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Bioherbicidal Effect of *Artemisia absinthium* Hydrolate on Some Field and Weed Species

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Abstract

Hydrolates have attracted increasing attention as environmentally compatible products with potential applications in weed management. This study evaluated the effects of *Artemisia absinthium* L. hydrolate on seed germination and early seedling growth of maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), red clover (*Trifolium pratense* L.), redroot pigweed (*Amaranthus retroflexus* L.), common lambsquarters (*Chenopodium album* L.) and common purslane (*Portulaca oleracea* L.) under in vitro conditions. Seeds were treated with 10, 20, 50 and 100% hydrolate. Germination was assessed using GP, CVG, GI, T_{50} and GRI, while germination and seedling growth data were analysed using one-way and factorial ANOVA. Higher hydrolate concentrations inhibited germination and early seedling growth, whereas 10% hydrolate stimulated seedling growth. Changes in germination indices indicated altered germination speed and timing even when final germination remained relatively high. Relative growth analysis showed that field crops were generally less affected than weeds, with redroot pigweed exhibiting the greatest susceptibility. The phytotoxic effects of *A. absinthium* hydrolate depended on concentration and target species, indicating potential selectivity in weed management.

Keywords: biological activity; inhibition; phytotoxicity; stimulation; hormesis

1. Introduction

Hydrolates, also referred to as hydrosols or aromatic waters, are by-products generated during the hydrodistillation or steam distillation of plant material. They contain predominantly water-soluble constituents, along with small amounts of dissolved essential oil components [1]. Hydrolates derived from numerous plant species exhibit various biological activities, including antioxidant [2], antibacterial [3], antifungal [4], insecticidal, nematicidal, repellent [5] and antiviral properties [6]. Their phytotoxic effects on seed germination and early plant growth indicate their potential as plant-derived bioherbicides. For example, *Haplophyllum tuberculatum* hydrolate inhibited seed germination in wheat and radish [1], whereas *Thymus vulgaris* hydrolate reduced germination and early growth in several economically important weed species [7].

The biological activity of hydrolates is largely determined by their chemical composition, particularly their volatile constituents derived from plant secondary metabolism. Their composition generally differs quantitatively and often qualitatively from that of the corresponding essential oils, with oxygenated monoterpenes usually occurring in higher

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proportions. Chemical characterization is therefore essential for understanding and comparing their biological properties, particularly because hydrolate composition varies with plant species, plant organ, extraction conditions and other factors influencing distillation [8].

Artemisia absinthium L. (common wormwood) is a source of secondary metabolites with documented phytotoxic activity. Aqueous leaf extracts of this species reduced seed germination and seedling development, including epicotyl and hypocotyl growth, in Santa Maria feverfew (*Parthenium hysterophorus*) [9]. Inhibitory effects on seed germination and seedling growth have also been reported for dandelion (*Taraxacum officinale*) and hitch hikers (*Bidens pilosa*) [10]. Plant extracts and essential oils of *A. absinthium* significantly inhibited germination and early seedling growth in redroot pigweed (*Amaranthus retroflexus*) and green foxtail (*Setaria viridis*), with the magnitude of inhibition depending on both the tested product and the target plant species [11].

Compared with essential oils and plant extracts, considerably less information is available on the chemical composition of *A. absinthium* hydrolates. The biological activity of *A. absinthium*-derived products is closely related to their chemical composition, including their volatile and phenolic constituents [8,12]. Four major essential oil chemotypes have been identified in plant material originating from different European countries, characterized by the predominance of sabinene and myrcene, thujone isomers, epoxyocimene or *trans*-sabinyl acetate [13]. Chlorogenic acid was the most abundant identified phenolic compound in an *A. absinthium* plant extract, followed by kaempferol-3-O-glucoside and rutin, whereas the corresponding essential oil contained predominantly oxygenated monoterpenes, with *trans*-thujone, *cis*-epoxyocimene and bicyclogermacrene as the major constituents [11]. Analysis of the volatile fraction (recovered oil) obtained from *A. absinthium* hydrolate over three consecutive growing seasons identified *trans*-thujone and *trans*-sabinyl acetate as the dominant constituents of both the recovered oil and the corresponding essential oil [14]. These findings indicate that the chemical composition of *A. absinthium*-derived products varies with product type and geographical origin of the plant material, emphasizing the need for product-specific chemical characterization when interpreting biological activity.

Although *A. absinthium* extracts and essential oils have been extensively investigated for their phytotoxic activity, considerably less information is available on its hydrolate. Previous studies demonstrated strong nematicidal activity of *A. absinthium* hydrolate, indicating its potential as a plant-derived biopesticide [15,16]. However, its effects on seed germination and early seedling growth in cultivated and weed species remain insufficiently investigated. Therefore, this study aimed to evaluate the effects of different concentrations of *A. absinthium* hydrolate on seed germination and early seedling growth under in vitro conditions in three field crops (maize, soybean and red clover) and three weed species (redroot pigweed, common lambsquarters, and common purslane). It was hypothesized that the hydrolate would affect germination and early seedling growth in a concentration-dependent manner, with responses varying among plant species.

2. Results

2.1. Effects of A. absinthium Hydrolate on Seed Germination

Final germination percentage was significantly affected by plant species ($F(5, 114) = 42.868$, $p = 0.0000$), hydrolate concentration ($F(4, 115) = 4.215$, $p = 0.0032$), and their interaction ($F(20, 90) = 11.448$, $p = 0.0000$) (Figure 1A, Figure 1B, and Figure 1C, respectively).

