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The exogenous application of salicylic acid and biochar to mitigate chromium stress in Salvia hispanica L.

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ABSTRACT

Heavy metal contamination, particularly chromium (Cr) stress, poses a serious threat to agricultural productivity and food safety because of its toxic effects on plant growth and metabolism. This study investigated the individual and combined effects of salicylic acid (SA) and biochar in mitigating chromium-induced stress in *Salvia hispanica* L. under controlled pot conditions. Plants were exposed to different chromium concentrations (0, 50, 100, and 150 µM), together with foliar application of salicylic acid (10 µM) and soil application of biochar (2%), either individually or in combination. Chromium stress significantly reduced morphological parameters, biomass accumulation, and photosynthetic pigment content, indicating substantial oxidative and physiological damage. However, the exogenous application of SA (10 µM) and biochar (2%) significantly alleviated Cr toxicity by improving growth attributes, chlorophyll a and b levels, carotenoid content, and overall fresh and dry plant biomass. Among all treatments, the combined application of SA and biochar was the most effective. It produced the greatest improvements in plant height, root length, fresh and dry weights, and pigment stability, even under high Cr stress. The enhanced tolerance was attributed to reduced metal bioavailability and improved soil conditions. These findings highlight the synergistic potential of salicylic acid and biochar as an eco-friendly and sustainable strategy for mitigating chromium toxicity and improving the growth performance of *Salvia hispanica* L. in contaminated soils.

Contribution/Originality: This study contributes to the existing literature by providing a comprehensive evaluation of the individual and combined effects of salicylic acid and biochar on chromium-induced stress in Salvia hispanica L. The findings demonstrate a synergistic approach for enhancing growth and physiological performance under heavy metal stress.

1. INTRODUCTION

In recent years, soil contamination by heavy metals (HMs) has emerged as a critical environmental concern due to factors like rapid urban growth, industrial expansion, intensive farming and excessive mineral resource exploitation, which severely impact agricultural lands. These metals accumulate in soil and are readily taken up by plant roots, leading to oxidative damage [1, 2]. Heavy metals are non-biodegradable toxic elements that pose risks to plants, animals and humans [3]. Plants are very sensitive to toxic metals and even low concentrations can disrupt normal physiological processes. High levels of HMs hinder seed germination, damage antioxidant enzymes and membrane systems, cause chromosomal abnormalities, and in severe cases lead to plant death [4].

Among various heavy metals, chromium (Cr) poses a significant threat due to its high mobility, widespread presence in contaminated soils and severe phytotoxicity. Cr stress causes delayed seed germination, root damage and stunted root growth, reduced biomass, decreased plant height, impaired photosynthesis, membrane damage, leaf chlorosis, necrosis and low grain yield. It also suppresses antioxidant enzyme activities (such as SOD, POD, APX, and CAT) and promotes excessive production of reactive oxygen species (ROS), which cause cellular damage and ultimately plant death [5, 6]. Chromium enters the food chain and affects the health of all living organisms either directly or indirectly [7].

To alleviate the harmful effects of these toxic metals, a variety of remediation strategies, including physical, chemical and biological methods have been proposed. Among these strategies, biochar is the most widely used solution for mitigating soil heavy metal pollution due to its quick activation, simple operation and low cost [8].

Biochar is produced through pyrolysis, which involves heating organic materials derived from plants or animals at temperatures ranging from 350 to 750 °C under limited oxygen conditions [9]. Compared with other organic amendments, biochar has a high cation exchange capacity (CEC), extensive surface area, and considerable porosity [10]. Its application enhances several soil properties, including structure, water and nutrient retention, porosity, water infiltration, pH balance, organic matter content, and the removal of inorganic and organic pollutants [11]. Heavy metal contamination reduces soil fertility; however, biochar application increases soil organic matter and enhances soil fertility [12]. Biochar added to soil immobilizes heavy metals, reducing their bioavailability and uptake by plants [13, 14].

Beyond soil amendments, plant growth regulators like salicylic acid (SA) activate plant defense mechanisms against abiotic stresses, including heavy metals. Salicylic acid (SA) is an important phytohormone that enhances plant tolerance to heavy metal stress by stimulating antioxidant defense mechanisms [15]. As a signaling molecule, SA is integral to various plant functions, including growth, photosynthesis, development and biochemical pathways. Exogenous application of SA has also been reported to improve plant growth and photosynthesis under various heavy metal stresses, including arsenic stress in several plant species, such as Oryza sativa [16], Glycine max [17] and Artemisia annua [18].

Understanding how such growth regulators and amendments interact with specific crops under stress, such as the nutritionally important Chia (Salvia hispanica L.), is crucial for developing integrated management strategies. Chia (S. hispanica L.), a member of the Lamiaceae family, is an annual herbaceous plant native to the region between Mexico and Guatemala [19]. Chia seeds possess high nutritional value. They contain approximately 15-25% protein, 26-41% carbohydrates, 30-39% lipids, 18-30% dietary fiber and 4-5% ash. They are also rich in vitamins, minerals and antioxidants, which reduce the risk of cardiovascular diseases [20]. Chia seeds are frequently referred to as a "functional superfood" because of their notable protein content, which falls between 15-26% [21]. One of the primary benefits of chia seeds is that they are naturally gluten-free. They also help to increase satiety and reduce the risk of inflammatory disorders, nervous system disorders, and diabetes [22].

