IECAG 2021

MDPI

Proceedings

Phytotoxic Effect of Essential Oil from Hyssop (*Hyssopus officinalis* L.) against Spring Wheat and White Mustard †

Beata Jop [1,*], Agnieszka Krajewska [2], Karolina Wawrzyńczak [2], Karolina Polaszek [1] and Agnieszka Synowiec [1]

[1] Department of Agroecology and Crop Production, The University of Agriculture in Krakow, 31-120 Kraków, Poland; karolina.polaszek@student.urk.edu.pl (K.P.); a.synowiec@urk.edu.pl (A.S.)
[2] Institute of Natural Products and Cosmetics, Lodz University of Technology, 90-924 Łódź, Poland; agnieszka.krajewska@p.lodz.pl (A.K.); karolina.wawrzynczak@dokt.p.lodz.pl (K.W.)
* Correspondence: beata.jop@urk.edu.pl
† Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://iecag2021.sciforum.net/.

Abstract: Hyssop essential oil is a rich source of biologically active compounds. This study aimed to characterize the chemical composition of essential oil from the hyssop herb and its phytotoxicity against germination and initial growth of wheat and mustard. The main compounds of the oil were isopinocamphone and pinocamphone. In a Petri dish experiment, the oil inhibited mainly germination and initial growth of wheat, whereas mustard was less affected. In conclusion, hyssop oil displays phytotoxic potential against the studied species and should be tested further.

Keywords: chemical composition**;** phytotoxicity; ED50

Citation: Jop, B.; Krajewska, A.; Wawrzyńczak, K.; Polaszek, K.; Synowiec, A. Phytotoxic Effect of Essential Oil from Hyssop (*Hyssopus officinalis* L.) against Spring Wheat and White Mustard. *Proceedings* **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Published: date

1. Introduction

Hyssop (*Hyssopus officinalis* L.) essential oil is one of the most valuable oils mentioned in the European Pharmacopeia [1]. It is rich in valuable active compounds that have antimicrobial and antioxidant activities [2]. This oil can also be used to control plant fungal diseases [3,4]. Moreover, it was showed that hyssop oil also displays herbicidal effects on the germination of some weeds and crops [5–7]. There has been a growing interest in using essential oils (EO) as natural, botanical herbicides [8] to replace the synthetic products that cause environmental pollution [9]. However, as many authors showed, the EOs also display phytotoxic effects against crops [6,10,11], and that is why studying the response of crops to EOs is justified. Hence, this study aimed to assess the phytotoxic potential of the essential oil obtained from the hyssop's herb (Oleum hyssopi officinalis) on germination and initial growth of seedlings of two crops: spring wheat (*Triticum aestivum* L.) and white mustard (*Sinapis alba* L.).

2. Materials and Methods

The essential oil was hydrodistilled from the hyssop's herb collected from central Poland. The EO was analyzed by gas chromatography coupled with mass spectrometry (GC-FID-MS), using a Trace GC Ultra gas chromatograph coupled with DSQ II mass spectrometer (Thermo Electron Corporation). The percentages of constituents were computed from the GC peak area without using a correction factor [10]. Identification of the components was based on comparing their mass spectra and linear retention indices (RI, nonpolar column) with those in [12] and computer libraries: NIST 2011 and MassFinder 4.1.

The hyssop EO was stored in a dark glass in a cool place. The biological tests were performed in three replications, two series and two seasons, 2018 and 2020. Seeds of

