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Research Article

Titanium dioxide nanoparticles (TiO_2-NPs) effect on germination and morphological parameters in alfalfa, tomato, and pepper

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Abstract

Titanium dioxide nanoparticles are used in different processes, derived from this their presence in wastewater is common, concentrating in residual sludge. These residues are used as agricultural soil improvers, being a source of crop exposure. In this study, the effect of TiO_2-NPs (450, 900, and 1800 mg L^{-1}) on the germination of alfalfa, tomato, and pepper seeds was evaluated. The germination parameters were not different ($p > 0.05$) except for, the seed vigor index in alfalfa and pepper, and the mean germination time in tomato. The germination index was below 80% in 450 mg L^{-1} in tomato, which showed moderate phytotoxicity. Morphological modifications with differences ($p < 0.05$) were found in the three crops, mainly in the root. In tomatoes, the length of the main root, root hairs, and the width of the root tip were reduced but increased the width of the main root, piliferous zone, and the length of root hairs were. For alfalfa, root length and number of secondary roots augment. However, the stem, root tip width, and root villi were reduced. Finally, for pepper, the length, and width of the root and the piliferous zone were modified. Additionally, a concerning trend has been observed in the length of root hairs. TiO_2-NPs affected germination and morphology in alfalfa, tomato and pepper seeds differently. Tomato was most negatively affected, with reduced root length and width. Alfalfa showed mixed effects, with positive impacts on some parameters but negatives on others. Pepper seeds responded positively overall, with improved germination and root length, despite some impacts on root morphology.

Keywords: germinative parameters; morphological parameters; nanomaterials; seeds; titanium

Abbreviations: Ag-NPs – silver nanoparticles; GI – germination index; GP – germination percentage; GR – germination rate; MGT – mean germination time; SVI – seed vigor index; TiO_2-NPs – titanium dioxide nanoparticles; ZnO-NPs – nanoparticles zinc oxide.

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Introduction

Titanium dioxide (TiO_2) is an oxidized form of this transition metal that is found naturally in the environment, originating from minerals such as anatase, rutile, or brookite (Cox *et al*., 2017). The synthesis of titanium dioxide nanoparticles (TiO_2-NPs) has been of great interest since they present improved properties and are different from those of macro-sized particles; nanoparticles are obtained in the order of nanometers (nm) (10^{-9} m) (Mosquera *et al*., 2015). TiO_2-NPs are considered the most widely used engineered nanomaterials at an industrial level (Kamali *et al*., 2021). It is estimated that world production by 2025 will be 2,500,000 tons (Robichaud *et al*., 2009). TiO_2-NPs have become the most produced nanomaterials, with an annual production capacity of 10,000 metric tons (Szymańska *et al*., 2016).

TiO_2-NPs present photocatalytic activity, high stability, and anticorrosive properties that give them a wide range of applications, they can be found in cosmetics, food, paints, and water treatment (Bakshi *et al*., 2019). It is reported that they are one of the most discarded nanomaterials into the environment (Zuverza-Mena *et al*., 2017). Therefore, its presence in wastewater bodies is frequent, which results in its agglomeration and concentration in sewage sludge (Kim *et al*., 2012).

They have recently been used in agriculture, to treat seeds, increase production (Maity *et al*., 2016), and protect crops under water stress (Jaberzadeh *et al*., 2013), thus revolutionizing conventional practices of agriculture (Chhipa, 2017). Seed treatment with TiO_2-NPs has generated higher dry matter production, increased photosynthetic rate, and generation of type a chlorophyll. Another benefit they offer is the elevation of the availability of nutrients, significantly increasing the germination and development of the plant (Qureshi *et al*., 2018).

The mechanisms of action of nanoparticles are based on their chemical composition, dimension of 0-100 nm, coverage surface, and reaction abilities (Chaudhary and Singh, 2020), as well as the efficient penetration into the cell interior, in addition to their rapid distribution once entered into organisms (Khodakovskaya *et al*., 2012). It has been found that nanoparticles smaller than 50 nm can cross the epidermis of the root and through a series of biochemical reactions reach the xylem and translocate to the stem, fruits, and flowers, also having foliar interaction through the stomata or cuticle (Rodríguez-González *et al*., 2019). This interaction capacity of TiO_2-NPs at the cellular level has also generated genotoxic and inhibitory effects on germination, changes in the length of the radicle, and an increase in reactive oxygen species (Rafique *et al*., 2018). Studies carried out in Nicotiana tabacum showed that exposure to TiO_2-NPs caused negative effects on root elongation (Frazier *et al*., 2014); in Fabaceae, a decrease in the number of secondary roots was observed and there was a delay in the formation of nodules (Fan *et al*., 2014); in maize seeds, contact with TiO_2-NPs caused germination inhibition after 24 h (Ruffini *et al*., 2011); in *Lactuca sativa* a decrease in root elongation was observed with 5000 $mg.L^{-1}$ of TiO2-NPs, while in concentrations of 100 to 2500 $mg.L^{-1}$ an increase was observed in this area (Song *et al*., 2013).

Currently, the incorporation of sewage sludge into agricultural soils has increased the concentration of TiO_2-NPs and other nanomaterials in this resource (Castillo-Michel *et al*., 2017), generating greater contact and exposure of plant species in different proportions (Tan *et al*., 2018). Previously, we have demonstrated the presence of TiO_2-NPs in the sludge from waste water treatment plants from Chihuahua, Mexico, waste that is used as an agricultural amendment (Reyes- Herrera et al., 2022).

