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Article

Effect of zinc oxide nanoparticles on radish development in organic substrates

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Abstract

In recent decades, nanotechnology applied to agriculture has attracted considerable interest due to its potential to boost crop growth and productivity. This research aimed to evaluate the effect of different concentrations of zinc oxide nanoparticles applied to radish plants grown on different organic substrates under low tunnel conditions. The research was conducted at the Chiapas Regional Academic Center of the Antonio Narro Autonomous Agrarian University in 2023. A randomized design with nine treatments and five replications (two plants per replication) was used. The substrates used were common soil, worm humus, and bokashi. During the development of the crop, three applications of zinc oxide nanoparticles of 5 ml per plant were made through the soil at concentrations of 0, 10 and 20 mg L^{-1}. Morphological variables of fresh and dry biomass were estimated, which are directly associated with yield. The results showed statistical differences in 10 of the 12 variables evaluated. The best values were obtained in treatments T5 and T8, which represent worm humus in combination with 10 and 20 mg L^{-1} of zinc oxide nanoparticles, respectively. On the contrary, the treatments (T7 and T9) that were grown on the bokashi substrate in combination with zinc oxide nanoparticles had the lowest values, even compared to the control. It is important to explore further the interactions that occur between nanoparticles with different organic substrates and the responses of agricultural crops.

Keywords:

Raphanus sativus L., nanomaterials, organic substrates, zinc oxide.

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Introduction

Globally, 12 million hectares of agricultural land are lost annually due to degradation caused by various factors, including the excessive use of agrochemicals (Liu *et al*., 2021). In this context, organic fertilizers have gained great relevance for their ability to restore degraded lands, offer environmental benefits, and provide essential nutrients to plants (Lestari *et al*., 2024).

The effectiveness of various organic materials, such as cattle manure, compost, biosolids, and biochar, in increasing carbon levels in the soil has been widely documented in scientific literature (Diacono and Montemurro, 2011). Recently, nanotechnology has emerged as a tool in agriculture, offering an alternative to conventional technologies (Nguyen *et al*., 2024). Its use ranges from seed germination, plant development, postharvest, to the storage and transport of agricultural products (Saritha *et al*., 2022).

In this sense, the potential of nanomaterials as possible nanofertilizers as an alternative to synthetic fertilizers continues to be explored (Semenova *et al*., 2024). Nanoparticles, due to their smaller size and larger surface area, possess unique properties, such as magnetism, electrical conductivity, physical resistance, and optical effects (Singh *et al*., 2023). Among the different nanoparticles, zinc oxide nanoparticles have stood out for their agricultural applications, such as nanofertilizers, nanopesticides, nanosensors, and as an antioxidant to improve crop protection (Priyanka *et al*., 2019).

These particles improve agronomic parameters under normal conditions and in the face of environmental stress (Mazhar *et al*., 2022), because zinc is crucial for cell division, chlorophyll and protein synthesis, and nucleic acid and carbohydrate metabolism in plants (Rehman *et al*., 2012). Recent studies showed that the application of Zn NPs in crops increases germination percentage (Sarkhosh *et al*., 2022), improves chlorophyll content (Ahmed *et al*., 2024), and promotes growth (Mahawar *et al*., 2024).

This research examined the interactions between zinc oxide nanoparticles (ZnO NPs) and different organic substrates, such as worm humus and bokashi, and their effect on radish (*Raphanus sativus* L.) crops. Radish is an economically important vegetable crop that is grown in various regions of the world, and its edible part (bulb) develops in the soil and is a rich source of calcium, phosphorus, potassium, vitamins, and polyphenolic compounds (Gui *et al*., 2017; Mahawar *et al*., 2024).

The annual production of radish is estimated at seven million tonnes per year, representing 2% of the total vegetable production in the world. It is projected that the planted area will increase in tropical areas due to tolerance to adverse environmental conditions and its short cycle duration (CABI, 2019). In addition, research suggests that optimal fertilization improves bulb yield and quality (Zhang *et al*., 2019), so it is relevant to explore technologies that increase productivity and quality with a lower environmental impact. The purpose of this study was to evaluate the effect of the application of different concentrations of ZnO NPs via drench on the growth and yield of *Raphanus sativus* L., in the substrates of common soil, worm humus, and bokashi.

