

Evaluation Of The Allelopathic Effects Of Residues And Aqueous Extract Two Wheat Cultivars On (*Avena fatua* L.) And (*Silybum marianum* L.)

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Abstract

The experiment was conducted in Wasit Province, Iraq, during the 2024 growing season to evaluate the allelopathic effects of two wheat (*Triticum aestivum* L.) cultivars, Jihan and Aba-99, on the suppression of weed growth. The results showed that both root and shoot residues of the cultivars markedly reduced the germination and growth of two major weeds wild oat (*Avena fatua* L.) and milk thistle (*Silybum marianum* L.) with variations in the magnitude of inhibition depending on the wheat cultivar and type of residue. The 'Jihan' shoot residues exhibited the most pronounced allelopathic activity, achieving complete inhibition (100%) of wild oat germination and a 44.6% reduction in its biomass. For milk thistle, the germination percentage was reduced by 76.1%, accompanied by a 68.6% decrease in biomass.

In contrast, the 'Aba-99' cultivar showed comparatively lower allelopathic potential, reducing wild oat and milk thistle germination by 91.3% and 52.3%, respectively, while decreasing their dry biomass by 22.6% and 56.3%, respectively. Furthermore, root and shoot length were also inhibited to varying degrees. HPLC chemical analysis identified eight allelochemicals in total six phenolic compounds and two terpenoids present in the wheat residues.

Keywords: wheat (*Triticum aestivum*), cultivars, allelopathic effect, phenolic compounds, Weed management, *Silybum marianum*, *Avena fatua*

INTRODUCTION

Allelopathy refers to a biochemical interaction that occurs between plants or between plants and soil microorganisms, whereby certain plants release allelopathic chemicals capable of inhibiting the germination, growth, or development of competing plant species. These allelopathic substances are secondary metabolic products synthesized by plants, microorganisms, viruses, and fungi, exerting regulatory effects either positive or negative in agricultural and ecological systems by modifying physiological and biochemical processes. Harnessing the allelopathic potential of crops to suppress weed growth represents a promising and sustainable alternative to synthetic herbicides within integrated weed management programs in field farming systems (Kostina-Bednarz et al., 2023; Jamil et al., 2009). Allelopathic crops are considered one of the promising modern strategies for controlling weed growth. Many studies have focused on them and have shown positive results in inhibiting weeds. (Chou, 1999; Hozayn et al., 2011). Many researchers are found that allelochemicals released from some crop residues can ably inhibit the germination and growth of weeds under field conditions. Some have suggested possibly using them as environmentally friendly natural pesticides that apply directly in pest control or in synthesis new pesticides that are less harmful to the environment (Jamil, 2004).

Consequently, researchers worldwide have conducted extensive screenings to identify wheat varieties with strong allelopathic traits. In this context, a study in Pakistan evaluated 35 wheat varieties to measure their allelopathic effects against wild oats (*Avena fatua*), identifying 11 varieties exhibiting high allelopathic activity (Mahmood et al., 2013; Zhang et al., 2016). Such studies form a critical foundation for developing sustainable weed management strategies. Allelopathically potent varieties can be integrated into crop rotations or utilized as genetic resources in breeding programs aimed at developing cultivars with enhanced weed-suppressing capabilities.

To validate the functional significance of allelopathic traits in weed suppression, Kashif (2016) conducted a two-year field experiment comparing three wheat varieties Shafaq-06, Faisalabad-08, and Sehar-06 for their ability to suppress (*Phalaris minor*) across four weed densities (0, 100, 200, and 300 plants/m²) using a replicated design (n=3). Quantitative analysis revealed that Shafaq-06 consistently produced the highest concentrations of allelopathic phenolic compounds across all weed densities, directly correlating with significant inhibition of (*Phalaris minor*) growth. Furthermore, Shafaq-06 demonstrated superior performance in key physiological and agronomic traits, achieving the highest grain yield. In contrast, Sehar-06 exhibited the weakest allelopathic effect against the target weed.

