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Effects of *Rhizobium* inoculation on *Trifolium resupinatum* antioxidant system under sulfur dioxide pollution

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Abstract

Introduction: Plant growth stimulating rhizobacteria are beneficial bacteria that can cause resistance to various stresses in plants. One of these stresses is SO_2 air pollution. SO_2 is known as a strong damaging air pollutant that limits growth of plants. The aim of this study is evaluation of the effects of bacterial inoculation with native and standard *Rhizobium* on Persian clover root growth and antioxidants activity and capacity under air SO_2 pollution.

Materials and methods: In this study, 31 days plants (no-inoculated and inoculated with two strains of *Rhizobium*) exposed to the different concentrations of SO_2 (0 as a control, 0.5, 1, 1.5 and 2 ppm) for 5 consecutive days and 2 hours per day.

Results: Results showed different concentrations of SO_2 had a significant effect on Persian clover root weight and antioxidant system. Increasing SO_2 stress decreased root fresh and dry weight and antioxidant capacities (IC50) and increased antioxidant activities (I%) of Persian clover leaves significantly in comparison to the control plants (under 0 ppm) and increased SOD, CAT and GPX activity. Inoculation of Persian clover plants with native and standard *Rhizobium* increased root weight and did not show a significant effect on antioxidants activity and capacity, but interaction between *Rhizobium* inoculation and SO_2 treatment reduced significantly the stress effects of high concentration of SO_2 on root growth and antioxidants activity and capacity. In fact, level of this change of root growth and antioxidant system under SO_2 pollution stress in inoculated plants was lower than in the non-inoculated plants.

Discussion and conclusion: As a result, an increase in SO_2 concentration caused a decrease in root weight, increase in antioxidants activity and capacity of Persian clover. Inoculation with *Rhizobium* strains could alleviate the effect of SO_2 pollution on antioxidant system by effects on root growth.

Key words: Antioxidant activity, Antioxidant capacity, Clover (*Trifoliumresupinatum*), *Rhizobium*, SO_2 pollution

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Introduction

Legumes are well known for their important roles in maintaining productivity in agricultural systems (1). They are rich in proteins, minerals, vitamins and sugars in seeds (2). Persian clover (*Trifolium resupinatum*) is an annual plant of the legume family and it has a high nutritive value as pasture or hay (3). Persian clover is among the most important forage crops native from the temperate regions cultivated in this regions to produce seeds (4). Persian clover can establish a symbiotic relation with the soil *Rhizobium* (5).

The *Rhizobium* is a genus of gram-negative, aerobic, rod-shaped bacteria that activate plant root nodulation in leguminous plants. Members of this genus are nitrogen-fixing and common soil inhabitants (6). *Rhizobium* is one of the most prominent Plant growth promoting rhizobacteria (PGPR) members. PGRP are a group of bacteria that can actively colonize plant roots and increase plant growth. These PGPR can prevent the deleterious effects of phytopathogenic organisms and environmental stressors (7). The use of PGPR to promote plant growth has increased in various parts of the world. PGPR can affect plant growth by producing and releasing secondary metabolites and facilitate the availability and uptake of certain nutrients from the root environment (8).

Sulfur dioxide (SO_2) is a major air pollutant that its concentrations increase in many metropolitan and industrial areas. It is a primary product of fossil fuels power plants combustion or from refining of sulfur-containing ores; it influences human health and theglobal ecology (9). SO_2 occures normally 0.05–0.5 ppm in the urban areas and up to 2.0 ppm or more around point sources of air pollution and it is the major source of atmospheric sulfur (10). The result of second world war that followed by the post war economic expansion is an unprecedented absolute rate of SO_2 emissions. Global emissions peaked in the 1970s, and have declined overall since 1990, with an increase between 2002 and 2005, largely due to strong growth of emissions in China. The 40% of SO_2 global emissions originated from Asia and it is growing (11).

In very low concentrations, SO_2 especially in sulfur-deficient soils can cause positive effects on physiological and growth characteristics of plants (13). But, high concentrations of SO_2 causes toxicity and growth reduction due to sulphite and sulphate accumulation within the plant. The main factors that determinethe phytotoxicity of SO_2 are: environmental conditions, duration of exposure, atmospheric SO_2 concentration, sulphur status of the soil and the genetic constitution of the plant (13, 14). SO_2 can easily penetrate into chloroplasts and affect plant growth and development. Even when stomata are closed, SO_2 can react with water to produce bisulfite and enter the leaf through the cuticle. In the chloroplasts, SO_2 is mainly converted into sulfite, which causes a reduction of net CO_2 assimilation, inhibits photosynthetic enzymes and decreases the photosynthetic electron transport rate (9). SO_2-toxicity is mainly attributed to produce high reactive intermediates such as the sulphur trioxide radical (HSO_3^-), the superoxide radical and the hydroxyl radical, which are generated during the radical-initiated oxidation of SO_2. Chloroplasts can initiate oxidation of SO_2 and hence, may be a primary site of radical production during SO_2 treatment. To counteract the toxicity of reactive oxygen species (ROS), a high efficient antioxidative defence system, composed of both non-enzymatic (e.g. α-tocopherol, β-carotene, glutathione and ascorbate) and enzymatic constituents (e.g. superoxide

dismutase SOD, catalase, peroxidase and enzymes of the ascorbate-glutathione cycle) is present in all plant cells (15). In the present study, effects of *Rhizobium* inoculation on antioxidant activity and capacity of *Trifolium resupinatum* under different concentrations of SO_2 (0, 0.5, 1, 1.5 and 2 ppm) were evaluated.

