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Nitric oxide and hydrogen sulfide alleviates salt incited oxidative stress in *Coriandrum sativum* L

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ABSTRACT

Soil salinity accelerates osmotic, ionic and oxidative pressure that suppresses plant growth and crop production. The objective of of the present investigation was to analyse protective function of nitric oxide (NO) and hydrogen sulfide (H_2S) against salt (NaCl) stress in coriander plants. Different morphological, biochemical parameters, enzymatic and non-enzymatic antioxidants were analysed. Exposure of *Coriandrum sativum* L. to NaCl reduced length of root and shoot, biomass, relative water content and biochemical attributes. Exogenous use of SNP and NaHS individually or in combined treatment increased all the above-mentioned growth variables and inhibited NaCl incited biochemical alterations and oxidative damages in *Coriandrum sativum* L. Synergistic treatment (SNP + NaHS) exhibited increase in the morphological parameters, pigment and osmolyte contents. The oxidative stress biomarkers, hydrogen peroxide and superoxide generation were declined by SNP and NaHS as evidenced by reduced peroxidation of lipids. Activities of both glyoxylase as well as antioxidant enzymes were enhanced with presence of SNP and NaHS. Coriander seedlings treated with NaCl + SNP + NaHS exhibited 21 %, 7 %, 31 % and 59 % increase in superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), respectively over NaCl treated plants. Level of methylglyoxal was decreased in *Coriandrum sativum* with rise in glyoxalase I and II enzymes. Therefore, synergistic supplementation of NO and H_2S alleviates salt stress in coriander plants more efficiently than individual treatment and it might be because of crosstalk mechanism that facilitate salt stress resistance in plants.

1. Introduction

Salinization of soil has become a major threat which adversely affects plant development and crop production and reduces cultivable areas all over the world (Kaya et al., 2020a). The continuous increase in salinization due to rise in excessive use of fertilizers, industrialization and irrigation with contaminated water which may cause 50 % depletion of agrarian areas up to 2050 (Raju et al., 2020). Salinity negatively affected soil texture which resulted in deterioration of approximately 800 million hectare of arable land at a global level (Tariq and Shahbaz, 2020). Salt stress incites ionic and osmotic distress in plants which promotes secondary pressure by free radicals accretion, enhances peroxidation of lipids, disruption of ionic balance, uptake of water and nutrients, water utilization ability (Khan et al., 2021). Sodium chloride is mainly present in soil in dissolved form and enhances sodium and chloride ions, which are responsible for inhibition of uptake of mineral nutrients like K^+, Ca^{2+} etc. Salinity enhanced intake of Na^+ by plasma membrane depolarization resulted in K^+ loss which inhibits potassium ions and ratio of K^+/Na^+, disrupts equilibrium of ions and ultimately apoptosis (Basu et al., 2021). The prolonged exposure of salt stress causes adverse effect on cell division and growth, osmotic imbalance, production of free radicals that generates oxidative stress, inhibits biochemical processes, causes DNA damage and membrane lipids peroxidation (Kaya et al., 2020a).

Nitric oxide and hydrogen sulfide are lipophilic, colorless, organic molecules and these were initially regarded as phytotoxic in nature. Nitric oxide induces nitro-oxidative stress at high concentration and show signaling behaviour at low concentration (Huang et al., 2020). Hydrogen sulphide is hazardous in nature for plant cells at high concentration but exhibits protective response under environmental

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pressure, mitigates nitro-oxidative deterioration at low concentration (Corpas, 2019). H_2S acts as a growth regulator for the plants against environmental pressure. Nitric oxide contribution in regulatory processes under salt stress like mitogen activated protein kinase, calcium dependent, salt overly sensitive, G-protein associated signalling has been reported (Saddhe et al., 2019).

Nitric oxide and hydrogen sulfide are significant gaseous signalling molecules which function together to enhance resistance against abiotic stresses and H_2S work downstream or upstream of NO which depends on its involvement in biochemical events under abiotic stresses (Corpas et al., 2019). Both hydrogen sulfide and nitric oxide are dynamic molecules which can cross through cell membrane and can function separately from the receptor system. Corpas et al. (2019) observed pragmatic relationship in functions of NO and H_2S and their signaling interplay in plants promotes stress resistance.The crosstalk between NO and H_2S was responsible for the resistance in *Capsicum* against salt stress (Kaya et al., 2020).

Coriander (*Coriandrum sativum* L.; family Apiaceae), is widely used as an important plant with nutritional and medicinal properties. The essential oils and extracts of coriander have shown antibacterial, anti-inflammatory, antioxidant, antidiabetic, anticancer, antimutagenic and neuroprotective properties. There is an urgent need to solve salinity problem to feed the global population under limited agricultural land. Different approaches used to mitigate soil salinization are expensive and may rise the risk of secondary salinization. We hypothesize that combined impacts of NO and H_2S may improve physiological and biochemical variables of coriander to enhance its tolerance to combat salt stress. To best of our knowledge, combined treatment of signaling molecules i.e. SNP and NaHS on the antioxidative and glyoxalase system of coriander seedlings for mitigation of salt stress have not been reported earlier. Considering the protective functions of SNP and NaHS against salt stress, this investigation was planned to (i) interrogate individual and synergistic impacts of SNP and NaHS supplementation on growth attributes (ii) analyse oxidative variables (iii) understand the probable mechanism of action of SNP and NaHS on activation of antioxidant and glyoxylase defense system for protection of coriander seedlings under salt stress. Thus, present study reveals that crosstalk between SNP and NaHS is essential for resistance against salt stress in coriander.