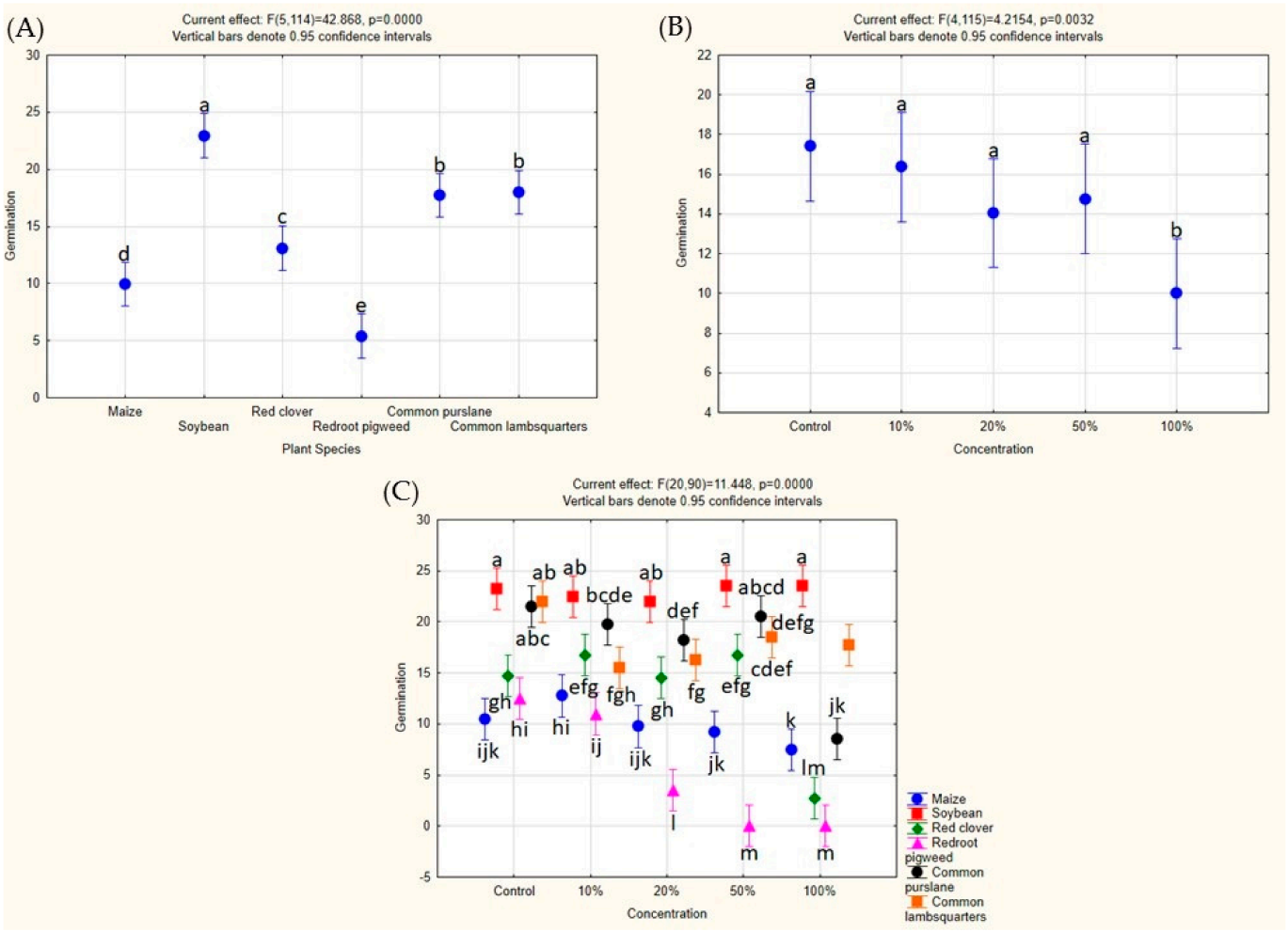

Figure 1. Final germination percentage as affected by plant species ((**A**), one-way ANOVA), hydrolate concentration ((**B**), one-way ANOVA), and their interaction ((**C**), factorial ANOVA). Data are presented as mean ± SD. Different letters (a–m) indicate statistically significant differences for $p < 0.05$ (Duncan's multiple range test).

Soybean (a) exhibited the highest germination percentage, whereas redroot pigweed (e) was the most sensitive species in regard to germination. The statistically significant differences were noted between the 100% hydrolate concentration (b) compared to the homogeneous group consisting of all the other tested concentrations and the control (a). The significant interaction indicated that the effect of the hydrolate concentration depended on the tested plant species. The calculated germination indices (GP, CVG, GI, T_{50} and GRI) demonstrated species-specific responses to *A. absinthium* hydrolate (Table 1).

Table 1. Effects of different concentrations of *A. absinthium* hydrolate on germination parameters of selected field and weed species.

Test Plant	Hydrolate Concentration	GP (%)	CVG (% Day^{-1})	GI	T_{50} (Time, in Days)	GRI (% Day^{-1})
maize	control	42	26.58	304	2.94	12.29
	10%	51	25.63	362	2.88	14.24
	20%	39	28.89	294	2.65	11.85
	50%	37	19.37	216	4.42	8.05
	100%	30	20.27	182	4.20	6.82

Table 1. *Cont.*

Test Plant	Hydrolate Concentration	GP (%)	CVG (% Day^{-1})	GI	T_{50} (Time, in Days)	GRI (% Day^{-1})
soybean	control	93	24.50	638	2.98	28.30
	10%	90	22.44	586	3.37	26.34
	20%	88	20.66	542	3.61	22.80
	50%	94	21.03	587	3.86	27.75
	100%	94	21.12	589	3.82	23.64
red clover	control	59	22.96	417	2.32	14.26
	10%	67	26.27	482	3.05	21.83
	20%	58	33.53	721	1.91	22.59
	50%	67	28.63	503	2.45	23.45
	100%	11	17.46	58	5.25	2.10
redroot pigweed	control	50	16.34	244	5.73	9.78
	10%	44	14.01	170	6.71	6.52
	20%	14	13.33	49	7.25	1.98
	50%	-	-	-	-	-
	100%	-	-	-	-	-
common lambsquarters	control	86	23.18	743	1.65	38.85
	10%	79	27.82	585	2.77	26.67
	20%	73	29.67	557	1.99	26.67
	50%	82	23.03	436	4.75	18.78
	100%	34	22.08	220	1.80	9.36
common purslane	control	88	18.14	483	4.71	18.53
	10%	62	19.93	371	4.35	13.83
	20%	65	18.21	359	4.60	13.52
	50%	74	16.97	378	5.11	14.15
	100%	71	17.97	386	4.96	14.33

GP—Germination percentage; CVG—Coefficient of velocity of germination; GI—Germination index; T_{50}—Median germination time; GRI—Germination rate index. Germination indices for redroot pigweed at 50 and 100% *A. absinthium* hydrolate are not presented because no germination occurred at these concentrations.

