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Yield, quality, and phytochemicals of two strawberry cultivars in response to foliar calcium nanofertilization

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ABSTRACT

Objective: to evaluate the foliar application of Calcium (Ca^{2+}) nanofertilizer on the yield, fruit quality, total phytochemicals and capacity of two strawberry cultivars 'Monterrey' and 'Albion' of *Fragaria × ananassa* Duch.

Design/Methodology/Approach: three foliar calcium treatments were established (two commercial foliar fertilizers, one nanofertilizer). The doses used were 2.5, 5 and 7 mEq L^{-1} and were applied at the stages of beginning of flowering, full flowering, end of flowering, fruit setting and full production. The design of the experiment was randomized blocks in four replicates, each replicate consisted of a 1 m^2 area, with 16 plants. An analysis of variance was performed and the Tukey's test ($p \leq 0.05$) was applied for comparison of means.

Results: the results indicated that doses of 2.5 mEq L^{-1} for the 'Monterrey' cultivar and 5 mEq L^{-1} for the 'Albion' cultivar favored increases in total phenols, total flavonoids, total anthocyanins, antioxidant capacity and higher yields, by obtaining 13 kg m^{-2} and 12.59 kg m^{-2} from those cultivars, respectively.

Findings/Conclusions: this indicates that the use of nano-calcium as a foliar fertilizer could be a suitable alternative that helps improving the bioactive compounds and yields of strawberry fruits.

Keywords: *Fragaria × ananassa* Duch, phenols, flavonoids, anthocyanins, DPPH.

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INTRODUCTION

The strawberry (*Fragaria × ananassa*) crop is popular around the world, occurring in China, the United States and Mexico (FAOSTAT, 2022). Mexico ranks third in the world with a cultivated area of 17 400 hectares and a production of 861 337 Megagrams (SIAP-SADER, 2020), produced mainly in the states of Michoacán, Baja California, Baja California Sur, Guanajuato, Jalisco, and Tlaxcala. A successful strategy has been the foliar application of macronutrients and micronutrients to improve nutrition, increasing quality and disease resistance (Zouari *et al.*, 2016; de Dios-Delgado *et al.*, 2006). However, these practices at present are not particularly successful at improving simultaneously in plants, the uptake, transport, and efficiency in the use of those nutrients (Adnan *et al.*, 2020).

The shelf life of strawberries is one of the problems to be solved in this crop and is reported to be directly related to problems from calcium (Ca^{2+}) deficiency. Since this element, being found in low concentrations, affects the thickness of the cell wall, turgor and the interaction in adhesion of pectate lyases between cells (Lanauskas *et al.*, 2006); all of these are characteristics that impact fruit quality. One of the characteristics of Ca^{2+} is immobilization, so a relevant strategy can be presented with technological advances at the nanoscale. Specifically, foliar nanofertilizers that present a targeted and immediate absorption through the stomatal cells in plant tissues (Kopittke *et al.*, 2019).

This is due to the size of its nanostructures (100 nanometers, nm) which facilitate their intake speed and favorably stimulate physiological and biochemical responses (Lira-Saldívar *et al.*, 2018). This macroelement is a secondary messenger and important precursor in the composition of cell walls. Also it is a promoter of the firmness of the fruits because of its role in the formation of structural polysaccharides. Therefore, complementary studies are required to compare the action mode of Ca^{2+} foliar nanofertilizers vs. conventional sprays in organoleptic qualities such as quality and phytochemicals; phenols, flavonoids, anthocyanins, and antioxidant capacity.

MATERIALS AND METHODS

The experiment was established in March 2022, at the Faculty of Agrotechnological Sciences Campus Cuauhtémoc, of the Autonomous University of Chihuahua. The plants of the cultivars 'Monterrey' and 'Albion' had been established for a year in microtunnel conditions. Vegetative materials were placed in beds, which were delimited by a space of 0.80 m between lanes, 12 m long by 1.20 m wide, at a height of 0.40 m. Black plastic mulch was established on the beds and then perforations were made every 0.25 m to carry out the planting. A drip irrigation system was installed, two drip-lines were used per bed between two plant rows. Each drip-line had drippers every 15 cm, with an average flow of 2 L h^{-1} .

