Heliyon 10 (2024) e38007

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Plant and soil characteristics affected by the allelopathic pathways of *Avena fatua* and *Lolium temulentum* weeds

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ARTICLE INFO

Keywords:
Allelopathy
Avena fatua
Lolium temulentum
Leachates
Root exudates
Decayed residues
Decomposition
Phenolic acids
Triticum aestivum
Soil parameters

ABSTRACT

The potential of the most prevalent weeds should be characterized biologically and chemically in infected soil and crops for sustainable agriculture. Therefore, the allelopathic potential of *Avena fatua* L. and *Lolium temulentum* L. weeds were compared via leachates, root exudates, decayed residues in soil, and the decomposition in water pathways. Chemical measurements were taken on wheat (*Triticum aestivum* L.), and soil decomposed solution. Based on EC_{50}, the allelopathic effect of leachates were higher in aboveground parts than in subterranean parts, influenced by plant parts and concentrations. The root exudates show EC_{50} by 655.9 μg. ml^{-1} for *A. fatua* and 625.66 μg. ml^{-1} for *L. temulentum*in the seedling biomass fresh weights of *T. aestivum*. The systematic inhibition by decayed residues was affected by plant types, concentration, and time and correlated with soil parameters and crop performance. The decomposition rate was higher under aerobic conditions than anaerobic conditions, with the inhibition pattern showing the reverse trend. These finding highlight the importance of environmental conditions in mediating allelopathic effects. The highest quantities of phenolic acids determined by LC-ES/MS in decomposed solutions were citric acid, with concentrations of 7.71 and 13.31 μg/ml in *A. fatua* under aerobic conditions, and coumaric acid, with concentrations of 9.21 and 16.99 μg/ml in *L. temulentum* under aerobic conditions. The allelopathic potentials of *A. fatua* and *L. temulentum* may play a crucial role in *T. aestivum* crop growth and soil parameters. In general weed residues can suppress crop growth and negatively affect soil parameters based on their quantity and type, therefore they should be managed carefully for sustainable crop production.

1. Introduction

The Poaceae family is an important grass family with great economic importance which includes the three most valuable crops in the world such as wheat such as rice and maize. It also encompasses six of the seven most planted crops globally, including three types of cereals as well as barley, sorghum, and millet. Cereals are considered staple crops as they are grown in large quantities and provide

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https://doi.org/10.1016/j.heliyon.2024.e38007
Received 22 June 2024; Received in revised form 10 September 2024; Accepted 16 September 2024
Available online 18 September 2024

more food energy worldwide than any other type of crop [1,2]. Bread wheat (*Triticum aestivum*) is a strategic crop, widely produced and essential for human nutrition [3]. However, Poaceae is one of the best producers of alien invaders globally [4]. Weed causes 10–65 % yield losses in wheat depending up on weed species [5]. Poaceous weeds are prolific and competitive in winter crops [6]. In Egypt, the grasses of the Poaceae family are the largest flowering plants with 284 species belonging to 103 genera and 22 tribes [7]. *A. fatua*, and *l. temulentum* species belonging to the family Poaceae represent the highest plentiful numbers in wheat fields in Egypt [8]. The negative interactions between weeds and crops might be due to a combination of competition and allelopathic factors [9].

Wild oat (*Avena fatua* L.) is considered to be among the world's worst crop weeds [10]. The major weed threat in cereal cropping system in Mediterranean countries is *Avena* sp [11]. It produces severe yield and quality losses in cereal and oil seed crops [12], reducing wheat yields by 16–46 % [13]. *A. fatua* shows a high competitive ability and is often more competitive than wheat [14]. Wild oat produces a deep and extensive root system and responds significantly to a high supply of nitrogen [15]. It also exhibits strong allelopathic effects on wheat through its allelochemicals [16].

Lolium temulentum L. originated in the Mediterranean and has spread widely across temperate areas where wheat and cereals are grown [17,18]. This spread is due to its biological characteristics, including high adaptability, potential for rapid reproduction, and toxicity, which lead to its establishment in new habitats, often mixed with wheat, and can cause food poisoning in humans and livestock [19]. *L. temulentum* can reduce wheat and barley yields by up to 17 %, and wheat infested with *L. temulentum* may respond less effectively to nitrogen fertilization [20,21]. The seeds of *L. temulentum* have poisonous effects when consumed [22]. *Bromus rigidus*, *Bromus diandrus*, *Lolium multiflorum* and *Lolium temulentum* have allelopathic effects via watery shoot extracts on the germination and growth of winter wheat [23]. *L. multiflorum* parts affected seed germination and seedling growth of *Oryza sativa* L. and *E. oryzoides* through polyphenolic allelochemicals [24]. On the other side, seeds of *L. temulentum* contain 20 % of an oil which can give more stability to flax oil due to its high tocopherol content [25]. Also, *A. fatua* and *L. temulentum* can reduce soil erosion [26].

The bioactivity of Poaceae plants is often attributed to allelopathy [27]. Most of the allelopathic substances are released into the environment through root exudation, leaching, volatilization and decomposition of plant residues [28]. Allelopathy involves communication via allelochemicals [29]. Roots release phytotoxic secondary metabolites that inhabit or promote the growth of nearby plants [30]. Decomposition in most ecosystems results from the activities of microorganisms and animals which break down non-living organic matter into simpler forms [31]. The decaying weed residue effect depends upon the release of allelochemicals into the soil or may result from microbial activity during decomposition [32,33]. The decomposition of plant debris is a critical process producing short-term changes in water quality that quickly return to background levels level [34]. Litter quality and microbial community capacity are the key drivers of decomposition [35]. Chemically different litter structures impact decomposition rates and pathways [36].

Despite recent advancements in our understanding of allelopathy, there remains a need to compare the allelopathic potential of species within the Poaceae family, specifically *Avena fatua* and *Lolium temulentum*, on wheat crops. Additionally, there is limited knowledge about the efficiency of different pathways such as volatilization, leaching, root exudation, and residue decomposition that produce phytotoxic compounds. Specifically, we need to understand the potentials of the plant parts, the timing of phytotoxic effects during residue decomposition in water or soil, and the concentrations of allelochemicals, as well as their interactions affecting crop seed germination and seedling development. Thus, our hypothesis suggests that within the Poaceae family, there are variations in allelopathic potential on crops and soil properties, depending on weed species and allelochemical pathways. These differences extend to the qualitative and quantitative aspects of allelochemicals and their influence on soil properties. This study aims to characterize allelopathic potentials of *A. fatua* and *L. temulentum* by analyzing leachates, root exudates decayed residues in soil, and decomposition in water. We will examine their chemical changes and quantify their allelopathic effects on the seeds and seedlings of *T. aestivum* crops.

