

Alleviation Effects of Nitric Oxide on the Growth Rate and Photosynthetic Pigments and Reducing Sugar Content in NaCl-Stressed Coriander (*Coriandrum sativum* L.)

Roghayyeh Babri Bonab¹, Sara Saadatmand^{1*}, Hossein Nazemiyeh², Ali Reza Iran Bakhsh¹

¹Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.

²Research Center for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

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ABSTRACT

In this study the influence of sodium nitroprusside (SNP, the donor of NO) on several physiological parameters in *Coriandrum sativum* L. which has grown in saline and non-saline conditions was investigated. fifteen days old of coriander seedlings were treated with 50 and 100 mM NaCl and 50,75 and 100 μ M sodium nitroprusside during 3 months. The growth parameters, photosynthetic pigments and reducing sugars content were analyzed.

The outcomes showed that NaCl-induced ionic toxicity caused to momentous decrease in growth parameters and photosynthetic pigments of coriander. Application of 50 μ M SNP could improve growth parameters and photosynthetic pigments of coriander, while the application of 75 and 100 μ M SNP had the opposite effects in this condition. Under NaCl salinity, carbohydrate content increased sharply as to compare with control plants. Application of different concentration of SNP showed different results. These results suggested that 50 μ M of SNP is compatible molecule for reduce damage caused by salt stress.

KEYWORDS: Nitric Oxide, NaCl Salinity, *Coriandrum sativum*, Growth, Photosynthetic Pigment, Reducing Sugar Content.

1. INTRODUCTION

From many years ago, herbal drugs were effective drugs with limit or no side effects. Herbal plants synthesize innumerable compound in their system; hence, these are often reported as storehouse for bioactive compounds [1]. One of the important medical and food herbs is coriander (*Coriandrum sativum* L.) from umbelliferae family, which is native to the Mediterranean region, Bangladesh, Russia, Asia and Central Europe [2]. All parts of this popular herb are eatable and the fresh leaves and the dried seeds use widely in cooking [3]. The seeds are used for many goals such as food, drugs, cosmetics and perfumery [4].

Normally plants are exposed to various environmental stresses which restrict their growth and agriculture productions [5]. Currently twenty percentage of the world's cultivated lands and about half of all irrigated lands are under the influence of salt stress [6]. High salinity is one of the harmful factors which led to disorders in growth, protein synthesis, photosynthesis and energy and lipid metabolism [7]. According to Sreenivasulu [8], the adverse effects of salts on plant growth categories into three broad: (i) a devaluation in soil solution osmotic potential that reduces plant available water and thus cause a water stress in plants, (ii) a decay in the soil physical structure so that aeration of soil and water permeability are reduced, and (iii) rise the concentration of the certain ions that these ions have an adverse effect on plant metabolism and also imbalance the ion content of soil or (iv) a combination of these factors.

Salinity tolerance in plants might increase or decrease depending on the environmental factors and plant species. Plants have numerous mechanisms to adapt to salinity, including in molecular level they alteration gene expression and also it could utilize exogenous substances like nitric oxide. It is suggested that nitric oxide is as signal transducer or messenger in plants, thus it could have a protective effect on plants under NaCl salinity [9]. Nitric oxide (NO) is a bioactive signaling molecule, which plays a crucial role in throughout the plant life from germination to death including growth and development, flowering, ripening of fruit, senescence of organs and adaptive responses to environmental stress [10]. It has been shown that in abiotic stress condition, application of NO donor, SNP alleviate salinity adverse effects [11]. Furthermore, NO as a signaling molecule can alter antioxidant gene expression and scavenge ROS, thus protects plant cells from oxidative damage [10]. However, different studies

*Corresponding Author: Sara Saadatmand, Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran. Email: s_saadatmand@srbiau.ac.ir

on NO effects in plants have shown that this molecule have either protective or toxic effect on cells that it depends on concentration of NO and the location of NO action [12].

