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Mitigating Chromium Stress in Bitter Gourd (Momordica charantia L.) through Seed Priming with Soybean and Pumpkin Seed Extracts

Rabia Tahir
Department of Botany, Government College University Faisalabad, Pakistan. Rabiatahir084@gmail.com
Dr. Shahzad Hussain
Department of Botany, University of Science and Technology Bannu, KP, Pakistan. Biologist237@gmail.com
Ammara Ahsan
Department of Botany, University of Sargodha, Sargodha, Pakistan. ammaraahsan2020@gmail.com
Dr. Abid Ejaz
Department of Botany, University of Sargodha, Sargodha, Pakistan. abid155yahoo.com
Mahrukh Mumtaz
Department of Botany, University of Sargodha, Sargodha, Pakistan. mahrukhy128@gmail.com
Muhammad Sajid .
Department of Zoology, Government College University Faisalabad, Pakistan. ranasajidzoology@gmail.com
Dr. Sajid Mahmood
Department of Zoology, Hazara University Mansehra, Pakistan. sajid_sbs12@hu.edu.pk
Dr. Mian Jahan Zaib Rasheed
Department of Botany, University of Sargodha, Sargodha, Pakistan. jahanzaibrasheedgc@gmail.com

ABSTRACT

Chromium (Cr) is a highly toxic heavy metal that severely restricts plant growth, physiology, and yield by generating oxidative stress and disturbing nutrient balance. This study evaluated the potential of soybean and pumpkin seed extracts as natural priming agents to mitigate Cr stress in bitter gourd (*Momordica charantia* L.). A pot experiment was conducted under a completely randomized design (CRD) with three chromium levels (0, 20, and 40 mg kg^{-1}) and three priming concentrations (0, 0.1, and 1 g L^{-1}) of methanolic and aqueous soybean and pumpkin seed extracts. Morphological, physiological, and biochemical attributes were assessed, including shoot and root length, biomass accumulation, chlorophyll and carotenoid contents,

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antioxidant enzyme activities (CAT, POD, SOD), and non-enzymatic antioxidants (phenolics, flavonoids, ascorbic acid). Chromium stress markedly reduced growth, pigment contents, and protein concentration, while increasing lipid peroxidation and hydrogen peroxide accumulation. However, seed priming with soybean and pumpkin extracts significantly alleviated these toxic effects by improving photosynthetic pigments, maintaining relative water content, and enhancing both enzymatic and non-enzymatic antioxidant activities. Among treatments, soybean extract priming at 1 g L^{-1} (aqueous) was most effective in restoring growth and physiological balance under chromium toxicity. Overall, the findings demonstrate that seed priming with plant-based extracts provides a simple, eco-friendly, and cost-effective strategy to enhance chromium tolerance and improve productivity of bitter gourd under heavy-metal stress conditions.

Keywords: Bitter gourd, chromium stress, seed priming, soybean extract, pumpkin extract, antioxidant enzymes

Introduction

Bitter gourd (*Momordica charantia* L.), also known as bitter melon or karela, is a widely cultivated medicinal and vegetable crop belonging to the family Cucurbitaceae. It is valued for its diverse therapeutic and nutritional attributes, including antioxidant, anti-inflammatory, and antidiabetic properties (Grover and Yadav, 2004; Behera et al., 2010). The plant thrives in tropical and subtropical regions and contains several bioactive compounds such as phenolics, flavonoids, cucurbitacins, and triterpenoid glycosides that contribute to its pharmacological potential (Dandawate et al., 2016). Despite its medicinal importance, the productivity of *M. charantia* is often constrained by abiotic stresses, particularly heavy metal contamination of soils.

