

Zinc oxide nanoparticles and their effect on melon yield and quality

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Abstract

The research consisted of the foliar application of zinc oxide nanoparticles (NPs ZnO), two commercial sources (Z40 and GZ), in addition to a control treatment and their effect on yield and commercial and nutraceutical quality in cv Cruiser melon fruits (*Cucumis melo* L.). The treatments consisted of the foliar application of five doses of NPs ZnO: 50, 100, 150, 200 and 250 mg L⁻¹. The results indicated that the highest yield was for the treatment with 200 mg L⁻¹ of NPs ZnO, with an increase of 53 t ha⁻¹ in relation to the control, the same trend was for the weight of the fruit that showed values of 1.74 kg, 1.45 kg and 1.33 kg, respectively. Concerning flavonoid content, doses of 50 and 100 mg L⁻¹ exceeded the control treatment and commercial sources; however, as the concentration of NP increased, flavonoids decreased. Phenols, antioxidant capacity (DPPH) and vitamin C decreased by 17, 10.8 and 9% with the control, respectively. The highest concentration of zinc in pulp was observed with 200 mg L⁻¹ of NPs ZnO. The use of zinc nanoparticles is a good alternative to improve the yield and nutraceutical content and the concentration of Zn in melon fruits, being an option to combat the malnutrition of this micronutrient.

Keywords: *Cucumis melo* L., DPPH, flavonoids, nanoparticles, phenols.

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Introduction

Melon (*Cucumis melo* L.) is one of the vegetables grown in many regions of the world, in 2019, 1 039 691 hectares were reported, with China, Iran and Turkey being the main producing countries (FAOSTAT, 2020). In Mexico, it is planted in places that present warm climates and low precipitation, in 2019 about 20 thousand ha were reported (SIAP, 2021). The state of Coahuila is in the north of the country, where 1 800 producers planted more than 6 thousand ha with a high degree of specialization, quality and safety, which has allowed its export (Arellano *et al.*, 2017).

Melon is a fruit characterized by providing a significant number of benefits for both health and the economy, which is why it is one of the most consumed worldwide. Melon pulp is rich in water, proteins, lipids, vitamin C source and beta-carotenes (Maietti, 2012). It is a source of antioxidants and bioactive polyphenols, which provide important health benefits, particularly for the cardiovascular system (López *et al.*, 2007). In addition, it is slightly diuretic, as it removes toxins from the body (Bayer, 2018).

The use and application of metal nanoparticles in agriculture has been successful, especially in crops that are produced in dry climates, since they stimulate their growth, increase the yield and commercial quality of their fruits (Prasad *et al.*, 2014). They are also reported to induce oxidative stress in plants (Chandra *et al.*, 2020) and have an influence on photosynthetic and antioxidant activity (Kumar *et al.*, 2020). In general, the effects of nanomaterials on plants vary greatly not only because of the nature of the materials but also depending on the species studied (Wang *et al.*, 2015). Zinc is an essential micronutrient for plants and humans, worldwide more than 30% of the population is deficient in this element (Amarakoon *et al.*, 2012), negatively affecting the cognitive, brain and reproductive development of people (Salgueiro *et al.*, 2004).

It has a direct effect on growth, neurological, behavioral development and the immune system (López de Romaña *et al.*, 2010), in addition, it promotes the intellectual development of children, accelerates the growth of adolescents, affects the palate, appetite and male fertility (Yin *et al.*, 2012). Strategies have been proposed to mitigate this problem, one of them has been biofortification, where it is possible to obtain fruits enriched with minerals (Restrepo *et al.*, 2020). Reports have shown that it is possible to obtain biofortified fruits with minerals, increasing their concentration in the edible portions of plants (Neeraja *et al.*, 2017).

In the case of zinc in plants, this is a micronutrient involved in protein synthesis, is a stabilizing agent for membranes of wide propagation and cell elongation (Mousavi *et al.*, 2014), maintains protection against environmental stress (Sturikova *et al.*, 2018), and helps adaptability to harsh environmental conditions (Cakmak, 2017). The application of zinc oxide nanoparticles (NPs ZnO) has been shown to have positive effects on nutritional and physiological parameters in some crops (Rizwan *et al.*, 2018), in addition to increasing their content in the edible part of the plant (Subbaiah *et al.*, 2016), these changes can induce stress and toxicity in plants and stimulate antioxidant systems (García-Gómez *et al.*, 2017).