The species studied in this research, alfalfa (*Medicago sativa* L. var 'Cuf 101'), saladette tomato (*Solanum lycopercicum* L. var 'Rio Grande') and jalapeño pepper (*Capsicum annuum* var 'Grande'), are of great importance for agriculture in the region; the first is used in feeding dairy cattle in the United States of America and Mexico (Rojas *et al*., 2016); the second and third are popular vegetables and accepted throughout the world (Hernández *et al*., 2013). Therefore, exposure and contact with TiO_2-NPs are unavoidable, and knowing the positive and negative effects on germination and/or possible morphological changes in seedlings will help identify the benefits or harms that TiO_2-NPs cause. In the case of alfalfa where there is little information, in tomato saladette where evidence of nanoparticles has been found in cortical cells of the root (Tiwari *et al.*, 2017), and in jalapeño pepper in which structural damage has been reported on the surface of this tissue (Smith *et al.*, 2015). This will allow in the future, to propose solutions to ensure production and food safety of consumers.

Materials and Methods

Site of study

The study was carried out in Chihuahua, Mexico, at the Autonomous University of Chihuahua, Faculty of Agrotechnological Sciences, from January to April 2021.

Material description

The alfalfa (*Medicago sativa* L. var 'Cuf 101') and saladette tomato (*Solanum Lycopersicum* L. var 'Rio Grande') seeds used in the experiments were the KristenSeed brand. In contrast, jalapeño pepper (*Capsicum annuum* var 'Grande') was produced in Southern Star Seeds S. de R.L. of C.V. from Mexico City.

The titanium dioxide (TiO_2) nanoparticle material was Aeroxide TiO_2 P25 from Evonik; characterized by having a purity > 99.5%, and it is a combination of the anatase and rutile crystalline phases from the United States; this reagent was provided by the Advanced Materials Research Center S.C. from the city of Chihuahua, Chih. Mexico.

Experimental design

The established experimental design was completely randomized, and three treatments of TiO_2-NPs in suspension and control with distilled water were considered, with five replicates for each (4 × 5). The concentrations of TiO_2-NPs were 450 mg L^{-1} (treatment 1), 900 mg L^{-1} (treatment 2), and 1,800 mg L^{-1} (treatment 3), estimated considering the concentrations of titanium found in amended agricultural soil with sludge from the northern wastewater treatment plant of the city of Chihuahua (Reyes-Herrera *et al.*, 2022).

Five replicates were considered for each germinative parameter and biomass calculation. Meanwhile, fifteen observations were taken into account for the morphological parameters.

Seed management

The seeds of each cultivar were disinfected with 10% sodium hypochlorite for 5 min, rinsed with distilled water three times, and allowed to dry.

In each treatment, 20 seeds per replicate (100 seeds per treatment) were used, including the control. These seeds were placed on grade 41 sterile filter paper of approximately 8.2 cm in diameter. Inside each 9 × 1.5 cm Petri dish on the filter paper, 3 mL of the solutions prepared with TiO_2-NPs were added, as described by Rodríguez *et al.* (2014). The boxes were sealed with paper similar to Parafilm to avoid desiccation and artificial light was provided. Photoperiod was 16-8 h (light/dark), 22-28 °C (day/night) monitored with a Fisher Scientific brand mercury thermometer.

Calculation of germination parameters

The number of germinated seeds was recorded daily, the seed was considered germinated when the radicle was visible. For the calculation of the germination percentage (GP), the germination rate (GR), the mean germination time (MGT), the seed vigor index (SVI), and the germination index percentage (GI) formulas reported in the literature (Table 1).

Table 1. Formulas and definitions used for the calculation of seed germination parameters

Index	Definition	Calculation formula	Used references
Germination percent	Difference between the total number of germinated seeds and the number of seeds tested.	$GP=100 \times \frac{GN}{SN}$ (1)	(Gao-Lin *et al.*, 2009 and Wang *et al.*, 2009)
Germination rate	Number of seeds per day	$GR = \sum \frac{Gi}{I}$ (2)	(Bu *et al.*, 2007, Mahmoodzadeh *et al.*, 2013 and Figueroa and Armesto, 2001)
Mean germination time	The number of days was calculated based on the sum of germinated seeds per day from the start to the end of the experiment.	$MGT = \frac{\sum_i Gi \times i}{\sum_i Gi}$ (3)	
Seed vigor index	Evaluation of the length of the radicle and the stem of the germinated seeds.	$SVI = GP \times (Lr + Lt)$ (4)	(Hernándz-Valencia *et al.*, 2017)
Germination index	The relationship between the number of seeds germinated under experimental conditions compared to the control.	$GI = \frac{(PGR)(CRR)}{100}$ (5) Which are calculated as follows: $PGR = \frac{number\ of\ seeds\ in\ the\ sample}{number\ of\ seeds\ in\ the\ control} * 100$ $CRR = \frac{Sample\ root\ elongation}{control\ root\ elongation} * 100$	(Huerta *et al.*, 2015)

GN: Total germinated seeds; SN: number of seeds during the test; Gi: Total germinated seeds; I: Number of days since the experiment has begun; i: Number of days since the seeds were sown (day 0); Gi: Number of germinated seeds on a day i (note that only germinated seeds were included in the calculation); Lr: Root length; Lt: Stem length; PGR: Relative germination percentage and CRR: Relative radicle growth.

