

Phenolic Composition and In Vitro Allelopathic Potential of *Inula graveolens* Extracts

Mesut Sırrı¹ and Melih Yılar^{2*}

¹Siirt University, Kurtalan Vocational School, Department of Crop and Animal Production, Siirt, Türkiye

²Department of Plant Protection, Faculty of Agriculture, Kırşehir Ahi Evran University, Kırşehir, Türkiye

*Corresponding author. E-mail: melih.yilar@ahievran.edu.tr

ABSTRACT

Inula graveolens (L.) Desf. is an aromatic plant that grows naturally in the wild. Several studies have shown that this species is rich in bioactive compounds, including phenols, terpenoids, tannins, and flavonoids. In this context, the present study aimed to investigate the allelopathic potential of aqueous, methanol, and ethanol extracts of *I. graveolens* against wheat (*Triticum aestivum* L.) and several ecologically significant invasive plant species. The phytochemical composition of the plant was analyzed by LC-MS/MS method using a Shimadzu Nexera model ultra-high-performance liquid chromatograph (UHPLC), enabling quantitative evaluation of 56 phytochemicals. Based on the HPLC results, chlorogenic acid (8.444 mg/g extract), quinic acid (1.702 mg/g), and gentisic acid (1.183 mg/g) were identified as the predominant constituents in the methanol extract of the plant. In allelopathy experiments, aqueous extracts of *I. graveolens* (1%, 3%, 5%, 10%, and 20%) and methanol and ethanol extracts (150, 300, 600 ppm) were tested for their biological activity against *Triticum aestivum* L., *Amaranthus palmeri* S. Watson, and *Cephalaria syriaca* (L.) Schrad., *Ipomoea purpurea* (L.) Roth, and *Vaccaria pyramidata* Medik. The extracts demonstrated significant inhibitory effects on both seed germination and biomass production of the test species.

Keywords: plant extracts, phenolic compounds, phytotoxic effect, *Inula graveolens*.

INTRODUCTION

The rapid growth of the global population has increased the need for sustainable management of agricultural lands, placing significant pressure on agricultural production. Ensuring global food security, utilizing existing arable lands efficiently, and obtaining high-quality yields while maintaining ecological balance are among the most critical goals of modern agriculture. However, weeds pose a significant threat to crop production by competing with cultivated plants for essential resources such as nutrients, water, and light, thereby significantly reducing both yield and quality (Özer et al., 2001). Although synthetic herbicides are undeniably effective in weed management, their negative environmental, soil, and human health impacts necessitate the development of more environmentally friendly and sustainable alternatives (Kropff and Walter, 2000; Tekeli et al., 2006).

Allelopathy has emerged as a promising strategy in agroecosystems. Allelopathy is defined as the effect of chemical compounds released by a plant on the growth, development, or germination of neighboring plants (Önen, 2003). Plant-derived allelochemicals can interfere with seed germination and early seedling development and therefore may contribute to the suppression of undesirable vegetation. Laboratory bioassays using aqueous plant extracts have demonstrated that the magnitude of allelopathic effects may vary according to the plant species, plant organ, extract concentration, and target species (Bilalis et al., 2013; Konstantinović et al., 2014; Horuz and Üremiş, 2024). *Inula graveolens* (L.) Desf. [Synonym: *Dittrichia graveolens* (L.) Greuter] (Asteraceae) is an aromatic species native to the Mediterranean climate zone and notable for its allelopathic potential, represents a key example from Türkiye's rich plant biodiversity.

Inula graveolens is a naturally growing aromatic plant in Mediterranean regions (Seca et al., 2014). In addition to its traditional uses, it has recently gained attention in agriculture for its allelopathic effects (Omezzine et al., 2011a). Research has shown that extracts derived from the aerial parts of the plant can inhibit the germination and growth of various weed seeds, suggesting its potential as a natural herbicide (Omezzine et al., 2011b). The allelopathic activity is primarily attributed to monoterpenes (volatile oils) and other secondary metabolites such as alkaloids, tannins, and glycosides (Kruse et al., 2000). These compounds can suppress the germination of weed seeds, prevent their dominance in the soil, and thus contribute to preserving biodiversity in agricultural lands.

Seed germination is a critical stage in the impact of weeds on agricultural productivity (Özer et al., 2001). Allelochemicals with inhibitory properties can control weed populations by interfering with germination. Essential oils, phenolic compounds, and terpenoids found in allelopathic plants can disrupt metabolic processes during germination, reducing germination rates (Önen, 2003; Uludağ, 2006; Uludağ et al., 2017). Root growth is frequently particularly sensitive to plant-derived phytotoxic compounds, making root and shoot measurements useful complementary parameters in allelopathic bioassays. The use of germination and seedling-growth bioassays has also been successfully applied to evaluate the phytotoxicity of plant extracts obtained with different solvents (Perisoara et al., 2023). Studies have shown that extracts of *I. graveolens* can significantly inhibit or completely suppress the germination of specific weed species (Omezzine et al., 2011a, b). These extracts, especially effective in controlling invasive species, are considered environmentally friendly alternatives to conventional herbicides. The allelochemicals present in the plant decompose rapidly in soil, reducing the risk of toxic accumulation and enabling effective weed control without harming soil microbiota. Furthermore, the potential

applicability of *Inula* species extracts in both organic and conventional farming systems offers valuable opportunities for developing sustainable agricultural strategies (Omezzine et al., 2011c; Dor and Hershenhorn, 2012; Bayhan et al., 2017; Horoz and Üremiş, 2024).

Despite the increasing evidence concerning the allelopathic potential of *I. graveolens*, information is still limited regarding the relationship between its phenolic composition and the phytotoxic effects of aqueous, methanol, and ethanol extracts on both cultivated and invasive plant species. Therefore, the present study aimed to investigate the phenolic composition of *I. graveolens* naturally growing in Siirt Province, Türkiye, using LC-MS/MS analysis, and to evaluate the phytotoxic effects of aqueous, methanol, and ethanol extracts prepared from its aerial parts (stems, leaves, and flowers) on seed germination and seedling development of *Triticum aestivum* L., *Amaranthus palmeri* S. Watson, *Cephalaria syriaca* (L.) Schrad., *Ipomoea purpurea* (L.) Roth, and *Vaccaria pyramidata* Medik.

MATERIAL AND METHODS

Materials

The primary plant material used in this study, *I. graveolens*, was collected during its flowering period in July 2024 from the Kezer Campus of Siirt University (Figure 1). The plant material was dried under shaded conditions in the laboratory and stored for later use. Weed seeds used as test plants in the study were collected during the growing seasons between 2020 and 2023 from agricultural fields in the Siirt provinces (Figure 1). The plant materials used in the experiment were collected and identified by Mesut Sırrı.

Methods

Determination of phenolic compounds in *Inula graveolens*

Preparation of Plant Extracts for LC-MS/MS: Ten grams of powdered plant material were extracted using the maceration method at room temperature in 50 mL of

methanol separately and in triplicate (each for six hours). After filtration, the solvents were evaporated under vacuum at 30°C using a rotary evaporator, yielding methanol extracts. Internal standards Ferulic acid D3 (20 mg/L), Rutin D3 (1 mg/L), and Quercetin D3 (5

mg/L) were added to the dried extracts. These were diluted with methanol to a final concentration of 1000 mg/L and filtered through 0.2 µm syringe filters before LC-MS/MS analysis (Yılmaz, 2020).

