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**GREEN SYNTHESIS AND CHARACTERIZATION OF ZnO  
NANOPARTICLES USING *Coleus forskohlii* LEAF EXTRACT AND ITS  
EFFECT ON TOMATO GROW THUNDER DROUGHT STRESS**

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**ABSTRACT**

Biological methods for nanoparticle synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods. In this study, we report on the synthesis of nanostructured zinc oxide particles by biological method using plant leaves extract. Highly stable and spherical zinc oxide nanoparticles are produced by using zinc nitrate and *Coleus forskohlii* leaf extract. Greater than 95% conversion to nanoparticles has been achieved with *Coleus forskohlii* broth concentration 20%. Structural, morphological and optical properties of the synthesized nanoparticles have been characterized by using UV-Vis spectrophotometer, FTIR spectrometer, transmission electron microscopy TEM and X-ray diffraction (XRD) analysis. TEM analysis shows that the zinc oxide nanoparticles prepared were poly dispersed and the average size ranged from 46 to 91nm. The particles obtained have been found to be predominantly spherical. This study explored the effectiveness of green zinc oxide

nanoparticles (ZnO-NPs) foliar spray on tomato growth under drought stress. Tomato plant subjected to four water regimes (100%, 75%, 50% and 25% FC), and in the same while seedlings were sprayed with 25, 50 and 100  $\mu\text{g/l}$  green ZnO-NPs. The results showed that tomato growth parameters reduced gradually by increasing drought stress level, while ZnO-NPs enhanced plant growth under all studied drought levels. 25 and 50  $\mu\text{g/l}$  ZnO-NPs proved to be the optimum treatments for alleviating drought stress. They increased shoot and root length, chlorophyll a and b, proteins, carbohydrates and free amino acid concentrations at higher levels comparable to well-irrigated seedlings. Also, application of green 25 and 50  $\mu\text{g/l}$  ZnO-NPs reduces proline level in tomato plants grown under drought conditions compared green ZnO-NPs untreated controls. Therefore, the application of green ZnO-NPs should be considered as a promising agricultural practice treatment in locations prone to suffering from water shortage.

**Keywords: Green nanoparticles, characterization, drought stress, growth factors, stress indicator**

## INTRODUCTION

Drought stress is a term indicating that a plant has experienced a water deficit situation due to limited water availability from growing media [38]. Between the abiotic stresses, drought is a major factor affecting the growing and production of crops worldwide [42]. The effects of plant stress differ for different types of plants [30]. Early recognition of the symptoms of water stress can be decisive to maintaining crop growth. The furthest common symptom of water stress is wilting. When the plant is subjected to water stress, the water potential inside the leaves declines and the plant wilts. Drying and wilt will decrease growth in almost any plant [4].

Drought stress inhibited plant growth before reducing photosynthesis [50]. The

energy balance in plant is regulated by photosynthetic pigments and hence they are involved at the adaptation of plants and their survival in drought [15]. Nonetheless, Chlorophyll biosynthesis Inhibition, activation Chlorophyllase and/or chloroplast degradation decreased the pigment content under the abiotic stress [44]. Furthermore, Mejri *et al.* [28] recorded that drought stress reduced chloroplast activity and it was contributed to chlorophyll breakdown. Under conditions of drought, plants accumulate vast quantities of different osmo-protectants, such as soluble sugars, which eventually preserve the status of tissue water. In osmotic adjustment and carbon storage, carbohydrates perform various

functions [55]. The osmotic adjustment maintains cellular water balance with active accumulation of the substances dissolved in the cytoplasm, the maintenance of high swelling increases photosynthesis and growth rate [1]. Protein synthesis, coupled with a decrease in plant growth and crop yield under water stress conditions [22]. In general, drought conditions increase the content of free amino acids in plants, these compounds are considered osmoprotectants [49]. As a signaling controller molecule, wheat plants accumulate proline than the other osmoregulators, activating several mechanisms that assist in drought adaptation. Proline is an important amino acid that prevents cells from oxidation and serves as a water regulator that helps plants to absorption water from the atmosphere [26]. Recently, nanotechnology has opened new and interesting horizon for plant physiologists in order to improve plant performance under stress conditions [6]. Moreover, researchers believe that the uptake of nanoparticles (NPs) in plants is more than the similar chemicals added to the plant in bulk form [5]. The foliar application of NPs is deemed more effective and easier since the plants can directly absorb it and thereby reduce soil pollution, as opposed to the soil application of chemical fertilizers. The second main advantage of the foliar application of nano-

fertilizers is that very little fertilizer is required compared to the addition of these materials to the soil [2]. Green ZnO-NPs is an environmental friendly material that can be used to avoid negative effect of drought [25, 35, 52]. Hence, the current study aims to study the effect of green ZnO-NPs foliar spray on ameliorating drought stress in tomato plant.

## MATERIALS AND METHODS

### Green synthesis of ZnO Nanoparticles using *Coleus forskohlii* leaf extract

Fresh and healthy leaves of *C.forskohlii* (commonly known as Alshara) will be collected from Jeddah region, KSA.



