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Chemical Composition of *Ambrosia trifida* Essential Oil and Phytotoxic Effect on Other Plants

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This study aimed to identify the main components of an essential oil produced from leaves of *Ambrosia trifida* and to evaluate its potential allelopathic effect on seed germination and seedling growth of lettuce, watermelon, cucumber and tomato. The essential oil was obtained by hydrodistillation and characterized chemically by gas chromatography (GC) coupled with both mass spectrometry (MS) and flame ionization detector (FID). Total 69 compounds were identified, with limonene (20.7%), bornyl acetate (15.0%), borneol (14.7%) and germacrene D (11.6%) as the major components. The working solutions of the essential oil emulsified with Tween 20 and dissolved in distilled water were prepared at four concentration levels (0.01, 0.1, 0.5% and 1%, v v^{-}1). The results obtained showed that increase in essential oil concentration leads to decrease in seed germination, as well as shoot and radical length of lettuce, watermelon, cucumber and tomato. The obtained data revealed a highly significant effect ($p<0.05$) between control and 1% and 0.5% oil concentrations in all treatments. The essential oil of *A. trifida*, exhibited more powerful phytotoxic effects on lettuce, watermelon and tomato than on cucumber regarding germination and early seedling growth.

Keywords: chemical composition • essential oil • *Ambrosia trifida* • phytotoxic effects

Introduction

Biological invasions are currently receiving more attention due to their increasing ecological and economic impact, yet the mechanisms of successful plant invasions are not well understood. Invasive weeds possess allelopathic, defensive and antimicrobial chemicals, to which native organisms have not been adapted. [1] Allelopathy is an interaction phenomenon in which living or dead plants produce and release allelochemicals that exert an effect (mostly negative) on the surrounding plants and other species. [2,3] Allelochemicals produced by plant secondary metabolism are essential for the interaction of plants with the biotic part of the environment, and the ability to produce and release allelopathic compounds into the environment, or even to tolerate the presence of allelochemicals released by other plants, determines the ability of species to survive and reproduce. [4] Plant phenolics and mono- and sesquiterpenes are allelochemicals which show great chemical diversity and are involved in a number of metabolic and ecological processes. In agroecosystems, these allelochemicals may be employed for managing weeds, insects and diseases in field crops. Therefore, they are considered as important parts of biologically active ingredients from plants as a potential source of biopesticides. Allelopathic effects of some essential oils containing terpenes as their main constituents, have been investigated in various aromatic plants, weeds or crops and the phytotoxic potential for inhibiting seed germination and seedling growth of many different plants has been revealed. [5 - 8]

Giant ragweed (*Ambrosia trifida* L. syn. *A. aptera* DC., *A. integrifolia* Muhl. ex Willd) originates from North America and it has been introduced into Europe, Asia, and South America. [9, 10] According to Vrbničanin *et al*., [11], *A. trifida* has been included on the list of invasive weeds in Serbia. It exhibits a high degree of morphological and reproductive plasticity in response to encroachment by neighboring plants. [12] The allelopathy of giant ragweed, which releases allelochemicals that inhibit the growth of other plants, also plays a significant role in an environment. Plants in the genus *Ambrosia* are able to biosynthesize many types of secondary metabolites, including flavonoids, sesquiterpene lactones, phenolics, ambrosin, isabelin, and psilostachyin. [13, 14] Although only a few research studies on the phytochemistry of giant ragweed have been conducted so far, some very specific compounds, such as caroten, sesquiterpenes, thiarubrines and thiophenes, have been identified in giant ragweed. [15 - 18] These phytochemicals are biologically active against insects, microbes, and nematodes. [18, 19] Some of them are allelochemicals that inhibit the growth of other plants. [17, 20]

Chemical control is relatively poorly developed in vegetable crops as they tend to be grown in relatively small areas, hence making the use of herbicides expensive and uneconomical. Also, with the increasing significance of organic horticulture, some future research should focus on developing new bioherbicides and optimizing their use in production systems. To our best knowledge there had been no reports on phytotoxic effects of essential oils of the invasive weed species *A. trifida* on germination and seedling growth of vegetable crops. Therefore, this study focused on identifying the chemical composition and evaluating the phytotoxic effect of an essential oil isolated from leaves of giant ragweed (*Ambrosia trifida*) on seed germination and seedling growth on lettuce (*Lactuca sativa*), watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*) and tomato (*Solanum lycopersicum*).

Results and Discussion

Composition of A. trifida essential oil

The essential oil of *A. trifida* leaves was hydrodistillated to yield 0.15% (v/w, calculated on dried plant material). The oil was a yellowish liquid with a characteristic strong aromatic fragrance, and its chemical composition is presented in Table 1. The data show 69 identified compounds accounting for 98.87% (v/w) of total oil mass. The predominant components were limonene (20.7%) as monoterpene hydrocarbon, bornyl acetate (15.0%) and borneol (14.7%) as oxygenated monoterpenes and germacrene D (11.6%) as sesquiterpene hydrocarbon. These four compounds made 62% of total oil mass. As Table 1 shows, the content of all other compounds was much lower, whereby oxygenated sesquiterpenes and sesquiterpene hydrocarbons predominated (13.1 and 10.4%, respectively), followed by oxygenated monoterpenes (6.0%), monoterpene hydrocarbons (4.8%), phenylpropanoids (0.8%) and other compounds (1.7%).

