The Effect of Zinc Oxide Nanoparticles on Safflower Plant Growth and Physiology

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***Abstract*—In this paper, a study of the effect of ZnO nanoparticles on safflower growth and physiology was performed. Each of these elements plays a particular role in the plant life, the presence of these elements is necessary for plant's life cycle and growth. Zinc deficiency causes the biggest problems in safflower's production. Considering the importance of nanoparticles in today's world, this research investigated the effect of Zinc oxide nanoparticles on the concentration of guaiacol peroxidase, polypeptide oxidase, dehydrogenase and malondialdehyde in four plant sample groups in greenhouse and laboratory conditions. Results of showed that malondialdehyde enzyme increased with different treatments of various concentrations of Zinc oxide. The enzyme guaiacol oxidase increased at concentrations of 100 mg/L and polyphenol oxide at concentrations of 10 and 500 mg/L and dehydrogenase in 1000 mg/L and decreased in other treatments. In addition to showing the effect of nanoparticles in plants, these findings determine the beneficial concentrations of nanoparticles that have a positive effect on the level of enzymes in plants.**

Keywords-nanoparticles; malondialdehyde; guaiac peroxidase; polyphenol oxidase; dehydrogenase

I. INTRODUCTION

Nanotechnology is a science and technology that has the ability to produce new materials, tools, and systems with atomic and molecular levels and has the potential to revolutionize agriculture. Nanotechnology properties, their use, and their probable harmful effects in the environment, have created concerns [1]. Most studies have been conducted on the effects of nanoparticles on aquatic systems. Nanoparticles enter the food chain through plants. Although the level of these nanoparticles is not remarkable at present, one day it can reach a critical level. Some of the aims of the use of nanoparticles are plant pathology, specific agricultural problems related to pathogens, and the creation of new ways of product's protection [2]. Nanoparticles are used in many agricultural and biomedical departments [3]. Nanoparticles are also used in a variety of biological programs [4]. Nanotechnology, as the science of working with the smallest particles, has increased hopes for overcoming the problems the agricultural sector is facing. Nanotechnology has significant impact on important areas of the agricultural sector, such as the modernization of new cultivars, the development of intelligent chemical delivery systems and pesticides, the integration of intelligent systems for food processing and packaging, the removal of herbicide debris from plants and soil [5].

Safflower (Carthamus tinctorius L.) raceme is of dense clump type, covered by a number of involucres [6, 7]. The cultivation of safflower was initially done to extract the color of its petals in order to color the fabric and decorate the food, but today it is also cultivated for the production of oils, meal, pharmaceuticals, cosmetics and ornament [8]. Safflower has 8 species and 50 different cultivars and high alkaloids content. In the past, its infused leaves have been used to treat bee stings and prevent uterine bleeding [9]. All plant parts have alkaloids. Ajmalicine, placed at the root of this plant, is used to treat high blood pressure [10]. Serpentine is also used to treat circulatory system abnormalities [11]. Among the 300 types of terpenoid indole alkaloids identified in safflower, Vinblastine and Vincristine are of particular importance: these two alkaloids have antitumor activity by binding to the microtubules and stopping cell division during mitotic metaphase and they are used in chemotherapy of many cancers for over 40 years [12-14]. A small amount of these two alkaloids in the safflower plant, complexity and multi-stage synthesis path of these two alkaloids, the lower effectiveness of semi-synthesis drugs and high demand for this two drugs are some of the most important reasons for attracting researchers to the use of biotechnology and tissue culture techniques to increase the production of these vital alkaloids in the plant. Finding the factors affecting the increase in the biosynthesis of Vinblastine and Vincristine or their monomers, such as Catharanthine and Vindolin, are always important research objectives [15].

II. THEORETICAL FOUNDATION

A. Zinc in Plants

1) Physical and chemical Zinc characteristics

Zinc is a transition metal with an atomic number of 30 and it is the 23rd element on the earth's crust in terms of frequency. The Zinc element has five stable isotopes. Heavy isotopes exist at the roots and light isotopes exist in the stems of the plant. 30 Zinc radioisotopes have been identified. Among these radioisotopes, Zn^{65} radioisotope has the highest half-life and is used as a tracer in plants. Zinc has the characteristic of Lewis acid, that is, its radius relative to rainfall is smaller than that of other elements. The electron configuration of the Zinc organic complexes is in the form of a hexagonal octahedral, although there are pentagonal and tetragonal shapes [16]. In solutions, Zinc is in the form of Zn^{2+}, and unlike Fe^{2+} and Cu^{2+} in physiological conditions, it has a stable oxidation and resuscitation mode due to the fact that its organic electrons are complete [16, 17]. Zinc creates salts with halides, sulfates, nitrates, ferrates, acetates, thiocyanates, perchlorates, flocyls, and cyanides [16, 18].

