Research Article Open Access

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# Composition and herbicidal effect of *Heracleum sosnowskyi* essential oil

DOI 10.1515/biol-2015-0044
Received April 25, 2015; accepted August 10, 2015

**Abstract:** Plants of *Heracleum sosnowskyi* Manden. are rich in essential oil which might be of herbicidal potential. In this study, we examined 1) the content and chemical composition of *H. sosnowskyi* essential oil (EO) distilled from seeds and 2) the herbicidal effect of EO in a bioassay against germination of maize and five weed species. As a result, a 5.1% EO yield was obtained from the seeds of *H. sosnowskyi*. We identified sixty-two compounds of EO that constituted 96% of the total oil. Aliphatic esters were the main constituents of the EO, followed by aliphatic alcohols. The tested seeds showed different susceptibilities to the tested concentrations of EO (0.2 – 7.2 g $L^{-1}$). The most susceptible were *Bromus secalinus* and *Avena fatua*, and the most resistant were *Echinochloa crus-galli* and maize. Kernels of maize germinated normally up to a 0.6 g $L^{-1}$ dose of EO, and 20% of the seedlings were still germinating in the presence of 7.2 g $L^{-1}$ of EO. These findings suggest that the EO of *H. sosnowskyi* and its main compounds should be studied further in soil conditions for its herbicidal properties against *A. fatua* and weeds in maize.

**Keywords:** Petri-dish bioassay, herbicidal effect, Sosnowsky hogweed, essential oil composition, germination, maize, wild oat

## 1 Introduction

*Heracleum sosnowskyi* (Manden, Apiaceae), or Sosnowsky hogweed, is one of the most dangerous invasive species in Central and Northern Europe [1-3]. One of the main reasons for its harmfulness is its phototoxic effect, which causes skin burns from direct contact with the plant in the presence of sunlight. Substances responsible for this effect are called furanocoumarins and are present in the above-ground parts of the plant, especially the leaves and stalks [4]. Moreover, a strong, competitive effect of *H. sosnowskyi* against neighboring plants has been shown [5]. This effect may be mediated by changes in the composition of soil biota, which may be due to the allelopathic interactions of *H. sosnowskyi* [6].

The allelopathic potential of *H. sosnowskyi*, which is due to the high concentration of biologically active compounds found in its tissues [7], might be a reason for the invasiveness of this species. All parts of *H. sosnowskyi* contain high amounts of phenolics [8], which are the main compounds responsible for allelopathic potential [9,10]. Baležentienė and Bartkevičius [8], in a series of bioassays, showed that phenolic compounds from the aqueous extracts of *H. sosnowskyi* inhibited the germination of rapeseed and perennial ryegrass. The tissues of *H. sosnowskyi* are also rich in other biologically active compounds, such as coumarins and esters, and have a chemical composition that is very similar to giant hogweed (*H. mantegazzianum*) [11].

*Heracleum sosnowskyi* also contains essential oil (EO) that could be an interesting source of allelopathic compounds. To date, *H. sosnowskyi* EO has not been tested for its phytotoxic properties. However, Burgieł et al. [12] tested the antifungal properties of *H. sosnowskyi* EO and found strong biological activity against *Fusarium culmorum*. Mycelium of *F. culmorum* was totally inhibited in the presence of EO [12].

Recently, different types of EOs have been intensively studied as a potential source of natural substances against pests and weeds in agriculture and horticulture [13-15]. They exhibit potential as natural herbicides, and some have been commercialized, such as clove oil and lemongrass oil [16]. In relation to these experiments, our work focused on assessing 1) the chemical composition of *H. sosnowskyi* essential oil and 2) the influence of this oil on the germination of monocot and dicot weeds and maize in a laboratory experiment.

