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The ferrite nanoparticles (Fe_2O_3) impact on germination, growth and lignine content of carrot (*Daucus carota*)

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Abstract

This study investigated the effect of ferrite (Fe_2O_3) Nanoparticles (NPs) on carrot plantlets (*Daucus carota).* Seeds were soaked during twelve hours in NPs solutions at 50, 500 and 1000 mg L^{-1} (T1, T2, T3), and a control treatment (TC) during 14 days. It was measured the root length and germination percentage at 3, 7 and 14 days; Kotowski's coefficient was estimated at 14 days. Raman spectroscopy was used to analyze samples TC, T1 and T3 at 7 days of germination. Results showed an effect of NPs on plantlets. Inhibition of germination measured as germination percentage was found with NPs, but the growth rate was higher at 500 mg L^{-1}. There was an inhibition of total seeds treated with solution at 1000 mg L^{-1} but the maximun value of root length was obtained in seeds soaked at 50 mg L^{-1}. Finally, in Raman spectroscopy it was observed a positive relationship between NPs concentration and lignin content in plant tissue; this represent a possible modification on the composition and concentration after to addition of NPs on wall cell of plant.

Vegetable, Raman spectroscopy, Kotowski's coefficient

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Introduction

Nowadays nanotechnology has opened doors in the differents fields worldwide, e.g., agriculture, biotechnology, industries, medicine, because it is common to use Nanoparticles (NPs) that have unique caracteristics, and physical and chemical properties with millions of applications (Siddiqui, 2015). This NPs can interact with plants causing morphological and physiological changes (Siddiqui, 2015). NPs interact with roots and shoots; size and surface properties of NPs can influence the pathways (Dietz and Herth, 2011). Another way is for xylem; xylem diameter determines the size of the particle that can be transported and also the speed of water transport (Ma *et al.*, 2010).

Ma *et al.*, 2010, and other suggested that these nanomaterials have both positive and negative effects on plant growth and depends on the composition, concentration, size, and physical and chemical properties of NPs as well as plant species. The unique propierties of NPs could affect crop plants or stimulate the food and agriculture production. Some examples of this nanomaterials are the metal based nanomaterials: Fe, Cu, Zn, Ti, etc (Zurveza-Mena *et al.*, 2016).

E. g. the utilization of iron based nanomaterials is being widely studied. The use of this NPs is many common because it is simple and the synthesis has a low cost so it makes them available from the industrial producers (Deliyanni *et al.*, 2004; Waychunas *et al.*, 2005; Mohapatra and Anand, 2010; Mueller *et al.*, 2012). The main use of this NPs is about remediation purposes from contaminated waters and soils (Gómez-Pastora *et al.*, 2014), because the iron oxidez NPs can also be easily separated and recovered due their magnetic properties (Gómez-Pastora *et al.*, 2014).

According to different experiments this NPs are considered non-toxic to the environment because are poorly absorb by organism (Zhu *et al.*, 2008) but their potencial adherence to the root surface cause adverse effects in plants when administered in relatively high concentrations (Zuverzá-Mena *et al.*, 2016).

Material and methods

Seed conditioning

Carrot seeds were stored at 25 ºC, after the seeds were washed with a solution of sodium hypoclorite 0.6% and washed out three times with distilled water.

Nanoparticles

Ferrite NPs (~30 nm) were selected to carried out the study. NPs acquired from the laboratory Materiales nanoestructurados S.A. de C.V.

Treatments

We established four treatments: a) treatment control (TC), distilled water without Fe_2O_3 nanoparticles; b) treatment T1: Solution of NPs-Fe_2O_3 at 50 mg L^{-1}; c) treatment T2: NPs- Fe_2O_3 solution at 500 mg L^{-1}; d) treatment T3: NPs-Fe_2O_3 solutionat 1000 mg L^{-1}.

Incubation in Petri dishes

The washed seeds were placed into the nanoparticles solutions during six hours and put in petri dishes. Each treatment was carried out per triplicate. The petri dishes were incubated at 28±5°C during 14 days. The total number of experimental units was 15.

Sampling and measurements

The samples were taken at 3, 7, 14 days. It was calculated the germination percentage and the germination rate coefficient (Kotowski coefficient); the root length was measured.

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The Raman spectroscopy was used in order to determine the effect of the NPs on the surface of plant.

Statistical analysis

The data obtained were analysed by using the statistical software XLStat®. For the germination rate and Kotowski´s coefficient ANOVA were performed assuming 95% of confidence range and tolerance of 0.0001; the graphics of standardized coefficient were used to proble statistical differences. For the analysis of the length root measurements a Principal Component Analysis (PCA) was performed takin the factors with most impact on the behaviour o the active observations. The plots of variables and factors, factors and observations was showed and clusters were built. The PCA analysis was performed considering 5% of significance.