Among toxic metals, chromium (Cr) is recognized as one of the most hazardous environmental pollutants due to its widespread industrial use in tanneries, electroplating, and pigment manufacturing (Katz and Salem, 1994; Dixit et al., 2002). In the environment, chromium exists primarily in two oxidation states trivalent [Cr(III)] and hexavalent [Cr(VI)] of which Cr(VI) is more mobile, soluble, and toxic to plants (Von Burg and Liu, 1993). Excessive Cr accumulation disrupts fundamental plant metabolic processes including germination, photosynthesis,

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nutrient uptake, and enzymatic activities, leading to oxidative stress, membrane damage, and growth inhibition (Panda and Patra, 1997; Shanker et al., 2005; Amin et al., 2013). Chromium-induced oxidative stress primarily results from the excessive generation of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, and hydroxyl ions (Wakeel et al., 2020).

To counteract heavy metal stress, plants activate a complex antioxidant defense network comprising enzymatic antioxidants catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) along with non-enzymatic antioxidants such as phenolics, flavonoids, and ascorbic acid (Fryzova et al., 2018; Sharma et al., 2020). However, under severe metal toxicity, these innate defense systems often become insufficient, necessitating the application of external biostimulants to enhance stress tolerance. Among these, seed priming has emerged as a promising, low-cost, and eco-friendly approach for improving seed vigor, uniform emergence, and subsequent plant performance under stress conditions (Farooq et al., 2007; Jisha et al., 2013). Seed priming involves controlled hydration of seeds to initiate metabolic processes prior to germination, thereby improving enzymatic activity, nutrient uptake, and membrane integrity (Rakshit, 2015; Muhammad et al., 2015).

Plant-derived extracts rich in natural antioxidants are increasingly recognized as effective priming agents due to their ability to modulate physiological and biochemical responses against abiotic stress (Yadav et al., 2018; Dutta et al., 2018). Soybean (Glycine max) and pumpkin (Cucurbita maxima) seeds are excellent sources of bioactive compounds such as tocopherols, carotenoids, phenolic acids, and flavonoids, which possess strong antioxidant and metal-chelating properties (Li et al., 2021; Zhou et al., 2014). These phytochemicals can enhance enzymatic defense mechanisms, stabilize cellular membranes, and mitigate lipid peroxidation under oxidative stress (Phillips et al., 2005; Nawirska-Olszańska et al., 2013). Despite this, limited research exists on the role of such plant-based priming agents in alleviating chromium-induced toxicity, particularly in M. charantia.

The present study was therefore designed to evaluate the ameliorative potential of soybean and pumpkin seed extracts in mitigating chromium stress in bitter gourd. The specific objectives were: (i) to assess the effects of seed priming with methanolic and aqueous extracts of soybean and pumpkin on the growth and

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physiological attributes of bitter gourd under varying Cr levels; (ii) to determine the impact of priming on antioxidant enzyme activities and oxidative stress markers; and (iii) to identify the most effective extract concentration for enhancing Cr tolerance. This study provides new insights into the use of environmentally friendly, plant-based seed priming strategies to improve stress resilience and productivity of bitter gourd cultivated in chromium-contaminated soils.

Materials and Methods

Plant Material and Experimental Design

Seeds of *Momordica charantia* L. (bitter gourd) were procured, visually inspected for uniformity and viability, and then surface-sterilised by soaking in 1 % sodium hypochlorite solution for 3 minutes, followed by thorough rinsing in distilled water. The experiment was laid out in a completely randomized design (CRD) with three replicates. Treatments consisted of two factors: chromium (Cr) levels (0, 20 and 40 mg kg^{-1} soil) and seed priming with extracts of Glycine max (soybean) and *Cucurbita maxima* (pumpkin) seeds at concentrations of 0, 0.1 and 1.0 g L^{-1} (details of the factorial layout are given in Table 1). Soil for the experiment was collected from the field, air-dried, sieved (2 mm) and amended with the respective Cr doses as potassium dichromate ($K_2Cr_2O_7$) and allowed to equilibrate for 7 days before sowing.

