Phytotoxic Effects of Aromatic Plants' Hydrodistillation Water Residues on Germination and Growth Characteristics of *Avena sterilis*, *Echinochloa crus-galli*, and *Zea mays*

Pinelopi N. Liontou , Anastasia V. Badeka , Thomas K. Gitsopoulos , Georgios Patakioutas ,
Nicholas E. Korres *

Posted Date: 2 April 2026

doi: 10.20944/preprints202604.0216.v1

Keywords: natural herbicides; phytotoxicity; liquid residues of distillation; weed control; thyme; laurel; oregano; lavender

Article

Phytotoxic Effects of Aromatic Plants' Hydrodistillation Water Residues on Germination and Growth Characteristics of *Avena sterilis*, *Echinochloa crus-galli*, and *Zea mays*

Pinelopi N. Liontou [1], Anastasia V. Badeka [2], Thomas K. Gitsopoulos [3], Georgios Patakioutas [1] and Nicholas E. Korres [1,*]

[1] Department of Agriculture, School of Agriculture, University of Ioannina, Kostakii, 47100 Arta, Greece
[2] Laboratory of Food Chemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece
[3] Hellenic Agricultural Organization - DIMITRA, Athens, Greece
* Correspondence: nkorres@uoi.gr

Abstract

The demand for sustainable weed management and the limited discovery of new herbicide molecules have led to high interest in plant-derived bioherbicides, such as the water residues (WRs) from the hydrodistillation of aromatic plants, which contain biologically active secondary metabolites. Here, the phytotoxic potential of WRs of four aromatic plant species was investigated. Chemical composition of WRs was determined by SPME–GC–MS, and their effect was assessed on seed germination and seedling growth characteristics of *Avena sterilis*, *Echinochloa crus-galli,* and *Zea mays*. Five concentrations, i.e., 0, 10, 20, 50, and 100, with 100 representing pure WR were tested. Phenolic monoterpenes dominate WRs in oregano and thyme, and oxygenated monoterpenes in laurel and lavender. Germination and growth responses were dose-dependent and species-specific. Oregano and lavender WRs exhibited the strongest phytotoxicity, reducing weed germination by 82% and 79%, respectively. In contrast, laurel extracts showed weaker germination inhibition. Across all tested species, germination delays were observed, making WRs a promising candidate for weed control. The results also showed that WR affected root growth by up to 95% shoot by 70–80%. Maize exhibited greater tolerance than the weed species maintaining higher germination. Overall, WRs represent a promising tool for integrated weed management.

Keywords: natural herbicides; phytotoxicity; liquid residues of distillation; weed control; thyme; laurel; oregano; lavender

1. Introduction

Herbicides have been the primary method of weed control worldwide since the late 1960s. However, the excessive use of the same modes of action (MoA) has led to the development of weed resistance [1]. Recently, progress toward more sustainable weed management approaches has attracted the attention of various stakeholder groups [2]. These efforts are part of the European Green Deal and the Farm to Fork strategy, which aims to reduce chemical herbicide use by 50% by 2030 [3]. Only a limited number of new MoAs have been discovered in recent years [4]. Natural compounds emerge as a promising source for new chemistry, because of their structural diversity that differs from synthetic herbicide molecules produced through conventional organic synthesis [5]. Among natural sources, aromatic plants and essential oils produced by processing raw plant material have gained considerable attention for the development of the next-generation herbicides due to their pronounced phytotoxic properties [6].

The Epirus region in northwestern Greece is characterized by high plant biodiversity, a significant part of which is endemic. Differences in altitude, temperature, and soil conditions have led to the development of distinct plant ecotypes. Studies have shown that altitude influences the content and composition of essential oils and other secondary metabolites in aromatic plants, resulting in variation in their biological activity and chemical profiles [7].

Among the aromatic plant species included in the present study i.e., thyme (*Thymus vulgaris*), lavender (*Lavandula angustifolia*), oregano (*Origanum vulgare*), and laurel (*Laurus nobilis*), substantial chemical-ecological variation has been documented in Greece. Oregano, for example, has been shown to produce high essential oil yield with dominant carvacrol and p-cymene chemotypes [8]. A study of multiple Greek thymus species documented substantial variation in volatile profiles (e.g., linalool-rich, carvacrol-rich chemotypes), illustrating strong chemical polymorphism in relation to habitat [9]. This ecological and chemical diversity attracts the interest of scientific research towards aromatic and medicinal plants in Epirus region as a potential source for discovering new chemistries for sustainable weed control.

Distillation of essential oils results in the production of two main by-products (i) plant biomass, which includes solid residues from leaves, flowers, and stems that represents about 60–65%, and (ii) condensed water, referred to as water residues (WRs), which accounts for 30–35% of the total output (Figure 1) [10]. These contain various secondary metabolites, including terpenes, phenolic compounds, and flavonoids, which have been shown to act as germination and growth inhibitors of several weed species [11,12]. By-products from the distillation of thyme (*Thymus vulgaris*) and lavender (*Lavandula angustifolia*), for instance, contain potent bioactive molecules such as thymol and carvacrol, which have exhibited phytotoxic activity against a range of weed species [13]. In addition, residues from the distillation of rosemary (*Rosmarinus officinalis*) contain flavonoids such as luteolin and rosmarinic acid, which affect the growth of several weed species [14,15]

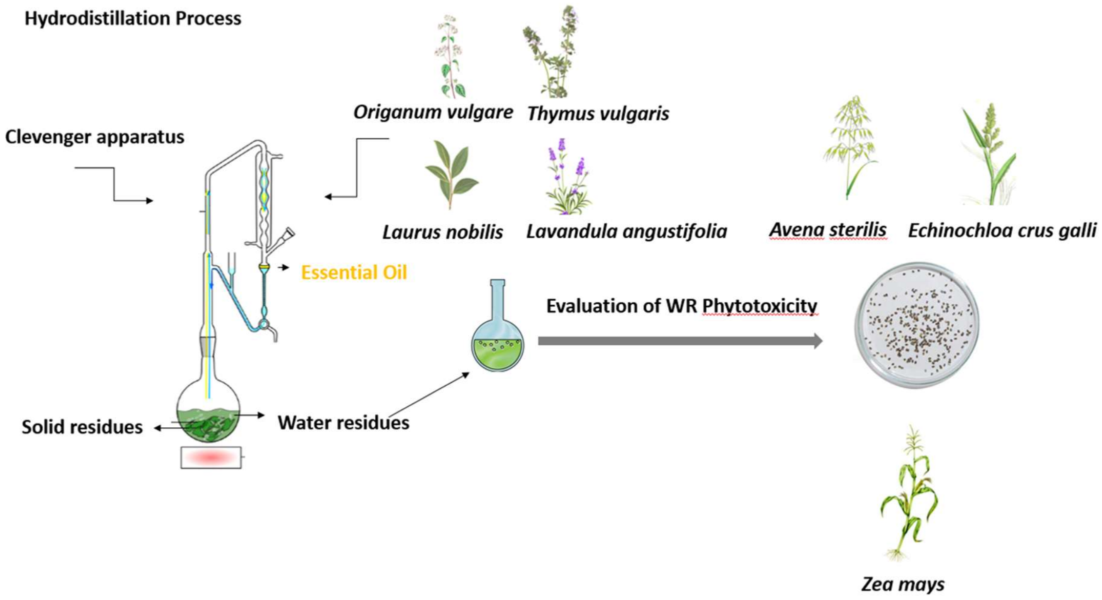

Figure 1. Graphical abstract of hydrodistillation process for extracting WRs and testing their phytotoxicity.

To our knowledge, research on the agricultural use of WRs from essential oil extraction remains underexploited. This study aims to investigate the phytotoxic effects of liquid residues produced by hydrodistillation of selected aromatic and medicinal plant species from the Epirus region on the germination and seedling growth of various grass weed species.

2. Materials and Methods

2.1. Plant Material

Aromatic plants, including oregano, thyme, lavender, and laurel were collected in July 2025 from various locations in the Epirus region, Greece (Table 1). For each species, plant material was sampled from two different altitudes (low and high) to evaluate the potential influence of altitude on water residue composition. The aerial parts of the aromatic plants were collected during the flowering stage, air-dried at room temperature (25 ± 2 °C) in a shaded and well-ventilated area, and stored in paper bags until distillation.

Table 1. Stratified sampling design of aromatic plant species from various altitudes in the Epirus region.

Aromatic Plant Species	Location	Altitude (m)	Sample Name
Origanum vulgare (Oregano)	Arta	1100	Oregano H
	Arta	30	Oregano L
Thymus vulgaris (Thyme)	Ioannina	800	Thyme H
	Arta	30	Thyme L
Laurus nobilis (Laurel)	Ioannina	680	Laurel H
	Arta	30	Laurel L
Lavandula angustifolia (Levander)	Preveza	650	Lavender H
	Arta	30	Lavender L

Note: H=High altitude, L=Low altitude.

