Ochradenus arabicus and Maerua oblongifolia

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Zincoxide nanoparticles (ZnO NPs) are among the most produced and used nanomaterials worldwide, and in recent times these nanoparticles have also been incorporate in plant science and agricultural research. The present study was planned to synthesize ZnO NPs biologically using Ochradenus arabicus leaves and examine their effect on the morphology and physiology properties of Maerua oblongifolia cultured in vitro. ZnO NPs were characterized by UV–visible spectroscopy (UV–vis), X-ray diffractometer (XRD), Fourier transform infrared spectroscopy (FT-IR), and transmission electron microscopy, which demonstrated hexagonal shape nanoparticles of size ranging from 10 to 50 nm. Thus, the study uncovered an efficient, eco-friendly and simple technique for biosynthesis of multifunctional ZnO NPs using Ochradenus arabicus following growth of Maerua oblongifolia shoots in different concentrations of ZnO NPs (0, 1.25, 2.5, 5, 10, or 20 mg L−1) in Murashige and Skoog medium. Remarkable increases in plant biomass, photosynthetic pigments, and total protein were recorded up to a concentration of 5 mg L−1; at the same time, the results demonstrated a significant reduction in lipid peroxidation levels with respect to control. Interestingly, the levels of proline and the antioxidant enzyme catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR) activities were increased significantly in response to all ZnO NP treatments. These findings indicate that bioengineered ZnO NPs play a major role in accumulation of biomass and stimulating the activities of antioxidant enzymes in plant tissues. Thus, green-synthesized ZnO NPs might be of agricultural and medicinal benefit owing to their impacts on plants in vitro.

ZnO NPs green-synthesize M. oblongifolia chlorophyll proline

antioxidant enzymes

1. Introduction

Nanotechnology nowadays is the focus of scientific community interest and has taken hold in all fields of science due to the necessity of applications of nanomaterials in many aspects of human endeavor, such as industry, business, medicine, public health, and agriculture [1]. In general, nanotechnology comprises synthesis of nano-sized (1–100 nm) particles. Particles of this size are referred to as engineered nanomaterials (ENMs) [2]. While many researchers have stated various techniques for the manufacturing of metal oxide nanoparticles (NPs), biological synthesis using plant extracts and microorganisms is simpler, less costly, and more eco-friendly as compared to physicochemical procedures [3]. Additionally, the NMs synthesized using plants are more steady, less toxic, and biocompatible [4]. Many studies have been carried out on the vastly used nanomaterials (NMs), such as fullerenes, ZnO, TiO_2, CuO, and Ag [5]. Among these important nanomaterials, zinc oxide (ZnO) engineered nanoparticles (ENPs) have a unique position [6]. In manufacturing industries worldwide, ZnO NPs are among the most produced and used, in recent times these nanoparticles have also been incorporated in plant science and agricultural research, although contradictory results on their benefits are reported [7]. A variety of synthetic methods are used for the synthesis of ZnO NPs; these methods can be broadly divided into three types; that is, chemical, physical, and biological techniques [8]. ZnO NPs have been effectively synthesized using the biological technique. The main idea behind the green synthesis of ZnO NPs is that the natural materials (plants and microorganism) contain phytochemicals which act as both reducing as well as stabilizing (capping) agents. They reduce the metal (zinc) to the 0-valence state and then through calcinations, oxide may be added to the metal [9]. Different examples have been reported for synthesis of ZnO NPs using bio-extracts and their applications in plant species such as fodder maize (*Zea mays*) [10], *Allium cepa* [11], and *Sesamum indicum* [12]. At certain concentrations in plant cell cultures, ZnO NPs are reported to play an essential role in enhancing growth, seed germination, photosynthetic efficiency, chlorophyll content, starch content, and notably secondary metabolites production [13][14][15][16][17].

Maerua oblongifolia is a rare plant found in Saudi Arabia. The plant is a member of the family Capparaceae. It used to cure diseases such as stomach ache, fever, cough, and skin infections, urinary calculi, diabetes, epilepsy and abdominal colic [18]. Because of overexploitation for feed, food, lumber, and medicinal usages as well as its slow regeneration rate, wild populations of this plant are quickly decreasing [19]. Therefore, there is a considerable demand to enhance the regeneration of *M. oblongifolia* with micropropagation [20]. This can be attained successfully with the application of NPs [19].

Reports pertaining to the morphophysiological characteristics of plants under exposure of synthesized ZnO NPs are scarce [21]. Hence, in the present investigation it was planned to synthesize and study the influence of ZnO NPs on the regeneration, biomass, and antioxidant enzyme activities of *M. oblongifolia* raised in vitro.

