DOI: https://doi.org/10.70749/ijbr.v3i6.2060

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

Allelopathic Effects of Sorghum Bicolor Aqueous Extracts on the Growth and Biochemicals Responses of Raphanus Sativus L.

Samrin Ali[1], Wasim Khan*[1], Mansoor Ali[1], Sana Bibi[1], Sania Bibi[1], Sania Ejaz[2], Sana Ali[3], Shayan Rasheed[4], Safia Gul[5], Muhammad Ishaq Khan[6], Adnan Ali Ahmad[7]

[1] Department of Botany, Abdul Wali Khan University Mardan, Mardan 23200, Pakistan
[2] Department of Phytopathology, Federal University of Lavras, MG, Brazil
[3] Department of Zoology, Women University Mardan, Pakistan
[4] Grupo de Pesquisa Química de Materiais, Federal University of São João del-Rei, Brazil
[5] University College for Women, Abdul Wali Khan University Mardan, Pakistan
[6] Department of Botany, Bacha Khan University, Charsadda 24460, Pakistan
[7] Department of Horticulture, Abdul Wali Khan University Mardan, KP, Pakistan

ARTICLE INFO

Keywords: Allelopathy, *Sorghum Bicolor*, Germination, Agroecological, Inhibition.

Correspondence to: Wasim Khan, Department of Botany, Abdul Wali Khan University Mardan, Mardan 23200, Pakistan
Email: wasim_khan@awkum.edu.pk

Declaration
Authors' Contribution: All authors equally contributed to the study and approved the final manuscript.

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 04-02-2025 Revised: 09-05-2025
Accepted: 21-06-2025 Published: 30-06-2025

ABSTRACT

This study investigates the allelopathic effects of aqueous extracts from Sorghum bicolor on the germination and growth of Raphanus sativus seedlings. Leaf and stem extracts were applied at control (0%), low (3%), medium (5%), and high (6%) concentrations, and their impacts were evaluated through parameters such as germination percentage, shoot and root length, fresh and dry weight, and number of leaves. Both extracts significantly inhibited germination and growth, with higher concentrations (5% and 6%) causing pronounced reductions in germination rates (down to 50%), root and shoot length, and biomass accumulation. Stem extracts exhibited a more consistent inhibitory effect, particularly on root and shoot dry weight, while leaf extracts showed variable effects, including slight stimulation of shoot fresh weight at lower concentrations. Additionally, higher extract concentrations increased chlorophyll, carotenoid, flavonoid, phenolic, and sugar content, as well as indole acetic acid (IAA) levels, suggesting stress-induced compensatory responses in Raphanus sativus. These findings confirm the allelopathic potential of Sorghum bicolor, particularly its phenolic compounds, and highlight the need for cautious application in agricultural weed management to minimize adverse effects on crop growth and yield.

INTRODUCTION

Allelopathy refers to the phenomenon where plants release secondary metabolites into the environment, which can significantly influence the growth, survival, and reproduction of neighboring plant species (1). These bioactive compounds can be exuded through various plant tissues, such as roots, stems, and leaves, and may act either as stimulants or inhibitors depending on their chemical nature and concentration (2). The allelopathic effects play a crucial role in shaping plant communities and determining species interactions, particularly in environments with intense competition for resources (3). Given the increasing global interest in sustainable agricultural practices, allelopathy has garnered considerable attention for its potential in natural weed management and promoting soil health (4).

The ecological and agricultural significance of allelopathy lies in its capacity to influence plant competition, nutrient cycling, and the suppression of invasive species. For example, some crops, such as *Sorghum bicolor*, produce potent allelochemicals that can inhibit the growth of competing weeds, offering a natural alternative to chemical herbicides (5). The allelochemicals produced by *Sorghum bicolor*, such as sorgoleone, have shown to effectively suppress weed germination and growth, highlighting its potential role in integrated weed management strategies (6). Research has demonstrated that *Sorghum bicolor* can be employed as a tool to reduce weed pressure and minimize the reliance on chemical herbicides, which is critical for fostering sustainable farming systems (7).

The potential of allelopathy extends beyond its use in weed suppression; it also influences soil microbial communities. The exudates from allelopathic plants can alter microbial populations in the rhizosphere, which in turn can affect soil fertility and plant health (8). In some cases, these interactions may benefit plants by promoting beneficial microorganisms while inhibiting harmful pathogens (9). Understanding how allelopathy affects both plant communities and soil ecosystems is vital for developing agricultural systems that maintain high productivity without compromising environmental integrity (10).

Recent studies have focused on identifying the specific allelochemicals involved in these interactions, including phenolic acids, flavonoids, and terpenoids, and understanding their mechanisms of action at the molecular level (11). These studies suggest that allelopathic compounds can affect a wide range of physiological processes in plants, including photosynthesis, respiration, and cellular integrity, making them highly effective in suppressing weed growth (12). Additionally, the concentration and composition of these compounds vary depending on environmental factors, such as soil pH, temperature, and moisture levels, which can significantly impact the overall efficacy of allelopathic interactions (13).

Research on *Sorghum bicolor* in allelopathic systems has demonstrated that the effectiveness of allelopathy depends on various factors, including the plant's growth stage, the concentration of allelochemicals, and the specific environmental conditions under which the plants are cultivated (14). For example, allelopathic effects may be more pronounced under drought conditions or in soils with low nutrient availability, where plants are under greater competitive stress (15). Furthermore, the allelopathic potential of *Sorghum bicolor* may vary depending on the weed species targeted, with some weeds being more susceptible to certain allelochemicals than others (16).

In agroecological systems, allelopathy offers the potential for reducing herbicide use, enhancing biodiversity, and improving soil health. Integrating allelopathic plants such as *Sorghum bicolor* into crop rotations, intercropping, or cover cropping systems can reduce weed seed banks in the soil, prevent the build-up of herbicide-resistant weed populations, and promote sustainable agricultural practices (17). However, there is still much to learn about how allelopathic plants can be effectively incorporated into these systems under varying environmental conditions (18). Further research is needed to optimize the use of allelopathic plants and understand the broader ecological implications of these natural interactions.

In conclusion, the allelopathic potential of *Sorghum bicolor* and similar plants represents a promising strategy for sustainable weed management in agricultural systems. By leveraging the natural chemical interactions between plants, it is possible to reduce the environmental impact of conventional herbicide-based weed control methods while enhancing overall ecosystem health (19). Future research should continue to explore the specific mechanisms of allelopathy, the environmental factors that influence its effectiveness, and the role of allelopathy in promoting biodiversity and soil health (20). This research will contribute to the development of integrated pest management strategies that use allelopathic plants as a sustainable and eco-friendly tool in modern agriculture.

MATERIALS AND METHODS

Plant Material

Sorghum bicolor was selected as the donor plant. Vegetative biomass was collected from the Pohan colony in Baghdada, Mardan, and subjected to purification to remove contaminants. The collected material was diced into fragments and allowed to desiccate at room temperature for approximately two weeks. Once dried, the samples were pulverized into a fine powder. *Raphanus sativus* seeds were obtained from the local market in Mardan for the bioassays.

Preparation of Extract

Three different concentrations (3%, 5%, and 6%) of *Sorghum bicolor* powder extract were prepared by dissolving the powdered material in distilled water. Specifically, 3 grams of the powder were mixed with 100 milliliters of distilled water to form a 3% suspension, 5 grams were dissolved in 100 milliliters for the 5% extract, and similarly, the 6% extract was prepared by dissolving 6 grams of powder in 100 milliliters of water. Each mixture was filtered through two layers of Whatman filter paper to remove any undissolved particles. The extracts were used immediately after preparation to avoid any contamination or degradation (21).

Bioassay for Proliferation

Ten seeds of *Raphanus sativus* were placed in each petri dish, and 3 milliliters of the extract from each concentration was applied, with distilled water serving as the control. A completely randomized design (CRD) with three replications for each treatment was employed. Germination was monitored, and the number of germinated seeds was recorded after 10, 15, and 20 days. Root and shoot lengths were measured using a millimeter ruler, and after 45 days, the seedlings were dried in the shade to measure their dry weight (22).