2. Materials and methods

2.1. Plant materials and chemical compounds

Coriander (*Coriandrum sativum* L. variety Kasturi) seeds were obtained from local seed market of Ghaziabad. Sodium nitroprusside (SNP; a NO donor, molecular weight: 261.9 g.mol^{-1}) and Sodium hydrosulfide (NaHS; a H_2S donor, molecular weight: 56 g.mol^{-1}) were procured from Merck.

2.2. Plant growth and stress treatment

Seeds of coriander were treated with mercuric chloride (0.01 %) solution for five minutes and rinsed by deionized water and kept in nutrient rich substrate in tray for 8 days and properly irrigated. Two groups of pots were kept and amended with salt (NaCl) 0 g for control and 10 g/kg for treatment was mixed with soil during pot filling. Initially different concentrations of SNP and NaHS were used for the experiments but 50 μM concentration of SNP and NaHS was selected due to its better protective role against salt stress. Total 24 plastic pots (8 treatment x 3 replicates) were arranged for experiment. The eight different treatments were utilized for the study: (1). Control (2). NaCl (3). 50 μM SNP (4). 50 μM NaHS (5). 50 μM SNP + 50 μM NaHS (6). NaCl + 50 μM SNP (7). NaCl + 50 μM NaHS (8). NaCl + 50 μM SNP + 50 μM NaHS. One seedling was transferred to each plastic pot (14 x 8 cm) and kept under 150 μmol photons $m^{-2}\bullet s^{-1}$ photosynthetically active radiation for 16:8h day and night regime at 27 ± 2 °C under 90 % relative humidity. The solution of 50 μM SNP and 50 μMNaHS (10 ml per pot) prepared in Hoagland solution was sprayed on every alternate day up to 15 days and control seedlings (without NaCl) were sprayed with distilled water. Twenty-three days old coriander seedlings (two weeks after SNP and NaHS treatment) were taken for estimation of biochemical parameters as given below:

2.3. Estimation of growth and relative water content

Seedling length and biomass were analyzed in control and treatment. For RWC, fresh weight of coriander leaves was measured and disc was put in distilled water at 25 ± 2 °C for 12 h under dark condition. After turgid weight estimation, discs were placed in oven at 80 °C for two days and dry weight was analysed.

$$RWC = (FW\text{-}DW) / (TW\text{-}DW) \times 100 \quad (1)$$

2.4. Measurement of pigment, sugar, proline, protein

Coriander leaves were crushed with 80 % acetone. After centrifugation, optical density of filtrate was taken by spectrophotometer (Shimadzu 1800, Kyoto, Japan) at 663, 646 and 470 nm for determination of chl *a, b,* total chl *(a + b),* carotenoid as per procedure of Lichtenthaler (1987).

Total chlorophyll (mg/g) = (20.2 x OD_{645} + 8.02 x OD_{663}) x V/100 x W

Chlorophyll *a* (mg/g) = (12.7 x OD_{663} − 2.69 x OD_{645}) x V/100 x W

Chlorophyll *b* (mg/g) = (22.9 x OD_{645} − 4.68 x OD_{663}) x V/100 x W

Carotenoid (mg/g) = (1000 OD_{470} − 1.82 Chl*a* − 85.02 Chl*b*)/198

where V = supernatant volume (ml).

W = Leaves fresh weight in g, OD = absorbance.

Sugars, protein and proline were assessed by following Hedge and Hofreiter (1962).-, Lowry et al.(1951) and Bates et al. (1973) respectively.

2.5. Assessment of electrolyte leakage, hydrogen peroxide and superoxide level

Lutts et al. (1996) procedure was used in estimation of electrolyte leakage in coriander leaves. Calculation of electrolyte leakage was done by given formula: EC = EC1/EC2 × 100. Velikova et al. (2000) method was used for estimation of hydrogen peroxide. Optical density was calculated at 390 nm and standard curve was used for estimation of hydrogen peroxide. Yang et al. (2013) method was used for estimation of the superoxidelevel. Absorbance was calculated at 530 nm, and superoxide level was analyzed by standard curve of sodium nitrite.

2.6. Lipid peroxidation and methylglyoxal content

Leaves of coriander (two hundred milligrams) were fused in trichloro-acetic acid (0.1 %) and filtrate was mixed in 0.5 % TBA and kept at water bath at 95 °C for half an hour and after centrifugation, optical density of filtrate was taken at 532 nm and then reducing with non-specific absorbance at 600 nm. Malondialdehyde amount was calculated with extinction coefficient at 155 $mM^{-1}cm^{-1}$ (Heath and Packer, 1968).

For methylglyoxal level, leaves of coriander were fused with perchloric acid (5 %) and filtrate was treated with charcoal, K_2CO_3, N-acetyl-L-cysteine, NaH_2PO_4. As per Wild et al. (2012) method N- α-acetyl -S- (1-hydroxy-2-oxo-prop-1-yl) cysteine synthesis was measured at 288 nm.

2.7. Contents of nitric oxide and hydrogen sulfide

Nitrite amount was measured for estimation of NO by procedure of Zhou et al. (2005). Optical density was calculated at 540 nm, nitric oxide amount was measured by the standard curve by applying sodium nitrite as standard.

Hydrogen sulfide amount in leaves of coriander was analysed by procedure of Xie et al. (2014). Standard curve was prepared with various sodium hydrosulfide concentrations and denoted by nmol/g fresh weight.

2.8. Assessment of glyoxalase I and II

Activity of glyoxalase I was estimated by Kaya et al., (2020b) and glyoxalase II activity was measured by Principato et al. (1987).

