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*Research Article*

# CHROMIUM TOXICITY AMELIORATED BY 24-EPIBRASSINOSTEROIDS IN CORIANDRUM SATIVUM

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## Abstract

Cr (VI) stress poses a significant challenge to plant growth and development, resulting in reduced yields and economic losses in agriculture globally. Coriander is the oldest spice of the world. It is basically a leafy vegetable rich in protein, vitamins and dietary products. 24-Epibrassinosteroids (BRs) have been identified as regulators of stress responses, by associating with the expression of genes associated with stress. This study aimed to investigate the effects of 24-Epibrassinosteroid on Chromium (VI) toxicity in (Coriandrum sativum) plant. An experiment is designed to study the effect of Antioxidant hormone 24-epibrassinosteroid to eliminate the toxicity of chromium (50 µmol and 100 µmol). The experiment consisted of two varieties (Dhania and coriander) under Cr stress. The findings revealed that the application of 24-Epibrassinosteroids mitigate the negative impacts of heavy metal chromium on plant growth. It resulted in significant improvements in various parameters related to plant growth, yield and biochemical processes. Overall, the result suggested that the application of 24-Epibrassinosteroid could effectively alleviate the detrimental effects of heavy metal stress, ultimately enhancing the growth and productivity of coriander plants. Molecular docking presented that there is strong affinity between 24-epibrassinosteroid (24-Ebr) and defense protein (1-Psd) with four hydrogen bonds observed through autodock Vina. This prediction shows improved defense mechanism in peas with the application of brassinosteroid to cope with chromium stress which needs to be farther investigated.

**Keywords:** Heavy metal stress, Negative consequences of Cr exposure, Brassinosteroid E, Docking, Coriander plant

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## Introduction

Due to rapid urbanization and industrialization, there has been a significant surge in the usage of heavy metals. Industrial effluents, carrying hazardous heavy metals, are causing irreparable destruction in the ecosystem. Among these metals, Chromium (Cr) stands out as it is abundant in industrial discharges, mainly existing in dichromate and chromate ions, and is widely considered to possess high toxicity (Desmarias and Costa, 2019). The oxidative states of chromium range from Cr (II) to Cr (IV), but the trivalent and hexavalent forms of the metal are most frequently found, are extremely stable, and have significant impacts on plants (Jamil *et al.*, 2022). Chromium significantly hinders plant growth and development. Suwalsky *et al.* (2008) identified that the concentration of chromium above 0.05 mg/L in plants is hazardous for human health. Chromium-induced oxidative stress leads to lipid peroxidation, causing severe cell membrane damage (Panda and Choudhury, 2005). Heavy metals, including chromium, are responsible for severe plant ailments. For example, oxidative stress lowers overall plant development and initiates the breakdown of chemicals related to photosynthesis. As chromium concentration exceeds the allowable limit, the chloroplast structure is affected.

Coriander is the oldest spice of the world. It is frequently grown in China, Morocco, India, Pakistan, Turkey, Myanmar, Romania, France, Italy, Spain and extent in the UK and USA (Sharma & Sharma, 2012). Over the past decade, extensive research has been conducted on three plant hormones, namely auxins, abscisic acid, and Brassinosteroids. Their roles have been elaborated in in plant growth, development, and stress alleviation (Ali *et al.* 2020). The well-known class of steroidal lactones known as Brassinosteroids (BRs) provides plants with resilience to a variety of biotic and abiotic stresses (Bukhari *et al*., 2016). Brassinosteroids are frequently used as a modulator to reduce the harmful effects of heavy metals on plants (Ahanger *et al*. 2018). Molecular study is one of the key tools to assess the affinity of applied treatment as ligand with proteins involved in defense mechanisms against biotic or abiotic stress (Ferreira et al., 2015). Defensin protein family and defensin like peptide molecules have been reported to play crucial role in biological and biotechnological applications in plants under stressful conditions (Carvalho & Gomes, 2011). Hence this study uses defensing proteins to assess the role of 24-epibrasinosteroid in mitigating the effect of heavy metals. The goal of the current study was to assess and characterize the detrimental effects of chromium as well as the corresponding ameliorative effects of 24-epibrassinolide in terms of growth, physiological characteristics, nutrient uptake, antioxidant defense, and glycosylate system in coriander plants.

## Materials and Methods

### Seed Germination Experiment

A field experiment was carried out at Seed Center, University of the Punjab, Quaid e Azam campus, Lahore, during 2022-23. Seeds of two cultivars i.e., Dhania and Coriander were subjected to two different chromium stress levels (50 µM and 100 µM) in a factorial design with randomized complete block arrangement with three replications. The experiment was conducted in 12-inch pots weighing around 0.35Kg and accommodating 4.28Kg of garden soil. The stress treatment was applied 7-10 days after emergence to avoid any confounding effects of poor germination rate.

### Foliar application of 24-Epibrassinolids

A solution of 0.01% 24-Epibrassinolide was applied as topical spray. The plants under heavy metal stress received two doses of 24-Epibrassinosteroids. However, the control plants, did not receive any 24-epibrassinosteroids. The first dose was applied to the stressed plants 20 days after sowing, while the second dose was administered after 40 days of flowering.

### Growth Parameters and Biochemical Analysis

After foliar application of 24-Epibrassinolide various growth parameters such as 1$^{st}$ cutting fresh weight, 1$^{st}$ cutting dry weight, 2$^{nd}$ cutting fresh weight, 2$^{nd}$ cutting dry weight, number of leaves, root length, shoot length etc. were measured and recorded carefully. Similarly, biochemical analysis such as chlorophyll content, total soluble protein, fats, ash percentage, free nitrogen extract percentage were analysed for each plant and its replicate.

