

## The allelopathic activity of *Festuca arundinacea* Shreb. rhizospheric soil is exacerbated under drought stress

Item Type	Journal article
Authors	Motalebnejad, Masuod;Karimmojeni, Hassan;Baldwin, Timothy;Majidi, Mohammad Mahdi
Citation	Motalebnejad, M., Karimmojeni, H., Baldwin, T.C. et al. The Allelopathic Activity of <i>Festuca arundinacea</i> Shreb. Rhizospheric Soil Is Exacerbated Under Drought Stress. <i>Journal of Soil Science and Plant Nutrition</i> , 23, 5495–5512 (2023). <a href="https://doi.org/10.1007/s42729-023-01417-x">https://doi.org/10.1007/s42729-023-01417-x</a>
DOI	<a href="https://doi.org/10.1007/s42729-023-01417-x">10.1007/s42729-023-01417-x</a>
Publisher	Springer
Journal	<i>Journal of Soil Science and Plant Nutrition</i>
Download date	2026-05-19 04:06:01
Link to Item	<a href="http://hdl.handle.net/2436/625283">http://hdl.handle.net/2436/625283</a>

# Journal of Soil Science and Plant Nutrition

## The allelopathic activity of *Festuca arundinacea* Shreb. rhizospheric soil is exacerbated under drought stress --Manuscript Draft--

<b>Manuscript Number:</b>	JSSP-D-23-00486R2
<b>Full Title:</b>	The allelopathic activity of <i>Festuca arundinacea</i> Shreb. rhizospheric soil is exacerbated under drought stress
<b>Article Type:</b>	Original Paper
<b>Funding Information:</b>	
<b>Abstract:</b>	<p>The aims of the current study were to (i) investigate the allelopathic activity of 16 <i>F. arundinacea</i> genotypes and identify the genotypes with the greatest inhibitory effect (ii) to evaluate the allelopathic activity of the rhizospheric soil of <i>F. arundinacea</i>, under conditions of normal irrigation and severe drought stress, as well as investigating the allelopathic activity of <i>F. arundinacea</i> shoot residues of in the soil (iii) the identification of the allelopathic phenolic compounds present in the soil, directly caused by the activity of the roots or released from the shoots residues of <i>F. arundinacea</i>. The results obtained showed that the genotype, extract concentration and the application of drought stress all significantly reduced the germination of <i>L. sativa</i>. As the concentration of the extract increased, a corresponding decrease was observed in the seed germination and growth of the <i>L. sativa</i> seedlings. The highest concentration of the extract (100%) caused the greatest decrease in germination percentage (85%), shootlet length (72.9%), rootlet length (77.04%) and seedling dry weight (63.7%). Drought stress was shown to produce a marked increase in the allelopathic activity of the extracts. The extract obtained from the 23M genotype was shown to exhibit the most inhibitory effect upon the growth of <i>L. sativa</i>. HPLC analysis, showed the presence of phenolic compounds in both the rhizosphere and <i>F. arundinacea</i> residues. The identified compounds included p-coumaric acid, apigenin acid, ferulic acid, 4-hydroxybenzoic acid, gallic acid, syringic acid, caffeic acid, vanillic acid, and chlorogenic acid. The results show the presence of more phenolic compounds in the <i>F. arundinacea</i> plant residues compared to the rhizosphere. These data demonstrate the considerable diversity in allelopathic activity of the <i>F. arundinacea</i> genotypes tested, and that it may be feasible to select and breed this species for the purpose of allelopathic weed management.</p>
<b>Corresponding Author:</b>	Hassan Karimmojeni Isfahan University of Technology IRAN, ISLAMIC REPUBLIC OF
<b>Corresponding Author Secondary Information:</b>	
<b>Corresponding Author's Institution:</b>	Isfahan University of Technology
<b>Corresponding Author's Secondary Institution:</b>	
<b>First Author:</b>	Masuod Motalebnejad
<b>First Author Secondary Information:</b>	
<b>Order of Authors:</b>	Masuod Motalebnejad Hassan Karimmojeni Timothy C. Baldwin Mohammad Mahdi Majidi
<b>Order of Authors Secondary Information:</b>	
<b>Author Comments:</b>	
<b>Response to Reviewers:</b>	Dear Editor, We are grateful for your thorough review and comments upon our manuscript entitled

"The allelopathic activity of *Festuca arundinacea* Shreb. rhizospheric soil is exacerbated under drought stress" (manuscript number: JSSP-D-23-00486).

We have followed your suggestions closely. As you will see, we have attempted to address all the remarks and suggestions indicated. We hope that this revised version of our manuscript will now be acceptable for publication in your Journal.

A list of our responses to each of your comments is provided below, our responses are highlighted in yellow.

Responses Editor's comments:

1-Please provide an adequate response to each comment and submit a list of responses to the comments indicating specifically in which lines the improvements were done, or explaining why they were not considered. Furthermore, I inform you that due to JSSPN policies, we do not accept changes in the authorship of the manuscript once the peer review process has begun.

Answer: This has been done

2-Please change the format of units. For example, replace mg/g with mg g<sup>-1</sup>

Answer: The format of all the units has been amended, as suggested.

3-Scientific names must be written in italics, even in references. Please correct it.

Answer: The scientific names throughout the manuscript have been amended (including the references) as requested.

The legends of tables and Figures are not all self-explainable; the content does not help the quick and easy interpretation of the results. Remember that tables and figures must be self-explanatory. That is, statistics and abbreviations used must be clearly explained. All tables and figures must present standard error and suitable statistical analysis when appropriate.

Answer:

Tables:

- The legends of all tables and figures have been reviewed and amended.
- The title and legends of tables 2, 3, 4, 5, 6, 7, 8, 9, and 10 have been amended.
- Also, explanations of legends were added under tables 8, 9, and 10 for better self-explanation.
- Standard errors were added to all tables and figures.

Figure:

- The legends and abbreviations in figures 1 and 2 were reviewed and modified.
- In Figure 1, explanations of abbreviations were added to the figure.
- In Figure 2, the description of the Figure of modification and figure self-explanatory.

4-The conclusions section should illustrate the mechanistic links of findings obtained under applied treatments. The authors should avoid repeating what has already presented in results and discussion. Please, avoid using abbreviations and acronyms in the conclusions section. Remember that the conclusions must be self-explanatory

Answer: This has been done

[Click here to view linked References](#)

1 The allelopathic activity of *Festuca arundinacea* Shreb. rhizospheric soil is exacerbated under  
2 drought stress

3 Masuod Motalebnejad <sup>a</sup>, Hassan Karimmojeni<sup>a\*</sup>, Timothy C. Baldwin<sup>b</sup>, Mohammad Mahdi Majidi<sup>a</sup>

4  
5 <sup>a</sup> Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of  
6 Technology, Isfahan 84156-83111, Iran

7  
8 <sup>b</sup> Faculty of Science and Engineering, University of Wolverhampton, Wolverhampton, WV1 1LY,  
9 United Kingdom

10

11

12 \*Corresponding author: Hassan Karimmojeni, Department of Agronomy and Plant Breeding,

13 College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran Email:

14 **[kmojeni@iut.ac.ir](mailto:kmojeni@iut.ac.ir)**

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34 **Abstract**

35 The aims of the current study were to (i) investigate the allelopathic activity of aqueous extracts  
36 obtained from the shoots of 16 *F.arundinacea* genotypes and to identify those with the greatest  
37 inhibitory effect (ii) to evaluate the allelopathic activity of the rhizospheric soil of *F.arundinacea*,  
38 under conditions of normal irrigation and severe drought stress, as well as investigating the  
39 allelopathic activity of *F.arundinacea* shoot residues in the soil (iii) the identification of the  
40 allelopathic phenolic compounds present in the soil, directly caused by the activity of the roots or  
41 released from the shoots residues of *F.arundinacea*. The results obtained showed that the  
42 genotype, extract concentration and the application of drought stress all significantly reduced the  
43 germination of *Lactuca sativa*. As the concentration of the extract increased, a corresponding  
44 decrease was observed in the seed germination and growth of the *L. sativa* seedlings. The highest  
45 concentration of the extract (100%) caused the greatest decrease in germination percentage (85%),  
46 shootlet length (72.9%), rootlet length (77.04%) and seedling dry weight (63.7%). Drought stress  
47 was shown to produce a marked increase in the allelopathic activity of the extracts. The extract  
48 obtained from the 23M genotype was shown to exhibit the most inhibitory effect upon the growth  
49 of *L. sativa*. HPLC analysis showed the presence of phenolic compounds in both the rhizosphere  
50 and *F.arundinacea* shoot residues. The identified compounds included p-coumaric acid, apigenin  
51 acid, ferulic acid, 4-hydroxybenzoic acid, gallic acid, syringic acid, caffeic acid, vanillic acid, and  
52 chlorogenic acid. The results show the presence of more phenolic compounds in the *F.arundinacea*  
53 plant residues compared to the rhizosphere. These data demonstrate the considerable diversity in  
54 allelopathic activity of the *F.arundinacea* genotypes tested, and that it may be feasible to select  
55 and breed this species for the purpose of allelopathic weed management.

56

57 **Keywords:** allelopathy, secondary metabolites, phenolic compounds, abiotic stress, plant residues

58

59 **1 Introduction**

60 Allelopathy refers to processes involving secondary metabolites produced by plants,  
61 microorganisms and fungi, that affect the growth and development of agricultural and biological  
62 systems, including both positive and negative effects (Rice 1984). Allelochemicals from plants,  
63 are released into the environment by leaching from roots, stems and leaves, or by the  
64 decomposition of plant material (Lovett and Ryuntyu 1992; Rice 1984; Rizvi and Rizvi 1992;

65 Scavo et al. 2018; Singh et al. 2021). In recent decades, this subject has become the focus of  
66 numerous studies in the field of sustainable agriculture (Callaway and Maron 2006; Jovanović et  
67 al. 2010; Khamare et al. 2022; Sahrir et al. 2022). Allelopathy affects plant communities and alters  
68 interspecies interactions, either through direct effects upon plant growth and development, or  
69 through indirect effects on soil fertility and beneficial soil microorganisms (Hierro and Callaway  
70 2021; Inderjit et al. 2011; Kaur et al. 2009; Wu et al. 2022). Some plants suppress the growth of  
71 neighbouring plants through physical, chemical, and biological inhibition, i.e., resource and light  
72 competition, weed life cycle disruption and, most importantly, the release of allelochemicals (Xiao  
73 et al. 2019). When allelochemicals are released into the soil, they affect the soil environment,  
74 causing simultaneous and continuous allelopathic activity, which subsequently affect the  
75 germination and seedling establishment of target plants (Gerhards and Schappert 2020; Xu et al.  
76 2019). When allelochemicals are released into the soil, they interact with the soil's organic and  
77 inorganic phases, as well as with soil associated microorganisms (Aldrich 1984; Inderjit and  
78 Weiner 2001; Scavo et al. 2019).

79 The degree of phytotoxicity is influenced by the chemical, physical and biological properties of  
80 the soil environment, which exert multiple effects upon the retention, transport and transformation  
81 processes of allelochemicals within the soil (Blum 2006; Cheng 1992; Dao 1987). As is the case  
82 with herbicides, allelochemicals are continuously removed and/or immobilised from soil solutions,  
83 by leaching, microbial degradation, adsorption to soil particles, and plant uptake (Cheng 1994;  
84 Inderjit et al. 2001; Weidenhamer 1996). However, the behaviour of allelochemicals in the soil is  
85 more complex than herbicides, as the former are continuously released by the donor plant with  
86 significant differences in relation to the target plant organs and developmental stage of the target  
87 plant species (Abu-Romman 2016; Aslam et al. 2016; Iqbal et al. 2002; Suksungworn et al. 2016).  
88 Allelopathic crops can be used to manage weeds in agro-ecosystems by including them in  
89 rotational sequences or intercropping in close proximity to a cash crop, cover cropping as living  
90 or dead mulches, incorporation of crop residues into the soil, or by using their allelochemicals as  
91 bioherbicides (Jabran et al. 2015; Khanh et al. 2005; Scavo and Mauromicale 2021). Allelopathy  
92 of cover plants and their residues, especially Poaceae species, has been demonstrated to be an  
93 effective means of biological weed management (Hammermeister 2016). Managing weed growth  
94 in this manner, is therefore a sustainable solution in agricultural systems, in order to reduce the  
95 chemical pollution caused by the application of herbicides (El-Metwally et al. 2022; Ojija et al.

96 2019; Xiao et al. 2017). Recent research has shown that *Festuca* species -as an important cover  
97 species- can be used in this manner to suppress weed growth in an agricultural setting (Lipinska et  
98 al. 2019).

99 *Festuca arundinacea* Shreb. (tall fescue) is a cool-season, perennial, C3 grass native to Europe,  
100 that is widely recognized as an important forage and cover crop (Ahmed and Escobar-Gutiérrez  
101 2022). This species belongs to the genus *Festuca*, family Poaceae (Festuceae), subfamily Pooidea  
102 Festucoideae and class Poaceae grass and is used to produce fodder, for soil conservation and the  
103 development of ornamental lawns (Carrow and Duncan 2003; Cougnon et al. 2014; Ge and Wang  
104 2015; Hand et al. 2012). Tall fescue is also known to exhibit allelopathic activity (Peters and Luu,  
105 1985; Buta and Spalding, 1989; De Bertoldi et al. 2012a). *F. arundinacea* root secretions as well  
106 as the compounds present in the shoots, have been shown to exhibit strong allelopathic properties  
107 (Hammermeister 2016).

108 *F. arundinacea* is known to tolerate both heat and drought stress better than the majority of cold-  
109 season forage grass species (Amini et al. 2011; Kosmala et al. 2012; Taleb et al. 2023). Plants  
110 under stress also exhibit protective systems to overcome oxidative damage, by the synthesis of  
111 secondary metabolites such as phenolics and flavonoids (Blokhina 2003; Makaure et al. 2022;  
112 Misra et al. 2023; Romani et al. 2002; Yang et al. 2018). The accumulation of phenolic compounds  
113 is crucial for plants to physiologically adapt to the harmful effects of drought stress. The main  
114 factor which seems to influence differences in the total phenolic content of plants grown under  
115 drought stress conditions, is related to the differences between genotypes (Wagay et al. 2023).

116 *Amaranthus retroflexus* L. is a common weed species which affects many agricultural regions of  
117 the globe. *A. retroflexus* is a C4, annual weed, that originated in tropical America and has  
118 subsequently become widely distributed, worldwide (Evon et al. 2019; Sheibany et al. 2009;  
119 Hamidzadeh Moghadam et al. 2023). With its phenotypic flexibility and high genetic diversity,  
120 this species is able to survive in a wide variety of agricultural settings, as well as in the presence  
121 of other weeds (Hamidzadeh Moghadam et al. 2023; Orłowski and Czarnecka 2009). The  
122 physiological characteristics of *A. retroflexus* have made it widely recognised as a troublesome  
123 weed species in a variety of agricultural settings; and it has recently been reported to exhibit  
124 herbicide resistance (Brankov et al. 2022).