Soybean proved to be the most tolerant field species, maintaining high germination and relatively stable germination dynamics across all *A. absinthium* hydrolate concentrations. Maize exhibited a concentration-dependent decline in both germination and germination performance, whereas red clover maintained high germination up to 50% *A. absinthium* hydrolate despite changes in germination dynamics, while the highest *A. absinthium* hydrolate concentration markedly reduced both germination and germination performance. Among the tested weed species, redroot pigweed was the most susceptible, showing a progressive reduction in germination as hydrolate concentration increased. At 10 and 20% hydrolate concentration germination was delayed relative to the control, as indicated by lower CVG, GI and GRI values and higher T_{50}, while at 50 and 100% hydrolate concentration no germination occurred. In contrast, common lambsquarters and common purslane retained relatively high germination and showed only moderate changes in germination dynamics, indicating considerably greater tolerance to *A. absinthium* hydrolate than the redroot pigweed.

2.2. Absolute Growth of Epicotyl and Hypocotyl as Affected by Hydrolate Concentration and Plant Species

One-way ANOVA confirmed the existence of the statistical significance for the epicotyls and hypocotyls growth as the dependent variables in comparison with the tested hydrolate concentration as the independent variable ($p = 0.00000$ for $p < 0.05$). In case of the epicotyls,

the highest growth was noted when 10% concentration was applied (a), while the lowest growth was observed for the highest applied concentration (c) (Figure 2A).

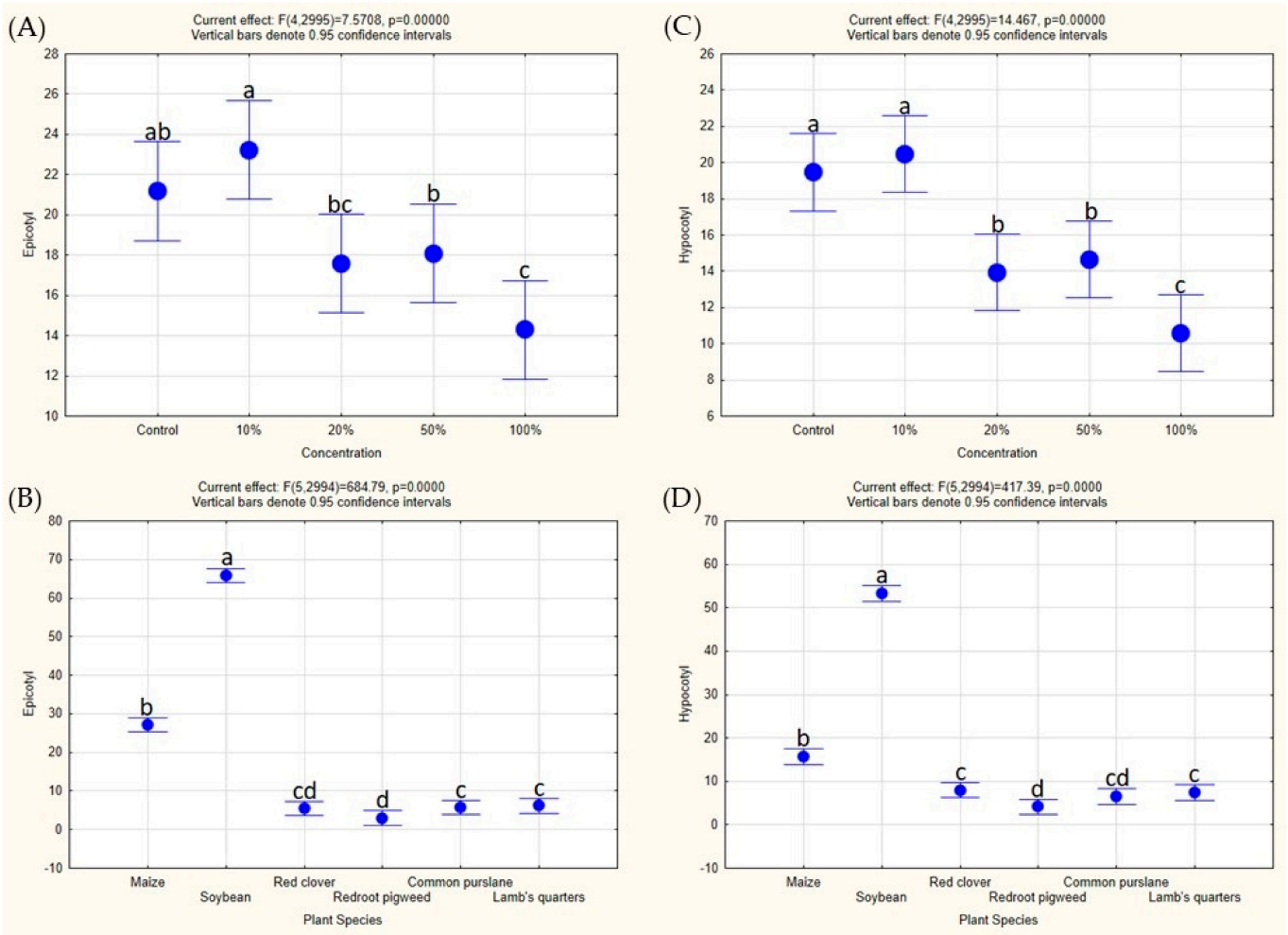

Figure 2. Absolute growth of epicotyl and hypocotyl as affected by hydrolate concentration (a; b) and plant species (c; d) (one-way ANOVA): (**A**) epicotyl growth depending on hydrolate concentration; (**B**) hypocotyl growth depending on hydrolate concentration; (**C**) epicotyl growth depending on plant species; (**D**) hypocotyl growth depending on plant species. Data are presented as mean ± SD. Different letters (a–d) indicate statistically significant differences for $p < 0.05$ (Duncan's multiple range test).