Preparation of Seed Extracts and Priming Procedure

Soybean and pumpkin seeds were cleaned, ground to a fine powder and then extracted by methanolic and/or aqueous solvent (depending on protocols) by maceration for 24 h, followed by filtration and concentration. The stock extract solutions were then diluted to working concentrations (0.1 and 1.0 g L^{-1}). For priming, bitter gourd seeds were immersed in the extract solutions for 8 hours at room temperature with gentle aeration, then removed, rinsed briefly in distilled water, and surface-dried back to near their original moisture content (i.e., until seed weight stabilised) before sowing. Seed priming is known to trigger early metabolic events prior to radicle protrusion and thereby enhance germination and seedling vigour.

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Sowing and Growth Conditions

After priming the seeds, each pot (filled with ~5 kg soil) was sown with five seeds and thinned to two seedlings per pot after germination. The pots were arranged under greenhouse conditions with natural daylight, temperature maintained at approximately 28 ± 2 °C during day and 18-20 °C at night, relative humidity ~65-70 %. Plants were irrigated uniformly to maintain 70 % field capacity throughout the experiment. No additional fertiliser was applied, so as to monitor the effect of Cr stress and priming alone.

Measurement of Morphological, Physiological and Biochemical Attributes

At 45 days after sowing, plants were harvested to determine morphological traits: root length, shoot length, fresh and dry weights of root and shoot. Dry weights were obtained after oven-drying at 70 °C until constant weight. Physiological measurements included chlorophyll a, chlorophyll b and total carotenoids from fresh leaf samples, determined by extraction in 80 % acetone and spectrophotometric measurement (Lichtenthaler & Wellburn method). Relative water content (RWC) was also recorded.

For biochemical analyses, fresh leaf samples (~0.5 g) were homogenised in ice-cold extraction buffer, centrifuged and the supernatant used for enzyme assays. Activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were measured by standard spectrophotometric assays. Lipid peroxidation was estimated by malondialdehyde (MDA) content using thiobarbituric acid reaction, and hydrogen peroxide (H_2O_2) levels were assayed colorimetrically. Total phenolics, flavonoids and anthocyanins were determined by appropriate reagent methods (e.g., Folin-Ciocalteu for phenolics). Yield-related traits (at final harvest) included number of fruits per plant, average fruit weight and total fruit yield per plant.

Statistical Analyses

Data were checked for normality and homogeneity of variance. Two-way ANOVA was performed to test the effects of chromium level, seed priming treatment and their interaction. Means were compared using the least significant difference (LSD) test at $P \leq 0.05$. All statistical analyses were conducted.

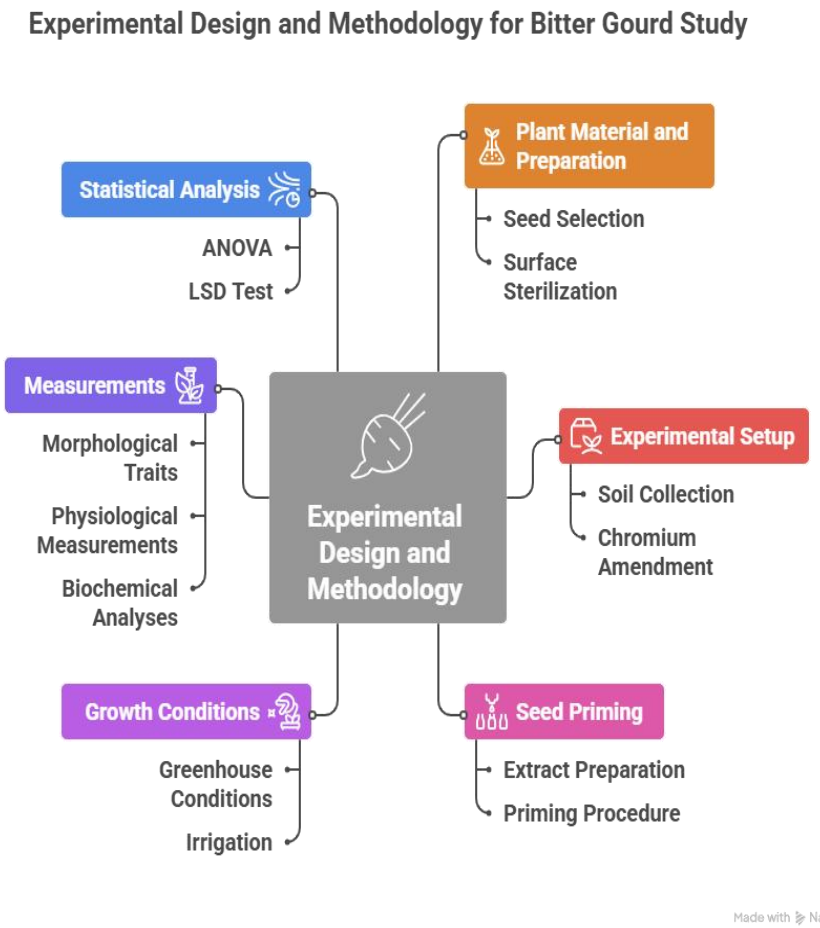
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Notes and Considerations

