***Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi*, 31 (3), 664-682**
Mustafa Kemal University Journal of Agricultural Sciences, 31 (3), 664-682
https://dergipark.org.tr/tr/pub/mkutbd
e-ISSN: 2667-7733

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Dose-response analysis and selectivity ındices of certain Lamiaceae essential oils on seed germination and root elongation in selected weeds and cucumber (*Cucumis sativus*)

Bazı Lamiaceae uçucu yağlarının belirli yabancı otlar ve hıyar (*Cucumis sativus*) bitkisinde tohum çimlenmesi ve kök uzaması üzerindeki doz-tepki analizi ve seçicilik indeksleri

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ARTICLE INFO

Article history:
Recieved / Geliş: 02.04.2026
Accepted / Kabul: 05.06.2026

Keywords:
Bio-herbicide
Selectivity Index (SI)
Log-logistic model
Dose-response

Anahtar Kelimeler:
Biyo-herbisit
Selektiviteİndeksi (SI)
Log-lojistik model
Doz-tepki

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ABSTRACT

The intensive use of synthetic herbicides has led to severe environmental issues and the rapid evolution of resistant weed biotypes, making the development of eco-friendly bioherbicides imperative. This study aimed to evaluate the phytotoxic potential and physiological selectivity of essential oils extracted from plants belonging to the *Origanum*, *Thymus*, *Lavandula*, and *Rosmarinus* genera of the Lamiaceae family on four major agricultural weed species (*Amaranthus palmeri*, *Portulaca oleracea*, *Chenopodium album*, *Solanum nigrum*) and a crop species, cucumber (*Cucumis sativus*). Data obtained on the seed germination and root elongation were analyzed using log-logistic dose-response models (LC_{50}), Selectivity Index (SI), and Principal Component Analysis (PCA). Experimental results revealed that the inhibitory effects of the essential oils on seed germination increased dose-dependently; furthermore, Pearson correlation analysis indicated a highly negative relationship ($r = -0.92$) between essential oil dose and root length. *Origanum* essential oil exhibited the highest phytotoxic effect, requiring only 0.37–1.58 µl/petri to inhibit weed germination by 50% (LC_{50}). In contrast, the crop plant, cucumber, demonstrated a remarkably high tolerance to all tested oils. Selectivity analysis revealed that Origanum essential oil provided the widest safety margin, producing values (SI: 10.13–43.24) well above the safety threshold of SI > 2.

ÖZET

Sentetik herbisitlerin yoğun kullanımı, ciddi çevresel sorunlara ve dirençli yabancı ot biyotiplerinin hızla gelişmesine yol açarak çevre dostu biyo-herbisitlerin geliştirilmesini zorunlu kılmaktadır. Bu çalışma, Lamiaceae familyasına bağlı *Origanum*, *Thymus*, *Lavandula* ve *Rosmarinus* cinsine ait bitkilerinin uçucu yağının, tarım alanlarında önemli sorun oluşturan dört yabancı ot türü (*Amaranthus palmeri*, *Portulaca oleracea*, *Chenopodium album*, *Solanum nigrum*) ve bir kültür bitkisi olan hıyar (*Cucumis sativus*) üzerindeki fitotoksik potansiyelini ve fizyolojik seçiciliğini değerlendirmeyi amaçlamıştır. Çalışmalarda tohum çimlenmesi ve kök uzaması üzerine elde edilen veriler log-lojistik doz-tepki modelleri (LC_{50}), Selektivite İndeksi (SI) ve Temel Bileşenler Analizi (PCA) kullanılarak analiz edilmiştir. Denemeler sonucunda, doz artışına bağlı olarak uçucu yağların tohumların çimlenmesini engelleyici etkileri ortaya çıkmış; Pearson korelasyonu sonucunda ise uçucu yağ dozu ile kök uzunluğu arasında yüksek düzeyde negative bir ilişki ($r = -0.92$) belirlenmiştir. *Origanum* uçucu yağı, yabancı ot çimlenmesini %50 (LC50) oranında engellemek için yalnızca 0.37–1.58 µl/petri doza ihtiyaç duyarak yüksek fitotoksik etki göstermiştir. Buna karşılık, hıyar tüm uçucu yağlara karşı çok yüksek oranda tolerans göstermiştir. Selektivite analizi sonucunda Origanum uçucu yağı, SI > 2 güvenlik eşiğinin çok üzerinde değerler (SI: 10.13–43.24) üreterek en geniş güvenlik aralığını sunan uygulama olarak öne çıkmıştır.

Cite/Atıf Yalçın, L., Taşkan, E., Pesen, Ö., Uz, Ş., & Tursun, N. (2026). Dose-response analysis and selectivity indices of certain Lamiaceae essential oils on seed germination and root elongation in selected weeds and cucumber (*Cucumis sativus*). *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 31*(3), 664-682. https://doi.org/10.37908/mkutbd.1919110

INTRODUCTION

Weeds are recognized as one of the most important biotic factors limiting agricultural productivity worldwide and causing major economic losses by competing with crops for nutrients, water, space, and light (Patterson, 1985; Tursun et al., 2021). Traditionally, synthetic chemical herbicides have been intensively used in weed management. However, the continuous and intensive use of these synthetic substances has lead to serious global problems such as the rapid development of herbicide-resistant weed biotypes, contamination of groundwater and the environment, and residual toxicity in the food chain (Araniti et al., 2017; Mutlu et al., 2011). In recent years, increasing environmental and health concerns have accelerated the search for eco-friendly, biodegradable, and sustainable alternatives, such as bioherbicides and biopesticides derived from plant secondary metabolites (Tworkoski, 2002; Sertkaya et al., 2010a,b; Kaya et al., Özen et al., 2017; Kaya et al., 2018; Üstüner et al., 2018b; Bozkurt et al., 2020; Kara et al., 2022).

Allelopathy, known as the phenomenon where plants affect the germination and growth of one another by releasing bioactive compounds, offers a powerful mechanism for the natural suppression of weeds (Rice, 1984). Among aromatic plants, the Lamiaceae family is particularly notable for its high concentrations of essential oils with strong phytotoxic properties (Kordali et al., 2007). These oils are complex mixtures of secondary metabolites, primarily consisting of monoterpenes and sesquiterpenes. Numerous studies to date have reported that essential oils belonging specifically to *Origanum*, *Thymus*, *Lavandula, Cuminum, Mentha, Allium, Dittrichia*, and *Rosmarinus* species significantly inhibit the seed germination and seedling growth of weeds (Angelini et al., 2003; Üremiş et al., 2009; Atak et al., 2016; Al Sakran et al., 2020; Almhemed et al.,2021).

The herbicidal efficacy of these essential oils is structurally attributed to their main chemical constituents, such as carvacrol, thymol, linalool, and 1,8-cineole (Kordali et al., 2008; Yılar et al., 2019). These compounds exert phytotoxicity through multiple physiological pathways by disrupting the structural integrity of cellular membranes, leading to electrolyte leakage and oxidative stress (Araniti et al., 2017). Furthermore, these volatile compounds exhibit phytotoxicity in the gas phase, enabling them to penetrate the soil microenvironment and effectively target weed seeds before their emergence (Azirak & Karaman, 2008; Aydın & Tursun, 2010; Almhemed & Üstüner, 2023).