125 The most common method of commercial weed control is the use of herbicides. However, the  
126 disadvantages of this traditional method of weed management are well established

127 (Bagavathiannan et al. 2014; Poudyal and Cregg 2019). The accumulation of chemical herbicides  
128 has been shown to cause both environmental pollution and damage to the biological food chain.  
129 Residues of chemical herbicides seep into the ground, which can lead to the pollution of  
130 groundwater (Dar et al. 2019; Eevers et al. 2017; Kughur 2012). Moreover, the more herbicides  
131 are used, the more herbicide resistant the weeds become (Hulme 2023; Piñar Fuentes et al. 2021).  
132 For the reasons mentioned above, it is widely recognised that it would be environmentally  
133 beneficial to produce natural herbicides, with low toxicity and high efficiency which are also  
134 biodegradable (Batish et al. 2006; Scavo et al. 2020a; Scavo and Mauromicale 2021). For example,  
135 nanoencapsulated DiS-NH<sub>2</sub> bioherbicide mimics have been shown to exhibit significant weed-  
136 suppressive ability (WSA) in weeds commonly associated with the cultivation of wheat, at three  
137 different sites. Also, both broadleaf weeds and grasses were controlled by the DiS-NH<sub>2</sub>  
138 bioherbicide mimic, allowing broad-spectrum weed control (Scavo et al. 2023). In addition to  
139 WSA, the DiS-NH<sub>2</sub> bioherbicide mimic had no phytotoxic effects on the wheat plants after its  
140 application (by visual assessment), but also had beneficial effects on wheat plant production  
141 (Sabella et al. 2020). Another recent study found that polymeric nanoparticles of disulfide acted  
142 as potent inhibitors of *Plantago lanceolata* L. seed germination (Macías et al. 2019). Furthermore,  
143 a study of seven aminophenoxazinone derivatives showed a significant reduction in root length of  
144 the weed *P. oleracea* with phytotoxicity equal to, or greater than, that of the herbicide  
145 pendimethalin (Díaz-Franco et al. 2023).

146 Research into the field of allelopathy is therefore of major significance in order to develop a more  
147 sustainable and ecologically friendly approach to agricultural practices. To date, relatively little  
148 research has been conducted on the allelopathic activity of *F.arundinacea*. In light of which, the  
149 aims of the current study were to (i) investigate the allelopathic activity of 16 *F.arundinacea*  
150 genotypes and identify the genotypes with the greatest inhibitory effect (ii) to evaluate the  
151 allelopathic activity of the rhizospheric soil of *F.arundinacea*, under conditions of normal  
152 irrigation and severe drought stress, as well as investigating the allelopathic activity of  
153 *F.arundinacea* shoot residues of in the soil (iii) the identification of the allelopathic phenolic  
154 compounds present in the soil, directly caused by the activity of the roots or released from shoot  
155 residues of *F.arundinacea*.

156

## 157 **2 Materials and methods**

## 158 2.1 Plant material and its preparation

159 The tall fescue (*F.arundinacea*) genotypes (Table 1), were cultivated under two environmental  
160 conditions, namely normal irrigation (control) and severe drought stress (90% soil moisture  
161 depletion) at the research farm of Isfahan University of Technology, located in Lavark, Najafabad,  
162 Iran (40 km south west of Isfahan, 32° 32 'N, 51° 23 'E and 1630 meters above sea level) (Majidi  
163 et al. 2009). Fresh samples of *F.arundinacea* from three replicates were collected for the two  
164 irrigation treatments (control and severe drought stress) in the late spring and at the flowering stage  
165 of plant development in 2020. In each plot consisted of three cultivated rows. Five plants from the  
166 middle row were randomly selected and were manually separated from the soil. The plant samples  
167 were then dried at room temperature, ground in a pestle and mortar and stored in closed plastic  
168 bags, at room temperature, prior to use. Under control conditions, irrigation was non-restrictive,  
169 and plants were irrigated when 50% of the total water available from the root zone was depleted.  
170 Severe drought stress irrigation was performed when 90% of the total available water was depleted  
171 from the root zone (Allen and Pereira 1998). Water stress was applied alternately during the  
172 growing season from the first of May to the first of October. The irrigation intervals between the  
173 two irrigation treatments during the growing season varied depending on the weather conditions.  
174 The irrigation interval was 5-9 days in control conditions and 14-28 days under conditions of  
175 severe drought stress. To determine the gravimetric soil–water content, three soil samples were  
176 taken from each plot at the depth of 0-20, 20-40, and 40-60 cm for both treatments of water  
177 conditions. The irrigation depth was determined using the following equation:

$$178 I=[(\theta_{FC}-\theta/100)]D\times B$$

179 where I is the irrigation depth (cm),  $\theta_{FC}$  is soil gravimetric moisture percent at field capacity,  $\theta$   
180 is soil gravimetric moisture percentage at irrigating time, D is the root zone depth, and B is the  
181 root zone soil bulk density (1.4 g/cm<sup>3</sup>).

182 Water was applied via a basin irrigation system. The field was supplied with water via a pump  
183 station and polyethylene pipes. The volume of water applied to each treatment was measured with  
184 a volumetric counter.

185 The seeds of *L. sativa* and the weed species *A. retroflexus*, were used as the target species for the  
186 study. Commercial seeds of *L. sativa* were obtained from Pakan Bazr Co., Isfahan. *L. sativa* is  
187 considered as a biological indicator species due to its sensitivity to chemical growth inhibitors and  
188 promoters, it has been used in many previous allelopathic studies, as it displays rapid seed

189 germination and uniform initial seedling growth, both of which were favorable traits for the current  
190 study (Reigosa et al. 2013). Seed of the weed species *A. retroflexus*, were collected from Lavark  
191 Farm, Department of Agriculture, Isfahan University of Technology, and their germination  
192 percentage and dormancy were tested under Petri-dish conditions in a growth chamber.

193

## 194 **2.2 Preparation of aqueous shoot extracts of *F. arundinacea* genotypes and seed germination** 195 **experiments**

196 To prepare the aqueous extracts, shoots of the selected *F. arundinacea* genotypes were harvested  
197 at the flowering stage of development. Prior to extraction, the samples were air-dried. The method  
198 of Bali et al. (2017) was then used to prepare the aqueous extract.

199 First, the samples were ground in a pestle and mortar and 12.5, 25, 50, 75 and 100 grams of the  
200 ground sample added into 100 ml of deionized water and incubated for 24 hours, at a temperature  
201 of 25°C. The extracts were then passed through a three-layer muslin fabric once, and thrice through  
202 Whatman #1 filter paper, then centrifuged (3000 rpm, 20 minutes) and the obtained solution was  
203 passed through filter paper once more and the resultant aqueous extracts were stored at 4°C until  
204 use.

205 The initial allelopathy experiment was performed in a factorial manner, in the form of a completely  
206 randomized design, in 3 replications, in a Petri dish with a diameter of 9 cm, in a growth chamber  
207 with the seed of *L. sativa* used as the target species. The *L. sativa* seeds were first surface sterilized  
208 with a 10% sodium hypochlorite solution for 10 minutes, then washed with sterile distilled water  
209 for 10 minutes and then rinsed in deionized water for 5 minutes.

210 Fifty seeds were placed on a filter paper in a petri dish. The seeds were then irrigated with the  
211 prepared extracts (10 ml for each Petri dish) and the Petri dishes placed in a growth chamber at a  
212 temperature of 25°C, with 12 hours of light radiation. Irrigation with distilled water was used as a  
213 control. According to the percentage of seed germination, root length, stem length and dry weight  
214 of the target plant, the genotype that produced the most inhibitory effect upon the growth of the  
215 target plant was selected and used for the subsequent test of allelopathic activity of the rhizospheric  
216 soil.

217

## 218 **2.3 Sampling of the rhizospheric soil and measurement of the growth of the target species**

219 The main objective of this set of experiments was to evaluate the allelopathic effect of rhizospheric  
220 soil and to identify the phenolic allelochemicals present in rhizosphere of the genotype 23M, which  
221 had displayed the highest level of allelopathic activity in the previous screening experiment.

222 The soil samples were taken according to (Xu et al. 2009). Five different soil samples were  
223 collected from a depth of approximately 30 cm around the root of genotype 23M; in plants  
224 cultivated both under the control and severe drought stress treatments. Then, a sieve (2 mm mesh)  
225 was used to remove the root tissue. The resultant rhizosphere soil samples, were then air-dried and  
226 stored in polyethylene containers at 4°C.

227 The RSM (Rhizosphere Soil Method), proposed by (Fujii et al. (2005), and (Karmegam et al.  
228 (2014), was used to determine the presence of allelochemicals isolated from the root system in the  
229 rhizospheric soil. This experiment was performed in factorial manner, with a completely  
230 randomized design, with six replications. The culture medium treatments include pots (with a  
231 capacity of 100 grams of soil), containing farm soil in which *F. arundinacea* did not grow (as a  
232 control), pots containing rhizospheric soil of *F. arundinacea* under control (normal) irrigation  
233 conditions, pots containing rhizospheric soil under severe drought stress conditions and the pots  
234 containing 50 wt.% mixture of *F. arundinacea* plant powder and 50% control soil (leaf powder  
235 amended soil). These pots were moistened with 120cc of distilled water (Bhowmik and Doll 1982).  
236 The test seeds were surface sterilized with 10% sodium hypochlorite for 10 minutes, and washed  
237 3 times with sterile, distilled water. Twenty-five seeds of *L. sativa* and *A. retroflexus* were planted  
238 in pots. The pots were then transferred to a plant growth chamber at a temperature of 25°C and  
239 incubated for 30 days. The percentage seed germination, root length and stem length were  
240 recorded. To calculate the dry weight of seedlings, the samples were dried in an oven at 70°C for  
241 48 hours and then weighed (Fragasso et al. 2012).

242

#### 243 **2.4 Measurement of total phenolic content**

244 The total concentration of phenols and flavonoids, present in plant shoots and rhizospheric soil,  
245 were estimated using gallic acid as a standard and the Folin–Ciocalteu colorimetric method as  
246 detailed in Alinian et al. (2016). The results were expressed in gallic acid equivalents (GAEs).

247 To summarize the methodology used, 3 grams of air-dried sample (leaf powder and soil samples)  
248 were extracted with 10 ml 80% (v/v) methanol using an orbital shaker incubator (Jaltajhiz, Iran,  
249 Karaj, JTSL20) (110 rpm) at 25°C for 24 h. A 0.5 ml aliquot of the methanol extract was then

250 filtered and combined with 2.5 ml of the Folin–Ciocalteu reagent (diluted with 1:10 amount of  
251 distilled water) and 2 ml sodium carbonate at 7.5% (v/v). It was heated at 45°C for 15 minutes and  
252 the absorbance was measured at 765nm against a blank, by spectrophotometry (HITAGHI-Japan  
253 model U-1800). The phenolic content of shoots was recorded as gallic acid equivalents per 1 g of  
254 shoot dry matter, and for soils, as gallic acid equivalents per 1 g of dry soil.

255

## 256 **2.5 Phenolic compound content of rhizospheric soil samples**

257 Soil samples were extracted with 80% (v/v) methanol. A polar solvent, HPLC-grade methanol,  
258 was used to extract the free phenolic acids from the soil due to its high extraction efficiency for  
259 these compounds (Kong et al. 2006). In addition, methanol has a protective function as it can  
260 prevent phenolic compounds from being oxidized by enzymes such as phenol oxidase (Proestos et  
261 al. 2006). 100 grams of each oven-dried soil sample was extracted with 300 ml of methanol 80%  
262 (HPLC grade, Merck) (agitation, 25°C for 48 hours, centrifugation, 1200 x g for 15 minutes). The  
263 extract was then concentrated on a rotary evaporator (40°C.) and the residue dissolved in HPLC  
264 grade methanol. Extracts were analyzed on an HPLC system (model Agilent 1090). The instrument  
265 consisted of an Agilent 1100 HPLC, diode detector, and mass spectrometer (MSD, SL mode)  
266 (Agilent Technologies, Palo Alto, CA, USA).

267 Filtration of the extract was performed using a 0.22 µm nylon Acrodisc filter and injection onto  
268 the analytical column was performed using 20 µl of the filtered extract. HPLC grade methanol was  
269 used as the solvent for dissolving the standards.

270 The stationary phase consisted of a 250 mm × 4.6 mm (5 µm) Symmetric C18 column (Waters  
271 Corp., Milford, MA, USA) (10 mm × 4 mm ID) and the mobile phase was formic acid (0.1 %).  
272 Acetonitrile (99.8%) was used at a flow rate of 0.8 mL/min and the wavelength was set between  
273 200 and 400 nm. Implementation of gradient conditions was characterized by the following  
274 specifications: 10 to 26% solvent B (v/v) for 40 min, 65% solvent B for 70 min, and finally 100%  
275 solvent B for 75 minutes. The DAD was set at 350, 310, 270, and 520 nm, extreme peaks were  
276 read in real time, and determination of soil constituents was achieved by continuous recording of  
277 the entire spectrum (190–650 nm) (Lin and Harnly 2010). Analysis of allelopathic compounds was  
278 repeated three times using triplicate extracts for each sample.

279

## 280 **2.6 Statistical analyses**

281 Prior to carrying out an analysis of variance (ANOVA), the Kolmogorov-Smirnov test was  
282 performed to examine the normality of the distribution of the data. Subsequent to which, data from  
283 the first bioassay, i.e. the total phenolic content in 3 independent replicates, and the second  
284 bioassay in 6 independent replicates, were subjected to ANOVA using SAS 9.4. Means were  
285 compared individually with the least significant difference (LSD) test, tested with a probability of  
286 5%. Principle component analysis (PCA) was conducted based on the correlation matrix of  
287 measured traits using XLSTAT software version 2019.2.2.

288

### 289 **3 Results**

#### 290 **3.1 Germination percentage**

291 The results of the first experiment, indicate that all three factors namely genotype, extract  
292 concentration and drought stress, as well as the interaction between the three, have a significant  
293 effect upon the germination percentage of *L. sativa* seed (Table 2). The largest decrease in  
294 germination percentage was observed at the highest extract concentration (-85.3%) and using  
295 extracts from plants grown under drought stress conditions (-24.84%) compared to the control  
296 (Tables 3). The PCA analysis showed that the aqueous extracts obtained from genotypes 6L, 11M,  
297 4E, 20L, 23M had the greatest inhibitory effect upon the target species, of which the 23M genotype  
298 showed the highest inhibition percentage (-51.51) (Figure 1 and Table 4,5,6).

299 The findings of the second phase of the study, indicated that the percentage seed germination is  
300 strongly influenced by the target species and the culture medium, and that the effect of these two  
301 factors on the germination rate is significant at the 1% level. Also, the interaction between these  
302 two factors has an effect upon the germination percentage at the 1% level (Table 7). The highest  
303 decrease in seed germination percentage in comparison with the control for both *L. sativa* and *A.*  
304 *retroflexus* (-33.82%, -14.82%) was recorded for the soil amended with plant powder from the  
305 23M genotype. The smallest decrease in percentage seed germination was recorded for the culture  
306 medium containing plant material from plants grown under severe drought stress the decrease in  
307 germination percentages relative to the control for which were *L. sativa* (0.15%) and for *A.*  
308 *retroflexus* (0.54%) respectively. (Table 8).