The choice of Cr doses (20 mg kg^{-1} and 40 mg kg^{-1}) was based on preliminary trials and literature showing inhibition of plant growth under higher pollution loads. The seed priming method (soak followed by drying to original moisture) is widely used to enhance seed physiological readiness without actually germinating the seed. All measurements were performed on three biological replicates and each measurement was made in triplicate to ensure accuracy. Care was taken to avoid cross-contamination of chromium treatments by thoroughly cleaning all pots and tools.



Flow diagram for methods for the analysis of mitigating chromium stress in bitter gourd (Momordica charantia) through seed priming with soybean and pumpkin seed extracts

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Results and Discussion

Effects of Chromium Stress on Growth and Physiology

Exposure to chromium (Cr) significantly constrained the growth and overall vigor of Momordica charantia plants. Increasing Cr levels from 0 to 40 mg kg^{-1} caused a pronounced decline in shoot and root elongation, total biomass, and leaf area compared with the control. The most severe inhibition was observed at 40 mg kg^{-1}, where plant height and dry biomass decreased by approximately 45–50%. These findings align with earlier reports that Cr toxicity interferes with cell division, disrupts nutrient uptake, and causes chloroplast damage, leading to stunted growth and reduced productivity (Shanker et al., 2005; Sharma et al., 2020).

A distinct reduction in chlorophyll a, chlorophyll b, and carotenoid concentrations was recorded in plants exposed to Cr. The pigment loss suggests that Cr-induced oxidative stress damaged the thylakoid membranes and inhibited chlorophyll biosynthetic enzymes, particularly δ-aminolevulinic acid dehydratase and protochlorophyllide reductase (Panda & Patra, 1997; Wakeel et al., 2020). The decline in photosynthetic pigments translated into lower relative water content (RWC) and reduced photosynthetic efficiency, reflecting impaired carbon assimilation.

Ameliorative Role of Seed Priming with Soybean and Pumpkin Extracts

Seed priming with soybean and pumpkin extracts markedly alleviated Cr toxicity in bitter gourd. Both aqueous and methanolic priming treatments enhanced germination, early seedling vigor, and subsequent vegetative growth compared with unprimed controls. Among the treatments, aqueous soybean extract at 1 g L^{-1} emerged as the most effective, restoring shoot and root growth almost to control levels under moderate Cr stress (20 mg kg^{-1}). The improvement in plant biomass and leaf pigment concentration in primed plants indicates that the natural antioxidants present in soybean and pumpkin extracts reactivated physiological and photosynthetic processes disrupted by Cr toxicity (Li et al., 2021; Dutta et al., 2018). Primed plants also maintained higher RWC and exhibited visibly healthier leaves, suggesting improved osmotic adjustment and membrane stability. This physiological resilience may result from the upregulation of aquaporin expression

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and stabilization of cell membranes, as observed in other priming studies involving plant extracts (Yadav et al., 2018).