The design of the experiment was randomized blocks in four replicates, each replicate consisted of a 1 m^2 area which included 16 plants. The plants of all treatments were fertigated with a "Triple 18" solution at a dose 150 mg L^{-1} in Spring after the onset of vegetative growth, and 220 mg L^{-1} in the flowering stages and full production. Likewise, minor elements such as boron, zinc and copper were also applied at a dose of 40 mg L^{-1} . Foliar sprays consisted of three commercially available calcium preparations, one of them a nanofertilizer (Table 1). Those were applied during early flowering, full flowering, late flowering, fruit set and full production, to improve the quality traits of the fruit. The experiment included three treatments and one control (Table 1). The physicochemical and phytochemical parameters described in Tables 2 and 3, respectively, were evaluated post-harvest.

Data analysis

An analysis of variance was performed to identify significant variables due to treatments. Also, the multiple comparison of means was done with the Tukey's test ($p \leq 0.05$), corresponding to 95% confidence level, using the statistical software Minitab[®] 19.

Table 1. Description of treatments with its active Calcium ingredient, chemical composition and doses applied.

Treatments	% Active ingredient Ca	Chemical composition and density	Dose
Control	Non calcium	-	-
Calcium oxide: SuperCALCIO®	20% Ca	Mg=0.10 % Carboxylic acid=0.30 % Amino acids=5.00 % B=200 ppm ***Density=1.244 a 1.284 g/mL	2.5, 5.0 y 7 mEq/L
Calcium oxide: CALBIT C®	15% Ca	Cd=≤0.5 mg/ kg Pb=≤5 mg/ kg Hg=≤0.5 mg/ kg As=≤10 mg/ kg ***Density=1.45 g/cc	2.5, 5.0 y 7 mEq/L
Nanofertilizer: PHC® NANO Ca	41% Ca	***Density=1.65 g/cm	2.5, 5.0 y 7 mEq/L

Table 2. Physicochemical quality traits in two strawberry cultivars, 'Monterrey' and 'Albion'.

Physicochemical Quality Parameters	Metodología	Unidad de medida
Yield	It was expressed as the sum of the unit weight of the fruits during the production cycle lasting 25 weeks; starting in the month of June and ending in October. Weighing in their entirety the fruits of the 16 plants per 1 m ² .	Kg/m ²
Fruit weight	The number of fruits was calculated by dividing the total weight. The harvested fruits were weighed on a Trupper digital scale (BASE-5EP China mod).	g
Firmness	It was measured using a texture analyzer (Texture Analyzer CT3 Brookfield Engineering Laboratories, MA, USA), equipped with a 4 mm conical tip; which compressed the fruit to 8 mm at a speed of 5 mm/s, two perforations were made per fruit and the results were expressed in newtons (N).	N
Hydrogen potential	The juice of 10 strawberries was extracted using a manual juicer. Subsequently, the pH was determined from the mixture of the juice obtained, with an OHAUS potentiometer pH meter (Starter 3100).	pH
Titratable acidity	It was determined based on the citric acid content (%) according to the volumetric method of AOAC 935.57 (1990), in a sample of 5 fruits per treatment, 20 g of pulp were weighed individually, homogenizing with 100 mL of distilled water, subsequently the mixture was filtered and a 5 mL aliquot was taken, which was titrated with 0.1N NaOH, using 2 drops of phenolphthalein in 1% alcohol solution as an indicator.	citric acid (%)
Soluble solids	Soluble solids concentrations were determined in an Abbe refractometer at 20 °C.	°Brix

Table 3. Phytochemical parameters and antioxidant capacity in two strawberry cultivars, 'Monterrey' and 'Albion'.

Parameters of phytochemicals and DPPH	Methodology	Units
Total phenols	Five grams of strawberry were homogenized with Ultraturax (Ultra-Turrax, Model Micra D-9 KT, Digitronic 132 GmbH, Bergheim, Germany). After that, the extract was measured with the Folin - Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using gallic acid as a standard.	Milliequivalents of gallic acid (GAE)/100 g fresh weight
Total flavonoids	The total flavonoid content was measured using a colorimetric assay, according to the method of (Zhishen <i>et al.</i> , 1999). Flavonoids were extracted 5% NaNO ₂ 10% AlCl ₃ and 1 mol L ⁻¹ NaOH and measured spectrophotometrically at 510 nm using quercetin as a standard.	Milliequivalents of quercetin (QE)/100 g of fresh weight
Total anthocyanins	The total anthocyanin content was determined using the pH differential method (Lee <i>et al.</i> , 2005).	Milliequivalents of Cyanidin-3-glucoside (C3G)/100 g fresh weight
Antioxidant Capacity	Antioxidant capacity was evaluated by radical scavenging activity using DPPH (2-diphenyl-1-picrylhydrazyl) as detailed by Velderrain-Rodríguez <i>et al.</i> (2018).	Milliequivalents of trolox (TE)/100 g fresh weight

