was conducted at the Experimental Fruit Field of the Montecillo Campus, Colegio de Postgraduados, located in Montecillo, Texcoco, Estado de México, from April 14, 2021, to March 2022. The experiment was carried out under a chapel-type greenhouse, with a 25% opacity milky plastic covering, surface area of 60 m^2, without controlled air conditioning; the average temperature conditions inside the greenhouse ranged between 15 and 23 °C (Figure 1), and the photosynthetically active radiation values ranged between 700 and 900 μmol m^{-2} s^{-1} measured at noon, except on cloudy days when radiation values decreased.

Short photoperiod strawberry plants of the 99.717 selection were used, developed at the National Institute of Forestry, Agricultural and Livestock Research (INIFAP), medium size, vigorous and medium-sized fruits, which were transplanted in 10 L black polyethylene bags, in a substrate prepared from a mixture of soil, peat and agrolite, in a 2:2:1 ratio, with pH of 5.9, electrical conductivity of 0.4 dS m^{-1}, organic matter of 33.1%, and good fertility (Table 1 and Figure 4).

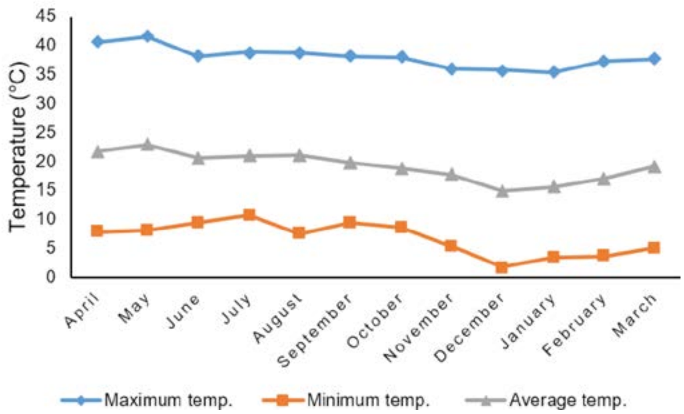

Figure 1. Record of average, maximum and minimum monthly environment temperatures during the experimentation period with greenhouse strawberry (April 2021-March 2022).

Irrigation was carried out using a pressurized drip system, by which fertigation was also carried out with Steiner's nutrient solution (1984) at 50%, with an EC of 1 dS m^{-1} and pH of 5.5, excluding the element zinc, while maintaining the required balance of anions and cations in the solution. Two applications of LOBI® 44 foliar fertilizer (2.5 g L^{-1}) were carried out to correct nitrogen deficiency, applied during growth and flowering.

The experiment used a completely randomized increased factorial design with eight replications, where the variation factors were: form of application (foliar and on substrate); and fertilization levels: dose of ZnO NPs (100, 200, 500 and 1000 mg L^{-1}) and Zn-EDTA (2.5 mL L^{-1} of water) as a conventional source of fertilization. The complete factorial design (2×5) was extended to accommodate a control, which consisted of not applying zinc, forming a 2×5+1 factorial; one plant per bag was considered as an experimental unit.

Zinc oxide nanoparticles (ZnO NPs) were used, provided by the Applied Chemistry Research Center (CIQA) in Saltillo, Coahuila, which have a polyhedral morphology and a diameter size between 20 to 40 nm (Figure 2).

Solutions with ZnO NPs and Zn-EDTA were prepared in deionized water and dispersed with a Branson 2510 sonicator for 30 minutes, in two 15-minute periods, separated by a

Table 1. Physicochemical analysis of the substrate prior to experimentation.

Chemical analysis													
pH	EC (dS m^{-1})	OM (%)	N - NH_4^+	N - NO_3^-	P	K	Na	Fe	Cu	Zn	Mn	Ca	Mg
			(ppm)									(meq 100 g^{-1})	
5.9	0.4	33.1	25	212	26	1.0	3.3	111	0.7	3	22	27	12

Physical analysis					
Total porosity %	Aeration porosity %	Moisture retention %	Bulk density Mg/m^3	Field capacity %	Permanent wilting point %
78	32	43	0.31	78	45

EC=Electrical conductivity, OM=organic matter, N-NH_4^+ =ammonium, N-NO_3^- =nitrate.