In this work, we investigate the effect of different concentration of nitric oxide on some growth parameters, photosynthetic pigments and carbohydrate content of coriander in terms of both salt and no salt condition for determination the best concentration of NO against the salt stress.

2. MATERIALS AND METHODS

2.1. Plant growth and treatment : Sterilized coriander seeds (*Coriandrum sativum* L.) were germinated on moist filter paper in petri dishes in the dark and kept at 25 °C for 15 days, then germinated seedlings were grown in a hydroponic solution in plastic pots. The plants were left to grow in a controlled greenhouse under the following growth conditions: 14-h light period, 50-60% relative humidity with day/night temperature of 25°C/20°C, respectively). Sodium chloride (0, 50 and 100 mM) without or with SNP (0, 50, 75 and 100 µM) was added to Hoagland solution during the ten weeks of seedling growth. SNP (Merck, Germany) was used as a donor of NO. Pots were randomly arranged in greenhouse during the treatment period. The solutions were renewed every 2-3 days during treatment [13]. The control plants have only been watered with Hoagland solution. Each treatment was replicated three times. At the end of growth stage, plants were harvested and the roots and shoots were separated and washed with deionized distilled water. For determination of some parameters, root and shoot fresh and dry weight was measured and fresh plant materials were frozen in -20 °C until use.

2.2. Growth parameters assay: For growth parameters assay [14], before starting treatment, 3 plants were randomly selected and those fresh weight, dry weight and leaf area were measured. At the end of treatments, 3 plants from each group were divided into separate shoot and root fractions. Fresh weights (FW) of shoot and root were weighed, and leaf area were measured. Dry mass was determined from material oven-dried at 70 °C for at least 48 h. Leaf area was determined by leaf area meter apparatuses.

Net assimilation rate (NAR): NAR is meaning the amount of dry matter produced by plants per leaf area and time unit.

$$NAR = \frac{1}{L} \times \frac{dw}{dt} \quad NAR = \frac{W_2 - W_1}{L_2 - L_1} \times \frac{\ln L_2 - \ln L_1}{t_2 - t_1}$$

L₁: leaf area amount in t₁ ; L₂: leaf area amount in t₂ ; W₁: plant dry weight (root and shoot) in T₁; W₂: plant dry weight in T₂; t₁: start of treatment time; t₂: start of harvest time.

Relative growth rate (RGR): RGR is the most fundamental part of the growth analysis. Because the amount of increased material in the plant during the considered period is assayed, without the requirement to net assimilation rate. It is the increase in size relative to the size of the plant present at the start of a given time interval.

$$RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \quad RGR = \frac{1}{W} \times \frac{dW}{dt} = \frac{d}{dt} (\ln W)$$

Ln: Napier logarithm; W₁: plant dry weight (root and shoot) in T₁; W₂: plant dry weight in T₂; T₁: start of treatment time; T₂: start of harvest time.

Relative leaf growth rate (RLGR): RLGR is the amount of leaf growth relative to the time. $RLGR = \frac{1}{L} \times \frac{dL}{dt}$

$$RLGR = \frac{\ln L_2 - \ln L_1}{t_2 - t_1}$$

Ln: Napier logarithm; L₁: leaf area amount in t₁ ; L₂: leaf area amount in t₂ ; t₁: start of treatment time; t₂: start of harvest time.

Leaf water content area (LWCA): It is the accumulation of water in the leaf. $LWCA = \frac{LFW - LDW}{L}$

LFW: leaf fresh weight; LDW: leaf dry weight, L: leaf area.

2.3. Determination of photosynthetic pigments: The content of chlorophylls and carotenoids (xanthophylls and carotenes) were determined according to the procedure described by Lichtenthaler and Wellburn (1983) [15]. The photosynthetic pigments were extracted from 0.1 g leaf fresh weight by 80% acetone. Then the homogeneous mass with Whatman No. 1 filter paper was smooth. Using a spectrophotometer, absorbance at wave lengths 2/663

nm(chlorophyll a), 8/646 nm (Chlorophyll b) and 470 nm(carotenoids) was measured and 80% acetone as the controls to set zero absorbance spectrophotometer was used.