RESULTS AND DISCUSSION

Yield and fruit weight

The PHC® NANO Ca treatment obtained the best yields, the 'Monterrey' cultivar produced 13 kg m⁻² at a 2.5 mEq L⁻¹ dose, while for the 'Albion' cultivar the optimal dose was 5 mEq L⁻¹ with 12.59 kg m⁻² (Table 4). Similar data have been reported in other species and it is mentioned that the significant differences among treatments are related to concentration levels, rather than to the type of fertilizer applied. Among those studies, a trial that evaluated apple production showed improvements in the amount of fruits from trees treated with nano-Ca²⁺ 2% (Ranjbar and Ramezanian, 2020). Similarly, applications of calcium and boron resulted favorable in pomegranate fruits, promoting yield increase (El-Salhy *et al.*, 2022).

Table 4. Fruit yield (kg m⁻²) of two strawberry cultivars ('Monterrey' and 'Albion') under foliar fertilization, with nano-Ca²⁺ and two other conventional commercial products.

	Foliar fertilization treatments with Ca ²⁺					
	superCALCIO®		CALBIT C®		PHC® NANO Ca	
Dose (mEq/L)	Monterrey	Albión	Monterrey	Albión	Monterrey	Albión
2.5	8.78 cd	11.46 ab	8.10 d	10.18 bc	13.00 a	11.79 ab
5	9.55 c	9.84 c	10.55 bc	8.89 c	11.66 ab	12.59 a
7	8.06 bc	8.26 bc	8.56 bc	7.47 c	10.05 a	9.17 ab

* Different letters within columns indicate statistical difference (Tukey, *p*≤0.05).

Despite no significant differences were observed in fruit development and weight (Table 5); a higher fruit development was obtained in August with nano-calcium foliar fertilization treatments. The average value of the three doses (2.5, 5 and 7 mEq L⁻¹) evaluated was 24.22 g for the cultivar 'Monterrey' and 23.96 g for 'Albion'. This indicates that even though the average number of fruits in the production season (June-October) did not represent statistically significant differences in weight, it did have an impact on overall fruit weight in the month of maximum production (Figure 1).

Physicochemical Quality Traits

Firmness, soluble solids, titratable acidity and pH were all statistically similar, with no differences among treatments and cultivars studied ('Monterrey' and 'Albion') (Table 6). This coincides with studies implemented on pomegranate fruits, where El-Salhy *et al.* (2022) reported that nano-calcium fertilizers, alone or in combination, did not present benefits

