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Original Research Article

Antioxidant Response *of Vicia faba* Plant to Foliar Spray of Green Synthesis Zirconium Oxide Nanoparticles

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Abstract: Previous studies have reported a positive influence of zirconium oxide nanoparticles on plants, the present effort was conducted to investigate the impact of two distinct concentrations of biosynthesized zirconium oxide nanoparticles (75 and 150 mg L^{-1}) on the physiological and antioxidant responses of *Vicia faba* Plants. Zirconium oxide nanoparticles (ZrO_2NPs) were synthesized using *Matricaria chamomilla* L. plant extracts. And their morphology and extent were determined Using X-Ray Diffraction and Transmission electron microscopy The diffuse reflectance spectra were measured using UV-Vis spectroscopy, the response of *V. faba* plants to foliar sprays regarding concentrations of (0, 75 and 150 Mg / l^{-1}) M-ZrO_2NPs was studied. The largest concentrations of chlorophyll a and b, carotenoids. Total pigments were documented in plants receiving 75mg / l^{-1} of M-ZrO_2NPs associated to the control, secondary metabolite (Phenolics, Flavonoids, and Tannins) were gathered in response to M-ZrO_2NPs treatment Glycosides, Terpenoid, Alkaloids (Mayer reagent) and Fuocoumarins. Moreover Tannins, Resins and Saponins, in addition to the levels of enzymatic and non-enzymatic antioxidants, rose by about two-fold in plant exposed to M-ZrO_2NPs compared to control plants. The nitrogen, phosphorus, the contents of bean plants in terms also potassium, calcium, zinc, iron were noted in response to two applied quantities of M-ZrO_2NPs. Spraying shrubberies by 75 Mg /l^{-1} of M-ZrO_2NPs promoted plant development in addition to the buildup of antioxidants, osmolytes, and secondary metabolites that can help plants cope with the harmful consequences of environmental stress.

Keywords: Green synthesis, zirconium, nanoparticles, *Matricaria chamomilla* L., and *Vicia faba* Plants.

INTRODUCTION

Nanotechnology is an emerging field of science and has diverse applications in the fields of health, environment, and agriculture (Kor and Sinh, 2020). This Technology utilizes several substances, and its goal is to create materials with nano meter dimensions that have better functions (Otero-Gonzalez *et al*., 2013). Nanotechnology deals with materials with structures whose size range is from 1 to 100 nanometers (Shaimaa *et al*., 2021). These materials are characterized by having different structural, physical, and chemical properties when compared to their larger counterparts, and because of the surface area, size, and shape at the nano level, the materials Nanoparticles possess optical, electrical, and magnetic properties (Amal *et al*., 2020). Many nanoparticles possess different chemical and physical properties when compared to their natural size, and this makes them enter many fields, starting from electronics and not ending in the aggrecluter field (Ahmed *et al*., 2017). Nanoscale zirconium oxide (ZrO_2) has emerged as a promising and interesting nanomaterial in the world of metals (Li *et al*., 2021) ZrO_2 NPs have attracted much attention by researchers because they possess optical, thermal, electrical, etch and mechanical properties which are biocompatible (Mallakpour and Nezamzadeh, 2017; Shinde *et al*., 2018) Zirconium (Zr) is one of the transition metal elements with atomic number 40 (Cazado *et al*., 2021). Zirconium dioxide (ZrO_2) is considered One of the constant oxides that are produced by thermal compounds of zirconium (Hassan and Jalil, 2022) dependent on the method of production, zirconium can exist in the crystal-like state, which includes cubic, tetragonal, and monoclinic (Zhang *et al*., 2018), due to high stability and low toxicity, zirconium (ZrO_2) has demonstrated a wide range of practical technologies. Green synthesis uses available and low cost sources such as plants, fungi, bacteria, and algae to synthesize ZrO_2 NPs (Rana *et al*., 2020; Nguyen *et al*., 2021). Plants are the most widely available and locally effective source in terms of nanoparticle synthesis (Yadi *et al*., 2018). When related to additional biosynthetic ingredients for example algae, fungi, and bacteria (Saravanan *et al*., 2021), plants contain a variety of biomolecules that have a major role in stabilizing ZrO_2 NPs, and these molecules include Phenols, Flavonoids, Sugars, and Tannins. Amino acids and enzymes found in extracts are medically important (Zarghani and Akhlaghinia, 2016; Ghotekar, 2019). (*Vicia faba*) a significant crop in North Africa is a great source of organic matter and quartzes (proteins, carbohydrates, folic acid, thiamine and tocopherols), is among the most important sources of nourishment for humans and animals, and it's employed in the food sector (Hassanin *et al*., 2012). Faba plants improve soil fertility and production by introducing nitrogenous substances into the soil (Qadous *et al*., 2015).

