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RESEARCH ARTICLE
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ALLEVIATION OF CHROMIUM TOXICITY BY SALICYLIC ACID AND MORINGA SEED EXTRACT IN WHEAT

Adeela Farooq[1*], Khizar Hayat Bhatti[1], Syed Abdul Majid[2]

[1]Department of Botany, University of Gujrat, Gujrat-Pakistan
[2]Department of Botany, University of Azad Jammu and Kashmir, Muzaffrabad
Email: khizar.hayat@uog.edu.pk

***Corresponding Author:** Adeela Farooq
*Department of Botany, University of Gujrat, Gujrat-Pakistan

Abstract
The most important crop in the world due to its nutritional worth is wheat. It can serve as an indicator for the economic health of a nation. Pakistan is an agrarian country whose economy is based on cash crops, one of which being wheat. Wheat annual growth should be 44 million tones to feed the people in 2030. Experiments on pot studies were conducted in the research field, University of Gujrat, throughout 2020-2022. Research was done to look at the effects of chromium (Cr) toxicity on variety (Sehar-06) of wheat (*Triticum aestivum* L.) by seed priming with salicylic acid (SA) and topically foliar spray of Moringa seed extract (MSE). The findings which are displayed as graphs and tables, represent the average of two years of wheat (2021 and 2022) using the applied treatments. Morphological characteristics showed clearly toxic effects of chromium unaccompanied or combine with salicylic acid and moringa seed extract. All morphological parameters frequently enhanced with the application of SA as well as MSE. Chromium was also shown to have an inhibitory effect in cases of decreased photosynthetic activity and biochemical parameters. Both (0.75, 1.0 mM) level of chromium significantly decreased chlorophyll contents, and flavonoids contents but there is increase in phenolics. Additionally, biochemical characteristics revealed an unfavorable reaction with both chromium levels. With various treatments, both enzymatic and non-enzymatic antioxidants exhibited distinct behavior at wheat variety, with as well as without chromium. DPPH activities also effected by high level of chromium stress in plants and also increased with application of SA and MSE. MSE (3%) condensed the antagonistic effects of chromium (Cr) stress in wheat, improving all of the attributes more than SA (3 mM).

Key words: Enzyme, Plant Growth, Biochemical, Antioxidant

Introduction
The family Poaceae, which includes sweet grasses, includes the cultivated wheat (*Triticum aestivum* L.) (Gupta & Ranjan, 2020). Due to its widespread use as the primary cereal crop, the term "king of cereals" is sometimes used to wheat. Due to its wide adaptability and higher nutrient content than other cereal crops, it is the most significant and widely farmed food cereal in the world (Khaliq et al., 2023). Wheat is a winter crop that is farmed throughout Pakistan. In Pakistan, wheat production is about 23888 kg ha^{-1}, which is quite low compared to other wheat-growing locations throughout the world (Bashir et al., 2021). Reduced and unequal fertilizer use, local climate, decreased soil

fertility, a lack of micro and macronutrients, illiteracy and the use of advanced technology by the former are the causes of the lower yield of wheat (Esilaba et al., 2021).

The issue of heavy metal poisoning is growing more significant due to ecological, evolutionary, dietary, and environmental factors. Environmental pollutants that were significant were heavy metals (Javaid et al., 2020). Growing crops in agricultural soils suffer greatly from rising levels of heavy metal pollution (Qin et al., 2021). Reduced photosynthesis, poor nutrient absorption, damaged roots, and finally plant mortality are some of the impacts of heavy metal stress on plants (Rehman et al., 2021). One of the most troublesome heavy metals in crops is chromium (Cr). Together with other human operations like mining and electroplating, the main source of chromium emissions into the soil environment is the tanning of leather (Ullah et al., 2023). Depending on the plant species, higher levels of Cr stress reduce plant growth and cause physiochemical and ultrastructural changes in plants (Singh et al., 2021). Through the food chain, chromium can enter the human body and cause a number of dangerous disorders (Sonone et al., 2020).

Salicylic acid (SA), a phenolic phytohormone, regulates a range of growth and developmental processes, including seed germination, photosynthesis, respiration, blooming, and senescence (Arif et al., 2020). Research has shown that exogenous SA application and seed priming offer plants substantial defense against abiotic stresses in a number of ways, such as by upregulating genes that respond to stress and serving as a signaling molecule to impart resistance and/or affect metabolic processes (Tiwari et al., 2021). Research has definitely demonstrated that exogenous application of SA, or its usage as a pre-treatment to combat oxidative stress caused by heavy metal toxicity, can enhance plant physiological responses, redox metabolism, and antioxidant activities (Mazumder et al., 2022).

Moringa oleifera seed extract is a chemical that may aid the plant in overcoming a variety of stresses, including heavy metal or water stress (Buthelezi et al., 2022). The goal of Desoky et al. (2019) study was to assess the possible impacts of exogenous Moringa extract of seed supplements added to foliar spray and drip irrigation water (SA) on changes in yield, physio-chemical components, growth, accumulation of heavy metals, mineral nutrients and the antioxidant defense system of pepper plants which was grown in soil (saline) contaminated with chromium (Cr) (Rady et al., 2023). Salicylic acid, ascorbic acid, and glutathione are some of the antioxidants and osmoprotectants found in (*Moringa oleifera* L.) seed extract (Elrys et al., 2021). A 0.5% MSE foliar spray increased yield while decreasing the buildup of trace metal elements in *Capsicum annuum* (Desoky et al., 2019).

Materials and Methods

Procurement of the materials: Pot study experiments were carried out in the research area, University of Gujrat, Gujrat-Pakistan during 2020-2022. Under the natural conditions of light and temperature according to the season of the crop i.e November to April for two consecutive years. Wheat seeds were purchased from Punjab seed cooperation. By seed priming with salicylic acid (SA) and applying Moringa seed extract (MSE) topically, research was carried out to examine the impact of chromium toxicity on variety (Sehar-06) of wheat (*Triticum aestivum* L.). Three replications of the experiment were set up using a Completely Random Design. Levels of Cr treatments, i.e., 0 (control), 0.5 mM, 0.75 mM and 1.00 mM by plant rooting medium after establishment of seedling. To preserve homeostasis, water was delivered on regular basis. Seeds were soaked in Salicylic acid (0mM, 3mM) for 25 minutes before sowing, with vigorously shaking. Salicylic acid was used as seed priming agent. Moringa seed extract (Thrice) was applied by a manual sprayer early in the morning. MSE (0%, 3%) was applied exogenously to leaves at three leaf seedling stage with an interval of about 10 days by using few drops of Tween-20 as a surfactant (Romano et al., 2022).