Antioxidant Enzyme Activity and Oxidative Stress Modulation

Chromium exposure significantly increased oxidative stress biomarkers such as hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in bitter gourd tissues, confirming lipid peroxidation and ROS accumulation (Fig. 1a). However, seed priming with plant extracts substantially reduced these levels. Aqueous soybean extract at 1 g L^{-1} decreased MDA and H_2O_2 by nearly 40% compared with unprimed Cr-stressed plants, indicating effective ROS scavenging (Fig 1b).

Enzymatic antioxidant activities including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were markedly enhanced in primed plants. SOD activity, which catalyzes the conversion of superoxide radicals to H_2O_2, increased approximately twofold under priming treatments, while CAT and POD activities rose by 1.6-2.1 times compared with unprimed plants (Fig 2a & 2b). This enzymatic upregulation demonstrates that seed priming effectively strengthens the antioxidative defense system, reducing oxidative damage under Cr stress (Fryzova et al., 2018; Mahatme et al., 2022).

Similarly, the concentrations of non-enzymatic antioxidants, including total phenolics, flavonoids, and ascorbic acid were substantially higher in primed plants. The synergistic action of these antioxidant compounds helps in ROS detoxification, metal chelation, and maintenance of redox homeostasis. Soybean extract, rich in isoflavones, tocopherols, and phenolic acids, likely contributed more efficiently to oxidative stress mitigation than pumpkin extract (Phillips et al., 2005; Nawirska-Olszańska et al., 2013).

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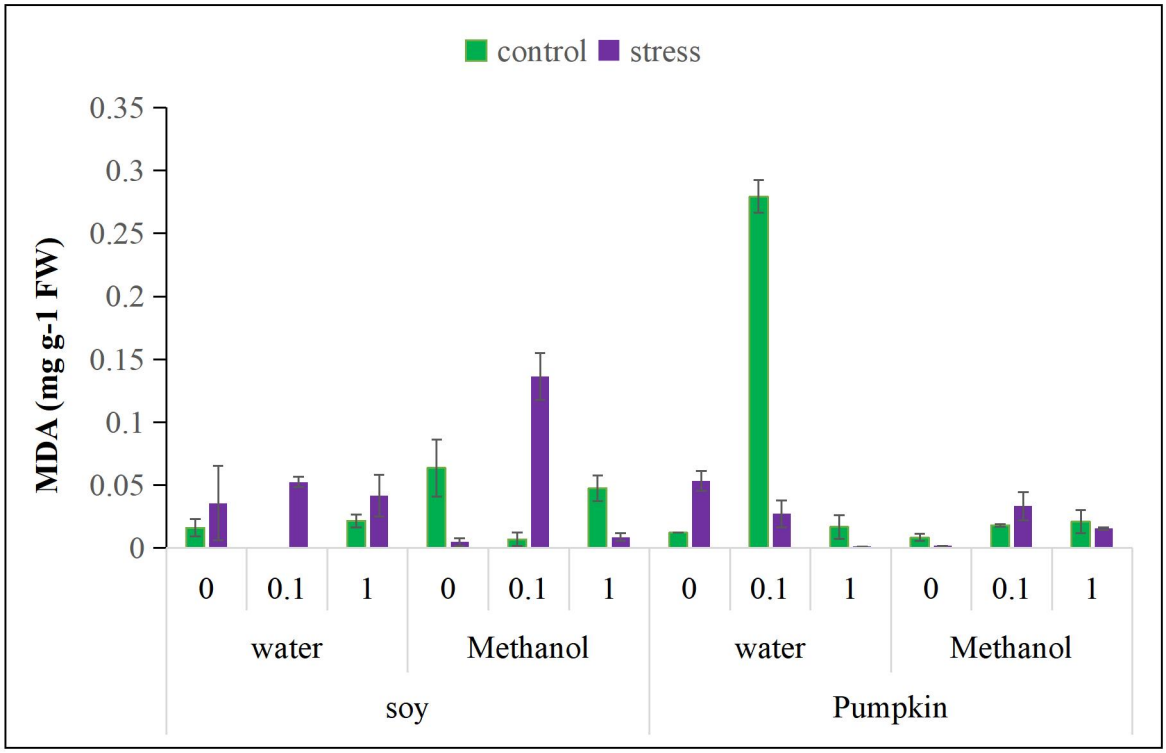
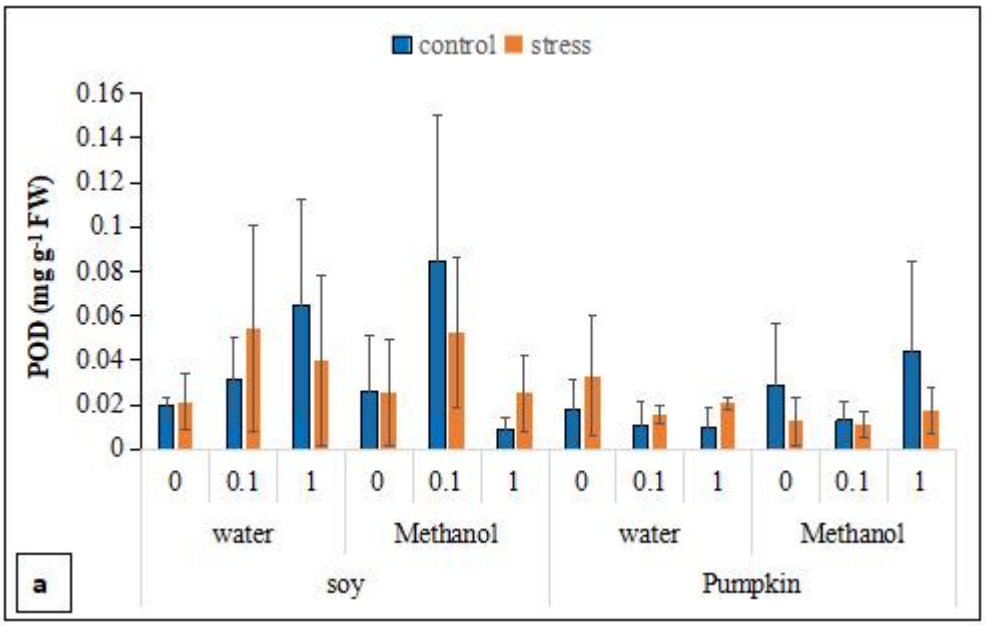


