



Article

Mentha suaveolens as Allelopathic Biomass for Weed Control: Phenolics, Organic Acids, and Volatile Organic Compounds Profiles

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Abstract: The need to find alternatives to synthetic herbicides has led to the evaluation of the use of allelopathic biomass from different agroforestry species as soil amendments for weed control. *Mentha suaveolens* Ehrh. (apple mint) could be a suitable candidate as an ecoherbicide due to its availability in the agroecosystem, well-studied biological activities, and known chemical composition. For the present study, two greenhouse pot experiments were conducted by incorporating flowering aerial biomass of apple mint into the soil. In the first one, the potential phytotoxic effects of *M. suaveolens* at doses 1 and 2% were evaluated on the germination and growth of maize and its accompanying weeds. In a second temporary assay, the duration of phytotoxicity and the effects of apple mint (0.5% dose) on the physicochemical properties of the soil were elucidated. The soluble (phenolics and organic acids) and volatile compounds potentially releasable from the allelopathic biomass were identified. The apple mint exerted adverse effects on the germination and growth of dicotyledonous weed species, especially *Amaranthus retroflexus* and *Solanum nigrum*, with almost 100% inhibition, as well as on the growth of monocotyledonous weeds such as *Digitaria sanguinalis*, with a reduction of more than 95%. On the contrary, maize yield and soil properties pH, CECe, organic matter, and exchangeable cations were improved by the ecoherbicide. Chemical analyses of apple mint aerial biomass revealed the presence of 7 phenolic compounds, 9 organic acids, and 32 volatiles. For this study, the effects of incorporating *M. suaveolens* aerial biomass into soil were evaluated for the first time, and it was demonstrated that it has potential as an eco-friendly plant-based tool for Integrated Weed Management.

Keywords: allelopathy; biological weed control; ecoherbicide; HS GC-MS; green manure; soil amendment; *Zea mays* L.



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1. Introduction

The need to find alternatives to synthetic herbicides has led scientists to explore and develop allelopathic crops with applications in rotations, intercropping, or the residues incorporated into soil as green manure [1]. However, not long ago, a wave of studies on the use of allelopathic biomass from agroforestry species as a soil amendment for weed control emerged [2–5]. Unlike cover crops, these do not need to be cultivated for this particular aspect of weed management since they are already found in nature. Others are forestry crops grown for different purposes that produce abundant residual biomass with allelopathic properties. For example, eucalyptus trees are mainly used for pulpwood or in

the timber industry; thus, Puig et al. [6,7] proposed the incorporation of harvest residues from *Eucalyptus globulus* Labill. plantations into soil as green manure for weed control.

Species with invasive capacity, rapid growth, and high biomass production, or those with reported evidence of allelopathy, are of interest for their potential use as herbicidal biomass. The main attraction is their production of allelopathic compounds capable of interfering with the development of other plants and therefore with agricultural weeds. For the matter at hand, incorporating allelopathic biomass into soil allows for a potential release of a cocktail of the allelochemicals present in the plant material, which can be slowly released as the decomposition process occurs [8]. Among the known allelochemicals, phenolic and volatile organic compounds (VOCs) have been widely studied for their phytotoxicity and allelopathic implications [9].

Among the great variety of plant species in the agroforestry system that meet the abovementioned requirements for use as a potential tool against weeds, we find *Mentha suaveolens* Ehrh., commonly known as apple mint. This species is widely distributed in Europe, North Africa, and in temperate countries of the Southern Hemisphere [10]. It is frequently found in relatively abundant biomass in agricultural plots and around greenhouses, forming monospecific patches of considerable extent. The genus *Mentha* includes aromatic species that are highly valued for their pharmaceutical properties and by the farm and food industry [11–13]. In their updated review, Kadoglidou and Chatzopoulou [14] discussed several works on the herbicidal activity of different species of mint, most of which were related to in vitro bioassays involving their essential oils. Still, none of them referred to *M. suaveolens*. Apple mint is known to have an extensive range of biological activities, including cytotoxic, antibacterial, antifungal, or insecticidal activities, among many others [15–18]. However, it is one of the least tested mint species in the world [19]. A few studies have addressed the phenolic composition of its ethanolic or methanolic extracts [20–22]. Ćavar Zeljković et al. [19] analyzed the total phenolic content of methanolic extracts from 13 different species of *Mentha*, finding the highest range from *M. suaveolens*. On the contrary, the bibliography of this paper shows a higher number of studies related to its essential oil and chemical composition [19,23–26]. However, all these references are focused on different properties, such as bactericidal, fungicidal, or antioxidant properties. As far as we are concerned, the phytotoxicity/herbicidal activity of *M. suaveolens* has been tested exclusively by using its essential oil in in vitro bioassays [27,28]. Otherwise, only *M. × verticillata* [29] and *M. spicata* [30] have been assayed as allelopathic green manures.

Zea mays L. is one of the most important cereals for human and animal consumption, and it is grown for grain and forage. Presently, about 1200 million tons of grain from about 206 million ha of land is produced worldwide [31]. Moreover, improvements in maize double cropping for grain production and stover biofuel conversion have become increasingly important in recent years [32]. Among the biotic (insects, pests, predators, weeds, etc.) and abiotic (drought, salinity, heat, etc.) factors that hinder maize production, weeds are considered one of the main factors limiting crop yields. In general, weeds significantly reduce maize yields and sometimes lead to total crop failure [33]. In the coming years, the use of authorized synthetic herbicides is expected to decline, meaning that the sustainability of crop production will rely on alternatives included in Integrated Weed Management strategies, where plant-based weed control should play a crucial role [34,35]. Weed control in maize is critical in the initial growth phases; if pre-emergence weed control is optimized, the need for post-emergence control may be reduced. Like cover crops used as green manures, biomass-based herbicides can help suppress weeds by altering nutrient availability, modifying soil environmental conditions, altering soil microbial activity, and releasing allelochemical compounds ([36] and literature cited). There is evidence in the literature of the use of *M. spicata* in intercropping [37] and of *M. spicata* and *M. × piperita* in rotation crops with maize [38]. As far as we know, this study is the first of its kind in which *M. suaveolens* is discussed in relation to its use as a biomass-based ecoherbicide, with *Z. mays* being the main crop.

Based on the above, the objectives of the current study were to investigate, under greenhouse conditions, the effects of incorporating *M. suaveolens* biomass into soil on (1) the germination, establishment, and growth of maize and its associated weed species, (2) the duration of its herbicidal effects on the target species, (3) the resilience of its effects on the soil, and (4) the compositions of the allelochemicals potentially releasable from the plant material and responsible for the observed phytotoxicity.

2. Materials and Methods

2.1. Plant Materials

Flowering aerial biomass of wild *M. suaveolens* was collected from several sites surrounding the campus of the University of Vigo (Galicia, NW Spain, 45.17° N, 8.69° W). Immediately after harvest, the relevant plant materials were air-dried in the dark at room temperature. A sample of *M. suaveolens* was deposited at the herbarium of the University of Santiago de Compostela (Ref: SANT 77123).

