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Co-application of EDDS and ZnO nanoparticles with TiO_2Ag nanoparticles on rye

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ABSTRACT

The aim of this study was to determine the effects of $nTiO_2Ag$, co-application of nZnO and $nTiO_2Ag$ and co-application of EDDS as an organic acid with $nTiO_2Ag$ on seed germination, seedling vigor, plumule and radicle length of rye. Ten seeds were placed in petri-dishes with double layer of filter paper which used as an inert material. Then 5 mL $nTiO_2Ag$, nZnO-$nTiO_2Ag$ and EDDS-$nTiO_2Ag$ suspensions were added to every petri dish with different concentrations (control, 50, 100 and 200 mg/L). Treatment of nZnO-$nTiO_2Ag$, especially at concentration of 50 mg/L, promoted the germination rate, seedling vigor, plumule and radicle elongation. The highest seedling vigor index (SVI) was observed at concentration of 50 mg/L nZnO-$nTiO_2Ag$. Seedling vigor index of rye seeds was decreased after treatment of $nTiO_2Ag$. The plumule elongation increased with treatment of all test chemicals and radicle elongation was increased nZnO-$nTiO_2Ag$ and EDDS-$nTiO_2Ag$ exposure compared to control. This is the first report on the effect of co-application of ZnO-TiO_2Ag nanoparticles and co-application of EDDS-TiO_2Ag nanoparticles on rye growth.

1. Introduction

Nanotechnology is a manipulation of nature that has emerged through the use of basic sciences, material science and engineering at nano-scale. Nowadays, nanomaterials are widely used in different fields such as cosmetics, agriculture, water and air purification and wastewater treatment, environmental remediation, and food additives as shelf life extender and packaging agents. Their unique properties (shape, size, huge specific surface area, high reaction activity, etc.) [1,2] make nanoparticles different from the other chemicals and contaminants. Toxicological studies have been increased because of increasing production, usage and disposal of nanoparticles pose a threat to the environment [3].

Plants are used to determine the potential toxicity of nanoparticles due to they can uptake and accumulate the nanoparticles easily [4]. Uptake and accumulation of nanoparticles by plants may change germination rate, shoot and root length [3]. The use of nanoparticles in agriculture was mostly theoretical in last decade [5] but using in fields increase day by day. Although, nanomaterials have some advantage, the negative effects of nanoparticles on plant productivity, soil microbes and environment should not overlook [6].

The interaction between two different nanoparticles, nanoparticles and organic materials and/or nanoparticles and biological environment is not yet well understood. Co-application of nanoparticles may cause them to accumulate in the soil and could be threatened plants. Plants can generally be exposed to both different nanoparticles and inorganic and organic contaminants, such as chelating agents through the soil in many ways [7].

Titanium dioxide nanoparticles ($nTiO_2$), zinc oxide nanoparticles (nZnO) and silver (nAg) nanoparticles are the most widely used nanoparticles in many industries. Previous reports have shown these nanoparticles responsible for toxicity in plants [8]. Cai et al. [7] indicated that, $nTiO_2$ will accumulate in soil and its concentration will increase accordingly. Silver nanoparticles are mostly used as antimicrobial agents in plant protection process [2,9,10]. Several papers reported effects of TiO_2, ZnO, and Ag nanoparticles in plants. Doğaroğlu and Köleli [11] reported that $nTiO_2$ and titanium dioxide-silver nanoparticles ($nTiO_2Ag$)

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Note: This study was presented at International Advanced Researches and Engineering Congress 2017 (IAREC'17) enhanced the seed germination of lettuce *(Lactuca sativa)*. Jian et al [12] investigated impact of ZnO nanoparticles on pearl millet, tomato, and wheat. Authors determined that 1000 mg/L nZnO inhibited wheat seed germination around 60%.

Chelating agents such as ethylene-diaminedisuccinic acid (EDDS) are highly effective in remediating heavy metal-contaminated soils.

The impact of nanoparticles on living organisms has attention from researchers. Determining these impacts is important to sustainable and healthy environment. It should be noted that the amount of nanoparticle-contaminated field increases day by day because of using nano-products. Plants are the most important component between soil and nanoparticles. Using plants for soil remediation has some advantages such as, sustainability, low cost and environmentally friendly, as against other soil remediation techniques. Seed germination rates and seedling growth are most measured to assess nanoparticles effects [13]. Thus, the first and most important step of this study was to investigate seed germination rate of rye under exposure of different nanoparticles combinations and nanoparticles-chelating agent (EDDS). It was not only investigated seed germination, but also seedling vigor, plumule and radicle length of rye and the effectiveness and impacts of EDDS due to its low cost, good degree of biodegradability and high efficacy of metal extraction on seed germination and seedling growth of rye.

