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Eco-Friendly Synthesis Of A Zno/Cuo Nano-Composite Mitigates Water Stress In *Pisum Sativum* By Promoting Antioxidative Defense Systems

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Abstract

Water scarcity is a significant environmental stressor that adversely affects plant development. This research examines the beneficial role of ZnO/CuO nano-composites in alleviating the adverse impacts of water scarcity on improving pea plants. The foliar spraying of ZnO/CuO nano-composite solutions (25 and 50 ppm) enhanced the growth parameters of pea plants cultivated under both optimal circumstances (100% available water) and water stress circumstances (80 and 60% water available). Plants subjected to ZnO/CuO nano-composites (50 ppm) exhibited elevated concentrations of significant osmolytes, including proline, phenol, flavonoid, and tocopherol. They preserved their photosynthetic pigments more effectively than others applied under both stress and non-stress circumstances. Moreover, the external foliar spray of ZnO/CuO nano-composites decreased the levels of OH, H_2O_2, O_2, and lipid peroxidation in pea plants exposed to water deficiency. The treatments enhanced the results, illustrating the advantageous effects of ZnO/CuO nano-composites, at a concentration of 50 ppm in mitigating the detrimental effects of soil water deficiency stress in pea plants.

Keywords: Antioxidant activity- chlorophyll- Drought- Essential elements- Nanocomposites- Oxidative stress

1. Introduction

Drought, an abiotic stressor, significantly hinders crop output and adversely impacts plants during seed germination and early seedling development. Drought seems to be the primary factor limiting agricultural output due to climate change, ultimately leading to food insecurity [1]. Drought conditions are more prevalent worldwide owing to modified precipitation patterns and less rainfall. In recent decades, drought has caused significant declines in the yields of several plant species, anywhere from 30% to 90%, contingent upon the crop [2]. Furthermore, dryness influences plant growth and yield, thus altering mineral nutrition and nutrient density. Nutrient transport and intake are contingent upon soil moisture since dryness reduces the transpiration rate and membrane permeability as well as disrupts active transport [3]. The severity of drought repercussions is contingent upon the plant growth stage at which it occurs and the length of the drought. Drought stress diminishes seedling vigor and impedes germination due to reduced water absorption, which is the most vulnerable phase of plant development [4].

Drought stress adversely influences plant development by modifying interactions with water-soluble nutrients, disrupting the photosynthetic approach, and thus resulting in a substantial decrease in crop output [5]. Furthermore, dry-induced oxidative stress damages macromolecules, including lipids, proteins, DNA, biological membranes, and pigments essential to photosynthetic activities [6]. There are several ways in which plants adapt to their environment to drought, including morpho-physiological and molecular alterations.

Nanotechnology pertains to the fabrication of materials ranging from one to 100 nanometers, potentially resulting in substantial alterations in their physical and chemical characteristics. Nanotechnology significantly enhances product management methodologies, with advanced nanomaterials serving as innovative agricultural instruments to mitigate stress, diagnose diseases, and augment nutrient absorption and transport, ultimately improving crops' quality and quantity [7]. The enhanced accessibility of nanoparticles characterized by exact dimensions, elevated stability, high solubility, tailored surfaces, diverse shapes and functionalities, unrestricted dispersion, and diffusion has garnered significant interest in nano-composites [8]. In the nano-composite synthesis technique, several carriers transport the material to the target cell and release it consistently and equitably inside the cell [9].

The ZnO/CuO mixed metal oxide semiconductor photocatalyst is one of a number of ZnO-linked semiconductors. Mageshwari *et al.,* [10] used reflux condensation to synthesize ZnO/CuO and degrade methylene blue (MB) and methyl

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orange (MO) in 120 and 180 minutes, respectively. Under an Xe lamp, ZnO/CuO nano-composites are synthesized, and MO is photodegraded. Liu *et al.,* [11] presented an efficient oxidative homogeneous co-precipitation method with 88% efficiency after 60 minutes [12]. Methylene blue (MB) degradation using hollow spherical structured ZnO/CuO under 800 W Xe-arc lamp illuminations [12].

The hollow spherical structure reduces the rate at which photoinduced charge carriers are recombined, enhancing photodegradation efficiency [13]. ZnO and CuO's appropriate band edge positions are responsible for the higher activity of ZnO/CuO nano-composites. Charge carriers may be transported to the surface of a semiconductor and begin participating in redox reactions with organic contaminants that have been adsorbed, as well as being mineralized by active species like superoxide radicals and hydroxyl radicals, holes if recombination of charge carriers is maintained [14]. The hydrothermal technique is a gentle, low-temperature chemical process in which morphological and structural characteristics change depending on the manufacturing conditions, affecting the method's activity [15]. Considering the studies mentioned above, the photocatalytic performance of ZnO/CuO nano-composites was improved using a low-cost metal nitrate source material and a low-temperature hydrothermal synthesis process.

Nowadays, the environment represents a crucial factor in the future of agriculture. Growing peas in crop fields is critical enough to be considered a great carbon sink. Peas have a protein content that is two and a half times higher than traditional cereals like durum wheat, maize, rice, and soy, so they are classified as an excellent alternative source of protein [16]. They are legumes and, therefore, have great value in agricultural practices for the mechanical production of biological nitrogen. Cultivation of such sensitive crops includes many factors that can lead to poor yields, such as abiotic stresses [17].

This research presents the synthesis of novel ZnO/CuO nano-composites. First, the prepared ZnO/CuO nano-composites were characterized via Fourier-Transform Infrared Spectroscopy (FT-IR), X-ray diffraction analysis (XRD), transmission electron microscope (TEM), and Dynamic Light Scattering (DLS) analysis. Then, the effectiveness of ZnO/CuO nano-composites was investigated, and the effect on some morphological and physiological aspects of pea plants exposed to a water deficit was investigated.

2. Materials

$ZnCl_2$, $CuSO_4\cdot 5H_2O$, and NaOH were sourced from Merck (Germany) and utilized without additional purification. Deionized water was utilized consistently during the study.

2.1. Synthesize of CuO/ZnO nano-composite.

A simple common approach was applied to develop the CuO/ZnO nano-composite [10]. The following procedure used specific quantities of the precursors. The reaction chamber was a glass plate with a volume of 60 ml that was under stirring. 30 ml of deionized H_2O was added to the plate to create the aqueous medium. The aqueous medium was then given 0.4 g (10.0 mmol) of NaOH, and the mixture was then stirred until it completely dissolved. The reaction chamber was then filled with 0.3 g of $ZnCl_2$ (2.0 mmol) and $CuSO_4\bullet 5H_2O$ (0.2497 g; 1.0 mmol), respectively. The resulting solution was kept in a sealed glass bottle and boiled to 80°C for 24 hrs after full dissolution. It was then cooled to allow the sediment to settle down naturally. The acquired material was then thoroughly cleaned with alcohol and distilled water to remove any remaining contaminants. The sediment was left in the oven for 12 hours at 80°C to ensure its dryness.

