



Effects of Foliar Application of Zinc Oxide Nanoparticles on Traits of Several Medicinal Plants Under Aeroponic System Conditions

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ARTICLE INFO

Article history:

Received: 9 Jan 2021,

Received in revised form: 21 Nov 2021,

Accepted: 15 Dec 2021

Article type:

Research paper

Keywords:

Chicory,
Purple coneflower,
Soiless culture,
Valerian,
Withania

ABSTRACT

As a micronutrient, zinc (Zn) plays an essential role in various physiological processes of plants. Here, acclimatized samples of valerian (*Valeriana officinalis* L.), chicory (*Cichorium intybus* L.), withania (*Withania coagulans*) and purple coneflower (*Echinacea purpurea* L.) were evaluated under aeroponic system conditions in order to explore the effects of zinc oxide nanoparticles (ZnO NPs). The plants were foliar sprayed with ZnO NP biofertilizer (0, 1, 2 and 3 g L⁻¹) for 20, 40 and 60 days after transplanting. The experiments were performed based on a completely randomized design with five replications. The valerian showed that 3 g L⁻¹ ZnO NPs caused the highest plant height, root length, leaf number per plant, root volume per plant, as well as fresh and dry weights of roots and shoots. The mean values in chicory showed that the plants that were treated with ZnO NPs (3 g L⁻¹) had the highest amount of biomass and photosynthetic pigments. Based on the results of mean values in purple coneflower, ZnO NPs (3 g L⁻¹) caused the largest increase in morphological values. Furthermore, comparing the mean values in the withania showed that the highest plant height, root length, leaf number per plant, root volume per plant, fresh and dry weights of roots and shoots were observed in plants treated with 3 g L⁻¹ ZnO NPs. Therefore, Zn is considered as an essential micronutrient in the growth of these plants and could be prepared in nanosized form.

Introduction

Medicinal plants are a large group of economically important plants. Valerian is a herbal medicine which is produced from the dried roots of *Valeriana officinalis* L. and has been used as a medicinal herb since ancient Greece and Rome. The phytotherapeutic properties of valerian were described by Hippocrates as a sedative and anti-anxiety agent. Valerian contains over 150 chemical constituents, many of which are physiologically active (Jiang et al., 2007).

So far, 23 species of the withania genus have been recognized. Withania is a significant member of the

Solanaceae family. The roots of these species contain some alkaloids with medicinal value. A related species, *W. coagulans*, is commercially important due to the ability of its berries to coagulate milk. The *Withania* species are distributed in the Eastern Mediterranean and South Asia (Negi et al., 2006).

Purple coneflower (*Echinacea purpurea* L.) is a genus of herbaceous perennials, including nine species, primarily indigenous to the Americas (McGregor, 1968). Several *Echinacea* species showed significant economic value and, for instance, *E. purpurea* has been long used as a

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popular garden ornamental plant and known as a top-selling perennial (Rhodus, 1995).

Chicory (*Cichorium intybus* L.) is a member of the Asteraceae family. Based on a study by Vavilov (1951), the species originates in the Mediterranean region. *C. intybus* is a medicinal plant used to promote appetite and digestion, as it contains bitter-tasting sesquiterpene lactones. Its roots contain eudesmanolides, germacranolides, and guaianolides, which are accumulated mainly as glycosides (Pyrek, 1985; Malarz et al., 2002).

Producing medicinal and aromatic plants in controlled environments improves their biomass, yield quality, and bioactivity, which can yield high-quality herbs and raw material free from accidental adulteration by weeds, pathogens, and soil or environmental toxins (Dorais et al., 2001). Nutrient solution management is key for succeeding in the production of these plants in soilless cultures such as aeroponic systems.

Zinc (Zn) is one of the vital trace elements for both plants and humans (Kaya et al., 1999; Asad and Rafique, 2000; Hao et al., 2007). Developing efficient nano-fertilizers can be a major contribution by nanotechnology to the production phase of agriculture. Nano-fertilizers can replace conventional fertilizers to gradually supply the nutritional requirements of plants in a controlled manner. Nanotechnology can enhance the efficiency of fertilizers and reduce soil pollution, while leveling down environmental risks in chemical fertilizers (Naderi and Danesh-Shahraki, 2013). Nanomaterial can be used for designing more soluble and diffusible sources of Zn fertilizer for increasing plant productivity. The effects of Zn fertilization on the growth and yield of many plants such as wheat, maize, barley, and cotton have been widely evaluated in previous research and have caused a significant increase in the yields of Zn-treated plants (Xi-Wen et al., 2011; Efe and Yarpuz, 2011).