Treatments: Following were the treatments:

- T_0 = 0.0mM Cr+ 0mM SA+ 0% MSE
- T_1 = 0.0mM Cr+ 0mM SA+ 3 % MSE

- T_2 = 0.0mM Cr+ 3mM SA+ 0 % MSE
- T_3= 0.0mM Cr+ 3mM SA+ 3 % MSE
- T_4 = 0.5mM Cr+ 0mM SA+ 0 % MSE
- T_5= 0.5mM Cr+ 0mM SA+ 3 % MSE
- T_6= 0.5mM Cr+ 3mM SA+ 0 % MSE
- T_7= 0.5mM Cr+ 3mM SA+ 3 % MSE
- T_8 = 0.75mM Cr+ 0mM SA+ 0 % MSE
- T_9= 0.75mM Cr+ 0mM SA+ 3 % MSE
- T_{10}= 0.75mM Cr+ 3mM SA+ 0 % MSE
- T_{11}= 0.75mM Cr+ 3mM SA+ 3 % MSE
- T_{12} = 1mM Cr+ 0mM SA+ 0 % MSE
- T_{13}= 1mM Cr+ 0mM SA+ 3 % MSE
- T_{14}= 1mM Cr+ 3mM SA+ 0 % MSE
- T_{15}= 1mM Cr+ 3mM SA+ 3 % MSE

Analytical tests

Morphological parameters like shoot and root lengths were measured by using iron meter scale. Fresh and dry weights of root and shoot weight were noted with the help of electric balance. At maturity the crop was manually harvested and threshed. Weight of 100-grains was determined (Yasmeen et al., 2013). Ethanolic extract of moringa seed extract was used. Seeds of moringa were collected and cleaned properly, and then seeds were air-dried. The moringa seeds were dehusked and ground into powder (Sowunmi & Gonzo, 2023).

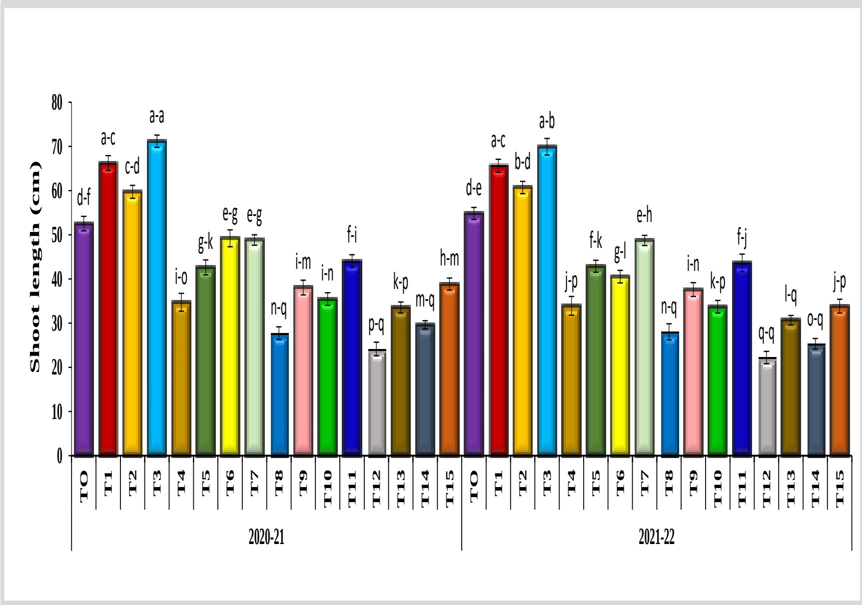
One kg of ground-dried seeds were weighed, powder was transferred into a flask and extracted with 1 litter of 70% (v/v) ethanol (eq) for 48 h at 4 C. The extract was filtered with Whatman filter paper and dried using a rotary evaporator at 40 ^{o}C and stored at-20 ^{o}C (Hamza, 2010), the method was used with slight modification. Before administration the crude extract was diluted to make required percentage with distilled water .Te extract was freeze-dried and stored at -20 °C for future use (Hamza, 2010).

Concentrations of chlorophyll a, total chlorophyll, chlorophyll b as well as carotenoid were detected by using the Arnon Methods (Nazeer et al., 2020). Antioxidant activities such as Superoxide dismutase (SOD) was detected through estimating restriction in photo reduction of nitroblue tetrazolium according to Giannopolitis and Ries method as used by Banakar et al. (2022). Catalase (CAT) was determined by Aebi method as used by Ijaz et al. (2021). Total phenolic contents were measured with the help of Folin Ciocalteu's method (Sulistiany et al., 2016) using gallic acid as the standard. Total contents of flavonoids were determined using a spectrometric assay according to Moldovan et al. (2021) method. Determination of Antioxidant Potential by the method of Brand-Williams as described by Mira-Sanchez et al. (2020).

Statistical analysis: The data were reported as the mean ± SE of three replicates and were then analyzed using the two-way ANOVA approach with Minitab software version-19. Tukey's HSD test was used to separate the means at $P < 0.05$, with a 95% confidence interval (Silverman, 2018). MS Excel was used to exhibit the data visually.

Results
Morphological characters

(A) Shoot Length (cm)



B) Root Length (cm)