From elemental analysis, the dried apple mint material showed C and N contents of 42.9 and 1.6%, respectively (elemental analyzer, EA1108 Fison Instruments Ltd., Crawley, UK) and values of PO_4^- , K^+ , Mg^{2+} , Mn^{2+} , and Ca^{2+} of 3.5, 14.8, 3.1, 0.06, and 13.0 mg g⁻¹, respectively (ICP-OES, Optima 4300DV, Perkin-Elmer, Shelton, CT, USA).

Assays were performed using *Zea mays* (L.) cv. Anjou '387' (Limagrain Ibérica S.A., Elorz, Spain) as a model summer target crop in allelopathy studies; redroot pigweed (*Amaranthus retroflexus* L.), field bindweed (*Convolvulus arvensis* L.), common purslane (*Portulaca oleracea* L.), and black nightshade (*Solanum nigrum* L.) were used as the target dicot weeds, and barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) and large crabgrass (*Digitaria sanguinalis* (L.) Scop.) were chosen as the monocot weeds. As a pre-germination treatment, redroot pigweed and barnyard grass seeds were synchronized via imbibition in distilled water at 4 °C for 15 days, and large crabgrass seeds were left under light at 4 °C for 56 days. Seed weed species were purchased from Herbiseed© (Twyford, UK, United Kingdom RG10 0NJ). No pretreatment was carried out on the maize seeds.

2.2. Greenhouse Assays

Two experiments were performed according to the procedure described by Puig et al. [6]. The first study focused on measuring the impact of apple mint biomass on the initial establishment and early growth of maize as a model target crop and some representative associated weeds for 30 days after incorporation (DAI); the second aimed to assess the duration of the observed phytotoxic effects.

The soil used was a sandy loam top soil (A horizon) with the following physicochemical characteristics: pH (1:2.5 H₂O) 4.6; EC < 0.13; an organic matter of 3.12%; total N 0.17%; concentrations of Ca^{2+} , K^+ , Mg^{2+} , Na^+ , and P^{3+} of 234, 71, 23, <15, and 115 mg kg⁻¹, respectively; and a maximum water retention capacity (WRC) of 316 mL kg⁻¹ DW.

Both experiments were conducted in summer under greenhouse conditions with natural light and temperature below 26 °C (maintained via a cooling system).

2.2.1. Establishment and Initial Growth of Maize and Weeds

For this trial, 5-litre pots (20 cm diameter) were filled with a mixture of 4 kg of soil and 82 or 41 g of dried and crushed apple mint biomass, corresponding to doses of 2 and 1% on a dry mass basis, respectively. The control treatment consisted of apple mint-free soil with drinking plastic straws cut in 1 cm pieces to simulate the plant material's padding effect [39], meaning that an inert material that could not release any potential phytotoxic compounds that could have interfered with the study was used. Patent PK (K+S KALI GmbH, Kassel, Germany) (P₂O₅ 12%, K₂O 15%, MgO 5%) was added to every pot at a dose of 800 kg ha⁻¹. Three replicates per treatment were performed.

Pots were sown with five maize seeds, five field bindweed seeds, and 24 mg of each of the other weed species, resulting in densities of the small-seeded weed seed bank in infested maize fields of g m⁻² [29]. Pots were watered to maximum water retention capacity

and weighed for the first time. After that, the pots were weighed every other day, and the water lost by evapotranspiration (ET) was replaced.

In addition, every two days, plant emergence was recorded until the control pots were overcrowded (13 days after sowing). Thirty days after apple mint incorporation, emerged seedlings were harvested, separated by species, and counted. Harvested plants were dried at 70 °C for 72 h to obtain each species biomass production (g DW). Maize yield was calculated as follows:

$$\text{Yield (\%)} = \left[\frac{\text{maize aerial biomass}}{(\text{maize aerial biomass} + \text{total weed aerial biomass})} \right] \times 100$$

Finally, the soil pH and electrical conductivity (EC) of each pot were determined in a 1:2.5 soil/water ratio.

2.2.2. Temporary Phytotoxic Effects of Apple Mint Biomass

According to the results obtained from the previous assay, a second pot experiment was performed to estimate the duration of the phytotoxic effects of apple mint on the germination and early development of maize in addition to redroot pigweed and barnyard grass (as two representative maize field weed species).

For this experiment, 1 L pots containing 800 g of soil mixed with 15 g of apple mint biomass (at a 0.5% dose) or drinking straws (control treatment) were used. In this case, all the pots corresponding to 4 replicates per treatment for 10 sowing times (80 pots) were concurrently prepared and watered as described in the previous assay. At this time (0 DAI) and every 3 days (3, 6, 9, 12, 15, 18, 21, 24, 27, 30 DAI), four pots were sown with 25, 25, and 15 seeds each of redroot pigweed, barnyard grass, and maize, respectively. Ten days after each sowing time, the emerged seedlings were counted and harvested to obtain the percentage of germination, shoot length, and total aerial biomass (g DW) for each species and the length and biomass of the maize roots (Figure 1).

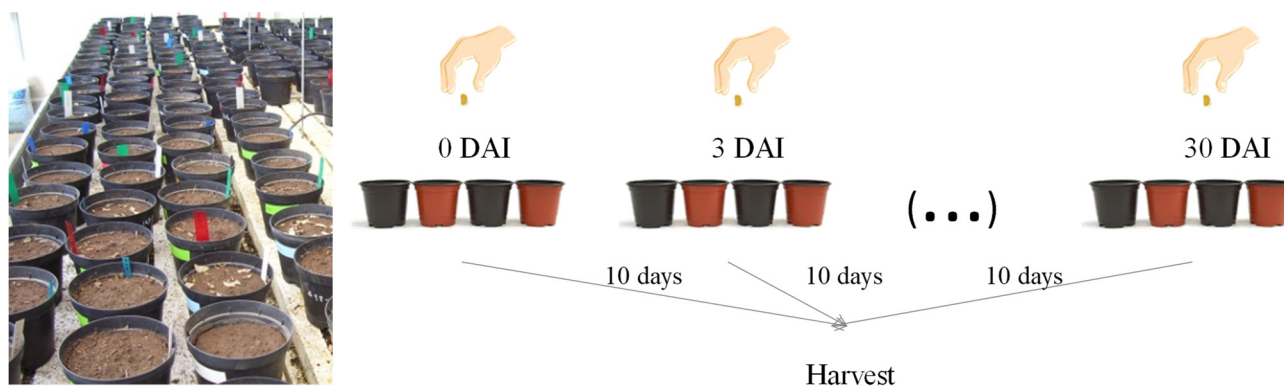


Figure 1. Experimental design of the temporary assay of the apple mint phytotoxic effects. (left) Image of all the pots prepared at the same time at the beginning of the experiment; (right) schematic visualization of the different sowing times after biomass incorporation, and for this assay, four replications per sowing time and treatment were performed. Pots corresponding to each sowing time were harvested ten days after sowing. DAI: Days after apple mint biomass incorporation.

Soils from the control and apple mint pots corresponding to 9 and 30 DAI were air-dried and sieved through a 2 mm mesh for physicochemical analyses. Soil pH was measured using 2:1 water/soil extracts [40]. Organic matter was determined using a muffle furnace (ELF 11/14B, Carbolite Gero Ltd., Hope Valley, UK) at 360 °C for 3 h. The cation exchange capacity (CEC) and exchangeable cation contents were determined according to Peech et al. [41]. Analyses were carried out three times for each treatment and sampling time.