Calculation of morphological parameters

After seven days of germination of each culture, three seedlings were randomly chosen per Petri dish. The morphology was evaluated through the length of the primary root and stem, the width of the main root, the width and length of the cap, the width of the root and vascular cylinder in the elongation zone, the number of secondary roots, the width of the piliferous zone and length of root hairs. Likewise, the dry biomass of 5 seedlings was determined for each treatment, including the control. Figures 1 show the sites where the measurements were made for the morphological determinations.

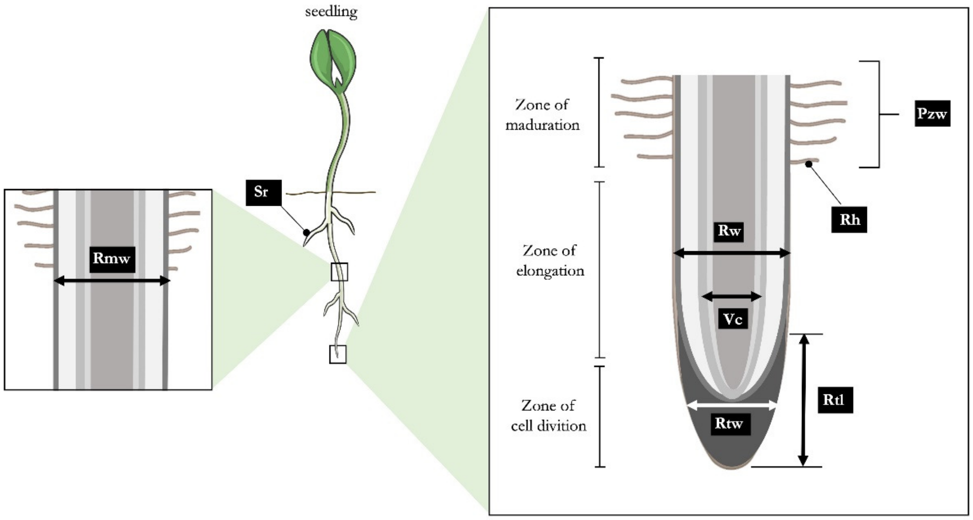

Figure 1. Scheme of measurements taken on the roots of seedlings treated with TiO2-NPs *in vitro*
Rmw= Main root width, Sr= secondary roots, Pzw= Piliferous zone width, Rh= root hairs, Rw= Root width in the elongation zone, Vc= vascular cylinder in the elongation zone, Rtl= root tip length and Rtw= root tip width.

Images of seedlings were taken with the LEICA DM1000 microscope from the United States of America in the Top View. Ink program. These images were subsequently analyzed with the ImageJ 1.52v program, where measurements of the tissues in mm were obtained. The microscope was calibrated with a 0.01 mm millimeter slide, and a 4x objective was used. Photographs of the control and treatments were taken to obtain visual differentiation (Kamali et al., 2021). The measurement of the length of the radicle was conducted using a measuring tape, as it was visible in comparison to other morphological measurements obtained through images captured with the microscope.

Statistical analysis

Statistical tests of analysis of variance (ANOVA) and Tukey were performed with the data obtained to assess whether or not there were significant differences ($p \leq 0.05$) between treatments. In addition, for the evaluation of the length of root hairs, it was necessary to perform a quadratic regression. The data was processed in Excel version 2016 and IBM SPSS Statistics 25, 2017.

Results and Discussion

The germinative parameters that showed significant differences ($p < 0.05$) between the control and the treatments with the TiO_2-NPs were in tomato, the mean germination time (MGT), and in alfalfa and pepper seedlings, the seed vigor index (SVI). On the other hand, for the three crops the percentage (GP) and the germination rate (GR) were without significant differences ($p > 0.05$) (Table 2).

Table 2. Germination parameters of three different crops treated with TiO_2-NPs

Parameter	Alfalfa					Tomato					Pepper				
	TiO_2-NPs concentration (mg L^{-1})					TiO_2-NPs concentration (mg L^{-1})					TiO_2-NPs concentration (mg L^{-1})				
	0	450	900	1800	P	0	450	900	1800	p	0	450	900	1800	p
GP (%)[1]	82 ± 0.15	84 ± 0.05	87 ± 0.07	89 ± 0.05	ns	71 ± 0.07	80 ± 0.11	80 ± 0.15	85 ± 0.08	ns	91 ± 2.24	91 ± 2.24	91 ± 2.24	88 ± 4.47	ns
GR (seeds/ days)[1]	11.17 ± 6.03	7.87 ± 5.12	12.40 ± 5.59	11.67 ± 5.00	ns	9.70 ± 3.11	9.23 ± 4.68	5.05 ± 1.053	12 ± 5.16	ns	3.66 ± 0.83	3.52 ± 0.30	4.58 ± 1.50	3.59 ± 0.61	ns
MGT (days)[1]	1.07 ± 0.07	1.12 ± 0.07	1.04 ± 0.03	1.05 ± 0.06	ns	4.04 ± 0.03 a	4.20 ± 0.16 ab	4.37 ± 0.12 b	4.13 ± 0.18 ab	*	6.64 ± 0.23	6.83 ± 0.34	6.79 ± 0.37	6.47 ± 0.24	ns
SVI[2]	3.37 ± 0.79 a	4.42 ± 1.34 ab	4.89 ± 1.11 b	5.46 ± 0.98 b	*	6.84 ± 1.46	5.74 ± 1.37	6.30 ± 1.67	6.05 ± 1.97	ns	7.48 ± 1.20 a	8.66 ± 1.53 ab	10.06 ± 2.77 b	9.09 ± 1.81 ab	*

