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# The alleviating effect on the growth, chlorophyll synthesis, and biochemical defense system in sunflowers under cadmium stress achieved through foliar application of humic acid

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## Abstract

**Background** With the progress of industrialization and urbanization, cadmium (Cd) pollution in farmland is increasingly severe, greatly affecting human health. Sunflowers possess high resistance to Cd stress and great potential for phytoremediation of Cd-contaminated soil. Previous studies have shown that humic acid (HA) effectively mitigates plant damage induced by Cd; however, its alleviating effects on sunflower plants under Cd stress remain largely unknown.

**Results** We employed four different concentrations of HA (50, 100, 200, and 300 mg $L^{-1}$) via foliar application to examine their ability to alleviate Cd stress on sunflower plants' growth, chlorophyll synthesis, and biochemical defense system. The results revealed that Cd stress not only reduced plant height, stem diameter, fresh and dry weight, and chlorophyll content in sunflower plants but also altered their chlorophyll fluorescence characteristics compared to the control group. After Cd stress, the photosynthetic structure was damaged and the number of PSII reactive centers per unit changed. Application of 200 mg $L^{-1}$ HA promotes sunflower growth and increases chlorophyll content. HA significantly enhances antioxidant enzyme activities (SOD, POD, CAT, and APX) and reduces ROS content ($O_2^-$, $H_2O_2$ and $^-OH$). Totally, Application of 200 mg $L^{-1}$ HA had the best effect than other concentrations to alleviate the Cd-induced stress in sunflower plants.

**Conclusions** The foliar application of certain HA concentration exhibited the most effective alleviation of Cd-induced stress on sunflower plants. It can enhance the light energy utilization and antioxidant enzyme activities, while reduce ROS contents in sunflower plants. These findings provide a theoretical basis for using HA to mitigate Cd stress in sunflowers.

**Keywords** Alleviating effect, Cd-induced stress, Foliar application, Humic acid

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## Background

Recently, the rapid growth of industries has caused a significant increase in heavy metal contamination in agricultural soil, mainly due to human activities and industrial emissions. Cadmium (Cd), an extremely toxic metallic element, can disrupt the normal physiological functions of living organisms and is particularly difficult to biodegrade [1]. Generally, Cd can pose toxicity risks to plants, even at low concentrations [2]. The Cd accumulation in farmland soil and its involvement in the food chain pose a serious threat to both agricultural products and public health [3]. Therefore, it is urgent to solve soil Cd pollution promptly. Accordingly, phytoextraction is a cost-effective and eco-friendly approach compared to physical and chemical methods [4]. Sunflower (*Helianthus annuus* L.), a member of the Compositae family, ranks as the fourth most significant oil crop globally. The robust root system grants it high stress resistance, while its diverse genetic makeup allows it to thrive in various environments [5]. As one of the most significant economic and ornamental crops, sunflowers are characterized by its substantial biomass and ability to grow in heavy metal-contaminated soil, and have immense potential for phytoremediation of Cd pollution [6, 7].

The absorption of Cd by plants through their roots in the soil typically causes initial root damage, altering cell membrane permeability and hindering the element's transport within the membrane [8]. Consequently, it curtails the plant roots' ability to absorb vital mineral nutrients [9]. Cd is commonly found as $Cd^{2+}$ and also exists in the form of Cd-chelates in the soil solution [10]. The Cd-chelate is generally stable across an extensive pH range, and can help plants reduce the damage of $Cd^{2+}$. Due to identical charge, and similar ionic radius and chemical behavior, excess $Cd^{2+}$ in soil has negative effect on the $Ca^{2+}$ uptake [11]. $Cd^{2+}$ can also impede the activities of nitrate reductase and nitrite reductase in plants, subsequently diminishing the capacity of their roots to adsorb nitrate [12]. Moreover, Cd stress can increase the production of reactive oxygen species (ROS) and cause oxidative damage to biofilms, proteins, or DNA [13]. For instance, the production of MDA and DNA damage caused by Cd stress can decrease the photosynthesis, and hence seriously affect the growth and development of plants [14, 15]. The antioxidant enzyme system concurrently responds to excess ROS by increasing the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) under Cd stress [16]. The continuous exposure to Cd stress reduces plants' antioxidant capacity and photosynthesis, thereby impacting their growth, development, and yield formation [14, 15].

Numerous strategies are currently being investigated to enhance plant tolerance to environmental stress, such as the utilization of nanoparticles [17, 18], chelators [6], cell signaling molecules [19, 20] and micronutrient fertilizers [21]. Recently, the use of plant growth regulators (PGR) in high Cd-resistant plants has gained significant attention for protecting them from heavy metal-induced damage [22]. The organic compound humic acid (HA) is commonly found in soil, water, weathered coal, peat, and lignite, making it a prevalent component of the natural environment [23, 24]. On the one hand, the increase in HA level enhances nutrient absorption, cell permeability, and the plant growth stimulation response mechanism [25–27], thereby promoting the plant germination, growth and yield, and enhancing abiotic tolerance [26–28]. On the other hand, the large granular colloid HA, with a negative surface charge, can absorb pollutants through electrostatic interaction. After forming complexes, these contaminants struggle to cross cell membranes due to their non-polarity, significantly reducing their potential impact on crops [29, 30]. The role of HA in alleviating Cd stress during plant growth is significant, with mechanisms including chelation, oxidative pressure reduction, promotion of soil microbial activity, and regulation of plant growth hormone [31, 32]. Therefore, whether HA can serve as an effective PGR candidate for enhancing sunflower growth and stress tolerance under Cd stress conditions needs further investigation.