Figure1: *Coleus forskohlii* plant

### Preparation of *C. forskohlii* leaf extract

The fresh leaves of *C. forskohlii* will be washed with running tap water in order to remove the impurities adhering on the surface. Then the leaves will be gently wiped by the filter paper, known amount of leaves (50 g) will be added to 100 ml distilled water and kept at 60°C on hot plate until the color of the water turned into green. Then the extract will be cooled

at room temperature, filtered, and stored for further experimental analysis [39].

### Preparation of ZnO nanoparticles

A known amount (50 ml) of filtered leaf extract of different concentrations will be taken in the beakers and heated at 60°C on a hot plate. Then 5 g of zinc nitrate [ $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ] will be added to the heated leaf extract and stirred well using a

glass rod until the mixture will be turned to paste form yellow color. The paste will be collected carefully in ceramic crucibles and annealed at 500°C for 2 h in order to remove the organic impurities present in the paste. After 2 h, the synthesized nanoparticles present in the crucible will be taken and stored in an airtight container for future experimental work [37].



Figure 2: Flow chart for preparation of zinc oxide nanoparticles (ZnO-NPs)

### Characterization of ZnO nanoparticles

Optical properties of ZnO-NPs were characterized using UV-Vis spectrophotometer. The chemical composition was studied by using FTIR spectrometer. The shape, size, microstructures of the products, size distribution and the average size of the NPs were estimated on the basis of transmission electron microscopy (TEM) micrographs with the assistance of Sigma-Scan Pro software. Phase purity and grain size were determined by X-ray diffraction (XRD) analysis recorded by diffractometer. All experiments were done in triplicates

and the results were recorded for 20% *C. forskohlii* leaf extract concentration.

### Effect of Biosynthesized ZnO NPs on drought stress in tomato plant:

#### Experimental Design

This experiment was performed at the experimental station at King Abdulaziz University in the Kingdom of Saudi Arabia during the autumn season, starting from the 1<sup>th</sup> of October until the 26<sup>th</sup> of November of 2019. The weather during this period of time was slightly windy with gradual drop in temperature and moderate humidity. The average daily minimum temperature was

24°C at night and in the early morning. While the average daily maximum temperature was 32°C during the day. Tomato seeds were planted in plastic containers filled with 3kg of homogeneous mixed sand: clay soil (2:1). The plants were grown in normal daylight circumstances in the greenhouse and were irrigated with tap water at field capacity regularly every two days. After the appearance of the fourth leaf, the pots were divided into four sets; one set was not treated with ZnO-NPs, while the other three were treated with ZnO-NPs (25, 50, 100µg/l) separately. For drought stress treatments, each group subdivided into four sub-groups, these sub-groups irrigated by 100% FC (control), 75%, 50% and 25% FC for and grown to two weeks. Then, regular watering with 100%FC was resumed after the water stress period, to allow the plants to recover. The experiment was carried out with a complete random design with three replicates.

#### **ZnO-NPs Treatments**

Zinc oxide nanoparticles were foliar sprayed in different concentrations (25, 50, 100µg/l) on tomato leaves directly with drought treatments. As a control, plants spraying with tap water were utilized.

#### **Shoot and root length**

The length of freshly harvested shoots and roots was recorded using a ruler at the end of the experimental period. At the end of

the experimental phase, Plant samples were collected and taken to the laboratory for analysis. For all physiological analyzes, Immediately, the plant samples were freezing in liquid nitrogen and then kept at -80°C.

#### **Preparation of plant extract**

In liquid N<sub>2</sub>, 0.5g samples of fresh leaves were crushed into fine powder. Then, it was suspended with 5ml of distilled water, then centrifuged and completed to a certain volume. Soluble carbohydrates, proteins and free amino acids were determined in this extract.

#### **Plant analysis:**

##### **Determination of Photosynthetic pigments**

Chlorophyll a and Chlorophyll b were measured by UV-VIS Spectroscopy according to Hiscox and Israelstam [20] with some modifications by Su *et al* [45]. They were extracted from 0.05g fresh leaves samples. In a test tubes, at 60°C, the leaves were suspended in 5ml of 95% ethyl alcohol until being colorless. Then, the total volume was completed to 5ml with 95% ethyl alcohol. The absorbance readings were measured at wavelengths of 664, 649 and 470nm.

##### **Determination of soluble carbohydrates**

The soluble carbohydrate content was estimated by anthrone method [13-40]. In a conical flask, 0.02g anthrone, 30ml distilled

water, 8ml absolute ethyl alcohol and 100ml concentrated H<sub>2</sub>SO<sub>4</sub> were blended under continuous cooling in an ice bath, respectively. In a test tube, 0.2ml of the leaves extract was supplemented with 4.5ml of anthrone reagent mixture. The test tube was boiled for 7 minutes in a 100°C water bath, then directly cooled for 5 minutes. The absorption of the blue-green color produced was measured at 620nm.

#### **Determination of soluble proteins**

The total soluble proteins content was estimated according to the Lowry method for protein quantitation [27]. 5ml of the alkaline reagent solution was applied with one ml of leaves extract. Both were thoroughly mixed and allowed to stand for 10 minutes at room temperature. After that, 0.5ml of Folin diluted reagent (1:2v/v) was added and quickly mixed. After 30minutes, The spectrophotometer was read for absorbance against a blank at 750nm.