Materials and methods

The research was conducted at the facilities of the Chiapas Regional Academic Center (CAR, for its acronym in Spanish)-Antonio Narro Autonomous Agrarian University (UAAAN), for its acronym in Spanish. It is located in the municipality of Cintalapa in the west of the state of Chiapas in southern Mexico. Its geographical coordinates are 16° 39’ north latitude and 93° 44’ west longitude, at an altitude of 540 m. The semi-warm subhumid climate predominates, with an average temperature of 24.5 °C and an average annual rainfall of 800 mm.

The experiment was carried out in a low tunnel covered with anti-aphid netting. The sowing took place on October 17, 2023, and the Champion radish variety was used. Two seeds were sown in each 10 x 30 cm polyethylene bag. During the development of the crop, direct irrigation was applied based on the plant’s requirements.

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Experimental design and treatments

A completely randomized design (CRD) consisting of nine treatments and five replications was used. The treatments were defined based on the different substrates (common soil, bokashi, and worm humus) and different concentrations of zinc oxide nanoparticles (0, 10 and 20 mg L^{-1}) (Table 1). Each replication was represented by two radish plants (10 per treatment). The bags were distributed in the low tunnel at a distance of 15 cm between bags and 40 cm between rows.

Table 1. Treatments with different substrates and concentrations of ZnO NPs.

Treatment	Description
T1	Common soil
T2	Worm humus
T3	Bokashi
T4	Soil+10 mg L^{-1} ZnO NPs
T5	Humus+10 mg L^{-1} ZnO NPs
T6	Bocashi+10 mg L^{-1} ZnO NPs
T7	Soil+20 mg L^{-1} ZnO NPs
T8	Humus+20 mg L^{-1} ZnO NPs
T9	Bocashi+20 mg L^{-1} ZnO NPs

Preparation and application of zinc oxide nanoparticles

The ZnO NPs were synthesized and provided by the Center for Research in Applied Chemistry (CIQA), for its acronym in Spanish located in Saltillo, Coahuila, Mexico. The morphology and structure of the nanoparticles were analyzed by transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM), showing quasi-spherical nanoparticles, with an average diameter of 16.49 nm and crystalline appearance (Garza-Alonso *et al*., 2023).

ZnO NPs are obtained functionalized with citric acid to improve their dispersion in aqueous media; the value of the Zeta potential obtained in distilled water was -64.6 ±0.1 mV (Toledo-Manuel *et al*., 2024). The ZnO NPs were prepared in the basic sciences laboratory of the CAR. First, 10 mg of ZnO NPs were dispersed in one liter of distilled water. Subsequently, AF Óptimos® dispersant was added to facilitate the stability and dispersion of the ZnO NPs in the solution.

This same process was repeated for preparing the dose of 20 mg of ZnO NPs. The solution of ZnO NPs was applied with a syringe at a dose of 5 ml per plant (10 ml per replication) via drench. Three applications were made, on 11, 19 and 26 days after sowing (DAS).

Response variables evaluated and data analysis

During the development of the experiment, four data recordings were made to determine the effects of the nanoparticles on different parameters of radish growth and yield. In the first three (11, 19 and 26 DAS), morphological data of the plant were recorded, such as the variables plant height (PH), number of leaves (NL), and stem diameter (SD). At harvest, 34 DAS, the following variables were estimated: bulb diameter (BD), bulb length (BL) and root length (RL).

Subsequently, the root, bulb, and leaves of radish plants were cut to calculate fresh root mass (FRM), fresh leaf mass (FLM) and fresh tuber mass (FTM). Root dry mass (DRT), dry leaf mass (DLM) and dry tuber mass (DTM) were also calculated. To do this, the root, bulb and leaves were dried in an Ecoshel 9052 oven at a temperature of 70 °C for 48 h.

The fresh and dry weights of the evaluated variables were obtained with a VE-204 precision analytical balance. PH, LB, and RL were measured with a graduated ruler, whereas SD and BD were measured with a manual vernier. The data collected were subjected to an analysis of variance (Anova). The homogeneity of variances of each response variable was verified with the Bartlett and

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Levene tests. Also, the Tukey mean comparison test (*p*# 0.05) was applied using the SAS® Studio statistical package.