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# 2 Experimental procedures

## 2.1 Essential oil distillation and chemical analysis

Essential oil was obtained from the seeds of the main umbels of *H. sosnowskyi*, which were harvested from several plants from a monoculture in Garlica Murowana near Krakow, Poland. Dried seeds (0.5 kg) were ground using an electric grinder and subjected to hydrodistillation using a Clevenger-type apparatus. The analysis of essential oil was performed using a gas chromatograph Trace GC Ultra coupled with a DSQII mass spectrometer (Thermo Electron) equipped with a Rtx-1 MS capillary column (60 m × 0.25 mm i.d., film thickness 0.25 µm). Simultaneous GC-FID and MS analyses were performed using a MS-FID splitter (SGE Analytical Science). The temperature was held at 50°C for 3 min, raised to 300°C at 4°C/min and held at 300°C for 30 min. The injection temperature was 280°C, and the detector (FID) temperature was 300°C. The carrier gas was helium at constant pressure (300 kPa). The mass range was 33-550 amu, ion source heating was 200°C, and ionization energy was 70 eV. The components were identified by comparing the retention indices and mass spectra with those of the NIST09, Wiley 275, and MassFinder 4.1 libraries, alongside our laboratory's own database. Retention indices (RI) of the components were determined in relation to the retention times of a series of n-alkanes with linear interpolation. Percentages were obtained from FID response without the use of correction factors.

## 2.2 Biological test

The germination of maize kernels (*Zea mays* cv. 'Wilga') and seeds of five weed species in the presence of *H. sosnowskyi* EO was tested. The tested weeds included three monocots, i.e., wild oat (*Avena fatua* L.), rye brome (*Bromus secalinus* L.) and barnyard grass (*Echinochloa crus-galli* (L.) Beauv.), and two dicots, i.e., redroot pigweed (*Amaranthus retroflexus* L.) and cornflower (*Centaurea cyanus* L.). Weed seeds were collected from local populations in arable fields near Krakow from June-August 2013.

Oil-in-water (O/W) solutions of *H. sosnowskyi* EO in distilled water containing 5% acetone were prepared. Five concentrations of EO (0.2; 0.6; 1.2; 2.4. and 7.2 g $L^{-1}$) were used. The control treatment was composed of distilled water and acetone. Two layers of filter paper were placed on the bottom of 9-cm-diameter Petri dishes. Then, 6 mL of the essential oil solution was added, and 30 seeds were immediately placed into each dish. Each dose of EO was replicated three times. Petri dishes were sealed with parafilm to protect the oil from evaporation. All Petri dishes were put randomly in the growth cabinet at a constant temperature of 18°C and left in darkness for 6 days. The number of germinated seedlings was counted, and their hypocotyl and root length were measured. Seedlings with at least a 1-mm-long hypocotyl and a visible root were recognized as germinated. The results were expressed as a percentage of germinated seeds in relation to the control treatments. Additionally, a median effective dose (ED50) was calculated, which is defined as the dose of EO required to achieve 50% inhibition of seed germination.

## 2.3 Statistical analysis

The results of the percentage of germinated seeds were analyzed using a dose-response non-linear analysis with the *drc* package [17] of the statistical software R ver. 3.1.2. The three-parameter model for fitting curves was used according to Knezevic et al. [18], where the lower limit is equal to zero:

$$Y = d / 1 + \exp [b(\log x - \log e)],$$

where e is the ED50, the upper limit is d and b denotes the relative slope around e. The above model is a very useful and widely accepted method for addressing the response of plants to increasing doses of any type of xenobiotic [17, 18].

Seedling hypocotyl and root length data were tested for equality of variances (by Levene test) and further analyzed in a one-way ANOVA using the STATISTICA ver. 10.0 software (StatSoft 2011). For statistical comparisons, means were separated by a Duncan test at a significance level $P = 0.05$.