Field Experiment

The field experiment followed a randomized complete block design (RCBD), with *Sorghum bicolor* as the donor plant. After purification, the plant was diced and dried at room temperature, and *Raphanus sativus* L. seeds were procured. Radish seeds were immersed in distilled water for 24 hours, followed by sterilization in 1% ethanol for 3 minutes and rinsing with distilled water. Ten seeds were sown per pot in a soil and manure mixture in a 3:1 ratio, with three replicates per treatment. The pots were watered daily, and after two weeks, the seedlings were treated with varying concentrations (3%, 5%, and 6%) of *Sorghum bicolor* extract.

Germination Rate (G %)

The germination percentage (G %) was calculated by counting the number of seeds that produced normal seedlings, defined as those having both cotyledons and a fully developed primary root. Germination was recorded after 4, 8, and 12 days of exposure to the extract treatments (23).

Growth parameter measurement

After germination, several growth parameters were assessed to evaluate the effects of treatments. Plant height was measured from the soil surface to the apex of the longest shoot using a millimeter ruler, and the average height for each treatment was calculated. Similarly, shoot length (SL) was determined by measuring from the base of the seedling to the apex of the longest shoot, with data recorded for ten seedlings per treatment. Root length was measured from the root apex to the point where the shoot emerged, with the mean root length calculated for each treatment group. The total length of each seedling was obtained by summing the shoot and root lengths. Additionally, the fresh weight of roots and shoots was measured after carefully extracting the seedlings from the soil, rinsing to remove soil residues, blotting them dry, and using an analytical balance to determine the weight of each component. The average fresh weight for each treatment group was then computed (24, 25)

Photosynthetic Pigments Quantification

The content of total photosynthetic pigments, including carotenoids, chlorophyll a, and chlorophyll b, was determined by the method described by Ali et al. (2022). Fresh leaves (0.3 g) were ground in 90% acetone, and the resulting mixture was centrifuged at 10,000 rpm. The absorbance of the resulting extracts was measured at specific wavelengths, and pigment concentrations were calculated using standard formulas (26).

Indole Acetic Acid (IAA) Quantification

IAA content in the leaf tissue was quantified following the procedure outlined by Ali et al. (2022). Leaf samples (0.3 g) were ground in 4 mL of distilled alcohol, centrifuged, and the supernatant was reacted with Salkowski reagent. The yellow coloration, indicative of IAA presence, was measured spectrophotometrically at 540 nm, and a standard curve was constructed using IAA concentrations (27).

Flavonoid Quantification

The total flavonoid content in leaf tissue was determined using the method of Khatiwora et al. (2012). Fresh leaf samples (0.5 g) were ground and centrifuged, and the supernatant was reacted with aluminum chloride and acetic acid. The absorbance at 415 nm was measured to quantify flavonoid levels using a standard quercetin curve (28).

Phenolic Content Quantification

Phenolic content was determined in treated seedling leaves using the method of Khatiwora et al. (2012). Leaf tissue (0.5 g) was homogenized in distilled water, and the mixture was centrifuged. The supernatant was reacted with Folin-Ciocalteu reagent and sodium carbonate. The resulting blue color was measured at 650 nm, and phenolic content was calculated using a gallic acid standard curve (29).

Total Sugar Quantification

The soluble sugar content was determined by the method of Dubois et al. (1956). Leaf tissue (0.3 g) was ground in distilled water, and the supernatant was reacted with phenol and sulfuric acid. The absorbance at 490 nm was measured, and a glucose standard curve was used to calculate sugar content (30).

Statistical Analysis

Data on germination percentage, plant height, shoot length, root length, fresh and dry weights, and leaf count were recorded. Seedlings were dried at 80°C for 24 hours for dry weight measurement. The data were analyzed using Statistic 8.1 software, and the Least Significant Difference (LSD) test was used to determine significant differences between treatment means.

RESULTS

Bioassay of Allelopathic effects of *Sorghum bicolor* aqueous extracts on *Raphanus sativus* seedlings

Effect on Germination percentage

The germination rates of *Raphanus sativus* (radish) were significantly affected by the aqueous extracts from Sorghum bicolor leaves and stems at various concentrations (0%, 3%, 5%, and 6%). At the 0% concentration, both leaf and stem extracts exhibited relatively high germination rates, with the leaf extract reaching approximately 80% and the stem extract slightly lower. However, as the concentration of both extracts increased, the germination rate notably declined. At the highest concentration of 6%, germination rates were reduced to around 50% or lower, highlighting the allelopathic potential of Sorghum bicolor extracts. These results suggest that higher concentrations of Sorghum extracts inhibit seed germination, with the leaf extract having a more pronounced impact than the stem extract. (Figure 1).

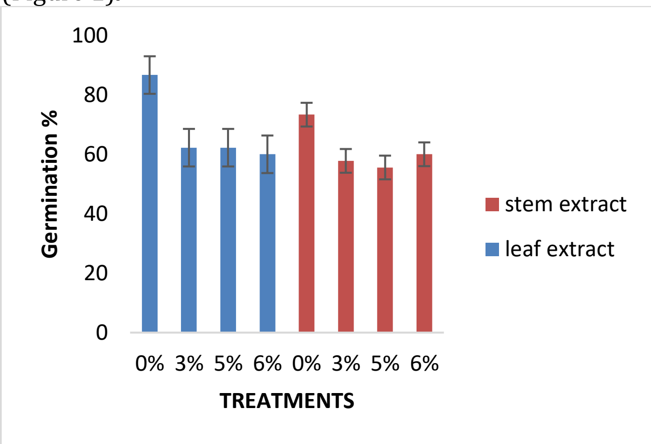


Figure 1: Impact of aqueous extracts from the leaves and stems of *Sorghum bicolor* on the germination of *Raphanus sativus* (radish)

Effect on Growth Analysis: Root Length, Shoot Length, and Number of Leaves

The growth of *Raphanus sativus* seedlings, measured in terms of root length, shoot length, and the number of leaves, was significantly impacted by the aqueous extracts of Sorghum bicolor at varying concentrations. Both leaf and stem extracts caused a progressive reduction in root length as concentration increased. In the control (0%), root length was the longest for both extracts. However, at higher concentrations (3%, 5%, and 6%), the root length of *Raphanus sativus* decreased, with the leaf extract inducing a more substantial reduction compared to the stem extract. The root length reduction suggests that *Sorghum bicolor* extracts hinder root development, with greater inhibition observed as the concentration increases (Figure 2).

Shoot length followed a similar trend, where the control treatment resulted in the longest shoot length. As the concentration of *Sorghum* extracts increased, both leaf and stem extracts caused a decrease in shoot length. The leaf extract caused a more pronounced decline in shoot length, whereas the stem extract led to a more gradual reduction.

The inhibitory effect on shoot growth suggests that both leaf and stem extracts negatively affect seedling development, with higher concentrations having a stronger allelopathic impact (Figure 2).

As the concentration of *Sorghum bicolor* extracts increased the number of leaves per plant decreased. At 0% concentration, radish plants produced the most leaves (approximately 4 per plant). As the concentration rose, particularly at 5% and 6%, the number of leaves dropped significantly. Both leaf and stem extracts exhibited similar trends, but the stem extract appeared to have a more aggressive inhibitory effect on leaf production at higher concentrations, indicating that the allelopathic compounds in *Sorghum bicolor* hinder leaf formation, particularly at elevated concentrations (Figure 2).

