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Morphological and phytochemical responses of *Coleus Amboinicus* Lour. towards chromium toxicity

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Abstract
Chromium is a potential toxic heavy metal which does not have any essential metabolic function in plants. Chromium is a natural pollutant occurring in soil and water imposing adverse effects on plant growth. In addition to imparting growth retardation, many plants are capable of accumulating Cr ions from soil/water. *Coleus amboinicus* is an important medicinal plant extensively used in traditional and Ayurvedic medicines. Toxic effects of Cr on *C.amboinicus* cultivated in Hoagland medium artificially contaminated with 150µM $K_2Cr_2O_7$ resulted in growth retardation. Growth rate and Cr toxicity levels are assessed in terms of shoot length, root length, leaf area and Tolerance index. Secondary metabolites analysis using GC-MS and the impact of Cr on growth pattern and distribution of secondary metabolites are discussed.

Keywords: *Coleus amboinicus*, chromium, tolerance index, GC-MS, secondary metabolites

Introduction
Heavy metals are major inorganic environmental contaminants that are toxic to the living system. Heavy metals inclusive of chromium (Cr), are toxic to plants and impart morphological, physiological and molecular symptoms of toxicity (Pradhan *et al*., 2017). In addition to cytotoxic effects mutagenic and carcinogenic impacts also have been reported due to Cr (Sankar *et al*., 2005; Kovacik *et al*., 2013).
Chromium in the soil/water adversely affect growth and development of plants. Effect of Cr on plants include alterations in morphological (Moral *et al*., 1995; Samantara *et al*., 1996; Iqbal *et al*., 2001) [12, 5], biomass production (Sankar *et al*., 2005; Panda, 2007) [14], biochemical (Samantara *et al*., 1996; Sankar *et al*., 2005), bioaccumulation potential (Sankar *et al*., 2005; Yadav *et al*., 2005; Ratheeshchandra *et al*., 2010; Kowacik *et al*., 2013; Pradhan *et al*., 2017) [28], production of Reactive oxygen species (Sankar *et al*., 2004), production of enzymic and non-enzymic antioxidants (Panda *et al*., 2003; Rai *et al*., 2004) [16].
Coleus amboinicus Lour. is a potential medicinal herb belonging to the family Lamiaceae. It is distributed in tropical and warm regions of Asia, Africa, and Australia. *Coleus amboinicus* is a large fleshy succulent perennial herb with aromatic pubescence and inherent medicinal power due to the presence of phytochemicals such as flavonoids, esters, phenolics and terpenoids (Arumugam *et al*. 2016) [1]. According to those authors, these phytochemicals attribute antibacterial, antifungal, antioxidant, anti-inflammatory, analgesic, antiepileptic, allelopathic, antihelminthic and larvicidal, properties to the plant.
The objectives of the present study is the elucidation of growth and morphological adaptations of *C.amboinicus* towards the toxicity of Chromium. Since *C.amboinicus* is a medicinal plant containing large number of secondary metabolites (Arumugam *et al*., 2016) [1], effect of Cr toxicity on the distribution of secondary metabolites and resultant impact in the medicinal property of the plant is proposed to undertake.

Materials and Methods
Healthy twigs of *C.amboinicus*, approximately 15cm length consisting of about 8-10 leaves were collected from the Botanical Garden, SNGS College, Pattambi. Cuttings were grown in water for root initiation. Rooted propagules were grown in Hoagland nutrient medium artificially contaminated by 150µM $K_2Cr_2O_7$. This optimal concentration was determined by trial-and-error method by different concentrations of $K_2Cr_2O_7$ and 150µM of Cr is found to be the optimum concentration in which the plants survived but exhibited significant growth retardation. Rooted propagules of *C.amboinicus* were cultivated for 20 days and samples were collected at an intervals of 4 days and used for the studies.

