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Allelopathic effect of some weeds on germination of lettuce (*Lactuca sativa* L.) seeds

Marul (*Lactuca sativa* L.) tohumlarının çimlenmesi üzerinde bazı yabancı otların allelopatik etkisi

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A B S T R A C T

Weeds can affect the reproductive success and survival of cultivated plants through both direct and indirect mechanisms. Among these, allelopathy, mediated by allelochemicals released from plants, is a key indirect factor influencing seed germination. This study aimed to evaluate the allelopathic effects of three common weed species (*Amaranthus retroflexus* L., *Sinapis arvensis* L. and *Diplotaxis tenuifolia* (L.) DC) on the germination and early growth of lettuce (*Lactuca sativa* L.) seeds. Aqueous extracts were prepared by soaking 10 grams of powdered plant material in 100 mL of distilled water for 24 hours (10% w/v). This stock extract was considered as 100%, and diluted to 50%, 25%, and 12.5% concentrations. These extracts (6 mL per Petri dish) were applied to 10 lettuce seeds and incubated at 18°C for 6 days under controlled conditions. The evidence clearly indicates that *D. tenuifolia* and *S. arvensis* were more aggressive than *A. retroflexus* in inhibiting lettuce germination and early growth. The distinction makes the point that not all weed species are equal in their capacity to exert allelopathic pressure, and such distinctions are valuable for understanding when pursuing alternative weed management strategies that are less reliant on synthetic herbicides.

INTRODUCTION

The term *weed*, which has Germanic origins, was popularized by Jethro Tull—an English agronomist and a pioneer of the British Agricultural Revolution (Başaran 2022). The Weed Science Society of America (WSSA) defines a weed as "a plant that grows where it is not wanted" (Buchholtz 1967). The interaction between weeds and cultivated crops generally involves two primary modes of competition: direct and indirect. Direct competition encompasses resource-based interference—such as competition for nutrients, water, light, and space—whereby one plant seeks dominance over another. Indirect competition, in contrast, occurs through *allelopathy*, in which plants influence each other's growth via chemical interactions (Başaran 2022).

Allelopathy is a biological phenomenon in which certain plants release biochemicals—termed *allelochemicals*—that affect the growth, reproduction, or survival of neighboring plants and associated soil organisms (Alam and Islam 2002, Cheng and Cheng 2015, Jabran et al. 2015). These allelochemicals are typically secondary metabolites found in various plant tissues including flowers, leaves, stems, and fruits (Rice 1984, Telci 2006). They may be released into the environment through volatilization, leaching, root exudation, or the decomposition of plant residues (Weston 2005, Housny 2023). Once in the surrounding soil or atmosphere, they may suppress germination and inhibit the growth of nearby plant species, potentially reducing crop productivity (Putnam and Tang 1986). Common allelochemicals include a diverse array of secondary compounds such as phenolics, terpenoids, alkaloids, aldehydes, ketones, esters, oxides, fatty acids, and steroids (Rice 1984, Waller 1987, Inderjit et al. 1995). Demonstrating allelopathic effects requires assessing the direct or indirect release of these compounds and evaluating their effects on other plant species through residue analysis or bioassays (Putnam and Tang 1986).

It has been reported that when used appropriately, plants containing allelochemicals are used appropriately, they will help reduce environmental degradation caused by pesticides and contribute to the biological control of pest and weed species (Xuan and Tsuzuki 2001), improve soil quality, enhance nutrient availability through the decomposition of crop residues, promote a favorable soil microbial environment, and increase crop diversity through crop rotation. (Xuan and Tsuzuki 2002), and provide additional benefits, including the development of biological pesticides and herbicides derived from allelochemicals isolated from plants exhibiting herbicidal activity. (Xuan and Tsuzuki 2004). The use of allelopathy to address challenges encountered in agricultural practices, and the utilization of allelopathic plants for sustainable agricultural production, have become increasingly important, particularly under current conditions of rising environmental pollution and intensive pesticide use that pose risks to human health, making the integration of such environmentally friendly approaches into agricultural production systems inevitable. It is known that some crop plants suppress weed growth and are incorporated into crop rotation systems for sustainable weed management. Phytotoxic chemicals leached from the roots of these plants or released from plant leaves and stems have been reported to strongly suppress weed germination and growth (Xuan and Tsuzuki 2002, Üremiş et al. 2009). Yarnia et al. (2011) reported that the field density of *Amaranthus retroflexus* may be reduced by the allelopathic activity of *Secale cereale* L. Interestingly, *A. retroflexus*, *Diplotaxis tenuifolia*, and *Sinapis arvensis* are prevalent in agricultural fields and have been reported to exhibit high allelopathic potential (Shams et al. 2014, Bendimerad et al. 2007, Baziar et al. 2014). It has been demonstrated that some species of *Amaranthus* are able to suppress root development, which can, in turn, have negatively affect on germination and early growth of subsequent crops (Mlakar et al. 2012, Harvey and Malcicka 2015). Allelochemical volatiles released by *Amaranthus* spp. have been associated with reduced germination, inhibited photosynthetic activities, and stunted short growth in neighboring plants (De Souza et al. 2011). For instance, *A. palmeri* has been found to inhibit the tomato seed germination (Connick et al. 1987), while its residues in the soil have been said to inhibit the growth of sorghum, cabbage, carrot, and onion for a period of 16 weeks owing to the presence of volatile and degrading allelochemicals (Menges 1987, Menges 1988, Bradow and Connick 1987).