# 3 Results

## 3.1 Chemical composition of *Heracleum sosnowskyi* essential oil

EO of *H. sosnowskyi* seeds was obtained with a yield of 5.1%. Sixty-two compounds were identified and constituted 96% of the total oil (Table 1). Aliphatic esters (82.9%) were the main constituents of the oil, followed by aliphatic alcohols (11%). Octyl acetate (39.5%), hexyl 2-methylbutanoate (14.4%), hexyl 2-methylpropanoate (5.9%), hexyl butanoate (5.4%), and octanol (8.6%)

**Table 1.** Constituents of essential oil of *Heracleum sosnowskyi* seeds.

| No. | Compound | $RI_{exp}$ | $RI_{lit}$ | % |
|---|---|---|---|---|
| | hexanol | 851 | 854 | 1.3 |
| | izopropyl 2-methylbutanoate | 873 | - | 0.1 |
| | izopropyl 3-methylbutanoate | 880 | 880 | 0.1 |
| | isobutyl 2-methylpropanoate | 901 | 901 | tr |
| | butyl 2-methylpropanoate | 937 | 936 | tr |
| | heptanol | 953 | 954 | tr |
| | sabinene | 965 | 966 | tr |
| | octanal | 980 | 981 | 0.7 |
| | isobutyl 2-methylbutanoate | 989 | 991 | 0.1 |
| | isobutyl 3-methylbutanoate | 991 | 993 | tr |
| | hexyl acetate | 994 | 997 | 1.0 |
| | 2-methylbutyl 2-methylpropanoate | 1002 | 1001 | tr |
| | p-cymene | 1011 | 1011 | 0.5 |
| | 1,8-cineole | 1019 | 1024 | tr |
| | butyl 2-methylbutanoate | 1026 | 1026 | 0.2 |
| | butyl 3-methylbutanoate | 1030 | 1031 | 0.1 |
| | isopentyl butanoate | 1043 | 1044 | tr |
| | (E)-oct-3-en-1-ol | 1044 | 1042 | 0.1 |
| | γ-terpinene | 1048 | 1047 | 0.3 |
| | **octanol** | 1055 | 1054 | **8.6** |
| | cyclooctanone | 1076 | - | tr |
| | nonanal | 1084 | 1083 | tr |
| | linalool | 1087 | 1086 | tr |
| | hexyl propanoate | 1087 | 1085 | 0.2 |
| | 2-methylbutyl 2-methylbutanoate | 1089 | 1090 | 0.1 |
| | heptyl acetate | 1095 | 1094 | tr |
| | pentyl 3-methylbutanoate | 1092 | 1093 | 0.1 |
| | **hexyl 2-methylpropanoate** | 1133 | 1132 | **6.0** |
| | hex-3-enyl butanoate | 1143 | - | tr |
| | lavandulol | 1151 | 1150 | tr |
| | p-cymen-8-ol | 1161 | 1169 | tr |
| | octanoic acid | 1164 | - | tr |
| | α-terpineol | 1172 | 1176 | tr |
| | **hexyl butanoate** | 1174 | 1176 | **5.4** |
| | oct-3-enyl acetate | 1178 | - | 0.8 |
| | decanal | 1184 | 1184 | 0.4 |
| | **octyl acetate** | 1196 | 1195 | **39.5** |
| | dec-3-en-1-ol | 1202 | - | 1.0 |
| | **hexyl 2-methylbutanoate** | 1223 | 1220 | **14.4** |
| | hexyl 3-methylbutanoate | 1225 | 1224 | 2.6 |
| | lavandulyl acetate | 1270 | 1275 | 0.2 |
| | octyl propanoate | 1284 | 1284 | 0.2 |

continued Table 1. Constituents of essential oil of *Heracleum sosnowskyi* seeds.

| No. | Compound | $RI_{exp}$ | $RI_{lit}$ | % |
|---|---|---|---|---|
| | nonyl acetate | 1293 | 1293 | tr |
| | heptyl 2-methylbutanoate | 1321 | 1317 | tr |
| | **octyl 2-methylpropanoate** | 1328 | 1329 | **2.4** |
| | hexyl hexanoate | 1367 | 1370 | 2.0 |
| | octyl butanoate | 1370 | 1372 | 1.9 |
| | dodecanal | 1389 | 1388 | tr |
| | decyl acetate | 1389 | 1389 | 0.3 |
| | **octyl 2-methylbutanoate** | 1418 | 1421 | **4.0** |
| | octyl 3-methylbutanoate | 1420 | 1420 | 0.5 |
| | butano-1,2,4-triol triacetate | 1442 | - | 0.2 |
| | octyl hexanoate | 1562 | 1567 | 0.5 |
| | tetradecanal | 1593 | 1596 | tr |
| | octyl octanoate | 1762 | - | tr |
| | etyl tetradecanoate | 1778 | 1778 | tr |
| Total identified | | | | 95.9 |
| Esters | | | | 82.9 |
| Alcohols | | | | 11.0 |