The objectives of this study are:

- Assess the allelopathic effects of wheat residues (root and shoot) incorporated into soil on the growth of wild oats (*Avena fatua*) and milk thistle (*Silybum marianum*) in plastic pots under a shade net.
- Investigate the inhibitory effects of aqueous extracts of wheat residues on seed germination of (*Avena fatua*) and (*Silybum marianum*) using in vitro bioassays in Petri dishes.
- Conduct a comparative study on the impact of varying residue concentrations on embryonic root and shoot growth in both target species to identify effective thresholds for potential integration into integrated weeds management programs.

MATERIALS AND METHODS

Plant materials

One of the agricultural fields in Wasit province was prepared for the 2024 season. The field was subdivided into two plots and sown with two wheat cultivars (Jihan and Abaa-99). After two months of growth from germination, the aboveground shoot and belowground root plant parts were harvested. The samples were carefully washed to remove adhering soil particles, oven-dried, ground to a fine powder, and subsequently prepared for extraction procedures.

Additionally, seeds of the weed species milk thistle (*Silybum marianum*) and wild oat (*Avena fatua*) were obtained from agricultural fields in proximity to Wasit University.

Preparation of aqueous extracts

Respective grinded plant parts were weighed Three different weights (5, 10, 20 g) were each separately mixed with 100 mL of distilled water and soaked for 24 h at room temperature. The mixtures were then blended using an electric blender for 5 minutes, the mixture was then filtered using Whatman No. 42 filter paper to be used fresh in the bioassay, this procedure yielded three different extract concentration 5%, 10%, 20%.

Shade-house experiment

The wheat shoot and root residues were ground and mixed with soil at two concentrations (3 and 6 g) of shoot and root residues separately. The mixtures were placed in plastic pots containing 1200 g of soil. Control treatments were also used by adding peat moss at the same concentrations as the residues to ensure equal amounts of added material.

Experimental Design and Treatments

The experiment was used a three-replication randomized complete block design (RCBD). The least significant difference (LSD_{0.05}) test proceeded to compare and separate the treatments Each treatment included 20 seeds per replication, totalling 660 seeds (360 in plastic pots and 300 in Petri dishes). The treatments were:

Jihan shoot, Jihan root, Abaa-99 shoot, Abaa-99 root

Germination tests/bioassay

Seeds were sown in the pots at a rate of 20 seeds per species, with three replicates for each treatment. The pots were kept in a shade house and watered as needed with equal amounts of running water. Ten days after sowing, the percentage emergence of weed seedlings was recorded. Additionally, the emerged weed seedlings were thinned to five seedlings per pot and left to grow for 67 days. After this period, whole plants were carefully removed, washed, and dried. The dry weights (mg) of the shoot and root parts were then measured. For the extract experiments, 20 weed seeds were placed individually in Petri dishes lined with filter paper. Three replicates were used for each concentration (5%, 10%, 20%). 10 mL of the extract were added to each

dish, and rehydration was performed as needed using the respective extracts. After 7 days, the germination percentage was recorded.(Ghaleb et al. 2022)

Radicle and plumule lengths(cm) were recorded 15 days after the standard germination period, five seedlings were randomly selected from each dish. The radicle was separated at its point of attachment to the seed, and the plumule was separated from the hypocotyl. Their lengths were measured using a ruler (AOSA, 1983; Association of Official Seed Analysts).

Statistical analysis

were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 26 and Microsoft Office Excel 2010. Numeric data were presented as mean, standard deviation after performance of Kolmogorov-Smirnov normality test and making decision about normally and non-normally distributed variables. One way anova test was used to study difference in mean among more than two groups provided that the variable is normally distributed. The level of significance was considered at P-value of less 0.05 and highly significant level at 0.01 or less (Daniel, 2018).