Application of NPs ZnO via foliar minimize the use of fertilizers, increase efficiency and greater availability of nutrients in plants (Nandhini *et al.*, 2019), in addition the effects on plant growth and development are greater compared to traditional forms of Zn (Kolčencík *et al.*, 2019). However, the information of the effects of its application in this way is still scarce, so the objective of this research was to determine the effect of foliar spray of zinc nanoparticles on the production, nutraceutical quality and their concentration of this micronutrient in fruits of melons developed under open field conditions.

Materials and methods

Study site

The experiment was carried out on agricultural land in the Rosas ejido, Municipality of Tlahualilo, Durango, Mexico, it is located at 1 095 masl. It is located at the coordinates 26° 06' 38'' north latitude and 103° 26' 17'' west longitude. According to the classification of Köppen modified by García (2004): dry desert or warm steppe climate with rains in the summer and cool winters. The rainfall is 258 mm, and the average annual temperature is 22.1 °C, with ranges of 38.5 as the maximum average and 16.1 as the minimum average. The average annual evaporation is approximately 2 396 mm.

Zinc oxide nanoparticles

The nanoparticles used were zinc oxide (NPs ZnO), with a size between 20 and 60 nm, a purity of 97%, white and structurally hemispherical and polygonal (Figure 1). NPs ZnO were synthesized through controlled precipitation as reported by Ramírez Barrón *et al.* (2019), using the chemical hydrolysis method. Twenty-four liters of ethanol and 530.4 g of zinc acetate $(\text{CH}_3\text{CO}_2)_2\text{Zn}$ were poured into a 200 L reactor with heating by a boiler at a temperature of 80 °C, using a temperature controller. The reaction remains in agitation for 3 h.

Subsequently, a solution of 0.22 M of NaOH is added to the reactor. It is left in agitation for 1 h to achieve the precipitation of NPs. The precipitate obtained was washed several times with distilled water for subsequent characterization by transmission electron microscopy (TEM) to determine the particle size of the material obtained.

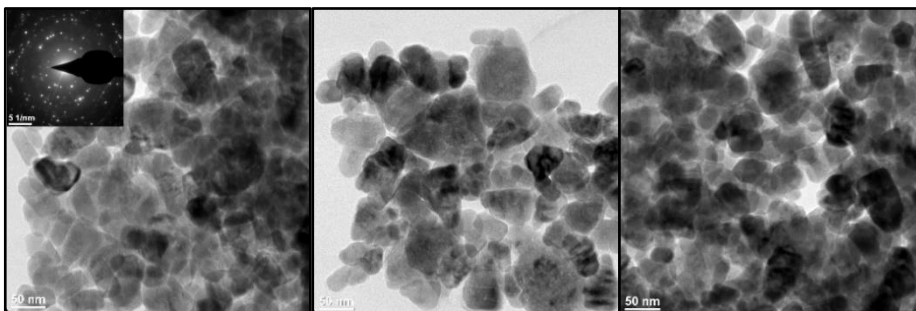


Figure 1. Structural form of zinc oxide nanoparticles (NPs ZnO).

Plant material and cultivation

The vegetative material used was hybrid melon (*Cucumis melo* L.) cv crusier type cantaloupe (Harris Moran Seed Company[®]). Direct sowing took place on March 20, 2019. Double-row ridges were built forming a microplot (melon bed) at 4 m between ridges and a separation between plants of 30 cm for a density of 16 665 plants per hectare. According to the technological package for this crop (Cano *et al.*, 1992), soil preparation was carried out which consisted of plowing at a depth of 30 cm, double harrowing, followed by leveling, construction of melon beds and ridges for conduction and retention of irrigation water.

Fertilization was mechanical using the chemical formula 120-60-00 (N-P₂O₅-K₂O); all the phosphorus and half of the nitrogen were incorporated at the time of planting and the rest of the nitrogen at flowering. The fertilizers were monoammonium phosphate (NH₄H₂PO₄) and ammonium sulfate [(NH₄)₂SO₄]. The irrigation was with water from the Francisco Zarco dam, a pre-sowing irrigation of 30 cm was applied, during the cultivation cycle six auxiliary irrigations were given with water levels of 15 cm each, in total a water level of 120 cm was applied.

