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# Role of EDDS and ZnO-nanoparticles in wheat exposed to $TiO_2$Ag-nanoparticles

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**Abstract:** Nanotechnology is a manipulation of nature that has emerged through the use of basic sciences, material science and engineering at the nano-scale. The interaction between biological environment and nanoparticles--nanoparticles or nanoparticles-organic materials is not yet well-understood. The toxic effects of nanoparticles on plants were investigated and it was proved that they caused morphological and physiological changes in plants. This study aimed to determine the effects of $TiO_2$Ag nanoparticles alone, co-application of ZnO nanoparticles--$TiO_2$Ag nanoparticles, and co-application of EDDS-$TiO_2$Ag nanoparticles on seed germination, seedling vigor, radicle and plumule elongation of two different wheat species. In the experimental stage, ten seeds were placed in petri-dishes with a double layer of filter paper which was used as an inert material. Then 5 mL of $TiO_2$Ag, ZnO+$TiO_2$Ag, and EDDS+$TiO_2$Ag suspensions were added to every petri dish. Results showed that the maximum SVI was determined at the concentration of 50 $mg \cdot L^{-1}$ $TiO_2$Ag+EDDS for bread wheat and the minimum SVI was observed at 100 $mg \cdot L^{-1}$ $TiO_2$Ag nanoparticles concentration for durum wheat. The effect of both nanoparticles--nanoparticles interaction and the other chemicals-nanoparticles interaction on the ecosystems should be evaluated.

## Introduction

The use of nanoparticles in many industrial products is constantly increasing and thus nanoparticles release into the environment due to waste disposal and/or accidents (Cambier et al. 2018, Cox et al. 2017, Krzyżewska et al. 2016). As a result of this existence, the environmental impacts of agricultural applications of nanotechnology have recently been investigated by many researchers (Baker et al. 2017, Bobik et al. 2017, Josko et al. 2017, Savithramma et al. 2012, Servin and White 2016). Therefore, it is necessary to monitor the effects of nanoparticles on ecosystems (Krzyżewska et al. 2016). The effects of nanoparticles can vary by plant species and nanoparticles type, exposure pathways and also environmental conditions. Titanium dioxide ($TiO_2$), zinc oxide (ZnO) and silver (Ag) nanoparticles are the most used types of nanoparticles in different industries such as cosmetics, pharmaceutical, painting and also agricultural production in the worldwide. $TiO_2$ and ZnO nanoparticles are used generally as effective opacifiers due to their chemical properties, and Ag nanoparticles are generally used as antifungal and antibacterial materials in many additives (Cox et al. 2017, Krzyżewska et al. 2016). Also, these three nanoparticles widely used in agricultural products as fertilizers or pesticides (Baker et al. 2017, Du et al. 2017, Shalaby et al. 2016, Tarafdar et al. 2013, Zapor 2016). Tareq et al. (2017) showed that the synthesized Ag nanoparticles give fruitful results against microbes and pathogens.

Chelating agents are commonly used in many industrial and agricultural applications like nanoparticles. Synthetic chelating agents including ethylenediamine-tetraacetic acid (EDTA), ethylene diamine-N,N'-disuccinic acid (EDDS), diethylene triamine penta acetic acid (DTPA), etc. are the best known. These biodegradable synthetic materials are used to help the removal of critical metals from a soil-plant system or to hinder the metal precipitation. Thus they can enhance the essential metallic nutrients' uptake by plants (Pinto et al. 2014) and are preferred to substitute the non-biodegradable chelating agents. Among these agents, EDDS has a relatively low molecular weight, is biodegradable and non-toxic to living organisms.

Soil type, pH value, and soil organic matter content are the most influencing parameters to determine the effects of contaminants on plants. Metal extraction with plants may be increased by synthetic chelating agents (Bech et al. 2014, Sidhu et al. 2017) because of their strong complexes with metal ions (Pinto et al. 2014). Sidhu et al. (2017) investigated the effects of ammonium molybdate and EDDS on Pb mobilization and extraction by *Coronopus didymus*. Researchers used contaminated soil at a concentration of 1200 and 2200 $mg \cdot kg^{-1}$ Pb. Moreover, 2 $mmol \cdot kg^{-1}$ ammonium molybdate and EDDS were used to determine the effects on Pb uptake and accumulation by *C. didymus*. Also, the authors determined if these chelators alleviate the uptake of lead from contaminated soil, or not. Results showed that the chelators enhanced the uptake and accumulation of Pb.