The weed species, wild oat and barnyardgrass, as well as a maize cultivar (P0937, Pioneer Hi-Bred International, Inc.), were used in this study. The weed samples were collected from naturally occurring weed populations at the experimental fields of the University Campus in Arta, Greece, during the 2024–2025. Both weed species were chosen due to their wide distribution and invasive nature in the region of Epirus.

2.2. Essential Oil Extraction and Water-Residue Preparation

Essential oils (EOs) were extracted from 40 g of dried plant material by hydrodistillation, employing a Clevenger-type apparatus for 4 h [16]. The WRs remaining beneath the boiler's lower grate after distillation were collected, filtered through Whatman No. 1 filter paper, and the resulting solutions were stored in dark containers at 4 °C until further analysis.

2.3. Sample Preparation and SPME Extraction

For the extraction of volatile compounds from the WRs, SPME was performed using a DVB/CAR/PDMS fiber (50/30 μm; Supelco, Bellefonte, PA, USA). Five milliliters of the WRs sample were placed into a 20 mL glass vial, sealed with PTFE-silicone septa and aluminum crimp caps, and equilibrated for 10 minutes at 45°C on a magnetic stirrer (100 rpm) with a PTFE-coated stir bar. After equilibration, the SPME fiber was exposed to the headspace for 10 minutes at 45°C to extract the volatile compounds. The fiber was then desorbed in the GC injector port at 250°C for 5 minutes.

2.3.1. GC-MS Analysis

Volatile compounds were analyzed using an Agilent 7890A GC system (Agilent Technologies, Santa Clara, CA, USA) coupled with an Agilent 5975-MS detector. Separation was performed on a DB-5MS capillary column (60 m × 0.32 mm i.d., 0.25 μm film thickness). The injector temperature of GC was set at 250 °C, while the oven temperature program started at 40 °C (held for 3 min) and increased to 260 °C at a rate of 8 °C min^{-1}. The transfer line temperature was maintained at 270 °C.

Helium was used as the carrier gas at a flow rate of 1.5 mL min^{-1}, with a split ratio of 10:1. For mass spectrometry, the ion source temperature was 250°C, the quadrupole temperature 150°C, and electron ionization at 70 eV, with a scan range of m/z 50–550. The volatile compounds were identified by comparing their mass spectra with the NIST 05 and Wiley 7.0 mass spectral libraries. Quantification was based on peak area integration from the GC-MS chromatogram.

2.4. In Vitro Experiment

The experiment was conducted in glass Petri dishes, 80 mm in diameter, to evaluate the effect of WRs at different concentrations on the germination and early growth characteristics of weed and maize seedlings. Before the experiment, seeds were surface sterilized by soaking them in 5% sodium hypochlorite for 3 minutes to eliminate possible fungi and spores' infections on their surface. After disinfection, the seeds were thoroughly rinsed with distilled water.

For each weed species, five seeds were placed on moistened Whatman No. 1 filter paper inside Petri dishes. The WRs solutions employed contained 100 (pure WR), 50 (one-part WR and one-part distilled water), 20 (one-part WR and three parts distilled water), and 10 (one-part WR and nine parts distilled water), and 0 (distilled water only), as a control (Table 2) [17]. Each Petri dish received 10 mL of the corresponding treatment solution to moisten the filter paper. Dishes were then sealed with parafilm to reduce evaporation. Also, they were checked daily and were getting moisturized with distilled water when necessary. Each combination of weed species, WR concentration, and aromatic plant species from which the WRs were obtained during hydrodistillation was replicated four times by two runs, resulting in a total of 960 Petri dishes.

Table 2. Doses of water residues from aromatic plants distillation.

Treatment	Doses	Description
T0	0	Distilled water only
TA	10	One-part WR and nine parts distilled water
TB	20	One-part WR and three parts distilled water
TC	50	One-part WR and one-part distilled water
TD	100	Pure WR

Note: WR=Water residue.

The Petri dishes were incubated in a growing chamber at 25°C under dark conditions. Germination was monitored every 24 hours, and the number of germinated seeds was recorded daily. Seeds were considered germinated when the radicle reached a length of at least 1 mm. After ten days of incubation, when no further germination was observed in control, the experiment was terminated and the germination percentage, shoot, and root length were recorded. These parameters were then used to calculate mean germination time, germination index, germination rate index, coefficient of velocity of germination, seedling vigor index. A detailed description of each parameter, along with the corresponding equations and their biological significance, is shown in Table 3.

Table 3. Description of the parameters used to study seed germination.

Germination Parameter	Calculation	Description	Notes & References
Germination Percentage (GP)	GP = (Number of germinated seeds / Total number of seeds) × 100	Ratio of germinated seeds to total seeds, expressed as a percentage	Indicates the final germination capacity under the tested conditions [18].
Mean Germination Time (MGT)	$MGT = \sum f \times X / \sum f$	f is the number of seeds germinated on day X, X is the number of days from the beginning of the experiment.	The lower the MGT, the faster the population of seeds has germinated [19].
Germination Index (GI)	$GI = \sum (Et / Dt)$	Et is the number of seeds that emerged in t days; Dt is the number of corresponding germination days	Higher GRI values indicate higher and faster germination [20].
Germination Rate Index (GRI)	$GRI = G1/1 + G2/2 + \cdots + Gx/x$	G1=Germination percentage × 100 at the first day after sowing, G2=Germination percentage × 100 at the second day after sowing	Reflects the percentage of germination on each day of the germination period. Higher GRI values indicate higher and faster germination [21].
Coefficient of Velocity of Germination (CVG)	$CVG = [\sum Ni / \sum (Ni \times Ti)] \times 100$	*Ni* is the number of seeds germinated at time *Ti*	Indicates the rate at which seeds emerge seedlings during an experimentation, higher values represent faster germination [19].
Seedling Vigor Index (SVI)	SVI = Seedling total length (cm) × GP (%)	Product of seedling growth and germination percentage	Evaluates overall seedling performance by combining growth and germination [20].

2.5. Experimental Design and Data Analysis

A stratified sampling design was employed in which distinct subgroups (aromatic plant spp.) based on altitude (>650 m and 30 m above sea level) were randomly collected for hydrodistillation (Table 1). A complete randomized design with four replicates and two runs was employed. Data were analyzed by the application of various statistical techniques due to the multilevel structure of the research described in this work. Specifically, these consisted of 4 levels of aromatic WRs based on the plant material used in hydrodistillation (i.e., lavender, laurel, oregano, and thyme), 2 levels of altitude (high vs low), five levels of WRs application rate (i.e., control, 10, 20, 50, and 100 parts per application dose), and 3 levels of treated plant material (i.e., wild oat, barnyardgrass, and maize). Analysis of variance (ANOVA) was used for the detection of significant differences in weed germination, root and shoot length, considering altitude as a random variable, while treatment (WRs application rate) was nested within aromatic plant species. In addition, the dose-response curve approach was used to determine the efficacy of WRs in reducing weed seed germination, using a logistic model with three parameters (Eq. 1).

$$\boldsymbol{y} = \frac{\boldsymbol{c}}{[1+\mathbf{exp}(-\boldsymbol{a}\times(\boldsymbol{treatment}-\boldsymbol{b}))]} \qquad \text{Equation 1}$$

Where y=percentage germination; c=asymptote; α=growth rate; b=inflection point

The data from the two runs were pooled and analyzed simultaneously, as no significant differences were found between them. JMP Pro v. 19.0.3 (SAS Institute, Cary, NC. USA) and Origin 6.0 (OriginLab Co., Northampton, MA, USA) were used for statistical analyses and curve fitting. Parallelism tests were performed to determine whether the curve fitting parameters (i.e., asymptote, inflection point and growth rate) are significantly different from each other. When p value is less than $p<0.05$ there is evidence that the curves are not parallel, meaning the parameters or some of these that control them are statistically different.

doi:10.20944/preprints202604.0216.v1

3. Results

3.1. Chemical Composition of Water Residues

The relative chemical composition (%) of the compounds identified in the WRs of laurel, lavender, oregano, and thyme (Figure 2). The GC-MS analysis detected constituents of oxygenated monoterpenes, monoterpene hydrocarbons, sesquiterpene hydrocarbons, and phenolic compounds, with qualitative and quantitative differences among aromatic plant species, while low and high altitude influenced the chemical profile of the collected samples (Figure 2).

