

## Uptake of Zinc Nanoparticles by *Prosopisfarcta* L.Plants Callus Cultures

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### ABSTRACT

The current study showed the ability of *Prosopisfarcta* L. plants to uptake and accumulate pollution caused metals present in the environment, and their role on the phytoremediation by indication of Zinc oxide nanoparticles uptake at various concentrations.

For this purpose Zinc oxide nanoparticles of 120 nm were used with concentrations of 1.0, 10, 50, 100 $\mu\text{g ml}^{-1}$ .

Results of this study referred to the formation of *Prosopisfarcta* plants stems callus on MS modified medium supplemented with 1.0 mg L<sup>-1</sup> NAA (Naphthalene acetic acid) and 4.0 mg L<sup>-1</sup> TDZ (Thidizurone). It was clear from the results that callus cultures of *Prosopisfarcta* had the ability to uptake and accumulate nanoparticles of Zinc oxide. Scanning electron microscope (SEM) photography showed that 100 $\mu\text{g ml}^{-1}$  of Zinc oxide nanoparticles was the higher to be accumulated by callus tissues, photographs showed a high density of these particles on the surface of the cells. In the same time a linear increasing of callus fresh weight with the increasing of Zinc oxide nanoparticles concentration was recorded. The study revealed that *Prosopis* seedlings had the ability to uptake Zinc oxide at 50  $\mu\text{g ml}^{-1}$  more than the other concentrations.

### استقطاب الدقائق النانوية لأوكسيد الزنك بواسطة مزارع كالس نباتات *Prosopisfarcta* L. الخرنوب

#### الخلاصة

أظهرت الدراسة الحالية قدرة نباتات الخرنوب *Prosopisfarcta* L. على استقطاب وتجميع العناصر الملوثة المتواجدة في البيئة ودورها في المعالجة النباتية بدلالة استقطابها عنصر الزنك المستخدم بهيئة دقائق نانوية لأوكسيد الزنك.

لتحقيق هذا الهدف استخدم أوكسيد الزنك النانوي بدقائق بحجم 120 نانوميتر وبتركيز مختلفة هي 1.0, 10, 50, 100 مايكروغرام مل<sup>-1</sup>.

أشارت نتائج الدراسة الى تكوين مزارع كالس سيقان نباتات الخرنوب على وسط MS المحور والمزود باضافة 1.0 ملغم لتر<sup>-1</sup> NAA<sup>1</sup> (Naphthalene acetic acid) و 4.0 ملغم لتر<sup>-1</sup> TDZ<sup>1</sup> (Thidizurone). وبدأ واضحاً من النتائج قدرة نسيج كالس الخرنوب على استقطاب وتراكم الدقائق النانوية لأوكسيد الزنك. وبينت نتائج فحص عينات الكالس باستخدام المجهر الإلكتروني

الماسح SEM Scanning Electron Microscope) ان التركيز 100 مايكروغرام مل<sup>-1</sup> كان الاكثر تراكما على اسطح خلايا الكالسوبكثافة عالية. وبذات الوقت لوحظ زيادة طردية للوزن الطري للكالس مع زيادة تراكيز اوكسيد الزنك النانوي. وبصورة مماثلة اوضحت الدراسة قدرة بادرات نباتات الخرنوب على استقطاب اوكسيد الزنك بتركيز 50 مايكروغرام مل<sup>-1</sup> اكثر من باقي التراكيز المستخدمة .

## INTRODUCTION

Nano particles refer to materials and components which have at least one dimension in the size range 1-100 nanometer (1). Combined with nanotechnology, Zinc oxide nanoparticles can be prepared, which possess some unique characters, such as small particle size and large area surface. Zinc oxide nanoparticles have selective toxicity and are generally regarded as a safe reagent to human and animals (2). Zinc oxide nanoparticles have their own importance due to their vast area of applications, e.g., gas sensor, chemical sensor, bio-sensor, cosmetics, storage, optical and electrical devices, window materials for displays, solar cells, and drug-delivery and in agriculture (3,4).