Plants are used to determine the potential toxicity of nanoparticles because they can uptake and accumulate nanoparticles easily (Narendhran et al. 2016). Uptake and accumulation of nanoparticles by plants may change germination rate, as well as shoot and root length (Siddiqi and Husen 2017). In plant's life, seed germination and embryonic development are important stages. In these stages, germinating seed (embryo, radicle, and plumule) interfaces with different environmental materials for the first time and is very sensitive to environmental conditions (Jaouani et al. 2018). Seed germination is the most valid test to determine the toxic effects of different environmental contaminants on plants because it is sensitive, fast and effective. Seed germination and seedling growth generally inhibited by metals, but the inhibition degree changes depending on plant species, metal types, and metal concentrations (Bae et al. 2016). Wheat is a recommended plant for the testing of organic and inorganic contaminants by the US Environmental Protection Agency (US EPA), the US Food and Drug Administration (US FDA), and the Organization for Economic Co-operation and Development (OECD) (Faraji and Sephri 2018, Priac et al. 2017). For this reason, in this study, two types of wheat (bread wheat and durum wheat) were selected as plant material.

The overall objective of the present work was to investigate the efficacy of EDDS and ZnO nanoparticles to alleviating $TiO_2$Ag nanoparticle stress in wheat seeds.

## Materials and methods

### Chemicals

$TiO_2$Ag and ZnO nanoparticles were synthesized using a combination of sol-gel and hydrothermal methods with a small change as described by Doğaroğlu and Köleli (2017) and Ito et al. (2008). Ethylenediamine-disuccinic acid (EDDS) was prepared at the concentration of 10 mg·L$^{-1}$ from commercial EDDS (~35% in $H_2O$, Sigma-Aldrich). The average size of ZnO and $TiO_2$Ag nanoparticles was determined using zeta-sizer Ver. 6.32 (Malvern Instruments Ltd.).

### Experiments

Two different wheat species were used in this study. Bread wheat (*Triticum aestivum* L. İkizce-96) and durum wheat (*Triticum durum* Desf. Ankara-98) were purchased from Mersin Province, Turkey. The seeds of the uniform size were randomly selected to minimize error in the germination stage. The seeds of bread and durum wheat were soaked in 70% ethanol for 30 s and then 5% sodium hypochlorite solution for 10 min. Sterilized seeds were rinsed by running deionized water 5 times for 5 min. The seeds were placed on a double layer of filter paper in petri dishes (10 seeds per dish). The petri-dishes were exposed to 5 mL of test chemicals in the manner as follows: C (control), TA50, TA50+E, TA50+Z, TA100, TA100+E, TA100+Z, TA200, TA200+E, TA200+Z where; E, Z, and TA refers to EDDS, ZnO nanoparticles, and $TiO_2$Ag nanoparticles respectively.

It was proved in our previous study that the wheat seeds have good resistance to different nanoparticles. For example, seed germination and plumule-radicle elongation parameters have not affected by $TiO_2$ nanoparticles at low concentrations (5, 10, 20, 40, and 80 mg kg$^{-1}$) (Doğaroğlu and Köleli 2017). For this reason, the concentrations of $TiO_2$Ag nanoparticles were chosen as 50, 100, and 200 mg·L$^{-1}$. Three different applications were used in the experiments. In the first application, bread wheat and durum wheat seeds were treated with different concentrations of 50, 100 and 200 mg·L$^{-1}$ $TiO_2$Ag nanoparticles.

Control seeds were treated with 5 mL of distilled water only. In the second application, the seeds were treated with co-application of $TiO_2$Ag+ZnO nanoparticles. In this step, ZnO nanoparticles (10 mg·L$^{-1}$) and $TiO_2$Ag nanoparticles (50, 100 and 200 mg·L$^{-1}$) were co-applied in a total 5 mL test chemical. And in the third application, the seeds were exposed to $TiO_2$Ag nanoparticles (50, 100 and 200 mg·L$^{-1}$) and EDDS (10 mg·L$^{-1}$) in a total 5 mL test chemical. Dishes were stored in dark at 25ºC for seven days.