2. Materials and methods

2.1. Collection of plant materials and soil

Avena fatua and *lolium temulentum* were collected at the beginning of the flowering stage during 2020–2022, from Borg Al-arab at 30 .52. 84°N, 29. 28 6 87°E Alexandria governorate, Egypt. The identification was confirmed by plant specialist Desert Research Center, Cairo, Egypt. All the steps of experimentation on *A. fatua* and *L. temulentum* weeds, including the collection of plant material, were in compliance with relevant institutional, national, and international guidelines. The studies were conducted in accordance with local legislation and with permissions from the Desert Research Center and they complied with the IUCN Policy Statement. The plants were dried in the shade, chopped, ground and then stored in paper bags at room temperature. Seeds of Wheat (*Triticum aestivum)* (Giza 193) were used as the test species. The used soil was collected from the same place which has the following anions; 1.51 meq/l l sodium, 0.78 meq/l potassium, 1.01 meq/l calcium, 0.65 meq/l magnesium as well as cations by 1.50 meq/l HCO3, 1.46 meq/l, chloride, 1.15 meq/l sulfate with, pH 8.01 and electrical conductivity of 0.42 ds/m and have 77.25 % sand, 12.40 % silt, 10.35 % clay respectively according to FAO –UNESCO methods & Soil Taxonomy, its sandy loam texture soil [37]. Concerning elemental analysis, the soil materials were analyzed using the standard method described by **Robinson and Dekker** [38]**, Cottenie et al.,** [39] using the ICP-OES Spectrometer (PerkinElmer) while soil nitrogen was by Kjeldahl method [40]**.**

2.2. Leachates of Avena fatua and lolium temulentum vegetative and subterranean parts

The vegetative and subterranean parts of *A. fatua* and *L. temulentum* were ground and subjected to extraction by immersing 200g dry wt in 1000 ml of distilled water on an orbital shaker at 160 RPM for 12 h at room temperature in the laboratory. Subsequently, the resulting filtrates were passed through cheesecloth to remove plant residues, followed by centrifugation at 3000 RPM for 15 min. The

filtrate was then passed through a 0.22 μm pore micro-filter to sterilize before bioassays. The filtrate was considered a solution with a concentration of 20 % (g 0.100 ml^{-1}) and was further diluted to varying concentrations (0, 1, 2, 4, 6, 8, 10 g/100 ml) for bioassays on *T. aestivum* seeds. The seeds were sterilized with 0.3 % V/V sodium hypochlorite for 1 min and rinsed with sterile distilled water. The sterilized seeds were then arranged on filter papers in 9-cm Petri dishes, each dish receiving a 10 ml aliquot of water extract from the respective concentration. The petri dishes were maintained at room temperature (25 °C ± 2) in a completely randomized design with three replications. Each experiment was repeated three times under the same conditions, considering each repetition as one replicate.

2.3. Root exudates of A. fatua and L. temulentum extraction and bioassay on T. aestivum seeds and seedlings

The seeds of *A. fatua* and *L. temulentum* were sterilized by rinsing in 0.3 % V/V sodium hypochlorite for 1 min, followed by followed by several washes with sterile distilled water. They were then grown in solid Murashige and Skoog (MS) basal medium and allowed to germinate until roots and shoots emerged. Then some seedlings were transferred to 10 ml liquid media in tissue culture tubes where the roots were submerged [41,42] and placed on an orbital platform shaker set at 50 rpm laboratory for 28 days at 25 °C ±2 with a photoperiod of 16 h light and 8h dark. Formerly the liquid media were collected, filtrated and subjected to the refrigerator (-20 °C) for one night before lyophilizing by freeze-drying. The residues were re-suspended in 50 ml distilled water and adjusted pH ≤ 4, followed by partitioning by equal volume with ethyl acetate three times. These root exudates were collected from 1000 seedlings of *A. fatua* and *L. temulentum.* The extract evaporated to dryness and dissolved in methanol to give 0, 250, 500, 750, 1000, 1250 and 1500 μg/ml concentrations and tested against *T. aestivum* seeds and seedlings in Complete Randomized Design, while total biomass was recorded after seven days from treatment.

2.4. Greenhouse decayed and bioassay

The sand soil was mixed with vegetative parts (chopped less than 1 cm) of *A. fatua* and *L. temulentum*, at concentrations of 0, 1.25 %, 2.5 %, and 5 %, soil. This mixture was placed in plastic pots and subjected to periods of 0, 1, 3, 7, 21, 28, 35, 42, 49, and 56 days. Simultaneously, ten seeds of *T. aestivum* were planted every time starting from 0 until 56 days, during decayed times five replicated pots were set in a Complete Randomized Design at 25 ± 5 °C in a greenhouse. After three weeks, the germination, shoot and root lengths were individually measured, and the percentage of growth inhibition was calculated by comparing it to the control.

2.5. Laboratory decomposition and bioassay

The decomposing of *A. fatua* and *L. temulentum* vegetative parts was conducted according to the procedure outlined by **Bonanomi et al.,** [43]. The ground dried plant materials were immersed in distilled water at concentrations of 6 % (60 g dry weight per liter water under both aerobic and anaerobic laboratory conditions at 25 °C ± 2 in side glass conical for 30 days. Under aerobic decomposition, an air pipeline (2 × 3.5 L/min) was connected to an air pump and inserted into the solution. However, during anaerobic decomposition the container is kept closed. Aqueous suspension samples were collected at intervals of 0.5, 1, 2.5, 5, 10, 15, 20, and 30 days. These samples were filtered through nylon cloth to remove plant residues, followed by centrifugation at 5000 RPM for 15 min. Subsequently, electrical conductivity (EC) and pH were measured in the filtrated solution. The resulting filtrate was sterilized by passing it through a 0.22 μm pore micro-filter before being utilized in bioassays. Ten seeds of *T. aestivum* were then treated with concentrations of 0, 1.25, 2.5, and 5 g/100 ml, with each treatment replicated four times. These extractions and bioassays were carried out until the completion of the 30-day decomposition period. Petri dishes were arranged in a Complete Randomized Design within a culture room maintained at 25 ± 2 °C for 5 days, Then the germination %, shoot and root lengths were measured.