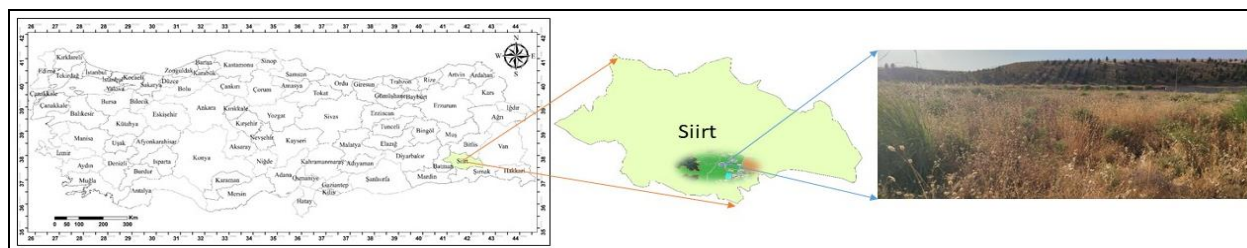


Figure 1. The study site was selected in the Kezer region, where *I. graveolens* grows naturally in the wild

Preparation of the Test Solution and Chromatographic Conditions: An LC-MS/MS analytical methodology was developed and optimized specifically for *I. graveolens* extracts in this study, based on the method reported by Yılmaz (2020). A total of 56 phenolic standards were used as references.

Allelopathy studies

Preparation of Water Extracts: The dried plant material, consisting of stems, leaves, and flowers, was ground into powder using a milling machine. To prepare 1%, 3%, 5%, 10%, and 20% extracts, 10, 30, 50, 100, and 200 grams of powdered plant material were added to 1000 mL of distilled water in glass containers. These mixtures were shaken on a rotary shaker at 120 rpm for 24 hours. After filtration through coarse filter paper to remove solids, the extracts were centrifuged at 4000 rpm for 15 minutes to achieve full separation of the remaining residues. The resulting supernatants were stored at +4°C in a refrigerator until used in the bioassays.

Preparation of Ethanol and Methanol Extracts: One hundred grams of powdered plant material (stems, leaves, and flowers) were weighed into 1000 mL Erlenmeyer flasks, and 600 mL of ethanol or methanol was added. The mixtures were shaken at 120 rpm at room temperature (24°C) for 48 hours. The extracts were filtered through coarse filter paper and centrifuged at 4000 rpm for

15 minutes to separate solid residues. Solvents were removed by evaporation using a rotary evaporator at 32°C until solid residues were obtained. The dried extracts were dissolved in DMSO + distilled water to prepare concentrations of 150, 300, and 600 ppm (Kombıçak et al., 2023).

Effect of Plant Extracts (Ethanol, Methanol, and Water) on Seed Germination and Seedling Development of Test Plants: To eliminate possible pathogen contamination, seeds of the test species [*Triticum aestivum* L., *Amaranthus palmeri* S. Watson, *Cephalaria syriaca* (L.) Schrad., *Ipomoea purpurea* (L.) Roth, and *Vaccaria pyramidata* Medik.] were surface-sterilized by immersion in 1% sodium hypochlorite solution for five minutes, followed by rinsing three times with sterile distilled water (Horuz and Üremiş, 2024). The effects of *I. graveolens* extracts on the test plants were evaluated in 9-cm sterile Petri dishes lined with double-layer filter paper. Twenty-five morphologically uniform seeds of each species were placed in each Petri dish. Aqueous extracts (1%, 3%, 5%, 10%, and 20%) and ethanol/methanol extracts (150, 300, and 600 ppm) were applied at a volume of 6 mL per Petri dish. Control groups received only distilled water or distilled water with DMSO (Sigma 99.9% purity; 1mg/1ml). The dishes were incubated at 24°C, the optimum temperature for germination, for one to three weeks. At the end of the

incubation period, germination rate, shoot length, and root length were measured to determine the phytotoxic effects of the extracts (Önen, 2003). The experiment was conducted with three replicates and two independent repetitions.

Data Evaluation: The inhibition rate of seed germination caused by plant extracts was calculated using the following formula:

$$IR(\%) = (C - U/C) \times 100$$

where IR represents the inhibition rate, C is the number of seeds germinated in the control group, and U is the number of seeds germinated in the treatment group exposed to the plant extract.

Data Analysis: The experimental data were analyzed statistically using ANOVA (Analysis of Variance) in the SPSS 15 statistical software. Differences between means were determined using Duncan's multiple range test.

RESULTS AND DISCUSSION

Phytochemical content (of *Inula graveolens* extract)

In our study, 56 phenolic compounds were used as reference standards to identify the phenolic profile of *I. graveolens* methanol extract in LC-MS/MS analysis. Eleven compounds were identified in the methanol extract (Table 1). The average concentrations of these compounds are shown in Table 1. The main phenolic compounds in the methanol extract of *I. graveolens* were chlorogenic acid (8.444 mg/g extract), quinic acid (1.702 mg/g), gentisic acid (1.183 mg/g), and protocatechuic acid (1.062 mg/g). Other identified compounds included gallic acid, protocatechuic aldehyde, cynarin, caffeic acid, salicylic acid, hesperidin, and acacetin. However, the remaining reference phenolics were not detected in any of the extracts of *I. graveolens* (Table 1 and Figure 2).

Table 1. Phytochemical composition of *Inula graveolens* extract

No	Analytes	(mg/g extract)	No	Analytes	(mg/g extract)
1	Quinic acid	1,702	29	Salicylic acid	0,711
2	Fumaric aid	N.D.*	30	Cyranoside	N.D.
3	Aconitic acid	N.D.	31	Miquelianin	N.D.
4	Gallic acid	0,034	32	Rutin-D3-IS	N.A.
5	Epigallocatechin	N.D.	33	Rutin	N.D.
6	Protocatechuic acid	1,062	34	isoquercitrin	N.D.
7	Catechin	N.D.	35	Hesperidin	0,078
8	Gentisic acid	1,183	36	o-Coumaric acid	N.D.
9	Chlorogenic acid	8,444	37	Genistin	N.D.
10	Protocatechuic aldehyde	0,056	38	Rosmarinic acid	N.D.
11	Tannic acid	N.D.	39	Ellagic acid	N.D.
12	Epigallocatechin gallate	N.D.	40	Cosmosiin	N.D.
13	Cynarin	0,185	41	Quercitrin	N.D.
14	4-OH Benzoic acid	N.D.	42	Astragalin	N.D.
15	Epicatechin	N.D.	43	Nicotiflorin	N.D.
16	Vanillic acid	N.D.	44	Fisetin	N.D.
17	Caffeic acid	0,318	45	Daidzein	N.D.
18	Syringic acid	N.D.	46	Quercetin-D3-IS	N.A.
19	Vanillin	N.D.	47	Quercetin	N.D.
20	Syringic aldehyde	N.D.	48	Naringenin	N.D.
21	Daidzin	N.D.	49	Hesperetin	N.D.
22	Epicatechin gallate	N.D.	50	Luteolin	N.D.
23	Piceid	N.D.	51	Genistein	N.D.
24	p-Coumaric acid	N.D.	52	Kaempferol	N.D.
25	Ferulic acid-D3-IS ^h	N.A.**	53	Apigenin	N.D.
26	Ferulic acid	N.D.	54	Amentoflavone	N.D.
27	Sinapic acid	N.D.	55	Chrysin	N.D.
28	Coumarin	N.D.	56	Acacetin	0,034

*N.D.: Not detected, **N.A.: Not applicable.