Due to the increasing heavy metal contamination in agricultural soils, there is an urgent need for sustainable strategies to protect crops and improve soil health. Although chia is a nutritionally valuable superfood, it remains underexplored with respect to its response to heavy metal stress. Investigating the combined application of biochar and salicylic acid could provide an effective approach to reduce heavy metal uptake, enhance plant growth, and improve antioxidant defense. Therefore, this study aimed to: (1) evaluate the impact of varying chromium concentrations on the growth, physiological parameters and antioxidant defense system of chia plants; (2) investigate the individual and combined effects of biochar and salicylic acid application on alleviating chromium-induced stress in chia; and (3) assess the efficacy of these amendments in reducing chromium accumulation in different chia plant tissues.

2. METHODOLOGY

2.1. Experimental Layout

To evaluate chromium (Cr) stress tolerance in S. hispanica L. and the mitigating effects of biochar and salicylic acid (SA) a controlled pot experiment was conducted at the Botanical Garden of Government Sadiq College Women University, Bahawalpur, Pakistan. Seeds were obtained from the Agriculture Research Institute, Faisalabad. Sandy loamy soil was collected from the botanical garden and 2 kg of soil was weighed for each pot using a digital balance to ensure uniformity. Biochar (2 g) was applied once and thoroughly mixed into the soil before sowing. Seeds were rinsed with distilled water and five to eight seeds were sown in each pot in November 2025. Thinning was performed at the seedling stage to maintain five uniform plants per pot.

The experiment was arranged in a completely randomized design (CRD) with three biological replicates per treatment. The experiment was conducted to investigate the individual and combined effects of biochar, foliar-applied SA and Cr stress Figure 1. Chromium stress was applied at four concentrations (T1, T2, T3 and T4; 0, 50, 100, and 150 µM). Treatments were structured as follows: (i) Cr stress applied alone to assess the baseline response of the plants; (ii) Cr stress combined with biochar to evaluate whether biochar alone can mitigate Cr toxicity; and (iii) Cr stress combined with both biochar and SA to determine whether the combined application has a greater mitigation effect compared to biochar alone. Foliar-applied SA (10 µM) and Cr stress were applied three times and the first application was done 12 days after seed sprouting. Each treatment was replicated three times to ensure data reliability.

2.2. Treatments

To evaluate the mitigating potential of salicylic acid and biochar against Cr induced stress, chia plants were exposed to different Cr concentrations applied alone or in combination with SA and BC. The detailed treatment combinations are presented in Table 1.

Table 1. Experimental treatment combinations of chromium (Cr), salicylic acid (SA) and biochar (BC) used in the study.

Chromium Level	Chromium (µM)	Salicylic Acid (10 µM)	Biochar (2 g)	Salicylic Acid (10 µM), Biochar (2 g)
0 µM (Control)	T_1 (0 µM)	T_5 (0 µM, 10 µM)	T_9 (0 µM Cr, 2 g)	T_{13} (0 µM, 10 µM, 2 g)
50 µM	T_2 (50 µM)	T_6 (50 µM, 10 µM)	T_{10} (50 µM, 2 g)	T_{14} (50 µM, 10 µM, 2 g)
100 µM	T_3 (100 µM)	T_7 (100 µM, 10 µM)	T_{11} (100 µM, 2 g)	T_{15} (100 µM, 10 µM, 2 g)
150 µM	T_4 (150 µM)	T_8 (150 µM, 10 µM)	T_{12} (150 µM, 2 g)	T_{16} (150 µM, 10 µM, 2 g)

2.3. Preparation and Application of $CrCl_2$

To induce chromium stress, S. hispanica L. plants were subjected to four chromium chloride ($CrCl_2$) levels: 0 µM (T_1: control), T_2 (50 µM), T_3 (100 µM), and T_4 (150 µM). The solutions were prepared by dissolving 0.00615 g of $CrCl_2$ in 1000 mL of distilled water for the 50 µM solution, 0.01229 g of $CrCl_2$ in 1000 mL of distilled water for the 100 µM solution, and 0.01844 g of $CrCl_2$ in 1000 mL of distilled water for the 150 µM solution.

Each treatment was applied three times in total with a 10-day interval between applications. The first application was carried out after 12 days of seed germination. For each application, 100 mL of the respective $CrCl_2$ solution was added to each pot through soil irrigation, while control plants received an equal volume of water.

2.4. Salicylic Acid Application

To assess the alleviating effect of exogenous salicylic acid (SA) on chromium-induced stress in S. hispanica L., plants were subjected to different concentrations of chromium stress in combination with SA application. A 10 µM SA solution was prepared by dissolving 0.0013 g of salicylic acid in 1000 ml of distilled water. Both chromium stress and salicylic acid were applied simultaneously, with a total of three applications at 10-day intervals during the experimental period. A total of 5 to 6 sprays of SA solution were uniformly applied to ensure complete coverage of the leaf surface and effective absorption.

2.5. Biochar Application

To evaluate the mitigation potential of biochar and the stress tolerance of S. hispanica L. under chromium stress, plants were subjected to $CrCl_2$ treatments at concentrations of 0 µM, 50 µM, 100 µM, and 150 µM in combination with biochar application. Biochar was applied at a rate of 2% (2 g per pot) and thoroughly mixed into the soil before sowing. Chromium stress was applied after plant establishment. All pots received equal volume (100 mL) of $CrCl_2$ solution according to their respective treatment levels.

2.6. Biochar and Salicylic Acid Combined Application

To evaluate the combined mitigating potential of salicylic acid and biochar against Cr induced stress in

S. hispanica L. Plants were exposed to different Cr levels in combination with SA and BC treatments, as detailed in Table 1. Biochar was incorporated into the soil at a rate of 2% (2 g pot^{-1}) prior to sowing. Chromium chloride ($CrCl_2$) solutions and SA were applied simultaneously three times at 10-day intervals. Salicylic acid (10 µM) was administered as a foliar spray, with 5 to 6 sprays per plant to ensure uniform leaf coverage and efficient uptake.

