

Morpho-physiological and phytochemical responses of basil (*Ocimum basilicum* L.) to toxic heavy metal cadmium

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Abstract

Cadmium (Cd) is a particularly noteworthy metal that may change the secondary metabolism efficacy of pharmaceutical valuable plants. This study aimed to explore the influence of Cd (0, 25, 75, 100, and 150 μ M) on the morpho-physiological traits, mineral contents, essential oil composition, and morphology of secretory glands in basil (*Ocimum basilicum* L.). The exposure to Cd reduced significantly shoot and root dry weight, shoot and root lengths, total chlorophyll, number, and length of secretory gland compared to control plants, while increased peroxidase activity, proline content, and number of stomata. Essential oil compositions were varied in the Cd-treated plants in a dose-dependent manner. The main compounds of the essential oil were Estragole followed by Linalool and Geranial which the highest percentages were observed in the plants treated with Cd 150 μ M. The Cd-treated plants exhibited reductions in calcium (Ca) and iron (Fe) concentrations in both leaves and roots. Cd contents in roots and shoots progressively enhanced with increasing Cd concentrations. In general, basil showed good tolerance to Cd stress, so that 50% reduction in shoot dry weight occurred in above 100 μ M treatments, which can be related to physiological reactions such as increased antioxidant activity, proline content, number of leaves stomata, and changes in other morpho-physiological factors.

Keywords: chlorophyll; essential oil; mineral contents; secondary metabolites; secretory glands

Introduction

Taking agriculture into account, environmental pollutants, in particular, heavy metals (the non-biodegradable elements) have become a significant concern (Chmielowska-Bąk et al., 2018; Iranbakhsh et al., 2018). These pollutant agents might adversely influence various living organisms, like plants and humans. Environmental contamination with pollutants like cadmium (Cd) is a common issue that may restrict the crop productivity. Cd is a major toxic heavy metal with an unknown biological role in human life (Fattahi et al., 2019). Cd is a high-mobility element, and exposure to Cd can be, therefore, highly toxic (Chmielowska-Bąk et al., 2018). In this concern, various attempts have been made to mitigate Cd-induced phytotoxicity (Hussain et

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al., 2018; Rizwan *et al.*, 2018). It is well established that Cd is commonly associated with increases in the overproduction of reactive oxygen species (ROS) and subsequent induction of oxidative stress in cells (Chmielowska-Bąk *et al.*, 2018). In adaptive response to heavy metals, reception, and signal transduction are followed by changes in transcriptions of genes (Safari *et al.*, 2018), organ development (Melato *et al.*, 2012), and metabolism (Babajani *et al.*, 2019b).

Common basil (*Ocimum basilicum* L.) as a member of the Lamiaceae family is an aromatic multifunctional vegetable with economical great essential oils (terpenoid derivatives) (Alemu *et al.*, 2018). Essential oils are secondary metabolites with isoprenoid structure. The accumulation of essential oils in plants is usually limited to specialized secretory structures which are present in several different types, namely glandular trichomes. As shown in Figure 1, leaves secretory glands consist of several cells providing different functions. The essential oils secreted by secretory cells are passed through a space situated at the apex of the subcuticular trichome and get accumulated; however, the basal cells provide attachment to the skin structure (Hazzoumi *et al.*, 2017). Basil essential oil compounds are exploited in the food and medicine industries owing to their multiple functions, especially antimicrobial, antioxidant, flavor, perfume, and preservative traits (De Araujo Couto *et al.*, 2019). Besides, these natural terpenoid-derivatives displayed outstanding characteristics to control parasites, fungi, bacteria, viruses, and insects (Predoi *et al.*, 2018). Furthermore, diverse isoprenoids exhibit considerable pharmaceutical functions to cure several diseases, including malaria, Alzheimer's, cardiovascular disorders, and cancer (Lu *et al.*, 2016).

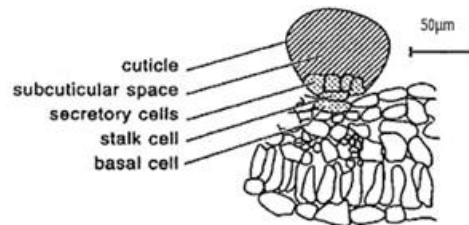


Figure 1. Glandular structure, drawn from a photograph by Gershenzon *et al.* (1989)

The content and chemical composition of the basil essential oils has been the subject of many studies. The yield from different plant parts varies between 0.2-1.9% with the main components being linalool, methyl chavicol, eugenol, and methyl cinnamate, as well as 1,8-cineole, methyl eugenol, geraniol, geranial, neral and α -bergamotene (Ilic *et al.*, 2019). Fattahi *et al.* (2019) reported that sweet basil cultivation in the Cd contaminated soils could cause an undesirable effect on the morphological traits, but might have a positive influence on the essential oil yield, composition, and phytoremediation of the soil.

In plants, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) are active isoprene units by which a multitude of terpenoids during the secondary metabolism are synthesized (Figure 2). Productions of these C5 precursors (isoprenes; IPP and DMAPP) are mediated through two independent routes, including the mevalonic acid (MVA) and the methylerythritol phosphate (MEP) (Lu *et al.*, 2016). Prenyl transferases are enzymes that contributed to the condensation process of C5 isoprene units by which structure of terpenoid derivatives, including monoterpenoids, diterpenoids, sesquiterpenoids, and triterpenoids are formed (Lu *et al.*, 2016).

Most research has focused on the mechanisms of Cd-induced phytotoxicity. However, there is little documented convincing evidence toward the possible Cd effect on secondary metabolism, and in particular, the quality of essential oils which have an industrial interest. Furthermore, tissue differentiation and organ development epigenetically may be influenced by environmental factors, like irrigation, nutrition, pollutant, etc. (Seddoghina *et al.*, 2019; Moghanloo *et al.*, 2019a). The negative impact of contaminated soil with heavy metals on plant and human health is an important global concern. In this concern, Cd may cause variations in morphology and distribution of secretory structures in leaves. Hence, the aim of this experiment was an

examination of the possible influence of different Cd concentrations ranging from 25-150 μM on the growth, distribution of leaves secretory glands, essential oil composition, and nutritional status in basil.