[1] Values represent the mean ± SD of 5 observations. [2] Values represent the mean ± SD of 15 observations

*Show significant differences $p < 0.05$; ns shows no significant differences

The values in the same row that present a different letter differ significantly in the Tukey test ($p < 0.05$)

In the morphological variables, the alfalfa seedlings presented significant differences ($p < 0.05$) between the control and the treatments with the TiO_2-NPs in the length of the stem and main root, root tip width, and the number of secondary roots, however, the width of the main root, length of the root tip, the width of the root and of the vascular cylinder in the elongation zone, the width of the piliferous zone and biomass in dry weight were not significantly different ($p > 0.05$) (Table 3).

Table 3. Morphological parameters and biomass of three different crops treated with TiO_2-NPs

Parameters	Alfalfa					Tomato					Pepper				
	TiO_2-NPs concentration (mg L^{-1})					TiO_2-NPs concentration (mg L^{-1})					TiO_2-NPs concentration (mg L^{-1})				
	0	450	900	1800	P	0	450	900	1800	p	0	450	900	1800	p
Stem length (cm)[1]	0.99 ± 0.27 b	0.70 ± 0.16 a	0.76 ± 0.20 a	0.70 ± 0.14 a	*	2.91 ± 0.41	2.59 ± 0.55	2.67 ± 0.70	2.55 ± 0.749	ns	1.49 ± 0.50	1.54 ± 0.43	1.80 ± 0.51	1.46 ± 0.39	ns
Main root length (cm)[1]	3.13 ± 0.97 a	4.57 ± 1.61 b	4.90 ± 1.37 b	5.46 ± 1.01 b	*	6.73 ± 1.76 b	4.54 ± 1.24 a	5.13 ± 1.36 a	4.49 ± 1.32 a	*	6.70 ± 1.01 a	8.02 ± 1.62 ab	9.23 ± 2.86 b	8.87 ± 1.99 b	*
Main root width (μm)[1]	2.19 ± 0.52	2.65 ± 0.99	2.16 ± 0.33	2.16 ± 0.27	ns	2.02 ± 0.160 a	2.00 ± 0.19 a	2.32 ± 0.17 b	1.96 ± 0.15 a	*	2.35 ± 0.26 b	2.20 ± 0.15 ab	2.13 ± 0.22 a	2.11 ± 0.17 a	*
Root tip length (μm)[1]	1.11 ± 0.23	1.01 ± 0.12	0.98 ± 0.27	1.09 ± 0.24	ns	0.65 ± 0.08 b	0.56 ± 0.15 ab	0.48 ± 0.12 a	0.54 ± 0.12 ab	*	0.79 ± 0.19	0.70 ± 0.12	0.75 ± 0.15	.076 ± 0.17	ns
Root tip width (μm)[1]	1.01 ± 0.17 b	0.90 ± 0.13 ab	0.84 ± 0.11 a	0.87 ± 0.06 a	*	0.69 ± 0.10 b	0.62 ± 0.09 ab	0.57 ± 0.08 a	0.55 ± 0.07 a	*	0.92 ± 0.15	0.91 ± 0.09	0.84 ± 0.13	0.87 ± 0.15	ns
Number of secondary roots[1]	1.46 ± 0.97 b	3.38 ± 1.19 a	3.08 ± 2.25 a	3.46 ± 1.45 a	*	3.87 ± 1.77	5.07 ± 1.71	5.27 ± 1.58	4.73 ± 1.47	ns	5.00 ± 2.62	4.47 ± 2.56	5.47 ± 2.97	3.40 ± 2.16	ns
Root width in elongation zone (μm)[1]	1.51 ± 0.26	1.40 ± 0.20	1.45 ± 0.41	1.27 ± 0.35	ns	1.22 ± 0.13	1.19 ± 0.21	1.17 ± 0.16	1.11 ± 0.30	ns	1.31 ± 0.21	1.22 ± 0.10	1.27 ± 0.14	1.29 ± 0.17	ns
Cylinder vascular width in the elongation zone (μm)[1]	0.98 ± 0.21	1.05 ± 0.17	1.03 ± 0.36	0.99 ± 0.15	ns	0.70 ± 0.11	0.67 ± 0.21	0.60 ± 0.28	0.71 ± 0.11	ns	0.79± 0.15	0.68 ± 0.11	0.74 ± 0.13	0.71 ± 0.13	ns
Roots hairs length (μm)[1]	1.48 ± 0.14	1.25 ± 0.19	0.99 ± 0.15	1.15 ± 0.17	na	1.52 ± 0.41	1.22 ± 0.18	1.57 ± 0.09	1.18 ± 0.17	na	1.44 ± 0.27	0.90 ± 0.14	0.77 ± 0.150	0.61 ± 0.10	na
Piliferous zone width (μm)[1]	2.14 ± 0.47	2.34 ± 0.60	2.13 ± 0.29	2.21 ± 0.35	ns	2.02 ± 0.19 ab	1.95 ± 0.17 a	2.17 ± 0.22 b	1.90± 0.16 a	*	2.32 ± 0.19 b	2.18 ± 0.14 ab	2.08 ± 0.18 a	2.10 ± 0.19 a	*
Biomass dry (g)[2]	0.01 ± 0.001	0.01 ± 0.002	0.01 ± 0.001	0.01 ± 0.003	ns	0.01 ± 0.001	0.01 ± 0.002	0.01 ± 0.001	0.01± 0.002	ns	0.02 ± 0.002	0.02 ± 0.004	0.02 ± 0.002	0.02 ± 0.004	ns

