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Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiflorum* L.

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Abstract

Ocimum tenuiflorum L. exposed to various concentrations of chromium (0.0, 10.0, 20.0, 50.0, 100.0 μM) accumulated high amount of chromium in various plant parts in a concentration and duration dependent manner. Chromium induced lipid peroxidation coupled with potassium leakage. Toxic effects of chromium on *O. tenuiflorum* were reflected by the reductions in photosynthetic pigments, protein, cysteine, ascorbic acid and non-protein thiol contents. In addition, chromium toxicity resulted in the reduction of nitrate reductase activity through impaired substrate utilization. However, chromium treated *O. tenuiflorum* leaves showed increased proline content. Hyperactivity of the superoxide dismutase (SOD), guaiacol peroxidase (GPX) and catalase indicated that antioxidant enzymes played an important role in protecting the plant from chromium toxicity. However, APX took a little part in detoxification of H_2O_2 due to its sensitivity to chromium. Therefore, reduced ascorbate peroxidase activity in chromium treated *O. tenuiflorum* plants was recorded. This reflects the sensitivity of the enzyme to the chromium. Further, chromium treated plants yielded more eugenol (a major component of *Ocimum* essential oil) in comparison to the control (14.46, 24.61, 16.80, 3.83% more eugenol from 10, 20, 50 and 100 μM chromium exposed plants, respectively). The study concludes that plant could grow under chromium stress and protect themselves from phytotoxicity of Cr by altering various metabolic processes. Further, the use of leaves collected from chromium polluted habitat in medicinal preparation is not safe for human health due to high chromium contents.

Keywords: Chromium accumulation; Medicinal plant; Antioxidant enzymes; Eugenol and proline contents; Photosynthetic pigments and nitrate reductase activity

1. Introduction

Toxic heavy metals (Cd, Cr, Ni, Hg, etc.), find their ways into environment through variety of sources such as metal smelters, industrial effluents including uses of fertilizers and pesticides, etc. [1]. They are present in the soils as free metal ions, soluble metal complexes (sequestered to ligands), exchangeable metal ions, organically bound metals, precipitated or insoluble compounds such as oxides, carbonates and hydroxides, or they may constitute a part of the structure of silicate materials (indigenous soil content) [2]. Based on chemical and physical properties of toxic metals, three different molecular mechanisms of heavy metal toxicity in plants can be distinguished (a) production of reactive oxygen species by autooxidation and Fenton reaction (b) blocking of essential functional groups in biomolecules (c) displacement of essential metal ions from biomolecules [3]. Chromium is a toxic carcinogen and released in soil mainly from leather tanning, textile, carpet and electroplating industries [4]. It occurs in soil as chromic (Cr^{3+}) or chromate (Cr^{6+}) ions. Chromium(VI) remains stable for several months in the soil without changing its oxidation state. In fact, oxidative

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behaviour of the chromium in soils is of ecological significance, since Cr(VI) is found more toxic to plants and animals than Cr(III) [5]. Chromium is accumulated by plants and its accumulation is biomagnified at different trophic levels through food chain [6]. Chromium interferes with several metabolic processes, causing toxicity to the plants as exhibited by reduced growth and phytomass, chlorosis, impaired photosynthesis, stunting and finally plant death [7]. Further, plants growing in chromium-stressed environment face a potential risk from reactive oxygen species (ROS) like superoxide (O_2), hydroxyl radicals (OH) and hydrogen peroxide (H_2O_2). Their presence causes oxidative damage to biomolecules such as lipids and proteins [4]. However, plants possess their own defense mechanisms like cellular antioxidants and antioxidant enzymes which protect various vital physiological processes from damage by ROS produced under various heavy metal stresses. The hyperactivity of antioxidant enzymes and accumulation of cellular antioxidants in various plants under copper, lead, zinc stress have been reported [8,9]. However, the role of antioxidant defense system in protecting plants from toxic effects of ROS under chromium stress has not been much elucidated [4,10].

The medicinal plants constitute, a large group of plants (both lower and higher) providing raw materials for use in drug formulation and related industries. If such plants are either naturally grown or cultivated in metal contaminated regions, there is a danger that the heavy metal accumulation by plants of medicinal value may cause serious health hazards to patients using metal adulterated herbal drugs. In last decade, there are a few reports on heavy metal accumulation by some essential oil yielding and other medicinal plants [11,12]. The contamination of heavy metals in market samples of some plant-based drugs has also been reported [13]. Hence, it becomes necessary that medicinal plants are first tested for metal contamination before exploiting them for medicinal uses. In this context, it becomes also relevant to study the impact of chromium on antioxidant defense system and other biochemical changes including secondary metabolite production in medicinal plants.

Ocimum tenuiflorum L. syn. *O sanctum* (Lamiaceae) is a plant of high medicinal value and is distributed throughout India and other parts of Asian subcontinent. The leaves contain an essential oil, in which various compounds (eugenol, eugenal, carvacrol, methyl-chavicol, limatrol and caryophylline) of medicinal value are present. The seeds also contain an oil composed of fatty acids and sitosterol. Further, the plant has antiseptic, antispasmodic, antibacterial and insect repellent properties [14]. Therefore, considering the medicinal importance of the *O. tenuiflorum*, the present study has been designed to find out the chromium accumulation potential of *O. tenuiflorum* and its impact on photosynthetic pigments, nitrate reductase activity (NRA), nitrate and proline contents and role of cellular antioxidants and antioxidant enzymes in protecting plant from chromium toxicity. Apart from it, effect of chromium on yield of eugenol (a major component of *Ocimum* oil) was also investigated.

