

Role of Zinc Oxide Particle Size in Modulating Growth and Cadmium Toxicity in *Allium sativum* L.: A Comparative Study of Quantum Dots, Nanoparticles, and Bulk Forms

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Abstract

Cadmium (Cd) adversely affects plant growth, necessitating the development of effective strategies to mitigate its toxicity for sustainable agriculture. This study evaluates the effectiveness of zinc oxide (ZnO) quantum dots (QDs), nanoparticles (NPs), and bulk particles (BPs) in promoting the growth of *Allium sativum* L. and alleviating Cd stress. The synthesised ZnO particles were characterised using FE-SEM, FTIR, XRD, Raman and fluorescence spectroscopy to confirm their structural and optical properties. Treatment with ZnO particles significantly improved overall growth and photosynthetic parameters, and reduced Cd bioaccumulation and oxidative stress. QDs demonstrated the highest efficacy compared to NPs and BPs. Correlations among the various physiological and biochemical parameters were established through mathematical modelling, encompassing clustered heatmap analysis, the Pearson correlation mapping, and principal component analysis. Additionally, the environmental risk quotient (RQ) assessment of Cd indicated an enhanced ecological safety following the incorporation of ZnO particles. The findings clearly demonstrate that the size of the particles has a significant impact on the efficacy of ZnO. In summary, this research underscores the crucial role of ZnO particle dimensions in promoting plant growth and mitigating Cd toxicity. Furthermore, it emphasises the promise of ZnO QDs as a viable nanoscale strategy for sustainable agricultural management.

Keywords: Zinc oxide; Growth promotion; Heavy metal; Oxidative stress; Food crop; Protective material

58 **List of Abbreviations**

59

60 AF – Assessment factor

61 CAT- Catalase

62 ENM – Engineered nanomaterials

63 ETR – Electron transport rate

64 FESEM – Field emission scanning electron microscopy

65 FTIR – Fourier transform Infrared spectroscopy

66 MDA – Malondialdehyde

67 MEC - Measured environmental concentrations

68 PCA – Principal component analysis

69 PNEC - Predicted no-effect concentration

70 PS II - Photosystem II

71 RQ – Risk quotient

72 ROS – Reactive oxygen species

73 SOD – Superoxide dismutase

74 XRD – X-ray diffraction spectroscopy

75 ZnO BPs - Zinc oxide bulk particles

76 ZnO NPs - Zinc oxide nanoparticles

77 ZnO QDs – Zinc oxide quantum dots

1. Introduction

Anthropogenic activities such as smelting, mining, fertiliser production, plastic and dye manufacturing, have made cadmium (Cd) an environmentally and agronomically hazardous pollutant [1]. According to the report released by the Agency for Toxic Substances and Disease Registry (ATSDR), Cd is ranked among the top 10 hazardous substances [2]. High Cd levels in agricultural soil and groundwater are contributed mainly by phosphate fertiliser, in which Cd is present as an impurity. Additionally, the use of sewage sludge and biomass ash as fertilisers contributed significantly to raising Cd levels up to 90 and 30 mg/kg in agricultural soils, respectively [3, 4]. Owing to its high mobility and solubility in water, Cd can be easily absorbed by plants and can be accumulated in various aerial parts. Cd is known to be toxic even at low concentrations and affects plants by disrupting photosynthesis, retarding growth, and reducing the uptake of essential nutrients [5]. It was reported that Cd supplied at 50 mg/kg in soil significantly reduced plant height and biomass in 11 out of 20 alfalfa cultivars being tested [6]. In another study conducted by Rizwan et al. (2008), Cd at 50 and 100 μ M reduced photosynthetic pigment levels, net photosynthetic rate and gas exchange efficiency [7]. Additionally, it is well-documented that Cd can induce oxidative stress in plants by promoting the generation of reactive oxygen species (ROS), as supported by the findings presented by Tao et al. (2013). They demonstrated that Cd exposure (20 – 80 μ M) increased malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity in leaves and roots of kenaf seedlings (*Hibiscus cannabinus* L.) [9]. Similarly, Jawad Hassan et al. (2020) reported that Cd exposure induced oxidative stress and altered the activity of antioxidant enzymes in sorghum. Nevertheless, Cd contamination in agricultural soils not only affects plants but also poses a serious health risk to humans through the food chain. Thus, addressing Cd pollution with robust and sustainable strategies is crucial for safeguarding the terrestrial ecosystem.

Engineered nanomaterials (ENMs) have garnered significant interest due to their effective integration into agricultural practices, which enhances plant productivity and facilitates the efficient delivery of essential nutrients to crops [11]. Among the various nanomaterials, zinc oxide nanoparticles (ZnO NPs) are extensively investigated due to their unique properties and potential applications. ZnO nanoforms rank as the third most extensively utilised metal-based nanomaterials, with global annual production estimates ranging from 550 to 33,400 tons [12, 13]. The anticipated environmental levels of ZnO NPs are estimated to range between 76 and 760 µg/L in aquatic environments and from 3.1 to 31 mg/kg in terrestrial soils [14]. The application of ZnO NPs to promote plant growth and productivity has a long-standing history in agricultural practices. For example, treating wheat seeds with a concentration of 10 mg/L of ZnO NPs has been shown to enhance the vigour index and improve germination rate. Seed priming with ZnO NPs also enhanced the level of photosynthetic pigments [15]. In another investigation, the foliar application of ZnO NPs at 50 and 100 mg/L improved the growth of both tomato and *Sophora alopecuroides* plants [16]. Similarly, the application of 1.5 mg/L of ZnO NPs to chickpea plants resulted in improved biomass and shoot dry weight, which can be attributed to low ROS generation resulting in lesser membrane damage [17]. Alongside NPs, bulk particles (BPs) have also been used to improve plant health, but their effect on plant growth is often insignificant compared to nano-scale forms [18, 19]. Although NPs and BPs have numerous applications in agriculture, their usage is limited by their prolonged sonication duration, low adsorption capacity, and low solubility in water, making quantum dots (QDs) a more favourable alternative for improving plant productivity [20–22]. In addition to traditional nano-ZnO, there has recently been enhanced emphasis on ZnO QDs (2-20 nm), attributed to their diverse functional groups, improved biocompatibility, and enhanced solubility in water. Their utilisation spans multiple biological domains, including biomedical applications, sensor

technology, and agriculture, making their entry into agricultural ecosystems unavoidable [23]. Studies have indicated that ZnO QDs demonstrate a superior capacity to stimulate plant growth and enhance productivity relative to ZnO NPs. For example, the foliar spraying of 50 mg/L ZnO QDs on pumpkin increased its biomass by 24.6% and also enhanced the levels of photosynthetic pigments and nutrients [24]. Similarly, Sun et al. (2023) reported that the foliar application of 50 mg/L of ZnO QDs increased the fresh weight, plant height, and protein levels in tomatoes. In another study, it was demonstrated that the foliar spraying of ZnO QDs on lettuce in the range of 50-500 mg/L increased the plant biomass and chlorophyll content at lower concentrations (50-100 mg/L), while adversely affecting the plant growth by inducing oxidative stress at higher concentrations (200-500 mg/L) [23]. Recent research has demonstrated that the utilisation of ZnO NPs is effective in mitigating heavy metal stress, particularly in reducing Cd toxicity and regulating its absorption in plants. The application of ZnO NPs at 100 mg/L via foliar treatment alleviated Cd stress in wheat by enhancing the activity of leaf antioxidant enzymes. Similarly, the foliar spraying of ZnO NPs at 50 mg/L alleviated Cd stress by regulating photosynthetic efficiency and antioxidant defence mechanisms in rice and tomato. Although numerous studies have investigated the role of ZnO NPs in mitigating Cd stress, there remains a notable research gap regarding the application of ZnO QDs for addressing Cd stress, which requires further exploration. In addition, the evaluation of size-dependent effects of ZnO particles on the growth and physiology of Cd-challenged plants requires critical investigations to gain insights into how particle size could significantly influence the stability, accumulation, and their underlying effects.

In this research, *Allium sativum* L. (garlic) serves as a model plant to examine the influence of ZnO particles of varying sizes (QDs, NPs, and BPs) on plant growth and their potential role in mitigating Cd stress. *A. sativum* roots were exposed to ZnO particles ranging from 1.25-20

150 mg/L concentrations. Among these, the 10 mg/L concentration was found to be optimal and
151 was subsequently used for alleviating Cd stress. Garlic is a high-value food crop with a rich
152 culinary and medicinal heritage. Due to its short chromosome number ($2n = 16$), easy
153 propagation, high sensitivity, and ability to absorb and accumulate heavy metals from the
154 environment, *A. sativum* serves as an excellent bioindicator to understand the dynamics of
155 heavy metal-induced damage in food crops. This understanding is crucial for developing
156 innovative strategies to alleviate abiotic stress, ultimately ensuring food safety and quality.
157 Following an extensive review of the existing literature, the authors contend that this study
158 represents the first investigation into the size-dependent impacts of ZnO particles (QDs, NPs
159 and BPs) on a food crop (garlic) at environmentally relevant concentrations. This research
160 addresses a critical gap in the literature by elucidating the distinct biological responses elicited
161 by various forms of ZnO particles in an edible plant. The current study examines these
162 responses under both normal growth and Cd-stress conditions, thereby providing insights into
163 nano-plant interactions to improve nutrient delivery and food safety. The primary focus of this
164 research involved, i) evaluating the individual effects of QDs, NPs, and BPs on various growth
165 and oxidative stress parameters of *A. sativum* and analysing the effect of leached Zn^{2+} on the
166 major growth and stress parameters of *A. sativum*, ii) evaluating the role of the differently-sized
167 ZnO particles (QDs, NPs, and BPs) in mitigation of Cd stress, iii) examining the accumulation
168 of Zn and Cd in *A. sativum* roots, iv) evaluating the environmental risk associated with Cd,
169 both individually and in the presence of ZnO particles. Variations in plant growth and
170 physiological responses were evaluated using several biological indicators, including cell
171 viability, levels of oxidative damage, photosynthetic performance, and activity of antioxidant
172 enzymes. This research presents an innovative and sustainable strategy to mitigate Cd stress,

thereby preserving food quality and protecting human health while ensuring a seamless, clean continuum from agricultural production to consumption.

2. Materials and methods

2.1 Chemicals used

Zinc oxide bulk powder ($< 5 \mu\text{m}$ particle size) was procured from Sigma-Aldrich, and $\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ was purchased from SRL. The treatments involving CdSO_4 will be referred to as Cd hereafter. Details of other chemicals and solvents used in this study are provided in Supplementary Information (Methods S1).

2.2 Synthesis and Characterisation of ZnO particles

2.2.1 Synthesis of ZnO QDs and NPs

The protocol for the synthesis of ZnO QDs and NPs was adopted from Saber et al. (2025) and Al-luhaibi and Sendi. (2022). Details of the synthesis methods are provided in Supplementary Information (Methods S2).

2.2.2 Physico-chemical characterisation of ZnO particles (in the presence and absence of Cd)

The ZnO particles (QDs, NPs, and BPs) were characterised by FE-SEM, FTIR, XRD, Raman and fluorescence spectroscopy. Furthermore, zeta potential and contact angle measurements were performed using a zeta analyser and a goniometer, respectively, by dispersing the samples in Hoagland nutrient solution. The detailed description of various characterisation techniques is provided in the Supplementary Information (Methods S3).

2.3 Stock preparation and exposure concentrations

A stock suspension of 100 mg/L ZnO particles (QDs, NPs, and BPs) dispersed in Milli-Q water was prepared by ultrasonication for 20 min, and a 200 mg/L stock suspension of Cd was prepared. For the pristine studies, the working concentrations of QDs, NPs, and BPs were fixed

at 1.25, 2.5, 5, 10 and 20 mg/L, which lie within the environmentally relevant concentration (ERC) of Zn nanoforms [14]. To evaluate the EC₅₀ value for Cd, concentrations of 0.5, 1, 2, 4, 8, 16, and 32 mg/L were employed, corresponding to the available Cd concentrations in soil [28]. Based on the EC₅₀ data, highly toxic concentrations (10 and 20 mg/L) of Cd were chosen for further studies involving the binary mixtures of Cd and ZnO particles. An optimal dose of 10 mg/L of QDs, NPs, and BPs was maintained throughout the binary exposure experiments, pertaining to the significant stimulatory effect observed. Therefore, in this study, any reference to the concentrations of QDs, NPs, and BPs in the binary exposure experiments will specifically pertain to a concentration of 10 mg/L.

Garlic cloves (*Allium sativum* L.) germinated in half-strength Hoagland nutrient medium were used for the bioassays. All the assays were performed after 120 h of interaction in Hoagland nutrient medium supplemented with ZnO particles (QDs, NPs, and BPs) and their binary mixtures with Cd. During the interaction period, the plants were maintained at 25 ± 2 °C with a 16:8 h light:dark photoperiod, and the set of cloves grown in Hoagland nutrient medium served as the control group [29]. Further, ZnSO₄·7H₂O (1.23 × 10⁻⁴ mol/L) served as the ionic control which is equivalent to 10 mg/L of Zn on particle mass basis. The detailed composition of the Hoagland nutrient medium is given in the Supplementary Information (Table S1).

2.4 Determination of EC₅₀, cytotoxicity, and accumulation of Zn and Cd

The cell viability of *A. sativum* root cells exposed to pristine QDs, NPs, and BPs and their mixtures with Cd was determined by the Evans blue staining method [30]. The details of the adapted procedure are outlined in the Supplementary Information (Methods S4). The EC₅₀ of Cd and its mixtures with QDs, NPs, and BPs was determined by testing several concentrations of Cd ranging from 0.5 to 32 mg/L in combination with 10 mg/L of ZnO particles. Additionally, the effect of leached Zn²⁺ on cell viability was assessed at 10 mg/L.

To quantify the uptake of Zn and Cd in *A. sativum* roots, the protocol adapted from Rossi et al. (2017) was followed [32]. More details regarding the procedure are provided in the Supplementary Information (Methods S5).

2.5 Determination of mitotic index

The protocol followed by Mangalampalli et al. (2018) was adopted to determine the mitotic index in the actively dividing cells of *A. sativum* using the acetocarmine stain. Regarding the procedure, details are provided in the Supplementary Information (Methods S6).

2.6 Root length measurement

After 120 hours of interaction, the changes in the root length of *A. sativum* exposed to QDs, NPs, and BPs and their mixtures with Cd were evaluated by measuring the root lengths from each replicate, and the values were then compared with those of the control [34].

2.7 Determination of changes in root morphology

Roots exposed to pristine ZnO particles and their mixtures with Cd were carefully excised at the tips, measuring 1-2 cm and were subjected to vacuum oven drying at 60 °C for 48 h. Following this, the roots were then coated with gold and analysed for the changes in their morphology using FE-SEM (Thermo Fisher FEI Quanta 250 FEG) coupled with Energy Dispersive X-ray spectrometer (EDS) [35].

2.8 Visualisation of suberin deposition through fluorescence microscopy

The changes in suberin deposition lining the Casparian strip are visualised using Nile red staining, following the procedure described by Waduware et al. (2008). The details of the procedure followed are briefed in the Supplementary Information (Methods S7).

2.9 Evaluation of oxidative stress markers

The protocols outlined by Giri and Mukherjee. (2022) and Kumar et al. (2023) were followed to determine the total ROS and the degree of lipid peroxidation. The details of the procedure are provided in the Supplementary Information (Methods S8).