The number of germinated seeds was recorded every day at the same hour for 7 days. After 7 days, each seedling's radicle and plumule length was measured by millimetric paper (3 replicates per treatment). Germination percentage (%) was calculated as described by Afrakhteh et al. (2013), Mahmoodzadeh et al. (2013), Manesh et al. (2018) in the following Equation (1) and seedling vigor index (SVI) was calculated as described by Faraji and Sepehri (2018), Prasad et al. (2012) in the following Equation (2).

$$\text{Germination Percentage (GP)} = \text{TNSG}/\text{TNST} \times 100 \quad (1)$$

TNSG is the total number of seeds germinated; TNST is the total number of seed tested.

$$\text{Seedling Vigor Index (SVI)} = \text{GP}(\%) \times \times (\text{Radicle Length+Plumule length}) \quad (2)$$

### Statistical analysis

The statistical significance levels of difference for all measurements were evaluated using SPSS Version 22.0 software (SPSS, USA) at the level of $p \leq 0.01$ with one-way analysis of variance (ANOVA).

## Results and discussion

### Nanoparticles characterization

The average size of ZnO and $TiO_2$Ag nanoparticles was determined using a Zeta-sizer and it was smaller than 100 nm for both nanoparticles types. The FE-SEM image showed that the ZnO nanoparticles have the amorphous structure with a primary particle size of 31.54 nm and $TiO_2$Ag nanoparticles have a rounded structure with a primary size of 78.17 nm. Also, EDX analysis which indicates the elemental content of ZnO and $TiO_2$Ag nanoparticles as shown in Figure 1a and b. The EDX analyses showed that the ZnO and $TiO_2$Ag nanoparticles are composed of Zn and oxygen in Figure 1a, and, Ti, Ag, and oxygen (Fig. 1b).

### Germination percentage

Seeds were the first and relatively sensitive part of the plant which interacted with organic and/or inorganic contaminants in environmental growth medium (Bae et al. 2016, Shah et al. 2010). The seed treated with $TiO_2$Ag nanoparticles-alone had no effect on germination percentage (GP) of bread wheat but at the concentration of 50 and 200 mg·L$^{-1}$ $TiO_2Ag$ nanoparticles increased the germination percentage of durum wheat. However co-application results showed that the GP of bread wheat (Fig. 2a) seed treated with $TiO_2Ag$+ZnO and $TiO_2Ag$+EDDS decreased at high concentration (TA200+Z and TA200+E) in contrast to durum wheat (Fig. 2b) ($p \leq 0.01$). The minimum GP was determined at TA100+E and TA200+E concentration in bread wheat, on the other hand, the minimum and maximum GP was observed at TA100 concentration and TA200 and TA200+Z concentration in durum wheat, respectively ($p \leq 0.01$). Nanoparticles effect on seed germination processes change depending on plant species, as well as nanoparticles type, concentration, and shape. Doğaroğlu and Köleli (2016) reported that $TiO_2Ag$ nanoparticles-alone enhanced the seed germination of lettuce, like the result of durum wheat.

### *Plumule and radicle growth*

Primary roots (radicles) are the first organs of growing plant embryo and the first organs exposed to pollutants in plants' life, hence, toxicity in plants may be less apparent in shoots than in roots (Faraji and Sepehri 2018). In this study, plumule and radicle elongation results showed that durum wheat is more sensitive than bread wheat to $TiO_2Ag$ nanoparticles-alone, application of TA+Z, and application of TA+E. The plumule of durum wheat seed treated with $TiO_2Ag$ nanoparticles-alone increased with respect to control except 100 mg·L$^{-1}$ $TiO_2Ag$ nanoparticles (TA50) concentration. On the other hand, the presence of EDDS with $TiO_2Ag$ caused better elongation of plumule than co-application of $TiO_2Ag$ nanoparticles with ZnO nanoparticles (Fig. 2c) ($p \leq 0.01$). For bread wheat, the growth of plumule increased with increasing all the test chemicals concentrations, the lowest plumule length was measured at TA200 as 11.1 cm (Fig. 2d) ($p \leq 0.01$).