2.2. Characterization of fabricated CuO/ZnO nano-composite

The transmission electron microscope (TEM) was conducted using a 200 kV JEOL-JEM-1400 high-resolution. The X-ray diffraction analysis (XRD), TEM, Fourier-Transform Infrared Spectroscopy (FT-IR), and Dynamic Light Scattering (DLS) images of the CuO/ZnO nano-composite (Figure 1, 2(a-d))

2.3. Experimental design

The palatable seeds of *Pisum sativum* L. cv. Master B was given by the Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. To ensure sterilization, the seeds were treated with a sodium hypochlorite solution (1%) for 2 minutes and then rinsed multiple times with sterilized distilled water. Sterilized seeds were placed in distilled water with endophytic bacteria for 8 hours after air-drying for two hours.

The effectiveness of a CuO/ZnO nano-composite in reducing drought stress is investigated in an open-field pot experiment conducted in 2022. Six kilograms of air-dried clay loam soil, including 28.48% sand, 20.1% silt, and 51.42% clay, were placed in a 30-cm-diameter polypropylene container (Table 1). Six seeds per pot were planted using surface-sterilized pea seeds. Ten days after seeding, pots were irrigated once with tap water, using around one liter from a can per pot. Ten days after the seed was planted, four identical plants per pot were selected to continue the experiment after complete germination.

Table 1. Chemical properties of soil.

Total Suspended Solids (TSS) ppm	**pH**	**Electrical Conductivity (EC) mm hos/cm**	**Cations meq/L**				**Anions meq/L**			
			Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4	HCO_3	CO_3
747	7.3	1.23	1.15	0.54	1.08	0.76	3.1	0.81	0.77	0

2.4. Irrigation Water Applied (IWA)

In order to calculate irrigation water administered (IWA), pea plants were assessed based on their soil moisture content (SMC within their effective root zone. In each treatment, IWA was quantified using the percentage of available water in the effective root zone. The SMC was evaluated using a pressure plate apparatus at vacuum pressures of −15.00 bar (ongoing wilting point, "$^{\theta}$PWP"), −0.33 bar (field capacity, "$^{\theta}$FC") in accordance with the methodology outlined by Klute and Dirksen [18]. Before each water addition, soil samples were obtained from strata that corresponded to the effective root length of pea plants using a soil auger. The moisture content at the ongoing withering point was subtracted from that at field capacity to ascertain the available water. For each irrigation level, the following equation was used to measure the volume of water treated in each plot:

$$IWA = \frac{(\theta_{FC} - \theta_{PWP}) \times D \times A}{E_a}$$

IWA represents the amount of water used for irrigation (m³), "$^{\theta}$FC" is the level of SMC ($m^3\ m^{-3}$), D is the depth of the root zone that is effective (m), "$^{\theta}$PWP" indicates the SMC at the ongoing wilting point ($m^3\ m^{-3}$), "E_a" stands for the irrigation efficiency and A is the each plot's surface area (m²)

The groups were split into:

T1	Full irrigation with 100%.
T2	Full irrigation with 100% + foliar treatment of 25 ppm CuO/ZnO nano-composite.
T3	Full irrigation with 100% + foliar treatment of 50 ppm CuO/ZnO nano-composite.
T4	Irrigation with 80%.
T5	Irrigation with 80% + foliar treatment of 25 ppm CuO/ZnO nano-composite.
T6	Irrigation with 80%+ foliar treatment of 50 ppm CuO/ZnO nano-composite.
T7	Irrigation with 60%.
T8	Irrigation with 60% + foliar treatment of 25 ppm CuO/ZnO nano-composite.
T9	Irrigation with 60% + foliar treatment of 50 ppm CuO/ZnO nano-composite.

2.5. Plant growth and biomass

Various plant growth variables were assessed during the experiment, including root and shoot length and dry and fresh weights of both root and shoot. A plant was systematically removed from each pot. The plants were subsequently dried at 80 °C for 72 hours and reweighed to calculate the dry weight.

2.6. Biochemical characters

2.6.1.Chlorophyll content

In order to extract chlorophyll (chl) pigments, 0.25 g of freshly cut leaf tissue was placed in a Falcon tube with 80% acetone (10 mL). The leaf material was then left in the dark for three days until it became white. A spectrophotometer was used to calculate the resultant Chl solution at wavelengths 470, 645, and 663 nm. The amounts of carotenoids, total pigment, Chl a, and Chl b were then measured using the technique [19,20].

2.6.2.Determination of total soluble sugars, protein, proline, phenol, flavonoids, α tocopherol

Using an anthrone reagent and the modified method of Irigoyen *et al.,* [21], the amount of total soluble sugar was measured, and the absorbance was measured at 625 nm. A Folin phenol reagent was used to quantify soluble protein using the method of Lowry *et al.,* [22]

2.6.3.Proline Content

The content of proline in the fresh leaves was analyzed [23]. 500 mg of leaf samples were crushed with sulfosalicylic acid (3%) and centrifuged for 10 min at 4000 × g. After adding toluene, the samples were measured at 520 nm for optical densities.

2.6.4.Quantification of Phenolics and Flavonoids

According to Dihazi *et al.,* [24], free phenol levels were assessed using sodium carbonate and Folin-Ciocalteu. 6.5 mL of 50% methanol was added to 100 milligrams of dried pea shoots. A supernatant of 1 mL was mixed with phosphoric acid (5 mL; 85%), Folin-Ciocalteu reagent (0.8 mL; 25%), and hydrochloric acid (10 mL). A spectrophotometer was used to calculate the absorbance at 765 nm.

The Zhishen *et al.,* [25] technique examined total flavonoid content. Ground samples were extracted with 1% HCl-methanol. Spectrophotometric measurement of upper aqueous phase absorption values was done at 340, 530, and 657 nm.

Kivçak and Mert [26] were used to quantify α-tocopherol. Prechilled chloroform homogenized fresh leaves. One milliliter of extract, ferric chloride reagent, and 2,2-dipyridyl were mixed for 10 seconds. The mixture's absorbance was 522 minutes and 50 seconds after adding ferric chloride.