2. Development and Findings

Characterization of synthesized ZnO NPs is commonly performed using UV–Visible spectroscopy, XRD, FTIR spectroscopy, and TEM microscopy. These techniques provide the information on the formation, size, structure, and elemental composition of nanoparticles. Optical properties of nanosized particles is being commonly assessed by UV–visible absorption spectroscopy [22]. The absorption peak (380 nm) found in the present study obviously demonstrates the presence of ZnO NPs in the reaction mixture and it is in agreement with the earlier results of [23][24]. The FTIR results demonstrated a band around 3400 cm^{-1}, potentially resulting from OH stretching vibrations; meanwhile, the peak at 2078 cm^{-1} for C=H suggests a strong stretch assigned to the alkyl methylene group. The peak at 1634 cm^{-1}, corresponding to amide I, appears to be caused by carbonyl stretching in proteins [25]. The peak at 704 cm^{-1} was for the C=H bond assigned to strong a mono-substituted aromatic benzene group [26]. Bearing in mind the FWHM of the plane in (101), the crystalline size of the engineered ZnO NPs was recorded using Scherrer’s formula; and the average particle size of the sample was found to be 351 nm. XRD pattern analysis proved the characteristic hexagonal wurtzite crystalline structure of ZnO NPs, which is in line with the earlier result reported by [27]. TEM was used to characterize the shape and size of ZnO NPs that were synthesized using *O. arabicus* leaf extract. The TEM images clearly showed that the synthesized ZnO NPs were almost hexagonal in shape, with the average diameter of nanoparticles ranging from 10–50 nm approximately, in accordance with the earlier result reported by [22]. In general, XRD is mostly used to estimate the particle size of nanoparticles, however TEM is the preferable technique for the measurement of nanoparticle size. The Scherrer formula for calculating particle size gives an average value of the entire particle responsible for diffraction,.while when using TEM, besides directly determining particle size, the morphology of the particles can also be noted [28].

To determine the growth enhancing effects of ZnO NPs applied to the culture medium of *Maerua oblongifolia* plant, morphological characteristics such as fresh weigh, dry weight, shoot length, shoot number, and leaf number were studied. Exposure of ZnO NPs to in vitro shoots of the *M*. *oblongifolia* significantly boosted the vegetative growth in the plant, including improving plant height, number of plants per pot, and plant biomass. This enhancement can most likely be attributed to the role of Zn in the production of tryptophan—the precursor of indole-3-acetic acid phytohormone [29]. In addition, ZnO NPs can alter the phytohormone biosynthesis of cytokinins and gibberellins, which can drive to an expansion in the number of internodes per plant [10]. Concentration at 5 mg L^{-1} ZnO NPs achieved the greatest growth of all morphological attributes, while the growth was reduced at 10 and 20 mg L^{-1}. This decrease in growth it might be due to a higher concentration of nanoparticles reaching toxic levels in stem and leaves, which reduced the plant growth; in an earlier study high concentrations of ZnO NPs drastically affected the growth of tomato plants [7]. The positive effect of ZnO NPs exposed to plants was also reported in wheat [30], cotton [26], cluster bean [31], and ryegrass (*Lolium perenne*) seedlings at 2 mg L^{-1} [32]. Silver nanoparticles and other nanomaterials' exposure have also been reported to enhance the growth of *M. oblongifolia* [19]. Iron-based NPs improved the growth of maize [33]. Contrary to our findings, ZnO NPs were reported as being toxic in several plants species such as *Allium cepa* roots [34], rice [35], and wheat seedlings [36]. These contradictions might be closely related to the chemical composition, chemical structure, particle size, and surface area of the NPs [37][38]. The response of the photosynthetic pigments' content (chl. a, chl. b and carotenoids) to ZnO NP treatment in the present study correlates with earlier reported findings [39] in which chlorophyll and other photosynthetic pigments in cilantro (*Coriandrum sativum*) were remarkably increased after application of ZnO NPs. Similar results were found in several plant species, according to [16] and [40], and the exposure of the ZnO NPs improved the photosynthetic pigments and protein in *Phaseolus vulgaris* and *Lupinus termis*. The reason behind the enhanced chlorophyll and other photosynthetic content in our study is most likely due to the presence of zinc as a vital nutrient for the plants, essential for protochlorophyllide formation. Zn plays an essential role in plant metabolism by influencing the activities of key important enzymes, such as carbonic anhydrase [39]. In addition, metal nanoparticles are powerful amplifiers of photosynthetic effectiveness that in parallel cause light absorption by chlorophyll, as they cause the transfer of energy from chlorophyll to nanoparticles [41][42]. Carotenoids act as antioxidant compounds soluble in plant cells. These compounds through a non-enzymatic pathway function to reduce oxidative damage to the plant. Carotenoids represent a vital class of antioxidant molecules, which are known to scavenge harmful free radicals, as well as protecting light-harvesting complex proteins and thylakoid membrane stability. In this study, it seems a certain amount of zinc induced oxidative stress increased carotenoid content synthesis [43]. Conversely to our findings, there are some studies which reported that ZnO NPs decrease the chlorophyll content in some plants species, such as kidney bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) [44][45]. This ambiguity may be because some plant species may have different responses to Zn exposure than others.