Morphological measurements
Growth of plants were assessed in terms of root length, shoot length and leaf area. The sampled propagules were washed, blotted and length of root, shoot and leaf area were measured manually using a graduated scale and graph paper. Measurements of all parameters using minimum five propagules were recorded each sampling.

Tolerance index
Tolerance index percentage was calculated according to the method of Turner (1994) [27] comparing root length of experimental and control plants.

GC-MS analysis
Fresh leaves of *C.amboinicus* were collected after 20 days of growth then shade dried and powdered. Five g of powder was taken in duplicates which was subjected to extraction using Methanol in Soxhlet apparatus. After running several cycles, the extract obtained was concentrated and volume was noted and was used for GC-MS analysis. GC-MS was performed using Shimadzu GC-MS, with model number QP2010S, at Kerala Forest Research Institute, Thrissur, Kerala.

Result and Discussion

Morphologically significant variations were observed in *Coleus amboinicus* treated with Chromium (Fig-1). Data on root length revealed same pattern of stem growth inhibition (Table-1). Gradual increase in stem length was observed due to Cr treatment and the differences were insignificant compared to the control. More or less same trend was seen in all intervals, but on 20th day, inhibition of stem elongation was increased significantly. *Coleus amboinicus* exhibited growth retardation in terms of leaf area also due to Cr toxicity. Increase in leaf area was gradual during all intervals (Table-1).

Fig 1: Morphological variations of *Coleus amboinicus* (control) and chromium treated plants

Table 1: Effect of Cr on shoot length, Root length and leaf area in *C.amboinicus*

Parameters		Interval-days					
		0	4	8	12	16	20
Root length	Control	4.5±0.40	8.21±0.32	10.53±0.34	13.67±0.88	15.81±1.5	17.92±1.2
	Cr (150µM)	4.32±0.5	8.32±0.53	9.9±0.42	11.57±0.82	12.72±0.99	13.53±1.8
Shoot length	Control	17.82±1.3	20.21±1.2	24.53±2.4	27.67±1.8	30.81±2.5	35.92±3.4
	Cr (150µM)	18.32±1.2	19.32±1.3	21.9±2.2	25.57±2.2	27.72±1.9	30.53±1.8
Leaf area	Control	5.62±0.21	8.83±0.12	9.91±0.14	10.93±0.08	11.43±0.05	12.83±0.04
	Cr (150µM)	5.7±0.3	6.12±0.13	7.36±0.02	8.89±0.02	9.94±0.09	10.78±0.18

Tolerance index was calculated in terms of root length in the treatment and control. When compared to control a decreasing trend in Tolerance index was observed (Table-2). Only negligible difference was occurred in samples of all intervals.

Chronological increase in root length is more or less equal in control and treatments during the period of 20 days. Cr has been reported as a strong inhibitor of root growth up to very low concentrations (Iqbal, 2001; Sharma *et al*., 2016) [5, 21]. As a tolerance mechanism in plants, stress-induced root length reduces the region of exposure to metal stress (Miras-Moreno *et al*. 2014) [11]. The reduction in root length of plants on Cr treatment was also reported in *Cicer arietinum* (Medda and Mondal, 2017) [10] and *Citrus aurantium* (Shiyab, 2019) [23]. During the period of 8-20 days, root growth reduction is found to be more than the control indicating the role of time/period of exposure to Cr to induce more inhibition of root growth proportional to the period of exposure (Table 1). In plants chromium toxicity depends on exposure time and concentration (Shanker *et al*., 2005; Kovacik *et al*., 2013) [20].

