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Effects of phytostabilized Zinc Sulfide nanocomposites on growth and Arsenic accumulation in Wheat (*Triticum aestivum* L.) under Arsenic stress

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ABSTRACT

This study employed phytostabilized zinc sulfide (ZnS) nanocomposites, a novel and environmentally friendly material (orange peels), to mitigate arsenic (As) toxicity and its accumulation in wheat (*Triticum aestivum* L. cv. Pishgam). Wheat plants were exposed to sodium arsenate (via fertigation) at concentrations of 75 and 150 mg/L. These treatments caused significant reductions in shoots and roots biomass, chlorophyll contents; with concomitant higher tissue As levels, oxidative stress markers, along with enhanced antioxidant enzyme activity, compared to non-stressed controls. Supplementation with ZnS nanocomposites at concentrations of 75 and 150 mg/L significantly reduced As accumulation in both roots and shoots and alleviated the As toxicity. This was evidenced by increase of up to 29 % in shoot fresh weight, 76 % in root fresh weight, 27 % in foliar chlorophyll contents, 38 % in proline levels, and 21 % in total soluble protein contents. There was notable increase in zinc concentration (up to 122 %) and enzymatic activities (peroxidase (POD) increased by 98 %, ascorbate peroxidase (APX) by 28 %, and catalase (CAT) by 39 %) in plants exposed to ZnS nanocomposites levels of 75 and 150 mg/L compared to As-stressed counterparts. Furthermore, ZnS nanocomposites reduced the As accumulation in roots by up to 41 % and in shoots by up to 30 %, while enhanced the hydrogen peroxide (H_2O_2) level by 80 % under As stress. These findings highlighted the potential of ZnS nanocomposites (especially at 75 mg/L) as a phytostabilizing, non-toxic, and environmentally friendly solution to ameliorate As toxicity in wheat plants. This study further helps to enhance identify critical avenues for focusing on the integrated application of nanoparticles in soil management to promote sustainable agricultural practices.

1. Introduction

Arsenic (As) naturally occurs in the Earth's crust and can be distributed in the environment through human activities. This metalloid exists in two main forms: organic and inorganic, which can be converted into one another through biotic and abiotic processes (Gosh et al., 2022). Arsenate and arsenite are recognized as the two major inorganic forms of As encountered in the natural environment (Souri et al., 2017). When poorly managed, agricultural practices in As-contaminated environments can pose a significant threat to human health. Arsenic contamination of drinking water from shallow tube wells caus harmful health effects on millions of people in certain regions of Asia (Tabassum et al., 2019; Biswas et al., 2023; Alamgir et al., 2024). Additionally, several areas in Iran have been reported to be contaminated with As (Hamidian et al., 2015). Previous investigations indicated that the primary source of As in contaminated regions of Iran is geogenic, resulting from the dissolution of As-containing compounds in the Earth's crust. However, some anthropogenic activities, such as mining in gold-As deposits, have significantly contributed to soil, water, and air pollution in these areas (Karimi et al., 2010). One of the major issues regarding As distribution in agricultural and household contexts is polluted groundwater. The extensive use of As-contaminated groundwater for all forms of cultivation can increase the contamination of surface soil, which subsequently affects plants and grains of cereals (Dehbandi et al., 2019).

Wheat (*Triticum aestivum* L.) is major group of cereals in the world. The physiological activities of wheat seedlings, including seed

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germination, biomass production, and the lengths of roots and shoots, are affected negatively under elevated As levels (Mahdieh et al. 2013; Zaheer et al., 2022). Wheat, a crucial agricultural commodity, holds significant importance in the As-contaminated regions of Iran's western territories. Therefore, the As uptake, toxicity and accumulation in wheat is a major concern of human health. Hence, examining the way and presenting solutions to reduce the absorption, transport and accumulation of As in this valuable crop is very important for decrease of health risks associated with As (Saeed et al., 2024).

Nanotechnology, with valuable and extensive applications, has received global attention as an innovative scientific advancement. The recent progress in plant nanobiotechnology has led to greener, safer, and more stable methods for the synthesis of nanoparticles (Ahmed et al., 2022; Jalil et al., 2023; Ridhi et al., 2024). Metal-based green synthesis of nanoparticles has become an interesting area due to numerous advantages of nanoparticles such as reducing stress toxicity and enhancing plant resilience against various stresses like salinity, heavy metals and metalloids toxicity (Souri et al. 2020; Hanif et al., 2024).

Zinc (Zn) is classified as a micronutrient with the potential to bind with insoluble and poorly bioavailable compounds in the soil. Therefore, the use of Zn nanoparticles in agriculture, both for plant protection against heavy metals/metalloids, and enhancement of our understanding of Zn uptake and accumulation mechanisms, is crucial especially in the near future (Jalil et al., 2023). It has been determined that Zn nanoparticles have nanoscale size and high surface area, and exhibit unique physicochemical properties that influence their bioavailability, mobility, and interaction with plant root systems (Hassan et al., 2022). Moreover, Zn nanoparticles can be absorbed by plants through specific transporters, such as ZIP (Zinc-Regulated Transporter/Iron-Regulated Transporter-like Protein) family proteins, and their uptake pathways can be tracked to understand how Zn is translocated and accumulated in plant tissues (Wang et al., 2019). The use of Zn nanoparticles in mitigating heavy metal stress in plants has revealed their role in competing with toxic metals for uptake sites, thereby reducing heavy metal toxicity and improving plant health (Hassan et al., 2022). Wu et al. (2020) found that the application of ZnO nanoparticles delivered beneficial effects on plants such as germination rate, seedling growth, chlorophyll content, biomass, antioxidant enzymes, and nutrient levels under As stress, indicating the suitability of Zn nanoparticles for improving rice resistance to As stress.

Sulfur (S) is an essential macronutrient required for the synthesis of thiol (SH)-containing metabolites that play a crucial role in stress defense responses (Arianmehr et al., 2022; Yuce et al., 2024). Thiol compounds, such as cysteine (Cys), glutathione (GSH), and phytochelatins (PCs), are powerful nucleophilic agents that perform fundamental functions, including redox regulation, antioxidant activity, and signaling transduction under stress conditions (Souri et al., 2020; Salbitani et al., 2023; Yuce et al., 2024). Therefore, S-containing metabolites are vital for plant growth and development. Previous research has underscored the advantageous effects of S nanoparticles on the physiological and metabolic processes of plants (Thapa et al., 2019). Notably, studies have demonstrated that S nanoparticles can mitigate As toxicity while simultaneously promoted the growth of rice and Indian mustard (Meselhy et al., 2021). Furthermore, S nanoparticles have been shown to enhance the plant biomass by scavenging reactive oxygen species (ROS) and stimulating the activity of antioxidant enzymes in plants subjected to heavy metal stress. This is particularly evident in the case of mercury, manganese, copper, and silver, where S nanoparticles can help to alleviate the detrimental effects in plants imposed by these heavy metals (Yuan et al., 2021, 2023; Sharma et al., 2023).

While several studies have reported the ameliorative effects of Zn nanoparticles in plants, there is limited information regarding the impact of Zn nanoparticles modified by S compounds on the physio-biochemical responses of crops under As stress. Therefore, this study hypothesized the effects of ZnS nanocomposites in mitigating As stress in wheat, and provided new insights into their potential application as a protective strategy in As-contaminated environments. This study aimed to investigate the effects of ZnS nanocomposites on the physio-biochemical responses, and As accumulation in wheat plants subjected to As stress.

2. Materials and methods

2.1. Synthesis of green Zinc Sulfide orange peel nanocomposites

The orange peels were dried in an oven at 80 °C and milled. Then, 1 g of dry powder with a weight ratio of10 % of Zn to dry powder was added to 40 ml of aqueous solution containing 0.03 M Zn $(NO_3)_2$ (Sigma-Aldrich company) and 0.07 M thiourea $(SCNH_2)_{2)}$ (Sigma-Aldrich company) and allowed to stir for 2 h. Then, the resulting sample was transferred to a Teflon-lined stainless-steel autoclave of 50 ml capacity containing the mixed solution and was heated at 180 °C for 6 h. The products were cooled, centrifuged and sequentially washed with distilled water and finally dried at 100 °C. The obtained product was characterized by an X-ray diffractometer (XRD) (Inel Equinox 3000 X-Ray), scanning electron microscopy (FE-SEM) (TESCAN Company, Czech), and Fourier-transform infrared spectroscopy (FTIR) (Bruker FTIR Spectrometer, Model: ALPHA).