2. Materials and Methods

2.1 Chemicals

Dr. Birol Karakaya synthesized TiO_2Ag and ZnO nanoparticles using a combination of sol-gel and hydrothermal methods. Ethylen-diamine disuccinic acid (EDDS) was prepared at concentration of 10 mg/L from commercial EDDS (~ 35% in H_2O, Sigma-Aldrich). The average size of ZnO and TiO_2Ag nanoparticles was determined using zeta-sizer Ver. 6.32 (Malvern Instruments Ltd.) using a 1 mL disposable cuvette.

2.2 Preparation of Suspensions and Treatment

TiO_2Ag nanoparticles suspensions were prepared at concentrations of 50, 100 and 200 mg/L. 10 mg/L ZnO nanoparticles and 10 mg/L EDDS solution was prepared from 100 mg/L stock nZnO solution and from 40 mM EDDS stock solution.

Rye seeds were purchased from Mersin Province, Turkey. Rye was selected for the present study as a representative cereal crop. This test plant can resist to aridity, cold climates and high saline soil, due to having strong root systems.

The seeds of uniform size were selected to minimize error in germination assay. Experiments were performed using 10 seeds in 10 cm petri dishes for seven days. The seeds were sterilized in 70% ethanol for 30 s and then exposed to 3% sodium hypochlorite for 10 min. After the sterilization, seeds were shaken in ultrapure water five times for 5 min. Double-layer of filter paper cut and placed in petri dishes was used as inert material. Ten seeds were placed in every petri dish and were separately treated with 5 mL test chemicals. Petri dishes placed in a dark chamber till germination at 25 ^oC.

Three different applications were realized in the experiments. In the first application, rye seeds treated with $nTiO_2Ag$ at concentration of 50, 100 and 200 mg/L. Control seeds were treated only 5 mL distilled water; in the second application, the seeds treated with co-application of $nTiO_2Ag$ and nZnO. In this step 10 mg/L nZnO and the concentration of 50, 100 and 200 mg/L $nTiO_2Ag$ were co-applied. Control groups include only 10 mg/L nZnO. And in the last application, the seeds treated with co-application of the concentration of 50, 100 and 200 mg/L $nTiO_2Ag$ and 10 mg/L EDDS. Control groups include only 10 mg/L EDDS.

Number of germinated seeds was recorded every 24 hours for 7 days. After 7 days from the date of germination, primary root and shoot length of each seedling was measured by millimetric paper (3 replicates per treatment). Germination percentage (%) was calculated as described by Mahmoodzadeh et al [14]; Afrakhteh et al [15] in the following Formula (1) and seedling vigor index (SVI) was calculated by the formula described by Prasad et al [16] in the following Formula (2).

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

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

$$\text{Seed Vigor Index} = \text{Germination percentage (\%)} \times (\text{Root length} + \text{Shoot length}) \quad (2)$$

3. Results and Discussion

3.1 Nanoparticles Characterization

The average size of ZnO and TiO_2Ag nanoparticles was determined using a zeta-sizer. Average size of nZnO was 31.5 nm, and average size of $nTiO_2Ag$ was 78.2 nm.

3.2 Effect of $nTiO_2Ag$, Co-application of $nTiO_2Ag$-nZnO, and $nTiO_2Ag$-EDDS on Seed Germination

Seed germination and root elongation assays are most valid methods to assess the phytotoxicity of organic and inorganic chemicals [17]. The effects of $nTiO_2Ag$, co-application of nZnO-$nTiO_2Ag$, and EDDS- $nTiO_2Ag$ at various concentrations (50, 100 and 200 mg/L) were tested on rye seeds and shown in Figure 1. The results indicated that exposure of 10 mg/L nZnO and 10 mg/L EDDS was increased germination rate at concentration of

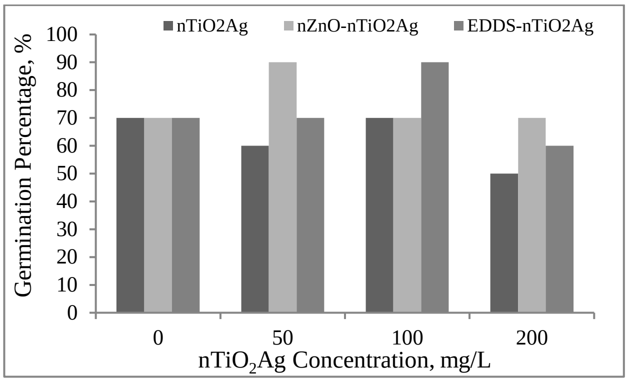

Figure 1. Seed germination percentage of rye; test chemicals include 10 mg/L nZnO or 10 mg/L EDDS and different concentration $nTiO_2Ag$, control groups include only distilled water.