RESULTS

Our study revealed significant allelopathic effects from the residues of both tested wheat varieties, demonstrating pronounced inhibitory impacts on (*Silybum marianum*) grass biomass as presented in **Table 1**. shoot residues from the Jihan variety exerted a substantially greater suppressive effect compared to those from Abaa-99, particularly on shoot biomass where Jihan residues applied at 3g and 6g concentrations induced dry weight reductions of (60.5, 68.6%) respectively, relative to the control treatment. In contrast, Abaa-99 shoot residues caused lower reductions of 57.1% and 56.3% at the same 3g and 6g concentrations. Regarding root biomass, Jihan shoot residues also showed stronger inhibition, reducing dry weight by 64.6% at the 6g concentration compared to 60.7% for Abaa-99 at the same concentration. In contrast, the inhibitory effects of root residues from both varieties were notably lower than their green residue counterparts. Root residues reduced shoot biomass within a range of 26.3% to 47.8% and root biomass within a range of 29.0% to 37.0% compared to the control. This is attributed to the presence of higher concentrations of allelopathic toxic compounds in the shoot residues compared to the root residues. Moreover, HPLC analysis results revealed that the Jihan variety contained higher levels of these compounds than the Abaa-99 variety, which in turn affected key physiological processes in the plant such as photosynthesis, protein synthesis, and respiration.

Treatments	Dry weight (mg)					
	Shoots		Roots		Whole plant	
	3g	6g	3g	6g	3g	6g
Jihan shoot	543.7	440.7	155.7	133.0	699.4	573.7
Jihan root	780.7	728.7	194.3	180.7	975.0	909.4
Abaa-99 shoot	580.3	607.0	189.0	146.7	769.3	753.7
Abaa-99 root	851.7	1030.3	170.0	248.7	1021.7	1279.0
Control	1376.3	1404.3	272.3	374.3	1648.3	1778.6
LSD ≤ 0.05	Residue shoot	Residue	Residue shoot	Residue	Residue shoot	Residue
	root		root		root	
Concentrations	235.2	122.8	53.5	48.8	177.8	174.4
Cultivars	271.2	182.4	73.3	56.4	148.3	199.3
Interaction	301.3	204.5	109.1	77.8	202.1	244.5

Table 1: Allelopathic effect of residue wheat cultivars on growth of (*Silybum marianum*) weed.

Table 2 illustrates the effects of wheat variety residues on the biomass of wild oat (*Avena fatua*). Shoot residues of both Jihan and Abaa-99 significantly reduced the dry weights of the aerial parts and roots of wild oat. At

the 6 g application rate, Jihan shoot residues produced a 44.6 % reduction in shoot dry weight compared to the control, while its root residues caused approximately a 41.3 % reduction. In contrast, Abaa-99 shoot residues at 6 g elicited a 51.2 % decrease, whereas its root residues did not exert a significant effect, with reductions ranging from (4 - 12 %.)

Regarding root biomass, Jihan residues led to decreases of 6.5 % to 22.7 %, whereas Abaa-99 residues produced a 15 % reduction. Moreover, the root residues of both varieties at the 3 g rate did not differ significantly from the control.

Treatments			Dry weight (mg)			
	Shoots		Roots		Whole plant	
	3g	6g	3g	6g	3g	6g
Jihan shoot	610.3	541.0	316.0	287.7	926.3	827.7
Jihan root	605.3	567.3	342.0	299.3	947.3	866.6
Abaa-99 shoot	636.7	471.3	285.3	290.3	922.0	761.6
Abaa-99 root	859.7	936.7	370.0	318.3	1229.7	1255.0
Control	984.0	976.3	338.0	370.0	1322.0	1346.3
LSD ≤ 0.05	Residue shoot	Residue	Residue shoot	Residue	Residue shoot	Residue
	root		root		root	
Concentrations	130.3	68.8	78.2	48.8	164.9	79.3
Cultivars	161.6	112.1	54.6	50.5	244.1	148.2
Interaction	199.2	141.5	92.1	78.2	289.3	179.7

Table 2: Allelopathic effect of residue wheat cultivars on growth of (*Avena fatua*) weed.

Table (3) presents the effect of aqueous extracts from the residues of two wheat cultivars (Jihan and Abaa-99) on the germination percentage of (*Avena fatua*) and (*Silybum marianum*). The results showed that both shoot and root extracts of Jihan completely inhibited wild oat germination at the 20% concentration, whereas the inhibition percentages for Abaa-99 shoot and root extracts at the same level were 91.3% and 73.4%, respectively. At the lower concentration (5%), variations in inhibition were observed, with the highest value recorded for Abaa-99 shoot extract (63.4%) and the lowest for its root extract (40%).