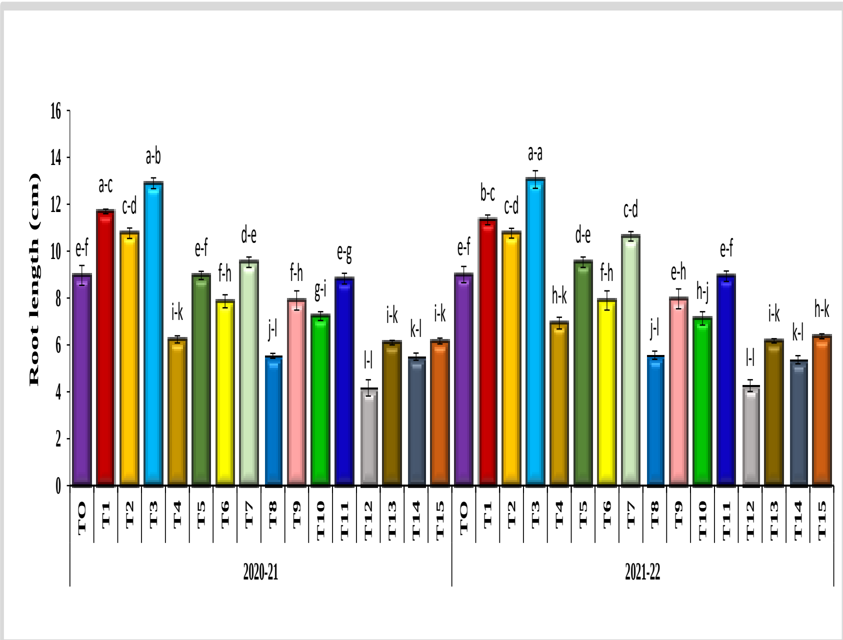


Figure 1: Effect of Foliar Treatments on morphological attributes T0- Control (0.0mM Cr+ 0mM SA+ 0% MSE), T1-(0.0mM Cr+ 0mM SA+ 3 % MSE), T2-(0.0mM Cr+ 3mM SA+ 0 % MSE), T3-(0.0mM Cr+ 3mM SA+ 3 % MSE), T4- (0.5mM Cr+ 0mM SA+ 0 % MSE), T5- (0.5mM Cr+ 0mM SA+ 3 % MSE), T6- (0.5mM Cr+ 3mM SA+ 0 % MSE), T7- (0.5mM Cr+ 3mM SA+ 3 % MSE), T8- (0.75mM Cr+ 0mM SA+ 0 % MSE), T9- (0.75mM Cr+ 0mM SA+ 3 % MSE), T10- (0.75mM Cr+ 3mM SA+ 0 % MSE), T11- (0.75mM Cr+ 3mM SA+ 3 % MSE), T12- (1mM Cr+ 0mM SA+ 0 % MSE), T13- (1mM Cr+ 0mM SA+ 3 % MSE), T14- (1mM Cr+ 3mM SA+ 0 % MSE) , T15- (1mM Cr+ 3mM SA+ 3 % MSE) were applied with Wheat (*Triticum aestivum* L.) Variety Sehar-06. **A)** Shoot Length **B)** Root Length

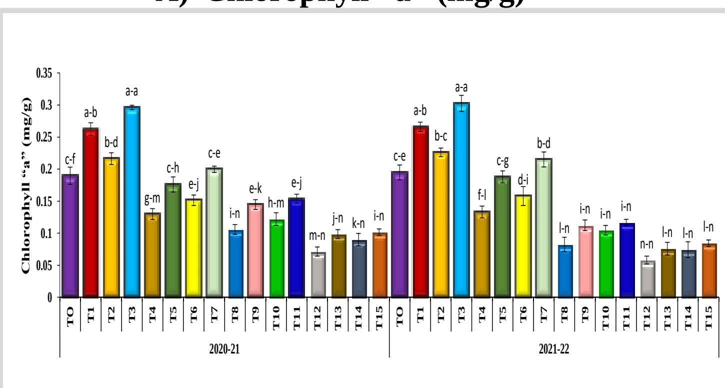
Chromium levels (0.5, 0.75, 1 mM) significantly reduced root length especially at the maximum level compared to the control by 53.53 % and 52.59 % in Sehar-06 during both seasons (Figure 1B).

The treatment of heavy metal (Chromium) stress resulted in a reduction in shoot length in variety (Sehar-06) of wheat (Figure 1A). Chromium levels (0.75, 1 mM) significantly reduced the shoot length by (59.457 %) in Sehar-06 notably at the highest level as compared to the negative control. Moreover, foliar spray of moringa seed extract and seed priming of salicylic acid significantly enhanced the shoot length in wheat variety under chromium stress. Moringa seed extract and salicylic acid in combination (38.86 cm and 33.87 cm) had a greater shielding effect against chromium stress, whereas shoot length was severely reduced to (24.17 cm and 22.23 cm) by chromium stress without salicylic acid and moringa seed extract in consecutive years in Sehar-06. On the basis of results, it was noted that variety Sehar-06 was sensitive to high chromium stress. Data showed that impact of salicylic acid and moringa seed were highly significant ($P \leq 0.001$) on wheat variety with its interactions. The interaction between treatments and year narrated non-significant ($P>0.05$) results.

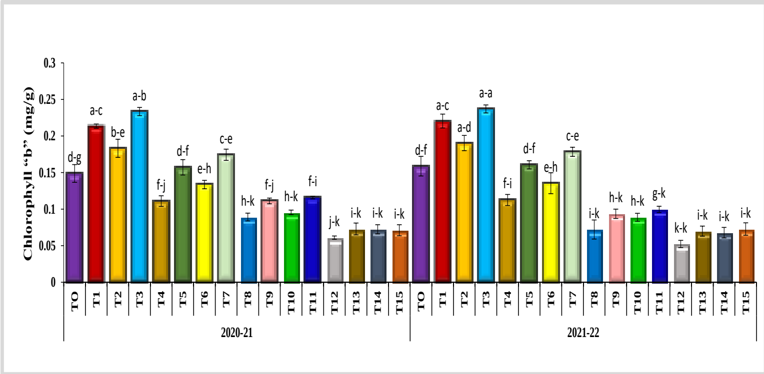
Physiological characters

Chromium stress at 1.0 mM concentration significantly reduced the chlorophyll a by 62.63 % and 70.26 % in Sehar-06 in both Years as compared to their respective unstressed control plants. Moringa seed extract (MSE) 3% solution showed positive impact on growth of wheat plants and enhanced the chlorophyll a by 38.42 % in Sehar-06 as compared to their control (0.0 mM) plants. Similarly, individual application of salicylic acid (SA) 3 mM significantly enhanced the by chlorophyll a 13.68 % as compared to negative control in Sehar-06. Furthermore, the application of MSE and SA either alone or in combination with each other significantly mitigate the chromium stress or enhanced the chlorophyll a by 43.66 % in Sehar-06 as for 1.0 mM Cr + 3mM SA+ 3 % MSE, by 45.28 % in Sehar-06 for 0.75 mM Cr + 3mM SA+ 3 % MSE, by 53.85 % in Sehar-06 for 0.5 mM Cr + 3mM SA+ 3 % MSE as compared to positive control plant. Results from Figure 2B also justified that highest values of chlorophyll b contents (0.233, 0.237) were obtained at non-stressed plants with application of salicylic acid as well as moringa seed extract at both years in Sehar-06 in comparison with negative control. While, minimum results (0.061, 0.052) in Sehar-06 was recorded with highly stressed plant as chromium reduced the chlorophyll 'b' contents badly. The application of MSE either alone or in combination with SA significantly mitigate the chromium stress or enhanced the chlorophyll b by 42.48 % in Sehar-06 as for 0.5 mM Cr + 3 % MSE. On the basis of results it is illustrated that variety Sehar-06 is sensitive to high chromium stress. Chromium stress at 1.0 mM concentration significantly reduced the total chlorophyll by 61.06 % and 68.93 % in Sehar-06 in both years as compared to their respective unstressed control plants. On the other hand, moringa seed extract (MSE) 3% solution showed positive impact on growth of wheat plants and enhanced the total chlorophyll by 37.57 % (0mM Cr) in Sehar-06 as compared to non-treated control. Chromium levels (0.50, 0.75, and 1 mM) significantly reduced the carotenoids by (54.65 %) in Sehar-06, notably at the 0.50 mM Cr as compared to the non treated control plant. It was also concluded from the results that salicylic acid and moringa seed extract application proved beneficial to the wheat under significant metal stress.