#### **Determination of total free amino acid**

In the leaves extraction, the free amino acid content was determined according to the Moore and Stein procedure [31]. One ml of stannous chloride reagent was added to 0.5 ml of leaves extract. The mixture was then boiled for 20 minutes and cooled in a water bath. After that, 4 ml of diluent solvent was applied and blended quickly. The absorbance of violet color was registered

spectrophotometrically at wavelength 570nm.

#### **Determination of Proline**

Proline was estimated according to technique of Bates et al.(7), the frozen plant leaves (0.5g) is homogenized in 1.5ml 3% aqueous sulphosalicylic acid and the residue is removed by centrifugation at 10,000g for 10 minutes. 1ml of the homogenized tissue reacts with 1ml ninhydrin reagent and boiled for 1 hour at 100°C. The reaction mixture is released with 2ml toluene. Its spectral density is calculated at 520nm.

#### **STATISTICAL ANALYSIS**

The data was analyzed by statistical software kit SPSS version 21.0. Variances between unprocessed and processed plants at each water regime were evaluated with a one-way variance analysis (ANOVA), followed by a 5%(P < 0.05) significance level (Duncan) test. All values were expressed using their standard deviation (SD) as a mean value of three replicates.

#### **RESULTS**

##### **Characterization of ZnO Nanoparticles: UV-Visible spectrum**

The ZnO-NPs' room temperature UV-Vis absorption spectrum can be seen in Fig. 3. With a concentration of 0.1 wt.%, the ZnO-NPs are distributed in water and then the solution is used to test UV-Vis. At a wavelength of 380 nm, the spectrum shows

a characteristic absorption peak of ZnO-NPs that can be assigned to ZnO-NPs's intrinsic band-gap absorption due to electron transfers from the valence band to the conduction band.

#### FTIR Spectrometer

In order to find out the functional groups present in the particles, synthesized zinc oxide nanoparticles from leaf extract (20 %) were analyzed using FTIR spectroscopy technique. The fingerprint area of zinc oxide nanoparticles exhibited at bandwidth of 1600-600  $\text{cm}^{-1}$  (Figure 4). The large peak around 2300  $\text{cm}^{-1}$  showed the vibration of the OH stretching bond due to water adsorption on the surface of zinc oxide nanoparticles, while the Zn-O stretching vibration was attributed to the peak at 500  $\text{cm}^{-1}$  (Figure 4).

#### XRD analysis

The crystalline size and structural features of the ZnO-NPs are released via Powder X-ray diffraction. The XRD layout of bio-synthesized ZnO-NPs from 20% leaf extract of *C. forskohlii* is illustrated in Figure 4. From the result (Figure 4) the peaks were recognized at 31.78°, 34.46°, 36.29°, 47.62°, 56.53°, 62.89°, 66.53°, 67.96°, 68.85°.

#### Transmission electron microscopy of ZnO-NPs

Transmission electron microscopic studies documented the size and shape of

synthesized ZnO-NPs. The synthesized ZnO-NPs with few elongated particles with particle size variations varying from 46 to 91 nm were mainly spherical in shape (Figure 5).

#### Growth parameter:

##### Shoot and root length

As demonstrated in figure (7), under all water levels, shoot length significantly increased when treated with 25 and 50  $\mu\text{g/l}$  ZnO-NPs compared to untreated control. 25  $\mu\text{g/l}$  ZnO-NPs treatment increased shoot length by about 7.37, 27.63, 46.18 and % 47.65 under all levels of water (100%, 75%, 50% and 25%FC) respectively higher than un-treated controls. Moreover, 50  $\mu\text{g/l}$  ZnO-NPs increased shoot length by about 7.75, 13.60, 46.67 and % 69.41 at all water regimes systems (100%, 75%, 50% and 25%FC) respectively. Nonetheless, significant reduction in shoot length was recorded due to the applied of 100  $\mu\text{g/l}$  ZnO under water stress and unstressed conditions (Figure 7).

The length of the tomato root decreased gradually by rising drought stress conditions to the lowest values at 25% FC (Figure 8). In full watered plants, root length significantly increased (by about 17.07%) when leaves spraying with 50  $\mu\text{g/l}$  ZnO-NPs. Under all drought levels, root length significantly increased upon application of 25 and 50  $\mu\text{g/l}$  ZnO-NPs

more than their untreated controls. 25µg/l ZnO-NPs treatment increased root length by about 28.58%, 13.85% and 33.96% at 75%, 50% and 25%FC respectively higher than un-treated controls. 50µg/l ZnO-NPs treatment increased root length by about 18.19%, 64.62% and 92.46% under all water stress levels of 75%, 50% and 25% FC respectively compared to their corresponding controls. Alternatively, 100µg/l ZnO-NPs negatively affected the root length compared to untreated control (**Figure 8**).

#### **Plant Pigments:**

The variation in growth response of tomato plant in terms of chlorophyll a (Chl a) content is presented in figures (9). No significant differences were detected in Chl a content by spraying ZnO-NPs at all studied concentrations in water unstressed plant. Nevertheless, all studied ZnO-NPs concentrations significantly enhanced Chl a content under all drought conditions (75%, 50%, 25%FC) compared to their corresponding controls. 25µg/l ZnO-NPs treatment increased Chl a content by about 25.75%, 23.67% and 51.55% under drought levels of 75%, 50% and 25% respectively compared to un-treated controls. Likewise, 50µg/l ZnO-NPs increased Chl a content by about 15.76%, 54.53% and 62.86% at 75%, 50% and 25%FC respectively of their corresponding

controls. 100µg/l ZnO-NPs concentrations significantly enhanced Chl a content by about 27.64%, 49.44% and 52.05% under 75%, 50% and 25% FC respectively of untreated controls (**Figure 9**).