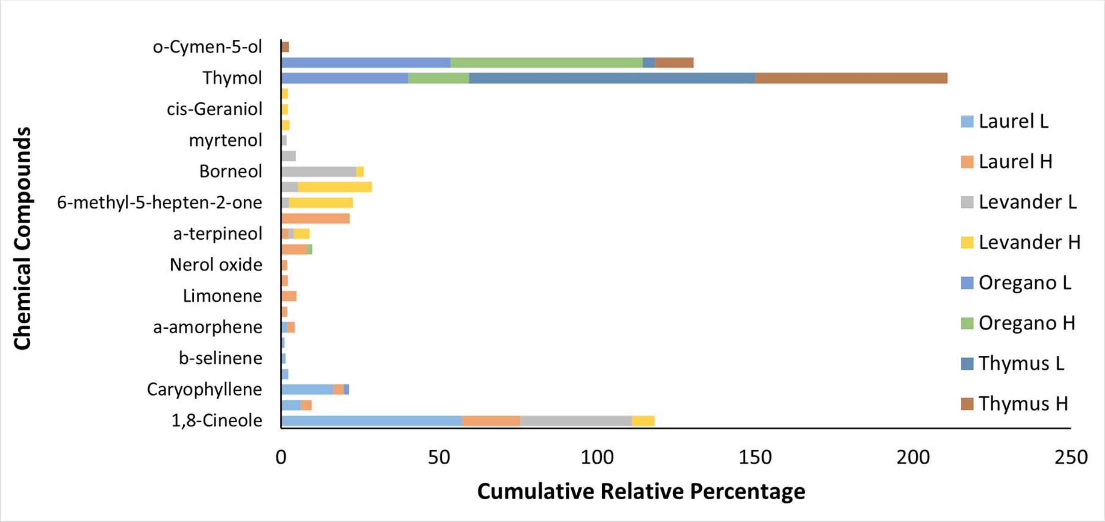

Figure 2. Chemical composition of water residues obtained from laurel, lavender, oregano, and thyme from various altitudes in the Epirus region. Note: H=High altitude, L=Low altitude.

The WRs of laurel were dominated by 1,8-cineole**,** which accounted for 57.22% in laurel L and 18.42% in laurel H. Laurel L contained higher relative percentages of several sesquiterpene hydrocarbons, including caryophyllene (16.47%), β-elemene (6.30%), α-humulene (2.28%), β-selinene (1.51%), α-selinene (1.14%), and α-amorphene (1.87%). In contrast, laurel H was characterized by the presence of limonene (4.92%), hotrienol (2.06%), nerol oxide (1.87%), α-terpineol (8.15%), and camphene (21.72%). These results indicate that laurel H displayed higher chemical diversity, whereas higher concentrations of selected major constituents characterized laurel L.

The chemical profile of lavender WRs was characterized mainly by oxygenated monoterpenes, as in the case of 1,8-cineole, which was the major constituent in lavender L (35.29%), while a lower percentage was observed in lavender H (7.21%). Borneol, on the contrary, was abundant in Lavender L (23.83%) but decreased markedly in lavender H (2.26%). Conversely, lavender H exhibited higher relative percentages of 6-methyl-5-hepten-2-one (20.32%) and linalool (23.39%) compared to lavender L (2.43% and 5.30%, respectively). Minor constituents, including α-terpineol, cis-verbenone, myrtenol, lavandulol, cis-geraniol, and geraniol, were detected at low levels in one or both samples. Overall, the two samples exhibited similar classes of compounds but differed substantially in the relative abundance of individual constituents.

WRs of oregano showed a phenolic-dominated composition. In oregano L, carvacrol (53.67%) and thymol (40.16%) were the predominant constituents. Oregano H exhibited a higher concentration of carvacrol (60.80%), while thymol was detected at a lower percentage (19.37%).

The WRs of thyme were characterized by a narrow phenolic profile. Thymol was the dominant compound in both samples, accounting for 90.58% in thyme L and 60.76% in thyme H. Carvacrol was detected at lower levels, with higher relative abundance in thymus H (12.17%) than in thyme L (3.92%). Additionally, o-cymen-5-ol was detected exclusively in thymus H (2.54%).

Across all plant species, low and high altitude collected samples shared similar major compounds within each species. The quantitative and qualitative variations in the chemical profiles can be attributed to species-related factors and altitude.

3.2. *Germination Attributes*

Germination tests were performed to examine the germinability of untreated seeds among all species tested in this research. The germination of these was recorded between 85% for barnyardgrass and wild oat, and 98 % for maize.

The phytotoxicity, in ascending order, on weed species exposed to WRs was laurel< thyme<lavender<oregano. In contrast, maize was generally more tolerant to WR treatments than the weed species, maintaining relatively higher germination percentages across extract types and concentrations (Figure 3a) often remaining above above 50% even at moderate concentrations. At low concentrations (10%), laurel and lavender WRs resulted in an increased germination of weed species by approximately 5–15% compared with the corresponding controls. However, germination declined progressively at higher concentrations. Overall, oregano and lavender WRs exhibited the strongest phytotoxic effects, followed by thyme, whereas laurel showed the weakest inhibitory activity. At 50% concentration, oregano and lavender WRs markedly reduced germination, in several cases less than 30%, while at 100% concentration, germination of weed species approached zero.

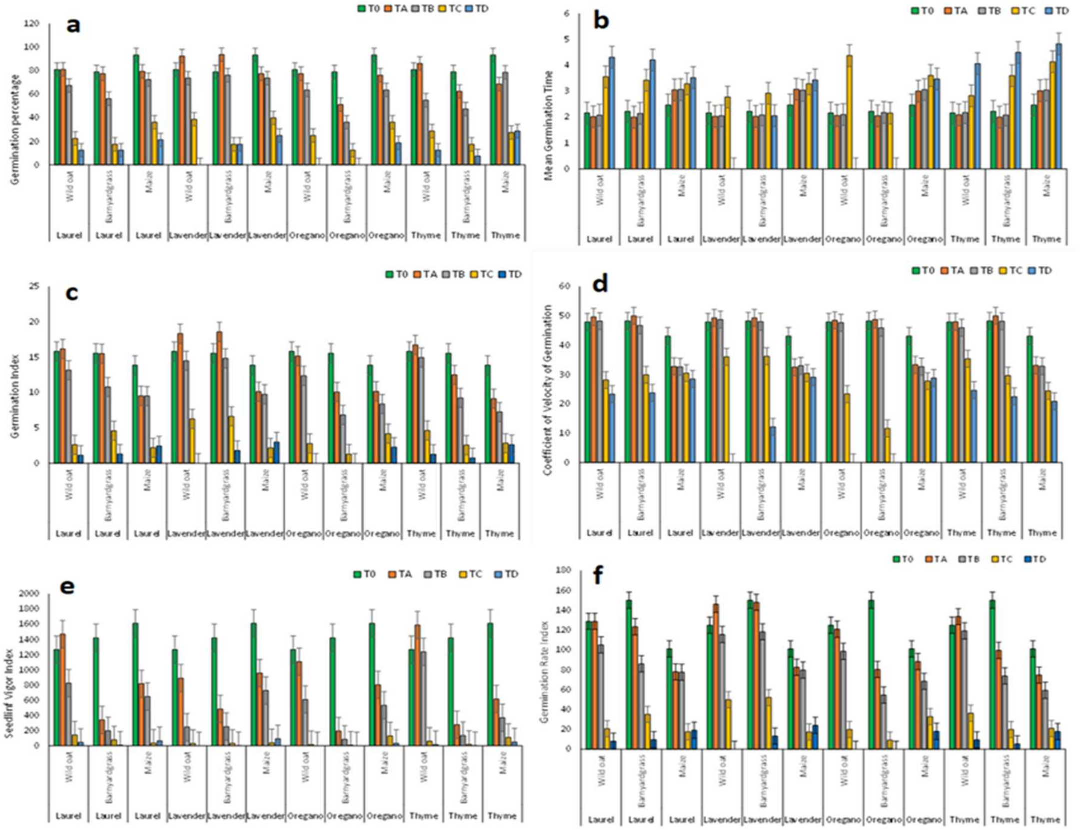

Figure 3. Effects of different concentrations (0–100%) of laurel, lavender, oregano, and thyme water residues (WRs) on germination indices GP (3a), MGT (3b), GI (3c), CVG(3d), SVI(3e), GRI (3f) of maize, wild oat, and barnyardgrass. Bars represent LSD values at p<0.05. Note: T0=control, TA=10% of WR, TB=20% WR, TC=50% of WR, TD=100 WR.

The effects of laurel, lavender, oregano, and thyme WRs on germination parameters are shown in Figure 3b-f.

Mean germination time increased significantly ($p<0.05$) at high concentrations of WRs, indicating delayed germination (Figure 3b). The same trend in relation to WR treatments, irrespective of the aromatic species used, was recorded for GRI and GI, demonstrating a substantial reduction in germination speed and cumulative rate (Figs 3c, f). The CVG was also decreased significantly ($p<0.05$) at higher WR concentrations (Figure 3d), confirming the inhibitory phytotoxic effects of aromatic plant treatments on germination rate.

The seedling vigor index (SVI) was also significantly ($p<0.05$) decreased with increasing WR concentration, following a trend similar to GRI and CVG, indicating that reduced germination rate translated into lower seedling vigor (Figure 3e). Significant effects were observed at 50% and 100% WR concentrations, particularly under oregano and lavender treatments. Overall, the inhibitory effects were more pronounced in weed species than in maize (Figure 3b–f)

3.2.1. Germination Curves

Germination of wild oat and barnyardgrass seeds exhibited similar sigmoidal patterns under all aromatic plant WRs treatments (Figure 4a-c) despite the estimated statistical differences between curve parameters (Table 4).

