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Exogenous NaHS Treatment Alleviated Cd-induced Stress in *Ocimum basilicum* Plants through Modulation of Antioxidant Defense System

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Hydrogen sulfide (H_2S) regulates many critical processes of plants. The effect of sodium hydrogen sulfide as H_2S agent was investigated in basil plants under cadmium stress. A completely randomized design with three different concentrations (0, 50 and 100 μM) of $CdCl_2$ and two levels of NaHS (0 and 100 μM) was used in this study. Cadmium exposure reduced growth parameters and relative water content. Cd also caused a significant increase in ion leakage and higher oxidative stress in terms of lipid peroxidation and H_2O_2 production. Although exogenous NaHS used in non-stressed control plants negatively affected growth and physiological parameters, it improved the root/shoot length ratio and fresh weight in basil plants under Cd 50 μM exposure. Moreover, NaHS alleviated deleterious effects of cadmium on ion leakage, relative water content and photosynthetic pigments of leaves. The activity of antioxidant enzymes like catalase, peroxidase and ascorbate peroxidase were also enhanced by NaHS in plants under moderate cadmium stress. Our results show that NaHS 50 μM ameliorates growth retardation induced by cadmium 50 μM stress in basil plants, probably through regulating physiological parameters such as photosynthetic pigments content, relative water content and the activity of antioxidant enzymes.

Key words: antioxidant compounds, green basil, hydrogen sulfide, heavy metals

INTRODUCTION

Cadmium is the most toxic element with no biological role in plants. Cd, mainly through industrial processes and phosphate fertilizers, enters the environment and pollutes food chains (Peralta-Videa et al., 2009). Like some other heavy metals such as Ni and Pb, Cd is easily absorbed through the roots of the plant. Cd ions form complexes with organic compounds such as proteins, and thereby prevent the basic activity of the cells (Benavides et al., 2005). Cd is a bivalent cation which competes with elements such as magnesium in the chlorophyll structure and with ferroelectric ions. Its exposure also increases membrane dissociation by encouraging production of reactive oxygen species and lipid peroxidation (Verbruggen et al., 2009). One of the major damages in plant tissues that occur due to exposure to heavy metals like cadmium is production of reactive oxygen species (ROS) and oxidative stress. ROS are lethal to cellular constituents like unsaturated fatty acids, proteins and nucleic acids (Hasanuzzaman et al., 2012).

To cope with heavy metals, sulfur-induced defense mechanisms are among the most important strategies of plants. Plant cells assign a number of anti-oxidative reactions to scavenge metal-induced free radicals (Mostofa et al., 2015). It has been shown that hydrogen sulfide (H_2S) is produced in small amounts in plant cells and contributes to many physiological processes as a bio-stimulator (Zhang et al., 2015). Hydrogen sulfide production is involved in various growth and developmental

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processes - from seed germination to aging and also plant responses to biotic and abiotic stresses (Chang et al., 2014; Li et al., 2012). The most prominent feature of H_2S in plant cells is its antioxidant ability against the active radicals of oxygen and nitrogen (Stone and Yang, 2006). Many studies demonstrated that under environmental conditions H_2S, at low concentration, detoxifies ROS effects in plant tissues (Mahanty et al., 2017; Fotopoulos et al., 2015). H_2S application has been proven to modulate cross-adaptation to heavy metals, salt, drought, cold, heat and flooding stresses mainly through regulating the antioxidant system, osmolytes content, heat shock proteins (HSPs) synthesis, and also mineral nutrients homeostasis in plants (Fang et al., 2016; Li, 2013). In plant tissues, hydrogen sulfide activates the antioxidant defense system through amplification of antioxidant enzymes activity such as superoxide dismutase, catalase and ascorbate-peroxidase (Mostofa et al., 2015). It has also been shown that H_2S enhances H_2O_2 production and lipoxygenase activity in plant cells. These findings confirm the protective role of H_2S against oxidative stress (Kuźniak and Urbanek, 2000). Treatment of plants with NaHS, a H_2S agent (Yonezawa et al., 2007), amplifies H_2S production which in turn affects the enzymes involved in oxidative stress. In tomato plants, H_2S has been shown to effectively increase lateral roots formation, probably using auxin interference (Fang et al., 2014). Zhang et al. (2010) reported that wheat seed treatment with NaHS increased germination percentage under both normal conditions and heavy metal stress caused by aluminum, chromium and cadmium (Zhang et al., 2010). It has been suggested that H_2S improves plant growth by increasing the total thiol content, glutathione and cysteine content in plants (Chen et al., 2011).

This research was carried out to determine the main mechanism of NaHS as a H_2S donor in physiological and biochemical responses of basil (*Ocimum basilicum* L.) under cadmium stress. We mainly focused on growth parameters, photosynthetic pigments, membrane stability, relative water content, and antioxidant defense responses of the plant. The results obtained from this study elucidate feasible pathways of H_2S impact on plants under heavy metal stress. Green basil, an edible perennial herb of the Lamiaceae family, is widely used around the world due to its nutritional benefits. Basil plant is of particular interest of many physiological studies because of its phytochemicals and antioxidant capacities, besides its fast growing properties and high biomass production.