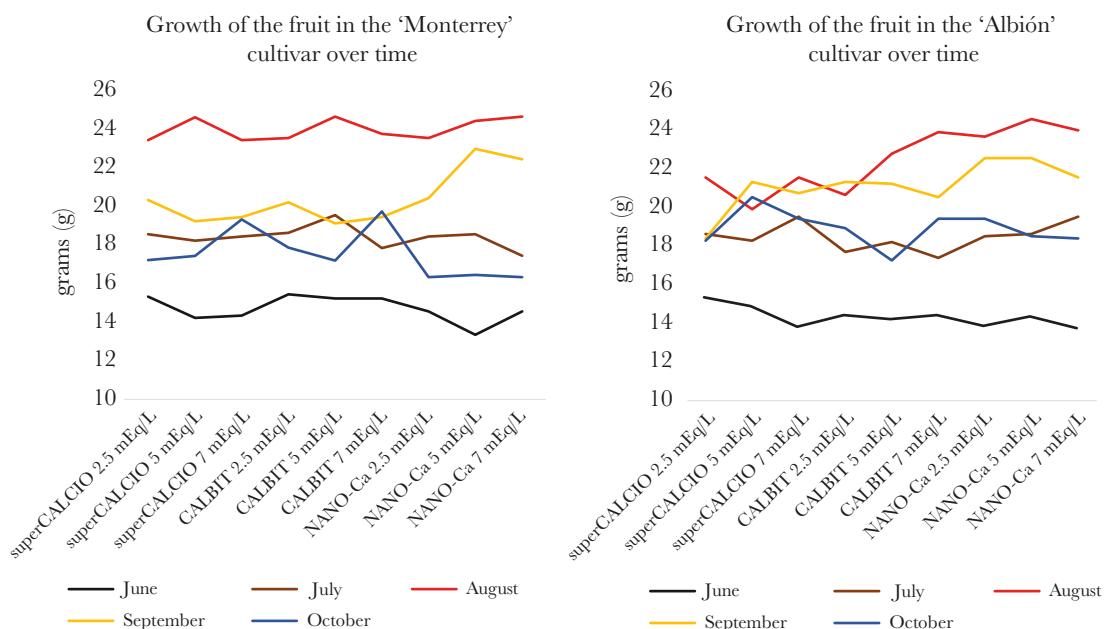


Figure 1. Fruit growth over time in two strawberry (*Fragaria × ananassa* Duch) cultivars, 'Monterrey' and 'Albion'. Different letters among Ca²⁺ treatments indicate statistical difference (Tukey, $p \leq 0.05$).

Table 5. Fruit weight (g) of two strawberry cultivars 'Monterrey' and 'Albion' under foliar fertilization, with nano-Ca²⁺ and two conventional commercial products.

	Foliar fertilization treatments with Ca ²⁺					
	superCALLOCIO®		CALBIT C®		PHC® NANO Ca	
Dose (mEq/L)	Monterrey	Albion	Monterrey	Albion	Monterrey	Albion
2.5	18.98 a	18.38 a	19.15 a	18.54 a	18.67 a	19.53 a
5	18.57 a	18.98 a	19.16 a	18.68 a	19.16 a	19.65 a
7	19.00 a	18.94 a	19.21 a	19.07 a	19.09 a	19.37 a

*Different letters within columns indicate statistical difference (Tukey, $p \leq 0.05$).

Table 6. Physicochemical quality traits of two strawberry cultivars ('Monterrey' and 'Albion') fruits, foliarly fertilized with nano-Ca²⁺ and two conventional commercial products.

Foliar fertilization	Variety	Dose (mEq/L)	Firmness (N)	Soluble solids (°brix)	Titratable acidity (citric acid)	pH
superCALCIO®	Monterrey	2.5	4.81 a	10.52 a	1.72 a	3.5 a
		5.0	5.04 a	10.11 a	1.88 a	3.6 a
		7	4.92 a	10.89 a	1.96 a	3.5 a
	Albión	2.5	4.63 a	10.90 a	1.78 a	3.6 a
		5.0	5.13 a	10.02 a	1.92 a	3.4 a
		7	5.06 a	10.14 a	1.77 a	3.5 a
CALBIT C®	Monterrey	2.5	4.88 a	10.08 a	1.66 a	3.5 a
		5.0	4.83 a	10.04 a	1.88 a	3.5 a
		7	5.12 a	10.07 a	1.91 a	3.6 a
	Albión	2.5	4.79 a	10.12 a	1.85 a	3.5 a
		5.0	5.27 a	10.98 a	1.97 a	3.5 a
		7	5.17 a	10.03 a	1.95 a	3.5 a
PHC® NANO Ca	Monterrey	2.5	4.95 a	10.02 a	1.94 a	3.4 a
		5.0	4.76 a	9.96 a	1.77 a	3.4 a
		7	4.83 a	10.74 a	1.93 a	3.4 a
	Albión	2.5	5.12 a	9.87 a	3.98 a	3.5 a
		5.0	5.18 a	10.02 a	3.87 a	3.5 a
		7	4.86 a	10.01 a	3.59 a	3.5 a

in the quality traits of that crop. Which also coincides with Davarpanah *et al.* (2018) who, when studying nano-Ca²⁺ fertilization, reaffirmed that fruit quality was similar in terms of titratable acidity, maturity and total sugars.

Overall, nanotechnology has been useful in improving crop growth, yield and quality; however, Zahedi *et al.* (2020) stated that more research should be done on the effects of nanofertilizers on fruit trees and more cultivars should be studied because the responses are very different among species and cultivars. Although foliar application of Ca²⁺ can be potentially effective in increasing the concentration of Ca²⁺ in fruit, spray of Ca²⁺ has been shown to have low efficiency in many cases. This has been attributed to limitations in Ca²⁺ absorption, intake by the fruit, epidermal characteristics, cuticle presence and composition, and may also be related to low rates of Ca²⁺ allocation through the phloem (Davarpanah *et al.*, 2018).