### Protocol for pigment content

Fresh leaf (0.5 g) from each experimental pots was crushed into small pieces with the help of pestle and mortar and added into the test tubes containing 10 ml of 80% acetone Aluminium foil was placed over test tubes to stop acetone from vaporizing. The samples were incubated at room temperature for 48 hours and were examined using a spectrophotometer after 48 hours. For chlorophyll a, chlorophyll b, and carotenoids, the colour intensity of each sample was measured at 663, 645, and 480 nm, respectively. The values were established using the Arnon, 1949, equation**.**

Chlorophyll content was determined from following formula

$$Chlorophyll\ a\ (mg\ g^{-1}) = 12.21\ (A663) - 2.81(A665)$$

$$Chlorophyll\ b\ (mg\ g^{-1}) = 20.13\ (A665) - 5.03(A663)$$

The carotenoid content was measured by recording the absorbance of sample at 480nm wavelength. Following equation was followed

$$Carotenoids\ (mg\ g^{-1}) = \frac{[1000(A480) - 3.27(Chl\ a) - 104(Chl\ b)}{227}$$

### Antioxidant activity

The gathered seeds were ground into a fine powder, and 2 g of that powder was agitated for 48 hours at room temperature in methanol (85%). The resulting extracts were then put through a filtering process using a funnel and filter sheets. The filtrates were concentrated under reduced pressure and at a temperature of 40 °C until dry. To obtain 100 mg per ml of stock solution, the extracts were re-suspended in methanol (the appropriate solvent). The DPPH (1.1-diphenyl-2-picryl-hydrazil) reagent was used to measure the activity of free radical scavenging according to the procedure described in Brand-Williams et al. (1995). Coriander seed extract was dissolved in methanol (85%, v/v), and 0.5 mL of the dissolved extract was added to 1.0 mL of freshly made DPPH solution (20 g/mL). The mixture was then agitated. Discoloration processes were measured at 517 nm against a blank control for 5 minutes after the reaction started.

$$Antioxidant\ activity = 100\ \times [(Contrrol - sample\ absorbance)]$$

### Proximate

The proximate analysis was carried out to determine the nutritional status of coriander leaves under heavy metal stress and to compare the nutritional improvement imparted due to the application of 24-Epibrassinosteroids. This analysis was done from the Food and Nutrition Laboratory of University of Veterinary and Animal Sciences, Lahore.

### Statistical analysis

Analysis of variance (ANOVA) was done to estimate the significant effects by using IBM SPSS Statistics 20. Duncan Multiple Range Test (DMRT) were computed to compare and rank different treatments. Principle component analysis was performed to determine the interrelationship among the parameters and treatments. Visual representation of data in the form of graphs was designed by using ORIGIN PRO 2023 and GRAPH PAD PRISM 8.0.1.

### Molecular docking:

Molecular docking is an effective tool to identify the best compound fit for protein (receptor) based on molecular docking energy. Autodock Vina extension of AMDock software was utilized for this purpose (https://github.com/Valdes-Tresanco-MS). Ligand i.e. 24-Epibrassinosteroids, ID_24-EBR, was retrieved from Pubchem and the receptor protein *Pisum sativum* defensin-1 (ID_IJKZ), was retrieved from protein data band (https://www.rcsb.org/structure/Psd). Both the structures were prepared and visualized for docking on PyMol software (https://pymol.org/) and pdbqt formats of these structures were utilized for docking through autodock Vina. Biovia Discovery studio was used for visualization of 3D and 2D receptor-ligand interactions. The bonds and interacting chains were observed from Protein-ligand interaction profiler (PLIP) (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index).

## Results and Discussion

Increased chromium levels, particularly hexavalent chromium (Cr(VI)), can inhibit plant growth. The most commonly apparent adverse effects can be stunted growth, reduced leaf size, and overall poor development of coriander plants. The number of seedlings emerged in control plants were significantly higher compared to chromium (Cr) stressed plants, as shown in Table 1. Chromium has been reported to have a negative impact on physiological and biochemical processes, hindering normal plant growth and development (Gill et al., 2015; Shanker et al., 2005; Yu et al., 2007). However, exogenous application of 24-Epibrassinosteroids enhances seedling emergence and growth in *Coriandrum Sativum* L. plants by regulating their metabolic system. Plants develop tolerance to metal stress through the activation of antioxidant systems, plant hormones (Kellős et al., 2008), phytochelatins (Diwan et al., 2010), and other biomolecules that protect against stress (Andre et al., 2010). As shown in Table 1, control plants emerge earlier than treated plants.

Plant height (cm) also showed a significant increase at control as compared to both stress levels (Table 1). Maximum plant height with exogenous application of 24-Epibrassinosteroids could be attributed to nutrient supply. Plant height may have increased due to an increase in internodal distance with the 24-Epibrassinosteroids application. Chromium toxicity can disrupt various physiological processes in coriander. This includes impaired nutrient uptake, and water balance, leading to overall weaker and less healthy plants. Furthermore, ( Abbaspour et al., 2020) and (Efimova et al., 2014) found that exogenous application increased plant height significantly. It could be because 24-Epibrassinosteroids plays a role in metabolic activity and physiological reactions, acting as a catalyzing enzyme in the transformation of carbohydrates, chlorophyll, and protein synthesis. The results are an analogy to the finding of Zafari et al. (2020)).

**Table 1: Means and standard error of day of emergence, germination percentage and plant height (cm) with control, Cr 50 µM and Cr 100 µM for foliar application.**

| | Treatments | Day to emergence | No. of seed germination | Plant Height (cm) |
|---|---|---|---|---|
| Foliar Application | T1 | $8.0^{a} \pm 3.74$ | $13.8^{a} \pm 1.32$ | $44.1^{a} \pm 3.89$ |
| | T2 | $14.0^{b} \pm 1.23$ | $6.82^{b} \pm 0.91$ | $26.0^{b} \pm 1.66$ |
| | T3 | $17.3^{c} \pm 0.95$ | $4.69^{c} \pm 0.76$ | $18.6^{c} \pm 1.62$ |

Different lowercase letter on each mean value shown the significant effect of each treatment on day of emergence, germination percentage and plant height.