2.2. Plant material, growing condition and treatment

Wheat seeds of the "Pishgam" cultivar were prepared from Kermanshah Agricultural Research Center, Kermanshah, Iran. The seeds were treated with a 1 % sodium hypochlorite solution for 10 min and subsequently rinsed with distilled water. Thereafter, sterilized seeds were sown at 1 cm depth of perlite in a container with dimensions of 18 × 40 cm. The water holding capacity of the container was kept up to 70 % of soil water storage capacity. Irrigation was done after every 48 h until seeds were germinated. After reaching a stem height of 5 cm, seedlings were transplanted to 1-liter hydroponic pots (one pot in each replication and 4 plants in each pot) consisting of modified Hoagland solutions (Karimi et al., 2009). The pots were arranged randomly in a growth chamber with alternating temperatures of 22 °C at night and 25 °C during the day, a relative humidity of 50 %, and a photoperiod of 16 h of light and 8 h of darkness. The nutrient solution in each pot was renewed every four days. Wheat seedlings, which had been cultivated with Hoagland nutrient solution for a duration of seven days, served as the experimental subjects. Subsequently, the plants were fertigated with varying concentrations of sodium arsenate $(Na_2HAsO_4{\cdot}7H_2O)$ and ZnS nanocomposites, specifically at levels of 0, 75, and 150 mg/L. To enhance the dispersion of the ZnS nanocomposite suspension, it was subjected to ultrasonic vibration at a power of 100 Watts and a frequency of 40 kHz for 30 min. The concentrations of sodium arsenate and ZnS nanocomposites were selected based on previous studies (Meselhy et al., 2021). Each experimental treatment was conducted with three replicates, with one pot designated for each replicate, and containing twelve plants per treatment. After growing the plants for 28 days, treated plants were harvested, the shoots and roots of each plant were separated and their fresh weight was recorded immediately.

2.3. Measurement of chlorophyll content

The wheat foliar chlorophyll content was measured from the mature leaves using a desk-top UV–visible spectrophotometer (Bausch & Lomb70). To prepare the chlorophyll extract, 0.5 g of fresh leaf tissue was progressively ground with 80 % acetone. The mixture was then centrifuged at 4000 rpm and 4 °C for 10 min to isolate the chlorophyll extract from the cellular debris. The absorbance of the supernatant was recorded at wavelengths of 663 nm and 646 nm. The concentrations of chlorophyll a, chlorophyll *b*, and total chlorophyll were calculated following the method outlined by Lichtenthaler (1987).

2.4. Measurement of hydrogen peroxide

For measuring the H_2O_2 levels, 0.05 g of root or shoot sample was homogenized in 0.1 % trichloroacetic acid (TCA). The resulting mixture was then centrifuged at 12,000 rpm for 15 min. After that, 0.5 ml of lubricating solution was mixed with a combination of potassium phosphate buffer (10 mM) and potassium iodide (1 M). Finally, the spectrophotometer (Bausch & Lomb70) was used to measure the absorbance of the resulting samples at 390 nm.

2.5. Assessment of total soluble protein content and antioxidant enzyme activities

For preparing extraction buffer, 1 ml of Tris–HCl buffer was added to 0.05 g of fresh leaf sample, then centrifuged at 12,000 rpm for 15 min. Thereafter, the upper phase was separated and kept at −20 °C for further steps (Bradford, 1976).

Bradford method (1976) was used to measure protein concentration. To prepare the reagent, 0.05 g of Kumasi Bryant Blue G250 dissolved in 50 ml of 96 % ethanol and then 25 ml of 85 % phosphoric acid was added drop by drop to this solution. To measure the total soluble protein concentration in each sample, 50 µl of the extracted sample was mixed with 2 ml of freshly prepared Coomassie Blue reagent. The absorbance of this solution was then recorded at a wavelength of 595 nanometers using a Bausch Lomb70 spectrophotometer.

2.6. Guaiacol peroxidase activity

POD activity was estimated based on Souri et al. (2018) proposed method. This method is based on guaiacol oxidation in the presence of H_2O_2. A volume of 100 µl of the supernatant was mixed with a reaction solution made up of 0.05 % guaiacol and 50 mM phosphate buffer at pH 7.0. The absorbance at 470 nm was measured over a period of 5 min.

2.7. Ascorbate peroxidase (APX) activity

For assessing ascorbate peroxidase (APX) activity, the method described by Chen and Asada (1989) was employed. This involved preparing a reaction buffer that included 50 mM phosphate buffer (pH 7.0), 10 mM ascorbic acid, 0.1 µM Na_2EDTA, and 1 mM H_2O_2, which was then mixed with 50 µl of the plant extract. The absorbance changes were then measured at 290 nm.

2.8. Catalase (CAT) activity

The method developed by Aebi (1984) was utilized to assess catalase (CAT) activity. A volume of 50 µl of the enzyme extract was combined with 1 ml of a reaction buffer containing 50 mM potassium phosphate (pH 7.0) and 15 mmol of H_2O_2. The activity was evaluated by monitoring the decrease in absorbance at 240 nm over a duration of 1 min.

2.9. Proline determination

For proline determination, the method described by Bates (1973) was employed. A sample of 0.1 g of fresh plant tissue was blended with 1.5 ml of 3 % sulfosalicylic acid, and the resulting mixture was centrifuged. Subsequently, 400 µl of the supernatant was combined with 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent, and the mixture was heated in a water bath for one hour. The reaction was then halted by placing it in an ice water bath. Following this, 4 ml of toluene was added, and the solution was stirred vigorously for about 20 s. The test tubes were then allowed to sit undisturbed until the two phases were fully separated. The absorbance of the pink phase in toluene was measured spectrophotometrically at 520 nm using a Tomas VTS 302.

2.10. Arsenic and Zinc determination

In this procedure, 0.25 *g* of powdered dry tissue were treated with 5 ml of concentrated nitric acid (HNO_3). After allowing the mixture to sit for 24 h, it was heated at 150 °C for 1 h. Following this, 2 ml of 30 % hydrogen peroxide was introduced to the solution, which was then heated at 150 °C for an additional 2 h. The digests were separated into two parts. The first part was diluted with distilled water and analyzed using atomic absorption spectrometer (AAS; Shimadzu, Tokyo, Japan) for Zn analyzes (Bankaji et al., 2023). The second diluted portion was combined with a solution containing 10 % hydrochloric acid (HCl), 5 % ascorbic acid, and 10 % potassium iodide (KI). The clear supernatant solution obtained was then analyzed using a Shimadzu AA-6200 atomic absorption spectrometer (HG-AAS; Shimadzu, Tokyo, Japan) (Karimi et al., 2024).

2.11. Translocation factor

The effect of ZnS nanocomposites on the bioavailability of As in solution culture was examined by calculating the translocation factor (Karimi et al., 2010):

Translocation factor (TF) = Shoot As concentration (mg kg^{-1})/Root As concentration (mg kg^{-1})

2.12. Statistical analysis

Complete randomize design (CRD) with the factorial arrangement was used for designing and analyzing data with three replications. Data presented as the means of all replicase ± SD (standard deviation). Subsequently, meaningful dissimilarities among the treatments were determined by means of Duncan's test, with a 5 % probability level using SAS software (SAS Institute Inc., Cary, NC, USA).

3. Result

3.1. Characterization of ZnS nanocomposites

ZnS nanocomposites appeared in spherical shape with a size of ~10–30 nm (Fig. 1A). However, as can be seen on the scale of 200 nm, ZnS nanocomposites were aggregated in a chain-like structure of orange peel nanocomposite. At the scale of 2.0 µm, the chain-like structure of orange peel nanocomposite showed a uniform spherical shape with a size of ~300–500 nm. Based on previous reports, the SEM images demonstrated the presence of ZnS nanoparticles with agglomeration on the orange peel nanocomposite surface (Nakarmi et al., 2020).