Application of ZnO NPs at the concentrations in our study caused an increase in total protein content compared to control: 5 mg L^{-1} concentration recorded the highest increase in protein, which may suggest the initiation of de novo synthesis of the enzymes [46]. On the other hand, protein level started decreasing at doses of 10 and 20 mg L^{-1} respectively. The increase in protein at certain concentrations indicates the optimum dose limit for the growth of *M. oblongifolia* plants. However, the reduction in protein beyond this concentration indicates the toxic effect of ZnO NPs. Similar results have been reported by a study on lettuce [47].

Lipids are a key components of cell membranes. They are sensitive to oxidation processes, and generating lipid peroxides is an indicator of an increase in production of toxic oxygen species [48]. MDA content is considered an indicator of oxidative damage. Our findings showed that MDA was diminished in ZnO NP treated plants. It is known that Zn has the ability to stabilize and protect the biomembranes against peroxidative and oxidative stress, integrity of plasma membrane loss, and change in the permeability of plasma membrane [49]. Our results at in line with the findings of previous reports in *Vicia faba* [50], soybean [17], and wheat [30].

The process of oxidative damage is due to the imbalance process of reactive oxygen species (ROS) metabolism in plants. Antioxidant enzymes, such as SOD, CAT, and GR, are known to be the major protective factors protecting against ROS in plants, through which plants can scavenge H_2O_2 (hydrogen peroxide) and O_2^- (superoxide radical) and other ROSs [51][52][53]. The activity of the antioxidant enzymes is significantly enhanced in plants upon exposure to ZnO NPs in a concentration dependent manner. In this study, SOD, CAT, and GR activities of *M. oblongifolia* plants exposed to ZnO NPS significantly increased with increasing of the doses. Boosted activity of antioxidant enzymes by supplementation of ZnO NPs may scavenge H_2O_2 and mitigate mineral uptake, reducing plant oxidative stress [54]. In addition, increasing CAT, SOD, and GR activity, notably with high concentrations of ZnO NPs, probably signals that the antioxidant enzyme system is adapting to counteract excessive production of ROS [55]. Our findings are consistent with the earlier studies documenting that the SOD, CAT, and GR activities were increased with increasing concentrations of ZnO NPs in rice seed [56]. Similarly, a previous trial documented that CAT, SOD, and GR activities were enhanced in *Arabidopsis thaliana* seedlings treated with gold nanoparticles [1]. Hence, enhanced antioxidant potential of *M. oblongifolia* plants under ZNO NPs treatment signifies better performance. Lu et al. [57] also observed that SOD, CAT, and GR activities of germinating seeds of soybean exposed to a mixture of nano-SiO_2 and nano-TiO_2 remarkably promoted seed germination and seedling growth. However, the antioxidant enzyme activities of SOD, CAT, and GR in nanoparticle exposed plants vary significantly according to the plant species, nanomaterial type, duration of treatment, and dose.

3. Conclusions

ZnO NPs were synthesized successfully using leaf extracts of *O. arabicus.* The existence of phytochemicals in the leaf extract of *O. arabicus* helped in the synthesis of ZnO NPs by inducing oxidation and reduction reaction. The average size of the synthesized NPs was 35 nm. The positive effect of the synthesized ZnO NPs is apparent from the promoting of plant biomass. Our findings showed that application of green synthesized ZnO NPs in MO presented morpho-physiolgical changes where, the exposure of 5 mg L^{-1} ZnO NPs to the culture media significantly promoted shoot formation and enhanced the plant weight, level of photosynthetic pigments, and total protein content. The plant growth retreated with higher ZnO NPs doses (10 and 20 mg L^{-1}), but increased the proline content and activated the production of antioxidant enzymes. On the other hand, MDA production was decreased in the plants.

This study indicates that the application of ZnO NPs to the in vitro culture media of plant tissues had positive influences; therefore, green-synthesized ZnO NPs hold promise for their judicious application in agriculture and medicine purposes. However, further investigation is indispensable for a better understanding of the molecular mechanism of ZnO NPs in cell developmental processes and secondary metabolism.

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