2.6.5.Non-antioxidant enzymes

The Jagota and Dani [27] technique tested pea leaf ascorbic acid (ASA). A 500 mg pea leaf completely expanded was extracted in 6% TCA (10 mL), 2 mL, 2% dinitrophenylhydrazine, and one drop of thiourea (10%) in ethanol (70%). The water bath heated the solution for 15 minutes. The samples were chilled, and H_2SO_4 (5 mL) was added. Spectrophotometry measured 530 nm absorbance.

Owens and Belcher [28] spectrophotometrically assessed the shoot's total glutathione (GSH). Fresh plant leaves (0.2 g) were homogenized in 6% metaphosphoric acid (2 mL) with EDTA (1 mM) using a cold pestle and mortar. To get the clear supernatant, the homogenate was centrifuged at 4 °C, 11,500×g for 15 minutes. To 0.4 ml supernatant, potassium phosphate buffer (0.5M) at pH 7.5 was added. A 412 nm absorbance was observed. One milliliter of the acid extract diluted 1:50 with forty liters of 2-vinylpyridine was incubated for one hr at 25 °C to calculate oxidized glutathione (GSSG). Incubated samples were used to estimate the GSSG content. Subtracting GSSG from total glutathione yielded reduced glutathione [28].

2.6.6.Determination of MDA, Relative water content (RWC).

Assessing Lipid Peroxidation (MDA), a lipid peroxidation product, was identified and quantified by Du and Bramlage [29]. Half-grams of pea leaves were homogenized in TCA (2 mL) and centrifuged for 20 minutes at 12,000 g. A 1 mL mixture of recovered supernatant and 10% TCA with 0.5% TBARS was boiled to 95 °C and refrigerated on ice. A spectrophotometer was used to calculate the absorbance of the supernatants at 532 and 600 nm. The MDA concentration was determined by deducting the non-specific absorbance at 600 nm from its extinction coefficient of 155 mM^{-1} cm^{-1}.

Five fresh predawn leaf discs were estimated for moisture to determine RWC. A high-precision balance measuring cm^{-1} disc fresh weight (FW) [30]. The total turgid weight (TW) was measured again after 24 hours in deionized water-filled Petri plates. The leaf disc was oven-dried at 80 ◦C until a consistent weight was reached, and the final dry weight (DW) was determined. The RWC is assessed in the following manner:

RWC = FW – DW / TW - DW × 100.

2.6.7.Assessment of Reactive Oxygen Species (ROS), Nitric oxide indicators

The method quantified hydrogen peroxide by Velikova *et al.,* [31]. To begin, 0.2 g of fresh leaf was homogenized in TCA (0.1%) and centrifuged at 4 °C at 12,000 × g for 20 minutes. Additionally, potassium phosphate buffer (10 mM) and KI (1 mM) were added to 0.8 mL of supernatant. The absorbance of each sample was measured at 390 nm using a spectrophotometer. Hydroxyl radical concentration was measured by Babbs *et al.,* [32]. In a 1 mL reaction mixture, deoxyribose, $FeCl_3$, EDTA, KH_2PO_4-KOH buffer (20 mM, pH 7.4), H_2O_2, and ascorbate were added. A spectrophotometer determined the optical density at 532 nm after 1 hour at 37 °C. The methodology described by [33] was used to assess the production rate of superoxide anion ($O_2^{\bullet-}$). A spectrophotometer measured absorbance at 530 nm after homogenizing one gram of fresh plant tissue with phosphate buffer (50 mM), PVP-30 (2%), and 0.5% Triton X-100. The standard $NaNO_2$ linear curve determines oxygen emission. Nitric oxide (NO) concentration was measured using the Adams *et al.,* [34] technique. Incubation of the supernatant with Griess reagent at room temperature for 30 minutes was performed after homogenization of the shoots in glacial acetic acid. Samples had 560 nm optical density.

2.6.8.Determination of antioxidant enzymes

Peroxidase was assayed in 5.8 mL phosphate buffer (pH 7.0), enzyme extract (0.2 mL), and 20 mM H_2O_2 (2 mL). Vetter *et al.,* [35] measured the absorbance increase of 2 mL pyrogallol, 25 °C at 470 nm and for 60 s using a UV-spectrophotometer (Model 6305, Jenway). A 1.0 g fresh pea leaf was extracted in phosphate buffer (100 mM; pH 7.8) with EDTA. The homogenate was centrifuged for 10 minutes at 15,000 × g. Enzyme activity was assessed in the supernatant. Beauchamp and Fridovich [36] measured SOD. The enzyme auto-oxidized epinephrine at 480 nm. For 3 min at 240 nm, Abogadallah [37] monitored catalase activity (CAT) and H_2O_2 disappearance. Ascorbate peroxidase activity (APX) was determined by measuring for 3 min at an absorbance of 290 nm by Nakano and Asada [38]. A milliliter of the test mixture contains 100 mM phosphate buffer, ascorbic acid (0.5 mM), H_2O_2, and enzyme extract (100 µL). Our calculations used an extinction coefficient of 2.8 mM^{-1} cm^{-1}. Jung *et al.,* [39] evaluated glutathione reductase activity (GR) at 470 nm after a minute of NADPH oxidation (extinction coefficient: 6.2 $mMcm^{-1}$) using a UV spectrophotometer (Model 6305, Jen The glutathione peroxidase (GPX) enzyme extract was mixed with K-phosphate buffer, decreased glutathione, Na_2HPO_4, and 5,5-dithiobis-2-nitrobenzoic acid. The absorbance after 5 min was measured at 412 nm, and GPX activity was assayed using an extinction value of 6.22 mM^{-1} cm^{-1}. Jung et al., [39]. Murshed *et al.,* [40] employed microplate assays to evaluate MDHAR and DHAR enzyme activity. Downs *et al.,* [41] measured nitrate reductase. Fresh leaves were treated in vials with isopropanol, phosphate buffer, and KNO_3 at 30 °C for 2 h. Incubation was followed by adding naphthyl ethylene diamine hydrochloride, sulfanilamide, and the solution. At 540 nm, the reaction was measured. Nitrate reductase activity (NR) was measured by Jaworski [42].

2.6.9.Elemental Analysis

Sen Tran *et al.,* [43] presented a method for measuring elements. To assess nitrogen, potassium, phosphorus, zinc, and copper levels, 100 mg of dry powdered sprouts were digested for 25 minutes at 185 ◦C in nitric acid (13 M). The glass nebulizer (0.4 mL/min) was measured using ICP-MS. External standards were calibrated using curves from 1 to 600 µg/L, and standard minerals were produced using 0.23 M nitric acid.