3.2. FTIR analysis

The FTIR- spectra of ZnS nanocomposites which were determined in the range of 400 - 4000 cm^{-1} have been demonstrated in Fig. 1B The FTIR spectra show a wide peak at 3400–3600 cm^{-1} which is due to the presence of N–H and O–H stretching vibration (Liu et al., 2017). The weak peak observed in 2868 and 2935 cm^{-1} is related to the symmetric and antisymmetric vibration of CH group. The peaks in the range of 450–650 cm^{-1} were related to the stretching vibrations of ZnS (Liu et al., 2017). Four bands around 1570, 1395, 1290 and 1072 cm^{-1} were associated with C—C, C–N–C, C–OH and C–O–C bands which show the presence of carbon, nitrogen and oxygen functional groups on the orange peel nanocomposite surface (Atchudan et al., 2022).

3.3. XRD pattern

The XRD patterns of ZnS – orange peel nanocomposites have been shown in Fig. 1C. The broadening peaks at 28.51°, 47.54° and 56.40° can be indexed to the cubic ZnS that indicated the nanocrystalline nature of the ZnS samples (Nazerdeylami et al., 2011). Also, three sharp peaks

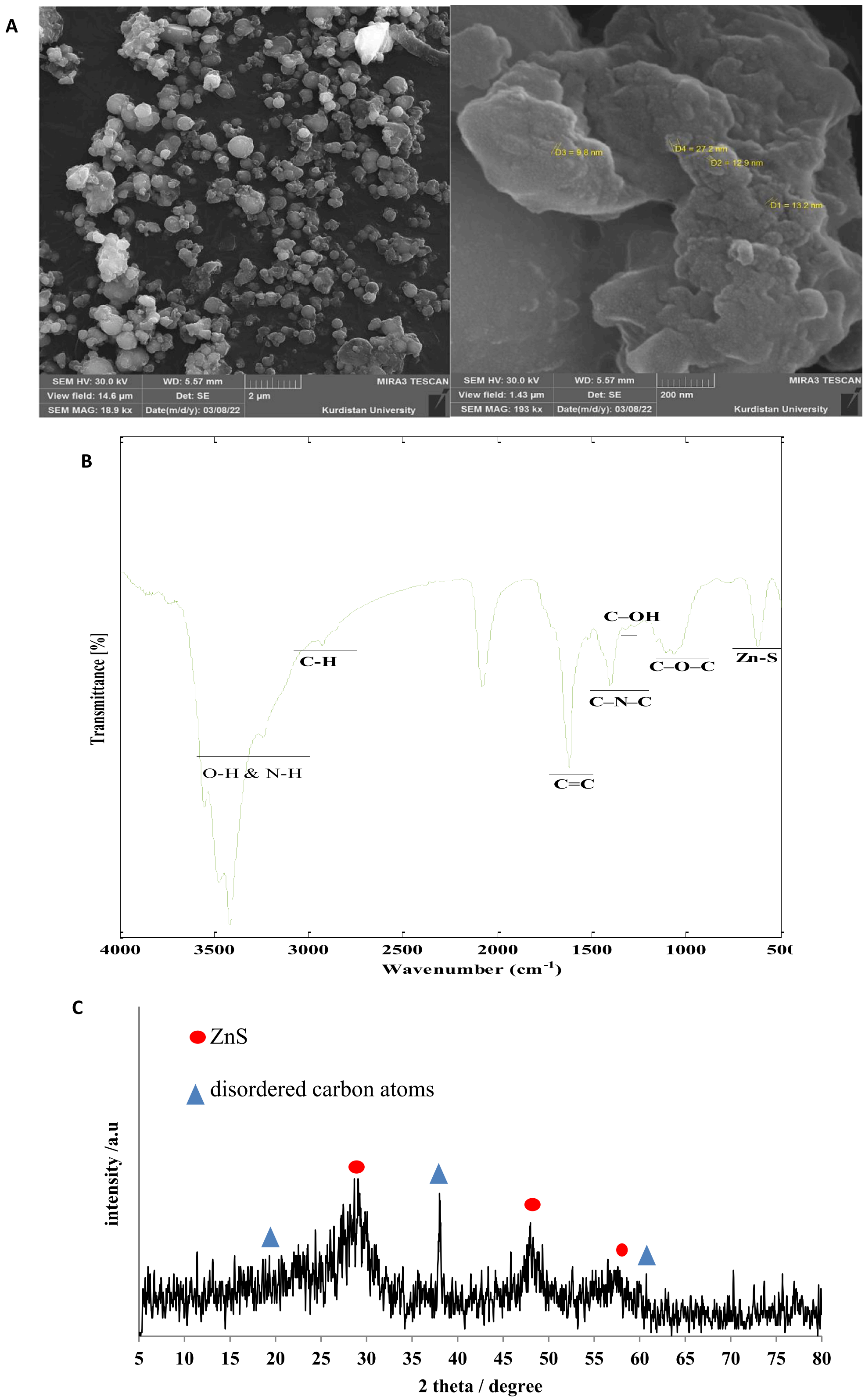

Fig. 1. The scanning electron microscope (SEM) images of ZnS nanocomposites (A); Fourier transform infrared spectroscopy (FT-IR) spectra of the ZnS – Orange peel nanocomposites (B); The X-ray diffraction (XRD) spectrum of the ZnS nanocomposites (C).

at 19.7°, 38.6° & 60.2° indicated the crystalline nature of disordered carbon atoms in the orange peel nanocomposite's structure (Rai et al., 2022).

3.4. The effect of Zinc Sulfide nanoparticle on wheat growth parameters during arsenic stress

Arsenic exposure led to a notable decrease ($p < 0.05$) in the fresh weight of both shoots and roots when compared to the control (Fig. 2). Plants treated with 75 mg/L and 150 mg/L of As showed a reduction in shoot fresh weight by 17.6 % and 46.1 %, respectively, while the fresh weight of roots decreased by 28.5 % and 60.1 % in comparison to their respective control plants. In contrast, the application of ZnS nanoparticles mitigated As toxicity in the parameters studied. For example, the treatment of 150 mg/L ZnS nanoparticles combined with 150 mg/L As resulted in a 10 % increase in shoot fresh weight compared to plants treated solely with 150 mg/L As. The highest growth rate was observed in plants treated with 75 mg/L ZnS nanoparticles (Fig. 2).

3.5. Foliar chlorophyll content

The results showed that the total chlorophyll contents of wheat reduced significantly under 75 (by 17.3 %) and/or 150 mg/L (by 32.1 %) As treatments, respectively over the control treatment, indicating the inhibitory effect of As on the chlorophyll content in plant leaves. The addition of ZnS nanocomposites demonstrated effectiveness in reducing the adverse impacts of As stress, leading to a notable increase in total chlorophyll content by 110 % and 73 % when compared to samples treated solely with 75 mg/L and 150 mg/L of As, respectively. Additionally, the independent application of 75 mg/L ZnS nanoparticles resulted in a significant rise in total chlorophyll content compared to the control group (Fig. 3). These results support the beneficial effects of ZnS nanoparticles on leaf chlorophyll content to a certain degree.