### Shoot length (g) and Fresh and dry shoot weight (g) of 1st cutting

Data indicates that both heavy metal stress levels exhibit a considerably negative impact on shoot length and weight of Coriander plant compared to the control (Table 1.2). The alleviation of Cr-induced phytotoxicity in mustard (Diwan et al. 2), radish (Choudhary et al. 2012 and Sharma et al. 2011), rice (Sharma et al. 2023), tobacco (Bukhari et al. 2016), is reported in literature. These research reports provide evidence of the harmful effects of chromium (Cr) on plant growth, revealing that the decline in growth is linked to intense competition among transporters responsible for uptake of both Cr (in its trivalent and hexavalent forms) and essential ions like iron (Fe) (Shanker et al. 2005). The exogenous application of 24-Epibrassinosteroids mitigate the stress. It has ability to enhance plant growth can be due to its significant processes involved in cell division and cell elongation such as increased activity of enzymes related to loosening of cell wall (xyloglucan transferase/hydrolase, XTH) via the over-expression of xyloglucan endo-transglycosylase gene (Leubner-Metzger, G. 2003).

## Antioxidant activity

Exposure to heavy metals in coriander plants triggered a cascade of detrimental effects, including the disruption of cellular structures, the activation of stress responses (notably oxidative stress), and ultimately, cell death, whereas control plants remain unaffected (Table 3). Heavy metal stress has a deleterious impact on plants (Alzahrani et al. 2018; Desoky et al. 2019; Rady et al. 2019b). Exogenous Br treatments, however, reduced the impacts of heavy metal stress by raising endogenous Br concentrations and lowering the concentrations of heavy metals in several plant sections (Rady et al. 2016).

Fresh and dry root weight (g) also showed a significant increase at control as compared to T2 and T3 (Table 3) in plants under metal stress, EBR promotes mitotic activity in the apical meristem of roots which improves seedling establishment (Yusuf et al. 2012). Similar experiments showing the beneficial effects of 24-EBR on growth in Cr-stressed plants (Hayat et al. 2009).

**Chlorophyll a, Chlorophyll b (mg/g) and carotenoid (mg/g)**:

Exogenous application of 24-Epibrassinoteroids significantly enhanced the chlorophyll and carotenoid content in coriander leaves (Table 4).

**Table 2 Means and standard error of shoot length, 1st cutting fresh weight and 1st cutting dry weight with control, Cr 50 µM and Cr 100 µM for foliar application.**

| | Treatments | Shoot length (cm) | 2nd cutting fresh weight (g) | 1st cutting dry weight (g) |
|---|---|---|---|---|
| Foliar Application | T1 | $22.4^{a} \pm 1.44$ | $6.01^{a} \pm 0.62$ | $0.57^{a} \pm 0.06$ |
| | T2 | $13.6^{b} \pm 1.90$ | $2.85^{b} \pm 0.34$ | $0.23^{b} \pm 0.02$ |
| | T3 | $7.98^{c} \pm 1.18$ | $1.96^{c} \pm 0.26$ | $0.13^{c} \pm 0.03$ |

Different lowercase letter on each mean value shown the significant effect of each treatment on shoot length, 1st cutting fresh weight (g) and 1st cutting dry weight.

The increase in chlorophyll content is due to an increased area providing more surface area for photosynthesis. Siddiqui et al. (2019) stated that it can be due to Brassinolides being involved in photosynthesis, assimilation, and translocation of photosynthates from source to sink. High levels of chromium can cause symptoms on coriander leaves, such as yellowing, necrotic spots, or leaf curling. These symptoms indicate stress and damage to the plant tissues and ultimately lower yield.

**Dry matter (%), Protein content (%)**

**Dry matter (%), Protein content (%)**:

An increase in dry matter production was observed in response to T2 and T3 over control (Table 5). An increase in dry matter production can be due to the movement of dry matter from source to sink in response to the application of micronutrients (Singh *et al*., 2023). Our results are also in line with the findings of Al-Maliki & Al Shammari, (2019)) who stated that a significant increase could be attributed to the role of Brassinolides, iron, and NPK in improved shoot and root development which leads to increased dry matter production. 24-Epibrassinosteroids is an essential part of several enzymes and plays role in nitrogen uptake, leading to better amino acid and protein production in plants. A significant increase in protein content was analysed in the control (Effimova et al., 2014). While in response to the sole and exogenous application of 24-Epibrassinosteroids, a significant decrease in protein content was observed.

Analysis of variance (Table 6) showed that there were significant variations among cultivars and effect of treatments was observed to be significant as well for all plant growth and biochemical attributes except for day of emergence (Figure 1b & 1c). where coriander cultivar outperformed dhania cultivar among all vegetative and biochemical attributes. The findings of our results were in analogy to the findings of Abbaspour et al. (2020) and Cakmak et al. (2010).

Using Pearson correlation analysis (Figure 1a) a heat map was generated to present the positive or negative relationship between the plant growth and biochemical parameters in response to the application of 24-Epibrassinosteroid. It demonstrated that an increase in chlorophyll concentration led to considerable rise coriander parameters. All the parameters were positively correlated except for antioxidant which negatively correlated with growth indices as well as chlorophyll contents.

