

Allelopathic influence of wheat (*Triticum aestivum*) extracts and residues on the physiology of *Lolium temulentum* L

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Abstract

The experiment was conducted in laboratories of the College of Science at the University of Wasit during the 2024 agricultural season to investigate the allelopathic effects of wheat crop residues and extracts on the germination and growth of *Lolium temulentum* L. The results demonstrated that the wheat extracts significantly inhibited the germination of *L. temulentum* seeds with the highest inhibition rate reaching 81.4%. The extracts also exhibited a significant suppressive effect on the length of both the plumule and radicle at most concentrations, particularly at higher levels. Furthermore, the application of wheat residues significantly reduced the dry weight of the plants two months after emergence. The greatest reduction in dry weight (64.7%) was observed with the residues of the Jihan cultivar, while the lowest reduction (19.04%) was recorded for the Abaa-99 cultivar compared to the control. The inhibitory effects varied between the plant parts used and the wheat cultivar. The Jihan cultivar exhibited the strongest allelopathic impact, which is attributed to its higher content of phytotoxic compounds, particularly those found in its shoot parts.

Keywords: Wheat (*Triticum aestivum*), Cultivars, Allelopathic effect, Phenolic compounds, Weed management, *Lolium temulentum* L

Introduction

Weeds inflict substantial economic losses on global agricultural production, with estimated field losses reaching 5% in developed countries, 10% in less developed nations, and up to 25% in developing countries. In response to this growing threat, reliance on chemical herbicides has intensified over recent decades. Herbicides now constitute approximately 37% of all pesticide use, compared to 24% for insecticides. However, this extensive dependence has led to the evolution of herbicide-resistant weeds, with 332 documented resistant biotypes encompassing 189 plant species (113 dicots and 76 monocots) identified across more than 300,000 fields worldwide.

Given the environmental concerns, increasing costs, and proliferation of resistant weed species associated with synthetic herbicides, there is a critical need to develop sustainable and eco-friendly alternatives for weed management. One promising approach involves harnessing allelopathy a biochemical phenomenon where plants or microorganisms release natural compounds that inhibit the germination and growth of competing vegetation.

Lolium temulentum L. is native to the Mediterranean region and has subsequently expanded throughout temperate zones where wheat and other cereals are cultivated. Its successful spread is largely attributed to its biological traits, including high ecological adaptability, rapid reproductive capacity and inherent toxicity, which facilitate its establishment in new environments, often in association with wheat fields, thereby posing risks of food poisoning to both humans and livestock (Holm et al., 1991; Yang et al., 2023). This weed species can reduce yields of wheat by up to 17%, and wheat stands infested with *L. temulentum* often show reduced responsiveness to nitrogen fertilization. The seeds of *L. temulentum* are toxic when ingested (Yang et al., 2022; Farnworth & Said, 1983). Previous studies have further demonstrated that *Bromus rigidus*, *Bromus diandrus*, *Lolium multiflorum*, and *L. temulentum* exert allelopathic influences through aqueous shoot extracts, negatively affecting germination and growth of winter wheat. Additionally, plant parts of *L. multiflorum* were reported to suppress seed germination and seedling growth of *Oryza sativa* L. and *E. oryzoides* via polyphenolic allelochemicals. On the other hand, the seeds of *L. temulentum* contain approximately 20% oil, which, owing to its high tocopherol concentration, can enhance the oxidative

stability of flax oil (Lehoczký et al., 2011; Vitalini et al., 2020).

Allelopathic interactions are mediated through secondary metabolites produced by plants, microbes, and fungi, which can positively or negatively influence physiological and biochemical processes in agricultural and ecological systems. The integration of allelopathic crops and plant residues into farming systems offers a viable strategy for suppressing weeds within integrated weed management frameworks. Numerous studies have demonstrated the potential of these natural compounds to serve as bioherbicides or as templates for developing new, environmentally benign herbicides, thereby providing a sustainable pathway toward reducing dependency on synthetic chemicals.