The *M. chamomilla* L. plant is one of the medical plants and economic importance (Abdel Baqi *et al*., 2001). The *M. chamomilla* L. plant contains many active substances such as Volatile oils, Phenolic compounds, Tannins, Glucosides, Coumarins, and Favonoids (Chevalia, 2003).

The objective of this work was to synthesize zirconium oxide nanoparticles from *M. chamomilla* L. Extract and examine the crystallite form and extent of the shaped nanoparticles. The investigations also centered on the application of ZrO_2 NPs to *Vicia faba* Plants for studying the physiological response of antioxidants in *Vicia faba* Plants and improve plant performance.

MATERIALS AND METHOD

Collect Plant Materials

The plants were collected from local markets in the Najaf city / Iraq. The plants were selected based on their human uses. They were classified by a specialist at the University of Kufa. Broad bean (*Vicia faba* L. cv. Nubaria, 1) was obtained from the Field Crops Research Institute, Agricultural Research Center, Najaf, and employed in the current investigations. This cultivar is distinguished by early blossoming, a large quantity of seeds per plant, recorded 100-seed mass (95 G), and a high protein and carbohydrate contents (24 and 58%, respectively) in seeds (Qabil *et al*., 2018).

Preparation of Aqueous Extract of *M. Chamomilla* L. Plant

The hot aqueous extract was prepared from the leaves of the *M. chamomilla* L. plant according to the method (Ali *et al*., 2020). The *M. chamomilla* L. leaves were washed, dry, grinding then the powder of the *M. chamomilla* L. leaves was created and stored in plastic containers until use.

50 grams of *M. chamomilla* L. leaf powder were weighed and added to deionized water (DW) in a ratio of (1:10) and vigorously swirled for half an hour at 70 °C. After that, were filtered the extract and kept at 4°C in until used.

Synthesis of Zirconium Nanoparticles (Green Synthesis)

M-ZrO_2NPs were manufactured according to the method (Al-Othman *et al*., 2017), where 10 ml of *M. chamomilla* L. leaf abstract was mix with 100 ml of zirconium nitrate solution, 1 mM, and observing the mixture color change as preliminary evidence for the nano-zirconium particles. The nanoscale properties were confirmed by scanning electron microscopy SEM, XRD and UV.

Plants Agriculture and Treatments

Equally sized and weighted broad bean seeds were sterilised in an aqueous solution of 0.1% HgCl2 for one minute while shaking constantly before being washed methodically with disttilled water several periods. 10 seeds (5 cm deep) were evenly distributed among the 25 cm diameter plastic pots, weighing 3.5 kg dry clay loam (80% sand; 15.5% silt; 4.5% clay; 0.45% organic substance, pH 7.8; EC 0.4 dSm-1). The pots were maintained in lighting and dark environments with a temperature range of 28°C/ 16°C, day / night and relative humidity 75%) in the glasshouse of the College of Science, University of Kufa. Every plant received watering to ensure growth throughout the experimentation, the study involved three treatments: the first treatment (T1) contained of spraying shrubberies with new water by way of a controller, the second treatment (T2) was foliar spraying with M-ZrO_2NPs (75 0 Mg / l^{-1}), and the third treatment (T3) was spraying with M-ZrO_2NPs (150 Mg / l^{-1}), each treatment consisted of five replicates randomly distributed. Zirconium oxide nanoparticles were sprayed two time each two week, plant samples were taken two months later antioxidants and other properties were measured.

Preliminary Chemical Detection of Active Compounds in Plant

Detection of glycosides according to the method (Ayoola *et al*., 2008). Detection of Triterpenoids depending on (Harborne, 1984). Discovery of tannins.

A. Lead acetate reagent: It was ready using the technique (Ahmed *etal*., 1998).
B. Ferric chloride reagent: Prepared depending on the method (Trease and Evans, 2002; Adedayo *et al*., 2001).

Detection of resins rendering to the method of (Al-Shami, 1982).
Discovery of flavonoids

A. Alcoholic potassium hydroxide reagent: It was prepared depending on (Al-Khazraji, 1991).
B. Concentrated sulfuric acid reagent: It was prepared according to (Al-Khazraji, 1991).