For hypocotyls, the highest growth was recorded for the homogeneous group consisting of control group and 10% concentration (a), while the lowest growth, as in the epicotyls, was confirmed for the highest applied concentration (c) (Figure 2B). The statistical significance was also confirmed for the epicotyls and hypocotyls growth as the dependent variables when compared with the plant species as the independent variable ($p = 0.0000$ for $p < 0.05$). The highest growth of both epicotyls and hypocotyls was recorded for soybean (a), while the lowest was noted for redroot pigweed (d) (Figure 2C,D).

The factorial ANOVA determined the existence of the statistical significance for the epicotyls and hypocotyls growth as the dependent variables in comparison with the combined effect of the hydrolate concentration and plant species as the independent variables ($p = 0.0000$ for $p < 0.05$). For both epicotyls and hypocotyls, the highest growth was noted for soybean, while the lowest was observed in case of the tested weed species (Figure 3).

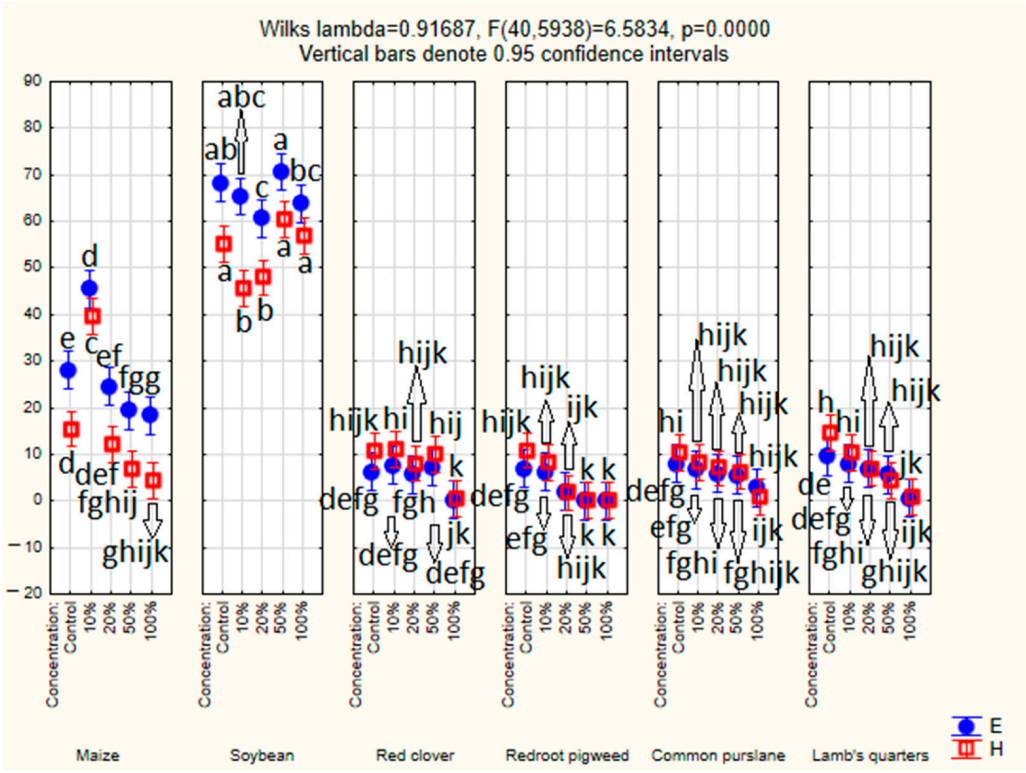

Figure 3. Absolute growth of epicotyl and hypocotyl as affected by combined effect of hydrolate concentration and plant species (factorial ANOVA). Different letters indicate statistically significant differences for $p < 0.05$ (Duncan's multiple range test). E denotes the absolute epicotyl and H denotes the absolute hypocotyl length.

The Duncan's multiple range test showed the statistically significant differences between the soybean epicotyls and hypocotyls growth and the growth observed in the other studied plant species. When 50% concentration was applied, soybean had significantly higher epicotyls growth compared to all the other combinations of hydrolate concentration and plant species, while the tested weeds had significantly lower, mutually non-significant values, with the lowest growth in redroot pigweed when 50% concentration was applied. For hypocotyls, compared to all the other combinations of hydrolate concentration and plant species, the highest growth was recorded in soybean for the homogeneous group consisting of the control group and 50 and 100% concentration, while the significantly lower growth was expressed by the weed species, with the lowest growth again observed in redroot pigweed, this time when 50 and 100% concentrations were applied (Figure 3).

2.3. Relative Growth of Epicotyl and Hypocotyl (Relative to Control) as Affected by Hydrolate Concentration and Plant Species

One-way ANOVA confirmed the existence of the statistical significance for the effect of the tested hydrolate concentration as the independent variable on the epicotyls and hypocotyls growth as the dependent variables ($p = 0.0000$ for $p < 0.05$). In case of the epicotyls, compared to the control group (a), the strongest inhibitory effect of the tested hydrolate was noted for the highest concentration applied (c), while a weak stimulatory effect, which was at the same level of the statistical significance as the control, was observed when 10% concentration was applied (a) (Figure 4A).