#### 309 **3.2 Shoot length**

310 The first experiment showed that all three factors, genotype, extract concentration and severe  
311 drought stress, as well as the interaction of these three factors, significantly affected the length of

312 the *L. sativa* shoot (Table 2). The largest decrease in shoot length, was observed at the highest  
313 extract concentration (100% concentration) (-72.9%) with extracts from plants grown under severe  
314 drought stress conditions (-23.89%) (Tables 3). The aqueous extract obtained from 23M genotype  
315 produced the greatest reduction on shoot length (-48.41%) (Table 4,5,6).

316 According to the results obtained from the second study, the effect of two factors, the target species  
317 and the culture medium, as well as the interaction of the two, on the shoot length, is significant at  
318 the level of 1% (Table 7). This means that the shoot length in each plant is not only influenced by  
319 the vigour of the plant, but also by the culture medium. Based on the results of mean comparison  
320 of interactions, the greatest decrease in shoot length compared to the control, was observed in the  
321 presence of plant residues in *L. sativa* and *A. retroflexus* (-31.18%, -26.25%) respectively (Table  
322 8). Meanwhile, the culture medium containing the rhizospheric soil from plants grown under  
323 drought stress, produced the least reduction of the stem length in *L. sativa* and *A. retroflexus*  
324 respectively (-0.49%, -0.11%) (Table 8).

### 325 **3.3 Root length**

326 In the first experiment, it was found that the effect of all three factors, genotype, plant extract  
327 concentration, severe drought stress and the interaction of these three factors, on *L. sativa* root  
328 length, is significant at the 1% level (Table 2). The use of extract at the 100% concentration caused  
329 the greatest decrease in root length (77.04%) compared to the control. In addition, the extracts  
330 obtained from plants grown under severe drought stress caused a 25.37% decrease in root growth  
331 compared to those grown under control conditions (Tables 3). The aqueous extract obtained from  
332 the 23M genotype was shown to produce the greatest inhibitory effect on *L. sativa* root length  
333 (-48.68%) (Table 4,5,6).

334 In the second experiment, the effect of two factors, the target species, and the culture medium, as  
335 well as the interaction of these two factors on root length, is significant at the 1% level (Table 7).  
336 The highest decrease in root length compared to the control, was observed in the soil amended  
337 with plant powder (23M genotype) for *L. sativa* and *A. retroflexus* plants (-31.09%, -21.27%),  
338 respectively (Table 8). Meanwhile, the culture medium containing rhizospheric soil under severe  
339 stress had the least effect on the reduction of root length in the target species *L. sativa* and *A.*  
340 *retroflexus* (-1.69%, -0.58%) (Table 8).

### 341 **3.4 Seedling dry weight**

342 The first experiment showed that all three factors of genotype, extract concentration and drought  
343 stress and the interaction of these three factors have a significant effect (at the 1% level) on *L.*  
344 *sativa* seedling biomass (Table 2). The greatest decrease in the dry weight of seedlings (-63.7%)  
345 was observed in the presence of the highest extract concentration (100%) and in plants grown  
346 under drought stress conditions (-19.9%) (Table 3). Also, the aqueous extract obtained from the  
347 23M genotype caused the largest reduction in dry weight of the *L. sativa* seedlings (-44.38%)  
348 (Tables 4,5,6).

349 The results of the second study showed that in addition to the effect of two factors, the target  
350 species and the culture medium, the interaction of these two factors on the dry weight of *L. sativa*  
351 and *A. retroflexus* seedlings was significant at the 1% level (Table 7). The results of the mean  
352 comparison of interaction, show that the treatment of soil mixed with plant residues led to the  
353 greatest decrease in the dry weight of seedlings in *L. sativa* and *A. retroflexus* respectively  
354 (-40.61%, -31.79%). Moreover, the culture medium containing the rhizosphere soil under severe  
355 drought stress caused the lowest decrease in the dry weight of seedlings in *L. sativa* and *A.*  
356 *retroflexus* respectively (-0.59%, -0.21%) (Table 8).

357 Figure 1 shows the principal component analysis (PCA) of the data obtained in the initial phase of  
358 the study. The results show that two components contributed to more than 97% of the total  
359 variation in control conditions and severe drought stress. The genotypes were divided into two  
360 groups: 1) a group of genotypes that displayed a small inhibitory effect on the studied traits and  
361 showed high germination percentage, root length, shoot length and dry weight (group a). These  
362 genotypes included 12L, 3M, 21M, 14E, 9E, 22M, 17M, 3E, 1M, 10E, 1E. 2) genotypes 6L, 11M,  
363 4E, 20L, 23M, which had a significant inhibitory effect upon the traits studied and thereby caused  
364 a significant decrease in germination percentage, root length, shoot length and seedling dry weight,  
365 which indicates the allelopathic activity of these genotypes (group b). The PCA of control  
366 conditions and severe drought stress were similar to each other and showed that the genotypes that  
367 had higher allelopathic activity under control conditions, also had a higher allelopathic activity  
368 than the genotypes of the first group grown under conditions of severe drought stress. Moreover,  
369 the genotypes that had weak allelopathic activity under normal conditions, were also weak  
370 genotypes in terms of their allelopathic activity when grown under severe drought stress. Based  
371 on the results obtained, the genotype with the high allelopathic activity (genotype 23M) and its'  
372 rhizospheric soil was selected for further study. The origin of the genotypes is shown in Table 1.

### 373 **3.5 Phenolic compounds**

374 The results indicate the presence of phenolic compounds in the shoots as well as the rhizospheric  
375 soil of genotype 23M (Table 9, Figure 2). The mean comparison data showed that the shoots of  
376 genotype 23M grown under drought stress conditions, have more phenolic compounds than in  
377 plants grown under control conditions (Figure 2). In addition, the total phenolic content of the  
378 rhizospheric soil from plants grown under drought stress was significantly lower than that from  
379 plants grown under control irrigation conditions (Table 9).

380 The results of HPLC analysis show the presence of phenolic compounds in the rhizospheric soil  
381 (Table 10). As mentioned above, in plants grown under conditions of severe drought stress, the  
382 total phenolic content of the rhizospheric soil was significantly lower than present in the  
383 rhizosphere of plants grown under normal conditions. Furthermore, the type and quantity of  
384 phenolic compounds detected in the control soil (farm soil in which *F. arundinacea* was not  
385 grown) and soil from plants grown under severe drought stress, were lower than the rhizospheric  
386 soil from plants grown under normal irrigation conditions (absence of drought stress) and soil  
387 mixed with plant powder (soil amended with powder 23M genotype). The results of HPLC show  
388 the highest quantity and number of phenolic compounds in soil mixed with *F. arundinacea* plant  
389 powder (Table 10).

390

### 391 **4 Discussion**

392 The results of the study presented, demonstrate that aqueous extracts of the *F. arundinacea*  
393 genotypes can significantly inhibit seed germination, root length, shoot length and dry weight of  
394 *L. sativa* and *A. retroflexus* seedlings, especially at a high dosage, but that the inhibitory effect of  
395 the *F. arundinacea* extract upon seed germination and seedling growth was dependent upon the  
396 genotype, extract concentration, irrigation conditions and their interaction (Tables 2,5,6). These  
397 data are in agreement with previous studies. For example, Smith and colleagues reported that  
398 aqueous extracts of both *Cynodon dactylon* (L.) Pers. and *F. arundinacea* reduced the leaf area  
399 index, leaf dry weight and total plant dry weight of *Carya illinoensis* when compared to the  
400 control (Smith et al. 2001). In a more recent study, Wang and co-workers evaluated the effect of  
401 an aqueous leaf extract of *Cinnamomum migao* at a variety of different concentrations on seed  
402 germination and seedling growth of *Liquidambar formosana* (Wang et al. 2019b). These authors

403 demonstrated that the extract inhibited the germination of *L. formosana* seeds at high  
404 concentrations.

405 The current study has also shown the effect of the presence of *F. arundinacea* residues in the soil  
406 upon the reduction of seed germination, shoot length, root length and biomass reduction in two  
407 test species, *L. sativa* and *A. retroflexus* (Table 8). Several previous studies have also shown that  
408 plant residues can also display allelopathic and growth inhibition properties (Shehata et al. 2022).  
409 Previous studies have also demonstrated that incorporation of *F. arundinacea* shoot residues into  
410 soil resulted in effective control of weed species (de Bertoldi et al. 2012). Furthermore, Bertin and  
411 colleagues studied 78 *F. arundinacea* genotypes and subsequently reported that 4 of them were  
412 highly allelopathic with potential for use as a biological control to suppress weed growth (Bertin  
413 et al. 2007). In another study, conducted by Sahoo and co-workers, it was found that when  
414 sunflower (*Helianthus annuus* L.) residues were added to the soil, the allelopathic potential  
415 increased due to the increase in the concentration of phenolic compounds, and as a result, the  
416 germination of rice (*Oryza sativa* L.) seeds was decreased (Sahoo et al. 2023). Powdered plant  
417 residue obtained from rat's-tail fescue (*Vulpia myuros*) was shown to inhibit root and shoot growth  
418 in *Lepidium sativum* (Yamamoto and Kato-Noguchi 2015). These authors reported that the degree  
419 of inhibition varied according to the amount of powder added to the soil, or the concentration of  
420 the extract. In general, the release of phytotoxins present in plant residues, into the soil surrounding  
421 the roots, may result in the release of significant quantities of phenolic containing compounds into  
422 the rhizosphere and thus have an inhibitory effect upon plant growth (Brady and Weil 2002;  
423 Castells et al. 2005; Raihan et al. 2018).

424 According to the results of our study, the rhizospheric soil also possessed an allelopathic and  
425 inhibitory effect upon seed germination, root and shoot growth and the dry weight of *L. sativa* and  
426 *A. retroflexus* seedlings (Tables 7 and 8). In other studies, researchers have also demonstrated the  
427 effect of root allelopathy, for example, Wang and colleagues stated that *Medicago truncatula* root  
428 secretions decreased the fresh weight and dry weight of *M. truncatula* and *M. sativa* plants (Wang  
429 et al. 2022a). In another recent study by Lipińska, it was found that plant cultivation in soils  
430 containing tall fescue residues significantly reduced plant growth (Lipinska et al. 2019).

431 It has also been reported that a rhizospheric soil extract of *Glycyrrhiza uralensis* had a phytotoxic  
432 activity on seedlings of *Lactuca sativa* L. In addition, six allelopathic compounds were identified  
433 to be present in the rhizosphere of this species (Ren et al. 2017).

434 According to the results obtained in our study, among the selected test plant species, the highest  
435 inhibition of seed germination, root and shoot growth and dry weight was observed to occur in *L.*  
436 *sativa* (Table 8). This species is known to be highly sensitive to allelopathic compounds and is  
437 therefore widely used as a bioindicator species (Reigosa et al. 2013). Several hypotheses have been  
438 proposed to explain why some species are more sensitive to allelopathic chemicals than others.  
439 Both *L. sativa* and *A. retroflexus* are known to produce small size seeds. Small-seeded plants have  
440 a greater root length per unit root mass, which provides a larger surface area for allelochemical  
441 uptake (Leishman et al. 2000; Mašková and Weiser 2019). Species with larger seeds, contain more  
442 endosperm reserves, which are positively correlated with seed size. Therefore, large seeds are less  
443 susceptible to allelopathic substances due to better seedling respiration in conditions of carbon  
444 deficiency caused by stress (Simpson et al. 2021; Westoby et al. 2002). The species with more  
445 seed reserves are therefore better adapted to tolerate and detoxify allelopathic chemicals (Liebman  
446 and Sundberg 2006; Zubay et al. 2021).

447 Our data are consistent with those presented in the literature, for example, the study of Wang and  
448 colleagues investigated the allelopathic effects of aqueous extracts of three species namely  
449 *Chnatherum splendens*, *Artemisia frigida* , *Stellera chamaejasme* on *L. sativa* and demonstrated  
450 that increasing the concentration of the extract significantly reduced seed germination and seedling  
451 growth (He et al. 2020; Wang et al. 2022b). In general, allochemicals are known to have a toxic  
452 effect on small seeds, prevent the absorption of water and nutrients, and stop the growth and  
453 development of seedlings (Cheng et al. 2021).

454 Another interesting data set obtained from our investigation, was the effect of drought stress on  
455 the allelopathic properties of aqueous extracts obtained from the shoots of *F. arundinacea*. The  
456 extracts obtained from the genotypes grown under conditions of drought stress, were shown to  
457 possess more allelopathic activity, and as a result, caused a marked decrease in seedling growth  
458 (Tables 2 and 3).

459 Plant species have been shown to protect their photosynthetic apparatus against excessive stress,  
460 by activating different defense mechanisms, including the synthesis of phenolic antioxidants  
461 (Dumanović et al. 2021; Hura et al. 2008).

462 Studies have shown that production of these phenolic compounds is increased under conditions of  
463 drought stress, and that the up regulation of these compounds occurs predominantly in drought-  
464 resistant cultivars (Qaderi et al. 2023; Rezayian et al. 2020). *F. arundinacea* is known to be a

465 relatively drought tolerant grass species (Fariaszewska et al. 2017), and the observed increase in  
466 phenolic compounds therefore correlates with the protective role of, these compounds in drought  
467 stressed plants (Wagay et al. 2023).

468 Drought stress has been shown to lead to an increase in the content of phenolic acid and flavonoids  
469 in nine varieties of five grass species (*Lolium perenne*, *Lolium multiflorum*, *Festuca pratensis*,  
470 *Festuca arundinacea* and *Festulolium braunii*) (Fariaszewska et al. 2017). In a study using  
471 aqueous extracts of leaves of *Rhus typhina* and *Sapindus mukorossi* grown under drought stress,  
472 on the grown and development of *L. sativa*, it was observed that all of these plant extracts had an  
473 allelopathic effect on seed germination and the growth of *L. sativa* seedlings, and drought stress  
474 increased the allelopathy of *R. typhina* and *S. mukorossi* (Zhong et al. 2023). Similarly, Sarker and  
475 colleagues stated that the concentration of phenolic acids and flavonoids in *Amaranthus tricolor*  
476 grown under conditions of drought stress were increased in comparison to those cultivated under  
477 control conditions (Sarker and Oba 2018). Plant allelopathic behaviour is modulated by abiotic  
478 and biotic stressors, which influence the quantity of allelochemicals released by the donor plant  
479 and the effect of these chemicals upon the target plant (Scavo et al. 2018, Scavo et al, 2020b). In  
480 our study, abiotic stress (drought stress) was shown to increase the quantity of allelopathic  
481 compounds produced in tall fescue.

482 In the current study, it was found that the rhizospheric soil of genotype 23M, produced the highest  
483 quantity of phenolic compounds and, as a result, produced the largest degree of growth inhibition  
484 of *L. sativa* and *A. retroflexus* seedlings in severe drought stress and non-stress conditions (Table  
485 9). The rhizospheric soil of plants grown under control conditions had more allelopathic properties  
486 and reduced the seed germination and growth of the seedlings, while the rhizospheric soil of plants  
487 grown in the conditions of severe drought stress, did not display an inhibitory effect upon the  
488 growth and development of the indicator species. HPLC analysis of these soils revealed that they  
489 differ both in terms of the type, and quantity of phenolic compounds present (Table 10).