The effects of zinc oxide nanoparticles (ZnO NPs) were evaluated on seed germination and root growth of different plants (Lin and Xing, 2008). In this regard, however, further research is required to reduce the risks and to clarify the extents of ecotoxicity. Emphasis should be placed on their successful transfer to the plant system. Although ZnO NPs are a novel elicitor, their use has not been reported in aeroponics. Therefore, the present study aims to explore the effects of foliar spraying of ZnO NPs on the growth of valerians, withania, purple coneflowers, and chicories in an aeroponic system, while also assessing the quantitative and qualitative characteristics of these plants.

Materials and Methods

The plants were obtained from seed culture (valerians, Withania, purple coneflowers, and chicories) in pots under greenhouse conditions for one month. Then, uniform plants (approximately 20 cm height) were transferred into an aeroponic system. The research was conducted in plastic-greenhouse conditions at day/night temperatures of 25/20 °C with 16-hour daylight in the research greenhouse of Malayer University in 2019.

Aeroponic system

An experimental aeroponic system (phytorhizotron) was used to produce the plants (Movahedi et al., 2012). This phytorhizotron system consisted of two compartments. The upper compartment was supplied with photoperiod control, and the lower was kept in darkness. The plants were cultured on the board of the upper compartment with a spacing of 13 × 13 cm² and about one-third of the length of the stems was placed inside the lower compartment. The shoots grew in the upper compartment. However, the roots were developed in the lower compartment in darkness. The lower compartment was a closed container (100 × 100 × 120 cm³; deep × width × length), which had a removable front panel for monitoring and harvesting. The plant roots were periodically sprayed (every 20 min for 20 sec) with a nutrient solution using 12 fog nozzles per m². The nutrient solution was renewed weekly. The remaining nutrient solution flowed back into a collecting tank to be recirculated.

Nutrient solution and growing conditions

A composition of the nutrient solution is given in Table 1. The electrical conductivity (EC) of the solution was set at 1.6 ± 0.2 dS/m. Typically, the nutrient tank was filled with filtered water followed by adding fertilizer. The EC meter probe was calibrated by using the buffer solution. The solution was diluted by adding water when the reading yielded a higher value, compared to the optimum level. The nutrient concentration was increased to the optimum level if the reading was lower than the optimum level. The pH was initially adjusted to 5.8 ± 0.2.

Treatments

The effect of foliar application of ZnO NPs (0, 1, 2, and 3 g L⁻¹) was assessed on the growth and development of the valerian, Withania, purple coneflower, and chicory plants. ZnO NPs (99% purity) were purchased from Pishgaman-e Nano Company.

Table 1. The components of the nutrients used in the aeroponic system (mg/l)

Elements	Concentration (mg/l)	Elements	Concentration (mg/l)
K	200	Fe	1
N	190	Mn	0.5
Ca	150	B	0.5
S	70	Zn	0.15
Mg	45	Cu	0.1
P	35	Mo	0.05

The nanoparticles had an average size of 10-30 nm, and 20-60 m²/g specific area.

The control plants were treated with distilled water. In all treatments, Tween 20 was used as the wetting agent at a rate of 0.05 mL/L. The plants were foliar sprayed with ZnO NPs 20, 40, and 60 days after transplanting (DAT) in an aeroponic system. The experiment was based on a completely randomized design (CRD) with five replications. Each replication included three plants, which were merged and considered as a biological replication.

Data recording

The plants were harvested six months after transplanting. Plant traits included height, root length, leaf number, root volume, fresh and dry weights of roots, fresh and dry weights of shoots, chlorophyll a and b, and carotenoids.

Fresh and dry weights of roots and shoots were measured. The roots and shoots were first weighed by a digital scale to record their fresh weight and were then oven-dried at 68 °C (48 h) to reach a constant weight. The dry weight was measured and the dry matter (%) was calculated accordingly.

