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Effect of Chromium Nitrate Stress on Secondary Metabolism of *Lantana camara* L.

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Abstract. *Lantana camara* L. is an invasive weed that spreads rapidly owing to its quick growth and easy distribution of seeds. Chromium, a common heavy metal contaminant, shows stunted plant growth, reduces root length and causes anatomical changes. Interestingly, *L. camara* can develop tolerance to chromium over time, making it potentially useful for phytoremediation. In this study, experiments are performed by growing it in soils containing different concentrations of Chromium Nitrate over varying times to monitor the trend of changes brought about in the secondary metabolite contents of the plant. This content was measured by performing the respective assays to quantify them. The study showed the undoubted potential of the plant to survive even at concentrations as high as 75 µM. The plant sample that was treated with 25 µM showed an initial increases in 3 days. This value increased and reached a peak in 7 days. The content further decreased in 14 days. Through these defence mechanisms, it has elevated the levels of available plant secondary metabolites.

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1 Introduction

Plant stress refers to any adverse biotic or abiotic condition that restricts normal growth and development. Biotic stresses arise from insects and pathogens, while abiotic stresses include water imbalance, salinity, temperature variation, and soil contamination. These factors impair actively growing tissues, causing structural and functional damage. Plants respond by synthesizing secondary metabolites such as terpenes, phenolic acids, and alkaloids. Lantana species, particularly *L. camara*, are well known for their ecological dominance [1].

Chromium, a major heavy-metal pollutant, delays germination, reduces root and shoot growth, and disrupts physiological balance. Key genes such as Phenylalanine Ammonia Lyase (*PAL*) and Metallothionein (*MT*) reflect oxidative stress responses specific to chromium exposure. In this study, *L. camara* was treated with 25, 50, and 75 μM chromium nitrate for 14 days to evaluate stress-induced secondary metabolite dynamics. Its invasive growth further disrupts agriculture by affecting soil microbes and beneficial fauna [2–5].

Plant stress refers to any unfavourable condition that restricts the normal growth and development of plants. These stresses are broadly classified into biotic and abiotic types. Biotic stresses include damage caused by insects and pests, while abiotic stresses arise from environmental factors such as water and salt imbalance, temperature fluctuations, and soil contamination. When plants are exposed to stress, their growth is adversely affected, particularly in actively growing tissues like roots and shoots. This results in structural deformities and reduced survival potential.

Plants possess natural defence mechanisms to cope with stress conditions. One such strategy involves the production of plant secondary metabolites. These compounds are synthesized only under stressful or unfavourable conditions and do not play a direct role in primary growth processes. Secondary metabolites are mainly classified into terpenes, phenolic compounds, and alkaloids, and they help plants tolerate and adapt to environmental stress.

Chromium is used in plant stress research because it is a common environmental heavy metal pollutant that disrupts plant growth and metabolism, induces oxidative stress, and alters secondary metabolite pathways. Studying chromium stress helps understand how plants respond and adapt at physiological and molecular levels under heavy metal toxicity. (20)

Lantana camara Linn., a member of the Verbenaceae family, was named by Linnaeus in 1753. The genus *Lantana* comprises approximately 50–270 species, with *L. camara* being the most widespread and dominant. Native to South America, it was introduced to India in 1809 as an ornamental plant. Over time, it became a highly invasive weed across most regions of India. Recognized by the IUCN as one of the world's 100 most invasive species, *L. camara* negatively impacts biodiversity, soil health, and agricultural productivity by disrupting ecological balance.

Chromium is a toxic heavy metal that accumulates in the environment due to natural processes and industrial activities, especially from tanning and leather industries. Chromium contamination affects plant health by inhibiting germination, reducing root and shoot growth, and disturbing nutrient balance.

Among its various forms, Cr(III) and Cr(VI) are considered the most toxic.

Key genes such as PAL, DFR, HQT, and CHS regulate the biosynthesis of secondary metabolites, while stress-related genes like MT and SOD indicate oxidative stress levels. In this study, *L. camara* plants were exposed to chromium nitrate concentrations of 25 µM, 50 µM, and 75 µM for 14 days. Observations were recorded at 3, 7, and 14 days. This research enhances understanding of secondary metabolite responses under heavy metal stress and may aid in weed management and utilization of stress-induced plant compounds [15 – 19].