Treatments and experimental design

The experimental design was random blocks considering eight treatments with four repetitions, forming 32 experimental units, each of them consisting of an area of 4 m wide by 10 m long (40 m²). The concentrations of NPs ZnO applied were as follows: T1= control, T2= 50 mg L⁻¹; T3= 100 mg L⁻¹; T4= 150 mg L⁻¹; T5= 200 mg L⁻¹; T6= 250 mg L⁻¹; T7= Z 40 (1 L ha⁻¹) and T8= GZ (1 ml L⁻¹ of water). Treatments T7 and T8 consisted of the application of commercial foliar fertilizers, zinc 40 (Z40: concentration 40%) Tec-Fort[®] and Gro-zinc (GZ: concentration 9.66%) Grodeco[®], both chelated fertilizers.

The nanoparticles were applied with a 20 L Lola manual sprinkler (Swissmex[®]). Each concentration of NPs ZnO was dissolved in eight liters of deionized water (Quimicrón[®]) and for better adhesion, a non-ionic acidifier - adherent (AF-optimus[®]) was added at a dose of 1 - 2 ml L⁻¹ of spray water. Three foliar applications of NPs ZnO and conventional fertilizers were made, the first at 20 days after sowing, when the crop presented expansion of the foliage, then every 15 days, on average 40 ml per plant were applied. The applications were made in the morning, without the presence of wind and they wet the entire plant (Trinidad and Aguilar, 1999).

Variables evaluated

Weight, soluble solids and fruit firmness

For the analysis of all the variables, eight plants per treatment were considered, two for each repetition and the harvested fruits considered were those that presented commercial maturity; that is, firm-mature state or 3/4 detached, between 1.2-1.5 kg and without physical damage (Beaulieu and Jeanne, 2007). For the fruit weight (PF), all the fruits harvested were weighed on a digital scale (Adir[®], USA) with a capacity of 5 000 g. The total soluble solids (SST) were measured with a Master T 0-53 brix AT-Master53T optical Refractometer (Twiligh[®], USA), for this the fruits were split, and a drop of juice was placed in the glass and the reading was taken. The results were

expressed as °Brix. The firmness of the fruit (FF) was measured in two melons of each repetition with a Penetrometer of the Exttech® Instruments brand model FHT200 (USA), the tip or plunger of 8 mm was used. The exocarp (peel) was removed and placed on a flat surface, four penetrations were made per fruit (Azam *et al.*, 2015) and the results were averaged, recording as maximum compression force (N).

Yield

For this variable, the weight of all fruits harvested per experimental unit, the planting density were considered and extrapolated for one hectare, the results were reported in t ha⁻¹.

Total flavonoids (FLVT)

Total flavonoid content was determined by the colorimetric method (Zhishen *et al.*, 1999) and the results were expressed in mg quercetin equivalents per 100 g⁻¹, based on fresh weight (mg equiv. Q 100 g⁻¹ FW).

Total phenolic compounds

Total phenolic content was measured using the Folin-Ciocalteu method (Singleton *et al.*, 1999). The determinations were made in triplicate and were reported in mg of gallic acid equivalent per 100 g of fresh weight (mg equiv GA 100 g⁻¹ FW).

Antioxidant activity

Antioxidant capacity was determined using the DPPH method (1,1-diphenyl-2-picrylhydrazyl), developed by Brand-Williams *et al.* (1995). Analyses were performed in triplicate and reported in µM Trolox equivalent per 100 g fresh weight (µM equiv Trolox 100 g⁻¹ FW).

Vitamin C

Ascorbic acid was determined by the technique of Doner and Hicks (1981). The readings were performed on a Varian ProStar 320 HPLC equipped with a UV-Vis detector (ProStar 210, Varian Prostar Inc., Greek Walnut, CA, USA), using a 10 cm Varian amine column and a 20 mL injection loop, the units are reported in mg per 100 grams of fresh weight (mg 100 g⁻¹ FW).

Zinc content in fruit

The zinc content in the peel, pulp and seeds of six melon fruits per treatment was determined. The method used was by atomic absorption spectrophotometry (AOAC, 1995), using equipment of the Perkin Elmer brand® model 3110. Results were reported in mg kg⁻¹.

Statistical analysis

The data of the variables were analyzed by analysis of variance and means separations test using the Tukey test ($p \leq 0.05$) with the Statistical Analysis System Institute (SAS) version 9.1 statistical package.

