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Effect of cadmium phytotoxicity and plant defence in *Ocimum basilicum* L.

Sheeba Naaz[1, 2], Nadeem Ahmad[2], M Irfan Qureshi[1]

[1] Department of Biotechnology, Faculty of Natural Sciences, Jamia Millia Islamia (A Central University), New Delhi, India

[2] Department of Biosciences, Faculty of Natural Sciences, Jamia Millia Islamia (A Central University), New Delhi, India

Abstract

Many sections of the world have heavy metal-contaminated soil, which has a number of negative impacts on plants and threatens the effectiveness of the global food supply. Hazardous and non-essential element cadmium disrupts physiological processes, also having a detrimental impact on a plant's growth, the photosynthetic properties and delaying plant development metabolism. To investigate the impact of cadmium toxicity in Ocimum, pot experiment was carried out. In this work, cadmium (Cd) effects by using the concentrations (0, 100, 150 mg $CdCl_2$ kg^{-1} of soil) on the physiological, morphological, and biochemical responses of Ocimum grown in greenhouse conditions were examined. Cadmium reached to the soil to decrease the membrane permeability, growth features, relative water content and photosynthetic attributes though the antioxidant enzyme activity augmented when the concentration of Cd increased. Malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and proline were also higher in the heavy metal stressed plants compared to control plants. By regulating enzyme activities and osmolyte buildup in response to heavy metal stress, this study indicated that Ocimum plants evolved well-developed defence mechanisms.

Keywords: cadmium, ocimum, antioxidant enzyme activity, proline, photosynthetic attributes

Introduction

Ocimum basilicum L. (Sweet basil), a member of the Labiatae family [1] that produces essential oils is a 20 to 70 cm tall annual herb that grows in tropical areas and warm states like southern Asia, Africa, and India. Its essential oils add flavors and smell to a range of products in the culinary and cosmetic industries. Since a long time, different parts of sweet basil has been used in folk medicine as a carminative, galactogogue, stomachic, and antispasmodic [2]. Heavy metal pollution in soil occurs due to various human activities, including the ongoing use of fertilizers, and sewage sludge [3]. Since Cd is a highly hazardous metal pollutant of soil, it degrades various factors such as morphological, physical, and biochemical characteristics in plants [4].

Through the ROS production, Cd is in charge of causing oxidative stress. These harmful ROS caused an oxidative injury. To mitigate these heavy metal-related effects, plants improve several defence systems. Antioxidant enzymes, including APX, SOD, AsA, CAT and GR and are part of these defence systems. These antioxidants and antioxidant enzymes defend against oxidative damage. The defence mechanism created by plants to alleviate stress depends in large part on antioxidant enzymes. The goal of the current experiments was to assess the potential effects of Cadmium hyper-accumulation on Ocimum's photosynthetic responses, growth and yield responses, oxidative stress level, internal Cd level, and effectiveness of antioxidant enzymes that may act as regulatory mechanisms against Cd-induced metabolic shift.

Materials and Methods

Experimental design, growth analysis

Ocimum seeds were obtained from the Indian Agricultural Research Institute, New Delhi, in good health. The seeds were surface sterilized with a 0.2 percent mercuric chloride solution for five minutes while being frequently shaken, and then they were washed with de-ionized water. The clay pots (25 cm in diameter and 25 cm in height) were filled with a uniform mixture of soil weighing 5 kg before seeding. The experiment was carried out in pots in a net house. Cd was added to the soil in the chosen pots (0, 100, 150 mg $CdCl_2$ kg^{-1} of soil) and watered on alternate days. The Ocimum's growth and biochemical characteristics were assessed 75 days after seeding (DAS). Analysis of the crop's growth, physiological, and biochemical characteristics was done. Four plants from each treatment were harvested at 75 DAS, and their roots were carefully cleaned with tap water to get rid of any adherent foreign material.

Chlorophyll content

The SPAD-502 chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan) was used to measure the chlorophyll content.

Membrane Permeability (MP)

Membrane Permeability (MP) is the electrical conductivity (EC) measurements taken in moist leaf tissues and is a marker of damage brought on by plant stress in leaf tissue, particularly in cell membranes. By calculating the ratio of EC1 to EC2, the values for permeability of membrane (MP) were determined.

Relative Water Content (RWC)

Relative Water Content (RWC) was determined by González and González, 2001 [5].

H_2O_2 and MDA

According to Liu *et al*., 2014 [6], MDA and H_2O_2 concentrations were calculated [6].

Antioxidant Activity

According to Liu *et al*., 2014 [6] antioxidant enzyme activities were carried out [6].

Proline content

Proline content in leaf was estimated through Bates *et al*., 1973 [7].

Results and discussion

The growth parameters of the Ocimum plants in our experiment were negatively impacted by heavy metal treatments (Table 1). Significant growth inhibition was seen in the Ocimum after exposure to Cd stress. As the Cd concentration increased, a considerable decrease in the fresh weight (FW) and dry weights (DW) of the shoots and roots occurred. Overall, the growth of the plant was severely slowed down by 150 mg kg^{-1} Cd. As compared to the control, the 150 mg kg^{-1} Cd dose inhibited the leaf FW, leaf DW, root FW, and root DW by 48.1%, 55.04%, 21.4%, and 33.3%, respectively. One of the most significant signs of Cd and other heavy metal exposure in plants is root development suppression [8]. Heavy metals induce oxidative damage to plants and interfere with their metabolism.