Material and methods

Bacterial strains and inoculant preparation

In this research two *Rhizobium* strains were used. A local strain isolated from Persian clover nodules of Arak region in central of Iran, a city of Iran that high levels of SO_2 has been reported (12), as native strain. Physiological and biochemical characters of the local isolated bacteria were examined according to methods described in Bergey's manual of systematic bacteriology (16). For this purpose, clover roots were sterilized with 70% ethanol and were washed with sterile distilled water (17). Then the pink nodules (containing active bacteria) isolated from roots, crushed in distilled water and cultured in solid medium of YMA (18). These cultures were transferred to incubator at 25^{o}C. After incubation, gram reaction and morphology of bacteria were studied under the microscope. Formation of convex prominent semi-transparent slimy and mucilage colonies and gram-negative reaction were considered a sign of successful isolation of *Rhizobium* (17).

Standard strain of *Rhizobium* (*Rhizobium meliloti* PTCC 1684) were obtained from the Persian type culture collection (PTCC, Iran). To activate these bacteria, 1 ml of liquid medium of YMA under sterile conditions added to bacteria. For proliferation of bacteria, one inoculation loop of these bacteria dissolved in 100 ml of liquid YMA and incubated on an orbital shaker at 200 rpm for 24 h.

Optimum amount of *Rhizobium* to stimulate clover growth was reported 10^5cells/mL (19). For this purpose two strains of *Rhizobium* (native and standard) were cultured separately in liquid medium of YMA (18) and incubated on an orbital shaker at 200 rpm for 24 h at 25^{o}C (20). Then these cultures were centrifuged at 1000g for 10 min and were resuspended with phosphate buffer. If, the optical density (OD_{620}) of this solution was 0.1 it means 10^8cells/mL (21)**.** To prepare optimum amount of inoculants (10^5cells/mL), this solution was diluted by phosphate buffer.

Seed preparation and its inoculation

The seeds of Persian clover (*Trifolium resupinatum* cv. Alashtar Lorestan) were prepared from Arak Agriculture Research Center. They were surface-sterilized by 70% ethanol for 2 min and 1% sodium hypochlorite for 5 min, after that washed with distilled water 5 times (22). There after, seeds were divided into three groups. First group of seeds inoculated with native inoculants (10^5cells/mL), second group of seeds inoculated with standard inoculants (10^5cells/mL) and third group soaked in sterile phosphate buffer. All of the groups were placed under vacuum and ambient temperature for 2 h (23)**.**

Hydroponic cultivation of seed

Inoculated and non-inoculated seeds were placed in plates system containing nutrient solution (without nitrogen) in the darkness for 24 h. The germinated seeds transferred to sterile microtubes in plastic container containing 2 L of Half-Hoagland solution (without nitrogen) (24). Containers were oxygenated by the air compressor. Each container was considered as a treatment. These containers were maintained under 12 h photoperiod, at 25^{o}C during day and 20^{o}C during night. The nutrient solution changed every five days (23).

SO_2 injection to plant

Sulfur dioxide 0.1 % gas prepared from Shazand Petrochemical Co. injected in different concentrations 0 (as control), 0.5, 1, 1.5 and 2 ppm into 31 days plants. Gas injection was performed by syringe for 5 days and 2 h daily to closed plastic containers (25).

Measurement of root fresh and dry weight

Root fresh weight of 41-days plants was measured, then these roots were placed in oven at 75 ± 2 ^{0}C for 24h and dry weight was measured.

Enzyme assays

A) Extraction

Leaf fresh materials (0.1g) was powdered by liquid nitrogen and homogenized in 1 ml of 50 mM phosphate buffer (pH=7) containing 1 mM ethylene diamine tetra acetic acid (EDTA) by a homogenizer into microtubes. Insoluble materials removed by Beckman refrigerated centrifuge at 13000 g for 20 min at 4°C, and the supernatant used as the source of enzyme extraction.

B) Assays

All activities of the enzymes determined with a spectrophotometer (PG T80 UV/VIS, Oasis Scientific Inc.).

Superoxide dismutase (SOD) assay

SOD activity was measured by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium according to the method described by Giannopolitis and Ries (1977), using a reaction mixture (3 mL) containing 50mM phosphate buffer (pH=7.8), 13mM methionine, 75 µM nitro blue tetrazolium, 20 µM riboflavin, 0.1 mM EDTA and 100 µl of the enzyme extract in absence of light. The reaction mixtures were illuminated for 15 min under fluorescent light. One unit of superoxide dismutase activity is defined as the amount of enzyme required to cause 50% inhibition of nitro blue tetrazolium reduction, which was monitored at 560 nm (26).