With regard to chlorophyll b (Chl b) content in **Figure 10**, data showed that there is negative effect of ZnO-NPs treatments on Chl b concentration in treated tomato plants compared to untreated controls at no and severe drought stress (25%FC). Although at low drought stress (75%FC), 25, 50 and 100µg/l ZnO-NPs increased Chl b concentration by about 54.28%, 45.31% and 44.61% respectively compared to their corresponding control. Also at moderate drought stress (50%FC) water stress, 50 and 100µg/l ZnO-NPs increased Chl b concentration by about 21.95% and 17.45% respectively of untreated control with no significant differences were detected in Chl b content by spraying 25µg/l ZnO-NPs at 50% FC (**Figure 10**).

#### **Carbohydrates**

According to the results represented in **Figure 11**, At 100%FC, carbohydrates content significantly increased (by about 6.93%) when tomato plants sprayed with 50µg/l ZnO-NPs. In the same context, under water stress conditions (75%, 50% and 25% FC), carbohydrates content increased significantly only when treated

with 25 and 50µg/l ZnO-NPs. By applying 25µg/l ZnO-NPs carbohydrates content increased by about 33.16%, 106.03% and 77.11% higher than untreated controls respectively at 75%, 50% and 25%FC. Also, 50µg/l ZnO-NPs increased carbohydrates content by about 29.02%, 164.66% and 220.48% higher than their corresponding control respectively under 75%, 50% and 25%FC. In contrast, 100µg/l ZnO-NPs dropped off of carbohydrates concentration at all studied water regimes (Figure 11).

#### Protiens

As shown in Figure 12, the most pronounced induction recorded for 50µg/l ZnO-NPs treatment, which increased proteins content by about 9%, 35.40%, 66.11% and 124.60% under all water regimes of 100%, 75%, 50% and 25%FC respectively compared corresponding controls. However, compared with untreated control, 100µg/l ZnO-NPs significantly decreased the content of proteins under all studied water levels (Figure 12).

#### Free amino acids

The results in Figure 13 indicated that, the application of lower ZnO-NPs concentrations significantly enhanced the free amino acid content in tomato leaves both in water unstressed and stressed plants. Foliar spraying with 25µg/l ZnO-

NPs increased free amino acid content by about 25.58%, 82.93%, 144.44% and 190.91% at water regimes of 100%, 75%, 50% and 25% FC consecutively compared with their respective controls. Moreover, 50µg/l ZnO-NPs enhanced free amino acids content by about 39.53%, 14.63%, 129.63% and 181.82% respectively higher than untreated control under 100%, 75%, 50% and 25%FC. It was noticeable that, no significant differences observed between the effect of 100µg/l ZnO-NPs and their corresponding control at 100%FC and all drought levels (Figure 13).

#### Stress indicators

##### Proline

As presented in Figures 14, proline concentration increased progressively by rising water stress in tomato leaves. No significant differences were detected in proline content by spraying with ZnO-NPs at lower concentrations (25 and 50µg/l) in water unstressed plants. While, in stressed plants, 25 and 50µg/l ZnO-NPs significantly reduced proline concentration lower than their untreated controls. By spraying with 25µg/l ZnO-NPs proline content decreased by about 40.58%, 45.35%, and 31.91% lower than their corresponding control respectively under water levels of 75%, 50% and 25%FC. Under all drought levels (75%, 50% and 25%FC), 50µg/l ZnO-NPs

decreased of proline content by about 50.72, 54.65 and 67.02% less than untreated controls respectively. Nonetheless, 100 $\mu$ g/l

ZnO-NPs associated with increased in proline content which presented at 100% FC and all drought levels (**Figure 14**).

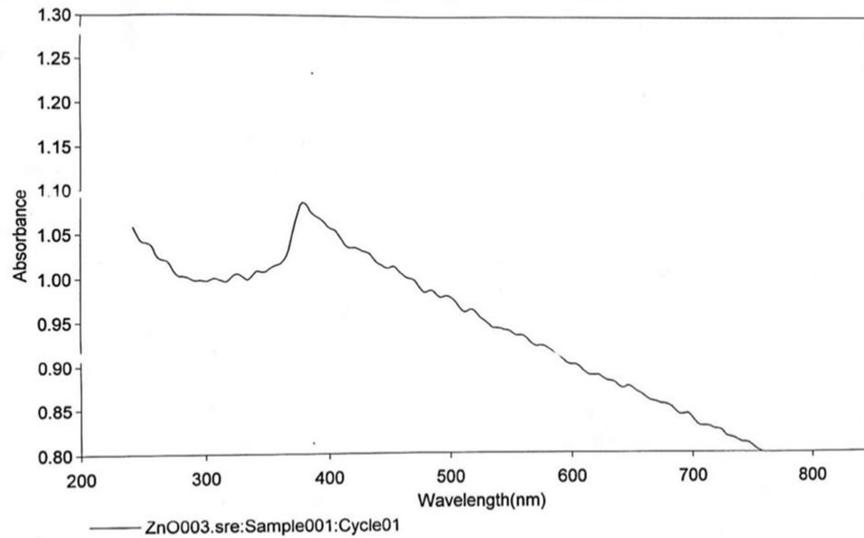


Figure 3: UV-visible spectroscopy of ZnO-NPs synthesized using *Coleus forskohlii* leaf extract showing absorption peak at 370 nm

