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Original Research Article

Impact of AM Fungi and *Azotobacter* in the Alleviation of Cd-Induced Growth Reduction and Activity of Antioxidants in *Coriandrum Sativum* L

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Abstract

The aim of the present study was to evaluate the role of arbuscular mycorrhizal (AM) fungi and *Azotobacter*on growth, oxidative effect and antioxidant defense mechanisms under cadmium (Cd) stresses in *Coriandrum sativum* L. (Coriander). Treatments consisted of mycorrhizal and *Azotobacter* treatments and threeconcentrations of cadmium treatments (0,50and 100 mg Cd kg^{-1} of soil). Metal induced oxidative damage through increased lipid peroxidation. Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) increased under stress and it was found that there was a direct correlation between root length, shoot length, plant fresh weight and plant dry weight and the activity of these enzymes in concentration dependent manner. The activity of antioxidants and growth increased in mycorrhizal and *Azotobacter* stressed plants. However, the combination of mycorrhiza (AMF) and *Azotobacter*acted synergistically and significantly arrested lipid peroxidation and led to increased growth as well as activity of activity of antioxidants. The interactions of cadmium and biofertilizers proved to be synergistic and the antioxidant activities further increased indicated the positive role of biofertilizers in increasing the antioxidant activity thereby decreasing oxidative stress and stimulating growth. Therefore, biofertilizers are advisable to enhance the tolerance to cadmium stress in medicinally important coriander.

Keywords: *Azotobacter*, Antioxidants, *Coriandrum Sativum*, mycorrhizal.

INTRODUCTION

Heavy metal stress is one of the major abiotic stresses in recent decades because unlike other organic pollutants, they are not degraded and converted into harmless compounds via biological processes [1]. Its persistence in the environment for a long time pose a risk for primary as well as secondary consumers and ultimately to humans through the entry into the food chain [2]. Cadmium (Cd) is one of the most toxic, nonessential, persistent environmental toxicant [3, 4], mobile and a non different indirect mechanisms. It is emanated in the arable field mainly due to the application of pesticides, industrial processes, fossil fuel combustion, cement manufacture, non-ferrous metal production, irrigation with wastewater, sewage sludge, metal-containing pesticides, municipal- based composts and phosphate fertilizers [5-10] and from the soil, despite its non-essentiality, it is readily taken up and translocated upward by the plants where it causesreduction of growth as well as phytotoxicitythroughthe production of toxic oxygen derivatives [11] and alsoa potential risk for human health when transferred from crops to human diet. Cd stress disrupts cellular homeostasis and enhances the generation of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($^{\cdot}OH$), singlet oxygen ($^1O2$) and hydrogen peroxide (H_2O_2) in plants [12]. Oxidative stress would arise if the balance between ROS generation and removal was broken [13] which induces damage to the biomolecules, causes lipid peroxidation by the production of malondialdehyde (MDA) which is one of the decomposition products of polyunsaturated fatty acids of membranes [14-20], disruption of some of the metabolic pathways such as electron transport chain and ATP production [21, 22] and a cumulative adverse effects on plant growth and yield [6, 23, 24]. Plants have the ability to combat negative consequences of heavy-metal stress and protect the cells from oxidative damage by complex ROS scavenging mechanisms at the molecular and cellular levels due to a series of detoxification reaction catalyzed by efficient antioxidant defense systems such as CAT, SOD and POD which inhibit the oxidation of biomolecules, oxidative chain reactions and cellular oxidative damage [25-29]. Superoxide dismutase (SOD; EC, 1.15.1.1) is a key enzyme of primary line of

defense that dismutates superoxide radicals to H_2O_2 and protects cells against oxidative stress [30, 31] Further, the accumulation of H2O2 is restricted through the action of catalase (CAT; EC, 1.11.1.6) [32, 33] and peroxidase (APX; E.C.1.11.1.11.) whichhas to be further detoxified [15] to water and O_2 [34, 35].

Mycorrhiza areobligate symbionts, non-pathogenic fungi, potential phosphate-solubilizing bio-fertilizers, a cheap and environment friendly alternative to expensive chemical fertilizers [36]. These are the most ancient and wide spread plant strategies to facilitate plant growth, enhance resistance to cope with the environmental stress and are able to regulate genes which cause accumulation of ROS in order to mitigate HM-induced oxidative stress [25, 37-40], enhance plant mineral nutrition, water acquisition [41], increase biomass and survival rate under different environmental stresses [42-46, 28].