Catalase (CAT) assay

Catalase activity was assayed by measuring the initial amount of hydrogen peroxide disappearance using the method of Cakmak and Marschner (1992) in a reaction mixture containing 2 ml of 25 mM phosphate buffer (pH=7.0, containing 10 mM H_20_2) and 100 µl of the enzyme extract. Decomposition of 1 µmol H_2O_2/min is equal to one unit of catalase activity (27).

Guaiacol peroxidase (GPX) assay

Guaiacol peroxidase activity was measured by Polle *et al*. (1994). The reaction mixture contained 100 mM phosphate buffer (pH=7.0), 20 mM guaiacol, 10 mM H_20_2 and 50 µl of the enzyme extract. Activity was determined by increasing absorbance at 470 nm due to guaiacol oxidation (28).

c) Measurement of DPPH-radical scavenging activity

Determination based on DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging activity Abe *et al*. (1998) method (29). Leaf fresh materials (100 mg) was powdered by liquid nitrogen, homogenized in 1 ml of 90% ethanol and then maintained at 4°C for 24 h. Insoluble materials removed by centrifuge at 3500 g for 5 min. 20 µl of extracting solution was mixed with 800 µl of DPPH (0.5 mM in ethanol). The absorbance of the resulting solution was measured at 517 nm after 30 min in darkness. The antiradical capacity (three replicates per treatment) was expressed as IC_{50} (mg ml^{-1}), the antiradical dose required to cause a 50% inhibition. A lower IC_{50} value corresponds to a higher

antioxidant capacity of plant extract (30). The ability to scavenge the DPPH·radical was calculated by:

$$I\% = \frac{A0-A1}{A0} \times 100$$

Where A0 is the absorbance of the control at 30 min, and A1 is the absorbance of the sample at 30 min.

Statistical analysis

All data were analyzed by variance analysis using SPSS 16. Experiments were tested using completely randomized design in factorial for three replicates. Mean comparisons were conducted using Duncan's test.

Results

Effect of bacterial inoculation and SO_2 pollution on root

Study of root morphology of inoculated clover plants indicated pink nodules on the roots of these plants. Root fresh and dry weight of inoculated plants with native *Rhizobium* increased significantly as compared with inoculated plants with standard *Rhizobium* and non-inoculated palnts. In inoculated plants with native *Rhizobium*, root fresh and dry weight increased until 85.2% and 87.57% respectively in compared with non-inoculated palnts. Also, in inoculated plants with standard *Rhizobium*, root fresh and dry weight increased until 44.44% and 35.71% respectively in compared with non-inoculated palnts (Table 1).

Root fresh and dry weight of Persian clover showed a significant increase in 0.5 ppm concentration of SO_2 but decreased significantly in high concentrations of SO_2 (1, 1.5 and 2 ppm) as compared with control plants (Table 2).

Interaction between bacterial inoculation and SO_2 on root fresh and dry weight was significant statistically. The highest levels of root fresh and dry weight were obtained at inoculated plants with native *Rhizobium* under 0.5 ppm of SO_2. The lowest levels of root fresh and dry weight were obtained at non-inoculated plants under 2 ppm SO_2. Reduction of 71.88% and 85% in fresh and dry weight of non-inoculated roots under 2 ppm SO_2 was changed to 25% and 45% respectively in inoculation with native *Rhizobium* and to 56% and 56% respectively in inoculation with standard *Rhizobium*. Namely, inoculation improves stress effects of high consentrations of SO_2 (Fig.1).

Table 1- Effects of bacterial inoculation (no-inoculation (-R), inoculation with native and standard *Rhziobium*) on root fresh and dry weight in 41-days plants. Similar words indicate not significantly difference according to Duncan's test. The data are the means of three replicates±SE and comparisons were performed separately for each index.

Index	No-inoculation(-R)	Inoculation with *Rhizobium*	
		native	standard
Fresh root weight (g)	0.27^{c}±0.04	0.50^{a}±0.05	0.39^{b}±0.05
Dry root weight (g)	0.014^{c}±0.002	0.025^{a}±0.002	0.019^{b}±0.002

Table 2- Effects of SO_2 pollution (0, 0.5, 1, 1.5 and 2 ppm) on root fresh and dry weight in 41-days plants. Similar words indicate not significantly difference according to Duncan's test. The data are the means of three replicates and comparisons were performed separately for each index.

Index	Concentrations of SO_2 (ppm)				
	0	0.5	1	1.5	2
Fresh root weight (g)	0.54^{b}±0.06	0.61^{a}±0.03	0.37^{c}±0.04	0.26^{d}±0.03	0.16^{e}±0.02
Dry root weight (g)	0.02^{b}±0.003	0.03^{a}±0.001	0.018^{c}±0.002	0.013^{d}±0.001	0.008^{e}±0.001