Evaluation of antioxidant enzyme activity

Superoxide dismutase (SOD) enzyme activity was assessed using the protocol described by Aebi. (1974), and catalase (CAT) enzyme activity was determined by the protocol mentioned by Zhang et al. (2014). A comprehensive description of the procedure is provided in the Supplementary Information (Methods S9).

2.10 Photosynthetic parameters

2.10.1 Measurement of chlorophyll and carotenoid levels

The chlorophyll and carotenoid levels were analysed by adapting the protocol mentioned by Dey et al. (2019). The complete description of the procedure followed is given in the Supplementary Information (Methods S10).

2.10.2 Measurement of Chlorophyll fluorescence

The maximum quantum yield of photosystem II (F_v/F_m) and the apparent electron transport rate (ETR) in the shoot were measured using the protocol described by Elshoky et al. (2021). Further details are mentioned in the Supplementary Information (Methods S11).

2.11 Effect on soluble sugars and protein levels

The soluble sugars and protein levels in the root cells were estimated based on the protocol followed by Krishnaraj et al. (2012). Additional details are provided in the Supplementary Information (Methods S12).

2.12 Environmental risk assessment

The present research aims to propose a risk quotient (RQ) for assessing the ecological hazards of Cd in the soil environment. The RQ is a conventional approach for evaluating the risk that pollutants present to the soil ecosystem. The RQ for Cd was determined using the formulae:

$$PNEC = \frac{EC_{50}}{AF} \quad (i)$$

$$RQ = \frac{MEC}{PNEC} \quad (ii)$$

In this study, the measured environmental concentrations (MEC) were sourced from existing literature [44–48]. The predicted no-effect concentration (PNEC) was derived from the EC_{50} values, applying an assessment factor (AF) for the evaluation. For this analysis, the AF was established at 1000, following the guidelines set by the European Chemicals Agency for evaluating acute toxicity [49]. The EC_{50} values of Cd were calculated both with and without the presence of ZnO particles.

2.13 Quality Assurance

All chemicals used in this study were of analytical grade. Ultrapure water (resistivity ≥ 18.2 M Ω ·cm) was used for stock solution preparation, which was performed in sealed containers to prevent contamination. Glassware was acid-washed with 10% HNO₃ and rinsed with ultrapure water. All procedures were conducted with sterilised instruments, and stock suspensions of ZnO particles and Cd were freshly prepared before each exposure experiment. To ensure colloidal stability and hydrodynamic size of the ZnO dispersions, zeta and DLS measurements were conducted immediately after sonication and before the exposure study. To minimise contamination during handling, laboratory coats, safety goggles, and nitrile gloves were worn. All experiments were performed in a sterile environment, using only healthy garlic bulbs for the biochemical assays to maintain consistent physiological health among replicates.

2.14 Statistical analysis

All toxicological experiments were conducted in triplicate ($n = 3$) and are presented as the mean $\pm$ standard deviation (SD). One-way ANOVA, followed by Bonferroni post-hoc tests, was performed using GraphPad Prism version 10.5.0 (673) to determine the significance level between the test samples and the control. Only results with $p < 0.05$ were considered statistically significant. All the graphs were made using GraphPad Prism and OriginPro 2025b software. To assess the effect of Cd in the presence and absence of ZnO particles on *Allium sativum*, cluster heatmap and Pearson correlation matrix analyses were carried out, along with principal component analysis (PCA), using OriginPro 2025b.

3. Results

3.1 Physico-chemical characterisation of ZnO particles and their interactions with Cd

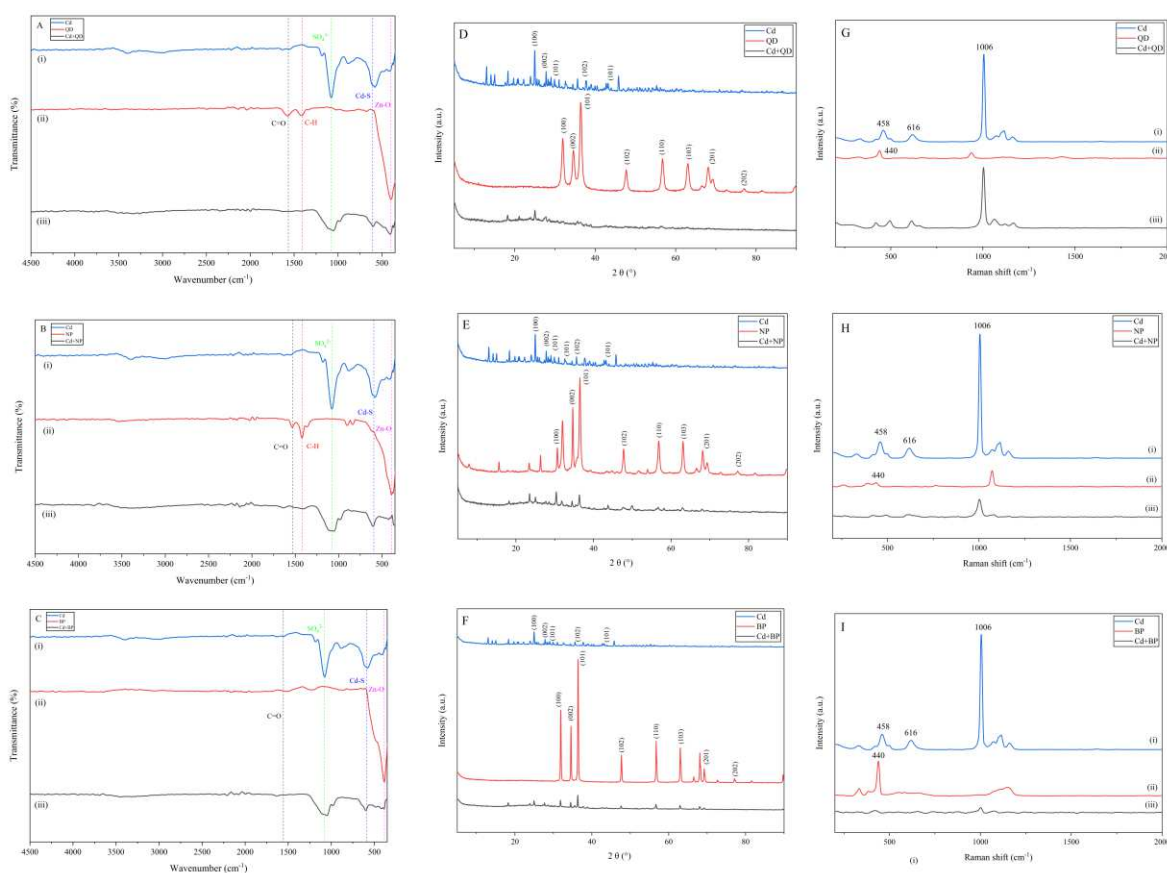


Fig. 1. FTIR spectra of A-i: ZnO QDs, A-ii: Cd, A-iii: QD + Cd, B-i: ZnO NPs, B-ii: Cd, B-iii: NP + Cd, C-i: ZnO BPs, C-ii: Cd, C-iii: BP + Cd. XRD spectra of D) pristine ZnO

QDs, Cd and QD + Cd, E) pristine ZnO NPs, Cd and NP + Cd, F) pristine ZnO BPs, Cd and BP + Cd. Raman spectra of G) pristine ZnO QDs, Cd and QD + Cd, H) pristine ZnO NPs, Cd and NP + Cd, I) pristine ZnO BPs, Cd and BP + Cd.

Fig. S1 represents the FESEM images of pristine ZnO QDs, NPs, and BPs and their mixtures with Cd. The QDs were observed to be well scattered and had a fine, dot-shaped structure with a size of ~ 15.35 nm. The NPs were found to be spherical in shape with well-defined borders, measuring around ~ 98 nm in average size. The BPs were found to have an irregular oval shape with a particle size of ~ 3 μ m. However, after interacting with Cd, the ZnO particles were observed to undergo agglomeration and appeared as large aggregates.

The FTIR analysis of pristine QDs, NPs, and BPs, as well as their binary mixtures with Cd, is represented in Fig. 1A-C. In the FTIR spectrum of ZnO QDs (Fig. 1A(ii)), the major peak noted around 396 cm^{-1} is ascribed to the stretching vibrations of the Zn-O bond, while for the NPs (Fig. 1B(ii)) and BPs (Fig. 1C(ii)), the peaks corresponding to Zn-O stretching were observed at 381 and 384 cm^{-1} , respectively. The band at around 1403 cm^{-1} arises due to the stretching and bending vibrations of the C-H bond, while for NPs, the C-H absorption peak occurred at around 1417 cm^{-1} . The absorption band around 1564 cm^{-1} for QDs can be attributed to the C=O stretching vibration. In contrast, for the NPs and BPs, the corresponding peaks were observed at 1527 and 1555 cm^{-1} , respectively. The CdSO_4 (Cd) absorption peak around 1085 cm^{-1} arises due to the sulphate ions (SO_4^{2-}) undergoing triply degenerate asymmetric stretching (ν_3), and the narrow peak centred at around 605 cm^{-1} is attributed to the Cd-S stretching [50–52].

Fig. 1(D-F) represents the XRD patterns of pristine ZnO particles and their respective mixtures with Cd. In the XRD patterns of ZnO QDs, NPs, and BPs (Fig. 1 D-(ii), E-(ii), and F-(ii)), the strong and sharp peaks corresponding to (100), (002), (101), (102), (110), (103), (201), and (202) reflections can be well indexed to the hexagonal wurtzite crystalline phase of the ZnO

particles supported by standard data (JCPDS File No. 043-0002). In the XRD spectrum of CdSO₄ (Cd), five major peaks corresponding to the (100), (002), (101), (102), and (101) planes were observed (Fig. 1D-(i)), confirming the crystalline nature of CdSO₄ (Cd), which matches the standard data (JCPDS file No. 41-1049) [53] [54].

Raman analysis was performed to determine the vibrational and crystalline properties of ZnO particles (Fig. 1 G-I). For the pristine ZnO particles, the fairly narrow peak around ~ 440 cm⁻¹ is attributed to the non-polar optical phonon *E*₂ (*high frequency*) mode, arising from the movements of oxygen atoms, which is typical of the wurtzite form of ZnO. In the case of CdSO₄ (Cd), the peak noted around ~ 458 cm⁻¹ is attributed to the bending mode (*v*₂) of the SO₄²⁻ ion, and the weak peak around ~ 616 cm⁻¹ is due to the triple degenerate oscillations of the free sulphate ion. The intense peak centred at ~ 1006 cm⁻¹ is characteristic of the SO₄²⁻ ion and corresponds to the symmetric stretching (*v*₁) mode [55, 56].

Fluorescence quenching analysis (Fig. S2 (A-C)) revealed that the fluorescence spectrum of pristine ZnO QDs exhibited the highest level of fluorescence emission, followed by NPs and BPs. Furthermore, the interaction of ZnO particles with Cd did not induce any significant alterations in the fluorescence properties. This indicates that the adsorption of Cd onto ZnO QDs, NPs, and BPs does not influence their inherent fluorescence characteristics.

The zeta potential of ZnO QDs, NPs, and BPs was measured at 0 and 120 h time intervals by dispersing them in the Hoagland nutrient medium (Table S2). At 0 h, among the ZnO particles, QDs were found to be comparatively stable with a zeta potential value of -16.05 ± 0.83 mV than NPs and BPs. After the addition of Cd to ZnO particles, a reduction in the zeta potential was observed, yielding a value of -15.93 ± 1.80 mV. This trend was consistently observed across both NPs and BPs dispersions. At 120 h, pristine QDs were found to be more stable than NPs and BPs, with a zeta potential value of -24.11 ± 1.92 mV. However, the zeta potential

values of QDs were observed to decrease to -22.11 ± 3.69 mV upon interaction with Cd (10 mg/L). A similar decrement in the zeta potential values was observed for NPs and BPs as well. The observed reduction in zeta potential was significantly more pronounced in binary mixtures containing 20 mg/L of Cd compared to those with 10 mg/L of Cd. This suggests a dose-dependent effect of Cd concentration on the stability of the mixtures. The contact angle analysis revealed a similar pattern to that of zeta potential results, as provided in Table S3. At 0 h, ZnO QDs were found to be hydrophilic in nature ($54.53 \pm 2.99^\circ$), followed by NPs and BPs. Upon the addition of Cd to pristine ZnO dispersions, the contact angle values decreased. The contact angle value of QDs was reduced to $52.20 \pm 5.37^\circ$ (at 10 mg/L Cd), and a similar trend was noted for NPs and BPs as well. At 120 h, ZnO QDs were noted with a contact angle value of $56.00 \pm 2.38^\circ$, comparatively making them less hydrophilic than NPs and BPs. However, after the addition of Cd (10 mg/L) to ZnO QDs, the contact angle values decreased to $54.47 \pm 1.27^\circ$, and this decrement was consistent across NPs and BPs as well. Following the trend of zeta potential, the decrement was more pronounced in the binary mixtures containing 20 mg/L of Cd than in those containing 10 mg/L of Cd.

3.2 Modulations in the cytotoxic effects

3.2.1 Evaluation of EC₅₀ and cytotoxicity

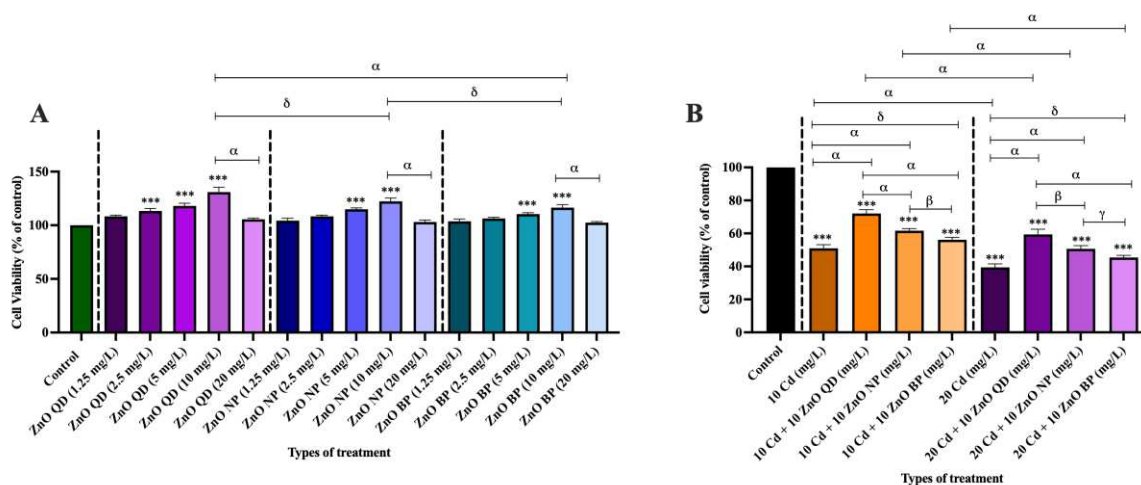


Fig. 2. Cell viability of *A. sativum* roots exposed to A) pristine ZnO particles and B) Cd + ZnO particles. All the bars represent mean $\pm$ SD with n=3. Significant differences between the control setup and the different treatments of ZnO particles and mixtures of Cd + ZnO particles are marked with ‘*’ ($p < 0.05$), ‘*’ ($p < 0.01$), ‘***’ ($p < 0.001$). ‘ α , β , γ and δ ’ signifies the notable difference between the treatment groups ($\alpha = p < 0.001$, $\beta = p < 0.01$, $\gamma = p < 0.05$, and $\delta = p > 0.05$).**

The EC_{50} values for Cd alone and in combination with ZnO particles (QDs, NPs, and BPs) were evaluated. The EC_{50} concentration for Cd was found to be 10.36 mg/L. However, in the presence of ZnO QDs, the EC_{50} values increased to 96.12 mg/L. A similar response pattern was observed for NPs and BPs, where the EC_{50} values increased to 69.07 mg/L and 11.21 mg/L, respectively. This demonstrates the size-dependent protective effects of the ZnO particles.