[1] Values represent the mean ± SD of 15 observations; [2] Values represent the mean ± SD of 5 observations;

*Show significant differences $p < 0.05$; ns: shows no significant differences

The values in the same row that present a different letter differ significantly in the Tukey test ($p < 0.05$)

na: not applicable, values calculated by quadratic regression.

Regarding the tomato seed, the length and width of the main root, the length and width of the root tip and the width of the piliferous zone were significantly different ($p < 0.05$); in contrast, the variables of stem length, number of secondary roots, root width, and vascular cylinder in the elongation zone and biomass in dry weight did not present significant differences ($p > 0.05$) (Table 3). For pepper, the variables where significant differences were found ($p < 0.05$) were the length and width of the main root and the width of the piliferous zone; on the other hand, in stem and root tip length, root tip width, number of secondary roots, root width, and vascular cylinder in the elongation zone and dry biomass, no differences were obtained ($p > 0.05$) (Table 3).

For the three species, trends in the length of root hairs were observed when comparing the treatments with TiO_2-NPs against the control (Figure 2). In alfalfa, the treatments decreased the length of the root hairs, specifically the 900 mg L^{-1} treatment; in tomato, a decrease was observed in the 450 and 1800 mg L^{-1} treatments, and a similarity between the control and the 900 mg L^{-1} treatment; and for pepper, the decrease in the length of the root hairs was proportional to the concentration of the TiO_2-NPs.

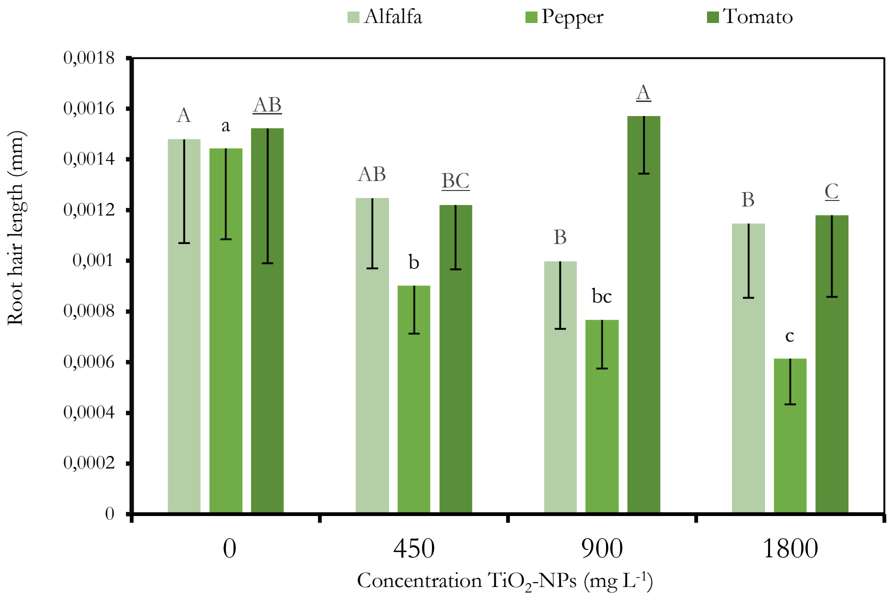

Figure 2. Root hairs length and TiO_2-NPs concentration in seedling emergence of alfalfa (A), tomato (B), and pepper (C)

Tukey test groups are separated in capital letters for alfalfa, lower case for pepper, and underlined capital letters for tomato.

In the alfalfa, tomato, and pepper seeds of the present study, the lowest germination percentages in the blocks treated with TiO_2-NPs were 84%, 80%, and 88%, respectively, and in the control, they were 82%, 71%, and 91%, however, there were no significant differences due to the addition of the TiO_2-NPs (Table 2). These results agree with those found in radish seeds (*Rafanus sativus* L. parvus) treated with 1, 10, 100, 200, 500 y 1000 mg L^{-1} of TiO_2-NPs Aeroxide P25 mixture of anatase-rutile structure, where a 93% germination percentage was obtained in treatments of 10, 200, 500 and 1000 mg L^{-1} while 100% in 1 mg L^{-1} and control without significant differences (Manesh *et al.*, 2018).

On the other hand, studies carried out with Mentha piperita showed negative effects of TiO_2-NPs on germination, obtaining total inhibition at the concentration of 300 mg L^{-1}, while in the treatments of 100 and 200 mg $L^{-1,}$ the percentage of germination was 10%, compared to the control of 30% (Samadi *et al.*, 2014). The negative effects in the germination stage suggest that the seeds are stressed by the presence of nanoparticles, not only TiO2 but also iron or carbon oxides, as in the study carried out on Cucumis sativus (Mushtaq, 2011).