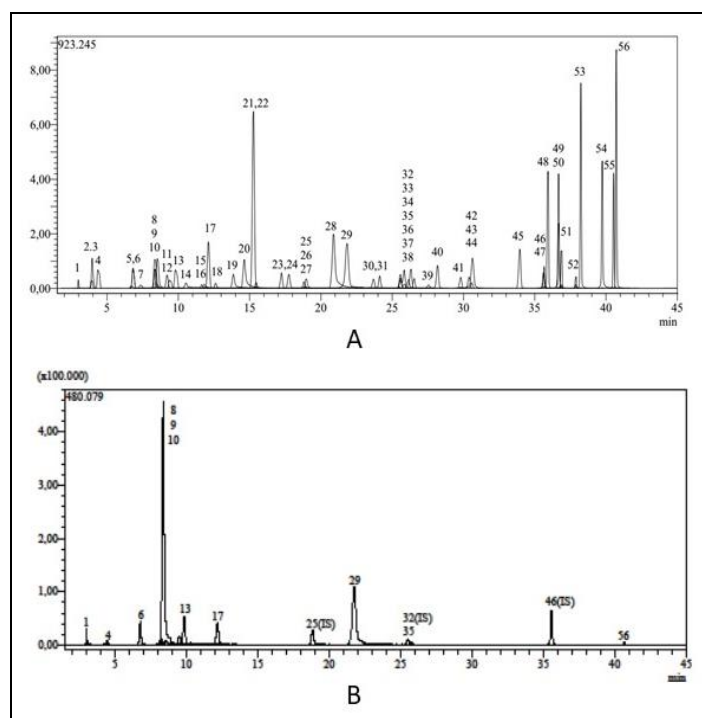


Figure 2. A) Chromatogram of all the standard phenolic compounds, B) Chromatogram of *I. graveolens* species methanol extract

Inula graveolens contains crucial phytochemical constituents closely associated with its observed biological activities. Studies on crude extracts of *I. graveolens* have shown that its antioxidant (Akpulat and Şahinler, 2021), cytotoxic, and antibacterial activities (Topçu et al., 1993) are attributed to the presence of bioactive compounds such as sesquiterpenes, eudesmane-type acids, aromatic compounds, and phenolics (Silinsin and Bursal, 2018; Koç et al., 2021). Silinsin and Bursal (2018) identified chlorogenic acid (2167 ± 106 ppb) and quinic acid (845 ± 41 ppb) as the major phenolic compounds in *I. graveolens* extract. Additionally, small amounts of hyperoside, protocatechuic acid, quercetin, 4-OH-benzoic acid, hesperetin, salicylic acid, p-coumaric acid, and trans-caffeic acid were detected, with 27 phenolic compounds identified. In samples collected from Tokat province, chlorogenic acid (3.5 mg/g), salicylic acid (0.055 mg/g), caffeic acid (0.054 mg/g), protocatechuic acid (0.026 mg/g), hesperidin (0.016 mg/g), and rutin (0.016 mg/g) were identified in the methanol extract (Erenler et al., 2023). These findings indicate a considerable phytochemical diversity in the plant, which may be linked to its potential therapeutic effects.

Allelopathic Effects of *Inula graveolens* Extract

The allelopathic activity of aqueous, methanol, and ethanol extracts of *Inula graveolens* was evaluated on various test plant species using Petri dish bioassays. According to the experimental results, *I. graveolens* exhibited allelopathic potential; however, the extent of this effect varied depending on the type of extract, application dose, and test plant species (Tables 2-4). In all extract treatments, seed germination and seedling growth inhibition in the test plants increased with rising extract concentrations. The inhibitory effects of *I. graveolens* extracts on seed germination, root elongation, and shoot development were statistically significant ($\alpha = 0.05$).

The aqueous extract of *I. graveolens* completely inhibited seed germination, root, and shoot development of *I. purpurea*, *V. pyramidata*, and *C. syriaca* at the 10% concentration, compared to the control. Among the test species, *A. palmeri* was the most sensitive to the aqueous extract; even the 3% concentration completely suppressed seed germination and seedling growth (Table 2; Figures 3-5). A comparison of germination and post-germination growth responses

revealed that, particularly at low and moderate concentrations, seedling development was more sensitive to *I. graveolens* extracts than seed germination. For example, the 1% aqueous extract did not cause any change in the germination rate of *V. pyramidata*, with germination reaching 100% in both the control and treatment groups. In contrast, the same treatment reduced root length from 65.20 mm to 28.75 mm, resulting in approximately 56% inhibition. Similarly, although the 150 ppm

methanol extract did not significantly affect the germination of *T. aestivum* and *I. purpurea*, it markedly suppressed root and shoot development. This difference was more clearly observed in *C. syriaca* treated with the 150 ppm ethanol extract. In this species, the germination rate decreased from 30% to 28%, and this reduction was not statistically significant; however, root and shoot lengths decreased by approximately 54% (Table 2; Table 3).

Table 2. Effect of plant water extract on seed germination and seedling development of test plants