At the intermediate concentration (10%), Jihan shoot extract exhibited the highest inhibition rate (91.3%), while Abaa-99 root extract recorded 46.05%.

For *S. marianum*, allelopathic effects were less severe than for wild oat. The germination percentage in the Jihan shoot extract treatment at 20% concentration was 15%, corresponding to 76.1% inhibition, while the Abaa-99 shoot extract resulted in 52.3% inhibition. Root extracts of both cultivars at the same concentration showed inhibition values ranging from 47.6% to 58.7%.

At the 5% concentration, both cultivars exhibited relatively low effects, with inhibition percentages ranging from 19.04% to 34.6%. At the intermediate concentration (10%), inhibition varied, with the highest value (44.4%) recorded for Abaa-99 root extract, and the lowest (26.1%) for Jihan root extract.

These findings are consistent with Wu et al. (2001), who reported that cultivars with higher phenolic acid content possess greater potential to inhibit the growth of various weed species. Similarly, (Chum et al. (2010) demonstrated that the effectiveness of allelopathic compounds increases with concentration.

Avena fatua					Silybum marianum			
Treatments	5%	10%	20%	Mean	5%	10%	20%	Mean
Jihan shoot	35.0	6.5	0.0	13.8	45.5	38.5	15.0	32.8
Jihan root	36.5	35.0	0.0	23.8	50.0	46.5	33.5	43.5
Abaa-99 shoot	26.5	10.0	6.5	14.3	38.5	43.8	30.0	37.3
Abaa-99 root	46.5	41.5	20.0	36.0	51.5	35.0	26.5	37.6

Control	76.5	76.5	76.5	76.5	63.5	63.5	63.5	63.5
LSD ≤ 0.05								
Concentrations	7.33				9.41			
Cultivars	7.99				8.81			
Interaction	11.66				12.62			

Table 3: Allelopathic effect of (*Triticum aestivum* L.) aqueous extract on germination percentage of test weeds on Petri dishes.