Fig. 2A represents the cell viability percentage of *A. sativum* roots exposed to pristine ZnO particles. In the case of pristine ZnO QDs treatment, the cell viability increased in a dose-dependent manner up to 10 mg/L, with the highest significant increase noted at 10 mg/L ($p < 0.01$, ~130.88%) compared to the control setups. This was followed by an insignificant increase ($p > 0.05$) in the cell viability at 20 mg/L (~105.60%) relative to the control. A similar response pattern was observed in cell viability for the test groups exposed to NPs and BPs as well.

Overall, ZnO QDs were found to be more effective than NPs and BPs in improving the cell viability. For the pristine ZnO particles exposure, the cell viability response pattern followed ZnO QDs > NPs > BPs. The results indicate that 10 mg/L of ZnO particles exerted maximum effect and was chosen for the binary mixture studies (Cd + ZnO particles) aimed at evaluating the efficacy of ZnO QDs in comparison to NPs and BPs in mitigating Cd stress.

Fig. 2B represents the cell viability percentage of *A. sativum* root cells exposed to Cd and the binary mixtures of Cd and ZnO particles. It was observed that Cd at 10 and 20 mg/L exhibited a significant reduction ($p < 0.001$) in viability of up to 50.96% and 39.43%, respectively, when compared to the control groups. Upon the addition of ZnO particles to the Cd solution (10 and 20 mg/L), the cell viability was significantly increased ($p < 0.001$) in all the binary mixtures. Under 10 mg/L of Cd stress, the highest cell viability was observed in the test groups containing ZnO QDs (~72.08%), followed by mixtures containing NPs (~61.61%) and BPs (~56.13%). A similar response pattern was observed in the case of 20 mg/L Cd-stressed treatment groups as well. In the case of binary mixtures, the order of response in cell viability was found to be QDs + Cd > NPs + Cd > BPs + Cd, with a significant difference between QDs, NPs, and BPs, demonstrating the greater efficacy of ZnO QDs in mitigating Cd stress.

3.2.2 Release of Zn^{2+} in the medium and its effects on cell viability

From Table S4, the nominal concentrations of pristine ZnO QDs, NPs, and BPs were observed to be 7.932 ± 0.025 , 7.399 ± 0.754 , and 6.865 ± 0.077 mg/L, respectively, with QDs having the highest Zn^{2+} concentration. After the addition of Cd to ZnO dispersions, the nominal concentrations of ZnO particles decreased; however, the QDs + Cd mixture was still noted to have the highest Zn^{2+} release, followed by the mixtures with NPs and BPs.

The amount of Zn^{2+} leached from the respective ZnO particles is provided in Table S4. The highest leaching was noted for QDs (0.917 ± 0.008 mg/L), and the lowest leaching was

recorded for BPs (0.646 ± 0.062 mg/L). The addition of Cd to three different types of ZnO dispersions reduced the leaching of Zn^{2+} . In the binary mixtures, the highest leaching was observed for the mixture containing QDs, and the lowest leaching was noted for the mixtures containing BPs.

Fig. S3-A depicts the alterations in the cell viability of *A. sativum* roots caused by the leached ions. The leached Zn^{2+} ions from pristine ZnO QDs, NPs, and BPs did not markedly affect the cell viability. A similar response was observed in root length and ROS generation as well (Fig. S3-C & E). The exposure of *A. sativum* to leached ions did not result in observed enhancement in cell viability, root length and in the amount of ROS generated when compared to the control setups. Conversely, in the case of exposure involving the leached ions from the binary mixtures of ZnO and Cd, (Fig. S3-B,D,F), a significant decline ($p < 0.001$) in cell viability and root length was noted relative to the control groups. Furthermore, a significant increase ($p < 0.001$) in ROS generation was also noted relative to the control setup.

3.2.3 Accumulation of Zn and Cd in *A. sativum* roots

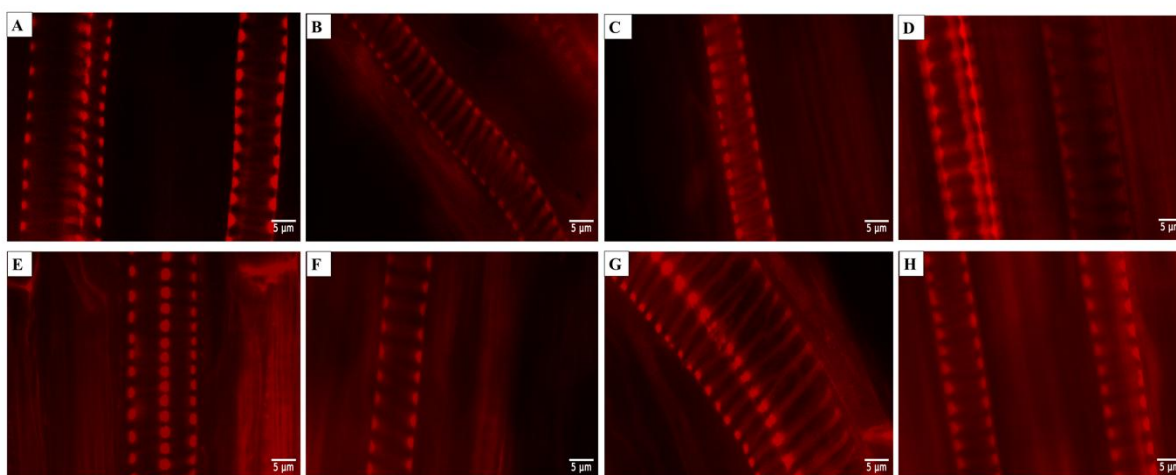


Fig. 3. Fluorescence imaging of suberin deposition in *A. sativum* roots exposed to A) control, B) pristine ZnO QDs, C) pristine ZnO NPs, D) pristine ZnO BPs, E) Cd alone (20mg/L), F) Cd + QDs, G) Cd + NPs and H) Cd + BPs.

At 120 h, among the pristine ZnO particles, roots treated with QDs exhibited the highest Zn accumulation (1.561 ± 0.114 mg/L) while those exposed to BPs exhibited the lowest Zn accumulation (0.964 ± 0.050 mg/L) (Table S4). The addition of Cd to ZnO particles caused a decrease in the Zn accumulation in roots, with the highest accumulation observed in the test groups exposed to the binary mixtures containing QDs, followed by the treatment groups exposed to the binary mixtures containing NPs and BPs. The accumulation of Zn in plant roots followed the order QDs > NPs > BPs, both in pristine and in combination with Cd, clearly indicating that QDs showed the highest growth-promoting potential.

The nominal concentrations of Cd at 0 h were recorded to be 3.668 ± 0.127 mg/L and 4.665 ± 0.037 mg/L for 10 and 20 mg/L of Cd, respectively. The addition of ZnO particles to Cd solutions has decreased their nominal concentrations. The binary mixtures involving QDs were found to have the lowest Cd concentrations when compared to those involving NPs and BPs. At 120 h, the accumulation of Cd in roots exposed to Cd solutions alone (10 and 20 mg/L) was noted to be 0.715 ± 0.009 mg/L and 0.742 ± 0.025 mg/L, respectively. The addition of ZnO particles to Cd solutions caused a decrease in the Cd accumulation, with the 20 mg/L Cd + BPs treatment groups showing the highest Cd accumulation (0.674 ± 0.032 mg/L), while the lowest Cd accumulation (0.306 ± 0.001 mg/L) was noted in the trial groups exposed to 10 mg/L Cd + QDs.

The accumulation data were also supported by the fluorescence staining image of suberin (Fig. 3(A-H)), which lines the Casparian strip and is deposited in response to increased Cd uptake. Fig. 3A denotes the suberin deposition in the control roots being visualised, and Fig. 3B-D depicts the suberin deposition on roots exposed to pristine ZnO QDs, NPs, and BPs. Under non-stressed conditions, suberin deposition was found to be meagre in all pristine particle exposures. The roots exposed to QDs exhibited the minimal suberin deposition indicative of

non-stressed conditions. In contrast, for the roots treated with NPs and BPs, there appeared to be a slight increase in suberin deposition. Under 20 mg/L of Cd stress (Fig. 3E), the suberin deposition increased in response to regulate the uptake of Cd. On the contrary, in the roots exposed to the binary mixtures, the suberin deposition was slightly reduced, indicating the stress-mitigating efficacy of ZnO particles. Among the roots exposed to three binary mixtures (Fig. 3F-H), the roots treated with Cd + QDs were observed with reduced thickness of suberin, while a higher deposition of suberin was visualised in the roots exposed to Cd + BPs.

3.3 Alterations in the mitotic index

Table 1. Alterations in the mitotic index of *A. sativum* root cells exposed to pristine ZnO particles and the binary mixtures of ZnO and Cd

Types of treatments	Mitotic (%)	Index Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)
Control	73.33 ± 1.79	34.43 ± 5.93	20.23 ± 1.35	12.93 ± 3.85	5.73 ± 2.76
1.25 mg/L ZnO QDs	83.9 ± 0.3	21.39 ± 26.85	15.07 ± 5.67	7.5 ± 3.04	4.6 ± 1.06
2.5mg/L ZnO QDs	85.23 ± 0.35	49.23 ± 11.13	15.37 ± 5.23	9.7 ± 2.23	10.93 ± 12.01
5 mg/L ZnO QDs	86.4 ± 0.7	45.7 ± 8.03	17.27 ± 6	11.6 ± 5.77	11.83 ± 9.86
10 mg/L ZnO QDs	88.97 ± 0.75	61.33 ± 4.2	9.47 ± 0.46	8.27 ± 8.73	9.9 ± 10.85
20mg/L ZnO QDs	82.77 ± 0.42	36.23 ± 7.89	24.67 ± 6.65	13.2 ± 5.06	8.67 ± 3.31
1.25 mg/L ZnO NPs	81.13 ± 0.81	30.07 ± 5.83	25.17 ± 5.29	16.13 ± 4.35	9.77 ± 6.39
2.5mg/L ZnO NPs	83.23 ± 1	34.6 ± 6.39	21.63 ± 8.75	12.77 ± 10.06	14.23 ± 3.39
5 mg/L ZnO NPs	84.77 ± 0.55	50.83 ± 9.38	17.07 ± 4.31	10.3 ± 8.25	6.57 ± 5.98
10 mg/L ZnO NPs	87.1 ± 0.9	41.53 ± 18.13	14.1 ± 1.51	20.4 ± 14.64	11.07 ± 3.35
20mg/L ZnO NPs	76.53 ± 0.78	33.77 ± 11.2	21.13 ± 5.22	13.2 ± 11.45	8.43 ± 9.13
1.25 mg/L ZnO BPs	74.57 ± 1.12	28.4 ± 7.51	28.33 ± 2.94	17.3 ± 3.82	5.83 ± 5.39
2.5mg/L ZnO BPs	81.8 ± 1.77	33.8 ± 5.34	22.63 ± 8.13	15.7 ± 3.36	9.67 ± 3.32
5 mg/L ZnO BPs	83.13 ± 0.8	34.33 ± 9.07	27.6 ± 1.15	11.7 ± 2.6	9.5 ± 6.13

10 mg/L ZnO BPs	85.1 ± 1.57	36.67 ± 8.29	26.17 ± 3.09	15.27 ± 2.67	7 ± 6.56
20mg/L ZnO BPs	74.57 ± 1.12	36.03 ± 4.9	27.37 ± 1.8	7.07 ± 6.28	4.1 ± 4.26
10 mg/L Cd	65.07 ± 2.9	27.77 ± 3.87	14.1 ± 6.15	9.33 ± 10.07	13.87 ± 8.47
20 mg/L Cd	59.73 ± 1.29	19.27 ± 1.9	20.83 ± 9.4	13.63 ± 4.72	6 ± 5.24
10 mg/L Cd + ZnO QDs	69.5 ± 1.23	33.53 ± 9.32	18.4 ± 2.86	7.37 ± 6.01	10.2 ± 7.9
10 mg/L Cd + ZnO NPs	67.77 ± 0.32	27.7 ± 7.4	19.57 ± 2.67	8.13 ± 3.5	12.37 ± 3.89
10 mg/L Cd + ZnO BPs	66.27 ± 0.5	33.07 ± 5.13	16.2 ± 3.61	14.43 ± 5.64	2.57 ± 2.24
20 mg/L Cd + ZnO QDs	65.27 ± 2.4	37.57 ± 4.26	16.83 ± 6.68	6.97 ± 6.28	3.9 ± 3.72
20 mg/L Cd + ZnO NPs	63.57 ± 1.15	32.23 ± 8.11	12.43 ± 10.86	8.93 ± 2.37	9.97 ± 8.95
20 mg/L Cd + ZnO BPs	62.17 ± 0.31	23.6 ± 7.02	12.8 ± 1.61	14.07 ± 7.39	11.7 ± 4.06

Table 1 represents the alterations in the mitotic index of *A. sativum* root cells upon exposure to pristine ZnO particles and in combination with Cd. It was observed that treatments with all pristine ZnO particles resulted in increased cell division at all supplied doses. Among the various concentrations of QDs exposure, the 10 mg/L concentration was marked with the highest MI percentage ($\sim 86.4 \pm 0.7 \%$), while 20 mg/L exhibited the lowest cell division percentage ($\sim 82.77 \pm 0.4\%$) in comparison with the control groups. A similar response pattern was noted in the case of NPs and BPs as well. Among all the test concentrations, exposure to 10 mg/L of ZnO was noted to cause the highest increment in MI percentage. Particularly, the test groups exposed to QDs exhibited the highest increase, compared to those observed in the test groups treated with NPs ($87.1 \pm 0.9\%$) and BPs ($85.1 \pm 1.57\%$).

Exposure to 10 and 20 mg/L of Cd significantly decreased the MI percentage, with values reaching $65.07 \pm 2.9\%$ and $59.73 \pm 1.29\%$, respectively, compared to the control setups. Upon the addition of ZnO particles to Cd solutions, the MI percentage tends to increase when compared to that observed in the test groups exposed to Cd solution alone. Among the test groups exposed to the binary mixtures containing 10 mg/L Cd, the highest MI percentage was observed in the treatment groups exposed to QDs ($69.5 \pm 1.23\%$), and the lowest MI percentage was noted in the trial groups supplied with BPs ($66.27 \pm 0.5\%$). A similar response was observed for the test groups exposed to the binary mixtures involving 20 mg/L Cd as well.

3.4 Effects on root length of *A. sativum*

The effect of pristine ZnO particles on the root length of *A. sativum* is depicted in Fig. S4-A. It is clearly seen that QDs have significantly ($p < 0.001$) increased the root length at all ZnO concentrations relative to the control. A similar effect on root length was also observed for NPs and BPs treatments as well. The highest increase in root length was observed at 10 mg/L for QDs ($\sim 143.69\%$), compared to the control groups. A similar response pattern was observed in

the test groups exposed to NPs and BPs as well. Notably, there exists a significant difference ($p < 0.001$) in root length at 10 mg/L of QDs vs BPs, as well as NPs vs BPs. Overall, the QDs were effective in increasing the root length by ~10.23% and ~24.50% when compared to their nano and bulk counterparts, respectively.