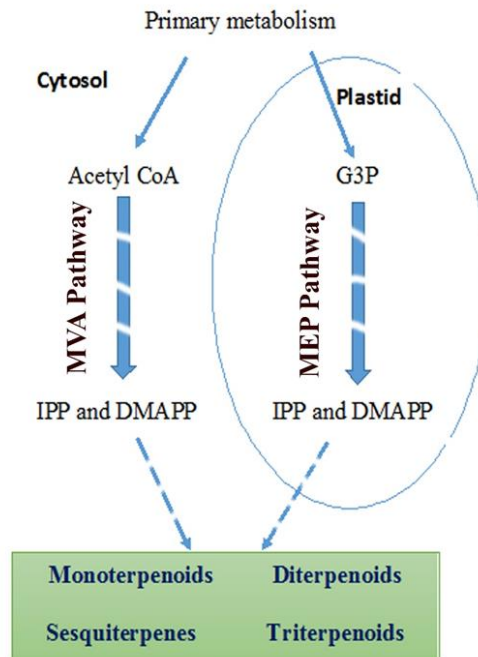


Figure 2. The schematic design on production of secondary metabolites with isoprenoid structure. Abbreviation: IPP- isopentenyl pyrophosphate; DMAPP- dimethylallyl pyrophosphate; MVA- mevalonic acid; MEP- methylerythritol phosphate

Materials and Methods

Plant material and experimental design

This experiment was conducted under greenhouse conditions at the Department of Horticultural Science, Shahed University of Tehran, Iran, in 2018. Common basil (*O. basilicum* L.) seeds of a local population were purchased from Pakan Seed Co., Isfahan, Iran. The seeds were sterilized using sodium hypochlorite and sown in a pot containing soil with traits described in Table 1. Seedlings were kept in a greenhouse under controlled temperature ($26\text{ }^{\circ}\text{C} \pm 4\text{ }^{\circ}\text{C}$), humidity ($50 \pm 10\%$), photoperiod (16/8 h light/dark), and light intensity ($160\text{ }\mu\text{mol}/\text{m}^2/\text{s}$). Following this, each pot was irrigated every 2 days (for ten times) with half strength of Hoagland's nutrient solution containing various levels of cadmium chloride ($\text{CdCl}_2 \cdot 5\text{H}_2\text{O}$) (25, 75, 100, and $150\text{ }\mu\text{M}$). Experiments were designed in a completely randomized design (CRD) with three replicates. Four-week-old plants were harvested to measure all morphological, anatomical, and physiological parameters.

Table 1. Physicochemical characteristics of the applied soil

Soil	Clay (%)	Silt (%)	Sand (%)	Pb mg/kg	Cd mg/kg	Mg mg/kg	O.C (%)	pH	EC (dS/m)
Sand-loam	15	32	53	0.2	0.1	15	0.7	8	3.5

Total chlorophyll content

In order to measure the total chlorophyll content, the samples were immediately frozen in liquid nitrogen and kept in a freezer at $-80\text{ }^{\circ}\text{C}$ before conducting the biochemical analyses. Then, the photosynthetic

pigments were measured by using Lichtenthaler (1987) method. Based on this method, 0.25 g of fresh tissue was extracted by using 5 ml 80% acetone. Then, the extract was centrifuged at 11000 rpm for 10 min. In addition, the optical density (O.D.) of the extract was measured at the wavelengths of 646.8 and 663.2 nanometer.

Peroxidase activity

To measure the enzyme activity of the total seedling, 0.1 g of fresh tissue was used. To extract protein, 0.2 g of fresh tissue plant was pulverized in a mortar using liquid nitrogen and then one mL of buffer Tris-HCl (0.05 M, pH = 7.5) was added. The obtained mixture centrifuged for 21 min at 13,000 rpm, at 4 °C and the supernatant was used for enzyme activity measurements (Aghighi Shahverdi *et al.*, 2017). The peroxidase activity was determined in a reaction of the mixture, which consisted of a suitable amount of 28 mM guaiacol, 5 mM H₂O₂, 25 mM Na-phosphate buffer (pH 6.8) and enzyme (Ghanati *et al.*, 2002).

Proline content

Proline was determined according to the method described by Bates *et al.* (1973). Approximately 0.5 g of fresh seedling tissue was homogenized in 10 ml of 3% aqueous sulfosalicylic acid. Then, this aqueous solution was filtered through Whatman's paper No. 2 and finally, 2 ml of filtrated solution was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 °C. The reaction mixture was extracted with 4 ml toluene, cooled to room temperature, and the absorbance was measured at 520 nm with a spectrometer.

Essential oil analysis

Essential oil samples were analysed using GC-MS (GC-2010 SHIMADZU), according to the method described in detail for the type, column, etc. by Fattahi *et al.* (2016). Basil leaves were collected and hydrodistilled using a Clevenger apparatus. Gas chromatography/mass spectrometry (GCMS) analysis was equipped with a split-splitless injector (split ratio; 30:1), scan time 1s, ionization energy 70 eV, and mass range of 40-300 amu. a column (60 m × 0.25 mm i.d., film thickness 0.25 μm); oven temperature was 60-230 °C at a rate of 7 °C/min, transfer line temperature 260 °C. The carrier gas was helium, with a linear velocity of 31.5 cm/s. The oils diluted in dichloromethane (2 μl of oil in the 2 ml solvent), the next 2 μl oil of each treatment was injected to GC/MS manually. The retention indices were calculated, for all volatile constituents using a homologous series of n-alkanes C8-C22 on the HB-5 column. The essential oil constituents were determined by comparing their GC retention indices, mass spectra with data published in the literature of Adams. Compounds were further identified using their mass spectra data compared National Institute of Standards and Technology mass spectra library data provided by the software of the GC-MS system. Essential oil components are shown as a relative percentage of the total oil.

Morphology and distribution of leaf secretory glands and stomata

Leaves secretory glands consist of several cells providing different functions. The Cd-mediated changes in morphology and distribution of leaf secretory glands were monitored by Scanning Electron Microscope (SEM) (Hazzoumi *et al.*, 2017). To measure the number of leaf stomata, the Radoglou and Jarvis (1990) method was used. For each leaf sample, 8 observations of a light microscope (Leica Galen III) in magnification 200 were prepared. The number of leaf stomata by GSA Image Analyzer V3.8.6 Software was determined.

