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To cite this article: Michel Esper Neto, David W. Britt, Kyle Alan Jackson, Carolina Fedrigo Coneglian, Vitor Rodrigues Cordioli, Alessandro Lucca Braccini, Tadeu Takeyoshi Inoue & Marcelo Augusto Batista (2021) Assessments in early growth of corn seedlings after hausmanite (Mn_3O_4) nanoscale seed priming, Journal of Plant Nutrition, 44:11, 1611-1620, DOI: [10.1080/01904167.2021.1871745](https://doi.org/10.1080/01904167.2021.1871745)

To link to this article: <https://doi.org/10.1080/01904167.2021.1871745>



Published online: 15 Jan 2021.



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Assessments in early growth of corn seedlings after hausmanite (Mn₃O₄) nanoscale seed priming

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ABSTRACT

Nanofertilizer application is becoming a sustainable alternative for plants micronutrients supply. Seed nutrient priming before seeding reduces non-target dispersion; although, applying nanofertilizer in correct concentration must be narrowly chosen to prevent germination and development issues. Here, we evaluated corn seedlings development and germination after seed priming with Mn₃O₄ nanoparticle (NP), Mn₃O₄ bulk and MnCl₂. Sterile seeds were soaked for 8 hours in priming solutions of 0, 20, 40, 80 and 160 mg L⁻¹ for each Mn sources. The seeds vigor and germination were evaluated after 7 days on germination paper. Root, shoot and total lengths were measured as well as root, shoot and total dry biomass. Compared to the control, the Mn₃O₄ NP and Mn₃O₄ bulk promoted beneficial effects. Mn₃O₄ NP seed-priming exhibited a concentration dependent profile in improving seedling growth, with greatest benefit around 20 mg L⁻¹, providing higher germination, vigor, dry biomass and length than control and the other source tested. Particle size plays an important role in the reactivity of Mn₃O₄ NP. On the other hand, seeds primed with soluble source did not differ from the control. These findings support NP-seed priming as an alternative to delivery micronutrients.

ARTICLE HISTORY

Received 4 May 2020

Accepted 21 December 2020

KEYWORDS

biomass; nanofertilizer; nanoparticle; plant nutrition; seed parameters

Introduction

Nanoparticle (NP) is any material having at least a single dimension between 1 and 100 (Dimkpa and Bindraban 2017). In last decades NP is being used in several areas, including agriculture. Normally in agriculture, these materials are used as fertilizers, mainly as micronutrients source and pesticides (Chhipa 2017). The NP plant application higher efficiency, compared to bulk equivalent molecules, is due to high reactivity and specific surface area (30–50 m² g⁻¹) (Esper Neto et al. 2020).

Despite that, there is a narrow difference between NP concentration which will cause environmental issues and concentration that will increase crops yield. In addition, thinking of nanofertilizers, different plants will respond the same NP concentration differently, as well as the application way which can be performed in different ways (Liu and Lal 2015). Therefore, it is important to know specific changes cause by nanoparticle to an economic interest crop.

There are evidences showing nanoparticles may have beneficial effects on seedling growth and development (Sheykhbaglou et al. 2010). Nanotechnology shows positive prospects for sustainable

agricultural practices and it is hoped that this will be a tool to improve management practices and yield (Monreal et al. 2016; Chhipa 2017). It is even possible to synthesize controlled nutrient release particles (Kottegoda et al. 2011).

Among the elements applied as nanofertilizers, Mn is being studied, since this is one of the essential micronutrients linked to photosynthesis, respiration and nitrogen metabolism conferring greater development and plants yield. On the other hand, Mn can also be toxic to the plants in high concentrations and according to the chemical characteristics of the soil like low pH and high potential redox that alter the Mn state of oxidation for toxic species to the plants (Millaleo et al. 2010; Dimkpa et al. 2018).

When compared to the application of other NPs as Zn-NP, Cu-NP, Fe-NP less attention is given to Mn-NP tests in crop systems (Dimkpa and Bindraban 2017). However, Pradhan et al. (2013) showed that green bean plants (*Vigna radiata*) increased shoot and roots length, chlorophyll and photosynthetic rate. Yuvaraj and Subramanian (2015) showed zinc uptake increase by rice plants (*Oryza sativa*) in the Mn-NP presence. Elmer and White (2016) found a 22% increase in eggplant (*Solanum melongena*) yield. Other authors did not find significant differences for the development of white mustard roots (*Sinapis alba*) (Landa et al. 2016), watermelon yield (*Citrullus lanatus*) (Elmer et al. 2018), and lettuce seed germination (*Lactuca sativa*) (Liu, Zhang, and Lal 2016).