Results and discussion

Quality parameters of melon fruits

The foliar applications of NPs ZnO had favorable effects on melon fruits, the concentration of 200 mg L⁻¹ was the one that promoted greater fruit weight, yield, soluble solids and firmness, exceeding by 32% the control treatment and with respect to the commercial sources of Zn (Z-40 and GZ), it exceeded them in 24% and 20%, respectively. On the other hand, the concentration of 250 mg L⁻¹ decreased the weight of the fruit by 11% (Table 1). Regarding yield, the treatment of 200 mg L⁻¹ reported the highest yield with 53 t ha⁻¹, the treatment with the commercial source of zinc (GZ) 43.95 t ha⁻¹ and the control 40 t ha⁻¹.

Table 1. Comparison of parameters of commercial quality of cantaloupe melon fruits by effect of foliar application of NPs ZnO at different concentrations.

NPs ZnO (mg L ⁻¹)	Fruit weight (kg)	Yield (t ha ⁻¹)	Soluble solids (°Brix)	Firmness (Newton)
Control	1.33 ±0.27 d*	40.09 ±8.35 d	11.26 ±0.38 b	17.75 ±1.25 b
50	1.49 ±0.11 bdac	44.95 ±3.64 bdac	11.37 ±0.22 b	21.25 ±3.59 ba
100	1.7 ±0.09 ba	51.63 ±2.93 ba	11.85 ±0.75 b	22.25 ±3.59 ba
150	1.61 ±0.23 bac	48.93 ±7.13 bac	11.76 ±1.05 b	23 ±5.83 a
200	1.74 ±0.18 a	52.96 ±5.59 a	12.73 ±0.31 a	24.5 ±6.45 a
250	1.64 ±0.16 bac	49.73 ±5.03 bac	11.94 ±0.48 b	20.5 ±5.68 ba
Z40	1.4 ±0.17 dc	42.53 ±5.36 dc	11.65 ±0.21 b	21.75 ±2.21 ba
GZ	1.45 ±0.17 bdc	43.95 ±5.39 bdc	11.6 ±0.13 b	21.75 ±3.77 ba

*= averages with different letters, within the same column, indicate significant difference (Tukey; $p \leq 0.05$). Where: LSD= minimum significant difference.

The average regional yields reported for this crop are 45.2 t ha⁻¹ and with a fruit weight of 1.7 kg (García-Mendoza *et al.*, 2019), in both cases the applications of the nanoparticles exceeded these values. Results report for *Vigna mungo* (L.), with doses of 200 ppm of NPs ZnO, the best results for seed germination and plant growth (Rajitha *et al.*, 2020). In lettuce, they report a better response when applying 20 ppm (NPs Ag) and when applying the double, the fresh weight of the leaves decreased (Jurkow *et al.*, 2020).

In plants of *Capsicum chinense*, foliar applications of 1 000 mg L⁻¹ had positive effects on growth and with 2 000 mg L⁻¹ there is a negative effect. Various studies point out that the positive effects of NPs ZnO can be attributed to the fact that zinc is indispensable for developing metabolic and catalytic activities in plant growth (Fageria *et al.*, 2016), regulates enzymatic activities and biochemical reactions that lead to the formation of chlorophyll and carbohydrates (Gawrońska *et al.*, 2018). However, the effect of nanoparticles varies depending on their properties such as their size, chemicals, structure, surface coating, speed and dose of application (Khodakovskaya *et al.*, 2012).

With respect to conventional sources of zinc, it is noted that foliar nanofertilizers may be more effective than these because their release can be slow and gradual (DeRosa *et al.*, 2010). Regarding the decrease in weight and yield of melon fruits, an ambivalent effect of nanoparticles has been

reported (Méndez-Arguello *et al.*, 2016). For example, in *Lycopersicon esculentum*, growth parameters decreased when 200 mg L⁻¹ of NPs ZnO were applied via foliar (Faisan and Hayat, 2019). The causes are diverse from a possible toxicity (Reed *et al.*, 2011; Jurkow *et al.*, 2020) or due to factors linked to the shape, size, seasonal period and number of applications of Nanoparticles (Kolenčik *et al.*, 2019).

Regarding soluble solids and firmness, the values reported for this vegetable in the region are between 6.5 to 11 °Brix and firmness of 38 N (Moreno-Reséndez *et al.*, 2014). The treatment of 200 mg L⁻¹ exceeded the regional value but was inferior in terms of firmness. According to international standards melons with sugar content of 12-14 °Brix are suitable for the international market and values of 9 °Brix are optimal for the optimal internal quality of melons (Bower *et al.*, 2002).

