Plant Archives Vol. 20 Supplement 1, 2020 pp. 3465-3468 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSE OF *LUPINUS ALBUS* PLANTS TISSUES FOR TREATMENT TO ZINC OXIDE NANOPARTICLE

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Abstract

The results of this study gave a scientific insight about the role of Zinc oxides (ZnO) nanoparticles at concentrations (20, 40, 60, 80, 100) μg/ml in germination of *Lupinus albus* L. plants seeds and growth of their seedlings after 21 days in solidified MS medium. The results showed the superiority of 100 μg/ml in seeds germination percentage were 100% after 5 days compared with the MS medium alone (control) were reached to 90% after 7 days. The same concentration has encouraged the best of average length of their root and shoot groups of 5.5 and 8 cm, respectively, accompanied by successes of multiple cell divided with resulted an increase in protein content of 3.2 mg / g after 21 days. The SEM (scanning electron microscope) tests explain the ability of the seedling tissues cells for up taking of Zinc oxide nanoparticles and accumulation on their surface especially at high concentration 100 μg/mL of Zinc oxide nanoparticles.

Key words: *Lupinus albus*, Zinc oxides (ZnO) nanoparticles, SEM.

Introduction

Nanotechnology is a new scientific field that allows a wide area of advanced research and various technological applications in the most fields and improvement of plant characteristics using different nanomaterials (Nair *et al*., 2010). The nanoparticles (Nano-scale particles NP) are molecular or atomic aggregates with dimensions ranging from 1-100 nm and have unique physical and chemical properties (Giraldo *et al*., 2014; Siddiqui *et al*., 2014). Nanoparticles interfere with plants causing many morphological and physiological changes depending on the properties of these particles whose effect depends on chemical composition, concentration, size, surface cover and effectiveness (Khodakovskaya *et al*., 2012). As scientists have assumed through many experiments the presence of positive and negative effects of these particles in the growth and development of plants and this varies depending on the type of plant and this effect includes its role in seed germination and plant growth (increase the biomass of the total shoot and roots) and photosynthesis (Ma *et al*., 2010).

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The positive effects of nanoparticles on plant growth have wide application in plant cultivation by releasing various nutrients that stimulate plant growth which are associated with target cells causing stimulation for example, silver nanoparticles affect the growth of higher plants and this effect depends on the type and age of these plants, the size and concentration of these particles, the experimental conditions such as temperature, conditions and method of treatment with these particles so these nanoparticles have a positive effect on the germination and growth of pea plants *Vicia faba* (Farooqui *et al*., 2016; Yahya, 2019) also in another study the nanoparticles of titanium oxide stimulated the photosynthesis and plant growth of spinach (Yang and Hong, 2006).

Lupinus albus plants belong to the Fabaceae family, a winter crop found in all sandy lands, there are several varieties of it depends on the color of its flowers are white, yellow and blue (Von Baer *et al*., 2009; Bhardwaj and Hamama, 2012). Lupine has nutritional benefits for humans and feed for livestock because it contains a high percentage of proteins and fats, It is also an oil crop and it has health benefits (Sirtori *et al*., 2004).

This study performed to explain the obvious role of different concentrations of Zinc oxide nanoparticles in response of lupine tissues in growth and in the seedling content of some biomolecules as protein, also confirmed the ability of this tissues to up take the nanoparticles on their surfaces.

Materials and Methods

Preparation of Zinc oxide nanoparticles

ZnO-NPs were obtained from Sigma Aldrich, UK according to the properties of these particles their size used is < 120 nm and a molecular weight of 81.39 g/mol. 1.0 g of ZnO dissolved in 1 liter of distilled water and the remaining concentrations approved in the study were 20, 40, 60, 80 and 100 μg/ml of the standard solution prepared.

Producing of sterile seedlings of *Lupinus albus*

Lupinus albus seeds (sweet type) were prepared from local markets / Erbil city. washed with running water and sterilized by submerging them in 96% ethyl alcohol solution for 2 minutes and then in 3% of NaOCl sodium hypochlorite solution for 20 minutes and then washed with sterile distilled water three times / minute (Sinha *et al*., 2003). the seeds were transferred to flasks containing solid MS (Murashige and Skoog, 1962) alone and supplemented with different concentrations of zinc nanoparticles at 20, 40, 60, 80 and 100 μg/ ml, which then kept in the growth incubator and in dark conditions at 22 ± 2°C. After the germination (which lasted 5 days) the flasks were transferred to the light conditions 16 hours light / 8 hours dark.

Estimation of seedling total protein

The protein content of lupine seedling were determined according to the Lowry method (Lowry *et al*., 1951).

Preparation of samples (tissues) for the microscopic photography

Seedling segments tissues of *Lupinus albus* which treated with Zinc oxide nanoparticles were dried at 80ºC using oven (Gallenkamp oven BS Model, England) for 24 hours (Al-Salih *et al*., 2013). These samples examined by Scanning Electron Microscope (SEM)(VEGA / TESCAN, Czech Republic) in Nanotechnology Center, University of Technology, Baghdad, Iraq.