There are not many literature data relating to the chemical composition of *A. trifida* essential oil. To our knowledge, only the composition of two *A. trifida* oils originating from Northeast China were available. [17, 18] Interestingly, there are some differences between those published data, as well as our present findings. Although both Chinese *A. trifida* oils contained bornyl acetate and borneol at very high percents, which is in agreement with our findings, the contents of other dominant components in all mentioned oils were different. Thus, for example, limonene as the most abundant component in our study (20.7%) was present at more than 10-fold smaller quantity (2.2%) in the essential oil of *A. trifida* collected from the Shenyang Experimental Station of Ecology in August 2005, [18] while the same compound was not detected in the oil produced from *A. trifida* leaves collected two years earlier on the same location. [17] Similarly, hexahydrofarnesyl acetate and caryophyllene oxide contents were more than 5-fold higher in both Chinese oils than in our study. The mentioned differences could probably be attributed to distinctive geo-climatic conditions existing in the respective regions.

On the other hand, comparing our findings with the composition of an essential oil of *A. artemisiifolia* collectedalso in Serbia (Belgrade district) [21] a conclusion was made that species belonging to the same genus and growing under the same geo-climatic conditions differed considerably in their composition (*Table 2*). Thus, the oil of *A. artemisiifolia* is much more abundant in hydrocarboned mono- and sesquiterpenes, while *A. trifida* oil contains significantly higher percents of oxygenated mono- and sesquiterpenes. Accordingly, the contents of borneol and bornyl acetate in *A. trifida* oil (*Table 1*) are much higher than the contents of these compounds in the oil of *A. artemisiifolia* (2.9 and 0.1%, respectively), [21] while germacrene D was present at a much higher percent in *A. artemisiifolia* oil (24.1%) than our current findings showed (11.6%). Although the content of limonene is slightly higher in *A. trifida* oil (20.7%), compared to the 16.8% reported for *A. artemisiifolia* oil, [21] all other monoterpene hydrocarbons found in *A. artemisiifolia* oil had higher contents than in *A. trifida* oil.

Table 1. Chemical composition of *Ambrosia trifida* essential oil

N°	Component	RI_{EXP}[a]	RI_{LIT}[b]	Content (%)
1	α-Pinene	932	932	1.3
2	Camphene	947	946	0.9
3	Sabinene	971	969	0.5
4	β-Pinene	975	974	0.3
5	β-Myrcene	987	988	0.7
6	α-Phellandrene	1003	1002	0.1
7	p-Cymene	1022	1020	0.2
8	Limonene	1026	1024	20.7
9	(Z)-β-Ocimene	1029	1032	0.6
10	(E)-β-Ocimene	1044	1044	0.2
11	cis-Sabinene hydrate	1064	1065	0.2
12	Linalool	1096	1095	0.1
13	Nonanal	1101	1100	0.1
14	α-Campholenal	1123	1122	0.8
15	cis-β-Terpineol	1137	1140	0.5
16	Camphor	1143	1141	2.4
17	Borneol	1164	1165	14.7
18	Terpinene-4-ol	1175	1174	0.3
19	α-Terpineol	1188	1186	0.1
20	cis-Piperitol	1194	1195	0.3
21	Verbenone	1198	1204	0.3
22	trans-Carveol	1217	1215	0.3
23	Thymol methyl ether	1229	1232	0.2
24	Carvone	1240	1239	0.3
25	Perilla aldehyde	1273	1269	0.2
26	Bornyl acetate	1283	1284	15.0
27	α-Cubebene	1343	1345	0.1
28	(2E)-Undecenol	1361	1365	0.1
29	Isoledene	1373	1374	0.2
30	β-Bourbonene	1387	1387	0.8
31	β-Caryophyllene	1418	1417	2.2
32	β-Copaene	1427	1430	0.1
33	trans-α-Bergamotene	1432	1432	0.8
34	β-Farnesene	1452	1454	1.1