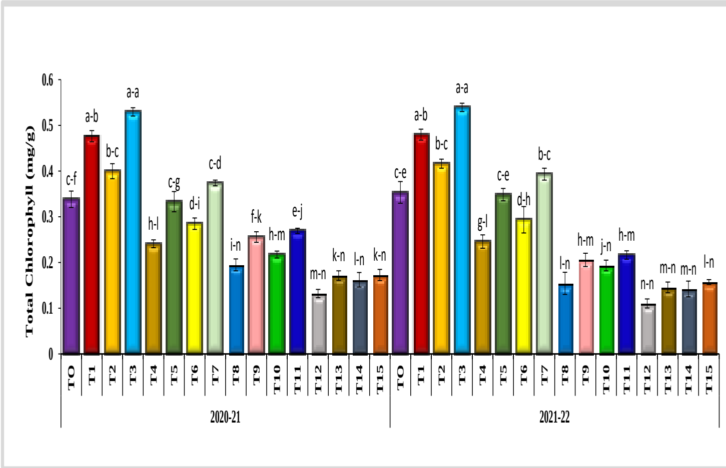
A) Chlorophyll "a" (mg/g)



B) Chlorophyll "b" (mg/g)



C) Total Chlorophyll



D) Carotenoids contents

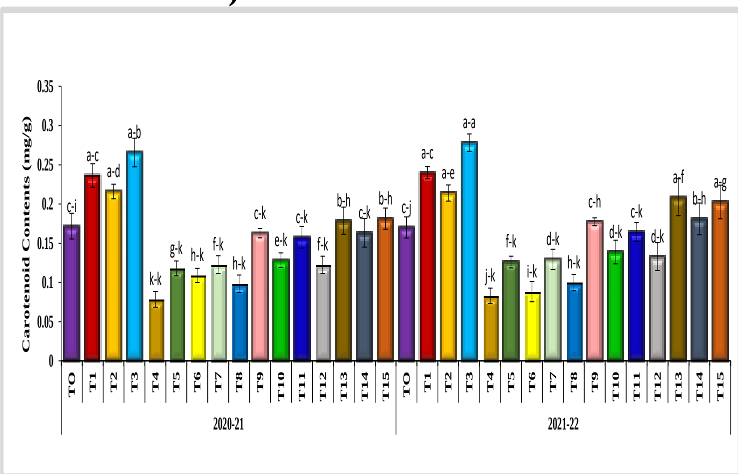
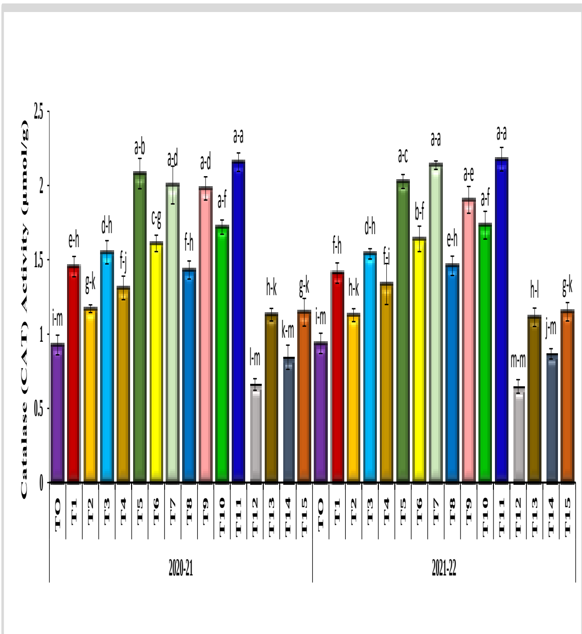
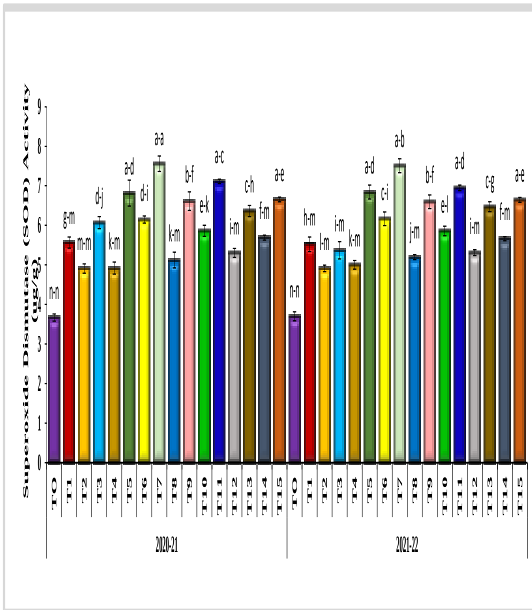


Figure 2: Effect of Foliar Treatments on physiological attributes T0- Control (0.0mM Cr+ 0mM SA+ 0% MSE), T1-(0.0mM Cr+ 0mM SA+ 3 % MSE), T2-(0.0mM Cr+ 3mM SA+ 0 % MSE), T3-(0.0mM Cr+ 3mM SA+ 3 % MSE), T4- (0.5mM Cr+ 0mM SA+ 0 % MSE), T5- (0.5mM Cr+ 0mM SA+ 3 % MSE), T6- (0.5mM Cr+ 3mM SA+ 0 % MSE), T7- (0.5mM Cr+ 3mM SA+ 3 % MSE), T8- (0.75mM Cr+ 0mM SA+ 0 % MSE), T9- (0.75mM Cr+ 0mM SA+ 3 % MSE), T10- (0.75mM Cr+ 3mM SA+ 0 % MSE), T11- (0.75mM Cr+ 3mM SA+ 3 % MSE), T12- (1mM Cr+ 0mM SA+ 0 % MSE), T13- (1mM Cr+ 0mM SA+ 3 % MSE), T14- (1mM Cr+ 3mM SA+ 0 % MSE) , T15- (1mM Cr+ 3mM SA+ 3 % MSE) were applied with Wheat (*Triticum aestivum* L.) Variety Sehar-06. **A)** Chlorophyll a **B)** Chlorophyll b **C)** Total chlorophyll **D)** Carotenoid contents