Determination of Fe, Ca, and Cd contents

Plants were dried in an oven at 65 °C. The dried samples were ground and digested using HNO₃, H₂SO₄, HCl. The Fe, Ca, and Cd contents of the solutions were determined by Inductively coupled Plasma Mass Spectrometry ICP- OES [Perkin Elmer ELAN 6100DRC-e] (Akpınar-Bayizit *et al.*, 2010).

Statistical analysis

Distribution normality of achieved data was done according to the Kolmogorov-Smirnov and Shapiro-Wilk test. Then the studied traits were statistically analysed by the Statistical Analysis System software (SAS Institute, Cary, NC, USA, and Version 9.2). The differences among means were separated using LSD test (least significant difference) at 0.05 statistical probability level.

Results

As shown in Table 2, the shoot and root lengths reduced significantly with the increasing Cd concentrations. This decrease in Cd150 treatment was 23.9 and 59.7% compared to the control treatment, respectively. However, there was no significant difference in root lengths between Cd100 and Cd150 treatments. Plant shoot dry weight decreased significantly in basil plants exposed to different concentrations of Cd. Plants subjected to 25, 75, and 100 μM Cd showed a reduction of 14, 28.5, and 39% of plant dry weight, respectively, relative to the control plants. The strongest decrease in plant shoot dry weight (57%) was observed at the highest Cd concentration. The exposure of basil seedlings to 25 to 150 μM Cd reduced significantly the root dry weight compared to the control plants (Table 2).

Table 2. Effect of Cd stress on morphological characteristics of basil (*Ocimum basilicum* L.)

Cd treatment (μM)	Shoot length (cm)	Root length (cm)	Shoot dry weight (g/plant)	Root dry weight (g/plant)
Control	55.6 \pm 0.6 a	6.73 \pm 0.2 a	2.73 \pm 0.09 a	0.25 \pm 0.01 a
25	52.6 \pm 0.5 b	4.0 \pm 0.1 b	2.33 \pm 0.05 b	0.20 \pm 0.01 b
75	50.6 \pm 0.5 c	3.35 \pm 0.1 c	2.09 \pm 0.1 c	0.17 \pm 0.004 c
100	46.6 \pm 0.5 d	2.98 \pm 0.1 d	1.73 \pm 0.8 d	0.08 \pm 0.006 d
150	42.3 \pm 0.5 e	2.71 \pm 0.1 d	1.19 \pm 0.6 e	0.08 \pm 0.003 d
LSD ($p \leq 0.05$)	1.06	0.32	0.12	0.014
Significance level	**	**	**	**

Average and standard deviation values with different letters in the same column are statistically different (LSD, $p = 0.05$). Level of significance of ANOVA: ** = $p \leq 0.01$.

The effect of Cd stress was significant on physiological traits, number of stomata, and secretory gland characteristics (Table 3). The Cd 150 treatment was caused decreases in total chlorophyll (51.0%), number of secretory glands (63.0%), and secretory gland length (16.9%) while increased peroxidase activity (71.4%), proline content (62.8%), and number of stomata (28.2%), in compared to the control treatment (Table 3). As shown in Figures 3 and 4, both distribution and morphology of secretory glands in leaves were influenced by Cd treatments in a dose-dependent manner.

Ca, Fe, and Cd contents in basil leaves and roots are presented in Table 4. Changes in Ca concentrations were more pronounced in leaves when compared to roots. The Cd-treated plants exhibited reduced Ca concentrations in both leaves and roots. In leaves and roots, Fe contents were influenced showing a significant decline in all Cd-treated plants. Cd contents in roots and shoots progressively were enhanced with increasing Cd concentrations. In general, with increasing Cd concentrations, the content of Fe and Ca absorption decreased in both root and stem organs, while the Cd content increased in both organs.

Results of the simple correlation presented in Table 5. According to the results, shoot dry weight showed a significantly positive correlation with shoot length, root length, root dry weight, total chlorophyll, number of secretory glands, secretory gland length, root, and shoot Ca contents, shoot and root Fe contents, while negatively correlated peroxidase activity, proline content, number of stomata, shoot and root Cd contents.

Table 3. Effect of Cd stress on some morpho-physiological characteristics of basil (*Ocimum basilicum* L.)

Cd treatment (μM)	Total chlorophyll (mg/g FW)	Peroxidase activity (U/mg protein.min)	Proline content ($\mu\text{mol/g}$ FW)	Number of stomata	Number of secretory glands	Secretory gland length (μm)
Control	1.45±0.04 a	0.06±0.002 e	7.29±0.3 e	36.3±1.5 d	15.3±1.5 a	104.4±3.5 a
25	1.31±0.1 a	0.10±0.005 d	8.61±0.2 d	37.0±1.0 d	13.0±1.0 b	101.1±4.3 a
75	1.05±0.03 b	0.12±0.005 c	10.7±0.3 c	40.0±1.0 c	10.0±1.0 c	95.2±2.1 b
100	0.88±0.06 c	0.18±0.01 b	14.6±0.2 b	46.3±1.5 b	7.66±0.5 d	90.3±1.3 bc
150	0.71±0.07 d	0.21±0.01 a	19.6±0.3 a	50.6±1.5 a	5.66±0.5 e	86.7±1.5 c
LSD ($P \leq 0.05$)	0.14	0.014	0.53	2.44	1.81	5.13
Significance level	**	**	**	**	**	**

Average and standard deviation values with different letters in the same column are statistically different (LSD, $p = 0.05$). Level of significance of ANOVA: ** = $p \leq 0.01$.