2. Materials and methods

2.1. Culture of the experimental plants

Seeds of *O. tenuiflorum* L. were obtained from Aurawan Research Centre, National Botanical Research Institute, Lucknow. Seeds were grown in earthen pots (12 in. diameter) filled with acid washed sand (0.01 M HCl), placed in field receiving normal day light, temperature and humidity. These pots were irrigated daily by 10% Hoagland solution. Plants having approximately same height and weight were carefully uprooted after 45 days of sowing and transferred into 250 ml conical flasks containing 5% Hoagland solution and allowed to grow for one week more in a growth chamber (light:dark, 14:10 h, temperature 28 ± 2 °C, 115 μmol m^{-2} s^{-1} illumination was provided through day florescent tube light).

2.2. Chromium treatment, estimation of chromium accumulation and biomass

Plants of the same age, weighing approximately 2.0 g, were selected for experimentation. Various concentrations (0.0, 10.0, 20.0, 50.0 and 100.0 μM) of Cr(VI), were prepared by diluting 1000 μM stock solution of $K_2Cr_2O_7{\cdot}7H_2O$ using 5% Hoagland solution. Two plants were transferred to 250 ml conical flasks containing 200 ml of nutrient medium (5% Hoagland solution) supplemented with different concentrations of chromium. Three sets, each of the five concentrations were placed separately in growth chamber under similar conditions as described above. Plants placed in 200 ml conical flasks with 5% Hoagland solution without chromium served as control. These flasks were aerated 6 h a day. One set of each concentration was harvested after 24, 48 and 72 h of treatment and washed three times with double distilled water. The oven dried (80 °C) plant tissues (leaves stem and roots) of treated and control plants were digested in HNO_3:$HClO_4$ (3:1, v/v) mixture at 80 °C. Chromium concentration was determined using a Flame Atomic Absorption Spectrophotometer (Perkin-Elmer 2380). Biomass of the chromium treated and untreated plants was observed by recording the dry weight of the whole plant and was expressed on dry weight (DW) basis.

2.3. Estimation of photosynthetic pigments

Photosynthetic pigments were extracted in 80% chilled acetone as per the procedure of Arnon [15] Carotenoid concentration in these extracts were calculated by the formula given by Duxbury and Yenstch [16].

2.4. Nitrate reductase activity, nitrate and protein contents

In vivo nitrate reductase (EC 1.6.6.1.2) activity (NRA) was assayed in fresh leaves (0.5 g) by the method of Srivastava [17]. NRA was expressed as nmol NO_2^- h^{-1} g^{-1} FW. The nitrate (NO_3^-) content in leaves was estimated as per procedure given by Shanker et al. [18]. The excised leaves (0.5 g) were boiled in 20 ml double distilled water for 30 min and filtered. The nitrate concentration in filtrate was measured using ion selective electrode of Thermo Orion Ion Meter (model: Orion 960 Auto Chemistry System). The protein content was determined in leaves following the procedure given by Lowry et al. [19].

2.5. Estimation of ascorbic acid, cysteine, proline and nonprotein thiol contents

The total ascorbic acid content in fresh leaves of chromium-treated plants was estimated as per method of Washko et al. [20]. Cysteine content in leaves was measured following the method of Gaitonde [21]. Free proline content in leaves was estimated according to the method given by Bates et al. [22]. For estimation of non-protein thiol (NP-SH) content, frozen plant tissue (ca. 700 mg fresh leaves) was extracted in 6.67% 5-sulfosalicylic acid and centrifuged at 13,000 × *g* for 10 min. Supernatant was reacted with Ellmans reagent and absorbance was recorded at 412 nm [23].

2.6. Lipid peroxidation and membrane permeability

The level of lipid peroxidation in fresh leaves was measured in terms of malondialdehyde (MDA) content by the thiobarbutaric acid (TBA) reaction method [24]. Membrane permeability was estimated in terms of K^+ leakage. Potassium content was estimated in the treated solution by using Flame Photometer (Mediflame).

2.7. Assay of antioxidant enzymes

Plant material (0.5 g leaves) was homogenized in 50 mM phosphate buffer (pH 7.0) containing 1% insoluble polyvinylpyrrolidone (w/v) at 4 °C with mortar and pestle (0.1 g FW/ml buffer), filtered through four layers of cheese cloth and centrifuged at 15,000 × *g* for 10 min. The supernatant obtained was designated as crude enzyme extract and was used for various antioxidant enzyme assays.

2.7.1. Catalase (EC 1.11.1.6)

The activity of catalase (CAT) was measured monitoring H_2O_2 decomposition at 240 nm in 3 ml reaction mixture containing 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2, 100 μl homogenate (1 mg/ml protein) and 0.1% (v/v) Triton X-100 [25]. The activity was expressed in terms of μmol of H_2O_2 reduced min^{-1} g^{-1} FW at 25 ± 2 °C.

2.7.2. Ascorbate peroxidase (EC 1.11.1.11)

The activity of ascorbate peroxidase (APX) was measured according to the method of Nakano and Asada [26] by estimating the rate of ascorbate oxidation (extinction coefficient: 2.8 mM^{-1} cm^{-1}). The 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM sodium ascorbate, 0.1 mM EDTA and 100 μl enzyme extract. The enzyme activity was expressed in terms of μmol of ascorbate oxidized min^{-1} g^{-1} FW at 25 ± 2 °C.