2.3. Chemical Analyses of Apple Mint Aerial Biomass

2.3.1. Reagents and Standards

Sulfuric and phosphoric acids, ethanol, methanol, and acetonitrile were obtained from LiChrosolv® (Darmstadt, Germany). Phenolic acids and flavonoid standards were purchased from Extrasynthèse (Genay, France); organic acids and VOC standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). The water was treated in a Mili-Q water purification system (Millipore, Bedford, MA, USA).

2.3.2. Phenolic Compounds: Extraction, Identification, and Quantification Using High-Performance Liquid Chromatography Coupled with a Diode Array Detector (HPLC-DAD)

The extraction was performed following the method described by Areias et al. [42]. Three grams of aerial biomass of *M. suaveolens* were ground to pass through a 910 µm sieve and extracted at room temperature via agitation using 50 mL ethanol for 15 min, followed by 50 mL for 10 min, and, finally, 25 mL for 5 min. The three extracts were combined, filtered through a Büchner funnel, and dried under reduced pressure via rotary evaporation (Rotavapor R-215, Büchi Labortechnik AG, Flawil, Switzerland) at 30 °C. The residue was dissolved in 10 mL of methanol and filtered using 0.45 µm PTFE membrane; then, 20 µL samples were analyzed via HPLC-DAD. This procedure was performed in triplicate.

The identification of phenolic compounds was carried out using an analytical HPLC unit (Gilson Medical Electronics, Villiers le Bel, France) with a reversed-phase Spherisorb ODS2 (250 × 4.6 mm, 5 mm particle size; Waters, Milford, MA, USA). The solvent system used was a gradient of water/phosphoric acid (999:1; A) and acetonitrile (B), starting with 17% B and installing a gradient to obtain 23% B at 35 min, 36% B at 37 min, 56% B at 57 min, 100% B at 59 min, and 100% B at 63 min at a solvent flow rate of 1 mL/min. Detection was achieved using a Gilson diode array detector. Spectral data from all peaks were collected in the 200–400 nm range, and chromatograms were recorded at 320 nm for chlorogenic, caffeic, and rosmarinic acids and 340 nm for flavonoids. The data were processed using Unipoint System software v 1.6 (Gilson Medical Electronics, Villiers le Bel, France). The compounds were identified by comparing their elution order and UV-vis spectra with authentic standards.

Peak purity was checked using the software contrast facilities. Phenolic compound quantification was carried out using the absorbance recorded in the chromatograms relative to external standards using the following equation:

$$C(c) = \frac{A(c)}{A(st)} \times C(st)$$

where $C(c)$ is the concentration of the compound in the sample, $A(c)$ is the peak area of the compound in the sample chromatogram, $C(st)$ is the concentration of the standard in the reference solution, and $A(st)$ is the area of the peak for the standard in the reference chromatogram. This procedure was performed in triplicate. The data presented are the average of three independent measurements.

2.3.3. Organic Acids: Extraction, Identification, and Quantification via HPLC-DAD

One gram of powdered material was mixed with 25 mL of H₂SO₄ 0.01 N for 20 min under stirring (200 rpm). According to a procedure described by Oliveira et al. [43], the obtained extracts were then filtered, evaporated to dryness under reduced pressure, and dissolved in H₂SO₄ 0.01 N (1 mL), followed by filtration using a 0.45 µm Nylon membrane. This procedure was performed in triplicate.

The organic acids were separated and quantified using a system consisting of an analytic HPLC unit (Gilson Inc., Middleton, WI, USA) with an ion exclusion Nucleogel Ion 300 OA (300 × 7.7 mm; MachereyNagel, Düren, Germany) column. Elution was performed in isocratic mode using H₂SO₄ (0.01 N) at a flow rate of 0.2 mL/min. Detection was achieved using a UV detector set at 214 nm. Organic acid quantification was achieved by

measuring the absorbance recorded in the chromatograms relative to external standards. This procedure was performed in triplicate. The data presented are the mean values of three independent measurements.

2.3.4. Volatile Organic Compounds: Extraction and Identification via Headspace (HS)-Gas Chromatography–Mass Spectrometry (GC-MS)

One gram dw of *M. suaveolens* flowering aerial biomass was transferred to a 20 mL headspace vial (Restek, Bellefonte, PA, USA). The vial was sealed using a crimp top cap with polytetrafluoroethylene (PTFE)–silicone headspace septa (Restek, Bellefonte, PA, USA). The vial was introduced in a headspace sampler (HP 7694, Hewlett Packard Corp., Hayward, CA, USA) and equilibrated for 45 min at 80 °C. After this time, 1 mL of headspace gas was transferred to an Agilent 7820A GC System gas chromatograph coupled to an Agilent 5975 MSD Series mass spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA) for analysis. Split injection was performed with helium as the carrier gas at a constant flow rate of 1 mL/min. Compounds were separated on an HP-5ms (60 m × 0.25 mm I.D.; film thickness 0.25 µm) capillary column (Agilent, Palo Alto, CA, USA). The column was kept at 40 °C for 2 min after injection and then programmed at 8 °C/min to 280 °C, and then this temperature was held for 10 min. All mass spectra were acquired in electron impact (EI) mode. The injector and transfer line temperatures were 250 and 290 °C, respectively. The ionization voltage was 70 eV, and the mass range was 35–350 *m/z*.

Compounds were identified by comparing the mass spectrum of the chromatographic peaks with the mass spectra included in the National Institute of Standards and Technology (NIST 2011) MS spectral database. Individual compounds' relative amounts (RAs) are expressed as per cent peak areas relative to the total peak area.

2.4. Statistical Analyses

Replicated experiments were performed according to a completely randomized design. Data were first tested for normality using the Kolmogorov–Smirnov test and homogeneity of variance using the Levene's test. When variances were homogenous, data were analyzed using a one-way ANOVA and LSD test for post hoc multiple comparisons. In the case of heteroscedasticity, the variance was analyzed using the Kruskal–Wallis H test and Tamhane's T2 test for post hoc multiple comparisons. For the phytotoxic temporary effect experiment, data obtained for each parameter and sowing time were expressed in percentage relative to the control and then compared using Student's *t*-test for independent samples. Statistical analyses were performed using the IBM SPSS Statistics 24 software (IBM SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Establishment and Early Growth of Maize and Weeds

The effects of incorporating apple mint biomass into soil on the emergence and initial growth of maize and weed seedlings are represented in Table 1. There were significant differences between the control and apple mint treatments for dicot weeds, but no differences between the doses were observed. The allelopathic biomass was able to control the germination and emergence of dicot seeds during the first 13 days of the experiment, reaching inhibitions from 100% (up to day 6 for the 2% dose) to reductions of more than 70% and almost 90% with respect to the control for the 1 and 2% doses, respectively. On the other hand, both doses significantly reduced monocot weed emergence by about 40% up to day 6 after sowing. Maize was not affected at any measurement time.