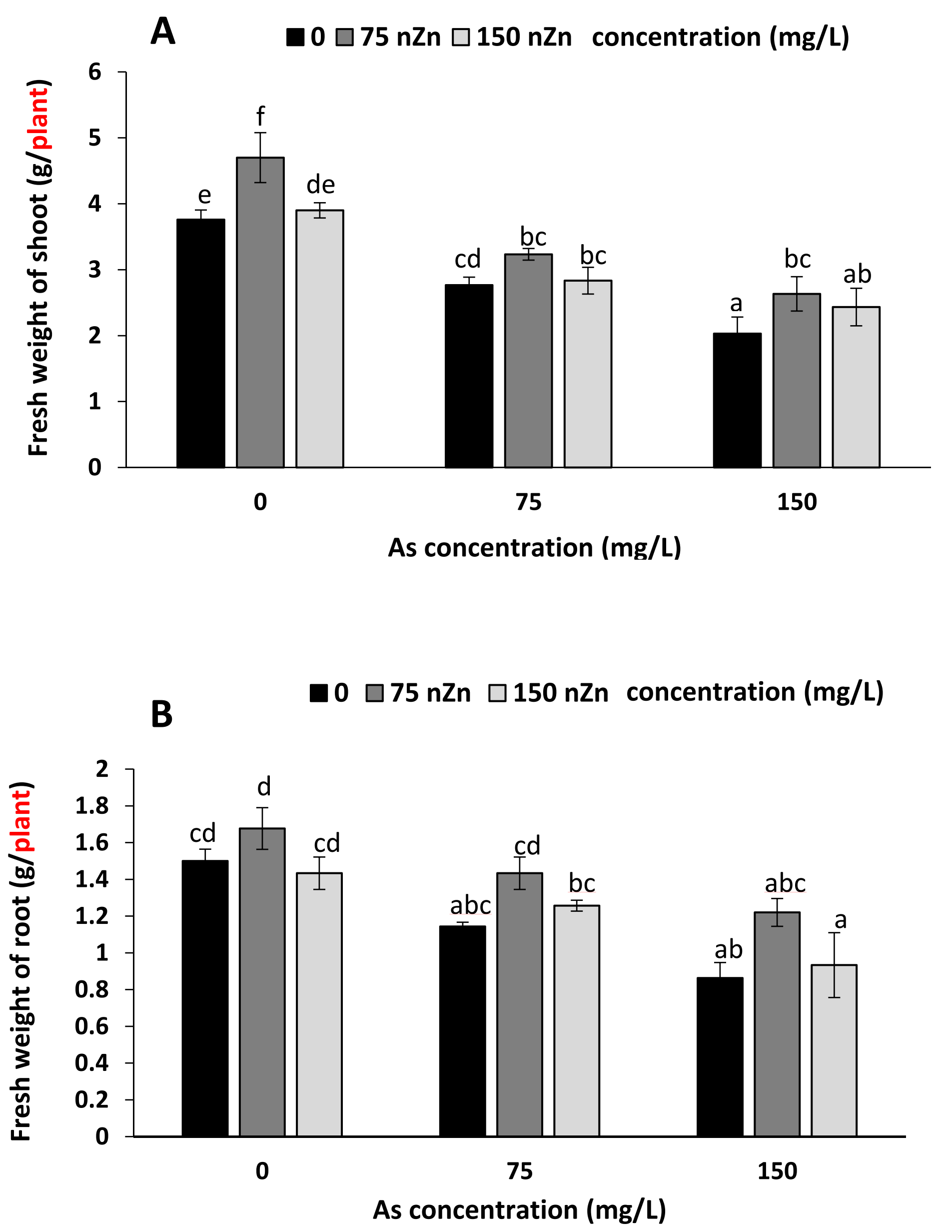

Fig. 2. The effects of 0, 75 and 150 mg/L ZnS nanoparticles on the biomass of the shoot (A) and root (B) of the wheat plants under 0, 75 and 150 mg/L sodium arsenate stress. Different letters indicate significant difference between the treatments at $p < 0.05$ "Duncan's test". The data are the means ± SE ($n = 3$).

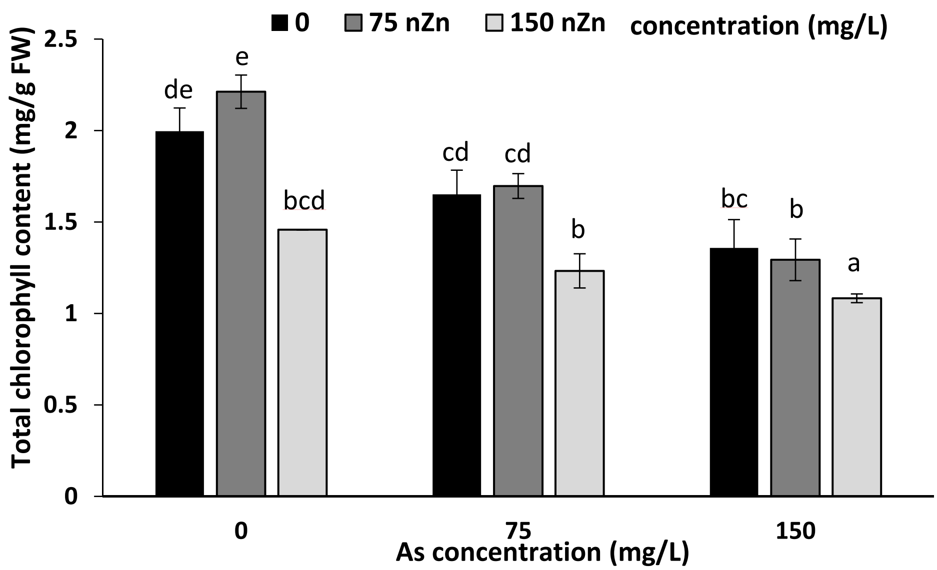

Fig. 3. The effects of 0, 75 and 150 mg/L ZnS nanoparticles on the total chlorophyll content of the shoot (A) and root (B) of the wheat plants under 0, 75 and 150 mg/L sodium arsenate stress. Different letters indicate significant difference between the treatments at $p < 0.05$ " Duncan's test ". The data are the means ± SE ($n = 3$).

3.6. Effect of ZnS nanoparticles on H_2O_2 and antioxidant enzyme activities under arsenic stress

In plant cells, H_2O_2 is introduced as an important indicator of oxidative stress. The As stress in both 75 and 150 mg/L levels significantly increased H_2O_2 content in root and shoot samples. In control plants, 75 and 150 mg/L ZnS nanocomposites had no significant effects on root and shoot H_2O_2 content, whereas ZnS nanocomposites reduced H_2O_2 content under As stress (Fig 4A). For instance, the H_2O_2 contents were enhanced by 2 and 2.5-fold in shoots and 1.5 and 2-fold in roots, respectively at sole 75 and 150 mg/L As treated plants compared to control samples. Compared to As treatment alone, 75 mg/L ZnS nanocomposites reduced root H_2O_2 content by 14.5 % and 21 %, and decreased H_2O_2 content in shoots by 39.6 % and 29.3 % in 75 and 150 mg/L As treated wheat seedlings, respectively (Fig. 4B).

To explore the role of ZnS nanocomposite in improving As-induced oxidative stress, the activity of POD, APX and CAT were analyzed in roots and shoots of wheat. Increasing As concentration in the medium from 75 to 150 mg/L significantly reduced the activity of POD in wheat shoots by 63 % and 42 %, respectively, compared to untreated treatments (Fig. 5A). Similarly, the reduction in POD activity of wheat roots was observed under As stress (Fig. 5B). However, shoot and root supplemented ZnS nanocomposites increased POD activity, in each As exposed level, compared to respective un supplemented treatments. For instance, in plant shoots treated with 75 mg/L As, the application of 75 mg/L and 150 mg/L ZnS nanocomposites enhanced POD activity 3.9 and 4.1-folds, respectively. Although, there was no ascertained trend in root POD activity under As × nano ZnS levels.

The activity of APX in both roots and shoots of plants treated with 75 and 150 mg/L As exhibited an increase compared to the control group (Fig. 5C and D). Specifically, APX activity in roots and shoots samples was elevated by 3.28 and 1.60-fold, respectively, at the higher As concentration (150 mg/L) compared to non-As-fed plants. For the As75+nZnS75 and As75+nZnS150 treatments, APX activity in roots increased by 45.7 % and 67.7 %, respectively, while in shoots, it rose by 18.7 % and 85.9 % when compared to the corresponding As treatments alone. Notably, the addition of 150 mg/L nano ZnS diminished APX activity in the As-treated plants (Fig. 5C and D). Regarding CAT activity, shoot samples demonstrated a rise to 1.98-fold with 75 mg/L As and 3-fold with 150 mg/L As, relative to the control, whereas root samples exhibited increases of 1.20 and 1.66-fold, respectively. The As × nano ZnS treatments further influenced CAT activity; for example, root samples showed a 29 % increase in CAT activity with the combination of As (150 mg/L) and nano ZnS (75 mg/L), while shoot samples experienced an 8 % decrease under the same treatment conditions compared to As treatments alone (Fig. 5E and F)

3.7. Effect of ZnS nanocomposites on proline and total soluble protein content under arsenic stress

The results indicated a substantial increase in proline levels corresponding to higher concentrations of As and ZnS nanocomposites. The combined application of ZnS nanocomposites with As resulted in enhanced the proline accumulation in both roots and shoots samples. Specifically, supplementation with 150 mg/L of ZnS nanocomposites markedly elevated proline levels in As-treated plants compared to treatment with 150 mg/L of As alone; however, proline content exhibited minimal variation at 75 mg/L of ZnS nanoparticles when compared to the control group (Fig. 6). Furthermore, the total soluble protein content showed a significant enhancement due to As application, with increases of 76 % and 75 % observed in shoots and roots, respectively, of plants treated with 150 mg/L of As compared to the control (Fig. 7).