The GR ranged from 7.87 to 12.40 germinated alfalfa seeds per day with an approximate MGT of 24 h after sowing, in the control and all treatments. In tomato, the GR was from 5.05 to 12 seeds/day, showing in this case significant differences in the MGT of the control (4.04 days) against treatments 1, 2, and 3 (4.20, 4.37 and 4.13 days, respectively). In pepper, the GR was from 3.52 to 4.58 seeds/day, but germination began from the fifth day, so the MGT was around 6 days in both the control and the treatments (Table 2).

The number of germinated seeds in this study differs from that obtained for lentil (*Lens culinaris*) seeds treated with TiO_2-NPs concentrations of 0, 100, 200, and 300 mg L^{-1}; reporting significant differences between the control of 14.75 seeds per day up to 23.90 in the 300 mg L^{-1} treatment. Also in this experiment, the MGT was different ($p < 0.05$) in the concentration of 200 mg L^{-1} obtaining 1.803 days against 1.065 and 0.9924 in the concentrations of 100 and 300 mg L^{-1} respectively (Feizi *et al.*, 2020).

However, the GR for alfalfa, tomato and pepper was similar to 6.4 seeds/day for fennel, which was placed in a Petri dish and irrigated with TiO_2-NPs solutions at concentrations of 5 to 80 mg L^{-1}; the authors of this research identified that low concentrations of nanoparticles improved germination speed (Feizi *et al.*, 2013); the opposite occurred with what was found with the species under study, where the concentration of TiO_2-NPs at 900 mg L^{-1} for alfalfa, pepper and 1800 mg L^{-1} for tomato increased the number of germinated seeds per day when compared to the treatment of 450 mg L^{-1} and with the control. According to the results of this study and what is found in the literature, GR and MGT will depend on the species investigated and the concentration of TiO_2-NPs, there is no clear pattern that strictly defines this behavior.

On the other hand, the SVI obtained was significantly different ($p < 0.05$) in alfalfa and pepper (Table 2); it was observed that treatments 2 (900 mg L^{-1}) and 3 (1800 mg L^{-1}) presented higher SVI than the control, this is because there is a direct relationship of the length of the root for said calculation, and in this pair of cultures the elongation of the tissue was increased by adding the TiO_2-NPs; this coincides with what was reported in experiments carried out with zinc oxide nanoparticles (ZnO-NPs) applied to corn seeds; the authors worked at concentrations of 10, 25, 50, 100 and 200 mg.L^{-1} of ZnO-NPs and found an increase in the vigor index of corn concerning the control in the treatment of 100 mg L^{-1}, presenting this concentration up to 0.7 cm more in root length concerning the other treatments (Itroutwar *et al.*, 2020). In tomatoes, this variable did not present differences.

For the analysis of the GI, the following criterion was considered: values less than 50% indicate high phytotoxicity of the treatment for the seeds, between 50% and 80% the phytotoxicity is moderate, and if the value is greater than 80% the treatment does not generate phytotoxicity (Huerta *et al.*, 2015). Therefore, it was determined that the TiO_2-NPs in alfalfa and pepper seeds did not generate phytotoxic effects when obtaining GI values greater than 80%. However, in tomatoes, treatment 1 (450 mg L^{-1}) presented moderate phytotoxicity (Figure 4) and, in general, the GI was much lower than in the other two plants. The results obtained from the seeds under study indicated that the toxicity of the nanoparticles will depend on the species and the concentration (Rizwan *et al.*, 2016). This coincides with what was published in an investigation carried out with seeds of desert plants *H. strobilaceum*, *H. aphyllum*, *N. choberi*, *Z. eurypterum,* and *H. glaucus*, where solutions with TiO_2-NPs of 0, 10, 100, 500 and 1500 mg L^{-1} were tested in a Petri dish, finding no effects on the germination of *H.strobilaceum* seeds. On the other hand, germination stimulation was observed at the concentration of 500 mg L^{-1} for *H. glaucus* and *H. aphyllum*, likewise, a phytotoxic effect of the concentration of 1500 mg L^{-1} in *H. aphyllum*, *N. Schober*, *Z.eurypterum,* and *H. glaucus* by significantly reducing the germination percentage compared to the control (Kamali *et al.*, 2021).

Then, the degree of toxicity of the TiO_2-NPs is related to their ability to remain in a colloidal state when interacting with matter, likewise, the possibility of forming agglomerates is a characteristic of the nanoparticles that controls and intervenes at the level of exposure (Bundschuh *et al.*, 2018). This agglomeration characteristic is appreciated in the same way in other nanoparticles such as silver nanoparticles (Ag-NPs), where it can be avoided by adding stabilizers (Pinheiro *et al.*, 2020), however in the present investigation the solutions of TiO_2-NPs were only shaken to continue in suspension.

Titanium is considered a strong acid with a high affinity for phosphate and ammonium; in this context, the presence of these bases in plant cell walls favors the association between TiO_2 and plants, promoting toxic effects (Pittol *et al.*, 2017).