**Table 3. Means and standard error of fresh root weight, dry root weight and Antioxidant activity with control, Cr 50 µM and Cr 100 µM for foliar application.**

| | Treatments | Fresh root weight (g) | Dry root weight (g) | Antioxidant activity |
|---|---|---|---|---|
| Foliar Application | T1 | $2.01^{a} \pm 0.17$ | $0.74^{a} \pm 0.10$ | $1.17^{a} \pm 0.50$ |
| | T2 | $1.43^{b} \pm 0.07$ | $0.44^{b} \pm 0.01$ | $2.35^{a} \pm 0.33$ |
| | T3 | $1.29^{c} \pm 0.04$ | $0.25^{c} \pm 0.07$ | $3.70^{b} \pm 0.45$ |

Different lowercase letter on each mean value shown the significant effect of each treatment on fresh root weight, dry root weight and Antioxidant activity.

**Table 4. Means and standard error of chlorophyll a, chlorophyll b and carotenoid with control, Cr 50 µM and Cr 100 µM for both foliar applications.**

| | Treatments | Chlorophyll a (mg $g^{-1}$) | Chlorophyll b (mg $g^{-1}$) | Carotenoid (mg $g^{-1}$) |
|---|---|---|---|---|
| Foliar application | T1 | $1.88^{a} \pm 0.23$ | $1.17^{a} \pm 0.02$ | $0.44^{a} \pm 0.93$ |
| | T2 | $0.60^{b} \pm 0.78$ | $0.10^{b} \pm 0.09$ | $0.28^{b} \pm 0.03$ |
| | T3 | $0.30^{c} \pm 0.46$ | $0.45^{c} \pm 0.19$ | $0.24^{bc} \pm 0.15$ |

Different lowercase letter on each mean value shown the significant effect of each treatment on chlorophyll a, chlorophyll b and carotenoid.

**Table 5. Means and standard error Dry matter %, Crude protein %, Crude fiber %, Fat % and Ash % with control, Cr 50 µM and Cr 100 µM for foliar application.**

| | Treatments | Dry Matter % | Crude Protein % | Crude Fiber % | Fat % | Ash% |
|---|---|---|---|---|---|---|
| Foliar application | T1 | $85.14^{a} \pm 2.77$ | $20.01^{a} \pm 1.53$ | $28.0^{a} \pm 6.4$ | $8.90^{a} \pm 1.58$ | $17.0^{a} \pm 1.42$ |
| | T2 | $63.72^{b} \pm 3.65$ | 17.61b ± 1.70 | $19.8^{b} \pm 3.18$ | $5.98^{b} \pm 0.88$ | $15.7^{b} \pm 1.24$ |
| | T3 | $48.81^{c} \pm 2.91$ | $15.54^{c} \pm 1.84$ | $18.2^{bc} \pm 3.00$ | $3.68^{c} \pm 1.13$ | $13.9^{c} \pm 1.36$ |

Different lowercase letter on each mean value shown the significant effect of each treatment on Dry matter %, Crude protein %, Crude fiber %, Fat % and Ash %. The results were given by University of Veterinary Sciences, Lahore.

**Table 6. Analysis of Variance to estimate probability of F for vegetative and reproductive parameters of coriander after foliar application of brassinolides.**

| Traits | Cultivar | Treatment | Cultivar× Treatment |
|---|---|---|---|
| **Day of emergence** | 0.01* | 0.00* | 0.55* |
| **No of seed germination** | 0.00* | 0.00* | 0.07* |
| **Plant height (cm)** | 0.00* | 0.00* | 0.00* |
| **1st cutting fresh weight (g)** | 0.00* | 0.00* | 0.01* |
| **1st cutting dry weight (g)** | 0.02* | 0.00* | 0.12* |
| **Shoot length (cm)** | 0.00* | 0.00* | 0.03* |
| **Chlorophyll a (mg/g$^{-1}$)** | 0.00* | 0.00* | 0.00* |
| **Chlorophyll b (mg/g$^{-1}$)** | 0.00* | 0.00* | 0.01* |
| **Carotenoid (mg/g$^{-1}$)** | 0.09* | 0.00* | 0.13* |
| **Antioxidant activity** | 0.00* | 0.00* | 0.03* |
| **Second cutting fresh weight (g)** | 0.00* | 0.00* | 0.01* |
| **Second cutting dry weight (g)** | 0.01* | 0.00* | 0.10* |
| **Fresh root weight (g)** | 0.01* | 0.00* | 0.00* |
| **Dry root weight (g)** | 0.00* | 0.00* | 0.18* |

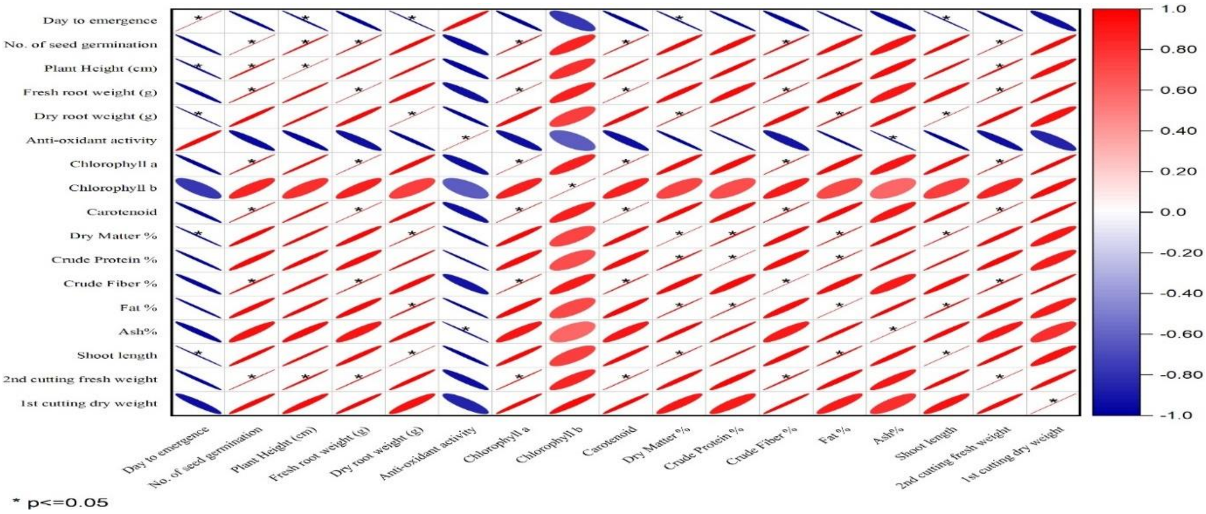


**Figure 1a. Graphical Illustration of correlation among vegetative and agronomic parameters in *Coriandrum sativum* L. by correlation heatmap.**