In the presence of Cd stress (10 and 20 mg/L), the root length was significantly decreased ($p < 0.001$) to ~49.35% and ~31.74%, respectively, relative to control setups, as shown in Fig. S4-B. However, with the addition of ZnO particles to 10 and 20 mg/L of Cd solutions, the root length seemed to increase significantly ($p < 0.001$). The highest increase (~78.16%) in root length under 10 mg/L Cd stress was observed in the test groups supplied with ZnO QDs, while the lowest increase (~69.28%) was noted for the trial groups exposed to BPs. A similar response pattern was observed in the treatment groups exposed to 20 mg/L of Cd stress as well. Under 10 mg/L of Cd stress, no significant differences were observed among the ZnO particles in the root length enhancement. Nevertheless, under 20 mg/L of Cd stress, a significant difference ($p < 0.05$) in root length was observed between QDs and BPs. The changes in root length followed an order similar to that of cell viability: QDs + Cd > NPs + Cd > BPs + Cd, and the pattern was consistent across all the growth, oxidative and photosynthetic parameters.

3.5 Changes in root surface morphology

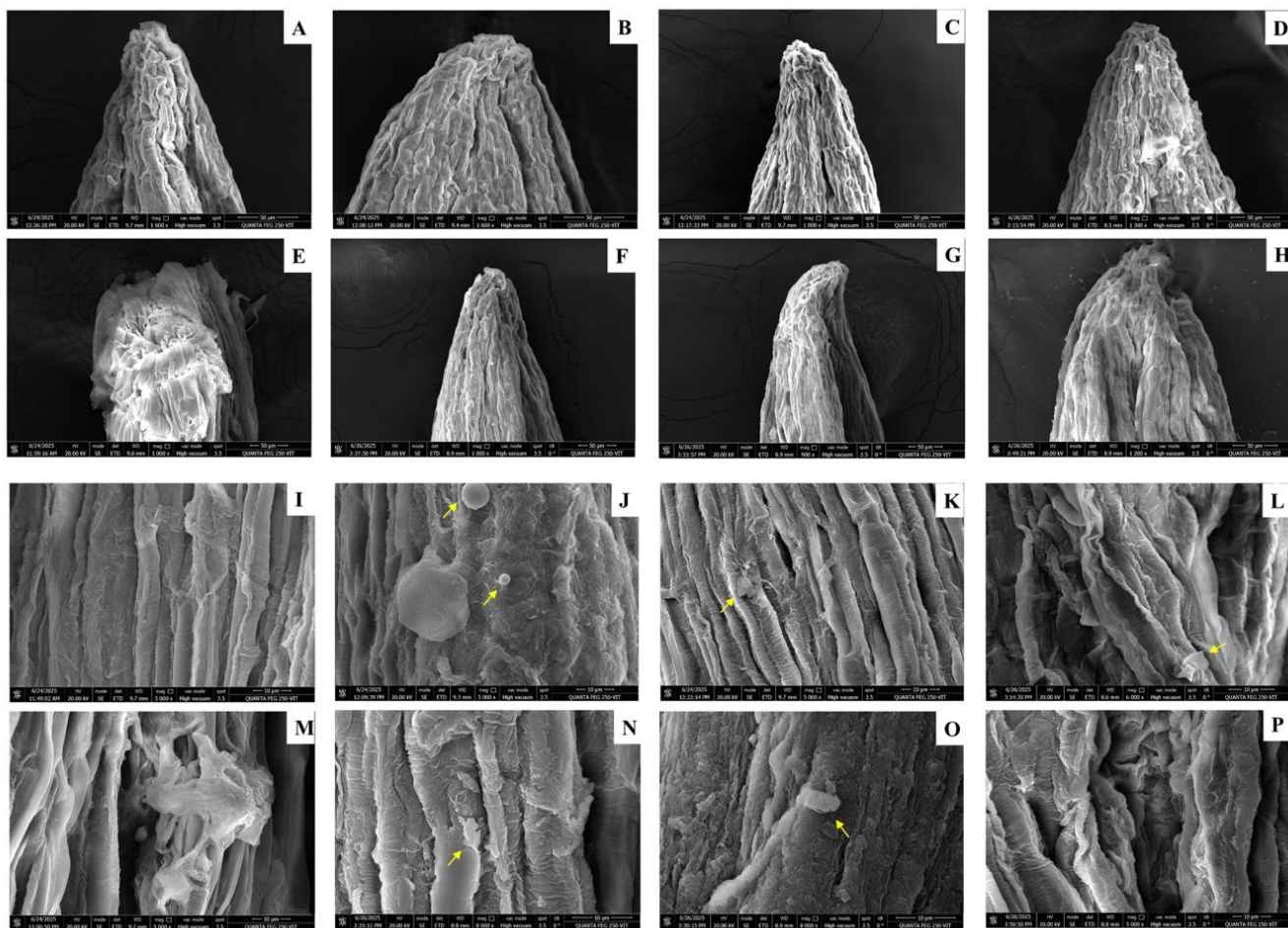


Fig. 4. FE-SEM images of *A. sativum* root tips exposed to A) control, B) pristine ZnO QDs, C) pristine ZnO NPs, D) pristine ZnO BPs, E) Cd alone (20mg/L), F) Cd + QDs, G) Cd + NPs and H) Cd + BPs. FE-SEM images of mid regions of *A. sativum* root exposed to I) control, J) pristine ZnO QDs, K) pristine ZnO NPs, L) pristine ZnO BPs, M) Cd alone (20mg/L), N) Cd + QDs, O) Cd + NPs and P) Cd + BPs. The adsorbed ZnO particles on *A. sativum* roots were indicated with arrows.

Fig. 4 illustrates the alterations in the morphology of the root surface of *A. sativum* upon exposure to pristine ZnO particles and the binary mixture involving ZnO particles and Cd.

From Fig. 4A, the root tip of the control group exhibited a healthy appearance, showing limited signs of damage alongside the presence of constricted ridges on its surface. In contrast, the root tip exposed to pristine ZnO particles (Fig. 4B-D) exhibited a uniform and well-defined root surface. Among the roots treated with pristine ZnO particles, the ones exposed to QDs exhibited a distinctly turgid surface, devoid of any irregular constrictions. In contrast, the root tip exposed to NPs and BPs exhibited minor aberrations and shrinkage of root walls, with these effects being more pronounced in the case of BPs. Fig. 4E shows the root tip of *A. sativum* exposed to 20 mg/L Cd. The root tip exhibited significant aberrations, with multiple internal layers of the root being sloughed off, indicating the highly damaging potential of Cd to the root. Upon the addition of ZnO particles to Cd solutions, the root morphology seemed to be restored, showing minimal damage and constrictions as depicted in Fig. 4F-H. Fig. 4I shows the internal grooves of the root belonging to the control setup. Fig. 4J-L shows the internal ridges and grooves of the root exposed to pristine ZnO particles, and it is evident that the ZnO particles adsorb onto the surface of the root (as indicated with pointers) to facilitate their subsequent absorption into the cellular structures. Fig. 4M depicts the constricted internal grooves of *A. sativum* roots exposed to 20 mg/L of Cd. Fig. 4N-P denotes the roots exposed to the binary mixtures of Cd and ZnO particles. The deposition of large aggregates of binary mixtures was noted; at the same time, damage in the internal grooves that was previously observed under Cd exposure appeared to be mitigated upon the addition of ZnO particles.

3.6 Effects on oxidative stress and antioxidant enzyme activity

3.6.1 Effects on ROS generation

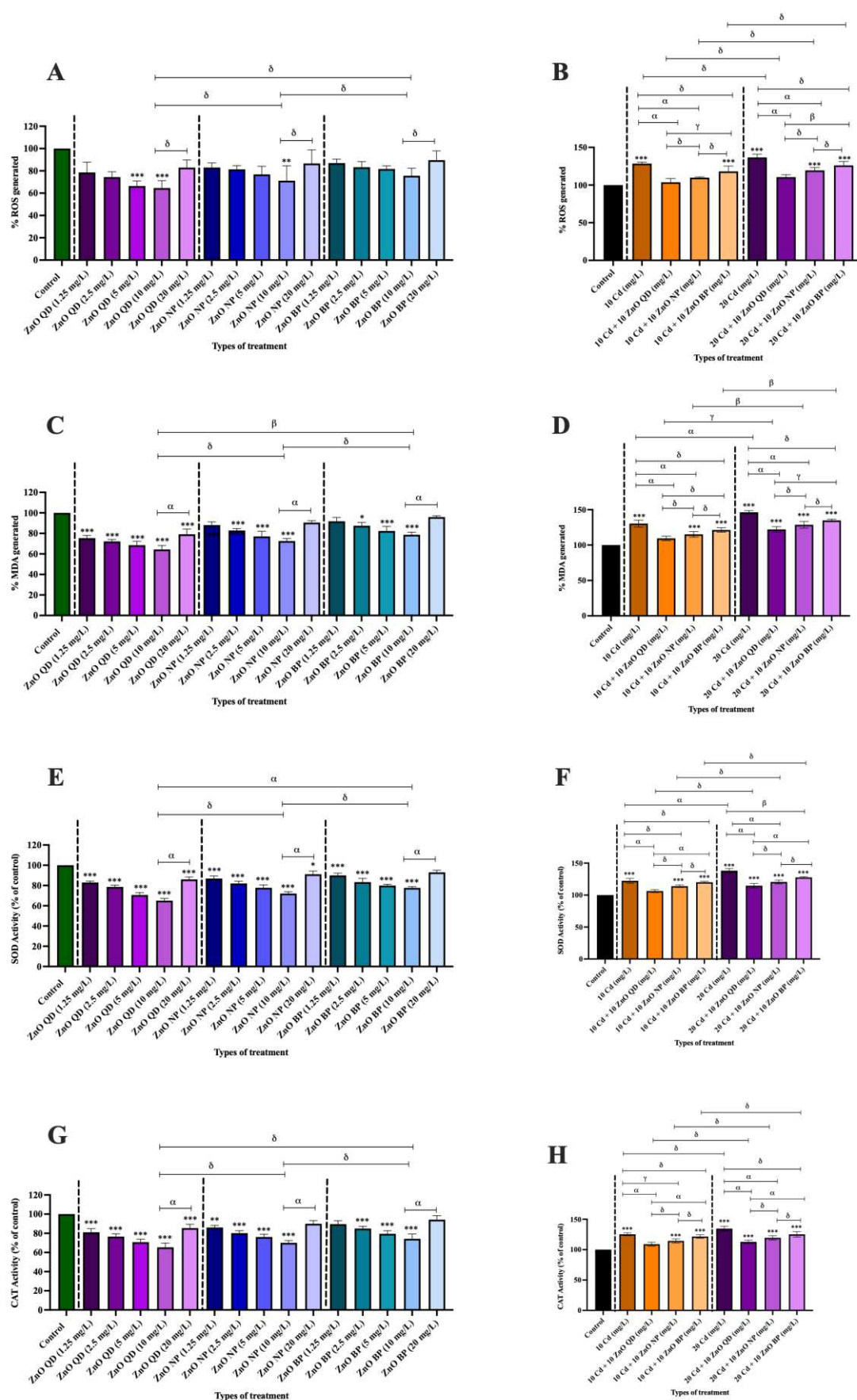


Fig. 5. Oxidative stress assessment in *A. sativum* roots after exposure to ZnO particles and Cd + ZnO particles. A) Amount of total ROS generated in *A. sativum* roots exposed to pristine ZnO particles, B) Amount of ROS generated in *A. sativum* roots exposed to mixtures of Cd + ZnO particles, C) Amount of MDA generated in *A. sativum* roots exposed to pristine ZnO particles, D) Amount of MDA generated in *A. sativum* roots exposed to mixtures of Cd + ZnO particles, E) Activity of SOD enzyme in *A. sativum* roots exposed to pristine ZnO particles, F) Activity of SOD enzyme in *A. sativum* roots exposed to mixtures of Cd + ZnO particles, G) Activity of CAT enzyme in *A. sativum* roots exposed to pristine ZnO particles, H) Activity of CAT enzyme in *A. sativum* roots exposed to mixtures of Cd + ZnO particles. All the bars represent mean $\pm$ SD with n=3. Significant differences between the control setup and the different treatments of ZnO particles and Cd + ZnO particles are marked with ‘*’ ($p < 0.05$), ‘’ ($p < 0.01$), ‘***’ ($p < 0.001$). ‘ α , β , γ and δ ’ signifies the notable difference between the treatment groups ($\alpha = p < 0.001$, $\beta = p < 0.01$, $\gamma = p < 0.05$, and $\delta = p > 0.05$).**

The amount of total ROS generated in *A. sativum* root cells upon exposure to the pristine ZnO particles is represented in Fig. 5A. Pristine ZnO particles at any given dose did not lead to an elevation in ROS levels in comparison to the control group. Among the different concentrations of QDs, the lowest ROS generation was noted at 10 mg/L ($p < 0.001$, ~ 64.60%) and the highest ROS production was observed at 20 mg/L (~ 90.75%). A similar response pattern was observed for NPs and BPs as well. Overall, the 10 mg/L ZnO exposure exhibited the lowest ROS generation, among which the treatments involving QDs resulted in less ROS production compared to NPs (~71.20%) and BPs (~75.62%).

Fig. 5B represents the amount of total ROS produced in *A. sativum* root cells upon exposure to the binary mixtures of Cd and ZnO particles. A significant increase ($p < 0.001$) in ROS production was observed at 10 and 20 mg/L of Cd, with increments of $\sim 128.47\%$ and $\sim 136.61\%$, respectively, compared to the control setups. The addition of ZnO particles to the Cd solutions significantly ($p < 0.001$) lowered the ROS levels when compared to those observed for Cd alone at 10 and 20 mg/L. Among the binary mixtures involving 10 mg/L Cd and ZnO particles, the test groups supplied with QDs exhibited the lowest ROS generation ($\sim 103.70\%$), while the treatment groups involving BPs showed the highest ROS production ($p < 0.001$, $\sim 118.09\%$), when compared to those ROS levels observed for Cd alone at 10 mg/L. A similar response pattern was observed for the test groups involving 20 mg/L Cd and ZnO particles. Overall, under Cd stress, the treatment with QDs resulted in less ROS generation compared to other ZnO forms.

3.6.2 Effects on lipid peroxidation

The degree of membrane damage observed in *A. sativum* root cells upon exposure to pristine ZnO particles is shown in Fig. 5C. The treatment with QDs at all doses resulted in significantly lower lipid peroxidation ($p < 0.001$). Among the various test doses of QDs, the dose at 10 mg/L showed the lowest MDA production ($\sim 64.34\%$), while the dose at 20 mg/L was noted to have the highest MDA level ($\sim 79.09\%$). A similar response pattern was noticed in the case of NPs and BPs as well. In general, the 10 mg/L ZnO exposure showed the lowest MDA generation. Among the test groups, those treated with QDs had less MDA production compared to those treated with NPs ($\sim 72.54\%$) and BPs ($\sim 78.68\%$).