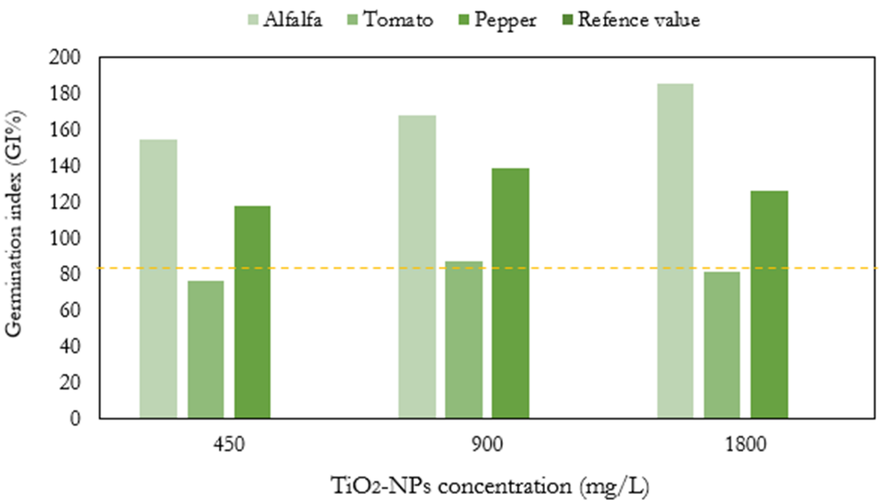

Figure 3. Germination index of alfalfa, tomato, and pepper seeds
The dashed line indicates the reference value of 80%. Results below this value imply that the material to which the plants are exposed generates phytotoxic effects (Huerta *et al.*, 2015).

In the three crops, the length of the radicle was modified, in alfalfa and pepper there was an increase in root length when increasing the concentration of TiO_2-NPs. Figure 4 shows images from alfalfa (Figure 4A) and pepper (Figure 4C) seedlings, respectively, starting from the control (a) and each of the treatments (b, c, d). These results can be compared with those reported in *H. glaucus* where a concentration of 500 mg L^{-1} of TiO_2-NPs increased root length from 8.32 to 10.17 cm. Otherwise, in *N. Schober* the opposite occurred, a concentration of 1500 mg L^{-1} reduced the radicle from 13.85 to 10.68 cm (Kamali *et al.*, 2021); the latter was obtained in the tomato seedling, where the treatments with the TiO_2-NPs reduced root growth. Also, Figure 4 shows tomato seedlings (Figure 4B) exposed to TiO_2-NPs, treatment 1 (450 mg L^{-1}) (b), treatment 2 (900 mg L^{-1}) (c), treatment 3 (1800 mg L^{-1}) (d) and the reduction in root length is observed when making the comparison against the control (a).

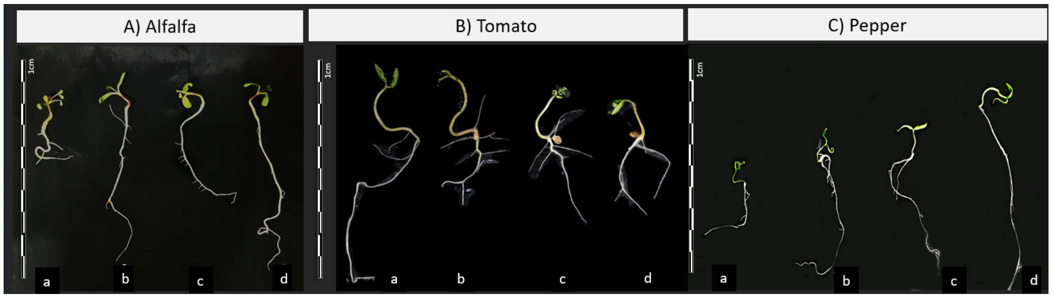

Figure 4. Alfalfa (4A), tomato (4B) and pepper (4C) seedlings 10 days after germination.
a) Control (without the addition of TiO_2-NPs), b) treatment 1 (450 mg L^{-1}), c) treatment 2 (900 mg L^{-1}), and d) treatment 3 (1800 mg L^{-1})

In the same way, the width of the main root and the width of the piliferous zone were evaluated; in tomato treatment 2 (900 mg L^{-1}) increased these variables significantly ($p < 0.05$), while in pepper both treatment 2 and treatment 3 (900 and 1800 mg L^{-1}) reduced them ($p < 0.05$) (Table 3). This assertion is according to the measurement of the control.

Authors report that TiO_2-NPs modify root growth either by increasing it, as in the case of zucchini with 1000 µg mL^{-1} and onion with treatments of 500 and 1000 µg mL^{-1}, or by reducing it in experiments with seeds of cabbage, corn, lettuce and oats, all these crops were tested at concentrations of 0, 250, 500 and 1000 µg mL^{-1} of TiO_2-NPs (Andersen *et al*., 2016). In addition, it has been described that the root length of *Arabidopsis* seeds increases in a concentration-dependent manner since a greater increase in this tissue was observed in concentrations of 500 to 1000 mg L^{-1} compared to those of 100 and 250 mg L^{-1}; in addition to this, it was reported that TiO_2 has an effect on the expression of genes related to auxins and thus interferes with root growth (Wei *et al*., 2020).