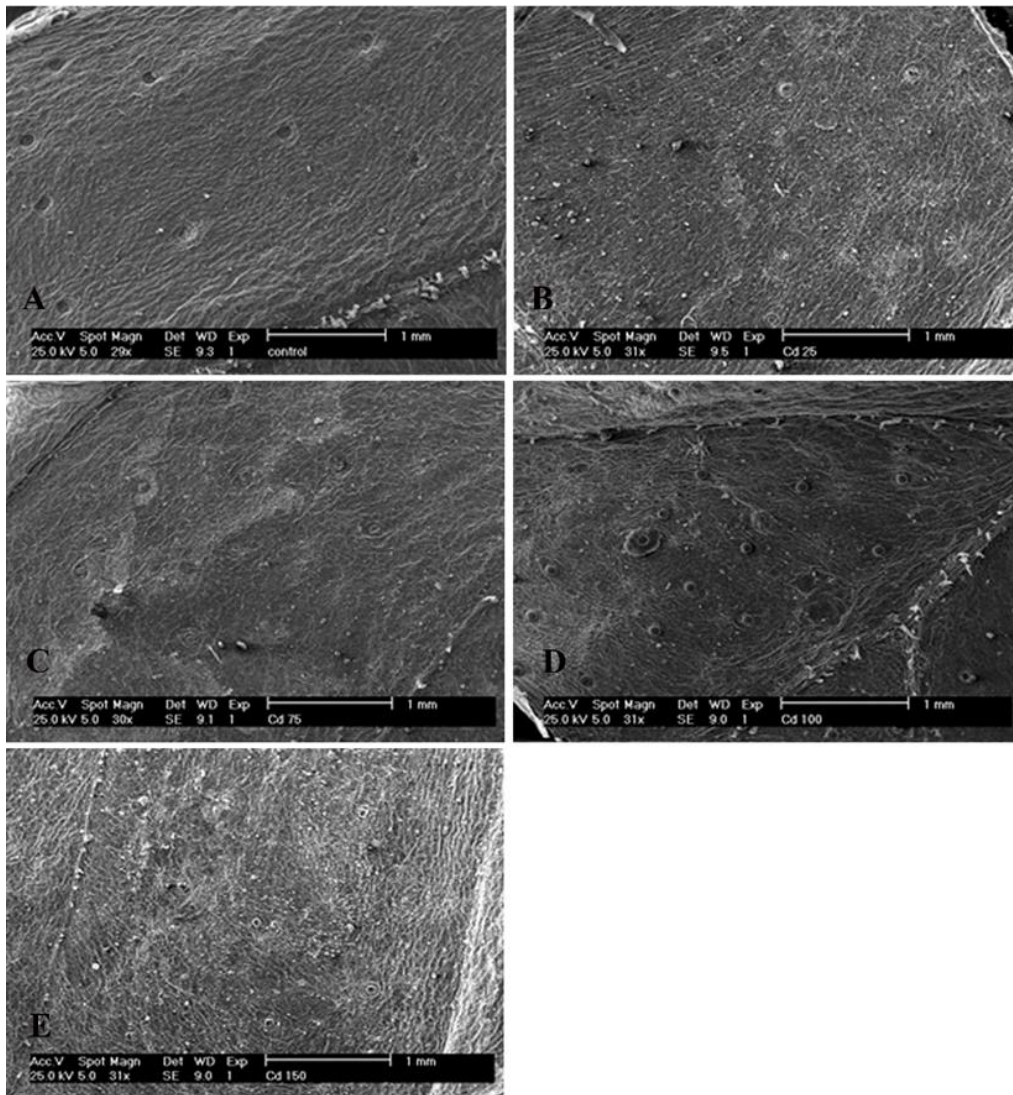


Figure 3. Cd-associated changes in distribution of secretory glands

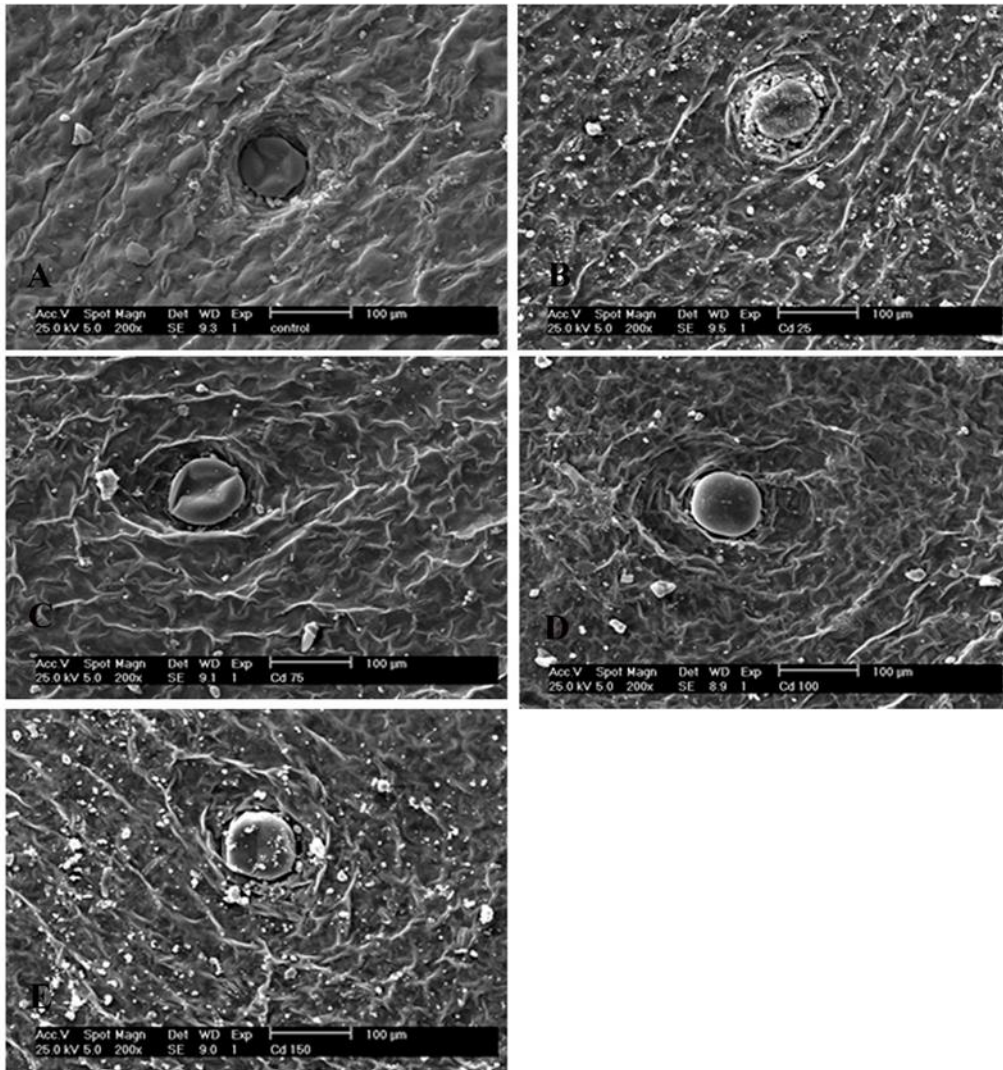


Figure 4. Differential morphology of secretory glands in Cd-treated basil seedlings

Table 4. Effect of Cd stress on Ca, Fe, and Cd content of basil (*Ocimum basilicum* L.)