2.9. Antioxidant enzymes

Enzyme extract was prepared by crushing leaves of coriander with sodium phosphate buffer (0.1 M) and polyvinyl pyrrolidone. After centrifugation (14,000g) at 4 °C for 30 min, filtrate was taken for estimation. NBT photochemical procedure was used for SOD assessment at 560 nm (Beyer and Fridovich, 1987). Catalase activity was estimated by H_2O_2 degradation for one minute at 240 nm by applying 39.4 mM^{-1} cm^{-1} extinction coefficient Cakmak and Marschner (1992). Method of Nakano and Asada (1981) was applied for APX measurement. Absorbance was taken at 290 nm in one minute with extinction coefficient 2.8 mM^{-1} cm^{-1}. Foster and Hess (1980) method was used for glutathione reductase estimation by changing optical density at 340 nm for three minutes.

2.10. Non- enzymatic antioxidants

For ascorbate content, coriander leaves were fused in 6 % TCA, 2 % dinitrophenylhydrazine and after centrifugation, 10 % thiourea were mixed in filtrate. The samples were properly cooled after heating for 15-minutes, and 80 % sulphuric acid was added. At 530 nm, optical density was calculated and ascorbate standard curve was used for analyses (Mukherjee and Choudhuri, 1983).

2.11. Statistical analysis

Treatment was denoted as randomized block design with 3 replicates. Values were assessed from ANOVA by applying SPSS and DMRT at $P < 0.05$ was used for calculation of standard error of mean.

3. Results and discussion

In present investigation, we aim to identify individual as well as interactive impact of nitric oxide and hydrogen sulfide for protection of coriander plants under salt stress. Coriander seedlings were exposed to saline conditions and supplemented with SNP (50 μM) and NaHS (50 μM) individually and in combination to verify their synergistic effects.

3.1. Morphological parameters

Coriander seedlings showed 65, 37, 54 and 64 % reduction in the length of root, shoot, biomass with NaCl treatment as compared to control. The SNP and NaHS treatment showed increase in seedling length and SNP + SPD combined application showed 17 and 16 % stimulation in shoot length and fresh weight over control in coriander seedlings. NaCl + SNP + NaHS treatment showed 173 and 304 % enhancement in root length and shoot dry weight, respectively as compared to salt exposed seedlings. Relative water content showed 21 % reduction in salt exposed plants and relative water content was enhanced to 29, 27 and 30 % in SNP, NaHS, SNP + NaHS treated coriander in comparison to NaCl treated plants. The relative water content was enhanced up to 24 % in coriander with NaCl + SNP + NaHS treatment as compared to salt exposed plants (Fig. 1).

3.2. Pigment content

Supplementation of SNP and NaHS increased total chlorophyll content 13 and 2.3 % and carotenoids by 18 and 9 % respectively in comparison to control (Table 1).Total chlorophyll and carotenoids contents were enhanced 20 and 22 % respectively under SNP + NaHS treatment as compared to control. Application of SNP and NaHS treatment enhanced pigment content and maximum alleviation was observed in combined treatment (SNP + NaHS) in coriander plants as compared to salt stress.

Values are mean ± SE of 3 replicates from 3 separate experiments. Values showing various letters exhibit variation among treatment at $P < 0.05$ significant level as per ANOVA and DMRT.

3.3. Osmolytes content

. Salt treatment showed 63 and 37 % increase in sugar and proline contents over control. Significant escalation in osmolyte contents of coriander seedlings 112 and 287 % was reported in sugar and proline respectively under NaCl + SNP + NaHS treatment as compared to control (Table 2).Significant reduction 18 % was reported in protein content of coriander plants under salt stress as compared to control. However, protein contents were enhanced to 24 and 13 % with SNP and NaHS treatment, respectively with maximum rise 42 % was observed under combined SNP + NaHS treatment.

Values are mean ± SE of 3 replicates from three different experiments. Values showing different letters show difference among treatment at $P < 0.05$ significant level as per ANOVA and DMRT.

3.4. Oxidative stress variables

Salt stress increased electrolyte leakage 57 %, production of hydrogen peroxide 140 % and superoxides 76.3 % in *Coriandrum sativum* L as compared to control. Sodium nitroprusside and sodium hydrosulfide amendment decreased electrolyte leakage by 45 and 42.3 % respectively as compared to salt stress. SNP + NaHS treatment significantly reduced hydrogen peroxide 68 % and superoxide production 61 % in coriander seedlings as compared to salt treatment hence inhibited oxidative stress (Fig. 2).

3.5. Malondialdehyde and methylglyoxal content

Coriander seedlings treated with SNP and NaHS showed 35 and 33 % decrease in MDA contents, respectively over NaCl treatment. Extraneous supply of SNP and NaHS individually as well as synergistically decreased methylglyoxal contents with significant decrease 39 % with SNP + NaHS treatment over NaCl treatment (Fig. 3).

3.6. Contents of nitric oxide and hydrogen sulfide

Corpas (2019) reported multifaceted connections between NO and H_2S as both participate in different biochemical processes. Salt stress increased NO and H_2S contents by 123 and 159 %, respectively, in the coriander leaves over control. SNP and NaHS individual treatment significantly enhanced 51, 27 % NO and 38 and 26 % H_2S contents in coriander leaves over control. The synergistic treatment (NaCl + SNP + NaHS) increased the contents of NO by 117 % and H_2S 67 % in coriander leaves over control (Fig. 4).