spring wheat cv. Harenda (breeder: MHR, PL) in 2018 or Blondynka (breeder: IHAR, ZD Grodkowice, PL) in 2020, and white mustard cv. Borowska (breeder: MHR, PL) was used. Oil in water (*o*/*w*) emulsions with the five EO doses: 0.6 g, 1.0 g, 1.4 g, 2,8 g, and 4.3 g L^{-1} were prepared. A 2.0 % aqueous solution of acetone was used as a carrier. The EO and water with acetone were weighed out (*w*/*w*). Dry and clean Petri dishes (11 cm diameter) were lined with two autoclaved filter paper pieces. Seven grams of *o*/*w* emulsion per dish was poured evenly. The emulsions contained: 0.004; 0.007; 0.01; 0.02 and 0.03 g EO per dish. The tested plants' seeds were surface-sterilized with 5% ethanol solution and abundantly rinsed with water. Next, 20 seeds of each species, separately, were placed in each dish. The dishes were put in thin polypropylene bags to reduce the EO evaporation and placed in a shadow place at a room temperature of 22 ± 3 °C. After seven days, the seedlings were counted, and their leaves/shoots and roots measured with a ruler. A dose-response test, using a 'drc' package in the R program ver. 3.5.3 [13] was performed. The ED50 value, i.e., a dose causing a 50% reduction of a plant trait, was calculated for the percentage of germination and seedlings' leaves/shoots and roots length. One-way ANOVA for a randomized design was applied to test differences between the EO concentrations, and means were separated using the Tukey test. Since the series in 2018 and 2020 were significantly different, they were analyzed separately.

3. Results and Discussion

In the analyzed hyssop EO, 57 compounds were identified by GC-MS (Table 1). The essential oil was rich in oxygenated terpene compounds. The main compounds were monoterpene ketones: isopinocamphone (42.1%) and pinocamphone (10.6%). Other important constituents were mononeterpene and sesquiterpene hydrocarbons e.g., β-pinene (8.8%), germacrene D (5.4%), bicyclogermacrene (2.7%), and (*E*)-β-caryophyllene (2.6%) and oxygenated constituents e.g., elemol (3.9%), myrtenyl methyl ether (3.6%).

The qualitative composition matched previous reports for isopinocamphone rich hyssop EO [14–18]. However, myrtenyl methyl ether was relatively rarely found [16–19]. The main constituents were within the requirements of the ISO 9841:2013 standard.

Table 1. Chemical composition of hyssop oil with the average content of main compounds [%].

Compound	RI $_{exp}$ [1]	RI $_{lit}$ [2]	[%]
α-Pinene	926	926	0.4
Camphene	948	950	0.1
Sabinene	972	973	1.6
β-Pinene	976	978	**8.8**
Myrcene	986	987	1.4
β -Phellandrene	1023	1024	2.4
Limonene	1024	1025	0.8
(*E*)-β-Ocimene	1038	1041	0.4
γ-Terpinene	1049	1051	0.1
trans-Sabinene hydrate	1051	1053	0.2
Terpinolene	1080	1082	tr [3]
α-Thujone	1087	1089	0.3
Linalool	1084	1086	0.4
β-Thujone	1099	1103	0.2
Pinocamphone	1138	1139	**10.6**
Myrtenyl methyl ether	1144	1145	**3.6**
Isopinocamphone	1153	1151	**42.1**
Terpinen-4-ol	1161	1164	0.1
Myrtenal	1170	1172	0.1
α-Terpineol	1174	1176	0.1

Myrtenol	1176	1178	0.9
Carvotanacetone	1218	1220	tr
Methyl myrtenate	1271	1275	0.1
Myrtenyl acetate	1303	1306	tr
δ-Elemene	1338	1340	1.2
Methyl eugenol	1370	1369	0.1
α-Copaene	1376	1379	0.1
α-Bourbonene	1380	1378	0.9
β-Bourbonene	1386	1386	0.3
α-Gurjunene	1409	1413	0.5
(*E*)-β-Caryophyllene	1418	1421	2.6
β-Copaene	1428	1430	0.2
Calarene	1439	1437	0.1
(*E*)-β-Farnesene	1446	1446	0.2
α-Humulene	1451	1455	0.5
Alloromadendrene	1456	1462	1.7
γ-Muurolene	1472	1474	tr
Germacrene D	1477	1479	**5.4**
Alloaromadendr-9-ene	1488	1489	0.2
Bicyclogermacrene	1491	1494	2.7
α-Muurolene	1495	1496	0.1
γ-Cadinene	1505	1507	0.2
cis-Calamenene	1512	1517	tr
δ-Cadinene	1516	1520	0.2
α-Cadinene	1531	1534	0.1
Elemol	**1538**	**1541**	**3.9**
(*E*)-Nerolidol	1549	1553	0.5
Spathulenol	1565	1572	0.4
Caryophylene oxide	1571	1578	0.3
Viridiflorol	1590	1592	tr
Ledol	1596	1600	0.3
10-*epi*-γ-Eudesmol	1607	1609	0.1
γ-Eudesmol	1616	1618	0.8
τ-Cadinol	1628	1633	0.2
β-Eudesmol	1637	1641	0.4
α-Cadinol	1640	1643	0.2
α-Eudesmol	1649	1653	0.2

[1] RI exp.—experimental retention index; [2] RI lit—standard retention index; [3] tr—trace content < 0.05%.