2.7.3. Guaiacol peroxidase (EC 1.11.1.7)

Guaiacol peroxidase (GPX) activity was assayed according to the method of Hemeda and Klein [27]. A 100 ml reaction mixture was prepared by adding 10 ml of 1% guaiacol (w/v), 10 ml of 0.3% H_2O_2 and 80 ml of 50 mM phosphate buffer (pH 6.6). 75 μl of enzyme extract was added to reaction mixture with a final volume of 3 ml). The increase in absorbance due to oxidation of guaiacol (extinction coefficient: 26.6 mM^{-1} cm^{-1}) was monitored for 3 min at 470 nm. Enzyme activity was expressed in terms of μmol of guaiacol oxidized min^{-1} g^{-1} FW at 25 ± 2 °C.

2.7.4. Superoxide dismutase (EC 1.15.1.1)

The activity of superoxide dismutase (SOD) was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) [28]. Reaction mixture lacking enzyme developed the maximum colour which decreased with increasing volume of added enzyme extract. The volume of enzyme extract corresponding to 50% inhibition of the reaction has been calculated by plotting a graph between enzyme concentration in reaction mixture and its absorbance at 560 nm and was considered as one unit enzyme. SOD activity was expressed as unit g^{-1} FW.

2.8. Thin layer chromatography (TLC) for eugenol content

The eugenol content was extracted by shaking one gram powdered leaves in dichloromethane for 15 min. The suspension was filtered and the clear filtrate evaporated to dryness. The residue was dissolved in 1 ml toluene and 25 μl aliquot was applied on TLC plates (E-Merck HPLTC precoated plates Silca Gel, E-Merck 60 F254) in triplicates. TLC plates were developed in CAMAG Twin trough Chamber using solvent system of toluene: ethyl acetate (93:7, v/v). The plates were sprayed by anisaldehyde sulphuric acid reagent and heated at 110 °C for 10 min. The eugenol content in treated and control plant were estimated by CAMAG densitometric Scanner by setting wavelength at 540 nm. The eugenol content was calculated using eugenol (Sigma) as standard and expressed as nl g^{-1} DW.

2.9. Statistical analyses

A two-way analysis of variance in complete randomized block design involving five treatments and three duration was performed to confirm the validity of the data except

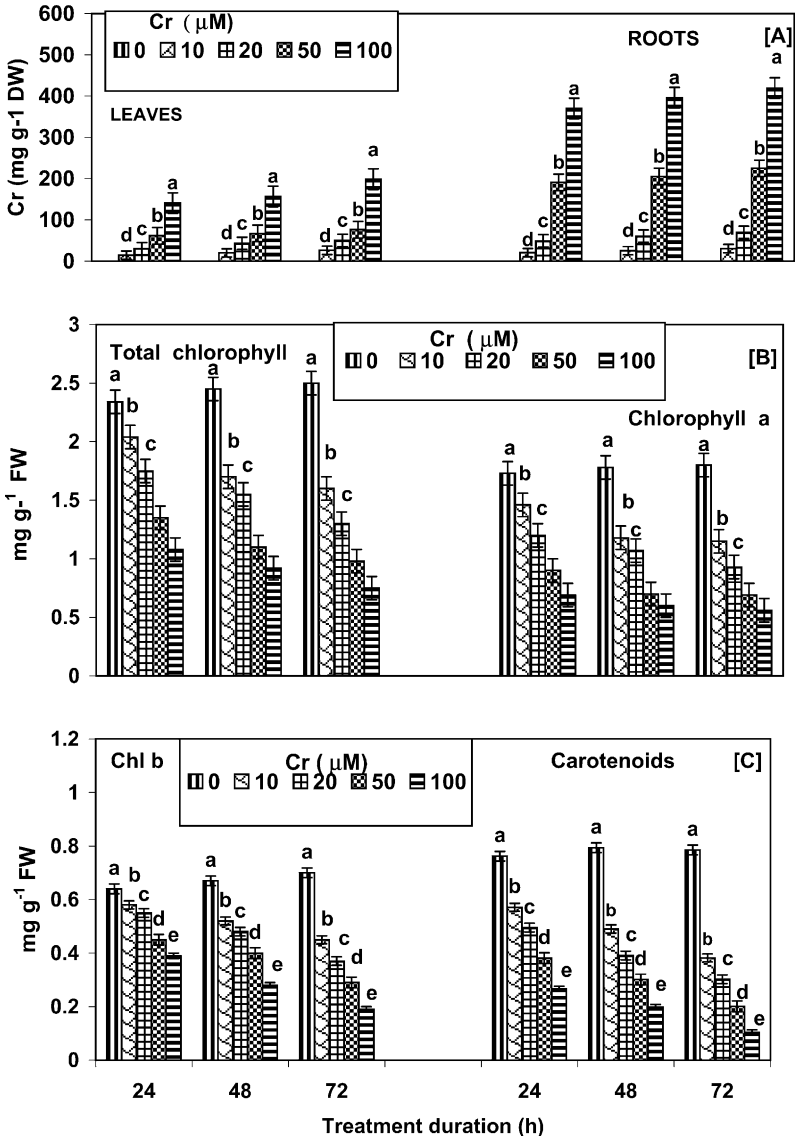

Fig. 1. Chromium accumulation (A) by *O. tenuiflorum* and its effect on total chlorophyll, chlorophyll *a*, (B) chlorophyll *b* and carotenoids (C). Mean ± S.D. ($n = 3$). ANOVA, $P < 0.05$. Different superscripts on bars (each duration) showed significant ($P < 0.05$) difference between the means according to Duncan's multiple range test.

for yield of eugenol content. Comparison from control and between means of the different treatments was done by Duncan's multiple range test [29]. A comparison of yield of eugenol content in chromium treated and untreated plants was done using *t*-test [30].