MATERIALS AND METHODS

Seeds of green basil (*Ocimum basilicum*) provided from Pakan Bazr Company (Isfahan, Iran) were cultivated on perlite medium in pots under greenhouse conditions. The seeds were planted in a completely randomized design with three replications. The seedlings were irrigated every other day with nutrient solution for 3 weeks. After full expansion of the third leaves, the plants were treated with 3 different concentrations of $CdCl_2$ including 0, 50 (as moderate stress) and 100 μM (high or severe stress). Two levels of NaHS 0, 50 μM were supplied in nutrient solution. After a week, growth parameters such as plant fresh weight and root/shoot length were recorded. The fully expanded third leaf of each plant was used to measure the relative water content, ion leakage and other physiological and biochemical parameters.

RELATIVE WATER CONTENT (RWC)

To measure RWC, leaf fresh weight, turgid weight (held 5 hours in deionized water) and the leaf dry weight (72 hours at 70°C) were recorded. The relative water content was calculated using the following equation (Mullan and Pietragalla, 2012):

$$\frac{\text{freshweight} - \text{dryweight}}{\text{torgidweight} - \text{dryweight}} \times 100$$

ELECTROLYTE LEAKAGE (EL)

The percentage of ion leakage was measured based on Lutts et al. (1996) and using the following equation:

$$\text{EL } (\%) = \frac{L1}{(L1+L2)} \times 100$$

L1 in the equation is ion leakage recorded for 0.3 g leaf fresh weight kept for 24 hours in 20 ml of distilled water at 25°C. After addition of 20 ml distilled water to the former aliquot and autoclaving at 120°C for 20 minutes, the ion leakage of cooled samples was recorded as L2.

PHOTOSYNTHETIC PIGMENTS

Lichtenthaler (1987) method was used to measure the content of photosynthetic pigments including total chlorophyll and carotenoids. 0.2 g frozen

leaves were extracted in 15 ml acetone 80% (Lichtenthaler, 1987). The absorbance of infiltrate solute was read by a spectrophotometer at 646.8, 663.2 and 470 nm and the contents of photosynthetic pigment were recorded based on μg/g fresh weight using the following equations:

Chla = 12.25 A663.2 – 2.79 A646.8
Chlb = 21.21 A646.8 – 5.1 A663.2
Total Chl = chla + chlb
Carotenoids = $(1000A_{470} - 1.8\ chla - 85.02\ chlb)/198$

PHENOLIC COMPOUNDS CONTENT

Leaf fresh weigh (0.1 gram) extracted in 5 ml 95% ethanol was stored in darkness for 24 hours. 1 ml 95% ethanol was added to 1 ml of the obtained extract and the volume was adjusted up to 5 ml using distilled water. 0.5 ml 50% Folin and Ciocalteu`s phenol reagent and 1 ml 5% sodium carbonate were added to the mixture and kept in the dark for 1 hour. The absorption of each sample was then read at 725 nm by a Cary 50 UV-Visible Spectrophotometer (Ainsworth and Gillespie, 2007). A standard curve of gallic acid was used to quantify the content of phenolic compounds content.

ANTHOCYANINS CONTENT

0.1 g leaf dry weigh was extracted in 10 ml acidic methanol (99:1; methyl alcohol 99.5%: pure hydrochloric acid). The extract was stored at laboratory temperature in a dark place for 24 hours. Samples were then centrifuged for 4 minutes at 4000 g and the absorption intensity of supernatants was read at 550 nm using a UV-Visible spectrophotometer. The anthocyanins content was recorded using 3300 $mM^{-1}cm^{-1}$ as the absorption coefficient (Wagner, 1979).

TOTAL FLAVONOIDS CONTENT

The total flavonoids content was measured according to the aluminum chloride colorimetric method (Toor and Savage, 2005). 10 ml of methanol were used to extract flavonoids from 0.1 g leaf tissues. The volume of 0.5 ml extracted solute was brought up to 5 ml using distilled water. 0.3 ml $NaNO_2$ 5% were added to the aqueous and left for 5 min. Then, 0.6 ml of $AlCl_3$ 10%, 2 ml NaOH 1M and 2 ml distilled water were added to the mixture. The concentration of total flavonoids was measured after reading the absorbance intensity of the samples at 510 nm and using the quercetin calibration curve.

LIPID PEROXIDATION

The content of malondialdehyde (MDA) was measured as lipid peroxidation index according to (Hodges et al., 1999). 0.2 grams of fresh leaf tissues were extracted in 5 ml 0.1% trichloroacetic acid (TAC) and centrifuged in 5000 ×g for 5 minutes. 5 ml 0.5% thiobarbituric Acid (TBA) in 20% TCA acid were added to 1 ml of supernatant. The mixture was heated at 95°C for 30 minutes. Then the test tubes were immediately cooled in an ice bath and centrifuged at 5000 ×g for 10 minutes. The absorbance of solutes was read at 523 nm. The absorbance of non-specific pigments determined at 600 nm was deducted from the former value. The extinction coefficient of 1.55 × 105 $M^{-1}cm^{-1}$ was used to measure MDA content. The results were expressed based on micro molar MDA in fresh weigh.

HYDROGEN PEROXIDE

Hydrogen peroxide was measured using the method described by Velikova et al. (2000). The aerial part of the plant was mauled in 0.1% TCA in an ice bath. The extract was centrifuged (Centrifuge 5804R, Germany from Eppendorf) at 1500 g for 10 minutes. 0.5 ml supernatant were added to 0.5 ml potassium buffer (10 mM, pH 7) and 1 ml KI 1M. The absorbance was read at 390 nm. Hydrogen peroxide concentration was calculated using the extinction coefficient 0.28 $M^{-1}cm^{-1}$.

