Uptake of Zinc Nanoparticles by Prosopisfarcta L.Plants Callus Cultures

Rana Tariq Yahya
College of Science, University of Mosul/ Mosul
Email:biology19802007@yahoo.com
Dr. Hanaa Saeed Al-Salih
College of Science, University of Mosul/ Mosul

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, 100µg 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$^{-1}$ NAA (Naphthalene acetic acid) and 4.0 mg L$^{-1}$ 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 100µg 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 µg ml$^{-1}$ more than the other concentrations.

استقطاع الدقائق النانوية لأوكسيد الزنك بواسطة مزارع كالس نباتات Prosopisfarcta L.

الخلاصة
انشأت الدراسة الحالية قدرة نباتات الخرنوب على استقطاع وتجميع العناصر الملوثة الموجودة في البيئة ودورها في المعالجة النباتية بدلالة استقطاعها عنصر الزنك المستخدم بيئة دقاع دقاعية لاوكسيد الزنك. لتحقق هذا الهدف استخدام أوكسيد الزنك النانوي بحجم 120 نانوميتري وتركيز مختلف في مختبرية 4.0 ملغات. وآتيت نتائج الدراسة إلى تكوين مزارع كالس سيفان نباتات الخرنوب على وسط المحمور MS والمزود بالإضافية. والمحور Naphthalene acetic acid$^{1}$ (1.0 ملغة) و NAA$^{1}$ (1.0 ملغة). ونحو تتراكم الدقائق النانوية لأوكسيد الزنك. ووصفت تتراكم فحص عينات الكالس باستخدام المجهر الإلكتروني.
Uptake of Zinc Nanoparticles by Prosopis farcta L. Plants Callus Cultures

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 melting 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 Prosopis farcta 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 Prosopis farcta 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, 2.0 cm of seedling hypocotyl stems excised and placed on MS* medium (10) (MS* = MS medium modified by increasing KNO₃ to 2000 mg L⁻¹).
Thiamine-HCl to 0.5 mg L\(^{-1}\), Pyrdoxine-HCl to 1.0 mg L\(^{-1}\) and supplemented with 1.0 mg L\(^{-1}\) NAA (Naphthalene acetic acid) and 4.0 mg L\(^{-1}\) TDZ(Thidizurone)(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\(^{®}\), 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\(^{-1}\) were prepared.

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

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

Whereas, the seeds were cultured on MSO supplemented with 50 µg ml\(^{-1}\) of Zinc oxide nanoparticles only.

**Determination of callus fresh weight**

Callus fresh weight determine 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 oxidenanoparticles were dried at 80ºC 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>
<thead>
<tr>
<th>Zinc oxide nanoparticles µg ml(^{-1})</th>
<th>callus fresh weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>2.09</td>
</tr>
<tr>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>50</td>
<td>3.4</td>
</tr>
<tr>
<td>100</td>
<td>5.7</td>
</tr>
</tbody>
</table>

The best growth of callus determined by fresh weight obtained when callus grown on MS\(^{*}\) medium with addition of 100µg ml\(^{-1}\) 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 50µg ml\(^{-1}\)also enhanced callus growth to reach 3.4 g in fresh weightafter 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 100µg ml⁻¹ of Zinc oxide nanoparticles as compared with the other concentrations 1.0, 10µg ml⁻¹ Figure (1-A, B). In comparison seedlings of this plant treated with 50 µg ml⁻¹ exhibited thickened leaves and shortened stems Figure (1-E).

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 (<10μg/ml\(^{-1}\)). In contrast, metal hyper accumulator plants can accumulate exceptionally high amounts of metals, in thousands of μg/ml\(^{-1}\) (16).

SEM examination of seedling samples treated with 50μg 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).

Where examination of callus cells using SEM showed that callus cells seem to be coated with particles of Zinc oxidenanoparticles which were used in this study. Callus cells treated with 1.0 and 10μg 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 (2) SEMphotographyat 50 μm of Seedlings cells of Prosopisfarcta Grown on MSO supplemented with 50 μg ml\(^{-1}\) of Zinc oxide nanoparticles, arrows referred to the particles of Zinc oxide.
Uptake of Zinc Nanoparticles by Prosopis farcta L. Plants Callus Cultures

Figure (3) SEM photography at 50 µm of callus cells of Prosopis farcta grown on MS* supplemented with different concentration of Zn$_2$O nanoparticles.
Zinc oxide. Nanoparticles Arrows indicated to Zinc nanoparticles cumulated on the surface of the cells.

100 µg 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 proof that callus cells of Prosopis farcta 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 concluded that this study succeeded in choosing Prosopis farcta 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.

REFERENCES
[12]. Al-Taee, R.T.Y. (2005). The effect of some growth regulators in initiation and growth of tissue and cell cultures of Chamomile (MatricariachamomillaL.) and measurement the level of some of their active compounds. Msc. Thesis, College of Science, University of Mosul, Iraq.

621