Test plants	Dose	Germination (%)	Root (mm)	Shoot (mm)
<i>Triticum aestivum</i>	Control	100.0 ^a ±0.0	85.23 ^a ±2.2	68.99 ^a ±1.3
	1%	96.0 ^{ab} ±1.6	43.23 ^b ±0.2	40.56 ^b ±0.1
	3%	92.0 ^{bc} ±2.8	33.72 ^c ±0.01	30.95 ^c ±0.02
	5%	85.0 ^c ±4.1	23.49 ^d ±1.7	20.54 ^d ±0.7
	10%	48.0 ^d ±2.8	9.64 ^e ±0.7	5.66 ^e ±0.6
	20%	40.0 ^e ±1.6	2.65 ^f ±0.4	2.80 ^f ±0.2
<i>Ipomoea purpurea</i>	Control	96.0 ^a ±1.6	58.24 ^a ±0.7	97.1 ^a ±1.5
	1%	84.0 ^b ±1.6	46.62 ^b ±5.9	76.86 ^b ±10.3
	3%	76.0 ^c ±4.3	33.22 ^c ±0.0	47.0 ^c ±0.02
	5%	72.0 ^c ±1.6	22.23 ^d ±1.1	20.8 ^d ±0.1
	10%	0.0 ^d ±0.0	0.0 ^e ±0.0	0.0 ^e ±0.0
	20%	0.0 ^d ±0.0	0.0 ^e ±0.0	0.0 ^e ±0.0
<i>Vaccaria pyramidata</i>	Control	100.0 ^a ±0.0	65.2 ^a ±3.2	35.20 ^a ±2.06
	1%	100.0 ^a ±0.0	28.75 ^b ±0.2	33.62 ^a ±0.0
	3%	20.0 ^b ±1.6	6.26 ^c ±0.7	15.40 ^b ±0.4
	5%	20.0 ^b ±0.0	5.23 ^c ±0.3	13.82 ^b ±0.3
	10%	0.0 ^c ±0.0	0.0 ^d ±0.0	0.0 ^c ±0.0
	20%	0.0 ^c ±0.0	0.0 ^d ±0.0	0.0 ^c ±0.0
<i>Amaranthus palmeri</i>	Control	100.0 ^a ±0.0	33.0 ^a ±1.6	24.56 ^a ±0.2
	1%	72.0 ^b ±2.82	23.58 ^b ±1.6	23.28 ^b ±0.8
	3%	0.0 ^c ±0.0	0.0 ^c ±0.0	0.0 ^c ±0.0
	5%	0.0 ^c ±0.0	0.0 ^c ±0.0	0.0 ^c ±0.0
	10%	0.0 ^c ±0.0	0.0 ^c ±0.0	0.0 ^c ±0.0
	20%	0.0 ^c ±0.0	0.0 ^c ±0.0	0.0 ^c ±0.0
<i>Cephalaria syriaca</i>	Control	64.0 ^a ±5.23	91.16 ^a ±7.5	60.44 ^a ±4.04
	1%	28.0 ^b ±3.65	25.32 ^b ±5.65	26.35 ^b ±5.93
	3%	10.0 ^{bc} ±5.7	4.02 ^{bc} ±2.32	5.33 ^c ±3.07
	5%	5.0 ^c ±3.0	3.45 ^{bc} ±2.04	4.24 ^c ±2.58
	10%	0.0 ^c ±0.0	0.0 ^c ±0.0	0.0 ^c ±0.0
	20%	0.0 ^c ±0.0	0.0 ^c ±0.0	0.0 ^c ±0.0

*Means in the same column with the same letter were not significantly different by ANOVA $\alpha = 0.05$).

The methanol and ethanol extracts, which exhibited less pronounced effects on the test plants than the aqueous extract, still demonstrated statistically significant inhibitory

effects on seed germination and seedling development relative to the control. Moreover, the degree of inhibition increased with higher extract concentrations (Table 3).

Table 3. Effect of plant methanol extract on seed germination and seedling development of test plants

Test plants	Dose (ppm)	Germination (%)	Root (mm)	Shoot (mm)
<i>Triticum aestivum</i>	Control	96.0 ^{a*} ±1.6	96.25 ^a ±3.9	71.10 ^a ±6.4
	150	96.0 ^a ±2.3	73.30 ^b ±6.1	50.93 ^b ±3.3
	300	80.0 ^a ±4.3	58.44 ^c ±2.5	44.04 ^b ±3.9
	600	74.0 ^a ±2.6	49.96 ^c ±3.6	37.33 ^b ±2.9
<i>Ipomoea purpurea</i>	Control	92.0 ^a ±1.6	54.49 ^a ±2.5	86.36 ^a ±4.8
	150	92.0 ^a ±0.0	47.0 ^{ab} ±4.9	64.94 ^{ab} ±4.3
	300	83.0 ^b ±1.9	43.71 ^{ab} ±3.2	53.56 ^{ab} ±1.9
	600	83.0 ^b ±1.9	36.28 ^b ±6.9	41.23 ^b ±1.2
<i>Vaccaria pyramidata</i>	Control	96.0 ^a ±1.6	69.77 ^a ±1.07	47.08 ^a ±4.8
	150	52.0 ^b ±2.8	45.45 ^b ±1.8	36.47 ^a ±0.7
	300	40.0 ^c ±1.6	34.23 ^c ±0.7	34.66 ^a ±1.1
	600	3.0 ^d ±3.0	0.93 ^d ±0.9	9.14 ^b ±3.1
<i>Amaranthus palmeri</i>	Control	100.0 ^a ±0.0	32.94 ^a ±1.03	25.94 ^a ±0.4
	150	64.0 ^b ±1.6	22.30 ^b ±0.7	24.42 ^a ±0.4
	300	48.0 ^c ±1.6	17.39 ^c ±0.5	19.55 ^b ±1.08
	600	16.0 ^d ±1.6	9.27 ^d ±1.6	11.05 ^c ±1.5
<i>Cephalaria syriaca</i>	Control	58.0 ^a ±10.8	90.10 ^a ±8.5	64.10 ^a ±2.7
	150	51.0 ^a ±14.0	61.52 ^b ±5.4	50.67 ^a ±5.4
	300	4.0 ^b ±2.3	19.80 ^c ±1.4	18.44 ^b ±10.6
	600	0.0 ^b ±0.0	0.0 ^c ±0.0	0.0 ^b ±0.0

*Means in the same column with the same letter were not significantly different by ANOVA ($\alpha = 0.05$).

At the highest concentration (600 ppm), the methanol extract of *I. graveolens* inhibited seed germination of *Cephalaria syriaca*, *V. pyramidata*, *A. palmeri*, *T. aestivum*, and *I. purpurea* by 100%, 96.87%, 84%, 22.91%, and 9.78%, respectively, than the control (Figure 6). Similar inhibitory effects were observed on root and shoot development of the test species (Figures 6 and 7). Among the tested plants, *I. purpurea* was the most tolerant to the methanol extract, followed by *T. aestivum*, *A. palmeri*, and *C. syriaca* (Figures 6, 7, and 8).

The ethanol extract of *I. graveolens* exhibited statistically significant ($\alpha = 0.05$) phytotoxic effects on the test plant species (Table 4). In terms of seed germination, *T. aestivum* was the most tolerant species to the ethanol extract, with only a 12% inhibition rate, while *A. palmeri* and *C. syriaca* were identified as the most sensitive species, both showing 100% inhibition (Figure 9). The ethanol extract also inhibited seed germination of *I. purpurea* and *V. pyramidata* by 29.16% and 96.87%, respectively (Figure 9).