Table 1. Effects of incorporating different doses of *M. suaveolens* into soil on the emergence and survival of maize seedlings and associated weeds in subsequent days after sowing in a pot experiment. Within each column and row, mean values of three replicates $\pm$ SD are shown. For each measurement day, distinct letters indicate significant differences between treatments ($p \leq 0.05$; LSD or Tamhane's T2 test for post hoc multiple comparisons).

Variable	Days after Sowing	Sig. ^a	0% (w/w)		1% (w/w)		2% (w/w)	
Total dicot weeds per pot (n)	4	*	40.0 $\pm$ 7.0	a	0.0 $\pm$ 0.0	b	0.0 $\pm$ 0.0	b
	6	*	67.7 $\pm$ 14.6	a	5.0 $\pm$ 2.6	b	0.0 $\pm$ 0.0	b
	8	*	76.0 $\pm$ 15.7	a	7.3 $\pm$ 5.0	b	0.7 $\pm$ 0.6	b
	10	*	77.7 $\pm$ 11.2	a	8.7 $\pm$ 5.0	b	2.3 $\pm$ 1.5	b
	13	***	60.0 $\pm$ 9.7	a	15.7 $\pm$ 8.1	b	6.3 $\pm$ 2.5	b
Total monocot weeds per pot (n)	4	*	9.0 $\pm$ 4.4	a	1.3 $\pm$ 0.6	b	1.0 $\pm$ 0.0	b
	6	*	26.7 $\pm$ 7.0	a	15.0 $\pm$ 2.6	b	16.0 $\pm$ 1.0	b
	8	n.s.	31.3 $\pm$ 5.9	a	21.7 $\pm$ 3.1	a	21.7 $\pm$ 4.5	a
	10	n.s.	31.7 $\pm$ 5.7	a	23.0 $\pm$ 5.3	a	24.0 $\pm$ 2.6	a
	13	n.s.	31.3 $\pm$ 6.1	a	23.0 $\pm$ 7.0	a	24.3 $\pm$ 3.1	a
Total maize seedlings per pot (n)	4	n.s.	0.0 $\pm$ 0.0	a	0.3 $\pm$ 0.6	a	0.0 $\pm$ 0.0	a
	6	n.s.	3.0 $\pm$ 1.0	a	4.0 $\pm$ 0.0	a	3.0 $\pm$ 2.0	a
	8	n.s.	3.3 $\pm$ 0.6	a	4.0 $\pm$ 0.0	a	4.0 $\pm$ 1.0	a
	10	n.s.	3.7 $\pm$ 0.6	a	4.0 $\pm$ 0.0	a	4.7 $\pm$ 0.6	a
	13	n.s.	4.0 $\pm$ 1.0	a	4.3 $\pm$ 0.6	a	4.7 $\pm$ 0.6	a

^a sig.: significant at * $p \leq 0.05$; *** $p \leq 0.001$; n.s.: not significant $p > 0.05$ (ANOVA or Kruskal–Wallis H test).

The effects of incorporating apple mint into soil on the establishment and growth of maize and weeds 30 days after sowing are summarized in Table 2. Consistent with the above data, both doses significantly reduced the total number of small dicot weeds with respect to the control, especially the seedlings of *A. retroflexus* and *S. nigrum*, where the highest apple mint dose showed complete inhibition. The same trend was observed for the aerial biomass, with reductions of 100% for both species. Regarding the other dicots, *C. arvensis* was not affected by the allelopathic biomass. For *P. oleracea*, despite its emergence being stimulated at 1%, the 2% dose could significantly reduce the aerial biomass by 94.5%. No effects were observed on the emergence of monocot species, but there were phytotoxic effects on aerial biomass, with reductions of 79.8% on *E. crus-galli* (at 2%) and 95% and 96.8% on *D. sanguinalis* (for the 1% and 2% doses, respectively). Consequently, both apple mint doses reduced more than 90% of the total aerial biomass of monocot weeds (Figure 2). Although maize was not statistically affected, there was a reduction in the aerial biomass provoked by the ecoherbicide; however, the weed control strategy led to a significant increase in the yield (induced by both apple mint doses).



Figure 2. Weed density in (A) control, (B) apple mint 1%, and (C) apple mint 2% pots at 30 days after incorporating allelopathic biomass into the soils.

Regarding soil, both doses increased the pH, but only the highest dose was also able to improve the EC significantly.

Table 2. Effects of incorporating different doses of *M. suaveolens* into soil on the establishment and growth of maize and associated weeds in a pot experiment. Within each column, mean values of three replicates $\pm$ SD measured at day 30 after sowing are shown. For each variable and/or species, distinct letters indicate significant differences between treatments ($p \leq 0.05$; LSD or Tamhane's T2 test for post hoc multiple comparisons).

Variable	Species	Sig. ^a	0% (w/w)		1% (w/w)		2% (w/w)	
Emerged seedlings per pot (n)	<i>C. arvensis</i>	n.s.	2.00 $\pm$ 0.00	a	2.00 $\pm$ 0.00	a	1.67 $\pm$ 1.53	a
	<i>A. retroflexus</i>	*	14.67 $\pm$ 3.22	a	0.33 $\pm$ 0.58	b	0.00 $\pm$ 0.00	b
	<i>S. nigrum</i>	*	14.33 $\pm$ 3.22	a	1.00 $\pm$ 1.00	b	0.00 $\pm$ 0.00	b
	<i>P. oleracea</i>	*	10.00 $\pm$ 6.25	a	20.33 $\pm$ 3.22	b	4.67 $\pm$ 3.51	a
	<i>E. crus-galli</i>	n.s.	9.67 $\pm$ 1.16	a	9.67 $\pm$ 0.58	a	7.33 $\pm$ 3.06	a
	<i>D. sanguinalis</i>	n.s.	23.67 $\pm$ 7.64	a	20.33 $\pm$ 5.13	a	17.00 $\pm$ 3.46	a
	Total small-seeded dicots	**	39.00 $\pm$ 11.53	a	21.67 $\pm$ 3.79	b	4.67 $\pm$ 3.51	c
	Total monocots maize	n.s.	33.33 $\pm$ 7.51	a	30.00 $\pm$ 5.57	a	24.33 $\pm$ 4.16	a
Aerial biomass per pot (mg dw)	<i>C. arvensis</i>	n.s.	17.73 $\pm$ 11.75	a	38.30 $\pm$ 3.37	a	18.90 $\pm$ 16.63	a
	<i>A. retroflexus</i>	*	11.20 $\pm$ 7.37	a	0.00 $\pm$ 0.00	b	0.00 $\pm$ 0.00	b
	<i>S. nigrum</i>	*	34.83 $\pm$ 13.12	a	0.50 $\pm$ 0.46	b	0.00 $\pm$ 0.00	b
	<i>P. oleracea</i>	*	7.27 $\pm$ 4.56	a	5.47 $\pm$ 2.22	ab	0.40 $\pm$ 0.40	b
	<i>E. crus-galli</i>	*	57.77 $\pm$ 28.78	a	23.13 $\pm$ 8.47	ab	11.67 $\pm$ 3.88	b
	<i>D. sanguinalis</i>	*	475.77 $\pm$ 213.77	a	23.33 $\pm$ 9.29	b	15.27 $\pm$ 6.55	b
	Total small-seeded dicots	*	53.30 $\pm$ 20.34	a	5.97 $\pm$ 2.35	b	0.40 $\pm$ 0.40	b
	Total monocots maize	**	533.53 $\pm$ 236.83	a	46.46 $\pm$ 17.73	b	26.94 $\pm$ 7.56	b
yield (%) ^b		*	66.41 $\pm$ 10.32	a	89.80 $\pm$ 2.07	b	95.03 $\pm$ 2.74	b
total ET (mL) ^c		*	6186.47 $\pm$ 76.65	a	6037.20 $\pm$ 40.93	ab	5990.57 $\pm$ 98.02	b
soil pH		***	4.38 $\pm$ 0.03	a	4.82 $\pm$ 0.01	b	5.10 $\pm$ 0.05	c
soil EC (dS m ⁻¹)		**	0.16 $\pm$ 0.03	a	0.21 $\pm$ 0.02	a	0.33 $\pm$ 0.06	b