Mn deficiency in soils is widely reported worldwide, especially in soils with pH above 6. In tropical region, soils are generally acidic; however, limestone application is performed superficially in no-tillage system to raise soybean and corn successively. This soil surface pH elevation can cause Mn deficiency, especially when there was no Mn fertilization (Favarin, Tezotto, and Ragassi 2008). However, Mn amount required by corn is relatively low, making it difficult to apply to the whole cultivation area using solid regular fertilizer. Therefore, seed priming is an interesting management alternative, which will promote rational use and greater nanofertilizer distribution uniformity in total area.

Therefore, this research tested the hypothesis that the Mn-NP application through corn seed priming is sustainable management and improve in seed germination, vigor and seedling growth, when compared to manganese soluble source and bulk Mn particle. In addition, we investigated different concentration range Mn rate applied to avoid toxic effects to the environment when using Mn-NP. In this context, the specific aim was to investigate changes in the growth and development of maize seedlings after Mn corn seed priming with different sources.

Material and methods

Nanoparticle synthesis

Mn₃O₄ nanofertilizer was prepared using 100 mL of MnSO₄·7H₂O (Sigma Aldrich Chemical Co[®], San Luis, MO, USA) 0.1 mol L⁻¹ and 100 mL of 0.4 mol L⁻¹ NaOH (Sigma Aldrich Chemical Co[®], San Luis, MO, USA). The reagents were done, and then mixed in a 500 mL Erlenmeyer. After that, the solution was shaken vigorously on a magnetic stirrer at 1500 rpm at temperature (22 °C ± 2) for 15 min. The flask was placed in a microwave oven for 2 min at a power of 700 W. The flask was cooled at temperature (22 °C ± 2). The flask was then centrifuged and washed many times with distilled water to remove excess SO₄²⁻ and Na⁺ residuals. The solution was frozen instantly at -70 °C using liquid nitrogen and dried by sublimation in a LS 3000 lyophilizer (Terroni[®], São Carlos, São Paulo, Brazil) and packed in a vacuum glass desiccator.

The Mn₃O₄ powder was analyzed by X-ray diffraction in an XRD 6000 system (Shimadzu[®], Kyoto, Japan). Diffractograms were obtained between 20 to 75° 2θ, in intervals of 0.02° 2θ for 0.6 s, step-mode, using CoKα radiation and nickel filter. The diffractogram peaks correspond to mineral hausmanite (Mn₃O₄) (Figure 1).

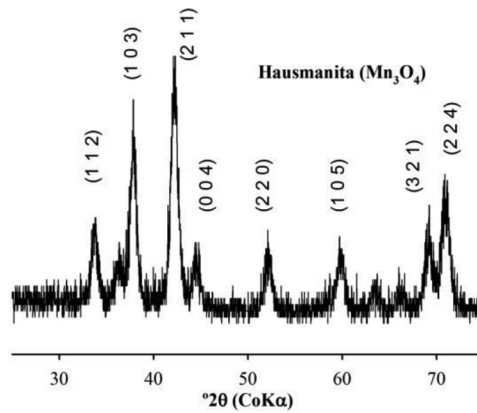


Figure 1. X-ray diffraction of the synthesized Mn_3O_4 (Hausmanite).