490 Two phenolic compounds, gallic acid and syringic acid were detected in the rhizosphere of  
491 genotype 23M cultivated under severe drought stress conditions. In soil harvested from plants  
492 grown under control conditions, five phenolic compounds were identified namely, coumaric acid,  
493 apigenin acid, ferulic acid, 4-hydroxybenzoic acid and gallic acid. However, since the highest total  
494 phenolic content was observed in the control soil mixed with plant powder, the HPLC analysis of  
495 the extract of this soil also showed the highest quantity of compounds and phenolic acid amounts.

496 This soil contained 9 phenolic compounds namely, syringic acid, caffeic acid, coumaric acid,  
497 apigenin acid, ferulic acid, vanillic acid, 4-hydroxybenzoic acid, chlorogenic acid and gallic acid.  
498 The increase in the type and quantity of phenolic compounds in this treatment is due to the presence  
499 of plant powder in the soil, which introduced allelopathic compounds into the soil during  
500 decomposition.

501 Different results were observed in terms of total phenol in the shoots and the rhizospheric soil of  
502 *F. arundinacea* grown under normal conditions and severe drought stress (Tables 10 and Figure  
503 2). While the results of the total phenolic and HPLC analyses showed the presence of small  
504 amounts of phenolic compounds in the soil from plants grown under severe drought stress (Table  
505 10), the quantity of phenolic compounds in the leaves of *F. arundinacea* from plants cultivated  
506 under severe drought stress conditions showed a significant increase compared to those from plants  
507 grown under control conditions (Figure 2).

508 The most important factor in determining the phytotoxicity of allelochemicals, is their  
509 concentration in soil water (Kobayashi 2004). When allelochemicals are released through root  
510 exudation, they enter complex plant-soil systems, where various factors influence their availability  
511 and thus their effective impact on target species (Blum et al. 1999; Kruse et al. 2000). In addition  
512 to the chemical nature of the allelochemicals produced, the phytotoxic activity of allelochemicals  
513 in soil is influenced by climatic conditions (e.g. temperature, solar radiation, rainfall), soil factors  
514 (e.g. texture, pH, moisture content, ion exchange capacity, nutrient dynamics, organic matter  
515 content and microbial ecology) and plant factors of both the donor and target plants (e.g. species,  
516 growth stages, plant parts, botanical variety) (De Albuquerque et al. 2011; Rice 1984; Scavo et al.  
517 2018).

518 Exudation from plant roots appears to decrease under severe drought stress, perhaps because the  
519 plants divert resources to essential processes under drought stress conditions, and the plant's  
520 defense mechanisms for survival are activated. For example, changes in osmotic regulation, cause  
521 the accumulation of solutes such as amino acids and saccharides in the leaves and roots of stressed  
522 plants, which helps to maintain cell turgor and reduce water loss from cells in which root secretions  
523 in the soil area are greatly reduced (Ahmed et al. 2014; Good and Zaplachinski 1994; Jones et al.  
524 2009; Wu et al. 2016).

525 In a study of two herbaceous species namely *Holcus lanatus* and *Alopecurus pratensis*, contrasting  
526 metabolic responses to drought stress were observed between shoots and roots. While the roots

527 remained active and produced high amounts of primary metabolites, the leaves showed a decrease  
528 in primary metabolites and an increase in some secondary metabolites (such as organic acids,  
529 terpenes, and phenols) (Gargallo-Garriga et al. 2014, 2015).

530 In our data, the allelopathic effect of phenolic compounds was clearly observed (Table 10).  
531 Phenolic compounds can lead to increased cell membrane permeability and also increase lipid  
532 peroxidation, which finally, leads to reduced growth or death of plant tissue. In addition, phenolic  
533 allelochemicals can prevent plants from absorbing nutrients from the environment and thereby  
534 adversely affect their growth and development (Adeboye et al. 2014; Li et al. 2010; Politycka  
535 1997). It has been shown that phenolic allelochemicals can prevent plant root elongation, cell  
536 division, change cell structure and disturb the normal growth and development of the plant (Li et  
537 al. 2010). Phenolic acids such as p-coumaric acid, ferulic acid, p-hydroxybenzoic acid and oxalic  
538 acid, which are produced after the decomposition of rice residues (Lin et al. 2013), can be stored  
539 in the rhizosphere and prevent the growth of rice seedlings and weeds (Chou 1989; Chou et al.  
540 1981; Li et al. 2020; Rice 1985). This strongly supports the hypothesis that phenolic acids are  
541 allelochemicals. Previous studies have shown that plant allelopathy is a complex, biological  
542 process of the rhizosphere, performed by allelochemicals (secondary metabolites) secreted by  
543 plant roots (Schandry and Becker 2020; Wenxiong et al. 2017). An experiment that investigated  
544 the rhizospheric soil of wild oats, determined that they produce allelopathic compounds (Iannucci  
545 et al. 2013). HPLC analysis showed seven phenolic compounds in wild oat rhizospheric soil:  
546 syringic acid, vanillin, 4-hydroxybenzoic acid, syringaldehyde, ferulic acid, p-coumaric acid and  
547 vanillic acid. These compounds are a group of the most important and common plant  
548 allelochemicals in the ecosystem (Li et al. 2010).

549

## 550 **5 Conclusions**

551 The present study has demonstrated that *Festuca arundinacea* possesses allelopathic chemical  
552 compounds in its shoot and root tissues. The results of the study presented also demonstrate that  
553 there is a significant difference in the level and type of phenolic compounds present in the shoots  
554 and rhizospheric soil of plants cultivated under control and drought stressed conditions. In  
555 summary, based upon the data presented and previous studies, the use of forage grasses such as *F.*  
556 *arundinacea* as a means of biological weed management, via the production of allelochemicals  
557 toxic to the target weed species is clearly worthy of further study. These future studies may provide

558 the basis for the commercial use of plant allelopathy as a component of a more ecologically  
559 friendly and sustainable approach to agriculture.

### 560 **Declaration of Competing Interest**

561 The authors declare that they have no known competing financial interests or personal  
562 relationships that could have appeared to influence the work reported in this paper. Data  
563 availability Data will be made available on request.

564

### 565 **Acknowledgment**

566 The authors greatly appreciate the personnel at the research field and lab facility of Agriculture  
567 College of Isfahan University of Technology for their assistance.

568

569

570

### 571 **References**

- 572 Abu-Romman S (2016) Differential Allelopathic Expression of Different Plant Parts of *Achillea*  
573 *Biebersteinii*. Acta Biologica Hungarica 2016 67:2 67:159–168.  
574 <https://doi.org/10.1556/018.67.2016.2.4>
- 575 Adeboye PT, Bettiga M, Olsson L (2014) The chemical nature of phenolic compounds determines their  
576 toxicity and induces distinct physiological responses in *Saccharomyces cerevisiae* in lignocellulose  
577 hydrolysates. AMB Express 4:1–10. <https://doi.org/10.1186/S13568-014-0046-7>
- 578 Ahmed LQ, Escobar-Gutiérrez AJ (2022) Tall fescue (*Festuca arundinacea* Schreb.) shows intraspecific  
579 variability in response to temperature during germination. Agronomy 2022, Vol 12, Page 1245  
580 12:1245. <https://doi.org/10.3390/AGRONOMY12051245>
- 581 Ahmed MA, Kroener E, Holz M, Zarebanadkouki M, Carminati A (2014) Mucilage exudation facilitates  
582 root water uptake in dry soils. Functional Plant Biology 41:1129. <https://doi.org/10.1071/FP13330>
- 583 Aldrich RJ (1984) Weed-crop ecology: Principles in weed management. Breton, North Scituate,  
584 Massachusetts, USA 465

585 Alinian S, Razmjoo J, Zeinali H (2016) Flavonoids, anthocynins, phenolics and essential oil produced in  
586 cumin (*Cuminum cyminum* L.) accessions under different irrigation regimes. *Ind Crops Prod* 81:49–  
587 55. <https://doi.org/10.1016/j.indcrop.2015.11.040>

588 Allen RG, Pereira LS (1998) Crop evapotranspiration guidelines for computing crop requirements. FAO  
589 Irrig. Drain. Report modeling and application. Article in *Journal of Hydrology*

590 Amini F, Mirlohi A, Majidi MM, ShojaieFar S, Kölliker R (2011) Improved polycross breeding of tall fescue  
591 through marker-based parental selection. *Plant Breeding* 130:701–707.  
592 <https://doi.org/10.1111/j.1439-0523.2011.01884.x>

593 Aslam F, Khaliq A, Tanveer A, Zahir ZA, Matloob A (2016) Wheat residue incorporation modulate  
594 emergence and seedling growth of canary grass by affecting biochemical attributes and soil  
595 properties. *Int J Agric Biol* 18:1033–1042. <https://doi.org/10.17957/IJAB/15.0205>

596 Bagavathiannan M V., Norsworthy JK, Smith KL, Neve P (2014) Modeling the simultaneous evolution of  
597 resistance to ALS- and ACCase-inhibiting herbicides in barnyardgrass (*Echinochloa crus-galli*) in  
598 clearfield rice. *Weed Technology* 28:89–103. <https://doi.org/10.1614/WT-D-13-00106.1>

599 Bali AS, Batish DR, Singh HP, Kaur S, Kohli RK (2017) Phytotoxicity and weed management potential of  
600 leaf extracts of *Callistemon viminalis* against the weeds of rice. *Acta Physiol Plant* 39:25.  
601 <https://doi.org/10.1007/s11738-016-2313-5>

602 Batish DR, Singh HP, Kohli RK, Dawra GP (2006) Potential of allelopathy and allelochemicals for weed  
603 management. *Handbook of sustainable weed management* 209–256.  
604 <https://doi.org/10.1201/9781482293593-16>

605 Bertin C, Weston LA, Huang T, Jander G, Owens T, Meinwald J, Schroeder FC (2007) Grass roots  
606 chemistry: meta-Tyrosine, an herbicidal nonprotein amino acid. *Proceedings of the National*  
607 *Academy of Sciences* 104:16964–16969. <https://doi.org/10.1073/pnas.0707198104>

608 Bhowmik PC, Doll JD (1982) Corn and Soybean response to allelopathic effects of weed and crop  
609 residues. *Agron J* 74:601–606. <https://doi.org/10.2134/AGRONJ1982.00021962007400040005X>

610 Blokhina O (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann Bot*  
611 91:179–194. <https://doi.org/10.1093/aob/mcf118>

612 Blum U (2006) Allelopathy: a soil system perspective. *Allelopathy: A Physiological Process with Ecological*  
613 *Implications* 299–340. [https://doi.org/10.1007/1-4020-4280-9\\_14/COVER](https://doi.org/10.1007/1-4020-4280-9_14/COVER)

614 Blum U, Shafer SR, Lehman ME (1999) Evidence for Inhibitory allelopathic interactions involving phenolic  
615 acids in field soils: concepts vs. an experimental model. *CRC Crit Rev Plant Sci* 18:673–693.  
616 <https://doi.org/10.1080/07352689991309441>

617 Brady NC, Weil RR (2002) The nature and properties of soils, 13th edition. In: *agroforestry systems*.  
618 [https://www.researchgate.net/publication/241010261\\_The\\_Nature\\_and\\_Properties\\_of\\_Soils\\_13th](https://www.researchgate.net/publication/241010261_The_Nature_and_Properties_of_Soils_13th_Edition_By_N_C_Brady_and_R_R_Weil)  
619 [h\\_Edition\\_By\\_N\\_C\\_Brady\\_and\\_R\\_R\\_Weil](https://www.researchgate.net/publication/241010261_The_Nature_and_Properties_of_Soils_13th_Edition_By_N_C_Brady_and_R_R_Weil). Accessed 11 Feb 2023

620 Brankov M, Simić M, Tabaković M, Vukadinović J, Djuric N, Branković-Radojčić D, Dragičević V (2022)  
621 Weed management practices for redroot pigweed (*Amaranthus retroflexus* L.) and smooth

622 pigweed (*A. hybridus* L.) control in maize. *Chil J Agric Res* 82:611–618.  
623 <https://doi.org/10.4067/S0718-58392022000400611>

624 Buta JG, Spaulding DW (1989) Allelochemicals in tall fescue-abscisic and phenolic acids. *J Chem Ecol*  
625 15:1629–1636. <https://doi.org/10.1007/BF01012389>

626 Callaway RM, Maron JL (2006) What have exotic plant invasions taught us over the past 20 years?  
627 *Trends Ecol Evol* 21:369–374. <https://doi.org/10.1016/J.TREE.2006.04.008>

628 Carrow RN, Duncan R. R (2003) Improving drought resistance and persistence in turf-type tall fescue.  
629 *Crop Sci* 43:978–984. <https://doi.org/10.2135/cropsci2003.9780>

630 Castells E, Penuelas J, Valentine DW (2005) Effects of plant leachates from four boreal understorey  
631 species on soil N mineralization, and white spruce (*Picea glauca*) germination and seedling growth.  
632 *Ann Bot* 95:1247–1252. <https://doi.org/10.1093/aob/mci139>

633 Cheng H, Wang S, Wei M, Yu Y, Wang C (2021) Effect of leaf water extracts of four Asteraceae alien  
634 invasive plants on germination performance of *Lactuca sativa* L. under acid deposition. *Plant Ecol*  
635 222:433–443. <https://doi.org/10.1007/s11258-021-01117-5>

636 Cheng HH (1992) A conceptual framework for assessing allelochemicals in the soil environment.  
637 *Allelopathy* 21–29. [https://doi.org/10.1007/978-94-011-2376-1\\_3](https://doi.org/10.1007/978-94-011-2376-1_3)

638 Cheng HH (1994) Characterization of the mechanisms of allelopathy. 132–141.  
639 <https://doi.org/10.1021/BK-1995-0582.CH010>

640 Chou C-H (1989) The role of allelopathy in biochemical ecology: experience from Taiwan. *Biol Plant*  
641 31:458–470. <https://doi.org/10.1007/BF02876219>

642 Chou C-H, Chiang Y-C, Chfng HH (1981) Autointoxication mechanism of *Oryza sativa*. *J Chem Ecol* 7:741–  
643 752. <https://doi.org/10.1007/BF00990306>

644 Cougnon M, Baert J, Van Waes C, Reheul D (2014) Performance and quality of tall fescue (*Festuca*  
645 *arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) and mixtures of both species  
646 grown with or without white clover (*Trifolium repens* L.) under cutting management. *Grass and*  
647 *Forage Science* 69:666–677. <https://doi.org/10.1111/GFS.12102>

648 Dao TH (1987) Sorption and mineralization of plant phenolic acids in soil. 358–370.  
649 <https://doi.org/10.1021/BK-1987-0330.CH033>

650 Dar MA, Kaushik G, Villareal Chiu JF (2019) Chapter 2 - pollution status and biodegradation of  
651 organophosphate pesticides in the environment. *Abatement of Environmental Pollutants: Trends*  
652 *and Strategies* 25–66. <https://doi.org/10.1016/B978-0-12-818095-2.00002-3>

653 De Albuquerque MB, Dos Santos RC, Lima LM, Melo Filho PDA, Nogueira RJMC, Da Câmara CAG, Ramos  
654 ADR (2011) Allelopathy, an alternative tool to improve cropping systems. A review. *Agron Sustain*  
655 *Dev* 31:379–395. <https://doi.org/10.1051/AGRO/2010031>