In terms of stem length, growth retardation pattern of *C.amboinicus* treated with chromium is more or less similar to that of root length (Table 1) revealing the toxicity of Cr in the shoot system also. Since *C.amboinicus* is exposed to $K_2Cr_2O_7$, translocation of Cr take place from the root to shoot as opined by Santana *et al*.(2012) who stated that uptake of Cr is affected by the oxidation state as more accumulation of Cr occurs in the shoot when Cr (VI) is applied. Cr (VI) is more toxic than Cr (III) (Shanker *et al*., 2005) [20] and the Cr stress-induced decrease in shoot growth may be due to ultrastructural damage in leaf mesophyll cells, which impairs photosynthesis and ultimately results in decreased shoot development (Singh *et al*. 2021) [24]. The reduction in shoot length and leaf area has been reported in *Myriophyllum spicatum* (Chandra and Kulshreshtha, 2004) [2], *Helianthus annus* (Fozia *et al*. 2008) [4], *Allium cepa* (Nematshahi *et al*. 2012) [13], *Camellia sinensis* (Tang *et al*. 2012) [26] and *Citrus aurantium* (Shiyab, 2019) [23].

Cr (VI) is a highly toxic metal that inhibits various morphological, anatomical, physiological and metabolic activities resulting in impaired growth of plants (Srivastava *et al*. 2021) [25]. As reported in many plants which exhibited inhibitory effect on growth, *C.amboinicus* also showed growth inhibition in terms of root length, shoot length and leaf area due to Cr treatment. Leaf growth rate is known to act as an appropriate bioindicator of heavy metal toxicity in general and Cr in particular. Even though the growth rate of root, stem and leaf are not much significant stage to stage during a period of 20 days, general growth reduction was evident. So, it seems that 150µM Cr causes only slight inhibition on growth rate in *Coleus amboinicus*.

Table 2: Effect of Chromium on Tolerance Index in *C.amboinicus*

		Interval-Days					
		0	**4**	**8**	**12**	**16**	**20**
Tolerance index	Control	100	100	100	100	100	100
	Cr(150 µM)	100	86.59±0.13	79.27±0.02	74.61±0.02	72.83±0.09	69.32±0.18

Primary toxic effects of metals on plants is root growth inhibition and this parameter is an ideal index to measure the degree of tolerance (Wong and Bradshaw, 1982). *Coleus amboinicus* exhibit negligible changes in tolerance index since the concentration of Cr given to the plant permit the plants survival with negligible growth retardation. According to Majeed *et al*. (2019) [9], tolerance indexes based on root length are regarded as a good metric for determining how resilient plants are to heavy metal stress.

Qualitative and quantitative distribution of secondary metabolites in *Coleus amboinicus* exposed to Cr toxicity is given (Table-3). Twelve components such as 5-isopropyl-2-methylphenol, Tetradecane, Neophytadiene, Hexahydrofarnesylacetone, Methylpalmitate, alpha-linolenic acid methyl ester, Phytol, 1, 6-Nonadien-3-ol, 3, 7-dimethyl, gamma-Sitosterol, gamma-Tocopherol, Vitamin E and Squalene present in the methanolic extract of control plants. Methyl octadeca-9, 12-dienoate, Methyl commate a, Beta sitosterol, Alpha – amyrin, Phenol, 2-methyl-5-1-methylethyl and Neophytadiene were the newly formed secondary metabolites due to the chromium treatment which were absent in control plants.

5-isopropyl-2-methylphenol is the major component of *C.amboinicus* and is commonly known as Carvacrol and it possesses antiseptic and antimicrobial properties and is commonly used in traditional medicine for its therapeutic benefits. Carvacrol possess a wide range of bioactivities putatively useful for clinical applications such antimicrobial, antioxidant and anticancer activities (Ismail *et al*., 2016; Arumugam *et al*., 2016) [6, 1]. carvacrol, a monoterpenoid phenol found in essential oils, and its various biological properties, including antimicrobial, anti-inflammatory, and antioxidant activity. Carvacrol is often found in plants alongside its isomer, thymol (Maczka *et al*., 2023). Chromium treatment resulted in a significant reduction of carvacrol.