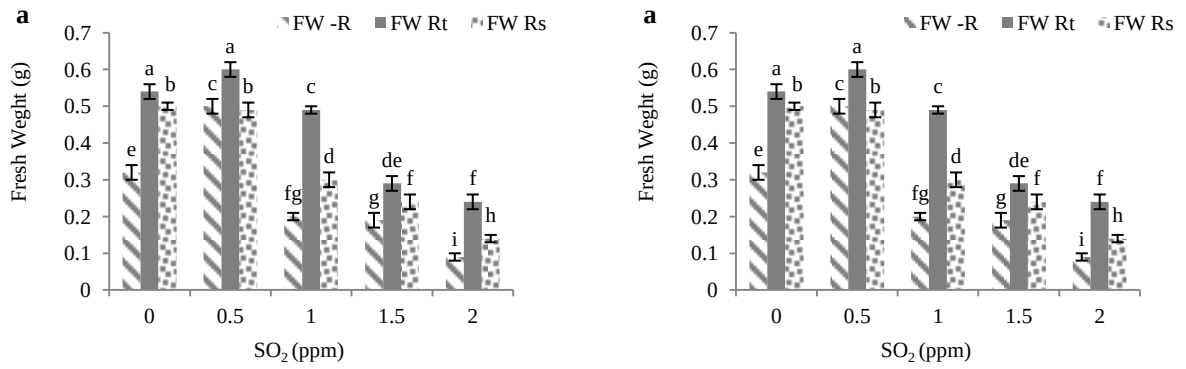

Fig.1- Interaction between of bacterial inoculation [no-inoculation (-R), inoculation with native (R_t) and standard *Rhizobium* (R_s)] and SO_2 on root weight, fresh (a) and dry (b), in 41 days *Trifolium resupinatum*. The data are the means of three replicates. Means followed by different letters are significantly difference ($P < 0.01$) as determined by Duncan's test.

Effects of bacterial inoculation and SO_2 pollution on total antioxidants:

In this study, the total antioxidant activities (I%) and capacities (IC_{50}) of the samples were determined by DPPH-radical scavenging activity test. The results showed that bacterial inoculation had no significant effect on total antioxidant activity and capacity.

The DPPH radical scavenging activity and capacity didn't change in 0 and 0.5 ppm concentrations of SO_2 gas. Increasing SO_2 stress changed I% and IC_{50} significantly. I% increased and IC_{50} decreased in high concentration of SO_2 (1, 1.5 and 2 ppm) as compared with control plant. I% was at its highest value in 2 ppm of SO_2 with 80.58% increase in comparison with the controls (Fig. 2a) whereas IC_{50} was at its lowest value in 2 ppm with 44.13% decrease in comparison with the controls (Fig 2b).

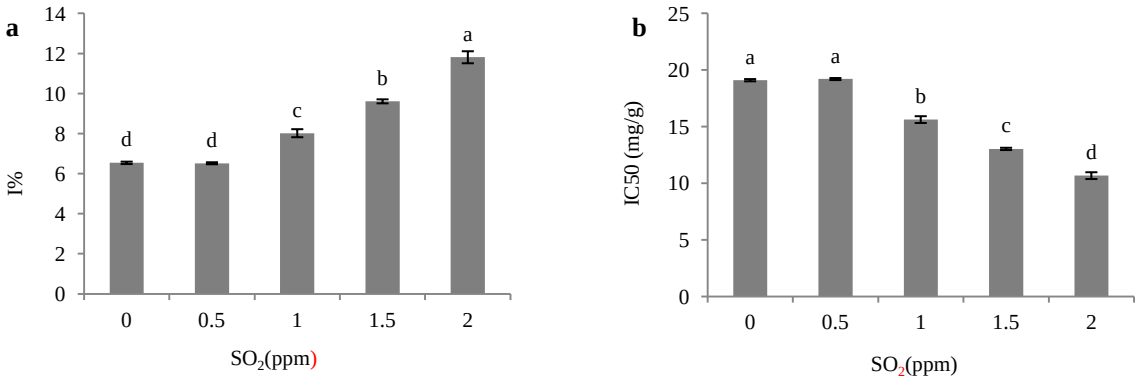

Fig.2- Effect of different concentrations of SO_2 on I% (a) and IC_{50} (b) of 41 days *Trifolium resupinatum*. The data are the means of three replicates. Means followed by different letters are significantly different ($P < 0.01$) as determined by Duncan's test.

To understand the protective action of antioxidants against SO_2 stress, Persian clover plants were treated with native and standard *Rhizobium* followed by measurement of the level of I% and IC_{50}. The effect of interaction between bacterial inoculation and SO_2 pollution on I% was significant as compared with control plants (no bacteri, no SO_2). Increasing SO_2 stress increased I% and decreased IC_{50} of Persian clover leaves significantly in comparison with control plants (Fig. 3). Inoculation of Persian clover plant with native and standard *Rhizobium* reduced the stress effects of high concentrations of SO_2 on I% and IC_{50} significantly. Among bacterial inoculation and SO_2 pollution treatments, non-inoculated plants under 2 ppm SO_2 and inoculated plants with native *Rhizobium* under 0 ppm SO_2 showed higher and lower levels of I% respectively. I% was lower in inoculated treatments compared with control treatments (Fig. 3a). Interaction between bacterial inoculation and SO_2 pollution indicated significant effects ($p \leq 0.01$) on IC_{50}. Increasing doses of SO_2 decreased IC_{50} significantly. The IC_{50} in the leaves of non-inoculated Persian clover plant under 2 ppm of SO_2 was 50.59% lower than control treatments. The level of decreasing in IC_{50} was slightly higher in inoculated plants. The effect of interaction between bacterial inoculation and SO_2 pollution indicated a decline in IC_{50} by 41.58% and 42.10% on inoculated plants with native and standard *Rhizobium* under 2 ppm SO_2 respectively. IC_{50} levels were lower in inoculated plants than non-inoculated plants (Fig. 3b).