2.7. Morphological Parameters

Morphological data of S. hispanica L. were recorded at the flowering stage. Plants were randomly selected from each treatment to determine plant height, root length, shoot length, leaf and plant fresh and dry weight of root, stem and leaves. Plant height was measured in cm using a measuring tape. Root length was recorded from the base of the main root to its tip and shoot length was measured from the base of the main stem to the top of the plant. Plants were carefully uprooted and cleaned to remove adhering soil, and fresh weight was recorded using a digital balance. For dry weight, plants were dried in an oven at 70 °C overnight and weighed thereafter.

2.8. Chlorophyll Estimation

Photosynthetic pigment contents, including chlorophyll a, chlorophyll b and carotenoid content were determined by using the method of Arnon [23]. Fresh leaves of *S. hispanica* L. (0.5 g) were weighed using an analytical balance and thoroughly washed with distilled water. The leaves were then ground in 80% acetone with a mortar and pestle to extract the chlorophyll pigments.

The resulting homogeneous mixture was transferred to labelled Eppendorf tubes and refrigerated at 4 °C for 24 hours to ensure complete pigment extraction. The homogenate was centrifuged at 10,000 rpm for five cycles of 30 minutes each to separate the chlorophyll extract from plant debris. The supernatant was carefully collected, and the residual debris was discarded.

The absorbance of the extract was measured at 480, 645, and 663 nm using a spectrophotometer, and the chlorophyll content was calculated as follows:

$$\text{Chlorophyll (a) } [(12.7 \text{ x O. D } 663) - (2.69 \text{ x O. D } 645)] \times V/1000 \times W \quad (1)$$

$$\text{Chlorophyll (b) } [(22.9 \text{ x O. D. } 645) - (4.68 \times \text{O.D. } 663)] \times V/1000 \times W \quad (2)$$

$$\text{Carotenoids (Car) } [\text{O. D } 480 + (0.114 \text{ x O.D } 663)] - (0.638 \times \text{O. D } 645) \quad (3)$$

Where W denotes the fresh sample weight (g), V represents the extract volume (mL), and A is the absorbance recorded at the specified wavelength(s) using a spectrophotometer.

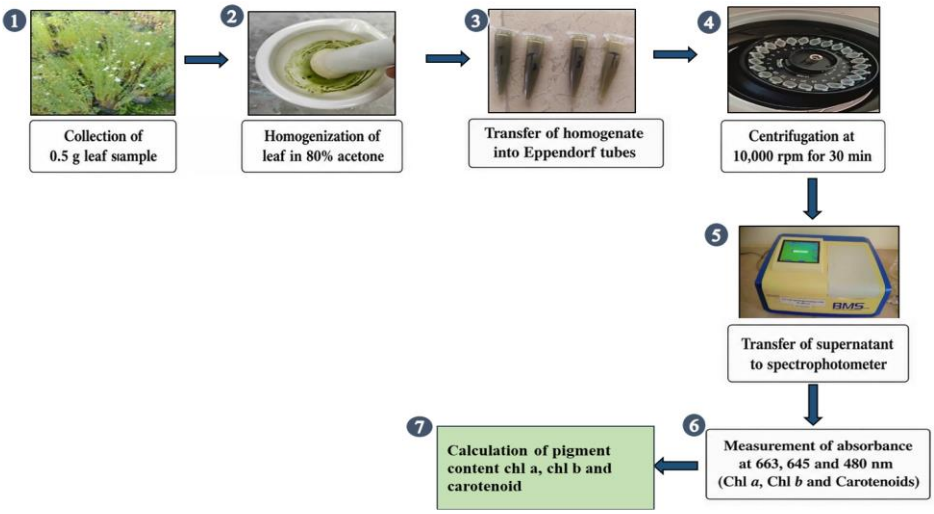

Figure 1. Schematic diagram for the estimation of photosynthetic pigments.