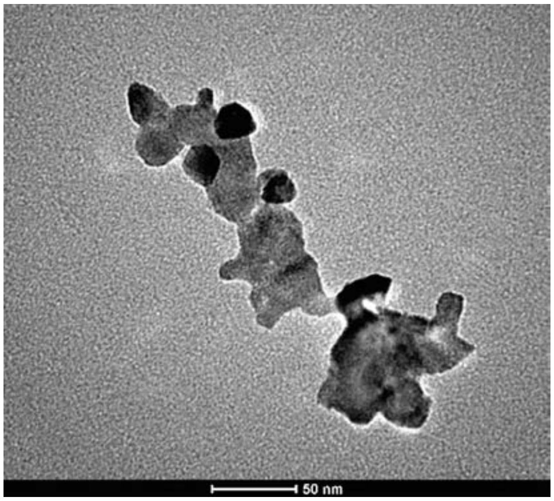

Figure 2. Morphology of ZnO NPs observed by transmission electron microscope (in bright field), Tecnai 2 Spirit brand, Thermo Fisher Fei Company, USA, operated at 120 keV. Electron Microscopy Unit at Colegio de Postgraduados (CP-UME), Montecillo, Texcoco, Mexico.

rest period of 5 minutes. A commercial adjuvant (DAP PLUS) was used for its application. Foliar treatments were applied by spraying 30 mL of solution per plant on the foliage and 30 mL of solution per bag on the substrate, every 15 days, starting on day 51 after transplant (June 4, 2021).

The variables under study were: a) **Plant height**, measured in centimeters at the end of the production cycle, using a tape measure from the base of the stem (crown) to the highest leaf of the plant. b) **Number of crowns per plant**, which were counted at the end of the production cycle, when they could be separated. c) **Number of flowers per plant**, by weekly counts to determine the total accumulated number of flowers during the production cycle. d) **Number of fruits per plant**, which was quantified periodically when the fruits reached harvest point (2/3 coloration); at the end of the cycle, the accumulated total was determined. e) **Fruit production per plant**, which was obtained by weighing all the fruits harvested during the experiment. f) **Zinc concentration in leaves and fruits per plant**, leaf samples (petiole together with leaflets) were taken during vegetative growth (June 14, 2021), flowering (November 22, 2021), and fruiting (February 16, 2022). The samples were washed with deionized water and dried in a forced air oven at 70 °C for 48 to 72 h, until a constant dry weight was obtained. Subsequently, each sample per plant was ground; then, 1 g of each pulverized sample was subjected to wet digestion with a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 2:1 (v/v) ratio. The liquid digest obtained was titrated with deionized water, from which samples were taken to be diluted and subjected to atomic absorption measurement with a GBC brand spectrophotometer, model SAVANTA PM, series FTSLA06. The fruit samples were analyzed in a similar way. g) **Fruit firmness**, measurements were carried out with a texturometer (Wagner, model FDV-30) with 8 mm conical strut; readings were taken on opposite sides at the equator of the fruit, thus obtaining the average value per plant. The data were expressed in Newtons (N). h) **Total soluble solids in fruits**, were determined in °Brix, using a digital refractometer model ATAGO-Pelette with a scale of 0 to 32%, using two drops of fruit juice per plant.

The data obtained were analyzed with generalized linear mixed models (GLIMMIX), and Tukey's means comparison was made ($P \leq 0.05$). The statistical analyses were performed with SAS software version 9.4.

RESULTS AND DISCUSSION

Plant height. Foliar application with 200 mg L^{-1} of ZnO NPs significantly increased the height compared to the control and the treatments with Zn-EDTA applied foliarly and on the substrate (Figures 3 and 4).

The increase in height caused by treatment with ZnO NPs may be due to the fact that zinc is involved in the formation of tryptophan, a precursor to the synthesis of auxins, associated with cell division and elongation, which stimulate growth in plants (Amezcua Romero and Lara Flores, 2017). Furthermore, nanometric-sized zinc particles can be absorbed by the plant (Rossi *et al.*, 2019) and penetrate more quickly than larger particles (Prasad *et al.*, 2012).