Detection of Saponins a ccording to the way (Kumar *etal*., 2009).
Discovery of alkaloids

A. Marquis reagent: prepared according to the method (Harpoon, 1973).
B. Dragendrov's reagent: It was prepared according to the method (Anthetden, 1969).

Detection of phenols

A. Ferric chloride reagent: prepared according to the method (Harbone, 1973).

Coumarin Detection according to the method of (1962, Geisman).

A. Detection of terpenes. A ccording to the method (Ayoola *etal*., 2008).

Estimation of Antioxidants:

* Estimation of chlorophyll a and b content:

Chlorophyll pigments in Brassica nigra L. were determined by the method (Arnon, 1949).

* Estimation of the content of α and β carotenoids:

The method used to determine the carotenoids' content was (Al-Sultani and Judah, 2022) based on the process of (Duxbury and Wintsch, 1956).

* Estimation of proline contented (Pro)

The free proline content in Brassica nigra L. plant was calculated using the method (Bates *et al*., 1973).

* Estimation of ascorbic acid contented (A.A):

The approach was used to estimate the ascorbic acid content. (Shalata and Newman, 2001).

* Estimation of Superoxide Dismutase (SOD) Activit .

Depending on (Marklund S and Marklund G, 1974).

* Estimation of total protein

Total proteins were estimated and measured using the technique of (Bishop *et al*., 1985).

RESULTS AND DISCUSSION

Preliminary Phytochemical Detection in *M. Chamomilla* L

The consequences shown in (table 1) the aqueous extract of *M. chamomilla* L. contains many active substances, as it gave a positive result for the appearance of glycosides, terpenoid, flavonoids, phenols, and coumarins, and provided negative results for tannins, resins, saponins. Chemical verification of the active ingredients in *M. chamomilla* L. extract was a preliminary step to identify its active ingredient content, which included the presence of flavonoids, phenols, and other active ingredients that possess biological characteristics like antibacterial and antioxidant activitiy, mutagenic activity, pro-apoptosis activity in cancer cells, and anti-inflammatory activity (Bouaoudia *et al*., 2017). Studies have shown the existence of active ingredients in *M. chamomilla* L., as Haghi *et al*., (2014) reported the existence of phenolic substances in the aqueous extract of *M. chamomilla* L. that was harvested from the Wargla region in April 2013. Also, Sureda and Sharifi-Rad (2018) reported the presence of flavonoids through a study conducted on *M. chamomilla* and *M. recutita*.

Table 1: Preliminary phytochemical detection in *M. chamomilla* L

N	Tests	Detection indicator	*M. chamomilla* L.
1	Glycosides Test	Brown ring	+
2	Terpenes	Reddish-brown layer	+
3	Trpenoid	Red purple ring	+
4	Tannins Test	Gelatinous precipitate Ferric chloride Test	-
		Bluish-green color Lead acetate Test	-
5	Resins Test	Turbidity	-
6	Flavonoids Test	Dark yellow color Potassium hydroxide	+
		Yellow colorsul furic acid test	+

N	Tests	Detection indicator	*M. chamomilla* L.
7	Saponins Test	Layer of foam	-
8	Alkaloids Test	Orange-colored precipitate/Dragendroff reagent	-
		TurbidityMayer reagent	+
9	Phenolate Test	Bluish-green color	+
10	Fuocoumarins Test	yellow color	+

M- ZrO_2NPs Nanoparticles Classification

The examination was carried out using field emission scanning electron microscopy (FESEM) morphological properties of the nanoparticles produced using *M. chamomilla* L. plant extract. The particle size ranges from 18.6 nm to 30.23 nm figure 1. The XRD pattern shows peaks for the tetragonal structure to M-ZrO2NPs samples. Peaks appear at 20 theta (degree) = 28.53◦, 35.42◦, 45.61◦, 70.44◦ which correspond to the (001), (110), (111) and (112) diagrams, respectively, which are consistent with the JCPDS card: (96-230-0613)., using Scherrer's formula (Janaki *et al*., 2015) figure 2. UV-visible spectra of *M. chamomilla* L. extracts and M-ZrO_2NPs are shown in Figure 3 Well-defined surface plasmon resonances (SPRs) are observed at around 280 nm in the plant extract but two peaks appear when the nanomaterial is synthesized using the extract, one peak at 280 nm and an additional peak at 328 nm indicating the presence of nano zirconium.

