Toxicity of Zinc Oxide and Titanium Oxide Nanoparticles on Lentil, Wheat, and Bean Seeds

Mohammad Reza Zare[1], Razieh Zolghadr[2], Somaye Yosaee[3], Mehdi Zare[4]

[1]Department of Environmental Health Engineering, School of Health, Larestan University of Medical Sciences, Larestan, Iran, [2]Department of Public Health, School of Health, Larestan University of Medical Sciences, Larestan, Iran, [3]Department of Nutrition Sciences, School of Nutrition and Food Sciences, Larestan University of Medical Sciences, Larestan, Iran, [4]Department of Occupational Health and Safety, School of Health, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

Abstract

Aim: Zinc oxide (ZnO) and titanium oxide (TiO_2) nanoparticles are used on a commercial scale in many countries. Despite numerous studies on the toxicity of nanoparticles, few have addressed their toxicity in edible grains. The aim of this study was to investigate the growth inhibition of ZnO and TiO_2 nanoparticles on lentil, wheat, and bean seeds. **Methods:** The ZnO and TiO_2 nanoparticles were investigated using transmission electron microscopy. Different concentrations of ZnO and TiO_2 nanoparticles (0.1, 1, 10, 100, and 1000 mg/l) were prepared in distilled water for irrigation of lentil, wheat, and bean seeds. The seeds were irrigated three times a day for 8 consecutive days, with 3 ml of solution per irrigation. To determine the toxicity of nanoparticles, the number of germinated seeds was counted, and the stem lengths were measured using a caliper. Data were analyzed to calculate the 50% lethal concentration (LC_{50}). **Results:** Exposure to all concentrations of both nanoparticles resulted in growth reduction in lentil seeds. Bean seeds showed decreased growth with ZnO nanoparticles and increased growth with TiO_2. Wheat seeds exhibited both growth increases and decreases at nanoparticle concentrations. **Conclusions:** This study showed that the toxic effect of nanoparticles depends on both the type of nanoparticle and the seeds. Furthermore, the concentration of nanoparticles plays a significant role in their toxicity. Therefore, more research is needed to explore the effects of different nanoparticles on plants in various growth environments to better understand their toxic effects on plant organs and their impact on plant growth and development.

Keywords: Plant grains, stem length, titanium oxide, toxicity, zinc oxide

Introduction

Nanomaterials have attracted considerable attention in recent years due to their favorable electrical, optical, mechanical, and chemical properties.[1] Many studies have been conducted on metal oxide nanoparticles with a focus on antimicrobial properties and self-elimination.[2] Altering microparticles to nanoparticles changes some of their properties. In this regard, the increase in the surface-area-to-volume ratio and the reduction of the particle size to the size of quantum particles are two important properties. Increasing the surface-to-volume ratio, which occurs as particle size decreases, causes the behavior of surface atoms to dominate over the internal atoms. This phenomenon affects the physical and chemical properties of the particle.[3-6] As the particles become sufficiently small, they begin to behave as quantum particles with new properties.[6] Among nanoparticles, Zinc oxide (ZnO) and titanium oxide (TiO_2) are widely used at the commercial scale in many countries.[7,8] ZnO nanoparticles are essential in various industries, including rubber, paint, electronics, glazes, and cosmetics.[7,9] They also have numerous sanitary and medical applications, as well as catalytic roles in the petroleum, gas, and petrochemical industries.[10] Zinc is toxic to aquatic animals at high concentrations, though it is necessary in low amounts for the production of nucleic acids and enzymes in the human body. Zinc is considered to be a heavy metal, which reduces the water quality.[11] Studies have shown that nanoparticles of ZnO can generate free radicals that damage DNA and potentially lead to cancer.[12] TiO_2 nanoparticles (10–20 nm

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Address for correspondence: Dr. Mehdi Zare, Department of Occupational Health and Safety, School of Health, Hormozgan University of Medical Sciences, Bandar Abbas, Iran. E-mail: mzare56@gmail.com

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in diameter) exhibit cytotoxic properties when exposed to UV-A rays.[13,14] TiO_2 nanoparticles, as one of the metallic oxide semiconductor nanocrystals, have received special attention in today's industrialized world and attracted the interest of many scientists.[15,16] TiO_2 nanoparticles have all the properties of TiO_2, and due to the small size of the particles, the contact surface with the material is increased and it works more effectively, so that these enhanced properties have caused concern about their potential effects on the environment.[17-20] Although TiO_2 is considered physiologically harmless for human, if it is inhaled, it can have harmful effects on rodents.[15] Nowadays, TiO_2 is used in the production of dyes, cosmetics, ceramics, and photocatalysts. It is also used in water and wastewater treatment.[21]

