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Effect of Titanium Dioxide and Titanium Dioxide-Silver Nanoparticles on Seed Germination of Lettuce (*Lactuca sativa*)

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Abstract

The aim of this study was to evaluate the effects of TiO_2 and TiO_2Ag nanoparticles on seed germination of lettuce (*Lactuca sativa*). Two parameters were examined in this study: number of seed germination and root-shoot elongation. Lettuce seeds in petri dishes were separately treated with different concentrations (control, 10, 20, 40, 60, 80 and 100 mg/L) of 5 mL TiO_2 and TiO_2Ag nanoparticle suspensions. Number of seed germination was increased at increasing TiO_2 nanoparticles concentration especially at 80 and 100 mg/L TiO_2 with compared to control and also determined that radicle increased with TiO_2 and TiO_2Ag treatment at 20 mg/L and 10 mg/L respectively.

Keywords:, Lactuca sativa, Nanotoxicology, Phytotoxicity, Seed germination

Titanyum Dioksit ve Titanyum Dioksit-Gümüş Nanopartiküllerinin Marul (*Lactuca sativa*)Tohumunun Çimlenmesine Etkisi

Öz

Bu çalışmanın temel amacı TiO_2 ve TiO_2Ag nanopartiküllerinin marul (*Lactuca sativa*) bitkisinin tohum çimlenmesine etkisinin değerlendirilmesidir. Çalışmada iki farklı parametre değerlendirilmiştir: tohum çimlenme sayısı ve kök-gövde uzaması. Marul tohumları petri kaplarına yerleştirildikten sonra farklı derişimlerdeki (kontrol, 10, 20, 40, 60, 80 ve 100 mg/L) 5'er mL TiO_2 ve TiO_2Ag nanopartikül süspansiyonlarına maruz bırakılmıştır. Sonuçta marul tohumlarının çimlenme sayılarının nanopartikül derişimi arttıkça arttığı ve bu artışın özellikle 80 ve 100 mg/L TiO_2 konsantrasyonlarında gerçekleştiği belirlenmiştir. Ayrıca kök radikula uzamasının 20 mg/L TiO_2 ve 10 mg/L TiO_2Ag nanopartikül derişimlerinde artış gösterdiği tespit edilmiştir.

Anahtar Kelimeler: Lactuca sativa, Nanotoksikoloji, Fitotoksisite, Tohum çimlenmesi

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1. INTRODUCTION

Nanotechnology is an application of scientific knowledge to manipulate the nature at the nanoscale, distinctively with bulk materials [1]. Nanoparticles (NPs) are sized between 1-100 nm which have some unique properties. Nanoparticles exist into the nature as a result of some human activities, this existence can be deliberate (e.g. soil and water remediation, fertilizer usage) and/or unintentional releases (e.g. water, air and application of sewage sludge to the soil) [2]. Physical and chemical properties of nanoparticles influence their impacts on environment and living organisms. There are less information in the literature about properties and application of nanoparticles and also due to concerns over possible adverse effects of nanotechnology on the living organisms so, many question marks about the nanoparticles [1,2].

Nanoparticles are present in the environment because of their increasing use and have the ability to enter, move into different parts of organism, and damage organisms. Overproduction, common use, and disposal of NPs will fatefully cause their release into atmospheric, aquatic and terrestrial environment [3]. Silver (Ag) and titanium dioxide (TiO_2) nanoparticles are the most widely used types of nanoparticles. On the basis of common applications, considerable fraction of Ag nanoparticles and TiO_2 nanoparticles will find their way into aquatic and terrestrial ecosystems in time [4].

The photosensitivity and biocidal properties of Ag and TiO_2 nanoparticles have been found to be toxic to various organisms. The previous studies provide valuable information for further understanding the fate and toxicological risks of Ag and TiO_2 nanoparticles. However, there is scarce information about the effects of coexisting Ag and TiO_2 nanoparticles on the fate and ecological risks. On the one hand, coexistence of both of these nanoparticles may exert enhancing toxicity due to synergistic effects of Ag and TiO_2 nanoparticles, on the other hand, antagonistic effects may also be observed because of the absorptive effects of TiO_2 NPs on Ag ions [4]. Ag nanoparticles are commonly used as an antibacterial agent, so this causes the presence of Ag nanoparticles in the environment via wastewater. Silver nanoparticles may easily enter the environment following their release from Ag nanoparticles and the major products which contained textiles, cosmetics, plastics, medical equipment, food containers, washing machines, pesticides and paints [5]. Ultimately, most nanoparticles will end up in the aquatic and terrestrial environment. Recently, Ag nanoparticles fate in the environment has been investigated and its potential eco-toxicological affect inspire the interests [6].