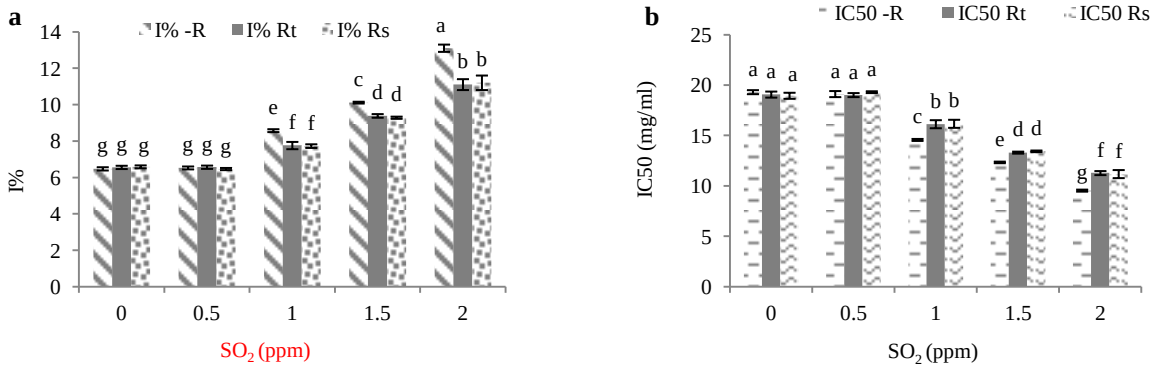

Fig.3- Interaction between bacterial inoculation [no-inoculation (-R), inoculation with native (R_t) and standard *Rhizobium* (R_s)] and SO_2 on values of I% (a) and IC_{50} (b) in 41 days *Trifolium resupinatum*. The data are the means of three replicates. Means followed by different letters are significantly different ($P < 0.01$) as determined by Duncan's test.

Effects of bacterial inoculation and SO_2 pollution on antioxidant activity (SOD, CAT, GPX)

The results showed that inoculation didn't create any significant effect on antioxidant activity (SOD, CAT, GPX) and there was no significant difference among inoculated and non-inoculated plants.

There was significant difference among treatments under different concentrations of SO_2 pollution in SOD, CAT and GPX activity. The change in SO_2 concentration caused an increase in antioxidant activity (Fig 4). SOD activity increased significantly with 0.5, 1, 1.5 and 2 ppm concentrations of SO_2. For instance, 2 ppm treatment showed a maximum effect (99.44% increase) on SOD activity, whereas 1.5, 1 and 0.5 ppm concentrations increased by 65.17%, 30% and 10.82% as

compared with control plants (under 0 ppm) (Fig. 4a). The effects of SO_2 pollution on Catalase activity was significant. CAT activity was affected by SO_2 treatments in comparison with the controls and was highest in the 2 ppm dose whereas it didn't change in other doses. The CAT activity in the leaves of Persian clover plant under 2 ppm 78.26% was higher than control treatments (0 ppm) (Fig. 4b). The antioxidant activity of Guaiacol peroxidase was not different under the influence of 0 and 0.5 ppm of SO_2 pollution, but increased in higher doses of SO_2 pollution. Among SO_2 pollution treatments, 2 ppm concentration showed highest level of GPX activity.The GPX activity in the leaves of Persian clover at 1, 1.5 and 2 ppm of SO_2 pollution was 20.58%, 50.33% and 68.23 % higher than 0 ppm of SO_2 pollution treatments, respectively (Fig 4c).