Cd treatment (µM)	Root Ca content (mg/g DW)	Shoot Ca content (mg/g DW)	Root Fe content (mg/g DW)	Shoot Fe content (mg/g DW)	Root Cd content (µg/kg DW)	Shoot Ca content (µg/kg DW)
Control	264.8±1.7 a	160.1±3.2 a	10.8±0.3 a	3.46±0.2 a	23.3±0.8 e	3.75±0.2 d
25	249.5±2.7 b	150.5±0.9 b	9.53±0.1 b	3.07±0.1 b	78.0±2.5 d	10.5±0.6 d
75	221.3±1.7 c	126.3±1.9 c	8.71±0.2 c	2.45±0.07 c	148.0±6.95 c	57.6±0.001 c
100	205.1±4.2 d	103.7±4.8 d	7.86±0.09 d	1.93±0.07 d	236.4±2.9 b	106.2±3.2 b
150	181.4±2.6 e	74.8±1.3 e	6.74±0.08 e	1.60±0.05 e	329.5±3.9 a	159.7±3.5 a
LSD (p ≤ 0.05)	5.04	5.18	0.35	0.28	7.26	13.3
Significance level	**	**	**	**	**	**

Average and standard deviation values with different letters in the same column are statistically different (LSD, $p = 0.05$). Level of significance of ANOVA: ** = $p < 0.01$.

Table 5. Variations in basil essential oil composition (% of total) following exposure to Cd stress (0, 25, 75, 100, and 150 μM)

Compound	R.T.	R.I.	Control	Cd 25	Cd 75	Cd 100	Cd 150
Linalool	11.741	1098.8	15.57	15.3	14.8	9.58	17.94
3-Thujen-2-one [Umbellulon]	13.548	1174.2	0.71	0.76	0.71	0.85	0.91
Estragole [Methyl chavicol]	14.202	1201.7	39.63	40.38	38.71	26.96	46.14
Neral [Z-citral]	14.771	1228	2.26	2.28	2.16	1.45	2.5
Geranial [E-citral]	15.074	1242	3.8	4.31	4.06	2.43	4.99
β -Caryophyllene	19.154	1433.6	1.64	6.03	3.39	6.18	1.7
Epi-Bicyclosesquiphellandrene	19.631	1457.8	1.65	1.72	0.64	1.82	2.0
δ -Cadinene	20.019	1477.4	0.97	1.26	0.55	9.71	1.2
δ -Amorphene	20.359	1494.7	2.24	3.84	4.34	4.17	1.86
Epizonaren	20.621	1508.5	1.71	1.95	1.99	3.88	1.05
Calamenene	20.948	1526.2	2.17	2.28	2.32	1.76	1.27
[E]- α -Bisabolene	21.263	1543.2	0.79	1.25	1.3	2.29	0.73
Caryophyllene oxide	22.343	1601.6	1.16	1.98	1.85	1.73	1.45
Cubenol	23.287	1655.2	2.55	4.35	4.33	5.55	3.02

R.T. Retention time; R.I. Retention index

The results of GC-MS analyses of the essential oil are shown in Table 5. Geranial concentration increased by Cd 25, 75, and 150 treatments, however, Cd100 showed a decrease over the control plant. The δ -Amorphene concentration recorded a progressive increase in Cd 25, 75, and 100 treatments compared to the control. On the contrary, Cd 150 declined δ -Amorphene content. The application of increasing Cd concentrations (25, 75, 100, and 150 μM) increased cubenol concentration. Linalool did not vary at 25 and 75 μM Cd treatments, while a significant decrease occurred at 100 μM . Our results showed that Cd enhanced Caryophyllene oxide production in basil and this effect was dose-dependent. Level of 25, 75, and 150 μM Cd did not induce changes in δ -Cadinene compared to the control, while 100 μM Cd increased the content of δ -Cadinene. The application of 25, 75, and 100 μM Cd-induced an increase of the α - Bisabolene and B-caryophyllene percentage. At 25 and 75 μM Cd, no changes in the percentages of Calamenene and Neral occurred.

Discussion

Cd stress reduced growth parameters and biomass accumulation. The results of the present study showed that a 50% reduction in the shoot dry weight (as the economic part of this plant) occurs in more than 100 μM Cd, and this indicates the optimal tolerance of this plant to the Cd stress. This can be due to physiological mechanisms such as the increased activity of antioxidant enzymes (peroxidase), proline content, number of leaves stomata. The overall results of this study showed that the presence of Cd in the culture medium affected the yield and growth characteristics of basil and reduced growth and disruption of various physiological processes in plant structure. It has been reported that the effect of Cd in peppermint reduced leaf area and dry weight of the aerial parts in Cd in comparison with control in peppermint (Amirmoradi *et al.*, 2012) and basil (Fattahi *et al.*, 2019).

With a similar trend, Cd treatments, especially at the high doses considerably decreased Ca and Fe concentrations in both leaves and roots. These results are in line with several other reports in different plant species, including wheat (Abbas *et al.*, 2018; Rizwan *et al.*, 2019), rice (Rizwan *et al.*, 2018), tomato (Ahmad *et al.*, 2018b), and okra (Rasheed *et al.*, 2018). Cd-induced disruption of membrane permeability, oxidative stress, photosynthesis performance, and nutrition may result in a decrease in plant growth. Hong *et al.* (2015) indicated that heavy metals prevent not only plant growth, but also adversely affect nutrient content. Heavy metals, including Cd, may inhibit or reduce the uptake of elements, including Cu, Fe, and Zn thereby blocking

some enzyme activation and negatively affecting photosynthesis. Hence, the inhibitory effect of heavy metals on water relations, nutrient uptake, and photosynthesis might lead to a decline in plant growth (Kilic *et al.*, 2017). Cd irreversibly prevents proton pumps involving in cell elongation and division owing to bonding with nucleophilic groups and consequent membrane permeability disruption. Moreover, Cd negatively affects photosynthetic pigments and the ultrastructure of the chloroplast, leading to decreased carbon assimilation (Ahmad *et al.*, 2018a).