2.7.Statistical Analysis

The data are expressed as the mean of five replicates ± standard error. Fisher's multiple range tests were employed to contrast the means, and statistical analysis was performed utilizing SPSS 27 software.

3. Result

3.1. Characterization of the CuO/ZnO nano-composite

The morphology of the CuO/ZnO nano-composite and the size distribution of the prepared CuO/ZnO nano-composite were determined using the transmission electron microscopy (TEM) technique. Figure 1 shows a substantially homogeneous CuO/ZnO nano-composite. Two different magnifications may be useful in determining the particle shape and the correct size distribution of the synthetic CuO/ZnO nano-composite. CuO/ZnO nano-composite was found in particle sizes ranging from 14 nm to 99 nm. The synthesized CuO/ZnO nano-composite was clearly in the nanoscale range.

The FT-IR spectrum of the synthesized CuO/ZnO nano-composite (Figure 2a). Distinctive selections may be associated with the referenced material. The two primary peaks observed at 3450 and 480 cm^{-1} (along with 630 cm^{-1}) can be ascribed to O-H stretching and metal-oxygen vibrations, respectively. The peak at 1640 cm^{-1}, characterized by low intensity, is associated with the OH bending of the water molecule.

The X-ray diffraction pattern of the fabricated CuO/ZnO nano-composites (Figure 2b) was in good agreement with the typical patterns of hexagonal wurtzite ZnO (JCPDS Card No. 36-1451). Such an arrangement could prove that this synthesis process was done correctly. It was clear that the copper oxide/zinc oxide nano-composite diffraction peaks were split into two sets of peaks. Each mentioned set is related to the monoclinic CuO and hexagonal wurtzite ZnO.

The preparation of CuO/ZnO nano-composite was recognized via laser diffraction (DLS), establishing that the particle size distribution accomplished through the fabrication of the nano-composites of CuO/ZnO, which designed a highly dispersed mixture was a maximum of 730 nm as shown in (Figure 2c). Furthermore, the prepared CuO/ZnO nano-composites were very stable, the zeta potential measurement showed a positive value, and it was 3.75 mV, as revealed in (Figure 2d). The positive value charge reveals the stability of the CuO/ZnO nano-composite without creating aggregates; these nano-composites do not convert to amorphous through the storage period.

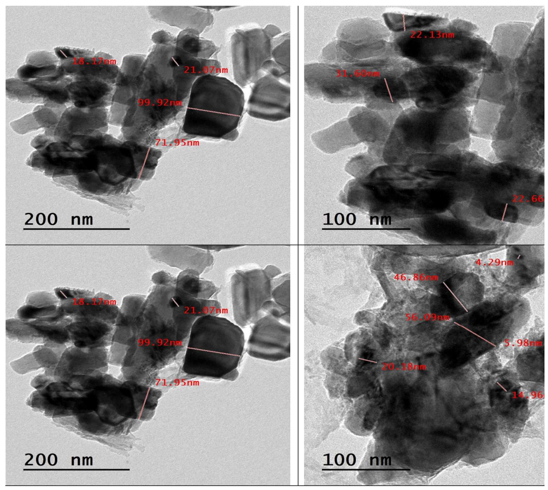

Figure 1. Transmission electron microscope (TEM) image of the prepared CuO/ZnO nanocomposite

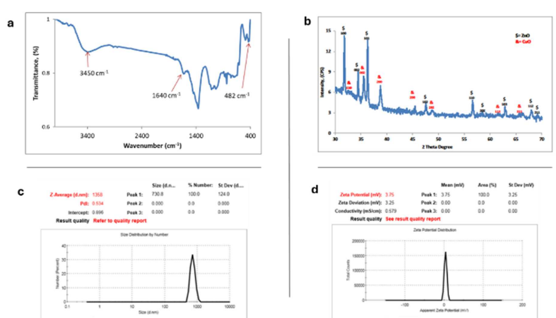

Figure 2. a) The Fourier-Transform Infrared (FT-IR) spectrum, b) X-ray diffraction analysis (XRD) pattern, Dynamic Light Scattering (DLS) analysis of the prepared CuO/ZnO nanocomposite, c) Particle size, d) Zeta potential

3.2. Morphological parameters

Figure (3a–f) demonstrates the pea plant's growth characteristics, like plant and root length, fresh and dry shoot, and fresh and dry root when treated with irrigation (60, 80, and 100% water applied), and ZnO/CuO nano-composite (25 and 50 ppm). In this regard, 80% and 60% of AW decreased shoot length about 37.57% and 60.38%, respectively; root length about 37.52% and 57.63%, respectively; FW of the shoot by 38.28 % and 55.25% respectively; DW of the shoot by 46.58 %, 73.68% respectively, FW of the root by 60.73 %, 79.28% respectively, DW of the root by 37.61%, 53.85% respectively compared to the unstressed control plant. These growth characteristics were significantly raised by treatment with ZnO/CuO nano-composite (25 and 50 ppm), and these enhancements were more noticeable in plants treated with ZnO/CuO nano-composite (50 ppm) than in plants not under stress. Applying ZnO/CuO nano-composite (25 and 50 ppm) to pea plants grown under deficit water stress significantly increased significantly than those growing under 80% and 60% water applied.

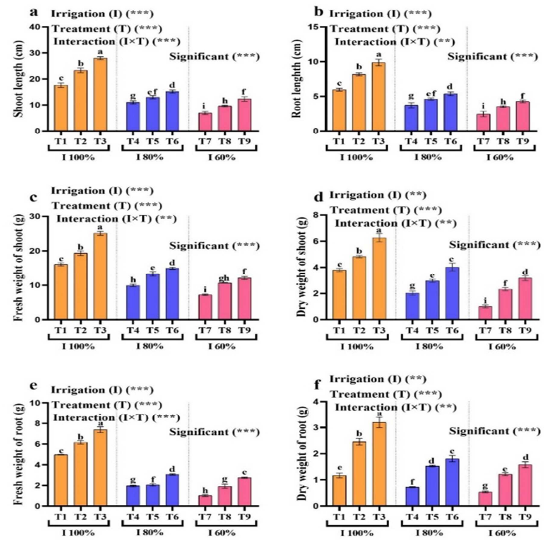

Figure 3. Effects of foliar application with ZnO/CuO nanocomposite (25 and 50 ppm), on a) shoot length, b) root length, c) fresh weight of shoot, d) dry weight of shoot, e) and fresh weight of root, and f) dry weight of root of pea grown under available water irrigation (80 and 60 %). Fisher's multiple range test indicates significant differences between means in each bar (P < 0.05). ** and *** indicate differences at p < 0.05 and p < 0.01probability level, respectively.