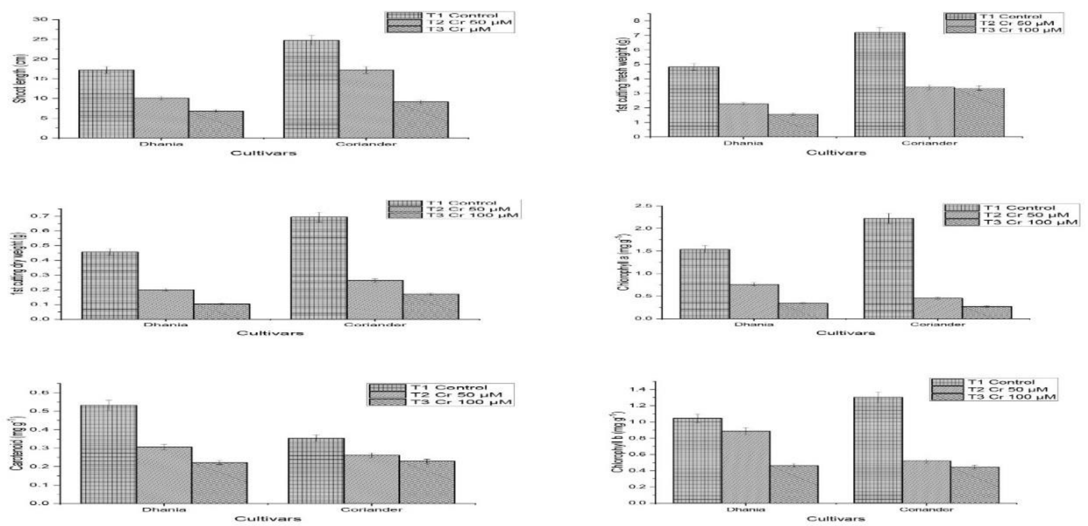


**Figure 1b. Graphical Illustration of effect of applied treatments on shoot length (cm), 1st cutting fresh and dry weight (g), chlorophyll a, b and carotenoid contents (mg g$^{-1}$) in cultivars (Dhania and Coriander) of *Coriandrum sativum*.**

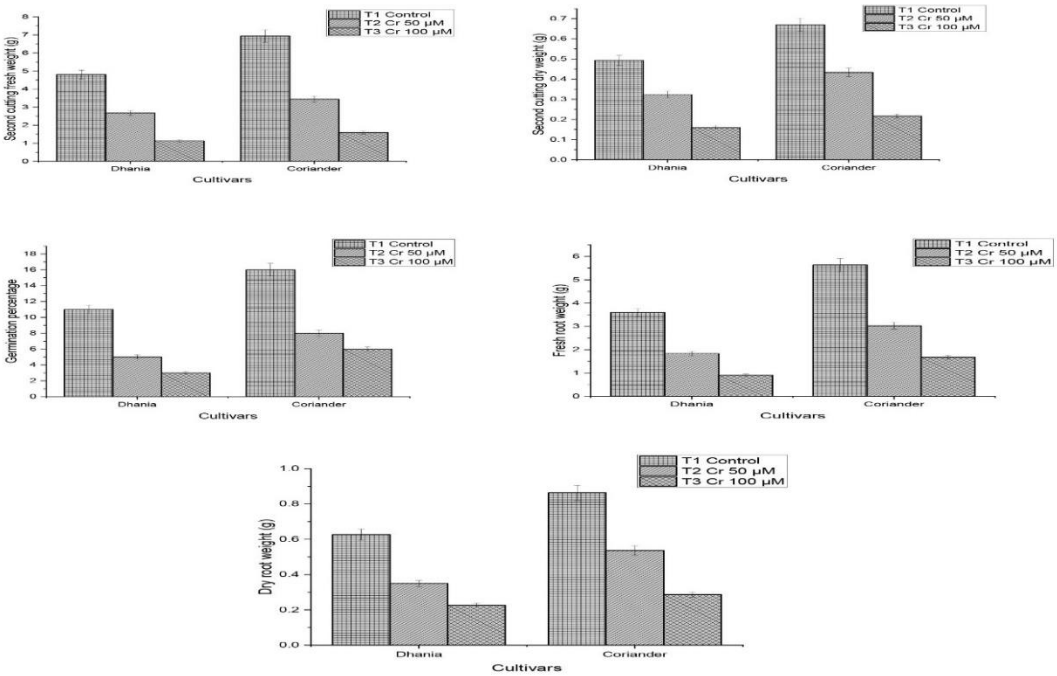


**Figure 1c. Graphical Illustration of effect of applied treatments on second cutting fresh and dry weight (g), germination percentage (%), Fresh root weight (g), and dry root weight (g) in cultivars (Dhania and Coriander) of *Coriandrum sativum*.**