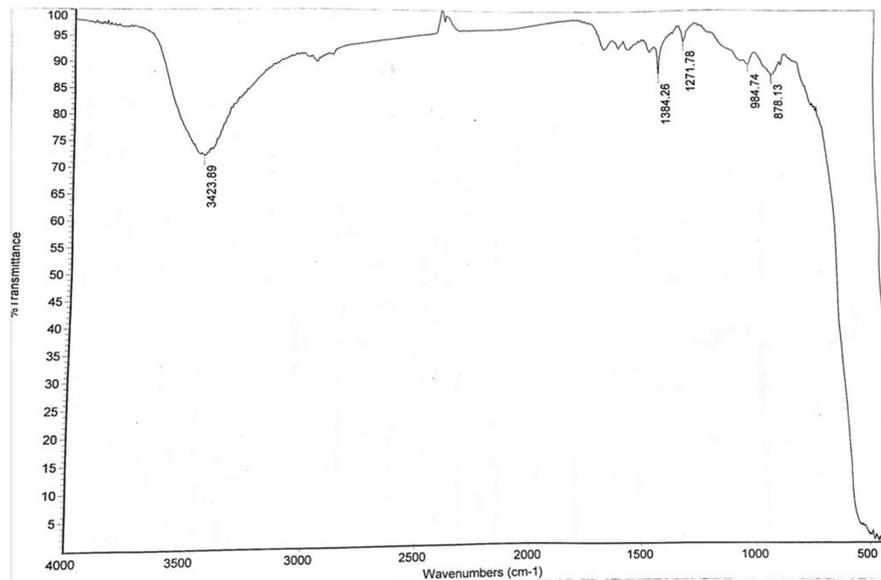


Figure 4: FTIR spectra of biosynthesized ZnO-NPs synthesized using *Coleus forskohlii* leaf extract

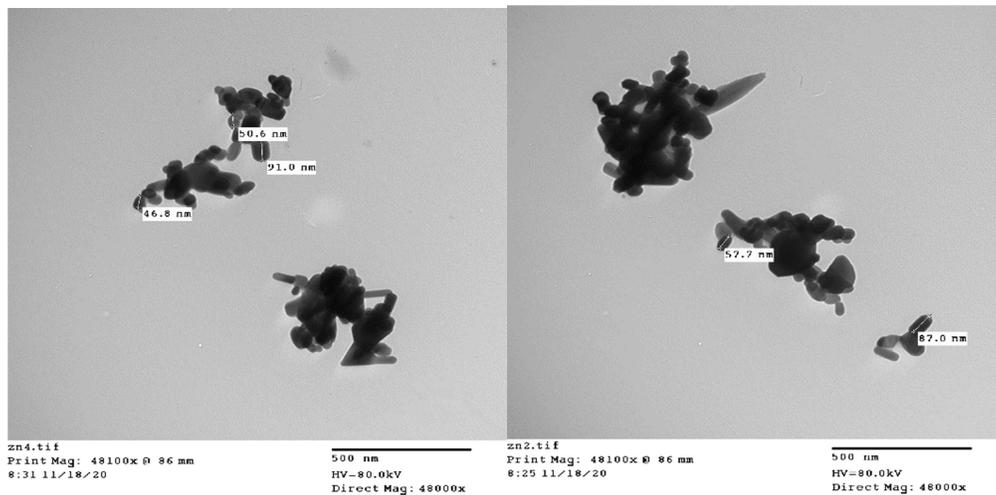


Figure 5: Images of Transmission electron microscopy (TEM) of ZnO-NPs synthesized using *Coleus forskohlii* leaf extract showing spherical or globular shaped with the undefined varied size of 46-91 nm. Bar scale—500 nm

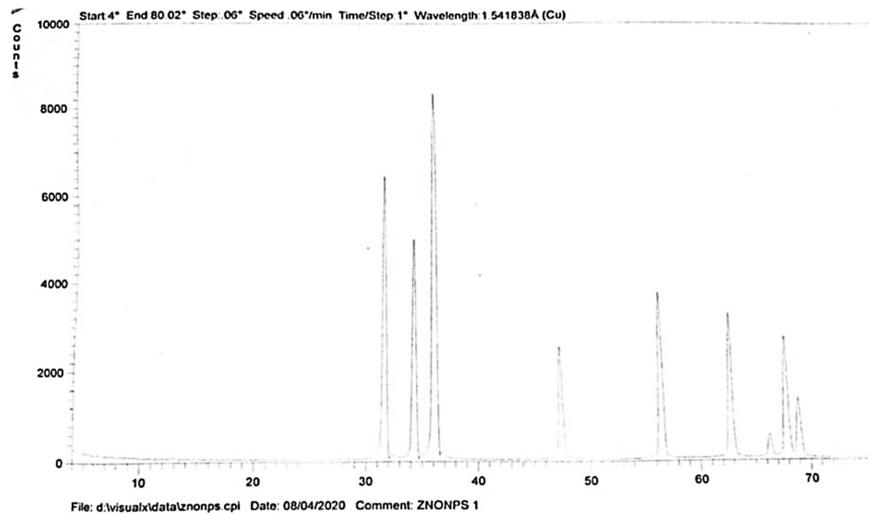


Figure 6: Powder X-ray diffraction pattern of ZnO-NPs synthesized using *Coleus forskohlii* leaf extract