Heavy metals are conventionally defined as elements with metallic properties (ductility, conductivity, stability as cations, ligand specificity, etc.). Common heavy metal contaminants are: Cd, Cr, Cu, Hg, Pb, and Zn. Contamination, however, resulted from industrial activities, such as mining and smelting of metalliferous ores, electroplating, gas exhaust, energy and fuel production, fertilizer and pesticide application, and generation of municipal waste (5). Heavy metals, such as Zinc (Zn) and copper (Cu) are required in trace amounts by higher plants to complete their life. In extended concentrations, however, all are toxic (6). Zn is found to be involved in many cellular functions such as protein metabolism, photosynthetic carbon metabolism and indole acetic acid metabolism, yet its higher concentrations cause toxicity (7). Phytoremediation is a promising technology using plants to remove contaminations as heavy metals and radioactive elements from the environment, it is a generic term for several ways in which plants can be used to clean up contaminated soil and water (8). According to the concept of phytoremediation and the facilities provided by nanotechnology, this study included the use of *Prosopisfarcta* plants for the treatment with Zinc nanoparticles as a heavy metal that causes problems in soil, and to be sure of which concentration is the most one to uptake and accumulate in this plant.

## MATERIALS AND METHODS

### Callus initiation from seedling hypocotyl stems

Seeds of *Prosopisfarcta* L. provided from mature fruits of plants grown naturally nearly in Hay Al-Arabi / Mousl. Were sterilized by soaking in ethyl alcohol 96% for two minutes, followed by 1:2 (V:V) sodium hypochlorite (NaOCl): water with stirring for five minutes, then rinsed thoroughly in sterilized distilled water three times (9). Sterilized seeds were placed on MSO (10) medium solidified with 0.8% agar and supplemented with 3% sucrose, pH adjusted to 5.8 before autoclaving. Cultures maintained in culture room at 25±2 °C in the dark. The seedlings produced after seven days of culture transferred to light condition with 16 hour light daily at 1500 lux.

For callus initiation, 2.0cm of seedling hypocotyl stems excised and placed on MS\* medium (10) (MS\* = MS medium modified by increasing KNO<sub>3</sub> to 2000 mg L<sup>-1</sup>

, Thiamine-HCl to 0.5 mg L<sup>-1</sup>, Pyridoxine-HCl to 1.0 mg L<sup>-1</sup>) and supplemented with 1.0 mg L<sup>-1</sup> NAA (Naphthalene acetic acid) and 4.0 mg L<sup>-1</sup> TDZ (Thidiazuron) (11). The callus aggregates formed were then subcultured on the same medium after 30 days for maintaining callus growth.

#### Preparation of Zinc oxide nanoparticles solution

1.0 gm of Zinc oxide (Zinc oxide *ReagentPlus*<sup>®</sup>, powder, <120 nm particle size, 99.9% , Sigma- Aldrich, UK) was dissolved in 1.0 liter of distilled water as stock solution, then other concentration of 1.0, 10, 50, 100 µg ml<sup>-1</sup> were prepared.

#### Culture of *Prosopisfarcta* seeds and callus on MS medium supplemented with the concentration of Zinc oxide nanoparticles

1.0 gm of callus was transferred to MS\* modified (MS\* = MS medium containing 1.0 mg L<sup>-1</sup> NAA and 4.0 mg L<sup>-1</sup> TDZ and supplemented with Zinc oxide nanoparticles at 1.0, 10, 50, 100 µg ml<sup>-1</sup> each of them alone.

Whereas, the seeds were cultured on MSO supplemented with 50 µg ml<sup>-1</sup> of Zinc oxide nanoparticles only.

#### Determination of callus fresh weight

Callus fresh weight determined after 30 days of cultured with different concentration of Zinc oxide by calculation the difference between the weight of flasks with culture and when it is with medium (12).

#### Preparation of samples (tissues) for the microscopic photography

Seedling segments and callus tissues of *Prosopisfarcta* which treated with Zinc oxide nanoparticles were dried at 80°C using oven (Gallenkamp oven BS Model, England) for 18 hours (13). Scanning Electron Microscope (SEM) (VEGA/ TESCAN, Czech Republic) in Nanotechnology Center, University of Technology, Baghdad, Iraq was used to detect Zinc oxide nanoparticles in *Prosopisfarcta* tissues.