Plants are the most important component between soil and nanoparticles so they may serve potential pathway for NPs transport and accumulation in the food chain [7,8]. Crop plants are more likely to be exposed to nanoparticles than other plants because of application of treatment sludge in soils and usage of Ag nanoparticles in pesticides. Seed exposure to nanoparticles may cause decrease in seed germination, poor healthy root-shoot growth and less evapotranspiration and after the root exposure, the roots uptake the nanoparticles caused phytotoxic and genotoxic effects [6]. There is an increasing amount of investigation effect of some nanoparticles on various organisms but there is much less knowledge on the effect of TiO_2 nanoparticles on plants [3,7,9-11] compared to animals [12]. In the literature some researchers studied phytotoxicity of different nanomaterials (Multi-walled carbon nanotubes, Aluminum oxide, Zinc oxide, Al, Zn Cerium dioxide, Lanthanum (III) oxide, Gadolinium (III) oxide, Ytterbium oxide) and effect on seed germination of some plants (rye grass, corn, radish, rape, tomato, lettuce, wheat, cabbage, and cucumber). They reported that the higher concentrations of nano sized ZnO and Zn nanoparticles inhibited the germination of corn and ryegrass, respectively [13]. Lin and Xing [3], reported that the nanoparticles species and concentrations most effective parameters in root growth. Other researchers reported that the CeO_2 nanoparticles affected root elongation only lettuce in high concentration and the other three types of nanoparticles significantly affected root elongation at the same concentration [13,14]. Barrena et al.

determined that metallic nanoparticles (Fe_3O_4, Ag and Au) had low effect on lettuce and cucumber [15]. Most of the nanoparticles cause toxic effects on plants in high concentrations and the toxicity threshold changes with species of plants and nanoparticles [3,16] also the surface characteristic of nanoparticles is important in the phytotoxic effects [17].

The main objective of this study is to determine the effect of TiO_2 and TiO_2Ag nanoparticles on seed germination of lettuce (*Lactuca sativa*), also we measured the root and shoot length of germinated plants.

2. MATERIALS AND METHODS

2.1. Chemicals

Titanium dioxide and TiO_2Ag nanoparticles (~30 nm) were commercially prepared by Dr. Birol Karakaya with sol-gel method and the characterization of the TiO_2 and TiO_2Ag nanoparticles suspension was performed by TEM technique.

2.2. Seed Germination and Root Elongation Assay

According to US Environmental Protection Agency, seed germination and root elongation assay are two standard indictors to determine the phytotoxicity [13]. The seeds selected were of uniform size to minimize error in seed germination and seedling vigor. Experiments were performed at 25^{o}C using 10 seeds in 10 cm petri dishes. Filter paper cut to fit regular petri dishes was used as inert material. A double-layer of filter paper was placed in the petri dish. Ten lettuce seeds were placed in every petri dishes and were separately treated with different concentrations (0 (control), 10, 20, 40, 60, 80 and 100 mg/L) of TiO_2 and TiO_2Ag NPs suspensions. After the 7 days seed germination, average lettuce plants were chosen in every petri dish and root-shoot length were measured. Germination was determined based on the number of seeds germination in a petri dish, and also root-shoot length was measured by millimetric paper. Two parameters are examined in this study: number of seed germination and root-shoot elongation.

3. RESULTS

Seed coat protects the embryo against adverse environmental conditions and it causes inhibition of nanoparticles through into the seed via semipermeable structure, but it may not be possible at any time [10,18]. Nanoparticles size and type are the principal factors at this stage. Titanium dioxide and TiO_2Ag NPs application significantly affected on lettuce seed germinations. The comparative of seed germination results can be observed from the table (Table 1). The results showed that the effect of nanoparticles can be change nanoparticles type and concentrations. As shown in Figure 1, at high concentration especially TiO_2Ag NPs application enhanced the seed germination.

Table 1. Number of the seed germinations after nanoparticles application

Nanoparticles Application, mg/L	**TiO_2Ag**	**TiO_2**
0	7	8
10	8	6
20	7	8
40	9	9
60	8	7
80	10	9
100	10	9

It is important that the healthier plants depend on having a healthy root system. The root and shoot elongation help to assess the health of plants, so in this study, the root and shoot length measurements was realized for understanding the effect of TiO_2 and TiO_2Ag nanoparticles on lettuce plants at seedling stage (Table 2).

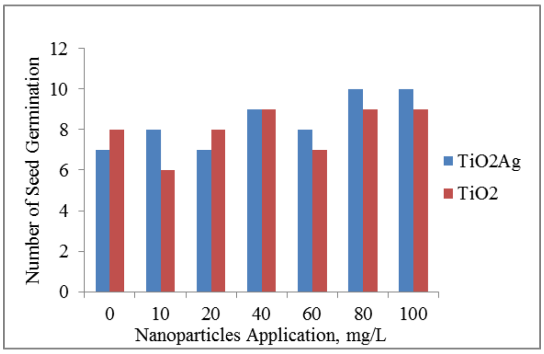

Figure 1. Effect of TiO_2 and TiO_2Ag NPs on lettuce seed germination

The effect of TiO_2 and TiO_2Ag nanoparticles on root and shoot elongation of lettuce was shown in Figure 2a and Figure 2b, respectively. TiO_2 nanoparticles had adverse effect on shoot elongation, after application of the 20 mg/L TiO_2 nanoparticles there was significant decreases in shoot length. On the other hand, TiO_2 nanoparticles promoted the root elongation, especially at 20 mg/L, as shown in Figure 2a.