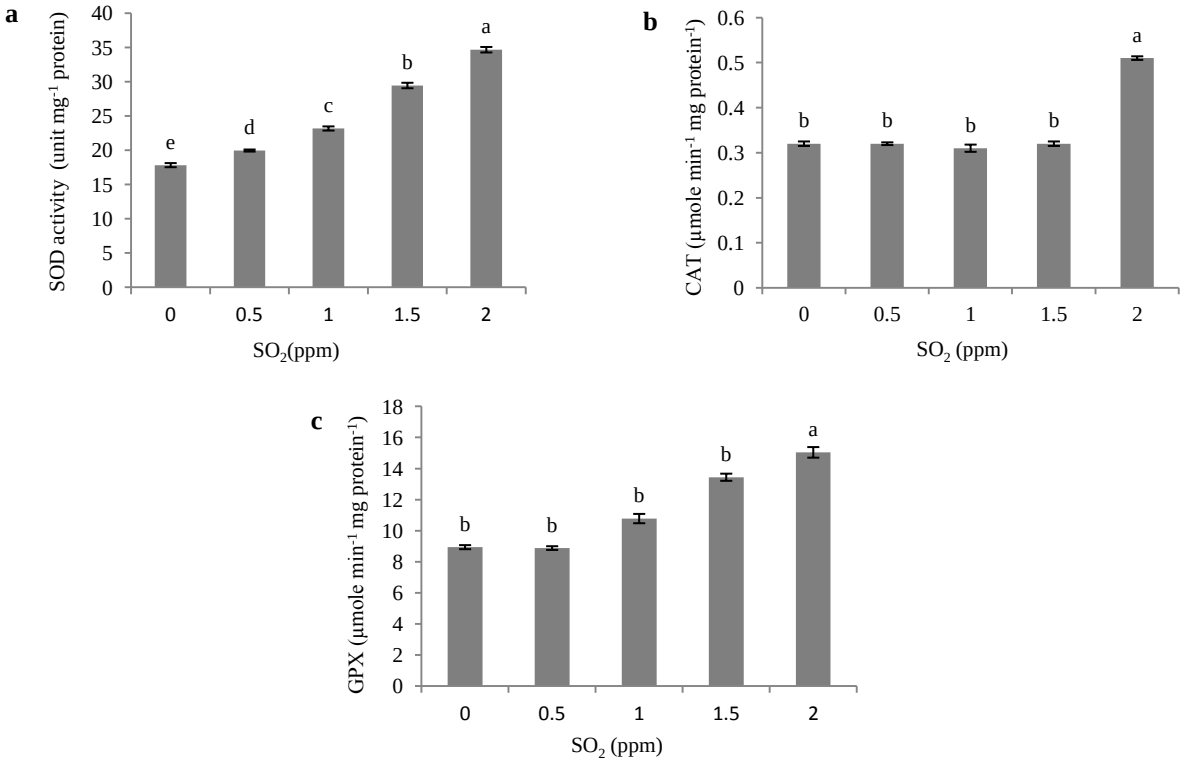

Fig. 4- Effect of different concentrations of SO_2 on activity of SOD (a), CAT (b) and GPX (c) of 41 days *Trifolium resupinatum*. The data are the means of three replicates. Means followed by different letters are significantly different ($P < 0.01$) as determined by Duncan's test.

Interaction of bacterial inoculation and SO_2 gas on antioxidant activity of SOD, CAT and GPX was significant statistically. The highest levels of SOD, CAT and GPX activity were obtained at non-inoculated plants under 2 ppm SO_2. In inoculated plants under high concentrations of SO_2 (1, 1.5 and 2 ppm) antioxidant activity was lower than non-inoculated plants. For instance, non-inoculated plants under 2 ppm of SO_2 gas treatment showed 84.98% increase in the GPX activity but inoculated plants with native and standard *Rhizobium* under 2 ppm of SO_2 gas treatment showed 64.27% and 63.13% increase, respectively. The level of increase in antioxidant activity under SO_2 pollution stress in inoculated plants was lower than in the non-inoculated plants (Fig. 5).