Key allelochemicals include ferulic acid, apigenin, and β-CD in *A. retroflexus* (Fiorito et al. 2017); quercetin, isorhamnetin, and cyanidin in *D. tenuifolia* (Jin et al. 2009); and 1-butenyl isothiocyanate, cubenol, and dimethyl sulfides in *S. arvensis* (Al-Qudah et al. 2011). Glucosinolates, isothiocyanates, alkaloids, and saponins are some of the most frequently reported phytotoxic secondary metabolites. Our inhibitory activity may largely part result from the presence of such compounds in the test species. *S. arvensis* and *D. tenuifolia* are particularly rich in glucosinolates and contain isothiocyanate and glucosinolate, respectively, which have been reported to cause inhibition of germination by disrupting cell division and alteration of membrane integrity (Al-Qudah et al. 2011, Jin et al. 2009).

Conversely, *A. retroflexus* is rich in alkaloids and saponins. These compounds are likely to interfere with germination processes, though often through alternative mechanisms and more frequently to lesser degrees (Fiorito et al. 2017). The presence of such phytochemicals is also presumably responsible for the most frequently met dose-response patterns of inhibition in allelopathic trials. For example, an aqueous 100% extract of *A. retroflexus* completely inhibited cumin seed germination (Shams et al. 2014), and reduced corn hypocotyl and epicotyl lengths (Konstantinović et al. 2014). The same effects were observed with a 75% S. arvensis extract, which profoundly inhibited Italian ryegrass germination (Baziar et al. 2014), and with a 15% *D. tenuifolia* extract which markedly suppressed the germination of wheat, barley, and *Poa* species. (Kombıçak et al. 2023, Yılar et al. 2019).

In Türkiye, recently, there has been growing interest in studies on the allelopathic effects of indigenous weed species as well as medicinal and aromatic herbs. For example, *Rosmarinus officinalis*, *Origanum syriacum*, and *Origanum majorana* essential oils have been found to inhibit germination of certain weed seeds (Cunedioğlu and Üremiş 2018, Efil and Üremiş 2019). In parallel, *Lythrum salicaria* has shown significant allelopathic potentiality, namely against seed germination of lettuce and seedling growth under different environmental conditions and concentrations of the extracts (Akın et al. 2017, Akın et al. 2019). These results propose the application of natural allelochemicals as environmentally friendly alternatives for weed control.

Further studies have expanded the allelopathy discussion to include less well-described organisms such as lichens - (e.g. *Xanthoparmelia somloensis*) and plants like *Sorghum bicolor*. Plant extracts have been reported exhibit inhibitory activities against a wide range of plant species. (Öten et al. 2022, Bingöl et al. 2022). In addition, compounds like juglone, thymol, and carvacrol, occurring in essential oils, have been found to exhibit dose-dependent phytotoxicity on weeds and crop plants (Kaymak 2018, Tutenocaklı et al. 2022, Tursun et al. 2022). These findings support the use of allelopathy in organic or reduced-input agriculture, and suggest that this approach can contribute toward more sustainable agriculture, particularly by reducing reliance on synthetic herbicides. (Uludağ et al. 2017).