## RESULTS AND DISCUSSION

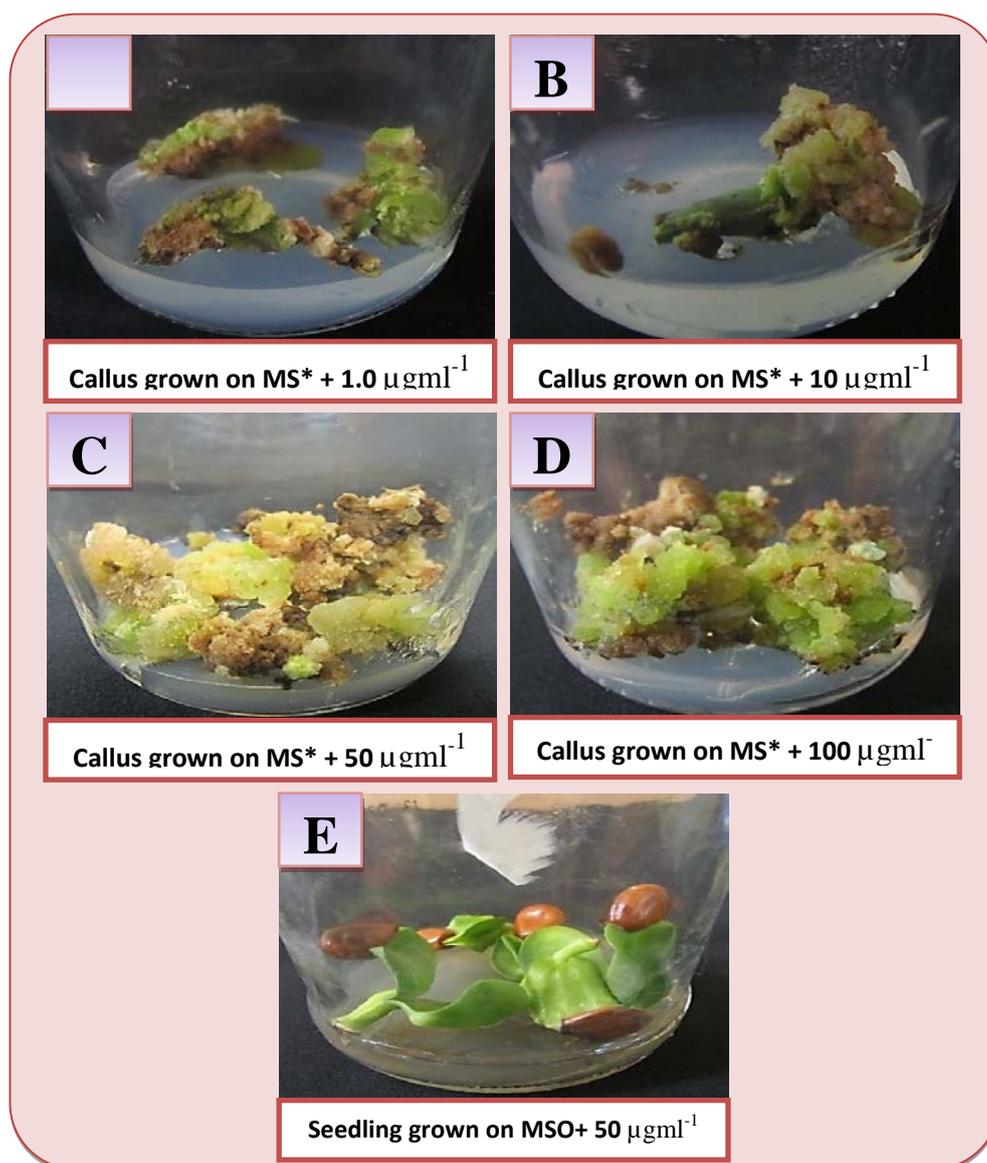
Results in table (1) showed that callus fresh weight of *Prosopisfarcta* varied with the variation of Zinc oxide nanoparticles concentrations.

**Table (1) Fresh weight of *Prosopisfarcta L.* callus grown on MS\* medium supplemented with different concentrations of Zinc oxide nanoparticles after 30 days of culture.**

Zinc oxide nanoparticles µg ml <sup>-1</sup>	callus fresh weight (gm)
1.0	2.09
10	2.5
50	3.4
100	5.7

The best growth of callus determined by fresh weight obtained when callus grown on MS\* medium with addition of 100 µg ml<sup>-1</sup> of Zinc oxide nanoparticles Figure (1-D) in which fresh weight of callus reached 5.7 g after 30 days of growth on this medium. The addition of 50 µg ml<sup>-1</sup> also enhanced callus growth to reach 3.4 g in fresh weight after 30 days. Results represented in Figure 1-C) referred to that callus cultures of *Prosopisfarcta* have the ability to grow even with the presence of high concentrations of Zinc oxide nanoparticles. Zinc was considered to be as a heavy

metal that is required in trace amounts by higher plants to complete their life (14). Generally results showed that callus seems to be actively grown on MS\* medium with addition of  $100\mu\text{g ml}^{-1}$  of Zinc oxide nanoparticles as compared with the other concentrations  $1.0$ ,  $10\mu\text{g ml}^{-1}$  Figure (1-A, B). In comparison seedlings of this plant treated with  $50\mu\text{g ml}^{-1}$  exhibited thickened leaves and shortened stems Figure (1-E).