3.3. Photosynthetic Characteristics

Figure (4a-d) shows that chlorophyll a, b, carotenoids, and total pigments were reduced in leaves with deficit water stress treatment compared to control plants. However, the contents of chl a, chl b, carotenoids, and total pigments were raised in pea plants by applying ZnO/CuO nano-composite (25 and 50 ppm) since ZnO/CuO nano-composite (50 ppm) mitigated the adverse effects of deficit water stress at levels 80 and 60%, where chlorophyll a increased by 84.72 % and 105.88 %, chlorophyll b raised by 128.58 % and 180.00 %, carotenoids raised by 63.46 % and 89.66%, and total pigments raised by 85.53 % and 119.28 %, compared to water stress (80, 60%) (Figure 4a-d).

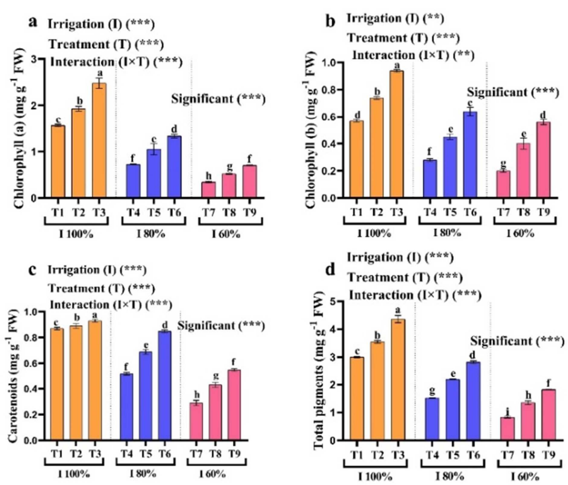

Figure 4. Effects of foliar application with ZnO/CuO nanocomposite (25 and 50 ppm) on a) chlorophyll a, b) chlorophyll b, c) carotenoids, d) total pigments of pea grown under available water irrigation (80 and 60 %). Fisher's multiple range test indicates significant differences between means in each bar (P < 0.05). ** and *** indicate differences at p < 0.05 and p < 0.01probability level, respectively.

3.4. Osmolyte content

The osmolyte content (carbohydrate, proline, phenol, flavonoids, α-tocopherol) rose significantly under water stress compared with control plants (Figure 5a-f). Nevertheless, protein content decreased significantly under water stress compared with non-stressed plants. In addition, under 80% and 60% water irrigation, a significant rise in carbohydrate, protein, proline, flavonoids, phenol, and α-tocopherol content was detected when pea plants were treated with ZnO/CuO nano-composite (50 ppm) in comparison to stressed plants, it extends to well-controlled plants.

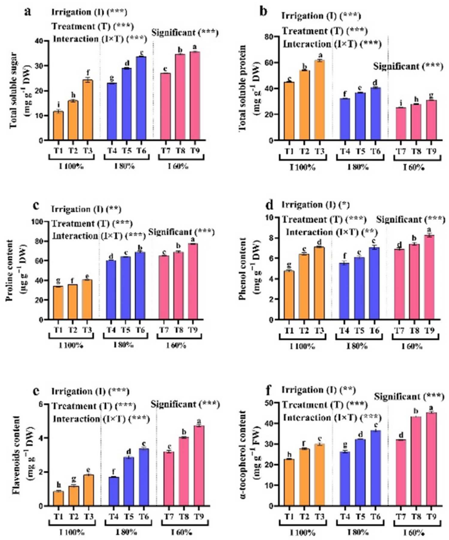

Figure 5. Effects of foliar application with ZnO/CuO nanocomposite (25 and 50 ppm) on a) carbohydrate, b) protein, c) proline, d) phenol, e) flavonoids, f) α tocopherol of pea grown under available water irrigation (80 and 60 %). Fisher's multiple range test indicates significant differences between means in each bar ($P < 0.05$). ** and *** indicate differences at $p < 0.05$ and $p < 0.01$probability level, respectively.

3.5. Non-enzymatic antioxidant compounds

The water deficit notably elevated non-enzymatic antioxidant levels (Figure 6a-c). However, applying the ZnO/CuO nano-composite to water-deficit-stressed plants resulted in significantly increased levels of AsA, GSH, and GSH/TGSH than the stressed plants. Furthermore, applying pea plants with 50 ppm ZnO/CuO nano-composite, as opposed to 25 ppm, resulted in a significant rise in their AsA, GSH, and GSH/TGSH levels (Figure 6a–c).

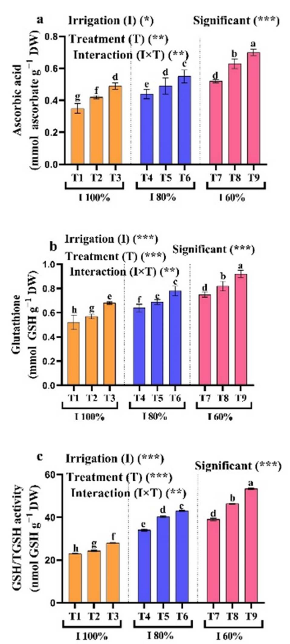

Figure 6. Effects of foliar application with ZnO/CuO nanocomposite (25 and 50 ppm), on a) ascorbic acid (ASA), b) glutathione (GSH), c) GSH/TGSH, of pea grown under available water irrigation (80 and 60 %). Fisher's multiple range test indicates significant differences between means in each bar ($P < 0.05$). ** and *** indicate differences at $p < 0.05$ and $p < 0.01$probability level, respectively.

3.6. Lipid peroxidation content and ROS production

Based on the results obtained, several important observations can be drawn regarding an indicator of ROS production (H_2O_2, OH, and O_2) and lipid peroxidation that has been developed using MDA, RNS (Nitric oxide), and RWC in pea leaves treated with ZnO/CuO nano-composite (25 and 50 ppm) under both drought stress conditions and normal conditions, as demonstrated in Figure (7). The results indicate that permitting pea plants to develop under drought-stress conditions led to a notable rise in MDA, H_2O_2, OH, and $O_{2,}$ as well as nitric oxide levels, accompanied by a reduction in RWC. Applying ZnO/CuO nano-composite at 25 and 50 ppm concentrations demonstrated a significant ability to mitigate drought stress by decreasing MDA, H_2O_2, OH, O_2, and nitric oxide levels. Conversely, applying ZnO/CuO nano-composite at 25 and 50 ppm concentrations resulted in a notable rise in RWC compared to stressed and unstressed plants.