Antioxidant activities

Catalase (CAT) enzymes protect plants from oxidative stress. The content of catalase enzymes in wheat increased after foliar application of moringa seed extract and seed priming of salicylic acid. Chromium (0.5, 0.75 mM) stress increased catalase activity significantly, followed by control (0.937) and the greatest amount of chromium stress (0.647) in Sehar-06. The study found that foliar spraying with 3% MSE and 3 mM SA resulted in the highest catalase enzyme concentrations (2.137, 2.177) in Sehar-06 in combination with levels (0.5 and 0.75mM) of chromium as compared to control. Results also narrated that moringa seed extract (3%) gave best results under heavy metals stressed plants and produced highest ratio of CAT activities in shoot as compared to SA (Figure 3A). The graphical depiction revealed that chromium stress increased the amount of superoxide dismutase activity (SOD) in the shoot with or without treatment in both years and at wheat variety. In Sehar-06 moringa extract alone gave better results (5.523) as in combination of moringa extract and SA (5.37). It was concluded that 0.75mM chromium treatment adversely affects plant by increasing SOD activity, which showed sensitivity for wheat cultivars at this level of chromium stress. High chromium increased SOD by 43.48% in Sehar-06. The results also revealed that there were few changes in the outcomes of SOD activities in shoots practically every year (Figure 3B). Overall, significant traits differences were seen for all treatments and chromium stress administered. It was shown by the results that DPPH activities were decreased in highly stressed plant as compare to non-stress treated plants. Levels of (0.5, 0.75, 1.0Mm) declined DPPH activity in shoot. From (Figure 3C), it was established that (0.5, 0.75mM) chromium treatment reduced DPPH activity in plants, which displayed sensitivity for wheat variety at this level of chromium stress particularly 1.0mM. However, 0.5 mM level of chromium stress may be overcome by foliar treatment of MSE (3%) as well as SA (3mM) to create tolerance in wheat plants. It was illustrated that DPPH activity (41.32 % and 38.19 %) in 0. 5 mM Cr+ 3mM SA+ 3 % MSE in Sehar-06 during both seasons.

<table>
<tr><td>CAT in Shoot

</td><td>SOD in Shoot

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<tr><td>c) DPPH in Shoot</td><td></td></tr>
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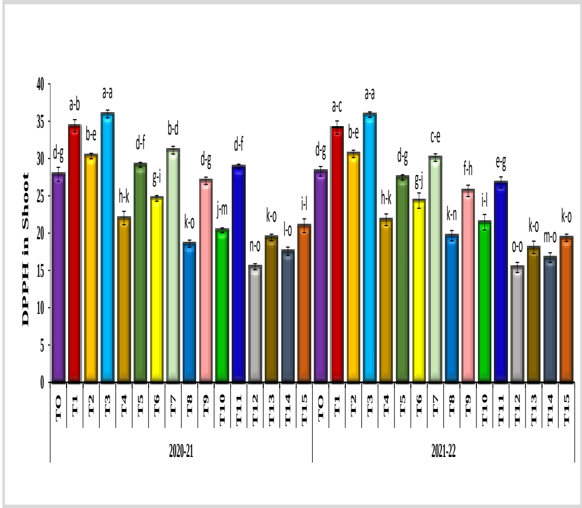

Figure 3: Effect of Foliar Treatments on antioxidant activities T0- Control (0.0mM Cr+ 0mM SA+ 0% MSE), T1-(0.0mM Cr+ 0mM SA+ 3 % MSE), T2-(0.0mM Cr+ 3mM SA+ 0 % MSE), T3-(0.0mM Cr+ 3mM SA+ 3 % MSE), T4- (0.5mM Cr+ 0mM SA+ 0 % MSE), T5- (0.5mM Cr+ 0mM SA+ 3 % MSE), T6- (0.5mM Cr+ 3mM SA+ 0 % MSE), T7- (0.5mM Cr+ 3mM SA+ 3 % MSE), T8- (0.75mM Cr+ 0mM SA+ 0 % MSE), T9- (0.75mM Cr+ 0mM SA+ 3 % MSE), T10- (0.75mM Cr+ 3mM SA+ 0 % MSE), T11- (0.75mM Cr+ 3mM SA+ 3 % MSE), T12- (1mM Cr+ 0mM SA+ 0 % MSE), T13- (1mM Cr+ 0mM SA+ 3 % MSE), T14- (1mM Cr+ 3mM SA+ 0 % MSE) , T15-(1mM Cr+ 3mM SA+ 3 % MSE) were applied with Wheat (*Triticum aestivum* L.) Variety Sehar-06. **A)** CAT in Shoot **B)** SOD in Shoot **C)** DPPH n shoot

Biochemical Attributes

Chromium treatment increased total phenolics in shoot, for (201.0 and 228.33) in 0.75 mM Cr+ 3mM SA+ 3 % MSE in Sehar-06, for (222.67 and 226.67) in 1.0 mM Cr+ 3mM SA+ 3 % MSE in Sehar-06. Moreover, lowest contents (111.33 and 116.67) in Sehar-06 was noted at non stressed plants at both years. Data from graphs elaborated that maximum total phenolic contents were obtained in highly chromium (1.0 mM) stress plants and also in foliar application of salicylic acid and moringa seed extract plants (Figure-4A). Increased total phenolic contents in shoot were noted at all levels of chromium (0.5, 0.75 and 1.0 mM) stress that increased with increasing concentration of chromium. It was established that (0.5, 0.75 and 1.0mM) chromium treatment decreased total flavonoid in shoot, which displayed sensitivity of wheat variety at this level of chromium stress. However, 0.5 mM level of chromium stress may be overcome by foliar treatment of MSE (3%) as well as SA (mM) to create tolerance in wheat plant. It was illustrated from Figure-4B that maximum total flavonoid (48.11 and 48.32) was found in 0.0 mM Cr+ 3mM SA+ 3 % MSE in Sehar-06. Moreover, lowest contents (19.14) in Sehar-06 was noted at highly stressed plants. TFC decreased with increasing Cr stress. Graphical representation elaborated that MSE helps in increase of TFC by 17.87% in Sehar-06 of non-chromium treated plants. High concentration of flavonoid contents in shoot were noted at combination of exogenous application of salicylic acid as well as moringa seed extract at both 2021 and 2022 years. Heavy metals reduce the contents of flavonoid in shoot more as compare to seed priming of salicylic acid as well as foliar spray of moringa seed extract in wheat variety on both 2021 and 2022 years.