3.7. Activity of glyoxalase enzymes

Salt treatment enhanced glyoxalase I and reduced glyoxalase II in

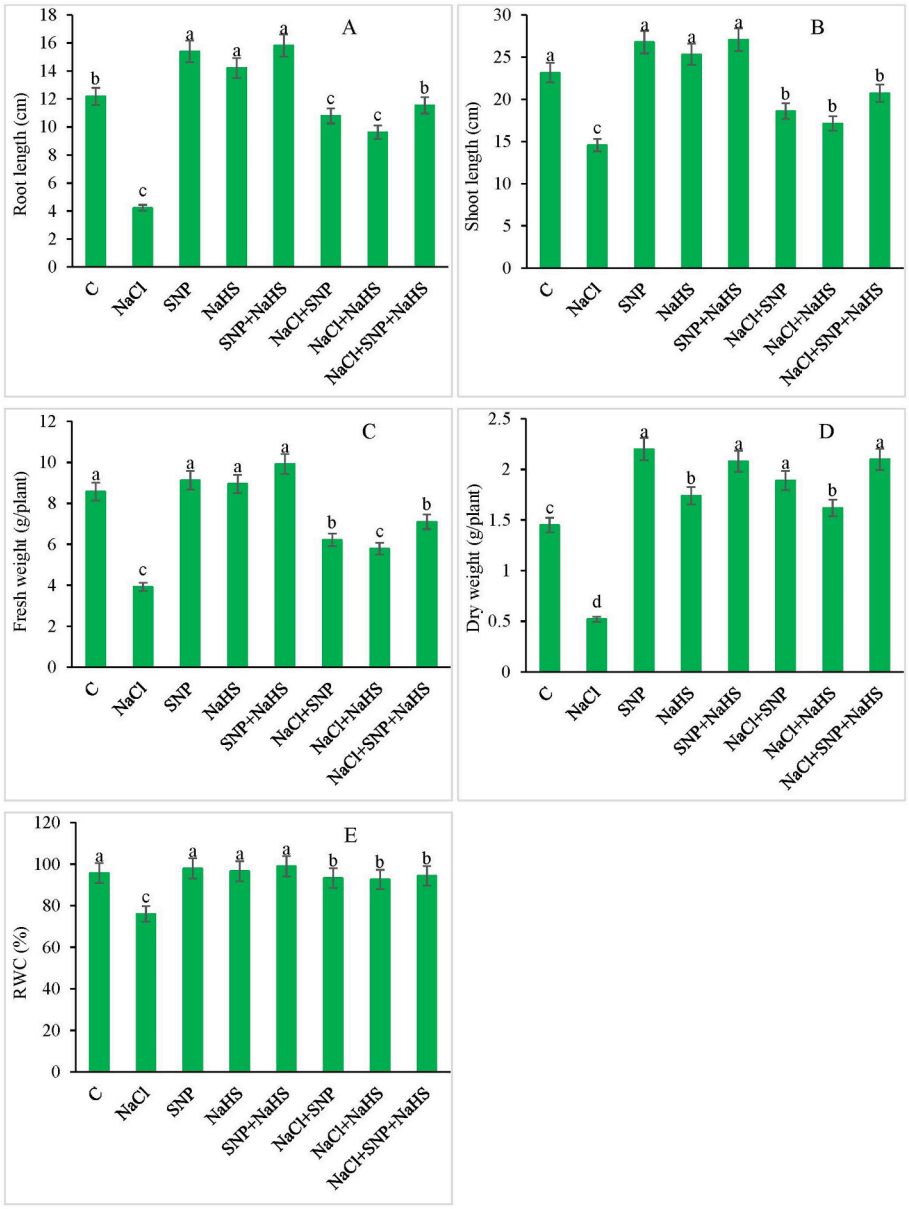

Fig. 1. Effect of salt stress on seedling length, fresh and dry weight, relative water content of *Coriandrum sativum* L. with or without SNP and NaHS. Values are mean ± SE of three replicates from 3 different experiments. Different letters show variation among treatment at $P < 0.05$ significant level as per ANOVA and DMRT.

coriander in comparison to control. Coriander seedlings exposed to NaCl treatment showed 136 % escalation in activity of Gly I over control. Addition of both SNP and NaHS in NaCl treated coriander seedlings enhanced Gly I activity upto 118 % over control. The SNP and NaHS supplementation in NaCl treated coriander seedlings escalated 133 % glyoxalase II activities in comparison to salt treated coriander seedlings (Fig. 5).

3.8. Antioxidant enzymes

The salt stress increased activities of antioxidant enzymes over control, further rise was recorded when both SNP and NaHS were added in NaCl treated coriander. Individual treatment of SNP and NaHS showed 11 and 2 % enhancement in SOD activity, however 5, 20 and 5.22 % reduction was reported with SNP and 10, 23 and 21 % decrease in CAT, APX, GR respectively with NaHS as compared to salt treated coriander plants. The seedlings treated with NaCl + SNP + NaHS reflected an enhancement of 21, 7 % SOD and CAT, 31, 59 % in APX, GR respectively over NaCl treated seedlings (Fig. 6). It was clearly observed that synergistic effect of SNP and NaHS was obvious as compared to individual application.

3.9. Ascorbate and glutathione contents

Salt stress decreased ascorbate cumulation but enhanced glutathione contents. Significant enhancement was reported with individual treatment of SNP and NaHS in ascorbate 10 and 4 % and glutathione contents 6 and 4.3 %, respectively. SNP and NaHS combined treatment enhanced 12 % ascorbate and 10 % glutathione contents in coriander seedlings over control. Application of SNP and NaHS significantly enhanced 95 % ascorbate and 24 % glutathione contents in salt treated coriander over salt stress alone (Fig. 7).

4. Discussion

Salt stress reduced growth of coriander seedlings, however

Table 1
Impact of salt stress on pigment composition of *Coriandrum sativum* L. with or without SNP and NaHS.