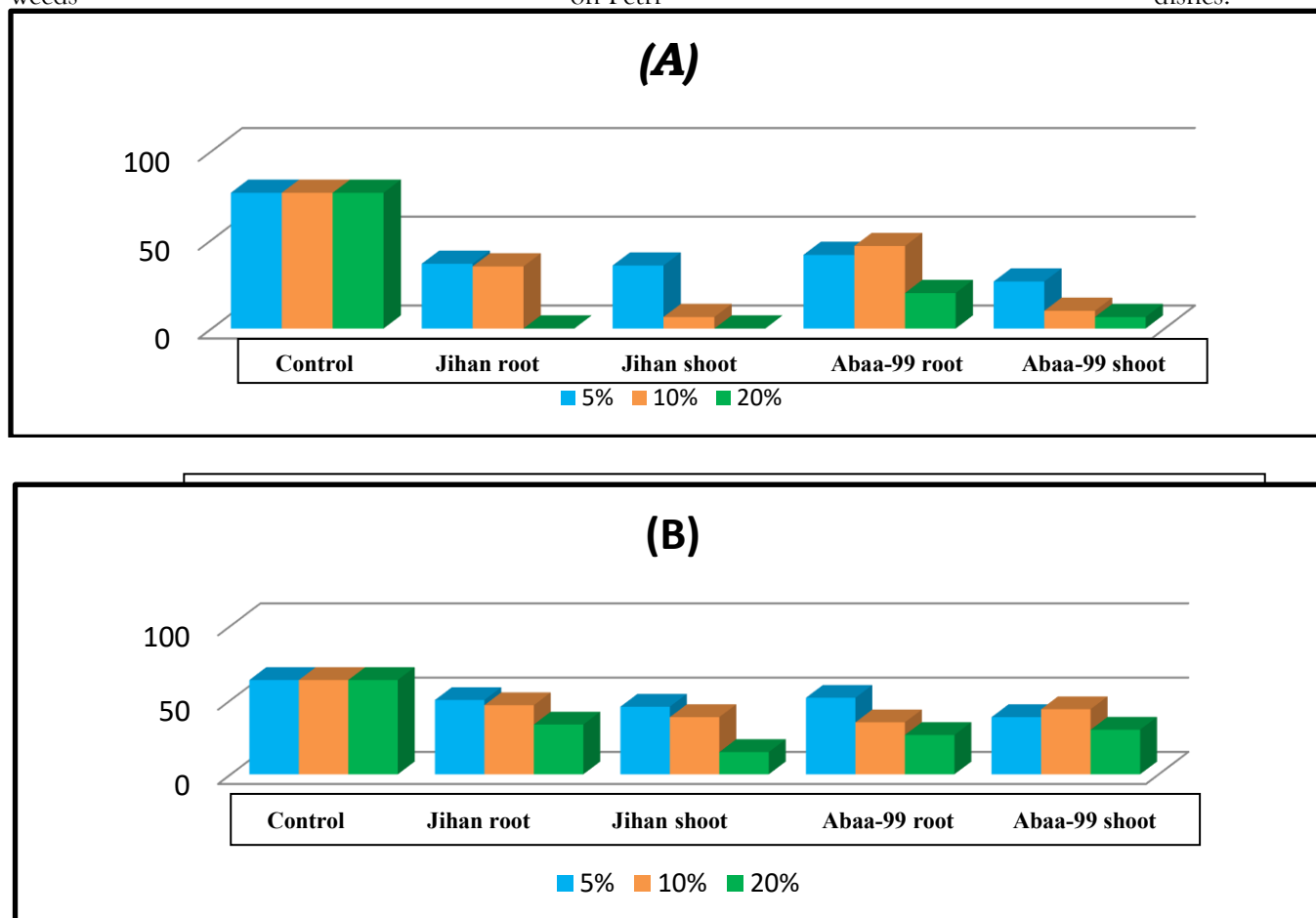


Figure 1. Allelopathic potential of (*Triticum aestivum* L.) extract against test species :(A) germination percentage of (*Avena fatua*) and (B) germination percentage of(*Silybum marianum*)
The results presented in **Table (4)** indicate that incorporating the plant residues of the two wheat cultivars (Jihan and Abaa-99) into the soil at application rates of 3 and 6 g resulted in varying inhibitory effects on the germination of (*A. fatua*) and (*S. marianum*). For wild oat, the shoot residues of the Jihan cultivar at 6 g exhibited the highest inhibition percentage (47.3%), whereas the root residues of the same cultivar at the same rate recorded 34.6%. In contrast, the shoot residues of Abaa-99 at 3 g showed an inhibition rate of 46.2%, while the inhibition percentages caused by its root residues at 3 and 6 g were 23.3% and 42.5%, respectively.

In the case of *S. marianum*, the shoot residues of Jihan achieved inhibition percentages of 50.1% and 46.5% at 3 and 6 g, respectively, while those of Abaa-99 ranged between 21.5% and 29.3%. Regarding root residues, Jihan showed inhibitory activity of 50.1% at 3 g and 46.2% at 6 g, whereas Abaa-99 recorded lower values of 23.07% and 1.9%, respectively.

These findings highlight the superiority of Jihan shoot residues in exerting allelopathic effects compared to other treatments, which aligns with the HPLC analysis results showing higher concentrations of active allelopathic compounds particularly phenolics and organic acids in these residues. Moreover, these outcomes are consistent with previous studies that have emphasized the pivotal role of phenolic compounds in inhibiting the germination and growth of weed seeds.

Treatments	(Avena fatua)				(Silybum marianum)			
	Residues Shoot		Residues Root		Residues Shoot		Residues Root	
	3g	6g	3g	6g	3g	6g	3g	6g
Jihan	56.5	41.5	53.5	51.5	25.0	31.5	28.5	28.5
Abaa-99	43.5	58.5	46.5	61.5	40.0	41.5	40.0	56.5
Control	80.0	78.2	80.0	78.5	51.5	58.5	52.0	57.5
LSD ≤ 0.05								
Concentrations	11.1		9.1		7.9		8.1	
Cultivars	10.9		9.8		12.4		11.02	
Interaction	13.4		12.1		14.3		14.3	