656 de Bertoldi C, De Leo M, Ercoli L, Braca A (2012) Chemical profile of *Festuca arundinacea* extract  
657 showing allelochemical activity. *Chemoecology* 22:13–21. <https://doi.org/10.1007/S00049-011-0092-4>  
658

659 Díaz-Franco C, Rial C, Molinillo JMG, Varela RM, Macías FA (2023) Synthesis of aminophenoxazinones  
660 and evaluation of their phytotoxicity in the search for new natural herbicides. *Agronomy* 13:568.  
661 <https://doi.org/10.3390/AGRONOMY13020568/S1>

662 Dumanović J, Nepovimova E, Natić M, Kuča K, Jačević V (2021) The significance of reactive oxygen  
663 species and antioxidant defense system in plants: a concise overview. *Front Plant Sci* 11:2106.  
664 <https://doi.org/10.3389/FPLS.2020.552969/BIBTEX>

665 Eevers N, White JC, Vangronsveld J, Weyens N (2017) Chapter seven - bio- and phytoremediation of  
666 pesticide-contaminated environments: a review. *Adv Bot Res* 83:277–318.  
667 <https://doi.org/10.1016/bs.abr.2017.01.001>

668 El-Metwally IM, Saady HS, Elewa TA (2022) Natural plant by-products and mulching materials to  
669 suppress weeds and improve sugar beet (*Beta vulgaris* L.) yield and quality. *J Soil Sci Plant Nutr*  
670 22:5217–5230. <https://doi.org/10.1007/S42729-022-00997-4/TABLES/7>

671 Evon P, Langalerie G De, Ramaux T, Labonne L, Ballas S, Véronèse T, Merah O, Talou T, Ouagne P (2019)  
672 Amaranth, a model for the future biorefinery of whole plants. *Symbiose-Flower Conference 0*

673 Fariaszewska A, Huylenbroeck J Van, Baert J, Aper J (2017) Mild drought stress-induced changes in yield,  
674 physiological processes and chemical composition in *Festuca*, *Lolium* and *Festulolium*. *J Agron Crop*  
675 *Sci* 203:103–116. <https://doi.org/10.1111/jac.12168>

676 Fragasso M, Platani C, Miullo V, Papa R, Iannucci A (2012) A bioassay to evaluate plant responses to the  
677 allelopathic potential of rhizosphere soil of wild oat (*Avena fatua* L.). *Agrochimica -PISA-* 56:120–  
678 128

679 Fujii Y, Furubayashi A, Hiradate S (2005) Rhizosphere soil method: a new bioassay to evaluate  
680 allelopathy in the field. *Proceedings of the 4th World Congress on Allelopathy, “Establishing the*  
681 *Scientific Base”, Wagga Wagga, New South Wales, Australia, 21-26 August 2005* 490–492

682 Gargallo-Garriga A, Sardans J, Pérez-Trujillo M, Oravec M, Urban O, Jentsch A, Kreyling J, Beierkuhnlein  
683 C, Parella T, Peñuelas J (2015) Warming differentially influences the effects of drought on  
684 stoichiometry and metabolomics in shoots and roots. *New Phytologist* 207:591–603.  
685 <https://doi.org/10.1111/NPH.13377>

686 Gargallo-Garriga A, Sardans J, Perez-Trujillo M, Rivas-Ubach A, Oravec M, Vecerova K, Urban O, Jentsch  
687 A, Kreyling J, Beierkuhnlein C, Parella T, Peñuelas J (2014) Opposite metabolic responses of shoots  
688 and roots to drought. *Scientific Reports* 2014 4:1 4:1–7. <https://doi.org/10.1038/srep06829>

689 Ge Y, Wang ZY (2015) Tall fescue (*Festuca arundinacea* Schreb.). *Methods Mol Biol* 1224:365–372.  
690 [https://doi.org/10.1007/978-1-4939-1658-0\\_29](https://doi.org/10.1007/978-1-4939-1658-0_29)

691 Gerhards R, Schappert A (2020) Advancing cover cropping in temperate integrated weed management.  
692 *Pest Manag Sci* 76:42–46. <https://doi.org/10.1002/PS.5639>

693 Good AG, Zaplachinski ST (1994) The effects of drought stress on free amino acid accumulation and  
694 protein synthesis in *Brassica napus*. *Physiol Plant* 90:9–14. <https://doi.org/10.1111/j.1399-3054.1994.tb02185.x>  
695

- 696 Hamidzadeh Moghadam S, Alebrahim MT, Mohebodini M, MacGregor DR (2023) Genetic variation of  
697 *Amaranthus retroflexus* L. and *Chenopodium album* L. (Amaranthaceae) suggests multiple  
698 independent introductions into Iran. *Front Plant Sci* 13:5377.  
699 <https://doi.org/10.3389/FPLS.2022.1024555/BIBTEX>
- 700 Hammermeister AM (2016) Organic weed management in perennial fruits. *Sci Hortic* 208:28–42.  
701 <https://doi.org/10.1016/j.scienta.2016.02.004>
- 702 Hand ML, Cogan NOI, Forster JW (2012) Molecular characterisation and interpretation of genetic  
703 diversity within globally distributed germplasm collections of tall fescue (*Festuca arundinacea*  
704 Schreb.) and meadow fescue (*F. pratensis* Huds.). *Theor Appl Genet* 124:1127–1137.  
705 <https://doi.org/10.1007/S00122-011-1774-6>
- 706 He Z, Yao L, Zhang X, Li Y, Wang D, Kang L, Cui C, Huang A, Yang R, Xiao Q, Guo Y (2020) Faba bean  
707 organs differed in their effects on maize seed germination rate and soil microbial activities as well  
708 as their decomposition patterns in a regosol soil. *J Soil Sci Plant Nutr* 20:367–379.  
709 <https://doi.org/10.1007/S42729-019-00117-9/METRICS>
- 710 Hierro JL, Callaway RM (2021) The Ecological importance of allelopathy. *Annu Rev Ecol Evol Syst* 52:25–  
711 45. <https://doi.org/10.1146/annurev-ecolsys-051120-030619>
- 712 Hulme PE (2023) Weed resistance to different herbicide modes of action is driven by agricultural  
713 intensification. *Field Crops Res* 292:108819. <https://doi.org/10.1016/J.FCR.2023.108819>
- 714 Hura T, Hura K, Grzesiak S (2008) Contents of total phenolics and ferulic acid, and PAL activity during  
715 water potential changes in leaves of maize single-cross hybrids of different drought tolerance. *J*  
716 *Agron Crop Sci* 194:104–112. <https://doi.org/10.1111/j.1439-037X.2008.00297.x>
- 717 Iannucci A, Fragasso M, Platani C, Papa R (2013) Plant growth and phenolic compounds in the  
718 rhizosphere soil of wild oat (*Avena fatua* L.). *Front Plant Sci* 4:  
719 <https://doi.org/10.3389/FPLS.2013.00509>
- 720 Inderjit, Kaur M, Foy CL (2001) On the significance of field studies in allelopathy. *Weed Technology*  
721 15:792–797. [https://doi.org/10.1614/0890-037x\(2001\)015\[0792:otsofs\]2.0.co;2](https://doi.org/10.1614/0890-037x(2001)015[0792:otsofs]2.0.co;2)
- 722 Inderjit, Wardle DA, Karban R, Callaway RM (2011) The ecosystem and evolutionary contexts of  
723 allelopathy. *Trends Ecol Evol* 26:655–662. <https://doi.org/10.1016/J.TREE.2011.08.003>
- 724 Inderjit, Weiner J (2001) Plant allelochemical interference or soil chemical ecology? *Perspect Plant Ecol*  
725 *Evol Syst* 4:3–12. <https://doi.org/10.1078/1433-8319-00011>
- 726 Iqbal Z, Hiradate S, Noda A, Isojima SI, Fujii Y (2002) Allelopathy of buckwheat: assessment of  
727 allelopathic potential of extract of aerial parts of buckwheat and identification of fagomine and  
728 other related alkaloids as allelochemicals. *Weed Biol Manag* 2:110–115.  
729 <https://doi.org/10.1046/J.1445-6664.2002.00055.X>
- 730 Jabran K, Mahajan G, Sardana V, Chauhan BS (2015) Allelopathy for weed control in agricultural systems.  
731 *Crop Protection* 72:57–65. <https://doi.org/10.1016/J.CROPRO.2015.03.004>

- 732 Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil–root  
733 interface. *Plant Soil* 321:5–33. <https://doi.org/10.1007/s11104-009-9925-0>
- 734 Jovanović EO, Marković S, Gavrilović Z, Dakić P (2010) The broadleaf weeds in corn of crop on area  
735 southwestern Banat and their control. *Banats J Biotechnol* 1:52–55
- 736 Karmegam N, Kalpana M, M.Prakash D (2014) Allelopathic effect of aqueous root bark extract of  
737 *Tamarindus indica* L. and rhizosphere soil on germination and seedling growth of *Oryza sativa* L. *Int*  
738 *J Curr Microbiol Appl Sci* 505–514
- 739 Kaur H, Kaur R, Kaur S, Baldwin IT, Inderjit (2009) Taking ecological function seriously: soil microbial  
740 communities can obviate allelopathic effects of released metabolites. *PLoS One* 4:e4700.  
741 <https://doi.org/10.1371/JOURNAL.PONE.0004700>
- 742 Khamare Y, Chen J, Marble SC (2022) Allelopathy and its application as a weed management tool: a  
743 review. *Front Plant Sci* 13:4766. <https://doi.org/10.3389/FPLS.2022.1034649/BIBTEX>
- 744 Khanh TD, Chung MI, Xuan TD, Tawata S (2005) The exploitation of crop allelopathy in sustainable  
745 agricultural production. *J Agron Crop Sci* 191:172–184. <https://doi.org/10.1111/J.1439-037X.2005.00172.X>
- 747 Kobayashi K (2004) Factors affecting phytotoxic activity of allelochemicals in soil. *Weed Biol Manag* 4:1–  
748 7. <https://doi.org/10.1111/J.1445-6664.2003.00112.X>
- 749 Kong CH, Li HB, Hu F, Xu XH, Wang P (2006) Allelochemicals released by rice roots and residues in soil.  
750 *Plant Soil* 288:47–56. <https://doi.org/10.1007/s11104-006-9033-3>
- 751 Kosmala A, Perlikowski D, Pawłowicz I, Rapacz M (2012) Changes in the chloroplast proteome following  
752 water deficit and subsequent watering in a high- and a low-drought-tolerant genotype of *Festuca*  
753 *arundinacea*. *J Exp Bot* 63:6161–6172. <https://doi.org/10.1093/JXB/ERS265>
- 754 Kruse M, Strandberg B, Strandberg M (2000) Ecological effects of allelopathic plants-a Review. Ministry  
755 of Environment and Energy National Environmental Research Institute 315:1–67
- 756 Kughur GP (2012) The effects of herbicides on crop production and environment in Makurdi local  
757 government area of Benue state, Nigeria. 206–216
- 758 Leishman M, Wright IJ, Moles A, Westoby M (2000) The evolutionary ecology of seed size. In: *Seeds: the*  
759 *ecology of regeneration in plant communities*, 2nd editio. CABI Publishing, UK, pp 31–57
- 760 Li Y, Xu L, Letuma P, Lin W (2020) Metabolite profiling of rhizosphere soil of different allelopathic  
761 potential rice accessions. *BMC Plant Biol* 20:1–21. <https://doi.org/10.1186/S12870-020-02465-6/TABLES/10>
- 763 Li ZH, Wang Q, Ruan X, Pan C De, Jiang DA (2010) Phenolics and plant allelopathy. *Molecules* 15:8933.  
764 <https://doi.org/10.3390/MOLECULES15128933>
- 765 Liebman M, Sundberg DN (2006) Seed mass affects the susceptibility of weed and crop species to  
766 phytotoxins extracted from red clover shoots. *Weed Sci* 54:340–345. <https://doi.org/10.1614/WS-05-54.2.340a>
- 767

- 768 Lin L-Z, Harnly JM (2010) Identification of the phenolic components of chrysanthemum flower  
769 (*Chrysanthemum morifolium* Ramat). Food Chem 120:319–326.  
770 <https://doi.org/10.1016/j.foodchem.2009.09.083>
- 771 Lin Z, Chun-lan Z, Qi-rong S, Yu YF and P (2013) Phenolic acids in decomposing organic materials. Acta  
772 Pedologica Sinica 38:471–475. <https://doi.org/10.11766/TRXB200004250403>
- 773 Lipinska H, Sykut M, Harkot W (2013) The effect of water extracts from leaves of *Festuca rubra*, *F. ovina*  
774 and *F. Arundinacea* on the initial growth and development of other grass species. Acta Agrobot  
775 66:61–70. <https://doi.org/10.5586/AA.2013.023>
- 776 Lipinska H, Sykut M, Kepkowicz A, Jackowska I (2019) Effects of decomposing biomass of *Festuca*  
777 *arundinacea*, *Festuca ovina* and *Festuca rubra* lawn cultivars on growth of other lawn grasses.  
778 Allelopathy Journal 46:107–120. <https://doi.org/10.26651/allelo.j/2019-46-2-1213>
- 779 Lovett J, Ryuntyu M (1992) Allelopathy: broadening the context. Allelopathy 11–19.  
780 [https://doi.org/10.1007/978-94-011-2376-1\\_2](https://doi.org/10.1007/978-94-011-2376-1_2)
- 781 Macías FA, Mejías FJR, Molinillo JMG (2019) Recent advances in allelopathy for weed control: from  
782 knowledge to applications. Pest Manag Sci 75:2413–2436. <https://doi.org/10.1002/PS.5355>
- 783 Majidi MM, Mirlohi A, Amini F (2009) Genetic variation, heritability and correlations of agro-  
784 morphological traits in tall fescue (*Festuca arundinacea* Schreb.). Euphytica 167:323–331.  
785 <https://doi.org/10.1007/S10681-009-9887-6/METRICS>
- 786 Makaure BT, Aremu AO, Gruz J, Magadlela A (2022) Phenolic acids and plant antioxidant capacity  
787 enhance growth, nutrition, and plant–microbe interaction of *Vigna unguiculata* L. (Walp) grown in  
788 acidic and nutrient-deficient grassland and Savanna soils. J Soil Sci Plant Nutr 23:190–203.  
789 <https://doi.org/10.1007/S42729-022-00967-W/METRICS>
- 790 Mašková T, Weiser M (2019) The roles of interspecific variability in seed mass and soil resource  
791 availability in root system development. Plant Soil 435:395–406. <https://doi.org/10.1007/S11104-018-3896-Y/FIGURES/14>
- 793 Misra D, Dutta W, Jha G, Ray P (2023) Interactions and regulatory functions of phenolics in soil-plant-  
794 climate nexus. Agronomy 2023, Vol 13, Page 280 13:280.  
795 <https://doi.org/10.3390/AGRONOMY13020280>
- 796 Ojija F, Arnold SEJ, Treydte AC (2019) Bio-herbicide potential of naturalised *Desmodium uncinatum*  
797 crude leaf extract against the invasive plant species *Parthenium hysterophorus*. Biol Invasions  
798 21:3641–3653. <https://doi.org/10.1007/s10530-019-02075-w>
- 799 Orłowski G, Czarnecka J (2009) Granivory of birds and seed dispersal : viable seeds of *Amaranthus*  
800 *retroflexus* L. recovered from the droppings of the grey partridge (*Perdix perdix* L.). Pol J Ecol Vol.  
801 57:191–196
- 802 Peters elroy J, Luu KT (1985) Allelopathy in tall fescue. pp 273–283
- 803 Piñar Fuentes JC, Leiva F, Cano-Ortiz A, Musarella CM, Quinto-Canas R, Pinto-Gomes CJ, Cano E (2021)  
804 Impact of grass cover management with herbicides on biodiversity, soil cover and humidity in olive