Fig. 5D depicts the amount of MDA formed in *A. sativum* root cells upon exposure to the binary mixtures involving Cd and ZnO particles. Exposure to 10 and 20 mg/L of Cd significantly ($p < 0.001$) increased MDA levels, with values reaching ~ 125.40 and 148.77% , respectively,

relative to the control groups. The addition of ZnO particles to Cd (10 and 20 mg/L) solution resulted in significantly ($p < .0001$) lower MDA generation in comparison with the MDA levels recorded under exposure to Cd solution alone. Among the binary mixtures containing 10 mg/L Cd, the lowest MDA formation was observed for the test groups exposed to QDs (~109.42%), while the test groups exposed to BPs showed the highest MDA level (~121.31%). A similar response pattern was noted for the mixtures supplied with 20 mg/L Cd as well. Overall, in the case of binary mixtures, treatments involving QDs were found to cause less membrane damage in the roots compared to those treatments involving NPs and BPs.

3.6.3 Effects on antioxidant enzyme activity

The influence of the pristine ZnO particles on the SOD activity of *A. sativum* root cells is shown in Fig. 5E. All the test concentrations of ZnO particles exhibited a significant ($p < 0.001$) decline in the SOD activity in comparison with the control setups. Among the test groups exposed to different concentrations of QDs, the test groups exposed to 10 mg/L of QDs showed the lowest SOD activity (~ 65.002%), while the treatments involving 20 mg/L exhibited the highest SOD activity ($p < 0.001$, ~ 83.21%). A similar response pattern in the SOD activity was noted for the test groups exposed to NPs and BPs as well. Overall, exposure to 10 mg/L of ZnO particles showed the lowest SOD activity, particularly the trial groups involving QDs resulted in lesser SOD activity compared to those with NPs (~72.09%) and BPs (~78.44%).

The effect of the binary mixtures of Cd and ZnO particles on the SOD activity of *A. sativum* root cells is given in Fig. 5F. A significant increase ($p < 0.001$) in the SOD activity was observed at 10 and 20 mg/L of Cd, with increments of ~122.29% and ~138.14%, respectively, relative to the control groups. The addition of ZnO particles to the Cd solutions lowered the SOD activity when compared to that observed for Cd alone at 10 and 20 mg/L. Among the binary mixtures involving 10 mg/L of Cd stress, the test groups exposed to QDs exhibited the

lowest SOD activity (~106.26%), while the treatment groups involving BPs exhibited the highest SOD activity (~119.93%) compared to those levels observed under the exposure to Cd alone at 10 mg/L. A similar response pattern was noted for the binary mixture treatments involving 20 mg/L of Cd as well. Overall, under Cd stress, the treatment with QDs resulted in less SOD activity compared to NPs and BPs.

Fig. 5G depicts the influence of pristine ZnO particles on the CAT activity of *A. sativum* root cells. The treatment with ZnO particles exhibited a significant ($p < 0.001$) decrease in CAT activity at concentrations up to 10 mg/L, compared to the control setups. Among the different doses of QDs, the 10 mg/L exposure showed the lowest CAT activity (~65.34%), while the treatments involving 20 mg/L QDs exhibited the highest CAT activity (~85.41%). A similar response pattern in the CAT activity was also noted for the NPs and BPs. Overall, exposure to 10 mg/L of ZnO particles exhibited the lowest CAT activity, among which the test groups supplied with QDs resulted in lesser CAT activity when compared to those supplied with NPs (~70.05%) and BPs (~74.25%).

The effect of the binary mixtures of Cd and ZnO particles on CAT activity is given in Fig. 5H. A significant increase ($p < 0.001$) in CAT activity was observed under 10 and 20 mg/L of Cd exposure, with increments of ~125.33% and ~134.55%, respectively, when compared to the control groups. The addition of ZnO particles to the Cd solutions reduced the CAT activity in the test groups. Among the binary mixtures involving 10 mg/L of Cd, the test groups exposed to QDs exhibited the lowest CAT activity (~108.99%), while the treatment groups involving BPs exhibited the highest CAT activity (~121.61%) compared to those observed under the exposure to Cd alone at 10 mg/L. A similar response pattern was noted for the binary mixture treatments involving 20 mg/L Cd as well.

3.7 Alterations in photosynthetic parameters

628 **3.7.1 Modulation in photosynthetic pigment levels**

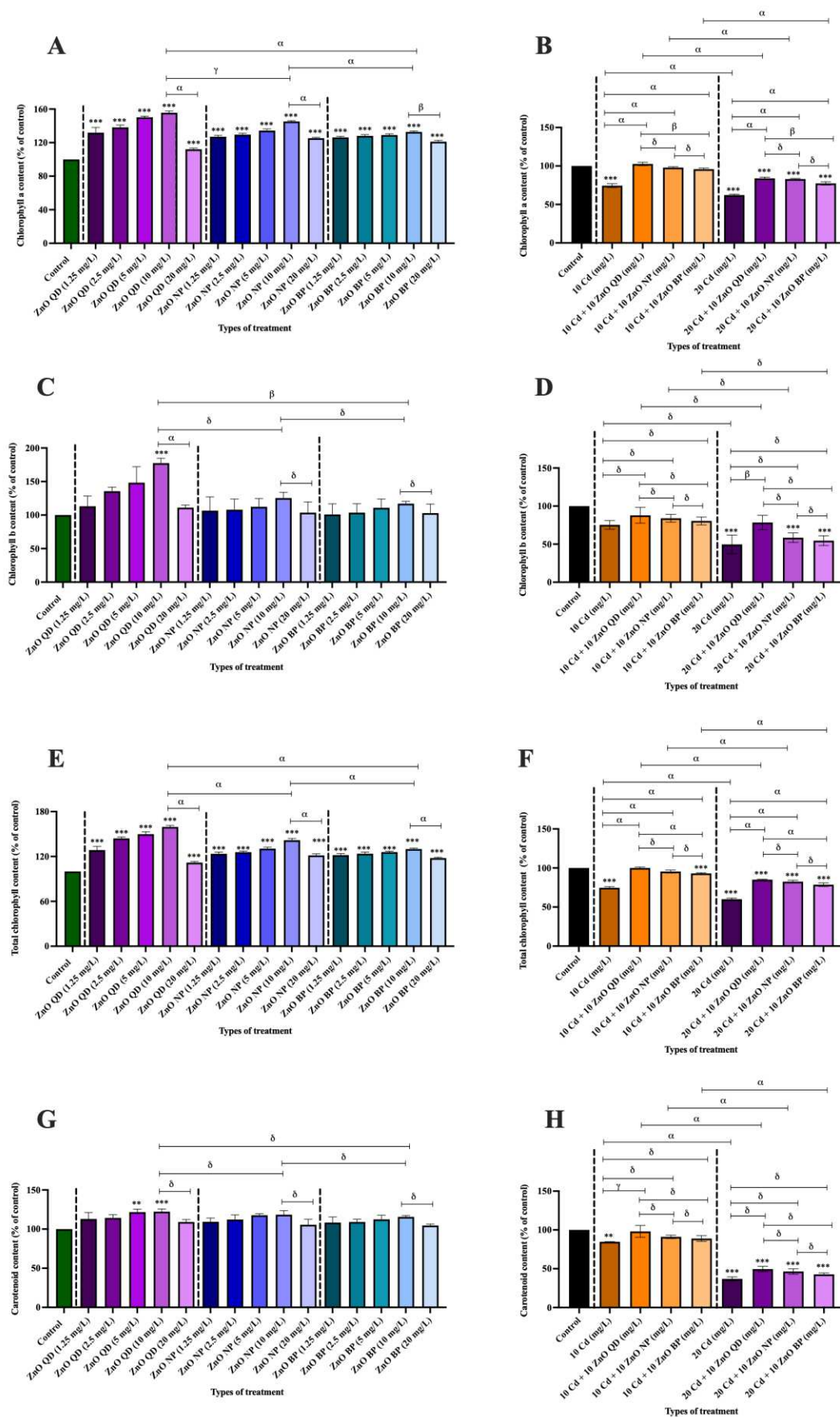


Fig. 6. Alterations in A) Chlorophyll *a* level of *A. sativum* leaves exposed to pristine ZnO particles, B) Chlorophyll *a* level of *A. sativum* leaves exposed to Cd + ZnO particles, C) Chlorophyll *b* level of *A. sativum* leaves exposed to pristine ZnO particles, D) Chlorophyll *b* level of *A. sativum* leaves exposed to Cd + ZnO particles, E) Total chlorophyll level of *A. sativum* leaves exposed to pristine ZnO particles, F) Total chlorophyll level of *A. sativum* leaves exposed to Cd + ZnO particles, G) Carotenoid level of *A. sativum* leaves exposed to pristine ZnO particles, H) Carotenoid level of *A. sativum* leaves exposed to Cd + ZnO particles. All the bars represent mean $\pm$ SD with n=3. Significant differences between the control setup and the different treatments of ZnO particles and Cd + ZnO particles are marked with ‘*’ ($p < 0.05$), ‘’ ($p < 0.01$), ‘***’ ($p < 0.001$). ‘ α , β , γ and δ ’ signifies the notable difference between the treatment groups ($\alpha = p < 0.001$, $\beta = p < 0.01$, $\gamma = p < 0.05$, and $\delta = p > 0.05$).**

Fig. 6A represents the effect of pristine ZnO particles on the chlorophyll *a* level of *A. sativum* shoot cells. It was observed that treatment with all three pristine ZnO particles had a positive impact on the chlorophyll *a* content, showing a significant ($p < 0.001$) increase at all applied doses. Among the different test concentrations of QDs, the treatment groups exposed to 10 mg/L showed the highest chlorophyll *a* content ($\sim 155.66\%$), while those treated with 20 mg/L showed the lowest chlorophyll *a* content ($\sim 112.04\%$). A similar response pattern was noted in the case of NPs and BPs as well. In general, the 10 mg/L ZnO concentration showed the highest chlorophyll *a* content, in which the test groups involving QDs resulted in the highest chlorophyll *a* content compared to NPs ($\sim 145.23\%$) and BPs ($\sim 132.74\%$). Furthermore, the alterations in chlorophyll *b* content exhibited a pattern analogous to that observed for chlorophyll *a*, indicating that the test groups exposed to 10 mg/L of ZnO particles showed the

654 maximum increment in chlorophyll *b* level, particularly the trial groups exposed to QDs
 655 exhibited highest increment ($p < 0.001$, ~177.50%) in chlorophyll *b* compared to those treated
 656 with NPs (~125.35%) and BPs (~117.09%), as depicted in Fig. 6C.

657 Fig. 6B depicts the alterations in the chlorophyll *a* content upon exposure to the mixtures
 658 involving Cd and ZnO particles. Exposure to 10 and 20 mg/L of Cd significantly ($p < 0.001$)
 659 decreased chlorophyll *a* level, with values decreasing to ~74.44% and ~62.14%, respectively,
 660 relative to the control setups. The addition of ZnO particles to Cd solutions (10 and 20 mg/L)
 661 was found to significantly ($p < .0001$) enhance chlorophyll *a* content compared to the levels
 662 observed under exposure to Cd solutions alone. Among the binary mixtures containing 10 mg/L
 663 of Cd, the highest chlorophyll *a* content was recorded for the test groups exposed to QDs (~
 664 102.60%), while the test groups exposed to BPs showed the lowest chlorophyll *a* content
 665 (~95.77%). A similar response pattern was noted for the mixtures supplied with 20 mg/L Cd.
 666 Overall, in the case of binary mixtures, treatments involving QDs were found to enhance
 667 chlorophyll *a* level compared to those treated with NPs and BPs. Furthermore, upon exposure
 668 to binary mixtures, the changes in chlorophyll *b* content exhibited a pattern consistent with
 669 those noted in chlorophyll *a* content (Fig. 6D).

670 Fig. 6E denotes the alterations in total chlorophyll levels of *A. sativum* shoot cells upon
 671 exposure to pristine ZnO particles. The treatment with QDs at all concentrations resulted in
 672 significant enhancement ($p < 0.001$) in the total chlorophyll content. Among the various applied
 673 concentrations of QDs, the dose at 10 mg/L showed the highest total chlorophyll level
 674 (~159.47%), while the dose at 20 mg/L was noted to have the lowest total chlorophyll level
 675 (~111.87%). A similar response pattern was noted in the case of NPs and BPs as well. For the
 676 10 mg/L of ZnO exposure groups also the ones containing QDs resulted in the highest total
 677 chlorophyll production compared to NPs and BPs.

Fig. 6F depicts the amount of total chlorophyll formed upon exposure to the binary mixtures of Cd and ZnO particles. Exposure to 10 and 20 mg/L Cd significantly ($p < 0.001$) decreased total chlorophyll content, with decrements of ~74.59% and 59.96%, respectively, relative to the control setups. The incorporation of ZnO particles to Cd (10 and 20 mg/L) solutions resulted in a significant ($p < .0001$) enhancement in the total chlorophyll levels in comparison with the levels recorded in test groups exposed to Cd solution alone. Among the binary mixtures involving 10 mg/L Cd, the highest total chlorophyll level was observed for the test groups exposed to QDs (~100.04%), while the test groups exposed to BPs showed the lowest total chlorophyll level (~93.10%). A similar response pattern was noted among the binary mixtures containing 20 mg/L of Cd as well. Overall, in the case of binary mixtures, test groups involving QDs were found to enhance total chlorophyll levels compared to those treated with NPs and BPs.

Fig. 6G illustrates the effect of pristine ZnO particles on the carotenoid content of *A. sativum* shoot cells. Exposure to pristine ZnO particles led to an increase in carotenoid content compared to the control group. Among the different test concentrations of QDs, the highest increment was noted at 10 mg/L ($p < 0.001$, ~124.49%) and the lowest carotenoid content was observed at 20 mg/L (~109.11%). It's important to mention that a similar response pattern was observed for NPs and BPs as well. Overall, the 10 mg/L ZnO exposure exhibited the highest elevation in carotenoid levels, among which the treatments involving QDs resulted in the highest carotenoid level compared to those exposed to NPs (~118.46%) and BPs (~115.62%).

Fig. 6H represents the changes in carotenoid content of *A. sativum* shoot cells upon exposure to the binary mixtures involving Cd and ZnO particles. A significant decrease was observed at 10 and 20 mg/L of Cd exposure, with decrements of ~84.67% ($p < 0.01$) and ~36.83% ($p < .0001$), respectively, compared to the control setups. Upon the addition of ZnO particles to the

Cd solutions, the carotenoid levels were enhanced; however, the increment was found to be significant only for the test groups involving QDs ($p < 0.05$). Among the binary mixtures exposed to 10 mg/L of Cd stress, the test groups containing QDs exhibited the highest increment in carotenoid content (~98.08%), while the treatment groups involving BPs showed the lowest carotenoid content (~88.90%), when compared to those levels observed under the exposure to Cd alone at 10 mg/L. A similar response pattern was noted for the binary mixtures involving 20 mg/L of Cd as well. Overall, under Cd stress, the treatment with QDs was more effective in enhancing the carotenoid levels than other ZnO particles.