Table 4. Effect of plant ethanol extract on seed germination and seedling development of test plants

Test plants	Dose (ppm)	Germination (%)	Root (mm)	Shoot (mm)
<i>Triticum aestivum</i>	Control	100.0 ^{a*} ±0.0	77.25 ^a ±2.9	49.2 ^a ±6.8
	150	96.0 ^{ab} ±0.0	58.11 ^b ±6.4	34.25 ^{ab} ±3.06
	300	94.0 ^{ab} ±2.5	43.26 ^c ±4.8	29.34 ^b ±6.05
	600	88.0 ^b ±4.3	33.07 ^c ±3.7	27.82 ^b ±5.71
<i>Ipomoea purpurea</i>	Control	96.0 ^a ±1.6	60.10 ^a ±1.8	69.32 ^a ±8.4
	150	84.0 ^b ±1.6	48.89 ^{ab} ±5.1	54.01 ^{ab} ±15.2
	300	76.0 ^c ±3.2	48.63 ^{ab} ±4.8	47.81 ^{ab} ±15.0
	600	68.0 ^d ±1.6	41.23 ^b ±0.03	18.90 ^b ±0.03
<i>Vaccaria pyramidata</i>	Control	96.0 ^a ±1.6	58.51 ^a ±2.5	24.67 ^a ±3.1
	150	52.0 ^b ±2.8	25.22 ^b ±0.9	20.56 ^a ±4.6
	300	40.0 ^c ±1.6	20.86 ^b ±3.07	10.74 ^b ±3.07
	600	3.0 ^d ±3.0	0.0 ^c ±0.0	0.0 ^c ±0.0

Table 4. (continued)

Test plants	Dose (ppm)	Germination (%)	Root (mm)	Shoot (mm)
<i>Amaranthus palmeri</i>	Control	96.0 ^a ±0.0	22.94 ^a ±0.8	18.34 ^a ±2.6
	150	56.0 ^b ±2.3	15.33 ^b ±3.2	13.86 ^a ±1.13
	300	19.0 ^c ±11.0	2.68 ^c ±1.58	2.43 ^b ±1.42
	600	0.0 ^d ±0.0	0.0 ^c ±0.0	0.0 ^b ±0.0
<i>Cephalaria syriaca</i>	Control	30.0 ^a ±1.1	78.38 ^a ±8.2	51.48 ^a ±7.4
	150	28.0 ^a ±1.6	36.07 ^b ±4.08	23.52 ^b ±0.4
	300	11.0 ^b ±6.4	3.15 ^c ±1.8	2.08 ^c ±1.2
	600	0.0 ^c ±0.0	0.0 ^c ±0.0	0.0 ^c ±0.0

*Means in the same column with the same letter were not significantly different by ANOVA (a = 0.05).

Regarding root and shoot development, the 600 ppm concentration of the ethanol extract completely inhibited root and shoot elongation of *V. pyramidata*, *A. palmeri*, and *C. syriaca*. At the same concentration, the ethanol extract inhibited root and shoot elongation of *T. aestivum* by 57.19% and 43.45%, and of *I. purpurea* by 31.39% and 72.73%, respectively, than the control (Figures 10 and 11). This study demonstrated

that aqueous, methanol, and ethanol extracts of *I. graveolens* exert significant allelopathic inhibitory effects on the germination, root, and shoot development of five tested weed species. The degree of inhibition varied depending on the type of extract and the applied concentration, with the highest concentrations consistently causing the strongest suppression across all parameters.

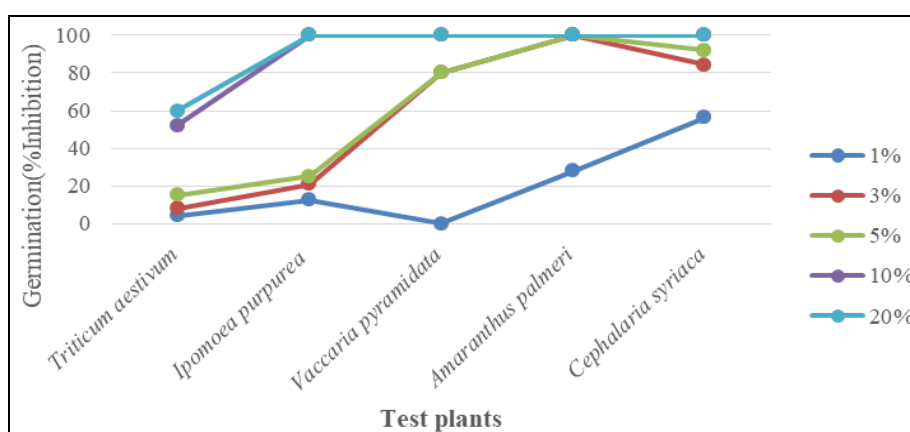


Figure 3. Percentage inhibition rate of water extract on seed germination of test plants

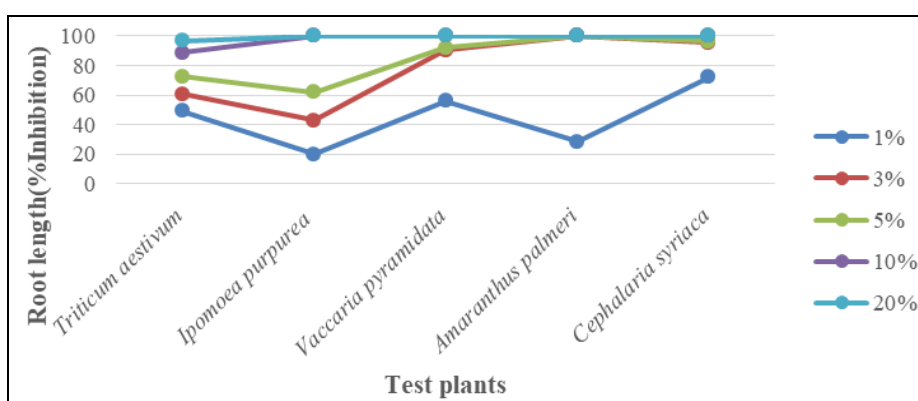


Figure 4. Percentage inhibition rate of water extract on root development of test plants

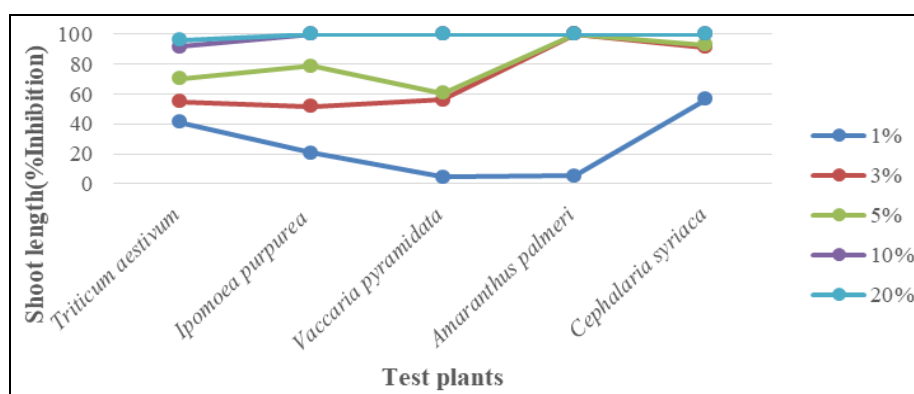


Figure 5. Percentage inhibition rate of water extract on the shoot growth of test plants