Azotobacter is a free-living, gram negative aerobic bacteria that can be an alternative to chemical fertilizers and pesticides [47], increase plant growth by synthesizing biologically active substances [48], increase root surface area, produce phytohormone precursors in N-free media [49, 50], provide N to the plants through N_2 fixation [51], provide essential nutrients [52, 53], increase the uptake of [54-56], synthesizes antioxidants to scavenge the toxicity of ROS generated by plants and play a pivotal role in protecting the plants from oxidative stress [57-59].

Coriander (*Coriandrum sativum* L.) is an important spice crop of family Apiaceae that has nutritional as well as medicinal properties and occupies a prime position in flavoring substances. Its volatile oil is rich in beneficial phytonutrients and the seeds are used as antispasmodic, carminative, stimulant, fungicidal, stomachic, hypoglycemic, hypolipidemic, antibacterial, antimutagenic, insecticidal activity and aflatoxin controlling effects. It is also used as an antioxidant in the control of swellings, diarrhea, mouth ulcers, anemia, menstrual disorders, small pox, eye care, and conjunctivitis and skin disorders.

The positive synergistic interactions among components of the tripartite symbiotic association (AMF-*Azotobacter- Coriandrum sativum* L.) alleviated toxic effects of ROS and maximized plant growth, its nutrition and yield [60]. However, this interaction under Cd stress has not yet explored and less attention was paid on this aspect. Therefore, proposed study was conducted as an acceptable approach, safe for human consumption than chemical fertilizers and also to appraise the effectiveness of synergistic microbes in the amelioration of tolerance in coriander against the inhibitory effects of cadmium.

MATERIALS AND METHODS

Seeds of coriander (*C. sativum* L.) were obtained from National Seed Corporation Ltd., New Delhi, India and the authentic culture of AM fungi and *Azotobacter* were procured from the Division of Microbiology, Indian Agriculture Research Institute, New Delhi. The seeds were surface sterilized with 0.5% hypochlorite for 5 min, rinsed thoroughly in double distilled water for 5-6 times and soaked overnight in sterile water for 12 h at 4 ^{0}C for uniform germination. *Azotobacter* culture was applied on the surface sterilized seeds by dipping in a sugary solution of bacterial powder containing *Azotobacter chroococcum* cells, 5×10^8 g^{-1} prepared in the ratio of 1:1for 15 min. After that, the seeds were dried for 30 min in shade at a temperature of 20°C.Five kg soil composed of peat and compost (4:1, w/w) mixed with sand (3:1,w/w)was filled in 23-cm-diameter earthen pots and mixed thoroughly with appropriate amount that is 0, 326.16 and 652.28 mg $CdCl_2$ kg soil to achieve 0, 50, 100 mg Cd kg^{-1} of soil (Cd_0, Cd_{50} and Cd_{100}) respectively and watered on alternate days. Spores were applied at the rate of thousand spores per pot as a thin layer below the seeds before sowing. The application rate was same in combined inoculation also. Plants were sampled at three stages (30, 60 and 90 DAS).

A completely randomized block design experiment with five replicates was arranged in the net house of Department of Botany, Aligarh Muslim University, Aligarh, India during September-February 2009–2010 under natural day/night condition (Photo synthetically active radiation >950 mmol m^{-2}s^{-1},average day and night temperature of 21 ± 3and 12 ±2°C respectively and relative humidity 75 ± 5%) which included two mycorrhizal treatments (with mycorrhizal fungi ‘‘M_+’’ and non-mycorrhizal fungi’M_-’’), two *Azotobacter* treatments (with *Azotobacter* ‘‘A_+’’ and *Azotobacter* ‘‘A_-’’)and three levels of cadmium treatments i.e.$CdCl_2$ at 0, 50 and 100 mg/kg of soil);. The total nine treatments T_0 - Cd_0 M_-A_-,T_1-Cd_0 M_+ A_- ,T_2-$Cd_0M_-A_+$, T_3- Cd_0 M_+ A_+,T_4- Cd_{50} M_+ A_-,T_5-$Cd_{50}M_-A_+$,T_6 - Cd_{50} M_+ A_+,T_7 - Cd_{100} M_+ A_-,T_8- Cd_{100} M_+A,T_9- Cd_{100} M_+ A_+,.