805 groves in the Southern Iberian. *Agronomy* 2021, Vol 11, Page 412 11:412.  
806 <https://doi.org/10.3390/AGRONOMY11030412>

807 Politycka B (1997) Free and glucosylated phenolics, phenol  $\beta$ -glucosyltransferase activity and membrane  
808 permeability in cucumber roots affected by derivatives of cinnamic and benzoic acids. *Acta Physiol*  
809 *Plant* 19:311–317. <https://doi.org/10.1007/S11738-997-0007-8/METRICS>

810 Poudyal S, Cregg BM (2019) Irrigating nursery crops with recycled run-off: a review of the potential  
811 impact of pesticides on plant growth and physiology. *Horttechnology* 29:716–729.  
812 <https://doi.org/10.21273/HORTTECH04302-19>

813 Proestos C, Sereli D, Komaitis M (2006) Determination of phenolic compounds in aromatic plants by RP-  
814 HPLC and GC-MS. *Food Chem* 95:44–52. <https://doi.org/10.1016/j.foodchem.2004.12.016>

815 Qaderi MM, Martel AB, Strugnell CA (2023) Environmental factors regulate plant secondary metabolites.  
816 *Plants* 12:447. <https://doi.org/10.3390/plants12030447>

817 Raihan I, Hirai N, Fujii Y (2018) Plant growth inhibitory activity of *Goniothalamus andersonii* bark  
818 incorporated with soil on selected plants. *Eur Exp Biol* 9:1. <https://doi.org/10.21767/2248-9215.100078>

820 Reigosa M, Gomes AS, Ferreira AG, Borghetti F (2013) Allelopathic research in Brazil. *Acta Bot Brasilica*  
821 27:629–646. <https://doi.org/10.1590/S0102-33062013000400001>

822 Ren X, Yan Z, He X, Li X, Qin B (2017) Allelochemicals from rhizosphere soils of *Glycyrrhiza uralensis*  
823 Fisch: discovery of the autotoxic compounds of a traditional herbal medicine. *Ind Crops Prod*  
824 97:302–307. <https://doi.org/10.1016/j.indcrop.2016.12.035>

825 Rezayian M, Ebrahimzadeh H, Niknam V (2020) Nitric oxide stimulates antioxidant system and osmotic  
826 adjustment in soybean under drought stress. *J Soil Sci Plant Nutr* 20:1122–1132.  
827 <https://doi.org/10.1007/S42729-020-00198-X/METRICS>

828 Rice EL (1984) *Allelopathy*. 2nd Edition, Academic Press, Orlando, 422. - References - Scientific Research  
829 Publishing. Elsevier Science

830 Rice EL (1985) *Allelopathy - An Overview*. *Chemically Mediated Interactions between Plants and Other*  
831 *Organisms* 81–105. [https://doi.org/10.1007/978-1-4757-9658-2\\_4](https://doi.org/10.1007/978-1-4757-9658-2_4)

832 Rizvi SJH, Rizvi V (1992) *Allelopathy : Basic and applied aspects*. 504

833 Romani A, Pinelli P, Galardi C, Sani G, Cimato A, Heimler D (2002) Polyphenols in greenhouse and open-  
834 air-grown lettuce. *Food Chem* 79:337–342. [https://doi.org/10.1016/S0308-8146\(02\)00170-X](https://doi.org/10.1016/S0308-8146(02)00170-X)

835 Sabella E, Aprile A, Negro C, Nicolì F, Nutricati E, Vergine M, Luvisi A, De Bellis L (2020) Impact of climate  
836 change on durum wheat yield. *Agronomy* 2020, Vol 10, Page 793 10:793.  
837 <https://doi.org/10.3390/AGRONOMY10060793>

838 Sahoo TR, Behera B, Paikaray RK, Garnayak LM, Sethi D, Jena S, Raza MB, Panda RK, Song B, Lal MK,  
839 Kumar A (2023) Effects of sunflower residue management options on productivity and profitability  
840 of succeeding rice under different crop establishment methods. *Field Crops Res* 290:108763.  
841 <https://doi.org/10.1016/j.fcr.2022.108763>

842 Sahrir MAS, Yusoff N, Azizan KA, Sahrir MAS, Yusoff N, Azizan KA (2022) Allelopathy activity under  
843 laboratory, greenhouse and field conditions: a review. *AIMS Agriculture and Food* 2023 1:78 8:78–  
844 104. <https://doi.org/10.3934/AGRFOOD.2023004>

845 Sarker U, Oba S (2018) Drought stress enhances nutritional and bioactive compounds, phenolic acids  
846 and antioxidant capacity of *Amaranthus* leafy vegetable. *BMC Plant Biol* 18:1–15.  
847 <https://doi.org/10.1186/S12870-018-1484-1/FIGURES/11>

848 Scavo A, Abbate C, Mauromicale G (2019) Plant allelochemicals: agronomic, nutritional and ecological  
849 relevance in the soil system. *Plant Soil* 442:23–48. <https://doi.org/10.1007/S11104-019-04190-Y>

850 Scavo A, Mauromicale G (2021) Crop allelopathy for sustainable weed management in agroecosystems:  
851 knowing the present with a view to the future. *Agronomy* 2021, Vol 11, Page 2104 11:2104.  
852 <https://doi.org/10.3390/AGRONOMY11112104>

853 Scavo A, Mejías FJR, Chinchilla N, Molinillo JMG, Schwaiger S, Lombardo S, Macías FA, Mauromicale G  
854 (2023) Wheat response and weed-suppressive ability in the field application of a nanoencapsulated  
855 disulfide (DiS-NH<sub>2</sub>) bioherbicide mimic. *Agronomy* 2023, Vol 13, Page 1132 13:1132.  
856 <https://doi.org/10.3390/AGRONOMY13041132>

857 Scavo A, Pandino G, Restuccia A, Mauromicale G (2020a) Leaf extracts of cultivated cardoon as potential  
858 bioherbicide. *Sci Hortic* 261:109024. <https://doi.org/10.1016/J.SCIENTA.2019.109024>

859 Scavo A, Restuccia A, Mauromicale G (2018) Allelopathy: principles and basic aspects for agroecosystem  
860 control. 47–101. [https://doi.org/10.1007/978-3-319-90309-5\\_2](https://doi.org/10.1007/978-3-319-90309-5_2)

861 Scavo A, Rial C, Molinillo JMG, Varela RM, Mauromicale G, Macías FA (2020b) Effect of shading on the  
862 sesquiterpene lactone content and phytotoxicity of cultivated cardoon leaf extracts. *J Agric Food*  
863 *Chem* 68:11946–11953.  
864 [https://doi.org/10.1021/ACS.JAFC.0C03527/ASSET/IMAGES/MEDIUM/JFOC03527\\_0007.GIF](https://doi.org/10.1021/ACS.JAFC.0C03527/ASSET/IMAGES/MEDIUM/JFOC03527_0007.GIF)

865 Schandry N, Becker C (2020) Allelopathic Plants: Models for Studying Plant–Interkingdom Interactions.  
866 *Trends Plant Sci* 25:176–185. <https://doi.org/10.1016/j.tplants.2019.11.004>

867 Shehata SA, El-Metwally IM, Abdelgawad KF, Elkhawaga FA (2022) Efficacy of agro-industrial wastes on  
868 the weed control, nutrient uptake, growth, and yield of onion crop (*Allium cepa* L.). *J Soil Sci Plant*  
869 *Nutr* 22:2707–2718. <https://doi.org/10.1007/S42729-022-00838-4/TABLES/8>

870 Sheibany K, Baghestani Meybodi MA, Atri A (2009) Competitive effects of redroot pigweed (*Amaranthus*  
871 *retroflexus*) on the growth indices and yield of corn. *Weed Biol Manag* 9:152–159.  
872 <https://doi.org/10.1111/j.1445-6664.2009.00333.x>

873 Simpson KJ, Atkinson RRL, Mockford EJ, Bennett C, Osborne CP, Rees M (2021) Large seeds provide an  
874 intrinsic growth advantage that depends on leaf traits and root allocation. *Funct Ecol* 35:2168–  
875 2178. <https://doi.org/10.1111/1365-2435.13871>

876 Singh AA, Rajeswari G, Nirmal LA, Jacob S (2021) Synthesis and extraction routes of allelochemicals from  
877 plants and microbes: a review. *Rev Anal Chem* 40:293–311. [https://doi.org/10.1515/REVAC-2021-0139-0139/ASSET/GRAPHIC/J\\_REVAC-2021-0139\\_FIG\\_005.JPG](https://doi.org/10.1515/REVAC-2021-0139/ASSET/GRAPHIC/J_REVAC-2021-0139_FIG_005.JPG)

- 879 Smith MW, Wolf ME, Cheary BS, Carroll BL (2001) Allelopathy of bermudagrass, tall fescue, redroot  
880 pigweed, and cutleaf evening primrose on pecan. *HortScience* 36:1047–1048.  
881 <https://doi.org/10.21273/HORTSCI.36.6.1047>
- 882 Suksungworn R, Sanevas N, Wongkantrakorn N, Fangern N, Vajrodaya S, Duangsrirai S (2016) Phytotoxic  
883 effect of *Haldina cordifolia* on germination, seedling growth and root cell viability of weeds and  
884 crop plants. *NJAS - Wageningen Journal of Life Sciences* 78:175–181.  
885 <https://doi.org/10.1016/J.NJAS.2016.05.008>
- 886 Taleb MH, Majidi MM, Pirnajmedin F, Maibody SAMM (2023) Plant functional trait responses to cope  
887 with drought in seven cool-season grasses. *Scientific Reports* 2023 13:1 13:1–13.  
888 <https://doi.org/10.1038/s41598-023-31923-y>
- 889 Wagay NA, Rafiq S, Khan A, Kaloo ZA, Malik AR, Pulate P V. (2023) Impact of phenolics on drought stress  
890 and expression of phenylpropanoid pathway genes. In: *Plant Phenolics in Abiotic Stress*  
891 *Management*. Springer Nature Singapore, Singapore, pp 265–285
- 892 Wang C, Liu Z, Wang Z, Pang W, Zhang L, Wen Z, Zhao Y, Sun J, Wang ZY, Yang C (2022a) Effects of  
893 autotoxicity and allelopathy on seed germination and seedling growth in *Medicago truncatula*.  
894 *Front Plant Sci* 13:2481. <https://doi.org/10.3389/FPLS.2022.908426/BIBTEX>
- 895 Wang C, Wu B, Jiang K (2019a) Allelopathic effects of Canada goldenrod leaf extracts on the seed  
896 germination and seedling growth of lettuce reinforced under salt stress. *Ecotoxicology* 28:103–116.  
897 <https://doi.org/10.1007/s10646-018-2004-7>
- 898 Wang D, Chen J, Xiong X, Wang S, Liu J (2019b) Allelopathic effects of *Cinnamomum migao* on seed  
899 germination and seedling growth of its associated species *Liquidambar formosana*. *Forests* 10:535.  
900 <https://doi.org/10.3390/f10070535>
- 901 Wang K, Wang T, Ren C, Dou P, Miao Z, Liu X, Huang D, Wang K (2022b) Aqueous extracts of three herbs  
902 allelopathically inhibit lettuce germination but promote seedling growth at low concentrations.  
903 *Plants* 11:486. <https://doi.org/10.3390/plants11040486>
- 904 Weidenhamer JD (1996) Distinguishing resource competition and chemical interference: overcoming the  
905 methodological impasse. *Agron J* 88:866–875.  
906 <https://doi.org/10.2134/AGRONJ1996.00021962003600060005X>
- 907 Wenxiong L, Changxun F, Linkun W, Sheng L (2017) Research on and application of rice allelopathy and  
908 crop allelopathic autotoxicity in China. In: *Agroecology in China*. CRC Press, Boca Raton, FL : Taylor  
909 & Francis, 2016. | Series: Advances in agroecology, pp 161–196
- 910 Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ (2002) Plant ecological strategies: some leading  
911 dimensions of variation between species. *Annu Rev Ecol Syst* 33:125–159.  
912 <https://doi.org/10.1146/annurev.ecolsys.33.010802.150452>
- 913 Wu GQ, Feng R-J, Shui Q-Z (2016) Effect of osmotic stress on growth and osmolytes accumulation in  
914 sugar beet (*Beta vulgaris* L.) plants. *Plant Soil Environment* 62:189–194.  
915 <https://doi.org/10.17221/101/2016-PSE>

916 Wu Z, Liu Q, Zhong Y, Xiao P, Yu F (2022) Additions of *Liriodendron sino-americanum* leaf powder change  
917 soil auality, improve *Sarcandra glabra* growth, and alter microbial community. J Soil Sci Plant Nutr  
918 22:4983–4995. <https://doi.org/10.1007/S42729-022-00975-W/METRICS>

919 Xiao Z, Le C, Xu Z, Gu Z, Lv J, Shamsi IH (2017) Vertical leaching of allelochemicals affecting their  
920 bioactivity and the microbial community of soil. J Agric Food Chem 65:7847–7853.  
921 <https://doi.org/10.1021/acs.jafc.7b01581>

922 Xiao Z, Lu S, Xu Z (2019) Biochemistry of allelopathic plant residues in soil. Ekoloji 28:2997–3006

923 Xu C, Ge Z, Li C, Wan F, Xiao X (2019) Inhibition of harmful algae *Phaeocystis globosa* and *Prorocentrum*  
924 *donghaiense* by extracts of coastal invasive plant *Spartina alterniflora*. Sci Total Environ 696:.  
925 <https://doi.org/10.1016/J.SCITOTENV.2019.133930>

926 Xu Y, Wang G, Jin J, Liu J, Zhang Q, Liu X (2009) Bacterial communities in soybean rhizosphere in  
927 response to soil type, soybean genotype, and their growth stage. Soil Biol Biochem 41:919–925.  
928 <https://doi.org/10.1016/j.soilbio.2008.10.027>

929 Yamamoto M, Kato-Noguchi H (2015) Potential of rattail fescue powder for weed management.  
930 Environmental Control in Biology 53:43–46. <https://doi.org/10.2525/ECB.53.43>

931 Yang L, Wen KS, Ruan X, Zhao YX, Wei F, Wang Q (2018) Response of plant secondary metabolites to  
932 environmental factors. Molecules 23:.. <https://doi.org/10.3390/MOLECULES23040762>