35	γ-Muurolene	1473	1478	0.3
36	Germacrene D	1480	1484	11.6
37	β-Selinene	1485	1489	0.5
38	(E)-Methyl isoeugenol	1495	1491	0.8
39	β-Bisabolene	1504	1505	2.4
40	γ-Cadinene	1514	1513	0.3
41	δ-Cadinene	1521	1522	0.6
42	trans-Cadina-1,4-diene	1529	1533	0.5
43	α-Cadinene	1535	1537	0.2
44	trans-Nerolidol	1555	1561	0.3
45	Muurol-5-en-4-α-ol	1560	1559	0.1
46	Germacrene B	1563	1559	0.3
47	Longipinanol	1571	1567	0.2
48	Spathulenol	1575	1577	0.1
49	Caryophyllene oxide	1582	1582	1.7
50	Globulol	1589	1590	2.0
51	Widdrol	1600	1599	0.8
52	Tetradecanal	1611	1611	0.2
53	1,10-di-epi-Cubenol	1616	1618	0.9
54	γ-epi-Eudesmol	1625	1622	1.2
55	Muurola-4,10(14)-dien-1-β-ol	1634	1630	0.5
56	Isospathulenol	1638	1640	0.3
57	β-Cedren-9-α-ol	1644	1645	0.6
58	α-Eudesmol	1651	1652	0.5
59	Intermedeol	1660	1665	1.5
60	(6Z)-Pentadecen-2-one	1669	1667	0.2
61	β-Bisabolol	1673	1674	0.3
62	epi-α-Bisabolol	1683	1683	0.3
63	Germacra-4(15),5,10(14)-triene-1-α-ol	1687	1685	0.3
64	Germacrone	1692	1693	1.0
65	2-Pentadecanone	1697	1697	0.3
66	Pentadecanol	1773	1773	0.5
67	γ-Eudesmol acetate	1783	1783	0.2
68	Hexahydrofarnesyl acetone	1845	1843	0.3
69	Heneicosane	2112	2100	0.3
	Total			98.8

Table 2. Main components and chemical classes of compounds detected in *A. trifida* and *A. artemisiifolia* (Chalchat et al., 2004) essential oils

Chemical class / main components	Content (%)	
	A. trifida	*A. artemisiifolia*
Monoterpene hydrocarbons	**25.5**	**37.2**
α-Pinene	1.3	8.0
β-Myrcene	< 1[a]	7.4
Limonene	20.7	16.8
(E)-β-Ocimene	< 1	3.3
Oxygenated monoterpenes	**35.7**	**5.3**
Camphor	2.4	nd[b]
Borneol	14.7	2.9
Bornyl acetate	15.0	< 1
Sesquiterpene hydrocarbons	**22.0**	**33.9**
β-Caryophyllene	2.2	2.7
trans-α-Bergamotene	< 1	1.3
β-Farnesene	1.1	< 1
Germacrene D	11.6	24.1
Bicyclogermacrene	nd	2.5
β-Bisabolene	2.4	< 1
Oxygenated sesquiterpenes	**13.1**	**9.3**
Longipinanol	< 1	1.6
Spatulenol	< 1	1.6
Caryophyllene oxide	1.7	< 1
Globulol	2.0	nd
γ-epi-Eudesmol	1.2	1.3
Isospatulenol	< 1	1.5
α-Eudesmol	< 1	1.4
Intermedeol	1.5	nd
Germacrone	1.0	nd
Phenylpropanoids	**0.8**	**/**
Others	**1.7**	**0.1**
Total	98.8	85.8

[a] Compounds with content <1%; [b] not detected.

Phytotoxic effects of Ambrosia trifida essential oil on seed germination and seedling growth

Chemical composition and major components of essential oils play a significant role in their biological activity. The presence of bioactive terpenoids suggest their potential role in plant establishment and proliferation in new habitats. Monoterpenes, the main constituents of essential oils, are known for their many phytotoxic activities. Furthermore, many researchers have reported that essential oils with the greatest phytotoxic potential contained oxygenated compounds as their main constituents. [8, 22 - 25] In our study, *A. trifida* oil contained 61.2% monoterpenes, whereas 35.7% belongs to the group of oxygenated monoterpenes (*Table 2*). To our best knowledge, there are no studies on allelopathic effects of *A. trifida* essential oil on vegetable crops and this is the first such report. The results showed that different concentrations

(0.01%, 0.1%, 0.5% and 1%) of the essential oil of *A. trifida* had significant impact on the germination of cucumber, tomato, lettuce and watermelon seeds (*Figure 1*). The highest oil concentration (1%) caused 100% inhibition of tomato, lettuce and watermelon, while 91% inhibition was observed for cucumber seeds. Even the 100-fold lower concentration (0.01%) significantly reduced the germination of lettuce, tomato and cucumber seeds (26%, 33% and 51%, respectively), and still more prominently of watermelon seeds (64%). Consequently, based on the calculated ED values, the concentration of ≤0.085% was found to be sufficient to cause 50% inhibition of seed germination of the test plants (*Table 3*).