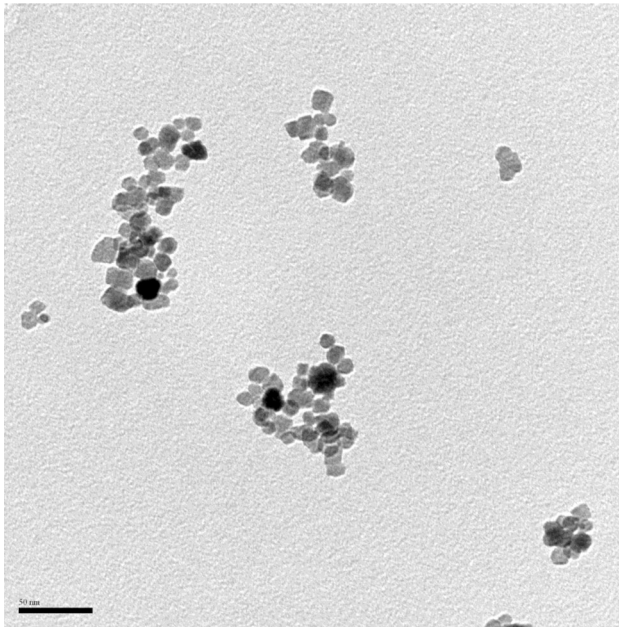


Figure 2. Transmission electron microscopy image of the Mn_3O_4 in the 50 nm scale.

The Mn_3O_4 NPs size and shape were assessed by transmission electron microscopy (TEM, CM200, Philips®, Amsterdam, Netherlands). TEM samples were prepared by depositing a small volume of a Mn_3O_4 in water suspension onto carbon grids coated with copper grids and allowed to dry overnight. Mn_3O_4 NP used in this assay had a diameter of approximately 20 nm and rounded shape (Figure 2).

Experiment characterization and assessments

The seeds of corn hybrid IAC 8046 were donated by a company authorized to commercialize seeds (Sella Sementes®, Astorga, Paraná, Brazil). The seeds were cleaned with sodium hypochlorite solution (1% v/v) and then washed several times with distilled water. The seeds were kept in a dry and dark place at a temperature of 16°C, until the experiment installation.

A cross-factor experiment (5×3) was carried out in a randomized design with 4 replications. The five-level factor was different concentrations (0, 20, 40, 80 and 160 mg L^{-1}) based on Mn level and the factor with three levels were the different Mn sources (Mn_3O_4 NP, MnCl_2 , and Mn_3O_4 bulk). Each plot was composed by 50 seeds. To prime with Mn, the seeds were immersed in the different solutions containing a total volume of 200 mL for 8 hr, then dried at room temperature for one hour.

The Mn_3O_4 NP was compared with two other Mn sources. The first one was a Mn ionic solution, which was prepared by dissolving MnCl_2 (Sigma Aldrich Chemical Co[®]. San Luis, MO, USA) in distilled water, the second one was Mn_3O_4 bulk with non-nanometric ($>300 \text{ nm}$) size (Sigma Aldrich Chemical Co[®]. San Luis, MO, USA). The nonionic sources (synthetic Mn_3O_4 NP and Mn_3O_4 bulk) were directly added in ultrapure water and dispersed using an ultrasonic shaker (100 kHz for 5 min).

Seed germination was assessed using Germitest paper (Germipel[®], Ribeirão Preto, São Paulo, Brazil). The seeds were placed in three sheets moistened with distilled water equivalent to 2.5 times the dry biomass of the paper and each seed was one cm or more away from another seed. After that, the seeds are taken to the Mangelsdorf germination chamber at a constant temperature of 25°C for 8 days. The results were showed as percentage of normal seedlings (perfect seeds with root and shoot well defined), abnormal (without shoot or root) and non-viable (non-germinated) seedlings. The vigor test was performed on the fifth day, by counting the number of vigorous seedlings (Brasil 2009).

The root length (distance in cm from the stem base to the root tip) and shoot length (distance in cm from the base of the leaf to the tip of the leaf) were measured in 15 seedlings randomly selected using a millimeter ruler. Then, the seedlings were transferred to Kraft paper bags, later these seedlings were taken to a drying oven at a constant temperature of 60°C for 72 hr to measure the dry biomass production.

Statistical analysis

The data were submitted to the basic statistical assumptions (normality of errors and homoscedasticity of variances) tests by Shapiro-Wilk and Bartlett tests, respectively ($p > 0.01$). The different concentrations and sources and possible interactions were tested by F test in the analysis of variance. The interaction was deployed only for concentrations within each source. Quantitative data was analyzed by regression and their coefficients submitted to the T test. While, the qualitative data were analyzed by Tukey average test ($p < 0.05$) (Zimmermann 2014).