These results are consistent with those reported by Kumar *et al.* (2017), who found that strawberry plants treated with ZnO NPs plus FeO NPs showed greater growth. Similarly, the application of ZnO NPs increased the height of chickpea, habanero chili, coffee and tomato plants (Pavani *et al.*, 2014; García-López *et al.*, 2019; Rossi *et al.*, 2019; Pérez Velasco *et al.*, 2020).

Number of crowns, flowers and fruits. A significant increase in the number of crowns (NC) was found with the different concentrations of ZnO NPs applied foliarly and on the substrate, obtaining averages of up to 10 crowns $plant^{-1}$, with the 200 mL L^{-1} treatment being statistically different from the treatments with foliar Zn-EDTA and the control (Table 2).

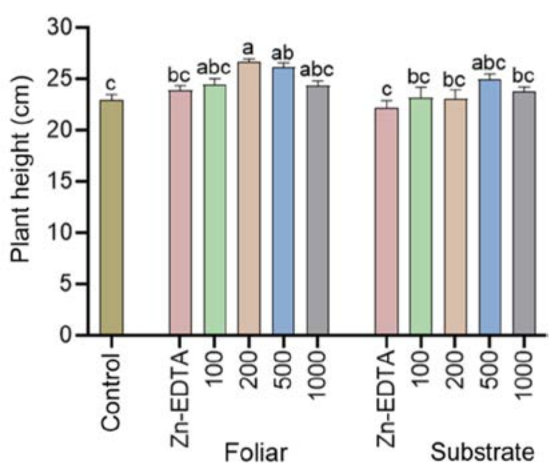

Figure 3. *Fragaria×ananassa* Duch. plant height grown under greenhouse conditions, treated with different doses of ZnO NPs and Zn-EDTA applied foliarly and on the substrate [Nanoparticle doses are expressed in mg L^{-1} and Zn-EDTA in mL L^{-1}; the vertical bars represent standard error (n=8). Bars with the same letter are statistically equal (Tukey, $P \leq 0.05$)].

Regarding the number of flowers (NFL), the concentration with 200 mg L^{-1} of ZnO NPs applied foliarly produced 113 flowers $plant^{-1}$ which was statistically different from the number of flowers produced with the foliar treatments and the Zn-EDTA substrate, as well as from the control, although not different from the applications of NPs to the substrate (Table 2 , Figure 4).

On the other hand, foliar application of ZnO NPs significantly influenced the number of fruits (NFR) per plant, positively correlating with the results obtained in case of number of flowers. Fruits increased with the foliar application of 200 mg L^{-1} of ZnO NPs, which was significantly different from the other treatments and the control (Table 2).

Table 2. Number of crowns, flowers and fruits per strawberry plant grown under greenhouse conditions, treated with different doses of ZnO NPs and Zn-EDTA applied foliarly and on the substrate.

Application	Treatments[3]	NC[4]	NFL	NFR
	Control	[2]6.6±0.18c[1]	93.2±4.18bc	75.9±9.00d
Foliar	Zn-EDTA	7.4±0.25bc	89.0±4.81c	76.5±5.18cd
	100	10.0±0.38a	107.4±11.69ab	91.6±4.66ab
	200	10.0±0.27a	113.0±10.38a	97.9±3.94a
	500	9.2±0.45a	97.0±11.95bc	91.6±14.04ab
	1000	9.1±0.44a	94.1±4.64bc	80.9±9.21bc
Substrate	Zn-EDTA	8.5±0.38ab	93.7±6.14bc	76.4±6.87cd
	100	9.1±0.29ab	99.4±5.03abc	77.1±7.25cd
	200	9.7±0.31a	98.5±7.13abc	81.4±14.91bc
	500	9.7±0.31a	100.9±5.32abc	80.5±6.82bc
	1000	9.0±0.50a	100.1±7.08abc	88.9±11.46abc

[1] The values followed by the same letter are not statistically different (Tukey, $P \leq 0.05$).
[2] Average values ± standard error.
[3] Nanoparticle concentrations are expressed in mg L^{-1} and Zn-EDTA in mL L^{-1}.
[4] NC=number of crowns; NFL=number of flowers; NFR=number of fruits.