3. RESULTS

3.1. Effects of Different Concentrations of Cr, SA and Biochar on Stem Length, Root Length and Leaf Area

The stem length of S. hispanica L. showed significant differences among the various concentrations of Cr, salicylic acid (SA; 10 µM) and biochar (2%). Under control conditions, the mean stem length was 21.33 cm, while under stress the mean stem length decreased progressively. At 50µM Cr, the mean stem length was 18.33 cm (14.1% lower than that of the control), at 100 µM Cr the mean stem length decreased to 16 cm (22.6% lower than the control). The stem length was lowest at 150 µM Cr (34.4 % decrease from the control). Application of SA (10 µM) enhanced the stem growth under both normal and chromium-stressed conditions. In non-stressed conditions, the SA-treated plants yielded a stem length of 26 cm, which was 21.9% higher than the untreated plants. Stem length under 50 µM Cr stress condition was 22 cm, lower than the SA control but higher than the plants under 50 µM Cr stress condition alone. In the same way, under high Cr stress 150 µM, the stem length of SA-treated plants was 17 cm, which was 21.5 % higher than the plants subjected to 150 µM Cr alone. The results suggest that Cr slightly inhibited stem growth and was partially improved with SA. The addition of biochar (2 %) had a more beneficial influence on stem growth than SA alone. The biochar-treated control plants had a stem length of 29 cm, which was 35.9 % greater than the untreated control plants. Stem length was recorded at 26.33 cm under 50 µM Cr stress. The results indicated that biochar had mitigated chromium toxicity, possibly due to its effect on soil properties and reducing the bioavailability of chromium. Stem growth was the best under the combined treatment of SA (10 µM) and biochar (2 %). For the combined treatment, the stem length was 34 cm. Due to the combined application of SA and biochar, the stem length under 150 µM Cr stress was increased by 25 %, which was higher than all the treatments applied at 150 µM Cr. The results indicate that SA and biochar have the best mitigating potential, as both SA increased plant physiological tolerance to Cr stress and biochar decreased the availability of Cr, resulting in overall improved protection against Cr stress Figure 2. The root length of S. hispanica L. exhibited a similar trend as stem length under all treatments. Under control conditions, the mean root length was 4.60 cm. Chromium stress progressively reduced root growth and decreased to 4 cm at 50 µM Cr, representing a 13 % reduction compared to the control. The greatest reduction was recorded at 150 µM Cr, where root length reduced to 2.7 cm, representing a 41.3 % decrease relative to the control. Application of salicylic acid (SA; 10 µM) improved root growth under Cr stress. At 50 µM Cr, root length increased to 4.50 cm, while under severe Cr stress (150 µM Cr), the SA-treated plant attained a root length of 3.5 cm, representing a 29.6 % increase compared with plants exposed to 150 µM Cr alone. Biochar (2 %) was more effective than SA alone and root lengths of 5.1 cm and 3.6 cm recorded under 50 and 150 µM Cr stress, respectively. However, the combined application of SA (10 µM) and biochar (2 %) produced the greatest improvement in root growth. Under control conditions, root length reached 6.43 cm, while values of 5.3 cm and 4.2 cm were recorded under 50 and 150 μM Cr stress, respectively. At the highest chromium concentration, the combined treatment increased root length by 25.8 % compared with the best individual treatment, indicating a synergistic effect of SA and biochar in mitigating Cr induced inhibition of root growth Figure 3.

Under control conditions, the mean leaf area was 13.07 cm^2. Chromium stress caused a progressive reduction in leaf area and reduced leaf area to 11.41 cm^2 at 50 μM Cr, representing a 12.7 % reduction compared with the control. The greatest decline was observed at 150 μM Cr, where leaf area decreased to 7.92 cm^2, representing a 39.4 % reduction. Application of SA (10 μM) partially alleviated the adverse effects of Cr stress by increasing leaf area to 13.36 cm^2 and 8.67 cm^2 under 50 and 150 μM Cr stress, respectively. Biochar (2 %) produced a greater improvement, maintaining leaf area of 15.76 cm^2 at 50 μM Cr and 11.50 cm^2 at 150 μM Cr. The combined application of SA (10 μM) and biochar (2 %) resulted in the highest leaf area across all treatments, recorded at 20.33 cm^2 under control conditions, 16.8 cm^2 at 50 μM Cr, and 12.83 cm^2 at 150 μM Cr. Under severe Cr stress, the combined application increased leaf area by 11.6 % compared with the best individual treatment, demonstrating the superior effectiveness of the combined application in alleviating Cr induced reductions in leaf growth Figure 4.

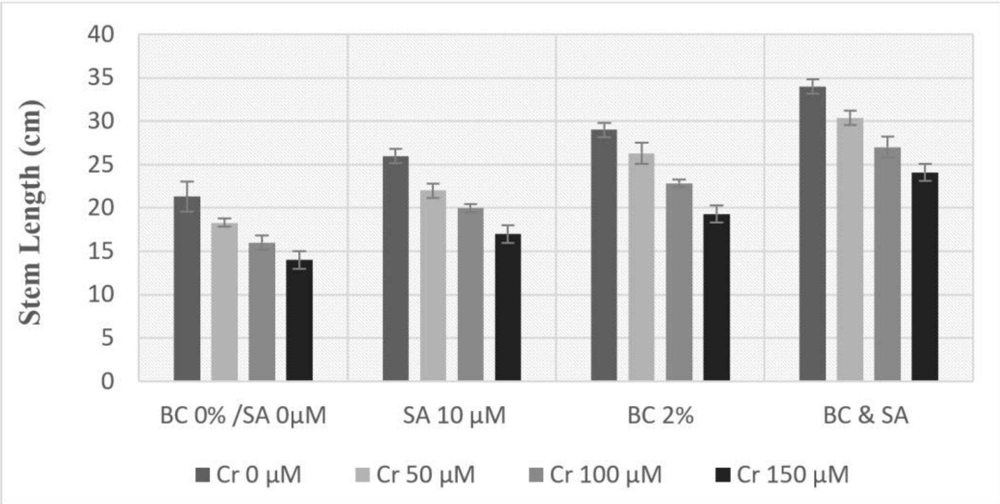

Figure 2. Effect of different concentrations of CrCl2, biochar and SA on the stem length of S. hispanica L.