3.7.2 Effect on yield of PS (II) and ETR

Fig. S5-A represents the influence of pristine ZnO particles on the maximum quantum yield of PS (II) and ETR of *A. sativum* leaves. It was observed that treatment with all three pristine ZnO particles had a positive impact on the Y(II) and ETR at all applied doses. Among the different doses of QDs, the 10 mg/L treatment showed the highest increment in Y(II) and ETR, with values increasing to ~120.74% and 117.64%, respectively, compared to the untreated control groups. However, the 20 mg/L treatment groups were noted to have the lowest increment, with values of ~103.71% and 102.96%, respectively, compared to the control setups. In the case of NPs and BPs, the Y(II) and ETR results followed a response pattern similar to that of QDs. In general, the 10 mg/L ZnO concentration showed the highest increase in Y(II) and ETR values, among which the test groups supplied with QDs showed the highest increment compared to those treated with NPs and BPs. The Y(II) and ETR values observed at 10 mg/L of NPs are ~113.78% and ~113.71%, and for BPs, the respective values of Y(II) and ETR at 10 mg/L were ~112.99% and 112.93%.

Fig. S5-B depicts the influence of binary mixtures of Cd and ZnO particles on the Y(II) and ETR values of *A. sativum* leaves. The treatment with 10 and 20 mg/L of Cd significantly ($p <$

0.001) decreased the Y(II) and ETR values. The Y(II) and ETR values observed at 10 mg/L Cd exposure were ~ 88.49% and 88.51%, respectively, and the values noted at 20 mg/L Cd treatment were ~ 80.88% and 81.08%. Upon the incorporation of ZnO particles into Cd solutions, the Y(II) and ETR values were found to be enhanced, with a significant ($p < .0001$) increase noted in Y(II) and ETR in the test groups exposed to QDs. Among the binary mixtures containing 10 mg/L of Cd, the highest increments in Y(II) and ETR values were observed in the trial groups exposed to QDs, with an increment of ~ 94.75% and ~94.92%, respectively, and the lowest Y(II) and ETR values were noted for the test groups exposed to BPs, having the increments of ~ 89.79% and ~89.96%, respectively. A similar response pattern was noted for the mixtures supplied with 20 mg/L of Cd as well. Overall, in the case of binary mixtures, treatments involving QDs were found to enhance Y(II) and ETR values compared to those treated with NPs and BPs.

3.8 Effects on total soluble sugars and protein levels

Fig. S6-A represents the effect of pristine ZnO particles on the total soluble sugar levels of *A. sativum* root cells. The treatment with ZnO particles had a positive influence on carbohydrate levels at all tested doses, particularly the treatments with QDs, which exhibited the highest increment in carbohydrate levels. Among the various test concentrations of QDs, the 10 mg/L exposure was noted to have the highest increment ($p < 0.001$, ~ 129.48%), while the 20 mg/L treatment showed the lowest increment (~109.15%) in carbohydrate level compared to the control group. A similar response pattern was noticed in the treatments involving NPs and BPs as well. Although the 10 mg/L exposure exhibited the maximum rise in carbohydrate content across all ZnO forms, the highest increment was observed in the test groups exposed to QDs compared to those treated with NPs (~123.89%) and BPs (~115.93%).

749 Fig. S6-B represents the impact of binary mixtures of Cd and ZnO particles on the sugar levels
 750 of *A. sativum* root cells. Exposure to 10 and 20 mg/L Cd resulted in a significant decrease ($p <$
 751 0.001) in carbohydrate levels, with decrements of $\sim 84.29\%$ and 78.55% , relative to the control
 752 groups. The carbohydrate levels were found to increase significantly ($p < 0.001$) upon the
 753 addition of ZnO particles to Cd solutions. Among the binary mixtures involving 10 mg/L of
 754 Cd, the highest increment in the carbohydrate level was noticed in the test groups exposed to
 755 QDs ($p < 0.001$, $\sim 106.17\%$), while the lowest increment was noticed in the trial groups exposed
 756 to BPs ($p < 0.001$, $\sim 93.37\%$), when compared to those levels observed at 10 mg/L of Cd alone.
 757 In the binary mixtures involving 20 mg/L of Cd, a similar response pattern was observed.

758 Fig. S6-C represents the influence of pristine ZnO particles on the protein content of *A. sativum*
 759 root cells. The application of ZnO particles significantly ($p < 0.001$) enhanced the protein
 760 content at all test concentrations studied. Among the different concentrations of QDs, the 10
 761 mg/L treatment exhibited the highest increase in protein level ($p < 0.001$, $\sim 145.70\%$), and the
 762 20 mg/L treatment showed the lowest increase ($p < 0.001$, $\sim 119.25\%$) relative to the control
 763 groups. A similar response pattern was observed with NPs and BPs as well. Overall, the 10
 764 mg/L dose was found to be effective in enhancing protein levels across all ZnO particle
 765 exposure. The highest increase was observed in the test groups supplied with QDs compared
 766 to those treated with NPs ($\sim 131.06\%$) and BPs ($\sim 125.25\%$).

767 Fig. S6-D depicts the influence of binary mixtures of Cd and ZnO particles on the protein levels
 768 of *A. sativum*. The treatment of roots with 10 and 20 mg/L of Cd significantly reduced ($p <$
 769 0.001) the protein content, with reductions of $\sim 82.06\%$ and 69.59% compared to the control
 770 setups. Whereas protein levels were found to increase significantly ($p < 0.001$) upon the
 771 addition of ZnO particles. Among the binary mixture treatments containing 10 mg/L Cd, the
 772 highest increment in protein content was noted in the treatment groups exposed to QDs ($p <$

0.001, ~ 93.99%). A similar response pattern was observed for the test groups containing binary mixtures of 20 mg/L Cd and ZnO particles.

4. Discussion

4.1 Physico-chemical interactions between ZnO particles and Cd

The FESEM images (Fig. S1) clearly show that the QDs possess a nearly spherical shape and remain monodispersed with better suspension stability. In their pristine state, NPs and BPs exhibited slight aggregation, with BPs being particularly characterised as thick, protrusive nanosheets, consistent with previous findings [57]. NPs and BPs generally tend to agglomerate into clusters when the van der Waals attractive forces dominate over the electrostatic repulsive forces [58]. Whereas, upon the interaction of ZnO dispersions with Cd, an increased agglomeration was noted, as seen in Fig. 1D-F. This pronounced agglomeration can be due to the attraction of Cd^{2+} ions to the negatively charged surface of the ZnO particles, forming a layer that effectively reduces the electrostatic repulsion forces that previously kept the ZnO particles apart. A similar agglomeration pattern was observed in a study conducted by Jassi et al. (2023), where the doping of Cd over ZnO samples led to agglomeration. Furthermore, the adsorption of Cd over ZnO particles is also confirmed by FTIR, XRD and Raman spectroscopic analysis. The shift in the frequency of the Zn-O bond peak and the reduction in intensity after interaction with Cd confirm the adsorption of Cd over ZnO particles. The findings by Thambidurai et al. (2023) reveal a similar shift in the absorption frequency of ZnO observed during FTIR analysis of Cd-doped ZnO nanocomposites. Additionally, distinct peaks at approximately 1085 cm^{-1} and 605 cm^{-1} in the absorption spectra of binary mixtures further validate the presence of adsorbed Cd over ZnO particles. A similar reduction in the intensity of the prominent ZnO peaks, and the emergence of additional peaks associated with Cd, was noted in the XRD patterns, as depicted in Fig. 1D-F. This indicates the successful adsorption

of Cd onto the ZnO particles. Moreover, in the XRD patterns of binary mixtures, there were no changes in the peak positions of ZnO, indicating that the ZnO crystal structure remains unaffected by Cd adsorption, which matches the similar findings put forth by Azqhandi et al. (2017). The adsorption of Cd over ZnO particles is further evidenced by the Raman spectroscopy (Fig. 1G-I), where the changes in the Raman spectra of binary mixtures were found to be consistent with FTIR and XRD results. More interestingly, the adsorption of Cd over ZnO particles followed a size-dependent order, i.e., QDs > NPs > BPs, as indicated by the varying peak intensities of the peak positioned at 1006 cm^{-1} in the binary mixtures. Thus, particle size significantly influences Cd adsorption, serving as an effective protective tool to combat heavy metal stress in plants.

The zeta potential of pristine ZnO particles was found to be comparatively stable in the absence of Cd (Table S2). However, after the interaction with Cd, a negligible decrease in the zeta potential values was observed, making the colloidal solution relatively less stable. This can possibly explain the agglomeration of ZnO particles upon Cd adsorption, which correlates with a slight decrease in the accumulation of ZnO particles in roots. Even though a decrease in the zeta potential was observed after interaction with Cd, QDs maintained their relative stability following the order QDs > NPs > BPs. The adsorption of Cd onto ZnO particles displays a consistent pattern of QDs > NPs > BPs, as evidenced by the decreased Cd accumulation in the roots.

From Table S3, as ZnO particles are inherently hydrophilic, the contact angle values further decreased slightly upon interaction with Cd, making them even more hydrophilic. The increased hydrophilicity led to an enhanced agglomeration, rendering the particles less stable, which is further correlated with the reduced zeta potential values. This increased agglomeration resulted in a slight reduction in Zn accumulation in roots, the total accumulation followed the

order QDs > NPs > BPs. Even though the contact angle values decreased upon interaction with Cd, the reduction was found to be marginal, which explains the possible stability of ZnO particles even after Cd adsorption. The order of adsorption of Cd over ZnO particles followed the order QDs > NPs > BPs. Furthermore, the greater the adsorption of Cd over ZnO particles, the lower the Cd accumulation in the root, which accounts for the improved growth observed in the test groups exposed to the binary mixtures.

4.2 Effect of pristine ZnO particles, Cd, and their binary mixtures on the growth parameters and metal accumulation in *A. sativum* roots

Zinc is an essential micronutrient that triggers several key physiological and molecular processes in plants, resulting in enhanced growth [62]. Exposure to ZnO particles resulted in increased cell viability and root growth at all supplied doses. Notably, the efficacy of QDs in increasing the cell viability/root growth surpassed that of the nano and conventional bulk forms. The improved efficacy of QDs in enhancing plant growth could be attributed to their enhanced colloidal stability and reduced hydrophilicity, as evidenced by zeta potential and contact angle results. This combination favours the greater accumulation of QDs in plant roots compared to nano and bulk forms. Previous findings reported by Broadley et al. (2007); Marschner. (2011) stated that Zn derived from ZnO particles enters root tissues predominantly through dissolution-mediated Zn^{2+} release, followed by the uptake via ZIP (Zinc-regulated/Iron-regulated transporter-like Protein) family transporters. Once inside the root, Zn is regulated by a coordinated network of heavy metal ATPases (HMAs) and sequestration mechanisms to maintain homeostasis while avoiding cytotoxic accumulation. [65, 66]. Several studies have reported on the effects of ZnO particles on plant growth and viability. For instance, previous studies have shown that the foliar spraying of 1000 mg/L ZnO NPs increased the cell viability of mustard plant (*Brassica juncea* (L.) Czern [67]. Similarly, in another study, the

application of 75 µg/mL of ZnO NPs to tomato plants resulted in an increase in root length of up to ~ 33%. Furthermore, it was reported that the foliar application of ZnO QDs at 50 mg/L to pumpkin (*Cucurbita moschata* Duch.) increased the total biomass of the plant by ~ 24%, resulting in overall improved plant growth [24]. A similar increase in fresh biomass is observed in lettuce supplied with 100 mg/L ZnO QDs through foliar application [23]. Existing studies have indicated that the mechanism by which ZnO nanoforms enhance growth may be attributed to the increase in the bioavailability of the micronutrient Zn²⁺. This augmentation of Zn²⁺ levels is thought to favour DNA replication and activate various transcription factors, ultimately leading to enhanced meristematic activity and growth in plant tissues [68, 69]. In our study, cell viability/root length increased dose-dependently up to 10 mg/L, after which a slight decrease (at 20 mg/L) in growth was observed; however, no toxic effects were noted, indicating the onset of a hormetic response. This highlights a key finding that exposure to ZnO particles at levels exceeding 20 mg/L could have toxic effects on garlic growth and development. A similar response was observed in *G. biloba* seedlings as well [70]. Even though numerous studies have demonstrated the role of Zn in promoting plant growth, further research is necessary to determine the threshold concentrations and elucidate the regulatory mechanisms underlying its effects.

Exposure to Cd at concentrations of 10 mg/L and 20 mg/L led to a significant decrease in both cell viability and root length of *A. sativum*. Notably, the extent of damage was more pronounced at the higher concentration of 20 mg/L. Cd is known to inhibit root growth by triggering excessive accumulation of ROS and membrane damage (Fig. 5(A-D)), eventually affecting the growth and viability of garlic cells [71]. Our results were consistent with previous findings, where exposure to Cd reduced root growth in garlic, decreased cell viability in synchronised

868 tobacco BY-2 cell cultures, and also resulted in a decrease in cell viability in small onion
869 (*Allium cepa* L.) and broad bean (*Vicia faba*) [72–74].

870 The addition of ZnO particles into Cd solutions markedly mitigated Cd toxicity, enhancing cell
871 viability and root length in the exposed test groups. Among the three forms of ZnO particles,
872 QDs exhibited a greater efficacy in alleviating Cd stress. This could be attributed to the
873 increased adsorption of Cd over QDs, which has led to a reduction in Cd accumulation in the
874 roots (Table S4), thereby reflecting a decrease in toxicity. Furthermore, among the binary
875 mixtures, the combinations involving QDs exhibited better stability, which also favoured an
876 increased accumulation of QDs, positively impacting growth by supplying Zn^{2+} . A similar
877 ameliorative effect was observed in the medicinal plant *Salvia miltiorrhiza*, where the foliar
878 application of 100 mg/L of ZnO QDs mitigated Cd stress by reducing the Cd accumulation and
879 improving overall biomass and plant growth [22]. In another study, the exposure to 25 μM of
880 ZnO NPs resulted in improved cell viability in chickpea under Cd stress [75]. Furthermore, the
881 application of 50 and 100 mg/L nano-ZnO to rice under As stress decreased the As
882 accumulation in roots and improved plant root biomass by enhancing the plant's resistance to
883 As stress [76]. In our study, the ameliorative effects of ZnO particles were more pronounced
884 under 10 mg/L of Cd stress as compared to 20 mg/L. This could be attributed to the increased
885 hydrophilicity and reduced colloidal stability of ZnO particles in combination with 20 mg/L of
886 Cd (Table S2). The observed phenomenon may be due to the potential sedimentation of ZnO
887 nanoparticles under gravitational forces, which in turn could contribute to a decrease in their
888 ameliorative efficacy under 20 mg/L of Cd stress [77].

889 The Leaching analysis revealed that ZnO QDs released higher concentrations of Zn^{2+} ions
890 compared to NPs and BPs. This finding aligns with the notion that smaller nanoparticles
891 possess greater surface area and reactivity, which accelerates ion release in aqueous

environments [78, 79]. However, the concentration of dissolved ions did not significantly enhance biological responses such as cell viability and root elongation (Fig. S3-A & C). This suggests that the leached Zn^{2+} ions alone were not the primary factor driving the observed growth responses [80, 81]. Conversely, exposure to particulate forms of ZnO (QDs, NPs, and BPs) led to notable improvements in garlic growth parameters, indicating that nano-specific effects extend beyond mere ionic nutrition. The smaller particle size and increased surface area of QDs and NPs likely promote better interactions with root surfaces, enhanced dispersion, and improved Zn availability. These factors contribute to enhanced physiological performance compared to larger bulk particles [82, 83]. Overall, the combined effects of Zn^{2+} release and particle-specific surface interactions play a crucial role in the enhanced growth-promoting effects of nanoscale ZnO particles.