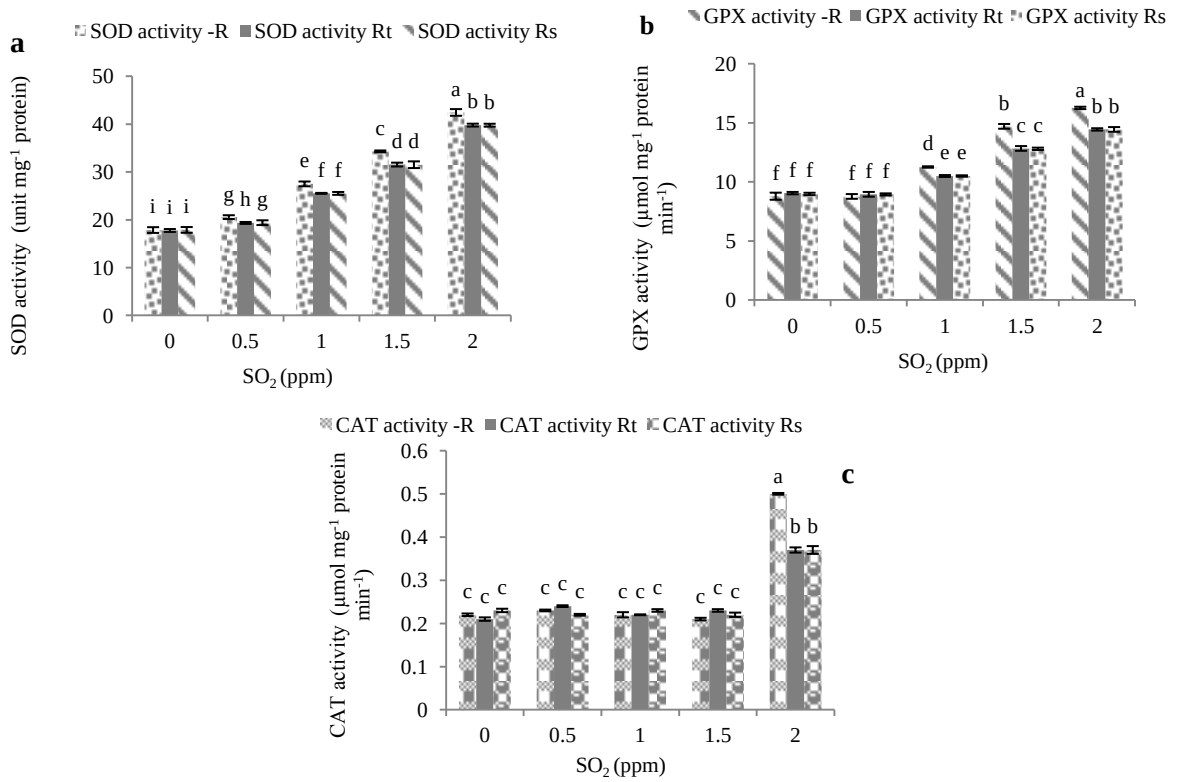

Fig. 5- Interaction between bacterial inoculation [no-inoculation (-R), inoculation with native (R_t) and standard *Rhizobium* (R_s)] and SO_2 on SOD (a), GPX (b) and CAT (c) activity of 41 days *Trifolium resupinatum*. The data are the means of three replicates. Means followed by different letters are significantly different ($P < 0.01$) as determined by Duncan's test.

Discussion and conclusion

In this study, root fresh and dry weight of Persian clover increased significantly in inoculation with *Rhizobium*. Similar results have been reported in *Vicia faba* (32). In this study, root fresh and dry weight of Persian clover increased in 0.5 ppm concentration of SO_2 but decreased significantly in high concentrations of SO_2. Similar results have been reported in *Calendula officinalis* (10).

Results of interaction between bacterial inoculation and SO_2 showed increased resistance and better growth of inoculated roots in high concentrations of SO_2. It can be concluded that *Rhizobium* can improve stress conditions by increasing of root growth. In *Vicia faba*, inoculation alone and coinoculation of *Rhizobium* and *Azotobacter* increased most of growth indexes such as root dry weight. Coinoculation of *Rhizobium* and *Azotobacter* can improve some of the *faba bean* growth indexes under the water stress conditions (1).

Rhizobacteria such as *Rhizobium* can promote plant growth. Mechanisms that use for this growth promotion can reduce stress conditions for plants. Plant growth promotion by rhizobacteria can occur directly and indirectly. There are several ways by which plant growth promoting bacteria can affect plant growth directly, e.g. by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores that solubilize and sequester iron, or production of plant growth regulators (hormones as auxin) that enhance plant growth at various stages of development. Indirect growth promotion occurs when PGPR promote plant growth by improving

growth restricting conditions. This can happen directly by producing antagonistic substances, or indirectly by inducing resistance to biotic and abiotic stresses (31). Indeed can be concluded that bacterium has been reduced antioxidant activity and capacity by reducing the effects of high concentrations of SO_2 and stress conditions.

Various abiotic stresses such as SO_2 pollution lead to the over-production of reactive oxygen species (ROS) in plants which are high reactive and toxic and cause damage to proteins, lipids, carbohydrates and DNA which ultimately result in oxidative stress (33). After entering SO_2 to the leaf, oxidation of sulphite to sulphate occurs in the chloroplast. This oxidation gives rise to formation ROS such as $O_2^{\cdot -}$ (34). In such conditions, plants develop a high efficient anti-oxidant enzymatic defense system to increase tolerance to different stress factors (33).

In this study, values of IC_{50}, I% and Guaiacol peroxidase activity indicated no significant difference at 0.5 ppm of SO_2 as compared with control plants (exposed to 0 ppm), because, stress conditions are not created in 0.5 ppm of SO_2, but indicated significant differences in higher concentrations. In higher concentrations of SO_2 (1, 1.5 and 2 ppm), IC_{50} value decreased with increasing stress intensity but I% increased. The antiradical activity was expressed as IC50 value, the concentration of sample that is required to scavenge 50% DPPH free radicals. I% value means inhibition of DPPH free radicals in percent. A lower IC50 value corresponds to a higher antioxidant activity of plant extract (35). Increasing of I% means more antioxidants have been produced with increasing stress intensity. Increase of DPPH-radical scavenging activity has been reported in many studies. DPPH-radical scavenging activity significantly increased as compared with control plants in *Cakile maritime* exposed to salinity stress (35).

Guaiacol peroxidase decomposes indole-3-acetic acid (IAA) and has a role in the biosynthesis of lignin and defence against biotic stresses by consuming H_2O_2 (33). Depending on plant species and stresses condition, activity of GPX varies. In this study, GPX activity increased in 1, 1.5 and 2 ppm of SO_2 as observed in *Zizyphus mauritian, Syzygium cumini, Azadirachta indica* and *Mangifera indica* (36) exposed to SO_2 pollution.

Catalase is tetrameric heme enzyme that can decompose H_2O_2 into H_2O and O_2 directly and is indispensable for ROS detoxification during stressed conditions (33). In this study, CAT activity indicated no significant difference at 0.5, 1 and 1.5 ppm concentrations of SO_2 as compared with control plants but in 2 ppm of SO_2 indicated a significant increase. CAT is an antioxidant that is activated in severe stress conditions (33). So increasing it is reasonable in 2 ppm of SO_2. Various studies have reported different results from the CAT activity. Increase in CAT activity indicated in *wheat* plant under drought stress (37). CAT activity of *Calandula officinalis* under high concentration of salinity (100 mM) increased in leaves but decreased in roots (38).