Lethal dose 50 (LD_{50}) values showed a moderate variation among WRs treatments. In the case of wild oat, for example, LD_{50} values ranged from 48% in oregano to 50% in laurel, with thyme and lavender exhibiting 50% of germination reduction at 60% of WRs dose (Figure 4a). Greater variability was observed in barnyardgrass (Figure 4b), with oregano WR exhibiting the lowest LD_{50} (10%), followed by thyme (22%) and laurel (30%). On the contrary, lavender required substantially higher concentrations (68% of WRs) to achieve 50% germination inhibition.

Regarding maize LD_{50} values under thyme and oregano WRs treatments were recorded at 20 and 38%, respectively. However, maize responded positively under higher doses of the aromatic plant species mentioned above WRs treatments (i.e., 50 and 100%), indicating a tolerance at higher concentrations. Instead, maize, at higher concentrations of laurel and lavender WRs treatment, showed the opposite response (Figure 4c), probably due to an inverse hormesis effect.

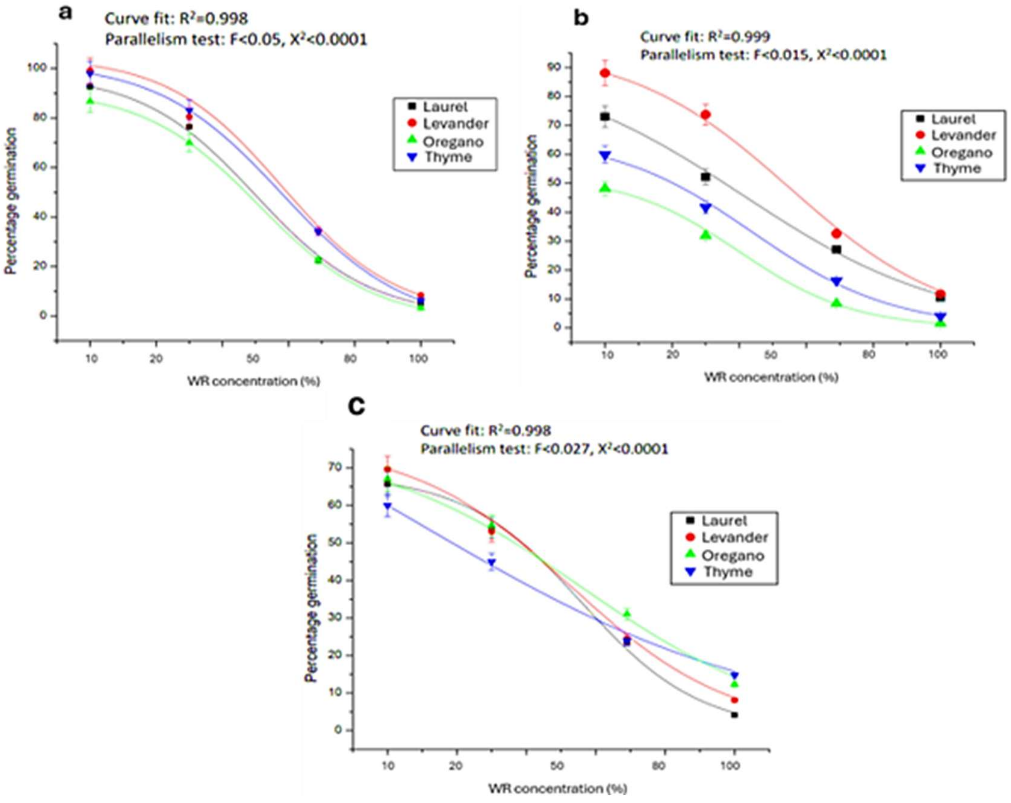

Figure 4. Curve fitting of the three-parameter sigmoidal model on the percentage germination of wild oat (a), barnyardgrass (b) and maize (c) for each aromatic plant WR regime treatment. Note: Vertical bars represent the standard error of the mean at $p<0.05$.

Comparisons of the curve parameters (Table 4) and parallelism tests (Figure 4a-c) indicate significant differences between the curve parameters for each treated plant species with WRs.

Table 4. Comparisons of the curve parameters between WRs concentrations as shown in Figure 4. Different letters within each group of each treated species denote significant differences between curve parameters at p<0.05. .

WRs origin	Growth rate	Inflection point	Asymptote
		Wild oat	
Laurel	-6.401^{a}	1.507^{b}	105.405^{a}
Lavender	-5.039^{a}	1.537^{b}	100.546^{b}
Oregano	-6.068^{a}	1.504^{b}	90.594^{c}
Thyme	-5.847^{a}	1.569^{b}	101.027^{a}
		Barnyardgrass	
Laurel	-3.119^{a}	1.368^{a}	95.839^{a}
Lavender	-4.662^{b}	1.558^{b}	94.989^{a}
Oregano	-5.256^{b}	1.358^{b}	55.425^{c}
Thyme	-4.184^{b}	1.382^{c}	71.844^{b}
		Maize	
Laurel	-5.425^{a}	1.559^{a}	68.353^{a}
Lavender	-3.993^{b}	1.475^{a}	70.949^{a}
Oregano	-3.775^{b}	1.591^{a}	73.835^{a}
Thyme	-2.347^{c}	1.266^{b}	92.399^{b}

Seed germination was monitored over 10 days under control and WR treatments (laurel, lavender, oregano, thyme) (Figure 5a–c). In all species, germination started within 48 h and reached a plateau by 3-4 days.

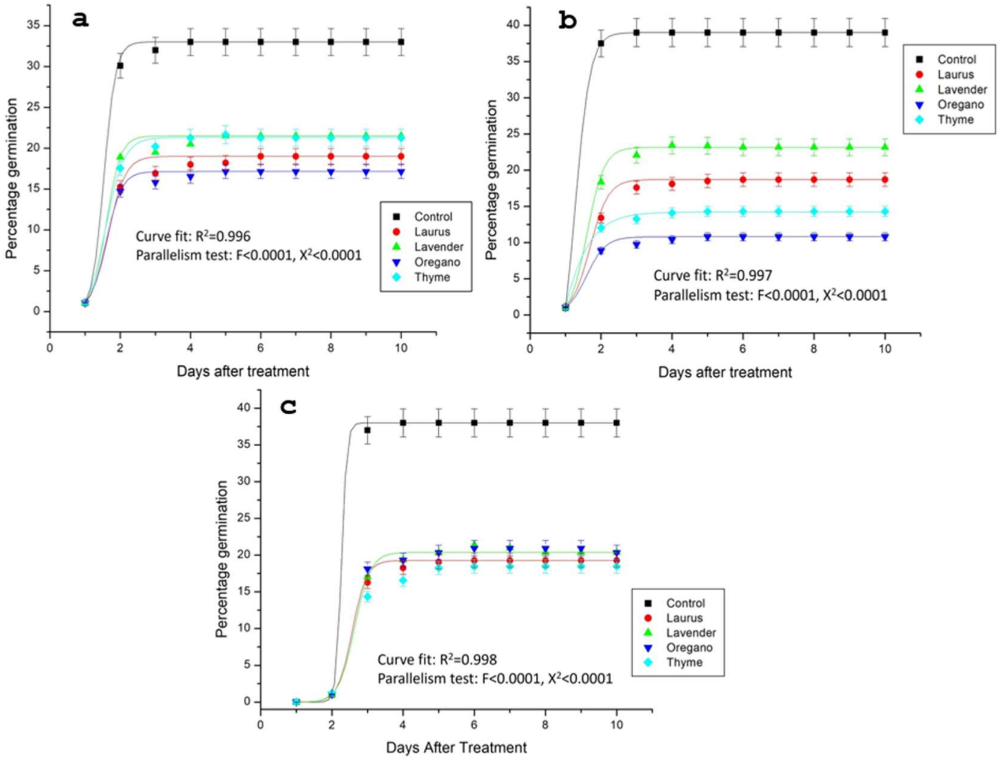

Figure 5. Curve fitting of the three-parameter sigmoidal model on the percentage germination of wild oat (a), barnyardgrass (b) and maize (c) for each aromatic plant WR during experimental period. Note: Vertical bars represent the standard error of the mean at p<0.05.

Across species, WRs consistently reduced germination compared to the control, mainly by lowering the asymptote, while effects on the inflection point (timing) were insignificant (Table 5).

Table 5. Comparisons of the curve parameters of wild oat, barnyardgrass and maize during the experimental period. Different letters within each group of each species denote significant differences between curve parameters at $p<0.05$. .