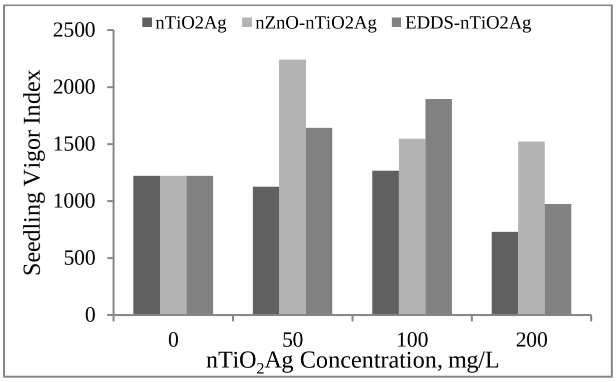

Figure 2. Seedling vigor index of rye; test chemicals include 10 mg/L nZnO or 10 mg/L EDDS and different concentration $nTiO_2Ag$, control groups include only distilled water.

50 and 100 mg/L $nTiO_2Ag$, respectively. The minimum seed germination was observed at concentration of 200 mg/L just $nTiO_2Ag$ as 50%, and the maximum germination percentage was observed both at concentration of 10 mg/L nZnO-50 mg/L $nTiO_2Ag$ co-application and 10 mg/L EDDS-100 mg/L $nTiO_2Ag$ co-application as 90%.

There are contradictory results about the plant-nanoparticles interaction in literature depending on plant species, nanoparticles types and application dose. Doğaroğlu and Köleli (2017) demonstrated that nZnO and nTiO2 nanoparticles had no effect on seed germination of barley [8], while exposing barley to nAg caused partial inhibition (13% germination rate) at concentration of 1500 mg/L was reported [18]. Xiang et al (2015) showed that nZnO did not affect germination rates at concentration of 1–80 mg/L chinese cabbage [19]. Another study showed that seed germination of ryegrass and flax was inhibited by nAg at 750 and 1500 mg/L [18].

3.3 Effect of $nTiO_2Ag$, Co-application of nZnO-$nTiO_2Ag$, and EDDS- $nTiO_2Ag$ on Seedling Vigor

Seedling vigor is one of the quality parameter of seed and it is a supplemental data for seed germination [12]. The seedling vigor index of rye was shown in Figure 2. Vigor index of control groups of rye was 1221.89. The maximum seedling vigor index was observed at concentration of 50 mg/L nZnO- $nTiO_2Ag$ (2239.0), and the minimum data was obtained at concentration of 200 mg/L $nTiO_2Ag$ (730.56). Treatment of 10 mg/L nZnO promoted the seedling vigor in all concentration of $nTiO_2Ag$. Similar result was observed by Prasad et al. (2012) when peanut seeds were treated with at concentration of 1000 mg/L nZnO [16].

Also treatment of 10 mg/L EDDS increased the seedling vigor except 200 mg/L $nTiO_2Ag$ concentration with respect to control rye seedlings.

Seedling vigor index of rye seeds was decreased after treatment of $nTiO_2Ag$. Co-application of nZnO-$nTiO_2Ag$ and co-application of EDDS-$nTiO_2Ag$ was promoted the seedling vigor. These results proved that the interaction between different nanoparticles and interaction between chelating agent and nanoparticles is important for the uptake of nanoparticles by plants and environmental fate and risk of nanoparticles.

3.4 Effect of $nTiO_2Ag$, Co-application of nZnO-$nTiO_2Ag$, and EDDS- $nTiO_2Ag$ on Seedling Growth

In order to understand the possible effects of co-application of different metal oxide nanoparticles and metal oxide nanoparticles-chelating agents, seedling growth (plumule and radicle elongation) was investigated in this study and shown in Figure 3.

Roots have an important role in plant development. The embryonic root called as radicle grows during the seed germination process. If the nanoparticles come into contact with seeds in the germination process, first translocation may realize on surface of radicle.

Plants exposed to nanoparticles can accumulate nanoparticles in their roots and can translocate these nanoparticles to other parts such as leaves, seeds or flowers. The first shoot which grows from seed is called as plumule. Nanoparticles may transportation from root to leaves and flowers via shoot. These accumulation and translocation factors depends on plant and nanoparticles type, exposure pathway and concentration, and agglomeration properties of nanoparticles [20]. Plumule and radicle is important for determination toxicity of nanoparticles so, the elongation of these parts were investigated in this study. Figure 3a and b present the effects of $nTiO_2Ag$, co-application of nZnO-$nTiO_2Ag$ and co-application of EDDS-$nTiO_2Ag$ on plumule and radicle elongation of rye. The plumule elongation increased with treatment of all test chemicals compared to control. The maximum plumule length was observed at concentration of 10 mg/L EDDS-50 mg/L $nTiO_2Ag$ as 10.29 cm, and the minimum data was obtained at concentration of 10 mg/L EDDS-200 mg/L $nTiO_2Ag$ as 6.08 cm. It was clear that EDDS and nZnO was more effective than $nTiO_2Ag$