Although the bioherbicidal effect of various essential oils has been proven, the greatest obstacle to their practical application is the lack of information regarding their selectivity between crop plants and weeds (Ibáñez & Blázquez, 2018). A successful bio-herbicide candidate must be able to make a clear distinction between the physiological sensitivities of the weed species and the target crop. The cucumber (*Cucumis sativus* L.) plant, which is of great economic importance worldwide due to its fresh consumption and industrial use, is highly sensitive to weed competition during its early developmental stages.

The integration of chemometric tools such as Principal Component Analysis (PCA) not only provides a holistic understanding of how species cluster based on their biochemical sensitivities but also facilitates the identification of the most promising bio-herbicidal formulations (Abd-ElGawad et al., 2021; Verdeguer et al., 2020). Although previous studies have generally focused on the inhibition of broad-spectrum weeds such as *Amaranthus palmeri* S. Watson, *Portulaca oleracea* L., *Chenopodium album* L., and *Solanum nigrum* L., comparative data on the Selectivity Index (SI) between these weeds and *C. sativus* are quite limited (Cunedioğlu & Üremiş, 2018; Efil & Üremiş, 2019). Therefore, this study was conducted to evaluate the pre-emergence herbicidal potential of essential oils from four different species belonging to the Lamiaceae family (*Origanum syriacum* L., *Thymus vulgaris* L., *Lavandula angustifolia* Mill., and *Rosmarinus officinalis* L.) on major weeds, as well as the selectivity of these oils on the cucumber plant. By calculating LC_{50} values and establishing selectivity profiles (safety margins), this research aims to provide a scientific foundation for sustainable and selective weed management in modern vegetable production.

MATERIALS and METHODS

The studies were conducted in 2026 at the Herbology Laboratory, Faculty of Agriculture, Malatya Turgut Özal University. In this study, seeds of four problematic weed species in agricultural areas (*Amaranthus palmeri* S. Watson, *Portulaca oleracea* L., *Chenopodium album* L., and *Solanum nigrum* L.) were collected from heavily infested agricultural fields in the Malatya region at the end of the growing season in 2025. Before setting up the experiments, the weed seeds were cleaned, dried, and stored in the dark at +4 °C to maintain their viability until the experiments began. Seeds of the crop plant, cucumber (*Cucumis sativus* L.), were obtained from commercial suppliers.

Extraction of essential oils

In this study, essential oils extracted from oregano (*Origanum syriacum* L.), common thyme (*Thymus vulgaris* L.), lavender (*Lavandula angustifolia* Mill.), and rosemary (*Rosmarinus officinalis* L.) plants obtained from Eastern Mediterranean populations were used. The aboveground parts of the plants were harvested during the full flowering period, dried in the shade at room temperature, and subjected to hydrodistillation for 180 minutes using a Neo-Clevenger type apparatus. The obtained essential oils were collected, dehydrated with anhydrous sodium sulfate, stored in amber-colored glass vials, and kept at +4 °C until use (Üremiş et al., 2009).

Bioassay procedure

The phytotoxic effects of the essential oils were evaluated using the vapor-phase method in plastic petri dishes with a 9 cm diameter (60 cm^3 volume). The experiment was established in a completely randomized design with four replications and was repeated twice independently. To ensure the permeability of the hard seed coats of the weed seeds, mechanical scarification (sanding) was first performed, followed by the application of 1000 ppm GA3. Two layers of sterile filter paper (blotting paper) were placed at the bottom of each petri dish, and 25 seeds of each plant species were distributed homogeneously. The filter papers were moistened with 3 mL of sterile distilled water. Due to the low water solubility of the essential oils, the applications were carried out in the gas phase. For this purpose, a 1×1 cm^2 piece of filter paper was attached to the inner center of the petri dish lids. Essential oils were applied onto this piece of paper using a micropipette at doses of 0.5, 1, 2, 4, 8, and 16 µl/petri. No essential oil was applied to the control groups; only 3 mL of distilled water was added to the bottom of the dishes. Immediately after the application, the petri dishes were tightly sealed by wrapping them with a double layer of parafilm to prevent the leakage of volatile components (Yaşar et al., 2024).

Germination conditions and measurements

The petri dishes were kept in an incubator (climate chamber) at a constant temperature of 25 ± 1 °C, determined as the optimum germination temperature for the tested species, under completely dark conditions for 21 days. At the end of the 21-day period, seeds with a radicle length reaching at least 0.5 cm were considered germinated (Uygur, 1985), and the final germination percentage and radicle lengths were recorded. The inhibition percentage was calculated using the following formula (Efil & Üremiş, 2019):

$$\text{Inhibition } (\%) = [(\mathrm{C} - \mathrm{T}) / \mathrm{C}] \setminus 100$$

*Where **C** is the germination in the control and **T** is the germination in the treatment.*

Statistical analyses

Since no statistically significant difference was found between the two independent trials, the obtained data were pooled, and mean values were used. Dose-response relationships were analyzed with a three-parameter log-logistic model using the *drc* package in R software (v.4.3.0). The concentration value required to inhibit germination by 50%

(LC_{50}) was calculated separately for each essential oil-plant species interaction. 95% confidence intervals (95% CI) for LC_{50} estimates were calculated using the delta method as implemented in the ED() function of the drc package. The dose-response data were analyzed using the three-parameter log-logistic model, mathematically expressed below:

$$y = (d)/(1 + \exp(b(\log(x) - \log(e)))$$

Where: *y*: The germination percentage (%) at a specific concentration *x*, *d*: The upper limit (fixed at 100% to represent germination in the control treatment), *x*: Essential oil concentration (µl/petri), *e*: The LC_{50} value (the effective concentration required to inhibit germination by 50%), *b*: The slope of the curve around the LC_{50} point.
To determine physiological selectivity, the Selectivity Index (SI) was calculated as the ratio of the LC_{50} value of the crop plant to the LC50 value of the target weed. Additionally, Principal Component Analysis (PCA) and a Pearson Correlation Matrix were applied to holistically visualize the multivariate relationships among dose, plant species, and growth parameters. PCA, Pearson correlation analysis, and Duncan's Multiple Range Test were performed using the agricolae and ggplot2 packages in R software (v.4.3.0), at a significance level of $P < 0.05$.

RESULTS

Inhibitory effects of essential oils on seed germination

The chemical compositions (GC-MS analysis) of the essential oils used in this study were previously conducted by Üremiş et al. (2009) and Atak et al. (2016). According to these analyses, the essential oils of plants belonging to the *O. syriacum* and *T. vulgaris* genera from this region exhibit a chemotype structure extremely rich in oxygenated phenolic monoterpenes (especially carvacrol and thymol) with high phytotoxic activity; whereas the main components of the oils from plants belonging to the *L. angustifolia* and *R. officinalis* genera consist of monoterpenes with lower toxicity, such as 1,8-cineole, linalool, and camphor.

Table 1. Effect of different essential oil concentrations on the seed germination (%) of four weed species and the crop plant, cucumber.