^a sig.: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s.: not significant $p > 0.05$ (ANOVA or Kruskal–Wallis H test). ^b yield (%): percentage of maize in the total yield (maize + weeds). ^c total ET (mL): total water loss by evapotranspiration.

3.2. Temporary Phytotoxic Effects of Apple Mint Biomass

Figure 3 describes the effects measured on maize seedlings sown at different days after apple mint incorporation and compared on a percentage basis to the apple mint-free control. At 3 DAI, inhibition of total germination, shoot length, and root biomass was observed, as well as a significant reduction in aerial biomass at 9 DAI. Except for the inhibition of germination at 18 and 21 DAI, after 12 DAI, there were no adverse effects, but significant stimulation in both aerial and root biomass and length was observed.

The temporary effects of incorporating apple mint into the soils on redroot pigweed and barnyard grass are represented in Figure 3. Redroot pigweed germination was inhibited by over 50% throughout the assay; however, those few seedlings that managed to germinate were highly stimulated by the ecoherbicide compared to the control. Despite this, redroot pigweed aerial biomass was controlled and practically nonexistent during the first 12 days of the assay. On the other hand, no general effects were observed in barnyard grass, but isolated inhibitions of germination at 12 and 24 DAI and of aerial biomass at 6 DAI were observed.

Results from soil analysis are represented in Table 3. The incorporation of apple mint biomass significantly increased pH, CECe, and exchangeable cations (Ca^{2+} , Mg^{2+} , K^+ , Na^+) with respect to the control soil at 9 DAI. Furthermore, the Al^{3+} cation was reduced by 69% compared to the control. These effects, together with an increase in the organic matter, were consistent after 30 DAI.

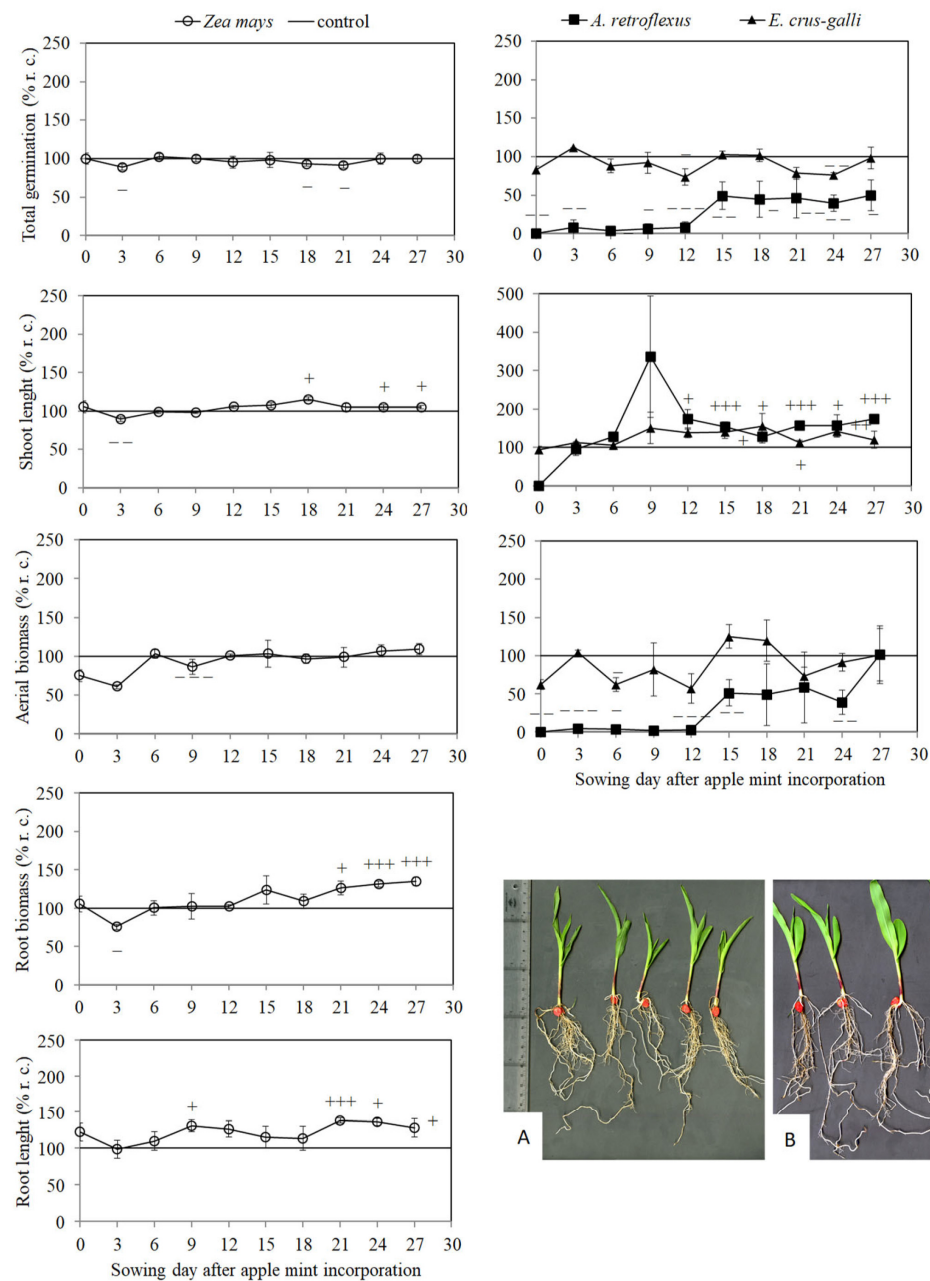


Figure 3. Growth parameters measured in 10-day-old maize, redroot pigweed, and barnyard grass seedlings sown at different times after the incorporation of *M. suaveolens* into the soils. For each parameter and time, signs represent inhibition (–) or stimulation (+) with respect to the control (line —): one sign, $p \leq 0.05$; two signs, $p \leq 0.01$; three signs, $p \leq 0.001$; no sign, $p > 0.05$ (independent samples *t*-test). Symbols represent mean values of four replicates $\pm$ SD. % r. c.: percentage with respect to the control. (right bottom) Images of maize seedlings recovered from (A) control pots and (B) from pots with apple mint incorporated into the soil at 0.5% showing the stimulating effects on maize growth.