2 Materials and methods

Three *Lantana camara* shrubs were grown in soils treated with three different concentrations of Chromium Nitrate ($Cr(NO_3)_3$), viz. 25 µM, 50 µM and 75 µM. For every respective concentration a set of 10 plants were used. The experiment was conducted at intervals of 3, 7, and 14 days for both the sample leaves and roots. After 14 days, the plants were uprooted to collect treated root samples. Untreated samples of leaves and roots were taken as the control.

2.1 Sample preparation

The plant extract was prepared following a standard protocol with minor adjustments [1].

2.2 Chlorophyll estimation

With a few minor adjustments, the total Chlorophyll was determined using the method of (Lichtenthaler, 1987).

2.3 Evaluation of overall polyphenol content

The Folin-Ciocalteu Reagent (FCR) technique (Singleton et al., 1999; Talukder et al. 2015) was used to determine the total polyphenol content with a few minor adjustments.

2.4 Evaluation of overall flavonoid content

The $AlCl_3$ colorimetric technique (Lin and Tang, 2007; Talukder et al., 2016) was used to determine the total flavonoid content of the samples, with a few minor changes.

2.5 Total antioxidant content estimation

The evaluation of overall activity of antioxidants from the extracts using the phosphomolybdenum technique, which was somewhat modified from (Prieto et al. 1999). For the antioxidant activity content phosphomolybdenum reagent was made using 28mM sodium phosphate, 0.6M sulfuric acid, and 4mM ammonium molybdate. 3 millilitre phosphomolybdenum reagent was combined with 0.3 ml of extract from each sample was mixed at incubated at 95°C for 90 minutes.

2.6 Gene expression analysis of *PAL* and *MT*

RNeasy Plant Mini Kit (cDNA (cDNA kit - Applied Biosystem, CA, USA) was used to extract RNA from plant samples. Procedure followed was following the manufacturer's guidelines with minimal adjustments. Then reverse transcription PCR was carried out by using a each of the specific primer (Livak and Schmittgen, 2001)
cDNA was prepared using mRNA to cDNA conversion kit (Applied Biosystems, CA, USA).

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Code Index	Sampl e Code	Treatment with $Cr(NO_3)_3$
1	LL (C)	*Lantana* leaf control (no treatment)
2	LL (I)	*Lantana* leaf treated with 25 µM
3	LL (II)	*Lantana* leaf treated with 50 µM
4	LL (III)	*Lantana* leaf treated with 75 µM
5	LR (C)	*Lantana* control root (no treatment)
6	LR (I)	*Lantana* root treated with 25 µM
7	LR (II)	*Lantana* root treated with 50 µM
8	LR (III)	*Lantana* root treated with 75 µM

2.6.1 Primer designing

The primers have been developed based on existing sequences identified in the NCBI database using the ClustalW multiple sequence alignment programme

3 Result

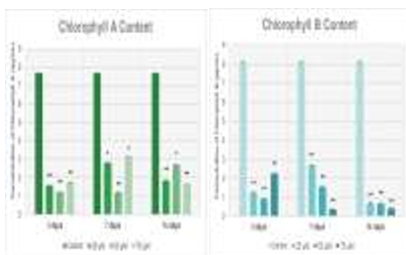

Fig. 1. (A) Chlorophyll A (B) Chlorophyll B Content in *L. camara*; *L. camara* (control); *L. camara* treated with 25 µM, 50 µM, and 75 µM of $Cr(NO_3)_3$ after 3,7, and 14 days of treatment.

Chlorophyll A content peaked at 3.14 mg/g at 7 days, and Chlorophyll B was highest at 2.24 mg/g at 3 days (Fig. 1).