Treatment	Chl *a* (mg/g FW)	Chl *b* (mg/g FW)	Total chl *(a + b)* (mg/g FW)	Carotenoids (mg/g FW)
Control	1.45 ± 0.12^{b}	0.76 ± 0.15^{b}	2.21 ± 0.21^{a}	0.45 ± 0.03^{b}
NaCl	1.02 ± 0.02^{d}	0.42 ± 0.04^{c}	1.44 ± 0.11^{c}	0.59 ± 0.07^{a}
SNP	1.69 ± 0.07^{a}	0.80 ± 0.07^{a}	2.49 ± 0.14^{a}	0.53 ± 0.06^{b}
NaHS	1.57 ± 0.15^{a}	0.69 ± 0.05^{a}	2.26 ± 0.02^{a}	0.49 ± 0.08^{b}
SNP + NaHS	1.82 ± 0.04^{a}	0.84 ± 0.03^{a}	2.66 ± 0.15^{a}	0.55 ± 0.04^{a}
NaCl + SNP	1.34 ± 0.07^{b}	0.61 ± 0.08^{b}	1.95 ± 0.23^{b}	0.52 ± 0.01^{a}
NaCl + NaHS	1.23 ± 0.11^{c}	0.48 ± 0.09^{c}	1.71 ± 0.44^{c}	0.54 ± 0.02^{a}
NaCl + SNP + NaHS	1.42 ± 0.13^{b}	0.68 ± 0.06^{b}	2.10 ± 0.21^{b}	0.67 ± 0.06^{a}

Table 2
Impact of salt stress on sugar, proline and protein contents of *Coriandrum sativum* L. with or without SNP and NaHS.

Treatment	Sugar (mg/g DW)	Proline (μM/g DW)	Protein (mg/g FW)
Control	3.45 ± 0.07^{d}	13.21 ± 0.34^{c}	12.43 ± 0.15^{c}
NaCl	5.61 ± 0.46^{b}	18.15 ± 0.37^{c}	10.14 ± 0.04^{c}
SNP	4.92 ± 0.08^{c}	27.23 ± 0.39^{b}	15.37 ± 0.14^{a}
NaHS	4.23 ± 0.01^{c}	25.92 ± 0.24^{b}	14.10 ± 0.46^{b}
SNP + NaHS	5.72 ± 0.24^{b}	33.75 ± 0.67^{b}	17.62 ± 0.11^{a}
NaCl + SNP	6.27 ± 0.28^{a}	38.91 ± 0.06^{a}	13.94 ± 0.42^{b}
NaCl + NaHS	5.99 ± 0.23^{b}	43.64 ± 0.04^{a}	13.17 ± 0.01^{b}
NaCl + SNP + NaHS	7.32 ± 0.27^{a}	51.18 ± 0.05^{a}	14.89 ± 0.07^{b}

supplementation of SNP and NaHS increased seedling growth (Fig. 1). Similar findings were observed in our experiments as salt stress reduced fresh and dry weight of coriander seedlings upto 54 and 64 % respectively over control. The presence of salt in soil decreased absorption of water by coriander seedlings and generated osmotic stress and accretion of excessive sodium and chloride ions in cells of plants inhibited K^{+} concentration and led to ionic toxicity. Under salt stress, nitric oxide was reported to increase germination and growth of sunflower and cucumber by mitigation of oxidative stress (Tailor et al., 2019). Nitric oxide recovered salt-induced reduction in growth, relative water content, photosynthesis and uptake of minerals in *Capsicum annum* (Shams et al., 2019). Function of nitric oxide in enhancing growth of plants under NaCl stress has been reported by Huang et al. (2020). Significant enhancement in germination, seedling growth, biomass and yield with treatment of nitric oxide releasing compounds (Kapoor et al., 2023). H_2S show significant function from germination to maturation of plants. The generation of H_2S in plant cells and its function become strengthen with the application of exogenous H_2S (Li et al., 2021).

. Exogenous application of H_2S induces generation of H_2S in cells of plants (Li et al., 2021). Nitric oxide and hydrogen sulfide induced signaling with jasmonic acid, strigolactone, auxin and ethylene promote root development and boost plants resistance against unfavourable environmental conditions (Mukherjee and Corpas, 2020). NO and H_2S contribute a promising function to boost plants resistance against unfavourable environmental conditions (Corpas et al., 2019). Maximum 98 % RWC was observed in combined treatment SNP + NaHS however, significant decrease 76 % in relative water content was observed under salt stress (Fig. 1).Cell membrane injury is significant indicator of NaCl stress and it can be analyzed by electrolyte leakage and generation of malondialdehyde by peroxidation of polyunsaturated fatty acids under salinity. Kharbech et al. (2020) observed H_2S crosstalk with nitric oxide for protection of integrity of plasma membrane. Our findings are consistent with their results, MDA content was reduced 34.8 and 32.9 % whereas hydrogen peroxide content was decreased 63.7 and 54.2 % with SNP and NaHS treatment respectively in coriander seedlings. The cells of plants accumulate osmolytes to maintain their water balance.