Table 2. Root and shoot length after application TiO_2Ag and TiO_2 nanoparticles

	TiO_2Ag		TiO_2	
NPs Application (mg/L)	**Root Length (cm)**	**Shoot Length (cm)**	**Root Length (cm)**	**Shoot Length (cm)**
0 (Control)	2.8	3.1	2.9	2.6
10	3.4	3.7	4.0	3.6
20	3.1	3.2	5.2	2.8
40	2.0	2.5	3.4	2.1
60	2.1	3.1	4.5	2.0
80	2.4	2.7	3.1	1.9
100	2.8	2.2	2.4	1.8

However TiO_2Ag nanoparticles promoted the seed germination, it had adverse effect on root-shoot elongation. Titanium dioxide-silver nanoparticles had adversely effect on root and shoot elongation, especially at high concentrations. Minimum elongation was observed at the 40 mg/L TiO_2Ag concentration as shown in Figure 2b. Both of these nanoparticles effects on lettuce as visual were shown in Figure 3, briefly.

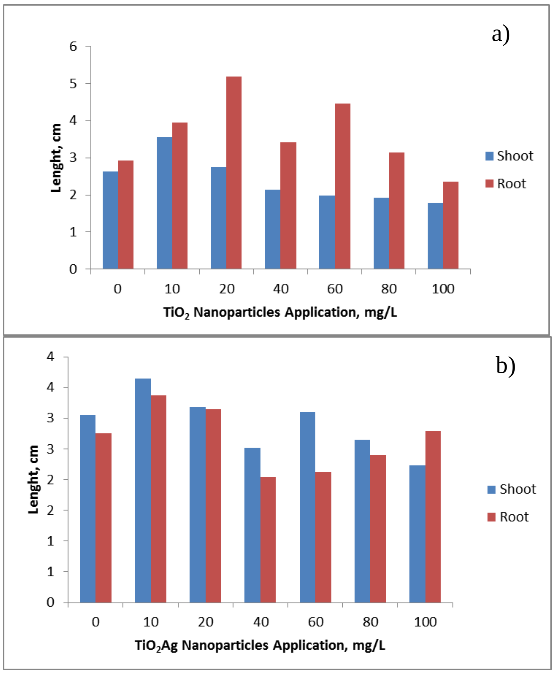

Figure 2. Effect of different TiO_2 (a) and TiO_2Ag NPs concentration on root and shoot elongation of lettuce

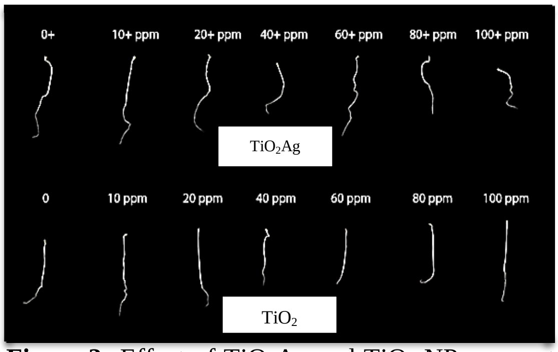

Figure 3. Effect of TiO_2Ag and TiO_2 NPs on root and shoot elongation of lettuce

It is known that the seed germination and root elongation tests are widely used for determining the phytotoxic effects in plants. These tests have some advantages such as rapid, sensitivity, low cost and suitability for different chemicals [3]. Limited reports in the literature emphasized the positive, negative or no effects of nanoparticles on plants. But it is clear that same nanoparticles have different effects on different plants.

4. CONCLUSION

Last decades of nanotoxicology research has been increased in the literature. MWCNT, fullerene and metallic (e.g. ZnO, TiO_2) nanoparticles are test materials which commonly used for determining and understanding better the nanotoxicity mechanisms of nanoparticles. Plants are the most important component between soil and nanoparticles. Seed coat is the most important part of the seed germination which has semipermeable structure. This structure allows or not allows nanoparticles to pass through the seed coat. If the nanoparticles pass through the coat, toxic effects may occur in the seed germination stage. If the nanoparticles cannot pass through the coat, the toxic effects may occur in the seedling stage. The pore size of the plants and nanoparticles size are the most affective parameters in this stage. Nanoparticles increase or inhibit the root and shoot elongation or it may be ineffective. In this study, we evaluated that the effect of ~30 nm TiO_2 and TiO_2Ag nanoparticles on seed germination and root-shoot elongation of lettuce. Titanium dioxide and TiO_2Ag nanoparticles application promoted the seed germinations, especially in high doses and also application of TiO_2 nanoparticles promoted the root elongation almost whole concentrations as compared with the control, but there was decreasing with increasing concentrations on shoot elongation. TiO_2Ag nanoparticles have inhibitory effects on root and shoot elongations. The lack of information, the possibility of adverse impacts on the environment, safety and sustainability, are still a challenge [2]. Transfer in the food chain of nanoparticles via plants should be considered in the life cycle analysis of nanoparticles, especially through the roots. Future studies should also need to clarify the nanotoxicology, possible uptake by plants and translocation of nanoparticles to different parts of plants [19].

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