Figure 4. Strawberry plants (*Fragaria×ananassa* Duch.) in development, showing in the image on the right the beginning of flowering which happened in the fall, given the short photoperiod habit of the selection under study.

The number of crowns in this crop is an indicator of vigor and rapid growth (Bish *et al.*, 2002) , since the crowns, as the growth center of strawberry, have the function of regulating the metabolic activities of the plant (Hancock *et al.*, 2008). A greater number of crowns correlates positively and significantly with plant growth, partly due to the fact that Zn participates in cell division (Rahman *et al.*, 2016) and that, as already mentioned, Zn assimilation is more efficient when nanometric-sized particles are used (Prasad *et al.*, 2012).

Zn is involved in the formation of flowers and fruits (Rahman *et al.*, 2016), since it is involved in genetic expression by being part of transcription factors related to the regulation of flower development (Amezcua Romero and Lara Flores, 2017). The increases in the number of flowers obtained are similar to those found by Kumar *et al.* (2017) and Mahmood and Al-Dulaimy (2021), agreeing that applications of zinc in nanometric size increase the number of flowers in strawberry plants.

Consequently, an increase in the number of fruits is also expected, due to more flowers (Amezcua Romero and Lara Flores, 2017). The results obtained coincide with those found by Davarpanah *et al.* (2016), García-López *et al.* (2019), Faizan and Hayat (2019), and Mahmood and Al-Dulaimy (2021), whose studies show that plants exposed to foliar application of ZnO NPs produce a greater number of fruits in pomegranate, chili pepper, tomato and strawberry, respectively.

Fruit production. Given the above results, plants exposed to foliar application with 200 mg L^{-1} of ZnO NPs showed a statistically different fruit production, compared to that obtained in the other treatments and the control (Figure 5).

Zn in nanometric size has greater assimilation, which facilitates the penetration capacity in the leaf (Prasad *et al.*, 2012). Treatments with different concentrations of nanoscale zinc oxide (ZnO NPs) leads to improved plant metabolism, increased growth, and increased fruit production (Davarpanah *et al.*, 2016). Thus, Faizan and Hayat (2019) found that foliar applications with 50 mg L^{-1} of ZnO NPs increase tomato yield; Subbaiah *et al.* (2016) point out that foliar applications with 400 mg L^{-1} of ZnO NPs

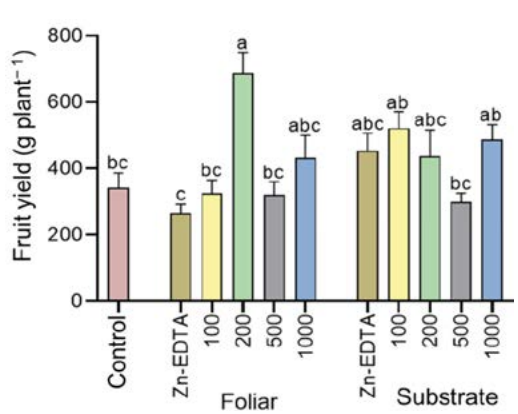

Figure 5. Fruit production of *Fragaria*×*ananassa* Duch. grown under greenhouse conditions, treated with different doses of ZnO NPs and ZnEDTA applied foliarly and on the substrate [Nanoparticle doses are expressed in mg L^{-1} and Zn-EDTA in mL L^{-1}. Vertical bars represent standard error (n=8). Bars with the same letter are statistically equal (Tukey, $P \leq 0.05$)].

increase corn yield; similar results were obtained in peanut and melon (Prasad *et al.*, 2012 ; Rivera-Gutiérrez *et al.*, 2021).