PREPARATION OF PROTEIN AND ENZYME EXTRACTS

Fresh leaf tissue was extracted in 5 milliliter 50 mM potassium phosphate buffer (pH 7.5) containing 1% PVP and 1 mM EDTA on ice. The extracts were centrifuged at 4000 ×g for 20 min. The supernatants were used for enzyme activity assays and protein content.

TOTAL PROTEIN CONTENT

Bio-Rad dye reagent was used to measure the total protein content based on Bradford (1976) protocol.

ENZYME ACTIVITIES ASSAY

Catalase (CAT): The activity was measured based on the reduction of H_2O_2 absorbance at 240 nm.

The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7), H_2O_2 15 mM and 100 μl enzyme extract. The activity of enzyme was expressed based on the enzyme unit per milligram protein. One CAT unit decomposes 1 mMol H_2O_2 within a minute (Velikova et al., 2000).

Ascorbate peroxidase (APX): (Nakano and Asada, 1981) method was used for APX activity measurement. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 150 μl enzyme extract. APX activity was measured based on absorbance reduction at 290 nm which occurs following oxidation of ascorbic acid. Using the extinction coefficient 2.8 $mM^{-1}cm^{-1}$ the oxidized ascorbate was estimated. One unit of APX oxidizes one micromole of ascorbic acid in 1 minute.

Guaiacol Peroxidase (GPX): 3 ml reaction mixture containing 2.77 ml potassium phosphate buffer (50 mM, pH 7), 100 μl H_2O_2 1%, 100 μl guaiacol 4%, and 30 μl enzyme extract was used. The spectrophotometric method was used and the guaiacol oxidation was followed at 470 nm for three minutes. The extinction coefficient 25.5 $mM^{-1}cm^{-1}$ was used for estimation of tetraguaiacol content (Zhang, 1990) in the samples. One unit of GPX oxidizes one micromole of guaiacol for 1 minute.

STATISTICAL ANALYSIS

One way ANOVA was used for data analysis on SPSS software (ver. 18). The Duncan's Multiple Range test was used to measure specific differences between the means of groups at $P < 0.05$.

RESULTS

The Duncan's test was performed at $p < 0.05$ on each parameter. The root/shoot length and dry weight decreased when $CdCl_2$ or NaHS were applied separately to plants (Fig. 1a-c). However,

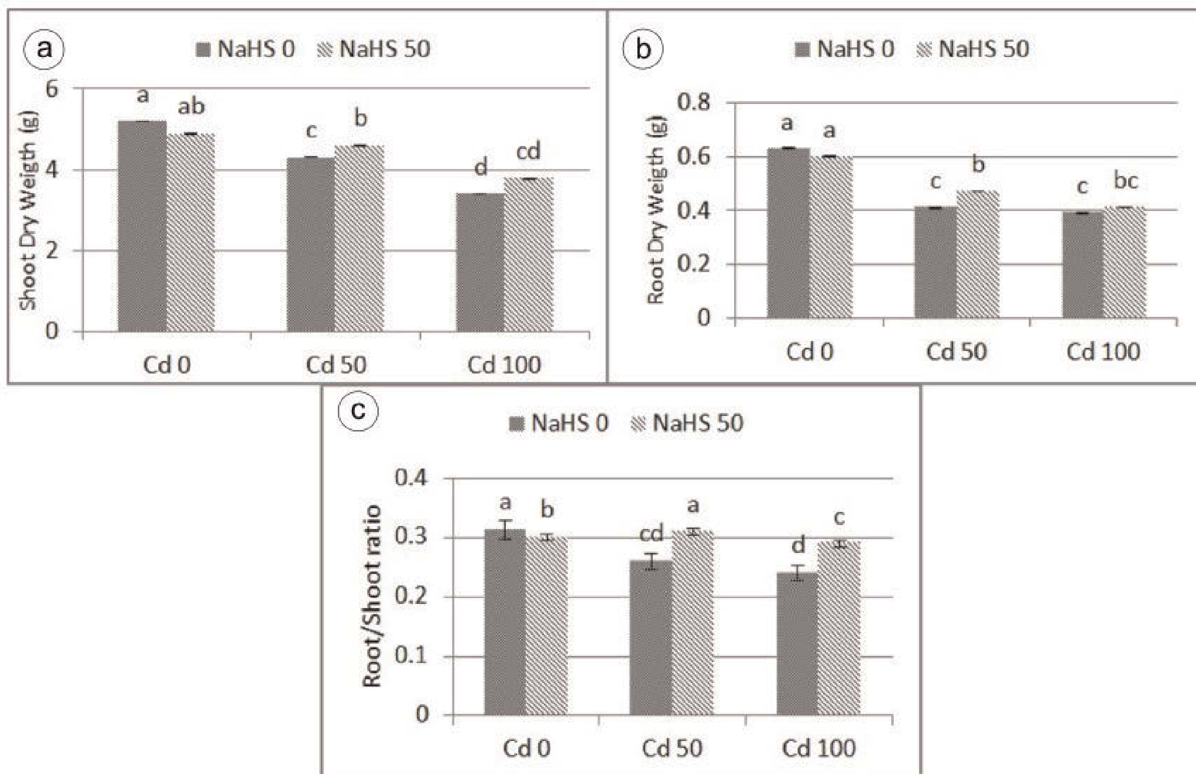

Fig. 1. Shoot dry weight (**a**), root dry weight (**b**), and root to shoot length ratio (**c**) of basil plants under Cd stress (50 and 100 μM $CdCl_2$) treated with NaHS. Means of three replicates were analyzed using the analysis of variances and Duncan's test ($p \leq 5\%$ level). Columns with the same letters do not show significant differences. The vertical bar represents the standard error of the mean.