Results and Discussion

Sterile seedlings produced

The results of surface sterilization of the lupines plants seeds showed their efficiency through their obtaining naturally grown seedlings completed in radicle and the smaller steams after 7 days of culture and characterized by elongation of their stems (Fig. 1A).The suitable surface sterilization method of *Lupinus albus* seeds used in this study showed its characteristics by obtaining healthy and good vitality seedlings. The use of various sterile materials depends on the time of exposure to the seeds as well as their concentrations, which are critical bases for the use of these seeds to produce sterile, healthy seedlings that are not affected by sterilization processes (Sen *et al*., 2013).

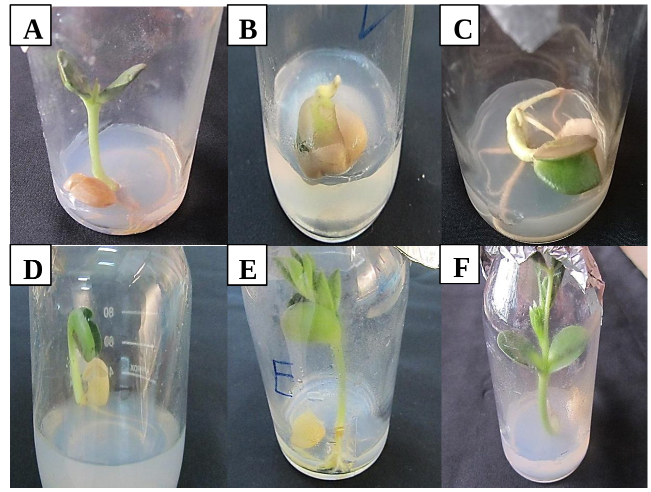

Fig. 1: Seedlings growth on MS alone and supplemented with different concentrations of Zinc oxide nanoparticles after 20 days.

The effect of Zinc oxide nanoparticles on the percentage and germination speed of *Lupinus albus* seedling

The results showed the positive role of the concentration 100 μg/ ml of ZnO <120 nm nanoparticles to encourage seed germination when added to the MSO medium with a shorter period of time, five days, compared to the time required for its propagation in comparison seedlings grown on MSO medium only that are seven days (Table 1), the stems of the seedlings showed clear length and thickening with many branches and good growth of branching roots (Fig. 1F) compared to the growth of comparative seedlings (Fig. 1A). The effect of zinc nanoparticles of various concentrations in

Table 1: Effect of different concentrations of Zinc oxide nanoparticles on percentage and germination speed of *Lupinus albus* seeds cultivated on MS medium.

MS + ZnO (μg / ml)	Germination percent (%)	Germination speed (day)
0 (Control)	90	7
20	30	8
40	50	8
60	65	8
80	85	5
100	100	5

Each value represents an average of five replication.

accelerating the growth of lupine seedlings accompanied by lengthening and thickening of stems and leafs due to the zinc mineralization and the ability of some plants to incorporate zinc as a growth-stimulating element, that similar to the study carried on the cattle plant in the positive effect of zinc and copper oxide nanoparticles in shoot seedling growth which may be due to a number of cellular proteins involved in regulating the intracellular transport of zinc and copper in the positive direction of growth (Dhoke *et al*., 2013).

This was followed by a medium supplemented with a concentration of 80 μg/ml which gave a germination speed of 85% in a period of 5 days (Fig. 1E) by elongation and thickening of the stems and the emergence of several branches and leaves, the next is the medium equipped with a concentration of 60 μg/ml ,which gave seedling speed of 65% (Fig. 1D),While concentrations of 20 and 40 μg/ ml were inhibited for germination speed to 30% and 50% over 8 days (Fig. 1C, B).

The effect of Zinc oxide concentrations in the growth of lupine plants seedling

The results showed the important role of the high concentration of Zinc oxide nanoparticles in stimulating the growth and development of lupine plants seedling for both the roots and shoot stems after 20 days of culturing (Table 2).

The data in the table above showed that the best average of both the shoot and root system that the seedling growth on the medium containing 100 μg\ml which reached to 5.5 and 8 cm respectively after 20 days of culture the next to that seedling growth on MS containing 80 ìg\ml the length reached to 4.5 and 4.8 cm respectively comparing with the MSO (control) 4.3 and 3.9 cm respectively while the medium supplemented with the concentration 20,40 and 60 μg\ml which inhibit the growth of seedling after 20 days of culture (Table 2) this explained the role of those nanoparticles on the growth of lupine seedling plants from the presence the number of inter location on the cellular membrane which represented the receptors which are soluble proteins called as the metal covered complained with the metal and transport it to the aim which can be enough for the necessary equipment for increasing the growth and protect the plant cell from the toxic of metals (O' Halloran and Culotta, 2000).

Table 2: Effect of different concentrations of ZnO <120 nm in the growth of *Lupinus albus* seedling plants (root and shoot total length) on MS medium.