Plants have antioxidant mechanisms to reduce the damage caused by ROS including non-enzymatic components such as ascorbate, glutathione, carotenoids, proline, and anthocyanin, and enzymes such as CAT, SOD, POD, etc. (Suzuki and Katano, 2018). Proline is an amino acid stored in the cytoplasm and is likely to protect macromolecules' structure inside the cell during stress conditions. Proline concentration in stress conditions may increase up to 100% under normal conditions. This osmoprotectant plays an essential role in regulating cell osmotic pressure under stresses such as drought, salinity, and toxicity (Slama *et al.*, 2015). Based on our results, the proline content and peroxidase activity were increased as a consequence of Cd stress.

Our results confirmed that the chemical composition of essential oils was altered in response to Cd treatments. Induction in secondary metabolism following exposure to Cd may be attributed to Cd-mediated changes in ROS levels and their subsequent signalling. It is well established that exposure to Cd is commonly associated with increases in the overproduction of reactive oxygen species (ROS) and subsequent induction of oxidative stress in cells (Chmielowska-Bak *et al.*, 2018). In addition to the possible ROS-mediated oxidation of biomolecules, these bioactive compounds can trigger specific signalling pathways and alter gene expression and cellular metabolism (Safari *et al.*, 2018; Babajani *et al.*, 2019a; Babajani *et al.*, 2019b). In response to heavy metal stress, ROS over accumulations associate with redox signalling, thereby changing developmental processes in plants (Berni *et al.*, 2018). In line with our results, heavy metals altered the chemical composition of secondary metabolites in *Melissa officinalis* (Babajani *et al.*, 2019b), *Hypericum perforatum* (Murch *et al.*, 2003), and *Cymbopogon citratus* (Lermen *et al.*, 2015). Changes in the chemical composition of secondary metabolites may affect the medicinal efficacy of these natural resources. Many studies reported linalool as the main compound of investigated basil essential oils (Silva *et al.*, 2015; Elgndi *et al.*, 2017). Sharafati-Chaleshtori *et al.* (2015) reported methyl chavicol as the main compound of basil essential oils while eugenol was detected as the compound with the highest abundance by Piras *et al.* (2018). In the present study, all of the compounds listed above had significant increases in the high level of Cd concentration.

Moreover, exposure to Cd changed both the morphology and distribution of the secretory gland. It has been well documented that tissue differentiation may vary in response to various environmental cues (Moghanloo *et al.*, 2019b; Seddignia *et al.*, 2019). Mizushima *et al.* (2019) indicated that *Avicennia schaueriana* can exclude toxic Cd in the form of Cd/Ca crystal through secretion of leaf salt glands, representing a fast mechanism for the Cd release. Choi *et al.* (2001) also showed that tobacco can eliminate Cd by excreting the Ca/Cd crystals through the trichome cells. Therefore, the Cd-associated changes in differentiation of secretory tissues may be considered as a potential defensive mechanism. Taken collectively, soil contamination with Cd may not only reduce growth but also may influence secondary metabolism and tissue differentiation.

Conclusions

Here, we determined the effect of Cd exposure on the morpho-physiological characteristics and the concentration of Ca, Fe, and Cd in various parts of basil. Application of 150 μM Cd reduced the morpho-physiological traits such as shoot and root length, shoot and root dry weight, total chlorophyll, number of secretory glands, secretory gland length, and concentration of Ca and Fe in the shoot and root organs, but increased peroxidase activity, proline content, number of stomata, and root and shoot Cd contents. In general, Cd stress increased the average essential oil composition of this plant compared to Cd-free treatment. Higher levels of Cd (100 and 150 μM) also showed the highest percentage of essential oils in the plant.

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Author's Contribution

MP collected and analyzed the data used in manuscript, and drafted the manuscript. AI was supervised of the project. ME and MHF were advisor of the project. All authors read and approved the final manuscript.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

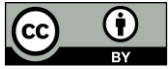
- Abbas T, Rizwan M, Ali S, Adrees M, Mahmood A, Zia-ur-Rehman M, ... Qayyum MF (2018). Biochar application increased the growth and yield and reduced cadmium in drought-stressed wheat grown in an aged contaminated soil. *Ecotoxicology and Environmental Safety* 148:825-833. <https://doi.org/10.1016/j.ecoenv.2017.11.063>
- Aghighi Shahverdi M, Omidi H, Tabatabaei SJ (2017). Morpho-physiological response of stevia (*Stevia rebaudiana* Bertoni) to salinity under hydroponic culture condition (A case study in Iran). *Applied Ecology and Environmental Research* 16(1):17-28. https://doi.org/10.15666/aecer/1601_017028
- Ahmad B, Jaleel H, Sadiq Y, Khan MM, Shabbir A (2018a). Response of exogenous salicylic acid on cadmium induced photosynthetic damage, antioxidant metabolism and essential oil production in peppermint. *Plant Growth Regulation* 86(2):273-86. <https://doi.org/10.1007/s10725-018-0427-z>
- Ahmad P, Ahanger MA, Alyemeni MN, Wijaya L, Alam P (2018b). Exogenous application of nitric oxide modulates osmolyte metabolism, antioxidants, enzymes of ascorbate-glutathione cycle and promotes growth under cadmium stress in tomato. *Protoplasma* 255(1):79-93. <https://doi.org/10.1007/s00709-017-1132-x>
- Akpinar-Bayizit A, Tiran MA, Yilmaz-Ersan L, Taban N (2010). Inductively coupled plasma optical-emission spectroscopy determination of major and minor elements in vinegar. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 38(3):64-68. <https://doi.org/10.15835/nbha3834618>
- Alemu AA, Garede W, Gebre A (2018). Essential oil yield and yield components of basil (*Ocimum basilicum* L.) as affected by genotype and intrarow spacing at Jimma, SW Ethiopia. *Acta Agrobotany* 71(3):1-10.
- Amirmoradi S, Moghaddam PR, Koocheki A, Danesh S, Fotovat A (2012). Effect of cadmium and lead on quantitative and essential oil traits of peppermint (*Mentha piperita* L.). *Notulae Scientia Biologicae* 4:101-109. <https://doi.org/10.15835/nsb448185>
- Babajani A, Iranbakhsh A, Ardebili ZO, Eslami B (2019a). Seed priming with non-thermal plasma modified plant reactions to selenium or zinc oxide nanoparticles: cold plasma as a novel emerging tool for plant science. *Plasma Chemistry and Plasma Processing* 39:21-34. <https://doi.org/10.1007/s11090-018-9934-y>
- Babajani A, Iranbakhsh A, Ardebili ZO, Eslami B (2019b). Differential growth, nutrition, physiology, and gene expression in *Melissa officinalis* mediated by zinc oxide and elemental selenium nanoparticles. *Environmental Science and Pollution Research* 26:24430-24444. <https://doi.org/10.1007/s11356-019-05676-z>
- Bates L, Waldren R, Teare I (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil* 39:205-207. [link.springer.com/article/10.1007/BF00018060](https://doi.org/10.1007/BF00018060)
- Berni R, Luyckx M, Xu X, Legay S, Sergeant K, Hausman JF, ... Guerriero G (2018). Reactive oxygen species and heavy metal stress in plants: impact on the cell wall and secondary metabolism. *Environmental and Experimental Botany* 161:98-106. <https://doi.org/10.1016/j.envexpbot.2018.10.017>