3.8. Effect of ZnS nanocomposites on As and Zn levels under arsenic stress

According to the results obtained, the As concentrations in the wheat roots and shoots were enhanced in a concentration dependent manner (Fig 8A and B). Nano ZnS application (150 mg/L) reduced the As concentration in wheat roots by 34 % and shoots by 18 % compared to the respective sole 150 mg/L As treated plants. The TF of As and Zn under Zn-S nanocomposite was calculated and results have been presented in Table 1. The results showed that both levels of ZnS (75 and 150 mg/L) significantly decreased the As TF values, which was more pronounced in 75 mg/L level whereas, exogenous ZnS nanoparticle application enhanced root to shoot Zn TF mainly in 150 mg/L As treatments. The supplementation of 150 mg/L ZnS reduced the TF of As from 0.67 To 0.28 in the 150-mg/L-As-stressed plants (Fig 8A and B).

The concentration of Zn in the roots of plants subjected to As stress at 150 mg/L decreased by 34.6 % relative to control plants. Additionally,

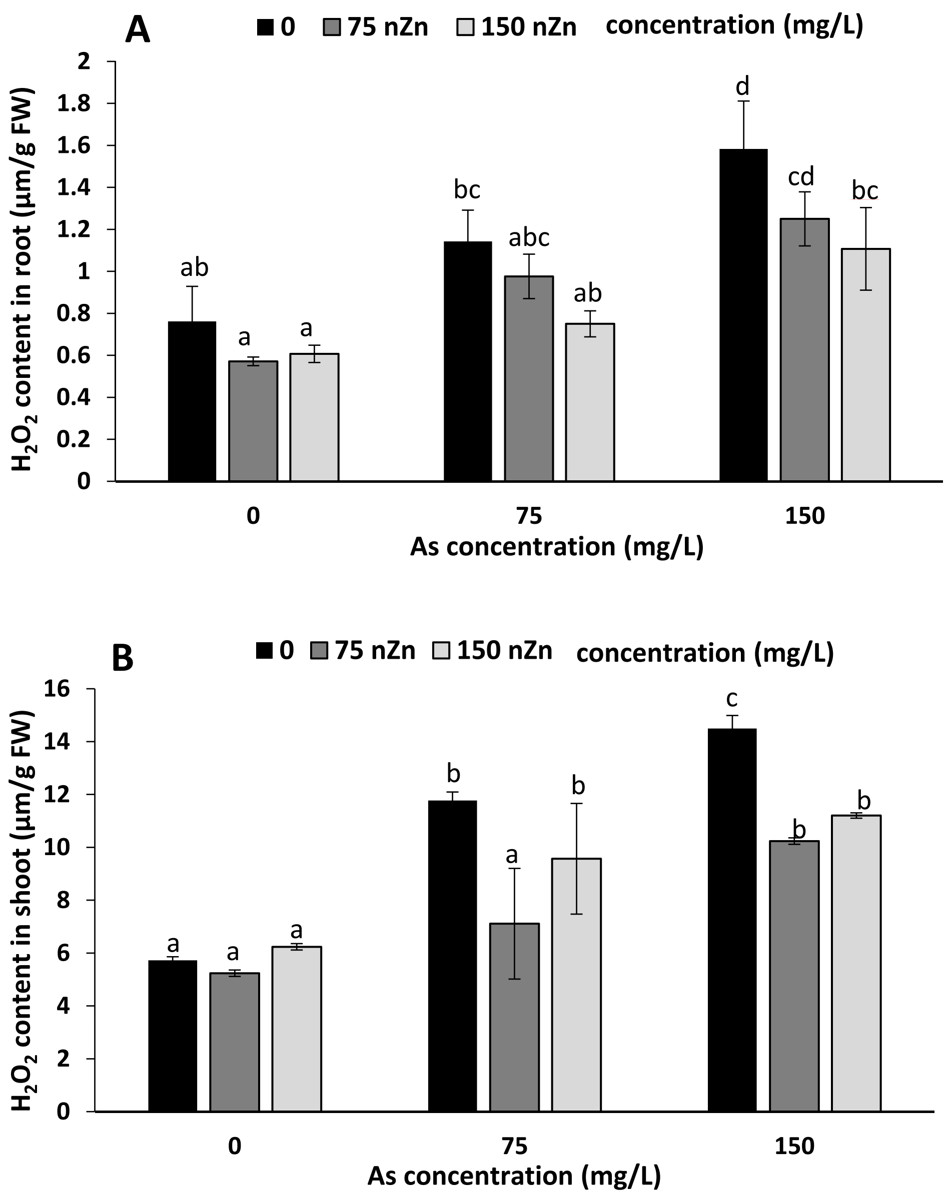

Fig. 4. The effects of 0, 75 and 150 mg/L ZnS nanoparticles on the H_2O_2 content of the shoot (A) and root (B) of the wheat plants under 0, 75 and 150 mg/L sodium arsenate stress. Different letters indicate significant difference between the treatments at $p < 0.05$ " Duncan's test ". The data are the means ± SE ($n = 3$).

shoot samples exhibited a notable reduction in Zn concentration when treated with 150 mg/L of As compared to the control group. Conversely, the application of ZnS nanocomposites, both independently and in conjunction with As treatments, significantly enhanced the Zn content in both roots and shoots tissues in comparison to control plants (Fig 8C and D).

4. Discussion

Arsenic toxicity is a global health and environmental crisis, posing significant challenges in the 21st century. As a potent carcinogen, arsenic induces cancer in various human organs and severely impacts plants, disrupting ecosystems and food chains. Its persistence in the environment and easy transfer from soil to humans via crops further endangers public health. Innovative solutions are urgently needed to mitigate exposure to this toxic metalloid. Conventional methods for managing As-induced phytotoxicity and reducing its bioaccumulation in plants face limitations, including high costs, inefficiency, and incomplete removal (Roy et al., 2025). Recent advances highlight the potential of nanoparticles as a promising alternative due to their high efficiency and selectivity in adsorbing As from soil, thereby alleviating phytotoxicity (Rahimzadehand Ghassemi-Golezani, 2025). This study explores the progress, challenges, and future prospects of ZnS nanocomposite in reducing As accumulation in plants, enhancing food security.