Results

The influence of nano, bulk and ionic Mn seed priming effects were evaluated as seedling vigor, germination percent, non-viable and abnormal seed percentages (Figure 3). There was no significant adjustment of simple regression models for concentrations, even after partitioning sources. On the other hand, the experimental results obtained after partitioning within each Mn concentration showed statistically significant difference. On concentration average there was statistical difference between the sources for variable germination (Table 1) in which the nanometric source obtained 5.5% more germination rate than the soluble source. At 20 mg L^{-1} Mn concentration, Mn_3O_4 -NP provided 90% vigor for maize seeds whereas MnCl_2 at the same rate, vigor was 72.5% only, although there were no differences in the other doses evaluated (Figure 3a). In addition, no negative impacts were seen.

Seed germination was significantly altered by treatments. In concentration average, there were differences among the sources tested, and the source Mn_3O_4 NP was 8.8% and 6.6% higher than Mn_3O_4 bulk and MnCl_2 , respectively (Table 1). At the concentration 20 and 40 mg L^{-1} , Mn_3O_4

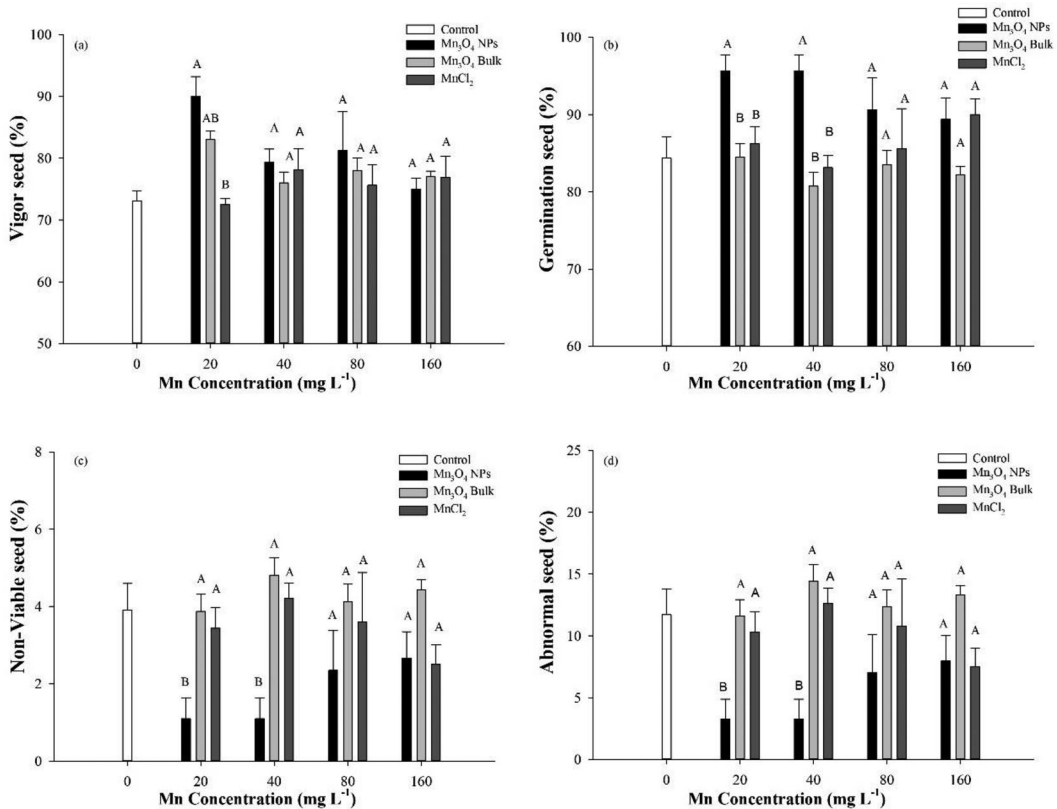


Figure 3. Corn (a) seed vigor, (b) seed germination, (c) non-viable seed and (d) abnormal after different sources and doses of Mn seed priming. Equal capital letters do not differ from each other in relation to the source at 5% probability by Tukey test.

Table 1. Manganese sources effects, independently of concentrations on vigor, germination, non-viable, abnormal seeds, seedling length and biomass production assessments.