Transpiration in plant organs tend to accumulate high levels of calcium, which is usually stored within vacuoles or is deposited in leaf trichomes to impose immobility through the phloem, leading to low calcium levels in leaves and fruits (Kumar *et al.*, 2015). The current study showed that applications with calcium nanofertilization had no significant influence on the quality of strawberry fruits, which could lead us to suppose that calcium in vacuoles imposes low mobility through phloem, causing nutritional disorders in fruits. It should be noted that reports in literature still do not show clarity on the effects of nanofertilizers on

fruit quality. Other proof of this is reported in a study by Bayat (2016) on pepper fruits where they highlighted that fruits treated with nano-calcium had lower levels of total soluble solids, lower pH and weight loss.

Phytochemical parameters and antioxidant capacity

Calcium is an essential structural, metabolic, and signaling element, which is required for nutritional signaling purposes (Demidchik *et al.*, 2018; Thor, 2019). Therefore, it shows a dual function, as a secondary messenger involved in plant growth and responses to biotic and abiotic stress (Xu *et al.*, 2015; Zhang *et al.*, 2017). This can trigger secondary metabolism responses, synthesizing bioactive compounds such as phenols and their derivatives.

Total phenols

Applications of the Ca^{2+} nanofertilizer increased the content of phenolic compounds in strawberry fruits (Figure 2). The varieties behaved somewhat differently; in the 'Monterrey' cultivar, the treatment of nanofertilizer- Ca^{2+} (PHC® NANO Ca) at a 2.5 mEq L⁻¹ dose achieved the highest value of total phenols 302.84 mg GAE per 100 g of fresh weight. On the other hand, the remaining two treatments with traditional foliar calcium fertilization achieved values of 186.80 and 196.23 mg GAE per 100 g of fresh weight for superCALCIUM® and CALBIT C® respectively. In the results for the 'Albion' cultivar, values were increased in the treatment at a 5 mEq L⁻¹ dose, which yielded 329.42 mg GAE per 100 g of fresh weight with PHC® NANO Ca (Figure 2).

It should be noted in the results of this study that for both varieties, the PHC® NANO Ca (nano- Ca^{2+}) treatment was superior. This is mainly due to the effect of the calcium nanofertilizer, which increased phenolic compounds by up to 50%, confirming the positive effect on the phytochemical composition of the fruits. The role of Ca^{2+} in phenol metabolism has been described by Ngadze *et al.* (2014) arguing an increase in the enzyme Phenylalanine Ammonium Liase (PAL), which could be due to a response elicited by a series of transduction signals that result in increased activity of the enzymes Polyphenol Oxidase (PPO) and Peroxidase (POD).

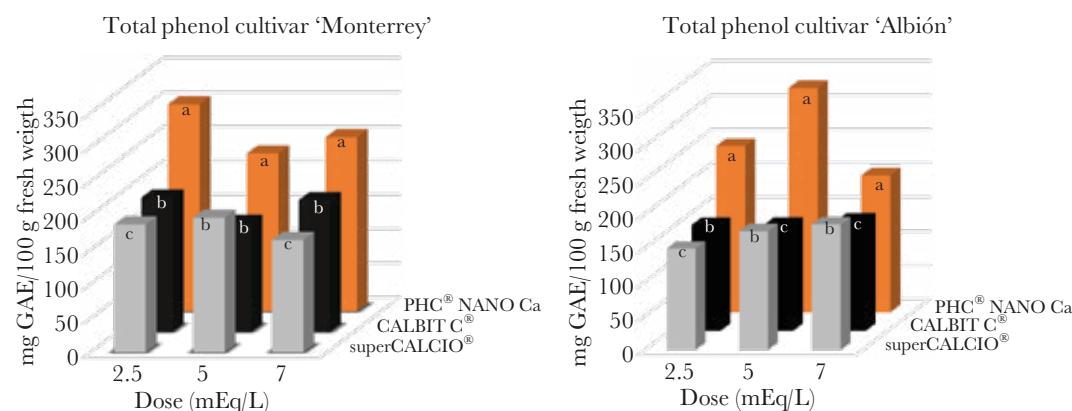


Figure 2. Total phenols in two strawberry (*Fragaria × ananassa* Duch) cultivars 'Monterrey' and 'Albion'. Different letters among Ca^{2+} treatments and doses indicate statistical differences (Tukey, $p \leq 0.05$).