NaHS improved growth parameters in plants under 50 and 100 μM Cd stresses. NaHS treatment increased the root to shoot length ratio by 17% and 18% in plants exposed to Cd 50 and 100 μM, respectively.

Both 50 and 100 μM $CdCl_2$ had significant negative effects on physiological parameters such as relative water content and membrane stability in terms of electrolyte leakage. NaHS treatment recovered the relative water content in plants under cadmium stress (Fig. 2a-b). As presented in Fig. 2a, NaHS enhanced RWC from 40% to 49% at 100 μM Cd. Fig. 2b shows that Cd at both 50 and 100 μM considerably disturbed membrane stability, while NaHS was somehow able to alleviate ion leakage in Cd-stressed plants. The value of EL% significantly increased by 44.3% in plants exposed to Cd 100 μM while NaHS reduced this value down to 41.1%.

Total chlorophyll content of plants was decreased by cadmium and NaHS when supplied separately. On the other hand, total Chl content was raised in Cd-stressed plants treated with NaHS (Fig. 3a). In plants under 100 μM Cd exposure, total

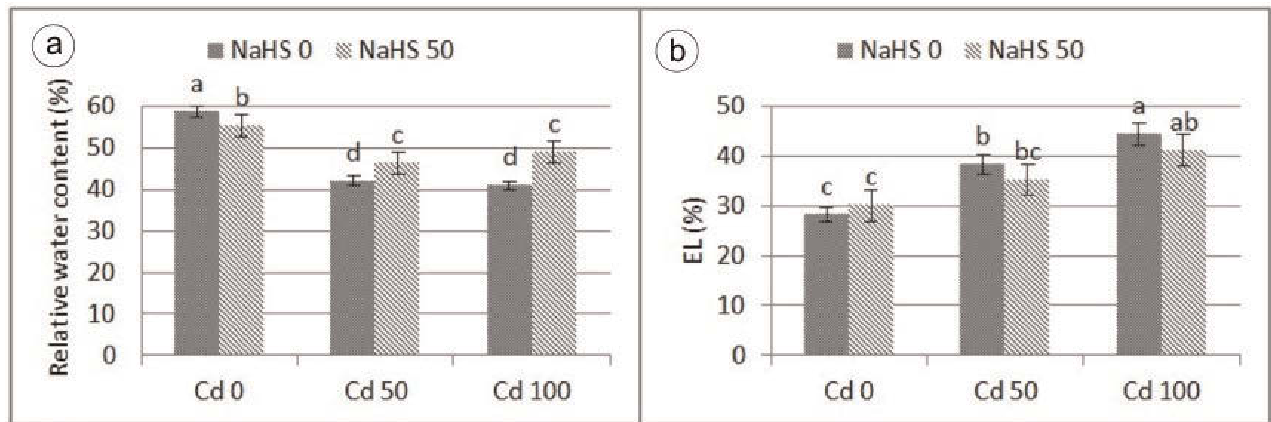

Fig. 2. Relative water content (**a**) and electrolyte leakage (**b**) of basil plants under Cd stress (50 and 100 μM $CdCl_2$) treated with NaHS. Means of three replicates were analyzed using the analysis of variances and Duncan's test ($p \leq 5\%$ level). Columns with the same letters do not show significant differences. The vertical bar represents the standard error of the mean.

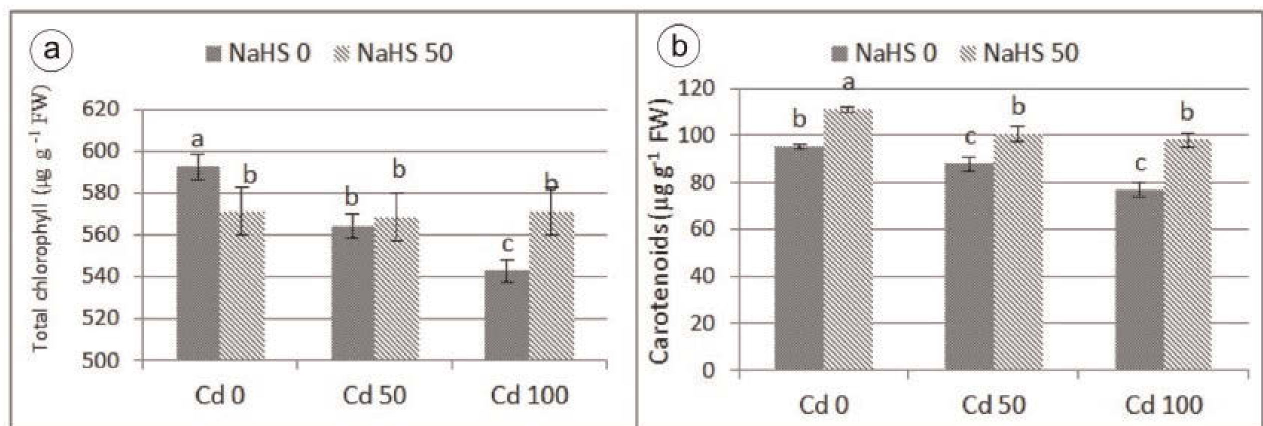

Fig. 3. Total chlorophyll content (**a**) and carotenoids content (**b**) of basil plants under Cd stress (50 and 100 μM $CdCl_2$) treated with NaHS. Means of three replicates were analyzed using the analysis of variances and Duncan's test ($p \leq 5\%$ level). Columns with the same letters do not show significant differences. The vertical bar represents the standard error of the mean.

chlorophyll content increased from 542 μg to 571 μg per gram fresh weight when treated with NaHS. Application of NaHS also increased carotenoids content in the control and Cd-stressed plants (Fig. 3b). The content of carotenoids under Cd 50 and 100 μM increased by 23% and 13%, respectively, in plants treated with NaHS.