2) Zinc and soil

Zinc concentrations in soils typically range from 10 τo 300 mg/g in organic and mineral soils respectively, and in most agricultural soils it varies from 50 to 66 mg/g [19, 20].

3) The sources of Zinc entry into soil

Zinc entry to soil is done by different sources:

- Zinc entry to soil can be the result of blasting of rocks like asphalite (Zns), smithsonite ($ZnCO_3$), hemorhphyte ($Zn_4\ (OH)_2\ Si2\ O_7\ H_2O$) [16].
- Zinc in the soil can be the result of natural phenomena such as volcanoes, forest fires, surface dust, etc. [21].
- Zinc enters soil through fossil fuels, mining waste, phosphate fertilizers, limestone, agricultural fertilizers and elastic coating [19, 22].

4) Zinc shapes in soil

Zinc can be seen in soil in the following forms:

- As a building part of some clay minerals, it can be replaced with magnesium in a crystalline network.
- A portion of Zinc in the form of cation is exchangeable with surface absorption of clay minerals.
- A part of Zinc in the soil is seen as a mixture of organic material.
- An amount of exists Zinc in the soil solution in the form of Zn^{2+} [23].

5) Factors of Zinc accessibility to plants

Some of the factors that control the accessibility of Zinc to plants are the total amount of Zinc in the soil, soil pH, the amount of organic material, the amount and type of clay, the amount of calcium carbonate, oxidation and regeneration conditions, microbial activity in the rhizosphere, soil moisture, other element accumulation and weather [24].

Soil pH: Soil pH is one of the most important factors for Zn access to plants. Zinc activity in the soil is controlled by pH. Increase of the soil pH causes a significant reduction in the Zinc dissolution. When PH increases, Zinc concentration decreases rapidly [18].

Organic material: Soil organic material plays an important role in the Zinc accessibility to growing plants. Soil organic material improves the physical properties of the soil, and also helps the maximum increase of the absorption of metal ions by plants with the formation of soluble complexes with metal ions. With the reduction of soil organic material, the dissolution of Zinc also reduces. The usage of organic material to increase the access of elements in soil depends on the type of source and the formation of organic material [25].

Calcareous soils: Zinc deficiency in calcareous soils is due to the strong absorption of Zinc on the surfaces of calcium carbonate ($CaCO_3$) because the levels of calcium carbonate are sites for deposition and adsorption of metal ions. Zinc emission factor in the calcareous soils is 5 times lower than that of acidic soils [26].

Soil Salinity: The problem of Zinc deficiency in the dry and semi-arid lands is due to soil salinity. Salinity increases the absorption of cadmium and reduces Zinc absorption. Because there is an antagonistic relationship between Zn and Cd, the increase of Cd absorption under salinity stress (NaCl) is due to the formation of the $CdCl_2$-n solution complex and is easily absorbed by the root. Application of Zn^{2+} under salinity conditions improves plant growth. Zinc controls the entry and exit of Sodium [28] due to its role in protecting the membrane [27]. In addition to this, Zinc takes part in the detoxification of reactive oxygen species (ROS), which may be due to salinity stress [29].

Clay soils: Zinc accessibility to the soil is also associated with clay material, the presence of Iron oxides and Manganese in the soil. The researchers observed that Zinc containment in the kaolinite clay soils is less than illite and montmorillonite, and this may be due to homogeneous material and permanent load of clay in the illite and montmorillonite compared to that of kaolinite. Due to its similarity with elements of two-capacity cations such as Mg, Cu, Fe, Mn, Zn accessibility can change physiological balance by local competition in different places [30].