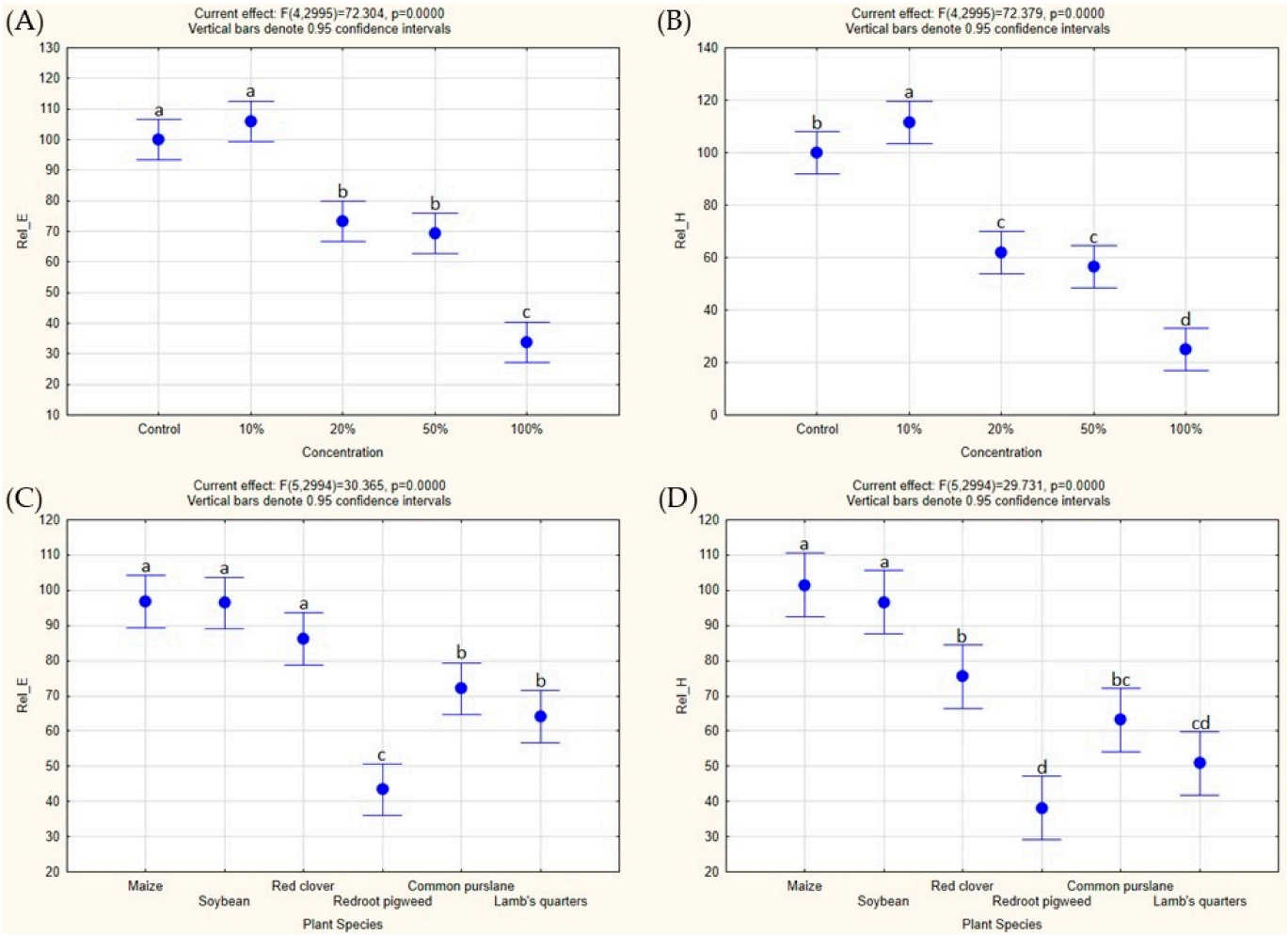

Figure 4. Relative growth of epicotyl and hypocotyl (relative to control) as affected by hydrolate concentration and plant species (one-way ANOVA): (**A**) relative epicotyl growth depending on hydrolate concentration; (**B**) relative hypocotyl growth depending on hydrolate concentration; (**C**) relative epicotyl growth depending on plant species; (**D**) relative hypocotyl growth depending on plant species. Data are presented as mean ± SD. Different letters (a–d) indicate statistically significant differences for $p < 0.05$ (Duncan's multiple range test).

Similar results were obtained in case of hypocotyls: compared to the control group (b), the strongest inhibitory effect was expressed after the application of the highest concentration (d), while a statistically significant stimulatory effect was observed at 10% concentration (a) (Figure 4B). The statistical significance was also confirmed for the effect of the plant species as the independent variable on the epicotyls and hypocotyls growth as the dependent variables ($p = 0.0000$ for $p < 0.05$). For epicotyls, the strongest inhibitory effect was confirmed for redroot pigweed (c), while the weakest effect of the studied hydrolate was observed for the field plant species (a) (Figure 4C). Similar results were noted for the hypocotyls. The strongest inhibitory effect was again confirmed for redroot pigweed (d), while the weakest effects were observed for the homogeneous group consisting of maize and soybean (a) (Figure 4D).

The factorial ANOVA confirmed the existence of the statistical significance for the combined effect of the hydrolate concentration and plant species as the independent variables on the epicotyls and hypocotyls growth as the dependent variables ($p = 0.0000$ for $p < 0.05$). For epicotyls, Duncan's multiple range test showed that, compared to the control group (bcd), the strongest inhibitory effect was observed for red clover, redroot pigweed and lamb's quarters when the highest concentration was applied (j), as well as for redroot pig-

weed at 50% concentration (j). Diametrically opposite results, which present the strongest stimulatory effect compared to the control (bcd), are noticed for maze treated with 10% concentration (a) (Figure 5).