Measurements of Growth

Plants were uprooted and growth characteristics such as shoot length, root length, plant fresh weight and plant dry weight were measured on a meter scale after 30 days of treatment. Shoot and root length were measured on meter scale. These plants were blotted in blotting sheets to remove the adhering water and weighed on electronic balance to record their fresh weight. Dry weight was determined after drying the samples in an oven for about 72 hours at 80°C till constant weight.

Determination of Lipid Peroxidation

The level of lipid peroxidation products in leaves was expressed by estimating thiobarbituric acid

reactive substances (TBARS content) expressed as equivalents of malondialdehyde (MDA; a byproduct of lipid peroxidation) using using 2-thiobarbituric acid (TBA) [61]. Frozen samples were taken and homogenized with two volumes of ice-cold 0.1 % (w/v) trichloroacetic acid (TCA) and homogenate was centrifuged at 12,000g for 15 min at 4 °C. Assay mixture containing 2 ml aliquot and 2 ml of 0.6% thiobarbituric acid (TBA) in 10% TCA was heated at 95^0 for 30 min and rapidly cooled in an ice bath to terminate the reaction. After centrifugation (12,000g for 15 min at 4 °C), absorbance (532 nm) was measured and values corresponding to nonspecific absorption (600 nm) were subtracted. The total MDA content was calculated using the extinction coefficient of155 $mM^{-1}cm^{-1}$ and expressed as Nano mole MDA per gram DW.

Extraction and Estimation of Antioxidatant Enzymes

Antioxidant enzymes were examined according to Zhang [62] with some modifications. The fresh leaves (0.3 g) were homogenizedin 5 ml of 50 mM potassium phosphate buffer (pH 7.8) containing 0.5 mM EDTA and centrifuged at 10,000 rpm at 4 °C for 15 min and the supernatant obtained were utilized for enzyme assays [63]. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction due to nitroblue tetrazolium (NBT) using the method of Sen Gupta *et al.*, [64]. A 3 ml of reaction mixture was prepared by taking 200 $mmolL^{-1}$ methionine, 2.25m $molL^{-1}$ nitro-blue tetrazolium (NBT), 3 m $molL^{-1}$ EDTA, 100 m $molL^{-1}$ potassium phosphate buffer, 60 µ $molL^{-1}$ riboflavin, distilled water and enzyme extract and it was illuminated with 30 W florescent lamps for 15 min at 28 °C. Blank and controls were run in the same manner but without illumination and enzyme. A unit of SOD is defined as the amount of enzyme required to cause 50% inhibition of the reaction of NBT measured at 560 nm. The activity of CAT (CAT, EC 1.11.1.6) was analyzed according to Aebi [65] in 3 ml of a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 2 mM Na_2-EDTA, 10 mM H_2O_2 and 0.1 ml of the enzyme extract. Decrease in absorbance was measured at 240 nm as a consequence of H2O2 consumption for 1 min (coefficient of absorbance of 39.4 mM^{-1} cm^{-1}). The activity of Peroxidase was estimated by the method of Bergmeyer *et al.*, [66]. In a 30.1 ml of reaction mixture consisting of pyrogallol phosphate buffer(pH 6.8),1% H2O2, deionized water and enzyme extract was incubated at 30°C Change in absorbance, due to catalytic conversion of pyrogallol to perpurogallin, was noted at an interval of 20 s for 2 h at 420 nm. A control set was prepared by distilled water instead of enzyme extract. The change in absorbance per seven minutes (4 times) over the linear portion of the curve was measured and this value was used in the calculation.

Statistical Analysis

Data of various parameters were subjected to analysis of variance (ANOVA) for two factor pot culture experiment i.e., microbial inoculants and cadmium stress, using SPSS software version 10.0. Duncan's multiple range test (DMRT) at 0.05 level of probability was used to evaluate the difference among treatment means. The 'F' test was applied to assess the significance of data at 5% level of probability ($P \leq 0.05$). A correlation coefficient value (r) was also undertaken.