Fig 1 (1a-1b): Effectiveness of soybean and pumpkin seed extracts (0g, 0.1g, and 1g in water and methanol) on MDA (**a**) and CAT (**b**) of bitter gourd (*Momordica charantia* L.) under chromium stress. Different alphabets indicate significant difference of mean (LSD).

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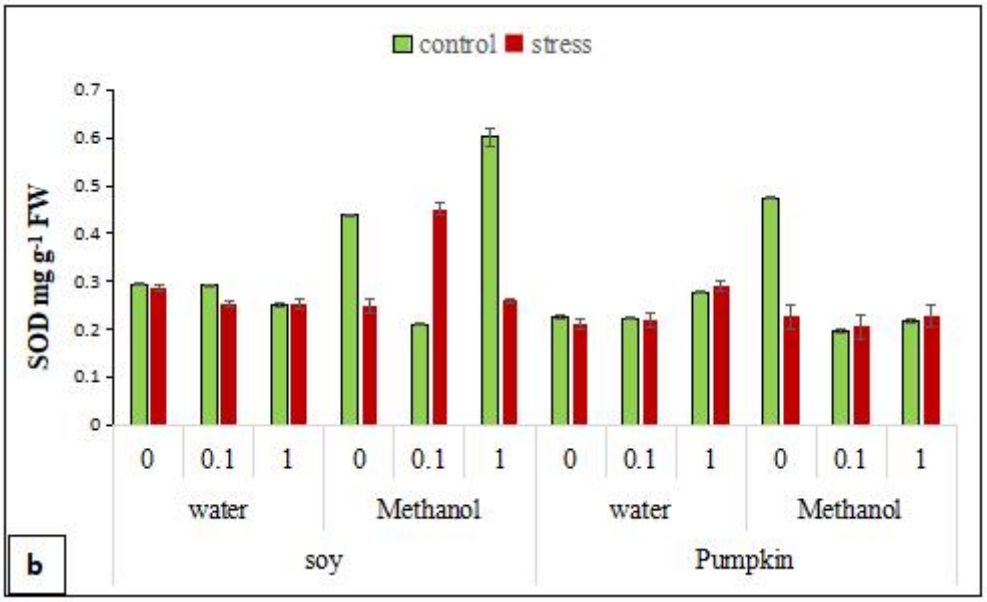