Table 3. Physicochemical properties of soils from control and *M. suaveolens* pots at 9 and 30 days after allelopathic biomass incorporation (DAI). Values denote means of three replicates $\pm$ SD.

	9 DAI			30 DAI		
	Sig. ^a	Control	<i>M. suaveolens</i>	Sig. ^a	Control	<i>M. suaveolens</i>
pH H ₂ O	**	4.9 $\pm$ 0.0	5.3 $\pm$ 0.1	***	4.77 $\pm$ 0.06	5.47 $\pm$ 0.06
Organic matter (%)	n.s.	3.4 $\pm$ 0.2	3.6 $\pm$ 0.3	*	3.3 $\pm$ 0.3	3.7 $\pm$ 0.1
CEC ^b	***	3.43 $\pm$ 0.13	4.63 $\pm$ 0.14	***	3.52 $\pm$ 0.06	4.42 $\pm$ 0.19
Ca ²⁺	***	1.00 $\pm$ 0.00	2.10 $\pm$ 0.10	**	1.00 $\pm$ 0.00	2.07 $\pm$ 0.15
Mg ²⁺	**	0.13 $\pm$ 0.00	0.47 $\pm$ 0.03	**	0.13 $\pm$ 0.00	0.58 $\pm$ 0.05
Na ⁺	**	0.15 $\pm$ 0.02	0.23 $\pm$ 0.02	**	0.18 $\pm$ 0.00	0.37 $\pm$ 0.02
K ⁺	**	0.18 $\pm$ 0.01	1.20 $\pm$ 0.05	***	0.17 $\pm$ 0.01	0.76 $\pm$ 0.03
Al ³⁺	***	1.97 $\pm$ 0.12	0.63 $\pm$ 0.06	***	2.03 $\pm$ 0.06	0.63 $\pm$ 0.06

^a sig.: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s.: not significant $p > 0.05$ (t -test for independent samples). ^b CEC: effective cation exchange capacity.

3.3. Chemical Analyses of Apple Mint Flowering Aerial Biomass

3.3.1. Phenolic Compounds and Organic Acids

The qualitative and quantitative composition of apple mint dry biomass is described in Table 4. The relative concentrations of identified compounds are presented in order of their retention time. The results of our HPLC-DAD analysis revealed seven phenolic compounds, of which were three phenolic acids—chlorogenic, caffeic, and rosmarinic acids—and four were flavonoids, of which three were luteolin derivatives, and one was an apigenine derivative. The major phenolic compound in our samples (83.4% of the total phenolic compounds identified) was rosmarinic acid. Regarding organic acids, nine were identified, and the most abundant compound found was citric acid, representing 57.9% of the total, followed by quinic and malic acid, ca. 15.7% and 13.2%, respectively.

Table 4. Qualitative–quantitative phenolic and organic acid composition of *M. suaveolens* aerial biomass (measured via HPLC-DAD analysis). The data shown are the mean values of three replicates $\pm$ SD.

Compound	RT ^a	Content ^b	% ^c
<u>Phenolic compounds</u>			
Chlorogenic acid	6	6.1 $\pm$ 0.05	0.2
Caffeic acid	9	11.1 $\pm$ 0.36	0.4
Luteolin hexoside	24	121.9 $\pm$ 0.56	4.4
Luteolin 7- <i>O</i> -rutinoside	25	59.1 $\pm$ 0.48	2.1
Luteolin 7- <i>O</i> -glucoside	27	57.3 $\pm$ 0.39	2
Rosmarinic acid	32	2333.2 $\pm$ 8.23	83.4
Apigenin 7- <i>O</i> -glucoside	35	208.1 $\pm$ 3.52	7.4
<u>Organic acids</u>			
Oxalic acid	19.2	74.7 $\pm$ 1.12	1.6
cis-Aconitic acid	24.5	2.4 $\pm$ 0.15	0.1
Citric acid	30.2	2697.8 $\pm$ 79.93	57.9
Pyruvic acid	32.2	360.1 $\pm$ 0.48	7.7
Malic acid	36.4	617.2 $\pm$ 21.24	13.2
Quinic acid	38.1	733.9 $\pm$ 6.97	15.7
trans-Aconitic acid	43.3	93.9 $\pm$ 9.34	2
Shikimic acid	47.1	55.2 $\pm$ 0.38	1.2
Fumaric acid	60.9	26.7 $\pm$ 0.17	0.6

^a RT = retention time in min. ^b Content in mg·kg^{−1} of plant material. ^c % of each compound with respect to the total of identified compounds.

3.3.2. Volatile Organic Compounds

By using a headspace extractor coupled to a chromatograph, it was possible to identify the potential volatile compounds capable of being released from the apple mint biomass into the soil pores. A total of 32 compounds belonging to different VOC classes were identified. More than half corresponded to terpenes; specifically, 16 were monoterpenes (α -pinene, β -pinene and (+)-sabinene as the main ones) and 5 were sesquiterpenes (Table 5).

Table 5. Composition of volatile organic compounds (VOCs) released from *M. suaveolens* aerial biomass (measured using HS/GC-MS). Compounds tentatively identified by comparison with NIST 2011.

Compound	RT ^a	RA ^b
<u>Sulfur compound</u>		
Dimethyl sulfide	4.3	1.26
<u>Aldehydes</u>		
2-methylpropanal	4.5	0.89
3-methylbutanal	5.6	0.86
2-methylbutanal	5.7	0.62
<u>Furans</u>		
2-ethylfuran	6.3	0.33
<u>Esters</u>		
2-methyl-propanoic acid ethyl ester	7.3	0.50
Methyl 2-methylbutyrate	7.7	2.55
Ethyl 2-methylbutyrate	9.2	6.00
Propyl 2-methylbutyrate	11.4	0.36
1-Octen-3-yl-acetate	14.8	0.93
<u>Aliphatic alcohol</u>		
3-Octanol	12.4	0.87
<u>Monoterpenes</u>		
β -thujene	11	3.76
α -pinene	11.2	15.06
Camphene	11.6	4.71
(+)-Sabinene	12.1	10.85
β -pinene	12.2	13.19
β -myrcene	12.4	6.50
Tricyclene	12.7	0.29
α -terpinene	13	1.29
D-limonene	13.2	6.32
β -ocimene	13.5	0.46
γ -terpinene	13.9	1.70
p-menthan-1-ol	14.1	8.38
Linalool	14.6	0.55
4-thujanol	14.7	0.75
Endo-borneol	16.1	1.00
Terpinen-4-ol	16.3	0.60
<u>Sesquiterpenes</u>		
α -bourbonene	20.1	0.41
Caryophyllene	20.7	1.20
Cadina-1(6),4-diene	21.1	0.69
β -cubebene	21.4	1.14
Germacrene D	21.7	5.99

^a RT = retention time in min. ^b RA = relative amount of individual compounds expressed as per cent peak area relative to the total identified compounds peak area.