Table 4: Allelopathic effect of wheat (*Triticum aestivum* L.) on germination percentage of test species on soil

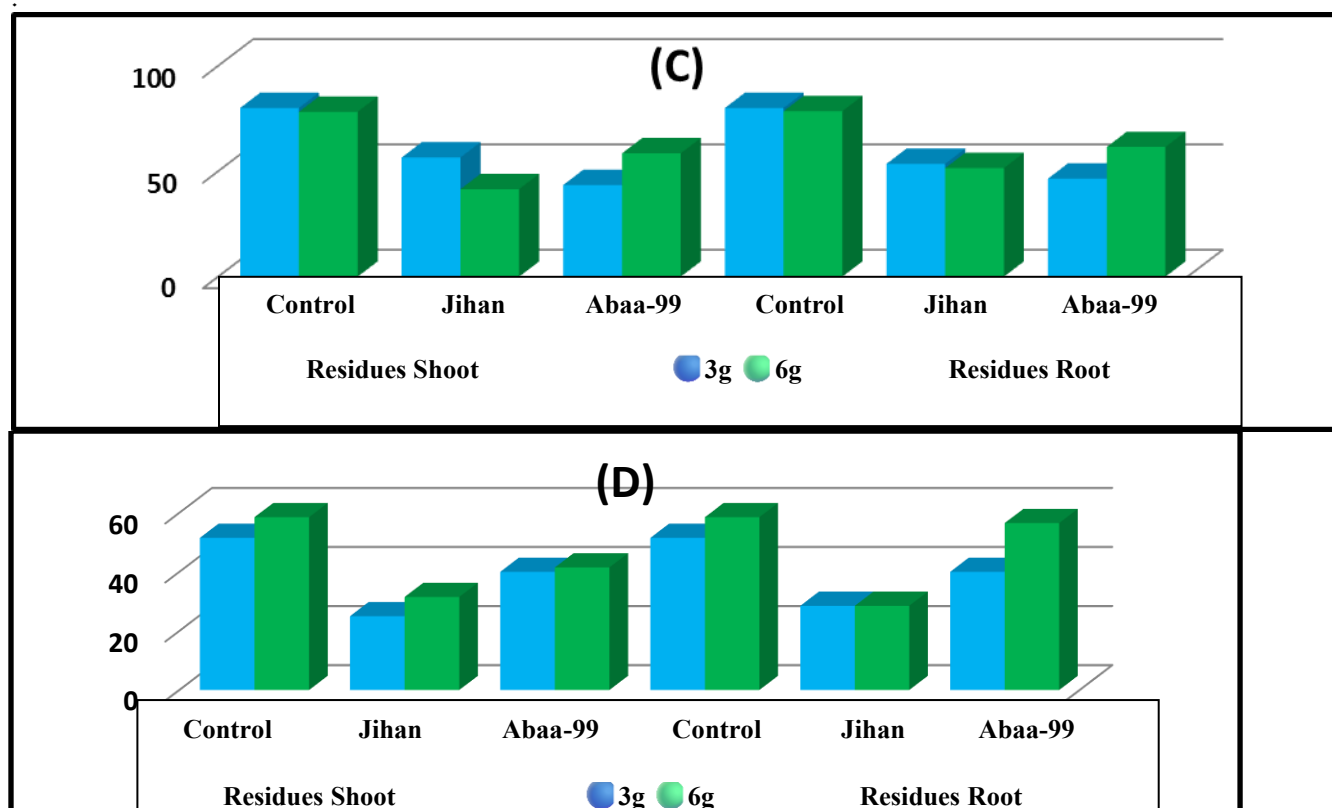


Figure 2: Allelopathic potential of (*Triticum aestivum* L.) residues (3 g ,6g) against test species :(C) germination percentage of (*Avena fatua*) and (D) germination percentage of (*Silybum marianum*) on soil.