- Chmielowska-Bąk J, Izbiańska K, Ekner-Grzyb A, Bayar M, Deckert J (2018). Cadmium stress leads to rapid increase in RNA oxidative modifications in soybean seedlings. *Frontiers in Plant Science* 8:2219. <https://doi.org/10.3389/fpls.2017.02219>
- Choi YE, Harada E, Wada M, Tsuboi H, Morita Y, Kusano T, Sano H (2001). Detoxification of cadmium in tobacco plants: formation and active excretion of crystals containing cadmium and calcium through trichomes. *Planta* 213(1):45-50. <https://doi.org/10.1007/s004250000487>
- De Araújo Couto HGS, Blank AF, Silva AMDO, deLima Nogueira PC, de Fátima Arrigoni-Blank M, deCastro Nizio DA, deOliveira Pinto JA (2019). Essential oils of basil chemotypes: Major compounds, binary mixtures, and antioxidant activity. *Food Chemistry* 293:446-454. <https://doi.org/10.1016/j.foodchem.2019.04.078>
- Elgndi MA, Filip S, Pavlič B, Vladić J, Stanojković T, Žižak Ž, Zeković Z (2017). Antioxidative and cytotoxic activity of essential oils and extracts of *Satureja montana* L., *Coriandrum sativum* L. and *Ocimum basilicum* L. obtained by supercritical fluid extraction. *Journal of Supercritical Fluids* 128:128-137. <https://doi.org/10.1016/j.supflu.2017.05.025>
- Fattahi B, Arzani K, Souri MK, Barzegar M (2019). Effects of cadmium and lead on seed germination, morphological traits, and essential oil composition of sweet basil (*Ocimum basilicum* L.). *Industrial Crops and Products* 138 111584. <https://doi.org/10.1016/j.indcrop.2019.111584>
- Fattahi B, Nazeri V, Kalantari S, Bonfill M, Fattahi M (2016). Essential oil variation in wild-growing populations of *Salvia reuterana* Boiss. Collected from Iran: using GC-MS and multivariate analysis. *Industrial Crops and Products* 81:180-190. <https://doi.org/10.1016/j.indcrop.2015.11.061>
- Gershenzon J, Maffei M, Croteau R (1989). Biochemical and histochemical localization of monoterpene biosynthesis in the glandular trichomes of spearmint (*Mentha spicata*). *Plant Physiology* 89:1351-1357.
- Ghanati F, Morita A, Yokota H (2002). Induction of suberin and increase of lignin content by excess boron in tobacco cell. *Soil Science and Plant Nutrition* 48(3):357-364. <https://doi.org/10.1080/00380768.2002.10409212>
- Hazzoumi Z, Moustakime Y, Joutei A (2017). Effect of arbuscular mycorrhizal fungi and water stress on ultrastructural change of glandular hairs and essential oil compositions in *Ocimum gratissimum*. *Chemical and Biological Technologies in Agriculture* 4(20):1-13. <https://doi.org/10.1186/s40538-017-0102-z>
- Hong J, Rico CM, Zhao L, Adeleye AS, Keller AA, Peralta-Videa JR, Gardea Torresdey JL (2015). Toxic effects of copper-based nanoparticles or compounds to lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*). *Environmental Science: Processes & Impacts* 17:177-185. <https://doi.org/10.1039/c4em00551a>
- Hussain A, Ali S, Rizwan M, urRehman MZ, Javed MR, Imran M, Chatha SAS, Nazir R (2018). Zinc oxide nanoparticles alter the wheat physiological response and reduce the cadmium uptake by plants. *Environmental Pollution* 242:1518-1526. <https://doi.org/10.1016/j.envpol.2018.08.036>
- Ilic AS, Antic MP, Jelacic SC, Solevicknudsén TM (2019). Chemical composition of the essential oils of three *Ocimum basilicum* L. cultivars from Serbia. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 47(2): 347-351. <https://doi.org/47.15835/nbha47111250>
- Iranbakhsh A, Ardebili ZO, Ardebili NO, Ghoranneviss M, Safari N (2018). Cold plasma relieved toxicity signs of nano zinc oxide in *Capsicum annuum* cayenne via modifying growth, differentiation, and physiology. *Acta Physiologiae Plantarum* 40(8):154-162. <https://doi.org/10.1007/s11738-018-2730-8>
- Kilic S, Kilic M (2017). Effects of cadmium-induced stress on essential oil production, morphology and physiology of lemon balm (*Melissa officinalis* L., Lamiaceae). *Applied Ecology and Environmental Research* 15(3):1653-69. https://doi.org/10.15666/aecr/1503_16531669
- Lermen C, Morelli F, Gazim ZC, da Silva AP, Gonçalves JE, Dragunski DC, Alberton O (2015). Essential oil content and chemical composition of *Cymbopogon citratus* inoculated with arbuscular mycorrhizal fungi under different levels of lead. *Industrial Crops and Products* 76:734-738. <https://doi.org/10.1016/j.indcrop.2015.07.009>
- Lichtenthaler HK (1987). Chlorophylls and carotenoids. Pigments of photosynthetic membranes. *Methods in Enzymology* 148:350-382. [https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1)
- Lu X, Tang K, Li P (2016). Plant metabolic engineering strategies for the production of pharmaceutical terpenoids. *Frontiers in Plant Science* 7:1647. <https://doi.org/10.3389/fpls.2016.01647>
- Melato FA, Regnier T, McCrindle RI, Mokgalaka NS (2012). Impact of metals on secondary metabolites production and plant morphology in vetiver grass (*Chrysopogon zizanioides*). *South African Journal of Chemistry* 65:178-183.