Chlorophylls content assay: For determination of the amount of chlorophyll a, chlorophyll b and total chlorophyll were used, respectively, the following relationships:

$$\text{Chlorophyll.a} = 12/25 A_{663.2} - 2/79 A_{646.8} \quad \text{Chlorophyll.b} = 21/51 A_{646.8} - 5/1 A_{663.2}$$

$$\text{Chlorophyll.T} = \text{Chla} + \text{Chlb}$$

$A_{663.2}$: absorbance at 663.2 nm ; $A_{646.8}$: absorbance at 646.8 nm

Carotenoids content assay :For assay of carotenoids, was used the following relation:

$$\text{Carotenoids (x+c)} = (1000 A_{470} - 1/8 \text{ Chla} - 85/02 \text{ Chlb})/198$$

A_{470} :absorbance at 470 nm

2.4. Determination of reducing sugar content: Determination of reducing sugars content was carried out by Cu^{+2} reduction test according to Nelson (1944)[16]-Somogyi (1952)[17] method. Two mL of the extract (.05 gr fresh leaf, 5mL water) was mixed with 2 mL copper reagent of Somogyi. The mixtures were transferred to boiling water bath for 20 minutes. After cooling 2mL of Nelson's arsenomolybdate reagent was added. After 10 minutes, the absorbance of the reaction mixtures was measured at 600 nm using Spectrophotometer. The amounts of reducing sugar was calculated with the help of standard curve obtained by using different concentration of standard glucose solution.

2.5. Statistical analysis: Analysis of variance (ANOVA) between treatment means was carried out with the spss 13 program using Duncan's multiple range tests at $P < 0.05$.

3. RESULTS

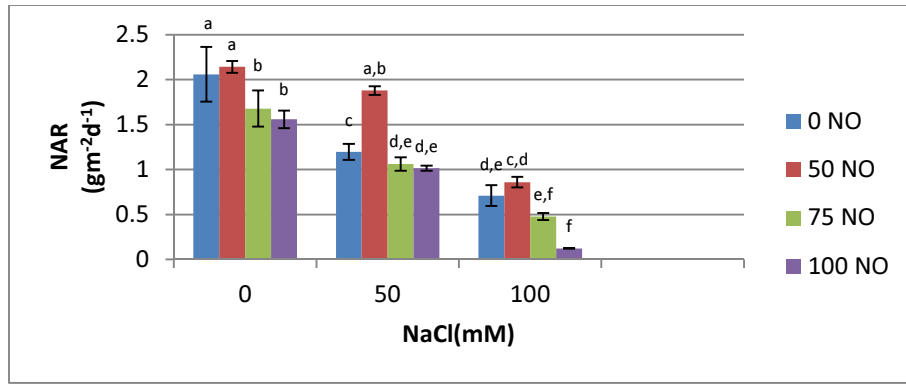
3.1. Growth analysis :Results in Figure 1 demonstrated that salt stress, particularly high salinity significantly decreased the all of growth parameters. Exogenous application of 50 NO donor improved The damaging effects caused by salt, while the application of 75 and 100 μM SNP had the reverse effects in this condition.

Net Assimilation rate (NAR) assay: Figure 1(a) demonstrates the significant inhibitory effects of NaCl on the net assimilation rate (NAR) of coriander plants. In this experiment, NAR of plants at 50 and 100 mMNaCl was decreased by 42% and 66% respectively, as compared with the control plants. Application of 50 μM NO could increase NAR under NaCl stress. This increasing is significant in 50 mMNaCl. Also, NAR decreased in the presence of 75 and 100 μM NO in all of cases.