Results and discussion

In the variables that evaluated the morphology of the plants (PH, NL, and SD), statistically significant differences were observed in NL and SD (Table 2). Regarding the NL variable, humus treatments in combination with the two doses of ZnO NPs (10 and 20 mg L^{-1}) expressed the highest values, although they were statistically equal to six of the treatments evaluated. This same trend was observed in SD; treatments with humus revealed a larger stem diameter. In both variables (NL and SD), the lowest values occurred in T9 (bokashi + 20 mg L^{-1} ZnO NPs).

Table 2. Effect of ZnO NPs on the morphology of radish plants in the different substrates evaluated.

Treatments	PH (cm)	NL	SD (mm)
T1: Soil	26.44 ±3.61^{a}	8.55 ±1.13^{ab}	0.93 ±0.13^{ab}
T2: Humus	24 ±2.34^{a}	8.6 ±1.07^{ab}	1.04 ±0.21^{ab}
T3: Bokashi	22.93 ±1.89^{a}	8.2 ±1.22^{ab}	0.8 ±0.2^{ab}
T4: Soil+10 mg L^{-1} ZnO NPs T5:	22.75 ±5.82^{a}	7.9 ±1.19^{ab}	0.82 ±0.33^{ab}
Humus+10 mg L^{-1} ZnO NPs T6:	24.65± 3.8^{a}	9.1 ±1.72^{a}	1.03 ±0.25^{ab}
Bocashi+10 mg L^{-1} ZnO NPs T7:	21.99 ±2.29^{a}	8 ±0.81V	0.81 ±0.23^{ab}
Soil+20 mg L^{-1} ZnO NPs T8:	22.11 ±3.2^{a}	7.77 ±0.83^{ab}	0.72 ±0.28^{ab}
Humus+20 mg L^{-1} ZnO NPs T9:	26.2 ±5.38^{a}	9.2 ±1.39^{a}	1.08 ±0.34^{a}
Bocashi+20 mg L^{-1} ZnO NPs	22.27 ±3.08^{a}	7 ±1.5^{b}	0.67 ±0.26^{b}

Means with different letters in the columns indicate significant differences between treatments (*p*< 0.05). PH= plant height; NL= number of leaves; SD= stem diameter.

Bokashi is a fertilizer that supplies macro and micronutrients to the soil, which are absorbed by plants. However, its effectiveness varies depending on the materials used in its preparation (Mendivil-Lugo *et al*., 2020). On the other hand, humus has been reported to have a positive effect on growth indicators in arugula (*Eruca sativa* Mill.) and peppermint (*Mentha piperita* L.) plants (Romero-Figueroa *et al*., 2013; Blanco-Villacorta, 2019). The positive effects are because humus increases soil fertility and contributes to the development of beneficial microorganisms (Lata-Álvarez and Llerena-Ramos, 2022).

Regarding the concentrations of ZnO NPs, Magdaleno-García *et al*. (2023) reported that the application of 100 mg L^{-1} of ZnO NPs increased plant height, leaf number and stem diameter in bell pepper (*Capsicum annuum* L.) seedlings. For their part, Fortis-Hernández *et al*. (2022) reported that ZnO NPs at concentrations of 20 and 25 mg L^{-1} caused an increase in height, leaf size, crown circumference, and fresh weight in the lettuce crop.

In this sense, positive effects could be found in both low and high concentrations. Nevertheless, the stimulating effect of nanoparticles on plants depends not only on their concentration, but also on their composition, size, physicochemical properties, and plant species (Juárez-Maldonado *et al*., 2019). As for the bulb variables, they showed statistical differences in the three variables evaluated (Table 3). The T3, which used only bokashi, presented a BD 29% smaller than that of the control, and the rest of the treatments were statistically equal to the control treatment.

Table 3. Effect of ZnO NPs on radish bulb variables in the different substrates evaluated.