Çizelge 1. Farklı uçucu yağ konsantrasyonlarının dört yabancı ot türü ve kültür bitkisi olan hıyar bitkisinin tohum çimlenmesi (%) üzerine etkisi

Weeds and crop	Essential oils	Control	0.5 µl	1 µl	2 µl	4 µl	8 µl	16 µl	F-value
Amaranthus palmeri	*O. syriacum*	100a	39.8b	9.6c	1.3c	0.5c	0.0c	0.5c	245.18***
	T. vulgaris	100a	91.2a	54.2b	28.3b	4.2c	1.5c	0.0c	112.45***
	L. angustifolia	100a	94.6a	92.5a	86.4a	32.4b	6.2c	7.1c	84.12***
	R. officinalis	100a	100a	98.6a	94.2a	88.4a	42.5b	17.1c	56.34***
Portulaca oleracea	*O. syriacum*	100a	32.6b	3.2c	0.0c	0.0c	0.0c	0.0c	312.05***
	T. vulgaris	100a	88.5a	67.4b	15.0c	11.6c	0.0d	0.0d	148.90***

Table 1 (continue). Effect of different essential oil concentrations on the seed germination (%) of four weed species and the crop plant, cucumber.

Çizelge 1 (devamı). Farklı uçucu yağ konsantrasyonlarının dört yabancı ot türü ve kültür bitkisi olan hıyar bitkisinin tohum çimlenmesi (%) üzerine etkisi

	L. angustifolia	100a	92.0a	56.8b	54.2b	20.2c	3.9c	0.0c	92.67***
	R. officinalis	100a	96.7a	98.4a	99.2a	99.8a	39.2b	34.0b	48.12***
Chenopodium album	*O. syriacum*	100a	99.2a	36.0b	0.8c	0.0c	0.0c	0.0c	286.40***
	T. vulgaris	100a	97.4a	96.1a	60.1b	0.0c	0.8c	0.0c	165.23***
	L. angustifolia	100a	93.2a	95.3a	86.4a	94.0a	35.6b	2.9c	78.45***
	R. officinalis	100a	96.6a	94.9a	91.5a	98.3a	88.1a	73.7b	12.56**
Solanum nigrum	*O. syriacum*	100a	99.6a	94.8a	16.4b	0.8c	0.0c	0.0c	210.15***
	T. vulgaris	100a	99.6a	98.8a	99.6a	89.6a	12.8b	0.0c	98.34***
	L. angustifolia	100a	100.0a	100.0a	99.2a	100.0a	99.6a	99.2a	1.05 ns
	R. officinalis	100a	100.0a	100.0a	99.2a	97.5a	99.2a	98.7a	1.12 ns
Cucumis sativus	*O. syriacum*	100a	96.6a	93.4ab	89.2ab	80.5bc	58.6c	51.6c	18.75***
	T. vulgaris	100a	89.6a	99.5a	94.2a	89.2a	82.2b	76.0b	12.40***
	L. angustifolia	100a	95.8a	97.9a	94.6a	91.3a	87.1a	85.1a	5.67*
	R. officinalis	100a	97.5a	90.0a	96.2a	96.2a	90.0a	81.4b	6.12*

Means followed by different letters (a, b, c, d) within the same row are statistically different according to Duncan's multiple range test (P < 0.05). Asterisks in the F-value column indicate statistical significance at ***: P < 0.001, **:P < 0.01, and *: P < 0.05; ns: non-significant.

Bioherbicidal effects of essential oils from four different species belonging to the Lamiaceae family on the seed germination of the weed and crop species were found to be statistically highly significant depending on the applied concentrations ($p < 0.001$). Analysis of variance (ANOVA) results revealed that *O. syriacum* and *T. vulgaris* essential oils, in particular, significantly inhibited germination in all weed species (Table 1). In parallel with the dose increase, significant decreases in germination rates were detected in the weed species compared to the control group, whereas the response of the crop plant *C. sativus* to these treatments remained more limited. Conversely, the effects of *Lavandula angustifolia* and *Rosmarinus officinalis* essential oils on *S. nigrum* were found to be statistically insignificant.

It was determined that the inhibitory effect of the essential oils on weed and cucumber seed germination was highly dependent on both the plant species and the essential oil concentration (Table 1). *O. syriacum* essential oil exhibited the strongest effect, reducing the germination of *A. palmeri* and *P. oleracea* to below 40% even at the lowest dose (0.5 µl/petri). While complete germination inhibition was achieved with *O. syriacum* oil at a dose of 2 µl/petri in most of the weed species used in the study, it was observed that *T. vulgaris* oil required higher doses (8–16 µl/petri) to reach similar suppression levels. On the other hand, *L. angustifolia* and *R. officinalis* essential oils showed a limited effect on *S. nigrum* across all doses. *C. sativus* continued to germinate even at the 8 µl/petri dose, where the germination of weeds had completely stopped; furthermore, a germination rate of over 50% was recorded even at the highest dose (16 µl/petri) of *O. syriacum* essential oil.

Statistically significant differences were determined among the inhibitory abilities of the essential oils on seed germination ($p < 0.001$) (Figure 1).

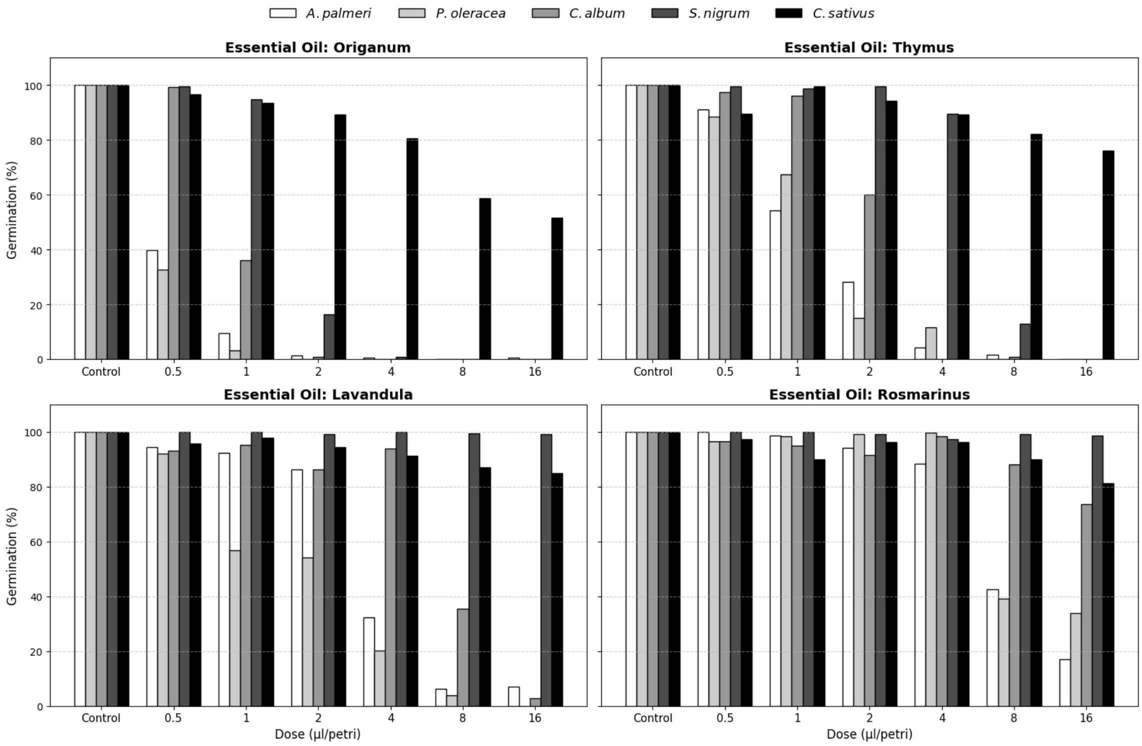

Figure 1. Comparative inhibitory effects of four Lamiaceae essential oils on the seed germination of weed and crop species.