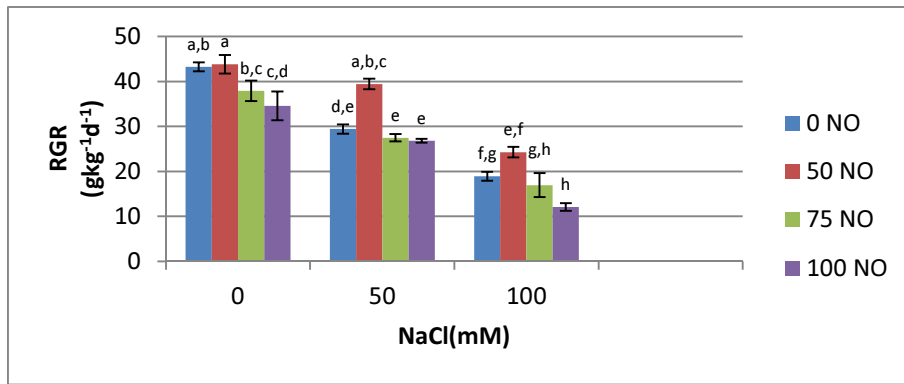
Relative growth rate (RGR) assay: Application of NaCl (50 and 100 mM) to coriander decreased significantly the relative growth rate (RGR) as compared with control plants (figure 1(b)).As indicated in figure, treatment of plants with 50 μM SNP increased significantly the RGR in coriander plants subjected to 50 and 100 mMNaCl. The application of 75 and 100 μM NO decreased the RGR.

Relative leaf growth rate (RLGR) assay: Relative leaf growth rate (RLGR) was decreased in *coriander* plants which were treated with salinity, especially in high concentration of NaCl salinity. Our data in figure 1(c) showed that in *coriander* treated with 50 mMNaCl, the application of 50 μM NO donor could significantly increase relative leaf growth rate, while on exposure to 100 mMNaCl, this parameter had reduce. The data have been supported that using 75 and 100 μM NO SNP decreased the RGR of *Coriander* in the both mediums containing of NaCl and not NaCl.

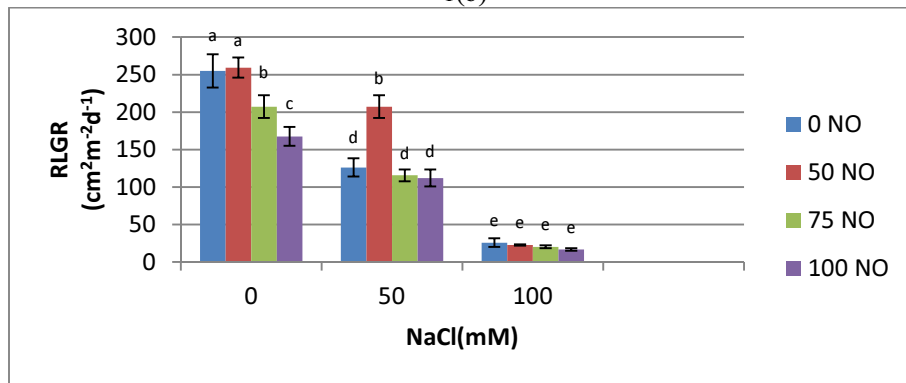
Leaf water content area (LWCA) assay: NaCl stress decreased the Leaf Water Content Area (LWCA) markedly (Figure 1(d)). In this work, application of 50 μM SNP in the absent of NaCl and in the presence of 50 and 100 mMNaCl increased LWCA. This increasing was significant in medium of 50 mMNaCl. Measurement indicated that salinity reduced the LWCA and 75 and 100 μM SNP had intensified this reduction.