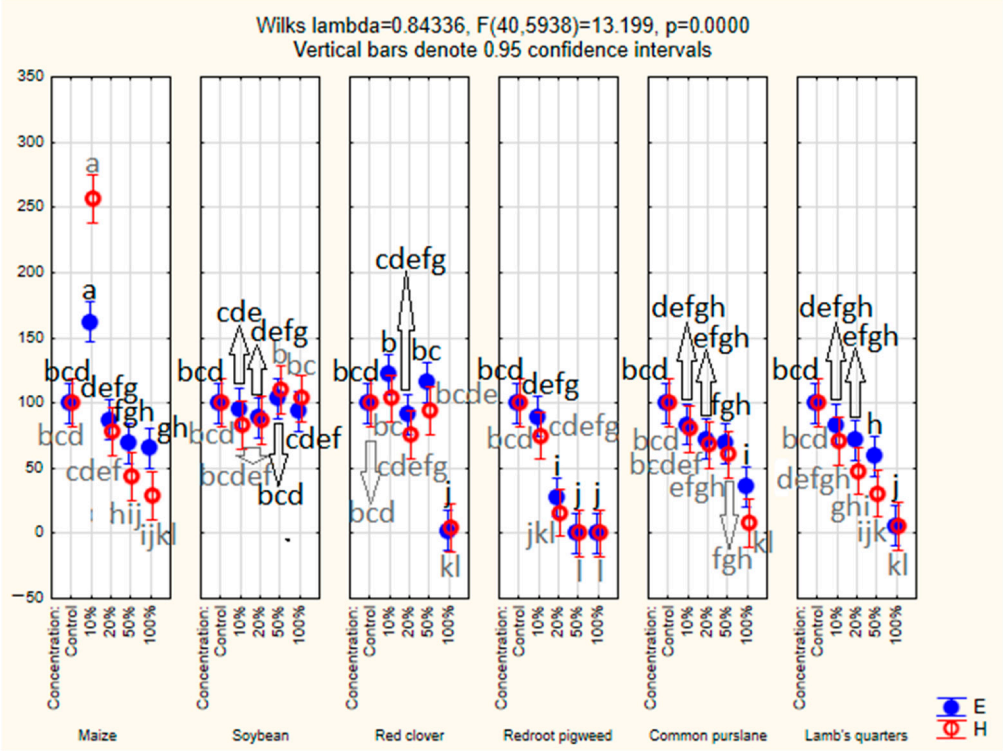

Figure 5. Relative growth of epicotyl and hypocotyl (relative to control) as affected by the combined effect of hydrolate concentration and plant species (factorial ANOVA). Different letters indicate statistically significant differences for $p < 0.05$ (Duncan's multiple range test). E denotes the relative epicotyl and H denotes the relative hypocotyl length.

For hypocotyls, compared to the control group (bcd), the strongest inhibitory effect was expressed in redroot pigweed for 50 and 100% concentrations (l). On the other hand, the strongest stimulatory effect, as in the epicotyls, was observed in maize at 10% concentration (a) (Figure 5).

3. Discussion

The present study demonstrated that the effects of *A. absinthium* hydrolate on seed germination and early seedling growth depended on both the applied concentration and the plant species. Soybean maintained high germination capacity, whereas redroot pigweed showed the greatest susceptibility. Similar selectivity was reported for *Thymus vulgaris* hydrolate, which had relatively little effect on soybean and maize but markedly inhibited several weed species, including redroot pigweed [7]. An aqueous extract of *A. vulgaris* inhibited germination and early growth of redroot pigweed, whereas maize germination remained unaffected, indicating differential sensitivity of cultivated and weed species to *Artemisia*-derived products [17]. The high susceptibility of redroot pigweed observed in the present study is consistent with the inhibitory activity previously reported for *A. absinthium* plant extracts and essential oils against this species [11]. However, the effects of *A. absinthium* hydrolate were not fully reflected by final germination percentage alone. In some species, relatively high GP was accompanied by changes in CVG, GI, GRI and T_{50}, indicating that the hydrolate altered germination speed and timing even when the final number of germinated seeds was only moderately affected. As germinability, germination rate and germination

time describe different properties of the germination process, their combined assessment provides a more complete interpretation of treatment effects than GP alone [18]. These findings indicate that *A. absinthium* hydrolate possesses phytotoxic activity, although its potential selectivity should be interpreted cautiously because responses varied among both field and weed species.

Early seedling growth showed a clear concentration- and species-dependent response to *A. absinthium* hydrolate. Although absolute growth values reflected inherent differences in seedling size among the tested species, relative growth more clearly demonstrated the magnitude of the hydrolate effect compared with the corresponding controls. The reduction in both epicotyl and hypocotyl growth at higher hydrolate concentrations indicated that post-germinative development was more sensitive to the treatment than final germination in several tested species. A similar pattern was reported for *A. absinthium* plant extract, with EC_{50} estimates indicating greater sensitivity of early seedling growth than seed germination in both redroot pigweed and green foxtail [11]. A comparable difference between germination and post-germinative growth was observed in maize treated with walnut leaf extract, where germination was less sensitive, whereas seedling growth was affected to an extent similar to that recorded for the tested weeds [19]. Species- and concentration-dependent responses were also reported for *Thymus vulgaris* hydrolate, with growth stimulation at low concentrations and inhibition at higher concentrations, depending on the target species [7]. The pronounced susceptibility of redroot pigweed observed in the present study is consistent with previous reports of inhibitory effects of *A. absinthium* and *A. vulgaris* products on germination and early seedling growth of this weed [11,17]. However, the comparatively weaker response of soybean and maize indicates that sensitivity cannot be generalised to all cultivated or weed species and that phytotoxic effects depend on both the target species and the applied hydrolate concentration.