Şekil 1. Dört Lamiaceae uçucu yağının yabancı ot ve kültür bitkisi türlerinin tohum çimlenmesi üzerindeki karşılaştırmalı engelleyici etkileri

Across all plant species, the strongest herbicidal activity was observed in *O. syriacum* essential oil, followed by *T. vulgaris*, *L. angustifolia*, and *R. officinalis* essential oils, respectively. The highest mean germination percentages were recorded in the *R. officinalis* essential oil treatments (Table 2).

The severity of the phytotoxic response was evaluated by comparing the slope parameters (*b*) of the dose-response curves for each essential oil (Figure 2).

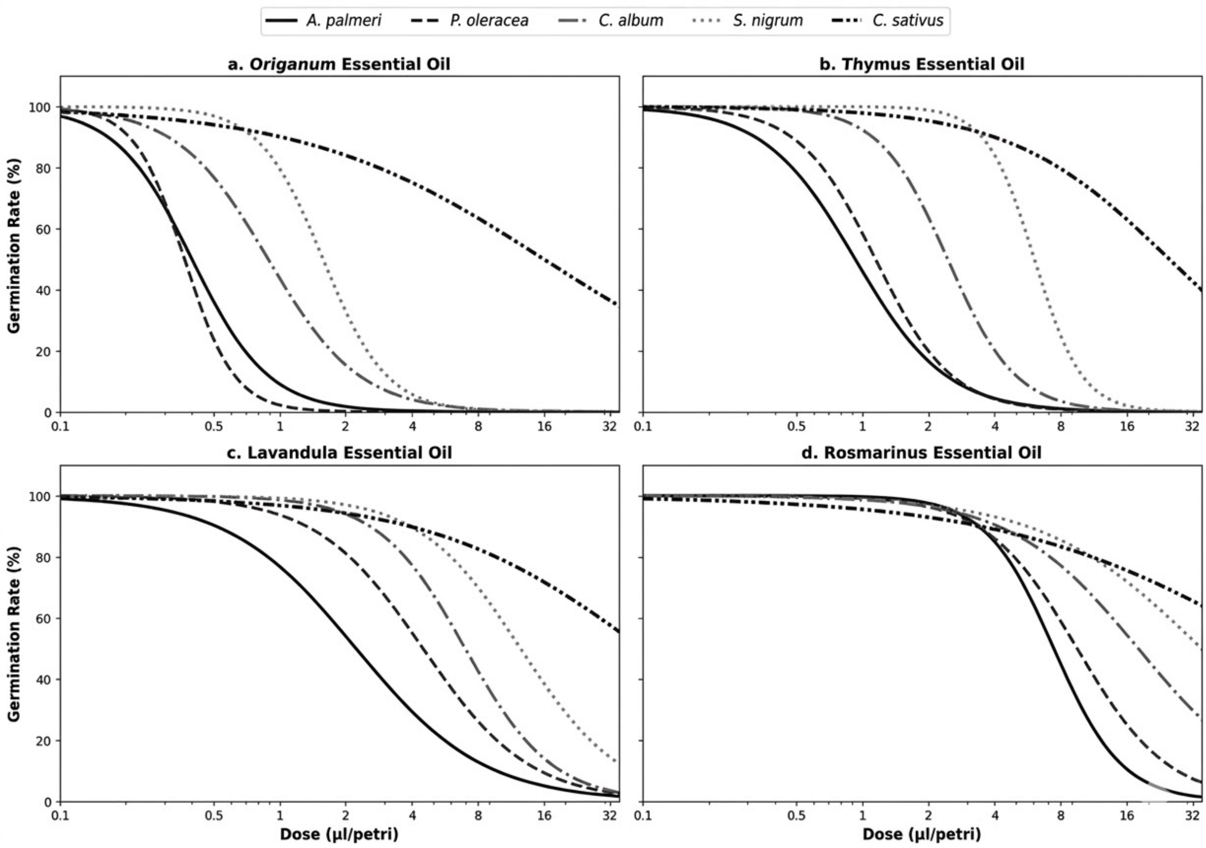

Figure 2. Comparison of slope values (*b*) derived from log-logistic dose-response models for the effects of Lamiaceae essential oils on weed seeds

Şekil 2. Yabancı ot tohumlarına Lamiaceae uçucu yağı etkileri için log-lojistik doz-tepki modellerinden elde edilen eğim değerlerinin (b) karşılaştırması

Statistically significant differences were determined among the essential oils, with *O. syriacum* and *T. vulgaris* exhibiting higher slope values compared to *L. angustifolia* and *R. officinalis*. These high slopes indicate that even small increases in concentration lead to a much sharper decline in germination, suggesting that once the toxic threshold is reached, these oils act rapidly to inhibit seedling development. In particular, the steep slope observed for *O. syriacum* essential oil reveals its stronger mechanism of action, whereas the lower slope values recorded for *R. officinalis* reflect a more gradual and less impactful inhibitory response across the tested dose range.

Table 2. Regression parameters and LC_{50} values, and 95% confidence intervals (CI) derived from the three-parameter log-logistic model regarding the effects of essential oils on the seed germination of weed and crop species

Çizelge 2. Uçucu yağların yabancı ot ve kültür bitkisi türlerinin tohum çimlenmesi üzerindeki etkilerine ait üç parametreli log-lojistik modelden elde edilen regresyon parametreleri, LC_{50} değerleri ve %95 güven aralıkları (GA)

Essential Oil	Weeds and crop	Slope (b)	LC_{50} (µl/petri)	95% CI Lower	95% CI Upper	R^2
O. syriacum	*A. palmeri*	2.50	0.40	0.35	0.45	0.98
	P. oleracea	3.80	0.37	0.25	0.49	0.99
	C. album	2.10	0.89	0.74	1.04	0.96
	S. nigrum	3.00	1.58	1.44	1.72	0.97
	C. sativus	0.80	16.00	12.92	19.08	0.95

Table 2 (continue). Regression parameters and LC_{50} values, and 95% confidence intervals (CI) derived from the three-parameter log-logistic model regarding the effects of essential oils on the seed germination of weed and crop species

Çizelge 2 (devamı). Uçucu yağların yabancı ot ve kültür bitkisi türlerinin tohum çimlenmesi üzerindeki etkilerine ait üç parametreli log-lojistik modelden elde edilen regresyon parametreleri, LC_{50} değerleri ve %95 güven aralıkları (GA)

Essential Oil	Weeds and crop	Slope (b)	LC_{50} (µl/petri)	95% CI Lower	95% CI Upper	R^2
T. vulgaris	*A. palmeri*	2.10	0.93	0.79	1.07	0.97
	P. oleracea	2.50	1.15	0.99	1.31	0.96
	C. album	2.80	2.45	2.17	2.73	0.95
	S. nigrum	4.00	6.08	5.62	6.54	0.98
	C. sativus	1.20	25.00	20.02	29.98	0.94
L. angustifolia	*A. palmeri*	1.50	2.23	1.60	2.86	0.94
	P. oleracea	1.80	4.50	3.05	5.95	0.93
	C. album	2.20	7.00	5.91	8.09	0.95
	S. nigrum	1.90	12.50	7.45	17.55	0.91
	C. sativus	0.90	45.00	31.84	58.16	0.89
R. officinalis	*A. palmeri*	2.80	7.44	6.26	8.62	0.94
	P. oleracea	2.10	9.50	7.64	11.36	0.92
	C. album	1.50	18.00	11.64	24.36	0.90
	S. nigrum	1.20	35.00	16.30	53.70	0.88
	C. sativus	0.70	80.00	39.33	120.67	0.85

95% CI: confidence intervals calculated using the delta method based on published model parameters.