## Molecular docking results

The ligand molecule, 24-PBR, exhibited a string binding affinity with receptor protein 1Psd which is a defense protein reported in *Pisum sativum* L. against abiotic stress. The binding energies of these compounds were -2.8 kcal/mol, at binding sites 2 and 8 respectively, indicating that the ligand-protein complex had higher binding energies than other compounds. The cartoon view, 3D view, and the surface view of these docking complexes are shown in Figure 2A–E Four hydrogen, ten van der waals bond, two pi-alkyl, one conventional carbon-hydrogen and one pi-sulphur bond was observed at various amino acids as shown in Figure 3. Hydrogen bonds were observed at ALA A:7, HIS A:S, THR A:34, and UNK (Table 7). These findings are supported by the Kumar et al. (2019) suggesting that the overexpression of 1-Psd proteins in *Pisum sativum* L. regulate the production of antioxidant enzyme which help to mitigate the effects of abiotic stress

## Conclusion

The study revealed that the Cr toxicity had a deleterious effect on the plant growth, exerting negative influences on the plant physiology and including chemical alternation in response to the Cr stress. However, the application of 24-epibrassinosteroids proved highly effective in mitigating this stress, leading to a significant increase in the growth rate. Thus, the promising results obtained from this study paved the way for the future utilization of 24-epibrassinosteroids. Molecular docking of 24-Ebr with 1-Psd showed that there is strong affinity of 24-epibrassinosteroids with defensin proteins in plants. Hence, it is strongly suggested that researchers should focus on studying the molecular patterns of effect of 24-epibrassinosteroids on expression patterns of proteins involved in defense against heavy metals in coriander.

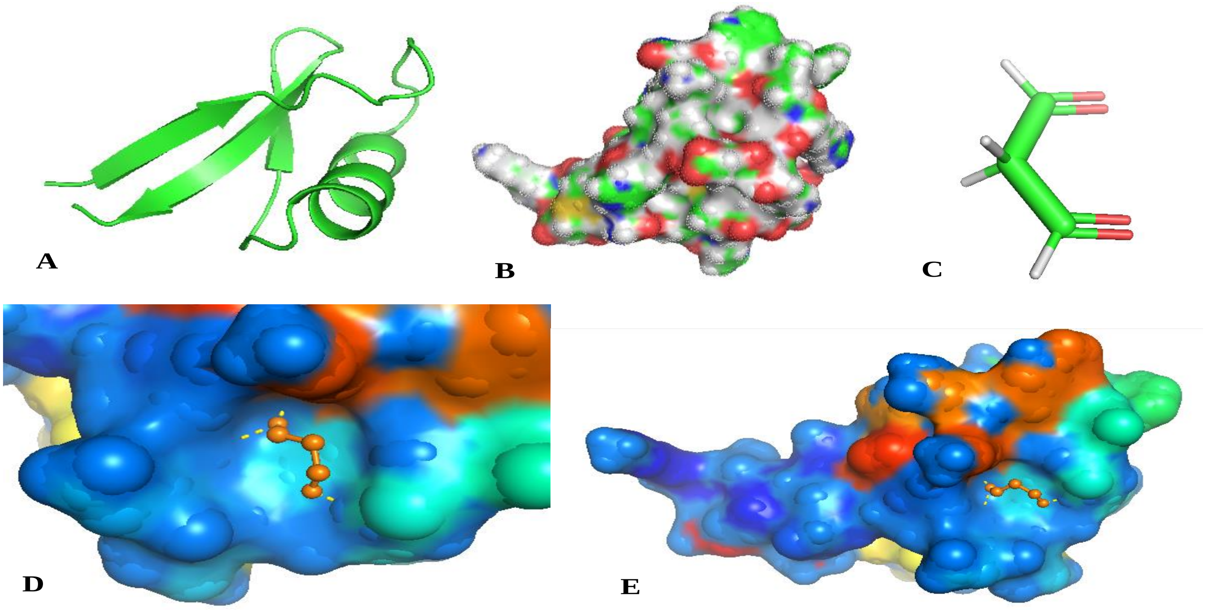


**Figure 2. Visualization of protein (ID_IJKZ) (A) cartoon view (B) Surface view (C) Ligand (D) Closer view of ligand (orange-dotted) and Protein (Surface view) interaction**

**Table 7. Hydrogen bonds between amino acids and ligand**

| Hydrogen Bonds | | | | | |
|---|---|---|---|---|---|
| **RESNR** | **RESTYPE** | **RESCHAIN** | **DIST** | **LIGCOO** | **PROT COO** |
| 10 | ALA | A | 3.66 | -2.166, 0.693, -8.833 | 1.249, 0.834, -7.528 |
| 39 | THR | A | 3.75 | -2.166, 0.693, -8.833 | -3.913, -2.535, -8.084 |
| 12 | HIS | A | 2.52 | -1.050, 2.708, -9.599 | 1.971, 2.329, -10.723 |
| 40 | UNK | | 1.96 | -1.979, 0.870, -6.419 | -1.211, -1.340, -4.672 |