Based on our observations, certain growth factors, including shoot and root length as well as fresh weight, along with chlorophyll content in wheat seedlings, were notably diminished in plants exposed to 75 and 150 mg/L of As, which resulted from increased oxidative stress. These results endorsed our previous finding (Mahdieh et al., 2013) and the findings of Tyagi et al. 2022, Shukla et al., 2022 and Boorboori et al. 2023 in As stressed wheat seedling. The reduced growth parameters of wheat affected by As can be due to inhibited metabolism through reaction with thiol groups of enzymes which lowered photosynthesis, enhanced oxidative stress, inhibited DNA biosynthesis and repair mechanisms, perturbed cell cycle, and restricted essential nutrients

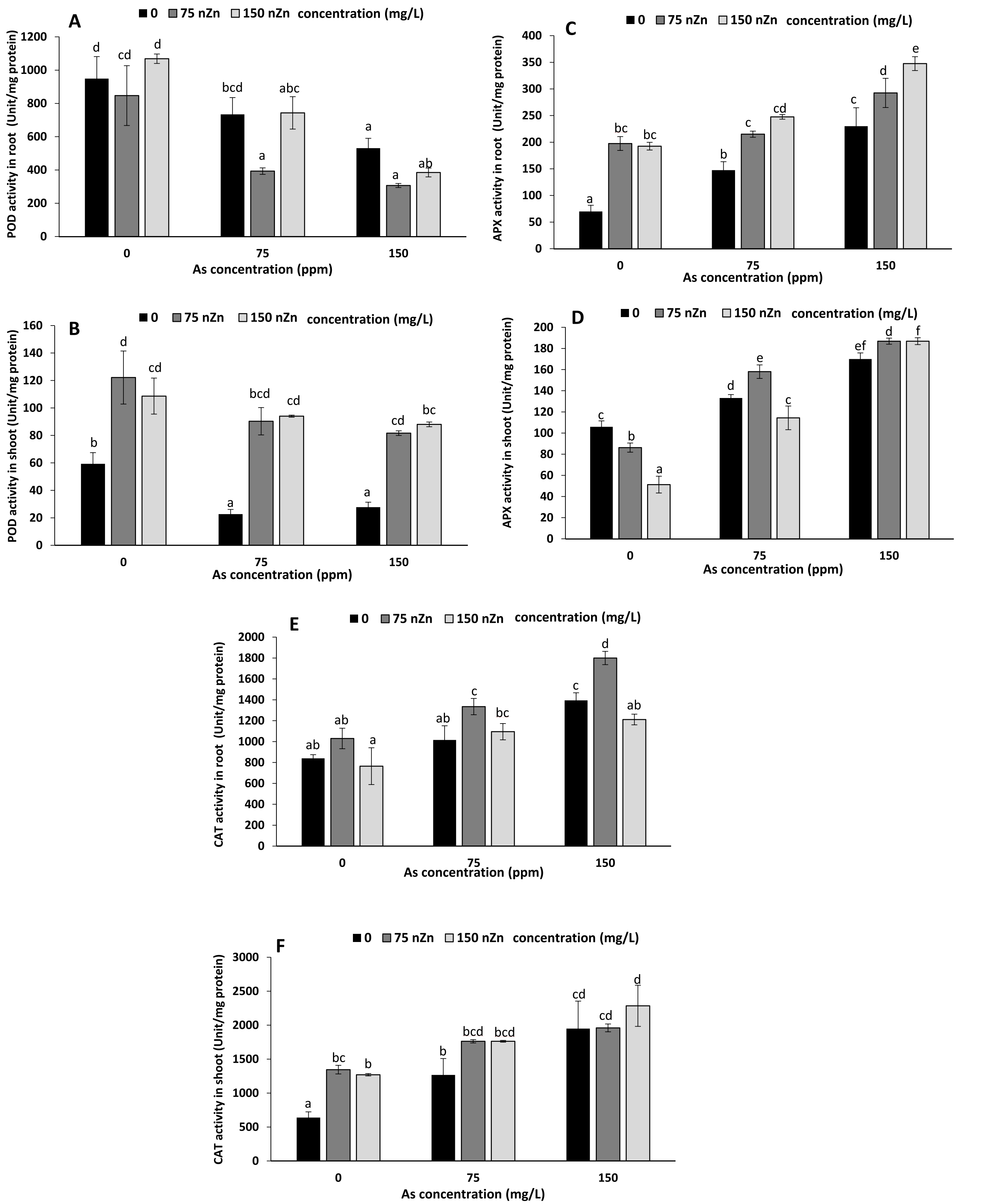

Fig. 5. The effects of 0, 75 and 150 mg/L ZnS nanoparticles on the POD activity of the root (A) and shoot (B); APX activity of the root (C) and shoot (D); CAT activity of the root (E) and shoot (F) of the wheat plants under 0, 75 and 150 mg/L sodium arsenate stress. Different letters indicate significant difference between the treatments at $p < 0.05$ "Duncan's test". The data are the means ± SE ($n = 3$).

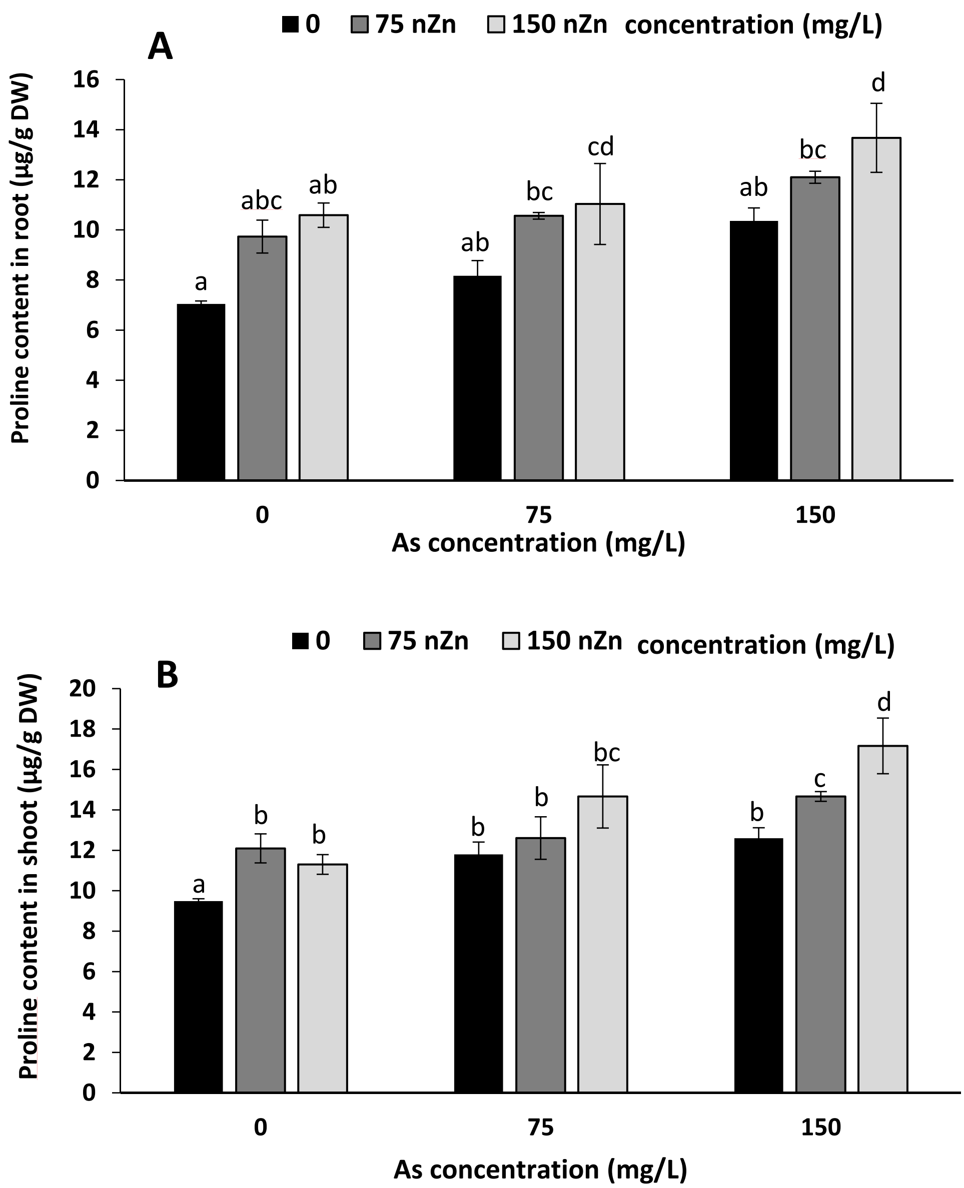

Fig. 6. The effects of 0, 75 and 150 mg/L ZnS nanoparticles on the proline content of the root (A) and shoot (B) of the wheat plants under 0, 75 and 150 mg/L sodium arsenate stress. Different letters indicate significant difference between the treatments at $p < 0.05$ "Duncan's test". The data are the means ± SE ($n = 3$).

uptake and assimilation by plants (Maghsoudi et al., 2020; Shukla et al., 2022; Boorboori et al. 2023; Saeed et al., 2024).