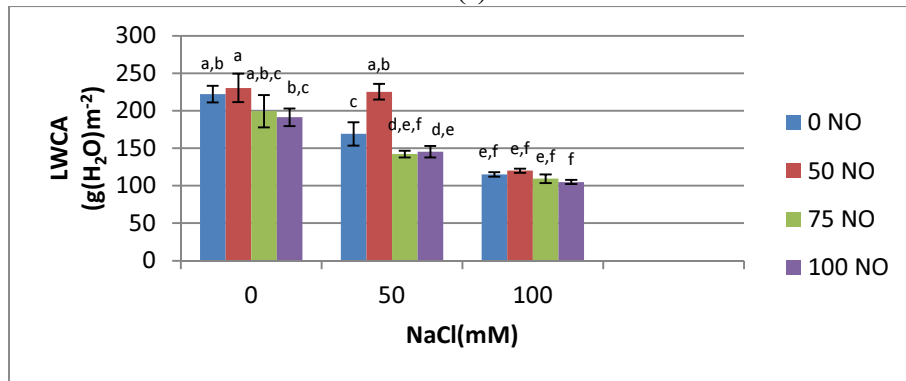
1(a)



1(b)



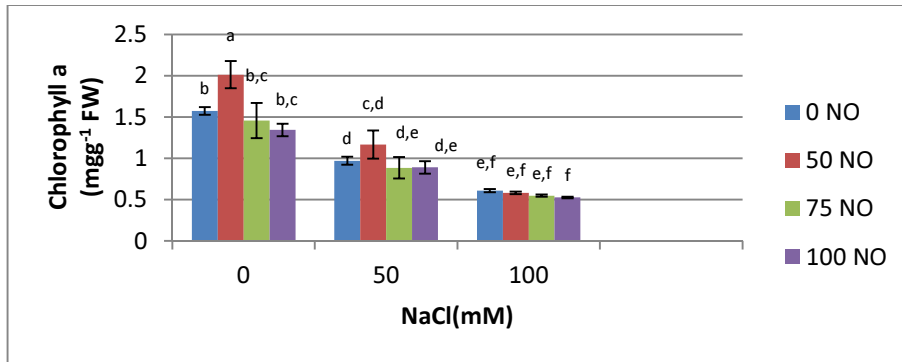
1(c)



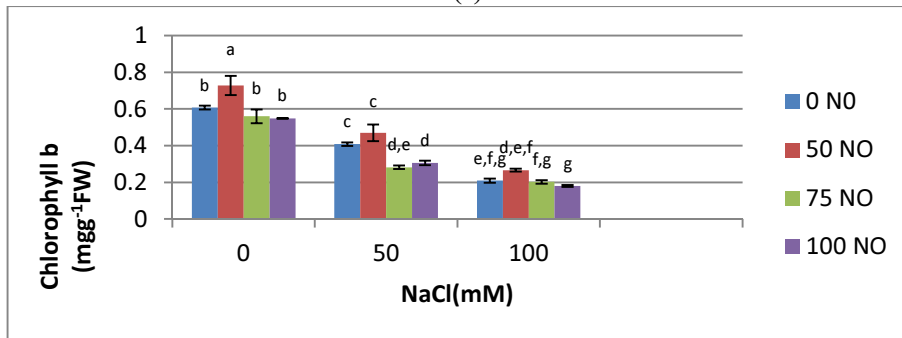
1(d)

Figure 1. Effects of NO on the NAR (a), RGR (b), RLGR (c) and LWCA (d) in Coriander under salin and non-salin conditions. Results are shown as Mean \pm SE ($p < 0.05$), obtained from three replicates.