RESULTS

Results pertaining to the effects of various concentrations of Cd on the growth attribute such as shoot length, root length, plant fresh weight and plant dry weight after 45 days of sowing was maximally and significantly reduced by the increasing concentration of cadmium. However, maximum reduction of toxicity in growth was reflected by 100 mg Cd kg^{-1} soil (Table-1). Roots were found to be more sensitive to toxic effects of Cd than shoots. The highest (100mg Cd kg^{-1}soil) and moderate(50mg Cd kg^{-1}soil) level of metal decreased root length by 22.8%,42.9%, shoot length by 19.3%,36.8%, plant fresh weight by 17.0%,51.6% and that of plant dry weight by 22.9%,52.0% respectively, over their controls. However, Dual inoculation of AMF and *Azotobacter* made the plants more adaptable to the stressed conditions. They together proved to be synergistic in the alleviation of toxic effects of cadmium stress and enhanced the growth attributes significantly as compared to their single inoculation. Their co-inoculation caused an increase of root length by 24.0 %, shoot length by 12.8%, plant fresh weight by 71.0% and that of plant dry weight by 31.0 % respectively, over their respective control at 100mg Cd kg^{-1}soil (Table-1).

Two way ANOVA showed that individual effects of inoculation and cadmium were significant ($p < 0.05$) for all the measured parameters except interaction (inoculation × Cd) effects on growth. The value for correlation between different parameters was presented in Table-2 showing positive correlation between various growth parameters.

In general, Cd had significant effects on the antioxidant activities of coriander. Metal stress significantly increased the antioxidant activity. In general, the toxicity of 100 mg Cd kg^{-1}soilwas much higher and led to higher increase in enzyme activity as compared to 50 mg Cd kg^{-1}soil. However, with the introduction of AM fungi and *Azotobacter* in the root, there was a significant improvement in this parameter. Their combined interactions further enhanced the activity of enzymes which clearly indicated the positive synergistic interaction of microbes in decreasing oxidative stress. Since, bacterial symbiosis and mycorrhizal colonization were relatively tolerant to different concentrations of metals, their effective symbiosis might have been responsible for bringing

tolerance in plants of heavy metal stress (SOD Fig-1A; CAT Fig-1B and POX Fig-3C).

In the present study, SOD activity had significant increase in the leaves of coriander exposed to 50 mg Cd kg^{-1} soil, however it further turned to decline at the highest dose of 100mg Cd kg^{-1}soil. Application of either AM fungi or *Azotobacter* singly increased the activity of this enzyme. Whereas, in case of co-inoculated plants, SOD activity showed a sharp and significant increase with the increase in cadmium concentration which reached its maximum level at 100 mg Cd kg^{-1}soil (Fig-1A).

The CAT activity enhanced significantly with the increase in the concentration of Cadmium in the soil in un inoculated control plants. The dual application of both the symbionts had profound synergistic effect on the activity of this enzyme as compared to control plants (Fig-1B).

The Cadmium stress caused a significant increase in the peroxidase (POD) activity of the coriander leaves, being maximum at100 mg Cd kg^{-1}soil as compared to control. Dual inoculation of AMF and *Azotobacter* gave enhancement in POD activity. Maximum activity of enzyme was reported when both the microbes were applied at the highest level of cadmium (Fig-1C).

Malondialdehyde (MDA) content, a phytotoxic product of lipid peroxidation, causes membrane disruption which becomes an index of membrane damage in leaves. Increase in Cd concentration in soil increases the lipid peroxidation and was highest at100 mg Cd kg^{-1}soil (Fig-1D). Its content in single and dual inoculated (AM fungi + *Azotobacter*) plants increased significantly ($p<0.05$) with the increase in Cd concentration being maximum at 100 mg Cd kg^{-1} soil but this increase is less as compared to their respective control plants. However, this decrease in the level of MDA was found to be maximum in combination treatment of AM fungi+*Azotobacter* (Fig-1D).

SOD activity had significant increase in leaves exposed to 50 mg/kg of cadmium and then turned to decline at 100 mg/kg. Whereas in case of inoculated plants SOD activity increases significantly with cadmium concentration. SOD activity reached maximum levels at 100 mg/kg of cadmium following co-inoculation with AM fungi and Azotobacter (Fig-1).

The catalase (CAT) activity increased significantly with Cd treatment in both inoculated and uninoculated plants, (Fig-1a). In contrast, the dual application of the AMF and *Azotobacter*had maximum effect on the CAT activity compared to the control.

The Cadmium stress and biofertilizer treatment caused a significant increase in the peroxidase (POD) activity of the leaves of plants compared to control. Maximum increase in POD activity was found in case of dual inoculation of AMF and Azotobacter at 100 mg/kg of cadmium.

Malondialdehyde (MDA) content which is an index of lipid peroxidation in leaves of both inoculated (AM fungi + *Azotobacter*) and non-inoculated plants increased significantly ($p<0.05$) by the increase in Cd concentration. The rate of this increase was maximum at 100 mg kg^{-1} of Cd. The decrease in the level of MDA was found to be maximum in combination treatment of (AM fungi+*Azotobacter* (Fig-2F).