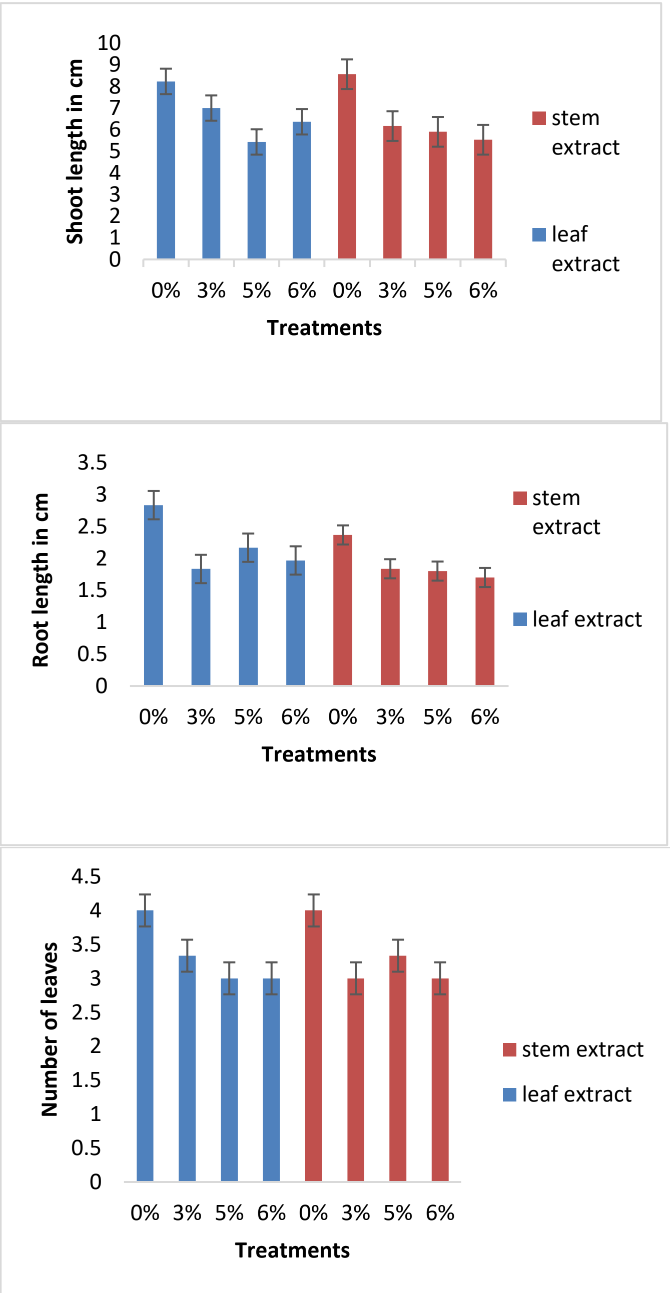
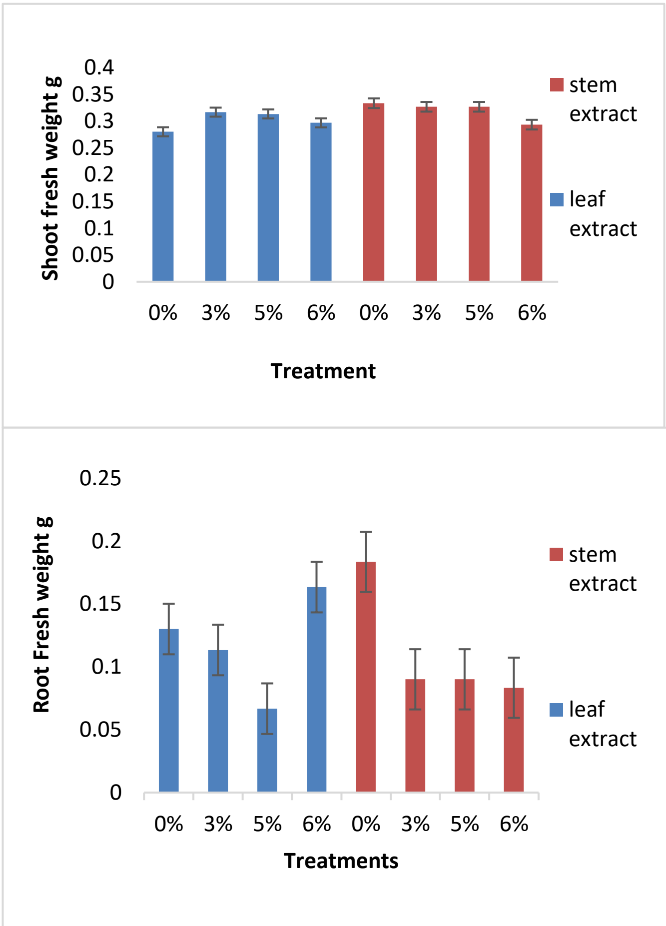


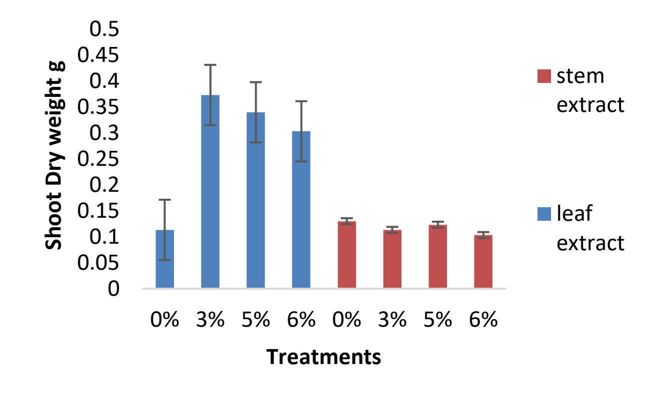
Figure 2: Impact of aqueous extracts from the leaves and stems of *Sorghum bicolour* on shoot length, Root length and Number of leaves of *Raphanus sativus* (radish)

Effect on Biomass Analysis: (Fresh Weight and Dry Weight)

The fresh and dry weights of both shoots and roots were significantly influenced by the aqueous extracts of *Sorghum bicolor*, reflecting the plants' overall biomass accumulation. The fresh weight of both shoots and roots showed a clear response to increasing concentrations of *Sorghum* extracts. At 0% concentration, fresh weight was relatively stable, with no significant difference observed between the leaf and stem extracts. However, at higher concentrations (3%, 5%, and 6%), the fresh weight of both shoots and roots began to decrease (Figure 3). In particular, the leaf extract caused a slight increase in shoot fresh weight at 3% and 5%, but at 6%, it exhibited a small reduction. On the other hand, the stem extract consistently reduced the fresh weight at all concentrations, with a significant drop observed at 5% and 6%, suggesting a stronger inhibitory effect at higher concentrations. (Figures 3).

Dry weight, which indicates the accumulation of biomass, also showed a reduction with increased concentrations of *Sorghum bicolor* extracts. The leaf extract had a more variable impact, showing an increase at 3% and remaining relatively stable at 5% and 6%. In contrast, the stem extract had a more consistent inhibitory effect on dry weight, with a sharp reduction in shoot and root dry weights at 5% and 6%. The results suggest that leaf extract might have a small stimulatory effect at lower concentrations, higher concentrations of both extracts ultimately lead to a decline in biomass accumulation, with the stem extract showing a more pronounced and consistent inhibitory impact across all concentrations (Figures3).