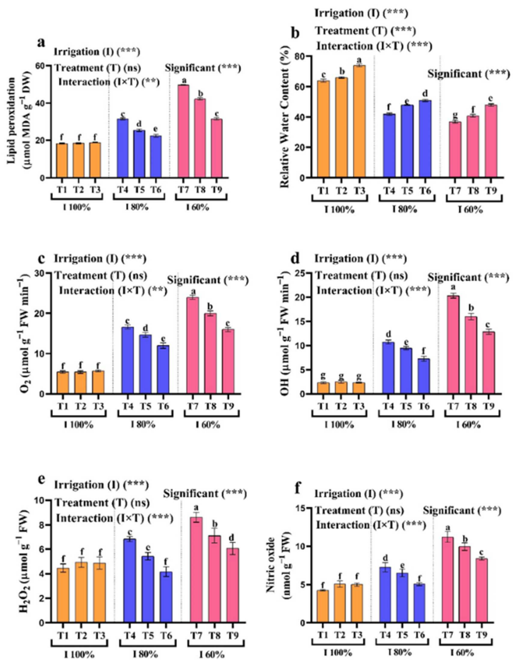

Figure 7. Effects of foliar application with ZnO/CuO nanocomposite (25 and 50 ppm) on a) lipid peroxidation, b) Relative water content (RWC), c) superoxide anion (O_2), d) Hydroxide (OH), e) Hydrogen Peroxide (H_2O_2), f) Nitric oxide of pea grown under available water irrigation (80 and 60 %). Fisher's multiple range test indicates significant differences between means in each bar ($P < 0.05$). ** and *** indicate differences at $p < 0.05$ and $p < 0.01$probability level, respectively.

3.7. Enzymatic Antioxidant Activity

Compared to full irrigation at 100%, water deficits lead to a rise in POX, SOD, APX, GR, CAT, GPX, and NR activities (Figure 8). The application of irrigation at 80% and 60% of the available water markedly enhances the antioxidant activities of SOD, achieving increases of (46.67% and 95.24%), respectively. POX (26.09%; 71.01%), CAT (28.46%; 81.36%), APX (33.87%; 49.43%), GR (69.05%; 138.89%), GPX (21.05%; 194.74%), and NR (8.97%; 14.00%), compared to the plants grown with full irrigation. Furthermore, ZnO/CuO nano-composite (25 and 50 ppm) Eg., pea plants increased with the foliar treatment of ZnO/CuO nano-composite (50 ppm) exhibited the highest SOD (14.94%, 18.54%), POX (17.82%, 17.80%), CAT (22.15%, 10.76%), APX (33.87%, 27.15%), GR (30.99%, 27.91%), GPX (113.33%; 57.14%), and NR (9.84%; 11.32%), activities as compared to plants with irrigation at 80% and 60%, respectively.

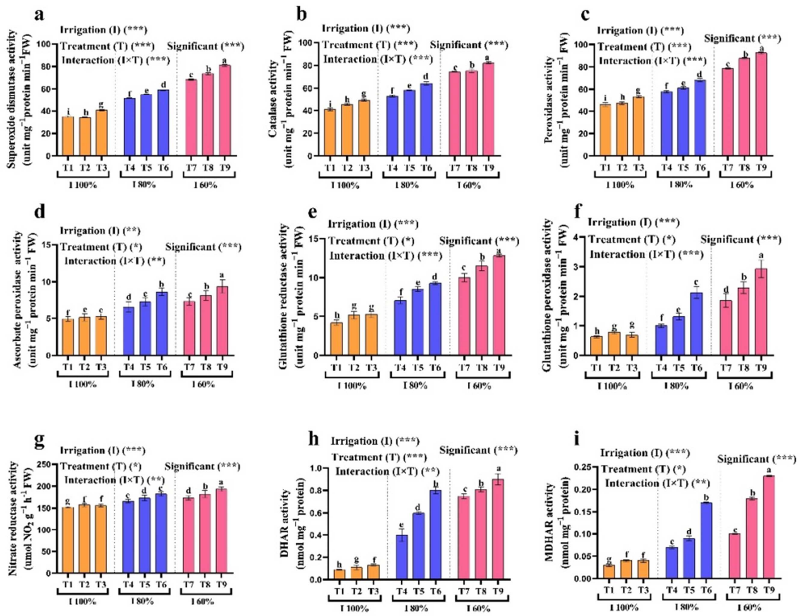

Figure 8. Effects of foliar application with ZnO/CuO nanocomposite (25 and 50 ppm) on a) superoxide dismutase (SOD), b) catalase (CAT), c) peroxidase (POX), d) Ascorbate peroxidase (APX), e) glutathione reductase (GR), f) glutathione peroxidase (GPX), g) nitrate reductase (NR), h)DHAR, i) MDHAR of pea grown under available water irrigation (80 and 60 %). Fisher's multiple range test indicates significant differences between means in each bar ($P < 0.05$). ** and *** indicate differences at $p < 0.05$ and $p < 0.01$probability level, respectively.

3.8. Mineral nutrients Concentration

Water deficit stress culminated in a significant decrease in P, N, K, Zn, and Cu content of pea plants compared to the non-water deficit-stressed plants in shoots (Figure 9). However, applying ZnO/CuO nano-composite (25 and 50 ppm) significantly raises P, N, K, Zn, and Cu levels in pea plants compared to non-water deficit-stressed plants in shoots.

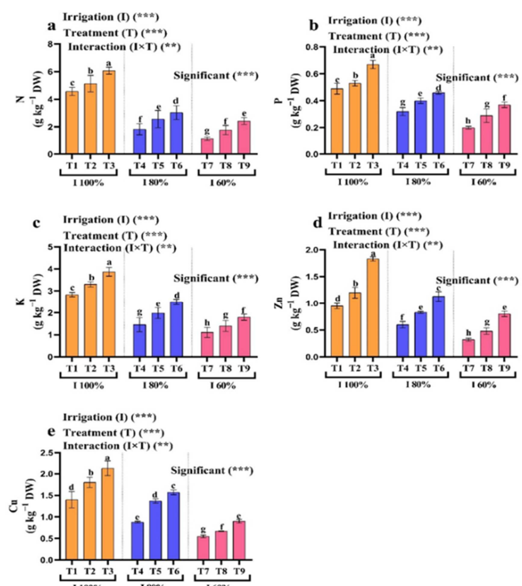

Figure 9. Effects of foliar application with ZnO/CuO nanocomposite (25 and 50 ppm), on a) nitrogen (N), b) phosphorous (P), c) potassium (K), d) zinc (Zn), e) copper (Cu) of pea grown under available water irrigation (80 and 60 %). Fisher's multiple range test indicates significant differences between means in each bar ($P < 0.05$). ** and *** indicate differences at $p < 0.05$ and $p < 0.01$probability level, respectively.