WRs origin	Growth rate	Inflection point	Asymptote
	Wild oat		
Control	5.776^{a}	1.588^{a}	32.876^{a}
Laurel	4.133^{b}	1.634^{a}	18.531^{b}
Lavender	5.058^{b}	1.579^{a}	21.127^{b}
Oregano	4.565^{b}	1.584^{a}	16.905^{b}
Thyme	4.383^{b}	1.645^{a}	21.188^{b}
	Barnyardgrass		
Control	6.851^{a}	1.530^{a}	38.997^{a}
Laurel	3.529^{c}	1.731^{b}	18.504^{c}
Lavender	4.301^{b}	1.688^{c}	23.099^{b}
Oregano	3.742^{b}	1.574^{a}	10.631^{d}
Thyme	4.174^{b}	1.589^{a}	14.154^{d}
	Maize		
Control	7.211^{a}	2.498^{a}	38.001^{a}
Laurel	4.556^{b}	2.618^{a}	19.114^{b}
Lavender	4.473^{b}	2.645^{a}	20.464^{b}
Oregano	4.908^{b}	2.591^{a}	20.543^{b}
Thyme	3.805^{b}	2.663^{a}	18.197^{b}

Oregano showed the strongest inhibition, whereas lavender generally showed the weakest. In wild oat (Figure 5a), germination declined from 32.9 (control) to 16.9–21.2 under WRs, with minimal differences in inflection point (1.579–1.645), indicating a synchronous germination timing. Growth rate was moderately reduced compared to the control. In barnyardgrass (Figure 5b), WR effects were stronger than in wild oat, with germination decreasing from 39.0 to 10.6–23.1. Oregano and thyme caused the greatest reductions. Unlike wild oat, small but significant differences in inflection point (1.530–1.731) suggest slight treatment-dependent germination delays. In maize (Figure 5c), germination decreased from 38.0 to 18.2–20.5 under all WRs, with no significant differences in inflection point (2.498–2.663), indicating stable germination timing despite reduced capacity. Overall, barnyardgrass was the most sensitive species, followed by wild oat and maize, with WRs primarily affecting final germination rather than germination dynamics.

3.3. Morphological Parameters

Following the evaluation of germination attributes, the effects of treatments, aromatic plant WRs, and altitude were examined for their impact during the early seedling growth stages i.e., shoot and root length.

ANOVA showed several significant interactions for shoot length (Figure 6a-d). Specifically, the interaction between treatment × species × aromatic plant WRs was highly significant ($p<0.0001$). In addition, treatment × aromatic plant WR × altitude ($p < 0.0001$), species × aromatic plant WR × altitude ($p=0.0038$), and the aromatic plant WR × altitude interaction ($p= 0.002$) were also significant.

Shoot length decreased progressively with increasing WR concentration across aromatic plant treatments and altitudes ($p<0.05$). The greatest reductions were observed under oregano and lavender treatments, where shoot length decreased by approximately 40–70% compared with the control (T0). In contrast, laurel and thyme exhibited weaker effects, with smaller reductions observed under the same concentrations.

Species-specific differences were also observed. In particular, wild oat exhibited the highest shoot length, followed by maize, whereas barnyardgrass showed reduced growth of approximately 55–70% under TD treatment compared with control across aromatic plant WRs and altitudes.

Altitude, along with species and aromatic plant WRs, also affected shoot length. In several combinations, particularly under laurel and thyme WRs, shoot length was significantly higher at high altitude compared with low altitude ($p<0.05$), although this pattern was not uniform across all treatments and species.

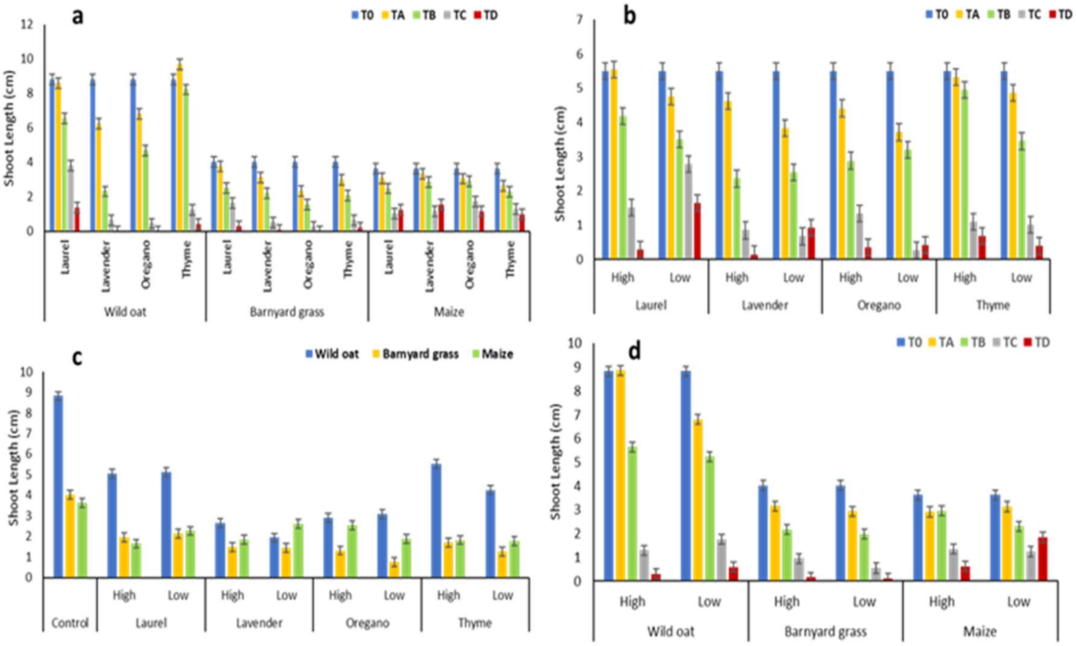

Figure 6. Effects of treatment, species, aromatic plant WRs, and altitude on shoot length under different three-way interactions: (a) Treatment × species × aromatic plant WRs, (b) Treatment × species × altitude, (c) Species × aromatic plant WRs × altitude, (d) Treatment × aromatic plant WRs × altitude. Vertical bars represent LSD values $p<0.05$ probability level.

Root length was also significantly influenced by treatment, species, aromatic plant WRs, and altitude. The interaction treatment × species × aromatic plant WR was highly significant ($p<0.0001$). Significant interactions were also observed for treatment × species × altitude ($p=0.0032$), treatment × aromatic plant WR × altitude ($p=0.002$), and species × aromatic plant WR × altitude ($p=0.0479$).

Across species and aromatic plant WRs (Figure 7a, d) treatments TA produced the highest root length, equaling or slightly exceeding the control, whereas TB resulted in moderate reductions (approximately 10–25% relative to control, depending on species and WR). In contrast, TC and TD suppressed root growth, with reductions ranging from approximately 35% to 65% compared with T0.

Wild oat exhibited the highest root length across most treatment × WR × altitude combinations, followed by maize, while barnyard grass consistently showed the lowest values. Under TD, barnyard grass root length was reduced by approximately 50–70% relative to control across aromatic plant WRs and altitudes. Similarly, for root growth, these observations revealed a species-specific effect.

When examined across altitude and aromatic plant WRs (Figure 7b, c), wild oat showed the highest root length at both altitudes, particularly under laurel and thyme WRs. Maize had intermediate root development, whereas barnyard grass maintained reduced root growth irrespective of altitude or WR type. Root length tended to be higher at high altitude, although this increase depended on treatment and WR identity, as shown by the three-way interactions.

Laurel and thyme WRs were generally associated with comparatively higher root length values under TA and TB, whereas oregano and lavender WRs were associated with reduced root growth, especially under TC and TD. These patterns were consistent across altitudes.

In general, the results demonstrate that root and shoot growth responses depend on concentration, species sensitivity, and altitude.

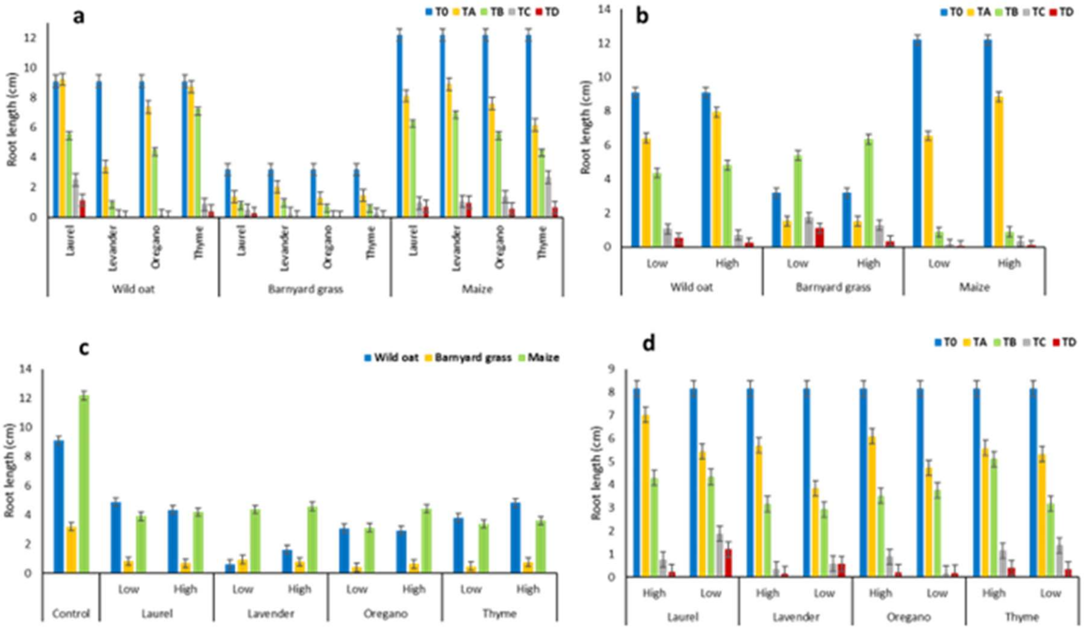

Figure 7. Effects of the treatment, species, aromatic plant WRs, and altitude on shoot length.(a) Treatment × species × aromatic plant WRs interaction, (b) Treatment × species × altitude interaction,(c) Species × aromatic plant WRs × altitude interaction,(d) Treatment × aromatic plant WRs × altitude interaction. Vertical bars represent LSD values p<0.05 probability level.