The photosynthetic pigments of the leaves were determined as described by Lichtenthaler (1987). For this purpose, 0.2 g of the fresh leaves was ground in a china mortar containing 15 mL of 80% acetone so as to allow the measurement of chlorophyll and carotenoid contents. After infiltration through a filter paper, the absorbance was read at 646.8, 663.2, and 470 nm by a spectrophotometer. The device was nullified by acetone 80%. The pigment concentrations were calculated by the following equations.

$$(1) \text{Chla} = 12.25 A_{663.2} - 2.79 A_{646.8}$$

$$(2) \text{Chlb} = 21.21 A_{646.8} - 5.1 A_{663.2}$$

$$(3) \text{Car} = (1000A_{470} - 1.8 \text{Chla} - 85.02 \text{Chlb})/198$$

where Chla (equations 1), Chlb (equations 2), and Car (equations 3) show the concentrations of chlorophyll a, chlorophyll b, and carotenoid, respectively. The concentrations were determined based on the mg/mL of plant extract and the results were finally reported based on the mg/g fresh weight (FW) of the tissue.

Data analysis

Primary statistical analyses included the normality test (Kolmogorov-Smirnov test) and homogeneity of variances (Levene test). The mean values of treatment combinations were compared by Duncan's Multiple Range Test (DMRT) after the analysis of variance (ANOVA). All statistical analyses were performed by SPSS version 21.

Results

Valerian

The ANOVA indicated highly-significant effects of Zn on all of the recorded traits ($P < 0.01$). ZnO NPs significantly influenced the total plant height. A comparison of the mean values indicated that ZnO NPs (3 g L⁻¹) enhanced the plant height by 46.5%, compared to the control (Table 2). ZnO NPs significantly ($p < 0.01$) affected root length and root volume. ZnO NPs (3 g L⁻¹) increased these traits by 69.9% and 95.6%, respectively, compared to the control (Table 2). Also, a significant effect was observed in leaf number. Plants treated with 3 g L⁻¹ ZnO NPs produced more leaves (72.6%) than the control (Table 2). Statistical analysis on the root fresh and dry weights showed that ZnO NPs functioned significantly by causing an increase in the fresh and dry weights of roots by 49.8% and 88.2%, respectively, using 3 g L⁻¹ ZnO NPs compared to the control (Table 2). The fresh and dry weights of shoots showed significant differences induced by ZnO NPs which caused an increase in the said values by 55.8% and 59.5%, respectively, when 3 g L⁻¹ ZnO NP was used, compared to the control (Table 2). Based on the statistical results of leaf chlorophyll content, using ZnO NPs affected the traits significantly. Using 3 g L⁻¹ ZnO NPs led to higher chlorophyll a and b contents (39.5%), while also generating a higher carotenoid content (26.5%), compared to the control (Table 2).

Chicory

The analysis of variance indicated the significant effect of ZnO NPs ($P < 0.01$) on all traits of chicory plants. Using 3 g L⁻¹ ZnO NPs caused a significant increase (75.8%) in the height of chicory (Table 3). As shown in Table 3, root length and root volume increased by 85% and 67.3%, respectively, by increasing ZnO NPs from 0 to 3 g L⁻¹ compared to the control. The experimental data on leaf number indicated a significant difference between the treatments ($p < 0.01$). Applying 3 g L⁻¹ ZnO NPs enhanced leaf number by 59.8%, compared to the control. As shown in Table 3, plants treated with 3 g L⁻¹ ZnO NPs developed higher values of root dry weight (60%) and higher values of root fresh weight (21.8%) compared to the control group.

Applying 3 g L⁻¹ ZnO NPs significantly increased the dry and fresh weights of shoots by 93% and 54.3%, respectively, compared to the control (Table 3). A significant effect of ZnO NPs was observed on photosynthetic pigments ($p < 0.01$) and, according to the comparison of mean values (Table 3), increasing the ZnO NPs content from 0 to 3 g L⁻¹ caused an increase in chlorophyll a and b contents, as well as a higher carotenoid content by 43.6% and 43.8%, respectively, compared to the control.