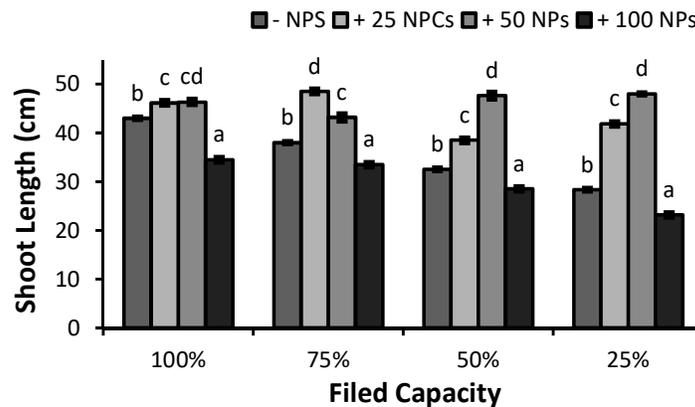


Figure 7: Shoot length of tomato plant as affected by foliar spraying with different concentrations of ZnO-NPs under different levels of drought stress. Each point represents a mean value of three replicates, and the vertical bars indicate  $\pm$  SD. Bars carrying different letters at each water level are significantly different at  $P < 0.05$ .

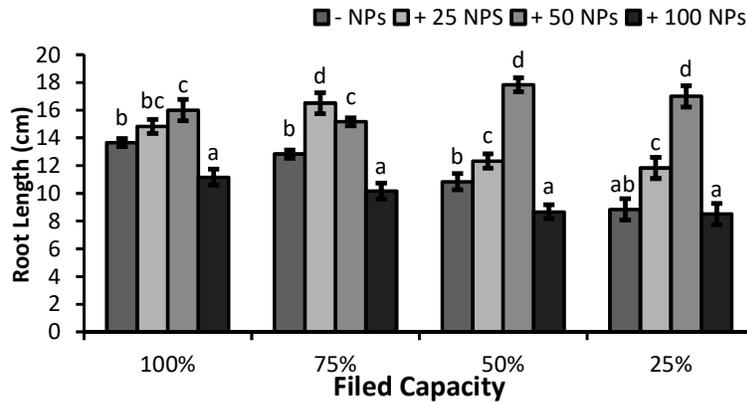


Figure 8: Root length of tomato plant as affected by foliar spraying with different concentrations of ZnO-NPs under different levels of drought stress. Each point represents a mean value of three replicates, and the vertical bars indicate  $\pm$  SD. Bars carrying different litters at each water level are significantly different at  $P < 0.05$

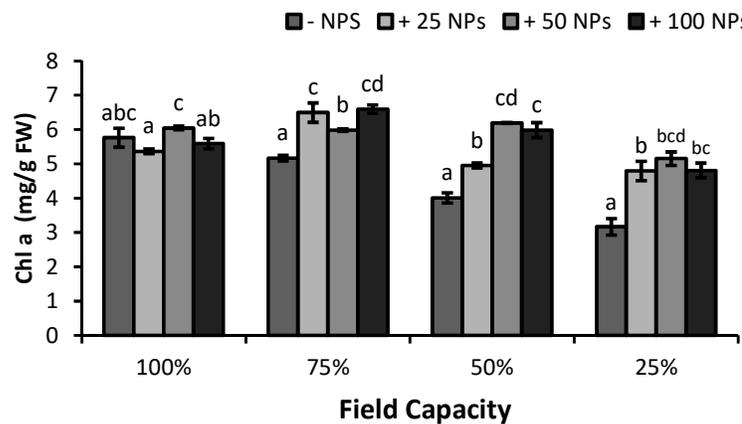


Figure 9: Chlorophyll a concentration in tomato leaves as affected by foliar spraying with different concentrations of ZnO-NPs under different levels of drought stress. Each point represents a mean value of three replicates, and the vertical bars indicate  $\pm$  SD. Bars carrying different litters at each water level are significantly different at  $P < 0.05$

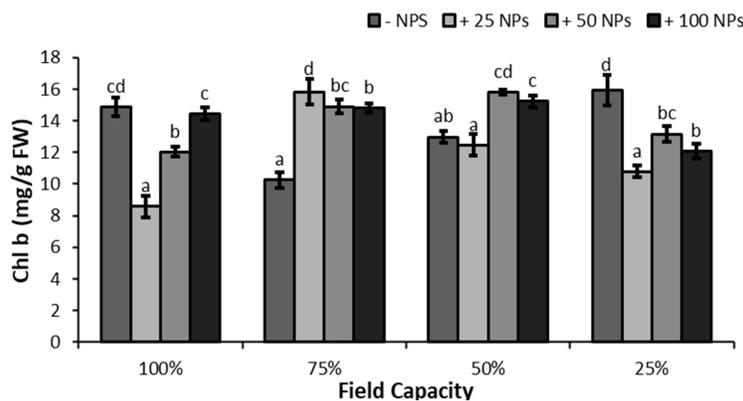


Figure 10: Chlorophyll b concentration in tomato leaves as affected by foliar spraying with different concentrations of ZnO-NPs under different levels of drought stress. Each point represents a mean value of three replicates, and the vertical bars indicate  $\pm$  SD. Bars carrying different litters at each water level are significantly different at  $P < 0.05$