B. The Role of Zinc in Plants

Zinc is an essential micronutrient element for organisms [31] and plays an important role in plant processes [24, 32]. Zinc is necessary for producing chlorophyll, pollen performance, fertility and germination [27, 33], as well as for lipid metabolites, nucleic acid, RNA metabolism, stability and DNA simulation and gene expression regulation. Zinc plays an important role in cell proliferation [27] and plant roughness [34]. Zinc tends to form tetragonal complexes with O, N, and especially S ligands, which affects the metabolic activity. Zinc as a catalyzer, has an activating or building role in many enzymes in plants [31]. Zinc is involved in the structure of more than 300 enzymes [35] and is the only metal that is present in all 6 enzyme grades [17].

III. METHODOLOGY

Fertile soil in the analogy of 50:50 soil and sand ratio was used in order to plant Isfahan cultivar safflower seeds, obtained from Isfahan's Pakan Co. Four pots were taken for each treatment, and 15 seeds were planted at a depth of 2 cm in each pot. The steps were done in the greenhouse and the laboratory of the Faculty of Science of Azad University of Mashhad. The greenhouse temperature was 25°C and the samples were irrigated once a day with urban water. In the two leaf stage of the plant, the first spray of Zinc oxide nanoparticles was given at concentrations 0, 10, 100, 500, and 100. The second and third sprays were performed at 14 day intervals.

A. Measurements

1) Measuring the activity of the enzyme guaiacolat oxidase

In order to measure the activity of the enzyme guaiacolat oxidase the method described in [36] was followed. In order to maintain the activity of the enzyme, all the steps were performed in temperature 1±1ºC [36].

2) Measuring the activity of the enzyme guaiac peroxidase

In order to measure the enzyme activity, phosphorus buffer solution of 0.1M and 50 µl of guaiacol followed by 50 µl of 3% hydrogen peroxide were added to 3mm enzyme extract. The change in optical absorption was recorded by a spectrophotometer (Shimaduz model UV/110) [36].

3) Measuring the activity of polyphenol oxidase enzyme

In order to maintain the activity of the enzyme, all enzyme extraction steps were carried out in 1±1ºC. Measuring the activity of polyphenol oxidase was done by the method described in [37].

4) Measuring malondialdehyde level

2.0 g of leaf tissue were eroded in 10 milliliters of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 10,000 rpm for 5 minutes. For each 1 mm of the resulting solution, 4 mm 20% TCA containing 0.1% thybutriracic acid were added. The product mixture was then placed at 90°C for up to 30 minutes. The samples were cooled for 5 minutes on ice and then centrifuged again for 10 minutes at 10000 rpm. The absorbance of the samples was measured at 532 nm and 600 nm by a spectrophotometer. The malondialdehyde value was obtained by differentiating the absorbance value of 600 from 532 and multiplying in 1.55x105 (unit: µmol/gFW).

5) Measuring the dehydrogenase level

The dehydrogenase's activity was measured using triphenyltetrazolium chloride (TTC) reduction to trifluoromethane (TPF). The level of young roots was washed and dried, and then the weight was measured. Roots were placed in tubes containing 2 mm 0.4% TTC and 1 ml phosphate buffer 0.6 M, with pH=7 and then were placed at 7°C for 3 hours. In the next step, 1 mm solution of sulfuric acid 1M was added to the tubes and TPF was formed. Then, the absorbing was read at 485 nm [38].

IV. RESULTS

A. Statistical Analysis

Data statistical analysis was done by using SPSS. For comparing averages, Duncan minimum significance level test was used at the error probability level of 5% ($p \leq 0.05$). Charts were drawn in Microsoft Excel. Data statistical analysis on the effect of different Zinc oxide nanoparticles levels on the level of malondialdehyde showed a significant increase compared to control. The highest rate in the treatment was at 100 mg/L treatment (Figure 1). Comparison on the amount of guaiac peroxidase in treatment of 100 mg/L ZnO and in treatments of 10, 500 and 1000 mg/L showed a decrease compared to control (Figure 2). Data analysis showed that the amount of polyphenol oxidase increased at concentrations of 10 and 500 mg/L and decreased at concentrations of 1000 and 100 mg/L, compared to control (Figure 3). Dehydrogenase increased in the treatment of 1000 mg/L and decreased in treatments of 10, 100 and 500 mg/L (Figure 4).