The combined application of plants and exogenous PGR or chelating agents can be effectively utilized for the management of heavy metal pollution [33–35]. To test the mitigating effect of HA on sunflower plants under Cd stress, this study extensively investigated the growth, fluorescence characteristics, and antioxidant system of sunflowers after spraying them with different concentrations of HA. The aim was to explore the optimal concentration and effectiveness of HA in alleviating Cd stress on sunflowers, providing essential information for future application in phytoremediation of Cd-contaminated soil.

## Results

### Effects of HA on growth of sunflower plants under Cd stress

Cd stress significantly inhibited the growth of sunflower plants, and the plant height, stem diameter, fresh and dry weight were significantly reduced by 21.45%, 33.65%, 38.37% and 28.51%, respectively, compared with those of CK (Fig. 1). In contrast, HA (HA1, HA2, HA3 and HA4 represent 50, 100, 200, and 300 mg $L^{-1}$ concentrations) can mitigate the growth inhibition effects of Cd stress on sunflower plants. For instance, plant height, stem diameter, fresh weight and dry weight were significantly

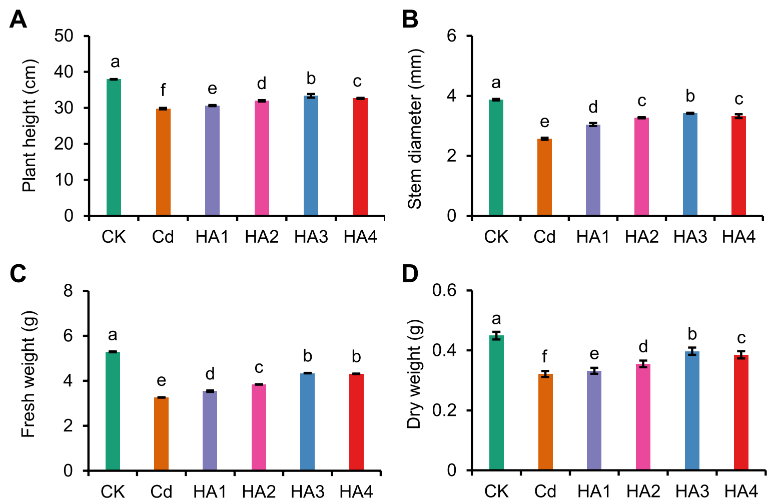


**Fig. 1** Effect of HA on growth of sunflower plants under Cd stress. The panel **A-D** represent the plant height, stem diameter, fresh weight, and dry weight of sunflower plants under normal conditions (CK), Cd stress conditions (Cd), and Cd stress conditions with foliar spraying of four different HA concentrations (HA1, HA2, HA3 and HA4). The significant differences in all detection indicators were determined using ANOVA, while the different letter on the top of any two columns represent significant differences between pairwise samples using pairwise Duncan's tests. The CK, Cd, HA1, HA2, HA3 and HA4 treatments were labeled using green (#189D76), orange (#DA6309), purple (#7F7AB8), magenta (#E9489B), blue (#4486BC) and red (#E41D1F), respectively

increased compared with those of Cd. With the increase of HA concentration, the growth indexes of sunflower plants first increased and then decreased. The HA3 treatment had the greatest effect on growth of sunflower plants.

### Effects of HA on chlorophyll content of sunflower plants under Cd stress

The chlorophyll content in sunflower plants exposed to Cd stress exhibited a significant reduction compared to the CK group (Fig. 2). The contents of chlorophyll a, chlorophyll b, and total chlorophyll have reduced by 36.06%, 58.83%, and 41.62% respectively. Conversely, it showed that HA significantly elevated the chlorophyll content of sunflower plants in these four treatments, especially HA3. When sunflower plants treated with 200 mg $L^{-1}$ HA (HA3), the contents of total chlorophyll, chlorophyll a, and chlorophyll b in the leaves exhibit a significant increase of 41.18%, 85.73%, and 49.85%, respectively, compared to Cd group.