- Mizushima MY, Ferreira BG, França MG, Almeida AA, Cortez PA, Silva JV, ... Mangabeira PA (2019). Ultrastructural and metabolic disorders induced by short-term cadmium exposure in *Avicennia schaueriana* plants and its excretion through leaf salt glands. *Plant Biology* 21(5):844-53. <https://doi.org/10.1111/plb.12992>
- Moghanloo M, Iranbakhsh A, Ebadi M, Ardebili ZO (2019a). Differential physiology and expression of phenylalanine ammonia lyase (PAL) and universal stress protein (USP) in the endangered species *Astragalus fridae* following seed priming with cold plasma and manipulation of culture medium with silica nanoparticles. *Biotechnology* 9(7):288-296. <https://doi.org/10.1007/s13205-019-1822-5>
- Moghanloo M, Iranbakhsh A, Ebadi M, Satari TN, Ardebili ZO (2019b). Seed priming with cold plasma and supplementation of culture medium with silicon nanoparticle modified growth, physiology, and anatomy in *Astragalus fridae* as an endangered species. *Acta Physiologiae Plantarum* 41(4):54-62. <https://doi.org/10.1007/s11738-019-2846-5>
- Murch SJ, Haq K, Rupasinghe HV, Saxena PK (2003). Nickel contamination affects growth and secondary metabolite composition of St. John's wort (*Hypericum perforatum* L.). *Environmental and Experimental Botany* 49:251-257. [https://doi.org/10.1016/S0098-8472\(02\)00090-4](https://doi.org/10.1016/S0098-8472(02)00090-4)
- Piras A, Gonçalves MJ, Alves J, Falconieri D, Porcedda S, Maxia A, Salgueiro L (2018). *Ocimum tenuiflorum* L. and *Ocimum basilicum* L., two spices of Lamiaceae family with bioactive essential oils. *Industrial Crops and Products* 113:89-97. [https://doi.org/10.1016/S0098-8472\(02\)00090-4](https://doi.org/10.1016/S0098-8472(02)00090-4)
- Predoi D, Iconaru S, Buton N, Badea M, Marutescu L (2018). Antimicrobial activity of new materials based on lavender and basil essential oils and hydroxyapatite. *Nanomaterials* 8(5):291-301. <https://doi.org/10.3390/nano8050291>
- Radoglou KM, Jarvis PG (1990). Effects of CO₂ enrichment on four poplar clones. II. Leaf surface properties, *Annals of Botany* 65:627-632.
- Rasheed R, Ashraf MA, Kamran S, Iqbal M, Hussain I (2018). Menadione sodium bisulphite mediated growth, secondary metabolism, nutrient uptake and oxidative defense in okra (*Abelmoschus esculentus* Moench) under cadmium stress. *Journal of Hazardous Materials* 360:604-614. <https://doi.org/10.1016/j.jhazmat.2018.08.043>
- Rizwan M, Ali S, Abbas T, Adrees M, Zia-ur-Rehman M, ... Nawaz R (2018a). Residual effects of biochar on growth, photosynthesis and cadmium uptake in rice (*Oryza sativa* L.) under Cd stress with different water conditions. *Journal of Environmental Management* 206:676-683. <https://doi.org/10.1016/j.jenvman.2017.10.035>
- Rizwan M, Ali S, Ali B, Adrees M, Arshad M, Hussain A, ... Waris AA (2019). Zinc and iron oxide nanoparticles improved the plant growth and reduced the oxidative stress and cadmium concentration in wheat. *Chemosphere* 214:269-277. <https://doi.org/10.1016/j.chemosphere.2018.09.120>
- Safari M, Ardebili ZO, Iranbakhsh A (2018). Selenium nano-particle induced alterations in expression patterns of heat shock factor A4A (HSFA4A), and high molecular weight glutenin subunit 1Bx (Glu-1Bx) and enhanced nitrate reductase activity in wheat (*Triticum aestivum* L.). *Acta Physiologiae Plantarum* 40(6):117-125. <https://doi.org/10.1007/s11738-018-2694-8>
- Seddighinia FS, Iranbakhsh A, Ardebili ZO, Satari TN, Soleimanpour S (2019). Seed priming with cold plasma and multi-walled carbon nanotubes modified growth, tissue differentiation, anatomy, and yield in bitter melon (*Momordica charantia*). *Journal of Plant Growth Regulation* 39:87-98. <https://doi.org/10.1007/s00344-019-09965-2>
- Sharafari-Chaleshtori R, Rokni N, Rafieian-Kapaei M, Drees F, Salehi E (2015). Antioxidant and antibacterial activity of basil (*Ocimum basilicum* L.) essential oil in beef burger. *Journal of Agricultural Science and Technology* 17(3):817-826.
- Slama I, Abdelly C, Bouchereau A, Flowers T, Savoure A (2015). Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Annals of Botany* 115(3):433-447. <https://doi.org/10.1093/aob/mcu239>
- Silva VA, Sousa JP, Guerra FQS, Pessôa HLF, Frietas AFR, Alves LBN, Lima EO (2015). Antibacterial activity of *Ocimum basilicum* essential oil and linalool on bacterial isolates of clinical importance. *International Journal of Pharmacognosy and Phytochemical Research* 7:1066-1071.
- Suzuki N, Katano K (2018). Coordination between ROS regulatory systems and other pathways under heat stress and pathogen attack. *Frontiers in Plant Science* 9: 490-498. <https://doi.org/10.3389/fpls.2018.00490>



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