Despite their benefits, nanoparticles can also cause serious harm to the organisms by passing through the cell membranes and the blood–brain barrier.[22,23] ZnO, in the form of both, nanoparticles and non-nanoparticles, is more toxic than TiO_2.[24] The study of Behbodi *et al*. showed that by increasing the concentration of ZnO and CuO nanoparticles, leaf vegetation, number of seeds per pod, number of pods per plant, pod weight and length, 100 seeds weight, grain yield, biological yield, harvest indices, shoot length, and grain protein decreased, and the concentration of copper and zinc in seeds increased.[25]

Today, the advancement of technology and human access to new ways to use natural resources brings achievements that, in addition to many positive effects on human life, will have negative effects on nature.[26] Few studies have been conducted on environmental behavior, the amount of release in aquatic environments, and the potential risks of nanoparticles.[24,27] Furthermore, common chemical and pharmaceutical sciences have not only been unable to explain the dangers imposed by nanoparticles but have declared an urgent need to evaluate their biological effects.[28]

Despite numerous studies on the toxicity of nanoparticles, few have focused on edible seeds.[28] Therefore, this study aims to investigate the toxic effects of ZnO and TiO_2 on edible plant seeds.

Materials and Methods

Before starting the experiments, the ZnO and TiO_2 nanoparticles were examined using transmission electron microscopy (TEM) [Figure 1]. Stock solutions of ZnO and TiO_2 nanoparticles with concentrations of 1000 mg/l were prepared.

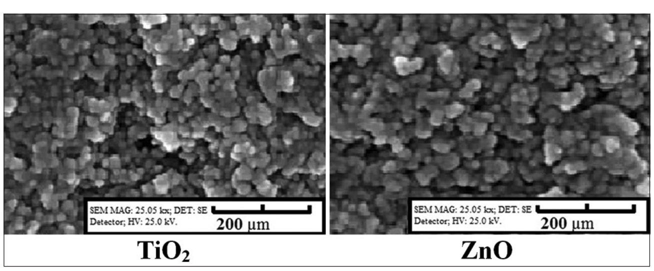

Figure 1: Transmission electron microscopy of the zinc oxide and titanium oxide nanoparticles. TiO2: Titanium oxide, ZnO: Zinc oxide

To prepare other concentrations (1000, 100, 10, 0.1, and 1 mg/l), the dilution method was used. To prepare the stock solution, 0.1 g of each of ZnO and TiO_2 nanoparticles were measured with a digital scale (with an error of ±0.0001 g), and 100 ml of distilled water was added to each of them. Three types of plant seeds including wheat, lentil, and beans were used in this study. Before their use in the tests, genetic purity was affirmed. For each grain, 14 plates were prepared, 4 plates as controls, and 5 plates for different concentrations of TiO_2 and ZnO. In each of the plates containing lentils and beans, 20 seeds were placed and 40 seeds were placed in each of the wheat plates. Control seeds were irrigated with distilled water and other seeds with different concentrations of ZnO and TiO_2 solutions. The seeds were irrigated three times a day with a pipette at certain time intervals, and the amount of solution in each irrigation was 3 ml for each type of seed. To maintain the moisture, the plates were covered by gauzes the before the germination, and after that the gauzes were removed and plates were placed in the sunshine for a specified period of time. The experiments were repeated twice, with each experiment lasting for 8 consecutive days. After the 8-day period, the number of germinated seeds was counted, and the stem length of the 5 seeds with the highest growth was measured using a caliper. The average test results were analyzed using probit analysis by the Statistical Package for the Social Sciences (SPSS) software, version 23 (IBM, Chicago, Illinois, USA) software. Probit analysis in SPSS found 50% lethal concentration (LC_{50}) from the log dose versus probit value graph using Equation 1.

$$y = mx + c \qquad (1)$$

Where:

y = 50% mortality (or 50% decline in growth)

m = The slope of log10 concentration-probit curve

c = The intercept

x = The log concentration).

Results

The TEM results showed that both nanoparticles had spherical shapes with an average size of 50 μm [Figure 1].

The results of the seed growth measurement are shown in Tables 1 and 2. The results of these tables were used for computing the LC_{50} as shown in Figure 2.

Zinc oxide nanoparticles

According to Table 1, in relation to lentil seeds, at a concentration of 10 mg/l, the reduction in growth was 4.6 cm, and at a concentration of 100 mg/l, this decrease was 5.04 cm. The lowest growth rate was observed at a concentration of 100 mg/l and the lowest growth was observed at a concentration of 1000 mg/l.