The current study investigates the allelopathic effects of aqueous extracts derived from three native broadleaf weed species: *A. retroflexus*, *S. arvensis*, and *D. tenuifolia*, on *Lactuca sativa* (lettuce) seed germination. The plants commonly occur in vegetable production systems, for instance, land employed in growing lettuce, where they compete with the crops (Soylu et al. 2017). Lettuce was chosen as the test crop since it is not only sensitive to phytochemicals, but it has also been widely used in previous allelopathy studies (Kocaçalışkan 2006, Akın et al. 2017, Akın et al. 2019).

The aim of this study was to evaluate the inhibitory effects of aqueous extracts derived from weeds on lettuce seed germination and to highlight any species-specific or concentration-dependent differences in allelopathic activity.

MATERIALS AND METHODS

Materials

In this study, the materials used were *S. arvensis*, *A. retroflexus* and *D.tenuifolia* plants containing stem, and leaf organs, as well as lettuce seed. Lettuce seeds (*Lactuca sativa* var. *crispa*) were obtained from the commercial cultivar labeled as 'Kışlık Kıvırcık' (Winter Curly Lettuce) supplied by Goldgen Pro Seed Tohumculuk, Türkiye. The other equipmentused in the study were paper bags, petri dishes, centrifuge tubes and centrifuge, beakers, an orbital shaker, paraffin, pure and distilled water, and an incubator.

Methods

Preparation of plant extracts

Sinapis arvensis, *Amaranthus retroflexus* and *Diplotaxis tenuifolia* plants used in this study were collected during their pre-flowering to early seed-setting stages from non-cultivated areas such as roadsides, open spaces within the grounds of the Ankara Plant Protection Central Research Institute. Only the above-ground green parts (leaves and stems) were used for the preparation of extracts. The collected plants were placed in paper bags and air-dried in the shade for 10 days. After the drying process was completed, the plants were finely chopped and powdered and 10-gram samples were weighed.

The powdered plant material (10 g) was added to 100 mL of distilled water to obtain a 10% w/v extract, and the mixture was transferred to Erlenmeyer flasks. (Figure 1). After the Erlenmeyer flasks were closed with paraffin, the resulting suspension was shaken in an orbital shaker for 24 hours. After the extraction process, the mixture was filtered with two layers of tulle and the filtrate was taken into centrifuge tubes and centrifuged at 4000 rpm for 30 minutes (Figure 2). The filtrate obtained after centrifugation was passed through Whatman No. 2 filter paper to obtain the crude aqueous extract, which was considered as the stock solution (10% w/v), hereafter referred to as the 100% extract (Figure 3). This stock solution was subsequently diluted with distilled water to prepare working concentrations of 50% (5% w/v), 25% (2.5% w/v), and 12.5% (1.25% w/v), respectively. These were the final application doses used in the bioassays (Reigosa et al. 2006, Modhej et al. 2013).

Figure 1. Preparation of plant extracts

Figure 2. Centrifugation of the filtrate for purification of plant extracts

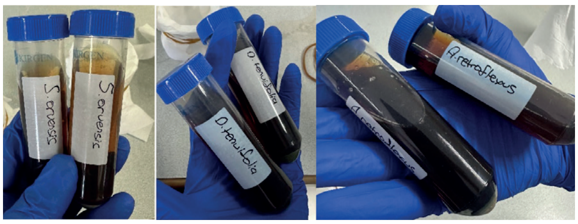

Figure 3. 100% buffer solutions prepared by passing the filtrate obtained after centrifugation through Whatman No.2 filter paper

Experimental design

The experiment was designed to have 2 repetitions and 4 replicates and was carried out in 90 mm diameter petri dishes with two layers of blotting paper inside. 10 *L. sativa* (Winter Curly Lettuce) seeds were added to each petri dish and 6 mL of extract solution was added (Figure 4). The prepared petri dishes were incubated in an incubator with 18 °C temperature and 70% relative humidity conditions under a 16-hour light and 8-hour dark cycle for 6 days (Modhej et al. 2013, Akın et al. 2017, Akın et al. 2019) (Figure 5). During the experiment, lettuce seeds with a germination tube length exceeding 5 mm were considered to have germinated.