Purple coneflowers

The ANOVA results indicated the significant influence of ZnO NPs ($P < 0.01$) on all traits of purple coneflower. ZnO NPs significantly influenced the total plant height and, by comparing the mean values, it was observed that using 3 g L⁻¹ ZnO NPs increased the plant height by 47.8%, compared to the control (Table 4). ZnO NPs significantly increased the root length and root volume ($p < 0.01$). Applying 3 g L⁻¹ ZnO NPs led to 82% and 84.9% enhancements in both traits compared to the control (Table 4). There was a significant effect of ZnO NPs on leaf number and the highest number of leaves was observed in plants treated with 3 g L⁻¹ ZnO NPs, which was 73.1% higher than that of the control (Table 4).

The use of ZnO NPs significantly affected the fresh and dry weights of roots, while comparing the mean values indicated that the fresh and dry weights of roots were enhanced by 79.1% and 63.7%, respectively, in plants treated with 3 g L⁻¹ ZnO NPs (Table 4). Evaluating the fresh and dry weights of shoots showed the significant influence of ZnO NPs on this trait. Calculating the fresh and dry weights of shoots revealed increases of 88.8% and 88% in the two traits, respectively, compared to the control group, as a result of ZnO NPs (Table 4). The statistical analysis of the leaf chlorophyll content showed the significant effect of ZnO NPs on this trait. Using ZnO NPs (3 g L⁻¹) increased chlorophyll a and b contents, as well as carotenoids, by 72.8% and 37.9%, respectively (Table 4).

Withania

Based on the ANOVA results, the effects of ZnO NPs were highly significant ($P < 0.01$) on all traits of *Withania*. As shown in Table 5, applying ZnO NPs (3 g L⁻¹) significantly increased the total height (87.8%), root length (84.2%), root volume (66.6%), leaf number (67.4%), root dry weight (88.1%), root fresh weight (98.2%), shoot dry weight (97.6%), shoot fresh weight (74.6%), chlorophyll a and b (63.8%), as well as carotenoids (46.8%), compared to the control (Table 5).

Discussion

Based on the results, ZnO NPs increased the root length and plant height of all four plants. Pavani et al. (2014) reported that Zn NPs promoted shoot and root growth of the *Cicer arietinum*. The foliar application of Zn may affect growth processes by influencing photosynthesis and other plant processes (El-Metwally et al., 2015). In confirmation of the current results, Reda et al. (2014) indicated that ZnSO₄ positively affected the height, branches, and shoots of faba bean (*Vicia faba* L.). The positive effects of Zn have been reported in the available literature, especially regarding plant height in various medicinal plants such as basil (Said Al-Ahl and Mahmoud, 2010), *Geranium* (Ayad et al., 2010), and *Salvia farinacea* (Nahed and Balbaa, 2007). Also, Rosramifard et al. (2012) stated that Zn-promoted effects on plant growth were a result of cell division and elongation.

In the current study, the fresh and dry weights of the roots and shoots increased as a result of the Zn in all four plants. Shokr et al. (2014) reported that the foliar application of Zn increased the fresh and dry weights of snap beans (*Phaseolus vulgaris* L. cv. Bronco), compared to the control. Furthermore, ZnSO₄ caused an increase in the shoot fresh weight of dry beans (*Phaseolus vulgaris* L.) (Ibrahim and Ramadan, 2015). According to a study by Jayarambabu et al. (2015), ZnO NPs significantly increased the fresh and dry weights of roots in *Vigna radiate*. The number of leaves increased by using Zn in the evaluated plants. Similarly, Vafa et al. (2015) indicated that the number of leaves increased significantly in *Satureja hortensis* L.) as a result of Zn treatment. Moreover, Khalifa et al. (2011) reported that the use of Zn caused an increase in the number of leaves in iris plants. In addition, considerable effects of Zn were observed in geranium (Ayad et al., 2010), wheat (Cakmak, 2008), and petunia (SalehiSardoei et al., 2014). The vital roles of trace elements include the activation of enzymes, biosynthesis of proteins, photosynthesis, cell division and differentiation.