	Vigor	Germination	Non-viable	Abnormal	Root length	Shoot length	Total length	Root biomass	Shoot biomass	Total biomass
	%				cm			g		
Mn ₃ O ₄ NPs	79.8 a	91.1 a	2.2 b	6.6 b	26.3 a	13.6 a	39.9 a	0.28 a	0.40 a	0.68 a
Mn ₃ O ₄ bulk	77.4 a	83.1 b	4.2 a	12.6 a	24.8 b	12.6 b	37.8 b	0.24 b	0.36 a	0.61 b
MnCl ₂	75.3 a	85.9 b	3.5 a	10.5 a	25.2 b	12.8 b	37.6 b	0.25 b	0.35 a	0.60 b
Average	77.1	86.2	3.4	10.3	25.4	13.0	38.5	0.25	0.37	0.63
CV ^a (%)	8.4	9.4	46.2	46.2	9.5	9.2	8.3	15.4	14.2	12.1

^aCoefficient of variation. Values followed by the same letter in a column do not differ from each other at 5% probability by the Tukey test.

NPs source promoted 95.6% germination of corn seeds, while Mn₃O₄ bulk promoted 84.5% and 80.4% for 20 and 40 mg L⁻¹, respectively, and MnCl₂ promoted 86.2% and 83.1%, for 20 and 40 mg L⁻¹, respectively. The other concentrations evaluated were not statistically significant for sources (Figure 3b).

The results for non-viable seeds after treatments were also significant. On the average of the concentration tested, Mn₃O₄ bulk and MnCl₂ were 91.0% and 59.2% higher than Mn₃O₄ NP (Table 1). At the concentration 20 and 40 mg L⁻¹, Mn₃O₄ NP seed priming promoted 1.0% of non-viable seeds, while Mn₃O₄ bulk promoted 3.9% and 4.8% for 20 and 40 mg L⁻¹, respectively, and MnCl₂ promoted 3.4% and 4.2%, for 20 and 40 mg L⁻¹, respectively. The other concentrations evaluated were not statistically significant for sources (Figure 3c). Abnormal seeds exhibited