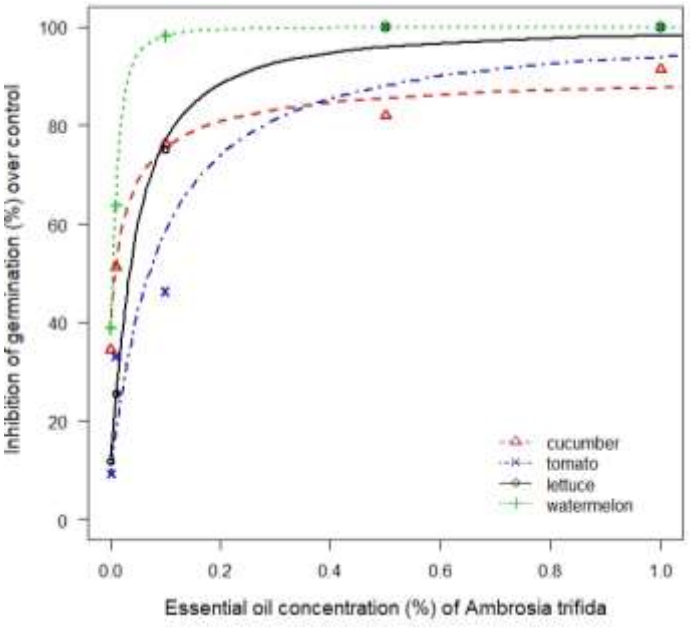

Figure 1. Inhibitory effects of different concentrations of *Ambrosia trifida* essential oil on seed germination of cucumber, tomato, lettuce and watermelon

In accordance with the results of the seed germination test, all concentrations of the essential oil showed significant inhibition of early seedling growth of cucumber, tomato, lettuce and watermelon. Inhibition at the concentrations of 0.5% and 1% was 100% for both parameters (shoot and radical length) in all plants excepting cucumber shoot length, which was 64% and 83%, respectively (*Figure 2 and 3*). At lower concentrations (0.01% and 0.1%), watermelon showed the highest sensitivity since its inhibition ranged from 45% to 100%. On the other hand, the reduction in shoot and radical length of tomato was 28-65%. Shoot and root length were found to be more sensitive parameters than seed germination, and the ED_{50} values were lower (≤ 0.012% and ≤0.015%, respectively) (*Table 3*).

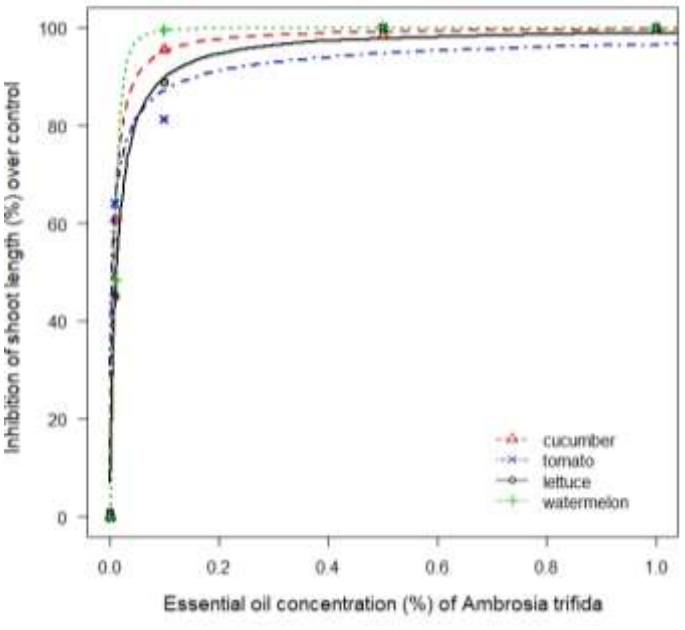

Figure 2. Inhibitory effects of different concentrations of *Ambrosia trifida* essential oil on shoot length of cucumber, tomato, lettuce and watermelon

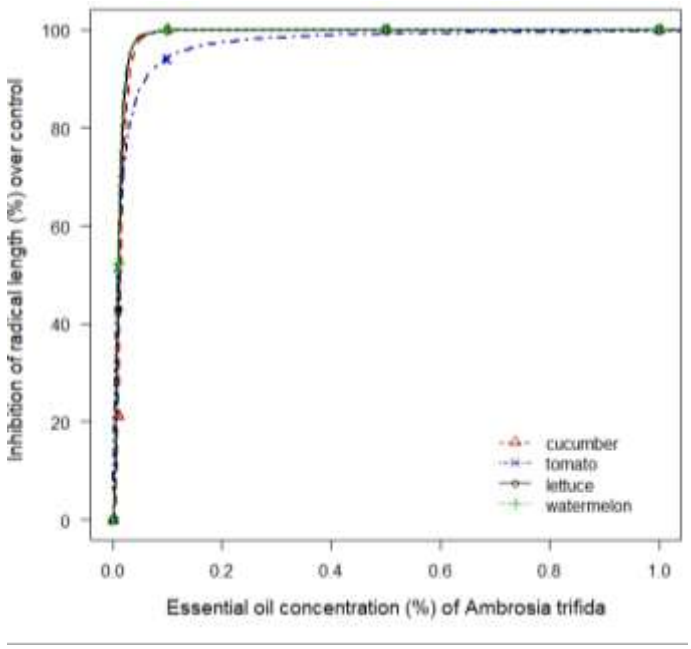

Figure 3. Inhibitory effects of different concentrations of *Ambrosia trifida* essential oil on radical length of cucumber, tomato, lettuce and watermelon