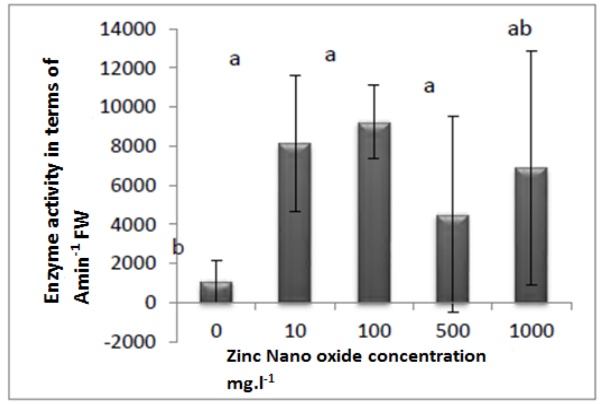

Fig. 1. The enzyme levels of malondialdehyde in safflower under Zinc oxide nano treatment, the columns with similar letters at the 5% probability level (p≤0/05) have no significant difference.

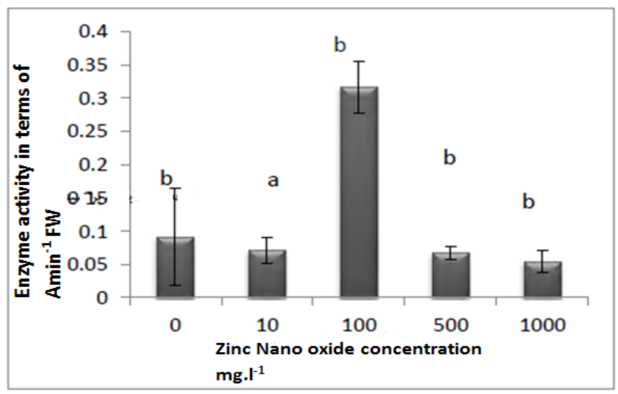

Fig. 2. The amount of enzyme of safflower guaiacol peroxidase under the treatment of Zinc oxide, the columns with similar letters at the probability level of 5% error (p≤0/05) have no significant difference.

V. DISCUSSION

The production of malondialdehyde due to lipid peroxidation has been reported in many plants under stress of various types of nanoparticles [39]. Reduced activity of antioxidant enzymes such as glutathione reductase and catalase and increased levels of malondialdehyde is believed to cause oxidative stress and, consequently, to reduce the plant growth under cadmium treatment [40]. Investigation of nanoparticles of iron oxide on the lawn and pumpkin was done in [41]. According to authors, iron oxide nanoparticles cause a higher oxidative stress and high induction of antioxidant activity in comparison to the mass of iron oxide in lawn and pumpkin. One of the reasons for oxidative stress in these two plants is the absorption of iron oxide nanoparticles on the root surface, or on the account of absorption of this nanoparticle, the physiological root functions like respiration are impaired while it causes instability on the two-layer lipid membrane. Due to increased activity of the catalase and superoxide dismutase enzymes, lipid peroxidation decreases followed by a reduction in the level of malondialdehyde (the index for determining the lipid peroxidation rate) [41].

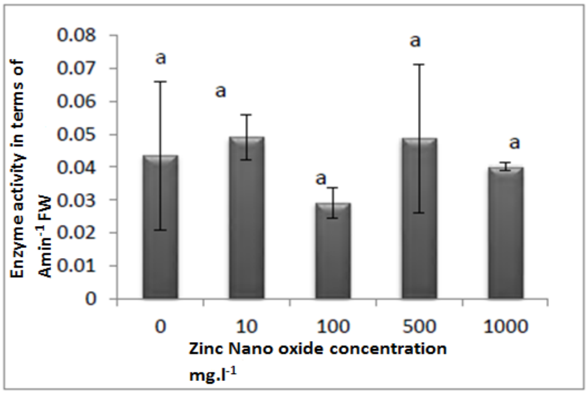

Fig. 3. The amount of safflower polyphenol oxidase under Zinc Nano oxide treatment, the columns with similar letters at the level of 5% error probability are not significantly different.