These results further found that the application of ZnS nanocomposites promoted the wheat growth parameters under As stress, especially at treatment level of 75 mg/L (Fig. 2). Many previous studies reported the ameliorative effects of Zn nanoparticles on rice (Yan et al., 2021), maize (Khan et al., 2022: Ramzan et al., 2024) and soybean (Ahmad et al., 2020) under As stress. It is postulated that Zn nanoparticles can enhance growth parameters through several mechanisms, including maintaining membrane stability and structural integrity, improving and restoring mineral absorption, decreasing oxidative stress caused by ROS, activating biochemical pathways related to biomass accumulation, increasing the production of photosynthetic pigments, and enhancing quantum yield, among others (Jalil et al., 2023). Furthermore, ZnS nanocomposites, as sulfur donor molecule, may play crucial roles in biosynthesis of some defensive metabolites e.g., cysteine, glutathione and phytochelatins. These metabolites are widely known for chelating and detoxifying As and then tolerate plant species against As toxicity (Shukla et al., 2022; Arianmehr et al., 2022).

It is well known that As toxicity can cause oxidative stress in wheat as indicated by the increase in H_2O_2 content and led to lipid peroxidation as an oxidative stress marker (Fig. 4). In this study, the supplementation of ZnS nanocomposites demonstrated a significant reduction in H_2O_2 accumulation, as illustrated in Fig. 4. These findings underscored the protective efficacy of ZnS nanocomposites in mitigating oxidative stress caused by As exposure. This result is in line with previous reports with different plant species (Liu et al., 2017; Shukla et al., 2022; Yuce et al., 2024). We proposed that ZnS nanoparticles application may reduce oxidative stress in wheat by enhancing antioxidant enzyme activity, antioxidant compounds production, modulating S containing metabolites biosynthesis and balancing mineral nutrition in As treated wheat.

It was observed that wheat develops higher antioxidant enzyme activity (POD, APX and CAT) when exposed to ZnS nanoparticles to combat As-induced oxidative damage. In the current study, As enhanced the activity levels of POD, APX and CAT, suggesting that enhanced activity in plants is a prerequisite of the strategy to overcome As toxicity

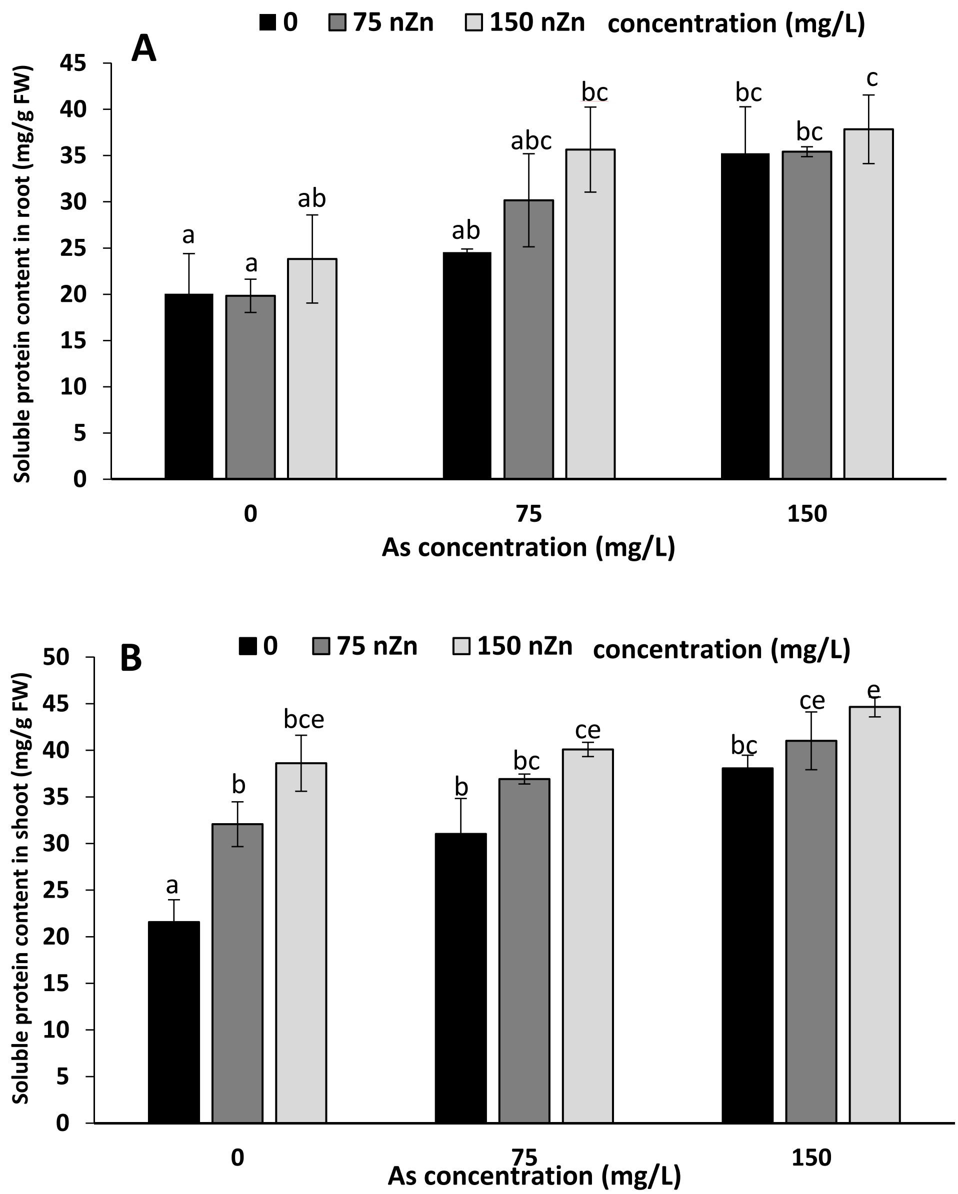

Fig. 7. The effects of 0, 75 and 150 mg/L ZnS nanoparticles on the total soluble protein content of the root (A) and shoot (B) of the wheat plants under 0, 75 and 150 mg/L sodium arsenate stress. Different letters indicate significant difference between the treatments at $p < 0.05$ "Duncan's test". The data are the means ± SE ($n = 3$).

(Fig. 5). The observed changes in antioxidant enzyme activities and the accumulation of oxidation products like H_2O_2 signify that the level of As-induced oxidative stress, as well as the consequent damage to cellular membranes and the detrimental impacts on plant growth, were notably reduced in seedlings treated with ZnS nanoparticles. This treatment resulted in enhanced enzyme activities and elevated levels of antioxidants when compared to seedlings exposed solely to As. This could be related to the shielding impact of ZnS nanocomposites against heavy metal pressure by means of antioxidant enzymes including POD, APX, and CAT, and may be associated with the assisting function of ZnS nanocomposites in enhancing nutrient levels as a co-factor for numerous antioxidative enzymes.

Some essential osmolytes like proline have a main role in alleviating damage effects by stress in plants. In this investigation, As significantly augmented the proline levels in treated wheat plants, suggesting a potential natural defense mechanism under these conditions (Fig. 6). Prior research has similarly demonstrated the impact of As on proline accumulation in rice (Bidi et al., 2021), soybean (Ahmad et al., 2020), and *I. cappadocica* (Karimi et al., 2024). Enhancing proline accumulation by wheat is the biochemical response of this crop to protect its metabolic pathways, scavenge ROS, activate different enzymes and maintain redox hemostasis in the presence of As stress (Sheteiwy et al., 2017; Ahmad et al., 2020). To enhance these natural defense responses in plants under stress, supplementation of some green synthesized nanoparticles can be very helpful for boosting the antioxidant system. Therefore, as we found in this study, exogenous supplementation of biosynthesized ZnS nanocomposites can help wheat to alleviate As stress.