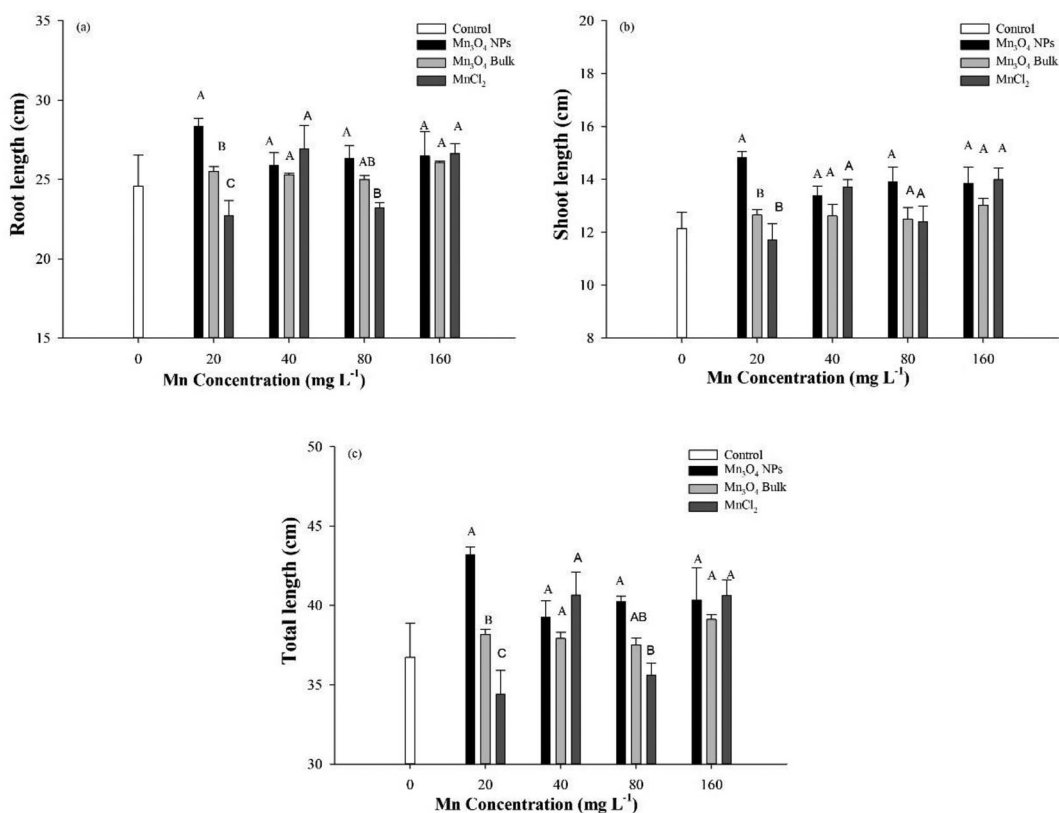


Figure 4. Corn seedlings root length (a), shoot length (b) and total length (c) after seed Mn different sources and doses of seed priming. Equal capital letters do not differ from each other in relation to the source at 5% probability by Tukey test.

a similar concentration trend for the Mn₃O₄ NP source. Considering the averages of concentrations, Mn₃O₄ bulk and MnCl₂ were higher than Mn₃O₄ NP (Table 1). At the concentration 20 and 40 mg L⁻¹ Mn₃O₄ NP promoted 3.3% of abnormal seeds, respectively, Mn₃O₄ bulk promoted 11.6% and 14.4% for 20 and 40 mg L⁻¹, respectively, and MnCl₂ promoted 10.3% and 14.6%, for 20 and 40 mg L⁻¹, respectively (Figure 3d).

No significant adjustments by regression test ($p < 0.05$) were observed for concentrations tested in any sources regarding shoot, root and total length. Figure 4 shows the effects of the Mn concentrations isolated in each source for length variables. The root seedlings length primed with Mn₃O₄ NPs was 20.0% (5.66 cm) and 10.1% higher than when seed was primed with Mn₃O₄ bulk and MnCl₂, respectively, for 20 mg L⁻¹. At this same concentration, seeds primed with Mn₃O₄ bulk also had 11.0% (2.9 cm) higher root length than seed primed with MnCl₂ (Figure 4a). Still regarding root length, 80 mg L⁻¹ concentration seed priming also showed differences between sources, in which Mn₃O₄ NPs was 11.81% (3.1 cm) higher than MnCl₂ seed priming (Figure 4a).

In relation to the shoot length of the seedlings there were also significant statistical differences (Figure 4b). At the average concentration, the seed primed Mn₃O₄ NPs promoted 7.4% and 5.9% higher shoot seedling length than seed primed with Mn₃O₄ bulk and MnCl₂, respectively. After concentration partitioning concentration, there were also differences for concentrations 20 and 80 mg L⁻¹ for each source. Mn₃O₄ NPs seed priming promoted better development of the seedlings 14.8 cm and 13.8 cm, for 20 and 80 mg L⁻¹, respectively, whereas the soluble particles (MnCl₂) obtained the length of 11.6 cm and 12.3 cm and Mn₃O₄ bulk obtained the length of 12.7 cm and 12.6 cm for concentrations 20 and 80 mg L⁻¹, respectively (Figure 4b).