4. Discussion

Water stress in semi-arid and arid climates can impact plant growth, resulting in restricted water availability to roots, elevated temperatures, and accelerated transpiration rates [44]. According to the results, peas were adversely affected by water stress. Additionally, water stress has been demonstrated to diminish the growth of barely plants [45], *Vicia faba* plants [46], and pea plants [47]. Water stress can lead to the deregulation of elongating cells due to disrupted water flow from the xylem to these cells, along with a reduction in mitosis during cell division, growth-promoting hormones, cell elongation, and cell expansion [48]. Water stress events are occurring globally due to the impacts of climate change [49]. Consequently, it will be necessary for new management to tackle this issue through the application of nanotechnology.

Water stress has been shown to reduce morphological parameters and yield across various species, including strawberries. Decreased growth parameters may result from elevated MDA levels, leading to cell shrinkage, reduced leaf growth, diminished meristematic cell division, accelerated senescence, obstruction of leaf production, and leaf drop [50,51].

The ingress of carbon dioxide into stomata that close as a result of drought can also be affected by water stress directly and indirectly. Water stress impairs photosynthetic materials' transport, constraining photosynthesis and diminishing vegetative plant development [52]. ZnO/CuO nano-composites have shown beneficial effects on drought tolerance and the dry and fresh weight of many species, including peas. The growth stimulation caused by ZnNPs is associated with increased antioxidative enzyme activity and enhanced photosynthesis under diverse environmental stress situations [53]. The components in these compounds might enhance assimilate synthesis and antioxidant enzyme activity, thereby mitigating stress and fostering vegetative growth [54].

Drought significantly alters biochemical and physiological processes, substantially reducing water absorption by plant roots [6]. Moreover, reactive oxygen species are produced expedited [55]. Consequently, the impairment of several biochemical characteristics and processes in plants exposed to water stress would inevitably compromise the effectiveness of photosynthesis. Our data corroborate this technique since photosynthetic pigments, namely Chl a, b, carotenoids, and total chlorophyll, exhibited a substantial drop in response to water stress. The findings indicate that the ZnO/CuO nano-composite effectively mitigates the detrimental impacts of elevated drought conditions on the photosynthesis of pea plants. Drought-sensitive plant species are particularly vulnerable to negative drought impacts, leading to a decrease in chlorophyll content [56].

It has been shown that ZnONPs can improve the manufacture of photosynthetic pigments in cotton plants under drought stress [57]. Photosynthesis pigments, chl a, chl b, and total photosynthesis pigments are positively impacted by ZnO/CuO nanocomposite. Muhammad *et al.,* [58] had previously documented drought-stressed wheat plants. Guha *et al.,* [59] indicated that the production of nano-composite pigments in soybean plants may be attributed to phosphorus, nitrogen, and potassium absorption alterations. The research demonstrated that treating *Pisum sativum* with a ZnO/CuO nano-composite considerably alleviated the detrimental effects of water stress on the photosynthetic apparatus by enhancing the production of pigments and their related constituents.

Applying ZnO/CuO nano-composite may significantly elevate plant phenols, flavonoids, and α-tocopherol levels, augmenting antioxidant capacity, stress resilience, and defensive mechanisms [60]. The ZnO/CuO nano-composite is essential for maintaining membrane stability and modulating phenolic compound synthesis in plants exposed to water stress. Optimal concentrations of ZnNPs mitigate the adverse impacts of water by safeguarding cell membranes from oxidative damage and improving the plant's antioxidant defense systems via synthesizing phenolic compounds [61]. The external application of ZnO/CuO nano-composite elevated the endogenous proline levels in pea plants exposed to varying water irrigation levels.

This discovery indicates that applying exogenous proline enhances plant accumulation of endogenous ZnO/CuO nano-composites. Proline is a prevalent endogenous osmolyte that accumulates in plants in response to numerous abiotic stressors like drought [62]. Exogenous proline application may enhance osmoprotectant concentrations in plants, hence improving their resilience to increased water irrigation [63]. Additionally, water stress induced the accumulation of endogenous proline in pea plants. Semida *et al.,* [64] noted that water stress led to heightened accumulation of endogenous proline in onion plants.

The maximum endogenous proline accumulation was seen when ZnO/CuO nano-composite (50 ppm) was applied to both water irrigation levels. Proline buildup in plants under water stress enhances membrane integrity and promotes the production of phenolic compounds. The accumulation of endogenous proline and the formation of phenolic products are essential for plant adaptation and tolerance to water conditions [65].

The findings indicate that MDA, H_2O_2, OH, O_2, and nitric oxide levels were markedly elevated in drought-stressed pea shoots compared to unstressed plants. ROS induces metabolic problems and cellular apoptosis by destroying DNA, lipids, and proteins [66]. Under water stress, the MDA concentration is markedly elevated in pea plants [67] and maize [68]. The elevation of malondialdehyde levels may be attributed to reducing the plant's enzyme activity under water conditions [69]. When the ZnO/CuO nano-composite is applied to drought-stressed plants, the MDA, H_2O_2, OH, O_2, and nitric oxide levels may be reduced, mitigating oxidative stress and membrane damage. Exogenous treatment of ZnO/CuO nano-composite considerably elevated endogenous levels of proline, phenol, GSH, and AsA under drought stress. ROS have been demonstrated to be generated uncontrolled in many sites inside plants subjected to environmental influences, abiotic stressors (drought, heavy metals, and salinity), etc. [70,71]. Elevated levels of ROS hinder enzyme function and induce oxidative damage to proline, phenol, lipids, ascorbic acid, and glutathione [72]. Plant cells are, therefore, protected from oxidative damage by antioxidant defences that scavenge ROS [73]. Our findings demonstrate that osmolytes accumulate to a greater extent during drought circumstances. In drought conditions, cells modify their osmotic equilibrium and experience a reduction in cellular damage due to proline buildup [74].