Similarly, the treatment of *A. sativum* with ZnSO_4 (ionic control) showed an insignificant decrease ($p > 0.05$) across all the growth, oxidative stress, and photosynthetic measurements, in comparison with the control (Fig. S7 and S8). Previous studies have reported that Zn^{2+} ions, when provided in an immediately soluble form, can more rapidly accumulate in plant tissues, disrupting cellular ion homeostasis and causing mild phytotoxic effects [84]. In contrast, ZnO particles function as a source of sustained Zn^{2+} ions at the root interface, thereby supporting plant physiological processes without inducing ionic stress. The findings of the current study align with those of previous research [85, 86].

The exposure of *A. sativum* to ZnO particles resulted in increased cell division, as indicated by an increase in MI%. The possible mechanism could be due to the triggering of enzymatic reactions by Zn^{2+} , which activates the cascade of events that ultimately induce cell division at the root meristems [87]. The results from our study are consistent with those from previously published studies, in which the treatment of *Vicia faba* with ZnO NPs (50 and 2000 mg/L)

916 induced a significant increase in MI [88]. Similarly, nanopriming the seeds of black gram with
917 80 mg/L of ZnO QDs resulted in a significant increase in meristematic division, leading to an
918 improved germination rate [89]. Exposure to ZnO nanoparticles affected the mitotic index (MI)
919 of *Vicia faba* seedlings, with low concentrations (10 and 25 mg/L) boosting MI, while higher
920 levels (50 and 100 mg/L) significantly decreased cell division [90]. Research indicates that Zn
921 may enhance cell division by enhancing auxin biosynthesis and upregulating genes involved
922 in the cell cycle and auxin transporters. In a study by Raskar and Laware. (2014), it was
923 demonstrated that ZnO NPs effectively stimulated cell division in root meristematic tissues at
924 low concentrations.. Similarly, in a study conducted by Zhao et al. (2025), it was observed that
925 ZnO NPs promoted cell division at low concentrations, which was attributed to the
926 upregulation of genes involved in auxin synthesis.

927 Cd exposure to *A. sativum* resulted in a significant reduction in MI. Cd is known to cause
928 excessive ROS generation, which damages the cell membrane, disrupts cell division and
929 inhibits root elongation [93]. Results from our study align with previous findings, which
930 demonstrated that exposure to 25 μ M Cd in onions (*Allium cepa*) resulted in a decrease in MI
931 [94]. In line with these findings, Alsam et al. (2014) demonstrated that supplying Cd (20-100
932 mg/L) to *Capsicum annuum* resulted in a decrease in MI [95].

933 MI has been noted to be enhanced in the *A. sativum* roots exposed to the binary mixtures. The
934 possible mechanism behind the improvement in MI in the binary mixture exposure can be
935 attributed to the noticeable decrease in ROS accumulation and MDA levels (Fig. 5 B and D),
936 which is consistent with the restored cell viability and root length results. Although there are
937 no extensive studies that explicitly discuss how ZnO particles restore MI in the presence of Cd
938 stress, a few studies are available that indirectly relate to restored cell division by analysing
939 physiological markers, such as restored root growth and meristem function. For instance, the

940 application of ZnO QDs to lettuce and *S. miltiorrhiza* under Cd stress enhanced plant biomass
941 and cell proliferation in roots, suggesting improved meristematic division [22, 23]. The exact
942 mechanism by which ZnO particles improve cell proliferation and restore root damage under
943 Cd stress warrants further investigation.

944 The FESEM images of *A. sativum* roots exposed to pristine ZnO particles revealed defined
945 structural morphology. Exposure to Cd distorted the root structure, resulting in an overall
946 damaged or rugged surface. This pronounced damage may be due to the accumulation of Cd
947 in root tips, which could potentially interfere with the key enzymatic reactions involved in cell
948 wall synthesis and inhibit them. Cd can damage root surfaces by generating ROS in root cells,
949 leading to compromised cell membrane integrity and disrupted cell wall structure [96].
950 However, after the addition of ZnO particles to Cd solutions, the root morphology was restored
951 to a certain extent. This could be attributed to the reduced Cd accumulation in the presence of
952 ZnO particles, resulting in decreased toxic effects of Cd.

953 The release of Zn^{2+} from ZnO particles was observed to be relatively minimal. As a result, the
954 ionic contributions to uptake were lower than those of pristine ZnO particles at 10 mg/L. A
955 similar effect was observed in tomato and *Raphanus sativus*, where the released Zn^{2+} did not
956 significantly reduce the root growth metrics [97, 98]. The leaching of Zn^{2+} was further reduced
957 upon interaction with Cd. This may be due to the adsorption of Cd over the ZnO surface, which
958 could have led to increased agglomeration of ZnO particles, thereby limiting their leaching.

959 The results of this study were consistent with previous findings, which demostarted that
960 treating *Salvia miltiorrhiza* and rice with ZnO QDs and NPs reduced Cd toxicity [22, 99].
961 Furthermore, the accumulation of Zn was higher in *A. sativum* roots exposed to pristine ZnO
962 particles. This increased Zn accumulation could possibly explain the enhanced growth and
963 viability of *A. sativum*. Furthermore, the amount of Cd accumulation was reduced in the

presence of ZnO particles, potentially decreasing its detrimental effects as well. Similar findings were reported in a previous study, where treatment of alfalfa seedlings with CaO NPs reduced Cd accumulation and toxicity [100]. While enhanced Zn accumulation contributes positively to plant growth, the intricate mechanisms governing its transport and regulation within plant systems warrant further investigation.

4.3 Effect of pristine ZnO particles, Cd, and their binary mixtures on the oxidative stress and photosynthetic parameters of *A. sativum*

ROS can serve as signalling molecules at low concentrations, but at high concentrations, ROS can cause damage to essential macromolecules, including proteins, lipids, and nucleic acids, leading to the accumulation of MDA. Antioxidant enzymes, such as SOD and CAT, are crucial for maintaining redox homeostasis [101]. In the present investigation, exposure to pristine ZnO particles at all the supplied concentrations did not increase ROS and MDA levels. These findings align with the decreased activity of key antioxidant enzymes, SOD and CAT, indicating that the ZnO exposure did not disturb the cell's redox balance [102]. Similar findings have been reported in previous studies on ZnO QDs and NPs [17, 23, 103]. Cd is known to disrupt cellular redox equilibrium, leading to the overproduction of ROS that can damage the cellular membrane. Exposure to Cd resulted in a significant increase in ROS and MDA levels in *A. sativum*. This correlates with the increased antioxidant enzyme activity in Cd-stress garlic roots. However, the addition of ZnO particles significantly reduced ROS and MDA levels under Cd stress. This demonstrates the protective effects of ZnO particles, particularly QDs, in effectively alleviating the Cd-induced oxidative damage. The proposed mechanism behind the mitigation effect of ZnO particles can be attributed to the adsorption of Cd onto ZnO particles, resulting in a subsequent reduction in Cd accumulation in roots and lower levels of ROS and MDA. This reduction in the stress markers has normalised the plant's necessity to produce

more antioxidant enzymes. Similar findings have been reported in previous studies where the levels have been reduced in the presence of ZnO QDs and NPs. Similar findings were reported for *S. miltiorrhiza* and *Lycopersicon esculentum* [22, 104]. The exact mechanism of how the incorporation of ZnO particles regulates redox homeostasis still warrants further investigation. Zinc is a cofactor for enzymes involved in photosynthesis, enhancing the synthesis of photosynthetic pigments by improving chlorophyll's ability to capture light and transfer energy [105]. The exposure of *A. sativum* to ZnO particles significantly increased its chlorophyll pigment levels and efficiency. The observed results were found to be consistent with previous findings reported for *Ligusticum chuanxiong* and soybean [106, 107]. Earlier studies have shown that the application of ZnO particles provide bioavailable Zn²⁺ ions, which serve as crucial cofactors for numerous enzymes involved in the biosynthesis of chlorophyll and carbon fixation processes [108]. Further, previous transcriptomic analyses indicate that treatment with ZnO NPs leads to the upregulation of genes linked to light-harvesting complexes and photosynthetic electron transport, resulting in enhanced photochemical efficiency of PSII. Additionally, it was reported that ZnO NPs elevate both the content and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), thereby promoting the fixation of CO₂. Collectively, these responses contribute to improved chlorophyll levels, enhanced PSII efficiency, and an overall increase in photosynthetic performance [109, 110]. In the present study, the exposure of *A. sativum* to Cd stress markedly reduced the photosynthetic pigment contents and efficiency (Y(II) and ETR). Similar results have been reported in previous studies as well [111, 112]. The supply of ZnO particles to Cd-stressed garlic improved the levels and efficiency of photosynthetic pigments, which is consistent with previous reports [22, 113]. Additionally, the beneficial impact of ZnO particles in alleviating Cd stress may be linked to a reduction in oxidative damage and Cd accumulation in plants subjected to binary mixtures.

Primary metabolites are essential for plants to overcome stress. The exposure of *A. sativum* to ZnO particles significantly increased the total soluble sugar and protein content [114]. Prior research has shown that Zn enhances the levels of proteins and carbohydrates by acting as a crucial cofactor for enzymes and transcription factors that play key roles in nitrogen metabolism and protein synthesis [115, 116]. Further, it was reported that higher photosynthetic performance induced by Zn leads to enhanced sugar and carbohydrate assimilation, which could be a possible mechanism by which Zn promotes sugar levels in plants [109]. Cd stress significantly decreased the soluble sugar and protein content in *A. sativum*, while the addition of ZnO particles to Cd-stressed plants improved protein and sugar levels. This could be attributed to the release of Zn^{2+} , which facilitates key metabolic functions, such as protein synthesis. This increase in primary metabolites may possibly benefit plants by helping maintain ROS balance and control photosynthetic activity.

4.4 Cluster Heatmap, Pearson correlation mapping and PCA

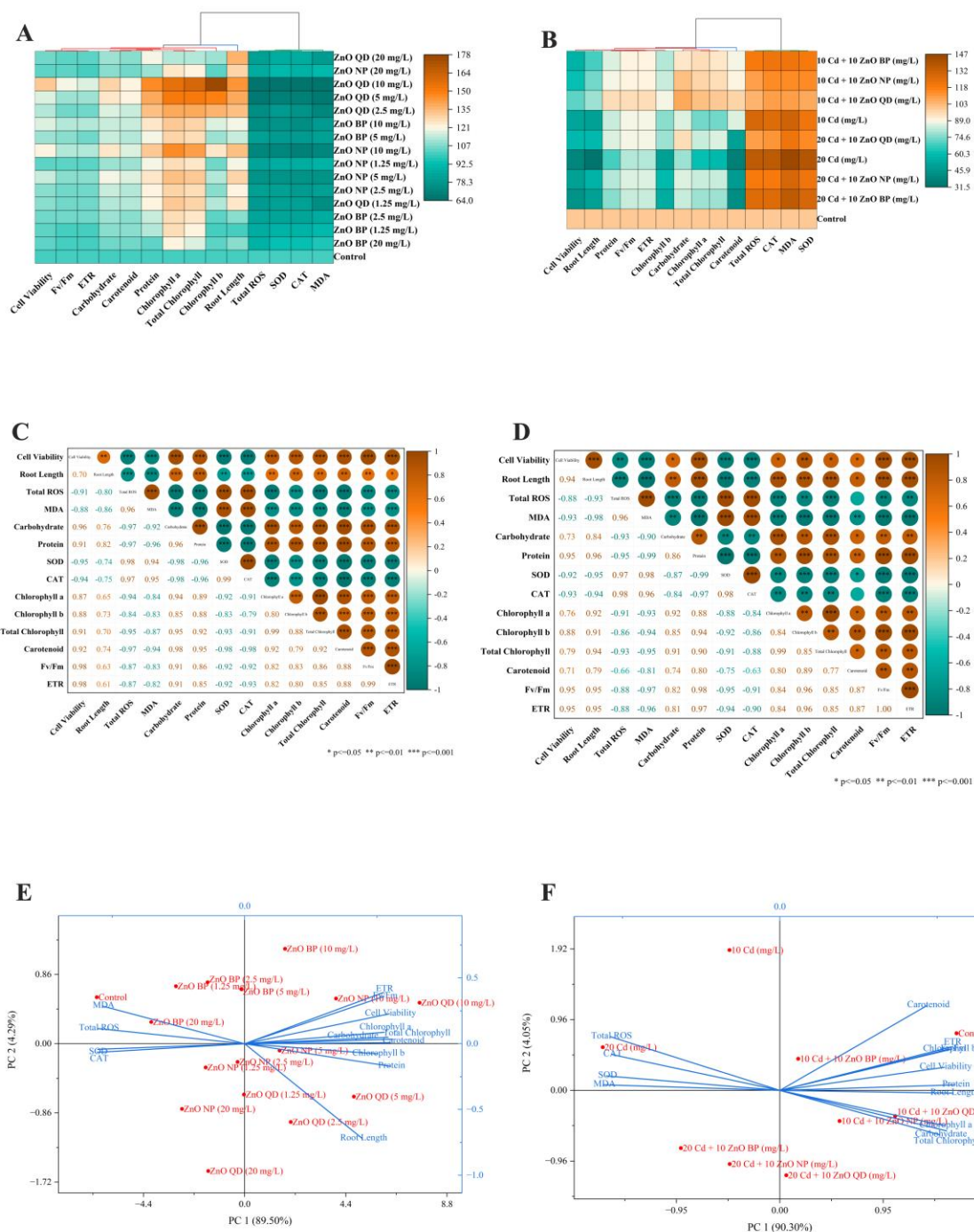


Fig. 7. Cluster heatmap of various biological parameters of *A. sativum* exposed to A) pristine ZnO particles, B) mixtures of Cd + ZnO particles. Pearson correlogram of various biological parameters of *A. sativum* exposed to C) pristine ZnO particles, D) mixtures of Cd + ZnO particles. “*” indicates the significant difference between the

control groups and various treatments. Principal component analysis (PCA) of various biological parameters of *A. sativum* exposed to E) pristine ZnO particles, F) mixtures of Cd + ZnO particles.

The clustered heat map analysis illustrating the effects of different pristine ZnO particles (QDs, NPs and BPs) on various growth, physiological, and biochemical parameters is presented in Fig. 7A. The data indicate that the treatments involving ZnO QDs and NPs at moderate concentrations, specifically at 5 and 10 mg/L, form a distinct cluster linked to enhanced positive responses. This is represented by an orange hue for metrics such as root length, chlorophyll content, carbohydrate content, protein content, and cell viability. Conversely, the control groups and treatments with lower concentrations are clustered separately, showing lighter greenish tones that suggest lower stimulation levels in these growth and biochemical metrics. Additionally, oxidative stress indicators, including total ROS, MDA, SOD and CAT, are grouped into a separate cluster characterised by dark green colouration. This finding suggests that exposure to ZnO particles did not induce oxidative stress in *A. sativum* cells. Hierarchical clustering analysis further indicates that treatments with ZnO QDs exhibit superior enhancement patterns, underscoring their greater impact on plant growth and physiological processes compared to nano and bulk particle treatments.

Fig. 7B illustrates the clustered heat map of various physiological and biochemical responses in *A. sativum* exposed to Cd stress alone and in combination with different ZnO particles, QDs, NPs, and BPs. Treatments exposed to Cd stress alone cluster with higher oxidative stress indicators (orange shades), reflecting elevated stress responses. However, treatments supplemented with ZnO particles, especially QDs and NPs, shift toward greener tones, indicating reduced oxidative damage and improved antioxidant activity. The clustering pattern suggests that ZnO treatments alleviate Cd-induced stress, likely through enhanced antioxidant

defense and improved physiological balance. Overall, the dendrogram indicates that ZnO-treated groups are more closely related in their stress-amelioration responses, distinguishing them from Cd-only conditions.