Table 2 presents results of 1-way ANOVA for germination and growth of wheat seedlings in the presence of hyssop EO in 2018 and 2020. Germination of wheat was visibly inhibited by hyssop EO at a dose of 0.02 g of EO per dish (equal to 2.8 g EO L^{-1}) and 0.01 g per dish (equal to 1.4 g EO L^{-1}) for cv. Harenda and cv. Blondynka, respectively. The ED50 dose for germination for each of those two cultivars was similar and equal to 0.017 and 0.016 g EO per dish. Seedlings of both wheat cultivars displayed different susceptibility to the hyssop EO, with wheat cv. Harenda being more susceptible. The growth of roots of both cultivars was more inhibited by the EO than leaves. In case of wheat cv. Harenda, a visible drop in the elongation of leaf and root was visible at doses 0.01 and 0.007 g of EO per dish, respectively. At the highest dose of hyssop EO wheat cv. Harenda did not germinate. Contrary, wheat cv. Blondynka germinate, and seedlings grew even at

the highest dose of hyssop EO; however, its growth was strongly inhibited. A visible inhibition of growth of seedlings cv. Blondynka was observed at EO dose 0.007 g per dish (equal to 1 g of EO per L^{-1}) for both leaves and roots.

Table 2. Germination and seedlings growth (mean value±standard error) of wheat cv. Harenda (an experiment in 2018) and cv. Blondynka (an experiment in 2020) in the presence of growing doses of hyssop essential oil.

Dose of EO (g per Dish)	cv. Harenda			cv. Blondynka		
	Germinated [%]	Leaf [mm]	Root [mm]	Germinated [%]	Leaf [mm]	Root [mm]
0	100 ± 0 a	62.0 ± 2.91 a	90.2 ± 5.57 a	90.0 ± 2.36 a	14.0 ± 1.42 a	45.0 ± 2.39 a
0.004	98.3 ± 1.36 a	52.8 ± 1.05 ab	66.1 ± 1.86 ab	90.0 ± 6.24 a	10.8 ± 0.67 ab	34.4 ± 1.15 ab
0.007	93.3 ± 3.6 a	49.7 ± 3.08 ab	60.8 ± 5.55 b	91.7 ± 1.36 a	7.31 ± 1.18 bc	28.9 ± 2.66 bc
0.01	93.3 ± 1.36 a	35.4 ± 10.36 b	42.3 ± 8.37 b	58.3 ± 11.9 b	4.97 ± 0.22 c	18.2 ± 1.23 cd
0.02	21.7 ± 9.53 b	3.41 ± 1.24 c	7.98 ± 2.72 c	41.7 ± 18.0 b	2.40 ± 1.02 c	11.5 ± 1.89 d
0.03	0 ± 0 c	0 ± 0 c	0 ± 0 c	11.7 ± 4.91 c	1.53 ± 0.62 c	5.40 ± 2.21 d
ED50	**0.017**	**0.011**	**0.009**	**0.016**	**0.008**	**0.009**

[1] diverse letters in the column denote a significant difference between means, according to the Tukey test at $p < 0.05$.

The germination and seedling growth of mustard was less affected by the hyssop EO than wheat (Table 3). The EO doses 0.02, and 0.03 g of EO per dish affected germination of mustard significantly in 2018 and 2019, respectively. That is why resulting ED50 doses are high, especially in 2020. As for the growth of mustard seedlings, similar values of ED50 doses point to similarities in their response to the EO in both study periods. In 2018 only the two highest doses of the EO caused a significant inhibition of mustard seedling growth. In contrast, in 2020—a significant drop in shoots and roots growth was observed already at 0.01 and 0.007 g of EO per dish, respectively.

Table 3. Germination and seedlings growth of white mustard cv. Borowska during the experiments performed in 2018 and 2020 in the presence of growing doses of hyssop essential oil.