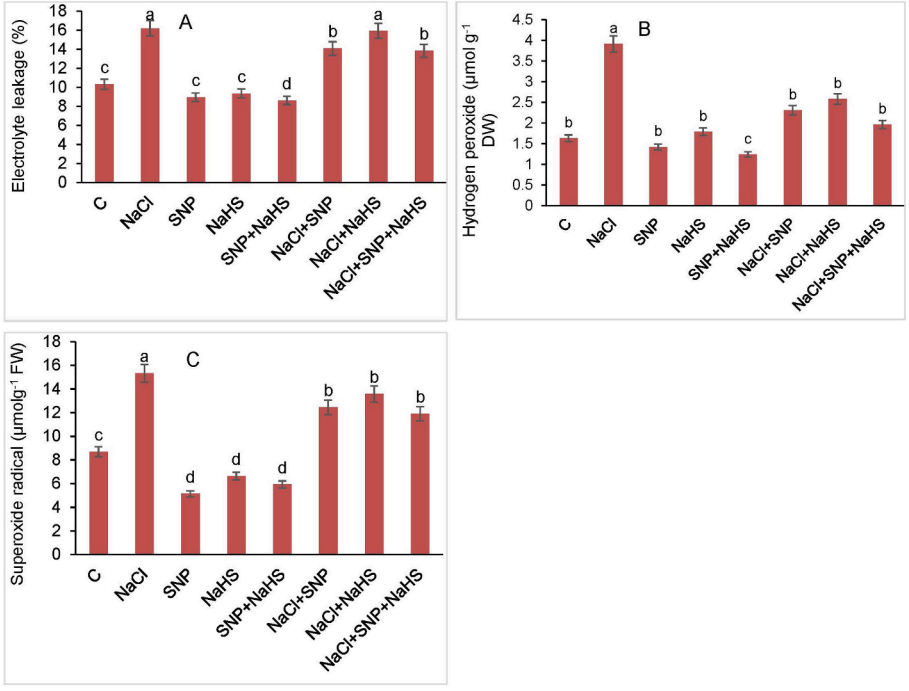

Fig. 2. (A). Electrolyte leakage (B) hydrogen peroxide (C) superoxide levels in *Coriandrum sativum* L. under salt stress with and without SNP and NaHS [SNP = 50 μM and NaHS = 50 μM concentrations]. The vertical data bars indicate mean ± SE of 3 replicates from three independent experiments. Different letters on the bars indicate difference among treatment at $P < 0.05$ significant level as per ANOVA and DMRT.

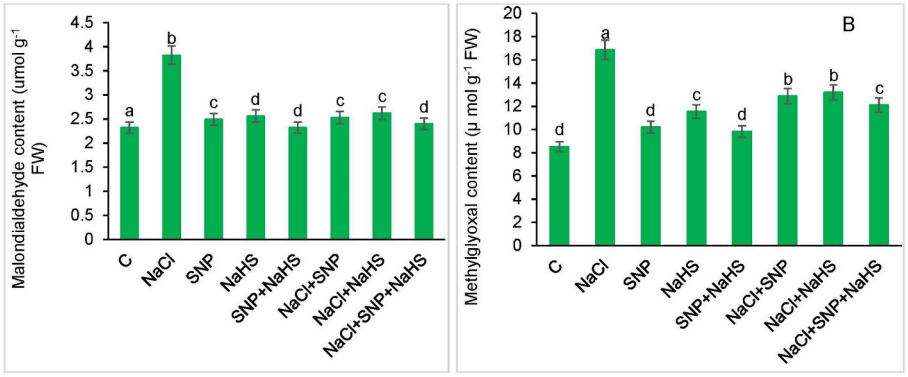

Fig. 3. (A) Lipid peroxidation and (B) methylglyoxal contents in *Coriandrum sativum* L. grown under salt treatment with and without SNP and NaHS [SNP = 50 μM and NaHS = 50 μM concentrations].Vertical data bars indicate mean ± SE of 3 replicates from 3 independent experiments. Different letters on the bars indicate difference among treatment at $P < 0.05$ significant level as per ANOVA and DMRT.

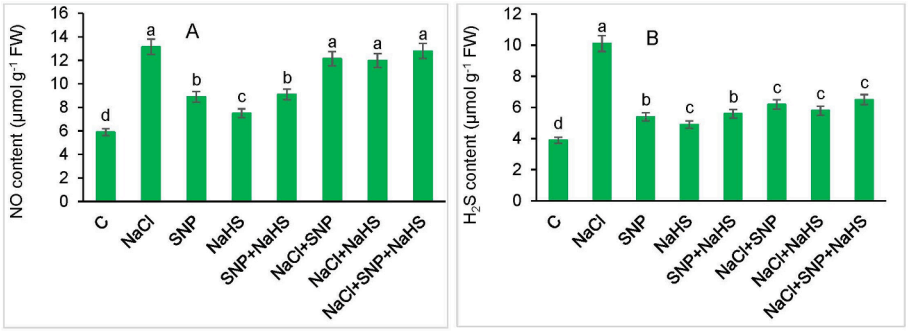

Fig. 4. Contents of (A) nitric oxide (NO) and (B) hydrogen sulfide (H_2S) in *Coriandrum sativum* L. exposed to NaCl treatment with and without SNP and NaHS [SNP = 50 μM and NaHS = 50 μM concentrations].The vertical data bars indicate mean ± SE of 3 replicates from 3 independent experiments. Different letters on the bars reflect difference among treatment at $P < 0.05$ significant level as per ANOVA and DMRT.

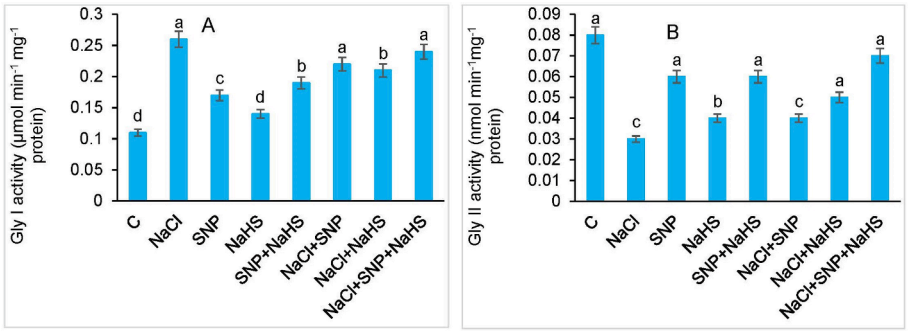

Fig. 5. Activities of (A) glyoxalase I and (B) glyoxalase II in *Coriandrum sativum* L. exposed to NaCl treatment with and without SNP and NaHS [SNP = 50 μM and NaHS = 50 μM concentrations]. Vertical bars show mean ± SE of 3 replicates from three independent experiments. Different letters on the bars indicate difference among treatment at $P < 0.05$ significant level as per ANOVA and DMRT.