4. Discussion

The phytotoxic activity observed among WRs was associated with their chemical composition. GC–MS analysis revealed qualitative and quantitative variation among plant species in relation to the altitude at which the aromatic plant species were collected. Differences in WR content and bioactivity can be attributed to geographical and climatic factors, soil properties, and variations in altitude [22–24]. In agreement with previous reports, oxygenated monoterpenes and phenolic compounds were the dominant chemical classes in aromatic plants [25].

Oregano and thyme WRs were characterized by high proportions of phenolic compounds, mainly carvacrol and thymol. Similar patterns have been reported for Greek populations of oregano and thyme, where carvacrol and thymol rich chemotypes were identified as dominant [9,26]. In laurel WRs, 1,8-cineole was detected at higher proportion, which is in accordance with previous research [27]. Likewise, lavender WRs were mainly composed of oxygenated monoterpenes such as linalool and borneol, in agreement with earlier studies describing linalool-dominated chemotypes in Mediterranean *Lavandula angustifolia* populations [10,28].

Altitude influenced the relative abundance of major constituents rather than altering the principal chemical identity of each aromatic plant species. High altitude samples generally exhibited greater chemical complexity, particularly in laurel and lavender, suggesting that harsh environmental conditions may enhance secondary metabolite diversification. Such ecological modulation of secondary metabolism has been reported for various Mediterranean aromatic species [7]. Differences in essential oil composition associated with altitude have also been documented in Greek Lamiaceae populations [9], which may explain the observed differences in phytotoxic responses between low and high-altitude aromatic species and subsequently the composition of WRs originating from these species.

Germination indices showed that WRs reduced the proportion of seeds able to germinate and delayed germination of viable seeds. The greatest inhibition was observed at 100% concentration, especially in the case of oregano and lavender WRs, demonstrating a strong dose-dependent phytotoxicity. The sigmoidal dose–response curves further confirmed this pattern, indicating a gradual transition from partial to complete inhibition as WR concentration increased. Both shoot and root length were influenced by treatment, species, aromatic plant, and altitude, indicating that morphological parameters are highly sensitive to chemical composition and environmental origin of WRs derived from aromatic plants. Similar results were found by Boukhalfa et al. [29], who reported a clear dose-dependent effect, where increasing concentrations of thymus essential oils progressively inhibited the seed germination and seedling growth of *Lolium perenne* and *Amaranthus retroflexus*, with the highest doses ceasing germination of these species. Another study showed that many oxygenated monoterpenes, such as 1,8-cineole, linalool, and borneol, inhibited seed germination and seedling growth of *Amaranthus retroflexus*, *Chenopodium album*, and *Rumex crispus* [30]. De Almeida et al. [31] observed that the essential oils (EOs) from various aromatic plants, including *Hyssopus officinalis*, *Lavandula angustifolia*, *Majorana hortensis*, *Melissa officinalis*, *Ocimum basilicum*, *Origanum vulgare*, *Salvia officinalis*, and *Thymus vulgaris* on *Raphanus sativus*, *Lactuca sativa* and *Lepidium sativum* showed satisfactory inhibitory activity against the germination and the radical length of the weed species mentioned previously, depending on the application dose of water residue.

Root length was more sensitive than shoot length at higher concentrations of WRs (Fig 7a-d). Phenolic compounds such as carvacrol and thymol have been shown to impair cell division and elongation processes in roots [32]. This observation aligns with studies on walnut leaf extract, where shoot elongation in *A. retroflexus* and *C. album* was less affected than root elongation [33]. Similarly, Alipour et al. [34] observed a greater effect of encapsulated rosemary essential oil on the root morphology compared to the aerial part of A. retroflexus. Roots are particularly sensitive due to their high permeability and direct exposure to compounds in the soil [35,36].

High concentrations of WRs significantly inhibited the germination and growth of weed species, while having only minor effects on the germination of maize. Seed germination efficiency is influenced by seed size and seed weight; larger seeds generally contain more nutrient reserves, enabling faster germination and seedling growth, as well as higher survival rates compared to smaller seeds [37,38]. This may explain the relatively good germination of maize seeds even at higher WRs concentrations. In line with the findings presented in this work previous studies have shown that the effect of plant-derived bioactive compounds are not necessarily generalizable to all weed species but can be markedly species-specific. Kitis [39] investigated the herbicidal activity of Origanum onites essential oil against five ALweed species and five wheat cultivars and observed that weeds were more sensitive, with germination and seedling growth inhibited by over 50 %, while wheat showed only up to ~30 % reduction, highlighting a species-specific herbicidal effect. Also, according to Konstantinović et al., [17] maize and sunflower showed the greatest resistance to Thymus vulgaris hydrolates among various weed species.

Lavender and laurel WRs showed weaker inhibition of seed germination and seedling growth than oregano WR, especially at lower concentrations. Their compounds are known to have phytotoxic properties but generally act less aggressively than phenolic monoterpenes [6,40]. Interestingly, low concentrations of lavender and laurel sometimes slightly stimulated germination or shoot growth. However, inhibition of seed germination and growth is strongly affected by increased concentration of WRs. Our results showed that lavender WRs exhibited stronger inhibition than laurel and thyme WRs. According to Poveda et al. [41] lavandin hydrolates were efficient at different concentrations to reduce seed germination and seedling development of different weeds and crops, monocotyledons and dicotyledons. Similarly, Pouresmaeil et al. [42] reported that 1,8-cineole exhibited strong phytotoxic activity against Avena fatua, leading to complete inhibition of seed germination at the tested concentration.

Based on our results (Figure 2), oregano and thyme share a similar chemical profile. The phytotoxic activity of *Thymus vulgaris* has been confirmed in various extracts and essential oils [43,44]. Our study showed that oregano WRs in most cases produced the higher suppression of weed germination and seedling growth. The estimated LD_{50} values showed that oregano WRs reached LD_{50} at lower concentrations, particularly in barnyardgrass, indicating higher phytotoxic effects. Moderate concentrations were sufficient to reduce germination significantly, while full-strength residues inhibited germination. Angelini et al. [45] tested different essential oils from *Rosmarinus officinalis*, *Thymus vulgaris* and *Satureja montana* and their main compounds on different weeds and crops. More particularly *S. montana* essential oil with 57% carvacrol was the most effective. The essential oil of *Thymbra capitata* and its main compound carvacrol inhibited the germination and seedling growth of *Setaria verticillata*, *Avena fatua*, and *Solanum nigrum* at 0.5 μL/mL [46].

Carvacrol, a monoterpenoid phenol present in the essential oils of many different plants, including oregano, had an effective phytotoxic effect against a wide variety of weeds [47]. De Mastro et al. [48] showed that carvacrol at the concentration of 0.3 μL mL-1 completely inhibited the germination of *A. retroflexus* and *L. perenne* seeds. Also, Kordali et al. [49] observed that thymol and carvacrol obtained from *Origanum acutidens* essential oil inhibited the seed germination and seedling development of *A. retfoflexus*. The phytotoxic activity of carvacrol is mainly attributed to its ability to disrupt cell membrane integrity, induce oxidative stress, and interfere with essential physiological processes during seed germination [6,50]. Thymol is a phenolic monoterpene also present in the essential oils of various plant species, being the main component of those obtained from oregano or thyme. The primary component of essential oils from lemon balm, sage, savory, thyme, and ajowan is thymol, which effectively prevents the germination and growth of the aerial and root parts of cocklebur, wild oat, short-spiked *Phalaris canariensis*, *Amaranthus retroflexus* and *Echinochloa crus-galli* [51]. There hasn't been much research done on the phytotoxic chemical thymol's modes of action. However, it has been reported that Mentha longifolia essential oil, rich in thymol, inhibited the germination and growth of *Cyperus rotundus* and *Echinochloa crus-galli* weeds as a result of cellular aberrations, including chromosomal bridges, c-mitosis, stickiness, vagrant chromosomes, and reduction of the mitotic index [52].