To quantitatively determine the phytotoxic effect of the essential oils, seed germination data were fitted to a three-parameter log-logistic regression model. Regression analysis yielded high coefficients of determination (ranging from R_2 = 0.85 to 0.99), indicating that the model accurately described the dose-response relationships (Table 2). Based on the calculated LC_{50} values, *O. syriacum* essential oil was identified as the most effective treatment, requiring a dose of only 0.37–1.58 µl/petri to inhibit weed germination by 50%. In contrast, *R. officinalis* essential oil exhibited the lowest efficacy, with calculated LC_{50} values reaching up to 18.00 and 35.00 µl/petri for *C. album* and *S. nigrum*, respectively. The LC_{50} values calculated for the crop plant *C. sativus* (ranging from 16.00$ to 80.00 µl/petri) showed effects above the maximum application concentration of 16 µl/petri (Table 2).

Selectivity index (si) and safety margin for cucumber (Cucumis sativus)

The selectivity of the essential oils between the weed species and the crop plant (*C. sativus*) was evaluated using the Selectivity Index (SI) based on LC_{50} values (Table 3). A clear distinction in safety margins was determined among the four essential oils.

The selectivity properties of the essential oils toward the cucumber plant were calculated using SI values (Table 3). *O. syriacum* essential oil exhibited the highest selectivity indices among all weed species. The highest SI values were recorded against *P. oleracea* (43.24) and *A. palmeri* (40.00). For *S. nigrum*, the most resistant weed, *O. syriacum* oil achieved an SI value of 10.13. *T. vulgaris* essential oil also showed high selectivity on *A. palmeri* (26.88) and *P. oleracea* (21.74), while its SI value on *S. nigrum* was determined as 4.11. In contrast, lower SI values were obtained for *L. angustifolia* and *R. officinalis* essential oils across all species, with the lowest value observed at 2.29 for the effect of *R. officinalis* on *S. nigrum*.

Table 3. Selectivity Index (SI) and safety margins of essential oils for cucumber (*Cucumis sativus*) against target weed species

Çizelge 3. Uçucu yağların hedef yabancı ot türlerine karşı hıyar (Cucumis sativus) için Seçicilik İndeksi (SI) ve güvenlik marjları.

Essential Oil	**Target weed**	**LC_{50} (Weed)**	**LC_{50} (*C. sativus*)**	**Selectivity Index (SI)**	**Interpretation**
O. syriacum	*A. palmeri*	0.40	16.00	40.00	Extremely Selective
	P. oleracea	0.37	16.00	43.24	Extremely Selective
	C. album	0.89	16.00	17.98	Extremely Selective
	S. nigrum	1.58	16.00	10.13	Extremely Selective
T. vulgaris	*A. palmeri*	0.93	25.00	26.88	Extremely Selective
	P. oleracea	1.15	25.00	21.74	Extremely Selective
	C. album	2.45	25.00	10.20	Extremely Selective
	S. nigrum	6.08	25.00	4.11	Moderate Selectivity
L. angustifolia	*A. palmeri*	2.23	45.00	20.18	Extremely Selective
	P. oleracea	4.50	45.00	10.00	Extremely Selective
	C. album	7.00	45.00	6.43	High Selective
	S. nigrum	12.50	45.00	3.60	Moderate Selectivity
R. officinalis	*A. palmeri*	7.44	80.00	10.75	Extremely Selective
	P. oleracea	9.50	80.00	8.42	High Selective
	C. album	18.00	80.00	4.44	Moderate Selectivity
	S. nigrum	35.00	80.00	2.29	Moderate Selectivity

SI classification: SI < 2, Low Selectivity; SI = 2–5, Moderate Selectivity; SI = 5–10, High Selectivity; SI > 10, Extremely Selective (Streibig et al., 1993; Ibáñez & Blázquez, 2018).

Effects of essential oils on root length

The inhibitory effects of the four Lamiaceae essential oils on the root growth of weed and crop seedlings are presented in Table 4. Statistical analysis results showed that root growth was significantly suppressed in all plant species in parallel with the increase in concentration ($P < 0.001$). In particular, *O. syriacum* and *T. vulgaris* essential oils brought root development in weeds to a near-halt even at the lowest doses (0.5–1.0 µl/petri). The root development of the crop plant *C. sativus* proved to be statistically more resistant to all oils compared to the weed species.

The results obtained from the study reveal that there are significant inhibition rates in germination across all plants depending on the dose. *O. syriacum* and *T. vulgaris* essential oils exhibited the strongest suppressive effect. At a dose of 1.0 µl/petri, *O. syriacum* essential oil completely inhibited (%100) the root growth of *A. palmeri* and *P. oleracea*. It was determined that *T. vulgaris* essential oil inhibited root development for *A. palmeri* at a dose of 4.0 µl/petri. Among the weed species, *S. nigrum* and *C. album* required higher doses (4.0–16.0 µl/petri) for root inhibition. In contrast, *L. angustifolia* and *R. officinalis* essential oils showed a more limited effect, especially at low doses; statistically significant reductions in root length were generally obtained from doses of 8.0 µl/petri and above. The crop plant *C. sativus* succeeded in maintaining root growth under all essential oils even at the 16.0 µl/petri dose.

Table 4. Effect of essential oil applications on the root length (cm) of weeds and crop

Çizelge 4. Uçucu yağ uygulamalarının yabancı ot ve kültür bitkisi türlerinin kök uzunluğu (cm) üzerindeki etkisi

Essential Oil	Weeds and crop	0 µl (Control)	0.5 µl	1.0 µl	2.0 µl	4.0 µl	8.0 µl	16.0 µl	F-value
O. syriacum	*A. palmeri*	4.50 ± 0.3a	1.25 ± 0.2b	0.00 ± 0.0c	0.00 ± 0.0c	0.00 ± 0.0c	0.00 ± 0.0c	0.00 ± 0.0c	345.12***
	P. oleracea	3.80 ± 0.2a	0.85 ± 0.1b	0.00 ± 0.0c	0.00 ± 0.0c	0.00 ± 0.0c	0.00 ± 0.0c	0.00 ± 0.0c	412.05***
	C. album	4.10 ± 0.4a	3.80 ± 0.3a	0.65 ± 0.1b	0.00 ± 0.0c	0.00 ± 0.0c	0.00 ± 0.0c	0.00 ± 0.0c	289.40***
	S. nigrum	5.20 ± 0.3a	5.05 ± 0.4a	3.10 ± 0.2b	0.45 ± 0.1c	0.00 ± 0.0d	0.00 ± 0.0d	0.00 ± 0.0d	215.18***
	C. sativus	8.50 ± 0.5a	8.35 ± 0.4a	7.90 ± 0.5a	6.80 ± 0.4b	5.10 ± 0.3c	2.45 ± 0.2d	1.10 ± 0.1e	45.75***
T. vulgaris	*A. palmeri*	4.50 ± 0.3a	3.65 ± 0.2b	1.80 ± 0.2c	0.65 ± 0.1d	0.00 ± 0.0e	0.00 ± 0.0e	0.00 ± 0.0e	156.45***
	P. oleracea	3.80 ± 0.2a	3.10 ± 0.3b	2.10 ± 0.2c	0.40 ± 0.1d	0.00 ± 0.0e	0.00 ± 0.0e	0.00 ± 0.0e	178.90***
	C. album	4.10 ± 0.4a	3.95 ± 0.4a	3.50 ± 0.3b	1.25 ± 0.2c	0.00 ± 0.0d	0.00 ± 0.0d	0.00 ± 0.0d	195.23***
	S. nigrum	5.20 ± 0.3a	5.10 ± 0.3a	4.85 ± 0.4a	4.50 ± 0.3a	2.80 ± 0.2b	0.55 ± 0.1c	0.00 ± 0.0d	112.34***
	C. sativus	8.50 ± 0.5a	8.10 ± 0.5a	8.45 ± 0.6a	7.50 ± 0.4b	6.80 ± 0.4b	5.40 ± 0.3c	3.25 ± 0.2d	32.40***
L. angustifolia	*A. palmeri*	4.50 ± 0.3a	4.65 ± 0.4a	4.10 ± 0.3a	3.80 ± 0.2b	1.45 ± 0.1c	0.20 ± 0.0d	0.00 ± 0.0e	98.12***
	P. oleracea	3.80 ± 0.2a	3.60 ± 0.2a	2.10 ± 0.2b	1.85 ± 0.1b	0.60 ± 0.1c	0.00 ± 0.0d	0.00 ± 0.0d	82.67***
	C. album	4.10 ± 0.4a	4.05 ± 0.3a	3.85 ± 0.4a	3.60 ± 0.3a	2.20 ± 0.2b	1.15 ± 0.1c	0.30 ± 0.0d	74.45***