The two way ANOVA showed that individual effects of inoculation and cadmium and their interaction (inoculation × Cd) were significant ($p < 0.05$) for all the measured parameters except interaction effects on CAT and MDA content. Antioxidant enzymes and MDA contents exhibited a negative correlation with growth parameters, presented in Table-2.

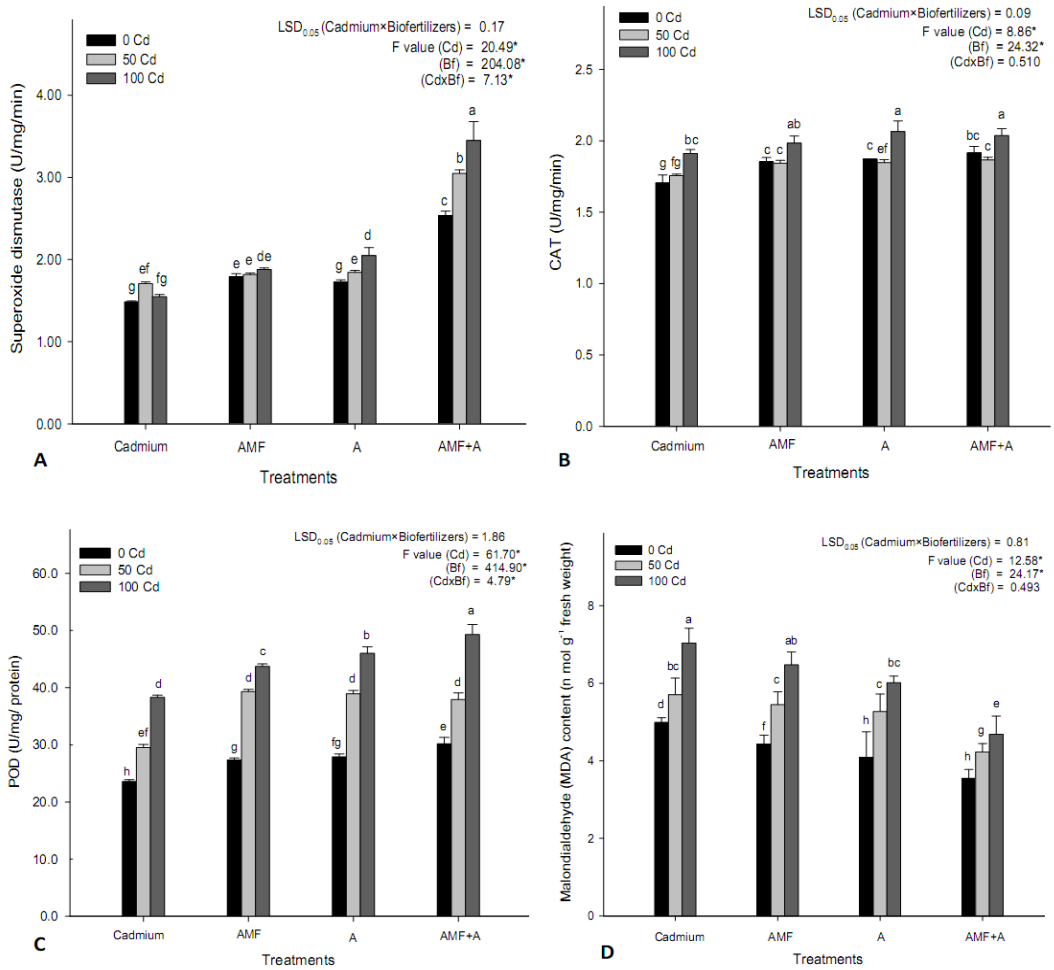

Fig-1

Table-1: Effect of inoculation with biofertilizers on growth parameters of coriander plants subjected to different soil cadmium concentrations