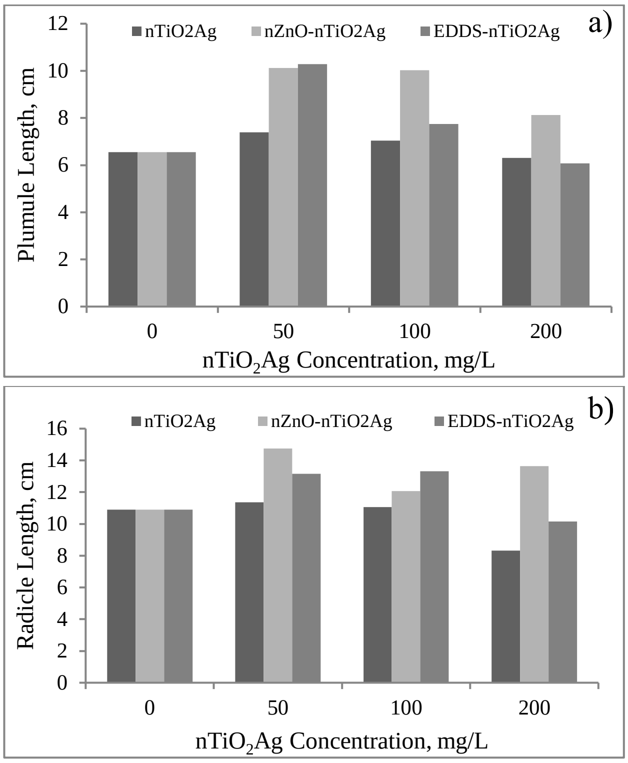

Figure 3. a)Plumule b)Radicles length of rye; test chemicals include 10 mg/L nZnO or 10 mg/L EDDS and different concentration $nTiO_2Ag$, control groups include only distilled water.

on plumule elongation of rye. On the other hand, the plumule elongation was more sensitive to co-application of EDDS-$nTiO_2Ag$ than co-application of nZnO-$nTiO_2Ag$, especially at high concentration. Yoon et al (2014) reported that the root and shoot length of soybean was inhibited by nZnO application as compared to control [21]. Figure 3b shows that the radicle elongation was increased nZnO-$nTiO_2Ag$ and EDDS-$nTiO_2Ag$ exposure at 50 and 100 mg/L concentration. At highest concentration $nTiO_2Ag$ (200 mg/L) and co-application of 10 mg/L EDDS-200 mg/L $nTiO_2Ag$ inhibited the radicle elongation of rye. The maximum radicle length was measured at concentration of 10 mg/L nZnO-50 mg/L $nTiO_2Ag$ co-application as 14.76 cm and the minimum length was measured at concentration of 200 mg/L $nTiO_2Ag$ as 8.31 cm, as Ghosh et al., (2010). The authors reported that 6 mM $nTiO_2$ reduced the root elongation of onion [22]. In Figure 3b, it is clear that the co-application process (nZnO-$nTiO_2Ag$ and EDDS-$nTiO_2Ag$) has positive impact on radicle elongation.

4. Conclusions

Last decades of nano-toxicology research has been increased in the literature. Metallic (e.g. ZnO, TiO_2, TiO_2Ag) nanoparticles are test materials which commonly used for determining and better understanding the nano-toxicity mechanisms. Today, a substantial amount of nanoparticles release inevitably into the environment and cause soil, air, and water pollution.

Plants are the most important component between soil and nanoparticles. Seed coat is the most important part of the seed germination. Its semipermeable 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. In this study, we evaluated that the effects of co-application of nZnO-$nTiO_2Ag$ and co-application of EDDS-$nTiO_2Ag$ on seed germination, seedling vigor index and plumule and radicle elongation of rye. We determined the nZnO promoted the seed germination, seedling vigor index and radicle-plumule elongation especially at concentration of 10 mg/L nZnO-50 mg/L $nTiO_2Ag$.

Future studies should also need to clarify the nano-toxicology, possible uptake and translocation of nanoparticles by plants. The potential toxic effects of commonly used nanoparticles on human health, plants, and animals are still unknown. This and the other studies in the literature demonstrate that there is too little data about the fate and effects of nanoparticles in the environment and need for more research in this area.

This is the first report on the effect of co-application of nZnO-$nTiO_2Ag$ and co-application of EDDS-$nTiO_2Ag$ on rye seed germination, seedling vigor and plumule and radicle elongation.

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