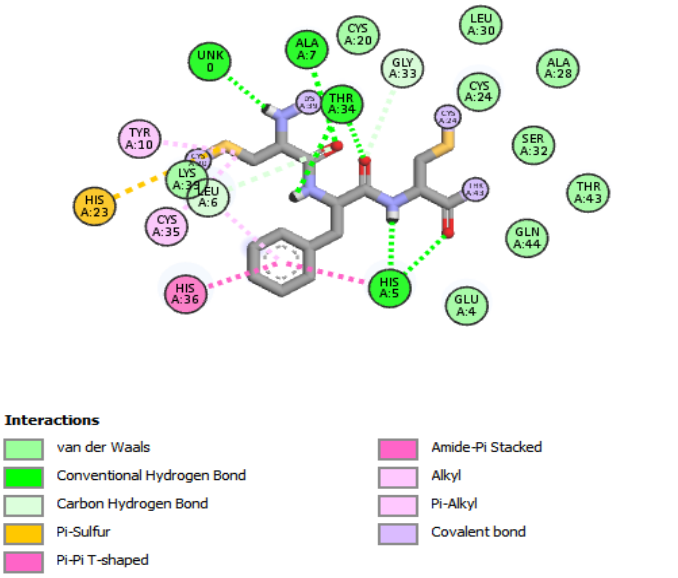


**Figure 3. Visualization (2-D) of 24-PBR (Ligand) and 1-Psd (Protein receptor ID_IJKZ) docking via. Discovery studio.**

Target: Protein

| Binding Site | Affinity(kcal/mol) | Estimated Ki | Ki Units | Ligand Efficiency |
|---|---|---|---|---|
| 2 | -2.8 | 8.86 | mM | -0.56 |
| 8 | -2.8 | 8.86 | mM | -0.56 |
| 1 | -2.5 | 14.71 | mM | -0.50 |
| 3 | -2.5 | 14.71 | mM | -0.50 |
| 4 | -2.5 | 14.71 | mM | -0.50 |
| 6 | -2.5 | 14.71 | mM | -0.50 |
| 5 | -2.3 | 20.61 | mM | -0.46 |
| 7 | -1.8 | 47.93 | mM | -0.36 |

**Figure 4. Statistics of molecular docking showing binding affinity through AutoDock Vina.**

## References


Abbaspour, H., Roudbari, N., Kalantari, K. M., & Aien, A. (2020). Effect of exogenous application of 24-epibrassinosteroids and hydrogen peroxide on some biochemical characteristics of Cuminum cyminum L. grown under drought stress. *Catrina: The International Journal of Environmental Sciences*, *20*(1), 49-57.

Abbaspour, H., Roudbari, N., Kalantari, K. M., & Aien, A. (2020). Effect of exogenous application of 24-epibrassinosteroids and hydrogen peroxide on some biochemical characteristics of *Cuminum cyminum* L. grown under drought stress. *Catrina: The International Journal of Environmental Sciences*, *20*(1), 49-57.

Ahanger, M. A., Ashraf, M., Bajguz, A., & Ahmad, P. (2018). Brassinosteroids regulate growth in plants under stressful environments and crosstalk with other potential phytohormones. *Journal of Plant Growth Regulation*, *37*(4), 1007-1024.

Ali, Q., Shahid, S., Nazar, N., Hussain, A. I., Ali, S., Chatha, S. A. S., ... & Hussain, S. M. (2020). Use of phytohormones in conferring tolerance to environmental stress. *Plant ecophysiology and adaptation under climate change: mechanisms and perspectives II: mechanisms of adaptation and stress amelioration*, 245-355.

Al-Maliki, O. A. L., & Al Shammari, M. Z. F. (2019). The effect of agricultural fertilizer IQ combi and brassinolide hormone on some growth characteristics, yield and active compounds of Petroselinum hortense plant. *Plant Archives*, *19*(1), 1-7.

Alzahrani, Y., Alshehri, F., El-Sorogy, A. S., & Alzahrani, H. (2023). Environmental assessment of heavy metals in soils around Al-Janabeen Dam, southwest Saudi Arabia. *Journal of King Saud University-Science*, *35*(2), 102503.

Andre, C. M., Larondelle, Y., & Evers, D. (2010). Dietary antioxidants and oxidative stress from a human and plant perspective: a review. *Current Nutrition & Food Science*, *6*(1), 2-12.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant physiology*, *24*(1), 1.

Bhuiyan, M. N. I., Begum, J., & Sultana, M. (2009). Chemical composition of leaf and seed essential oil of *Coriandrum sativum* L. from Bangladesh. ||| *Bangladesh Journal of Pharmacology*|||, *4*(2), 150-153.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1), 25-30.

Bukhari, S. A. H., Wang, R., Wang, W., Ahmed, I. M., Zheng, W., & Cao, F. (2016). Genotype-dependent effect of exogenous 24-epibrassinolide on chromium-induced changes in ultrastructure and physicochemical traits in tobacco seedlings. *Environmental Science and Pollution Research*, *23*, 18229-18238.

Bukhari, S. A. H., Wang, R., Wang, W., Ahmed, I. M., Zheng, W., & Cao, F. (2016). Genotype-dependent effect of exogenous 24-epibrassinolide on chromium-induced changes in ultrastructure and physicochemical traits in tobacco seedlings. *Environmental Science and Pollution Research*, *23*, 18229-18238.

Cakmak, I. (2010, August). Biofortification of cereals with zinc and iron through fertilization strategy. In *19th world congress of soil science* (pp. 1-6).

Carvalho, A., & Moreira Gomes, V. (2011). Plant defensins and defensin-like peptides-biological activities and biotechnological applications. *Current pharmaceutical design*, *17*(38), 4270-4293.

Choudhary, S. P., Kanwar, M., Bhardwaj, R., Yu, J. Q., & Tran, L. S. P. (2012). Chromium stress mitigation by polyamine-brassinosteroid application involves phytohormonal and physiological strategies in *Raphanus sativus* L. *PLoS One*, *7*(3), e33210.

DesMarias, T. L., & Costa, M. (2019). Mechanisms of chromium-induced toxicity. *Current opinion in toxicology*, *14*, 1-7.

Desoky, E. S. M., Merwad, A. R. M., Semida, W. M., Ibrahim, S. A., El-Saadony, M. T., & Rady, M. M. (2020). Heavy metals-resistant bacteria (HM-RB): Potential bioremediators of heavy metals-stressed *Spinacia oleracea* plant. *Ecotoxicology and Environmental Safety*, *198*, 110685.

Diwan, H., Ahmad, A., & Iqbal, M. (2012). Chromium-induced alterations in photosynthesis and associated attributes in Indian mustard. *Journal of Environmental Biology*, *33*(2), 239.

Diwan, H., Khan, I., Ahmad, A., & Iqbal, M. (2010). Induction of phytochelatins and antioxidant defence system in *Brassica juncea* and *Vigna radiata* in response to chromium treatments. *Plant Growth Regulation*, *61*, 97-107.