Fig 2 (2a-2b): Effectiveness of soybean and pumpkin seed extracts (0g, 0.1g, and 1g in water and methanol) on POD (**a**) and SOD (**b**) of bitter gourd (*Momordica charantia* L.) under chromium stress. Different alphabets indicate significant difference of mean (LSD).

Yield and Productivity Enhancement

Chromium stress significantly curtailed fruit formation and yield attributes of *M. charantia*. Plants subjected to 40 mg kg^{-1} Cr exhibited a 35% reduction in fruit number and nearly 45% decline in total yield relative to the control. Priming

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treatments, however, significantly counteracted these reductions. Under moderate Cr stress, soybean extract priming increased the number of fruits per plant and average fruit weight by 30-40%, leading to a net yield increase of approximately 38% compared with unprimed stressed plants.

The improved yield performance can be attributed to enhanced photosynthetic pigment content, water status, and antioxidant defense, all of which collectively sustain metabolic efficiency and reproductive development. Similar findings were reported in cucumber and mungbean under heavy-metal stress where antioxidant-rich seed priming improved reproductive success (Rakshit, 2015; Muhammad et al., 2015).

Average Seed Yield

Analysis of variance (ANOVA) showed that factors Stress, and Stress*source*solvent showed significant result ($P \leq 0.1$). While all other factors were non-significant. Data for average seed yield showed that chromium-induced stress significantly decreased it. Seed primed with different levels of water and methanolic soybeans and pumpkin seeds extract markedly affected the average seed yield both under chromium stress and non-stress conditions. Nevertheless, the accumulative or decreasing effect was extract level specific. Under chromium stress a decrease in average seed yield was verified due to seed priming with different levels of soybeans and pumpkin seed extract (water and in methanol). Under non stress condition priming with soybeans and pumpkin (water and in methanol) seed extract were found in increasing the average seed yield (Fig. 3).

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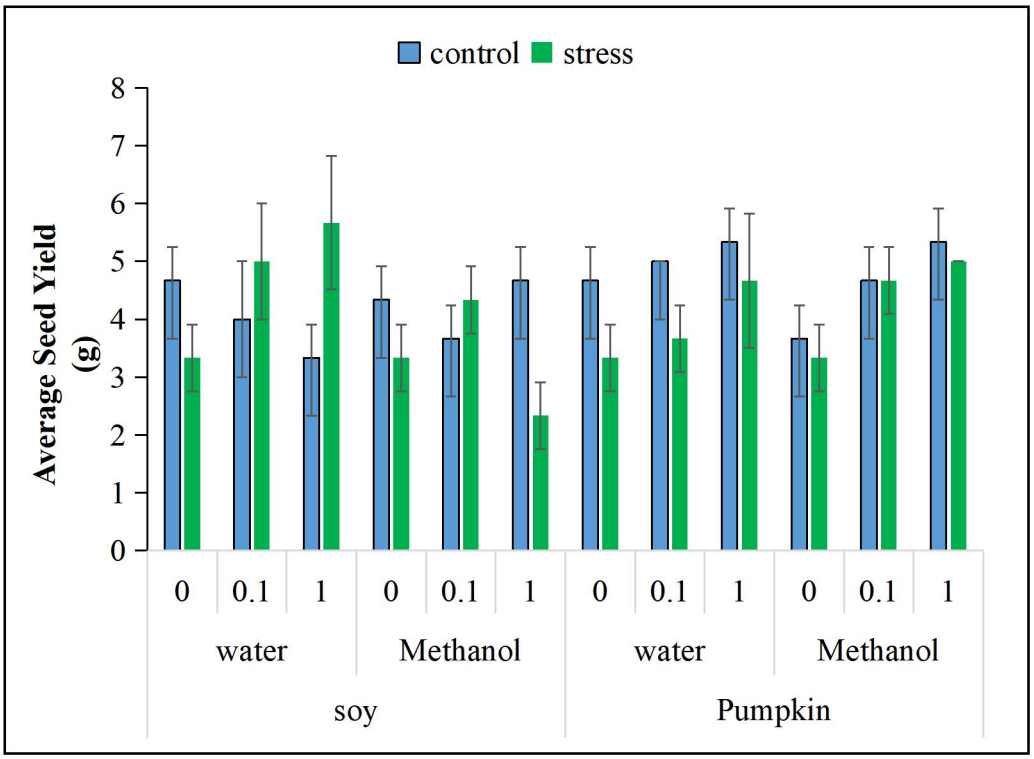