Comparing to the control, the amount of flavonoids was significantly increased by $CdCl_2$ 50 μM but it was reduced at Cd 100 μM (Fig. 4a). Treatment with sodium hydrogen sulfide reduced total flavonoids content by 5% and 8% in plants exposed to 50 μM and 100 μM Cd, respectively. Total phenolic compounds were significantly increased by cadmium stress. Total phenolic compounds content increased from 78 μg gr^{-1} FW under control condition to 110 μg gr^{-1} FW in plants irrigated with nutrient solution containing 100 μM Cd. NaHS treatment also caused 9.4% increase in the amount of phenolics in plants exposed to Cd 100 μM (Fig. 4b).

Cadmium toxicity significantly raised the indices of oxidative stress in basil plants (Fig. 2). Comparing to the control, lipid peroxidation increased by 80% and 105%, respectively, in plants exposed to Cd 50 μM and 100 μM (Fig. 5a).

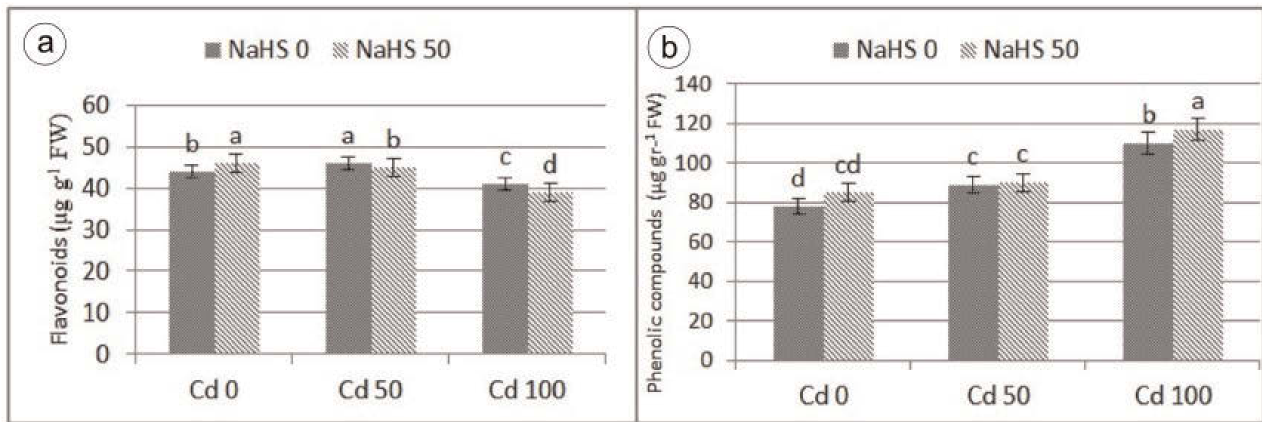

Fig. 4. Total phenolic compounds (**a**) and flavonoids content (**b**) of basil plants under Cd stress (50 and 100 μM $CdCl_2$) treated with NaHS. Means of three replicates were analyzed using the analysis of variances and Duncan's test ($p \leq 5\%$ level). Columns with the same letters do not show significant differences. The vertical bar represents the standard error of the mean.

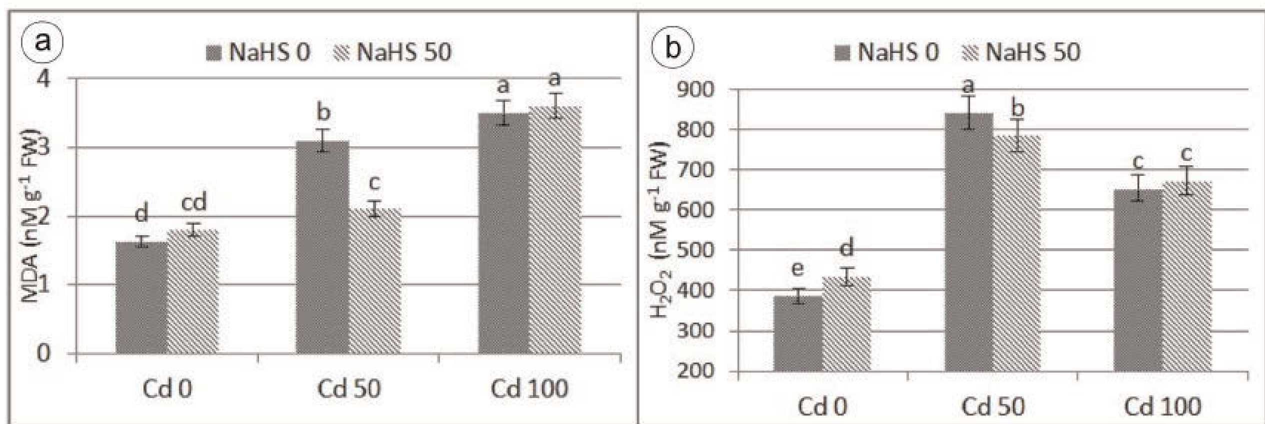

Fig. 5. MDA (**a**) and H_2O_2 content (**b**) of basil plants under Cd stress (50 and 100 μM $CdCl_2$) treated with NaHS. Means of three replicates were analyzed using the analysis of variances and Duncan's test ($p \leq 5\%$ level). Columns with the same letters do not show significant differences. The vertical bar represents the standard error of the mean.