2.6. Analysis and quantification of decomposing allelochemicals using LC-ESI-MS

For analysis, 10 ml of the decomposed solution was filtered, lyophilized using a freeze dryer, and then dissolved in 10 ml of absolute methanol. One ml of this solution was filtered again through a 0.22 μm pore micro-filter before being injected into the LC-ESI-MS at Ain Shams University. The LC-ESI-MS system (Waters, USA) was equipped with a DAD detector. A 150 × 4.6 mm C18 column was employed for compound separation. UV/V is spectra were obtained at wavelengths of 220, 240, 280, 330, and 350 nm. Gradient elution at a flow rate of 0.2 mL/min was employed for analysis. The mobile phase consisted of a multistep linear solvent gradient system, starting from 100 % H2O (adjusted to pH 3.2 with HCOOH) and reaching 100 % CH3CN in 30 min. Authentic samples were purchased from Sigma-Aldrich, USA including P-hydroxybenzoic acid, hydroxycinnamic acid, vanillic acid, salicylic acid, gallic acid, syringic acid, citric acid, caffeic acid, coumaric acid, salicylic acid, and ferulic acid were used for analysis [44]. Mass data and fragmentation patterns were compared with the literature and spectroscopic data of these authentic samples.

2.7. Data analysis and statistics

The data was subject to statistical analysis using univariate (three-way ANOVA) to separate the effect of types of plant extract, plant parts, and concentration, using Post hoc tests, specifically Duncan multiple range tests ($P \geq 0.05$.) were then applied using IBM SPSS 21 software to identify significant differences. The inhibition or stimulation response (RI %) was computed using the formula RI% = (C - T/C * 100), where C represents the control and T represents the treatment. RI% values equal to or less than 0 indicated inhibitory effects. Dose-response curves were generated by f = min + (max-min)/(1 + 10ˆ(logEC50-x)) using sigma plot software 12.5.

3. Results

3.1. Allelopathic potentials of A. fatua and L. temulentum leachates via water extracts

The vegetative and subterranean parts of *A. fatua* and *L. temulentum* were used prepare leachates through water extraction. For *A. fatua*, extracts, the most inhibitory plant parts were found to be the vegetative parts, with EC50 values of 5.64, 4.41, and 4.03 g/100 ml for germination, shoot length, and root length, respectively. A significant interaction effect of *A. fatua* plant parts × concentration was observed in *T. aestivum* germination ($F = 5.82$, $P \leq 0.025$), shoot length ($F = 7.20$, $P \leq 0.021$) and root length ($F = 11.04$, $P \leq 0.016$) respectively. For L. *temulentum* extracts, the most inhibitory plant parts were vegetative parts achieving EC_{50} values of 4.84, 4.31, and 3.52 (g.100 ml^{-1}) for germination, shoot length, and root length, respectively. A significant interaction effect of *L. temulentum* extracts plant parts × concentration was recorded ($F = 4.419$, $P \leq 0.03$) for germination, ($F = 9.75$, $P \leq 0.025$) shoot length and ($F = 13.34$, $P \leq 0.013$) root length of *T. aestivum* (Table 1).

3.2. Allelopathy of A. fatua and L. temulentum root exudates on T. aestivum seeds germination and seedling biomass

Root exudates of *A. fatua* yielded EC_{50} values for their extracts of 999.07 μg/ml for shoot length, 642.81 μg/ml for root length, 835.0 μg/ml for germination, and 655.9 μg/ml for seedling biomass fresh weight, respectively. Similarly, *L. temulentum* root exudates had EC_{50} values of 978.84 μg/ml for shoot length, 651.66 μg/ml for root length, 803.75 μg/ml for germination, and 625.73 μg/ml for seedling biomass fresh weight, respectively. The phytotoxicity of these root exudates significantly increased with higher concentrations for root length ($F = 360.3$, $p \leq 0.00$), shoot length, ($df = 6$, $F = 172.6$, $p \leq 0.00$) germination ($df = 6$, $F = 58.77$, $p \leq 0.00$) and seedling biomass ($df = 6$, $F = 390.68$, $p \leq 0.00$) in *T. aestivum* (Fig. 1).

3.3. Impact of decayed residues from A. fatua and L. temulentum on T. aestivum germination and growth

The data presented in Fig. 2 illustrates the impact of decomposed residues on the vigor index of *T. aestivum,* showing a gradual decline until reaching maximum phytotoxicity at 14 days from both *A. fatua* and *L. temulentum* weeds. Subsequently, the *T. aestivum* vigor growth began to rise again, continuing to increase until the completion of the decay process. Whereas, a gradual increase in EC values until reaching maximum at 35 days for both *A. fatua* and *L. temulentum* weeds and slightly stable during 42 and 49 days and began to decrease at the end of the experiment.

The effects of *A. fatua* and *L. temulentum* species, concentration, and time on *T. aestivum* germination and seedling development are shown in Table 2. Whereas, there is a significant effect of plant species on *T. aestivum* germination ($df = 1$, $F = 3.92$, $p \leq 0.049$). The effect of decayed time was significant on germination ($df = 10$, $F = 7.43$, $p \leq 0.000$), shoot length ($df = 10$, $F = 13.03$, $p \leq 0.00$), and root length ($df = 10$, $F = 8.55$, $p \leq 0.00$). The effect of concentration was significant on germination ($df = 3$, $F = 44.55$, $p \leq 0.000$) and shoot length ($df = 3$, $F = 97.23$, $p \leq 0.00$), and root length ($df = 3$, $F = 172.64$, $p \leq 0.00$) respectively. The interaction effect of time and plant species was found to be significant in root length ($df = 10$, $F = 2.51$, $p \leq 0.007$). Also, there is a significant interaction effect from time × concentrations for root length ($df = 30$, $F = 2.83$, $p \leq 0.00$), and germination ($df = 30$, $F = 2.83$, $p \leq 0.000$) respectively. The effect of times × plant species × concentration was significant on *T. aestivum* root length by ($df = 30$, $F = 2.77$, $p \leq 0.000$).