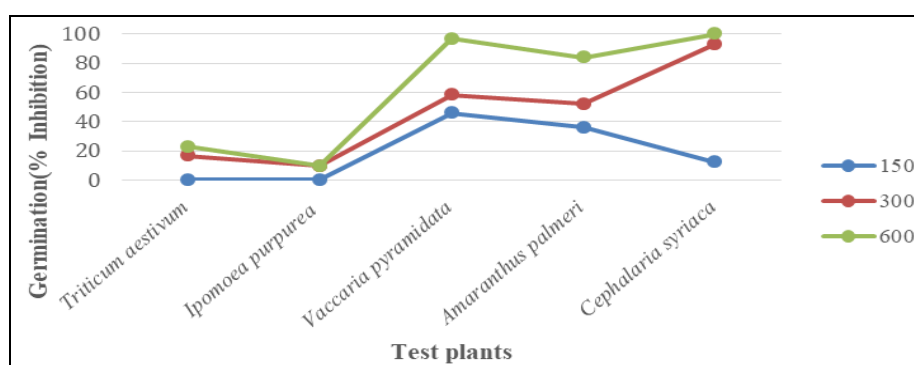


Figure 6. Percentage inhibition rate of plant methanol extract on seed germination of test plants

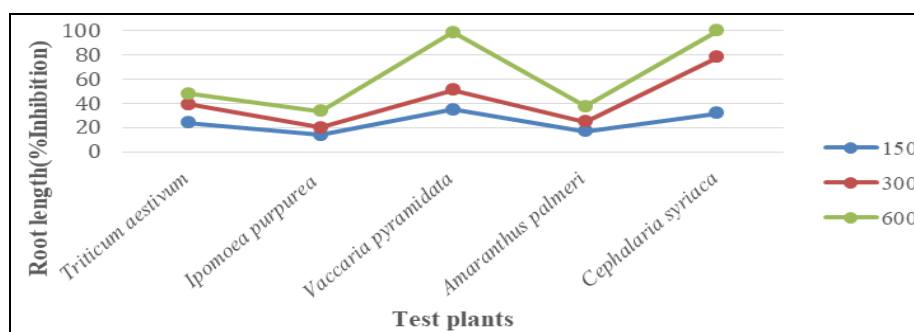


Figure 7. Percentage inhibition rate of plant methanol extract on root development of test plants

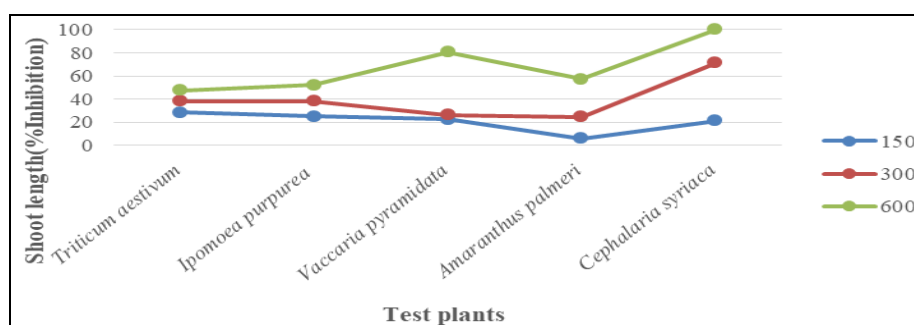


Figure 8. Percentage inhibition rate of shoot growth in test plants by plant methanol extract

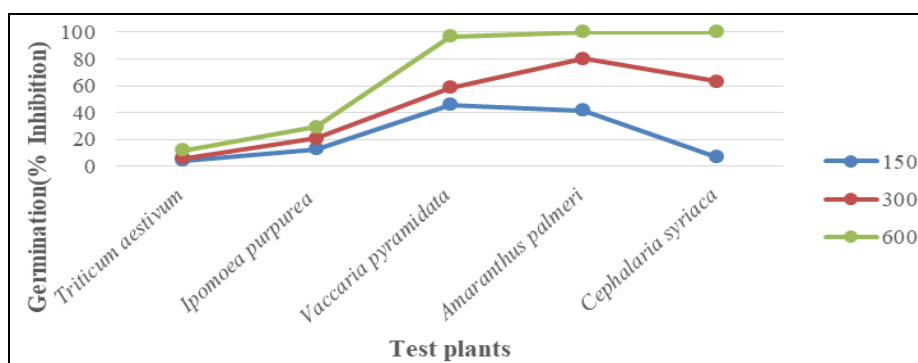


Figure 9. Percentage inhibition rate of plant ethanol extract on seed germination of test plants

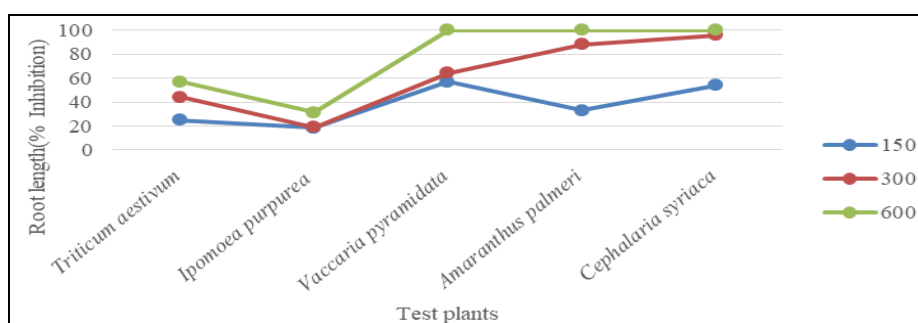


Figure 10. Inhibition rate of plant ethanol extract on root development of test plants

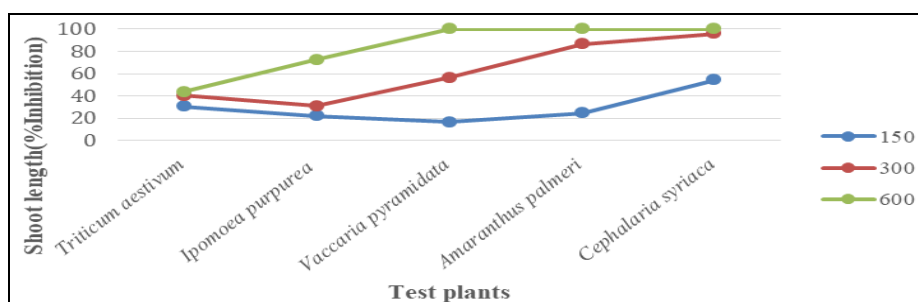


Figure 11. Percentage inhibition rate of shoot growth in test plants by plant ethanol extract

The allelopathic potential of *Inula graveolens* was evaluated on both a cultivated crop (*Triticum aestivum*) and various weed species (*I. purpurea*, *V. pyramidata*, *A. palmeri*, *C. syriaca*). Research has shown that extracts obtained using water and organic solvents have inhibitory effects on seed germination and seedling development. In a laboratory study, aqueous (10-40 g/L) and organic (3-6 mg/mL) extracts derived from the root, shoot, and flower parts of *I. graveolens* were tested on *Lactuca sativa*, *Raphanus sativus*, *Peganum harmala*, and *Silybum marianum*. The results revealed that especially shoot and flower extracts inhibited seedling growth by reducing germination rates, while root extracts stimulated growth in

some species. Moreover, flower extracts exerted more potent inhibitory effects than shoot extracts. Similar outcomes were reported in soil applications, where adding extracts significantly reduced germination rates and seedling length (Omezzine et al., 2011a).