Table 5 presents the plumule length response under the influence of wheat cultivar residues. Both Jihan and Abaa-99 residues significantly affected the cultivars and their interactions, while the effects varied with concentration. Jihan shoot extracts at 10% and 20% concentrations caused complete inhibition (100%) of plumule elongation, whereas the 5% concentration resulted in a 77.5% reduction. Root extracts of Jihan showed more variable effects ranging from 11.6% inhibition at 5% to 27.5% at 10% with a sharp increase to 81.6% at 20%.

In contrast, Abaa-99 shoot and root extracts exhibited relatively weaker allelopathic effects, with reductions ranging from 3.06% to 28.5%. However, inhibition increased with concentration, reaching 53.06% for shoot extracts and 36.5% for root extracts at 20%.

For *S. marianum*, all Jihan extract treatments resulted in highly significant reductions in plumule length. The greatest inhibition (90.5%) was recorded for the 20% shoot extract, while the lowest (72.4%) occurred with the 5% root extract. Abaa-99 extracts produced comparatively lower inhibition, with the highest value (53.1%) observed for the 20% shoot extract and the lowest (21.7%) for the 5% shoot extract, relative to the control.

Table 6 shows the effects of wheat residue extracts on radicle length in *A. fatua* and *S. marianum*. In *A. fatua*, Jihan shoot extracts at 10% and 20% concentrations caused complete inhibition (100%) of radicle growth, while the 5% concentration resulted in a 75.3% reduction. Root extracts of Jihan produced inhibition levels of 19.7%, 37.03%, and 74.2% at 5%, 10%, and 20% concentrations, respectively, compared to the control. For Abaa-99, shoot and root extracts exhibited similar effects at the 20% concentration, both resulting in approximately 49.4% inhibition. In the remaining treatments, inhibition values ranged from 4.9% to 28.3% relative to the control.

In *S. marianum*, Jihan shoot extracts demonstrated strong inhibitory activity, with 20% concentration reducing radicle length by 91.8%. The 5% and 10% concentrations also showed high inhibition rates of 88.6% and 81.1%, respectively. Jihan root extracts produced inhibition levels of 86.3%, 79.05%, and 69.4% at 20%, 10%, and 5% concentrations, respectively.

For Abaa-99, both shoot and root extracts had a pronounced effect at 20% concentration, reaching about 63.4% inhibition, whereas the 5% and 10% treatments produced lower values, ranging from 35.7% to 49.4%. Overall, these findings together with previous results indicate that all Jihan treatments significantly affected the physiological traits under investigation, likely due to their higher content of allelopathic compounds compared with Abaa-99, which contains lower levels. **Table (7)** presents the HPLC analysis of the alcoholic extracts of both Jihan and Abaa-99 cultivars

Table 5: Allelopathic effect of (*Triticum aestivum* L.) aqueous extract on plumule length (cm) of test species in Petri dishes lined with filter paper .

Treatments	Avena fatua				Silybum marianum			
	5%	10%	20%	Mean	5%	10%	20%	Mean
Jihan shoot	2.25	0	0	0.75	0.97	0.81	0.65	0.81
Jihan root	8.73	7.14	1.89	5.92	1.99	1.56	1.17	1.57
Abaa-99 shoot	8.95	6.99	4.64	6.86	5.42	4.89	3.24	4.52
Abaa-99 root	9.52	8.10	6.22	7.95	4.77	4.43	3.23	4.15
Control	9.80	9.80	9.80	9.80	6.98	6.98	6.98	6.98

LSD \leq 0.05

Concentrations	1.44	0.89
Cultivars	1.30	0.65
Interaction	1.57	1.02

Treatments	Avena fatua				Silybum marianum			
	5%	10%	20%	Mean	5%	10%	20%	Mean

Jihan shoot	2.0	0	0	0.67	1.79	1.08	0.77	1.21
Jihan root	6.58	5.1	1.74	4.44	2.91	1.99	1.31	2.07
Abaa-99 shoot	7.74	5.81	4.08	5.88	5.83	4.74	3.03	4.53
Abaa-99 root	7.53	6.18	4.09	5.93	6.12	5.80	3.90	5.27
Control	8.12	8.12	8.12	8.12	9.50	9.50	9.50	9.50

LSD \leq 0.05

Concentrations 1.27 0.97

Cultivars 1.39 1.33

Interaction 171 0.91

Table 6: Allelopathic effect of (*Triticum aestivum* L.) aqueous extract on radical length (cm) of test species in Petri dishes lined with filter paper .