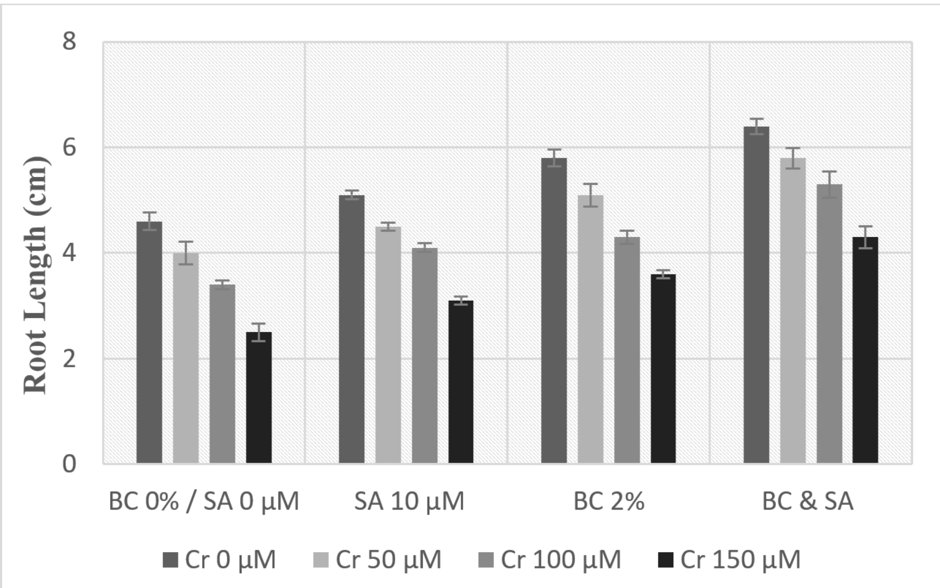

Figure 3. Effect of different concentrations of CrCl2 and biochar on the root length of S. hispanica L.

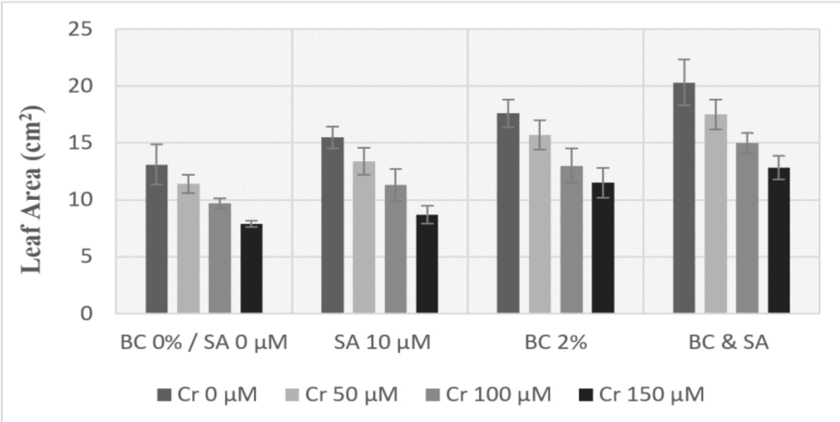

Figure 4. Effect of different concentrations of CrCl2, biochar and SA on the leaf area of S. hispanica L.

3.2. Effects of Different Concentrations of Cr, SA and Biochar on Plant Fresh and Dry Weight

Plant fresh weight of S. hispanica L. was significantly influenced by Cr stress, SA (10 µM) and biochar (2 %). Under control conditions, fresh weight was 11.60 g. Exposure to chromium caused a progressive decline in biomass, with a progressive decrease in fresh weight to 9.97 g (14 % decrease) at 50 µM Cr, 8.57 g (26.1 % decrease) at 100 µM Cr and 7.20 g (37.9 % decrease) at 150 µM Cr, which reflected the inhibitory effects of Cr on plant growth. The application of SA (10 µM) enhanced fresh weight under both normal and stress conditions and mitigated the Cr-induced reductions. Biochar (2 %) further improved biomass accumulation and maintained the relatively higher fresh weight at all Cr levels. Under the combined application of SA (10µM) and biochar (2 %), the plant fresh weight of 14.50 g was recorded under control. The combined application maintained the higher plant fresh weight values of 12.97 g, 11.33 g and 9.70 g at 50, 100 and 150 µM Cr, respectively. The results demonstrated a strong synergistic effect of SA (10 µM) and biochar (2 %) in alleviating Cr toxicity and promoting overall plant growth Figure 5.

Similarly, plant dry weight was significantly affected by chromium stress. Under control conditions, plant dry weight was 0.0667 g, which decreased to 0.0507 g (24 % decrease) at 50 µM Cr, 0.0413 g (38 % decrease) at 100 µM Cr and 0.0340 g (49 % decrease) at 150 µM Cr. This indicated the inhibition of biomass accumulation under heavy metal stress. The individual application of SA (10 µM) improved dry weight under stress, while biochar application showed even greater enhancement. The combined SA (10 µM) and biochar (2 %) application resulted in the highest plant dry weight of 0.1083 g under control. It also maintained relatively high values of 0.0983 g, 0.0943 g and 0.0837 g at 50, 100 and 150 µM Cr, respectively. These results highlighted the effective mitigation of Cr-induced growth inhibition through the synergistic action of salicylic acid and biochar, which not only supported the biomass accumulation but also improved plant resilience under heavy metal stress Figure 6.

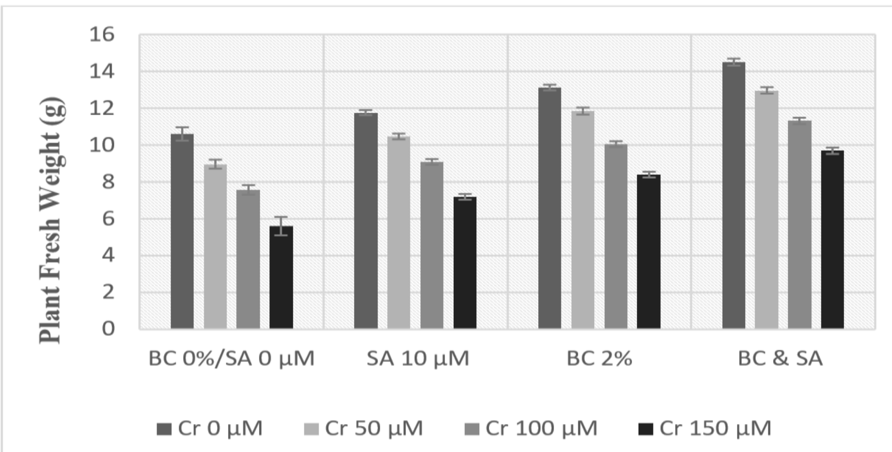

Figure 5. Effect of different concentrations of CrCl2, biochar and SA on fresh weight of S. hispanica L.