Dose of EO (g per Dish)	2018			2020		
	Germinated [%]	Shoot [mm]	Root [mm]	Germinated [%]	Shoot [mm]	Root [mm]
0	61.7 ± 5.44 a	22.3 ± 3.56 a	12.2 ± 1.54 a	93.3 ± 2.72 a	23.7 ± 1.14 a	32.9 ± 1.81 a
0.004	60.0 ± 2.36 a	18.3 ± 3.09 a	11.6 ± 3.11 a	81.7 ± 3.60 a	21.9 ± 1.89 a	24.9 ± 2.54 a
0.007	50.0 ± 4.71 ab	22.9 ± 6.47 a	15.2 ± 6.04 a	86.7 ± 3.60 a	22.1 ± 2.02 a	23.3 ± 0.85 ab
0.01	66.7 ± 7.20 a	23.2 ± 3.49 a	21.7 ± 2.14 a	81.7 ± 5.93 a	14.3 ± 1.17 ab	15.0 ± 1.58 bc
0.02	36.7 ± 8.28 bc	8.31 ± 1.32 b	5.35 ± 0.86 b	76.7 ± 9.53 ab	10.6 ± 0.48 b	10.9 ± 2.07 c
0.03	28.3 ± 3.60 c	5.87 ± 0.77 b	3.17 ± 0.52 b	65.0 ± 4.08 b	6.72 ± 0.16 b	8.56 ± 0.82 c
ED50	**0.03**	**0.02**	**0.02**	**0.09**	**0.02**	**0.01**

[1] diverse letters in the column denote a significant difference between means, according to the Tukey test at $p < 0.05$.

Hyssop oil displays herbicidal effect against germination and initial growth of some weed species, e.g., Lepidium sativum [5], which could be correlated with a high monoterpenes content in the oil [10,20]. In another study, the hyssop oil displayed a low herbicidal effect against rapeseed germination (*Brassica napus* L.) [6]. That result is compatible with our finding that the hyssop oil is less phytotoxic against the initial growth of Sinapis alba. Perhaps, this phenomenon could be connected with a higher content of oils in the seeds of both mustard and rapeseed, as [21] suggest.

4. Conclusions

The tested hyssop oil was rich in monoterpene ketones, e.g., isopinocamphone (42.1%) and pinocamphone (10.6%). The oil inhibits wheat and mustard germination, with wheat

being more inhibited than mustard. The hyssop oil also inhibits the elongation of seedlings of both crops in a dose-response manner. A visible inhibition of wheat seedlings occurs already at a dose of oil equal to 1.0 g per L^{-1}, whereas mustard—2.8 g per L^{-1}. Further testing of the phytotoxicity of hyssop essential oil should be carried out to assess the physiological background of different wheat and mustard seedlings' susceptibility to this oil.

Author Contributions: Conceptualization, A.S., B.J. and A.K.; methodology, A.S., A.K.; investigation, B.J., A.K., K.W., K.P.; data curation, A.S., B.J., K.W., K.P.; writing—original draft preparation, B.J., K.W., K.P.; writing—review and editing, A.S., A.K.; visualization, A.S.; supervision, A.S., A.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank Weronika Ciężka and Alicja Gąsiorowska for their technical support.