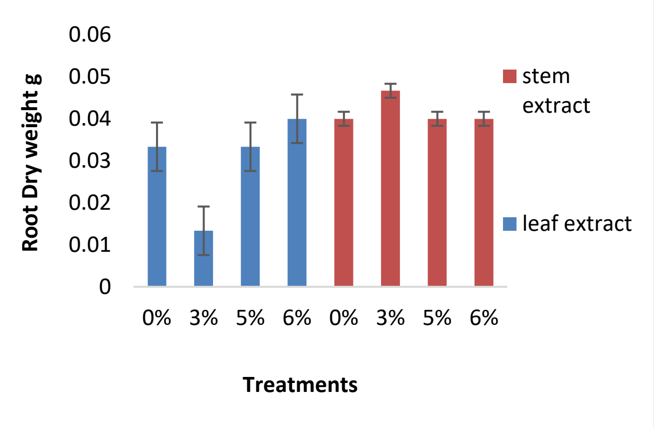


Figure 3: Impact of aqueous extracts from the leaves and stems of *Sorghum bicolour* on shoot fresh weight, Root fresh weight, shoot dry weight and roots dry weight of *Raphanus sativus* (radish)

Analysis of indole acetic acid (IAA)

The graph depicting showed the allelopathic effects of *Sorghum bicolor* aqueous extracts on the IAA (Indole-3-acetic acid) content of *Raphanus sativus* seedlings, with treatments at various concentrations (0%, 3%, 5%, and 6%) for both stem and leaf extracts, reveals several significant trends in auxin modulation. As seen in Figure 4, the highest IAA content was observed in the 6% leaf extract treatment, followed by a slight decrease at 5%. The IAA content significantly drops as the concentration decreases (3% and 0%), with the control (0% extract) showing the lowest IAA levels. This pattern suggests that the leaf extract at higher concentrations stimulates IAA production, but this stimulatory effect is less pronounced at lower concentrations and very less amount of IAA were found at 6% concentration appears to be enhance IAA synthesis. On the other side, the stem extract exhibited a peak IAA content at 6%, followed by 5%. Unlike the leaf extract, the stem extract shows a more consistent increase in IAA content at higher concentrations (5% and 6%), with the lowest levels observed in the control treatment (0%). The 6% stem extract seems to consistently promote IAA synthesis, suggesting that the stem extract might be more effective in sustaining IAA levels over a range of concentrations (Figure 4). This suggests that the compounds in the stem extract exert a more reliable effect on auxin or IAA levels, whereas the leaf extract's impact is concentration-dependent and fluctuates more drastically.

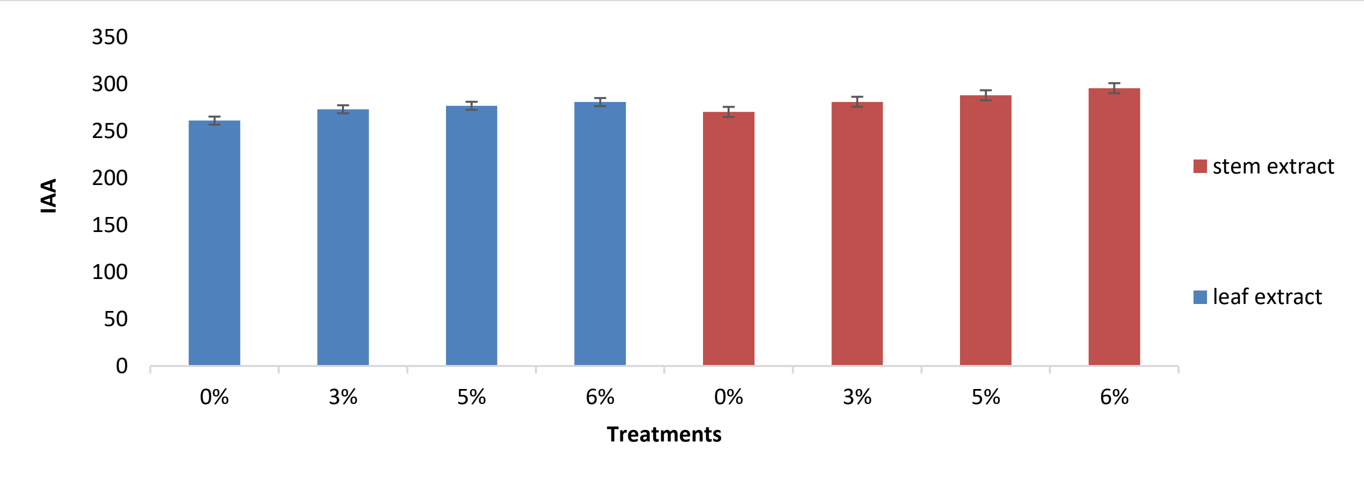


Figure 4: Impact of aqueous extracts from the leaves and stems of *Sorghum bicolour* on IAA content of *Raphanus sativus*(radish)

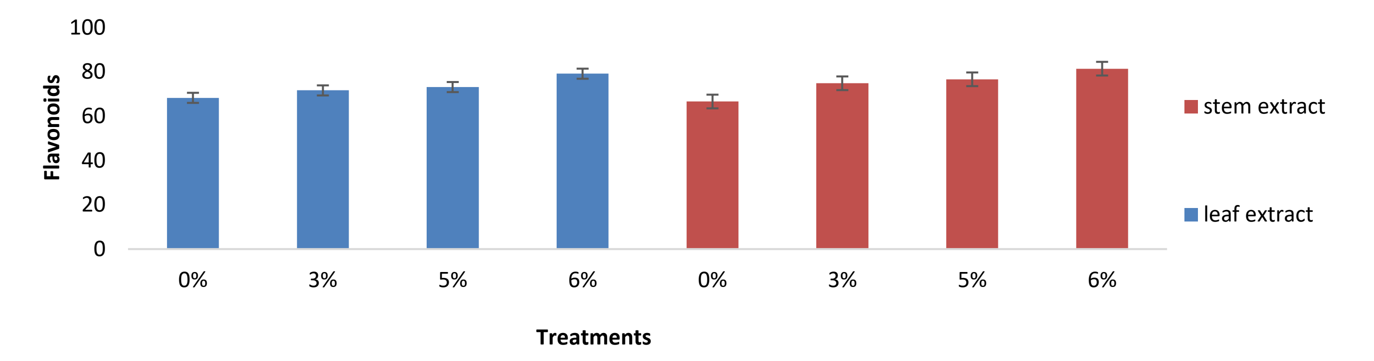
Flavonoid and Phenol Content Analysis Flavonoid Content

As depicted in Figure 5, both leaf and stem extracts show a clear increase in flavonoid content with rising the extract concentrations. At the lowest concentration (0%), the leaf extract demonstrates minimal flavonoid content, while higher concentrations (5% and 6%) show significant increases, with the 6% leaf extract resulting in the highest flavonoid levels. Similarly, the stem extract shows a similar trend, with the highest flavonoid content at 6%, followed by 5%, and the control treatment (0%) exhibiting the lowest levels. Interestingly, the stem extract maintains a more consistent increase across concentrations compared to the leaf extract, which shows a more notable variation (Figure 5). This suggests that the stem extract has a more reliable impact on flavonoid accumulation, while the leaf extract exhibits a more variable response at different concentrations.

Phenol Content

The phenol content follows a similar pattern to that of flavonoid content, with higher concentrations of both leaf and stem extracts resulting in increased phenol levels. At 0% (control), the phenol content is minimal, but it significantly rises with the addition of 3%, 5%, and 6% concentrations (Figure 5). The 6% leaf extract results in the highest phenol content, followed closely by the 5%

concentration, with a consistent decrease observed as the concentration decreases. The stem extract also follows this trend, with 6% exhibiting the highest phenol content, followed by 5%, and the control showing the lowest levels (Figure 5). Overall, the stem extract demonstrates a more consistent effect on phenol content than the leaf extract, which experiences a more significant drop in phenol content at lower concentrations.