Superoxide dismutase is the most effective intracellular enzymatic antioxidant which is ubiquitous in all aerobic organisms and in all subcellular compartments prone to ROS mediated oxidative stress (33). In this study, SOD activity increased significantly in 0.5, 1, 1.5 and 2 ppm of SO_2 as compared with control plants. SOD has been proposed to be important in plant stress tolerance and provide the first defense against the toxic effects of elevated levels of ROS (33), therefore production of it in low concentration of SO_2 can be due to this important role. Similar results have been reported in other studies. SOD activity

indicated a significant increase in *Phaseolus vulgaris* (15) exposed SO_2 pollution.

In this study, inoculation with native and standard *Rhizobium* had no significant effects on values of IC_{50}, I%, GPX activity, SOD activity and CAT activity, as reported by Gaballah and Gomaa (2005) (32). Their study showed that inoculation of two cultivars of *Vicia faba* with *Rhizobium* had no significant effects on SOD activity. Therefore, stress conditions are not created in inoculated plants.

Interaction between inoculation and SO_2 treatment in this study was significant. Indeed *Rhizobium* inoculation under SO_2 condition showed significant effect on the values of IC_{50}, I%, GPX activity, SOD activity and CAT activity. Different studies have expressed different conclusions about the interaction between bacterial inoculation and stress. Inoculation of Lettuce under salinity stress with *Rhizobium* sp. and *Serratia* sp.decreased enzyme activity, including GR and (APX), with increasing salinity stress (7). A clear decline in SOD activity in two cultivars of *faba bean* was observed with increasing salinity stress. Use of *Rhizobium* inoculation and sodium benzoate increased SOD activity in *faba bean* plants under salinity (32).

Stress resistance in plants has been related to better growth and more effective antioxidant systems. Low concentration of SO_2 (0.5 ppm) doesn't create stress conditions in Persian clover, therefore activity and capacity of most of antioxidants don't alter in this concentration and have a positive effect on root weight. In higher concentrations of SO_2 (1, 1.5 and 2 ppm), antioxidants activity and capacity of Persian clover increase with increasing stress intensity. *Rhizobium* inoculation of Persian clover under SO_2 treatment increased root growth and decreased antioxidant activity and capacity by reducing of stress conditions and thus reducing the amount of free radicals.

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اثرات تلقیح ریزوبیوم بر سیستم آنتی‌اکسیدانی گیاه شبدر تحت آلودگی گاز دی اکسید گوگرد

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چکیده

مقدمه: ریزوباکتری‌های محرک رشد گیاه، باکتری‌های مفیدی هستند که می‌توانند سبب مقاومت گیاهان به تنش‌های مختلف شوند. یکی از این تنش‌ها، آلودگی SO_2 هوا است. دی‌اکسیدگوگرد به عنوان یک آلاینده آسیب‌رسان قوی هوا شناخته شده که رشد گیاهان را محدود می‌کند. هدف از این مطالعه ارزیابی اثرات تلقیح باکتریایی با ریزوبیوم استاندارد و بومی بر رشد ریشه و فعالیت و کارایی آنتی‌اکسیدان‌های شبدر ایرانی تحت آلودگی SO_2 هوا است.

مواد و روش‌ها: در این پژوهش، گیاهان ۳۱ روزه (تلقیح‌شده و تلقیح‌نشده با ریزوبیوم) در معرض غلظت‌های مختلف گاز SO_2 (صفر به عنوان شاهد، ۰/۵، ۱، ۱/۵ و ۲ ppm) به مدت ۵ روز متوالی و هر روز ۲ ساعت قرار گرفتند.

نتایج: نتایج نشان داد که غلظت‌های مختلف SO_2 اثرات معنی‌داری بر وزن ریشه و سیستم آنتی‌اکسیدانتی شبدر دارد. با افزایش غلظت SO_2، به طور معنی‌داری نسبت به کنترل وزن تر و خشک کاهش، میزان فعالیت آنتی‌اکسیدانی (I%) افزایش، ظرفیت آنتی‌اکسیدانی (IC50)کاهش و فعالیت SOD، CAT و GPX افزایش یافت. تلقیح شبدر با ریزوبیوم بومی و استاندارد وزن ریشه را افزایش داد و اثر معنی‌داری بر فعالیت و کارایی آنتی‌اکسیدانت‌ها نشان نداد؛ ولی اثرات متقابل تلقیح ریزوبیومی و تیمار SO_2 بطور معنی‌داری اثرات منفی غلظت‌های بالای گاز را بر رشد ریشه، فعالیت و کارایی آنتی‌اکسیدان‌ها کاهش داد. شدت تغییر سیستم آنتی‌اکسیدانتی تحت تنش آلودگی SO_2 در گیاهان تلقیح‌شده کمتر از گیاهان تلقیح نشده بود.

بحث و نتیجه‌گیری: در نتیجه، افزایش غلظتSO_2 سبب کاهش وزن ریشه، افزایش فعالیت و کارایی آنتی‌اکسیدانتی شبدر شد. تلقیح با ریزوبیوم با افزایش رشد ریشه، اثرات آلودگی SO_2 بر سیستم آنتی‌اکسیدانی را کاهش می‌دهد.

واژه‌های کلیدی: آلودگی SO_2، ریزوبیوم، شبدر، فعالیت آنتی‌اکسیدانت، کارایی آنتی‌اکسیدانت

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