Phytotoxins	Concentration (ppm)			
	wheat cultivars			
	Jihan shoot	Jihan root	Abaa-99 shoot	Abaa-99 root
Gallic acid	88.9	84.23	74.58	74.12
Syringeic acid	83.6	50.11	70.33	40.6
Hydrobenzoic acid	53.23	71.45	42.65	60.22
p-coumaric acid	62.58	65.41	50.22	54.12
Ferulic acid	80.4	42.66	70.32	30.24
Rutin	60.22	90.25	50.56	75.66
Vanillic acid	74.89	55.08	67.11	46.25
Kaempferol	70.22	80.11	60.32	70.25
Total	573.84	539.30	486.09	451.46

Table 7: High-Performance Liquid Chromatography (HPLC) Analysis of Alcoholic Extract Concentrations from Wheat Residues.

DISCUSSION

Numerous previous studies in the field of allelopathy have demonstrated that certain crop species possess the ability to release chemical compounds with inhibitory effects on weeds, qualifying them as natural sources of bioherbicides (Alsaadawi et al., 1986a; Weir et al., 2004). The results of the current experiment confirm this concept by revealing the tangible allelopathic effects of plant residues from two wheat cultivars Jihan and Abaa-99 on a group of tested weed species. Despite the variation in the intensity of these effects between the two cultivars, Jihan exhibited the highest efficacy in suppressing growth, whether through its shoot or root residues, indicating its richness in bioactive compounds with direct physiological impacts. (Al Hamdi et al. 2001) (Einhellig, 2004; Yang, 2004).

Chemical analysis using HPLC identified eight distinct phenolic compounds in the plant residues. These compounds are well known for their inhibitory effects on vital biological processes in neighboring plants, including the suppression of chlorophyll synthesis, disruption of ion uptake, and reduction in photosynthetic efficiency (Ahmed et al. 2021) (El-Shora et al., 2022). The findings of this study suggest that one of the primary mechanisms by which these compounds contribute to weed suppression lies in their direct negative impact on ion absorption, resulting in ionic imbalance within plant cells and subsequent disruption of associated metabolic functions.

At the physiological level, treatments with wheat extracts and residues caused significant reductions in the germination rates of weed seeds, along with notable decreases in biomass as measured by dry weight.

Additionally, a clear inhibition in the growth of both coleoptiles and radicles was observed, indicating a direct effect of the phenolic compounds on cell division and elongation especially during early growth stages, which are most sensitive to chemical interference. Consistent with these findings, Hanwen Wu et al. (2003). Allelochemicals have been shown to contain endogenous growth regulators that exert a direct influence on auxin (IAA) concentrations within plant tissues, thereby leading to the suppression of seed germination and inhibition of seedling development (Singh et al., 1999), reported that wheat residues significantly affected weed germination, in some cases causing up to 100% inhibition, in addition to marked reductions in coleoptile and radicle length. (Altameme et al. 2015)

These results strengthen the hypothesis that wheat residues particularly from the Jihan cultivar can serve as an effective tool for sustainable weed management by leveraging their allelopathic properties. Further research is recommended to isolate and precisely identify the active compounds and investigate their molecular and physiological modes of action, paving the way for the development of environmentally friendly natural herbicides. (Li et al. 2010; Sarbout et al. 2024).

CONCLUSIONS

The germination percentage, plumule and radicle lengths, as well as dry weight of the tested seedlings, were significantly reduced, particularly in response to the aqueous extracts of the shoot residues of the Jihan wheat cultivar. The inhibitory effect increased proportionally with the concentration of the plant material used, and these findings are consistent with those reported by other researchers. The study revealed that wheat residues contain polar, water-soluble chemical compounds that significantly affected weed growth. Based on the current findings, it can be concluded that certain toxic allelochemicals are present in the examined plant parts, and are responsible for the inhibition of weed growth and development. Future studies are recommended to isolate and identify these compounds, which could potentially be utilized in the development of natural herbicides.

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