The objectives of this study are:

- Assess the allelopathic effects of wheat residues (root and shoot) incorporated into soil on the growth of (*Lolium temulentum*) in plastic pots under a shade net.
- Investigate the inhibitory effects of aqueous extracts of wheat residues on seed germination of (*Lolium temulentum*) 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 (*Lolium temulentum*) was 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 shoots 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 1000 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, 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; Jam et al., 2025)

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 and extract tested wheat varieties, Table (1) presents the effect of aqueous extracts from the residues of two wheat cultivars Jihan and Abaa-99 on the germination percentage of (*Lolium temulentum*). Most of the extract treatments had significant effects. The shoot extract of the Jihan cultivar caused the highest inhibition, reducing germination by 81.4%, while the lowest inhibition was observed with the root extract of the Abaa-99 cultivar, which reduced germination by 54.9%, both at a concentration of 20%. The other treatments varied in their effects however, most of the treatments derived from the Jihan cultivar outperformed those from the Abaa-99 cultivar in terms of inhibitory potential.

These results align with previous studies indicating that cultivars rich in phenolic acids tend to exhibit stronger allelopathic effects against different weed species (Wu et al., 2001). In addition, research by Chum et al. (2010) confirmed that the allelopathic activity of certain compounds is concentration-dependent, with higher concentrations leading to more pronounced inhibitory effects.

Table 1: Allelopathic effect of (*Triticum aestivum* L.) aqueous extract on germination percentage of *Lolium temulentum* on Petri dishes

<i>Lolium temulentum</i>				
Treatments	5%	10%	20%	Mean
Jihan shoot	50.0	33.5	15.0	32.8
Jihan root	65.0	53.5	20.0	46.1
Abaa-99 shoot	66.5	43.5	31.5	47.1
Abaa-99 root	68.5	75.0	36.5	60.0
Control	81.5	81.5	81.5	81.5
LSD ≤ 0.05				
Concentrations	7.33			
Cultivars	7.99			
Interaction	11.66			

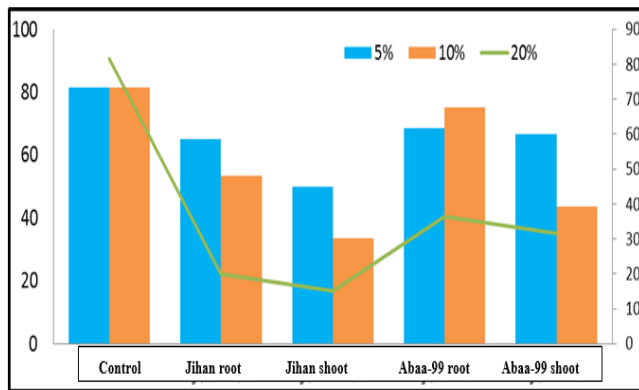


Figure 1. Allelopathic potential of (*Triticum aestivum* L.) extract against test species on germination percentage of (*Lolium temulentum*)

Table 2 In *L. temulentum* all extract treatments showed statistically significant effects with variations in the degree of inhibition. The shoot extracts of the Jihan cultivar exhibited the highest inhibitory effect at a concentration of 20%, reducing germination by 67.4%. This effect decreased to 34.9% at the 5% concentration. Similarly, root extracts from the same cultivar showed a maximum inhibition of 54.2% at 20%, which declined to 30.1% at 5%.

In comparison, the extracts of the Abaa-99 cultivar demonstrated considerably lower inhibitory effects. The highest reduction was recorded with the 20% shoot extract, which inhibited germination by 44.5%.

Other treatments ranged in their effects, with reductions between 32.5% and 8.43% relative to the control.

As for radicle length, most treatments showed significant effects, which increased with extract concentration. The highest reduction in radicle length was observed with the root extract of Jihan at 20%, causing a 75.1% decrease. This reduction lessened to 26.2% at the 5% concentration. Shoot extracts displayed a similar trend, with the 20% concentration resulting in a 72.5% reduction in radicle length, while the 5% concentration caused a 37.5% decrease.

On the other hand, the extracts of the Abaa-99 cultivar exhibited relatively milder effects compared to those of Jihan, although they still remained statistically significant. The strongest inhibition was recorded with the 20% root extract, which caused a 51.3% reduction in radicle length. Other treatments ranged from 3.7% to 36.7% in their inhibitory effects.

These results indicate that the Jihan cultivar contains higher levels of allelopathic compounds compared to Abaa-99. This conclusion is further supported by HPLC analysis of the extract samples, which confirmed the presence of greater concentrations of bioactive allelochemicals in Jihan.