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

References

1. European Pharmacopoeia Commission. *European Pharmacopoeia*, 9th ed.; (PhEur 9.0); European Directorate for the Quality of Medicines: Strasbourg, France, 2016.
2. Kizil, S.; Haşimi, N.; Tolan, V.; Kilinc, E.; Karataş, H. Chemical composition, antimicrobial and antioxidant activities of hyssop (*Hyssopus officinalis* L.) essential oil. *Not. Bot. Horti. Agrobot. Cluj-Napoca* **2010**, *38*, 99–103.
3. Letessier, M.P.; Svoboda, K.P.; Walters, D.R. Antifungal activity of the essential oil of hyssop (*Hyssopus officinalis*). *J. Phytopath*. **2001**, *149*, 673–678.
4. Fraternale, D.; Ricci, D.; Epifano, F.; Curini, M. Composition and antifungal activity of two essential oils of hyssop (*Hyssopus officinalis* L.). *J. Essent. Oil Res*. **2004**, *16*, 617–622.
5. De Almeida, L.F.R.; Frei, F.; Mancini, E.; De Martino, L.; De Feo, V. Phytotoxic activities of Mediterranean essential oils. *Molecules* **2010**, *15*, 4309–4323.
6. Hong, S.Y.; Choi, J.S.; Kim, S.M. Herbicidal activity of essential oil from Palmarosa (*Cymbopogon martini*). *Korean J. Weed Sci.* **2011**, *31*, 96–102.
7. Choi, H.J.; Sowndhararajan, K.; Cho, N.G.; Hwang, K.H.; Koo, S.J.; Kim, S. Evaluation of Herbicidal Potential of Essential Oils and their components under in vitro and greenhouse experiments. *Weed Turf. Sci*. **2015**, *4*, 321–329.
8. Benvenuti, S.; Cioni, P.L.; Flamini, G.; Pardossi, A.J.W.R. Weeds for weed control: Asteraceae essential oils as natural herbicides. *Weed Res*. **2017**, *57*, 342–353.
9. Nikolova, M.T.; Berkov, S.H. Use of Essential Oils as Natural Herbicides. *Ecol. Balk*. **2018**, *10*, 259–265.
10. Synowiec, A.; Kalemba, D.; Drozdek, E.; Bocianowski, J. Phytotoxic potential of essential oils from temperate climate plants against the germination of selected weeds and crops. *J. Pest Sci*. **2017**, *90*, 407–419.
11. Atak, M.; Mavi, K.; Uremis, I. Bio-herbicidal effects of oregano and rosemary essential oils on germination and seedling growth of bread wheat cultivars and weeds. *Roman. Biotechnol. Lett.* **2016**, *21*, 11149–11159.
12. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed.; Allured Publishing Corporation: Carol Stream, IL, USA, 2007.
13. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2020. Available online: https://www.R-project.org/ (accessed on).
14. Judžentienė, A. Hyssop (*Hyssopus officinalis* L.) oils. In *Essential Oils in Food Preservation, Flavor and Safety*; Preedy, V.R., Ed.; Academic Press: Cambridge, MA, USA, 2016; pp. 471–479.
15. Saeidi, K.; Jafari, S.; Samani, B.H.; Doodman, S. Effect of some novel and conventional drying methods on quantitative and qualitative characteristics of hyssop essential oil. *J. Essent. Oil-Bear. Plants* **2020**, *23*, 156–167.
16. Hristova, Y.; Wanner, J.; Jirovetz, L.; Stappen, I.; Iliev, I. Chemical composition and antifungal activity of essential oil of Hyssopus officinalis L. from Bulgaria against clinical isolates of Candida species. *Biotechnol. Equip*. **2015**, *29*, 592–601.
17. Moro, A.; Zalacain, A.; Hurtado de Mendoza, J.; Carmona, M. Effects of agronomic practices on volatile composition of *Hyssopus officinalis* L. essential oils. *Molecules* **2011**, *16*, 4131–4139.
18. Pandey, V.; Verma, R.S.; Chauhan, A.; Tiwari, R. Compositional variation in the leaf, flower and stem essential oils of Hyssop (*Hyssopus officinalis* L.) from Western-Himalaya. *J. Herb. Med*. **2014**, *4*, 85–89.

19. Kürkçüoğlu, M.; Eser, Ş.A.; Başer, K.H.C. Composition of the essential oil of the *Hyssopus officinalis* L. subsp. angustifolius (Bieb.) Arcangeli. *Nat. Volatiles Essent. Oils* **2016**, *3*, 15–19.
20. Abd-ElGawad, A.M.; el Gendy, A.El.G.; Assaeed, A.M.; Al-Rowaily, S.L.; Alharthi, A.S.; Mohamed, T.A.; Nassar, M.I.; Dewir, Y.H.; Elshamy, A.I. Phytotoxic Effects of Plant Essential Oils: A Systematic Review and Structure-Activity Relationship Based on Chemometric Analyses. *Plants* **2021**, *10*, 36.
21. Synowiec, A.; Krajewska, A. Soil or Vermiculite-Applied Microencapsulated Peppermint Oil Effects on White Mustard Initial Growth and Performance. *Plants* **2020**, *9*, 448.