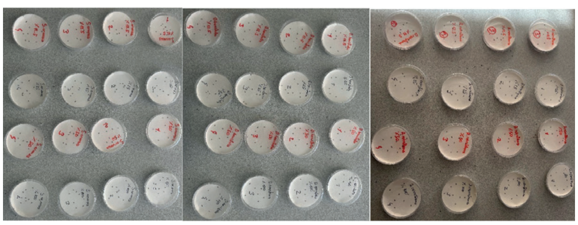

Figure 4. Adding 10 *Lactuca sativa* (lettuce) seeds to each petri dish

Figure 5. Incubating the prepared petri dishes in an incubator at 18 °C and 70% relative humidity

Statistical analysis

Data were analyzed using Generalized Linear Models (GLM) and One-Way Analysis of Variance (ANOVA) in the R Studio program (RStudio Team 2023); Duncan's multiple range test was used to determine differences between groups. Dependent variable was germination percentage, and independent variables were control (pure water) and *A. retroflexus*, *S.arvensis* and *D. tenuifolia* aqueous extracts at different concentrations (% w/v). Significant differences ($p < 0.05$) as a result of ANOVA were compared between groups using the Duncan's multiple range test, and statistically significant treatments were reported.

RESULTS

As shown in Figure 6, lettuce seed germination was significantly inhibited by increasing concentrations of aqueous weed extracts, with considerable variation among species.

Sinapis arvensis extract effects

Germination in the control group began on Day 1 (10%) and reached 100% by Day 4. At lower extract concentrations (1.25% and 2.5%), *S. arvensis* caused a moderate delay in germination. At 1.25%, germination started on Day 2 (50%) and reached 88% by Day 6. The 2.5% extract delayed initiation (8% on Day 2) but ultimately resulted in 80% germination. At higher doses, however, significant inhibition was observed: germination at 50% concentration was delayed until Day 5 and reached only 38% by Day 6, while the 10% extract completely suppressed germination. Statistical analysis showed that the 5% and 10% doses

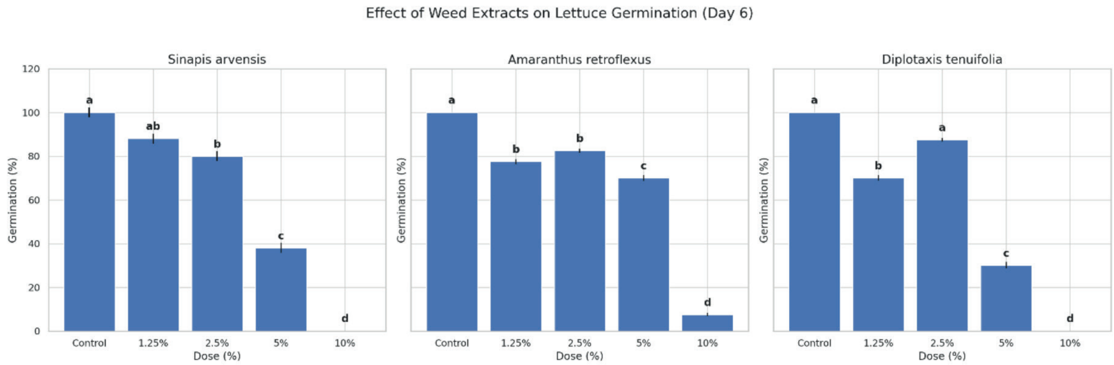

Figure 6. Effect of increasing concentrations (1.25%, 2.5%, 5%, and 10%) of aqueous extracts from *Sinapis arvensis*, *Amaranthus retroflexus*, and *Diplotaxis tenuifolia* on lettuce seed germination on Day 6. Data are presented as mean ± SE (n = 3). Different letters above bars indicate significant differences between treatments according to Duncan's multiple range test ($p < 0.05$)

significantly reduced germination compared to the control ($p < 0.05$), whereas the lower concentrations had no statistically significant effect ($p > 0.05$) (Figure 7).

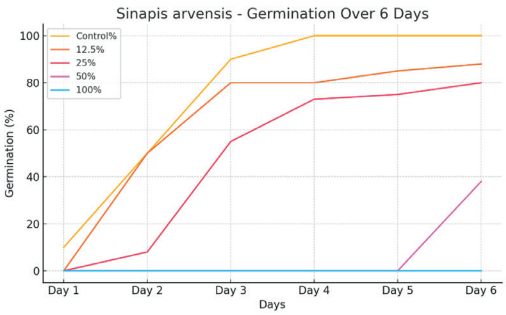

Figure 7. Germination percentage of lettuce seeds over six days under different concentrations of *Sinapis arvensis* aqueous extract