4. Discussion

The effects of incorporating *M. suaveolens* biomass into soil for weed control were evaluated for the first time for this study. In our first pot experiment, we observed that apple mint treatments effectively controlled germination from the beginning of the assay and reduced the aerial biomass of small-seeded dicotyledonous weeds, especially *A. retroflexus* and *S. nigrum*. Although *P. oleracea* seedling emergence was stimulated in the apple mint pots, the ecoherbicide negatively affected biomass production, resulting in the effective total control of dicots. Araniti et al. [27] also noted that, among the weed species tested with *M. suaveolens* essential oil, *A. retroflexus* was the most sensitive, showing the lowest ED₅₀ value for germination. It has also been reported that this essential oil significantly affected germination, being lethal to seed embryos, or decreasing vigour index, hypocotyl, and the radical growth of lettuce [28]. On the other hand, similar to common purslane, the ecoherbicide could not control monocotyledonous species' germination. However, it did reduce aerial biomass, especially that of *D. sanguinalis*, resulting in an inhibition of the total biomass of monocotyledonous weed species.

Regarding the second temporary pot experiment, the apple mint-based ecoherbicide controlled the dicot species redroot pigweed but not the monocot *E. crus-galli*. Dhima et al. [29] pointed out similar effects of *Mentha × verticillata* green manure on weed establishment and growth, where the development of the dicotyledon *P. oleracea* was more affected than the monocotyledon species *E. crus-galli*. Also, Chalkos et al. [30] observed that the emergence of broadleaf weeds (*A. retroflexus*, *C. album*, *P. oleracea*, and *Datura stramonium*) was more affected than grass weeds (*Cynodon dactylon* and *Sorghum halepense*) when their soil was amended with *M. spicata* compost. Similar to Puig et al. [6] when they incorporated eucalyptus leaves into the soil, our apple mint treatment controlled the germination of *A. retroflexus*, but the seedlings that managed to emerge grew more than those in the control pots. However, aerial biomass production was finally reduced.

Concerning maize, neither germination nor aerial biomass production were statistically affected by the ecoherbicide compared to the control. Similarly, the tolerance of maize to the phytotoxic activity of the essential oil of *M. × piperita* [44], *M. verticillata* green manure [29], and *M. spicata* when intercropped [37] have been previously demonstrated. Large seeds like maize generally tolerate changes in soil chemical properties caused by green manures better [45]. Seed size is relevant when assessing the phytotoxicity of a given material and/or the effects of allelochemicals, so the phytotoxic effect on small-seeded species can be higher than on large-seeded ones [46]. Also, Dhima et al. [47] found more substantial phytotoxic effects of winter cereal extracts on small-seeded weeds' germination and initial growth than large-seeded maize. The availability of more reserve substances in the grain can feed the seedling for longer without needing to absorb soil nutrients, including allelochemicals. This general fact is consistent with our results and could partly explain the tolerance of maize, and even its stimulation, and the sensitivity of weeds. Such tolerance represents an additional advantage of biomass-based weed control in systems based on "large-seeded" forage crops associated with "small-seeded" weeds [48].

On the contrary, in one study, it was observed that when *M. spicata* and *M. × piperita* were used in rotation with maize crops, maize plants' growth and dry biomass were negatively affected [38]. In our case, a slight reduction in maize aerial biomass reflected some phytotoxicity exerted by apple mint, which was compensated by higher maize yields due to weed control. The second temporary pot experiment helped us to find what physiological process of the maize might have been affected and at what time and therefore explain the observed aerial biomass reduction. Two phases in the growth of maize were differentiated: a first inhibitory phase and a second stimulatory phase. Puig et al. [6] reported similar dynamics when they used eucalyptus leaves as herbicides, blaming an initial release of VOCs ("volatile phase"), followed by a release of soluble phenolic compounds ("soluble phase"). Therefore, they proposed establishing a safety period for maize of 12 to 15 days of relay planting after incorporating eucalyptus into the soil. In the case of apple mint, setting

the safety period closer to 21 DAI would be more appropriate to avoid any possibility of the inhibition of maize germination.

The amounts of *M. suaveolens* biomass added to soil in our experiments (2, 1, and 0.5% (*w/w*) in soil on a dry mass basis) were consistent with those used for phytotoxic studies of other species ([4,6,7] and literature cited within). The dosages were consistent with the amounts of cover crop residues incorporated into soil when used as green manures, such as legumes, grasses, and their mixtures (i.e., 5–15 Mg ha⁻¹ dw) [49]. Because this study is the first of its kind to use the biomass of *M. suaveolens*, with a pretty high C/N ratio (26.8), two initial dosages were tested. However, a dose–response effect was not observed, but the phytotoxicity exerted by the plant material incorporated into the soil was statistically similar at 1 and 2% (*w/w*). Other authors have also observed that doubling the dosage does not increase the significance of weed control [6,50]. In our case, the phytotoxicity of *M. suaveolens* caused, even at 1%, some phytotoxic effect on maize aerial biomass compared to the control pots (Table 2); subsequently, the temporary assay was conducted with a reduced dosage of 0.5%.

The data obtained from our physicochemical analysis of the soil suggested that the *M. suaveolens* biomass incorporated as an amendment would have fertilizing capacity. The allelopathic biomass increased the values of pH, CECe, and exchangeable cations compared to the values measured at the beginning of the trial, and its effects lasted until 30 DAI. Significant increases in organic matter values were also observed at the end of the experiment. Together with the early nutrient supply, these results could explain the stimulatory phase observed in maize growth. Kadoglidou et al. [51] also reported increased organic matter by incorporating dried *M. spicata* tissues into seedbeds, improving tomato seedling production. Nevertheless, in contrast to our results, soil pH was not affected. The increase in pH values is agronomically interesting since soil pH is one of the critical environmental factors shaping soil bacteria communities and determining maize yields [52]. In addition, high pH values may reduce the toxic effect of the different aluminum species present in the soil on plant development [53,54]. In our experiment, the toxic Al³⁺ cation was reduced throughout the assay, thus neutralizing the possible toxicity.

The phytotoxic effect observed in our experiment could be attributed to the release of a cocktail of compounds during the decomposition of plant material. Among the variety of secondary metabolites that make up this cocktail, phenolic compounds and VOCs (mainly terpenoids) are the most studied and most cited sources of phytotoxicity; however, studies on their mode of action are still lacking, and the mechanism of only a few phytotoxic compounds has been described [55]. Phenolic compounds are known to inhibit germination and root elongation and interfere with the growth and development of plants [56–58]. The major phenolic compound found in our apple mint was rosmarinic acid. The *Mentha* genus is particularly rich in this compound, as well as in chlorogenic and caffeic acid, which were also present in our sample [13,19]. Several authors have also identified rosmarinic acid as the predominant phenolic compound in *M. suaveolens* extracts [21,59,60]. Rosmarinic acid has been shown to trigger a series of effects on cellular organization and ultrastructures that strongly inhibit the root growth and development of *Arabidopsis thaliana* [61], also leading to a high inhibitory effect on lettuce radicle elongation [62]. Additionally, caffeic and chlorogenic acids have been shown to be capable of suppressing the biomass of *Setaria viridis* and *P. oleracea*, and the latter compound is also capable of reducing the root length of both weeds [63]. Nevertheless, Kamran et al. [64] indicated that low concentrations of caffeic acid could increase cell division and cell enlargement by accelerating the rate of mitosis and cellulose synthesis, thus providing an opportunity for enhancing the growth of maize seedlings. The flavonoids luteolin, apigenin, and their derivatives present in our sample were also identified by other authors [13,19,65]. Recent studies have reported that luteolin reduced the growth of *Lemna gibba* plants [66] and can inhibit the germination of *Lactuca sativa* and *Agrostis stolonifera* [67]. Furthermore, some evidence suggesting that apigenin derivatives can delay germination and the cotyledon emergence of *Rumex crispus* has been found [68].