bold font – the main compounds of essential oil; tr (trace) – <0.05%; $RI_{exp}$ – experimental retention index; $RI_{lit}$ – literature retention index

predominated in the oil.

## 3.2 Herbicidal effect of essential oil of *Heracleum sosnowskyi*

The tested seeds exhibited different susceptibilities to *H. sosnowskyi* EO. Based on the ED50 value (Table 2), three groups of seeds with different germination susceptibilities to *H. sosnowskyi* EO were distinguished. The most susceptible were seeds of *Bromus secalinus* and *Amaranthus retroflexus*, where 0.63 and 0.67 g $L^{-1}$ of EO inhibited the germination of 50% of seeds of each species, respectively (Table 2). Seeds of *Avena fatua* and *Centaurea cyanus* were of moderate germination susceptibility, with ED50s of 1.26 and 1.68 g $L^{-1}$, respectively. The least susceptible were seeds of *Echinochloa crus-galli* and maize (*Zea mays*), in which the EO doses that affected ED50 were 2.37 and 2.48 g $L^{-1}$, respectively (Table 2).

Figure 1 presents the course of germination curves of the tested species. The analysis of a response of these species to a whole spectrum of doses of EO shows that in terms of germination, the most susceptible was *Bromus secalinus*. The three highest concentrations of EO (1.2 – 7.2 g $L^{-1}$) caused a total inhibition of germination of this weed (Fig. 1). The moderately susceptible species, *Avena fatua* and *Centaurea cyanus*, reacted differently to the increasing doses of *H. sosnowskyi* EO. As shown in Fig. 1, seeds of *Centaurea cyanus* germinated at the same level as did its control seeds up to a dose of 0.6 g $L^{-1}$ of EO. In contrast, seeds of *Avena fatua* germinated less as the doses of EO increased. As the third least susceptible to *H. sosnowskyi* EO, kernels of maize and seeds of *Echinochloa crus-galli* germinated normally up to 0.6 and 1.2 g $L^{-1}$, respectively. At the highest dose of *H. sosnowskyi* EO, none of the *Echinochloa crus-galli* seeds germinated, whereas approximately 20% of maize kernels were still able to germinate (Fig. 1).

**Table 2.** A median effective dose ED50 (g $L^{-1}$) for weeds and maize treated with essential oil of *Heracleum sosnowskyi*.

| Species | ED 50 dose |
|---|---|
| *Amaranthus retroflexus* | 0.67 |
| *Avena fatua* | 1.26 |
| *Bromus secalinus* | 0.63 |
| *Centaurea cyanus* | 1.68 |
| *Echinochloa crus-galli* | 2.37 |
| *Zea mays* | 2.48 |

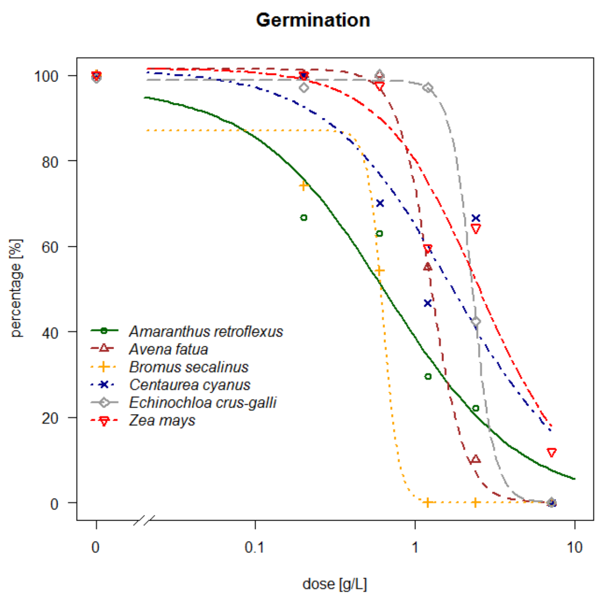


**Figure 1.** The dose-response curves for percentages of germinated seeds of weeds and kernels of maize in the presence of different doses of essential oil from *Heracleum sosnowskyi* (Manden.).