### Effects of HA on the PSII photochemical activity of sunflower plants under Cd stress

The potential photochemical activity (Fv/Fo) and the maximum photochemical efficiency of PSII (Fv/Fm) of sunflower plants reduced by 67.81% and 24.67% respectively under Cd stress (Fig. 3A and 3B). However, after treatment with HA, the Fv/Fo increased by 30.65% (HA1), 78.35% (HA2), 170.66% (HA3), and 123.63% (HA4), while the Fv/Fm increased by 9.32% (HA1), 19.03% (HA2), 29.76% (HA3), and 25.20% (HA4) compared to Cd group. HA could enhance photochemical efficiency sunflower plants under Cd stress. The number of active PSII reaction centers per unit area (RC/CSo) of sunflower plants under Cd stress reduced 54.99% compared to the CK group (Fig. 3C). Cd stress impaired the absorption and conversion capabilities of pigment molecules within PSII reaction centers. However, subsequent treatment with HA demonstrated a significant increase in RC/CSo compared to Cd, with increases of 18.49% (HA1), 34.96% (HA2), 40.20% (HA3), and 23.16% (HA4), respectively. The application of HA can facilitate the absorption and utilization of light energy by pigment molecules within PSII reaction centers. Compared to CK group, the photosynthetic activity ($PI_{abs}$) was significantly reduced after Cd stress (91.95%, Fig. 3D), leading to impairment of the structure and function of the PSII system. However, after spraying HA, $PI_{abs}$ was significantly increased. Totally, HA application can enhance the overall performance of the PSII system in sunflower leaves exposed to Cd stress, thereby fostering photosynthesis.

### Effects of HA on energy flow parameters of sunflower plants unit PSII reaction center under Cd stress

When compared to CK group, the absorption of light energy (ABS/RC), capture (TRo/RC), and dissipation (DIo/RC) per unit reaction center in sunflower plants under Cd stress exhibited increases of 51.24%, 16.50%, and 216.67%, respectively (Fig. 4). In contrast, the energy

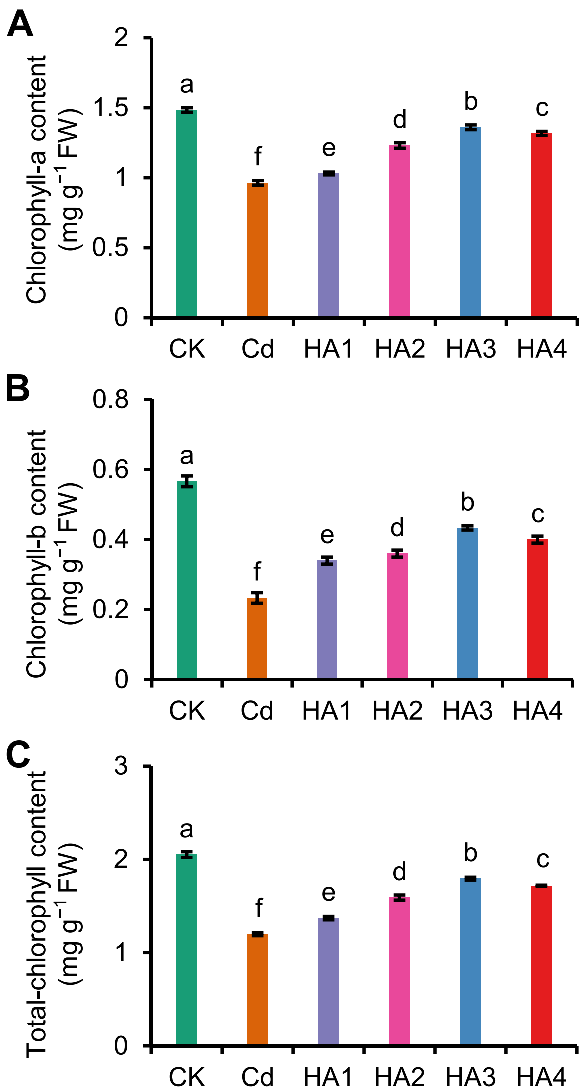


**Fig. 2** Effects of HA on chlorophyll content in sunflower plants exposure to Cd stress. The panel **A**-**C** represent the contents of chlorophyll a, chlorophyll b, and total chlorophyll of sunflower plants under CK, Cd, HA1, HA2, HA3 and HA4. The abbreviation FW stands for fresh weight. The same method of significance test and color as depicted in Fig. 1 was employed

for electron transport per unit reaction center (ETo/RC) experienced a significant reduction of 51.29%. Following the HA application, ABS/RC and DIo/RC decreased and ETo/RC increased compared to Cd group.

## Effects of HA on ROS of sunflower plants under Cd stress

Cd stress can significantly increase the $O_2^-$ production rate and $H_2O_2$ and $^-OH$ contents in the leaves of sunflower plants, which were 1.82 times, 1.18 times, and 1.30 times those of the control group, respectively (Fig. 5). With the elevation of HA concentration, the $O_2^-$ production rate and the content of $H_2O_2$ and $^-OH$ demonstrated a downward trend. Compared to the Cd group, the $O_2^-$ production rate, $H_2O_2$, and $^-OH$ content decreased the most in the HA3 group, with significant reductions of 44.91%, 13.29%, and 21.00%, respectively. Our results indicated that HA, especially HA3, could aid sunflower plants in eliminating excessive ROS.