Table 4 (continue). Effect of essential oil applications on the root length (cm) of weeds and crop

Çizelge 4 (devamı). Uçucu yağ uygulamalarının yabancı ot ve kültür bitkisi türlerinin kök uzunluğu (cm) üzerindeki etkisi

	S. nigrum	5.20 ± 0.3a	5.15 ± 0.4a	5.00 ± 0.5a	4.85 ± 0.4a	3.90 ± 0.3b	2.65 ± 0.2c	1.20 ± 0.1d	54.12***
	C. sativus	8.50 ± 0.5a	8.25 ± 0.4a	8.10 ± 0.5a	7.90 ± 0.4a	7.45 ± 0.3b	6.80 ± 0.4b	6.10 ± 0.3c	12.67**
R. officinalis	*A. palmeri*	4.50 ± 0.3a	4.80 ± 0.3a	4.45 ± 0.4a	4.20 ± 0.3a	3.95 ± 0.2a	1.80 ± 0.2b	0.65 ± 0.1c	46.34***
	P. oleracea	3.80 ± 0.2a	3.95 ± 0.2a	3.75 ± 0.3a	3.70 ± 0.2a	3.65 ± 0.2a	1.55 ± 0.1b	1.10 ± 0.1b	38.12***
	C. album	4.10 ± 0.4a	4.20 ± 0.4a	4.05 ± 0.3a	3.95 ± 0.4a	3.80 ± 0.3a	2.80 ± 0.2b	1.95 ± 0.2c	22.56***
	S. nigrum	5.20 ± 0.3a	5.35 ± 0.5a	5.10 ± 0.4a	5.05 ± 0.4a	4.95 ± 0.5a	4.10 ± 0.3b	3.40 ± 0.3c	18.12***
	C. sativus	8.50 ± 0.5a	8.65 ± 0.5a	8.20 ± 0.4a	8.40 ± 0.5a	8.35 ± 0.6a	7.90 ± 0.4ab	6.80 ± 0.4b	5.12*

Means in the same row followed by different letters (a, b, c, d, e) are statistically different according to Duncan's Multiple Range Test (P < 0.05). Asterisks in the F-value column indicate statistical significance at levels of ***: P < 0.001, **: P < 0.01, and *: P < 0.05.

The dose-response curves reveal a distinct sensitivity difference between the weed species and the crop plant (Figure 3).

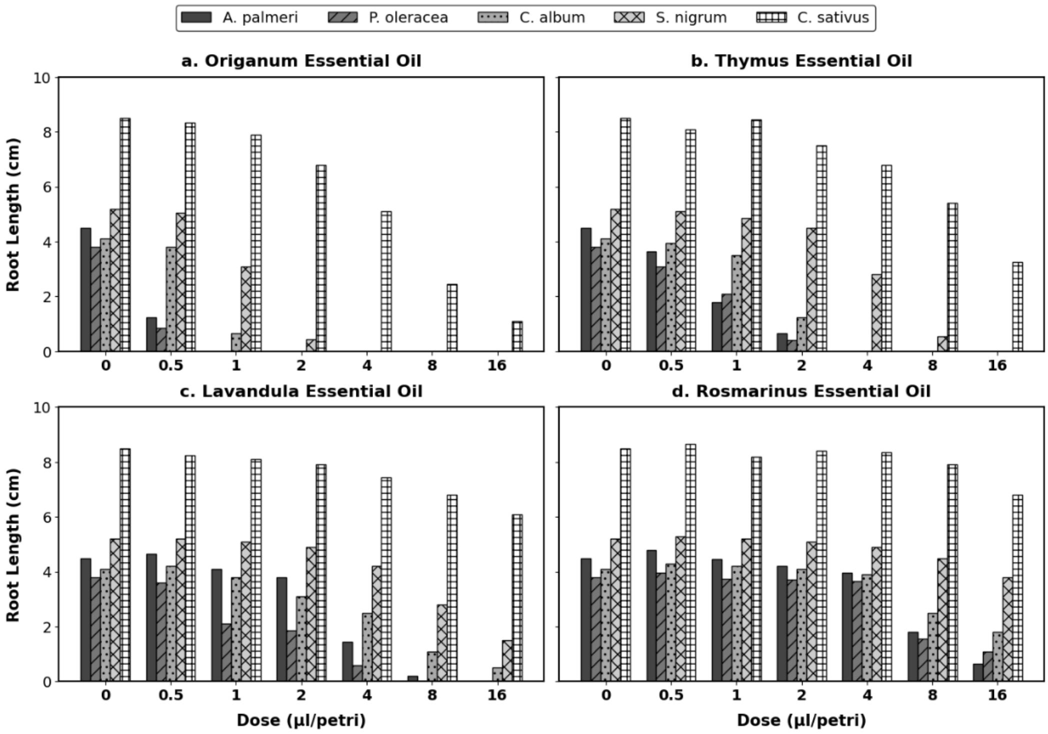

Figure 3. Dose-response curves showing the inhibitory effect of *Origanum* essential oil on the seed germination of weed species and the crop plant *Cucumis sativus*

Şekil 3. Origanum uçucu yağının yabancı ot türleri ve kültür bitkisi Cucumis sativus'un tohum çimlenmesi üzerindeki engelleyici etkisini gösteren doz-tepki eğrileri

While seed germination in the weed species *A. palmeri* and *P. oleracea* was completely inhibited at concentrations below 2 µl/petri, the germination rate of *C. sativus* remained above 80% up to the 8 µl/petri dose. The dose-response curve for the crop plant exhibited a distinctly right-shifted profile compared to the weed curves (Figure 3).