Methyl commate A is a newly formed secondary metabolites in *C.amboinicus* treated with Cr. This compound possess several pharmacological activities, including analgesic, anti-inflammatory, and antitumor effects and has also been used for neurological disorders such as Alzheimer's disease and Parkinson's disease (Li *et al*., 2016). Another newly formed compound after Cr treatment is Alpha-amyrin which possesses several biological activities, including anti-inflammatory, antitumor, and antidiabetic effects (Sautour *et al*., 2017). Neophytadiene also present in plants treated with Cr and it is characterized by anti-inflammatory and anti-tumor properties, which is used in the treatment of certain diseases (Huang *et al*., 2015).

Due to chromium treatment, Tetradecane, Neophytadiene, Hexahydrofarnesylacetone, alpha-linolenic acid methyl ester, Vitamin E, Squalene, 1, 6-Nonadien-3-ol, 3, 7-dimethyl and gamma.-Sitosterol were absent (Table 3) which also possess vital for medicinal properties like

Table 3: GCMS analysis of *Coleus amboinicus*

Compounds	Control	Chromium
5-isopropyl-2-methylphenol	55.88	17.53
Tetradecane	1.47	
Neophytadiene	2.55	
Hexahydrofarnesylacetone	1.33	
Methylpalmitate	2.24	4.62
alpha-linolenic acid methyl ester	1.52	
Phytol	16.79	6.58
1,6-Nonadien-3-ol, 3, 7-dimethyl	2.02	
gamma.-Sitosterol	4.07	
gamma.-Tocopherol	2.01	9.78
Vitamin E	1.02	
Squalene	9.09	
Methyl octadeca-9, 12-dienoate		5.38
Methyl commate a		18.72
Beta sitosterol		8.66
Alpha - amyrin		5.7
Phenol,2-methyl-5-1-methylethyl		13.34
Neophytadiene		9.96

Conclusion

Concentration of Cr at 150µM induces only negligible growth retardation of *C.amboinicus*. Treatment with Cr results in a significant reduction of many secondary metabolites and production of some new metabolites possessing medicinal properties maintaining the medicinal potential of the plant intact.

References

1. Arumugam G, Swamy MK, Sinniah UR. *Plectranthus amboinicus* (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance. Molecules,2016:21:369.
2. Chandra P, Kulshreshtha K. Chromium accumulation and toxicity in aquatic vascular plants. Bot. Rev,2004:70:313–327.
3. Dey U, Mondal NK. Ultrastructural deformation of plant cell under heavy metal stress in gram seedlings. Cogent Environ.Sci,2016:2:1.
4. Fozia A, Muhammad A, Muhammad A, Zafar MK. Effect of chromium on growth attributes in sunflower (*Helianthus annuus* L.). J. Environ. Sci,2008:20:1475–1480.
5. Iqbal MZ, Saeeda S, Muhammed S. Effects of chromium on an important arid tree (*Caesalpinea pulcherima*) at Karachi city, Pakistan.Ekol. Bratislava,2001:20:414-22.
6. Ismail M, Al Naqeeb G, Huq F. Antimicrobial and antioxidant activities of thymol from *Thymus vulgaris* L. J. basic appl. Sci,2016:12(1):25-30.