The effects of plant species, concentration, and time on soil macro-elements development were shown by the data in Table 2. The effect of plant species was significant for soil nitrogen ($df = 1$, $F = 71.42$, $p \leq 0.000$), phosphorus ($df = 1$, $F = 8.6$, $p \leq 0.004$), and potassium ($df = 1$, $F = 127.40$, $p \leq 0.00$) respectively. The effect of decayed time was significant for soil nitrogen ($df = 10$, $F = 33.80$, $p \leq 0.000$), phosphorus ($df = 10$, $F = 34.18$, $p \leq 0.00$) and potassium ($df = 10$, $F = 29.64$, $p \leq 0.00$) respectively. The effect of decayed plant concentration was significant for soil nitrogen ($df = 3$, $F = 169.53$, $p \leq 0.000$) and phosphorus ($df = 3$, $F = 101.94$, $p \leq 0.00$), and potassium ($df = 3$, $F = 236.79$, $p < 0.00$) respectively. The interaction effect of decayed time × plant species was significant for soil

Table 1
Allelopathic abilities of *A. fatua* and *L. temulentum* water extracts EC_{50} (g dry wt.100 ml^{-1} water) on *T. aestivum* crop.

		A. fatua		*L. temulentum*	
	Parameters	Vegetative	Subterranean	Vegetative	subterranean
	Germination	5.64 ± 0.78	6.61 ± 0.9	4.84 ± 0.74	5.26 ± 0.17
	Shoot length	4.41 ± 0.87	5.65 ± 0.53	4.31 ± 0.87	4.57 ± 0.90
	Root length	4.03 ± 0.31	4.99 ± 1.22	3.52 ± 0.30	3.71 ± 0.17
		F (p value)		F (p value)	
Plant parts	Germination	9.770 (0.000)		4. 58 (0.041)	
	shoot length	4.232 (0.049)		23.086 (0.000)	
	Root length	5.231 (0.030)		86.764 (0.000)	
Concentration	Germination	20.038 (0.000)		142.671 (0.000)	
	shoot length	30.511 (0.000)		224.182 (0.000)	
	Root length	80.675 (0.000)		265.868 (0.000)	
Plant parts × Concentration	Germination	5.825 (0.025)		4.419 (0.030)	
	shoot length	7.209 (0.021)		9.754 (0.025)	
	Root length	11.049 (0.016)		13.347 (0.013)	

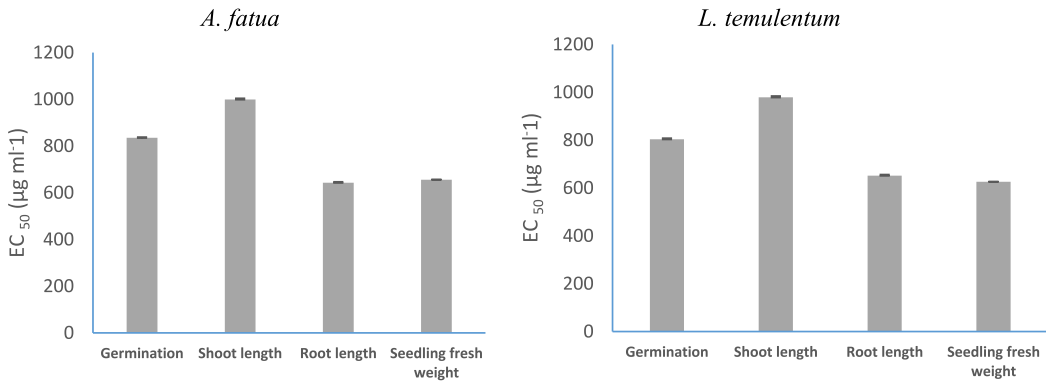

Fig 1. Allelopathic abilities of *A. fatua* and *L. temulentum* root exudates EC_{50} (µg ml^{-1}) on *T. aestivum* crop.

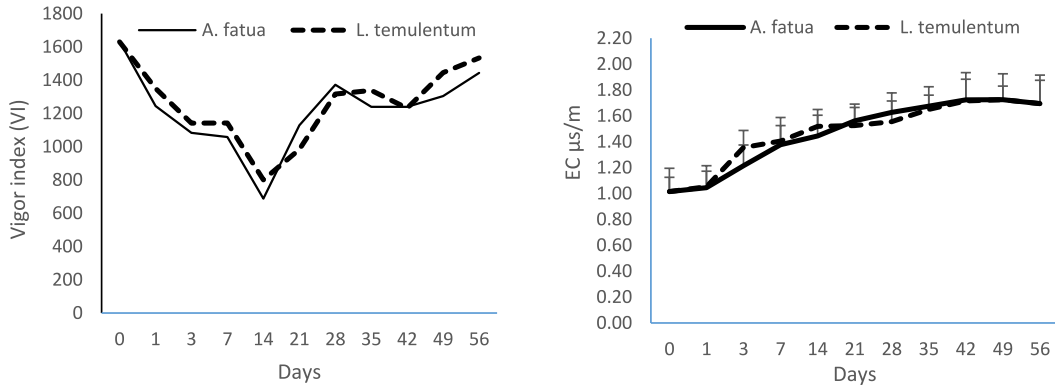

Fig 2. Effect of residues at 5 % concentration in *T. aestivuoam* vigor index and EC values during the decaying process.

nitrogen (df = 10, F = 14.95, $p \leq 0.00$), phosphorus (df = 10, F = 32.09, $p \leq 0.00$), and potassium (df = 10, F = 20.03, $p \leq 0.00$) respectively.The interaction effect of decayed time x concentrations was significant for soil nitrogen (df = 30, F = 23.03, $p \leq 0.00$), phosphorus (df = 30, F = 16.47, $p \leq 0.00$), and potassium (df = 30, F = 6.08, $p \leq 0.000$) respectively. Finally, there is a significant interaction effect of time × plant species × concentration on soil nitrogen (df = 30, F = 6.08, $p \leq 0.00$), phosphorus (df = 30, F = 13.70, $p \leq 0.00$), and potassium (df = 30, F = 5.95, $p < 0.000$) respectively.