The Pearson correlation analysis provided in Fig. 7C illustrates the relationship among various growth, physiological, and biochemical parameters in response to different treatments with pristine ZnO particles. A robust positive correlation ($p < 0.001$) is evident among growth-related traits, including cell viability and root length, alongside photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and total chlorophyll) and crucial biochemical constituents such as carbohydrate and protein levels. This positive correlation indicates that treatment with ZnO particles enhanced photosynthetic efficiency and biochemical composition, which are directly linked to improved plant growth and overall performance. Conversely, stress-related parameters, including total ROS, MDA, SOD, and CAT, demonstrate significant negative correlations ($p < 0.001$) with growth traits like cell viability and root length. This suggests that reduced stress conditions may enhance physiological performance in plants.

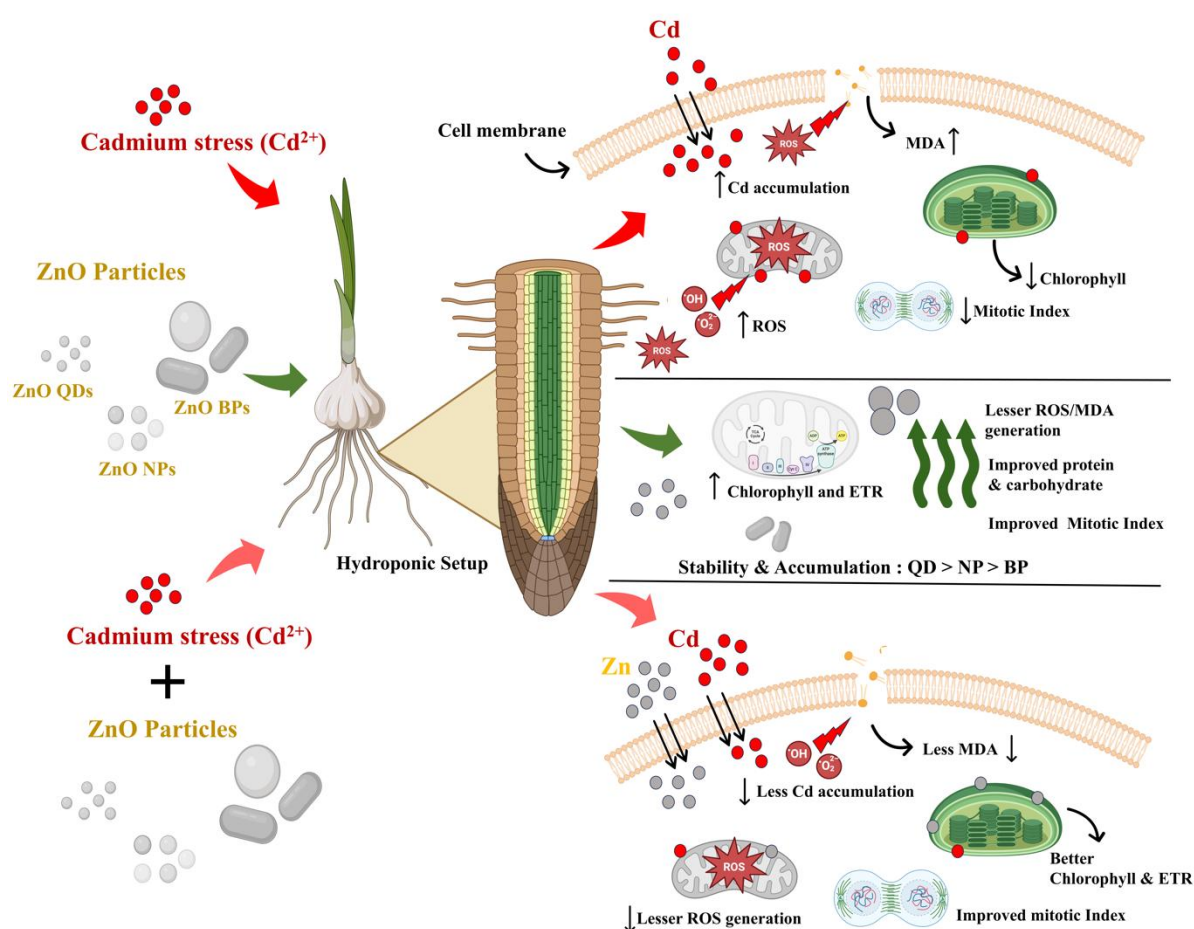
Fig. 7D illustrates interrelationships among the growth, physiological and biochemical parameters under Cd stress, which are alleviated by ZnO treatments. A pronounced positive correlation was observed between cell viability, root length, protein, carbohydrate, photosynthetic pigments (chlorophyll *a*, *b*, and total chlorophyll), and photosynthetic efficiency parameters (F_v/F_m , ETR), suggesting that improved biochemical and photosynthetic performance in plants treated with ZnO particles. Conversely, total ROS and MDA exhibited strong negative correlations ($r = -0.88$ to -0.98 , $p < 0.001$) with growth, pigment, and biochemical indices, indicating the Cd-induced oxidative stress and physiological damage. Their high positive association with antioxidant enzymes (SOD and CAT) suggests that elevated antioxidant activity is a protective response aimed at limiting oxidative damage.

Furthermore, the tight positive clustering among protein, chlorophyll, and Fv/Fm ($r > 0.95$, $p < 0.001$) indicates a coordinated increase in photosynthetic parameters and biochemical levels in ZnO-treated plants.

Principal component analysis (Fig. 7E) was performed to elucidate the size and concentration-dependent responses of *A. sativum* to different ZnO formulations, QDs, NPs, and BPs. The PCA biplot revealed a clear separation of treatments along the first principal component (PC1), which accounted for 89.50% of the total variance, indicating that the majority of the variation among treatments was driven by differences in particle size and concentration. The second component (PC2) accounted for an additional 4.29% of the variance, indicating minor differences among treatments. Treatments located on the positive side of PC1, mainly corresponding to ZnO QDs and NPs at moderate concentrations (2.5–10 mg/L), showed strong positive associations with growth-promoting and physiological parameters, including chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, protein, carbohydrate content, root length, and cell viability. This clustering pattern suggests that smaller ZnO particles, particularly QDs, at optimal doses, enhanced photosynthetic efficiency and biochemical levels, thereby supporting overall plant growth. Conversely, the control and higher concentration treatments of ZnO BPs and NPs (particularly 20 mg/L) were positioned on the negative side of PC1 and aligned with higher levels of oxidative stress markers such as ROS, MDA, SOD and CAT. This association suggests that excessive or larger-sized ZnO particles induce oxidative stress responses, potentially leading to cellular damage and reduced physiological performance.

Fig. 7F elucidates the interactions of *A. sativum* exposed to Cd stress alone and in combination with ZnO particles of varying dimensionalities- QDs, NPs and BPs on the physiological and biochemical responses of *A. sativum*. The biplot reveals a clear separation of treatments along

PC1 (90.30% of total variance), indicating that the primary variation arises from the differential response of plants to Cd toxicity and ZnO-mediated stress alleviation. Treatments exposed solely to Cd (10 and 20 mg/L) clustered distinctly on the negative side of PC1 and were closely associated with oxidative stress markers such as total ROS, MDA, SOD, and CAT, indicating severe oxidative damage. Conversely, treatments receiving combined Cd and ZnO particles (10 mg/L) were positioned toward the positive side of PC1, exhibiting strong positive associations with chlorophyll pigments, carotenoids, protein content, carbohydrate content, root length, and cell viability. Among these, the Cd + ZnO QD and Cd + ZnO NP groups exhibited the most favourable correlations with photosynthetic and biochemical parameters, indicating that smaller ZnO structures exhibited better efficacy in mitigating Cd-induced stress.



Scheme 1. Schematic representation of the overall mechanism by which ZnO QDs, NPs, and BPs alleviate Cd-induced stress and enhance the growth of *A. sativum*

4.4. Physiological mechanism underlying the ZnO particles-mediated alleviation of Cd-stress

Cd is a non-redox active metal that enhances the generation of ROS by interfering with the mitochondrial electron transport chain. This interference results in increased electron leakage, which subsequently raises ROS levels. When plants are subjected to Cd stress, the accumulation of ROS exceeds typical metabolic rates, causing oxidative damage, lipid peroxidation, and detrimental effects on growth and physiological functions (**Scheme 1**) [117]. It has been reported that Cd primarily affects PSII which is more susceptible than PSI. Furthermore, Cd disrupts the reaction centres of PSII and inhibits the activity of RuBisCo, resulting in reduced CO₂ assimilation. As a result, the production of photosynthates like glucose, sucrose, and starch declines, leading to a reduction in the total carbohydrate levels in plant tissues [118]. Previous research has shown that exposure to Cd downregulates psbA transcription and accelerates the degradation of the D1 protein [119]. A similar effect has been documented in lettuce, where Cd exposure altered the expression of photosynthesis-related proteins and inhibited the production of photosynthetic pigments [120]. Moreover, Cd has been shown to interfere with enzymes involved in nitrogen assimilation, consequently reducing the conversion of nitrate to amino acids [121]. The resultant scarcity of amino acids significantly hampers protein biosynthesis. Additionally, Cd can bind to sulfhydryl (–SH) groups on proteins and enzymes, altering their structure and function, further inhibiting protein synthesis [122]. In the current study, the exposure of garlic to Cd resulted in significant growth reduction, elevated ROS levels, and diminished photosynthetic pigment concentrations, adversely affecting overall plant growth. However, in the presence of ZnO particles, Cd-induced toxicity was found to be

alleviated. Among the various ZnO particles, QDs demonstrated superior efficacy in mitigating Cd stress, followed by nano and bulk forms. This differential biological response of *A. sativum* to ZnO QDs, NPs, and BPs under Cd stress can be elucidated by the size-dependent physicochemical properties of ZnO and their ensuing interactions with bioaccumulation. ZnO QDs, due to their ultra-small size, possess a higher surface area to volume ratio and enhanced colloidal stability, as evidenced by favourable zeta potential and contact angle values (Tables S2 & S3), allows them to remain well dispersed in the exposure medium, minimising aggregation and sedimentation and thus increasing the bioavailable Zn fraction at the root-solution interface and their subsequent bioaccumulation under both pristine and Cd-stressed conditions.

Under Cd stress, ZnO QDs demonstrated a more effective alleviation of stress compared to NPs and BPs. Previous research has suggested that this phenomenon may be linked to the enhanced competitive inhibition of Cd accumulation by Zn [123–125]. In particular, studies by Chen et al. (2024) and Sun et al. (2019) demonstrated that the adsorption of Cd onto ZnO particles resulted in a significant reduction of Cd accumulation in *Lemna minor* and *Medicago sativa* L., leading to diminished toxicity. Likewise, the present investigation observed lower Cd accumulation in the QDs-treated groups than in those exposed to NPs or BPs (Table S4). This disparity can be attributed to the superior adsorption capabilities of ZnO QDs, which are facilitated by their larger surface area-to-volume ratio when compared to their nano and bulk counterparts. The decrease in Cd accumulation correlates with reduced generation of ROS and lower levels of lipid peroxidation, as indicated by MDA formation. Additionally, there is evidence of enhanced photosynthetic function. In contrast, plants treated with ZnO NPs exhibited moderate protection, while those exposed to BPs showed minimal mitigation of Cd toxicity. The findings of the current work are in agreement with the previous findings put forth

by Chai et al. (2024); Xu et al. (2022). This antagonistic interaction between Zn and Cd has also been previously reported and offers a plausible explanation for the observed decrease in Cd accumulation and the improved physiological performance of garlic plants treated with QDs [108, 129, 130].

4.5 Environmental risk assessment

The RQ analysis (Table 2) indicated a significant decrease in the risks associated with Cd following treatments with ZnO particles, particularly with QDs. The RQ values for Cd alone were notably elevated across all regions studied, ranging from 3.86 to 791.77, highlighting a serious potential ecological threat. However, after applying ZnO QDs, these RQ values dropped significantly, ranging from 0.52 to 85.31, demonstrating effective risk reduction. While the RQ values for ZnO NPs and BPs in conjunction with Cd were slightly higher than those for QDs, they were still much lower than the values for Cd alone, indicating partial metal toxicity mitigation. The most significant reductions were observed in soils from the European Union, Japan, and New Zealand, where treatments combining QDs and Cd lowered RQ values to near-safe levels (< 10). Overall, the findings demonstrate that ZnO QDs have the greatest potential to reduce Cd-related environmental risks, followed by NPs and BPs, emphasising the importance of particle size in reducing environmental toxicity in contaminated soils.

1179 **Table 2: Risk quotient analysis of Cd in the presence and absence of ZnO particles (QDs, NPs and BPs) in different regions**

1180

Source	Available Concentration of Cd in soil (mg/kg)		RQ of Cd		RQ of QD + Cd		RQ of NP + Cd		RQ of BP + Cd	
	Lower range	Higher range	Lower range	Higher range	Lower range	Higher range	Lower range	Higher range	Lower range	Higher range
Germany	0.3	1	28.97	96.56	3.12	10.40	4.34	14.48	26.75	89.17
UK	0.6	0.7	57.93	67.59	6.24	7.28	8.69	10.13	53.50	62.42
China	0.25	6.92	24.14	668.18	2.60	71.99	3.62	100.19	22.29	617.08
European union	0.07	1	6.76	96.56	0.73	10.40	1.01	14.48	6.24	89.17
Japan	0.05	0.59	4.83	56.97	0.52	6.14	0.72	8.54	4.46	52.61
India	0.1	8.20	9.66	791.77	1.04	85.31	1.45	118.72	8.92	731.22
South America	0.04	3	3.86	289.67	0.42	31.21	0.58	43.43	3.57	267.52
North America	0.16	4.5	15.45	434.51	1.66	46.81	2.32	65.15	14.27	401.28
Belgium	1	1.5	96.56	144.84	10.40	15.60	14.48	21.72	89.17	133.76
New Zealand	0.1	2.03	9.66	196.01	1.04	21.12	1.45	29.39	8.92	181.02

5. Conclusion

This study demonstrated that ZnO particles distinctly influence the growth and Cd stress response of *A. sativum*, with particle size emerging as a critical determinant of biological efficacy. Among the three forms, ZnO QDs exhibited the most pronounced growth-promoting and stress-alleviating effect, significantly enhancing cell viability, pigment stability, and root morphology while reducing oxidative damage. Under Cd stress, the test groups exposed to ZnO QDs exhibited the lowest Cd accumulation and improved photosynthetic pigments and primary metabolites, indicating their potential for safe agricultural application in Cd-stressed conditions. The evaluation of the environmental RQ confirmed a minimal ecological risk associated with the optimised ZnO QD treatment compared to those with NPs and BPs. Collectively, these findings establish ZnO QDs as an effective nano-intervention for mitigating Cd-induced phytotoxicity and improving plant resilience. Governing Cd stress in food crops, such as *A. sativum*, is essential to ensure crop productivity, food quality, and long-term food security, making nanotechnology-based approaches a promising pathway for sustainable agricultural advancement.

1197 **Ethics, Consent to Participate, and Consent to Publish declarations:** Not applicable

1198

1199 **Availability of data and material:** The datasets used and/or analysed during the current study
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1216

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