Efimova, M. V., Savchuk, A. L., Hasan, J. A. K., Litvinovskaya, R. P., Khripach, V. A., Kholodova, V. P., & Kuznetsov, V. V. (2014). Physiological mechanisms of enhancing salt tolerance of oilseed rape plants with brassinosteroids. *Russian journal of plant physiology*, *61*, 733-743.

Ferreira, L. G., Dos Santos, R. N., Oliva, G., & Andricopulo, A. D. (2015). Molecular docking and structure-based drug design strategies. *Molecules*, *20*(7), 13384-13421.

Gill, R. A., Zang, L., Ali, B., Farooq, M. A., Cui, P., Yang, S., ... & Zhou, W. (2015). Chromium-induced physio-chemical and ultrastructural changes in four cultivars of *Brassica napus* L. *Chemosphere*, *120*, 154-164.

Hayat, S., Alyemeni, M. N., & Hasan, S. A. (2012). Foliar spray of brassinosteroid enhances yield and quality of Solanum lycopersicum under cadmium stress. *Saudi journal of biological sciences*, *19*(3), 325-335.

Jamil, N., Hyder, S., Valipour, M., Yasir, M., Iqbal, R., Roy, R., ... & Ahmed, A. (2022). Evaluation of the bioremediation potential of Staphlococcus lentus inoculations of plants as a promising strategy used to attenuate chromium toxicity. *Sustainability*, *14*(20), 13056.

Kellős, T. (2008). Effect of abiotic stress on antioxidants in maize. *Acta Biologica Szegediensis*, *52*(1), 173-174.

Kumar, M., Yusuf, M. A., Yadav, P., Narayan, S., & Kumar, M. (2019). Overexpression of chickpea defensin gene confers tolerance to water-deficit stress in Arabidopsis thaliana. *Frontiers in plant science*, *10*, 290.

Leubner-Metzger, G. (2003). Brassinosteroids promote seed germination. In *Brassinosteroids: bioactivity and crop productivity* (pp. 119-128). Dordrecht: Springer Netherlands.

Rady, M. M., Elrys, A. S., Selem, E., Mohsen, A. A., Arnaout, S. M., El-Sappah, A. H., ... & Desoky, E. S. M. (2023). *Spirulina platensis* extract improves the production and defenses of the common bean grown in a heavy metals-contaminated saline soil. *Journal of Environmental Sciences*, *129*, 240-257.

Rady, M. M., Mounzer, O. H., Alarcón, J., Abdelhamid, M., & Howladar, S. (2016). Growth, heavy metal status and yield of salt-stressed wheat (*Triticum aestivum* L.) plants as affected by the integrated application of bio-, organic and inorganic nitrogen-fertilizers. *J. Appl. Bot. Food Qual*, *89*, 21-28.

Shanker, A. K., Cervantes, C., Loza-Tavera, H., & Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment international*, *31*(5), 739-753.

Shanker, A. K., Cervantes, C., Loza-Tavera, H., & Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment International*, 31(5), 739-753.

Sharma, I., Pati, P. K., & Bhardwaj, R. (2011). Effect of 28-homobrassinolide on antioxidant defence system in *Raphanus sativus* L. under chromium toxicity. *Ecotoxicology*, *20*, 862-874.

Sharma, M. M., & Sharma, R. K. (2012). Coriander. In *Handbook of herbs and spices* (pp. 216-249). Woodhead Publishing.

Sharma, P., Singh, S. P., Tripathi, R. D., & Tong, Y. W. (2023). Chromium toxicity and tolerance mechanisms in plants through cross-talk of secondary messengers: An overview of pathways and mechanisms. *Environmental Pollution*, *320*, 121049.

Siddiqui, H., Sami, F., Faizan, M., Faraz, A., & Hayat, S. (2019). Brassinosteroid mediated regulation of photosynthesis in plants. *Brassinosteroids: Plant Growth and Development*, 185-217.

Singh, D., Keditsu, R., Hemanta, L., Yepthomi, G. I., & Kumar, N. (2023). Effect of plant growth regulators on the performance of African marigold (*Tagetes erecta* L.) cv. Pusa Narangi Gainda. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, 38.

Suwalsky M, Castro R, Villena F, Sotomayor CP (2008) Cr(III) exerts stronger structural effects than Cr(VI) on human erythrocyte membrane and molecular modes. J Inorg Biochem 102(4):842–849

Suwalsky, M., Castro, R., Villena, F., & Sotomayor, C. P. (2008). Cr (III) exerts stronger structural effects than Cr (VI) on the human erythrocyte membrane and molecular models. *Journal of*

*Inorganic Biochemistry*, 102(4), 842-849.
Suwalsky, M., Sánchez, S., Gallardo, M. J., Villena, F., Jemiola-Rzeminska, M., & Strzalka, K. (2016). Human health through traces of contaminants in water: structural effects induced by Cr (III) and Cr (VI) on red cells. In *Innovative Materials and Methods for Water Treatment* (pp. 91-108). CRC Press.
Yu, X. Z., Gu, J. D., & Huang, S. Z. (2007). Hexavalent chromium induced stress and metabolic responses in hybrid willows. *Ecotoxicology*, *16*, 299-309.
Yusuf, M., Khan, T. A., & Fariduddin, Q. (2016). Interaction of epibrassinolide and selenium ameliorates the excess copper in Brassica juncea through altered proline metabolism and antioxidants. *Ecotoxicology and environmental safety*, *129*, 25-34.
Zafari, M., Ebadi, A., Jahanbakhsh, S., & Sedghi, M. (2020). Safflower (Carthamus tinctorius) biochemical properties, yield, and oil content affected by 24-epibrassinosteroid and genotype under drought stress. *Journal of agricultural and food chemistry*, *68*(22), 6040-6047.