Data in Table 2 indicated the effect of plant species, concentration and time on soil parameters. The effect of plant species was significant (df = 1, F = 12.01, $p \leq 0.001$) on soil pH. The effect of decayed time was significant on soil pH (df = 10, F = 344.61, $p \leq 0.000$), and EC (df = 10, F = 75.11, $p \leq 0.00$) respectively. The effect of plant concentration was significant on soil pH (df = 3, F = 41.50, $p \leq 0.000$) and EC (df = 3, F = 129.55, $p \leq 0.00$) respectively. The interaction effect of time × plant species was significant on soil pH (df = 10, F = 12.96, $p \leq 0.00$), and EC (df = 10, F = 26.60, $p \leq 0.00$) respectively. The interaction effect of time × concentrations was significant on soil pH (df = 30, F = 7.93, $p \leq 0.00$), and in EC (df = 30, F = 6.78, $p \leq 0.000$) respectively. Finally, there is a significant interaction effect of times × plant species × concentration on soil pH (df = 30, F = 3.30, $p \leq 0.00$), in EC (df = 30, F = 6.76, $p \leq 0.000$) respectively.

3.4. Decompostion of A. fatua and L. temulentum vegetative parts in T. aestivum and soil parameters

During the decomposition of *A. fatua* and *L. temulentum*, the changes in dynamics were detected from their effect on *T. aestivum* growth and chemical parameters. These bioassays revealed a higher decay rate of weed residue in water under aerobic conditions, compared to anaerobic conditions as indicated by the lowest EC_{50} values in affected *T. aestivum* seedling parameters. The allelopathic activity started at a low level at 0.5 days and gradually increased from 1 to 5 days under aerobic conditions, extending until 10 days under anaerobic conditions. Subsequently, a gradual decrease in phytotoxicity starts from 10 days (aerobic) and 15 days (anaerobic) respectively condition towards the end of the 30-day decomposition process. The phytotoxicity of *A. fatua* and *L. temulentum* was higher in anaerobic than aerobic conditions. Notably, root length in *T. aestivum* exhibited greater susceptibility to allelochemicals during the decomposition process. Interestingly, there were only slight differences in phytotoxicity between the two weeds, with *L. temulentum* exhibiting higher phytotoxicity than *A. fatua* during the decomposition process under both anaerobic and aerobic

Table 2
Variance analysis of *A. fatua* and *L. temulentum* vegetative decayed residues under greenhouse conditions and their effect on T. *aestivum* and soil parameters.

	Parameters		Plant species	Time of decayed	Conc.	Times* plant species	Plant species* Concentration	Times * Concentration	Times * Plant species * Concentration
T. *aestivum*	Germination	df	1	10	3	10	3	30	30
		F	3.92	7.43	44.55	1.19	5.76	2.83	0.92
		Sig.	0.049	0.000	0.000	0.295	0.020	0.000	0.585
	Shoot length (cm)	df	1	10	3	10	3	3	30
		F	2.6	13.03	97.23	0.88	16.4	1.55	1.74
		Sig.	0.1080	0.0000	0.0000	0.5520	0.0030	0.2010	0.0110
	Root length (cm)	df	1	10	3	10	3	30	30
		F	0.72	8.55	172.64	2.51	6.76	2.83	2.77
		Sig.	0.396	0.000	0.000	0.007	0.001	0.000	0.000
Soil macro-elements	Nitrogen	df	1	10	3	10	3	30	30
		F	71.42	33.8	169.53	14.95	13.27	23.03	6.08
		Sig.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Phosphorus	df	1	10	3	10	3	30	30
		F	8.6	34.18	101.94	32.09	33.51	16.47	13.7
		Sig.	0.004	0.000	0.000	0.000	0.000	0.000	0.000
	Potassium	df	1	10	3	10	3	30	30
		F	127.4	29.64	236.79	20.03	18.41	6.08	5.95
		Sig.	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Soil parameters	pH	df	1	10	3	10	3	30	30
		F	12.01	344.61	41.5	12.96	5.6	7.93	3.3
		Sig.	0.001	0.000	0.000	0.000	0.001	0.000	0.000
	EC	df	1	10	3	10	3	30	30
		F	0.97	75.11	129.55	8.79	26.6	6.78	6.76
		Sig.	0.324	0.000	0.000	0.000	0.000	0.000	0.000

conditions (Fig. 3).

Data presented in Table 3 demonstrate the effects of plant species, concentration, and time on *T. aestivum* germination and seedling development. Specifically, plant species had a significant impact on *T. aestivum* germination (df = 1, F = 14.91, p ≤ 0.003), shoot length (df = 1, F = 13.79, p ≤ 0.01), and root length (df = 1, F = 10.35, p ≤ 0.02). The effect of decomposed time was significant on germination (df = 7, F = 26.92, $p < 0.000$), shoot length (df = 7, F = 24.46, $p \leq 0.00$), and root length (df = 7, F = 20.43, $p \leq 0.00$) respectively. The effect of concentration was significant on germination (df = 3, F = 120.59, $p \leq 0.000$) and shoot length (df = 3, F = 152.87, $p \leq 0.00$), and root length (df = 3, F = 157.13, $p \leq 0.00$). The interaction effect of time and condition was significant in germination (df = 7, F = 12.0, $p \leq 0.007$) and shoot length (df = 7, F = 7.18, $p \leq 0.00$), and root length (df = 7, F = 8.88, $p \leq 0.01$) respectively.The interaction effect of time × concentration was significant on germination (df = 21, F = 2.7, $p \leq 0.02$), shoot length (df = 21, F = 2.95, $p \leq 0.04$), and root length (df = 21, F = 4.67, $p \leq 0.00$) respectively. The interaction effect of plant species × condition × concentration was significant on *T. aestivum* root length by (df = 3, F = 3.34, $p < 0.000$). While, the interaction of time × condition × plant species was significant on germination (df = 21, F = 2.61, $p \leq 0.01$), and root length (df = 21, F = 3.34, $p < 0.00$) respectively.