Results

Kotowski's coefficient and germination percentage

The germination percentage was measured at 3, 7 and 14 days of incubation in petri dishes. Table 1 shows the results for this variable in which there is a significatively difference between the number of seeds germinated at control treatment TC (1000 mg L^{-1}) i.e, 94% at 14 days compared with treatment T3, i.e, 38%. A positive correlation is observed between the germination rate and the NPs concentration.

On the other hand, the growth velocity coefficient was estimated at 14 days. Table 2 shows that even with NPs the Kotowski coefficient is higher in T2 compared with others. TC and T3 obtained the same result.

Concentration (mg L^{-1})	Germination percentage		
	Day 3	Day 7	Day 14
TC	25.0 ± 2^{a}	76.0 ± 10^{a}	94.0 ± 3.5^{a}
T1	0.00 ± 0^{b}	89.0 ± 5.1^{a}	85.0 ± 8.4^{a}
T2	46.0 ± 9^{c}	50.0 ± 9.1^{b}	46.0 ± 4.7^{b}
T3	0.00 ± 0^{b}	33.0 ± 5.3^{c}	38.0 ± 5.2^{b}

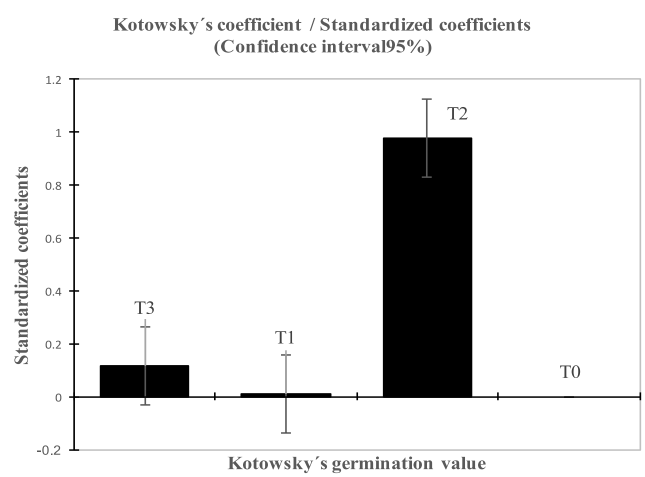
n = 9, 3 experimental units per triplicate
Same capital letters are not statistically different among treatments by the Tukey test ($p < 0.001$).

Table 1 Germination percentage at 3, 7 and 14 days of treatment

Concentration (ppm)	Kotowski's coefficient at 14 days
TC	9.90
T1	9.00
T2	14.1
T3	9.90

Table 2 Kotowski coefficient calculated at 14 days of germination

The above data were statistically analized by applying ANOVA analysis (XLStat®), confidence 95% and tolerance 0.001.
The graphic 1 shows the standardized coefficientºs and confirm the statistical difference between treatments.



Graphic 1 Mean value and standard deviation for the Kotowsky´s coefficient observed in the three treatments

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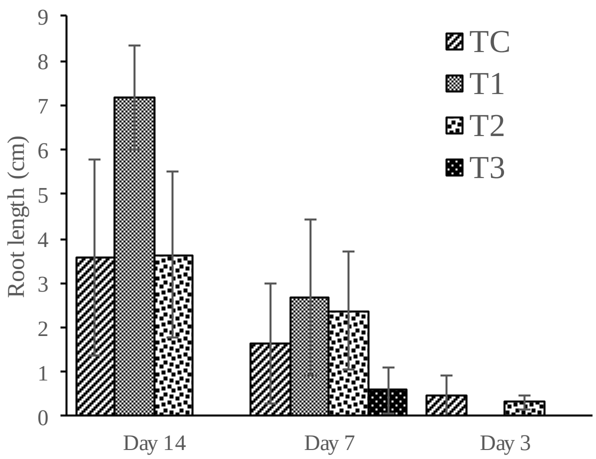
Root length

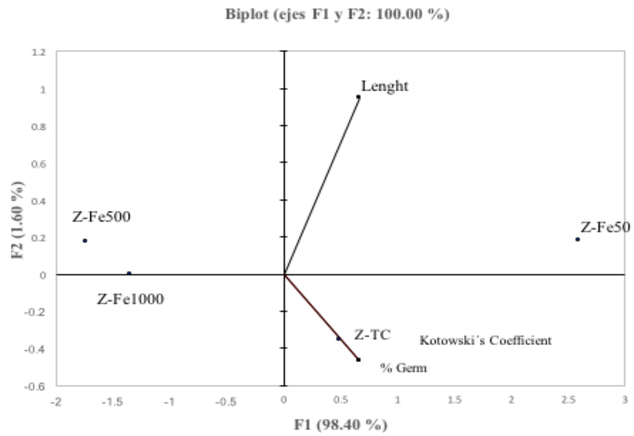
About the root length at 3 days we only could measure TC and T2. It took 7 days to observe a significant root length in four treatments. However, it was treatment T1 (50 mg L^{-1}) which reached the greatest length. At day 14th the seeds in T2 grew 7 cm; this was significant compared with control.