Bioactive compounds in fruits

The results indicate that the highest flavonoid content was obtained with the concentrations of 50 and 100 mg L⁻¹, exceeding the control treatment by 13.5% and the two sources of commercial Zinc by 10% (Z40) and 12% (GZ). It should be noted that as the concentration of nanoparticles increased, flavonoids decreased by 8%. The same behavior occurred for phenols, antioxidant capacity (DPPH) and vitamin C, decreasing by 17%, 10.8% and 9%, respectively (Table 2).

Table 2. Bioactive compounds present in melon fruits by foliar application of NPs ZnO.

NPs ZnO (mg L ⁻¹)	Flavonoids (mg QE 100 g ⁻¹ FW)	Phenols (mg gallic acid 100 g ⁻¹ FW)	DPPH (mg equiv Trolox 100 g ⁻¹ FW)	Vitamin C (mg 100 g ⁻¹ FW)
Control	122.65 ±0.22 e*	145.07 ±0.36 g	63.86 ±0.29 h	50.51 ±0.59 e
50	141.85 ±0.55 a	231.36 ±1.32 a	88.14 ±0.22 a	61.36 ±1.04 a
100	140.18 ±3.53 a	222.43 ±0.97 b	81.79 ±0.77 c	59.13 ±0.83 b
150	135.22 ±0.45 b	211.8 ±1.1 c	85.29 ±0.19 b	56.87 ±0.31 c
200	133.21 ±0.14 b	199.9 ±3.27 d	78.62 ±0.22 d	53.53 ±0.99 d
250	130.11 ±0.77 c	192.04 ±1.68 e	75.32 ±0.11 e	54.17 ±1 d
Z40	127.71 ±0.17 d	178.44 ±6.54 f	72.02 ±0.11 f	53.81 ±0.5 d
GZ	124.41 ±0.72 e	150.98 ±8.39 g	68.78 ±0.7 g	55.9 ±0.43 c

* = averages with different letters, within the same column, indicate significant difference (Tukey; $p \leq 0.05$). Where: PF= fresh weight; DPPH= 1,1-Diphenyl-2-Picrylhydrazil.

The applications of commercial zinc sources exceeded the control treatment for flavonoids by 4%, phenols by 18%, antioxidant capacity by 11.3% and vitamin C by 9.6%; however, these percentages were lower than those obtained by NPs ZnO. The quantity of flavonoids present in melon depends on the genotype, ripening of the fruit and the date of harvest (Tadmor *et al.*, 2010). They have various functions, it acts as an excellent antioxidant agent, it has antimicrobial compounds, UV protectors, insect protectors (Harborne and Williams, 2000).

Values of 106.18 mg 100 g⁻¹ (Morais *et al.*, 2015) up to 168 mg 100 g⁻¹ (Ismail *et al.*, 2010) have been reported, in our study, the average values were 141.85 with 50 mg L⁻¹ of NPs ZnO. These results may be associated with the foliar application of NPs ZnO has a greater transport potential, greater bioavailability and absorption that allows them to interact with intracellular structures that stimulate the formation of ROS (Ghosh *et al.*, 2016; García-López *et al.*, 2019).

Phenolic compounds also play a very important role in this crop, as they protect the fruit from insects and microorganisms, and determine the color and appearance of the fruits (Jeong *et al.*, 2004; Mallek-Ayadi *et al.*, 2019). Values of 168 mg 100 g⁻¹ have been reported in cantaloupe melon pulp (Ismail *et al.*, 2010). The phenolic content is associated with its antioxidant activities due to its redox properties that allows it to act as a reducing agent and oxygen donor (Chang *et al.*, 2001). In this sense, the results clearly indicate that the application of NP ZnO induces a higher content of antioxidant compounds (phenols, flavonoids and vitamin C) in melon fruits.

The content of vitamin C in melon fruits (*Cucumis melo* L.) showed a decrease when increasing the concentration of NPs ZnO between treatments. The highest value of vitamin C was found in fruits of sprinkled plants with 50 mg L⁻¹ of NPs ZnO, this concentration was 21.48% higher compared to the control treatment. Doses of 100, 150, 200 and 250 mg L⁻¹ of NPs ZnO showed increases of 17.06, 12.59, 5.97, 7.24%, respectively. While the commercial sources of zinc-40 (6.53%) and Gro-zinc (10.67%) compared to control treatment.

Results obtained by López-Vargas *et al.* (2018) showed that applications of 250 mg L⁻¹ of NPs of Cu significantly increased the concentration of vitamin C, which increased the nutraceutical quality of tomato fruits. Mathpal *et al.* (2015) state that zinc is involved in enzyme activation, protein biosynthesis and carbohydrates. Padayatty *et al.* (2003) mention that vitamin C is an antioxidant whose effects reduce human diseases such as atherosclerosis and cancer, which occur by oxidative damage to tissues, diets abundant in fruits and vegetables decrease the risk of cardiovascular disease, stroke and cancer.