Mammadov et al. (2023) reported that methanol extracts (20 mg/mL) of *I. graveolens* exhibited toxic effects on *Tubifex tubifex* comparable to the commercial anthelmintic drug andazole (10 mg/mL). Anžlovar and Anžlovar (2024) found that diluted doses of 10% aqueous *I. graveolens* extract reduced root length more than shoot length in Tartary buckwheat seedlings and caused morphological changes such as root

thickening and darkening, with effects shown to be dose-dependent. Similarly, Almhemed et al. (2021) reported that 2%, 6%, and 10% aqueous extracts of the plant suppressed germination and growth of weeds, including *Amaranthus retroflexus*, *Portulaca oleracea*, *Lolium multiflorum*, *Sorghum halepense*, and *Cuscuta campestris*. Although the degree of inhibition varied among species, the suppression of germination was generally more pronounced than that of growth.

Grašič et al. (2016) showed that 5% aqueous extracts delayed and significantly reduced germination of *T. aestivum* and *Ambrosia artemisiifolia*. Kitiş et al. (2017) reported that aqueous extracts of *I. graveolens* and *I. viscosa* exerted various levels of allelopathic effects on *Vicia sativa*, *A. retroflexus*, and *P. oleracea*. Dor and Hershenhorn (2012) highlighted the potential of dried leaves and leaf extracts of *I. viscosa* for organic farming and found high sensitivity in *C. campestris*, *A. retroflexus*, and *Sinapis arvensis*. Omezzine et al. (2011b) suggested that the phytotoxic compounds found in *I. graveolens* contribute to the ecological invasiveness of the *Inula* genus. *I. viscosa* leaf extract has been shown to exhibit vigorous antifungal activity (Bayhan et al., 2017) and allelopathic effects against weeds (Horuz and Üremiş, 2024). These results indicate that the allelochemicals present in *I. graveolens* extracts may exert stronger phytotoxic effects on early post-germination seedling development than on the initiation of germination. The relatively greater tolerance of seeds to the treatments may be associated with the physical protection provided by the seed coat and the utilization of internal reserves stored in the endosperm or cotyledons by the embryo during the early stages of germination. In contrast, the newly developing radicles are in direct and continuous contact with the extract, which may make metabolically active processes such as cell division and elongation, membrane integrity, enzymatic activity, and water and nutrient uptake more susceptible to the effects of allelochemicals. The present findings are also consistent with those of previous studies. Allelochemicals can inhibit

seed germination, restrict nutrient uptake, and consequently adversely affect seedling growth and development (Cheng et al., 2021).

Liu and Lovett (1993) reported that allelochemicals may have a greater effect on radicle development than on germination rate. Similarly, Bagheri and Cici (2015) found that although *Ailanthus altissima* extracts had a limited effect on the germination of *Lepidium sativum* seeds, they significantly reduced radicle length. Omezzine et al. (2011b) reported that the addition of *Inula* leaves and flowers to soil at a rate of 2.5 g/100 g inhibited root and shoot development of *Silybum marianum* by 86-100% and 88-100%, respectively. When considered together with these studies, the stronger suppression of root and shoot development than germination observed in the present study may be attributed to the direct and prolonged exposure of radicles to phytotoxic constituents, including the phenolic compounds identified in the extracts.

The findings demonstrate that the phytotoxic effects of *I. graveolens* extracts are not limited to the inhibition of germination, but also substantially affect physiological processes associated with early seedling development. Nevertheless, advanced physiological and biochemical analyses involving oxidative stress indicators, membrane damage, antioxidant enzyme activities, photosynthetic pigments, and nutrient uptake are required to elucidate the mechanisms underlying the observed growth inhibition. The literature indicates that the effects of allelopathy on seed germination and seedling growth may vary depending on the developmental stage and the response of the target species (Zhang et al., 2021; Wang et al., 2022). These findings highlight the potential of *Inula* species as natural herbicides.

CONCLUSIONS

In this study, the methanol extract of *Inula graveolens* was analysed by LC-MS/MS enabling the qualitative and quantitative characterization of its major detected phenolic compounds. Chlorogenic acid was identified as the predominant compound, followed by quinic acid, gentisic acid, and

protocatechuic acid. Furthermore, the methanol, ethanol, and aqueous extracts of the plant exhibited strong allelopathic activity depending on both the test plant species and the extract dose. The particularly pronounced effects observed against *Amaranthus palmeri*, *Cephalaria syriaca*, and *Vaccaria pyramidata* indicate that *I. graveolens* extracts have promising in vitro phytotoxic and allelopathic potential. However, further studies under soil, greenhouse, and field conditions are needed to elucidate the precise in vivo mechanisms underlying the action of these allelochemicals.