3.1. Total polyphenol content

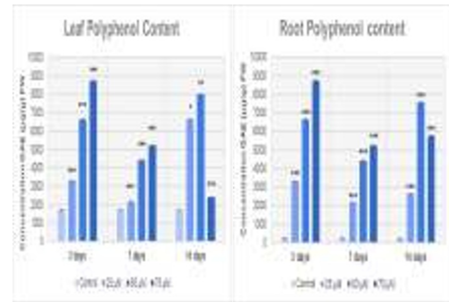

Fig. 2. Concentration of Total Polyphenol Content in (A) Leaf (B) Root in *L. camara*; *L. camara* (control); *L. camara* treated with 25µM, 50µM, and 75µM of $Cr(NO_3)_3$ after 3,7 and 14 days of treatment.

Total polyphenol content was expressed as GAE (µg/g FW), was highest in LL (III) at 3 days (8740.67 µg/g) (Fig. 2)

3.2. Total flavonoid content

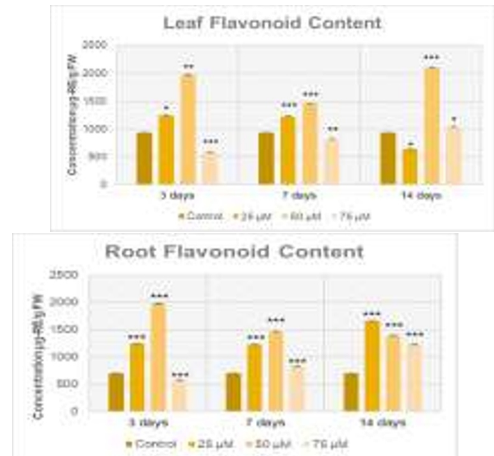

Fig. 3. Concentration of Total Flavonoid Content in (A) Leaf (B) Root in *L. camara*; *L. camara* (control); *L. camara* treated with 25µM, 50µM, and 75µM of $Cr(NO_3)_3$ after 3,7, and 14 days of treatment.

Flavonoid levels were expressed as Rutin Equivalent Concentration (µg-RE/g-FW), was highest in the leaf samples, LL (II) at 14 days 1965.38 µg/g (Fig. 3).

3.3 Evaluation of malondialdehyde (MDA) content using lipid peroxidation assay

A modified version of Heath and Packer's (1968) approach was used to estimate the extent of lipid peroxidation in the samples. Samples were prepared using thiobarbituric acid (TBA) and trichloroacetic acid (TCA). The samples were then spun at 15000 g for 10 minutes to measure the amount of malondialdehyde (MDA), which is a by-product of lipid peroxidation. The supernatant was mixed with the TBA-TCA mixed, boiled in a boiling water bath for half an hour, followed by rapid chilling on ice to stop the process. The absorbance at 532 nm was measured. The extent of lipid peroxidation was expressed in MDA equivalents, or µM/g FW. The extinction coefficient for MDA has been recorded to be 155 $mM^{-1}cm^{-1}$ (Fig. 4)

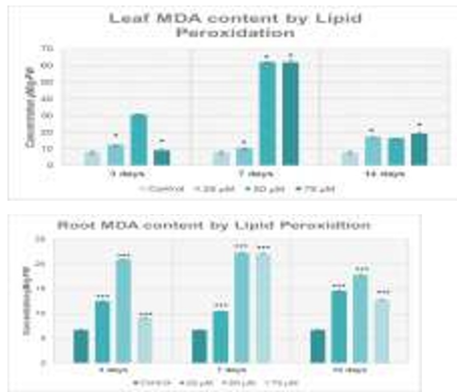

Fig. 4. Concentration of MDA in (A) Leaf (B) Root in *L. camara*; *L. camara* (control); *L. camara* treated with 25 µM, 50 µM, and 75 µM of Cr $(NO3)_3$ after 3,7, and 14 days of treatment

3.4 Gene expression analysis through Reverse Transcriptase (RT)-PCR

In the experiment, RNA was quantified from the sample plant tissues - leaves and roots. The forward and reverse primers for the genes *PAL* and *MT* were employed to assess how the genes showed expression in the samples in consecutive experimental intervals.

Primer Designing

The primers have been developed based on existing sequences identified in the NCBI database using the ClustalW multiple sequence alignment programme, leading to the identification of conserved domains. Using primer3 software, specific primers were created from the conserved regions. The following table contains a list of all the primer transcripts.