The H_2O_2 production was also elevated in plants under cadmium stress (Fig. 5a). NaHS treatment mitigated membrane damage and MDA production by 65% when it was used for plants under 50 μM $CdCl_2$. The H_2O_2 content declined from 841 nM to 765 nM in Cd 50 μM-stressed plants treated with NaHS. However, NaHS did not show any significant effect on MDA content and hydrogen peroxide production in plants under Cd 100 μM.

The obtained results from antioxidant enzymes activities are given in Table 1. Under control conditions, NaHS application increased the catalase activity. NaHS also increased CAT activity by 65% in plants exposed to Cd 50 μM but it decreased CAT activity from 2.7 U/mg protein to 2.1 U/mg protein in plants under Cd 100 μM. As in the case of CAT, the activity of GPX and APX was significantly increased by cadmium. NaHS treatment respectively increased the activity of GPX and APX by 12% and 13% in Cd 50 μM-stressed plants. NaHS had the reverse effects on plants under Cd 100 μM. A significant reduction of CAT, GPX and APX activity was observed in plants under Cd 100 μM treated with NaHS.

TABLE 1. Antioxidant enzymes activity of basil plants under Cd stress (50 and 100 μM $CdCl_2$) treated with NaHS. Means of three replicates were analyzed using the analysis of variances and Duncan's test ($p \leq 5\%$ level). Columns with the same letters do not show significant differences.

Groups		CAT (U mg^{-1} protein)	GPX (U mg^{-1} protein)	APX (U mg^{-1} protein)
Cd 0	NaHS 0	1.6 ± 0.10^{d}	3.5 ± 0.24^{d}	0.46 ± 0.065^{c}
	NaHS 50	1.67 ± 0.09^{d}	3.1 ± 0.12^{d}	0.35 ± 0.065^{c}
Cd 50	NaHS 0	2.4 ± 0.10^{bc}	4.7 ± 0.24^{b}	0.73 ± 0.076^{b}
	NaHS 50	3.7 ± 0.08^{a}	5.8 ± 0.28^{c}	0.98 ± 0.124^{a}
Cd 100	NaHS 0	2.7 ± 0.12^{b}	6.1 ± 0.31^{a}	0.81 ± 0.098^{a}
	NaHS 50	2.1 ± 0.21^{c}	5.7 ± 0.18^{c}	0.65 ± 0.074^{b}

NaHS 0 - control

DISCUSSION

Plant growth parameters were studied in Cd-stressed plants treated with NaHS as H_2S donor. In this study, both Cd 50 μM and 100 μM showed toxicity effects on growth and physiological parameters of basil plants. Our results also showed that NaHS, when used in controls, caused a slight reduction in the root/shoot ratio and RWC of leaves. It also caused a higher electrolyte leakage of the cell membrane. When NaHS was applied to plants suffering from Cd toxicity, it showed healing effects on growth parameters. Our results reveal a significant improvement on biomass production and the root to shoot ratio of Cd-stressed plants when exposed to NaHS. Significantly less water loss was also observed in NaHS-treated plants, comparing to the control. Electrolyte leakage was also reduced by NaHS treatment in comparison with untreated plants under moderate Cd-stress (Cd 50 μM). Therefore, our results imply that NaHS is able to maintain plant growth through regulating RWC of the plant and leaf ion leakage under cadmium stress.

As in the case of growth parameters, NaHS reduced the total chlorophyll content when it was used alone. For many years, H_2S has been known for its detrimental effects on plants. It has been reported that H_2S inhibits oxygen release from rice seedlings. Similarly, H_2S causes leaves injuries, and reduces growth of the plants such as *Medicago*, lettuce, grapes, sugar beets, pine and fir (Lisjak et al., 2013). It seems that H_2S may show phyto-toxicity effects on some plants. On the other hand, NaHS-treated plants under cadmium stress, accumulated higher photosynthetic pigments, especially carotenoids. These findings suggest that under stress conditions, NaHS recovers growth parameters and enhances Cd-resistance in basil

plants, probably through alleviating damages of chlorophylls and especially carotenoids content. Similar to our results, Chen et al. (2011) reported that hydrogen sulfide increased photosynthesis in *Spinacia oleracea* under drought stress. They reported that low concentrations of NaHS increased the chlorophyll content of grains and thus led to the development of chloroplasts. Taken together, our results suggest that NaHS may have growth retardation effects when it is used in normal situations but at the same time it suppresses stress injuries of plants by regulating physiological parameters.

Lipid peroxidation and MDA biosynthesis are the result of the ROS production in biological membranes, which increases under biotic and abiotic stresses. It was previously reported that peroxidation of membrane unsaturated fatty acids ensures lower cell membrane fluidity and an increase in ion leakage. It is well established that various abiotic stresses such as heavy metals, cold, and drought can lead to accumulation of ROS. In this project, the production of H_2O_2 as an experimental source of oxygen-derived free radicals was measured. Our data showed that H_2O_2 content significantly increased at both medium and high concentrations of cadmium, but NaHS was only helpful to reduce H_2O_2 in plants exposed to 50 μM $CdCl_2$. Moreover, lipid peroxidation and MDA content showed a remarkable decrease in the NaHS-treated plants at $CdCl_2$ 50 μM. These results are also supported by data from electrolyte leakage and the relative water content. These findings propose that NaHS is able to save basil plants against moderate cadmium concentrations probably through the maintenance of cell membrane integrity and preventing oxidative stress. In general, these studies confirm the probability of H_2S interaction with ROS metabolism. Just as in our results, it has been reported that pretreatment of *Pisum sativum* plants with NaHS significantly reduced the concentration of H_2O_2 in plants under stress of oxygen deficiency (Cheng et al., 2013). Comparable results have also been reported for *Dendrobium* under high light intensity where exogenous H_2S agent reduced the MDA production by increasing the activity of antioxidant enzymes (Fan et al., 2014).