3. Results

3.1. Chromium accumulation and its effect on biomass

O. tenuiflorum accumulated chromium in concentration and duration dependent manner (Fig. 1A). The amount of chromium accumulated by different plant tissues (roots > leaves) varied significantly (ANOVA, $P < 0.05$). Roots accumulated maximum amount (419.50 μg g^{-1} DW) of chromium when exposed to 100 μM chromium for 72 h. The maximum chromium (198.80 μg g^{-1} DW) content in leaves was also recorded at same concentration and treatment duration. Chromium uptake by *O. tenuiflorum* significantly (ANOVA, $P < 0.05$) affect the growth of the plant which was reflected by the decrease in biomass. Chromium reduced the biomass in dose and treatment duration dependent manner (Table 1).

Chromium significantly (ANOVA, $P < 0.05$) reduced the level of phototosynthetic pigments (total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoids) in *O. tenuiflorum* (Fig. 1B and C). The chromium toxicity to photosynthetic pigments enhanced with the increasing level of chromium in nutrient medium (DMRT, $P < 0.05$) and treatment duration. It was observed that 100 μM chromium treatment for 72 h reduced total chlorophyll, chlorophyll

Table 1
Effect of chromium on biomass (DW) of *Ocimum tenuiflorum*

Chromium (μM)	Treatment duration (h)		
	24	48	72
0.0	$0.4390^{a} \pm 0.022$	$0.4512^{a} \pm 0.020$	$0.4592^{a} \pm 0.024$
10	$0.3986^{b} \pm 0.012$	$0.3610^{b} \pm 0.016$	$0.3118^{b} \pm 0.012$
20	$0.3064^{c} \pm 0.010$	$0.2631^{c} \pm 0.012$	$0.2296^{c} \pm 0.010$
50	$0.2706^{d} \pm 0.014$	$0.2114^{d} \pm 0.010$	$0.1786^{d} \pm 0.004$
100	$0.2272^{e} \pm 0.012$	$0.1690^{e} \pm 0.006$	$0.1446^{e} \pm 0.008$

Mean ± S.D. ($n = 3$); ANOVA, $P < 0.05$; different superscripts denote significant ($P < 0.05$) difference between means in column according to Duncan's multiple range test.

a, chlorophyll *b* and carotenoids contents by 70.00, 68.89, 72.85 and 86.86%, respectively.

3.2. Nitrate reductase activity (NRA), nitrate and protein contents

A concentration and duration dependent (ANOVA, $P < 0.05$) inhibition of nitrate reductase activity was observed in chromium treatment to plants (Fig. 2A). A maximum inhibition of 64% in nitrate reductase activity was recorded when plants were exposed to 100 μM Cr for 72 h (DMRT, $P < 0.05$). It was also observed that impaired nitrate reductase activity resulted in a build-up of leaf nitrate content (Fig. 2B). The nitrate accumulation due to chromium toxicity was found to be dose and treatment duration dependent; a treatment by 100 μM Cr for 72 h resulted in ca. 3.59-fold accumulation of nitrate in leaves (DMRT, $P < 0.05$). The protein contents of the chromium treated plants were also

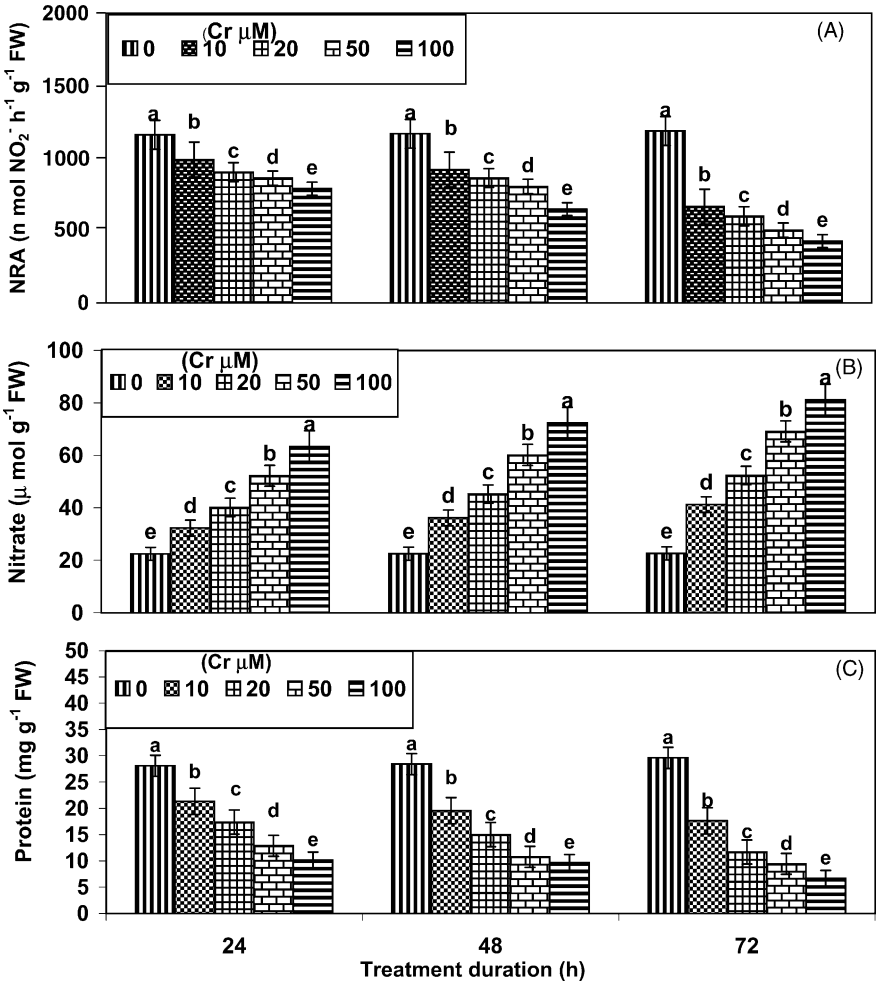

Fig. 2. Impact of chromium on nitrate reductase: NRA (A), nitrate content (B), protein (C), content of *O. tenuiflorum*. Mean ± S.D. ($n = 3$). ANOVA, $P < 0.05$. Different superscripts on bars (each duration) showed significant ($P < 0.05$) difference between the means according to Duncan's multiple range test.

found affected (ANOVA, $P < 0.05$) by chromium level in the nutrient medium (Fig. 2C). A maximum reduction of 77.45% in protein content was observed at 100 μM concentration and 72 h treatment duration.