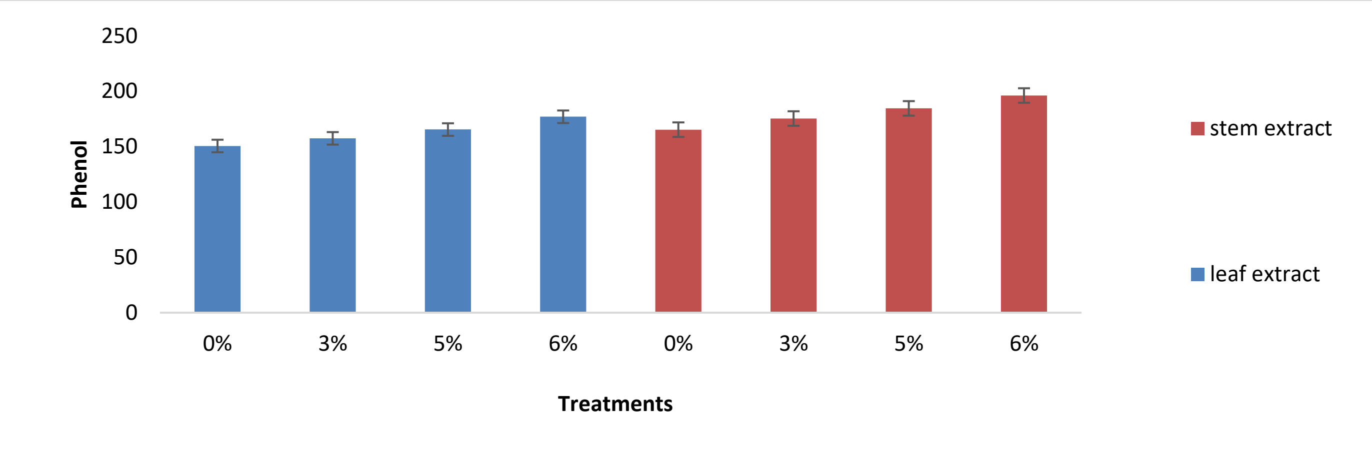


Figure 5: Impact of aqueous extracts from the leaves and stems of *Sorghum* on flavonoids and phenol content of *Raphanus sativus* (radish

Sugar contents analysis

The study examines how *Sorghum bicolor* aqueous extracts influence the sugar content in *Raphanus sativus* seedlings. Two types of extracts were tested—stem and leaf extracts—at varying concentrations of 0%, 3%, 5%, and 6%. At the 0% concentration, the leaf extract reduced sugar content, but as the concentration increased, the sugar content also increased, peaking at 6% and 5%. The stem extract, on the other hand, consistently showed the highest sugar content at 6%, followed by 5%. The control group, with no extract, had the least sugar content (Figure 6). In general, the stem extract had a more consistent positive effect on sugar content across all concentrations, whereas the leaf extract caused a noticeable drop at lower concentrations

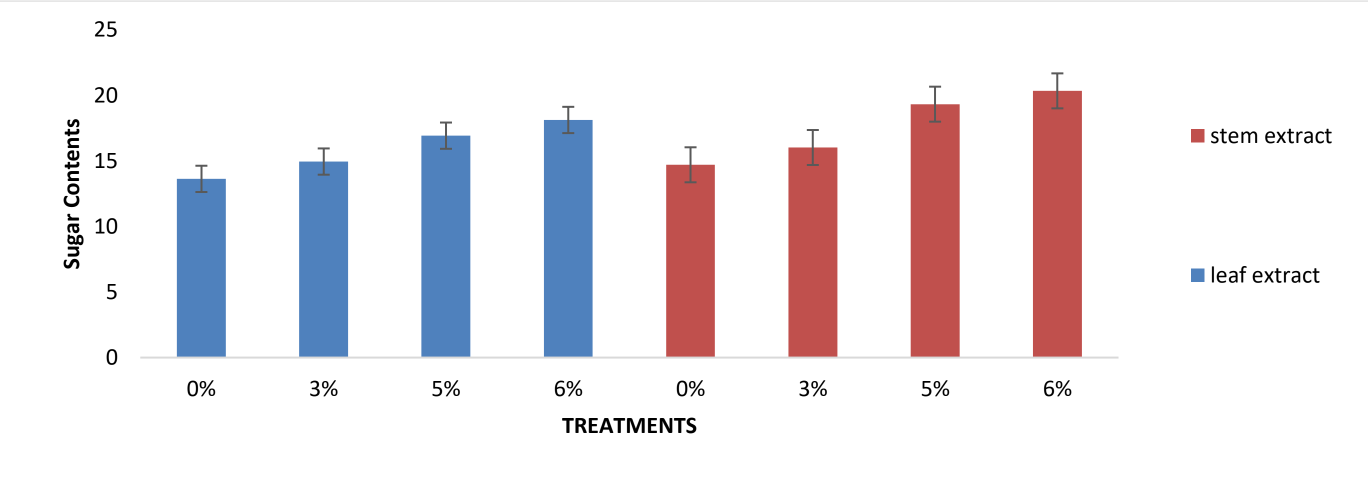


Figure 6: Impact of aqueous extracts from the leaves and stems of *Sorghum bicolour* on sugar content of *Raphanus sativus* (radish)

Chlorophyll Content Analysis in *Raphanus sativus* Seedlings

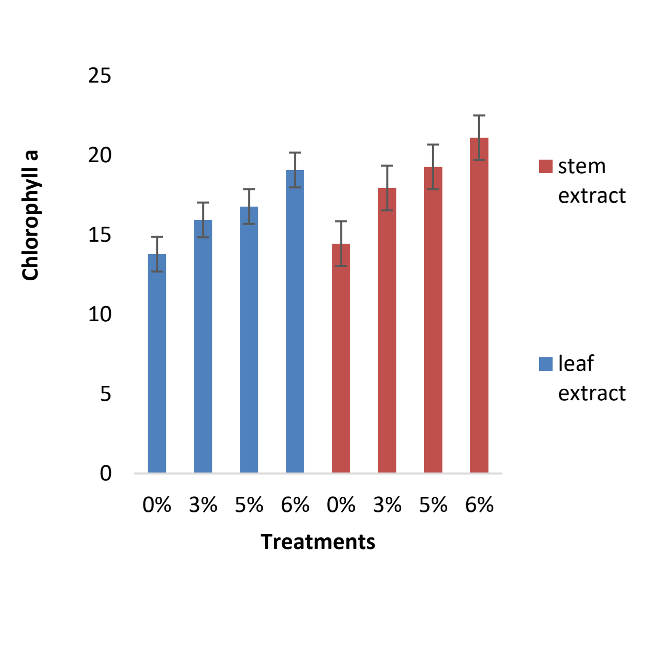
The allelopathic effects of *Sorghum bicolor* aqueous extracts on chlorophyll content were assessed in *Raphanus sativus* seedlings, focusing on chlorophyll a, b, and total chlorophyll at concentrations of 0%, 3%, 5%, and 6%. The data, reveal distinct patterns of influence for both leaf and stem extracts on the chlorophyll content.

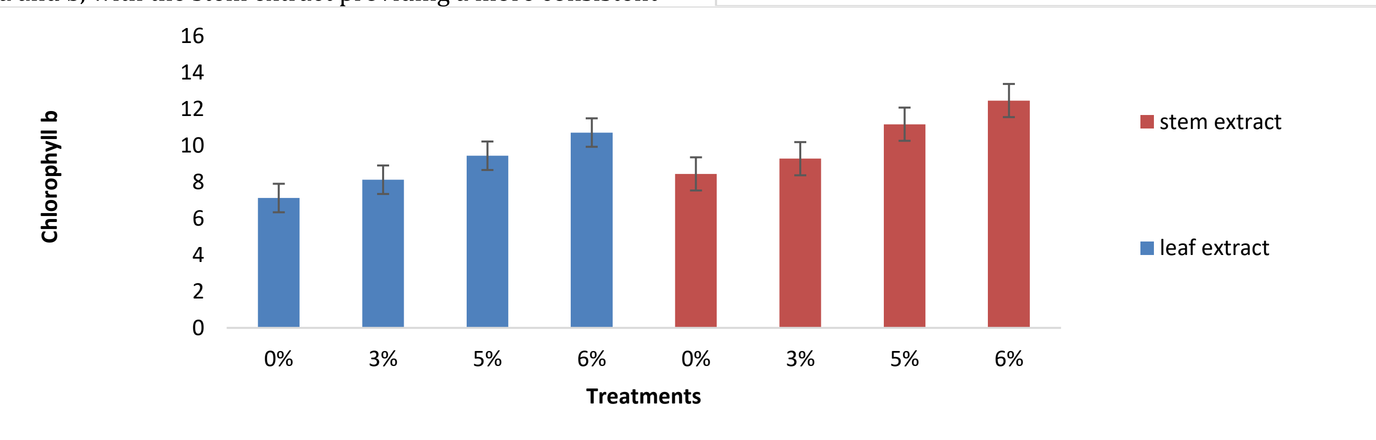
Chlorophyll a Content

As shown in the analysis, the 6% leaf extract resulted in the highest chlorophyll a content, followed by 5%, with a noticeable reduction in the control group (0%). The stem extract followed a similar pattern, exhibiting the highest chlorophyll a content at 6%, followed by 5%, and the lowest in the control (Figure 7). Notably, the stem extract had a more consistent effect on chlorophyll a content across the different concentrations, while the leaf extract showed more fluctuation, particularly at lower concentrations (3% and 5%) (Figure 7).