## Effects of HA on antioxidant enzyme activity of sunflower plants under Cd stress

Cd stress can activate the antioxidant enzymes, SOD, POD, CAT, and ascorbate peroxidase (APX), in sunflower plants, leading to significant elevations of 16.95%, 77.07%, 32.79%, and 40.00%, respectively, compared to the CK group (Fig. 6). As the HA concentration increased (except HA4), the activities of these enzymes continued to be upregulated. The HA3 group demonstrated the most robust enzyme activity, with SOD, POD, and CAT activities increased significantly by 13%, 207%, and 86%, respectively, compared to the Cd group. Hence, spraying 200 mg $L^{-1}$ HA can effectively enhance the activity of antioxidant enzymes to scavenge the excessive ROS and mitigate the deleterious effects of Cd stress on sunflower plants.

## Comparative analysis of physiological indexes of sunflower plants under Cd stress by varying HA concentrations

We conducted hierarchical cluster analysis (Fig. 7A) and K-means cluster analysis (Fig. 7B) based on all the tested physiological index, which could be categorized into three groups based on the relative level of each physiological index. The first group exhibited the highest values in the CK group and the lowest under Cd group. Among the varying HA concentrations, it reached their peak at HA3 concentration. These indicators included Fv/Fm, Fv/Fo, chlorophyll a, total chlorophyll, plant height, $PI_{abs}$, fresh weight, dry weight, ETo/RC, RC/CSo, and stem diameter and chlorophyll b. The second group showed the lowest values in the CK group and increased in Cd group. The index continued to rise with different concentrations of HA application, and reached its peak at HA3 concentration. Although declined at HA4 concentration, it still slightly higher than the CK group. These indicators included $O_2^-$, $^-OH$, ABS/RC, $H_2O_2$, and DIo/RC. The third group demonstrated the lowest values in the CK group and the highest in the Cd group. Indexes in this group decreased with varying concentrations of HA mitigation and reached their lowest point at HA3 concentration (although still slightly higher than the CK group). These indicators included SOD, TRo/RC, APX, POD, and CAT. Overall, 200 mg $L^{-1}$ HA was proved to be the most effective in alleviating Cd stress in sunflowers.

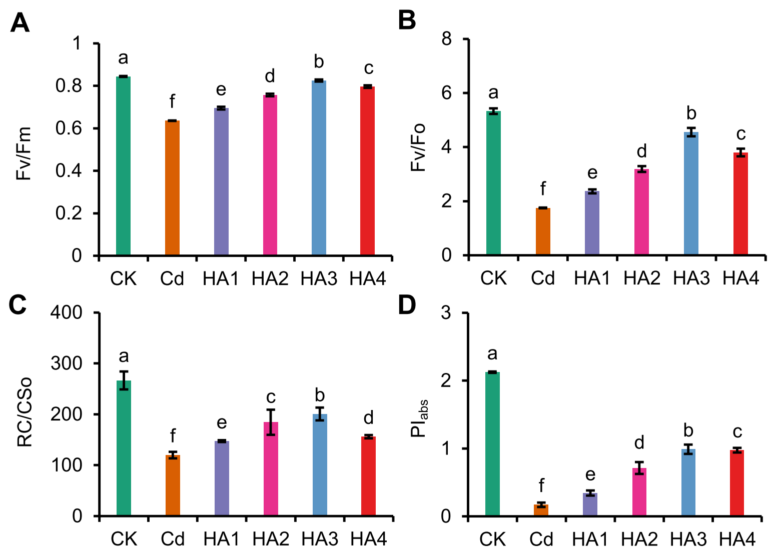


**Fig. 3** Effect of HA on chlorophyll fluorescence characteristics of sunflowers exposure to Cd stress. The panel **A-D** represent the values of Fv/Fm, Fv/Fo, RC/CSo and $PI_{abs}$ parameters of sunflower plants under CK, Cd, HA1, HA2, HA3 and HA4. The same method of significance test and color as depicted in Fig. 1 was employed

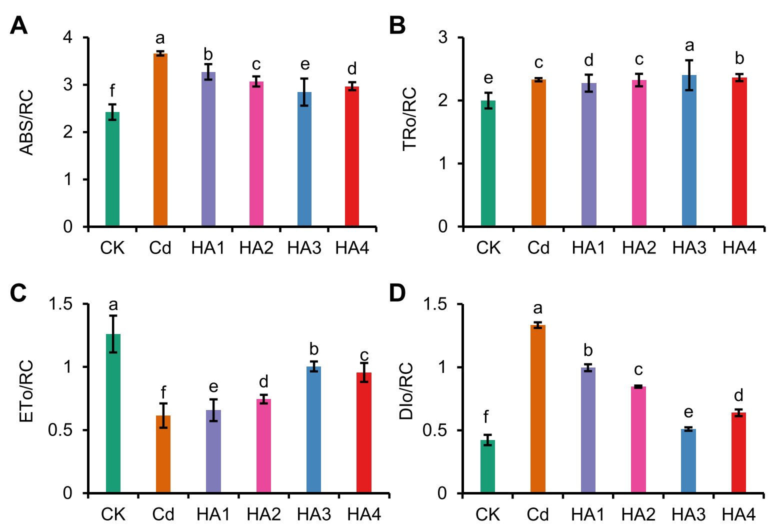


**Fig. 4** Effects of HA on energy fluxes per PSII reaction center of sunflowers exposure to Cd stress. The panel **A-D** represent the values of ABS/RC, TRo/RC, ETo/RC and DIo/RC parameters of sunflower plants under CK, Cd, HA1, HA2, HA3 and HA4. The same method of significance test and color as depicted in Fig. 1 was employed