3.3. Ascorbic acid, cysteine, proline and non protein thiol (NP-SH) contents

Chromium significantly (ANOVA, $P < 0.05$) reduced the ascorbic acid, cysteine and non-protein thiol contents of *O. tenuiflorum* leaves (Figs. 3A, D and E). The maximum inhibitions in ascorbic acid (52.83%), cysteine (77.33%) and NP-SH (60.87%) were recorded when plants were treated by 100 μM chromium for 72 h. However, a concentration and treatment duration (ANOVA, $P < 0.05$) related enhanced accumulation of proline content (leaves) was recorded in leaves of *O. tenuiflorum* grown in chromium-amended growth medium (Fig. 3B). A maximum build-up in foliar proline content (574.94 μmol g^{-1} FW) was recorded when plant were exposed to 100 μM Cr for 72 h (DMRT, $P < 0.05$).

3.4. Lipid peroxidation and membrane permeability

During present study, a significant (ANOVA, $P < 0.05$) increase in malondialdehyde (MDA) content of *O. tenuiflorum* leaves was observed initially at 10 μM chromium level after 24 h exposure (Fig. 3C) which continued to augment with an increase in chromium concentration in the nutrient medium and treatment duration (DMRT, $P < 0.05$). It was

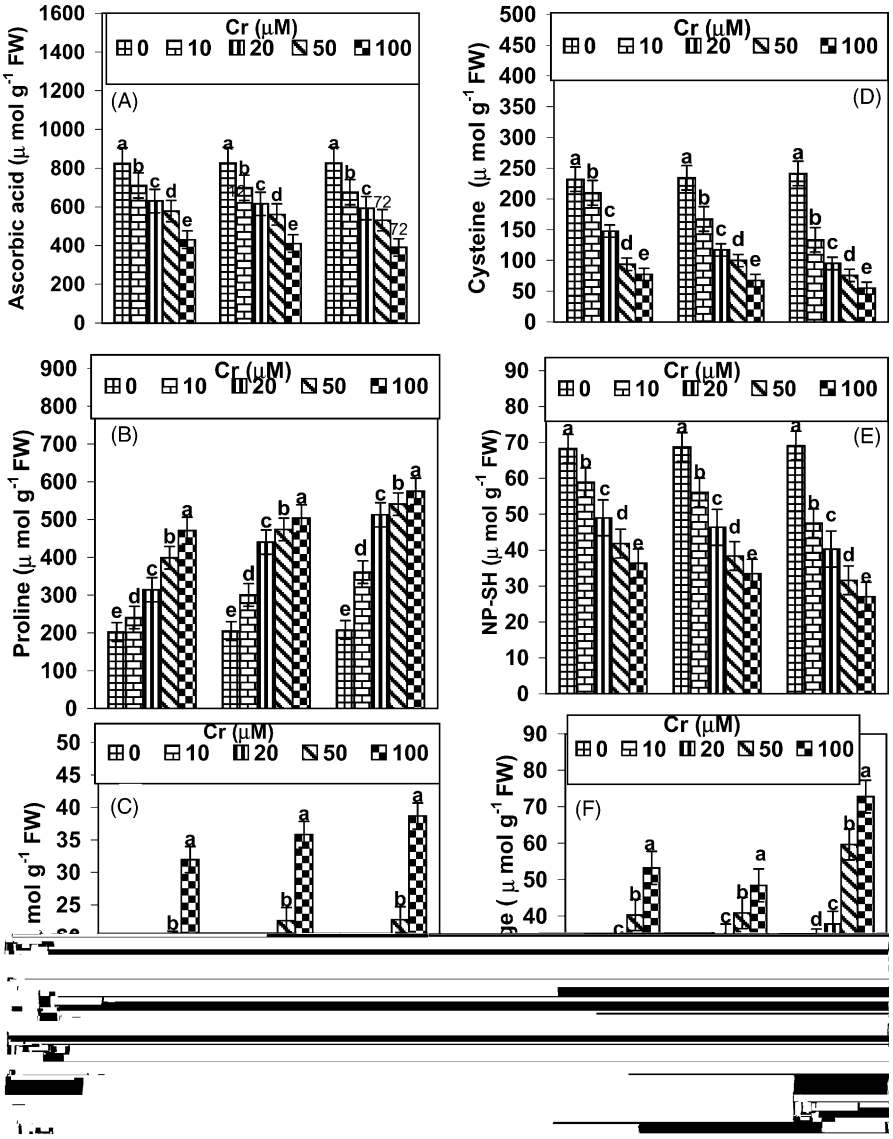

Fig. 3. Impact of chromium on ascorbic acid (A), proline (B), MDA (C), cysteine (D), non-protein thiol: NP-SH (E) content and K^+ of *O. tenuiflorum* (F). Mean ± S.D. ($n = 3$). ANOVA, $P < 0.05$. Different superscripts on bars (each duration) showed significant ($P < 0.05$) difference between the means according to Duncan's multiple range test.