Graphic 2 Root length for the four treatments at 3, 7 and 14 days of germination

Principal Component Analysis

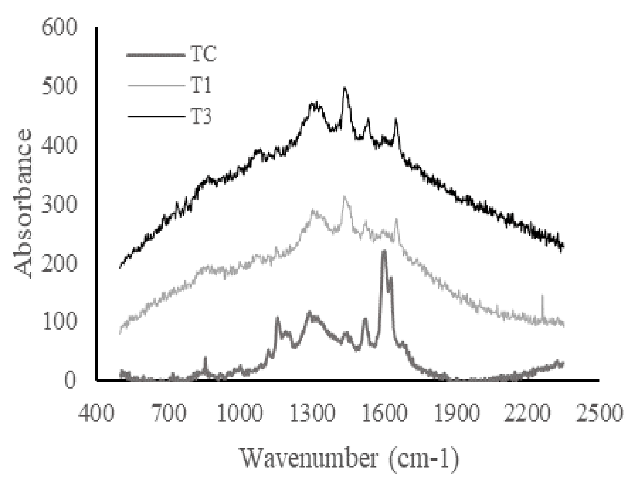
A Principal Component Analysis (PCA) was carried out for all the variables at day 14, in order to determine the percentage of explanation for variables involved in the experiment. The figure X. shows the behaviour of the variables i.e., root length, Kotowsky´s coefficient and percentage of germination (%).



Graphic 3 Principal Component Analysys for the most important variables after 14 days of experimental set up

Raman spectroscopy

It was found an effect of NPs on cell wall of plant according to the spectrum of Raman. Graphic 4 shows the differences between the peaks obtained to TC and T1, T3. At 1600 cm^{-1} exists a decrease in the intensity of peaks for T1 and T3. And the shape of the spectrum changes relatively.



Graphic 4 Raman spectroscopy for carrot at 7 days for treatments TC, T1, and T3

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Discussion

Many of studies provide evidence that plant uptake is a potential transport pathway of NPs in the environment (Zhu *et al.*, 2008). Libralato *et al.*, 2016, observed that germination depends on Fe form and plant species; they observed biostimulation effects on seedling length and biomass production using zero valent iron on germination in *Lepidium sativum* and *Sorghum saccharatum* in petri dishes. Wang *et al.*, 2016 found damages in root tissue and chlorophyll reduction with Fe NPs.

In this study, there was a statistical difference on the germination seeds percentage, we found that seeds treated with NPs had a low percentage compared with control treatment. However, the seeds soaked in 500 mg L^{-1} showed the maximun values for Kotowski's coefficient (14.1) compared with TC (9.90); TC and T3 had the same value.

It was our interest analyze the effect o f the interaction of other variables in the final value; a PCA was performed and it was observed that there was an effect of the Fe-NPs at 50 mgL^{-1} and it was related with the lengh of the plantlets in a positive form, contrarely, the treatments ant 500 and 1000 mgL^{-1} did not have any relation with the variable analyzed. The seeds under zero Fe-NPs treatments showed the maximum values for germination percentage and Kotowski´s coefficient after 14 days of experimentation, as is observed in Figure 3.

According to morphological analysis we found differences between treatments; analyzing the root length it was found that treatment T1 had the maximun value at 14 days of incubation while root length of T3 was reduced even we can assume that at this NPs concentration plants die.

Reported results of experiment carried out with Fe NPs at different concentrations i.e, nFe_2O_3 at 0.25, 0.5, 0.75 and 1 g L^{-1} have obtainded the follow data: in soybean the NPs increased leaf and pod dry weight; nFe_3O_4 at 0.67 mg mL^{-1} during 7 days it was found a total inhibition of germination in lettuce, radish, cucumber, spinach, tomato and peppers and at 0.5 g L^{-1} in less of 20 days in pumpkin plants its reported a translocation through out the plant tissues, NPs detected in stem and leaves and accumulation on the root surface (Sheykhbaglou *et al.*, 2010; García *et al.*, 2011; Zhu *et al.*, 2008).

Our research group found that were not statistical differences on length root and germination percentage in alfalfa (*Medicago sativa*) and broccoli (*Brassica oleracea italica*) plantlets after soaking in Fe nanoparticle at different concentrations (50 mg L^{-1}, 500 mg L^{-1} and 1000 mg L^{-1}) (Data no published).