Further, principal component analysis (PCA) was performed on each physiological index and the biological replicates of each sample were clustered together (Fig. 7C). This suggested that the physiological state of plants had significantly altered under various treatments, with the differences between samples being greater than those within samples. The proximity of the distances between the samples of Cd and HA1, HA2, HA3, and HA4, compared to the distance between the samples of CK, indicates that although different HA concentrations had a notable impact on alleviating sunflower plants, there were still some disparities from the CK group. Subsequently, we conducted correlation tests for each physiological index (Fig. 7D) and found a positive correlation between the main plant growth index and the photosynthetic index ($P < 0.001$). The three ROS and ABS/RC were positively correlated with DIo/RC ($P < 0.001$), and there was a positive correlation between the activities of the four antioxidant enzymes ($P < 0.001$). In contrast, plant growth indexes were negatively correlated with ABS/RC,

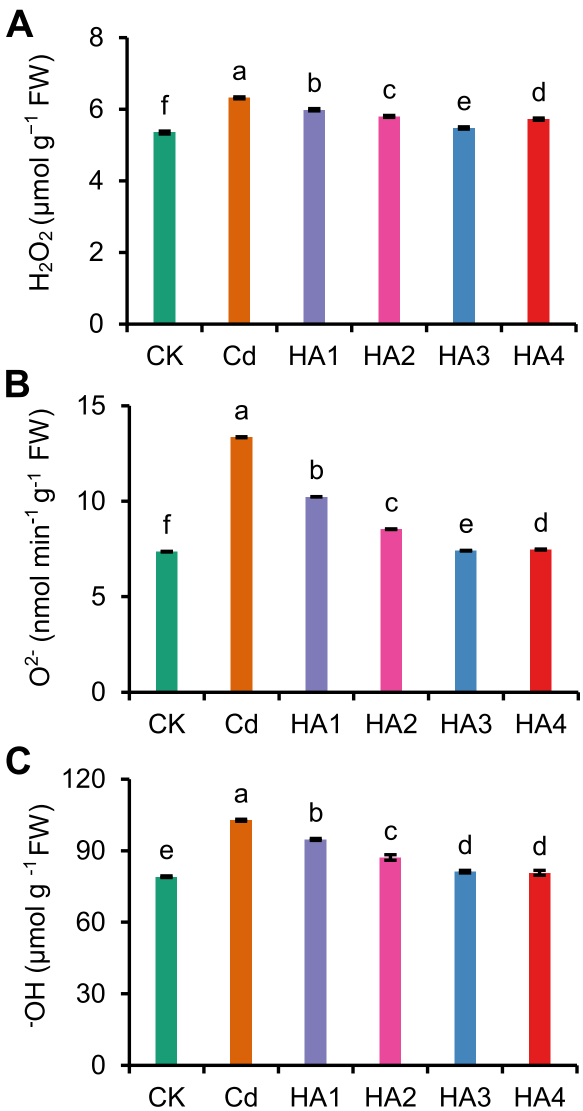


**Fig. 5** Effects of HA on ROS contents in sunflower plants exposure to Cd stress. The panel **A**-**C** represent the superoxide radical ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl ion ($^-OH$) contents of sunflower plants under CK, Cd, HA1, HA2, HA3 and HA4. The abbreviation FW stands for fresh weight. The same method of significance test and color as depicted in Fig. 1 was employed

DIo/RC, and TRo/RC ($P < 0.05$). There was also a negative correlation between plant growth index and the activity of four antioxidant enzymes, although the correlation was not significant except for SOD.

## Discussion

The issue of Cd pollution is garnering greater focus due to its unique properties of long-term persistence, bioaccumulation, irreversibility, high toxicity, and insidiousness when compared to other contaminants [36]. Phytoremediation, a cost-effective and eco-friendly approach, utilizes plants to efficiently extract and neutralize pollutants, significantly reducing their environmental impact [37]. Excessive Cd concentrations adversely affect essential physiological functions such as photosynthesis, respiration, and nitrogen metabolism, leading to stunted growth and diminished biomass accumulation [38]. Sunflower has demonstrated high tolerance and substantial biomass increase under cadmium stress, rendering it a promising solution for soil contamination mitigation [6, 7]. However, despite sunflowers surviving under a Cd treatment of 300 mg kg$^{-1}$, their growth parameters (plant height, stem diameter, fresh weight, and dry weight) were significantly reduced compared to the CK group (Fig. 1). Therefore, enhancing the tolerance of sunflowers to Cd stress is crucial for enhancing the efficiency of phytoremediation.