Zinc concentration in leaves. The results revealed that during vegetative growth there are no significant differences in foliar Zn concentration between treatments with ZnO NPs application and conventional fertilization treatments (Zn-EDTA), or without Zn application (Table 3). In the flowering stage, the control plants without Zn application had the lowest foliar concentration of Zn (18.83 μg g^{-1}), while the foliar treatments of 500 and 1000 mg L^{-1} of ZnO NPs reached the highest foliar concentrations of Zn (Table 3); however, it should be noted that these are excess foliar concentrations of Zn, as opposed to those determined for doses of 100 and 200 mg L^{-1}, which were within the range of Zn sufficiency (Becerril and Jaen, 2010) and are statistically equal to those observed in treatments with the highest doses of ZnO NPs. For the fruiting stage, plants treated with foliar and substrate doses of ZnO NPs had foliar concentrations of Zn significantly higher than the control (Table 3).

The results obtained confirm an increase in the concentration of zinc in strawberry leaves, in plants treated with ZnO NPs, which in principle indicates that the assimilation of Zn can be faster and more efficient by this method of application, and more so when nanometric-sized particles are used (Prasad *et al.*, 2012). These results agree with those found by Subbaiah *et al.* (2016) who point out that foliar applications with ZnO NPs increase foliar zinc concentration in corn, coinciding with what has been found in coffee and barley crops (Rossi *et al.*, 2019; Rajput *et al.*, 2021).

García-Gómez *et al.* (2020) indicate that high doses (>1000 mg L^{-1}) of ZnO NPs can be phytotoxic to plants and limit their yield; in this study the plants did not show symptoms of toxicity at concentrations of 500 and 1000 mg L^{-1} of ZnO NPs, even though the zinc

Table 3. Foliar zinc concentrations in strawberry plants grown under greenhouse conditions treated with different doses of ZnO NPs and ZnEDTA applied foliarly and on the substrate.

Application	Treatments[3]	Vegetative growth	Flowering	Fructification
		μg g^{-1} dry weight		
	Control	[2] 21.0±3.62a [1]	18.83±1.59c [1]	22.67±4.13b [1]
Foliar	Zn-EDTA	22.5±2.02a	21.83±6.93c	29.50±9.84ab
	100	21.5±2.31a	51.67±9.27abc	70.33±2.69ab
	200	32.0±6.65a	52.33±12.93abc	63.17±1.77ab
	500	31.6±5.35a	140.17±28.29a	106.00±197.8a
	1000	27.3±3.32a	125.00±18.05ab	103.33±32.49a
Substrate	Zn-EDTA	32.0±6.36a	23.67±0.44c	34.33±6.65ab
	100	22.3±0.73a	23.17±1.30c	34.50±3.55ab
	200	46.0±15.32a	21.83±1.30c	32.83±7.10ab
	500	26.0±4.62a	27.83±0.44bc	33.67±4.29ab
	1000	22.5±6.38a	24.50±3.61c	33.33±1.67ab

[1] The values followed by the same letter are not statistically different (Tukey, $P \leq 0.05$).
[2] Average values ± standard error.
[3] Nanoparticle concentrations are expressed in mg L^{-1} and Zn-EDTA in mL L^{-1}.

concentration was higher than the zinc sufficiency levels in leaf tissue, which according to Becerril and Jaen (2010), is 5-100 μg g^{-1} for strawberry crops.

Zinc concentration in fruit. The data showed an increase in the zinc concentration in the fruits with ZnO NPs and Zn-EDTA applications foliarly and on the substrate; however, there were no differences between treatments and the control (Figure 6).

Firmness and total soluble solids of fruits. The means comparison did not indicate significant differences between treatments for firmness, nor for total soluble solids of the fruit (Figure 7), although the values of both variables increased with the application of Zn.

Based on the results obtained, it is possible to predict that the use of ZnO NPs could be an alternative source of fertilizer, which, due to their size, is more efficiently absorbed than conventional Zn sources, and which could reduce possible losses when used in smaller quantities, with the consequent lower environmental impact in terms of pollution, in favor of improving the quality of crops.