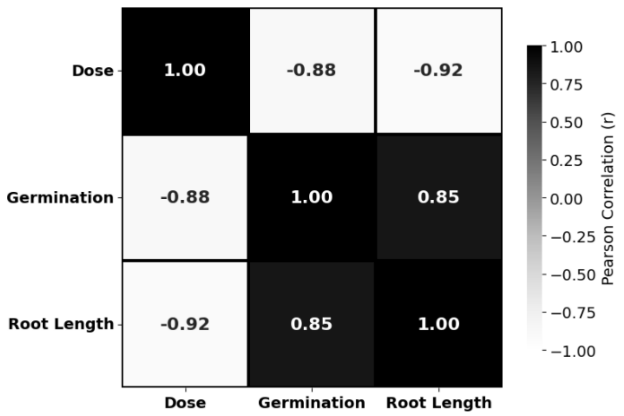

Figure 4. Pearson correlation matrix showing the relationships among essential oil dose, seed germination, and root length in all tested species

Şekil 4. Test edilen tüm türlerde uçucu yağ dozu, tohum çimlenmesi ve kök uzunluğu arasındaki ilişkileri gösteren Pearson korelasyon matrisi

The relationships among experimental variables across essential oils and plant species were evaluated using a Pearson correlation matrix. According to the analysis results, a statistically very strong and negative correlation (r = -0.92) was found between the applied dose and root length. Additionally, a high positive correlation (r = 0.85) was recorded between seed germination and root growth. These coefficients confirm that as the essential oil concentration increases, both germination and root development decrease in a highly consistent and synchronized manner (Figure 4).

Multivariate analysis of weed and cucumber seed germination in response to essential oils

PCA was performed on germination and root length data to evaluate the responses of plant species to essential oil applications through a holistic approach (Figure 5).

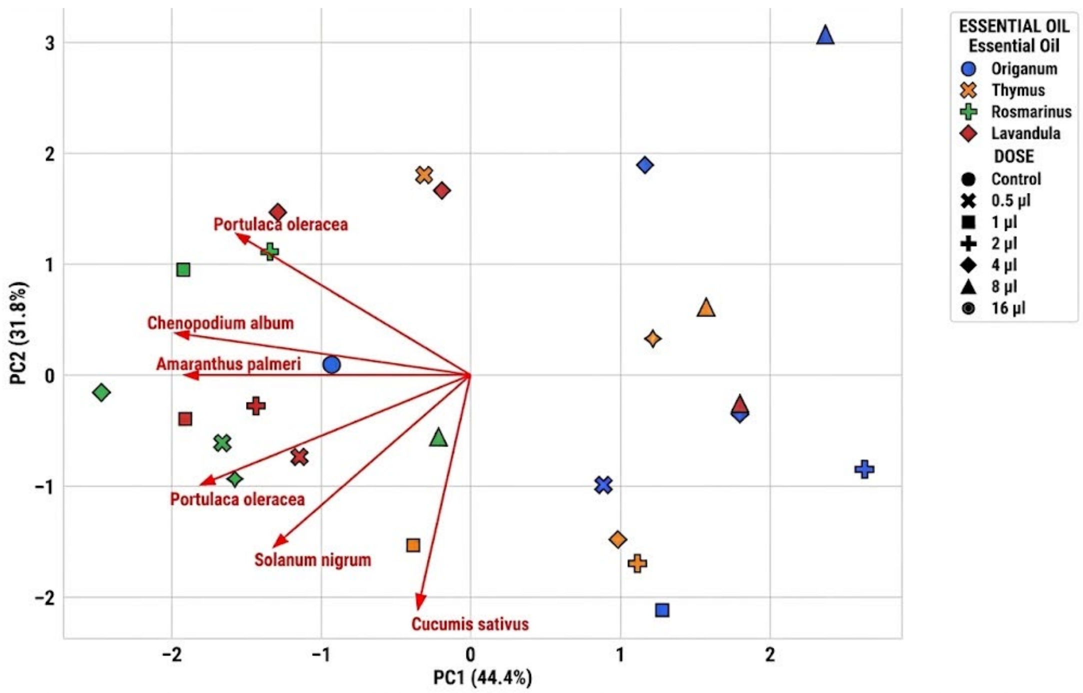

Figure 5. Principal Component Analysis (PCA) biplot showing the grouping of plant species based on their morphological and physiological responses (Seed Germination and Root Length) to different essential oil treatments

Şekil 5. Farklı uçucu yağ uygulamalarına karşı bitki türlerinin morfolojik ve fizyolojik tepkilerine (Tohum Çimlenmesi ve Kök Uzunluğu) gore gruplandırılmasını gösteren Temel Bileşenler Analizi (PCA) biplot grafiği

The resulting biplot reveals a distinct separation among species based on their multivariate response profiles. The crop plant, *C. sativus*, formed a homogeneous cluster on the positive (right) side of the first axis (PC1), completely independent of the weed species. In contrast, *A. palmeri*, *P. oleracea*, *C. album*, and *S. nigrum* clustered in the left and central segments of the plot. Increasing essential oil concentrations pushed these weed species toward the far-left (sensitive) region of the PC1 axis, reaching the points furthest from the crop plant cluster (Figure 4).

DISCUSSION

Previous studies have clearly demonstrated that essential oils obtained from aromatic plants, particularly species belonging to the *Lamiaceae* family such as *Origanum*, *Thymus*, *Lavandula*, *Rosmarinus*, *Mentha*, and *Satureja*, have a strong and distinct phytotoxic (allelopathic) effect on weeds (Angelini et al., 2003; Karaman et al., 2021; Yaşar et al., 2024) as well as on plant pathogens and pests (Sertkaya et al., 2010ab, Kaya et al., 2018; Bozkurt et al., 2020; Türkmen et al., 2022; Oğuz et al., 2024; Maral et al., 2025; Soylu et al., 2026). The observed inhibition of germination and seedling growth varies depending on the chemical tolerance threshold of the target species, as frequently reported in allelopathic studies. This situation is statistically consistent with the sudden drops recorded in weed germination rates in parallel with the increasing dose in our study, as evidenced by the high *F*-values (ranging from 210.15 to 312.05) in the analysis of variance. The herbicidal efficacy of essential oils follows a dose-dependent model (Cunedioğlu & Üremiş, 2018; Efil & Üremiş, 2019). While only delays in germination of weed seeds or reductions in seedling lengths are observed at low concentrations, the inhibition rate increases rapidly as the applied essential oil dose increases, and germination is completely halted after a certain threshold value (Atak et al., 2016). This dose-dependent response exhibited by the plants used in the experiment proves that essential oils can be used within standardized dose ranges, similar to a chemical bioherbicide.

This suppressive effect of essential oils on weeds is attributed to the complex secondary metabolites present in their composition, particularly terpenoids (Verdeguer et al., 2020). Essential oils of *Lamiaceae* family plants possess a rich chemical composition exhibiting phytotoxic properties, primarily monoterpenes and sesquiterpenes (Özen et al., 2017). The obtained results indicate that an increase in the amount of terpenoids in essential oils directly increases allelopathic activity. Among the essential oil components, the most successful results in terms of herbicidal effect have been obtained from oxygenated monoterpenes (Abd-ElGawad et al., 2021). Oxygenated monoterpenes (carvacrol, thymol, linalool, 1,8-cineole, etc.) demonstrate a much higher phytotoxic activity compared to monoterpene hydrocarbons (alpha-pinene, limonene, camphene, etc.) (Kordali et al., 2007; Üstüner et al., 2018a) This situation was also revealed by the steepness of the slope parameter (*b*) obtained from the dose-response model; the steep slopes observed for *Origanum* biometrically proved that a sudden metabolic collapse is induced in the target weeds once the threshold concentration is exceeded. The relatively higher water solubility of oxygenated monoterpenes increases their biological efficacy by enabling them to penetrate the soil microenvironment and seed coat more easily.