Based on the results of the present study, the use of ZnO NPs increased the photosynthetic pigments. Kaya and Higgs (2002) demonstrated that the foliar application of Zn enhanced the chlorophyll content in tomatoes. Similar results were reported regarding the positive effects of Zn on photosynthetic pigments in previous research. For instance, Song et al. (2015) and Fahad et al. (2014) assessed *Vitis vinifera* and *Gladiolus grandifloras* for this evaluative purpose.

Table 2. Comparison of mean values for the effect of different concentrations of ZnO NPs on morpho-physiological traits of valerian in the aeroponic system

ZnO (g L ⁻¹)	Shoot dry weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Root fresh weight (g)	Plant height (cm)	Root length (cm)	Leaf number	Root volume (cm ³ /plant)	Carotenoid (mg/g)	Chlorophyll a + b (mg/g)
Control (0)	18.5 d	91.5 d	10.2 d	64.2 d	90.4 d	41.3 d	22.3 d	18.2 d	1.39 b	2.98 b
1	21.2 c	109.2 c	13.1 c	72.8 c	115.2 c	54.2 c	29.2 b	25.4 c	1.44 b	3.41 a
2	26.8 b	130.8 b	16.4 b	83.4 b	124.7 b	61.5 b	35.4 a	30.2 b	1.47 b	3.54 a
3	29.5 a	142.6 a	19.2 a	96.2 a	132.5 a	70.2 a	38.5 a	35.6 a	1.94 a	3.77 a

Table 3. Comparison of mean values for the effect of different concentrations of ZnO NPs on morpho-physiological traits of chicory in the aeroponic system

ZnO (g L ⁻¹)	Shoot dry weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Root fresh weight (g)	Plant height (cm)	Root length (cm)	Leaf number	Root volume (cm ³ /plant)	Carotenoid (mg/g)	Chlorophyll a + b (mg/g)
Control (0)	48.6 d	243.2 d	58.6 d	193.5 d	110.6 c	68.37 c	47.1 d	25.4 d	1.14 d	3.05 d
1	65.9 c	314.5 c	65.9 c	202.5 c	172.3 b	117.5 b	58.2 c	29.3 c	1.27 c	3.25 c
2	79.1 b	343.2 b	79.1 b	220.3 b	177.7 b	119.4 b	62.5 b	34.6 b	1.33 b	3.59 b
3	93.8 a	375.4 a	93.8 a	235.8 a	194.5 a	126.5 a	75.3 a	45.2 a	1.64 a	4.38 a

Table 4. Comparison of mean values for the effect of different concentrations of ZnO NPs on morpho-physiological traits of purple coneflower in the aeroponic system

ZnO (g L ⁻¹)	Shoot dry weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Root fresh weight (g)	Plant height (cm)	Root length (cm)	Leaf number	Root volume (cm ³ /plant)	Carotenoid (mg/g)	Chlorophyll a + b (mg/g)
Control (0)	9.2 d	42.3 d	6.18 d	29.3 d	75.2 d	34.5 d	18.2 d	21.2 d	1.08 d	1.29 c
1	11.8 c	56.4 c	7.15 c	32.4 c	86.4 c	49.5 c	23.1 c	28.6 c	1.22 c	1.46 c
2	14.2 b	69.6 b	8.36 b	41.3 b	95.3 b	54.3 b	27.2 b	30.7 b	1.35 b	1.9 b
3	17.3 a	79.9 a	10.12 a	52.5 a	111.2 a	62.8 a	31.5 a	39.2 a	1.49 a	2.23 a

Table 5. Comparison of mean values for the effect of different concentrations of ZnO NPs on morpho-physiological traits of withania in the aeroponic system

ZnO (g L ⁻¹)	Shoot dry weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Root fresh weight (g)	Plant height (cm)	Root length (cm)	Leaf number	Root volume (cm ³ /plant)	Carotenoid (mg/g)	Chlorophyll a + b (mg/g)
Control (0)	5.01 c	21.3 d	2.86 c	9.13 d	58.3 d	30.5 d	21.2 d	6.12 cd	1.11 b	1.55 d
1	5.11 c	28.4 c	3.12 c	14.5 c	77.4 c	36.8 c	26.4 c	7.6 c	1.19 b	1.89 c
2	8.2 b	31.5 b	4.25 bc	17.2 b	86.2 b	40.7 b	30.7 b	8.14 bc	1.57 a	2.14 b
3	9.9 a	37.2 a	5.38 a	18.1 a	109.5 a	56.2 a	35.5 a	10.2 a	1.63 a	2.54 a

Singh et al. (2013) reported that chlorophyll content increased in response to ZnO NPs. Farrag (2015) demonstrated an increase of chlorophyll content in ZnO NPs-treated *Lemna gibba* L.