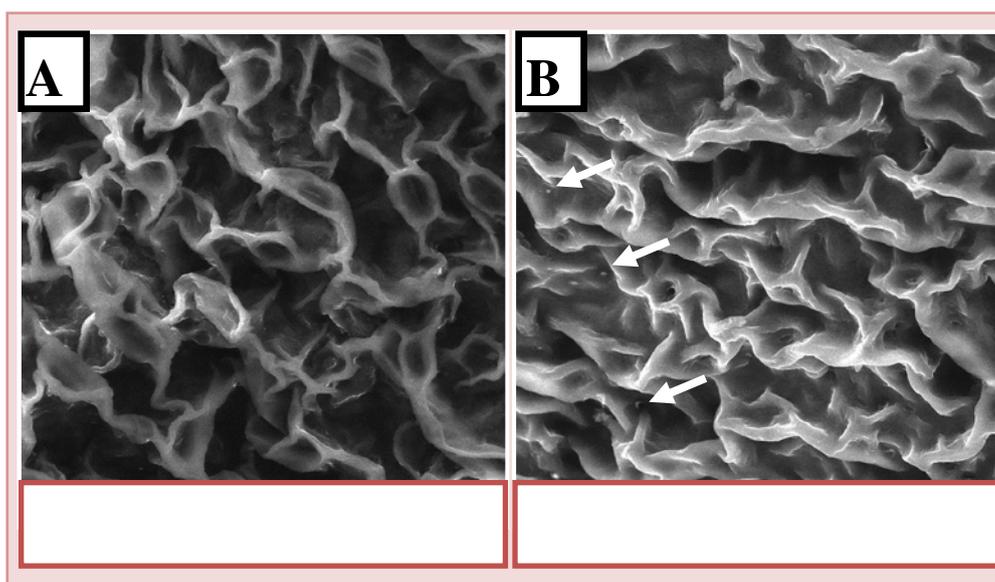


**Figure (1) Seedlings and callus cultures of *Prosopisfarcta L.* grown on medium supplemented with different concentrations of Zinc oxide nanoparticles after 30 days of culture.**

It was reported that Zn is found to be involved in many cellular functions such as protein metabolism, photosynthetic carbon metabolism and indole acetic acid

metabolism, yet its higher concentrations because toxicity (7). It was also reported that many metals such as Zn, Mn, Ni and Cu are essential micronutrients (15). In common nanoaccumulator plants, accumulation of these micronutrients does not exceed their metabolic needs ( $<10\mu\text{gml}^{-1}$ ). In contrast, metal hyper accumulator plants can accumulate exceptionally high amounts of metals, in thousands of  $\mu\text{gml}^{-1}$  (16).

SEM examination of seedling samples treated with  $50\mu\text{g ml}^{-1}$  of Zinc oxide nanoparticles showed that there were many particles participated on the surface of the cells compared with control Figure (2-A, B).



**Figure (2) SEM photograph at  $50\mu\text{m}$  of Seedlings cells of Prosopisfarcta Grown on MSO supplemented with  $50\mu\text{g ml}^{-1}$  of Zinc oxide nanoparticles, arrows referred to the particles of Zinc oxide.**

Where as examination of callus cells using SEM showed that callus cells seem to be coated with particles of Zinc oxidenano particles which were used in this study. Callus cells treated with  $1.0$  and  $10\mu\text{g ml}^{-1}$  of Zinc oxide nanoparticles Figure (3- B, C) were coated on their surfaces with less particles than the cell treated with the higher concentrations  $50$  and