Calcium activates PPO and modifies the conformational state of the enzyme, thus enhancing its activity (Ngadze *et al.*, 2014). Results of the current study are in agreement with the findings of Akladious and Mohamed (2018) who demonstrated a direct role of calcium in phenol synthesis. Since the essential role that phenol metabolism plays in many resistance responses to different stresses, rapid and effective manipulation of the metabolic process would improve plant resistance to adverse conditions. It should be noted that in this study, the treatments superCALLOCIO® and CALBIT C®, which are traditional foliar calcium fertilizers, acted differently on the varieties studied; the three doses evaluated (2.5, 5 and 7 mEq L⁻¹) generated different trends (Figure 2).

Total Flavonoids

In both cultivars, an increase in total flavonoids was generated, standing out the treatment with nano-Ca²⁺. The 'Monterrey' cultivar with doses of 2.5 mEq L⁻¹ obtained values of 30.33 mg QE per 100 g fresh weight, compared to 11.83 and 11.22 mg QE per 100 g fresh weight with the conventional calcium fertilizers superCALLOCIO® and CALBIT® respectively (Figure 2). Similarly, the 'Albion' cultivar reached 27.36 mg QE per 100 g fresh weight, the highest value with the 2.5 mEq L⁻¹ dose. While the superCALLOCIO® and CALBIT® treatments recorded 11.25 and 12.08 mg QE per 100 g fresh weight respectively (Figure 3).

Flavonoid compounds are among the most important antioxidant substances that initiate a number of secondary metabolites produced by the shikimic acid or malonic acid cycles, which serve as cell signaling agents (Michalak, 2006). The contribution of Ca²⁺ as a cation with multifunctional capacity as a secondary messenger in different responses is evident, antioxidants are free radical scavengers. Therefore, the antioxidant action of flavonoids depends on the availability of free OH groups. Enhanced flavonoid levels with nano-Ca²⁺ doses could be a form of defense (*i.e.*, oxidative loading).

The impact of Ca²⁺ is important according to Ma *et al.* (2019) who stated that the application of Ca²⁺ significantly improved phenol concentrations and enzyme activity used in phenol metabolism (PAL, PPO and POD). In addition, the increase in flavonoid

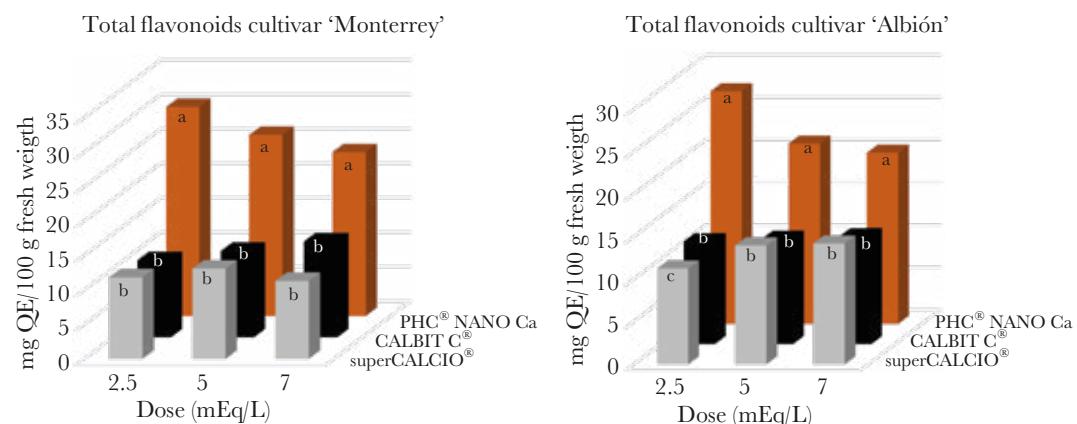


Figure 3. Total flavonoids in 'Monterrey' and 'Albion' strawberry cultivars. Different letters among Ca²⁺ treatments and doses indicate statistical differences (Tukey, $p \leq 0.05$).

compounds could be termed as enhanced phenylalanine ammonia-lyase activity, which is crucial for phenol production (Ma *et al.*, 2019). In this regard, foliar sprays of Ca^{2+} on wheat plants showed increases in flavonoid concentrations. Those findings suggest that Ca^{2+} may stimulate the production of secondary metabolites, which function as oxygen scavengers to minimize oxidative stress, thus boosting wheat growth and yield (Sadak *et al.*, 2023).