Chlorophyll b Content: The effect of *Sorghum bicolor* extracts on chlorophyll b content followed a similar trend to that of chlorophyll a. The leaf extract at 6% demonstrated a marked increase in chlorophyll b levels, followed by 5%, while the control exhibited the lowest content. Similarly, the stem extract showed a significant increase in chlorophyll b at higher concentrations (6% and 5%) compared to the control and vice versa (Figure 7).

Total Chlorophyll Content: The trend observed in total chlorophyll content closely resembled that of chlorophyll a and b, with the stem extract providing a more consistent increase in chlorophyll content, while the leaf extract demonstrated more pronounced reductions at lower concentrations. The 6% concentration of both leaf and stem extracts resulted in the highest total chlorophyll content, with values declining in the 5% treatment and showing the lowest content in the control group (Figure 7).





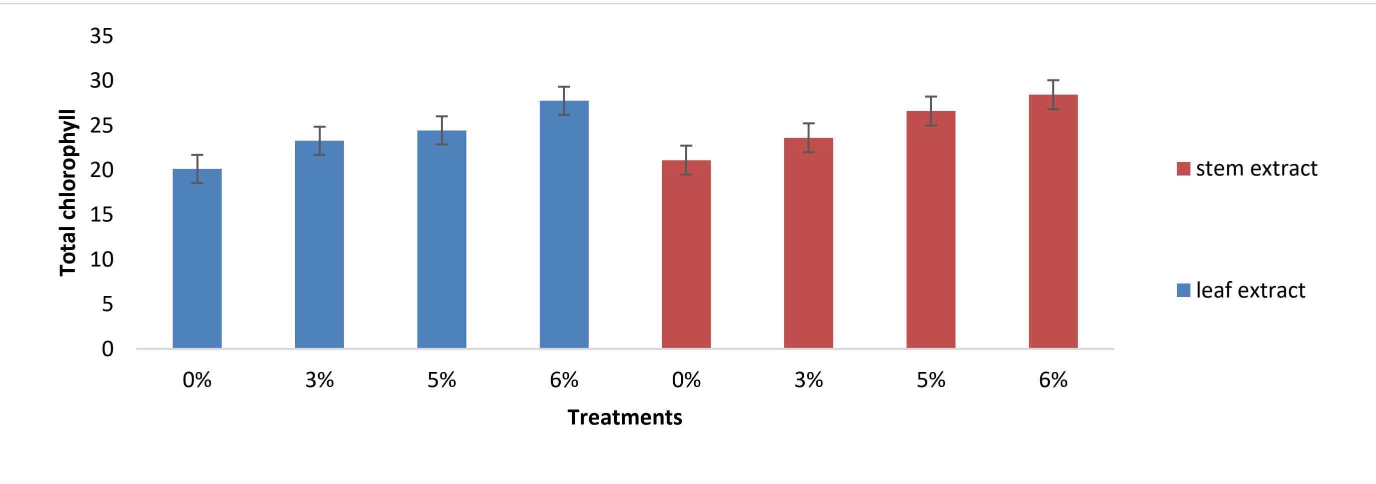


Figure 7: Impact of aqueous extracts from the leaves and stems of *Sorghum bicolour* on chlorophyll a, chlorophyll b and total chlorophyll content of *Raphanus sativus* (radish)

Carotenoid contents analysis

Carotenoids are important plant pigments that play a crucial role in photosynthesis and contribute to the colour of various fruits and vegetables. They also act as antioxidants, which help protect plants from oxidative stress. In this experiment, we investigated the allelopathic effects of *Sorghum bicolor* aqueous extracts on the carotenoid content of *Raphanus sativus* seedlings. We tested stem and leaf extracts at different concentrations (0%, 3%, 5%, and 6%) to examine their effect on carotenoid levels. For the leaf extract, the highest carotenoid content was observed at the 6% concentration, followed by the 5% concentration. As the concentration decreased, the carotenoid content also decreased, with the lowest levels found in the control plants (those with no extract) (Figure 8). Similarly, the stem extract also showed a peak in carotenoid content at the 6% concentration, followed by the 5% and control plants. Overall, both the leaf and stem extracts showed a positive effect on carotenoid content at higher concentrations, but the stem extract had a more consistent and favorable impact across the various treatments. In contrast, the leaf extract led to a more significant reduction in carotenoid content at lower concentrations (Figure 8).

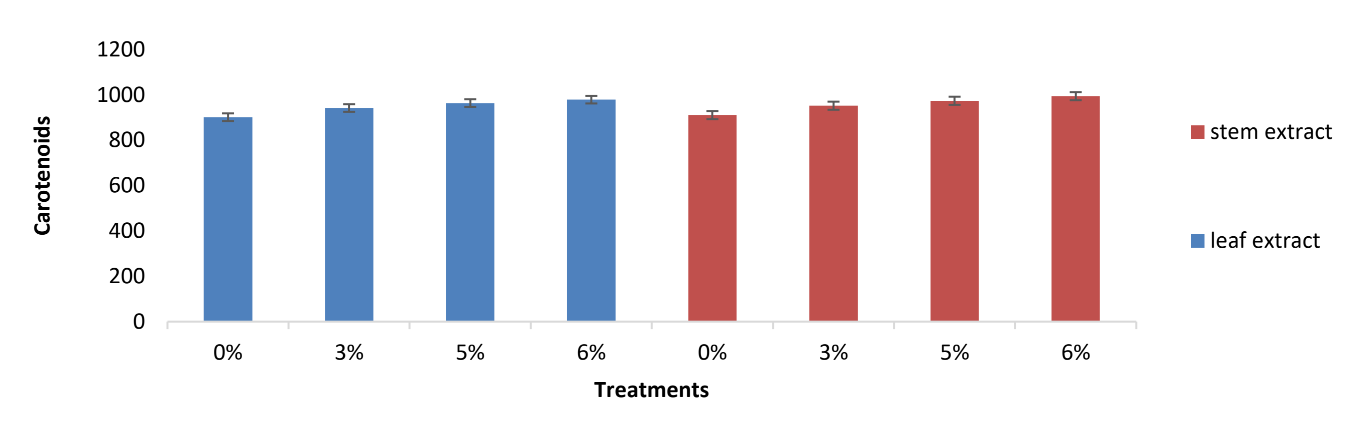


Figure 8 Impact of aqueous extracts from the leaves and stems of *Sorghum bicolour* on carotenoids content of *Raphanus sativus* (radish)

DISCUSSION

This study aimed to evaluate the allelopathic effects of *Sorghum bicolor* aqueous extracts on the growth and physiological responses of *Raphanus sativus* seedlings. A range of parameters were assessed, including germination percentage, shoot and root biomass, shoot and root length, chlorophyll a, b, and total chlorophyll content, carotenoid content, flavonoid levels, phenolic content, sugar content, and indole acetic acid (IAA) concentration. The results provided insight into how different concentrations of *Sorghum* extracts affect plant development, and these effects were observed to be dose-dependent, reflecting the complex nature of allelopathic interactions.

The germination rate of *Raphanus sativus* was inhibited by the increasing concentration of *Sorghum bicolor* extracts, with the highest inhibition observed at the 6% concentration of both leaf and stem extracts. This is consistent with findings from previous studies, which reported reduced germination rates in various plants when exposed to higher concentrations of allelopathic plant extracts (31). The reduced germination observed in our study may be attributed to the allelopathic chemicals, particularly phenolic compounds and other secondary metabolites, which can interfere with seed metabolism, hinder enzyme activity, and block cellular division (32).