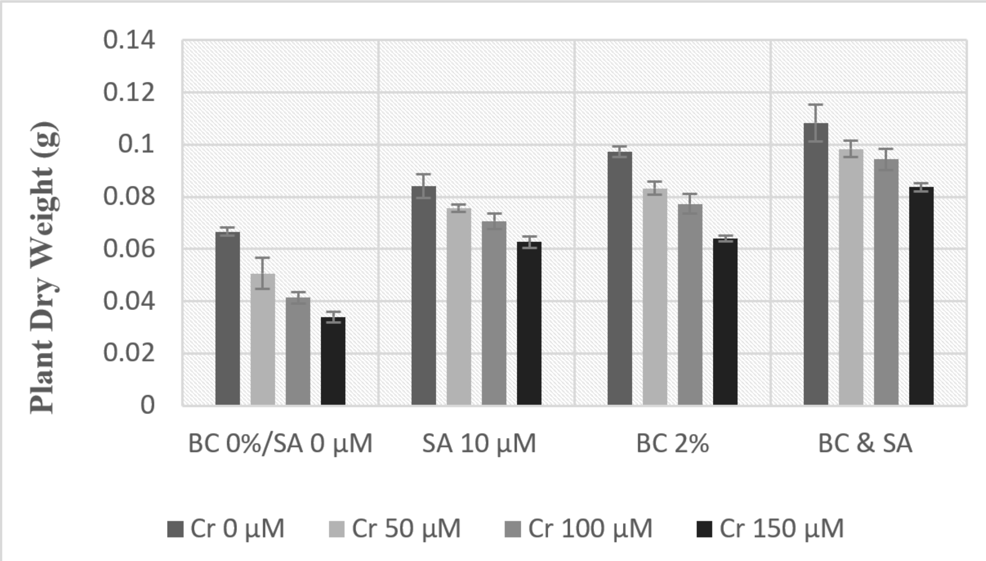

Figure 6. Effect of different concentrations of CrCl2, biochar and SA on dry weight of S. hispanica L.

3.3. Effects of Different Concentrations of Cr, SA and Biochar on chl a, chl b and Carotenoids

Under control conditions, carotenoid content was 0.0894 mg g^{-1}. Chromium stress caused progressive reductions of 0.0775, 0.0626 and 0.0449 mg g^{-1} at 50, 100 and 150 µM Cr, which represented 13.4%, 30.0%, and 49.8% reductions, respectively, compared with control. SA (10 µM) improved carotenoid content to 0.1103 mg g^{-1} under control and 0.0872 mg g^{-1} at 50 µM Cr. Biochar (2 %) further enhanced carotenoid content, recording 0.1403 mg g^{-1} under control and 0.1192 mg g^{-1} at 50 µM Cr. The combined application of SA (10µM) and biochar (2 %) produced the highest carotenoid content of 0.1617 mg g^{-1} under control and maintained relatively high levels under stress (0.1417, 0.1310 and 0.1150 mg g^{-1} at 50, 100 and 150 µM Cr), which demonstrated a strong synergistic mitigation potential of SA and biochar Figure 7.

Chlorophyll b content decreased with increasing Cr concentration from 0.0576 mg g^{-1} under control to 0.0505, 0.0427 and 0.0266 mg g^{-1} at 50, 100 and 150 µM Cr, representing 12.4 %, 26 %, and 53.8 % reductions. SA (10 µM) increased chlorophyll b to 0.0741 mg g^{-1} under control and 0.0620 mg g^{-1} at 50 µM Cr. Biochar treatment further enhanced it, with chlorophyll b content reaching 0.0860 mg g^{-1} under control and 0.0718 mg g^{-1} at 50 µM Cr. The combined application of SA (10µM) and biochar (2 %) showed the highest chlorophyll b content of 0.0923 mg g^{-1} under control and maintained 0.0623 mg g^{-1} even at 150 µM Cr, which highlighted the effective mitigation of Cr stress Figure 8.

Chlorophyll a followed a similar pattern and was 0.0656 mg g^{-1} under control. Chlorophyll content decreased with increasing Cr stress to 0.0570, 0.0436 and 0.0319 mg g^{-1} at 50, 100 and 150 µM Cr, representing 13.2%, 33.6%, and 51.4% reductions. The individually applied SA (10 µM) and biochar (2 %) improved chlorophyll a content, but the combined application of SA 10µM and biochar 2 % showed the highest chlorophyll a content. Chlorophyll a content under the combined application was 0.0975 mg g^{-1} and 0.0677 mg g^{-1} under 150 µM Cr. The combined application of SA (10 µM) and biochar (2 %) showed the highest pigment content, including carotenoids, chlorophyll a and b, under both control and Cr stress conditions. Even at 150 µM Cr, pigment levels remained relatively high, which indicated the strong protective role of SA and biochar in Cr stress alleviation Figure 9.