Data in Table 3 highlights the role of pH and electrical conductivity (EC) during the decomposition of weed residue in water under both aerobic and anaerobic conditions. The effect of decomposing time showed a significant impact on water pH (df = 7, F = 24.58, $p \leq 0.000$) and EC (df = 7, F = 89.03, $p \leq 0.00$) respectively. The condition also demonstrated a significant influence on water pH (df = 1, F = 149.54, $p \leq 0.000$) and EC (df = 1, F = 9.21, $p \leq 0.03$) respectively. Furthermore, the interaction effect of time and condition was significant on water pH (df = 21, F = 9.13, $p \leq 0.000$). Notably, the interaction effect of plant species, condition, and time was significant on both pH (df = 3, F = 3.54, $p \leq 0.032$) and EC (df = 3, F = 4.76, $p \leq 0.025$) of soil respectively.

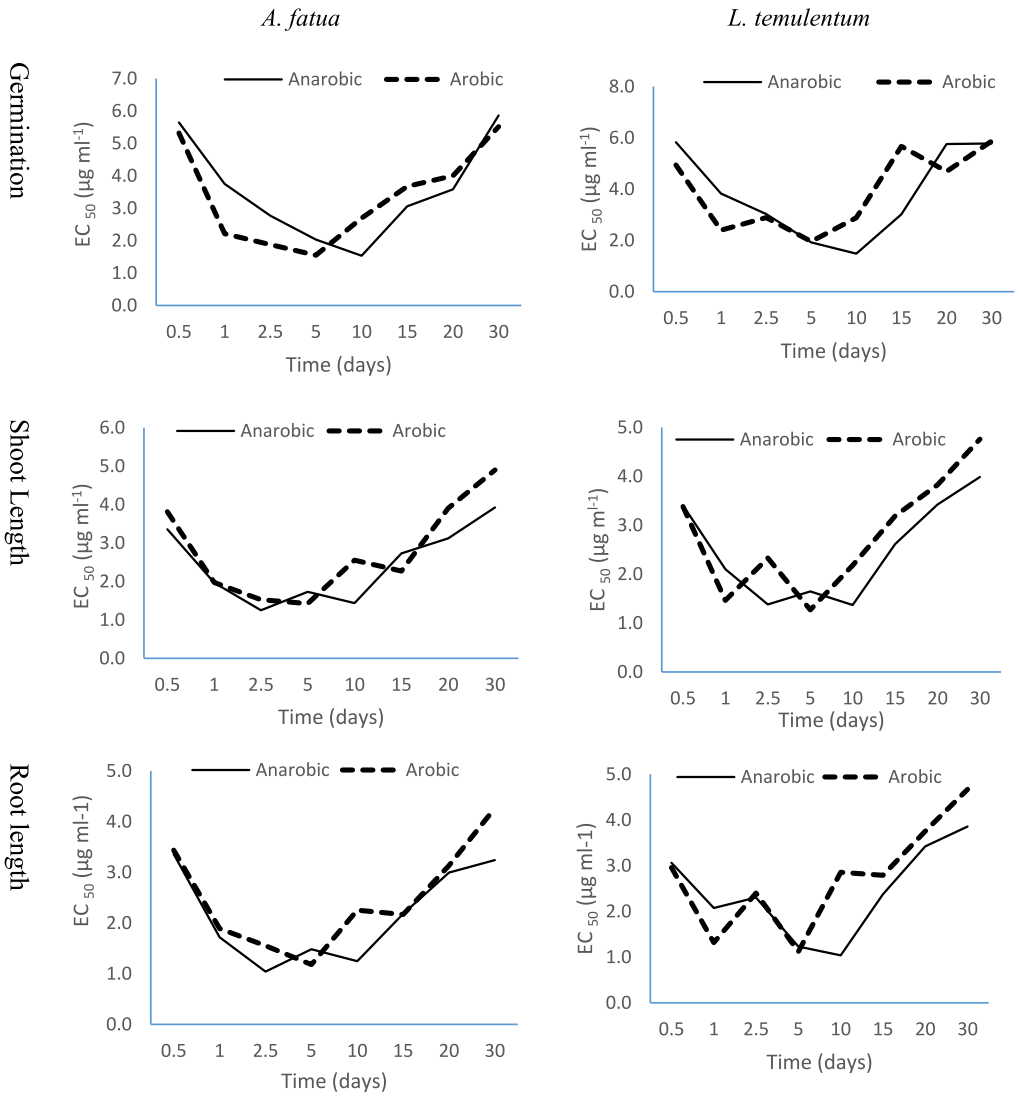

Fig 3. Decomposition of *A. fatua* and *L. temulentum* on *T. aestivum* germination and seedling development.

Table 3
Variance analysis of *A. fatua* and *L. temulentum* decompositions under aerobic and anaerobic conditions on *T. aestivum* and water parameters.

	Parameters	Effects	Plant species	Times of decomposition	Condition of decomposition	Concentration	Times* Condition	Times * Concentration	Plant species *Condition Concentration	Times * Condition * Plant species
T. aestivum	Germination	df	1	7	1	3	7	21	3	21
		F	14.91	26.92	2.68	120.59	12	2.7	3.36	2.61
		Sig.	0.03	0	0.39	0	0	0.02	0.26	0.01
	Shoot length	df	1	7	1	3	7	21	3	21
	(cm)	F	13.79	24.46	2.68	152.87	7.18	2.95	3.6	3.09
		Sig.	0.01	0	0.1	0	0	0.04	0.06	0
	Root length	df	1	7	1	3	7	21	3	21
	(cm)	F	10.35	20.43	1.07	157.13	8.88	4.67	5.06	3.34
		Sig.	0.02	0	0.52	0	0.01	0	0	0
water parameters	pH	df	1	7	1	21	21	1	3	21
		F	2.01	24.58	149.54	9.13	2.01	1.07	3.54	0.779
		Sig.	0.13	0	0	0	0.71	0.3	0.032	0.92
	EC	df	1	7	1	21	21	1	3	21
		F	0.44	89.03	9.21	0.61	0.75	0.61	4.76	1.924
		Sig.	0.341	0	0.003	0.748	0.432	0.622	0.025	0.536

Table 4
Quantitative determination of phenolic content in decomposition solutions using LC-ES/MS (μg ml^{-1}).