In contrast to the inhibition observed at higher concentrations, 10% *A. absinthium* hydrolate stimulated early seedling growth, with the most pronounced response recorded for maize. This pattern is consistent with a hormesis-like response, characterized by a biphasic dose–response relationship in which low concentrations of plant-derived phytotoxins stimulate plant responses, whereas higher concentrations are inhibitory [20,21]. Such responses may represent adaptive reactions to mild stress, allowing plants to temporarily compensate for the initial disturbance before inhibitory effects prevail at higher doses [21]. The stimulation observed at 10% *A. absinthium* hydrolate should therefore be interpreted as a concentration-specific response under the experimental conditions rather than evidence of a general growth-promoting effect. At higher concentrations, the progressive suppression of epicotyl and hypocotyl elongation may be associated with oxidative stress and disruption of ROS homeostasis induced by allelochemicals, which can affect membrane integrity and cellular metabolism [22]. However, these physiological mechanisms were not directly examined in the present study and remain possible explanations rather than confirmed modes of action.

Chemical characterization of the essential oil recovered from *A. absinthium* hydrolates over three consecutive years showed that *trans*-thujone (58.6–69.9%) was the major constituent, followed by *trans*-sabinyl acetate (4.4–6.7%), linalool (2.8–4.8%), 1,8-cineole (3.2–4.9%), and terpinen-4-ol (2.9–3.6%). Variations in their relative abundances were associated with differences in weather conditions [14]. These five oxygenated monoterpenes may contribute to the phytotoxic effects observed in the present study, although their individual activities were not evaluated. The reported phytotoxic activity of *Thuja occidentalis*, which is rich in thujones, suggests a possible role of these compounds [23]. Further studies should focus on the phytotoxic activity of individual compounds in their pure form and the determination of their effective concentrations. Based on these results,

hydrolates containing sufficient concentrations of the target compounds could be selected as potential bioherbicides. Nanoformulation and encapsulation techniques could subsequently be applied to improve their stability and herbicidal efficacy [24–27].

4. Materials and Methods

4.1. Hydrolate

Artemisia absinthium cv. Petrovački was cultivated at the Institute of Field and Vegetable Crops Novi Sad (IFVCNS), Department in Bački Petrovac (45.336676; 19.668637), during 2022. In August, the aboveground parts were harvested, dried in a solar dryer, and subsequently distilled using a custom-made pilot-scale steam distillation unit (IFVCNS, Bački Petrovac, Serbia). After 4 h of distillation, the essential oil was decanted, and the hydrolate was filtered through filter paper into sterile plastic containers and stored at 4 °C until the experiments were conducted in early 2024. To determine its chemical profile, the hydrolate was subjected to simultaneous distillation–extraction using a custom-made Likens–Nickerson apparatus (Faculty of Chemistry, University of Belgrade, Belgrade, Serbia). The resulting secondary essential oil was subsequently analyzed by the gas chromatography with mass spectrometry (GC–MS) using an Agilent 7890A GC coupled to an Agilent 5975C MSD (Agilent Technologies, Santa Clara, CA, USA) [14]. Compounds were identified using AMDIS and NIST software by comparing their mass spectra and linear retention indices (RI), calculated relative to C_8–C_{32} *n*-alkanes, with reference data (Wiley 7, NIST 17, and Adams 4). The corresponding chromatogram (Figure 6) shows the five major constituents from the secondary essential oil of *A. absinthium*: *trans*-thujone, *trans*-sabinyl acetate, linalool, 1,8-cineole, and terpinen-4-ol.

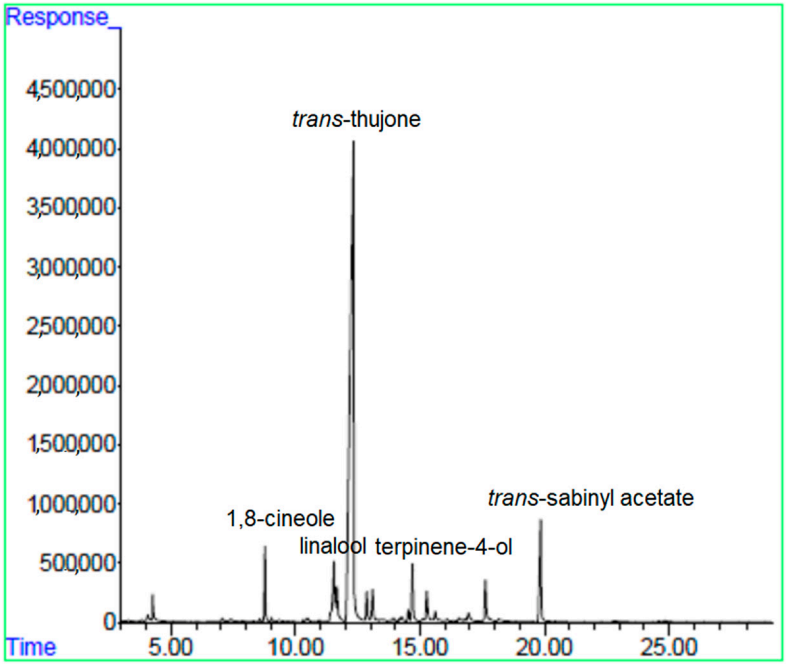

Figure 6. GC-FID chromatogram of secondary essential oil recovered from hydrolate of *Artemisia absinthium* cv. Petrovački.