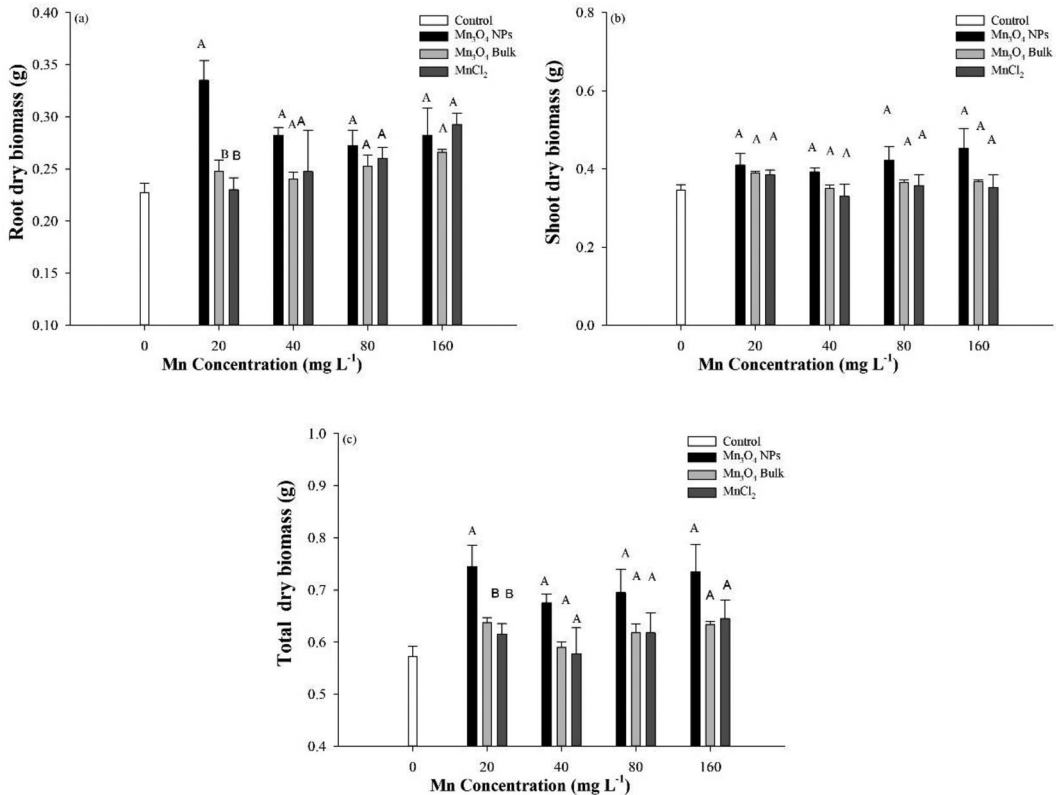


Figure 5. Corn seedlings (a) root dry biomass, (b) shoot dry biomass and total dry biomass (c) after seed Mn different sources and doses of seed priming. Equal capital letters do not differ from each other in relation to the source at 5% probability.

Total seedlings length exhibited a similar concentration trend for the Mn₃O₄ NP source. Considering the averages of concentrations, Mn₃O₄ bulk and MnCl₂ were lower than Mn₃O₄ NP (Table 1). At the concentration 20 and 80 mg L⁻¹ Mn₃O₄ NP seed priming promoted 41.1 cm and 40.2 cm of root length, respectively, whereas Mn₃O₄ bulk promoted 38.2 and 37.48 cm for 20 and 80 mg L⁻¹, respectively, and MnCl₂ promoted 34.4 and 35.6%, for 20 and 80 mg L⁻¹, respectively (Figure 4c).

Regarding dry biomass assessments, the results are shown in Figure 5. There were significant statistical differences for the root dry biomass (Figure 5a) and total dry biomass (Figure 5c); on the other hand, shoot dry biomass was not significantly changed by treatments (Figure 5b). NP increased root biomass by 10.7% when compared to soluble source and 14.3% compared to bulk. In addition, when fixing best Mn concentration (20 mg L⁻¹) the Mn₃O₄ NPs seed priming promoted 0.34 g seedlings root dry biomass, whereas root dry biomass after MnCl₂ seed priming was only 0.23 g and after Mn₃O₄ bulk seed priming was 0.25 g.

The shoot dry biomass seedlings were not significantly influenced ($p < 0.05$) by Mn sources or concentration (Figure 5b). The averages across all concentrations shoot dry biomass were 0.40 g, 0.36 g, and 0.35 g for Mn₃O₄ NP, Mn₃O₄ bulk and MnCl₂, respectively (Table 1).

Total seedlings dry biomass exhibited a similar trend as root dry biomass. Considering the averages of concentrations, Mn₃O₄ bulk and MnCl₂ were higher than Mn₃O₄ NP (Table 1). The use of Mn₃O₄ NP shows better results in all doses tested, but just at the concentration 20 mg L⁻¹ of Mn₃O₄ NP seed priming promoted 0.75 g of total length, while Mn₃O₄ bulk promoted 0.63 g and MnCl₂ promoted 0.57 g (Figure 5c).