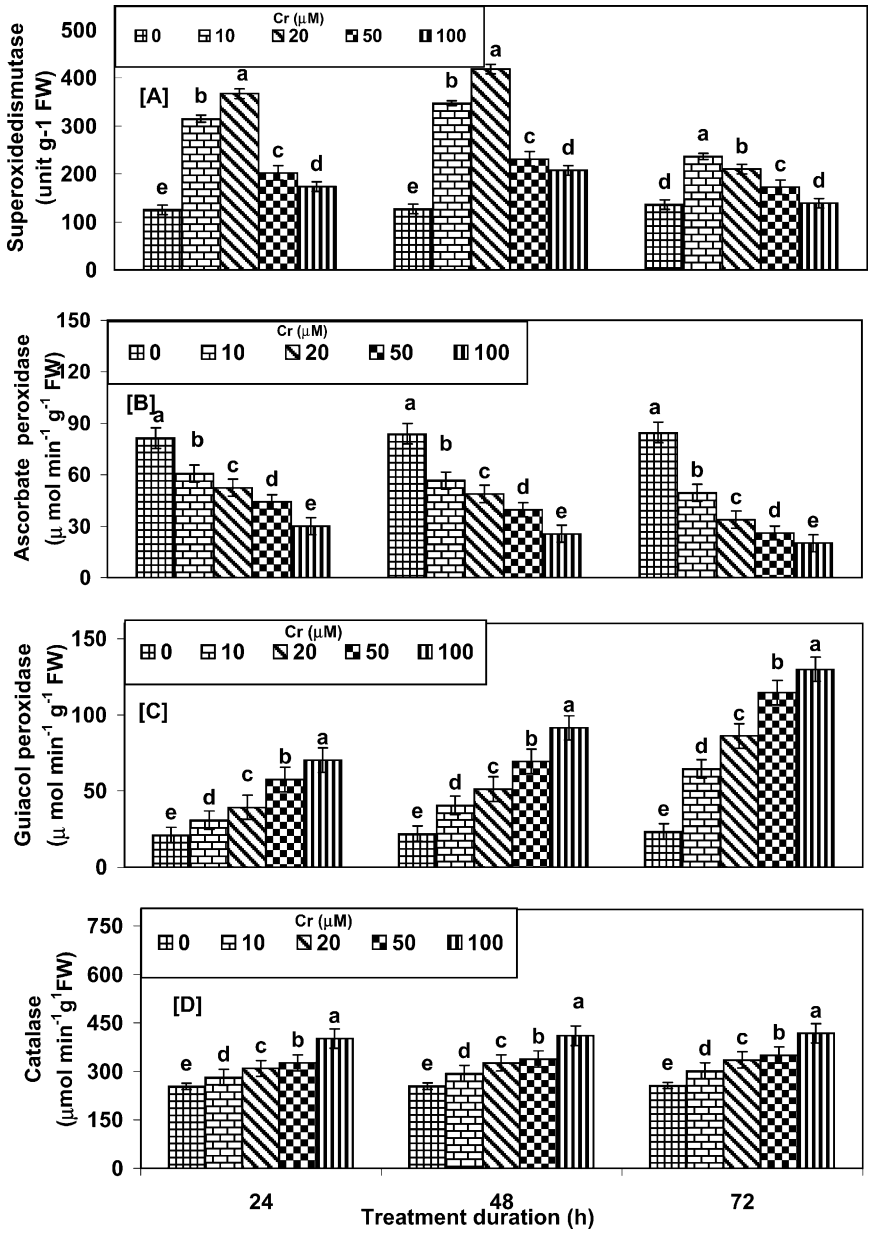

Fig. 4. Impact of chromium on superoxide dismutase (A), ascorbate peroxidase (B), guaiacol peroxidase (C), catalase activities (D) of *O. tenuiflorum*. Mean ± S.D. ($n = 3$). ANOVA, $P < 0.05$. Different superscripts on bars (each duration) showed significant ($P < 0.05$) difference between the means according to Duncan's multiple range test.

noted that 100 μM chromium (after 72 h) in nutrient medium resulted in the maximum build-up of MDA content (approximately five-fold in comparison to the control, DMRT, $P < 0.05$). Chromium also induced potassium leakage in test plants at 10 μM (DMRT, $P < 0.05$) concentration with 24 h exposure (Fig. 3F), which further increased in concentration and duration dependent manner (ANOVA, $P < 0.05$) with cell membrane disruption.

3.5. Antioxidant enzymes

Heavy metal-induced reactive oxygen species in plants are quenched by a number of antioxidant enzymes. During the present study, it was observed that chromium toxicity resulted in a significant (ANOVA, $P < 0.05$) hyperactivity of superoxide dismutase, guiacol peroxidase and catalase enzymes in *O. tenuiflorum* leaves (Figs. 4A, C and D). The maximum stimulation of SOD activity (3.29-fold) was observed when plants were exposed to 20 μM for 48 h and minimum at 100 μM chromium exposure for 72 h duration (DMRT, $P < 0.05$). However, peak stimulation of guiacol peroxidase (5.56-fold) and catalase activities (1.63-fold) was observed when plants were exposed to 100 μM chromium for 72 h (DMRT, $P < 0.05$). In contrast to these enzymes, chromium significantly (ANOVA, $P < 0.05$) reduced ascorbate peroxidase activity under chromium stress in a