Forward Primer	Reverse Primer
PAL (F)	*PAL* (R)
AAGAACGGCGAACATGAG AAG	TATTTGGCCCGGGTGATGC T
CHS (F)	*CHS* (R)
TCCCGGCCTCAAATCTAAG A	GGCTTCAGGGCCAGCTTAT C
DFR (F)	*DFR* (R)
CCAAAAGCGGATACAAAC TTGAC	TCAGGATCCAAGGAATCA AATTC
HQT (F1)	*HQT* (R1)
TGAGATCCTAGCTGCCCAC T	TGGCTGTGAACACCACATT T
ACT (F)	*ACT* (R)
ATCATGAAGTGTGATGTTG A	ACCTTAATCTTCATGCTGC T
MT (F)	*MT* (R)
TGCTCATGTGGCTCAAGCT G	CAGGTGCAAGGGTCGCAC
SOD(Cr) (F)	*SOD(Cr)* (R)
ATGGTGAAGGGTGTTGCAG T	GCCCTGAAGACCAATGATA CC

Table 3: - Details of primers used for Reverse Transcription PCR of PAL, CHS, DFR, HQT, ACT, MT, SOD(Cr)

Primer (Forward & Reverse)	Denaturation	Go to cycle for 35 times		Final Extension	Storage
		Annealing	Extension		
PAL	94°C ; 1 mins 30 seconds	68 °C ;1 min	72°C ; 1 min 30 secs	72°C ; 10 mins	4°C
HQT	94°C ; 1 mins 30 seconds	66 °C;1 min	72°C ; 1 min 30 secs	72 °C ; 10 mins	4°C
ACT	94°C ; 1 mins 30 seconds	60 °C;1 min	72°C ; 1 min 30 secs	72 °C ; 10 mins	4°C
CHS	94°C ; 1 mins 30 seconds	67 °C;1 min	72°C ; 1 min 30 secs	72 °C ; 10 mins	4°C
DFR	94°C ; 1 mins 30 seconds	63 °C;1 min	72°C ; 1 min 30 secs	72 °C ; 10 mins	4°C
SOD(Cr)	94°C ; 1 mins 30 seconds	66 °C;1 min	72°C ; 1 min 30 secs	72 °C ; 10 mins	4°C
MT	94°C ; 1 mins 30 seconds	68 °C;1 min	72°C ; 1 min 30 secs	72 °C ; 10 mins	4°C

This table represents the sequences of the forward and reverse primers used in a Reverse Transcription PCR (RT-PCR) experiment.

Table 4: - Details of Thermal Cycle for RT-PCR

This table represents three different conditions used for **Reverse Transcription PCR (RT-PCR)**, specifically for amplifying different genes (PAL, HQT, ACT, CHS, DFR, SOD(Cr), and MT).

Gel electrophoresis followed by visualisation was done using a gel documentation system (Bio-Rad Molecular Imager, GelDoc XR, Milan, Italy). The Gel Doc EZ imaging system software was utilised to analyse the data .

3.4.1 PAL gene expression

PAL expression analysis showed clear chromium-induced alterations. After 3 days, LL (I) LL (III) increased to 1.6-fold. At 7 days, LL (I) remained unchanged. By 14 days, LL (II) showed the strongest increase (1.57). (Fig.5)

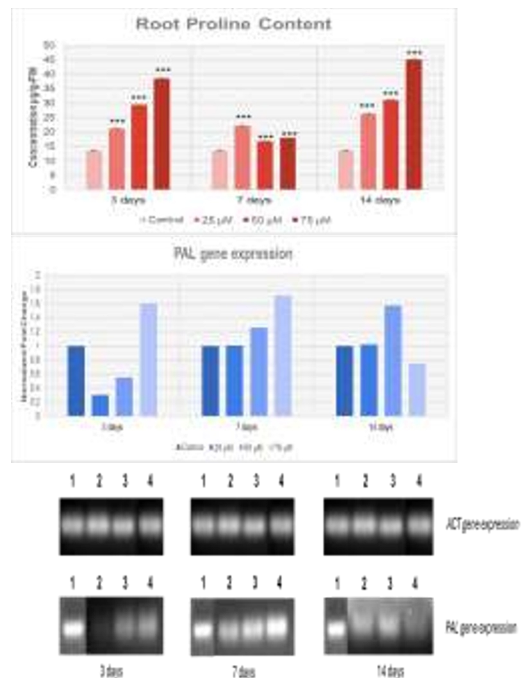

Fig. 5. Pictorial data of Gel electrophoresis and fold change in the expression levels of the PAL gene in the leaves of *L. camara*; *L. camara* (control); *L. camara* treated with 25µM, 50µM, and 75µM of Cr(NO3)3 after 3,7, and 14 days of treatment; Actin gene expression was used as positive control