Discussion

Plant early growth and development is a result cell enlargement at the apical meristem region due to continuous cell division. This process is highly responsive to the physical and chemical surrounding environment (Torrey 1956). In the present study, was showed that corn seed priming with aqueous suspension of Mn_3O_4 NPs at 20 mg L^{-1} concentration demonstrated to promote significant increases in early growth, such as higher seed germination and vigor, seedlings root length, shoot length total length, root dry biomass and total dry biomass, as well as decreasing in abnormal and non-viable seedlings when compared with the same 20 mg L^{-1} concentration of MnCl_2 soluble source, Mn_3O_4 bulk, and also the also to the control primed in pure water. Our results indicate enhancing in corn development after Mn_3O_4 NPs seed priming at relative low concentration. Therefore, synthetic Mn_3O_4 NP can be useful as a source of fertilizer for the growth and development seedlings since external supplementation during the priming process possibly facilitated the accumulation of adequate Mn inside seeds, which promoted plant well growth. Although seed priming can improve plant growth, little is known about the mechanisms that govern this whole process (Varier, Vari, and Dadlani 2010).

Liu, Zhang, and Lal (2016) evaluated the synthetic Mn-NP implications in germination of lettuce seeds in aqueous media as the present research. These authors found the Mn-NP improved the growth of lettuce seedlings by increasing the root elongation. On the other hand, seed germination was reduced from 84% to 63% in relation to the control, although this difference was not significant even at a high concentration of 50 mg L^{-1} . These authors also demonstrated that the application of the Mn soluble source between 20 and 50 mg L^{-1} decreased the roots development. In the same sense, even very low concentrations of Mn-NP application may significantly improve growth and yield parameters compared to the control with no Mn. Besides that, greater oxygen evolution and photophosphorylation is higher in Mn-NP treated plants, acting as a potential modulator (Pradhan et al. 2013).

The Mn element sometimes stands out more for its toxicity effects than for beneficial effect on plants (Barros and Calado 2014). In spite of that, the element plays a beneficial role for plants, such as seedling germination and emergence, establishment of the population, crop growth and development and yield (Farooq, Wahid, and Siddique 2012). The most important Mn functions in plants are related to nitrogen metabolism and water photolysis on photosynthetic process, and also are important in enzymatic complexes (Rasool, Ahmad, and Farooq 2019). Thus, corn plants originated from poor seed in Mn will have lower development mainly in soils with low Mn contents.

There are few manuscripts that test the efficiency of Mn-NP by means of seed priming, in corn specifically the researchers are even more scarce. Despite this, some studies show promising effects of Mn-NP for crops of economic interest (Pradhan et al. 2013; Liu, Zhang, and Lal 2016; Dimkpa et al. 2018) on the other hand these particles can also cause phytotoxic effects or even cause no effects (Landa et al. 2016). The determinant in relation to the deleterious effect of Mn-NP is related to the high concentrations, culture, time and way of application of these NPs.

Therefore, it is possible to infer that the responses to the use of Mn-NP are very specific. On the other hand, there is a tendency for Mn-NP applied at small concentration have beneficial effects for the plants, when compared to the normally used soluble sources of Mn and insoluble non-nano size.

Finally, in the context of sustainable agriculture, lower doses of well-positioned and tested Mn NPs may bring benefits to crops, since the amount applied is lower, utilization efficiency is increased, nutrient loss is lower, and adverse impacts on the environment are smaller, when compared to larger size particles and soluble sources. These factors are important, since the development of plants can be satisfactory with a smaller amount of fertilizers increasing the efficiency of the applications that is only of 30–50% in average (Liu and Lal 2015).

Therefore, the results confirm the hypothesis that Mn nanoparticle affects the development of maize seedlings when submitted to seed treatment. The best response was with the 20 mg L⁻¹ suspension rate. The Mn nanoparticle source proved to be more efficient in improving seedling growth when compared to other sources, since the doses to obtain positive results were lower. According to the answers obtained in this research it can be inferred that nanoparticles the seeds absorb the Mn supplied through the nanoparticle, through seed treatment, justifying new research with this theme, even with other elements and culture of interest. The micronutrient Mn can be supplied to seeds of maize seeds by means of the treatment of seeds, having as source Mn₃O₄ nanoparticle.

Acknowledgments

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico– CNPq (in Portuguese) PROC 141140/2017-8 and 427006/2016-3 for the scholarships. Grupo de Estudos em Solos e Plantas GESSO (in Portuguese) and Utah State University.

Conflict of interest: The authors declare no conflict of interest in any forms in this research.

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