Treatments	BD (cm)	BL (cm)	RL (cm)
T1: Soil	3.67 ±0.58^{a}	4.31 ±0.66##	9.55 ±3.46##
T2: Humus	3.88 ±0.72^{a}	4.47 ±0.8##	8.11 ±2.97##
T3: Bokashi	2.6 ±0.81^{b}	3.48 ±0.93^{a}	6.14 ±1.8^{a}

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Treatments	BD (cm)	BL (cm)	RL (cm)
T4: Soil+10 mg L-¹ ZnO NPs T5:	3.07 ±0.78ab	3.85 ±0.49ab	8.05 ±1.69ab
Humus+10 mg L-¹ ZnO NPs T6:	3.97 ±0.96a	5 ±0.83a	10.75 ±3.11a
Bocashi+10 mg LV¹ ZnO NPs T7:	3.36 ±0.46ab	3.91 ±0.76ab	6.69 ±1.44a
Soil+20 mg L-¹ ZnO NPs T8:	3.25 ±0.64ab	3.85 ±0.82ab	7.22 ±2.52ab
Humus+20 mg L-¹ ZnO NPs T9:	3.83 ±0.72a	4.42 ±1.63ab	9.76 ±3.47ab
Bocashi+20 mg L-¹ ZnO NPs	2.93 ±0.57ab	3.68 ±1.02ab	7.5 ±2.8ab

Means with different letters in the columns indicate significant differences between treatments ($p< 0.05$). BD= bulb diameter; BL= bulb length; RL= root length.

Regarding the variable's bulb and root length, the longest length was observed in T5 (humus + 10 mg L^{-1} ZnO NPs). On the other hand, the treatments that presented smaller BL and RL were T3. The positive effect of humus on radish bulb variables has been reported by Fleitas *et al*. (2013), who showed that the application of humus increased the polar and equatorial diameter and it also increased the weight of the radishes compared to the control.

Table 4 shows the impact of the different doses of ZnO NPs on the variables of fresh radish biomass (FRM, FLM, FTM). Treatments composed of bokashi substrate alone (T3) and with the application of 10 and 20 mg L^{-1} NPs (T8 and T9) showed the lowest average weights of FRM, 48.55, 46.8 and 31.25%, respectively, compared to T1.

Table 4. Production of fresh radish material subjected to ZnO NPs and organic fertilizers.

Treatments	FRM (g)	FLM (g)	FTM (g)
T1: Soil	4.16 ±1.17a	20.84 ±6.97a	28.99 ±10.11abc
T2: Humus	3.13 ±1.56ab	17.05 ±3.84ab	34.43 ±8.06ab
T3: Bokashi	2.14 ±0.89ab	14.74 ±4.02ab	16.78 ±6.87c
T4: Soil + 10 mg L-¹ ZnO NPs T5:	2.01 ±0.62ab	12.5 ±4.74b	20.51 ±8.58c
Humus + 10 mg L-¹ ZnO NPs T6:	4.13 ±2.02a	19.23 ±7.38ab	34.12 ±14.05ab
Bokashi + 10 mg L-¹ ZnO NPs	2.21 ±0.85ab	13.73 ±5.2ab	21.04 ±5.49ab
T7: Soil + 20 mg L-¹ ZnO NPs T8:	3.46 ±1.28ab	13.22 ±4.84ab	21.87 ±9.22ab
Humus + 20 mg L-¹ ZnO NPs T9:	3.14 ±1.37ab	20.99 ±7.05a	34.96 ±12.06a
Bokashi + 20 mg L-¹ ZnO NPs	1.3 ±0.33c	11.84 ±3.54b	18.79 ±6.04c

Means with different letters in the columns indicate significant differences between treatments ($p< 0.05$). FRM= fresh root mass; FLM= fresh leaf mass; FTM= fresh tuber mass.

Regarding the FLM variable, treatments T9 (bokashi + 20 mg L^{-1} of ZnO NPs) and T4 (soil + 10 mg L^{-1} of ZnO NPs) had the lowest average weights of fresh matter in leaves, with 43.18% and 40%, respectively, compared to the control. A trend of decrease in FLM was observed in the rest of the treatments, although without statistical differences compared to the control, except for humus + 20 mg L^{-1} of ZnO NPs (T8).

Concerning the FTM variable, treatments in which radish was grown with humus-based substrate, with or without nanoparticles, presented the highest average weight values. On the contrary, treatments T3 and T9 registered the lowest tuber weights. Nonetheless, all treatments were statistically the same as the control.