Amaranthus retroflexus extract effects

In the untreated control group, germination initiated at 20% on Day 1, reached 90% by Day 3, and achieved 100% by Day 4. Low concentrations (1.25% and 2.5%) exhibited minimal inhibitory effects. At 1.25%, germination was 25% on Day 2 and reached 77.5% by Day 6. At 2.5%, germination started earlier (45% on Day 2) and reached 82.5% by Day 6. The 5% extract caused a delay, initiating germination at 2.5% on Day 2 and reaching 70% by Day 6. Notably, the 10% dose caused substantial inhibition, with only 7.5% germination by Day 6. Statistically, only the 10% extract had a significant effect on germination suppression ($p < 0.05$), while lower doses showed no significant deviation from the control ($p > 0.05$) (Figure 8).

Diplotaxis tenuifolia extract effects

Control seeds showed 20% germination on Day 1, 80% by Day 3, and 100% by Day 4. At 1.25%, germination reached

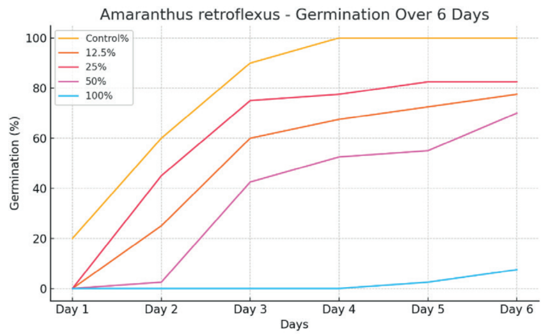

Figure 8. Germination percentage of lettuce seeds over six days under different concentrations of *Amaranthus retroflexus* aqueous extract

70% by Day 6. Interestingly, the 2.5% extract yielded higher germination (87.5% by Day 6), though with delayed initiation (22.5% on Day 3). A significant inhibitory effect was observed at higher concentrations: germination with the 5% extract began only on Day 5, reaching 30% by Day 6, while the 10% dose completely inhibited germination. As with *S. arvensis*, inhibition at 5% and 10% concentrations was statistically significant ($p < 0.05$), whereas the effects of lower concentrations were not ($p > 0.05$) (Figures 9-10).

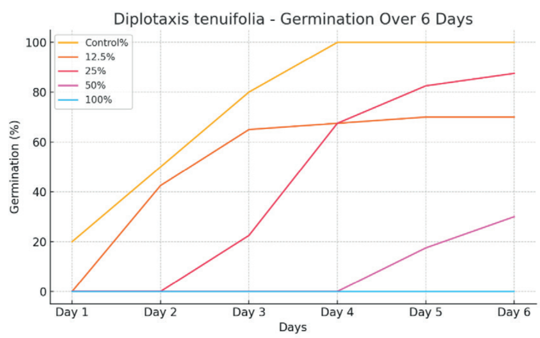

Figure 9. Germination percentage of lettuce seeds over six days under different concentrations of *Diplotaxis tenuifolia* aqueous extract

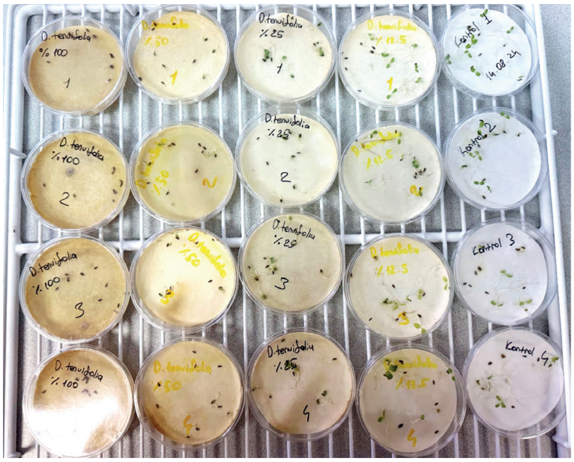

Figure 10. Germination response of lettuce seeds to different concentrations (Control, 1.25%, 2.5%, 5% and 10%) of *Diplotaxis tenuifolia* aqueous extracts at the end of the 6th day

The allelopathic impact of *S. arvensis*, *A. retroflexus*, and *D. tenuifolia* on lettuce germination demonstrated clear dose-dependent trends. While low extract concentrations (1.25% and 2.5%) induced moderate delays without statistically significant suppression, high doses (5% and 10%) caused substantial reductions in germination, with complete inhibition observed at 10% for *S. arvensis* and *D. Tenuifolia* (Figures 11-12, Table 1).