In available literature, there is a report about the volatile effect of *A. trifida* essential oil on plants. Wang et al. [17] revealed that the volatile oil of *A. trifida* significantly inhibited the seed germination and seedling growth of maize and wheat, noting, however, that the same oil significantly stimulated seed germination and seedling growth in barnyard (*Echinochloa crus-galli*). In two separated bioassays, Miranda et al. [26] evaluated the allelopathic potential of essential oils isolated from leaves and rhizomes of white ginger (*Hedychium coronarium*). They first estimated the volatile effect, and then the effect of direct contact of these oils on the germination of lettuce seeds. The results showed that volatile effects of the essential oils isolated from different parts of white ginger did not affect seedling first germination count or total germination, with respective mean percentages of 91.5 and 95% for the essential oil made from leaves, and 91 and 94% for the essential oil from rhizomes. When evaluating the effect of direct contact of essential oils on the germination and vigour of lettuce seedlings, both essential oils were seen to reduce the response of all test variables, while the rhizome oil additionally caused an even greater reduction in all variables than the leaf oil. In our tested essential oil isolated from leaves of *A. trifida* the predominant components were limonene (20.7%) as monoterpene hydrocarbon, bornyl acetate (15.0%) and borneol (14.7%) as oxigenated monoterpenes and germacrene D (11.6%) as sesquiterpene hydrocarbon. Ibrahim *et al.* [27] reported the effects of limonene on the growth and primary physiology of cabbage (*Brassica oleracea* L) and carrot (*Daucus carota* L) plants. Many species release phytotoxic monoterpenes (β-pinene, and limonene) which hinder the development of herbaceous species. [22, 28] Nishida *et al.* [29] demonstrated that monoterpenes (eucalyptol, α-pinene, camphor and camphene) inhibited mitosis through interference with DNA synthesis in meristematic cells. Areco *et al.* [30] reported that β-pinene reduced the speed of germination and seedling growth of maize. Also, in some publications[7, 31 - 33] germacrene D (9.9-69.7%), bornyl acetate (1.8-9.2%) and limonene (0.2-12.0%) have been described as the three major components of the essential oil of Canada goldenrod (*Solidago canadensis)*. Grulova et al. [7] examined the impact of essential oils of the invasive goldenrod species from five localitions on radical elongation of radish (*Raphanus sativus*) and garden cress (*Lepidium sativum*), and found that only one sample caused significant inhibition of radical elongation of garden cress (≤36% over control). The authors revealed that most of these essential oils significantly stimulated radical elongation in radish and garden cress test plants, although the composition of oils differed between localitions. Altogether, they identified 70 components, most of which were sesquiterpene hydrocarbons and monoterpene hydrocarbons. Undoubtedly, the activity of monoterpenoids seems to be selective. Whatever activity has been determined for a certain compound against a certain target species it will not necessarily be same against another target species, even of the same family or genus. It means that the indicator of essential oil phytotoxicity is in fact the test species. This is an important feature, particularly for application in agrophytocenoses of field crops, where specificity of activity is required. Therefore, further research on the impact of giant ragweed essential oil on other crops , as well as weeds, are needed in oreder to determine its potential for use as a natural-product based herbicide.

Table 3. Regression parameters (Equation 1) for each parameter (IG – inhibition of germination; ISL – inhibition of shoot length; IRL - inhibition of radical length) measured in four crops (c - cucumber, t - tomato, l - lettuce, w - watermelon) needed to obtain 30, 50 and 90% inhibition (ED_{30}, ED_{50} and ED_{90})

Parameter	crop	Regression parameters (±SE)			ED_{30} (±SE)	ED_{50} (±SE)	ED_{90} (±SE)
		B (±SE)	D (±SE)	I_{50} (±SE)			
IG	c	-0.785	91.25 (5.13)	0.029 (0.015)	0.010 (0.007)	0.029 (0.016)	0.470
	t	-1.070	100.00	0.085 (0.016)	0.038 (0.027)	0.085 (0.036)	0.662

	l	-1.239	100.00	0.043 (0.008)	0.022 (0.005)	0.043 (0.008)	0.256
	w	-1.671	100.00	0.013 (0.003)	0.008 (0.002)	0.013 (0.003)	0.047
ISL	c	-1.107	99.50 (0.29)	0.007 (0.001)	0.003 (0.002)	0.007 (0.002)	0.049
	t	-0.609	100.00	0.004 (0.002)	0.001 (0.001)	0.004 (0.001)	0.157
	l	-1.034	100.00	0.012 (0.001)	0.005 (0.001)	0.012 (0.001)	0.102
	w	-2.291	100.00	0.010 (0.003)	0.007 (0.011)	0.010 (0.003)	0.027
IRL	c	-3.204	100.00	0.015 (0.006)	0.012 (0.006)	0.015 (0.020)	0.030
	t	-1.193	100.00	0.010 (0.001)	0.005 (0.001)	0.010 (0.001)	0.060
	l	-2.824	100.00	0.011 (0.003)	0.008 (0.004)	0.011 (0.003)	0.024
	w	-2.521	100.00	0.010 (0.003)	0.007 (0.018)	0.010 (0.003)	0.023