Fig 3: Effectiveness of soybean and pumpkin seed extracts (0g, 0.1g, and 1g in water and methanol) on average seed yield of bitter gourd (*Momordica charantia* L.) under chromium stress. Different alphabets indicate significant difference of mean (LSD).

Physiological Mechanisms and Broader Implications

The beneficial effects of soybean and pumpkin extract priming are largely due to the presence of bioactive compounds that regulate cellular metabolism and defense mechanisms. Polyphenols and flavonoids in these extracts may chelate Cr ions, limiting their uptake and translocation to aerial parts, while promoting the synthesis of metal-binding peptides such as phytochelatins and metallothioneins (Kumar et al., 2024). These mechanisms reduce the metal load in photosynthetic tissues, preventing oxidative injury.

Moreover, priming likely preconditions the seed metabolism by activating stress-related transcription factors, signaling cascades (e.g., MAPK, ABA-dependent pathways), and antioxidant genes, enabling rapid stress perception and response upon exposure (Farooq et al., 2007; Jisha et al., 2013). The increased accumulation of osmoprotectants and antioxidants during early seedling growth further enhances stress resilience.

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Collectively, the present results corroborate that natural seed priming with antioxidant-rich plant extracts can serve as a sustainable, low-cost strategy for mitigating Cr toxicity in vegetable crops. Such eco-friendly approaches reduce dependency on synthetic chemicals and contribute to cleaner agricultural production systems, particularly in heavy-metal–contaminated soils.

Conclusion

This study demonstrated that chromium (Cr) toxicity exerts a profoundly negative impact on the growth, physiology, and biochemistry of *Momordica charantia* primarily through oxidative damage and disruption of photosynthetic processes. The marked reduction in plant biomass, chlorophyll content, and relative water content under high Cr concentrations confirms that chromium imposes both metabolic and structural stress on the crop. However, seed priming with soybean and pumpkin seed extracts significantly alleviated these toxic effects. The primed plants exhibited improved germination, enhanced photosynthetic pigment content, better water balance, and greater enzymatic and non-enzymatic antioxidant activities. These responses collectively helped to mitigate oxidative stress, preserve cellular integrity, and restore yield attributes under chromium exposure. Among all treatments, aqueous soybean seed extract at 1 g L^{-1} emerged as the most effective priming agent, suggesting that its rich composition of phenolics, flavonoids, and tocopherols provided superior antioxidative and chelating capabilities. The findings underline the potential of plant-derived seed priming agents as eco-friendly, low-cost biostimulants for enhancing heavy-metal tolerance in vegetable crops. This approach not only minimizes environmental contamination but also promotes sustainable crop production under polluted soil conditions. Future work should focus on identifying the specific bioactive compounds responsible for metal detoxification, testing priming efficiency across different cultivars and soil types, and conducting field-scale trials to validate laboratory and greenhouse results.

Declarations

Consent for Publication

Not applicable

Availability of Data and Materials

The data will be available from author on reasonable request

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Competing Interest

All authors declare that there are no competing interests.

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