			A. fatua		L. temulentum	
	RT	Phenolic compounds	Aerobic	Anaerobic	Aerobic	Anaerobic
1	8.7	P-Hydroxybenzoic acid	1.57 ± 0.02	2.78 ± 0.02	2.61 ± 0.11	3.54 ± 0.20
2	14.2	Hydroxicinnamic acid	5.26 ± 0.15	9.11 ± 0.01	5.25 ± 0.21	7.63 ± 0.31
3	15.0	Vanillic acid,	3.34 ± 0.01	5.82 ± 0.17	3.46 ± 0.13	5.70 ± 0.28
4	16.6	Syringic acid	1.28 ± 0.02	2.28 ± 0.03	1.61 ± 0.08	2.54 ± 0.047
5	17.5	Gallic acid	3.92 ± 0.16	6.81 ± 0.2	3.78 ± 0.07	6.25 ± 0.24
6	18.7	Coumaric acid	5.04 ± 0.34	8.73 ± 0.30	9.21 ± 0.35	16.99 ± 0.65
7	20.3	Ferulic acid	5.26 ± 0.23	10.53 ± 0.40	3.18 ± 0.41	5.23 ± 0.36
8	23.6	Caffeic acid	0.27 ± 0.001	0.55 ± 0.01	0.44 ± 0.02	0.67 ± 0.04
9	25.3	Citric acid	7.71 ± 0.13	13.31 ± 0.32	5.90 ± 0.11	9.03 ± 0.74
10	26.9	Salicylic acid	0.61 ± 0.04	1.12 ± 0.05	1.15 ± 0.04	1.46 ± 0.01
		F (p value)	4.85(0.02)	39.54(0.00)	6.73(0.01)	28.43(0.00)

± = standard deviations.

LC-ES/MS analysis revealed the presence of ten phenolic compounds (Table 4) in decomposed water solutions. Whereas citric acid showed substantial quantities of 7.71 and 13.31 μg m^{-1}, in *A. fatua* under aerobic and anaerobic conditions and coumaric acid in *L. temulentum* exhibited concentrations of 9.21 and 16.99 μg m^{-1} in aerobic and anaerobic conditions respectively. However, caffeic acid was measured in lower amounts by 0.27 and 0.55 μg m^{-1} in *A. fatua* and 0.44 and 0.67 μg m^{-1} in *L. temulentum* under aerobic and anaerobic conditions, respectively.

4. Discussion

The study explored the mechanisms by which grassy weeds, particularly *A. fatua* and *L. temulentum*, exert a significant negative impact on *T. aestivum* crops. These weeds are the most prevalent and pose a continuous risk of yield loss due to their allelopathic activities. Specifically, *L. temulentum* (Darnel ryegrass) and *A. fatua* (wild oats) are known to cause reductions in both grain yield and quality [45]. Therefore, their allelopathic pathways of leaching, root exudates, decayed residue in soil, and decomposition in water were investigated under laboratory and greenhouse conditions. Allelochemicals have four pathways to get into the environment [46]. Outstandingly in leachate effectiveness, the aboveground parts of *A. fatua* and *L. temulentum* weeds were identified as the primary source of allelochemicals, demonstrating higher activities compared to subterranean parts on *T. aestivum* germination and seedling development. These systematic activities were influenced by plant parts and concentrations whereas, root length is the most sensitive trait in *T. aestivum.* As indicated by EC_{50}, *L. temulentum* has a stronger inhibitory than *A. fatua* in *T. aestivum* traits. Phytotoxicity can be attributed to the characteristics of the materials [47]. Most allelochemicals are reported to be water soluble and hamper their germination and subsequent seedling growth [48]. Root growth is a more sensitive indicator of phytotoxicity than hypocotyl length [49]. The direct contact between the root and phytotoxic compounds present in the extract might inhibit cell division in the growing root tip [50]. Aerial parts of wild oats (*A. fatua*) had strong allelopathic potential through syringic acid, tricin, acacetin, syringoside, and diosmetin and could have different degrees of influence on surrounding plants [51]. While wheat varieties differ in susceptibility to *A. fatua* allelopathic effect [52].

Concerning root exudates of *A. fatua* and *L. temulentum*, their extract showed substantial phytotoxic potentials in *T. aestivum* germination and seedling growth due to the accumulation of allelopathic compounds. Although the effects of the two tested species are similar, this may be due to both weeds belonging to the same family. The root length, which was similar to the seedling biomass of *T. aestivum* crops, exhibited the most susceptible traits. Root exudates are the largest source of allelochemical inputs into the soil environment [53]. Exudation can be induced by biotic and abiotic factors [54].The allelopathic potential of wild oats (*Avena fatua*) via root exudates through toxic substances that suppress the growth of spring wheat in the absence of plant competition [55]. Wild oat roots exude allelopathic compounds of syringic acid, vanillin, 4-hydroxybenzoic acid, syringaldehyde, ferulic acid, p-cumaric acid and vanillic acid, and the levels of these phenolics in the rhizosphere soil vary according to plant maturity [56]**.** Phenolic compounds of p-coumaric, ferulic, 4-hydroxybenzoic, vanillic and syringic acids, vanillin and syringaldehyde were identified in the rhizosphere soil of the wild oat [57,58]. The root exudates vary significantly from plant species both in quantity and quality [59]. Root exudates play an important role in soil physicochemical properties, plant nutrient uptake, transformation and utilization, allelopathy, and environmental stress relief [60].