Significant increase 287.4 % in proline content was observed in combined treatment NaCl + SNP + NaHS over control (Table 2). The supplementation of SNP in salt-stressed *Capsicum annuum* enhanced proline content by maintaining osmoregulation, scavenging of free radicals and biomolecules and biomembranes stabilization (Kaya et al., 2020a). Being a source of carbon and nitrogen, proline removes free radicals and protects plants under salt stress. . Proline helps plants to retrieve from stress, preserves photosynthetic organelle and stores energy during NaCl stress and enhanced survival of plants.

The osmolytes and antioxidants accretion have been reported by NO and H_2S which mitigate NaCl stress in plants. Supplementation of NO maintains ionic equilibrium under NaCl stress by salt overly sensitive cycle with more cell membrane H^+-ATPase activity (Saddhe et al., 2019). Nitric oxide regulated stomatal conductance, energy quenching and quantum yield of photosystem II to enhance photosynthesis. H_2S supplementation increased chlorophyll precursors and carotenoid components. Application of SNP and NaHS enhanced 72.9 and 56.9 % total chlorophyll contents in coriander seedlings over salt stress. Chloroplast arrangement in mesophyll was disrupted and connection between grana was lost in *Sulla coronaria* under NaCl treatment (Hao et al., 2021). Salinity hindered function of oxygen-evolving and pigment-protein complexes on thylakoid membrane and decreased efficiency of electron transfer. . The combined action of NO and H_2S promoted melatonin which incited resistance against NaCl treatment was found to enhance growth and reduction in oxidative pressure in *Piper nigrum* (Kaya et al., 2020). Huang et al. (2020) stated enhancement in nitric

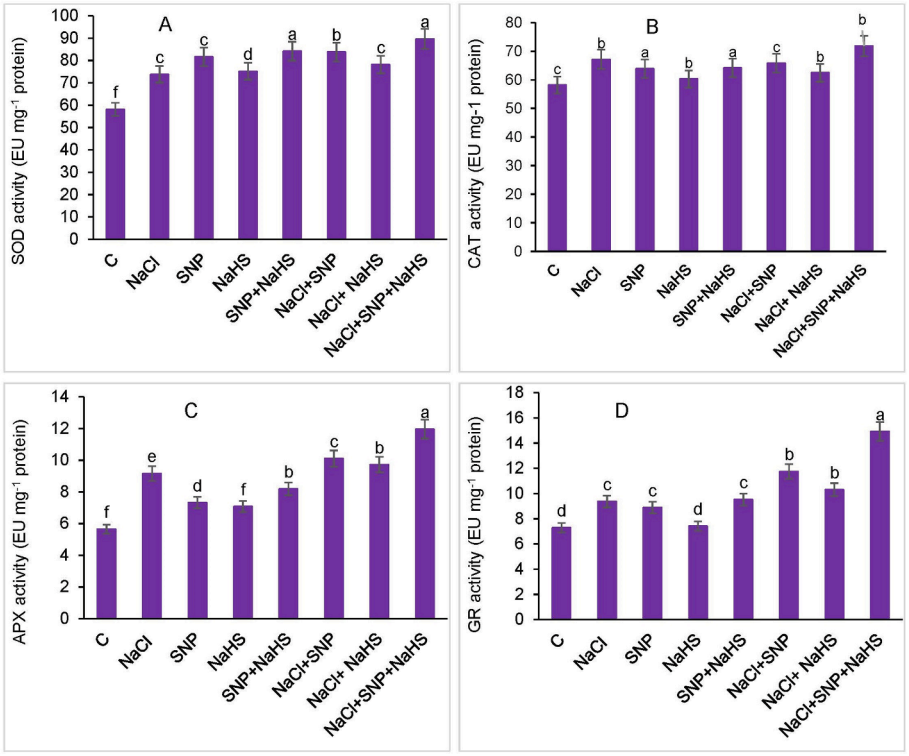

Fig. 6. (A) Superoxide dismutase (SOD) (B) catalase (CAT) (C) ascorbate peroxidase (APX) (D) glutathione reductase (GR) enzymes in *Coriandrum sativum* L. under salt stress with and without SNP and NaHS [SNP = 50 μM and NaHS = 50 μM concentrations]. Vertical data bars indicate mean ± SE of 3 replicates from three independent experiments. Different letters on the bars indicate significant difference among treatment at $P < 0.05$ significant level as per the ANOVA and DMRT.

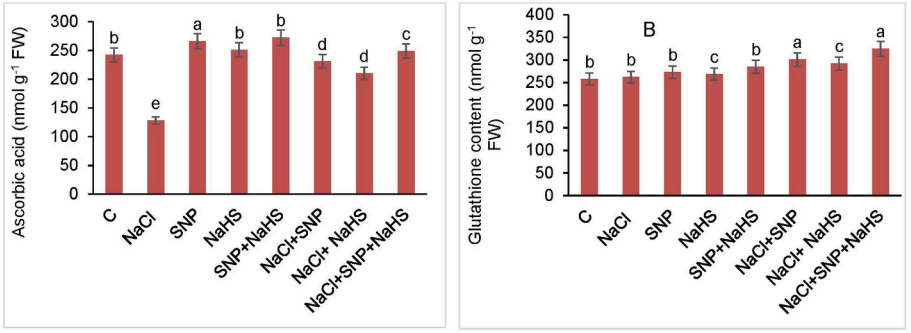

Fig. 7. Contents of (A) ascorbate (ASA) and (B) reduced glutathione (GSH) in *Coriandrum sativum* L. under salt stress with and without SNP and NaHS. [SNP = 50 μM and NaHS = 50 μM concentrations]. Vertical data bars indicate mean ± SE of 3 replicates from three independent experiments. Different letters on the bars show significant difference among treatment at $P < 0.05$ significant level as per the ANOVA and DMRT.

oxide in salt treated *Kandelia obovata*.Under salt stress, NO and H_2S contents were maximum in coriander seedlings (Fig. 4).