B - line slope at inflection point; I_{50}, oil concentration resulting in 50% response between the upper and lower limits; D - upper limit

Conclusions

The results of the present study revealed that the essential oil isolated from *A. trifida* leaves is rich in biologically active compounds: monoterpene hydrocarbons, oxigenated monoterpenes and sesquiterpene hydrocarbons. The obtained data are the first report of phytotoxic effects of this essential oil on seed germination and early seedling growth (shoot and radical length) of cucumber, tomato, lettuce and watermelon. The magnitude of inhibition depended on essential oil concentrations. The highest concentrations (0.5% and 1%) exhibited powerful phytotoxic effects on test species, and the most sensitive plant was watermelon. Shoot and root length were found to be more sensitive parameters than seed germination. Further studies are needed to identify and isolate the most effective allelochemicals from this invasive plant. Knowing the invasive potential of *A. trifida*, future studies should help us clarify to what extent its allelochemicals are responsible for its successful invasion. Another line of research should focus on examining its impact on other crop and weed species as a possible natural-product based herbicide.

Experimental Section

Material and methods

Plant material

Aerial parts (leaves) of *A. trifida* were collected in Despotovo (South Backa Distict of Serbia) during August 2016. Seeds of lettuce (Semenarna, Ljubljana, Slovenia), tomato (Mondial F_1, Enza Zaden), cucumber (Jazzer F_1, Enza Zaden) and watermelon seed (Semenarna, Ljubljana, Slovenia) were used in the trial.

Isolation of essential oil

Leaves of *A. trifida* were air-dried in shade at room temperature for two weeks, and then subjected to hydrodistillation for 2.5 h using a Clevenger type apparatus. The obtained essential oil was dried over anhydrous sodium sulphate and stored at 4 ° C until analysis.

Analysis of essential oil

The chemical composition of the essential oil studied was determined using a gas chromatograph (GC) equipped with two types of detectors. Quantitative analysis was performed using an Agilent GC (7890A model) equipped with a split/splitless injector, HP-5 capillary column (30 m, 0.32 mm i.d., 0.25 μm film thickness), and flame ionization detector (FID). Injector and detector temperatures were set to 250 and 300 ^{0}C, respectively, while the nitrogen flow rate was 1 ml/min. Column temperature was raised from 50 to 250 °C at a heating rate of 4 ^{0}C/min and then isothermally held for 10 min. Qualitative analyses was performed on the Varian CP-3800 GC coupled with Saturn 2200 mass spectrometer (MS). The column type, the injector and column temperatures were the same as for the GC-FID analysis. Helium was used as the carrier gas at a flow rate of 1 ml/min, while the ion trap and transfer line temperatures were set to 250 and 280 ^{0}C, respectively. The mass detector was operated in the electron impact (EI) mode (70 eV; 40–600 m/z range). In both cases, essential oil solution in n-hexane (1%, v/v) was injected in the split mode (1:20). Essential oil components were identified by comparison of experimental Retention indices (RI) with literature data, [34] and their mass spectra with those from Wiley 7.0 mass spectral library. RI values were determined in relation to a homologous series of n-alkanes (C6-C28), analyzed by both GC-FID and GC-MS under the same operating conditions as the essential oils. Quantitative data were expressed as area percent obtained by the GC-FID analysis.

Seed bioassay

The experimental treatments consisted of two factors: (i) test plants: cucumber, tomato, lettuce, watermelon; (ii) essential oil concentration: 0.01%, 0.1%, 0.5% and 1.0% v v^{-1}. The solutions were prepared with 0.5 ml essential oil emulsified with Tween 20 at the ratio of 1:1 (v v^{-1}) and dissolved in deionized distilled water to make 1% stock solution. The other concentrations (0.01%, 0.1% and 0.5% v v^{-1}) were prepared by dilution. Deionized water supplemented with a solution of Tween 20 at 1.0% was used as the control. Test seeds were surface sterilized with 5% sodium hypochlorite (NaOCl) and distilled water for three minutes and then rinced three times with distilled water to remove microorganisms. Twenty disinfected seeds were placed into each petri dish (90 mm dia). Five ml of each solution were also added to each

dish and kept in darkness in an incubator (Binder CE) at 25±1 °C. All dishes were sealed with parafilm to avoid evaporation. After eight days, the percentage of germination was calculated and early seedling growth (shoot and radical length) were measured. The experiment design was a randomized complete block with four replications, repeated twice, and data combined for analysis.

Statistical analysis

Data about the inhibition of germination and seedling length (shoot and radical) were subjected to a non-linear regression analysis and were analyzed using the four-parameter log-logistic model Streibig, [35]:

$$Y = C + \frac{D - C}{1 + exp[B(logX - logE)]} = \frac{D - C}{1 + (X/E)^B} \quad [1]$$

where Y is the response (e.g., percent of inhibition), C is the lower limit, D is the upper limit, X is the concentration of volatile oil of *Ambrosia trifida*, E is the dose resulting in a 50% response between the upper and lower limit (also known as the inflection point, I50 or ED50) and B is the slope of the line at the inflection point (also known as a rate of change). All statistical analyses and figures were performed with the R program (R Development Core Team, 2018) utilizing the dose response curves (drc) in the statistical addition package. [36, 37]
The data were analyzed by two-factorial analysis of variance (ANOVA) using STATISTICA 8.0. software package. When F values were statistically significant ($p<0.05$), treatments were compared using Fisher's Least Significant Difference (LSD) test.