In addition, the composition of organic acids was also identified in the present study. To our knowledge, only Park et al. [60] have analyzed the organic acids of several *Mentha* species, including *M. suaveolens*, and their reported organic acids match most of those present in our sample, where we found that citric acid turned out to be the predominant one. These types of compounds may also potentially be involved in phytotoxicity [69]. The application of citric acid as a natural herbicide is effective in controlling weeds, with 74.7% reductions in a potato field being observed in [70], and on *S. nigrum* and *Abutilon theophrasti* (with 95% control), but it did not substantially affect grass weeds in [71]. Agnello et al. [72] reported that citric acid negatively affected *Medicago sativa* germination. Likewise, Zhang et al. [73] demonstrated the phytotoxic effects of isolated citric and malic acids on melon growth. Also, the germination and early growth of *D. sanguinalis* and *Eleusine indica* were severely decreased by quinic acid in [74]. According to Chen et al. [75], oxalic acid could inhibit the activities of catalase and peroxidase, leading to the accumulation of reactive oxygen species and lipid peroxidation and impairing the leaf cells of *A. thaliana*.

Concerning volatile compounds, evidence of allelopathic and herbicidal activity has been reported extensively in the literature (e.g., [9,76]), with monoterpenes and sesquiterpenes being the most studied compounds since they are the main components of essential oils [55]. Belonging to the Lamiaceae family, mints synthesize volatile terpenes in glandular trichomes on leaves. The volatile profile of *M. suaveolens* has already been extensively identified, mainly from its essential oil [13,21]. However, when biomass is incorporated directly into soil, as in our case, it is of more interest to know which volatiles are “naturally” released into the soil pores. In this case, the use of headspace trapping techniques is suitable [77]. It must be considered that the compositions of VOCs of the same species vary significantly according to environmental, phenological, and genetic factors, as well as the different plant parts [19,21]. In general, the volatile terpenoids in the present study corresponded with those found in the literature [19,21,24–27]. These cited authors also found that α -pinene and β -pinene were among the major monoterpenes.

Focusing on the terpenes in our sample and the weed target species tested or species of the same genus, we found phytotoxic evidence in the literature for some compounds that could support the herbicidal effects observed after using our allelopathic biomass. For example, α -pinene negatively affected the germination and/or growth of *A. retroflexus*, *P. oleracea*, and *E. crus-galli* [55,78,79]. According to Singh et al. [80], this monoterpene caused lipid peroxidation when applied to young seedlings of *Cassia occidentalis* L., thus resulting in an increase in solute leakage. Furthermore and in order to explain the slight reduction in the aerial biomass of maize observed in our experiments, Graña et al. [81] pointed out that maize seedlings treated with α -pinene showed changes in mitochondrial respiration, with the activity of the electron transport chain being inhibited, and an increase in malondialdehyde levels. Other monoterpenes present in apple mint's VOC profile, such as β -pinene, sabinene, linalool, or terpinen-4-ol, were also tested on different weed species, showing herbicide potential on the germination and growth of our weeds of interest (*A. retroflexus*, *E. crus-galli*, and *D. sanguinalis* [78,79,82,83]). In particular, it is known that β -pinene reduces chlorophyll contents in *Oryza sativa* coleoptiles, cell respiration, the enzymatic activity of proteases and α - and β -amylases, and root and coleoptile length [81]. Linalool and terpinen-4-ol have a lipophilic nature that can alter cell permeability and also change membrane properties and functions by increasing membrane fluidity and changing cell permeability [84].

However, we do not attribute the allelopathic effects exerted by the apple mint-based ecoherbicide to a single allelochemical; instead, we attribute them to the mixture of compounds present in the plant material. It has been shown that synergy exists between compounds of the same class and between compounds of different chemical natures. Pardo-Muras et al. [85] found that the synergistic interactions among VOCs and water-soluble compounds explained the herbicidal potential of *Cytisus scoparius* (L.) Link residues when applied as a soil amendment. Sarheed et al. [86] proved that the com-

plete essential oils of *M. spicata*, *M. spicata crispa*, and *M. longifolia* had more powerful phytotoxic effects than any of their isolated monoterpenes (menthone, linalool, limonene, α -pinene, and β -pinene). Moreover, a chemical analysis of volatile and phenolic compounds from different parts (leaves, stems, and whole plant) of *M. suaveolens* not only indicated a higher content of the same compound in the whole plant compared to its parts but also the presence of different compounds or in different amounts among the parts [21]. Therefore, by using the whole aerial biomass of the flowering plant, a greater variety of allelochemicals would be available, which might result in different modes of action and target sites in the recipient plant.

5. Conclusions

At this point, it is time to address the feasibility of using *M. suaveolens* biomass as allelopathic biomass for weed control purposes in new plant-based strategies for Integrated Weed Management. In our study, the apple mint-based ecoherbicide exerted an adverse effect on the germination and growth of dicotyledonous weed species, especially *A. retroflexus* and *S. nigrum*, as well as on the growth of the monocotyledonous species *D. sanguinalis*. This herbicidal effect was achieved even at lower doses (0.5%) than those used for regular green manuring, which could be translated into moderate amounts of biomass to make further fieldwork easier and cheaper. In contrast, although maize was not statistically affected by the ecoherbicide and its relative yield was higher in pots with apple mint, it is suggested to establish a safety period of about 21 days of relay planting after the incorporation of the allelopathic biomass into the soil to mitigate any possible phytotoxic side effect on the crop.

At 30 days after incorporation, the herbicidal effect was still noticeable. The release of compounds must have been carried out gradually according to the decomposition process of the plant material. Both soluble and volatile allelochemicals must be available in the soil to be embedded by seeds or affect weed seedlings' radicles, and the continuous release of compounds must take place stepwise throughout the entire period of the gradual germination of weeds. Contrary to what may happen with essential oils (with low solubility and high volatility), this strategy would cause a long-lasting phytotoxic effect, and allelochemicals would also be at the right place and time for weed control.

The biomass of *M. suaveolens* has been shown to contain a cocktail of potentially releasable phytotoxic compounds that could exert synergistic and long-lasting actions. In addition, as a green manure, apple mint improved the physicochemical properties of the soil, increasing pH—thus diminishing the availability of toxic cations such as Al^{3+} —providing organic matter, increasing CECe and exchangeable cations, and reducing evapotranspiration.

However, this is by no means the end of the story; further studies on this strategy's potential side-effects on the diversity of crops, soil microorganisms, and invertebrates; the fate and evolution of the allelochemicals released during decomposition; and assays with different soil types and field trials are required to confirm the effects of incorporating *M. suaveolens* biomass into soil for weed control purposes.

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