Same results were obtained when carrot seeds were soaken in Ti-NPs and the length root was measured after 14 days of incubation, the enhanced effect on root length of Ti-NPs was observed in alfalfa seeds after 14 days of incubation (Data no published).

On the other hand, the Raman spectroscopy results showed a positive relationship between NPs concentration and the lignine content in the plants tissue. It is possible to observe a representative peak at 1600 cm^{-1}; Gierlinger *et al.*, 2007 reported the same results in plants tissues analysis at this wavenumber; it is associated with the lignin phenyl ring, which is the main component of the wall cell of plants; these results allow us to suppose that the plant tissues might have modification in the lignin composition and concentration after addition of NPs.

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Conclusions

The results obtained in this study show that metal oxide nanoparticles like iron NPs might have a potential effect on carrot seeds; we found that at moderate concentration the growth rate may be enhanced but the germination percentage and the root length at elevated concentration of NPs could be inhibited, however seeds treated with a small amount of NPs (50 mg L^{-1}) had a significant difference in root length at 14 days; this treatment had the higher root length. A reduction of the lignine amount in plant tissued was detected via spectroscopy analysis using Raman technique.

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References

Deliyanni, E.A., Lazaridis, N.K., Peleka, E.N., Matis, K.A. (2004) Metals removal from aqueous solution by iron-based bonding agents. Environ. Sci. Pollut. Res. 11, 18-21.

Dietz, K.J., Herth, S., (2011) Plant nanotoxicology. Trends Plant Sci. 16, 582-589. European Union, Comission Delegated Regulation No. 1363/2013 of 12 december 2013. Official Journal of the European Union, L 343/26-L343/28.

García, A., Espinosa, R., Delgado, L., Casals, E., Gonz_alez, E., Puntes, V., Carlos, B., Font, X., Sanchez, A., (2011) Acute toxicity of cerium oxide, titanium oxide and iron oxide nanoparticles using standardized tests. Desalination 269, 136-141.

Gierlinger, N., Schwanninger, M. (2007) The potential of Raman microscopy and Raman imaging in plant research. Spectroscopy 21, 69-89.

Gómez-Pastora, J., Bringas, E., Ortiz, I. (2014) Recent progress and future challenges on the use of high performance magnetic nano adsorbents in environmental applications. Chem. Eng. J. 256, 187-204.

Libralato, G., Costa Devoti, A., Zanella, M., Sabbioni, E., Micetic, I., Manodori, L., Pigozzo, A., Manenti, S., Groppi, F., Volpi, G.A., (2016) Phytotoxicity of ionic, micro- and nano-sized iron in three plant species. Ecotoxicol. Environ. Safe 123, 81-88.

Ma X, Geiser-Lee J, Deng Y, Kolmakov A (2010) Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. Sci Total Environ 408(16):3053–3061.

Mohapatra, M., Anand, S. (2010) Synthesis and applications of nano-structured iron oxides/hydroxides e a review. Int. J. Eng. Sci. Technol. 2 (8), 127-146.

Mueller, N.C., Braun, J., Bruns, J., _Cerník, M., Rissing, P., Rickerby, D., Nowack, B. (2012) Application of nanoscale zero valent iron (nZVI) for groundwater remediation in Europe. Environ. Sci. Pollut. Res. 19, 550-558.

Sheykhbaglou, R., Sedghi, M., Tajbakhsh Shishevan, M., Seyed Sharifi, R. (2010) Effects of nano-iron oxide particles on agronomic traits of soybean. Not. Sci. Biol. 2, 112-113.

Siddiqui M, Al-Whaibi M, Firoz M, Al-Khaishany Y (2015) Role of nanoparticles in plants. Siddiqui, Manzer H, Al-Whaibi, Mohamed H, Mohammad, Firoz. Nanotechnology and plant sciences. (pp. 19-21).

Wang, J., Fang, Z., Cheng, W., Yan, X., Tsang, P.E., Zhao, D., (2016) Higher concentrations of nanoscale zero-valent iron (nZVI) in soil induced rice chlorosis due to inhibited active iron transportation. Environ. Pollut. 210, 338-345.

Waychunas, G.A., Kim, C.S., Banfiled, J.F. (2005) Nanoparticulate iron oxide minerals in soils and sediments: unique properties and contaminant scavenging mechanisms. J. Nanopart. Res. 7, 409-433.

Zhu, H., Han, J., Xiao, J.Q., Jin, Y. (2008) Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. J. Environ. Monit. 10, 714-717.

Zuverza-Mena, N., Martínez-Fernández, D., Du, W., Hernandez-Viezcas, J., Bonilla-Bird, N., Lopez-Moreno, M., Komárek, M., Peralta-Videa, J., Gardea-Torresday, J. (2016) Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses-A review. Plant Physiology and Biochemistry, 1-29.