Total Anthocyanins

In addition to phenolic compounds and flavonoids, foliar fertilization with nano- Ca^{2+} also promoted the accumulation of water-soluble pigments such as anthocyanins. Results showed an average for the three doses of 35.31 mg C3G per 100 g fresh weight in 'Monterrey' and 36.10 mg C3G per 100 g fresh weight in the cultivar 'Albion'. It should be noted that the varieties and doses with the PHC® NANO Ca treatment of nano- Ca^{2+} maintained similarity in their behavior, finding no statistical differences (Table 7). For the rest of the studied treatments of commercial Ca^{2+} fertilization, it was possible to be certain of similar behaviors; these treatments did not affect this studied variable. It is important to note that anthocyanins are one of the main groups of polyphenols in strawberries (40.3% of total phenols) (Nowicka *et al.*, 2019). Hence, their outstanding contribution to the antioxidant properties they confer on these fruits. Therefore, increasing these pigments through foliar fertilization with nano- Ca^{2+} is important.

Table 7. Phytochemical composition of total anthocyanins and antioxidant capacity of strawberry fruits, foliarly fertilized with nano- Ca^{2+} and two conventional commercial products.

Foliar fertilization	Variety	Dose (mEq/L)	Total anthocyanins (mg C3G/100 g fresh weight)	Antioxidant capacity (DPPH) (mg TE/100 g fresh weight)
superCALCIO®	Monterrey	2.5	27.82 bc	261.70 ef
		5	25.67 bcd	235.03 g
		7	26.41 bcd	247.80 fg
	Albión	2.5	24.95 cd	157.05 i
		5	27.75 bc	165.53 hi
		7	29.09 b	186.51 h
CALBIT C®	Monterrey	2.5	28.00 bc	167.58 hi
		5	23.20 d	177.58 hi
		7	24.39 cd	158.41 i
	Albión	2.5	27.58 bc	176.76 hi
		5	25.98 bcd	187.50 h
		7	23.99 cd	167.02 hi
PHC® NANO Ca	Monterrey	2.5	34.75 a	377.97 a
		5	35.33 a	315.53 b
		7	35.87 a	277.25 de
	Albión	2.5	35.16 a	292.67 cd
		5	35.98 a	306.14 bc
		7	37.17 a	315.92 b

* Different letters within columns indicate statistical differences (Tukey, $p \leq 0.05$).

Results in this study differ from those reported by Zakaria *et al.* (2018) who indicated that strawberry fruits obtained from plants sprayed with Nano-Ca at 15 or 30 mg L⁻¹ doses showed the lowest values of anthocyanin concentrations. In this regard, those authors also argued that applications with nano-Ca generated a decrease in color development, thus fruits became less red, and were related to an increase in firmness. In other words, the firmer the fruits, the less red they were.

It should be noted that in this study there was no impact on firmness, which could explain why the results of total anthocyanins are opposite to what was reported by Zakaria *et al.* (2018). Another study implemented by El-Ramady *et al.* (2021) in apple cultivation showed that nano-Ca treatments significantly improved the total anthocyanins content, similar results to which is found in this research.

Antioxidant capacity

Table 7 shows that strawberry fruits foliarly fertilized with nano-Ca²⁺ (PHC® NANO Ca) at doses of 2.5 mEq L⁻¹ presented significantly higher values according to the DPPH tests. The lowest values corresponded to the 'Albion' cultivar with the superCALCIUM® treatment. Data ranged from 157.05 to 377.97 mg TE per 100 g fresh weight, which means a 41% increase with nano-Ca²⁺. In addition, this behavior of antioxidant activity is in agreement with the results of total phenols and total flavonoids in this study; therefore, they are responsible for conferring this activity to strawberry fruits.

CONCLUSIONS

The use of nano fertilizers in agriculture is offering great opportunities to improve plant nutrition, thus increasing yields. Nanomaterials, including nanoparticles, are characterized by rapid absorption and controlled delivery of nutrients to plants. The mechanisms of transport and assimilation are still unknown, although higher yields and an increase in the responses of antioxidant components were found.

Among these, phenols, flavonoids and total anthocyanins with nano-Ca²⁺ foliar fertilization stood out for both cultivars studied. Physicochemical quality traits such as firmness, soluble solids, titratable acidity, and pH did not show differences. They behaved similarly to conventional commercial Calcium foliar fertilizers.

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