7. Kováčik J, Babula P, Klejdus B, Hedbavny J. Journal of Agricultural and Food Chemistry,2013:61(33):7864-7873.
8. Kakkalameli SB, Daphedar A, Hulakoti N, Patil BN, Taranath TC. Azolla filiculoides lam as a phytotool for remediation of heavy metals from sewage. Int. J. Pharm,2018:8:282–287.
9. Majeed A, Muhammad Z, Siyar S. Assessment of heavy metal induced stress responses in pea (*Pisum sativum* L.). Acta Ecologica Sinica,2019:39:284–288.
10. Medda S, Mondal NK. Chromium toxicity and ultrastructural deformation of *Cicer arietinum* with special reference of root elongation and coleoptile growth, Annals of Agrarian Science,2017:15:396-401.
11. Miras Moreno B, Almagro L, Pedreño MA, Ferrer MA editors. Accumulation and tolerance of cadmium in a non-metallicolous ecotype of *Silene vulgaris* Garcke (Moench). Anales de Biologı´a: Servicio de Publicaciones de la Universidad de Murciam, 2014.
12. Moral R, Pedreno JN, Gomes I, Mataix J. Effects of Chromium on the nutrient element content and morphology of tomato.*J.Plant Nutri*,1995:18:815-822.
13. Nematshahi N, Lahouti M, Ganjeali A. Accumulation of chromium and its effect on growth of (*Allium cepa* cv. Hybrid). Eur. J. Exp. Biol,2012:2:969–974.
14. Panda SK. Chromium- mediated oxidative stress and ultra-structural changes in root cells of developing rice seedlings. Plant physol,2007:164:1419-1428.
15. Rai V, Mehrotra S. Chromium induced changes in ultramorphology and secondary metabolites of *Phyllanthus amarus* schum and Thonn-A hepatoprotective plant. Environ. Monitoring and Asses,2008:147(1-3):307-15.
16. Rai V, Vajpayee P, Singh SN, Mehrotra S. Effect of Chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiiflorm* L. Plant Sci,2004:167:1159-1169.
17. Ratheesh Chandra P, Abdussalam AK, Nabeesa Salim, Puthur JT. Distribution of bio accumulated Cd and Cr in two Vigna species and the associated histological variations. Stress physiol. Biochem,2010:6:4-12.
18. Samantary S, Rout GR, Das P. A study of soil plant and root-shoot relationship in rice (Oryza sativa L. Cv Pathan) grown on chromiferous mine soil. Proc.Natl.Acad.Sci.USA,1996:66:349- 357.
19. Santana KB, de Almeida AAF, Souza VL, Mangabeira PAO, Silva DdC, Gomes FP, *et al*. Physiological analyses of *Genipa americana* L. reveals a tree with ability as phytostabilizer and rhizofilter of chromium ions for phytoremediation of polluted watersheds. Environ.Exp.Bot,2012:80:35-42.
20. Shanker AK, Cervantes C, Loza Tavera H, Avudainayagam S. Chromium toxicity in plants. Environ.Intl,2005:31:739-753.
21. Sharma P, Kumar A, Bhardwaj R. Plant steroidal hormone epibrassinolide regulate-Heavy metal stress in Oryza sativa L. by modulating antioxidant defense expression.Environ.Exp.Bot,2016:122:1- 9.
22. Sharma P, Kumar A, Bhardwaj R. Plant steroidal hormone epibrassinolide regulate-Heavy metal stress in Oryza sativa L. by modulating antioxidant defense expression.Environ.Exp.Bot,2016:122:1- 9.
23. Shiyab S. Morphophysiological Effects of Chromium in Sour Orange (Citrus aurantium L.). HortScience,2019:54:829–834.
24. Singh D, Sharma NL, Singh CK, Yerramilli V, Narayan R, Sarkar SK, *et al*. Chromium (VI)- Induced Alterations in Physio-Chemical Parameters, Yield, and Yield Characteristics in Two Cultivars of Mungbean (*Vigna radiata* L.). Front. Plant Sci,2021:12:735129.
25. Srivastava D, Tiwari M, Dutta P, Singh P, Chawda K, Kumari M, *et al*. Chromium Stress in Plants: Toxicity, Tolerance and Phytoremediation. Sustainability,2021:13:4629.
26. Tang J, Xu J, Wu Y, Li Y, Tang Q. Effects of high concentration of chromium stress on physiological and bio-chemical characters and accumulation of chromium in tea plant (*Camellia sinensis* L.). Afr. J. Biotechnol,2012:11:2248–2255.
27. Turner AP. The responses of plants to heavy metals. In: S.M. Ross (Ed.). Toxic Metals in Soil-Plant Systems. John Wiley & Sons Ltd, 1994, 154-187.
28. Yadav S, Sukla OP, Rai UN. Chromium pollution and bioremediation. Environ.News Arch,2005:11:1-4.