Table 2: Allelopathic effect of (*Triticum aestivum* L.) aqueous extract on plumule length and radical length of *Lolium temulentum* in Petri dishes lined with filter paper

Treatments	Lolium temulentum							
	plumule length (cm)				radical length (cm)			
	5%	10%	20%	Mean	5%	10%	20%	Mean
Jihan shoot	5.41	4.4	2.72	4.18	5.03	3.87	2.23	3.71
Jihan root	5.87	4.14	3.83	4.61	5.96	3.77	1.99	3.91
Abaa-99 shoot	7.38	6.77	4.61	6.25	7.71	5.04	3.93	5.56
Abaa-99 root	7.69	6.76	5.67	6.71	7.64	6.75	5.86	6.75
Control	8.35	8.35	8.35	8.35	8.06	8.06	8.06	8.06
LSD ≤ 0.05								
Concentrations	1.02				1.34			
Cultivars	1.41				1.49			
Interaction	0.94				1.15			

The demonstrating pronounced inhibitory impacts on (*Lolium temulentum*) grass biomass dry as presented in Table 3 also shows the effect of residues on the biomass dry of *L. temulentum*. The green residues of the cultivar Jihan caused a reduction in

shoot biomass by 64.8% and in root biomass by 46.6%, which led to a decrease in total plant biomass. In contrast, the root residues of Jihan exhibited a more severe effect, reducing shoot biomass by 19.04% and root biomass by 20.8%.

Similarly, the shoot residues of the cultivar Abaa-99 reduced shoot biomass dry by 53.8% and root biomass by 15.2%. Meanwhile, its root residues had an inhibitory effect on shoot biomass and a stimulatory effect on root biomass, which ultimately resulted in a 27.5% reduction in total plant biomass 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.

Table 3: Allelopathic effect of residue wheat cultivars on growth of (*Lolium temulentum*) weed

Treatments			Dry weight (mg)			
	Shoots		Roots		Whole plant	
	3g	6g	3g	6g	3g	6g
Jihan shoot	251.3	221.3	113.7	113.7	365	316.3
Jihan root	526.7	563	257	257	783.7	799
Abaa-99 shoot	235.3	384.7	149	149	384.3	696.7
Abaa-99 root	336.7	581.7	149.3	149.3	468	789.7
Control	376	968.3	160	230.3	536	1198.6
LSD ≤ 0.05	Residue shoot Residue root	Residue shoot Residue root	Residue shoot Residue root	Residue shoot Residue root	Residue shoot Residue root	Residue shoot Residue root
Concentrations	65.6	131.2	32	56.2	164.1	124.7
Cultivars	84.2	112.54	26.4	68.5	158.4	148.2
Interaction	112.3	152.1	48.3	77.1	179.2	162.3

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 (*L. temulentum*) all treatments involving residues from the Jihan cultivar had significant effects on emergence rate. The shoot residues at a

concentration of 6 g caused a reduction in seedling emergence by 13.3%. In contrast, the shoot residues of the Abaa-99 cultivar showed no significant difference compared to the control. Regarding root residues, those from Jihan exhibited the strongest inhibitory effect, reducing emergence by 32.2%, while root residues of Abaa-99 resulted in a 17.7% reduction compared to the control.

Table 4: Allelopathic effect of wheat (*Triticum aestivum* L.) on germination percentage of (*Lolium temulentum*)

Treatments	<i>Lolium temulentum</i>			
	Residues Shoot		Residues Root	
	3g	6g	3g	6g
Jihan	76.5	78.5	60.0	61.5
Abaa-99	91.5	90.0	51.5	73.5
Control	91.5	90.0	91.5	90.0
LSD ≤ 0.05				
Concentrations	6.72		9.90	
Cultivars	8.91		8.87	
Interaction	10.12		12.51	

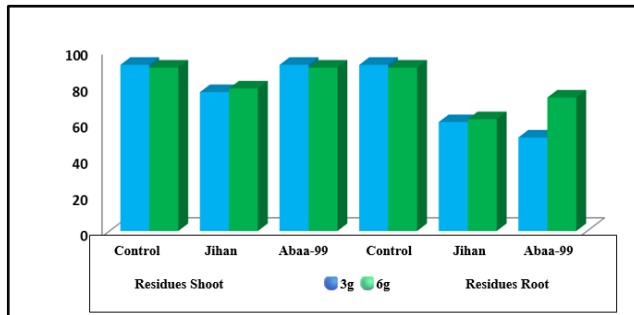


Figure 2: Allelopathic potential of (*Triticum aestivum* L.) residues (3 g, 6g) against test species germination percentage of (*Lolium temulentum*) on soil.