To remove ROS at the cellular level, plants utilize a variety of enzymatic and non-enzymatic antioxidant compounds. Among non-enzymatic compounds, phenolic compounds, anthocyanins and flavonoids are well recognized. Phenolic compounds carry out their antioxidant activity by regulating peroxidase enzymes activity and also prohibition of free radicals and lipid peroxidation (Chu et al., 2000). In this regard, the content of flavonoids and phenolic compounds has been studied. Our results showed that NaHS treatment of Cd 100 μM-stressed plants accumulates higher phenolic compounds, comparing to the plants not treated with NaHS. On the other hand, NaHS treatment reduced flavonoids content under stress conditions. Based on these findings, it could be suggested that NaHS implements its protective role against Cd stress partly by modulating total phenolic compounds content in *O. basilicum* plants.

Plant cells are able to achieve a balance between production and removal of ROS under normal and stressful conditions. The amount of reactive oxygen species and H_2O_2 content are controlled by enzymatic mechanisms (Sirikhachornkit and Niyogi, 2010). Antioxidant enzymes such as superoxide dismutase, catalase, guaiacol peroxidase and glutathione ascorbate cycle enzymes are involved in the detoxification process of free radicals and H_2O_2 production during stress (Teotia and Singh, 2014). It was reported by Zhang et al. (2009) that H_2S reduced the level of MDA in sweet potato plants under osmotic stress by regulating antioxidant enzymes activity and decreasing lipoxygenase enzyme activity (Zhang et al., 2009). In this project, we studied the activity of the main known antioxidant enzymes including catalase, guaiacol peroxidase and ascorbate peroxidase. Based on the results, the activity of CAT was increased in NaHS-treated plants under Cd 50 μM stress. We observed lower activity of GPX and APX in NaHS-treated plants under control conditions, but due to Cd 50 μM stress, the activity of the antioxidant enzymes increased in plants treated with NaHS. Moreover, plant treatments with H_2S releasing agent caused a decrease in the activity of CAT, GPX and APX under a higher concentration of cadmium (100 μM). Similar to our results, it has been shown that in rapeseed plants NaHS pretreatment under cadmium stress reduced H_2O_2 content by positive regulation of antioxidant enzymes (Savvides et al., 2016). Zhang et al. (2009) showed that spraying NaHS increased the activity of antioxidant superoxide dismutase enzymes, catalase and ascorbate peroxidase, and increased the concentration of H_2O_2 and lipoxygenase activity. These researchers argue that H_2S plays a role in oxidative stress and thereby it protects plants

against freezing stress. Fang et al. (2014) also reported that the exogenous H_2S application reduces production of H_2O_2 and other active oxygen species by inhibiting the activity of NADPH oxidase in Cr-induced stress in *Setaria italica* seedlings (Fang et al., 2014).

It can be concluded from our results that NaHS as a H_2S agent alleviates moderate cadmium stress in basil plants. NaHS is not effective under severe Cd toxicity. NaHS mediates protective responses against moderate cadmium stress mainly through regulating physiological responses such as protection of photosynthetic pigments against their decomposition, maintenance of the relative water content and cell membrane integrity in terms of reducing electrolyte leakage; it strengthens antioxidant system and regulates antioxidant enzymes. Thus, based on the results of our study, optimized concentration of NaHS under heavy metal stress is recommended. However, at the same time our results imply that NaHS may have limiting effects on plant growth under control conditions and its effects might be plant and dose dependent. Therefore, its application for agricultural purposes should be optimized before it is used.

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AUTHORS' CONTRIBUTIONS

Hakimeh Oloumi: designed and performed experiments, analyzed data and co-wrote the paper.

Mansooreh Khodashenas: performed experiments and co-wrote the paper. The authors declare that they have no conflict of interest.

REFERENCES

Ainsworth EA, and Gillespie KM. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols* 2: 875.

Benavides MP, Gallego SM, and Tomaro ML. 2005. Cadmium toxicity in plants. *Brazilian Journal of Plant Physiology* 17: 21-34.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.

Chang Z, Jingying S, Liqin Z, Changle L, and Qingguo W. 2014. Cooperative effects of hydrogen sulfide and nitric oxide on delaying softening and decay of strawberry. *International Journal of Agricultural and Biological Engineering* 7: 114-122.

Chen J, et al., 2011. Hydrogen sulphide enhances photosynthesis through promoting chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification in *Spinacia oleracea* seedlings. *Journal of Experimental Botany* 62: 4481-4493.

Cheng W, et al., 2013. Hydrogen sulfide alleviates hypoxia-induced root tip death in *Pisum sativum*. *Plant Physiology and Biochemistry* 70: 278-286.

Chu YH, Chang CL, and Hsu HF. 2000. Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture* 80: 561-566.