The decayed residues of *A. fatua* and *L. temulentum* were peaked at 14 days and their phytotoxicity affected significantly by soil, plant types, concentration and time and interaction between plant species × concentration × decayed times in *T. aestivum* root length. Also, the decayed residue has a positive effect on soil nitrogen, phosphorus and potassium which are significantly affected by (time), (concentration), (time × plant species) and (time × concentrations) and (times × plant species × concentration) respectively. The decayed residue concentration and time have a significant effect on soil pH and EC and there was a significant interaction between time x plant species, time × concentrations and times × plant species × concentration. The decayed residues showed positive correlations for *A. fatua* and *L. temulentum* as follows: 0.156 and 0.043 with decay time and crop vigor index, 0.738 and 0.46 with soil pH and crop vigor index, 0.125 and 0.422 with soil EC and crop germination, 0.977 and 0.109 with soil phosphorus and crop vigor index, and 0.305 and 0.398 with soil potassium and crop vigor index, respectively. Plants decomposition residues resulted in phytotoxicity in soil [61,

62]. Soil incorporation with crop residues resulted in an overall decline in the density and vigor of the weed community [48]. Allelochemicals released by decaying plant residues can regulate the soil microbial community and chemical and physical properties of the soil [63]. However, soil plays a role in the biological environment and has the potential to detoxify or toxified allelochemicals through microbial action [64]. The presence of phenolic acids in the soil, including syringic, vanillic, gallic, caffeic, p-coumaric, ferulic, p-hydroxybenzoic, and protocatechuic acids, was observed as a result of sorghum residue decomposition [65]. Phenolic acids are a diverse class of compounds that can act as agents in plant defense [66]. Plant phenolic acids (PAs) are extremely important in the soil C cycle, soil aggregation and measurement of soil ester-linked PA composition can provide an index of plant-derived C in soil [67]. Nevertheless, these components do not long stay because of decomposing and leaching from soil depth [68].

Concerning the decomposition of *A. fatua* and *L. temulentum* residue in water, their relative phytotoxicity peaked between 5 and 10 days under aerobic conditions and anaerobic conditions respectively. Whereas the higher decomposition process rate in water was observed under anaerobic than aerobic conditions, similarly the inhibitory on *T. aestivum* was higher under anaerobic than aerobic conditions respectively. The decomposition of *A. fatua* and *L. temulentum* caused a significant effect on *T. aestivum* germination, shoot length and root length. There was an interaction effect between time × concentrations, plant species × condition × concentration in *T. aestivum* germination and root length. These capabilities depend on plant parts, concentrations of aqueous extracts and conditions, times and concentrations during the decomposition process [69]. Changes over time in both the composition and quantity of allelochemicals can either increase or decrease the phytotoxicity [70]. While, the relative phytotoxicity of the invasive species decayed residues with a positive correlation with concentrations [71].Allelochemicals interact with the organic and inorganic soil phases, as well as with soilmicroorganisms. These interactions fix allelochemicals bioavailability and phytotoxic level [72]. Residue-mediated inhibition can occur only if the susceptibility period of the receptor plant coincides with the inhibitory allelopathic potential peak period [73]. Timing of phytotoxicity is variable, with some reporting it is greatest at the early [74,75] or increasing toxicity with increasing time after incorporation [76]. Chemical analysis revealed citric acid showing substantially higher quantities followed by Hydroxycinnamic acid in *A. fatua* under aerobic and anaerobic conditions. In the decomposed solution of *L. temulentum,* coumaric acid exhibited concentrations followed by citric acid in aerobic and anaerobic conditions respectively. Finally, there is a slight difference in the phytotoxicity of decomposed *A. fatua* compared to *L. temulentum*. It is important to identify the allelopathic compounds in soil or water substrates [77]. Water soluble phenolics, dissolved organic carbon and soil nutrients of litter influence phytotoxicity dynamics [78].

The study covered the allelopathic potentials of *A. fatua* and *L. temulentum* that pose a negative impact on *T. aestivum* germination and seedling growth especially from the aboveground parts of leachates by water extracts. The observed phytotoxic potential of root exudates from both *A. fatua* and *L. temulentum* are similar which may be attributed to their shared family. The positive correlations of decayed residues between crop growth and soil parameters emphasized the potential influence of allelopathic activities on soil conditions and crop performance, emphasizing the connected nature of weed residues with soil health, nutrient availability, and crop vigor. The differential phytotoxicity observed under aerobic and anaerobic conditions during the decomposition highlights the importance of environmental conditions in mediating allelopathic effects. These findings highlighted the importance of implementing sustainable agricultural practices to take into account the allelopathic potential of weed residue quantity and types to optimize both crop productivity and soil health. Allelopathic plants should be included in a crop rotation or as part of an intercropping system that may provide a nonherbicide mechanism for weed control [79]. Incorporating allelopathy as a tool in an integrated weed management plan could meaningfully bring down herbicide application [80]. Plant allelopathy could be applied as an ecological approach to integrated weed control in sustainable crop production [81]. Direct application of some leguminous plants to farmlands could suppress weed without using herbicides and also act as nitrogen fertilizer [82]. Therefore, allelopathy may be useful to minimize serious problems in the present agricultural production such as environmental pollution, unsafe products, human health concerns, depletion of crop diversity, soil sickness and reduction of crop productivity [83].

5. Conclusion

Based on these findings, it can be inferred that *A. fatua* and *L. temulentum*, weeds can release allelochemicals through various means, including leachates, root exudates, and decayed residues in soil, as well as the decomposition of residues in water. The concentration and plant species play a significant role in allelopathic potentials in most cases in *T. aestivum* germination and seedling growth. Plant species, concentration and processing time influence the dynamics either by increasing or decreasing the allelopathic potentials. Nonetheless, comparing the strength of allelopathic potentials across leachates, root exudates, and decayed pathways faces many challenges due to using different concentration units and the nature of bioassay tests if we use the same test plant. Despite this difficulty, the study offers considerations for soil and crop health in agricultural practices and highlights a notable suppressive and differentiated allelopathic effect of A. *fatua* and *L. temulentum* on *T. aestivum* germination, growth traits, and soil parameters. Thus, it is crucial to take into account plant residues particularly weeds to promote sustainable crop production and prevent changing soil properties. Future research should aim to, explore a wider range of weed species, and examine the long-term effects of allelopathic compounds on soil health and crop productivity.

Data availability statement

The datasets used during the current study are available from the corresponding author upon reasonable request.

Funding

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through a large group Research Project under grant number (RGP2/439/45).

CRediT authorship contribution statement

Rahmah Al-Qthanin: Methodology, Formal analysis, Conceptualization. **Asmaa M. Radwan:** Resources, Methodology, Investigation. **AbdElRaheim M. Donia:** Writing – original draft, Resources, Methodology, Formal analysis, Conceptualization. **Khaled A. Abou-zied:** Resources, Formal analysis. **Mohamed A. Balah:** Writing – review & editing, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors express their appreciation to the plant protection staff at the Desert Research Center, Cairo, Egypt.

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