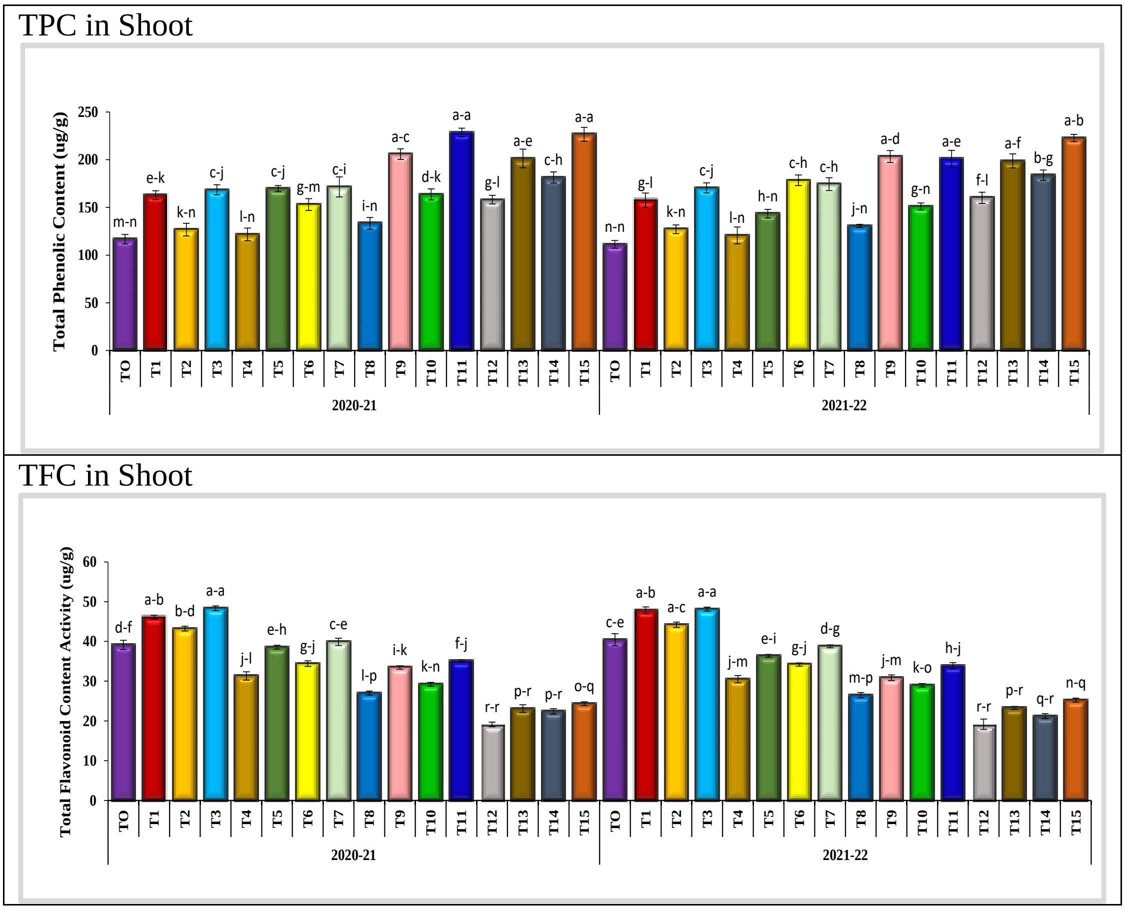

Figure 4: Effect of Foliar Treatments on Biochemical attributes T0- Control (0.0mM Cr+ 0mM SA+ 0% MSE), T1-(0.0mM Cr+ 0mM SA+ 3 % MSE), T2-(0.0mM Cr+ 3mM SA+ 0 % MSE), T3-(0.0mM Cr+ 3mM SA+ 3 % MSE), T4- (0.5mM Cr+ 0mM SA+ 0 % MSE), T5- (0.5mM Cr+ 0mM SA+ 3 % MSE), T6- (0.5mM Cr+ 3mM SA+ 0 % MSE), T7- (0.5mM Cr+ 3mM SA+ 3 % MSE), T8- (0.75mM Cr+ 0mM SA+ 0 % MSE), T9- (0.75mM Cr+ 0mM SA+ 3 % MSE), T10- (0.75mM Cr+ 3mM SA+ 0 % MSE), T11- (0.75mM Cr+ 3mM SA+ 3 % MSE), T12- (1mM Cr+ 0mM SA+ 0 % MSE), T13- (1mM Cr+ 0mM SA+ 3 % MSE), T14- (1mM Cr+ 3mM SA+ 0 % MSE) , T15- (1mM Cr+ 3mM SA+ 3 % MSE) were applied with Wheat (*Triticum aestivum* L.) Variety Sehar-06. **A)** TPC in Shoot **B)** TFC in Shoot

Yield Parameters

Chromium stress at 1 mM concentration significantly decreased the weight of grains by 43.44% and 43.15% in Sehar-06 in both years compared to their respective un-stress control plants. On the other hand, moringa Seed extract 3% solution showed positive impact on growth of wheat plants and enhanced the weight of grains by 43.32 % (0mM) in sehar-06 as compared to negative control plants. Similarly, individual application of salicylic acid 3mM significantly enhanced the weight of grains by 23.65 % (0mM) in Sehar-06 as compared to negative control plant (Figure 5A). Furthermore, the application of MSE and SA either alone or in combination with each other significantly mitigate the chromium stress and enhanced the weight of grains by 38.9% for 1mM Cr+ 3mM SA+ 3 % MSE in Sehar-06. When compared to control or non-stressed wheat varieties, chromium significantly decreased the 100 grain weight (g). With the maximum concentration of chromium (1.0 mM), the minimum weight (g) of 100 grains was achieved. The results showed that the treatment of chromium had a substantially detrimental effect on grain weight, but that seed priming of salicylic acid and foliar spray of moringa seed extract topically greatly increased grain weight.

A) 100 grain weight (g)