Methylglyoxal and free radicals are cytotoxic in nature, excessive production of ROS and MG can degrade biomolecules and induce apoptosis (Kaya et al., 2020b). Excessive free radicals and methylglyoxyl accretion was reported in plants under salt stress which may degrade proteins, sugars, lipids and nucleic acids. . Significant increase 98 % in methylglyoxyl content was observed in salt stress in comparison to control, however 28 % decrease was observed in NaCl + SNP + NaHS treatment over salt stress in coriander seedlings (Fig.3). Plants evolved enzymatic and non-enzymatic antioxidants for removal of free radicals and glyoxalase system for detoxification of toxic methylglyoxal for protection against abiotic stresses. Hydrogen sulfide and nitric oxide regulated CAT, POX and APX activities by post translational alterations in proteins, enhanced resistance of plants against oxidative pressure and maintained redox balance (Li et al., 2020). The salt tolerance was promoted by H_2S by activation of antioxidative enzymes and maintenance of Na^+/K^+ balance by decreasing elimination of potassium ions in *Spartina* (Li et al., 2020). Supplementation of H_2S mitigated NaCl incited toxic impacts in *Cucumis sativus* by regulating sodium and potassium equilibrium and H^+-ATPase expression by promoting antioxidant enzymes with reduction in free radicals aggregation and peroxidation of lipids (Jiang et al., 2019). Application of NaHS enhanced antioxidative defense system and photosynthesis by reducing malondialdehyde and hydrogen peroxide contents in *Triticum aestivum* under salinity(Corpas et al., 2019). H_2S and NO escalated activities of catalase, superoxide dismutase and ascorbate peroxidase to scavenge free radicals, decreased peroxidation of lipids and enhanced plant tolerance against salinity. Combined effects of NO and H_2S modulated multiple defense pathways in stressed plants to combat adverse conditions. H_2S and NO enhanced antioxidant enzymes activity via post-translational modifications (Corpas, 2019). H_2S increased NO content and upregulated SOD, APX and

CAT activities and maintained redox balance in plant cells (Ahmed et al., 2021). Similar interactions between endogenous NO and H_2S have been reported in tobacco against salt treatment (Da Silva et al., 2017). Figur 6 clearly depicted 21, 7, 31 and 59 % rise in activities of SOD, CAT, APX and GR in synergistic treatment of SNP and NaHS under salt stress. In tomato, NO-donor increased hydrogen sulfide level by 18 %, whereas H_2S donor showed 10 % increment of nitric oxide level in roots (da-Silva et al., 2018).

Nitric oxide and hydrogen sulfide suppressed cell membrane injury, oxidative damage and extrusion of H^+ by increasing H^+ -ATPase in plants at stress (Kharbech et al., 2020). NO and H_2S suppressed oxidative damage, recovered the loss in ascorbate content, increased GSH, glyoxalase I and II enzymes and regulated homeostasis of glutathione system and reduced cytotoxicity of methylglyoxal (Kharbech et al., 2020). Supplementation of SNP and NaHS under salt stress increased Gly II activity, however Gly I activity was slightly reduced in coriander seedlings as compared to salt treatment (Fig. 5). The 24 and 95 % escalation in reduced glutathione and ascorbate contents were observed in coriander seedlings under NaCl + SNP + NaHS treatment (Fig.7). Function of NaHS in mitigation of salt stress in tomato and eggplant was due to the increase in growth, moisture content, rubisco activity, anti-oxidative enzymes and maintenance of AsA-GSH cycle (Raju and Prasad, 2021). Mitogen-activated protein kinase (MAPK) which acts as defence strategy in plants against adverse climatic conditions. MAPK may enhance nitric oxide and hydrogen sulfide levels for plants preservation (Qi et al., 2019). Hence, results of present investigation showed the close relationship between NO and H_2S which helps to combat salt stress incited impairment in coriander plants.

5. Conclusion

In present investigation, we reported that individual or synergistic use of NO and H_2S alleviated detrimental impacts of NaCl treatment in coriander plants by regulation of ion homeostasis, osmoprotection and resistance against oxidative stress. Findings of the present paper revealed that crosstalk between NO and H_2S, assist salt stress resistance in *Coriandrum sativum*. More investigations are required to study the mechanism behind the synergistic effects of NO and H_2S and role of their biochemical conjugates on protection of plants under harsh environmental conditions.

Ethics approval

Not applicable.

Consent to participate

All authors consent to participate in the manuscript publication

Consent for publication

All authors approved the manuscript to be published.

Availability of data and material

The data supporting the conclusions of this article are included within the article. Any queries regarding these data may be directed to the corresponding author.

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Riti Thapar Kapoor: Conceptualization, Investigation, Resources, Supervision, Drafted the experimental design, Performed the experiments, Revised the manuscript. **Mohamed A Elsheikh:** Analysed the data and helped in writing of this manuscript. **Saleh Alansi:** Formal analysis, Funding acquisition, Analysed the data and helped in writing of this manuscript. **Awais Shakoor:** Analysed the data and helped in writing of this manuscript. **Parvaiz Ahmad:** Validation, Drafted the experimental design, Revised the manuscript.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

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