Radicle elongation of durum wheat increased with the application of $TiO_2Ag$ nanoparticles-alone and TA50+E and TA200+E in comparison with control, except at TA100 (2.63 cm). Figure 2e shows that TA50+Z and TA100+Z application decreased radicle elongation of durum wheat but it was increased at TA200+Z application. Co-application of $TiO_2Ag$ nanoparticles with ZnO nanoparticles (TA+Z) or $TiO_2Ag$ nanoparticles with EDDS (TA+E) decreased the adverse effects of $TiO_2Ag$ nanoparticles on radicle elongation of durum wheat. ZnO nanoparticles negatively affected radicle elongation of durum wheat in low concentration, in contrast to EDDS. The minimum radicle elongations of durum wheat were measured at 100 mg·L$^{-1}$ concentrations of all test chemicals (TA100, TA100+Z, and TA100+E) ($p \leq 0.01$). On the other hand, all test chemicals negatively affected radicle elongation of bread wheat at increasing concentrations, except at TA50+E application (18.20 cm), in comparison with control (15.52 cm) (Fig. 2f) ($p \leq 0.01$). Manesh et al. (2018) indicated that $TiO_2$ nanoparticles increased the root elongation of radish seedlings at 10 mg·L$^{-1}$ and 200 mg·L$^{-1}$, but the other concentrations (1 mg·L$^{-1}$, 100 mg·L$^{-1}$, 500 mg·L$^{-1}$, 1000 mg·L$^{-1}$, and control) caused its inhibition. At low concentration, co-exposure of $TiO_2$ nanoparticles and $CdCl_2$ significantly increased the root elongation of radish.