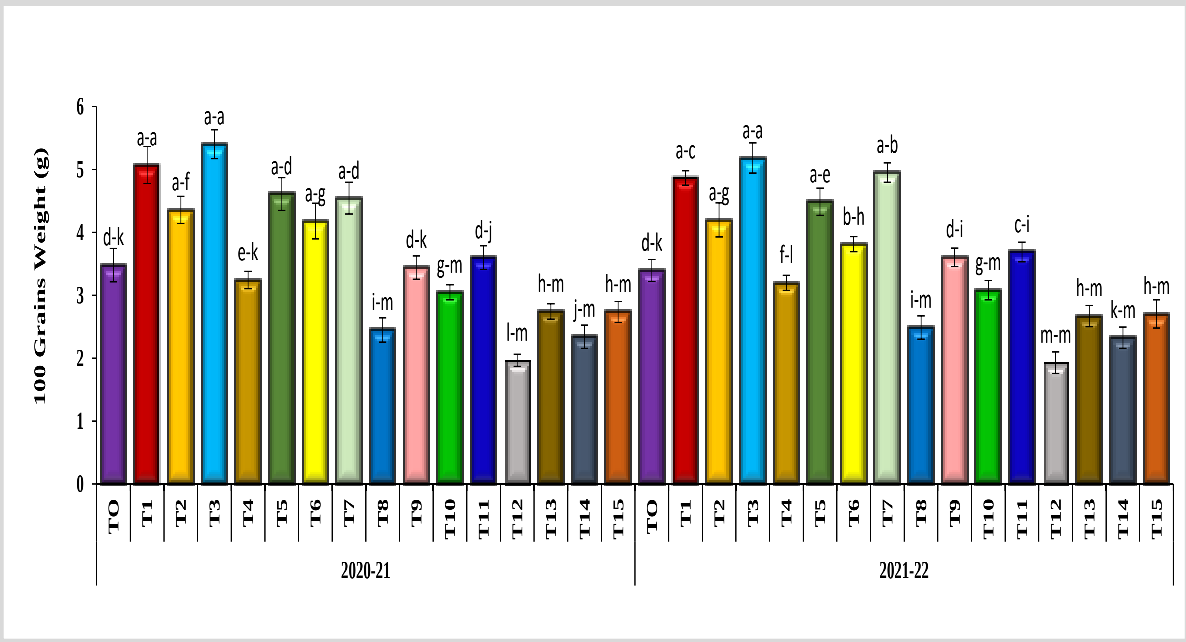

Figure 5: Effect of Foliar Treatments on Biochemical attributes T0- Control (0.0mM Cr+ 0mM SA+ 0% MSE), T1-(0.0mM Cr+ 0mM SA+ 3 % MSE), T2-(0.0mM Cr+ 3mM SA+ 0 % MSE), T3-(0.0mM Cr+ 3mM SA+ 3 % MSE), T4- (0.5mM Cr+ 0mM SA+ 0 % MSE), T5- (0.5mM Cr+ 0mM SA+ 3 % MSE), T6- (0.5mM Cr+ 3mM SA+ 0 % MSE), T7- (0.5mM Cr+ 3mM SA+ 3 % MSE), T8- (0.75mM Cr+ 0mM SA+ 0 % MSE), T9- (0.75mM Cr+ 0mM SA+ 3 % MSE), T10- (0.75mM Cr+ 3mM SA+ 0 % MSE), T11- (0.75mM Cr+ 3mM SA+ 3 % MSE), T12- (1mM Cr+ 0mM SA+ 0 % MSE), T13- (1mM Cr+ 0mM SA+ 3 % MSE), T14- (1mM Cr+ 3mM SA+ 0 % MSE) , T15- (1mM Cr+ 3mM SA+ 3 % MSE) were applied with Wheat (*Triticum aestivum* L.) Variety Sehar-06. **A)** 100 grain weight (g)

Discussion

Moringa contain more vitamin A, vitamin C, calcium, protein, potassium and iron than in carrots, oranges, milk, yoghurt, bananas, spinach (Rockwood et al., 2013). Based on the research findings, at 0.75 mM and 1.0 mM levels of chromium, respectively, shoot length decreased by up to 59.457 % in Sehar-06 at highest level of chromium. When applied topically to non-stressed wheat varieties, moringa seed extract (MSE) and seed priming with 3mM (SA) increased shoot length in by 27.6% in Sehar-06. Plant development is hindered when the concentration of Cr is higher because it expressively reduces the absorption of nutrients (Tauqeer et al., 2016). Moreover, foliar spray of moringa seed extract and seed priming of salicylic acid significantly enhanced the shoot length in wheat variety under chromium stress.

Growth and yield of wheat was affected by salt stress and with exogenously sprayed natural and synthetic stimulators decreased the damage caused by salt-induced. MSE and SA when used alone or in combination improved the growth attributes in cancer bush plants under of heat-stress as compared to control. This might be due to bioactive compounds present in MSE (Buthelezi et al., 2024).

The chromium badly affects the morphology of wheat particularly root, stem and leaf that in turn is also affected by form of chromium. Chromium inhibits cell division and cell elongation that cause root length and biomass reduction. It leads to the deformation of roots as adaptive strategy that may cause thick roots to reduce surface area to obstruct Cr uptake. Reduction in shoot length might be due to interrupted nutrients that also cause leaf necrosis and chlorosis. When chromium stress increased, root length likewise decreased. Additionally, plants treated with foliar sprays of MSE and SA seed priming did not demonstrate pronounced improvement in root length, but have impact on root length when used together indicating that foliar was more pronounced treatment, which was not available to the rooting media. The SA regulate the salt stress in wheat, SA and Ca2+ could be used together to improve growth of plant against stress (Yucel & Heybet, 2016). Reduced nutrition and water absorption as a result of metal toxicity-induced root architecture breakdown and root growth suppression results in stunted shoot development (Ulhassan et al., 2019).

The dried weight significantly decreased as the amount of chromium stress increased and remained increased when MSE and SA were applied externally. Additionally, another research showed that when choysum plants were exposed to growing media with increasing concentrations of Cr^{6+} (150 and 300 μM), there was a discernible decrease in the development of shoots as well as roots (Kamran et al., 2021). Chromium stress obstructs various physiological processes like photosynthesis, respiration, metabolism and absorption of nutrients. Similar results from earlier research showed that Cr limits photosynthetic rate and chlorophyll contents, which in turn inhibits stomatal conductance, carbon dioxide assimilation as well as transpiration rate (Atta et al., 2013).

In present work amount of caroenoids decreased but with application of MSE, SA and MES+SA improved the carotenoids and other photosynthetic pigments. The increase in carotenoids content may act as an antioxidant to scavenge ROS generated as a result of Cr toxicity. In response to toxicity of chromium, careteniods might increase to scavange ROS, that cause Lipid peroxidation which damages biological membranes making (Panda & Choudhury, 2005). Photosynthetic system is markedly affected by presence of excessive chromium by targeting its electron transport, thylakoid membranes and enzymes of Calvin cycle (Fatma et al., 2023). Two different sorts of reactions are activated in plants when chromium causes oxidative damage up to a certain point. Enzymatic antioxidants are one type of reaction, while non-enzymatic antioxidants are the other. MSE caused a rise in the levels of certain enzymatic antioxidants such as catalase activities (CAT) as well as superoxide dismutase (SOD) which is somewhat superior to salicylic acid. MLE treatment showed maximum activities of enzymatic antioxidants i.e. SOD, CAT and POD under moderate salinity as compared to non-saline and high saline conditions (Yasmeen et al., 2013).

According to Husak & Bayliak (2023), Cr^{6+} poisoning significantly reduces the catalytic activity of SOD and CAT, which are extremely vulnerable to heavy metal stressors. One more study related to our research indicated that the addition of JA to plants stressed by Cr increased the activities of SOD and CAT enzymes, indicating a significant contribution of these enzymes to the relief of Cr-induced choysum toxicity as seen by the reduction in H_2O_2 and MDA concentrations (Kamran et al., 2021).