Although most previous studies have focused on essential oils, the present results demonstrate that water residues retain considerable phytotoxic activity. This indicates that WRs contain sufficient amounts of water-soluble bioactive compounds or residual fractions of volatile constituents capable of exerting biological effects. Therefore, the phytotoxic effects observed in this study are indicative that WRs can exert similar behavior to that reported for essential oils, despite the lower intensity due to dilution and compositional differences.

5. Conclusions

The present study demonstrates that water residues (WRs) derived from the hydrodistillation of aromatic plants exhibit phytotoxic potential, affecting both weed seed germination and seedling growth. The observed effects were dose-dependent and species-specific, with weed species showing greater sensitivity than maize. Among the tested residues, oregano and lavender WRs exhibited the strongest inhibitory effects in most cases, whereas laurel showed comparatively weaker activity.

Chemical analysis revealed that phenolic monoterpenes, particularly carvacrol and thymol, were associated with stronger phytotoxic effects, whereas oxygenated monoterpenes showed more moderate activity. Furthermore, altitude influenced the relative abundance of bioactive compounds, contributing to variation in phytotoxic responses.

Dose–response curves indicated that WRs reduced final germination capacity rather than altering germination dynamics, as reflected by differences in curve asymptotes and LD_{50} values. The low LD_{50} values observed in weed species suggest that substantial inhibition can be achieved at moderate WR concentrations, highlighting their practical potential for weed management applications.

The greater tolerance of maize compared to weed species indicates a degree of selectivity, suggesting that WRs could be used to suppress weeds without severely affecting crop germination. This selective response may enhance crop-weed competition during early growth stages, offering an additional advantage to the crop.

Furthermore, the utilization of WRs is in line with the circular economy practices, as these residues represent an underexploited by-product of essential oil production that can be exploited for agricultural use. Further research is needed to evaluate their performance under field conditions and to optimize their application strategies.

Overall, these findings highlight the potential of WRs as a sustainable and selective tool for environmentally friendly weed management approaches. Their ability to inhibit weed germination while minimizing the negative effects on crop species suggests that WRs could be further explored as bioherbicidal agents in integrated weed management strategies.

Author Contributions: Conceptualization, Pinelopi N Liontou and Nicholas E Korres; Methodology, Pinelopi N Liontou, Anastasia V Badeka and Nicholas E Korres; Validation, Pinelopi N Liontou, Anastasia V Badeka, Thomas K Gitsopoulos, Georgios Patakioutas and Nicholas E Korres; Investigation, Pinelopi N Liontou, Anastasia V Badeka and Nicholas E Korres; Resources, Nicholas E Korres; Data curation, Nicholas E Korres; Writing – original draft, Pinelopi N Liontou and Nicholas E Korres; Writing – review & editing, Pinelopi N Liontou, Anastasia V Badeka, Thomas K Gitsopoulos, Georgios Patakioutas and Nicholas E Korres; Visualization, Pinelopi N Liontou, Thomas K Gitsopoulos, Georgios Patakioutas and Nicholas E Korres; Supervision, Nicholas E Korres; Project administration, Nicholas E Korres; Funding acquisition, Nicholas E Korres. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by INTERREG VI-A, GREECE-ITALY (2021-2027) Strengthening BIOdiversity Preservation through Sustainable Exploitation of the Bioresources CHAIN (BIOCHAIN) in the Program Area, grant number 6006563.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author(s).

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

CVG	Coefficient of velocity of germination
GRI	Germination rate index
MGT	Mean germination time
MoA	Mode of action
SVI	Seedling vigor index
GI	Germination index
GP	Germination percentage
WR	Water residue
LD_{50}	Lethal dose 50
EOs	Essential oils
L	Low altitude
H	High altitude

References

1. Shaner, D. L. (2014). Lessons learned from the history of herbicide resistance. Weed Science, 62, 427–431.
2. Korres, N. E., Burgos, N., Travlos, I., Vurro, M., Gitsopoulos, T., Varanasi, V., Duke, S., Kudsk, P., Brabham, C., Rouse, C., & Salas-Perez, R. (2019). New directions for integrated weed management: Modern technologies, tools and knowledge discovery. In Advances in Agronomy.
3. Montanarella, L., & Panagos, P. (2021). The relevance of sustainable soil management within the European Green Deal. Land Use Policy, 100, 104950.

4. Qu, R., He, B., Yang, J., Lin, H., Yang, W., Wu, Q., Li, Q. X., & Yang, G. (2021). Where are the new herbicides? Pest Management Science, 77(6), 2620–2625.
5. Duke, S. O. (2012). Why have no new herbicide modes of action appeared in recent years? Pest Management Science, 68(4), 505–512.
6. Abd-ElGawad, A. M., El Gendy, A. E. G., Assaeed, A. M., Al-Rowaily, S. L., Alharthi, A. S., Mohamed, T. A., Nassar, M. I., Dewir, Y. H., & Elshamy, A. I. (2021). Phytotoxic effects of plant essential oils: A systematic review and structure–activity relationship based on chemometric analyses. Plants, 10(1), 36.
7. Cheminal, A., Kokkoris, I. P., Strid, A., & Dimopoulos, P. (2020). Medicinal and aromatic Lamiaceae plants in Greece: Linking diversity and distribution patterns with ecosystem services. Forests, 11(6), 661.
8. Kosakowska, O., Węglarz, Z., Styczyńska, S., Synowiec, A., Gniewosz, M., & Bączek, K. (2024). Activity of common thyme (Thymus vulgaris L.), Greek oregano (Origanum vulgare L. ssp. hirtum), and common oregano (Origanum vulgare L. ssp. vulgare) essential oils against selected phytopathogens. Molecules, 29(19), 4617.
9. Kakouri, E., Daferera, D., Andriopoulou, A., Trigas, P., & Tarantilis, P. A. (2024). Evaluation of the essential oil composition of five Thymus species native to Greece. Chemosensors, 12(1), 7.
10. Lesage-Meessen, L., Bou, M., Sigoillot, J., Faulds, C. B., & Lomascolo, A. (2015). Essential oils and distilled straws of lavender and lavandin: A review of current use and potential application in white biotechnology. Applied Microbiology and Biotechnology, 99(8), 3375–3385.
11. Narayani, M., et al. (2016). Bioactive components of aromatic plant residues and their role in weed management. Journal of Environmental Management, 183, 346–354.
12. Özdemir, C., et al. (2019). Herbicidal activity of distillation by-products of lavender and thyme. Journal of Essential Oil Research, 31(4), 303–314.
13. Tavares, R., et al. (2020). Essential oils of thyme and their phytotoxic properties for weed management. Industrial Crops and Products, 147, 112–120.
14. Khan, M. A., et al. (2017). Allelopathic potential of rosemary and peppermint residues in weed suppression. Agricultural Sciences, 8(7), 1295–1306.
15. El Mahdi, J., Tarraf, W., Ruta, C., Piscitelli, L., Aly, A., & De Mastro, G. (2020). Bio-herbicidal potential of the essential oils from different Rosmarinus officinalis L. chemotypes in laboratory assays. Agronomy, 10(6), 775.
16. Economou, G., Panagopoulos, G., Tarantilis, P., Kalivas, D., Kotoulas, V., Travlos, I., Polysiou, M., & Karamanos, A. (2010). Variability in essential oil content and composition of Origanum hirtum L., Origanum onites L., Coridothymus capitatus (L.) and Satureja thymbra L. populations from the Greek island Ikaria. *Industrial Crops and Products*, *33*(1), 236–241. https://doi.org/10.1016/j.indcrop.2010.10.021
17. Konstantinović, B., Popov, M., Samardžić, N., Aćimović, M., Elez, J. Š., Stojanović, T., Crnković, M., & Rajković, M. (2022). The Effect of Thymus vulgaris L. Hydrolate Solutions on the Seed Germination, Seedling Length, and Oxidative Stress of Some Cultivated and Weed Species. Plants, 11(13), 1782. https://doi.org/10.3390/plants11131782
18. Scott, S., Jones, R., & Williams, W. (1984). Review of data analysis methods for seed germination. Crop Science, 24, 1192–1199.
19. Zafar-Ul-Hye, M., Naeem, M., Danish, S., Fahad, S., Datta, R., Abbas, M., Rahi, A. A., Brtnicky, M., Holátko, J., Tarar, Z. H., & Nasir, M. (2020). Alleviation of cadmium adverse effects by improving nutrients uptake in bitter gourd through cadmium tolerant rhizobacteria. Environments, 7(8), 54.
20. Allam, E., El-Darier, S., Ghattass, Z., Fakhry, A., & Elghobashy, R. M. (2024). Application of chitosan nanopriming on plant growth and secondary metabolites of Pancratium maritimum L. BMC Plant Biology, 24(1), 466.
21. Kader, M. A. (2005). A comparison of seed germination calculation formulae and the associated interpretation of resulting data. Journal and Proceedings of the Royal Society of New South Wales, 138, 65–75.
22. Moadeli, S. N., Rowshan, V., & Abotalebi, A. (2013). Comparison of the essential oil components in wild and cultivated populations of Salvia virgata. International Research Journal of Applied and Basic Sciences, 4, 337–340.