Table 1: Impact of heavy metal application on some growth characteristics of Ocimum plants

Treatment dose mg kg^{-1}	Stem diameter (mm)	Leaf fresh weight (g $plant^{-1}$)	Leaf dry weight (g $plant^{-1}$)	Root Fresh weight per plant (g)	Root Dry weight per plant (g)
Control (0)	3.68^{a}	7.56^{a}	1.09^{a}	1.82^{a}	0.18^{a}
Cd (100)	2.75^{bc}	4.61^{b}	0.54^{bc}	1.49^{b}	0.14^{c}
Cd (150)	2.67^{c}	3.92^{c}	0.49^{c}	1.43^{b}	0.12^{d}

Treatments with Cd resulted in a significant drop in leaf area, leaf number, and SPAD values (Table 2). A decrease in cadmium-induced chlorophyll concentration was observed [9]. Chlorophyll content decreases as a result of Cd stress, which is linked with suppressing the production of chlorophyll biosynthesis [10]. The inhibition of aminolaevulinic acid synthesis by the chlorophyll reductase protocol in the biosynthesis of chlorophyll is the primary cause of high Cd doses disrupting the chlorophyll biosynthesis. Table 2 displays the influence of varying Cd levels on the RWC and MP of the Ocimum plant. Applications of Cd raised the MP while lowering the RWC. Water content may drop as a result of membrane permeability being impacted by metal toxicity. Hydraulic conductivity losses induced through heavy metals may be the reason for the reduced RWC seen in our investigation. Similar to this, past studies demonstrated that heavy metal toxicity resulted in a decrease in RWC of a number of crops [11]. Our results concur with those of Alyemeni *et al*., 2017 [12], who claimed that Cd increased the MP in beans [12].

Table 2: Outcome of heavy metal application on leaf no, SPAD, leaf area, MP and RWC in Ocimum

Treatment mg kg^{-1}	Leaf number $plant^{-1}$	Chlorophyll SPAD	Leaf dry weight (g $plant^{-1}$)	Leaf area cm2 $plant^{-1}$	MP %	RWC %
Control (0)	3.68^{a}	7.56^{a}	1.09^{a}	1.82^{a}	0.18^{a}	70.15^{a}
Cd (100)	2.75^{bc}	4.61^{b}	0.54^{bc}	1.49^{b}	0.14^{c}	64.38^{b}
Cd (150)	2.67^{c}	3.92^{c}	0.49^{c}	1.43^{b}	0.12^{d}	61.28^{c}

The current investigation showed that Cd-treated plants had higher levels of MDA and H_2O_2 than control plants (Table 3). Due to increased peroxidation of lipid and H_2O_2 concentration, Cd treatments led to oxidative stress. The 150 mg kg^{-1} Cd treatment resulted in the greatest MDA and H_2O_2 levels. Plant oxidative stress is indicated by lipid peroxidation. In this investigation, the application of Cd led to an increase in the value of lipid peroxidation. In plant metabolism, cadmium generates significant oxidative stress and damage [13]. The lipid peroxidation increment caused by Cd treatment was observed earlier also in other crops, including maize, wheat, and peas.

Table 3: Effect of Cd application on MDA, H_2O_2 and proline content in Ocimum

Treatment mg kg^{-1}	MDA nmol g-1 dry wt	H_2O_2 mmol kg^{-1}	Proline µg g^{-1} fresh wt
Control (0)	3.79^{c}	9.59^{c}	163.92^{c}
Cd (100)	4.01^{b}	10.03^{b}	182.37^{b}
Cd (150)	4.82^{a}	11.88^{a}	218.99^{a}

Heavy metal treatments resulted in an increase in proline content (Table 3). The highest proline values was seen in Cd treatments at 150 mg kg^{-1} dose. Proline increases plants' ability to withstand stress. It's been noticed that plants that are tolerant to cadmium collect suitable osmolytes. Similarly Irfan *et al*., 2014 demonstrated that in Cd stress circumstances, tolerant mustard cultivars had more proline content than sensitive ones, supporting the findings of the present research.

In Table 4, the Cd treatments effects on CAT and SOD activity in Ocimum plants are displayed. More CAT and SOD activity was sustained in Cd-treated plants than in control plants (Table 4). When under stress, plants activate enzyme defence mechanisms like SOD, APX, GR, CAT, and GPX. Abiotic stress conditions make ROS in cells more potent, resulting in cellular homeostasis damage. Oxidative stress stimulates antioxidant enzymes, which reveals a general approach to managing stress [14]. It has been hypothesized that the activities of CAT and SOD reduce the lipid peroxidation process in Cd-stressed sunflower plants. Our findings are in agreement with Patel *et al*., 2016 [15], who showed increased activities of antioxidants, MDA, and proline synthesis in spearmint (*Mentha spicata*) under toxic metal stress [15].

Table 4: CAT and SOD activities under the influence of Cd in Ocimum

Treatment mg kg^{-1}	SOD (EU g leaf $^{-1}$)	CAT (EU g leaf $^{-1}$)
Control (0)	211.67 c	177.33 c
Cd (100)	222.67 b	190.67 b
Cd (150)	236.67 a	227.00 a

Conclusion

Plant growth, photosynthetic activity, chlorophyll concentration, and antioxidant enzyme activities were all negatively impacted by Cd treatment. By controlling enzyme activity and osmolyte buildup in response to Cd stress, Ocimum plants evolved defence mechanisms.

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