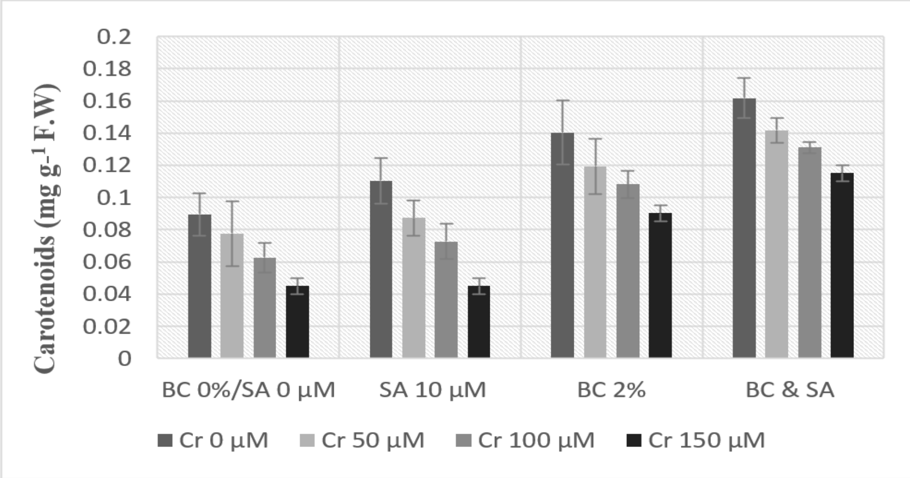

Figure 7. Effect of different concentrations of $CrCl_2$, biochar and SA on the carotenoid content of S. hispanica L.

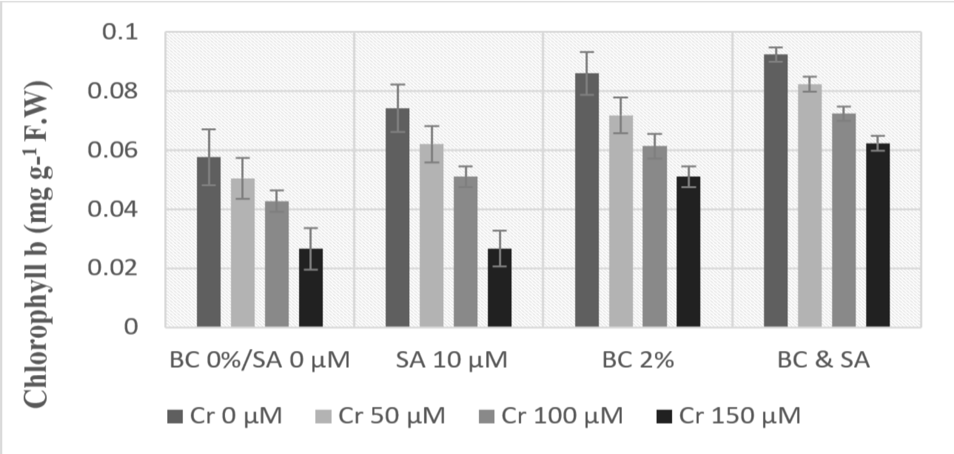

Figure 8. Effect of different concentrations of $CrCl_2$, biochar and SA on the chlorophyll b content of S. hispanica L.

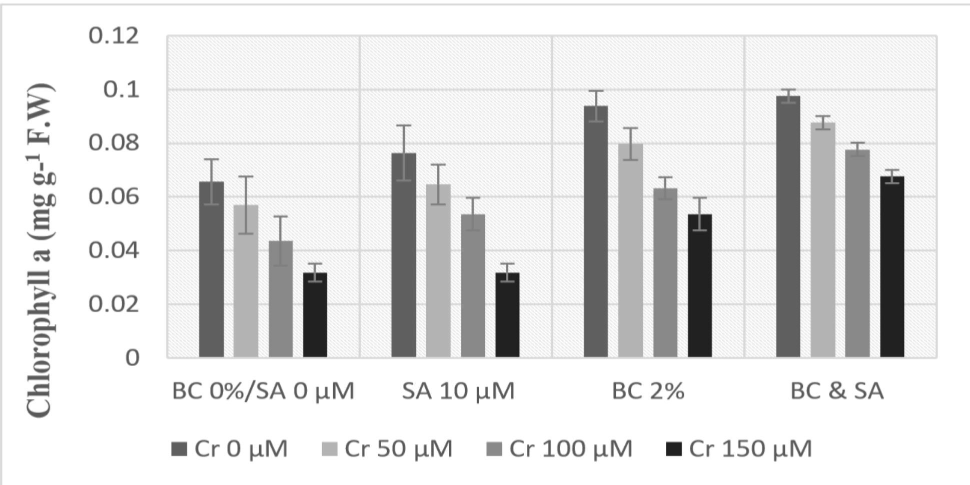

Figure 9. Effect of different concentrations of $CrCl_2$, biochar and SA on the chlorophyll a content of S. hispanica L.

4. DISCUSSION

The present study demonstrated that chromium (Cr) stress significantly impairs the growth and physiological processes of S. hispanica L. Exposure to Cr stress led to a notable decrease in stem length, root length, leaf area, and overall plant biomass, and the most pronounced effects were visible at higher Cr concentrations. Specifically, stem length was reduced by 14.1 % at 50 µM Cr and 25 % at 100 µM Cr, while root length and leaf area experienced similar reductions. Both the fresh and dry weight of the plant were reduced, which reflected the harmful impact of Cr on plant vigor. These results are consistent with earlier studies that have shown heavy metal stress disrupts nutrient uptake, inhibits cell division and elongation and causes oxidative stress, which ultimately results in stunted growth and lower biomass accumulation [24, 25]. These findings highlight that Cr affects both the structural and functional development of plants, which emphasizes the vulnerability of S. hispanica L. to heavy metal contamination.

Cr stress had a pronounced effect on photosynthetic pigments. The carotenoid content decreased from 0.0894 mg g^{-1} in the control to 0.0775 mg g^{-1} at 50 µM Cr, representing a 13.4% reduction. Chlorophyll a and b content also experienced a significant reduction under Cr stress. The results showed that Cr stress caused oxidative damage to the photosynthetic machinery and a disruption in pigment biosynthesis [26]. Carotenoids and chlorophylls play vital roles in light absorption, photoprotection and reactive oxygen species scavenging. Their reduction under Cr stress led to a decrease in photosynthetic efficiency and stunted growth [27]. These findings highlighted the importance of evaluating both structural and biochemical characteristics to comprehensively understand the effects of heavy metal stress on plant performance.