The seedlings of the tested species also expressed different susceptibilities to *H. sosnowskyi* EO (Figs. 2 and 3). C3 grasses, wild oat and rye brome showed an increasing susceptibility to increasing doses of EO, as reflected in the significant shortening of both hypocotyls and radicles. The other species' seedlings were unsusceptible to *H. sosnowskyi* EO up to the one or two highest doses at 2.4 and 7.2 g $L^{-1}$. Because the ED50 doses that reduced the germination of these seeds were well below 2.4 g $L^{-1}$, with the exception of barnyard grass, these weed species may be identified as susceptible to *H. sosnowskyi* EO. Among the tested seeds and seedlings, C4 grasses, barnyard grass, and maize in particular were tolerant to *H. sosnowskyi* EO. Maize seedlings, both their hypocotyls and radicles, were still able to grow, even at the highest dose of *H. sosnowskyi* EO (Figs. 2 and 3).

# 4 Discussion

The essential oil of *Heracleum sosnowskyi* was previously studied by Kwaśny et al. [19]. The authors obtained a 2.7% yield of oil and identified the same main compounds as in our work, though in different amounts: octyl acetate (29.5%), hexyl 2-methylbutanoate (7.4%), and octanol (16.2%). Tomaszkiewicz-Potępa and Vogt [20] found octanol and esters in the ethyl acetate extract of *H. sosnowskyi* seeds. Szumny et al. [21] found a 9.5% oil yield from *H. mantegazzianum* seeds and identified 21 constituents, with the main ones being octyl acetate (59.1%), octanol (8.8%), hexyl butanoate (7.9%), and anethole (6.6%). Jakubska-Busse et al. [11] analyzed the composition of the dichloromethane and pentane extracts of *H. sosnowskyi* and *H. mantegazzianum* seeds and did not find significant differences in their qualitative chemical compositions. Our results confirm the other authors' findings. Additionally, a similarity in the chemical composition of essential oils of *H. sosnowskyi*