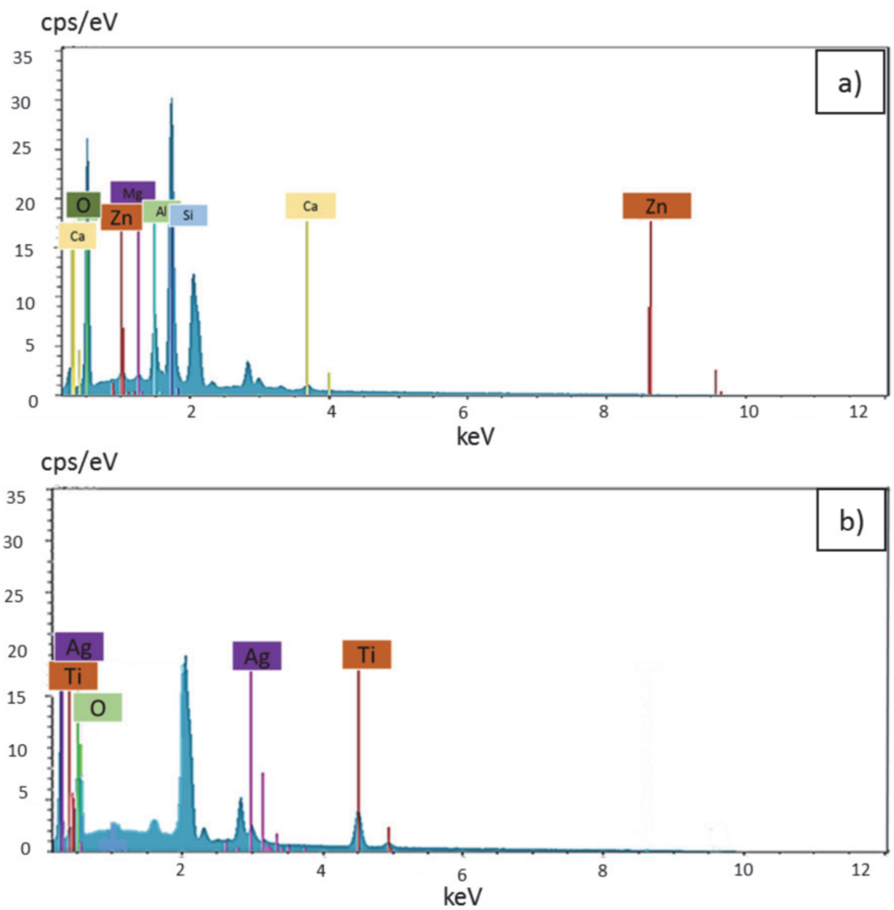


**Fig. 1.** The EDX pattern of a) ZnO and b) $TiO_2Ag$ nanoparticles used in experiments

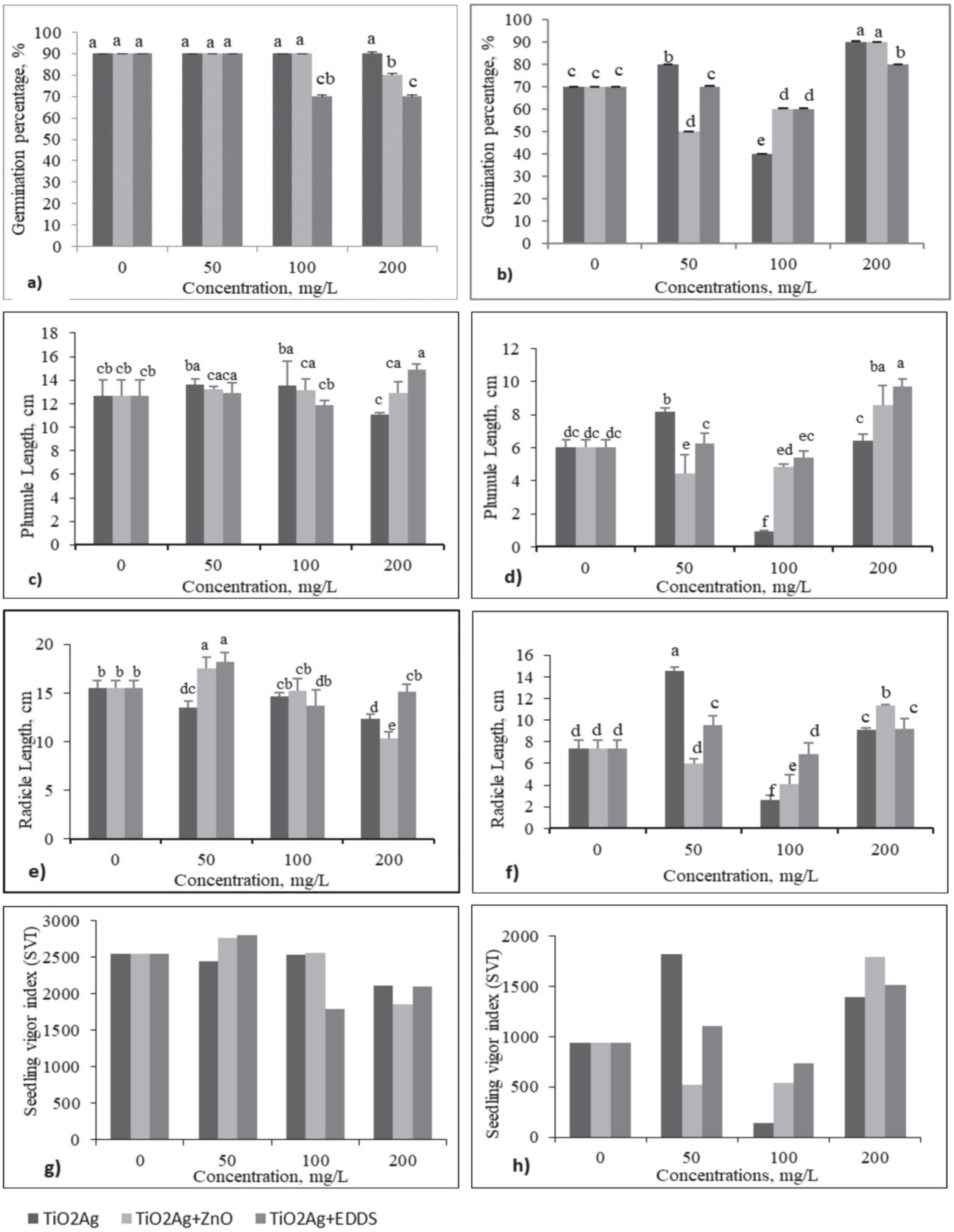


**Fig. 2.** Germination percentage, plumule length, radicle length and seedling vigor indexes of a, c, e, g) bread wheat and b, d, f, h) durum wheat at different test chemicals concentrations