Exogenous application of SA (10 µM) markedly mitigated the growth inhibition caused by Cr stress. In comparison to plants under Cr stress, those plants treated with SA (10 µM) showed an increase in stem length by 12-18 %, root length by 10-15 %, and leaf area by 15-20 %. Additionally, both plant fresh and dry weight also increased by approximately 18- 22%, which reflected enhanced physiological activity. This improvement in growth parameters is due to SA's role in activating antioxidant defense mechanisms, which reduces reactive oxygen species (ROS) buildup and safeguards cellular structures [28]. SA is also crucial for stabilizing chlorophyll synthesis and preserving membrane integrity, which leads to enhanced photosynthetic performance as observed in the present study. Previous studies have reported similar protective effects of SA under heavy metal stress, reflecting its function as a signaling molecule in stress tolerance pathways [29, 30].

Biochar (2 %) application demonstrated a more significant impact than SA alone application in reducing Cr toxicity. In comparison to plants under Cr stress, plants treated with biochar 2 % exhibited a 20-25 % increase in stem length, a 22- 28% increase in root length and a 25- 30% increase in leaf area. Both plant fresh and dry weights also increased by 30-35 %, indicating a notable restoration of plant health. The enhanced performance of plants treated with biochar is due to its capacity to immobilize Cr in the soil, thus minimizing its absorption and movement to the plants aboveground parts [31]. Furthermore, biochar improves soil structure, nutrient availability and water retention, which collectively foster better root development and plant growth. These results align with previous studies that have shown biochar effectively reduces the bioavailability of heavy metals and alleviates metal-induced oxidative stress [32]. The combined application of SA 10 µM with biochar 2 % resulted in the most notable improvements in both growth and physiological parameters. In comparison to plants subjected to Cr stress, this combined treatment led to a 30-40 % increase in stem length, a 35- 45% increase in root length and an approximately 40% increase in leaf area. Both plant fresh and dry weight showed significant recovery and increased by 40-50%. These findings suggested that biochar reduced the availability of Cr in the soil, while SA enhanced the plant's internal defense mechanisms and ultimately enhanced stress tolerance. Consequently, the combined application provided both external and internal (physiological) protection and resulted in maximum growth recovery. Cr stress caused a reduction in photosynthetic pigments; chlorophyll a and b decreased 40-60 % and carotenoid content decreased by 30-45 %. These reductions indicated oxidative damage to chloroplasts and impaired pigment biosynthesis. The application of SA 10 µM restored chlorophyll a and b content by 20-30 %, while carotenoid content increased by 25 % compared to Cr stressed plants. Biochar (2 %) application had a more pronounced effect, boosting chlorophyll and carotenoid content by 35-45 %. The combined application of SA (10 µM) with biochar (2 %) treatment remarkably increased the pigment recovery; chlorophyll a and b increased 50-60 % and carotenoid content increased 60-70 % relative to Cr stressed plants. This enhancement signifies improved photosynthetic efficiency, better protection against oxidative damage and increased light harvesting capacity. Similar improvements in pigment stability under combined mitigation strategies have been observed in previous studies involving heavy metal stress [33, 34]. The observed improvements in photosynthetic pigments under combined treatment are particularly noteworthy. Carotenoid and chlorophyll a & b contents were restored to near-control levels, suggesting that SA and biochar together protect the photosynthetic apparatus from Cr-induced oxidative damage. Enhanced pigment levels contribute to better light absorption, energy transfer, and ROS scavenging, which ultimately support growth and biomass accumulation. These biochemical responses highlight the dual role of mitigation strategies in preserving both structural and functional integrity under heavy metal stress [35]. This study showed important research gaps in the context of S. hispanica L. and Cr stress. While individual applications of SA or biochar have been studied in other crops, few investigations have examined their synergistic effects on multiple growth and biochemical parameters in S. hispanica L. By evaluating stem length, root length, leaf area, plant fresh and dry weight, carotenoid content, and chlorophyll a & b content, this research provided a comprehensive assessment of how integrated mitigation strategies influence both structural and physiological traits under Cr stress.

5. CONCLUSION

S. hispanica L. (chia) is an important oilseed and functional food crop. It is valued for its high nutritional content including proteins, essential fatty acids and antioxidants. However, heavy metal contamination, particularly chromium (Cr), severely affects its growth, biomass accumulation, leaf area and photosynthetic pigment content, posing a significant threat to crop productivity and quality. In this study, S. hispanica L. was exposed to different concentrations of $CrCl_2$ (0, 50,100 and 150 µM). Cr stress impaired plant growth, reduced stem and root length, leaf area, biomass and photosynthetic pigments, which reflected the severe physiological and biochemical disruption. To alleviate these negative effects, SA 10 µM and biochar (2 %) were applied individually and in combination. Biochar (2 %) showed stronger mitigating effects than SA (10 µM). Notably, the combined application of SA (10 µM) and biochar (2 %) showed the highest mitigation potential, significantly enhancing the stem and root length, leaf area, fresh and dry biomass and chlorophyll a, b and carotenoid contents. These results demonstrated a strong synergistic effect, where biochar reduced Cr bioavailability and SA boosted internal stress tolerance, providing an effective integrated strategy to alleviate Cr induced toxicity and improve overall growth and productivity of chia under contaminated conditions.

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