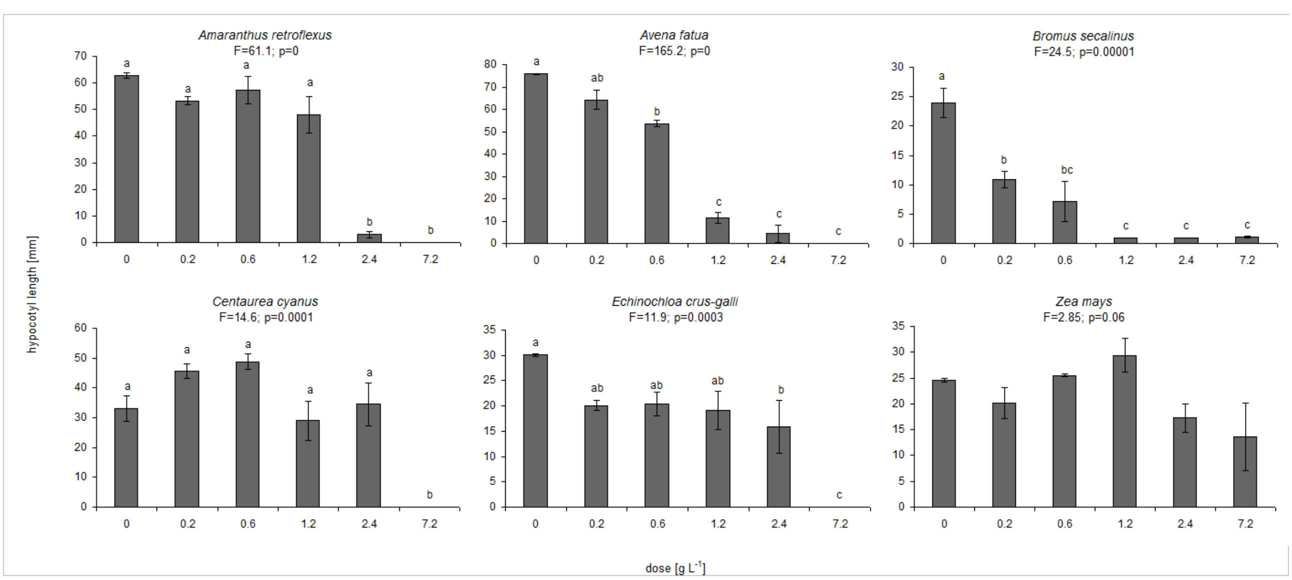


**Figure 2.** Seedlings' hypocotyl length [mm] 6 days after germination in the presence of *Heracleum sosnowskyi* essential oil at different concentrations. Bars with the same letter do not differ significantly ($p \le 0.05$) according to a Duncan test. Whiskers denote ± standard error. F – Fisher test value; p – level of significance.

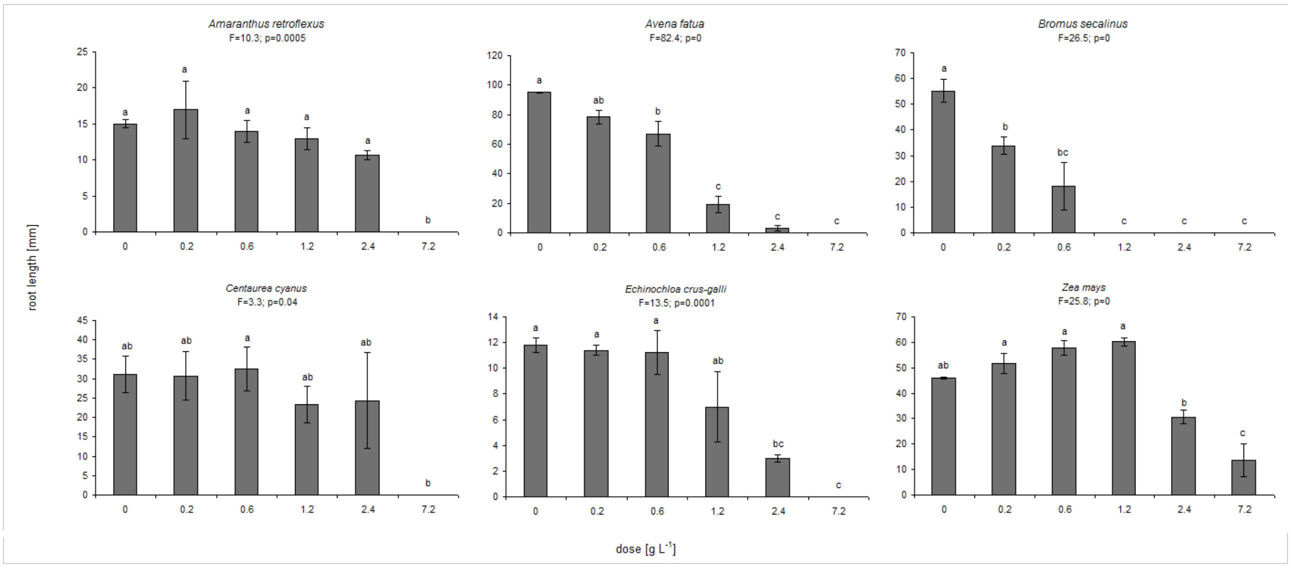

**Figure 3.** Seedlings' root length [mm] 6 days after germination in the presence of *Heracleum sosnowskyi* essential oil at different concentrations. Bars with the same letter do not differ significantly ($p \le 0.05$) according to a Duncan test. Whiskers denote ± standard error.