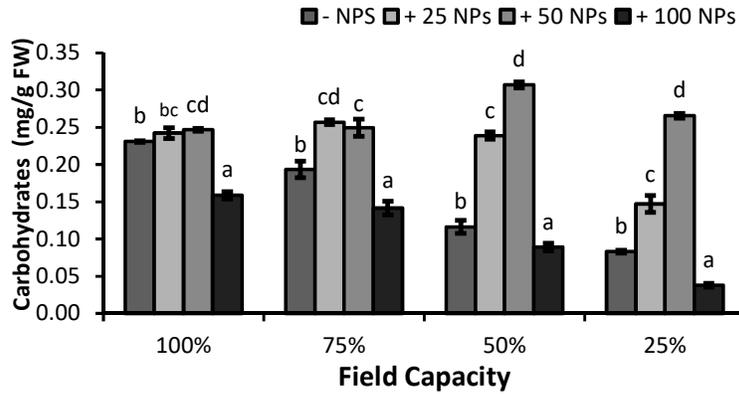


Figure 11: Carbohydrates concentration in tomato leaves as affected by foliar spraying with different concentrations of ZnO-NPs under different levels of drought stress. Each point represents a mean value of three replicates, and the vertical bars indicate  $\pm$  SD. Bars carrying different letters at each water level are significantly different at  $P < 0.05$

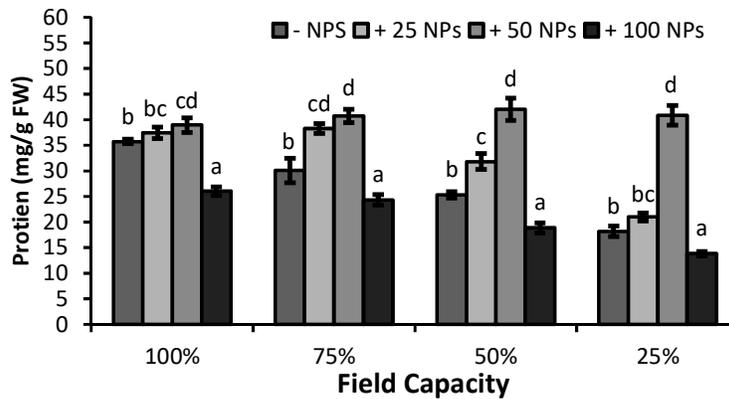


Figure 12: Protein concentration in tomato leaves as affected by foliar spraying with different concentrations of ZnO-NPs under different levels of drought stress. Each point represents a mean value of three replicates, and the vertical bars indicate  $\pm$  SD. Bars carrying different letters at each water level are significantly different at  $P < 0.05$ .

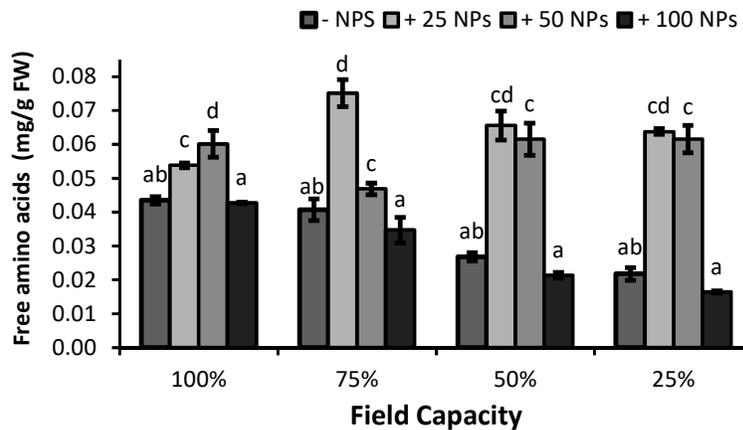


Figure 13: Free amino acids concentration in tomato leaves as affected by foliar spraying with different concentrations of ZnO-NPs under different levels of drought stress. Each point represents a mean value of three replicates, and the vertical bars indicate  $\pm$  SD. Bars carrying different letters at each water level are significantly different at  $P < 0.05$

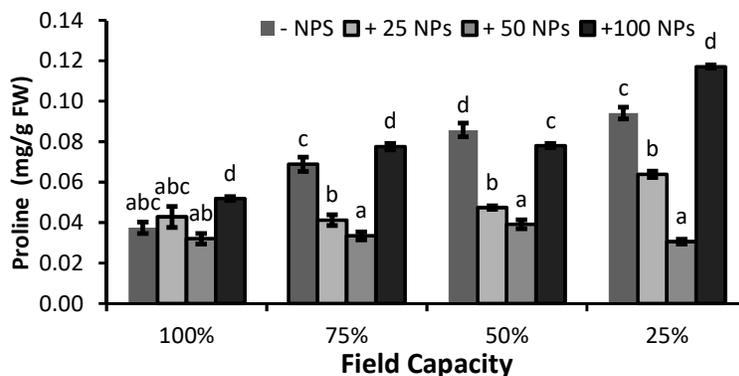


Figure 14: Proline concentration in tomato leaves as affected by foliar spraying with different concentrations of ZnO-NPs under different levels of drought stress. Each point represents a mean value of three replicates, and the vertical bars indicate  $\pm$  SD. Bars carrying different letters at each water level are significantly different at  $P < 0.05$