The application of ZnO NPs did not show a positive effect on the biomass production of radish plants. In contrast, an increase in fresh biomass has been reported in lettuce (*Lactuca sativa*) seedlings with applications of 50 mg L^{-1} of ZnO NPs (Galindo-Guzmán *et al*., 2022). On the other hand, the application of Zn nanoparticles can reduce root growth, because they accumulate in them and compete with other metals, which causes a phytotoxic effect (Yang *et al*., 2023).

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There are also factors that can alter the effect of nanoparticles on growth promotion, such as the dose used, the route of application, and the plant species used (Priyanca *et al*., 2019). In addition, according to the findings, it is important to consider the substrates or media in which the crops are established.

The DRM variable did not show statistical differences between treatments, although humus combined with nanoparticles (T5 and T8) presented the highest average weights. On the other hand, the DLM and DTM variables showed statistically significant differences between the treatments evaluated (Table 5). A trend of lower weight was recorded in the treatments with bokashi alone and with the two doses of ZnO NPs (10 and 20 mg L^{-1}), although they were statistically similar to the control.

Table 5

Dry matter production of radish plants under conditions of organic substrates and ZnO NPs.

Treatments	DRM (g)	DLM (g)	DTM (g)
T1: Soil	0.72 ± 0.16^{a}	1.42 ± 0.56^{ab}	1.05 ± 0.31^{ab}
T2: Humus	0.7 ± 0.24^{a}	1.33 ± 0.29^{ab}	1.06 ± 0.36^{ab}
T3: Bokashi	0.7 ± 0.21^{a}	1.07 ± 0.27^{ab}	0.7 ± 0.26^{b}
T4: Soil + 10 mg L^{-1} ZnO NPs T5:	0.56 ± 0.25^{a}	0.98 ± 0.33^{ab}	0.86 ± 0.26^{ab}
Humus + 10 mg L^{-1} ZnO NPs T6:	0.84 ± 0.33^{a}	1.52 ± 0.73^{ab}	1.33 ± 0.46^{a}
Bokashi + 10	0.63 ± 0.25^{a}	1.07 ± 0.29^{ab}	0.84 ± 0.2^{ab}
mg L^{-1} ZnO NPs			
T7: Soil + 20 mg L^{-1} ZnO NPs T8:	0.69 ± 0.29^{a}	1.05 ± 0.3^{ab}	0.98 ± 0.51^{ab}
Humus + 20 mg L^{-1} ZnO NPs T9:	0.92 ± 0.53^{a}	1.56 ± 0.79^{a}	1.17 ± 0.52^{ab}
Bocashi + 20	0.57 ± 0.17^{a}	0.85 ± 0.38^{b}	0.66 ± 0.22^{b}
mg L^{-1} ZnO NPs			

Means with different letters in the columns indicate significant differences between treatments ($p < 0.05$). DRM= dry root mass; DLM= dry leaf mass; DTM= dry tuber mass.

In this study, low doses of nanoparticles were evaluated in different substrates, observing slight effects on the morphological and productive behavior of radish plants. Future explorations could analyze higher concentrations, as it has been reported that the application of up to 1 000 mg L^{-1} of Zn nanoparticles in radish crops does not inhibit radish growth (Samuditha *et al*., 2024). Nanoparticles can inhibit or stimulate plants, although their effects on growth are not always clear (González Moscoso *et al*., 2022).

Reports on the negative effects of the interaction of bokashi substrate with ZnO NPs on biomass production variables are limited. Therefore, it is essential to research these interactions to understand the mechanisms that cause phytotoxicity. On the other hand, immature organic substrates maintain high microbial activity and may contain toxic compounds that could immobilize essential nutrients and therefore impair plant growth (Giang *et al*., 2024).

Conclusions

The application of the two concentrations of zinc oxide nanoparticles on different substrates showed statistically significant differences in some variables of morphology, biomass, and radish yield. In general, the best results occurred in treatments in which worm humus was used as a substrate, with 10 and 20 mg L^{-1} of ZnO NPs. In contrast, bokashi treatments, with or without nanoparticles, showed the lowest values.

This interaction has likely caused a phytotoxic effect. The results of this research provide a basis for the study of different doses of nanoparticles on organic substrates. In addition, future studies should focus on exploring the mechanism of joint interaction of ZnO nanoparticles with components present in organic fertilizers and agricultural crop responses.

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Effect of zinc oxide nanoparticles on radish development in organic substrates

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