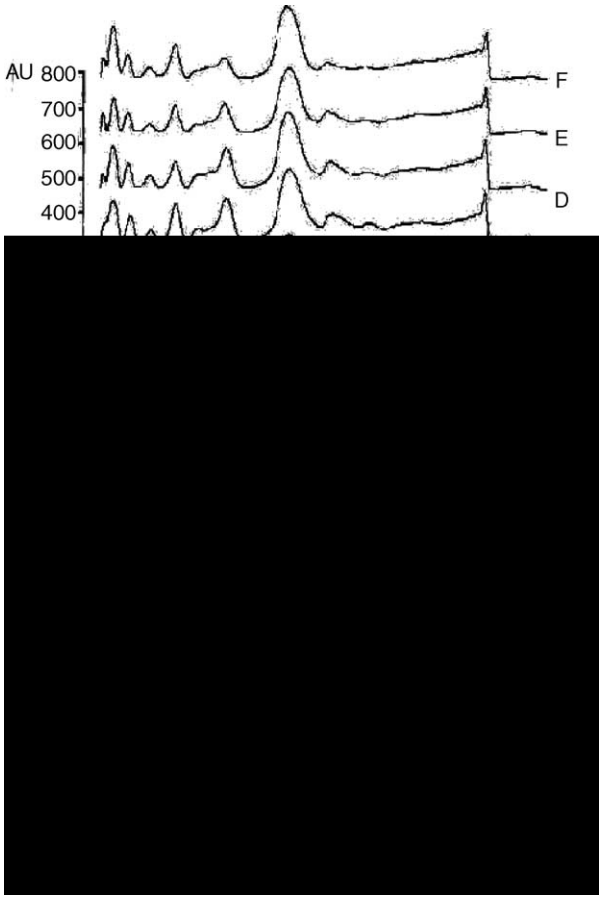

Fig. 5. Impact of chromium on eugenol content of *O. tenuiflorum* after 72 h. (a) Densitometric scan at 540 nm of control and treated plants (A: eugenol, B: control, C: 10 μM, D: 20 μM, E: 50 μM, F: 100 μM); (b) TLC fingerprint profile of extract of control and treated plants (1: control, 2: 10 μM, 3: 20 μM, 4: 50 μM, 5: 100 μM).

concentration and duration dependent manner (Fig. 4B). It was observed that 100 μM chromium exposure for 72 h reduced ascorbate peroxidase activity by 76.22% (DMRT, $P < 0.05$).

3.6. Eugenol content

Chromium treatment to *O. tenuiflorum* affected eugenol content—a secondary metabolite (*t*-test, $P < 0.05$). The chromium concentrations in nutrient medium enhanced the eugenol content significantly up to 100 μM in comparison to control after 72 h exposure (Figs. 5a and b, Table 2) (ANOVA, $P < 0.05$). A maximum increase of 24.61% in eugenol content was observed when plants were exposed to 20 μM chromium for 72 h (Fig. 5a and b, Table 2).

Table 2
Impact of chromium on eugenol content of *O. tenuiflorum*

Chromium (μM)	Eugenol (nl g^{-1} DW)
0.0	290.00 ± 12.40
10.0	331.92 ± 14.52
20.0	361.36 ± 17.07
50.0	338.72 ± 13.50
100.0	301.12 ± 12.50

Mean ± S.D. ($n = 3$), Student's *t*-test. Significant ($P < 0.05$) as compared to control.

4. Discussion

4.1. Chromium accumulation and its effect on biomass

O. tenuiflorum was able to tolerate 100 μM chromium with some physiological and biochemical changes. This suggests this plant has a high adaptability to cope-up with chromium stress. The roots accumulated more chromium than the leaves in all treatments. Comparatively lower accumulation of chromium in leaves than roots was probably due to reduction of Cr(VI) to Cr(III), which reduces its mobility from roots to shoots [31]. It has been reported that Cr(III) readily forms complexes with –COOH groups which inhibits the translocation of metal from roots to shoots [32]. Chromium reduced the biomass of the *O. tenuiflorum*. Reduction in biomass of *V. spiralis* plants exposed to varying concentrations of chromium has been reported [4].

4.2. Photosynthtic pigments

Chromium reduced the foliar contents of total chlorophyll, chl *a*, and chl *b* contents in *O. tenuiflorum*. This might be attributed to the toxicity of chromium to chlorophyll biosynthesis of the test plant. The impaired δ-aminolaevulinic acid dehydratase activity leading to reduced photosynthetic pigments has been observed in chromium-treated *Nymphaea alba* [33]. Besides, lipid peroxidation also causes degradation of the photosynthetic pigments [34]. In this case also, increased lipid peroxidation was observed in chromium-treated plants. Chromium also reduced carotenoid contents in *O. tenuiflorum* which served as accessory pigments for photosynthesis and also protected the plants from photo-oxidation. Similar, observations have also been made in other plants exposed to chromium [4].

4.3. Nitrate reductase activity, nitrate and protein contents

Nitrate reductase (NR) is a key enzyme of nitrogen metabolism. NR catalyses the first step in the assimilatory reduction of nitrate into ammonia, which reduces nitrate to nitrite [35]. During present study, NRA was found severely affected by chromium treatment. Further, a decline in NRA was found correlated to the decrease in total chlorophyll content which could affect photosynthesis process [35]. It is suggested that NRA depends upon active photosynthesis or production of photosynthates and requires photosynthetically generated reductant and energy [36]. Hence, reduction in NRA in chromium-treated *O. tenuiflorum* plants could be due to reduced photosynthesis as a result of inhibition of chlorophyll biosynthesis. Chromium affected photosynthetic rates have been reported in *Nelumbo lutea* [37]. It

was also observed that impaired nitrate reductase activity resulted in a build up of leaf nitrate content. The accumulation of nitrates under salt stress coupled with impaired NRA has been reported in seedling of *Phaseolus* sp. [18].