Reduced growth was observed in bean seeds in all concentrations, and this decrease continued by increasing the concentrations (except at the concentration of 100 mg/l). At

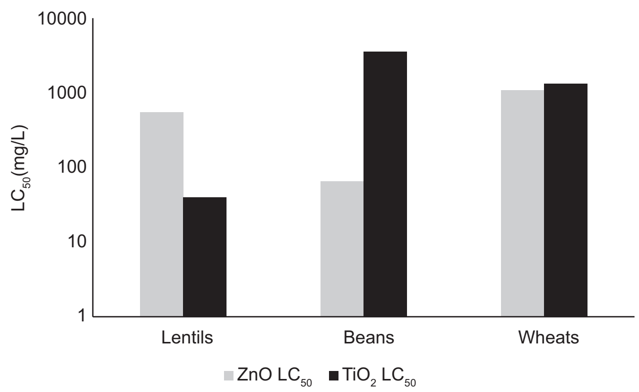

Figure 2: Titanium oxide and zinc oxide LC50 using lentil, bean, and wheat seeds after 8 days

a concentration of 0.1 mg/l, the plants had the lowest growth rate (1.82 cm) and the highest growth rate (7.46 cm) was observed at the concentration of 1000 mg/l.

In the case of wheat grains, in low concentrations (0.1, 1, and 10 mg/l), the longitudinal growth increased with increasing the concentration, but in higher concentrations (100 and 1000 mg/l), the longitudinal growth increased with increasing concentration.

Titanium oxide nanoparticles

According to Table 2, the growth of lentil seeds has decreased in all concentrations, but the decrease in growth did not have a linear trend. At the concentration of 100 mg/l, the greatest reduction in growth (4.23 cm) was seen and at the

Table 1: Average growth and decline in growth (cm) of lentil, bean, and wheat seeds in different concentrations of zinc oxide nanoparticles after 8 days

ZnO	The concentration of nanoparticles (mg/L)	Average growth (cm) after 8 days	Average growth in control group (cm) after 8 days	Decline in growth (cm) after 8 days	*P*
Lentils	0.1	3.68	5.25	1.57	0.01
	1	3.56	5.25	1.69	0.00
	10	4.6	5.25	0.65	0.07
	100	5.04	5.25	0.21	0.69
	1000	1.84	5.25	3.41	0.00
Beans	0.1	6.64	8.46	1.82	0.00
	1	4.62	8.46	3.84	0.00
	10	2.22	8.46	6.24	0.00
	100	5.7	8.46	2.76	0.00
	1000	1	8.46	7.46	0.00
Wheat	0.1	4.07	3.57	−0.5	0.47
	1	5.47	3.57	−1.9	0.01
	10	5.42	3.57	−1.85	0.00
	100	2.77	3.57	0.8	0.09
	1000	2.11	3.57	1.46	0.02

ZnO: Zinc oxide

Table 2: Average growth and decline in growth (cm) of lentil, bean, and wheat seeds in different concentrations of titanium oxide nanoparticles after 8 days

TiO_2	The concentration of nanoparticles (mg/L)	Average growth (cm) after 8 days	Average growth in control group (cm) after 8 days	Decline in growth (cm) after 8 days	*P*
Lentils	0.1	3.82	5.27	1.45	0.03
	1	3.76	5.27	1.51	0.01
	10	4.9	5.27	0.37	0.11
	100	1.04	5.27	4.23	0.00
	1000	1.52	5.27	3.75	0.00
Beans	0.1	11.36	5.08	−6.28	0.00
	1	6.56	5.08	−1.48	0.02
	10	6.22	5.08	−1.14	0.06
	100	6.8	5.08	−1.72	0.00
	1000	5.84	5.08	−0.76	0.09
Wheat	0.1	3.91	4.3	0.39	0.41
	1	4.96	4.3	−0.66	0.13
	10	5.35	4.3	−1.05	0.07
	100	4.53	4.3	−0.23	0.56
	1000	3.21	4.3	1.09	0.08

TiO_2: Titanium oxide

concentration of 10 mg/l, the lowest growth reduction was observed (0.37 cm). In the case of bean seeds, an increase in the growth was observed in all concentrations, and this increase in growth decreased with increasing the concentration (except in the concentration of 100 mg/l) so that the highest increase was in the concentration of 0.1 mg/l (−6.28 cm) and the lowest increase was observed at the concentration of 1000 mg/l (−0.76 cm). Regarding wheat grains, with increasing concentration, there was no linear trend in the growth reduction so that growth reduction was observed in concentrations of 0.1 and 1000 mg/l, and a growth increase was observed in concentrations of 1, 10, and 100 mg/l. At the concentration of 1000 mg/l, the highest reduction in growth rate was observed (1.09 cm) and at the concentration of 10 mg/l, the lowest reduction in growth rate was observed (−1.05 cm).