Treatments		**RL**	**SL**	**PFW**	**PDW**
Cadmium	Biofertilizers				
Control		16.87 ± 0.41 bc	39.95 ± 0.39 bcd	19.88 ±0.07 c	09.37 ± 0.21 ab
0	AMF_1A_0	19.09 ± 0.64 a	45.08 ± 0.71 a	21.95 ± 0.53 b	10.41 ± 0.66 a
	AMF_0A_1	18.59 ± 0.39 ab	45.29 ± 1.19 a	22.73 ± 0.38 b	09.74 ± 0.54 a
	AMF_1A_1	19.82 ± 0.62 a	45.28 ± 0.37 a	25.32 ± 0.90 a	11.96 ± 0.62 a
50	AMF_0A_0	13.03 ± 0.09 d	32.25 ± 1.68 f	16.50 ± 0.98 d	07.22 ± 0.61 c
	AMF_1A_0	16.29 ± 0.55c	37.89 ± 1.21 de	19.00 ± 0.57 c	07.34 ± 0.69 bc
	AMF_0A_1	16.09 ± 1.96 c	37.33 ± 1.86 e	18.81 ± 0.99 c	07.25 ± 0.38 c
	AMF_1A_1	16.38 ± 1.29 c	39.33 ± 1.76 cde	19.85 ± 0.44 c	07.30 ± 0.37 c
100	AMF_0A_0	09.63 ± 0.35 e	25.24 ± 1.19 j	09.62 ± 0.64 f	04.45 ± 0.29 d
	AMF_1A_0	10.94 ± 0.80 e	27.71 ± 0.36 ij	13.50 ± 0.76 e	05.67 ± 0.28 cd
	AMF_0A_1	10.80 ± 0.26 e	27.77 ± 0.69 hij	13.17 ± 0.60 e	05.67 ± 0.64cd
	AMF_1A_1	11.98 ± 0.07 de	28.47 ± 0.87 ghi	16.48 ± 2.30 d	05.83 ± 0.52cd
LSD at 0.05%		**1.78266**	**2.54856**	**1.8648**	**2.0424**
F- value					
BF	df (3)	7.229*	8.885*	16.155*	3.615*
Cadmium	df (2)	94.387*	210.481*	102.435*	96.410*
Interaction	df (6)	0.350	0.670	0.787	1.505

* Significantly different from control at $p \leq 0.05$

Table-2: Correlation among growth and antioxidant enzyme activity of coriander (Coriandrum sativum L.) under diferent treatments of cadmium and biofertilizers

Attributes	Root length	Shoot length	Plant fresh weight	Plant dry weight	Superoxide dismutase	Catalase	Peroxidase	Malanoaldehyde content
Root length	1							
Shoot length	0.909**	1						
Plant fresh weight	0.902**	0.892**	1					
Plant dry weight	0.848**	0.854**	0.853**	1				
Superoxide dismutase	-0.007ns	-0.064ns	0.131ns	-0.055ns	1			
Catalase	-0.394*	-0.446**	-0.361*	-0.328ns	0.487**	1		
Peroxidase	-0.641**	-0.703**	-0.570**	-0.706**	0.476**	0.692**	1	
Malainoaldehyde content	-0.742**	-0.721**	-0.769**	-0.635**	-0.402*	0.157ns	0.365*	1

**Correlation is significant at the 0.05 level, *Correlation is significant at the 0.01 level

DISCUSSION

In the present experiment, the application of bio-fertilizers (alone and in combination) was found favourable to abate the cadmium stress in *coriandrum sativum*. These results are in consistent withthe previous works that cadmium had a toxic effect on growth whereas addition of bio-fertilizer helps in promoting growth under cadmium stress [67-71]. The presence of cadmium in the soil inhibits plants growth by altering the plant metabolism even at low concentration [72]. The effect of inoculation of AM fungi and *Azotobacter* (alone or in combination) on the vegetative growth of coriander was significantly higher as compared to control plants. A possible mechanism of this eff ect is the ability of AMF to bind heavy metals by fungal hyphae outside and inside the roots [73]. *Azotobacter* is able to produce plant hormones or hormone-like substances which can promote plant growth [74-76]. AMF are known to affect plant growth and health by improving mineral nutrition [77] and increasing resistance or tolerance to biotic [78, 79] as well as abiotic stress [80]. Both AMF and *Azotobacter* complement each other in their role in N_2-fixation, phytohormone production, P-solubilization, and increasing surface absorption. The positive synergistic interactions between AM fungi and various N_2-fixing bacteria is the basis of application of these microbes as biofertilizer in the mycorrhizosphere [81]. These microbes are regulated for their own benefit, which in turn benefits the host plant.