Zn contributes to the stability of enzymes, proteins, and cell membrane lipid composition by binding to the 2-sulfhydryl group. Furthermore, Zn protects the 2-sulfhydryl group and, thus, promotes chlorophyll synthesis. Porphobilinogen is the precursor of chlorophyll, the formation of which requires Zn and Mg. Overall, Zn contributes to the protection, formation, and integrity of chlorophyll (Powell, 2000).

The results confirmed the applicable use of aeroponic systems for evaluating the effects of nutrients on plant growth, especially roots. The loss of roots in soil preparation during the harvest process may lead to a significant loss of yield in medicinal plants. In soilless farming, nutrients are dissolved in water and the solution enters the roots, which causes an uptake of water with minerals towards different parts of the plant. In soil-based production, the elements stick to the soil particles and pass into the soil solution, where they are absorbed by the plant roots. Nutrients are sprayed directly to the roots every few minutes and, thus, provide a thin layer of nutrients.

Minimizing the nutrient loss and increasing yields through better nutrient absorption and water management are the main aims of using nanoparticles in soilless farming. Nanoparticles, which have high specific surface and relative reactivity, can provide the plant with more soluble and usable forms of nutrients, while restricting precipitation and insolubilization processes. Therefore, nanoparticles are more effective nutrient carriers for plants, compared to the conventional fertilizers, thereby suggesting that nanoparticles are promising tools in general, particularly for soilless growing systems (Maluin et al., 2021).

The principles of aeroponics rely on the possibility of cultivating vegetables with roots that are not inserted in a substratum (i.e. in the case of hydroponics) or soil, but are instead inserted in containers filled with a flowing-liquid of plant nutrition. Roots can be exposed to the best conditions of oxygenation and moisture in these containers. These conditions enable better plant nutrition assimilation in a more balanced way, along with a faster growth of cultivated plants. Plant containers can be mounted on top of each other and can be easily moved if required, given their low weight and feasibility of relocation. The aeroponic system involves a continuous cycle in an enclosed space that reduces the agricultural labor into a series of mechanical routine operational tasks,

which can be performed daily and throughout the year. Therefore, workers can acquire considerable skills within a short period, maybe in a few months. Climate control in the greenhouse ensures optimal growing conditions and high yields. A limited use of water is the most prevalent advantage of aeroponic systems compared to traditional agriculture. This system has been commercially successful in arid areas. In aeroponic systems, agricultural success can occur regardless of land and soil qualities. Soil composition is irrelevant since the soil is never used in the process. The system allows intensive food production on a limited land area. It has offered the highest output per unit of land in a year among all known systems. The growing system can be constructed in the vicinity of consumers. The greenhouse can be constructed near the urban centers and markets to reduce freight costs and to supply consumers with fresh crops. In aeroponic systems, yields are less likely to be affected by seasonal adversity, e.g. chilling, hot windy days, or dry weather. Moreover, its non-stop production cycle ensures a constant market supply at more stable prices. The automation of most agricultural operations with a limited need for farm workers and investment in farm equipment can be another advantage of this system. The simplicity and reliability of the mechanical system usually permit the employment of unskilled or partially handicapped labor. Additionally, it is commercially applicable in areas without any agricultural tradition.

Conclusion

Based on the results, Zn had a significant effect on photosynthetic pigments, plant height, root length, leaf number per plant, root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight in the evaluated plants (i.e. chicory, *Withania*, valerian, and purple coneflowers). This positive effect was linear and the best results were achieved at the highest level of ZnO NPs (3 g L^{-1}). Furthermore, it seems that more positive results can be obtained at higher levels of applicable concentrations.

Conflict of interest

The authors report no conflict of interest in this study.

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