Chromium has been reported to reduce foliar protein content in plants [4,33]. In this case, also a reduction in protein content was also observed in chromium- treated *O. tenuiflorum*. As suggested by earlier workers, protein degradation might be the result of increased activity of the protease or other catabolic enzymes, which were activated under chromium stress. It is also likely that chromium induced lipid peroxidation in *O. tenuiflorum* and fragmentation of proteins due to toxic effects of reactive oxygen species led to reduced protein content [38].

4.4. Ascorbic acid, cysteine, proline and nonprotein thiol contents

Cellular antioxidants (thiols, carotenoids, ascorbate, etc.) may also play an important role in inducing resistance in plants to metals by protecting labile macromolecules against attack by free radicals which are formed during various metabolic reactions leading to oxidative stress [39]. During the present study, reduced levels of NP-SH, carotenoids and cysteine were observed in Cr-treated plants. This might be attributed to the toxicity of the chromium. Ascorbic acid plays an important role in α-tocopherol regeneration, which has been reported to act as the primary antioxidant [40]. Besides this, ascorbate plays many other roles in the antioxidant metabolism. Therefore, measured decline in the content of ascorbic acid in *O. tenuiflorum* could be totally or partially due to its consumption while acting as antioxidant to limit lipid peroxidation. Many environmental stresses have been reported to increase the level of proline in plants, such as heavy metals, UV radiation, temperature and drought [41,42]. During the present study, higher accumulation of proline in chromium-treated plants of *O. tenuiflorum* has been observed which might be attributed to the strategies adapted by plants to cope up with chromium toxicity as proline has multiple functions, such as, osmoticum, scavenger of free radicals, protecter role of cytoplasmic enzymes, source of nitrogen and carbon for post stress growth, stabilizer of membranes, machinery for protein synthesis and a sink for energy to regulate redox potential [42].

4.5. Lipid peroxidation and membrane permeability

The chromium accumulation in *O. tenuiflorum* leads to various physiological and biochemical changes. The accumulation of chromium in *O. tenuiflorum* promoted MDA production (a cytotoxic product of lipid peroxidation) through excessive generation of free radicals. Further, peroxidaton of membrane lipids resulted in potassium leakage, indicating a loss of membrane integrity. Chromium-induced loss of membrane permeability coupled with increased MDA production has also been observed in *Vallisneria spiralis* [4].

4.6. Antioxidant enzymes

Chromium induced reactive oxygen species in plants [4,10,43]. Further, to mitigate and repair the damage initiated by reactive oxygen species, plants have evolved a complex system involving of antioxidant enzymes. Ascorbate peroxidase (APX) is the member of the ascorbic acid–glutathione cycle, and plays a crucial role in eliminating poisonous H_2O_2 from plant cells. In this study, all chromium concentration used in the experiment inhibited APX activity in *O. tenuiflorum* at all the treatment durations. This is in contrast to the results earlier described in different plants growing under heavy metal stress [44,45]. This might be due to the harmful effects of the over production of H_2O_2 or its poisonous active oxygen derivatives, because of manifold increase in SOD activity of the chromium-treated *O. tenuiflorum*. Amongst various enzymes involved in quenching of reactive oxygen species, guaiacol peroxidase (GPX) and catalase have their importance in elimination of H_2O_2. The stimulated activities of these enzymes (GPX and catalase) and reduced APX activity found in this study led to the conclusion that elimination of H_2O_2 in *O. tenuiflorum* was achieved by GPX and catalase. While APX took a little part in detoxification of H_2O_2 due to its sensitivity to chromium. Furthermore, GPX participates in the lignin biosynthesis and might build up a physical barrier against poisoning of the heavy metals. Therefore, hyperactivities of GPX, catalase and SOD in *O. tenuiflorum* might be attributed to the strategies adopted by the *O. tenuiflorum* to overcome the toxicity of the chromium. High guaiacol peroxidase activity was also observed in *Nymphaea candida* growing in paper pulp mill effluent rich in chromium [46].

4.7. Eugenol content

The secondary metabolites are formed under various stresses as a defense mechanism [47]. During the present investigation, all the chromium concentrations increased eugenol (a phenylpropanoid) content (14.46, 24.61, 16.80, 3.83% by 10, 20, 50 and 100 μM chromium, respectively) after 72 h. It has been reported that cadmium, copper and zinc contamination of soils did not affect the essential oil in *Mentha piperita* L. and *Mentha arvensis*, *Lavendula angustifolia* Mill [9,10]. However, in our case, chromium induced the eugenol content in treated plants in comparison to control. This might be a part of defense strategy adapted by *O. tenuiflorum* against chromium toxicity to protect themselves.

5. Conclusion

It may be concluded from the present study that *O. tenuiflorum* could grow in chromium-amended nutrient medium

and accumulate high amount of chromium in roots followed by leaves. Chromium accumulation by *O. tenuiflorum* affects various physiological processes. Chromium-induced oxidative stress was tolerated by this plant through the hyperactivity of antioxidant defense system. The H_2O_2 formed by the superoxidation of active oxygen species was quenched by catalase and GPX. However, APX took a little part in quenching of H_2O_2 due to its sensitivity to the chromium. Therefore, reduced APX activity was recorded in chromium treated *O. tenuiflorum*. Chromium-stress induced the production of the eugenol (a major component of essential oil of *Ocimum*) in *O. tenuiflorum*. Therefore, *O. tenuiflorum* could be grown in chromium polluted soils for higher yields of essential oil. However, our results are laboratory based and before exploiting the results in field, a pilot field study is recommended. Because in field studies natural variables (temperature, pH, light, soil quality, etc.) may affect the results. However, the use of chromium containing leaves in medicinal preparations is not advised due to its toxic effects and health risk.

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