At the time of oxidative stress due to metal, membranes are considered as the primary sites of injury and its destabilization, lipid peroxidation and consequent disruption is directly correlated with the production of a phytotoxic product called as MDA [82, 83]. It is considered as themost damaging process known to occur in every living organism. Peroxidation of lipid further boosted gradually in proportion to increased concentration of Cd in the soil as compared to unstressed conditions [84]. MDA content of AM and *Azotobacter* inoculated plants was lesser than in the non-inoculated plants even under metal treatments. Synergistic interaction of microbes further decreased the production of MDA in stressed plants. Similar results were found in canola [85], wheat [86], Cajanus [87], isabgol [71].

Cd toxicity induced oxidative stress in plants by the formation of ROS [88, 89], Excess ROS production can cause oxidative damage to biomolecules such as lipid, protein and nucleic acids, and disrupt cellular metabolism [90, 91]. Plants cope with oxidative stress by using antioxidant enzymes such as SOD, CAT, and POD which are responsible for scavenging excessively accumulated ROS in plants under stress conditions [92, 93].

SOD is called the cell's first line of defense against ROS [94] because superoxide radical production via one electron reduction of triplet oxygen is a precursor to several other highly reactive species and is a starting point for ROS biosynthesis, oxidative stress, and redox regulation in plants [95]. So that control over the steady state of superoxide concentration by SOD constitutes an important protective mechanism [96]. In the present investigation, results showed that the SOD activity was first increased and declined slightly at higher concentration of cadmium. The SOD activity showed an increase and in some, a reduction under abiotic stresses [97]. An increase in SOD activity could possibly be the result of both a direct effect of heavy metalions and an indirect effect mediated via an increase in levels of superoxide radicals [98, 99], which in turn may be associated with an induction of genes of SOD by superoxide-mediated signal transduction [31, 100]. Liu *et al.*, [101] found that AM fungi improved the capability of ROS scavenging and reduced Cd concentration in plants to alleviate Cd stress. The changes in SOD activity under *Azotobacter* treatments can be also a consequence of an altered synthesis and accumulation of less active enzymes and/or of a higher turnover of SODs [102].

SOD activity reached maximum levels following coinoculation with AM fungi and bacteria [103].

In our study, an increased level in CAT activity was found with the increase in the concentration of Cd in soil. CAT participates in the main defense system against accumulation and toxicity of ROS, such as H_2O_2, and canplay a key role in controlling H_2O_2 levels in plant cells [29]. CAT catalyzes the dismutation of H_2O_2 into H_2O and O_2 and has been found to increase under stress which in turns protected plants from oxidative damage [104]. A further increase in CAT activity was observed in plants following the-inoculation of AMF and Azotobacter. The synergistic interaction of microbes further enhanced the activity of this enzyme. Our result coincides with karthikeyan [75], Azcon *et al*., [103] as well as Lin and Kao [105].

In the present investigation, POD activity was increased with the elevation in the level of Cd in soil. *Azotobacter* or AM fungi symbiosis increased the activity of enzyme, however their interaction had greater effect. These results suggest that symbionts colonization reduces ROS elevated stress. POD not only scavenges H_2O_2, but also catalyzes the synthesis of cell wall [106], modifies mechanical properties of the cell wall and cell membrane integrity of plant leaves under stress conditions [107]. Zhang *et al.*, [108] observed decreased oxidative stress in Vicia faba by intricating antioxidant defense system due to POD grown in soil contaminated with Cd following G. mosseae treatment.

All the antioxidant (SOD, CAT and POD) activities in case of inoculated plants were significantly higher than those of uninoculated plants at all concentrations, suggesting that biofertilizers could improve the antioxidant enzyme systems to alleviate destructive stress. Plants with high concentration of antioxidants have been reported to have greater resistance to this oxidative damage [109-111]. The antioxidant activities further increased with the application of biofertilizers was a significant improvement [29, 103, 112, 113].

CONCLUSION

Cd causes oxidative stress due to reduction in growth, alterations of antioxidant enzymes and increase of MDA content in plants. Use of biofertilizer in the form of AM fungi and *Azotobacter* played a potential role in the increment of growth and antioxidant activity and thereby decrease oxidative stress. Co-inoculation of both the microbes had positive synergistic interaction on growth and antioxidant activity. However, combined interaction declined the MDA content. Therefore, microbes ameliorated Cd stress by the increase of antioxidant activity and decline of membrane damage and application of biofertilizers is cost efficient, eco-friendly, easier to use, alleviates Cd stress and enhances the tolerance to cadmium stress in *Coriandrum sativum*.

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