933 Zhong S, Xu Z, Cheng H, Wang Y, Yu Y, Du D, Wang C (2023) Does drought stress intensify the allelopathy  
934 of invasive woody species *Rhus typhina* L.? Trees 1–9. [https://doi.org/10.1007/s00468-022-02385-](https://doi.org/10.1007/s00468-022-02385-y)  
935 [y](https://doi.org/10.1007/s00468-022-02385-y)

936 Zubay P, Kunzelmann J, Ittész A, Németh Zámboriné É, Szabó K (2021) Allelopathic effects of leachates of  
937 *Juglans regia* L., *Populus tremula* L. and juglone on germination of temperate zone cultivated  
938 medicinal and aromatic plants. Agroforestry Systems 95:431–442. [https://doi.org/10.1007/S10457-](https://doi.org/10.1007/S10457-020-00572-9/FIGURES/4)  
939 [020-00572-9/FIGURES/4](https://doi.org/10.1007/S10457-020-00572-9/FIGURES/4)

940

**Table 1** Background information on the tall fescue genotypes selected for the production of the aqueous shoot extracts

Origin	Variety	Genotype code
Iran, Kohkiluye, Yasuj	3Moderate-Half Sib	3M-HS
Hungary, unknown	11 Moderate -Half Sib	11M-HS
Iran, Isfahan, Fozve	17 Moderate -Half Sib	17M-HS
Iran, Kohkiluye, Yasuj	3Early-Half Sib	3E-HS
Iran, Isfahan, Mobarake	4Early-Half Sib	4E-HS
Poland, unknown	22Moderate-Half Sib	22M-HS
Iran, Isfahan, Fozve	9Early-Half Sib	9E-HS
Iran, Isfahan, Yazdabad	1Moderate-Half Sib	1M-HS
Iran, Kohkiluye, Yasuj	6Late-Half Sib	6L-HS
USA, New Jersey	10Early-Half Sib	10E-HS
Hungary, Csesznek	14Early-Half Sib	14E-HS
<b>Poland, unknown</b>	<b>23 Moderate -Half Sib</b>	<b>23M-HS</b>
Iran, Isfahan, Yazdabad	1Early-Half Sib	1E-HS
Hungary, unknown	12Late-Half Sib	12L-HS
Iran, Isfahan, Fozve	20Late-Half Sib	20L-HS
Iran, Isfahan, Fozve	21 Moderate -Half Sib	21M-HS

The genotype highlighted in bold (**23M**) was assessed for the allelopathic activity of its rhizospheric soil, as well as its aqueous shoot extract.

**Table 2** Analysis of variance of the effect of the tall fescue genotypes, irrigation conditions and extract concentrations upon the germination indices of *Lactuca sativa* L.

Source of variation	df	Mean square			
		Germination	Hypocotyl	Radicle	Dry weight
Genotypes	15	230**	719**	56.7**	17.5**
Irrigation conditions	1	33321**	20043**	2234**	7.56**
Extract concentrations	5	93179**	35814**	3823**	360**
Genotypes × Irrigation conditions	15	25.2**	43.5*	5.17*	0.523**
Genotypes × Extract concentrations	75	67.5**	127**	8.39*	2.78**
Irrigation conditions × Extract concentrations	5	2208**	1123**	165**	4.32**
Genotypes × Irrigation conditions × Extract concentrations	75	23.8*	30.2*	2.65**	0.611**
Error	384	2.4	1.91	0.2	0.06
Coefficient of variation		15.6	12.8	13.2	18.4

\*=significant at 5% level of probability. \*\*=significant at 1% level of probability.

**Table 3** Mean comparison for seed germination indices of *Lactuca sativa* L. affected by the extract concentration and irrigation conditions of the tall fescue genotypes

	Germination (%)	Hypocotyl (mm)	Radicle (mm)	Dry weight (mg plant <sup>-1</sup> )
Extract concentration				
Control	88.27 ± 0.17 <sup>a</sup>	68.43 ± 0.27 <sup>a</sup>	21.30 ± 0.09 <sup>a</sup>	8.02 ± 0.05 <sup>a</sup>
12.5	82.74 ± 0.66 <sup>b</sup>	58.34 ± 1.07 <sup>b</sup>	18.72 ± 0.33 <sup>b</sup>	7.54 ± 0.08 <sup>b</sup>
25	70.61 ± 0.82 <sup>c</sup>	52.18 ± 0.95 <sup>c</sup>	15.52 ± 0.37 <sup>c</sup>	6.51 ± 0.06 <sup>c</sup>
50	39.31 ± 1.43 <sup>d</sup>	36.24 ± 0.70 <sup>d</sup>	12.84 ± 0.31 <sup>d</sup>	5.86 ± 0.05 <sup>d</sup>
75	27.95 ± 1.45 <sup>e</sup>	26.95 ± 1.20 <sup>e</sup>	7.78 ± 0.30 <sup>e</sup>	4.42 ± 0.15 <sup>e</sup>
100	12.90 ± 0.90 <sup>f</sup>	18.54 ± 1.16 <sup>f</sup>	4.89 ± 0.28 <sup>f</sup>	2.91 ± 0.16 <sup>f</sup>
LSD(%5)	0.447	0.393	0.124	0.071
Irrigation conditions				
Control	61.26 ± 1.56 <sup>a</sup>	49.34 ± 1.10 <sup>a</sup>	15.49 ± 0.37 <sup>a</sup>	6.64 ± 0.11 <sup>a</sup>
stress	46.04 ± 1.85 <sup>b</sup>	37.55 ± 1.13 <sup>b</sup>	11.56 ± 0.35 <sup>b</sup>	5.32 ± 0.12 <sup>b</sup>
LSD(%5)	0.253	0.221	0.071	0.042

In each column, the values which have similar letters do not have significant difference based on LSD test. (data were reported as mean ± standard error)

**Table 4** Mean comparison of the seed germination indices of *Lactuca sativa* L. affected by the interaction effects of the tall fescue genotypes and the irrigation conditions

Irrigation conditions	Genotypes	Germination (%)	Hypocotyl (mm)	Radicle (mm)	Dry weight (mg plant <sup>-1</sup> )
Control	3M	61.77 ± 5.98	51.99 ± 3.69	16.01 ± 1.33	6.59 ± 0.36
	11M	61.72 ± 5.96	40.49 ± 4.46	13.42 ± 1.26	5.38 ± 0.39
	17M	62.55 ± 6.09	52.68 ± 3.79	16.43 ± 1.43	6.72 ± 0.38
	3E	62.77 ± 6.13	52.77 ± 3.79	16.54 ± 1.41	6.74 ± 0.38
	4E	60.61 ± 6.02	42.81 ± 4.13	15.06 ± 1.38	6.11 ± 0.31
	22M	62.44 ± 6.08	52.57 ± 3.77	16.37 ± 1.39	6.69 ± 0.38
	9E	62.38 ± 6.56	52.40 ± 3.75	16.28 ± 1.37	6.66 ± 0.37
	1M	62.77 ± 6.13	52.88 ± 3.81	16.63 ± 1.43	6.76 ± 0.39
	6L	61.61 ± 5.96	40.50 ± 3.71	12.69 ± 1.53	4.81 ± 0.59
	10E	62.83 ± 6.14	53.02 ± 3.83	16.72 ± 1.44	6.78 ± 0.39
	14E	62.27 ± 6.06	52.25 ± 3.72	16.19 ± 1.36	6.65 ± 0.37
	23M	50.50 ± 9.11	39.84 ± 7.11	12.43 ± 2.22	4.63 ± 0.81
	1E	63.22 ± 6.20	53.50 ± 3.89	16.83 ± 1.45	6.26 ± 0.29
	12L	63.00 ± 6.18	53.24 ± 3.86	15.72 ± 1.22	6.65 ± 0.36
	20L	57.72 ± 7.50	46.43 ± 5.75	14.48 ± 1.82	5.83 ± 0.67
21M	61.94 ± 6.02	52.16 ± 3.71	16.10 ± 1.35	6.62 ± 0.37	
	LSD(%5)	1.08	1.09	0.34	0.20
Drought stress	3M	46.33 ± 7.37	39.07 ± 4.09	11.64 ± 1.27	5.84 ± 0.37
	11M	44.83 ± 7.79	33.93 ± 4.79	10.00 ± 1.57	4.41 ± 0.53
	17M	47.22 ± 7.43	39.97 ± 4.16	12.20 ± 1.34	5.97 ± 0.39
	3E	47.27 ± 7.45	40.12 ± 4.17	12.31 ± 1.35	5.98 ± 0.39
	4E	46.27 ± 7.27	32.50 ± 4.45	12.08 ± 1.38	5.53 ± 0.40
	22M	47.16 ± 7.42	39.81 ± 4.15	12.09 ± 1.32	5.94 ± 0.39
	9E	46.88 ± 7.43	39.67 ± 4.14	11.99 ± 1.30	5.92 ± 0.38
	1M	47.33 ± 7.46	40.32 ± 4.19	12.43 ± 1.36	6.00 ± 0.40
	6L	44.72 ± 7.67	32.51 ± 5.50	10.53 ± 1.56	4.29 ± 0.56
	10E	47.38 ± 7.47	40.44 ± 4.19	12.53 ± 1.38	6.02 ± 0.40
	14E	46.77 ± 7.40	39.48 ± 4.12	11.88 ± 1.29	5.91 ± 0.38
	23M	41.11 ± 8.48	30.90 ± 5.93	9.16 ± 1.79	4.26 ± 0.75
	1E	47.72 ± 7.50	40.82 ± 4.24	12.66 ± 1.39	5.9 ± 0.36
	12L	47.66 ± 7.49	40.60 ± 4.22	12.32 ± 1.26	5.97 ± 0.38
	20L	41.38 ± 8.51	31.36 ± 5.99	9.31 ± 1.81	4.43 ± 0.78
21M	46.66 ± 7.37	39.24 ± 4.10	11.75 ± 1.28	5.89 ± 0.38	
	LSD(%5)	0.96	0.67	0.23	0.14

LSD at P = 0.05 level. (data were reported as mean ± standard error)

**Table 5** Mean comparison of the seed germination percentages of *Lactuca sativa* L. affected by the interaction effects of the tall fescue genotypes, irrigation conditions and extract concentrations.

Irrigation conditions	Genotypes	Extract concentrations					
		Control	12.5%	25%	50%	75%	100%
Control		88.74					
	3M		88 ± 0.57	78.23 ± 0.88	52.61 ± 0.66	42.12 ± 0.56	22.31 ± 0.34
	11M		87.63 ± 0.81	75.19 ± 0.64	50.56 ± 0.59	40.09 ± 0.45	19.04 ± 0.19
	17M		89.12 ± 0.88	79 ± 1.03	54 ± 1.11	42.27 ± 0.50	21.86 ± 0.37
	3E		89.67 ± 0.85	76.31 ± 0.90	54.20 ± 1.27	42.11 ± 0.48	22.37 ± 0.46
	4E		86.57 ± 2.11	74.93 ± 2.23	50 ± 0.18	38.95 ± 0.72	18.32 ± 0.16
	22M		89.29 ± 0.90	79 ± 0.99	53.55 ± 1.22	42 ± 0.44	21.62 ± 0.29
	9E		89.41 ± 0.82	79.09 ± 1.12	53.30 ± 1.45	42.17 ± 0.58	22.41 ± 0.35
	1M		89.71 ± 0.85	79.25 ± 0.91	54.35 ± 1.16	42.23 ± 0.33	21.93 ± 0.25
	6L		87.22 ± 1.20	76.04 ± 0.88	52.27 ± 0.78	39.38 ± 0.57	19.01 ± 0.11
	10E		88.78 ± 0.57	79.87 ± 0.76	54.53 ± 1.51	42.46 ± 0.88	22.53 ± 0.41
	14E		89 ± 0.64	79 ± 1.07	53.18 ± 1.38	42.01 ± 0.60	22.81 ± 0.44
	23M		87 ± 1.50	70.42 ± 0.33	48.74 ± 0.95	0 ± 0	0 ± 0
	1E		89.07 ± 1.07	79.11 ± 0.92	55 ± 1.11	43.21 ± 0.66	22.93 ± 0.50
	12L		89.35 ± 0.90 <sup>a</sup>	79.25 ± 0.92	54.42 ± 1.34	42.75 ± 0.64	22.70 ± 0.37
	20L		87.5 ± 1.18	75.57 ± 0.66	49.83 ± 0.88	38 ± 0.55	0 ± 0
21M		89 ± 0.55	78.17 ± 0.74	52.46 ± 0.64	41.97 ± 0.58	22.03 ± 0.28	
Drought stress		88.74					
	3M		76.29 ± 1.33	62.31 ± 0.69	25.75 ± 0.64	18.68 ± 0.15	7.73 ± 0.17
	11M		75.25 ± 1.30	61.15 ± 0.58	23.20 ± 0.38	17.08 ± 0.04	0 ± 0
	17M		77.59 ± 1.20	63.37 ± 0.33	25.71 ± 0.44	19.21 ± 0.07	7.63 ± 0.07
	3E		77.62 ± 1.16	63.24 ± 0.33	26.08 ± 0.57	18.93 ± 0.19	8.75 ± 0.09
	4E		73.31 ± 2.04	59.74 ± 0.09	23.86 ± 0.74	16.92 ± 0.04	5.25 ± 0.08
	22M		76.93 ± 1.18	63.40 ± 0.29	26.11 ± 0.55	19.23 ± 0.16	8.58 ± 0.06
	9E		78.18 ± 1.37	62.95 ± 0.52	25.54 ± 0.89	20 ± 0.29	7.91 ± 0.11
	1M		77.58 ± 1.24	63.70 ± 0.31	25.89 ± 0.57	18.78 ± 0.17	8.51 ± 0.09
	6L		75.59 ± 1.66	61.19 ± 0.59	23.33 ± 0.36	16.02 ± 0.13	0 ± 0
	10E		77.47 ± 1.20	64.22 ± 0.11	26.07 ± 0.59	17.90 ± 0.05	8.68 ± 0.19
	14E		77 ± 1.48	62.59 ± 0.45	25.24 ± 0.91	18.32 ± 0.23	8.40 ± 0.06
	23M		74.32 ± 1.54	58.47 ± 0.77	19.87 ± 0.40	0 ± 0	0 ± 0
	1E		78.17 ± 1.50	64.32 ± 0.30	25.98 ± 0.62	19.41 ± 0.09	8.74 ± 0.11
	12L		78.09 ± 1.52	64.19 ± 0.34	26.45 ± 0.68	18.94 ± 0.11	8.68 ± 0.09
	20L		75.43 ± 1.85	62 ± 0.60	22.51 ± 0.50	0 ± 0	0 ± 0
21M		76.49 ± 1.48	63 ± 0.62	25.30 ± 0.84	18.78 ± 0.08	7.84 ± 0.08	

LSD at P = 0.05 level for germination percentage = 0.72. (data were reported as mean ± standard error)

**Table 6** Mean comparison for the dry weight of *Lactuca sativa* L. affected by the interaction effects of the tall fescue genotypes, irrigation conditions and extract concentrations