In this work, declined DPPH activity (44.43%, 45.53%) in Sehar-06 with respect to respective negative control were noted at stressed plants (1.0 mM) at both years. However, 0.5 mM level of chromium stress might be minimized by foliar treatment of MSE (3%) as well as SA (3mM) and bring tolerance in wheat plants. Using MLE in combination with other growth regulators of plant (i.e., SA, H_2O_2 and AsA) boosts growth and show synergic effects. Salicylic acid affects several developmental processes like growth, metabolic processes, regulation of stomatal and photosynthesis (Khan et al., 2017).

When exposed to chromium, the levels of flavonoids dropped by 20–50%; however, at 1.0 mM, the decline rate for the majority of the characteristics was nearly twice as high and phenolics increased with increasing chromium level. In contrast to Zandi & Schnug (2022), who reported a rise in flavonoids and proline activity with saline stress, which give evidence for membrane protection from LPO, Arshad et al. (2015) also obtained the same findings under abiotic stress. The decrease may be due to disruptive antioxidative system or nutrient deficiency. Due to Cd in rhizosphere of wheat cultivars total flavanoids and tocopherols significantly dropped. Total flavanoids and tocopherols were increased with foliar application of SNP (Muhammad et al., 2019). Total phenol and total flavonoids were affected by different levels of drought stress and mycorrhizal fungus. In buckwheat, phenolic compounds were enhanced by drought stress and mycorrhizal inoculation (Mohammadi et al., 2022). In the defense mechanism, phenolic compounds play a key role, and conciliate the transport of auxins. Their increase production under heavy metal stress protects plants from oxidative damage (Ramzan et al., 2023).

Exposure to chromium affects yield parameters of wheat by damaging growth, root and shoot system, photosynthetic machinery etc. and ultimately reduced weight of grain. Cadmium stress decreases yield parameters like 100-grain weight. With the maximum concentration of chromium (1.0 mM), the minimum weight (g) of 100 grains was achieved. The results showed that the treatment of chromium had a substantially detrimental effect on grain weight, but that seed priming of salicylic acid and foliar spray of moringa seed extract topically greatly increased grain weight. Different yield attributes decreased by Cd^{2+} Stress (spike length, number of spikelets per spike, grain yield plant^{-1}, and 100-grain weight). When Si is applied exogenously it helped in mitigating the damaging effects of Cd^{2+} stress on spike length, number of spikelets spike^{-1}, number of gains spike^{-1}, grain yield plant-1, and 100-grain weight (Hussain et al., 2015). Furthermore demonstrating the value of foliar spraying in cases of cadmium stress were reported by Rizwan et al. (2017).

Pearson correlation

Correlation analysis among morphological and biochemical attributes were observed during two consecutive growing years (2021-22). Pearson regression analysis showed that chl a and chl b showed the most positive correlation r-0.99, DPPH and shoot length (SL), root length (RL) and shoot length (SL), root length and shoot length r= 0.96 also showed positive strong correlation. SOD and total chlorophyll showed negative correlation r= -0.082, SOD with chl b also showed negative correlation with r= -0.077. Morphological and biochemical attributes of wheat variety Sehar-06 showed both positive and negative, weak and strong regression trends. Which results could be used to analyze the effect of treatments on growth attributes.

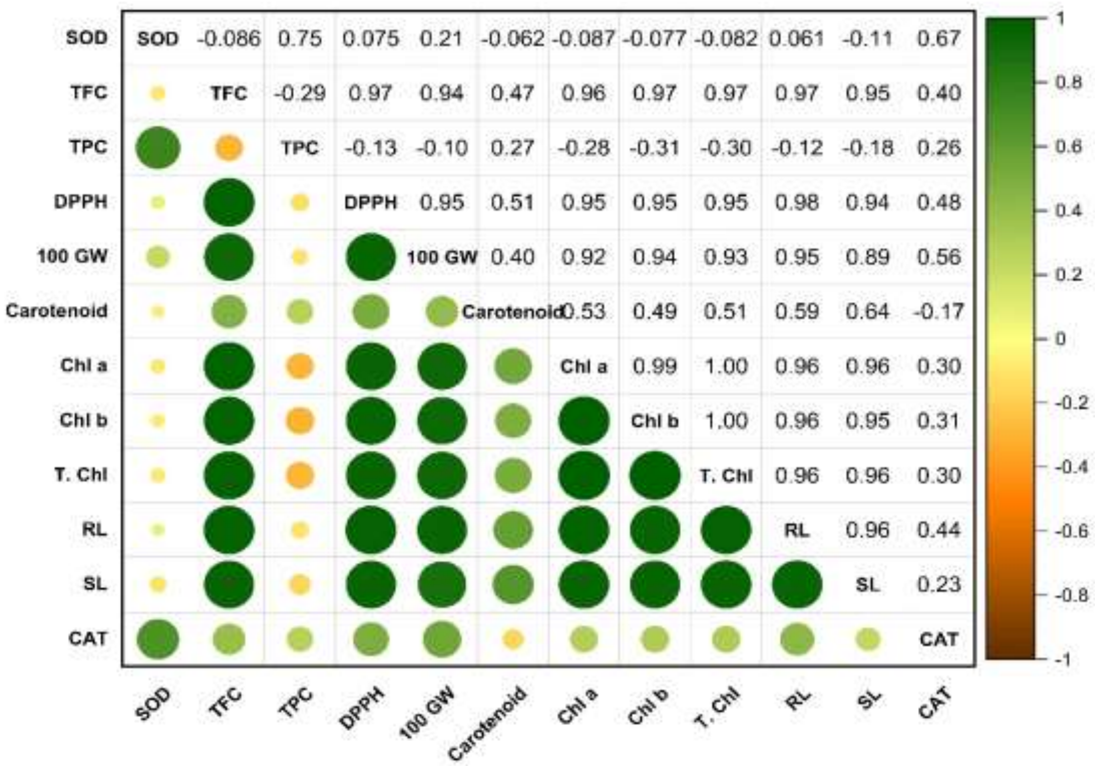

Figure 6: Pearson correlation regression analysis among morphological attributes root length (RL), shoot length (SL) and biochemical attributes superoxide dismutase (SOD), total flavonoid content (TFC), total phenolics content (TPC), DPPH, 100 grains weight (100 GW), carotenoids, chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (T. chl), and catalase (CAT) of wheat variety Sehar

Conclusion

Moreover, using moringa seed extract is advised to improve wheat plant growth, biochemical development, production and yield under conditions of heavy metal stress or not. It could help plants cope with the stress caused by heavy metals.

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