HPLC analysis identified six allelopathic phenolic compounds: gallic acid, syringic acid, p-hydroxybenzoic acid, p-coumaric acid, ferulic acid, and vanillic acid.

These phenolic compounds are known to exert significant effects on plant enzymes and hormonal balance. The concentration of these compounds varied significantly between cultivars and plant parts. The Jihan cultivar exhibited the highest total phenolic content in its shoot extracts at 443.34 µg/g, whereas the Abaa-99 cultivar showed the lowest total content in its root extracts at 305.78 µg/g.

Table 5: HPLC analysis of alcoholic extract from wheat residues

Phytotoxins(µg/g)	Jihan shoot	Jihan root	Abaa-99 shoot	Abaa-99 root
Gallic acid	88.7	84.24	74.57	74.13
Syringeic acid	83.4	50.13	70.32	40.71
Hydrobenzoic acid	53.22	71.45	42.64	60.23
p-coumaric acid	62.54	65.42	50.23	54.12
Ferulic acid	80.6	42.65	70.31	30.27
Vanillic acid	74.88	55.09	67.12	46.32
Total	443.34	368.98	375.19	305.78

Discussion

Previous research in the field of allelopathy has demonstrated that certain crop species possess the ability to release chemical compounds with inhibitory effects on weed growth, thereby qualifying them as potential natural sources of bioherbicides (Alsaadawi et al., 1986a; Weir et al., 2004). The findings of the present study reinforce this concept by revealing clear allelopathic effects of plant residues from two wheat cultivars, Jihan and Aba-99, on the tested weed species. Although some variation in the degree of inhibition was observed between the two cultivars, Jihan consistently exhibited the strongest suppressive activity, whether through shoot or root residues, suggesting that it is particularly rich in biologically active compounds with direct physiological impacts (Al Hamdi et al., 2001; Einhellig, 2004; Yang, 2004).

Analysis using HPLC identified six distinct phenolic compounds within the extracts. These phenolics are

well-documented for their inhibitory effects on essential biological processes in neighboring plants, including the suppression of chlorophyll biosynthesis, disruption of ion uptake, and reduction in photosynthetic efficiency (Sarbout et al., 2024; Alsaadawi et al., 1983). The results further suggest that one of the principal mechanisms underlying the suppressive activity of these compounds lies in their direct interference with ion absorption, which disrupts ionic balance within plant cells and subsequently impairs associated metabolic functions.

Collectively, these findings support the hypothesis that wheat residues particularly those of the Jihan cultivar may serve as an effective and sustainable tool for weed management through the exploitation of their allelopathic potential. Nevertheless, further investigations are required to isolate and precisely characterize the active compounds, and to elucidate their molecular and physiological mechanisms of action, thereby contributing to the development of environmentally friendly, natural herbicides (Hiero

& Callaway, 2021; Ahmed et al., 2021 Alsaadawi et al., 1993b).

Conclusions

The results revealed a significant inhibitory effect on germination percentage, plumule and radicle elongation, as well as seedling dry weight. This reduction was most evident in response to the aqueous extracts of the shoot residues of the Jihan wheat cultivar. It was further observed that the intensity of inhibition increased proportionally with the concentration of the applied plant-derived materials, which is consistent with findings reported in earlier studies. The results also indicated that wheat residues contain polar, water-soluble chemical compounds that not only exert negative effects on weed growth but also display autotoxic effects on wheat seeds themselves. Based on these observations, it can be concluded that the tested plant parts contain toxic allelochemicals that are primarily responsible for suppressing weed growth and hindering their development. Therefore, future research should focus on isolating and accurately characterizing these compounds using LC-MS techniques, which may provide a foundation for their potential application in the development of environmentally friendly natural herbicides.

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