REFERENCES

- Akpulat, H.A., and Sahinler, S.S., 2021. *Chemical composition and antioxidant activity of the essential oil and various extracts of Inula graveolens (L.) Desf.* International Journal of Plant Based Pharmaceuticals, 1(1): 52-55.
<https://dergipark.org.tr/en/download/journal-file/23546>
- Almhemed, K., Sakran, M., Ustuner, T., 2021. *Allelopathic Effect of Aqueous Extracts of Stinkwort (Dittrichia graveolens L.) on Germination and Growth of Some Weed Species.* Records of Agricultural and Food Chemistry, 1(1-2): 3-11.
<http://dx.doi.org/10.25135/rfac.2.2107.2151>
- Anžlovar, S., and Anžlovar, A.M., 2024. *Allelopathic effect of aqueous extract from selected invasive plants on germination and growth of tartary buckwheat.* Acta Biologica Slovenica, 67(1): 35-44.
<https://doi.org/10.14720/abs.67.1.18886>
- Bagheri, F., and Cici, S.Z.H., 2015. *Effects of Ailanthus altissima on the growth of weeds and agricultural plants, study on inhibitory.* Biological Forum - An International Journal, 7(1): 506-511.
- Bayhan, Ş., Özkan, E., Onaran, A., 2017. *Antifungal activity of Inula viscosa L. plant extracts against some plant pathogenic fungi.* Central Anatolia Region 3rd Agriculture and Food Congress (26-28 October 2017, Sivas).
- Bilalis, D.J., Travlos, I.S., Karkanis, A., Gournaki, M., Katsenios, G., Hela, D., Kakabouki, I., 2013. *Evaluation of the allelopathic potential of quinoa (Chenopodium quinoa Willd.).* Romanian Agricultural Research, 30: 359-364.
- Cheng, H., Wang, S., Wei, M., Yu, Y., Wang, C., 2021. *Effect of Leaf Water Extracts of Four Asteraceae Alien Invasive Plants on Germination Performance of Lactuca Sativa L. under Acid Deposition.* Plant Ecology, 222(4): 433-443.
- Dor, E., and Hershenhorn, J., 2012. *Allelopathic effects of Inula viscosa leaf extracts on weeds.* Allelopathy Journal, 30(2): 281-289.
- Erenler, R., Atalar, M.N., Yıldız, I., Gecer, E.N., Yıldırım, A., Demirtas, I., Alma, M.H., 2023. *Quantitative analysis of bioactive compounds by LC-MS/MS from Inula graveolens.* Journal of Integrative and Anatolian Medicine, 4(3): 3-10.
<https://doi.org/10.53445/batd.1278048>
- Grašič, M., Anžlovar, S., Strgulc Krajšek, S., 2016. *The impact of aqueous extracts of stinkwort (Dittrichia graveolens) and false yellowhead (D. viscosa) on germination of selected plant species.* Phytion, 56(2): 293-301.
[http://dx.doi.org/10.12905/0380.phytion56\(2\)2016-0293](http://dx.doi.org/10.12905/0380.phytion56(2)2016-0293)
- Horuz, H., and Üremiş, İ., 2024. *Allelopathic Effects of False Yellowhead (Inula viscosa L.) and Tress of Heaven [Ailanthus altissima (Miller) Swingle] Extracts On The Germination of Some Weed Seeds.* Turk. J. Weed Sci., 27(1): 9-22.
<https://dergipark.org.tr/tr/pub/tjws>
- Kitiş, Y.E., Öztürk, G., Çavuşoğlu, O., 2017. *Allelopathic effects of fleabane species (inula viscosa and i. graveolens) on seed germination of some weed species.* International Conference on Agriculture, Forest, Food Sciences and Technologies, 15-17 Mayıs, Cappadocia - Türkiye.
- Koç, K., Aysin, F., Ozek, N.S., Geyikoğlu, F., Taghizadehghalehjough, A., Abuc, O.O., Cakmak, O., Deniz, G.Y., 2021. *Inula graveolens induces selective cytotoxicity in glioblastoma and chronic leukemia cells.* Rev. Assoc. Med. Bras., 67(12): 1771-1778.
<https://doi.org/10.1590/1806-9282.20210614>
- Kombıçak, F.K., Arisoy, G., Kuşluk, B., Yılar, M., 2023. *Allelopathic Effect of Diplotaxis tenuifolia (L.) DC. (sand rocket plant) on Some Crops.* Journal Ahi Agri., 3(2): 235-243.
- Konstantinović, B., Blagojević, M., Konstantinović, B., Samardžić, N., 2014. *Allelopathic effect of weed species Amaranthus retroflexus L. on maize seed germination.* Romanian Agricultural Research, 31: 315-321.
- Kropff, M.J., and Walter, H., 2000. *EWRS and the challenges for weed research at the start of a new millennium.* Weed Research, 40: 7-10.
<https://doi.org/10.1046/j.1365-3180.2000.00166.x>
- Kruse, M., Strandberg, M., Strandberg, B., 2000. *Ecological effects of allelopathic plants a Review.* NERI Technical Report No. 315. Publ. Ministry of Environment and Energy National Environmental Research Institute, DK-1201 Copenhagen K Denmark.
- Liu, D.L., and Lovett, J.V., 1993. *Biologically active secondary metabolites of barley. II. Phytotoxicity of barley allelochemicals.* J. Chem. Ecol., 19: 2231-2244.
- Mammadov, R., Kaya, B., Atıcı, İ.C., Atay, M.Ö., 2023. *Investigation of Some Biological Activities of Inula graveolens (L.) Desf. Species from Turkey.* Ordu University Journal of Science and Technology, 13(2): 110-119.
<https://doi.org/10.54370/ordubtd.1240934>
- Omezzine, F., Ladhari, A., Rinez, A., Haouala, R., 2011a. *Potent herbicidal activity of Inula crithmoides L.* Scientia Horticulturae, 130(4): 853-861.
<https://doi.org/10.1016/j.scienta.2011.08.013>

- Omezzine, F., Ladhari, A., Rinez, A., Haouala, R., 2011b. *Allelopathic potential of Inula graveolens on crops and weeds*. Allelopathy Journal, 28(1): 63-76.
- Omezzine, F., Rinez, A., Ladhari, A., Haouala, R., 2011c. *Allelopathic potential of Inula viscosa against crops and weeds*. Int. J. Agric. Biol., 13: 841-849.
- Önen, H., 2003. *Bioherbicidal effects of some plant essential oils*. Turkish Journal of Weed Science, 6(1): 39-47.
- Özer, Z., Kadioğlu, İ., Önen, H., Tursun, N., 2001. *Herbology (Weed Science)*. Gaziosmanpaşa University Faculty of Agriculture Publications No: 20, Book Series No: 10, Tokat.
- Perisoara, A., Tihauan, B.M., Pirvu, L., Ene, D.M., Pavel, M., Cristea, S., 2023. *The study of some fenugreek extracts by germination bioassay*. Romanian Agricultural Research, 40: 573-583. <https://doi.org/10.59665/rar4054>
- Seca, A.M., Grigore, A., Pinto, D.C., Silva, A.M., 2014. *The genus Inula and their metabolites: From ethnopharmacological to medicinal uses*. Journal of Ethnopharmacology, 154(2): 286-310. <https://doi.org/10.1016/j.jep.2014.04.010>
- Silinsin, M., and Bursal, E., 2018. *UHPLC-MS/MS phenolic profiling and in vitro antioxidant activities of Inula graveolens (L.) Desf.* Natural Product Research, 32(12): 1467-1471. <https://doi.org/10.1080/14786419.2017.1350673>
- Tekeli, İ., Güler, Ç., Yerli, V.S., Algan, N., Vaizoglu, A.S., Kaya, D.A., Öztürk, B., Mutlu, B., Demirayak, F., 2006. *Turkey's Promises on the Environment*. Turkish Academy of Sciences (TÜBA) Publications, No. 13.
- Topçu, G., Öksüz, S., Shih, H.L., Cordel, G.A., Pezzuto, J.M., Bozok-Johansson, C., 1993. *Cytotoxic and Antibacterial Sesquiterpenes from Inula graveolens*. Phytochemistry, 33(2): 407-410. [https://doi.org/10.1016/0031-9422\(93\)85529-Z](https://doi.org/10.1016/0031-9422(93)85529-Z)
- Uludağ, A., 2006. *Studies a general view on allelopathy in Turkey*. Allelopathy Workshop (13-15 June 2006, Yalova) Proceedings: 37-46.
- Uludağ, A., Uremis, I., Rusen, M., Tursun, N., 2017. *Possible uses of allelopathy in weed control in organic farming in Turkey*. Acta Herbologica, 26(2): 87-93. <https://doi.org/10.5937/ActaHerb1702087U>
- Wang, K., Wang, T., Ren, C., Dou, P., Miao, Z., Liu, X., Huang, D., Wang, K., 2022. *Aqueous extracts of three herbs allelopathically inhibit lettuce germination but promote seedling growth at low concentrations*. Plants, 11(4), 486.
- Yilmaz, M.A., 2020. *Simultaneous quantitative screening of 53 phytochemicals in 33 species of medicinal and aromatic plants: A detailed, robust and comprehensive LC-MS/MS method validation*. Industrial Crops and Products, 149, 112347. <https://doi.org/10.1016/j.indcrop.2020.112347>
- Zhang, Z., Liu, Y., Yuan, L., Weber, E., van Kleunen, M., 2021. *Effect of allelopathy on plant performance: A meta-analysis*. Ecology Letters, 24(2): 348-362.