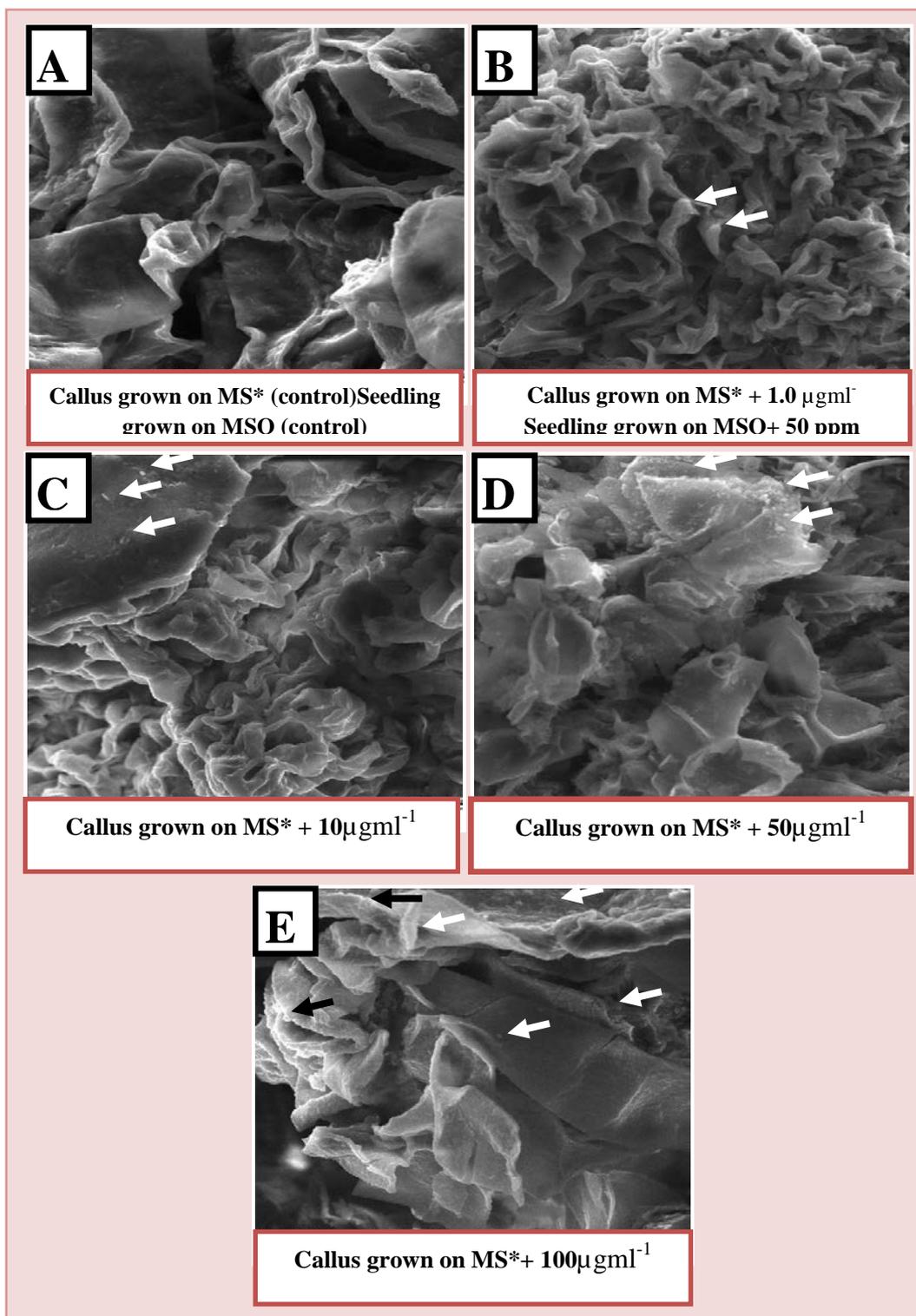


Figure (3) SEM photomicrography at 50 μm of callus cells of Prosopisfarcta grown on MS\* supplemented with different concentration of Zn<sub>2</sub>O nanoparticles.

Zinc oxide. Nanoparticles Arrows indicated to Zinc nanoparticles accumulated on the surface of the cells.

100  $\mu\text{g ml}^{-1}$  of this element Figure (1-D, E), whereas callus cells grown on MSO, control, were surface free from the particles Figure (3-A). These results were considered to be as prove that callus cells of *Prosopisfarcta* can accumulate high concentration of Zinc oxide nanoparticles without affecting the rate of growth (depending on the concentration). This concept agreed with the essentials of phytoremediation mentioned by (17). So we can conclude that this study succeeded in choosing *Prosopisfarcta* plants to be a model of plants that could be used in phytoremediation programs to clean the contaminated soils with Zinc as a heavy metals.

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