Fan H, Guan L, Li T, Wu Q, Wu M, Cai Y, and Lin Y. 2014. Hydrogen sulphide alleviates oxidative damage and enhances light energy transformation under high light for *Dendrobium officinale*. *Scientia Horticulturae* 177: 47-52.

Fang H, Liu Z, Jin Z, Zhang L, Liu D, and Pei Y. 2016. An emphasis of hydrogen sulfide-cysteine cycle on enhancing the tolerance to chromium stress in *Arabidopsis*. *Environmental Pollution* 213: 870-877.

Fang T, Cao Z, Li J, Shen W, and Huang L. 2014. Auxin-induced hydrogen sulfide generation is involved in lateral root formation in tomato. *Plant Physiology and Biochemistry* 76: 44-51.

Fotopoulos V, Christou A, Antoniou C, and Manganaris G. 2015. Hydrogen sulphide: a versatile tool for the regulation of growth and defence responses in horticultural crops. *The Journal of Horticultural Science and Biotechnology* 90: 227-234.

Hasanuzzaman M, Hossain MA, da Silva JAT, and Fujita M. 2012. Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: *Crop stress and its management: perspectives and strategies*, 261-315. Springer.

Hodges DM, DeLong JM, Forney CF, and Prange RK. 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* 207: 604-611.

Kuźniak E, and Urbanek H. 2000. The involvement of hydrogen peroxide in plant responses to stresses. *Acta Physiologiae Plantarum* 22: 195-203.

Li Z-G, Gong M, and Liu P. 2012. Hydrogen sulfide is a mediator in H_2O_2-induced seed germination in *Jatropha curcas*. *Acta Physiologiae Plantarum* 34: 2207-2213.

Li Z. 2013. Hydrogen sulfide: a multifunctional gaseous molecule in plants. *Russian Journal of Plant Physiology* 60: 733-740.

Lichtenthaler HK. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: *Methods in enzymology*, vol 148, 350-382. Elsevier.

Lisjak M, Teklić T, Wilson ID, Whiteman M, and Hancock JT. 2011. Hydrogen sulfide effects on stomatal apertures. *Plant Signaling and Behavior* 6: 1444-1446.

Lutts S, Kinet J, and Bouharmont J. 1996. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Annals of Botany* 78: 389-398.

Mahanty T, Bhattacharjee S, Goswami M, Bhattacharyya P, Das B, Ghosh A, and Tribedi P. (2017) Biofertilizers: a potential approach for sustainable agriculture development. *Environmental Science and Pollution Research* 24: 3315-3335.

Mostofa MG, Rahman A, Ansary MMU, Watanabe A, Fujita M, and Tran L-SP. 2015. Hydrogen sulfide modulates cadmium-induced physiological and biochemical responses to alleviate cadmium toxicity in rice. *Scientific Reports* 5: 14078.

Mullan D, and Pietragalla J. 2012. Leaf relative water content. In: *Physiological Breeding II: a field guide to wheat phenotyping*, 25-27. CIMMYT, Mexico.

Nakano Y, and Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology* 22: 867-880.

Peralta-Videa JR, Lopez ML, Narayan M, Saupe G, and Gardea-Torresdey J. 2009. The biochemistry of environmental heavy metal uptake by plants: implications for the food chain. *The International Journal of Biochemistry and Cell Biology* 41: 1665-1677.

Savvides A, Ali S, Tester M, and Fotopoulos V. 2016. Chemical priming of plants against multiple abiotic stresses: mission possible? *Trends in Plant Science* 21: 329-340.

Sirikhachornkit A, and Niyogi KK. 2010. Antioxidants and photo-oxidative stress responses in plants and algae. In: *The chloroplast*, 379-396. Springer.

Stone JR, and Yang S. 2006. Hydrogen peroxide: a signaling messenger. *Antioxidants and Redox Signaling* 8: 243-270.

Teotia S, and Singh D. 2014. Oxidative stress in plants and its management. In: *Approaches to plant stress and their management*, 227-253. Springer.

Toor RK, and Savage GP. 2005. Antioxidant activity in different fractions of tomatoes. *Food Research International* 38: 487-494.

Velikova V, Yordanov I, and Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Science* 151: 59-66.

Verbruggen N, Hermans C, and Schat H. 2009. Mechanisms to cope with arsenic or cadmium excess in plants. *Current Opinion in Plant Biology* 12: 364-372.

Wagner GJ. 1979. Content and vacuole/extravacuole distribution of neutral sugars, free amino acids, and anthocyanin in protoplasts. *Plant Physiology* 64: 88-93.

Yonezawa D, et al. 2007. A protective role of hydrogen sulfide against oxidative stress in rat gastric mucosal epithelium. *Toxicology* 241: 11-18.

Zhang H, Hu L-Y, Li P, Hu K-D, Jiang C-X, and Luo J-P. 2010. Hydrogen sulfide alleviated chromium toxicity in wheat. *Biologia Plantarum* 54: 743-747.

Zhang H, Ye Y-K, Wang S-H, Luo J-P, Tang J, and Ma D-F. 2009. Hydrogen sulfide counteracts chlorophyll loss in sweet potato seedling leaves and alleviates oxidative damage against osmotic stress. *Plant Growth Regulation* 58: 243-250.

Zhang J. 1990. Response of cell protective enzymes in corn leaf to water stress at seedling stage. *Acta Agriculture Boreali-Sinica* (Suppl 5): 19-23.

Zhang Y-X et al. 2015. The hydrogen sulfide donor NaHS delays programmed cell death in barley aleurone layers by acting as an antioxidant. *Oxidative Medicine and Cellular Longevity* 2015: 714756.