The active groups in HA, such as carboxyl, hydroxyl, methoxyl, and acyl, facilitate the removal of $Cd^{2+}$ from soil by ion exchange, chemisorption, and chelation [39, 40]. In our preliminary experiments, we employed HA through two methods: soil application and foliar spray. Among these exogenous treatments, foliar spray was superior in enhancing plant biomass, chlorophyll content, and various physiological and biochemical indices. Similar, previous studies also found that applying HA as a foliar treatment significantly enhanced plant biomass under stress [28, 41]. In this study, we confirmed that foliar HA spraying can promote the recovery of various morphological indexes of sunflower plants under Cd stress. HA can affect the processes including cell respiration, photosynthesis, protein synthesis, nutrient and water uptake, as well as enzyme activities, thereby enhance crop yield [26, 27, 42]. Humic substances can also work against environmental stresses by increasing dry biomass weight and promoting plant growth [41].

The pigment chlorophyll is essential for photosynthesis and plays a significant role in determining plant growth [43]. The excessive Cd can inhibit chlorophyll ester reductase and δ-amino ester dehydrogenase activities, leading to the degradation of chlorophyll membrane structure and exacerbating its decomposition [44, 45]. In this study, the contents of chlorophyll a, chlorophyll b, and total chlorophyll in sunflower plants under Cd stress were notably decreased compared to the CK group (Fig. 2), suggesting that Cd stress impedes chlorophyll synthesis in sunflowers. This result is similar with Kaya et al. [41]. HA also has the potential to stimulate the increase in plant biomass, chlorophyll content, mineral nutrition, and key antioxidant enzyme activity [46]. Thus, applying HA to the leaf surface can effectively mitigate the chloroplast damage in sunflowers induced by Cd stress. Moreover, chlorophyll fluorescence parameters in plants are widely regarded as crucial indicators

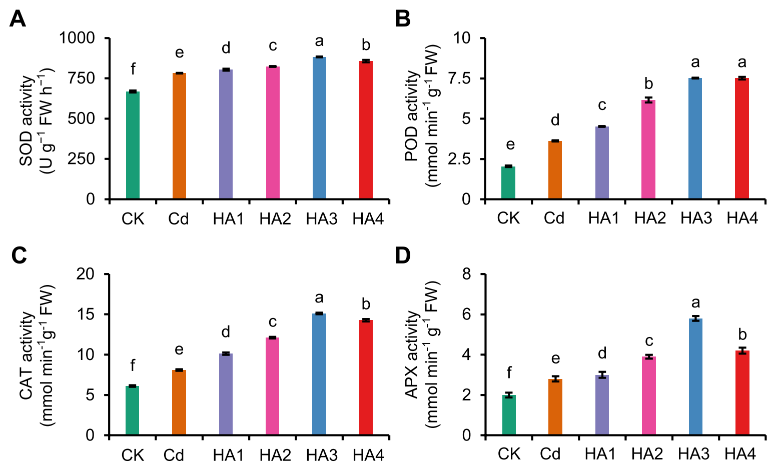


**Fig. 6** Effect of HA on antioxidant enzyme activities of sunflower plants exposure to Cd stress. The panel **A-D** represent the values of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) of sunflower plants under CK, Cd, HA1, HA2, HA3 and HA4. The abbreviation FW stands for fresh weight. The same method of significance test and color as depicted in Fig. 1 was employed