## DISCUSSION

The biological synthesis of zinc nanoparticles with *Euphorbia hirta* leaf extract offers an environmentally safe, simple and effective way of synthesizing nanoparticles. The use of plant extract prevents the use of reducing and stabilizing agents that are harmful and toxic [3]. The ZnO nanoparticles have been characterized using various techniques, such as UV-Vis, FTIR, TEM and XRD etc. The UV-visible spectroscopy of ZnO-NPs synthesized using *Coleus forskohlii* leaf extract showed absorption peak at 370 nm. The spectrum shows a characteristic absorption peak of ZnO that can be assigned to ZnO's intrinsic band-gap absorption due to electron transfers from the valence band to the conduction band [11]. In order to find out the functional groups present in the particles, synthesized zinc oxide nanoparticles from leaf extract (20 %)

were analyzed using FTIR spectroscopy technique. The fingerprint area of zinc oxide nanoparticles exhibited at bandwidth of 1600-600  $\text{cm}^{-1}$ . Add to that, the large peak showed the vibration of the OH stretching bond due to water adsorption on the surface of ZnO-NPs, while the Zn-O stretching vibration was attributed to the peak at 500  $\text{cm}^{-1}$  (48). The crystalline size and structural features of the ZnO nanoparticles are released via Powder X-ray diffraction. In the XRD layout of bio-synthesized ZnO-NPs from leaf extract of *C. forskohlii*, all the peaks are validated ZnO-NPs hexagonal phase (wurtzite structure) [41]. It was earlier stated that *C. forskohlii* extracts, during synthesis serve as an active template that prevents synthesized nanoparticles from aggregating [16].

Zinc is known to influence plant water relationships under drought stress, zinc might protect plant cells from damage as it

potentially affects the plant cell growth [51]. In this study it was revealed that foliar application of green ZnO-NPs at appropriate concentration (25 and 50µg/l) on tomato plant exhibited increased shoot and root lengths when dryness increases as compared to controls. While 100µg/l ZnO-NPs treated plants showed the highest length reduction rate. The recorded reduction in plant growth due to application of 100µg/l ZnO-NPs could related to Zn toxicity where above certain concentration, Zn becomes toxic to the degree that the growth and development of plant cells is retarded. Previous studies have documented exposure to ZnO-NPs led to increased shoot height in winter wheat [9].

Our results presented that by spraying tomato plant with 25, 50 and 100µg/l ZnO-NPs, chlorophyll degradation was not only avoided, but also in equally drought-stressed and unstressed plants, the Chl a content increased significantly. Also, Chl b increased by Applying 25, 50 and 100µg/l ZnO-NPs at low drought stress (75%FC). Chl b increased by spraying 50 and 100µg/l ZnO-NPs at moderate drought stress (50%FC), with negative effect of ZnO-NPs treatments on Chl b concentration in treated tomato plants compared to untreated controls at severe drought stress(25%FC). Enhancement of

chl a content by ZnO-NPs treatment are in line with the results found by Fathi *et al.* [14] and Kheirizadeh Arough *et al.* [24] who declared that ZnO-NPs treated plants exhibited higher amount of total chlorophyll content than the control. Additionally, Zarrouk *et al.* [53] displayed a positive relation between Zn levels and leaf chlorophyll concentration in plant. Chlorophyll concentration increased when ZnO-NPs is sprayed on peanuts (*Arachis hypogaea* L.) also [36].

In tomato plant, 25 and 50µg/l ZnO-NPs increased carbohydrates, proteins and free amino acids concentration at all studied drought levels (75, 50 and 25%FC) compared to untreated control. On the other hand, 100µg/l ZnO-NPs minimized carbohydrates, proteins and free amino acids in tomato leaves under all studied water levels. As discussed earlier, Zn is needed for plants to conduct many physiological activities, such as protein biosynthesis, chlorophyll, and the normal functioning of metabolic processes [32-43]. Via accumulating osmolytes, osmotic adjustment retains greater cell turgor, thus shielding plants from drought [46].

Zinc oxide nanoparticles (25 and 50µg/l) substantially decreased water stress effects in tomato, thereby reducing the accumulation of proline at all studied drought levels (75, 50 and 25% FC)

compared to untreated control. While 100µg/l ZnO-NPs promoted proline in tomato leaves under all water levels. In this regard, green ZnO-NPs was lowered proline in soybeans as well [17].

## CONCLUSION

As discussed above, the application of green ZnO-NPs as treatments should be considered as a promising agronomic practice to be tested safely at field scale in sites that suffer from water deficit as ZnO-NPs using optimum concentrations appear to be able to alleviate the drought stress which is one of the most dangerous stresses on plant growth by osmotic adjustment and maintaining plant water potential. Hence, foliar application of green ZnO-NPs is recommended for the growing of tomato under drought stress conditions. Future studies are important to broaden under varying agro-climatic conditions, various crop species at field level for the cost-effectiveness and adaptability estimation of green ZnO-NPs foliar treatment.

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