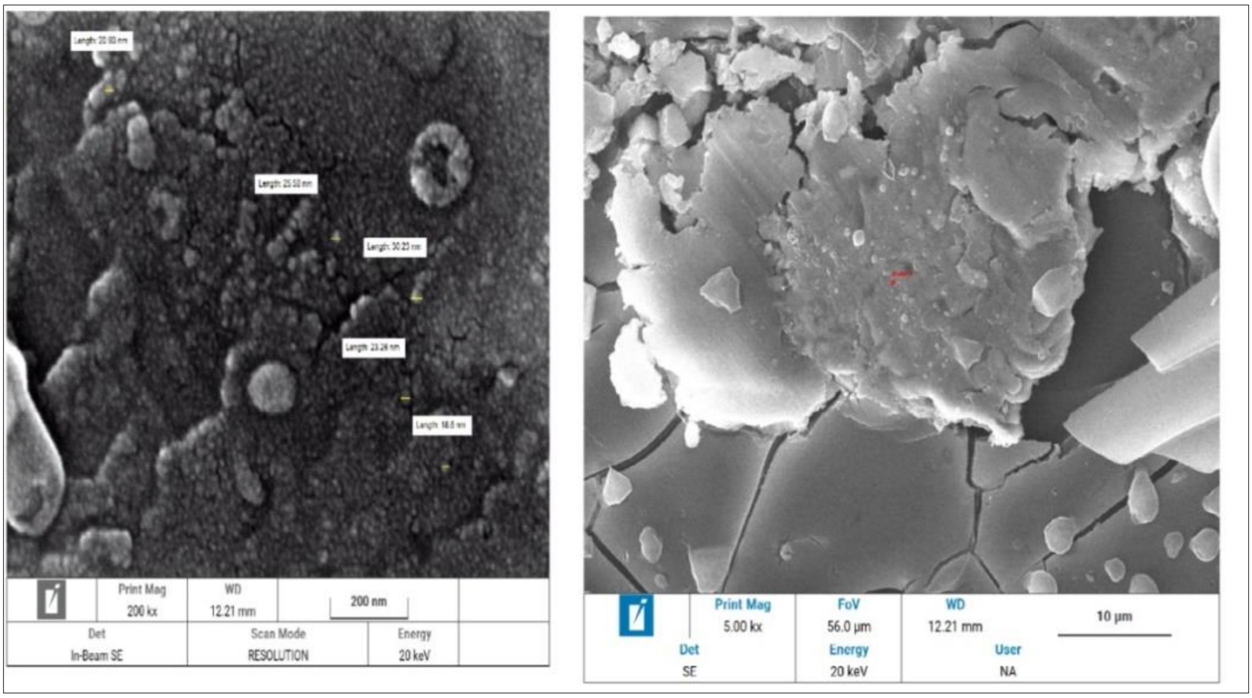

Fig. 1: FESEM micrograph of the synthesized M-ZrO_2NPs

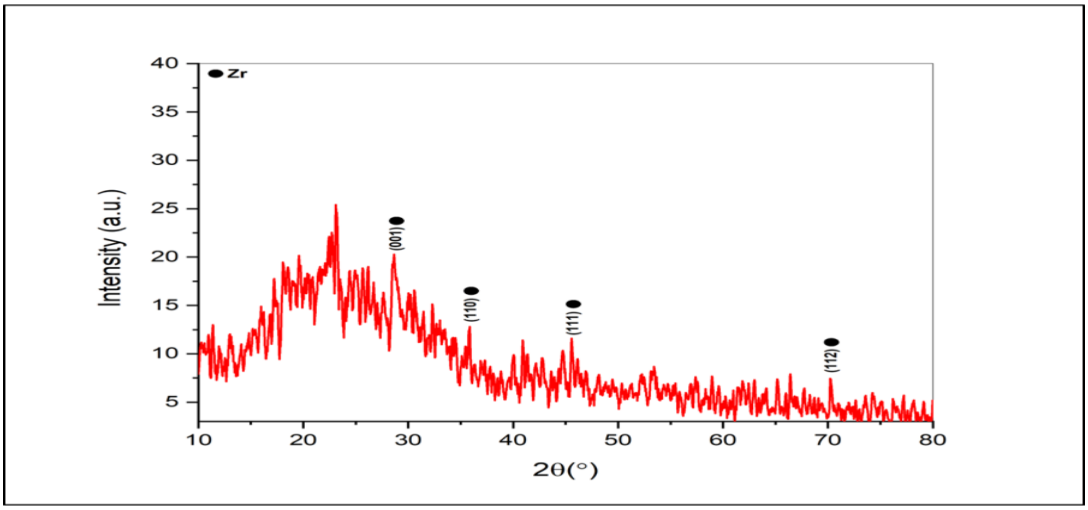

Fig. 2: XRD spectra of M-ZrO_2NPs utilizing extracts of *M. chamomilla* L

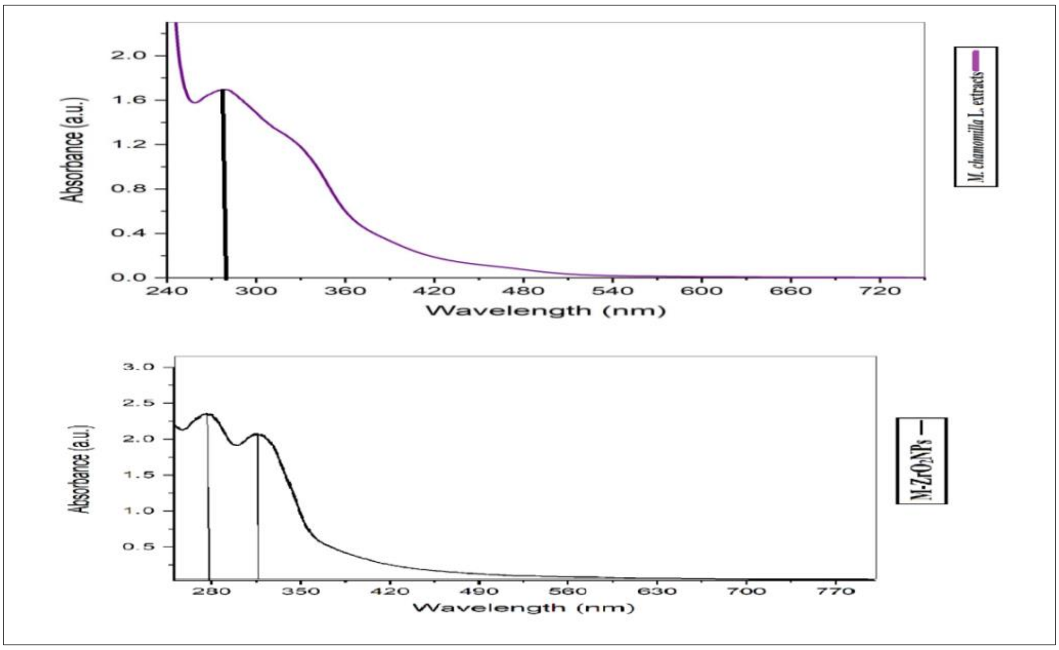

Fig. 3: UV-Visible Spectra of *M. chamomilla* L. extracts before and after the formation of M-ZrO_2NPs

Change in Growth Parameter

The data showed that the foliar spray influence of M-ZrO_2NPs increased leaf and root extent, Weight of fresh and dried beans in their natural environments compared to treatment of control. The minimum concentration of M-ZrO_2NPs (75 Mg / l^{-1}) caused about 27% and 20% increase in length, 35% and 31% in fresh weight, and 29% and 23% in dry weight of roots and shoots, respectivly, compared to untreated plant. However, plants cured by 150 Mg / l of M-ZrO_2NPs presented significant decrease relative to control plants in each of the aforementioned parameters table (2).

Table 1 Effect of different concentrations of M-ZrO_2NPs (75 and 150 mg L^{-1}) on *Vicia faba* plants on morphological parameters. (T1) Control, (T2) M-ZrO_2NPs treatment at 75 mg L^{-1}, (T3) M-ZrO_2NPs treatment at 150 mg L^{-1}.

Treatment	Shoot l. cm	Root l. cm	Shoot f.w. g	Root f.w. g	Shoot d.w. g	Root d.w. g
T1	62.23 ± 0.83 b	42.73 ± 0.75 b	9.37 ± 0.31 a	3.23 ± 0.41 ab	4.02 ± 0.15 a	2.04 ± 0.15 a
T2	68.13 ± 1.06 a	45.80 ± 0.55 a	9.33 ± 0.35 a	3.60 ± 0.06 a	4.12 ± 0.14 a	2.07 ± 0.01 a
T3	55.02 ± 2.10 c	35.80 ± 0.44 c	9.05 ± 0.20 b	3.07 ± 0.15 b	3.60 ± 0.20 b	2.02 ± 0.2 a

By using Duncan's multiple range test at $p < 0.05$, the mean values (n = 3) for each characteristic in the same column with the same lowercase letter do not differ substantially.