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Author Contribution Statement

Lj.R. and Lj.Š. collected the samples, M.S.-K., J.G.U., R.Đ-P. and M.R. performed the experiments and analyzed the data. All authors read and approved the final manuscript.

References

[1] R. M. Callaway, E. T. Aschehoug, 'Invasive plants versus their new and old neighbours: A mechanism for exotic invasions', *Science* **2000**, *290*, 521 - 523.
[2] A. Fitter, 'Making allelopathy respectable', *Science* **2003**, *301*, 1337 - 1338.
[3] S. Inderjit, S.O. Duke, 'Ecophysiological aspects of allelopathy', *Planta* **2003**, *217*, 529 - 639.
[4] J. Kroymann, 'Natural diversity and adaptation in plant secondary metabolism', *Curr. Opin. Plant Biol.* **2011**,*14*, 246 - 251.
[5] I. Amri, L. Hamrouni, M. Hanana, B. Jamoussi, 'Reviews on phytotoxic effects of essential oils and their individual components: news approach for weeds management', *Int.*
J. Appl. Biol. Pharmac. Technol. **2013**, *4*, 96 - 114.
[6] M. Atak, K. Mavi, I. Uremis, 'Bio-herbicidal effects of oregano and rosemar essential oils on germination and seedling growth of bread wheat cultivars and weeds', *Rom.*
Biotechnol. Lett. **2016**, *21*, 11149 - 11159.
[7] D. Grulova, B. Baranova, V. Ivanova, L. De Martino, E. Mancini, V. De Feo,' Composition and bio activity of essential oils of *Solidago* spp. and their impact on radish and garden cress', *Allelopathy J.* **2016**, *39*, 129 - 142.
[8] A. Synowiec, D. Kalemba, E. Drozdek, J. Bocianowski, 'Phytotoxic potential of essential oils from temperate climate plants against the germination of selected weeds and
crops', *J. Pest Sci.* **2017**, *90*, 407 - 419.
[9] I. J. Bassett, C. W. Crompton, 'The biology of Canadian weeds. 55. *Ambrosia trifida* L.', *Can. J. Plant Sci.* **1982**, *62*, 1002 - 1010.
[10] I. Washitani, S. Nishiyama,' Effects of seed size and seedling emergence time on the fitness componenets of *Ambrosia trifida* and *A. artemisiifolia* var. *elatior* in competition with grass perennials', *Plant Species Biol*. **1992**, *7*, 11- 19.
[11] S. Vrbničanin, B. Karadžić, Z. Dajić-Stevanović, 'Adventive and invansive weed species in Serbia', *Acta herbologica* **2004,** *31*, 1 - 12.
[12] T. W. Jurik, 'Population distributions of plant size and light environment of giant ragweed (*Ambrosia trijfida* L.) at three densities', *Oecologia* **1991**, *87*, 539 - 550.
[13] N.H. Fischer, J.D. Weidenhamer, J.M. Bradow, 'Inhibition and promotion of germination by several sesquiterpenes', *J. Chem. Ecol.* **1989**, *15*, 1785 - 1793.
[14] E. Wollenweber, K. Mann, M. Dorr, H. Fritz, J.N. Roitman, G. Yatakievych, 'Exudate flavonoids in three *Ambrosia* species', *Nat. Prod. Lett.* **1995**, *7*, 109 - 116.
[15] E. Bloszyk, U. Rychlewska, B. Szczepanska, M. Budesinsky, B. Drozdz, M. Holub, 'Sesquiterpene lactones of *Ambrosia artemisiifolia* L. and *Ambrosia trifida* L. species', *Collect. Czech. Chem. Comm.* **1992**, *57*, 1092 - 1102.
[16] T.S. Lu, F.J. Parodi, D. Vargas, L. Quuano, E.R. Mertooetomo, M.A. Hjortso, 'Sesquiterpenes and thiarubrines from *Ambrosia trifida* and its transformed roots', *Phytochemistry* **1993**, *33*, 113 - 116.
[17] P. Wang, W.J. Liang, C.H. Kong, Y. Jiang, 'Allelopathic potentials of volatile allelochemicals from *Ambrosia trifida* L. on other plants', *Allelopathy J.* **2005**, *15*, 131 - 136.

[18] P. Wang, C.H. Kong, C.X. Zhang, 'Chemical composition and antimicrobial activity of the essential oil from *Ambrosia trifida* L.', *Molecules* **2006**, *11*, 549 - 555.