23. Raut, J. S., & Karuppayil, S. M. (2014). Status review on the medicinal properties of essential oils. Industrial Crops and Products, 62, 250–264.
24. Yilar, M., Kadioglu, İ., & Telci, İ. (2015). Determination of essential oil compositions of some Salvia species naturally growing in Tokat Province. Turkish Journal of Agricultural and Natural Sciences, 2, 313–319.
25. Duke, S. O., & Dayan, F. E. (2015). Discovery of new herbicide modes of action with natural phytotoxins. In Discovery and synthesis of crop protection products (pp. 79–92).
26. Węglarz, Z., Kosakowska, O., Pióro-Jabrucka, E., Przybył, J. L., & Bączek, K. (2020). The quality of Greek oregano (Origanum vulgare L. subsp. hirtum) and common oregano (O. vulgare L. subsp. vulgare) based on their essential oil composition. Foods, 9(11), 1671.
27. Ovidi, E., Laghezza Masci, V., Zambelli, M., Tiezzi, A., Vitalini, S., & Garzoli, S. (2021). Laurus nobilis, Salvia sclarea, and Salvia officinalis essential oils and hydrolates: Evaluation of liquid and vapor phase chemical composition and biological activities. Plants, 10, 707. https://doi.org/10.3390/plants10040707
28. Dong, G., Bai, X., Aimila, A., Aisa, H. A., & Maiwulanjiang, M. (2020). Study on lavender essential oil chemical compositions by GC–MS and improved pGC. Molecules, 25(14), 3166. https://doi.org/10.3390/molecules25143166
29. Boukhalfa, R., Ruta, C., Messgo-Moumene, S., Calabrese, G. J., Argentieri, M. P., & De Mastro, G. (2025). Assessment of the bioherbicidal potential of Thymus sp. pl. essential oils in weed control. Industrial Crops and Products, 227, 120856.
30. Kordali, S., Cakir, A., & Sutay, S. (2007). Inhibitory effects of monoterpenes on seed germination and seedling growth. *Zeitschrift Für Naturforschung C*, *62*(3–4), 207–214. https://doi.org/10.1515/znc-2007-3-409
31. De Almeida L.F.R., Frei F., Mancini E., De Martino L., De Feo V. Phytotoxic activities of Mediterranean essential oils. Molecules. 2010;15:4309–4323. doi: 10.3390/molecules15064309.
32. Verdeguer, M., Sánchez-Moreiras, A. M., & Araniti, F. (2020). Phytotoxic Effects and Mechanism of Action of Essential Oils and Terpenoids. *Plants*, *9*(11), 1571. https://doi.org/10.3390/plants9111571
33. Đorđević, T., Đurović-Pejčev, R., Stevanović, M., Sarić-Krsmanović, M., Radivojević, L., Šantrić, L., & Gajić-Umiljendić, J. (2022). Phytotoxicity and allelopathic potential of Juglans regia L. leaf extract. Frontiers in Plant Science, 13, 986740.
34. Alipour, M., Saharkhiz, M. J., Niakousari, M., & Seidi Damyeh, M. (2019). Phytotoxicity of encapsulated essential oil of rosemary on germination and morphophysiological features of amaranth and radish seedlings. Scientia Horticulturae, 243, 131–139.
35. Nishida, N., Tamotsu, S., Nagata, N., Saito, C., & Sakai, A. (2005). Allelopathic effects of volatile monoterpenoids produced by Salvia leucophylla: Inhibition of cell proliferation and DNA synthesis in the root apical meristem of Brassica campestris seedlings. Journal of Chemical Ecology, 31, 1187–1203.
36. Islam, A., & Kato-Noguchi, H. (2013). Plant growth inhibitory activity of medicinal plant Hyptis suaveolens: Could allelopathy be a cause? Emirates Journal of Food and Agriculture, 25, 692.
37. Sadeghi, H., Khazaei, F., Sheidaei, S., & Yari, L. (2011). Effect of seed size on seed germination behavior of safflower (Carthamus tinctorius L.). Journal of Agricultural and Biological Science, 6, 5–8.
38. Moles, A. T., & Westoby, M. (2006). Seed size and plant strategy across the whole life cycle. Oikos, 113, 91–105.
39. Kitis, Y. E. (2023). Chemical composition and herbicidal activity of oregano (Origanum onites) essential oil on weeds and wheat. Acta Agriculturae Scandinavica, Section B—Soil & Plant Science, 73(1), 142–151.
40. Tursun, N., Işıkber, A. A., Alma, M. H., & Usanmaz Bozhüyük, A. (2022). Inhibitory effect of oregano and laurel essential oils and their main components on seed germination of some weed and crop species. Selcuk Journal of Agriculture and Food Sciences, 36(2), 275–281.
41. Poveda, J., Vítores, D., Sánchez-Gómez, T., Santamaría, Ó., Velasco, P., Zunzunegui, I., Rodríguez, V. M., Herrero, B., & Martín-García, J. (2024). Use of by-products from the industrial distillation of lavandin (Lavandula x intermedia) essential oil as effective bioherbicides. *Journal of Environmental Management*, *373*, 123723. https://doi.org/10.1016/j.jenvman.2024.123723
42. Pouresmaeil, M., Movafeghi, A., Sabzi-Nojadeh, M., Kosari-Nasab, M., & Maggi, F. (2024). Exposure of Avena fatua L. seedlings to Artemisia austriaca Jacq. essential oil, 1,8-cineole, and camphor induces

oxidative stress and reduces cell viability. Industrial Crops and Products, 222, 119636. https://doi.org/10.1016/j.indcrop.2024.119636

43. Hemada, M., & El-Darier, S. (2011). Comparative study on composition and biological activity of essential oils of two Thymus species grown in Egypt. American-Eurasian Journal of Agricultural & Environmental Science, 11, 647–654.
44. Arouiee, H., Ghasemimohsenabad, S., Azizi, M., & Neamati, S. H. (2010). Allelopathic effects of some medicinal plants extracts on seed germination and growth of common weeds in Mashhad area. In Proceedings of the 8th International Symposium on Biocontrol and Biotechnology (p. 37). Pattaya, Thailand.
45. Angelini L.G., Carpanese G., Cioni P.L., Morelli I., Macchia M., Flamini G. (2003). Essential oils from Mediterranean Lamiaceae as weed germination inhibitors. Journal of Agricultural Food Chemistry, 251, 6158–6164. doi: 10.1021/jf0210728
46. Verdeguer, M., Torres-Pagan, N., Muñoz, M., Jouini, A., García-Plasencia, S., Chinchilla, P., Berbegal, M., Salamone, A., Agnello, S., Carrubba, A., Cabeiras-Freijanes, L., Regueira-Marcos, L., Sánchez-Moreiras, A. M., & Blázquez, M. A. (2020). Herbicidal Activity of Thymbra capitata (L.) Cav. Essential Oil. Molecules, 25(12), 2832. https://doi.org/10.3390/molecules25122832
47. Fouad, R., Bousta, D., El Ouali Lalami, A. E., Chahdi, F. O., Amri, I., Jamoussi, B., & Greche, H. (2015). Chemical composition and herbicidal effects of essential oils of Cymbopogon citratus (DC.) Stapf, Eucalyptus cladocalyx, Origanum vulgare L., and Artemisia absinthium L. cultivated in Morocco. Journal of Essential Oil Bearing Plants, 18, 112–123.
48. Da Mastro, G., Fracchiolla, M., Verdini, L., & Montemurro, P. (2006). Oregano and its potential use as bioherbicide. Acta Horticulturae, 723, 335–346. Vaughn, S. F., & Spencer, G. F. (1993). Volatile monoterpenes as potential parent structures for new herbicides. Weed Science, 41, 114–119.
49. Kordali, S., Kabaagac, G., Sen, İ., Yilmaz, F., & Najda, A. (2022). Phytotoxic effects of three Origanum species extracts and essential oil on seed germinations and seedling growths of four weed species. Agronomy, 12, 2581.
50. Vaughn, S. F., & Spencer, G. F. (1993). Volatile monoterpenes as potential parent structures for new herbicides. Weed Science, 41, 114–119.
51. Chung, I. M., Khanh, T. D., Lee, O. K., & Ahmad, A. (2007). Chemical constituents from ajwain seeds (Trachyspermum ammi) and inhibitory activity of thymol, lupeol and fatty acids on barnyardgrass and radish seeds. Asian Journal of Chemistry, 19, 1524–1528.
52. Singh, N., Singh, H. P., Batish, D. R., et al. (2020). Chemical characterization, phytotoxic, and cytotoxic activities of essential oil of Mentha longifolia. Environmental Science and Pollution Research, 27, 13512–13523.