Root tip width decreased in alfalfa and tomato in treatments 2 and 3 (900 and 1800 mg L^{-1}) of TiO_2-NPs when compared to the control. The image presented in Figure 5 shows the alfalfa root tip (Figure 5A) taken with the Leica DM1000 microscope with its respective measurement. The control is identified in subsection (a), treatment 1 in (b), and the concentrations of treatments 2 and 3 in (c) and (d) respectively, likewise it can be seen in Figure 5B the root tip of the tomatoes. The modification in the root tip was appreciated in previous studies carried out in *Lactuca sativa* with 100 mg L^{-1} of Ag-NPs. In this experiment, damage to the root surface, such as necrosis and deformation, was observed through microscope analysis. It is worth mentioning that there were no differences in the elongation zone (Pinheiro *et al*., 2020). The root tip is extremely important for root penetration to the substrate, protecting the apical meristem, sensing environmental conditions (Shi *et al*., 2018), and participating in the life of the plant in general (Kumpf and Nowack, 2015) its damage can cause plant deterioration, reduced production, disease, and even death.

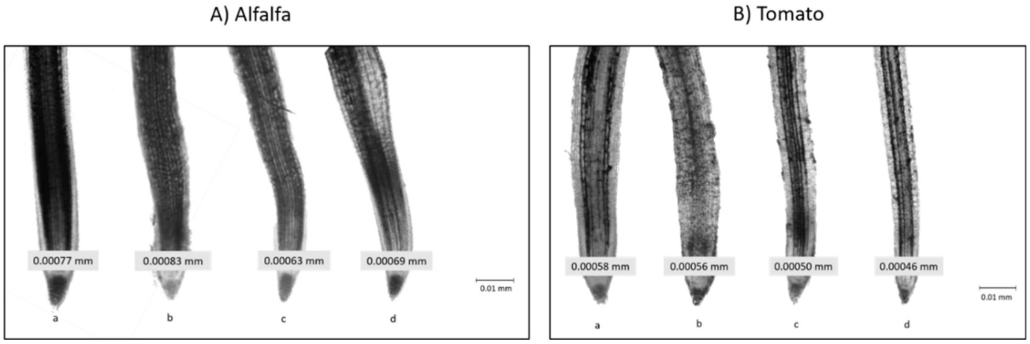

Figure 5. Root tip and its measurement in alfalfa (5A) and tomato (5B) seedlings 7 days after germination. a) Control (without the addition of TiO_2-NPs), b) treatment 1 (450 mg L^{-1}), c) treatment 2 (900 mg L^{-1}), and d) treatment 3 (1800 mg L^{-1})

Another variable studied was stem length and in this only alfalfa showed a decrease of 0.2 cm in seedlings exposed to TiO_2-NPs about the control (Table 3). These results are similar to those reported in *Mentha piperita* stems irrigated with 100 and 200 mg L^{-1} of TiO_2-NPs in germination tests. Here the differences were 0.5 to 1 cm respectively when compared to the control (Samadi *et al*., 2014). In contrast to this stem reduction, in *Vitex agnus-castus* exposed in Petri to TiO_2-NPs in concentrations of 0, 10, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 mg L^{-1}, an increase in stem length was observed when comparing against the control from 2,433 to 3,667 cm, however, the differences were not significant (Farahi *et al*., 2019).

In the particular case of the number of secondary roots, only the alfalfa seed was stimulated in the treatments with the TiO_2-NPs when compared to the control, finding an approximate difference of 2 rootlets. This effect was also found with polystyrene nanoparticles (PS-NPs) in *Oryza sativa* L.; where the number of lateral roots was stimulated in the treatments of 10, 50, and 100 mg L^{-1} PS-NPs, presenting 2 to 3 units more than those counted in the control experiment (Zhou *et al*., 2021). Likewise, it is important to mention that the presence of elements in excess or of toxic relevance for plants is particularly manifested in the formation of secondary roots (Pabón *et al*., 2020).

Conclusions

The TiO_2-NPs presence had an impact on the germination of alfalfa, tomato, and pepper seeds, resulting in morphological changes. However, the effects whether positive or negative varied depending on the species and concentration. Tomato was the most adversely affected, despite some parameters showing high values in nanoparticle treatments, the plant exhibited moderate phytotoxicity with the lowest GI. Additionally, the TiO_2-NPs presence led to reductions in main root length, root hairs, and root tip width. Alfalfa showed positive effects on SVI and GI in the germination phase, as well as increases in main root length and the number of secondary roots. However, parameters such as stem length, root tip width, and root hair length were negatively impacted. Pepper seeds responded positively to TiO_2-NPs treatments, with enhancements observed in SVI, GI, and main root length. Nevertheless, the width of the main root, length of root hairs, and width of the hair zone were influenced by nanoparticle exposure. Overall, pepper exhibited the best outcomes and the least negative effects among the three species.

Whence, further studies on various phenological stages of crops grown with TiO_2-NPs from anthropogenic sources are necessary, as their common presence in the environment may have detrimental effects on crops.

Authors' Contributions

Conceptualization: DAS, MCVA, HCM and JRH; Data curation: JHH and DAS; Formal analysis: DAS; Funding acquisition: MCVA; Investigation: DAS and JRH; Methodology, DAS, ACGF, JHH and MCVA; Project administration: MCVA and ESCH; Supervision: MCVA, ACGF, and JHH; Visualization: DAS, MCVA, HCM., and JRH; Writing - original draft: DAS; Writing- review and editing: MCVA, ACGF, JHH, ESCH, HCM, JRH., and DAS. 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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