[19] Y. Tamura, M. Hattori, K. Konno, Y. Kono, H. Honda, H. Ono, M. Yoshida, 'Triterpenoid and caffeic acid derivatives in the leaves of ragweed, *Ambrosia artemisiifolia* L. (Asterales: Asteraceae), as feeding stimulants of *Ophraella communa* Le Sage (Coleoptera: Chrysomelidae)', *Chemoecology* **2004**,*14*, 113 - 120.

[20] C.H. Kong, P. Wang, X.H. Xu, 'Allelopathic interference of *Ambrosia trifida* with wheat (*Triticum aestivum*)', *Agric. Ecosyst. Environ.* **2007**, *119*, 416 - 420.

[21] J. C. Chalchat, Z. A. Maksimovic, S. D. Petrovic, M. S. Gorunovic, S. Dordevic, M. Mraovic, 'Chemical Composition and Antimicrobial Activity of *Ambrosia artemisiifolia* L. Essential Oil', *J. Essent. Oil Res.* **2004**, *16*, 270 - 273.

[22] L.G. Angelini, G., Carpanese, P.L. Cioni, I. Morelli, M. Macchia, G. Flamini, 'Essential oils from Mediterranean Lamiaceae as weed germination inhibitors', *J. Agr. Food Chem.* **2003**, *51*, 6158 - 6164.

[23] D. Vokou, P. Douvli, G. J. Blionis, J. M. Halley, 'Effects of monoterpenoids, acting alone or in pairs, on seed germination and subsequent seedling growth', *J. Chem. Ecol.*
2003, *29*, 2281 - 2301.

[24] L. De Martino, E. Mancini, L. F. R. de Almeida, V. De Feo, 'The antigerminative activity of twenty-seven monoterpenes', *Molecules* **2010**, *15*, 6630 - 6637.

[25] M. Sarić-Krsmanović, J. Gajić Umiljendić, Lj. Radivojević, Lj. Šantrić, I.Potočnik, R. Đurović-Pejcev, 'Bio-herbicidal effects of five essential oils on seed germination and early
seedling growth of velvetleaf (*Abutilon theophrasti* Medik.)', *J. Environ. Sci. Health B* **2019**, *54*, 247 - 251.

[26] C. A. S. F. Miranda, M. G. Cardoso, M. L. M. Carvalho, A. C. S.Figueiredo, J. Andrade, 'Chemical characterisation and allelopathic potential of essential oils from leaves and rhizomes of white ginger', *Rev. Ciênc. Agron.* **2015**, *46*, 555 - 562.

[27] M. A. Ibrahim, E. J. Oksanen, J. K. Holopainen, 'Effects of limonene on the growth and physiology of cabbage (*Brassica oleracea* L) and carrot (*Daucus carota* L) plants', *J. Sci. Food Agric.* **2004**, *84*, 1319 - 1326.

[28] A. Pawlowski, E. Kaltchuk-Santos, M. C. Brasil, E. B. Caramao, C. A. Zini, G. L. G. Soares, 'Chemical composition of *Schinus lentiscifolius* March. essential oil and its phytotoxic and cytotoxic effects on lettuce and onion', *S. Afr. J. Bot.* **2013**, *88*, 198 - 203.

[29] N. Nishida, S. Tamotsu, N. Nagata, Ch. Saito, A. Sakai, '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', *J. Chem. Ecol.* **2005**, *31*, 1187 - 1203.

[30] V. A. Areco, S. Figuero, M. T. Cosa, J. S. Dambolena, J. A. Zygadlo, M.P. Zunino, 'Effects pinene isomers on germination and growth of maize', *Biochem. Syst. Ecol.* **2014**, *55*, 27 - 33.

[31] C. S. Chanotiya, A. Yadav, 'Natural variability in enantiomeric composition of bioactive chiral terpenoids in the essential oil of *Solidago canadensis* L. from Uttarakhand,
India', *Nat. Prod. Commun.* **2008**, *3*, 263 - 266.

[32] D. Mishra, S. Joshi, S. P. Sah, G. Bisht, 'Chemical composition, analgesic and antimicrobial activity of *Solidago canadensis* essential oil from India', *J. Pharm. Res.* **2011**, *4*, 63
- 66.

[33] M. El-Sherei, A. Khaleel, A. A. Motaal, P. Abd-Elbaki, 'Effects of seasonal variation on the composition of the essential oil of *Solidago canadensis* cultivated in Egypt', *J. Essent.*
Oil Bear. Pl. **2014**,*17*, 891 - 898.

[34] R. P. Adams, **'**Identification of Essential Oil Components by Gas Chromatography / Mass Spectrometry', Allured Publishing Corporation, Illinois, 2007.

[35] J. C. Streibig, **'**Herbicide bioassay', *Weed Res.* **1988,** *28*, 479 - 484.

[36] C. Ritz, F. Baty, J. C. Streibig, D. Gerhard, **'**Dose-Response Analysis Using R', *Plos One* **2015,** *10*, 1 - 13.

[37] S. Z. Knezevic, J. C. Streibig, C. Ritz, **'**Utilizing R software package for dose-response studies: the concept and data analysis', *Weed Technol.* **2007,** *21*, 840 - 848.

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