Discussion

This study aimed to investigate the toxic effects of ZnO and TiO_2 nanoparticles on lentil, wheat, and bean seeds. The results showed that the toxicity of the nanoparticles did not have a direct linear relationship with their concentrations. It was also found that ZnO and TiO_2 nanoparticles exhibit different toxic behaviors [Figure 2]. For example, the differences in LC_{50} values between ZnO and TiO_2 were more pronounced in beans compared to wheat.

The different toxic behaviors of ZnO and TiO_2 were also observed in wheat seeds. At a concentration of 1000 mg/L of ZnO nanoparticles, the growth reduction was 1.46 cm, whereas for TiO_2 nanoparticles, the reduction was 1.09 cm [Tables 1 and 2].

Zinc oxide nanoparticles

Regarding the effect of ZnO nanoparticles on lentil seeds, it was found that the growth reduction increases with increasing the concentration, except at 10 and 100 mg/l, where the growth reduction was lower [Table 1]. In the case of bean seeds, growth reduction was observed at all concentrations except at 100 mg/l. In contrast to the results of this study, Behbodi *et al*. showed that with increasing the concentration of ZnO nanoparticles, grain yield and length of the stem were decreased.[25] This discrepancy might be due to the adhesion and accumulation of nanoparticles at certain concentrations which increases the diameter of nanoparticles and causes less penetration of nanoparticles into organs and cells and, as a result, the toxicity of these substances will be recorded less than their actual toxicity.[29]

Regarding the effect of ZnO nanoparticles on wheat grains, growth reduction was observed at higher concentrations (100 and 1000 mg/l). This finding is parallel to the study of Lin and Xing that reported high concentrations of ZnO nanoparticles reduce the growth and biomass of wheat.[30] One of the reasons for this reduction in growth can be the reduction of root growth and subsequently the reduction of nutrient absorption.[25] The different toxicity effects are not only dependent on the solubility and size of the nanoparticles but also on their transfer and uptake in plant cells.[31] Generation of reactive oxygen species (ROS) by nanoparticles, also plays a role in their toxicity. The mechanism ROS generation differs in different nanomaterials and has yet to be fully explored.[31]

Titanium oxide nanoparticles

Regarding the effect of TiO_2 nanoparticles on bean seeds, an increase in growth was observed. This finding is consistent with the results of a study by Chehregani Rad *et al*. that investigated the effect of aluminum oxide nanoparticles on bean seeds and showed that these nanoparticles increase the germination percentage and root length of the bean seeds.[32] The study of Gao *et al*. also showed that exposure of spinach to TiO_2 nanoparticles stimulates the growth.[33] It seems that TiO_2 nanoparticles exert both toxic and nontoxic effects on plants by influencing redox homeostasis, leaf ultrastructure, morphology, stomata, photosynthesis, and the expression of genes associated with key metabolic pathways.[34] The mechanism of toxic effects can be elucidated through the stress induced by TiO_2 nanomaterials, which inhibits chlorophyll content and photosynthetic assimilation. TiO_2 nanoparticles can induce oxidative stress by increasing lipid peroxidation and ROS levels. In addition, TiO_2 nanomaterials may disrupt pyruvate metabolism and chlorophyll biosynthesis pathways.[34]

In the case of lentil seeds exposure to TiO_2 nanoparticles, growth reduction was observed but not in a linear trend.

In wheat seeds, exposure to TiO_2 nanoparticles decreased and also increased the growth at different concentrations, and similar to lentil seeds, growth changes did not follow a linear trend [Table 2]. This finding is consistent with Bina *et al*.'s study which showed that nanoparticles of TiO_2 at various concentrations and conditions could eliminate certain bacterial species; they also reported that TiO_2 nanoparticles show different toxic effects at different concentrations.[35]

Conclusions

This study showed that the toxic effect of nanoparticles depends on both the type of nanoparticle and the seeds. However, it can be generally claimed that the toxicity of ZnO nanoparticles is more than TiO_2 nanoparticles. By increasing the concentration of TiO_2 and ZnO nanoparticles, especially ZnO nanoparticles, the growth of plant seeds is more affected and, in most cases, it causes a decrease in the growth of seeds. Further research is essential to explore the effect of nanoparticles on plants in different plant growth environment to better understand their toxic impact on plant organs, growth, and development and their potential entry into the food chain.

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Ethics code

The authors approved this study in Ethical Number: IR.LARUMS.REC.1397.005.

Conflicts of interest

There are no conflicts of interest.

Author contributions

Mohammad Reza Zare: Study conception and design, Data collection, Writing – review and editing; Razieh Zolghadr: Analysis and interpretation of results, Draft manuscript preparation; Somaye Yosaee: Study conception and design, Analysis and interpretation of results, Draft manuscript preparation; Mehdi Zare: Study conception and design, Analysis and interpretation of results, Draft manuscript preparation, Writing – review and editing; All authors reviewed the results and approved the final version of the manuscript;

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