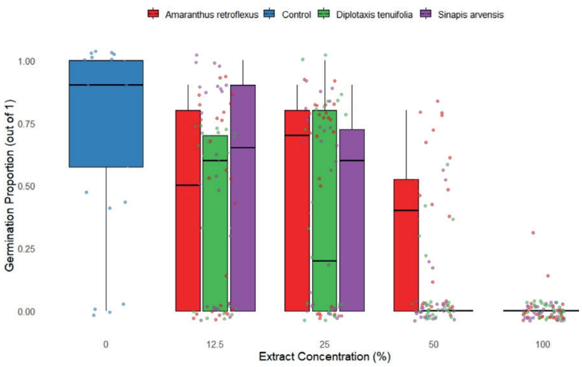

Figure 11. Effect of different concentrations of extracts obtained from *Amaranthus retroflexus*, *Diplotaxis tenuifolia* and *Sinapis arvensis* plants on the germination rate of *Lactuca sativa* (lettuce) seeds

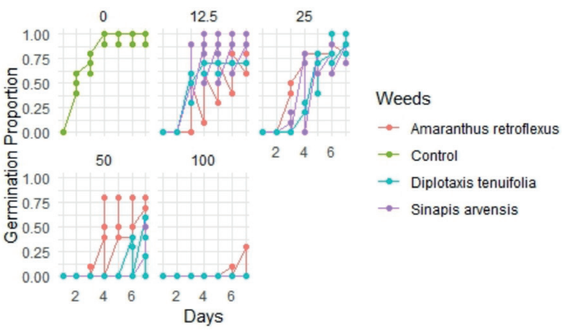

Figure 12. Variation in germination rates of *Lactuca sativa* (lettuce) seeds in different concentrations of extracts obtained from *Amaranthus retroflexus*, *Diplotaxis tenuifolia* and *Sinapis arvensis* plants for 6 days

DISCUSSION AND CONCLUSION

The results of this study indicated that allelochemicals released by the three above mentioned weed species significantly inhibited lettuce seed germination in a dose-dependent manner, confirming their strong allelopathic potential. It was found that Sinapis arvensis, also recognized as an allelopathic weed, moderately inhibits lettuce seed germination at lower concentrations (1.25% and 2.5%), whereas it causes severe inhibition at higher concentrations (5% and 10%). This is consistent with the findings of Al-Qudah et al. (2011), who reported that isothiocyanates and phenolic compounds secreted from the roots and leaves of *S. arvensis* inhibited lettuce seed germination. *S. arvensis* also has the ability to modify soil microflora and suppress germination in certain crop species. Similarly, Baziar et al. (2014) reported that these compounds inhibit cell division and enzymatic activity, thereby delaying or preventing seed germination. *A. retroflexus*, a common weed in agricultural lands, is known to have allelopathic properties. Our results show that aqueous extracts of *A. retroflexus* were relatively less effective on lettuce germination at low concentrations (1.25% and 2.5%), but inhibition was enhanced at high concentrations (50% and 100%). Surprisingly, even at 10% concentration, germination fell drastically to 7.5%. These results are in line with earlier reports by Fiorito et al. (2017), Mlakar et al. (2012) and Harvey and Malcicka (2015) of analogous inhibitory activity brought upon by phenolic acids and terpenoids in *A. retroflexus*. Furthermore, Menges (1987) reported the persistent effect of *A. retroflexus* in the soil, and emphasized the need to considersuch species when designing crop rotations.

Among the three weed species tested, D. tenuifolia exhibited the strongest allelopathic effect on lettuce seed germination, even at low concentrations. For example, the 1.25% dose inhibited about 30%, while the 2.5% dose still inhibited about 15%. Germination was significantly inhibited (about 70%) at 5%, and there was no germination at 10%. This agrees with Jin et al. (2009), who indicated that allelochemicals in *D. tenuifolia* may promote germination at low concentrations but become inhibitory at some threshold concentration. Kombıçak et al. (2023) and Yılar et al. (2019) reports also refer to the tremendous phytotoxic potential of this species on sensitive crop plants.