Furthermore, GSH is crucial for the ASC-GSH cycle [75,76], which may facilitate the regeneration of the water-soluble antioxidant AsA and elevate its levels in response to water stress. Treatment with ZnO/CuO nano-composite may enhance and prompt the activities of many antioxidant enzymes, including APX, CAT, GPX, SOD, POX, GR, DHAR, NR, and MDHAR, therefore enabling the pea plant to endure drought stress. Environmental stress, such as drought, induces a breakdown in cellular homeostasis, producing excess ROS in plants. Oxidative stress arises when the production of ROS exceeds the cell's immune system capability, resulting in diminished nucleic acid oxidation, protein oxidation, enzyme function, activation of the main apoptotic pathway, MDA formation, and, ultimately, cell death [77].

Antioxidant enzymes may minimize damage by eliminating excess reactive oxygen species generated during water stress. Numerous studies have shown that enhancing the expression of certain antioxidant enzymes may enhance stress tolerance. SOD serves as the primary and most effective defense against reactive oxygen species in several cellular compartments, including chloroplasts, peroxisomes, cell walls, and apoplast hence protecting against abiotic stress [78,79]. SOD activity has been elevated in chickpeas due to drought stress. SOD is an essential antioxidant enzyme that neutralizes O_2, OH, and H_2O_2 in the Haber–Weiss process [80]. The pea plant has been safeguarded against free-radical-induced membrane dysfunction by enhancing the activity of the SOD, CAT, POX, APX, GPX, GR, NR, DHAR, and MDHAR enzymes. The markedly elevated POX activity in maize plants treated with ZnO/CuO nano-composite may be linked to enhanced sensitivity to drought stress, attributed to advancements in lignin and other antioxidant compounds that mitigate production. Shinde *et al.,* [81] discovered that POX activity in peas was elevated during drought stress. Reduced H_2O_2 concentrations may correlate with elevated CAT activity in pea leaves exposed to water stress [82,83].

Furthermore, H_2O_2, a potent and deleterious oxidizing agent, was transformed into H_2O and O_2 by APX and CAT . Treatment with nano-composite significantly altered the activity of APX in maize plants subjected to water stress [84,85]. Following the exogenous delivery of ZnO/CuO nano-composite nanoparticles, glutathione reductase activity increased, suggesting its involvement in the recycling and enhancement of endogenous glutathione levels. The external foliar spray of ZnO/CuO nano-composite enhanced GR activity in pea plant shoots. This indicates that GSH may preserve the integrity of biological macromolecules and protect structural proteins and the sulfhydryl (-SH) groups of enzymes from oxidative damage [86,87].

Furthermore, the applied ZnO/CuO nano-composite concentration improves the AsA-GSH cycle, APX, and GR activities, in addition to boosting the levels of AsA. ROS was eradicated as a result of the synergistic actions of these enzymes [88].

The exogenous ZnO/CuO nano-composite at 50 ppm showed greater efficacy compared to the 25 ppm variant in enhancing the activities of APX, CAT, GR, SOD, POX, GPX, NR, DHAR, and MDHAR, indicating that the exogenous ZnO/CuO nano-composite more efficiently activates the AsA–GSH cycle [89]. Drought stress significantly reduced pea plants' phosphorus, nitrogen, and potassium levels relative to those without drought stress. In contrast, the concentrations of P, N, K, Zn, and Cu of water-stressed pea plants were significantly elevated with the exogenous ZnO/CuO nano-composite treatment. El-Beltagi et al., [57] demonstrated that nutrient absorption was reduced under water stress due to hydraulic conductivity limitations, increased root thickness, decreased root branching, and root length. The restricted water supply resulted in a notable decrease in the P, N, K, Zn, and Cu content in pea plants. This resulted in restricted nutrition absorption due to reduced transpiration, immobilized ions, decreased membrane permeability, and impaired root nutrient uptake [90]. Reports indicate that N, P, K, Zn, and Cu intake diminishes with prolonged water shortage duration [91]. Inadequate osmotic stress and soil moisture are two conditions that decrease nutrient absorption. The decreased solubility of nutrients near root hairs is responsible for this phenomenon [92]. During drought conditions, applying ZnO/CuO nano-composite by foliar spraying enhanced N, P, K, Zn, and Cu levels, improving water retention in the rhizosphere and promoting nitrification. A water shortage diminishes phosphorus content and fixing in alkaline soils.

Furthermore, the ZnO/CuO nano-composite may inhibit leaf water depletion and enhance potassium absorption in the leaves. Basavegowda and Baek [93] observed that applying nano-composite enhanced peas' potassium, phosphorus, and nitrogen concentrations under water deficiency circumstances. In their research, Jafir *et al.,* [94] discovered that ZnNPs enhanced phosphorus absorption in stressed plants, while Abbasi *et al.,* [95] observed that applying ZnNPs facilitated potassium accumulation in stressed plants. Muthulakshmi *et al.,* [96] showed that applying nano-composite enhances osmoregulatory chemicals, antioxidant activity in plant tissues, and the concentration of several mineral components, including potassium. In another investigation, Zn availability was shown to enhance the wheat harvest index and nitrogen usage efficiency. According to Mahmood *et al.,* [97], priming sunflower seeds can enhance drought resistance by providing energy and nutrients. In pea seedlings subjected to water stress, exogenous ZnNPs increase NPK concentrations [98].

4. Conclusion

The adverse impacts of dryness on growth, biochemical characteristics, and traits were significantly mitigated by treating ZnO/CuO nano-composites to pea plants. Inhibition or reduction of water accumulation in pea plants and decreased ROS-induced damage to the membrane system may be responsible for mitigating water stress caused by ZnO/CuO nano-composites. Furthermore, the uptake of essential nutrients, such as nitrogen, phosphorus, potassium, zinc, and copper, critical for plant growth, was enhanced by the ZnO/CuO nano-composites. The ZnO/CuO nano-composites enhanced the activities related to photosynthesis. Furthermore, ZnO/CuO nano-composites regulated the activity of stress enzymes to enhance maize plants' resilience to drought stress. This study may elucidate the probable mechanisms behind the resistance to drought toxicity conferred in peas by ZnO/CuO nano-composites. As a result of our study, ZnO/CuO nano-composites at 50 ppm mitigated drought-induced adverse effects and improved growth, biochemical and physiological traits, as well as P, N, K, Zn, and Cu concentrations, with ZnO/CuO nano-composites at 25 ppm following closely behind.

Author contribution statement

Mohamed A. Ghaleb, Mahmoud S. Abu-Shahba, Ahmed M. Youssef, Mohamed A. Mousa, Mahmoud R. Sofy methodology, writing—review and editing, supervision, project administration, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data availability

The data sets generated and analyzed in this study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interest.

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