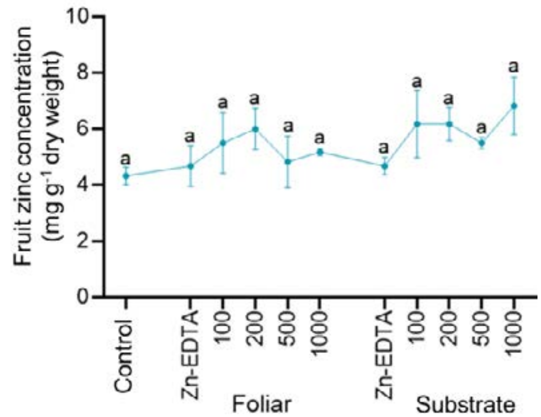

Figure 6. Zinc concentration in *Fragaria×ananassa* Duch. fruits from the effect of foliar or substrate applications of zinc oxide nanoparticles and Zn-EDTA. [Nanoparticle concentrations are expressed in mg L^{-1} and Zn-EDTA in mL L^{-1} . Vertical bars represent standard error (n=6). Bars with the same letter are statistically equal (Tukey, P≤0.05)].

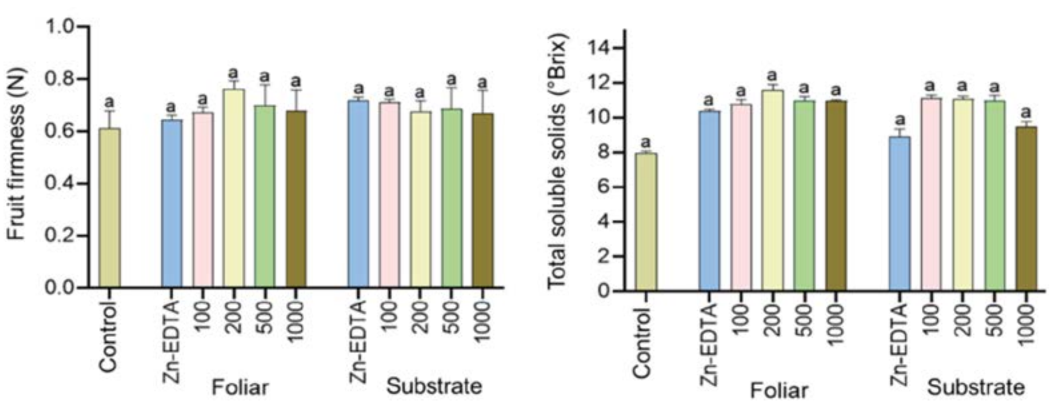

Figure 7. Fruit quality variables of *Fragaria×ananassa* Duch. grown under greenhouse conditions, treated with different doses of ZnO NPs and Zn-EDTA applied foliarly and on the substrate [Nanoparticle doses are expressed in mg L^{-1} and Zn-EDTA in mL L^{-1}. Vertical bars represent standard error (n=4). Bars with the same letter are statistically equal (Tukey, P≤0.05)].

The results obtained also demonstrate the potential of ZnO NPs applied foliarly to improve the growth and productivity of strawberry crops, since, in the case of Zn, it is required in smaller quantities and, in the vast majority of cases, its applications are complementary. Consequently, ZnO NPs can be an alternative to improve crop productivity, depending on the species and the concentration of nanoparticles applied. In the case of soil applications, it is necessary to carry out studies in field conditions to find out how Zn is distributed in the soil and to analyze its availability for the plant.

Overall, based on experimental evidence, nanometric fertilizers have great potential to improve the efficiency and sustainability of agriculture while reducing the use of conventional fertilizers, particularly in future precision agriculture systems. Therefore, it is important to continue researching and evaluating their safety and efficacy before their large-scale implementation, since it is also necessary to consider the potential risks, challenges, costs and profitability associated with their use, the appropriate regulation of their production and application, as well as the acceptance of this technology.

CONCLUSIONS

This study suggests that the application of ZnO NPs improves the development of strawberry plants with foliar dose applications of 200 mg L^{-1}, since they promoted plant growth and increased the number of crowns, flowers and fruits per plant, as well as fruit production.

High doses of nanoparticles can cause an increase in the leaf concentration of zinc, but they generate excess levels which could cause phytotoxicity, while doses of 100 and 200 mg L^{-1} promote levels within the sufficiency range.

The application of Zn fertilization positively affected the fruit quality variables studied.

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