MS + ZnO (μg / ml)	Average of root system length (cm)		Average of shoot system length (cm)	
	After 10 days	After 20 days	After 10 days	After 20 days
0	2.5	4.3	3.3	3.9
20	1.4	1.5	1.1	2.1
40	2.0	2.1	2.1	2.3
60	2.1	2.8	2.6	3.4
80	2.8	4.5	3.7	4.8
100	4.2	5.5	3.8	8.0

Each value represent the average of five replications.

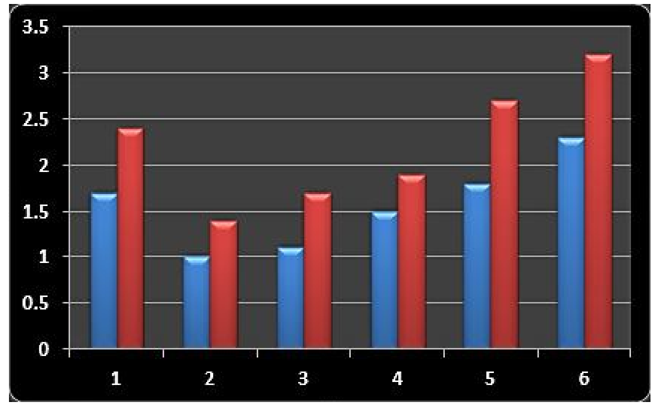

Fig. 2: Protein content in lupine seedlings cultivated on the MS medium supplemented with different concentrations of Zinc oxide nanoparticles after 10 and 20 days, each value represented average of five replications.

(1) MSO, (2) MS + 20 μg/ ml of ZnO, (3) MS + 40 μg/ ml of ZnO, (4) MS + 60 μg/ ml of ZnO, (5) MS + 80 μg/ ml of ZnO, (6) MS + 100 μg/ ml of ZnO.

Determination the total protein content in lupine plant seedlings

The data of the total protein content of the seedling growth on MS medium supplemented with 20, 40, 60, 80 and 100 μg\ml from Zinc oxide nanoparticles after 10 and 20 days conclude the importance of some concentration used in the biosynthesis of the important biomolecules for the cell. From the following (Fig. 2) explained the stimulated role for the concentration 100 μg\ml when its added to the MS medium in the protein biosynthesis in the content 3.2 mg\g after 20 days (Fig. 2, 6). The next medium are those which supplemented with 80 mg\ml from Zinc oxide nanoparticles in MS alone. The two researcher Finny and O' Halloran pointed to the presence of many protein groups controlled the activity of toxic atoms inside the cell and remove the metabolic and accumulation activity without toxic effect by integral transmembrane transporters or may be this metals in the form of nanoparticles are necessary in trace amount for the higher plants for accumulation their lifecycle and stimulation the biosynthesis pathway for the biomolecules (Sridhar *et al*., 2007). On the other side the concentration 20, 40 and 60 μg\ml reduced the protein content that produced from the seedlings after 20 days from culture in the content 1.4, 1.7 and 1.9 mg\gm subsequently (Fig. 2).

Detection the uptake of Zinc oxide nanoparticles by lupine tissues

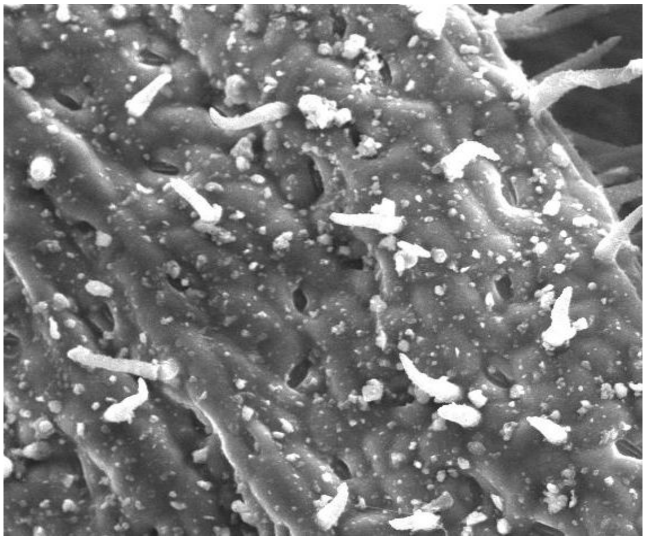

Fig. 3: SEM photography at 50 µm of seedlings cells of *Lupinus albus* grown on MSO supplemented with 100 µg ml^{-1} of Zinc oxide nanoparticles.

SEM examination of seedling samples showed different uptake of Zinc oxide nanoparticles according to the concentration of nanoparticles added to the seedling medium, the seedlings sample treated with 100 µg ml^{-1} surprised in the uptake of Zinc oxide nanoparticles and showed that there were many particles participated on the surface of the cells compared with control (Fig. 3). This concept agreed with the essentials of phytoremediation mentioned by (Raskin and Ensley, 2000).

Acknowledgment

The author is very grateful to the University of Mosul, College of Science for their provided facilities, which helped to improve the quality of this work.

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