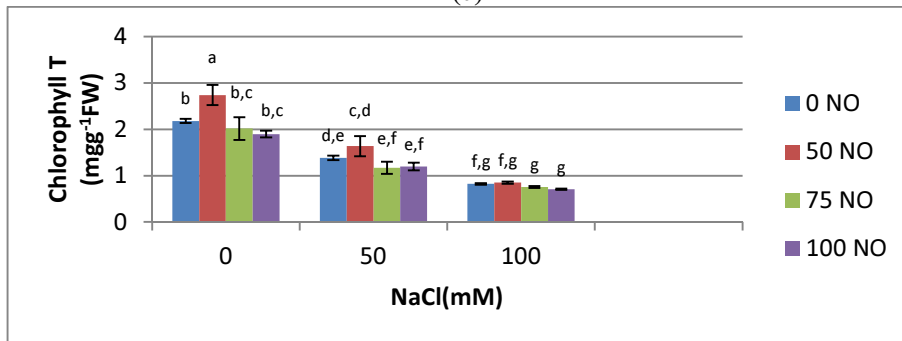
3.2. Photosynthetic pigments contents: As shown in Figure 2(a-d) when NaCl concentration increases, levels of photosynthetic pigments reduce significantly. Under salinity and non-salinity conditions, the amount of chlorophylls and carotenoids were increased by 50 μM SNP. In this condition, 75 and 100 μM SNP, decreased concentration of chlorophylls and content of carotenoids.



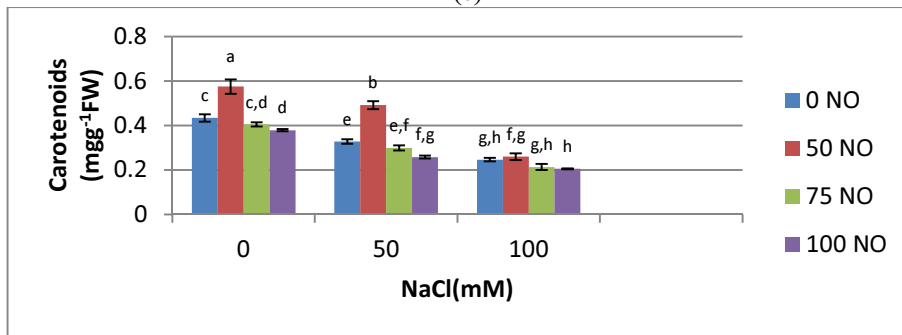
2(a)



2(b)



2(c)



2(d)

Figure 2. Effects of NO on the chlorophyll a content (a), chlorophyll b content (b), chlorophyll total content (c) and carotenoids content (d) in Coriander under saline and non-saline conditions. Results are shown as Mean \pm SE ($p < 0.05$), obtained from three replicates.

3.3. Reducing sugar content :As shown in Figure 3, a significant increase in reducing sugars content was observed in response to 50 and 100 mMNaCl salinity as compared with control plants. Our data showed that application of 50 μ M SNP enhanced the reducing sugars content in non-salin condition, also it had the opposite effects in salin condition. In the all concentration of NaCl, 75 and 100 μ M SNP, enhanced the reducing sugar content of plants.

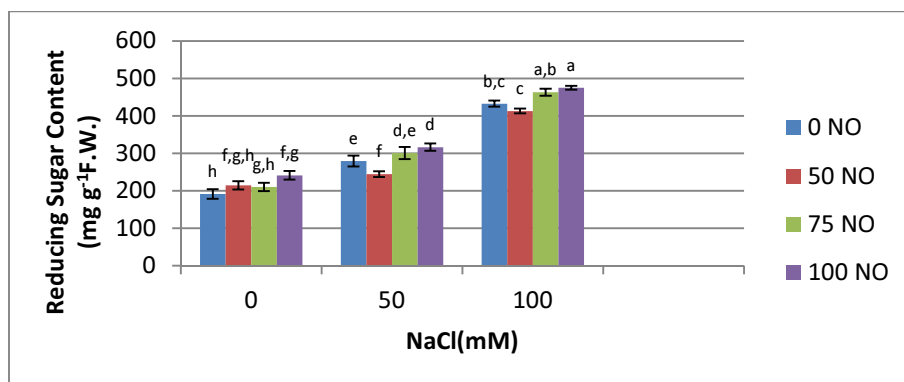


Figure 3. Effects of NO on the reducing sugar content in Coriander under salin and non-salin conditions. Results are shown as Mean \pm SE ($p < 0.05$), obtained from three replicates.