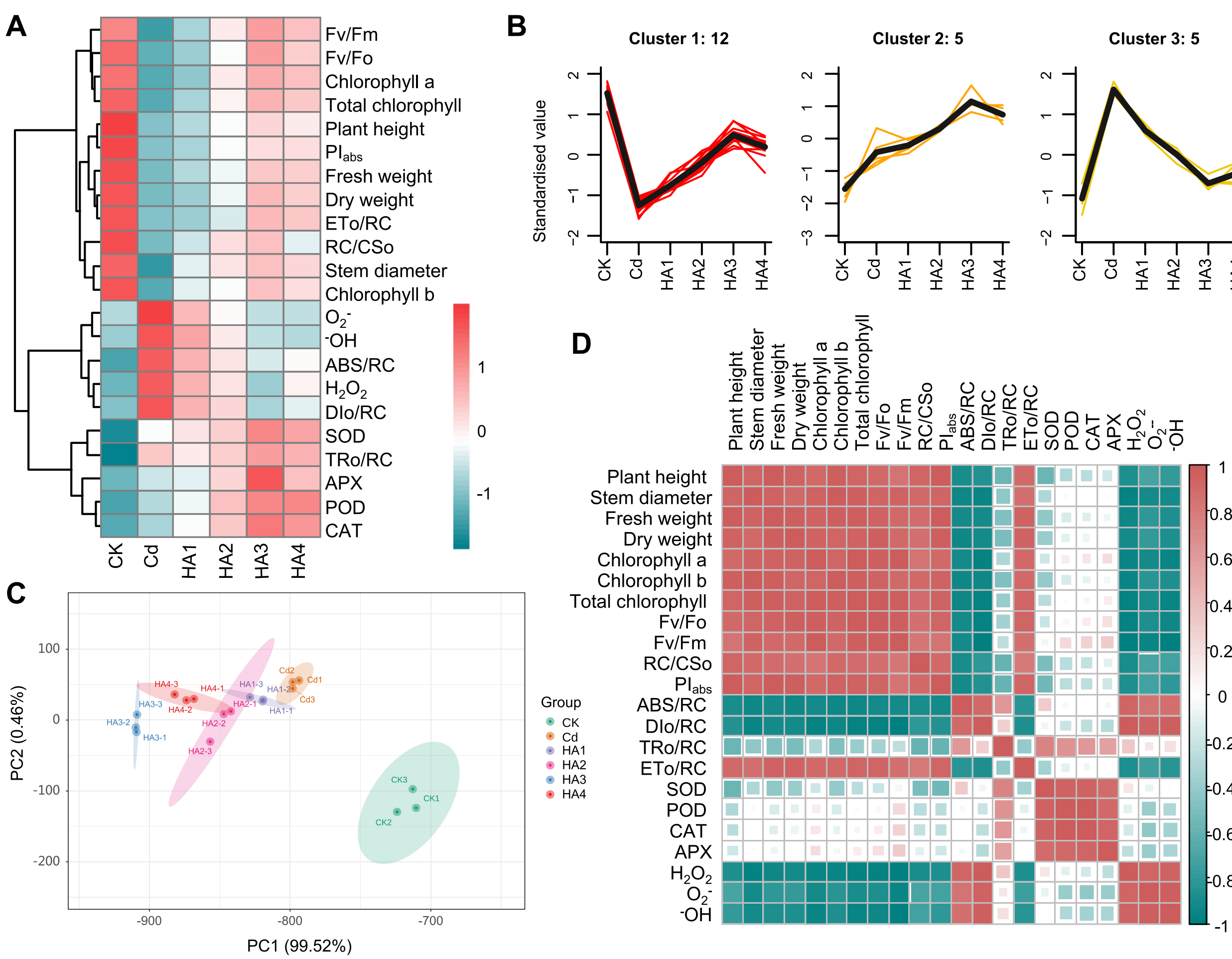


**Fig. 7** Comparative analysis of different physicochemical indexes under Cd stress by HA. **A** Hierarchical cluster analysis of physiological indexes of sunflower plants under Cd stress induced by HA. **B** K-means cluster analysis of physiological indexes of sunflower plants under Cd stress by HA. **C** Principal component analysis (PCA) of physiological indexes of sunflower plants under Cd stress. **D** Correlation analysis of physiological indexes of sunflower plants under Cd stress by HA

for assessing the effects of environmental stress on photosynthesis, which can effectively reveal the degree of Cd stress on photosynthesis [47, 48]. Under Cd stress, the performance of PSII system in sunflowers significantly declined, but increased after applying 200 mg $L^{-1}$ HA (Fig. 3). These results suggested that foliar application of 200 mg $L^{-1}$ HA can significantly elevate the photosynthetic efficiency.

Abiotic stresses can lead to detrimental changes in morpho-physiological and bio-molecular processes in plants such as the generation of ROS, membrane damage, loss of photosynthetic efficiency, etc. and thereby result in reduced growth and yield penalty [20]. The ROS are byproducts of lipid peroxidation in plants and their levels reflect the extent of plant damage, making them a valuable indicator for assessing cell damage [49]. Our results demonstrated that Cd stress significantly increased the production rate of $O_2^-$ and the contents of $H_2O_2$ and $^-OH$ in sunflower leaves (Fig. 5), indicating the detrimental effects of Cd stress on sunflowers. Plants can scavenge the excessive ROS from the body by producing antioxidant enzymes, thus reducing its potential damage [50]. When exposed to Cd, sunflowers' antioxidant defense system effectively enhanced the activity of antioxidant enzymes, especially after spraying the HA application (Fig. 6). SOD efficiently eliminates free radicals, while POD catalyzes the oxidative decomposition of active oxygen species [51]. CAT, in turn, removes $H_2O_2$ from plant cells, safeguarding them from oxidative stress [52]. This is a sensitive indicator reflecting the impact of heavy metal pollution stress [53]. The results of this study demonstrate that spraying 200 mg $L^{-1}$ HA can significantly boost the activities of SOD, POD, CAT, and APX in sunflowers following Cd stress (Fig. 6). This intervention helps sunflowers clear excessive ROS (Fig. 5), thereby effectively mitigating the damage caused by Cd stress on sunflower.

In summary, we extensively investigated the alleviating effect of different HA concentrations on the growth, chlorophyll synthesis, and biochemical defense system in sunflower plants under Cd stress through foliar application. Under Cd stress, HA enhanced the fluorescence characteristics and antioxidant enzyme activity, protected plant photosynthesis, and hence promoted plant growth and development (Fig. 8). Our results suggest that spraying exogenous HA at varying concentrations had a mitigating effect on deleterious effects caused by Cd stress on sunflower plants; particularly at a concentration of 200 mg $L^{-1}$. However, the concentration of 300 mg $L^{-1}$ HA had a weaker effect than 200 mg $L^{-1}$. From the results, we may infer that it may induce moderate osmotic stress through foliar spray application when exposed to an elevated concentration of HA (300 mg $L^{-1}$), the alleviating effect could be mitigated consequently. Certainly, the present inference is speculative in nature, and further studies is required for its validation.