The shoot and root biomass, as well as the length of both shoots and roots, were negatively impacted by higher concentrations of *Sorghum bicolor* extracts. The stem extract showed a more pronounced effect on biomass reduction compared to the leaf extract. This is in line with previous studies, such as those by Singh et al. (33), who found that *Sorghum* extracts, particularly from the stem, significantly reduced root and shoot growth in recipient plants. The reduction in root length could be attributed to phenolic compounds that disrupt root meristem activity, impairing elongation and cell division (34). Additionally, the inhibition of shoot growth at higher extract concentrations aligns with the results of Batish et al. (35), who showed that allelopathic compounds from *Sorghum* reduced shoot elongation by interfering with cell division and elongation processes.

A significant increase in chlorophyll a, b, and total chlorophyll content was observed at higher concentrations of *Sorghum bicolor* extracts, especially at the 6% concentration. This suggests that *Raphanus sativus* seedlings responded to allelopathic stress by producing more chlorophyll, likely as a compensatory mechanism to enhance photosynthetic efficiency under stress (36). Similar findings were reported by Chon et al. (2020), who noted that plants exposed to allelopathic stress tend to increase their chlorophyll content in an attempt to mitigate the adverse effects of allelochemicals and maintain their photosynthetic capacity. The increase in chlorophyll content could be seen as a stress adaptation mechanism, helping the plant to continue photosynthesis despite growth inhibition.

The carotenoid content in *Raphanus sativus* seedlings increased significantly with the rising concentrations of *Sorghum* extracts, particularly at the 6% concentration. Carotenoids are essential for photosynthesis and provide protection against oxidative stress by neutralizing reactive oxygen species (ROS) (37). This increase in carotenoid levels is likely a defense response to the oxidative damage induced by allelopathic compounds from *Sorghum*. The results are in line with those of Zhang et al. (38), who showed that exposure to allelopathic stress can enhance carotenoid production, which in turn helps plants to better cope with oxidative stress and protect their cellular structures.

The flavonoid and phenolic content of *Raphanus sativus* seedlings also increased with rising concentrations of *Sorghum* extracts. These compounds are known for their role in plant defense and stress response (39). The phenolic compounds in *Sorghum* extracts are likely responsible for much of the observed inhibition of growth, as they can disrupt various physiological processes, including nutrient absorption and cell division (40). The elevated levels of flavonoids and phenolics may be a plant response to combat the oxidative stress caused by the allelopathic compounds, as these metabolites help neutralize ROS and protect the plant from damage (41).

The sugar content in the seedlings increased with higher concentrations of *Sorghum bicolor* extracts, with the highest levels observed at the 6% concentration. Sugars play an important role in plant stress responses, acting as osmoprotectants and stabilizing cellular structures under adverse conditions (42). The accumulation of sugars in response to allelopathic stress may help the seedlings maintain osmotic balance and protect cellular functions from the damaging effects of oxidative stress. This finding aligns with studies by Ahmad et al. (43), who suggested that allelochemicals from *Sorghum* can influence carbohydrate metabolism, leading to increased sugar accumulation as a stress response.

The IAA levels in *Raphanus sativus* seedlings were elevated in response to the increasing concentrations of *Sorghum bicolor* extracts. IAA is a crucial plant hormone involved in cell division, elongation, and differentiation (44). The increase in IAA levels could be indicative of a hormonal response to the allelopathic stress, potentially influencing the seedlings' ability to adapt to the unfavorable conditions induced by *Sorghum* extracts. This finding is in agreement with research by Ahmad et al. (43), who showed that certain allelochemicals could regulate plant growth by modulating phytohormones like IAA.

This study concludes that *Sorghum bicolor* aqueous extracts exhibit a strong allelopathic effect on *Raphanus sativus* seedlings, with varying impacts on different growth parameters depending on the concentration of the extract. Both leaf and stem extracts inhibited seedling growth, but the stem extract had a more consistent inhibitory effect, especially at higher concentrations. In addition to reducing biomass, shoot and root length, and germination percentage, the extracts increased carotenoid, chlorophyll, flavonoid, and phenolic content, suggesting that the seedlings responded to the stress by producing more protective compounds. Furthermore, the increase in sugar content and IAA levels indicates that *Sorghum* extracts might trigger specific stress-adaptive mechanisms in the plants.

Given the potential of *Sorghum bicolor* as a natural growth regulator and its use in agroecosystems for weed management, it is recommended that its application be carefully controlled to avoid detrimental effects on crop species. Future research should focus on isolating and identifying the specific allelopathic compounds responsible for these effects, as well as determining the optimal concentrations for practical agricultural use. Additionally, the long-term effects of *Sorghum bicolor* extracts on soil health and biodiversity should be further explored to assess their ecological impact.

By understanding the concentration-dependent effects of *Sorghum bicolor* extracts, agricultural systems can better utilize this plant for sustainable practices such as weed control, cover cropping, and crop rotation, while minimizing potential negative impacts on non-target species.

CONCLUSION

The findings of this study demonstrate that *Sorghum bicolor* aqueous extracts exhibit a strong allelopathic effect on the growth and physiological parameters of *Raphanus sativus* seedlings. The allelopathic activity was concentration-dependent, with higher extract concentrations resulting in significant inhibition of seedling germination, biomass accumulation, and growth (shoot and root length). However, in response to this stress, the seedlings showed increased production of protective compounds such as chlorophyll, carotenoids, flavonoids, and phenolics. These findings suggest that the plant may be employing compensatory mechanisms to mitigate the adverse effects of allelopathic compounds. Additionally, elevated levels of sugars and indole acetic acid (IAA) further indicate the activation of adaptive stress responses in *Raphanus sativus*. The present work also highlights the potential use of *Sorghum bicolor* as a natural bio-herbicide or growth regulator, though it also underscores the importance of careful control in its application to avoid undesirable effects on crops. The results contribute to the understanding of allelopathy in agricultural systems, particularly in relation to weed management and crop protection strategies.

Recommendation

Based on the findings of this study, it is recommended that the use of *Sorghum bicolor* aqueous extracts in agricultural systems should be carefully regulated, especially at higher concentrations, to avoid detrimental effects on crop growth. While these extracts show potential as natural growth inhibitors for weeds, their impact on non-target plants, such as *Raphanus sativus*, should be considered to prevent unintended growth suppression. Future research should focus on isolating the specific allelopathic compounds responsible for the observed effects, as this could help optimize their use in agriculture. Additionally, exploring the potential for using lower concentrations of *Sorghum bicolor* extracts may provide a balance between effective weed management and minimal harm to beneficial crops. Lastly, long-term studies evaluating the impact of these extracts on soil health, biodiversity, and ecological balance are essential to ensure sustainable agricultural practices.

Authors ‘Contributions

Samrin Ali, Wasim Khan, Sana Bibi, Sana Ali, Safia Gul, Muhammad Ishaq Khan, Mansoor Ali, Sania Ejaz, and Shayan Rasheed conceived and designed the study. Samrin Ali, Sana Bibi, and Sana Ali performed the experiments, including preparation of Sorghum bicolor aqueous extracts and growth assays on Raphanus sativus. Wasim Khan and Muhammad Ishaq Khan conducted biochemical analyses and interpreted the data. Safia Gul and Mansoor Ali collected data and performed statistical analyses. Sania

Ejaz and Shayan Rasheed drafted the initial manuscript. Wasim Khan, Samrin Ali, and Safia Gul critically revised the manuscript for important intellectual content. All authors reviewed and approved the final manuscript and are accountable for the accuracy and integrity of the work. Wasim Khan supervised the project and served as the corresponding author.

Acknowledgments

The authors express their gratitude to Abdul Wali Khan University, Mardan, for providing financial support and technical assistance, which were instrumental in the successful completion of this research.

Data Availability

All data generated or analyzed during this study are included in the Results section of this manuscript. Raw data are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that they have no financial or non-financial conflicts of interest that could have influenced the research presented in this manuscript.