4.2. Collection of Plant Material

The seeds of field species maize (*Zea mays* L.; BUNS voucher number 2-0682), soybean (*Glycine max* (L.) Merr.; BUNS voucher number 2-0657), and red clover (*Trifolium pratense* L.; BUNS voucher number 2-0191) were obtained in 2023 from the Institute of Field and Vegetable Crops, Novi Sad, Serbia and stored in paper bags at room temperature until the

experiment. The seeds of weed species, redroot pigweed (*Amaranthus retroflexus* L.; BUNS voucher number 2-0680), common lambsquarters (*Chenopodium album* L.; BUNS voucher number 2-0655), and common purslane (*Portulaca oleracea* L.; BUNS voucher number 2-0192), were collected from several localities during 2023 and stored in paper bags at room temperature until the experiment. All field and weed species were tested concurrently in early 2024. The weed seeds were identified and deposited in the Herbarium of The Department of Environmental and Plant Protection, Faculty of Agriculture, University of Novi Sad. The voucher specimens are deposited in the Herbarium BUNS, Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia.

4.3. Seed Germination and Seedlings Length

Following surface sterilization, twenty-five seeds of each tested plant species were placed in Petri dishes (12 cm diameter) lined with filter paper. The hydrolate dilutions (10, 20, and 50%) were freshly prepared with distilled water immediately before the experiment, while undiluted hydrolate (100%) was used directly. The filter paper in each dish was moistened with 10 mL of the respective hydrolate solution (10, 20, 50 and 100%), or distilled water for the control treatment. Each treatment was conducted in four replicates [28]. The seeds of the field and weed species were incubated in a climate chamber (KBWF 720, Binder, Tuttlingen, Germany) under controlled conditions of 22/20 °C (day/night), a 12 h photoperiod, and 60 ± 2% relative humidity for 10 days. Seed germination and seedling growth were assessed daily throughout the 10-day incubation period at regular intervals. The effects of *A. absinthium* hydrolate (AH) on early seedling growth were evaluated by measuring epicotyl and hypocotyl lengths [29], whereas the final germination percentage was recorded on the last day of the experiment. Several germination indices, such as germination percentage (GP), coefficient of velocity of germination (CVG), germination index (GI), median germination time (T_{50}) and germination rate index (GRI) were subsequently calculated to assess germination responses.

4.3.1. Germination Percentage

Germination percentage (GP) represents the proportion of germinated seeds relative to the total number of seeds, expressed as a percentage (%), and was calculated using Equation (1) [30]:

$$\mathrm{GP} = \frac{Ng}{Nt} \times 100 \quad (1)$$

where Ng is the number of the germinated seeds and Nt is the total number of the seeds.

4.3.2. Coefficient of Velocity of Germination

Coefficient of velocity of germination (CVG) is an indicator of germination speed, expressed in % day^{-1}, with higher values indicating faster germination. It was calculated using Equation (2) [31]:

$$\mathrm{CVG} = \frac{\sum_{i=1}^{k} Ni}{\sum_{i=1}^{k} Ni \times Ti} \times 100 \quad (2)$$

where Ti is the time from the start of the experiment to the ith interval; Ni is the number of the seeds germinated in the ith interval (the number corresponding to the ith interval, not the accumulated number); and k is the total number of the intervals.

4.3.3. Germination Index

Germination index (GI) reflects germination speed, with higher values indicating faster germination. It was calculated using Equation (3) [32]:

$$\mathrm{GI} = (10 \times n_1) + (9 \times n_2) + \cdots + (1 \times n_{10}) \quad (3)$$

where n_1, n_2, ... n_{10} represent the number of seeds germinated on days 1, 2, and subsequent days up to day 10, respectively, and 10, 9, ..., 1 are the corresponding weighting factors assigned to each day.

4.3.4. Median Germination Time

Median germination time (T_{50}) represents the time required to reach 50% of the final number of germinated seeds, expressed in days. It was calculated using Equation (4) [33]:

$$T_{50} = Ti + \frac{\left(\frac{N}{2} - Ni\right) \times (Tj - Ti)}{Nj - Ni} \quad (4)$$

where N is the final number of germinated seeds; N_i and N_j are the total number of seeds germinated in adjacent counts at time T_i and T_j, when $N_i < \frac{N}{2} < N_j$.

4.3.5. Germination Rate Index

Germination rate index (GRI) is an indicator of germination speed, expressed in % day^{-1}, with higher values indicating faster germination. It was calculated using a modified equation (Equation (5)) [34]:

$$\text{GRI} = \frac{G_1}{1} + \frac{G_2}{2} + \cdots + \frac{G_x}{\text{x}} \quad (5)$$

where G_1 is the germination percentage × 100 on the 1st day after sowing; G_2 is the germination percentage × 100 on the 2nd day after sowing, etc., and G_x is the germination percentage × 100 on the xth day after sowing.

4.4. Statistical Analysis

Absolute growth was expressed as the measured epicotyl and hypocotyl lengths. Relative growth (%) was calculated by dividing each length value used in the analysis by the mean length of the same organ in the control treatment for the same species and multiplying by 100. The value of 100% corresponds to the control mean. The effects of the hydrolate concentration, plant species, as well as their combined effect, on germination and early seedling absolute and relative growth were analysed using one-way and factorial analysis of variance (ANOVA), followed by Duncan's multiple range test (Statistica 14.1.08; TIBCO Software Inc., Palo Alto, CA, USA, University License). The values followed by the same letters indicate the same level of significance ($p < 0.05$).

5. Conclusions

The results supported the proposed hypothesis that *Artemisia absinthium* hydrolate affected both seed germination and early seedling growth in a concentration- and species-dependent manner. Higher hydrolate concentrations progressively inhibited germination performance and early seedling growth, while 10% hydrolate led to stimulatory effects. Relative growth analysis demonstrated that the inhibitory effects were generally less pronounced in the field species than in the tested weeds, with redroot pigweed exhibiting the greatest susceptibility to the hydrolate. Overall, the results indicate that *A. absinthium* hydrolate has potential for use as a bioherbicide owing to its generally stronger inhibitory effects on the weeds than on the field species.

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Abbreviations

The following abbreviations are used in this manuscript:

GP	germination percentage
CVG	coefficient of velocity of germination
GI	germination index
T_{50}	median germination time
GRI	germination rate index

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