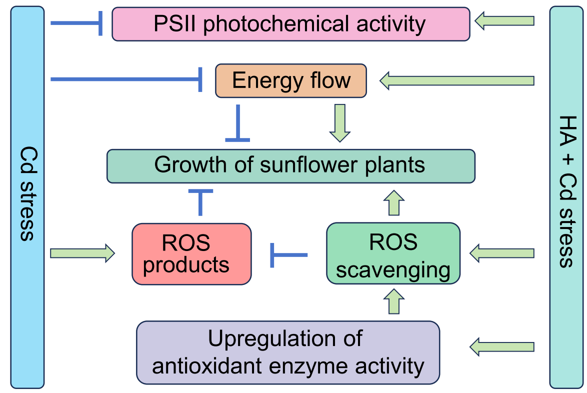


**Fig. 8** A schematic model for the HA-induced mitigation of Cd adverse effects in sunflowers

## Conclusion

The present study confirms that HA is a plant growth regulator, which can promote the sunflower growth when exposed to Cd stress. Our investigation advances our understanding of the alleviating effect of HA underpin the variations in plant growth, physiology and biochemistry of sunflowers under Cd stress through foliar application. It further demonstrated the potential role of HA in protecting the sunflower plants against Cd phytotoxicity through the following two major mechanisms (a) up-regulating the light energy utilization and chlorophyll content to enhance photosynthesis, (b) enhancing the antioxidant defense system, especially the antioxidant enzyme activity, to efficiently scavenge the generated ROS to reduce its oxidative damage. Our results suggest that spraying exogenous HA with certain concentration had a significantly mitigating effect on deleterious effects caused by Cd stress on sunflowers. Our study would provide valuable strategies for the application of HA in promoting the phytoremediation of cadmium-contaminated soil by sunflower plants.

## Materials and methods

### Plant cultivation and experimental treatment

The effect of HA on sunflowers' physiological and biochemical indices under Cd stress was investigated through a pot experiment conducted in the biochemical laboratory of Changzhi University. The sunflower cultivar (MH8361) seeds were purchased from Hebei Maohua seed industry Co., LTD. HA was obtained from Shandong Xiya Chemical Industry Co., LTD; Other biochemical regents were obtained from Beijing Solarbio

Science & Technology Co., Ltd. The consistent and full sunflower seeds were sowed in porous plastic pots filled with nutrient-rich soil. The experimental conditions included a daytime temperature of 28 °C and a nighttime temperature of 26 °C, a light–dark cycle of 16 h and 8 h, and a humidity level of (60 ± 5) %. After reaching two true leaves, the healthy and consistently growing sunflower plants were selected and exposed to a concentration of 300 mg $kg^{-1}$ $CdCl_2$. Meanwhile, the control was set as normal growth condition (CK). After 7 days of Cd stress, the plants were evenly sprayed each day with HA solutions (0, 50, 100, 200, and 300 mg $L^{-1}$) on both sides of the leaves until water droplets formed. The plants treated with these five HA solutions were designated as Cd, HA1, HA2, HA3, and HA4, respectively. The CK treatment was sprayed with distilled water. Relevant indices of sunflower plants were measured after a 7-day treatment period. All the six treatments had three biological replicates, each with a minimum of three sunflowers to minimize bias from individual variations.

### Determination of plant growth and physiological indexes

The plant growth indexes (including plant height, stem diameter, fresh weight, and dry weight) as well as chlorophyll content were measured following the method by Shen et al. [25]. The activities of SOD, POD, CAT, and APX were determined according to the method by Xu et al. [6]. The chlorophyll fluorescence parameters of sunflower plants were analyzed using the Handy-PE portable plant efficiency analyzer (Hansha Scientific Instruments Ltd.) following the method by Zhao et al. [54]. The content of $H_2O_2$ and the $O_2^-$ production rate were determined by the method of Velikova et al. [55] and Jiang et al. [56], respectively. The content of $^{-}OH$ was measured using a hydroxyl radical kit (Solebol, Beijing).

### Date statistics and analysis

The significant differences in all detection indicators were determined using Analysis of variance (ANOVA) in R (v4.3.1), while pairwise Duncan's tests were used to assess the significant differences between pairwise samples. All data presented in the figures were depicted as mean values ± standard deviations, with lowercase letters of distinct data denoting significant disparities among various treatments ($P < 0.05$). The pheatmap package in R was utilized for heatmap visualization, while the psych package in R was employed for conducting Pearson's correlation test. Principal component analysis (PCA) was conducted using the online tools of Metware cloud (https://cloud.metware.cn/#/home).

### Authors' contributions

X.W., A.L. and H.S. designed the study and revised the manuscript. X.W., J.Z. and J.S. performed the experiments. X.W., J.Z., L.Z., P.W. and H.S. analyzed data. X.W., J.Z. and A.L. wrote the manuscript. All authors read and approved the final manuscript.

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### Availability of data and materials

Data is provided within the manuscript.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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