### *Seedling vigor index (SVI)*

Vigor index of bread wheat was not significantly affected by $TiO_2Ag$ nanoparticles-alone, the maximum and minimum vigor index was determined at the control group (2540 SVI) and 200 mg·L$^{-1}$ (2104 SVI) concentration, respectively. The seedling vigor index decreased with increasing TA+Z and TA+E concentrations, except 50 mg·L$^{-1}$. The maximum and minimum SVI were determined at 50 mg·L$^{-1}$ (2767.20) and 200 mg·L$^{-1}$ (1858.67) for treatment with TA+Z and also treatment with 50 mg·L$^{-1}$ TA+E caused the maximum SVI as 2796 and the minimum SVI was determined as 1788.15 at 100 mg·L$^{-1}$ TA+E concentration (Fig. 2g). Faraji and Sepehri (2018), investigated effects of $TiO_2$ nanoparticles (500, 1000 and 2000 mg·L$^{-1}$) and sodium nitroprusside (SNP, as nitric oxide donor) (100 µM) on germination and seedling growth of wheat under cadmium stress (0, 50, and 100 mM), and the authors showed that co-application of $TiO_2$ and SNP dramatically affected seed vigor index. It was reported that the vigor index decreased at increasing Cd concentration.

Vigor index of durum wheat seedlings negatively affected by $TiO_2Ag$ nanoparticles-alone and minimum and maximum vigor index was determined at 100 mg·L$^{-1}$ (142.49 SVI) and 50 mg·L$^{-1}$ (1820 SVI) concentrations, respectively. Such inhibitory effects have been shown by Mahmoodzadeh et al. (2013) on canola seeds. The authors reported that the maximum and minimum vigor index were determined at 2000 mg·L$^{-1}$ and 1500 mg·L$^{-1}$ $TiO_2$ nanoparticle concentration, respectively.

It was also reported that the vigor index of canola was negatively affected at 100 mg·L$^{-1}$ $TiO_2$ nanoparticle concentration. In the presence of EDDS, the minimum SVI was determined at 100 mg·L$^{-1}$ TA+E concentration as 737 and the maximum SVI was 1511.20 in durum wheat at 200 mg·L$^{-1}$ concentration. On the other hand, the presence of ZnO nanoparticles (TA+Z) caused a decrease in low concentration and then the vigor index increased in high concentration. The lowest and highest SVI was determined at 50 mg·L$^{-1}$ (521.92 SVI) and 200 mg·L$^{-1}$ (1796.50 SVI) TA+Z concentration, respectively (Fig. 2h).

## Conclusion

Within my knowledge, no study has been conducted to determine the effects of the interaction of $TiO_2Ag$ nanoparticles with other nanoparticles and/or organic acids on the seedling growth of plants. In this study, possible effects of the interaction of $TiO_2Ag$ nanoparticles with ZnO nanoparticles and EDDS on the elongation of radicle and plumule length of bread and durum wheat during seed germination were investigated. It was clear that the nanoparticles have toxic effects on seed germination and plant growth, but at the same time, co-application of different nanoparticles or chemicals may reduce the adverse effects of just one nanoparticle, such as $TiO_2Ag$. It was known that the effects of nanoparticles may change with plant species. This study proved that the effects of test chemicals can change between durum wheat and bread wheat. The durum wheat was more sensitive to $TiO_2Ag$ nanoparticles than the bread wheat and also co-application of $TiO_2Ag$ nanoparticles with ZnO nanoparticles or EDDS reduced the adverse effects of $TiO_2Ag$ nanoparticles. As a result, it is necessary to investigate nanoparticles-nanoparticles or nanoparticles-other chemicals interaction on soil-plant systems, in order to be able to obtain a clearer knowledge of the effects of nanoparticles on the environment. The widespread application of nanoparticles in many industries must be carefully evaluated for environmental impacts before using.

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