4. DISCUSSION

High salinity causes both hyperionic and hyperosmotic stress effects. It restricts growth and development of plants in several ways including by influence on several main metabolic processes [18]. Also, salinity changes the activities of many enzymes including those that involve in nitrate and sulfate assimilation pathway in plants, lowers the these enzymes energy status, and increases the demand for nitrogen and sulfur [19]. Salinity stress is one of the reasons for injury at cellular level which associated with oxidative damage due to ROS. Studies have shown that plants possess a several mechanisms to protect themselves from oxidative damage [20]. So that evidences show that NO plays crucial roles in plant tolerance to environmental stress involving salt stress [21], drought stress [22], heavy metal toxicity [23] and so on. Some studies provided documentations that NO could counteract the salt stress inhibitory effects in the step of germination. NO mitigates abiotic stress in many ways, it was reported that this is due to NO modulation in antioxidant reactions [24]. The function of exogenous NO to plants has been used as an instrument to study how this molecule influences physiological process [25]. Plant growth is a prominent indicator of plant strategy with respect to productivity as related to environmental stress and disturbance regimes, thus its measurement is the best method for assay of damaging effects of stress.

Our findings indicated that nitric oxide acts as plant growth regulator that can counteract NaCl-induced oxidative stresses. The results showed that NaCl salinity, decreased net assimilation rate (NAR), relative growth rate (RGR), relative leaf growth rate (RLGR) and (LWCA). Furthermore, the effect of severe salinity is higher than low salinity. For two reasons the salinity inhibits plant growth: first, shortage of water and second salt-specific or ion-excess effects [26]. These results are in agreement with recent researches. It has been shown that plants accepted to high salinity (75 and 100 mM NaCl) reduced both leaf and stem dry masses [27]. In this work, application of exogenous 50 μ M SNP extremely improved the inhibition effects of growth induced by salt stress, also application of 75 and 100 μ M SNP, had a reverse effect and reduced all of growth parameters. These results accepted with the outcomes of Lopez *et al.*. They showed that in plants treated with 100 mM NaCl, leaf biomass production and RGR sharply decreased. However, application of the 0.25 and 0.5 mM SNP together in 100 mM NaCl condition increased both parameters in compared with the application of 100 mM NaCl alone. Beside this, the 1 mM dosage of SNP applied together with 100 mM NaCl further reduced both parameters. These results cover the literature which SNP applied at low and high concentrations has conflicting effects, since at low concentrations it can be useful but at high concentrations it can be toxic to plants [11]. One of the most important features of NO is the dual role as a powerful oxidant and an effective antioxidant. These dual effects of NO completely depend on the concentration and site of NO in the plant cells [28]. NO can interact with a different kind of intracellular and extracellular targets. In some cases, these interactions are cytotoxic and outcome is cell death [29]. Also, NO can act as an antioxidant and an anti-apoptotic modulator that inhibits cell death [30]. This protective role of NO may result, from its interaction with lipid hydroperoxyl radicals or high activation of superoxide which both increase lipid peroxidation [31]. Another role for NO is its role in promoting stomata closure [32]. Thus, NO can reduce the damaging effects of the

reactive oxygen species (ROS), and reacts with other target molecules, and affects on the expression of stress responsive genes under various stress conditions [33].

Chlorophyll (Chl), an important pigment to maintain plant growth, is mainly composed of Chl a and Chl b . Measurement of photosynthetic pigments under salinity conditions on Coriander explains that NaCl salinity has the negative effects on these pigments and treatment with NO decreases damages induced by salt stress. Our results showed that salinity reduced the chlorophylls and carotenoids content in leaves of Coriander plants. Furthermore, the effect of severe salinity is higher than low salinity. It has been observed that exogenous application of 50 μ M SNP increases the content of photosynthetic pigments under 50 mMNaCl salinity, but it had not positive effect under 100 mMNaCl salinity. Our finding showed that high concentrations of SNP (75, 100 μ M) not only had a positive effect but will exacerbate the damaging effects of salt.