Carvacrol and thymol, which are the main components of *Origanum* and *Thymus* essential oils in particular, stand out as bioherbicidal agents with a highly aggressive and rapid mechanism of action (Kordali et al., 2008; Yılar et al., 2019). Furthermore, GC-MS analyses conducted by Üremiş et al. (2009) have proven that these species contain carvacrol and thymol at rates exceeding 70%, and that these phenolic monoterpenes can completely halt the seed germination of important weeds such as *Amaranthus retroflexus* and *Chenopodium album* even at very low concentrations. In contrast, although oils containing mostly 1,8-cineole, camphor, or linalool, such as *Rosmarinus* or *Lavandula*, are also phytotoxic, they require higher doses to achieve the same level of inhibition (Üremiş et al., 2009; Isik et al., 2016).

The mechanism of action of these allelochemicals at the cellular level is highly complex and targets multiple physiological pathways. After penetrating the seed coat, essential oils disrupt the structural integrity of cellular membranes and degradation of intracellular organelles. It has been reported that oxygenated monoterpenes also lead to the accumulation of lipid globules within the cell, inhibit DNA synthesis, and arrest plant development at its roots by stopping cell division (Kordali et al., 2007; Mutlu et al., 2011). The most sensitive morphological response exhibited by plants to essential oil stress is observed on root (radicle) development (Atak et al., 2016). Root apical meristems are highly vulnerable to lipophilic terpenes because they are centers of intense mitotic division and are in direct contact with the environment, lacking the protective coverings found in the seed. Indeed, in this study, the very strong negative correlation ($r = -0.92$) between dose and root length reveals that the inhibition of root development is the most sensitive indicator of essential oil toxicity. This is confirmed by the fact that even in seeds that succeed in germinating, root length is severely weakened, exhibiting a strong negative correlation with the essential oil dose (Aydın & Tursun, 2010). On the other hand, the slight increase in root length detected at the lowest dose (0.5 µl/petri) of *Rosmarinus* oil indicates that it may exhibit a low-dose stimulatory effect defined as "hormesis", as reported in previous studies.

The degrees of sensitivity exhibited by different weed species to essential oils also vary significantly. Small-seeded broadleaf weeds such as *A. palmeri*, *P. oleracea*, and *C. album* are highly sensitive to essential oil applications, and their germination is rapidly inhibited (Angelini et al., 2003; Cunedioğlu & Üremiş, 2018). However, it has also been observed that in some species with hard seed coats, chemical resistance can partially increase because the diffusion rate of the essential oil to the embryo is limited by a physical barrier.

Selectivity, which is one of the most critical stages in the bio-herbicide development process, is the main factor determining the practical agricultural usability of these essential oils. Previous studies prove that essential oils create a distinct physiological difference between crops and weeds. Crops such as cucumber (*C. sativus*), cotton, wheat, or pepper have a much higher tolerance threshold to essential oils than weeds (Tursun et al., 2022). In agricultural applications, an SI value greater than 1 indicates basic selectivity, while SI > 2 is generally accepted as the minimum threshold for practical field safety to account for application variability and environmental stress (Streibig et al., 1993; Ibáñez & Blázquez, 2018)**.** Values exceeding SI > 10 are considered indicative of high selectivity, suggesting a wide and reliable safety margin under field conditions (Ibáñez & Blázquez, 2018)**.** According to the SI values calculated in our study, *Origanum* oil achieved SI values of 10.13–43.24 across all target weed species, placing it firmly in the **"**extremely selective" category. *T. vulgaris* essential oil likewise demonstrated high selectivity (SI: 4.11–26.88), confirming its potential for safe use in cucumber production. In contrast, *L. angustifolia* and *R. officinalis* oils produced lower SI values (2.29–20.18), with *R. officinalis* against *S. nigrum* (SI = 2.29) approaching the minimum practical threshold, indicating a narrow safety margin for this combination. This structural tolerance in crops can be explained by their ability to detoxify exogenous monoterpenes or their physical advantages in seed size (Tursun et al., 2022). It has been determined that other aromatic plants from the *Lamiaceae* family, such as pennyroyal (*Mentha pulegium*) and summer savory (*Satureja hortensis*), possess similarly strong allelopathic properties (Karaman et al., 2021; Yaşar et al., 2024). Even at low doses, the essential oils of these species exert severe pre-emergence suppression on summer and winter weeds, while causing less harm to crops like wheat and pepper. This indicates that these aromatic plants can be evaluated as weed-suppressive cover crops in polyculture or crop rotation systems. In addition to essential oils, hydrosols, which emerge as by-products during the distillation of these oils, have also been found to have significant bio-herbicidal potential (Efil & Üremiş, 2019). Hydrosols, containing dissolved allelochemicals, successfully inhibit the germination of problematic weeds. This demonstrates that all materials resulting from aromatic plant processing can be utilized in weed management with a zero-waste principle.

When considered in the context of environmental sustainability, the greatest advantage offered by plant-derived essential oils compared to synthetic herbicides is their rapid degradation in nature (biodegradability) and the absence of toxic residues (Tworkoski, 2002). These natural molecules, which do not pollute soil and groundwater, offer a unique weed control mechanism for organic farming systems (Arslan & Uremis, 2015). Despite these outstanding successes achieved under laboratory and controlled climate conditions, there are practical limitations that need to be overcome in the direct application of essential oils under field conditions, such as volatility and low water solubility (Kordali et al., 2022). Consequently, advanced formulation technologies are required to integrate these oils, which exhibit high selectivity and strong herbicidal activity, into practical agriculture. Future research focusing on encapsulation techniques to stabilize the volatility of active terpenes will make a lasting contribution to the sustainability of modern agriculture (Abd-El Gawad et al., 2021).

CONCLUSION

This study highlights the significant phytotoxic potential of essential oils from different species of the Lamiaceae family as eco-friendly alternatives to synthetic herbicides. Dose-response and multivariate analyses revealed that *Origanum* and *Thymus* essential oils strongly and dose-dependently inhibited both the seed germination and root elongation of weed species that cause major problems in agricultural areas. The most important finding of this research is the highly potent physiological selectivity exhibited by *Origanum* essential oil. While this essential oil severely suppressed target weeds at very low concentrations, cucumber (*Cucumis sativus*) exhibited a high level of tolerance, with calculated LC_{50} values reaching up to 80.00 µl/petri. This high disparity resulted in an extremely favorable dose range (with SI values upto 43.24) and proved that *Origanum* can provide effective weed control without causing phytotoxicity in the crop. In contrast, *Lavandula* and *Rosmarinus* essential oils did not demonstrate sufficient efficacy and selectivity, leading to the conclusion that these essential oils are inadequate for targeted weed management in cucumber cultivation. Based on the obtained results, *Origanum* essential oil stands out as a highly promising, natural bioherbicide candidate for sustainable and organic farming systems. Future research should focus on translating these *in vitro* results to greenhouse and open field trials by utilizing advanced formulation technologies.

STATEMENT OF CONFLICT OF INTEREST

The authors declare no conflict of interest for this research

AUTHOR'S CONTRIBUTIONS

The contribution of the authors is equal.

STATEMENT OF ETHICS CONSENT

Ethical approval is not required as there are no studies with human or animal subjects in this article.

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