3.4.2 MT gene expression

MT gene expression analysis from the sample leaves revealed that after 14 days, a different pattern was observed wherein LL (I) showed maximum increase (17.42). From the MT gene expression analysis in the roots, it was revealed that LR (II) showed a significant increase of 1.87 times (Fig. 6)

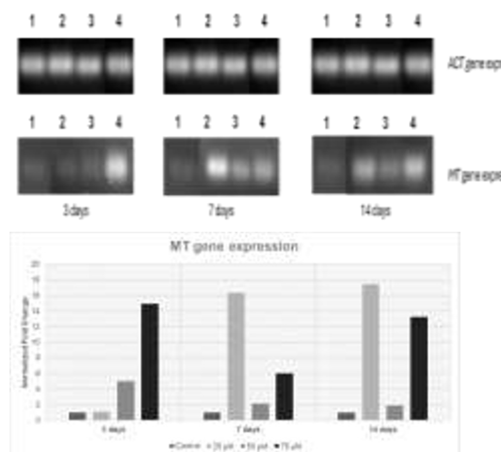

Fig. 6. Pictorial data of Gel electrophoresis and fold change in the expression levels of the MT gene in the leaves of *L. camara*; *L. camara* (control); *L. camara* treated with 25 µM, 50 µM, and 75 µM of $Cr(NO_3)_3$ after 3,7, and 14 days of treatment; Actin gene expression was used as a positive control**.**

4 Discussions

Lantana camara L. is recognized as one of the most invasive plant species globally, posing significant threats to agriculture and natural ecosystems [2]. Introduced for its ornamental value, it now thrives aggressively across tropical and subtropical regions [3, 4]. Its invasiveness is largely driven by potent allelopathic effects and strong competitive ability [5]. According to [6], the weed exploits stress-related metabolites produced by other plants to suppress competitors, while its allelochemicals directly slow the growth of surrounding vegetation. Chromium toxicity further impedes plant growth and development, prompting plants to secrete secondary metabolites that support survival under stress through complex molecular interactions [7].

In this study, leaves exhibited a gradual rise in polyphenol content with increasing chromium concentration, with 50 µM showing optimal production. Flavonoid accumulation displayed a similar, more pronounced trend. HPLC analysis confirmed elevated gallic acid (GA) and chlorogenic acid (CGA) levels under stress. Antioxidant activity peaked at 50 µM in leaves, whereas roots showed their highest activity at the same concentration. Lipid peroxidation (MDA) increased with treatment intensity, with 50 and 75 µM showing the highest levels. Proline accumulation also peaked at 50 and 75 µM after 3 days. Chlorophyll B declined more sharply than chlorophyll A. Gene expression analysis revealed increased PAL expression up to 50 µM before declining at 75 µM, while MT expression was highest at 25 and 75 µM treatments.

5 Conclusion

This study demonstrates that *L. camara* enhances the production of secondary metabolites to tolerate heavy metal–induced stress. It survived chromium exposure by increasing compounds such as chlorogenic and gallic acids, which also hold value in food, pharmaceutical, and processing industries. Although *L. camara* negatively impacts biodiversity through allelopathic effects, its strong phytochemical responses reveal potential for resource utilization. The observed rise in polyphenol and flavonoid levels with increasing metal concentrations indicates the threshold at which the plant maximizes defensive metabolite production. These stress-induced allelochemicals show considerable promise for various industrial applications, offering opportunities for sustainable upcycling.

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Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.
Author contribution statement: PT conceptualized, supervised and cross-checked the manuscript. All the authors contributed to the experiment, analysis and write-up of this work.

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