Our observations are further supported by several allelopathy studies conducted in Türkiye. *Rosmarinus officinalis*,

Table 1. Effect of weed extracts on lettuce seed germination rate on Day 6 (mean ± SE). Different letters in the same row indicate statistically significant differences among treatments according to Duncan's multiple range test ($p < 0.05$)

Species	Key Allelochemicals	Control	1.25%	2.5%	5%	10%
S. arvensis	Glucosinolates, Isothiocyanates	100±2.3[a]	88±2.3[ab]	80±2.3[b]	38±2.3[c]	0±0.0[d]
A. retroflexus	Saponins, Alkaloids	100±0.0[a]	77.5±1.1[b]	82.5±0.9[b]	70±1.4[c]	0±0.0[d]
D. tenuifolia	Glucosinolates, Isothiocyanates	100±0.0[a]	70±1.2[b]	87.5±0.8[a]	30±1.5[c]	0±0.0[d]

Origanum syriacum, and *Origanum majorana* plant extracts significantly inhibited seed germination of the weeds (Cunedioğlu and Üremiş 2018, Efil and Üremiş 2019). Akın et al. (2017, 2019) in their study reported inhibitory effects of *Lythrum salicaria* extracts on the seeds of lettuce, which also confirms our findings with *D. tenuifolia*. Additionally, allelopathy of *Sorghum* species and certain lichens such as *Xanthoparmelia somloensis* have been demonstrated (Bingöl et al. 2022, Öten et al. 2022), corroborating the use of natural phytotoxic compounds for weed control.

It is suggested that the allelopathic properties of certain weed species should be taken into account in the development of sustainable crop production systems. Screening dominant weeds for allelopathic activity can help anticipate yield losses and guide weed management strategies, particularly in low-chemical-input or organic farming systems. Allelopathic weeds with strong activity, such as *D. tenuifolia*, may also serve as potential sources of natural herbicides. The identification and isolation of these allelochemicals could reduce the reliance on synthetic herbicides and promote ecologically sustainable agriculture, in line with the European Green Deal (Xuan and Tsuzuki 2004).

In conclusion, this study provides insight into the allelopathic effects of weed species, particularly on lettuce germination. Further research should explore the applicability of these findings on a broader scale for a range of crops and environments, resulting in the creation of new, sustainable methods of weed control.

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ÖZET

Yabancı otlar, kültür bitkilerinin üreme başarısını ve hayatta kalma yeteneklerini doğrudan veya dolaylı yollarla etkileyebilir. Bu etkilerden biri olan allelopati, bitkilerin salgıladığı allelokimyasallar aracılığıyla gerçekleşir ve özellikle tohum çimlenmesi üzerinde önemli bir rol oynar. Bu çalışmada, yaygın olarak görülen üç yabancı ot türünün (*Amaranthus retroflexus* L., *Sinapis arvensis* L. ve *Diplotaxis tenuifolia* (L.) DC) marul (*Lactuca sativa* L.) tohumlarının çimlenmesi ve fide gelişimi üzerindeki allelopatik etkileri araştırılmıştır. Allelopatik ekstraktlar, 10 gram kuru ve öğütülmüş bitki materyalinin 100 mL saf suda 24 saat bekletilmesiyle hazırlanmıştır (%10 w/v). Elde edilen bu stok çözelti %100 kabul edilmiş ve sırasıyla %50, %25 ve %12,5 oranlarında seyreltilerek uygulanmıştır. Her Petri kabına 6 mL ekstrakt ve 10 adet marul tohumu konulmuş, 18°C sıcaklıkta, kontrollü ışık ve nem koşullarında 6 gün inkübasyona bırakılmıştır. Sonuçlar, *D. tenuifolia* ve *S. arvensis* türlerinin çimlenme ve fide gelişimi üzerinde belirgin baskılayıcı etkilere sahip olduğunu, *A. retroflexus*'un ise daha düşük düzeyde allelopatik etki gösterdiğini ortaya koymuştur. Bu bulgular, bazı yabancı ot türlerinden salınan allelokimyasalların kültür bitkilerinin çıkışı üzerindeki kritik etkisini vurgulamakta ve sürdürülebilir yabancı ot yönetimi yaklaşımlarına katkı sağlayabilecek potansiyellerini ortaya koymaktadır.

Anahtar Kelimeler: Allelokimyasallar, *Amaranthus retroflexus*, *Diplotaxis tenuifolia*, ekstraksiyon, *Lactuca sativa*, *Sinapis arvensis*

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