Zinc content in fruits

Foliar applications of NPs ZnO influenced the zinc content present in the peel, pulp and seeds of melon fruits. In the control treatment, the amount of zinc was 52.68 mg kg⁻¹ the largest amount of zinc is present in the pulp (76%), then in the seed (21%) and a small amount in the peel (2.5%). On the other hand, the treatment of 200 mg L⁻¹ presented 67.43 mg kg, with values of 78%, 19% and 2.5%, respectively. While in fruits with commercial fertilization (GZ), the amount was 57.6 mg kg, with values of 77% (pulp), 20% (seed) and 2.5% (peel) (Figure 2).

The treatment of 200 mg L⁻¹ NPs ZnO was the one that presented the highest zinc content in pulp (52.96 mg kg⁻¹), surpassing the control treatment by 24% and GZ by 17%. However, as a higher concentration was applied, zinc pulp values decreased by 6%. Other studies have reported zinc content of 22 mg kg⁻¹ dry weight in melon pulp (López-Zaplana *et al.*, 2020).

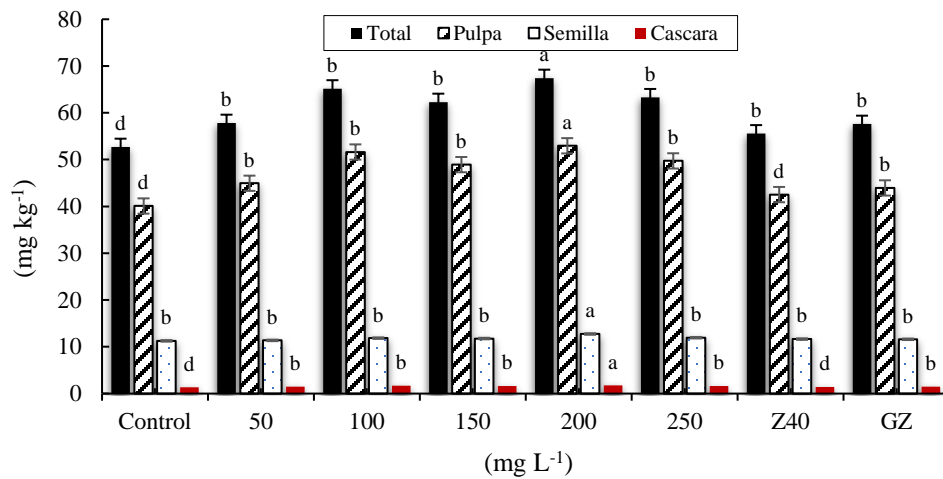


Figure 2. Zinc content in peel, pulp and seeds of melon fruits with foliar applications of zinc nanoparticles and conventional commercial fertilizers. Averages with different letters indicate significant difference according to Tukey's test ($p \leq 0.05$).

On the other hand, Wang *et al.* (2019) found that applying NPs Cu increased the copper content in wheat grains between 18.86% and 30.45% compared to the control and with a dose of 500 mg kg⁻¹ of NPs TiO. Rizwan *et al.* (2018) observed that when applying Nanoparticles of Zn and Fe there was an increase in the concentrations of both elements in *Triticum aestivum*. The Institute of Medicine (2002) reports a requirement of 11 mg day⁻¹ of zinc in adolescents from 14 to 18 years and 9.4 mg for people over 19 years, it is not recommended to ingest doses greater than 25 mg, as it can cause anemia and copper deficiency (Lenntech, 2021).

In this sense, the use of NPs with zinc is an effective way to enrich crops, since moving Zn through plant tissues will cause an accumulation of this micronutrient, which could help solve Zn deficiency in the human diet (Cakman and Kutman, 2018).

Conclusions

The foliar application of NPs ZnO improves the yield and commercial and nutraceutical quality of melon fruits. The yield and quality of the melon crop depend on the concentration used of NPs ZnO, since high doses favor the yield and a higher concentration of Zn in pulp; on the other hand, with the application of intermediate doses of NPs ZnO, a greater accumulation of bioactive compounds is favored. Foliar spray of NPs ZnO is a practical way to enrich melon fruits and mitigate the deficiencies of this micronutrient in the population.

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