F – Fisher test value; p – level of significance.

and *H. mantegazzianum* proves that both species are closely related [1].

The compositions of both invasive hogweeds' EOs are unique compared with the compositions of the majority of known EOs, which generally contain monoterpenes [22,23]. Both aliphatic esters and aliphatic alcohols, which are found in the EOs of invasive hogweeds, possess a strong toxic potential [24,25]. Moreover, octyl acetate, one of the main compounds of hogweed EOs, was found in the secretions of rice bug (*Leptocorisa oratorius*). Males and females of this species produce a combination of substances that primarily contains octyl acetate but also contains octanol to produce toxic, repellent and alarm properties [26]. This proves that the substances that are present in the *H. sosnowskyi* EO have strong, biologically active potential.

To date, the available literature lacks information on the herbicidal effect of *H. sosnowskyi* EO on the seed germination of weeds and maize. The only report is by Baležentienė and Bartkevičius [8], who discussed the inhibitory effect of aqueous extracts of *H. sosnowskyi*, which contain phenolic compounds against the germination of rapeseed oil and perennial ryegrass. Our results show that *H. sosnowskyi* EO displayed herbicidal action against germination and early seedling growth in the tested species. Both C3 grasses were especially susceptible to this EO and displayed significant reductions in germination and seedling growth in the presence of increasing doses of EO. Kernels of maize and seeds of *Echinochloa crus-galli*, both of which are C4 grasses from the Panicoideae botanical subfamily [27,28], were the most tolerant to *H. sosnowskyi* EO. This phenomenon of differences in C3 and C4 grass susceptibility to *H. sosnowskyi* EO requires further examination and may be of practical importance. Wild oat (*Avena fatua*) is one of the most aggressive grass-weeds worldwide [29] and competes effectively with spring cereals and peas [30,31]. Moreover, many biotypes of this species are herbicide-resistant [32,33]. In this light, the susceptibility of wild oat to *H. sosnowskyi* EO is worth testing to determine whether this weed is also susceptible to its main compounds, also in field conditions. However, the lower susceptibility of kernels of maize to this EO also sounds promising and could be used for weed management in maize fields. Previous research has shown that maize kernels are less susceptible to different essential oils [34]. Vaughn and Spencer [35] suggested that the tolerance to essential oils may result from the seed size; the bigger the seed is, the more tolerant it is.

Nevertheless, in this experiment, the influence of *H. sosnowskyi* EO on the germination of maize and weeds was examined in the laboratory as a Petri-dish bioassay. Another experiment in natural soil conditions is necessary to confirm these results and to assess the stability and possible adsorption of bioactive compounds of *H. sosnowskyi* EO in soil.

In summary, the *Heracleum sosnowskyi* essential oil contains mostly aliphatic esters with octyl acetate as the main component. EO shows different activity against the germination of weeds and maize. The most susceptible weeds are *Bromus secalinus* and *Avena fatua*. In the case of *Bromus secalinus*, even a dose of 0.2 g $L^{-1}$ of *H. sosnowskyi* EO causes a significant reduction in germination and seedling growth. The most resistant weed, *Echinochloa crus-galli*, germinates normally up to a dose of 1.2 g $L^{-1}$. The results of the bioassay show that the germination of weeds is more inhibited than that of maize kernels. Maize kernels are tolerant to a dose up to 0.6 g $L^{-1}$, and 20% of the seeds are still able to germinate in the presence of 7.2 g $L^{-1}$ of oil. *H. sosnowskyi* essential oil might be a potential agent for a natural herbicide against weeds in maize and against *Avena fatua*. Research on the herbicidal activity of *H. sosnowskyi* essential oil should be continued in soil conditions.

**Acknowledgements:** The authors thank Ms. Dagmara Brzozowska and Magdalena Dańda for technical assistance. The study was supported by the University of Agriculture in Krakow statutory research programme No. DS 3124.

**Conflict of interest:** Authors declare nothing to disclose.

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