Irrigation conditions	Genotypes	Extract concentrations					
		Control	12.5%	25%	50%	75%	100%
Control		8.46					
	3M	8.36 ± 0.19	7.39 ± 0.15	6.32 ± 0.19	5.41 ± 0.13	4.19 ± 0.11	
	11M	7.05 ± 0.02	6.04 ± 0.02	5.42 ± 0.01	4.73 ± 0.29	2.42 ± 0.31	
	17M	8.64 ± 0.18	7.49 ± 0.16	6.44 ± 0.20	5.38 ± 0.12	4.21 ± 0.10	
	3E	8.68 ± 0.19	7.22 ± 0.18	6.37 ± 0.21	5.47 ± 0.17	4.30 ± 0.12	
	4E	7.57 ± 0.21	6.30 ± 0.19	6.08 ± 0.04	4.90 ± 0.06	3.13 ± 0.05	
	22M	8.58 ± 0.14	7.18 ± 0.15	6.43 ± 0.21	5.42 ± 0.12	4.20 ± 0.11	
	9E	8.55 ± 0.16	7.35 ± 0.11	6.38 ± 0.20	5.28 ± 0.11	4.16 ± 0.14	
	1M	8.71 ± 0.18	7.24 ± 0.18	6.47 ± 0.19	5.53 ± 0.13	4.25 ± 0.08	
	6L	7.07 ± 0.07	6.14 ± 0.02	5.02 ± 0.34	4.04 ± 0.09	0 ± 0	
	10E	8.74 ± 0.19	7.25 ± 0.17	6.36 ± 0.19	5.52 ± 0.14	4.28 ± 0.08	
	14E	8.50 ± 0.18	7.13 ± 0.13	6.29 ± 0.18	5.45 ± 0.16	4.15 ± 0.17	
	23M	7.25 ± 0.25	6.18 ± 0.18	4.78 ± 0.14	0 ± 0	0 ± 0	
	1E	8.50 ± 0.20	6.97 ± 0.18	6.50 ± 0.16	5.39 ± 0.12	4.32 ± 0.09	
	12L	8.37 ± 0.35	7.26 ± 0.17	6.51 ± 0.17	5.43 ± 0.11	4.17 ± 0.15	
	20L	8.24 ± 0.17	7.02 ± 0.14	6.27 ± 0.18	4.84 ± 0.14	0 ± 0	
21M	8.43 ± 0.18	7.42 ± 0.19	6.35 ± 0.15	5.37 ± 0.10	4.19 ± 0.10		
Drought stress		8.46					
	3M	6.88 ± 0.01	6.30 ± 0.04	5.67 ± 0.06	4.69 ± 0.15	3.36 ± 0.02	
	11M	6.69 ± 0.34	5.57 ± 0.01	5.02 ± 0.01	3.72 ± 0.32	0 ± 0	
	17M	7.14 ± 0.04	6.42 ± 0.03	5.73 ± 0.05	4.70 ± 0.14	3.59 ± 0.04	
	3E	7.19 ± 0.08	6.44 ± 0.05	5.75 ± 0.04	4.70 ± 0.16	3.40 ± 0.06	
	4E	6.35 ± 0.05	6.01 ± 0.02	5.06 ± 0.01	3.71 ± 0.02	2.08 ± 0.01	
	22M	7.10 ± 0.07	6.39 ± 0.07	5.73 ± 0.07	4.64 ± 0.13	3.38 ± 0.07	
	9E	7.08 ± 0.06	6.28 ± 0.03	5.59 ± 0.05	4.56 ± 0.11	2.92 ± 0.03	
	1M	7.20 ± 0.07	6.46 ± 0.04	5.81 ± 0.04	4.79 ± 0.16	3.47 ± 0.05	
	6L	6.27 ± 0.33	5.56 ± 0.02	4.42 ± 0.02	2.52 ± 0.33	0 ± 0	
	10E	7.25 ± 0.09	6.49 ± 0.06	5.78 ± 0.04	4.67 ± 0.18	3.50 ± 0.04	
	14E	7.06 ± 0.06	6.35 ± 0.08	5.71 ± 0.05	4.53 ± 0.14	3.32 ± 0.05	
	23M	6.19 ± 0.03	5.04 ± 0.29	3.67 ± 0.03	0 ± 0	0 ± 0	
	1E	7.41 ± 0.05	6.53 ± 0.07	5.80 ± 0.08	4.58 ± 0.12	3.41 ± 0.03	
	12L	7.28 ± 0.10	6.48 ± 0.02	5.63 ± 0.04	4.65 ± 0.17	3.25 ± 0.02	
	20L	6.73 ± 0.02	5.72 ± 0.03	5.59 ± 0.03	0 ± 0	0 ± 0	
21M	6.99 ± 0.05	6.32 ± 0.01	5.70 ± 0.05	4.78 ± 0.15	3.52 ± 0.02		

LSD at P = 0.05 level for dry weight = 0.17. (data were reported as mean ± standard error)

**Table 7** Analysis of variance for the effect of target species (*Lactuca sativa* L. and *Amaranthus retroflexus* L.) and culture medium (rhizospheric soil of tall fescue genotype 23M) on seed germination indices in the pot experiments

Source of variation	Degree of freedom	Germination	Hypocotyl length	Radicle length	Dry weight
Target species	1	658.74**	5658**	15.7**	5.07**
Culture medium	3	1505.2**	546**	57.3*	19.71**
Target species × Culture medium	3	337.72**	48.2**	1.54**	0.147**
Experimental error	40	0.079	0.006	0.01	0.004
CV%		15.4	11.2	13.1	13.8

\*=Significant at 5 % level of probability. \*\*=Significant at 1 % level of probability.

**Table 8** Mean comparison for seed germination indices of *Lactuca sativa* L. and *Amaranthus retroflexus* L. affected by the interaction effects of target species and culture medium (rhizospheric soil of tall fescue genotype 23M).

Target species	Culture medium	Germination (%)	Hypocotyl length (mm)	Radicle length (mm)	Dry weight (mg plant <sup>-1</sup> )
<i>Lactuca sativa</i> L.	Soil (control)	98.67 ± 0.25 <sup>a</sup>	44.41 ± 0.32 <sup>d</sup>	21.87 ± 0.09 <sup>b</sup>	8.42 ± 0.06 <sup>c</sup>
	Soil (drought stress)	98.52 ± 0.51 <sup>a</sup>	44.19 ± 1.07 <sup>d</sup>	21.50 ± 0.07 <sup>b</sup>	8.37 ± 0.09 <sup>c</sup>
	Soil (normal)	77.42 ± 0.63 <sup>c</sup>	40.13 ± 0.96 <sup>e</sup>	17.92 ± 0.26 <sup>d</sup>	7.19 ± 0.03 <sup>d</sup>
	Soil amended with plant powder	65.29 ± 0.95 <sup>f</sup>	30.56 ± 1.80 <sup>f</sup>	15.07 ± 0.41 <sup>e</sup>	5.11 ± 0.13 <sup>f</sup>
<i>Amaranthus retroflexus</i> L.	Soil (control)	82.42 ± 0.23 <sup>b</sup>	69.47 ± 0.29 <sup>a</sup>	22.23 ± 0.05 <sup>a</sup>	9.12 ± 0.05 <sup>a</sup>
	Soil (drought stress)	81.97 ± 0.86 <sup>b</sup>	69.39 ± 0.66 <sup>a</sup>	22.10 ± 0.07 <sup>a</sup>	9.1 ± 0.05 <sup>a</sup>
	Soil (normal)	75.49 ± 0.21 <sup>d</sup>	60.04 ± 0.57 <sup>b</sup>	19.38 ± 0.22 <sup>c</sup>	8.62 ± 0.04 <sup>b</sup>
	Soil amended with plant powder	70.14 ± 0.95 <sup>e</sup>	51.23 ± 0.82 <sup>c</sup>	17.50 ± 0.35 <sup>e</sup>	6.22 ± 0.11 <sup>e</sup>

In each column, the values which have similar letters do not have significant difference. soil control= farm soil in which *F. arundinacea* did not grow, soil drought stress= rhizospheric soil of *F. arundinacea* (genotype 23M) under severe drought stress conditions, soil normal= rhizospheric soil of *F. arundinacea* (genotype 23M) under normal irrigation conditions, soil amended with plant powder= 50 wt.% mixture of *F. arundinacea* (genotype 23M) plant powder and 50% soil control. (data were reported as mean ± standard error)

**Table 9** Total phenolic content of aqueous extracts from culture medium (rhizospheric soil of tall fescue genotype 23M)

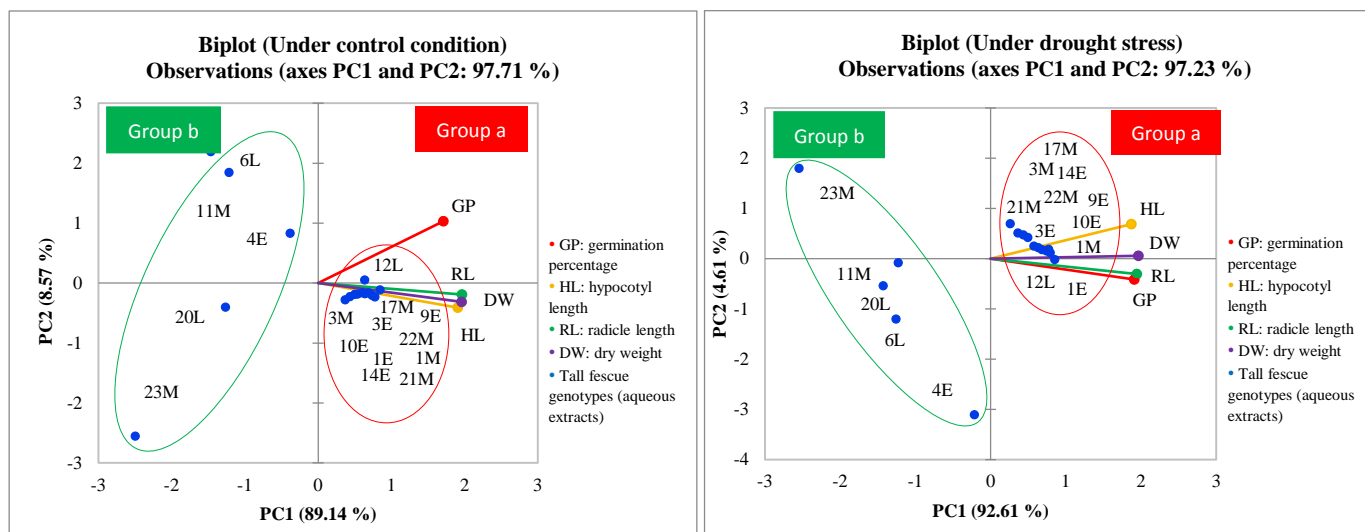
Culture medium	Total phenol (mg GAE g <sup>-1</sup> soil dry matter)
Soil (control)	0.06 ± 0.01 <sup>d</sup>
Soil (drought stress)	0.62 ± 0.03 <sup>c</sup>
Soil (normal)	2.19 ± 0.15 <sup>b</sup>
Soil amended with plant powder	5.15 ± 0.17 <sup>a</sup>
LSD(%5)	0.38

In each column, the values which have similar letters do not have significant difference based on LSD test. soil control= farm soil in which *F. arundinacea* did not grow, soil drought stress= rhizospheric soil of *F. arundinacea* (genotype 23M) under severe drought stress conditions, soil normal= rhizospheric soil of *F. arundinacea* (genotype 23M) under normal irrigation conditions, soil amended with plant powder= 50 wt.% mixture of *F. arundinacea* (genotype 23M) plant powder and 50% soil control. (data were reported as mean ± standard error)

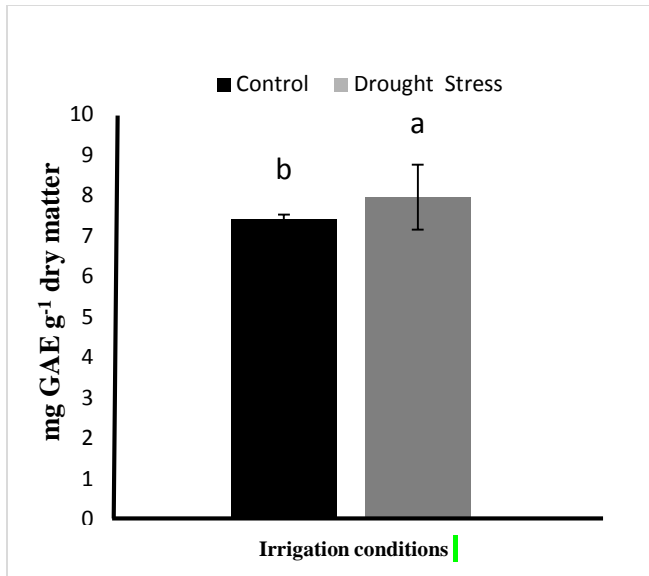
**Table 10** The phenolic acid compound composition of aqueous extracts from culture medium (rhizospheric soil of tall fescue genotype 23M)

Phenolic acids compound	Soil (control)	Soil (drought stress)	Soil (normal)	Soil amended with plant powder
Gallic acid	13.6	12.6	28.6	22.2
Chlorogenic acid	nd	nd	nd	61.1
Caffeic acid	nd	nd	nd	224.5
<i>p</i> -Cumarinic acid	nd	nd	134.8	112.7
Ferulic acid	nd	nd	90.3	108.2
Apigenin acid	nd	nd	104.9	111.1
Vanillic acid	nd	nd	nd	98.3
Syringic acid	nd	198.6	nd	255.4
4- Hydroxybenzoic acid	nd	nd	79	77.8
Total	13.6	211.2	437.6	1071.3

(nd): Not detected. The values are expressed in µg g<sup>-1</sup> soil dry matter. soil control= farm soil in which *F. arundinacea* did not grow, soil drought stress= rhizospheric soil of *F. arundinacea* (genotype 23M) under severe drought stress conditions, soil normal= rhizospheric soil of *F. arundinacea* (genotype 23M) under normal irrigation conditions, soil amended with plant powder= 50 wt.% mixture of *F. arundinacea* (genotype 23M) plant powder and 50% soil control. (data were reported as mean ± standard error)



**Fig. 1** Principal component analysis (PCA) of seed germination indices in 16 tall fescue genotypes under both control and drought stress conditions. GP: germination percentage, HL: hypocotyl length, RL: radicle length, DW: dry weight. The blue and red dots indicate the genotypes and trait vectors (arrows), respectively. The two components contributed to more than 97% of the total variation in both the control conditions and severe drought stress. The PCA categorized the genotypes into two groups, group a and group b. Group a included the genotypes 12L, 3M, 21M, 14E, 9E, 22M, 17M, 3E, 1M, 10E, 1E that all displayed a small inhibitory effect on the studied traits. Group b comprised of genotypes 6L, 11M, 4E, 20L, 23M, which had a significant inhibitory effect upon the traits studied. From group b, genotype 23M was selected for further study due to its strong allelopathic activity. The origin of the genotypes is shown in Table 1.



**Fig. 2** Comparison of the mean total phenolic content of aqueous extracts from the 23M genotype of tall fescue, cultivated under (a) drought stress and (b) control irrigation conditions. The vertical bars indicate the standard error. LSD=0.23



Click here to access/download  
**Supplementary Material**  
graphical abstract (1).docx





Click here to access/download  
**Supplementary Material**  
text track - Copy.docx