It has been suggested that exogenous NO led to the promotion of protective reactions to the photosynthetic pigments and induce a pre-adaptive response to salt stress [34], which confirm our results. Also Lee *et al.* (2004) [35] in their study on *Paspalumvaginatum* (L.) and Siler *et al.* (2007) [36] in their study on *Centaureaerythraea* (L.) reported that chlorophyll a,b and total chlorophyll decreased with the increase of salt concentrations [37]. The significant decrease of Chlorophyll content in the plants in salt stress is attributed to the increased degradation of photosynthetic pigments and the inhibited synthesis of pigments in this condition [38]. Sharma and Hall (1991)[39] showed that salt stress induces degradation of β -carotene and thus reduces the carotenoids content that are integrated constituents of thylakoid membranes and act in absorption and light transfer to chlorophyll and protect chlorophyll from photooxidation. Thus, destruction carotenoid led to degradation of chlorophylls [40].

Salt stress increases ROS in the leaves and causes damage to the membranes due to excessive generation of free radicals. The over-production of ROS under saline conditions attacked biomolecules such as lipid, protein, DNA, and some small molecules and damages to the biological membranes and even the death of the plants. Thus, the disintegration of biological membranes led to the chlorophyll degradation under salt stress, but NO performed as an efficient ROS scavenger or membrane stabiliser. Exogenous NO protected photosynthetic capacity inhibition by oxidative stresses through decreasing the H₂O₂ and ROS contents and alleviating the salt induced damage[41]. Shi *et al.* (2005) showed the protective effect of NO on thylakoid membrane proteins through reduced ROS under UV-B radiation in bean[42].

Similarly, Ruan *et al.* (2002)[43] have reported that in salt stress, NO treated wheat leaves showed less destruction in comparable to those of control. The reaction of NO with ROS prevented the chlorophyll disintegration and injury to membranes by preventing the increase in thiobarbituric acid reactive substances content. The treatment of NO served as releasing compound to reduce the chlorophyll loss; thus, it protects the damage of photosynthetic apparatus through the balance in the PS II complex proteins [44]. This finding was supported by the Laspina *et al.* (2005) [44]. They showed that in cadmium stress condition, the NO protects chlorophyll against oxidative damage. High Na⁺ content is viewed as the most important factor for salt toxicity. High content of Na⁺ restrained the chlorophyll synthesis because the accumulation of Na⁺ content inhibited the protein synthesis and weakened the links of chlorophyll and started decreasing chlorophyll content [45]. However, supplementation of NO at 50 μ M alleviated the salt toxicity and maintained chlorophyll content. The enhancement in photosynthesis by NO application was mediated through NO-induced changes in various photosynthetic characteristics. That is also correlated with an increase in activity of rubisco, efficiency of PS II and net photosynthesis indicating that NO scavenges the H₂O₂ content to protect the plant cells from oxidative damage and increases photosynthetic efficiency [46]. Our study showed that exogenous application of NO (50 μ M) attenuated salt injured plants by acting as an efficient ROS scavenger breaking the oxidative chain. In contrast, high concentration of NO (75 and 100 μ M) reduced the photosynthetic pigments. Thus, it can be inferred that appropriate concentration of NO supplementation acts as a stress factor in promoting photosynthesis [41].

Accumulation of sugars has been associated with drought and salinity-tolerant mechanisms in many species. The most important molecule which effect on different physiological responses is sugar [47]. Accumulation of carbohydrate in plants tissue under condition of environmental stress is a result of regulating and modification in current stress [48]. Generally, the increase in cellular osmolality which achieves from accumulation of compatible solutes is associated with the influx of water into cell[49].So, increasing of sugar under environmental stress could be the result of starch decay, sugar synthesis by non-photosynthesis pathways, non-converting of sugar to other productions and decreasing of transporting from leaves to other parts of plant[50].

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