Changes in Photosynthetic Pigments

Table 3 showed, M-ZrO_2NPs treatment plants had a significant influence on the pigment contents of plant when related with the control group. The content of Chl a, Chl b, Carotenoid and full Chlorophyll in M-ZrO_2NPs cured plants increased in 45, 56, 23 and 46.3%. The contented of the stated dyes presented a significantly increase after plant were expose to a concentrations of 75 Mg / l^{-1} (5.41, 3.15, 0.780 and 6.95 Mg / g $^{-1}$), compare with the controller. At higher concentrations of M-ZrO_2NPs nanoparticles, the step of reserve was significant extra marked when compare with the corresponding controls.

In table 3 Influence of altered concentrations of M-ZrO_2NPs (75 and 150 Mg /l^{-1}) on *Vicia faba* plant's photosynthetic pigment concentration. (T1) control, (T2) M-ZrO_2NPs treatment at 75 mg L^{-1}, (T3) M-ZrO_2NPs treatment at 150 mg L^{-1}.

Treatments	Chl.a mg/g^{-1}	Chl.b mg/g^{-1}	Car. mg/g^{-1}	Total Pig. mg/g^{-1}
T1	5.07 ± 0.11 b	3.05 ± 0.02 b	0.641 ± 0. 26 b	5.26 ± 0.19 b
T2	5.41± 0.02 a	3.15± 0.16 a	0.780 ± 0. 13 a	6.95 ± 0.16 a
T3	5.19 ± 0.22 c	3.03 ± 0.19 c	0.745 ± 0.05 c	5.70 ± 0.18 c

Duncan's multiple range test ($p \leq 0.05$) found no significant difference between mean values (n = 3) in the same column for each feature using the same lowercase letter.

Variations in the Content of Secondary Metabolites

The found results designated that TPC, TFC and TC were significant increase in contrast to the controller plant in reaction to M-ZrO_2NPs treatments at concentration of 75 Mg / l^{-1}, with a two-fold increase in TPC and TFC contents while a five-fold increase in (TC) contents was detected (table 4). Conversely, in contrast to plants in control, exogenous application of M-ZrO_2NPs at a concentrations of 150 Mg / l^{-1} significantly increased 10.1, 23.9, and 5.3 µg g-1 for TPC, TFC, and TC, respectively, in *Vicia faba* plants' secondary metabolite content.

Table 4 Influence of differents concentrations of M-ZrO_2NPs (75 and 150 Mg / l^{-1}) on bean plants on the contented of secondary metabolites. (T1) Control, (T2) M-ZrO_2NPs treatment at 75 mg L^{-1}, (T3) M-ZrO_2NPs treatment at 150 mg L^{-1}.

Treatments	TPC µg /g^{-1}	TFC µg /g^{-1}	TC µg/ g^{-1}
T1	8.90 ± 0.52 a	17.87 ± 0.49 a	2.00 ± 0.26 a
T2	10.17 ± 0.31 b	23.90 ± 1.51 b	5.33 ± 0.38 b
T3	10.01 ± 0.60 c	23.9 ± 1.10 c	5.30 ± 0.29 c

According to Duncan's multiple range test, there is no significant difference between the mean values (n = 3) for any feature in the same column that has the same lower-case letter at $p < 0.05$.

CONCLUSION

The effects of the research show that spraying with M-ZrO2NPs nanomaterial causes the accumulation of nanomaterial in plant tissues, which leads to an increase in antioxidant content, osmotic content and by-product contented, plant growing indicators and photosynthetic dye contented were improved through spraying at a concentration of 75 mg /l^{-1}, by increase the production of antioxidants, by-products and osmotic, little amount of bio synthesised M-ZrO2NPs particles supports improve growth on bean plants. However, high concentrations of M-ZrO2NPs particles cause a toxic effect to plants. These results can be clarified in light of the possible role of zucconium in keeping the structure of the cell membrane and regulatory the entrée of nutrient ions to the cell and its role in enzyme action as it has a positive charge that allows plants to engage NKP and rise enzyme action and secondary metabolite manufacture.

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