An elevated concentration of ZnS nanoparticles led to a decrease in As levels in both the roots and shoots of wheat (Table 1). Therefore, the positive effects of ZnS nanocomposites in mitigating As toxicity can be ascribed to a marked reduction in As accumulation within both the shoots and roots of wheat plants that were co-exposed to arsenic and ZnS nanocomposites. This reduction stands in contrast to the higher levels of As observed in plants subjected solely to As exposure (Yuan et al., 2021; Khepar et al., 2024). The inhibitory effect of ZnS nanocomposites on As accumulation in wheat may be attributed to the elevated concentrations

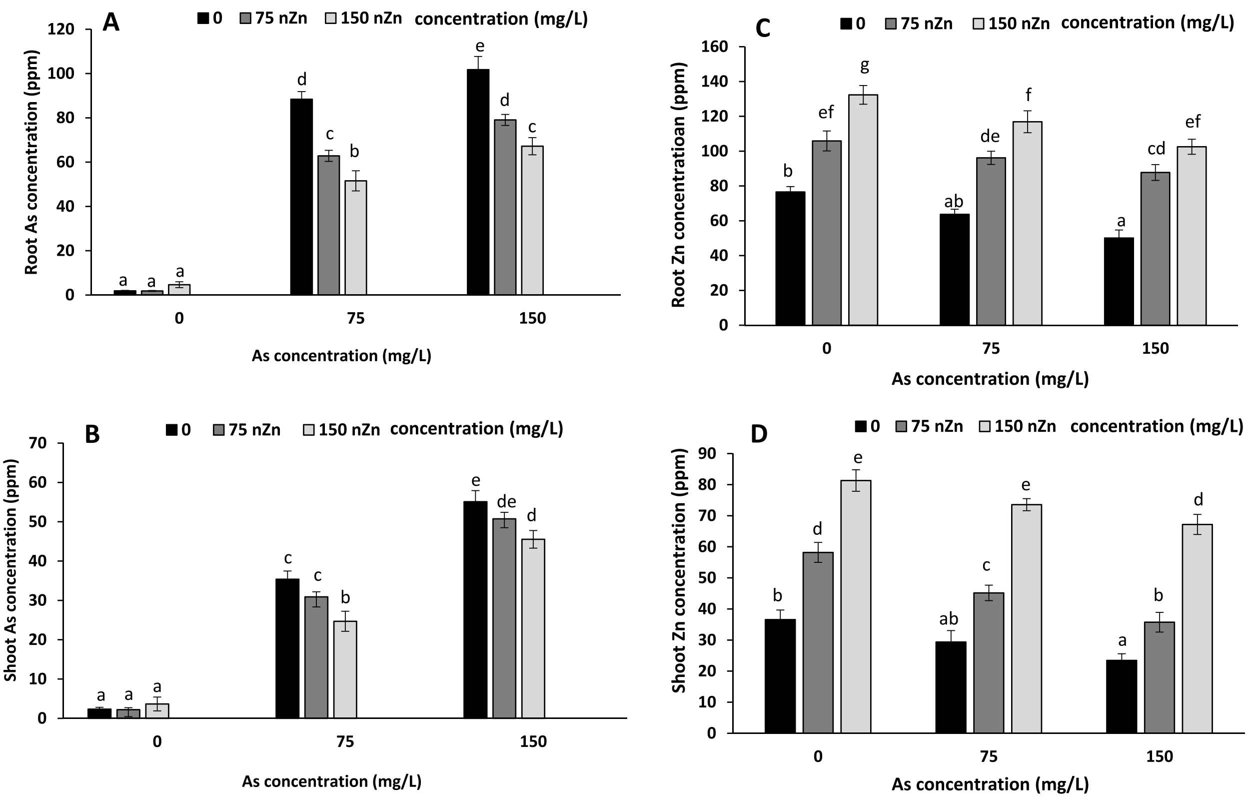

Fig. 8. The effects of 0, 75 and 150 mg/L ZnS nanoparticles on the As concentration of the root (A) and shoot (B); the Zn concentration of the root (C) and shoot (D) of the wheat plants under 0, 75 and 150 mg/L sodium arsenate stress. Different letters indicate significant difference between the treatments at $p < 0.05$ "Duncan's test". The data are the means ± SE ($n = 3$).

Table 1
The influence of Zn-S nanoparticles (nZn) on root to shoot arsenic and zinc translocation factor of *Triticum aestivum* under arsenic (As) stress.

nZn (ppm)	As translocation factor		
	0 ppm As (Control)	75 ppm As	150 ppm As
0	0.87±0.01a	0.40±0.02d	0.541±.04bc
75	0.82±0.05a	0.49±0.03c	0.64±0.07b
150	0.78±0.08a	0.47±0.04c	0.67±0.06b
Zn translocation factor			
0	0/47±0.05c	0/46±0.06cd	0/47±0.09c
75	0/57±03ab	0/46±0.09c	0/63±05a
150	0/61±16a	0/40±0.08d	0/62±0.09a

The values are means ± standard error of three independent replications ($n = 3$). The means sharing the same letters, for a parameter, don't differ significantly at $p < 0.05$ according to the Duncan's multiple tests.

of Zn and S in the nutrient solution, which can reduce As levels through the robust adsorption of ZnS nanoparticles (Yan et al., 2021). Additionally, Zn nanocomposites have the potential to interact with ionic As, resulting in the formation of relatively insoluble arsenic sulfide (AsS) compounds (Gupta et al., 2021). Furthermore, decreasing As TF in ZnS treated nanocomposites showed that As translocating to the shoots was suppressed, thus lowering As accumulation in the shoots. It was plausible that the accumulation of Zn enhances the shoot growth resulting in a dilution of As concentration within the shoots due to the corresponding increase in biomass (de Bang et al., 2021). The essential nutrients have crucial roles in enhancing plant adaptations against abiotic stress including heavy metal stress (de Bang et al., 2021). We observed that As decreased Zn uptake in wheat plants. This was probably related to the toxic effect of As on root nutrient uptake and Zn homeostasis (Liu and Li, 2022). The reduction in levels micronutrients such as Zn in crops treated with As will affect plant physiology by modifying enzymatic activity, stomatal conductance and osmotic adjustment (Vezza et al., 2018; Jalil et al., 2023). ZnS nanoparticles play a crucial role in promoting root growth by releasing Zn, an essential element in the synthesis and accumulation of auxin, specifically indole-3-acetic acid as vital phytohormone for root growth, cell division and expansion (Gao et al., 2024). Singh et al. (2024) indicated that the application of Zn enhanced this structural integrity of cellular membranes, facilitating the uptake of both micro- and macronutrients, particularly under stress conditions. Furthermore, zinc supplementation has beneficial effects in plants by the enhanced absorption of critical nutrients such as potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), and phosphorus (P). This restoration is vital for maintaining the structural and functional integrity of various organelles, including chloroplasts and mitochondria (Ramzan et al., 2024; Zeeshan et al., 2024).

5. Conclusion

In the overall view of this study, we have found that ZnS nanocomposites may become an effective substance to alleviate As toxicity and acquire sustainable crop production in As polluted medium. Generally, plants grown in an As-amended hydroponic medium exhibited signs of toxicity, as evidenced by decreased growth, reduced levels of chlorophyll and carotenoids, and a decline in the accumulation of essential nutrients. However, the ZnS nanocomposites alleviated As toxicity by enhancing essential nutrient content, restricting As translocation from roots to shoots, decreasing ROS production and modulating plants' antioxidant system in wheat. The findings of this study indicated that ZnS nanocomposites alleviated the As stress and enhanced the growth in wheat subjected to As stress. However, further molecular and proteomic investigations are necessary to draw definitive conclusions from the current research results. Furthermore, despite promising results, further research is needed to understand ZnS nanocomposites interactions with cellular biomolecules, optimize dosages to prevent its toxicity, and assess long-term ecological impacts. Standardizing nanoparticles concentrations and application methods is crucial for sustainable agriculture while minimizing environmental risks. Addressing these knowledge gaps will ensure safer food production and improved crop quality.

CRediT authorship contribution statement

Naser Karimi: Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Conceptualization. **Hadis Pakdel:** Writing – original draft, Software, Methodology, Investigation. **Zahra Souri:** Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Leila Norouzi:** Methodology, Formal analysis, Data curation. **Muhammad Rizwan:** Writing – review & editing, Visualization, Data curation. **Jean Wan Hong Yong:** Writing – review & editing, Validation, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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Data availability

Data will be made available on request.

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