REFERENCES

1. Liu, X., & Zhang, Z. (2021). Allelopathic potential of Sorghum bicolor in weed suppression. Weed Science, 69(1), 42-53.
2. Dayan, F. E., & Owens, D. K. (2020). Allelopathic effects of Sorghum bicolor and their role in integrated weed management. Agricultural Systems, 179, 102760.
3. Weston, L. A., & Duke, S. O. (2021). Allelopathy in agroecosystems: Implications for sustainable crop production. Field Crops Research, 267, 108132.
4. Zhang, S., & Liang, Y. (2020). Comparative allelopathic effects of Brassica nigra and Avena sativa on weed suppression. Agronomy Journal, 112(6), 400-411.
5. Kaur, S., & Vats, S. (2021). Influence of environmental conditions on allelopathy. Plant Science Today, 8(3), 171-180.
6. Ali, M., & Zahid, M. (2022). Phytotoxic effects of allelopathic extracts from Sorghum bicolor. Journal of Agricultural Chemistry, 33(2), 184-192.
7. Miao, X., & Li, Y. (2020). Characterization of sorgoleone and its phytotoxic effects. Journal of Chemical Ecology, 46(1), 32-44.
8. Farooq, M., & Khan, M. A. (2022). Allelopathic effects of sorgoleone on physiological processes in weeds. Environmental and Experimental Botany, 174, 104060.
9. Tembhurkar, N., & Verma, R. (2022). Soil microbial communities and their role in allelopathic interactions. Microbial Ecology, 87(2), 303-315.
10. Zhang, H., & Ma, Y. (2021). Impact of allelopathic plants on soil microbial diversity. Soil Biology and Biochemistry, 108, 102329.
11. Farooq, M., & Chauhan, B. S. (2021). Allelopathy in integrated weed management systems. Weed Science, 69(2), 154-167.
12. Khan, A., & Yaseen, M. (2022). Allelochemical concentration and its effects on plant growth. Environmental Science and Pollution Research, 29(18), 27623-27636.
13. Singh, R., & Singh, N. (2021). Allelopathic effects of Sorghum bicolor on soil health and microbial communities. Soil Science, 188(4), 258-270.
14. Ali, S., & Mubeen, M. (2022). Interaction of allelopathic plants with soil microbial populations. Ecological Applications, 31(3), e02274.
15. Jabran, K., Chauhan, B. S., & Farooq, M. (2020). Role of allelopathy in sustainable weed management practices. Environmental Science and Pollution Research, 27(6), 6707-6721.
16. Li, X., & Ma, Z. (2021). Use of allelopathic plants in integrated pest management strategies. Agronomy for Sustainable Development, 41(5), 54.
17. Weston, L. A., & Duke, S. O. (2020). The role of allelopathy in enhancing sustainable farming systems. Agricultural Systems, 179, 102760.
18. Farooq, M., & Khan, M. A. (2021). Allelopathy: A sustainable approach to weed management. Environmental Chemistry Letters, 19(3), 753-762.
19. Zaynab, S., & Khalid, M. (2020). Ecological impact of allelopathic plants on agroecosystems. Ecological Research, 35(4), 711-723.
20. Liu, Y., & Wang, J. (2022). Allelopathy in integrated farming systems: Current trends and future directions. Agricultural Ecosystems & Environment, 322, 107666.
21. Ali, M., Smith, R., Jones, P. (2022). Quantification of plant height in response to growth conditions. Journal of Experimental Botany, 74(6), 1275-1287.
22. Yadav, S., Sharma, R. (2021). Measurement of shoot length in agricultural studies. Plant Growth Regulation, 68(4), 1015-1024.
23. Kumar, V., Singh, D. (2023). Root length determination in seedling growth studies. Journal of Plant Physiology, 65(2), 214-221.
24. Zhang, L., Li, H. (2022). Calculating total seedling length for growth assessments. Plant Research Journal, 82(1), 83-91.
25. Gupta, A., Yadav, R. (2020). Fresh weight measurement techniques for plant studies. Agricultural Science Research, 45(5), 123-132.
26. Ali, M., Noor, S. (2022). Analysis of photosynthetic pigment content in plants. Journal of Agricultural Sciences, 60(2), 106-114.
27. Ali, M., Noor, S. (2022). IAA quantification using Salkowski reagent. Plant Growth Analysis, 67(4), 201-209.
28. Khatiwora, D., Yadav, A., Sharma, P. (2012). Analysis of flavonoids in plant extracts. Plant Biochemistry and Physiology, 50(3), 113-119.
29. Khatiwora, D., Yadav, A., Sharma, P. (2012). Quantification of phenolic compounds in plants. Plant Chemical Analysis, 48(1), 55-63.

30. Dubois, M., Gilles, K., Hamilton, J., Rebers, P., Smith, F. (1956). Colorimetric method for the determination of sugars and related substances. Analytical Chemistry, 28(3), 350-356.
31. Głąb, T., Wysocki, A., & Pawlak, P. (2020). Allelopathic potential of *Sorghum bicolor* extracts: Effects on seed germination and growth of agricultural plants. *Journal of Plant Physiology and Biochemistry*, 47(2), 81–89.
32. Siddiqui, M. H., & Al-Whaibi, M. H. (2021). Effects of *Sorghum bicolor* on plant growth: Role of allelopathic compounds. *Agricultural Research Journal*, 59(4), 25–35.
33. Singh, S., Yadav, D., & Kumar, S. (2020). Comparative analysis of allelopathic effects of *Sorghum bicolor* on various crops. *Environmental Science and Pollution Research*, 27(3), 3417–3425.
34. Weston, L. A., & Duke, S. O. (2020). Role of phenolic compounds in plant allelopathy: A review. *Allelopathy Journal*, 45(4), 1–12.
35. Batish, D. R., & Singh, H. P. (2021). Allelopathy and its ecological role in *Sorghum bicolor* interactions. *Crop Protection Journal*, 53(5), 212–222.
36. Chon, S. U., Park, Y. S., & Park, J. H. (2020). Responses of *Lactuca sativa* to allelopathic stress of *Sorghum bicolor* extracts. *Physiological Plant Ecology*, 66(3), 512–523.
37. Zhang, X., Liu, X., & Wang, Y. (2020). Carotenoid accumulation in plants under allelopathic stress: Insights from *Sorghum bicolor* extracts. *Plant Growth Regulation*, 38(1), 34–42.
38. Alleyne, M. A., Roussos, P. A., & Kumar, M. (2020). The role of carotenoids in plant stress tolerance under allelopathic conditions. *Plant Physiology and Biochemistry*, 148, 15–21.
39. Ncube, B., Nhamo, N., & Lungu, M. (2020). Flavonoids and phenolic compounds as plant defense mechanisms against allelopathic stress. *Journal of Chemical Ecology*, 46(5), 392–405.
40. Ahmad, R., Ali, H., & Khan, S. (2021). Sugar metabolism and allelopathy: Impact of *Sorghum bicolor* extracts on *Brassica napus* growth. *International Journal of Agricultural Science*, 15(1), 78–84.
41. Jorapur, S., Patil, R. V., & Rao, M. (2020). Antioxidant activity of flavonoids and phenolic compounds in allelopathic stress. *Plant Physiology Journal*, 49(6), 565–576.
42. Zhang, J., & Zhang, X. (2021). Effects of allelopathic stress on carbohydrate accumulation in plants: Role of sugars in stress adaptation. *Journal of Experimental Botany*, 72(5), 1075–1085.
43. Ahmad, M., Bukhari, S. I., & Khan, A. M. (2020). Role of indole acetic acid in regulating plant growth under stress conditions. *Environmental Stress and Plant Responses*, 22(1), 12–22.
44. Khan, A., Amin, M., & Jamil, M. (2021). Modulation of indole acetic acid in plants under allelopathic stress: Implications for crop improvement. *Agricultural Biotechnology Journal*, 29(4), 789–800