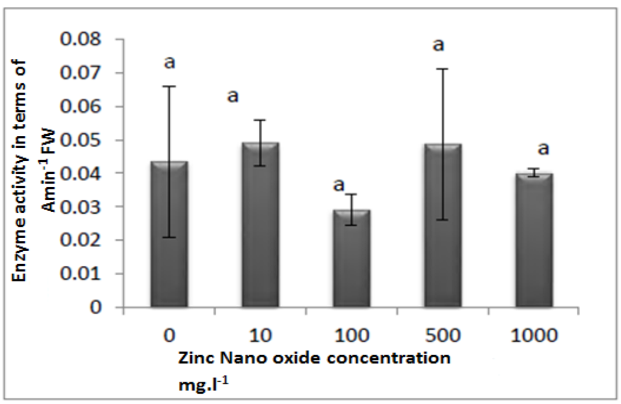

Fig. 4. The amount of safflower dehydrogenase enzyme under Zinc Nano oxide treatment, the columns with similar letters at the 5% probability level are not significantly different.

A. *Guaiacol Peroxidase*

Peroxidases belong to a large family of enzymes found in plants and fungi. These proteins usually include a prostatic group of protopourfirin IX, which oxidizes several substrates in the presence of hydrogen peroxide (H_2O_2) [42]. Many of the internal cellular and external cellular oxidases are easily identified [43]. Peroxidase catalyzes the oxidation reactions of the resuscitation and restores H_2O_2. Therefore, polyoxides are oxidoreducts that use H_2O_2 as an electron receptor to catalyze different oxidative reactions [44]. After applying a mixture of two nano particles of copper oxide less than 50 nm in size and Zinc oxide with a size less than 100 nm with different degrees, the root growth was significantly decreased. Oxidative stresses were observed in the plant under the treatment of nanoparticles. By increasing lipid peroxidation and glutathione oxidation in the roots, the chlorophyll level decreased and the activity of peroxidase and catalase increased. Nano particles increased ROS production [45]. Authors in [46] showed that during the germination stage of bean, cadmium treatment caused the increase of the induction peroxidase guaiacol enzyme activity. Generally, guaiacol peroxidase is known as a tensile enzyme and the increase of its activity has been reported under stress conditions such as cadmium treatment [47].

B. *Polyphenol Oxidase*

Authors in [48] also observed the increase of the antioxidant enzymes of guaiacol peroxidase and polyphenol oxidase in labrad purpurcus plant under cadmium treatment. Polyphenol oxidase activity in Arabidopsis thaliana plants also increased under cadmium stress. The enzyme uses polyoxyethylene peroxidation as a coagulant substrate [49]. Polyphenol oxidase causes oxidation of phenolic compounds to quinones and thus reduces the amount of these compounds in the leaves and roots of plants under stress. This action, by changing the peroxidation, leads to a decrease in the fluidity of the membrane and prevents the diffusion of the released radicals and thereby increases the plant's stress [48].

C. *Dehydrogenase*

Analysis of data variance showed that treatment with 1000 mg/L nanoparticles of Zinc oxide showed a significant increase compared to control, but treatments of 10, 100 and 500 showed an insignificant decrease. Dehydrogenases are a group of enzymes that play a role in biological cycles and synthesis pathways of various compounds in plants. Pyruvate dehydrogenase, molathase dehydrogenase, isocyte dehydrogenase, suction dehydrogenase and alpha-keto glutarate dehydrogenase are dehydrogenase in the TCA cycle. Dehydrogenases are responsible for water absorption and biomass increase in plants, and thus contribute to plant growth. Dehydrogenase regulates the absorption of the substances in the plants. The activity of dehydrogenase is an important indicator for root canal survival [50]. Dehydrogenase activity in plants increased affected by titanium dioxide [51]. Authors in [38] investigated the effects of multi-wall oxidized carbon nanotubes on wheat. Increase of electron transport reactions by the enzyme dehydrogenase was caused by these nanoparticles on wheat.

VI. Conclusion

In this study, the effect of different concentrations of Zinc oxide on some biochemical properties of safflower was investigated. The purpose of this stress was to study plant response to these particles. According to the results, it was found that the presence of these particles increased the level of malondialdehyde in the safflower plant. The amount of guaiacol peroxidase increased at concentration of 100 mg/l and decreased at concentrations of 10 and 500, and the concentration of 1000 mg/Ln of Zinc oxide increased the enzyme dehydrogenase, while the other concentrations had a reduced effect on the control plant.

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