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ORIGINAL PAPER

Phytotoxic potential of essential oils from temperate climate plants against the germination of selected weeds and crops

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Abstract This work aimed to assess the phytotoxic potential of 12 essential oils (EOs) collected from plants growing in natural or cultivated stands in a temperate climate, i.e., *Achillea millefolium*, *Acorus calamus*, *Carum carvi*, *Chamomilla recutita*, *Foeniculum vulgare*, *Lavandula angustifolia*, *Melissa officinalis*, *Mentha* × *piperita*, *Salvia officinalis*, *Solidago canadensis*, *Tanacetum vulgare* and *Thymus vulgaris*. The germination of four weed species, i.e., *Amaranthus retroflexus*, *Avena fatua*, *Bromus secalinus* and *Centaurea cyanus*, was tested against all 12 EOs, and the germination of three crops, i.e., *Avena sativa*, *Brassica napus* and *Zea mays*, was tested in the presence of six EOs. The influence of five doses of each EO against the germination of the tested species was assessed in a petri dish experiment. The results were analyzed using dose-response non-linear analysis, the effective dose (ED50) and multivariate analysis. As a result, four groups of EOs of contrasting phytotoxicity were distinguished. The most phytotoxic group consisted of four EOs, namely *C. carvi*, *T. vulgaris*, *M.* × *piperita* and *S. officinalis*. These EOs were composed mainly of oxygenated monoterpenes in a range of 64.1–93.3 %. The least phytotoxic group consisted of *S. canadensis* EO, composed mainly of mono- and sesquiterpene hydrocarbons (92.3 %). In addition, principal component analysis indicated that the phytotoxic effect of the EOs also depended on the sensitivity of the plant species. Crops are more tolerant than weeds to the majority of EOs. Small-seeded species, namely *A. retroflexus* and *C. cyanus*, were the most sensitive to the EOs, while the kernels of *Z. mays* and the seeds of *A. fatua* were the most tolerant.

Keywords Chemical composition · ED50 · Essential oil toxicity in vitro · Germination · Oxygenated monoterpenes · Seed size

Key message

- The phytotoxic potential of essential oils of temperate climate species against weeds and crops of the same origin needs to be recognized.
- Essential oils may serve as natural herbicides for weed control.
- The essential oils of *C. carvi*, *T. vulgaris*, *M.* × *piperita* and *S. officinalis*, i.e., those rich in oxygenated monoterpenes, successfully inhibit weed germination under laboratory conditions.

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- Crops are more tolerant than weeds to the majority of essential oils.
- The larger the seed size is, the lower the susceptibility to the essential oil.
- In summary, the above-mentioned essential oils of temperate climate species may serve as a source of natural herbicides for the selective control of weeds in future.

Introduction

Among natural products, essential oils are very promising substances that exhibit a wide range of biological effects (Buchbauer 2009). During the last few decades, essential oils have also been explored and implemented in agriculture as a source of natural pesticides, mostly natural fungicides (Kumar et al. 2014) and insecticides (Isman et al. 2011; Abbad et al. 2014). Recently, attention has been paid to the phytotoxic potential of different essential oils, e.g., Amri et al. (2013) list more than 80 essential oils and in excess of 50 constituents with phytotoxic activity. Due to the complexity and structural diversity of their constituents, essential oils affect a variety of physiological and biochemical processes in treated plants (Amri et al. 2013). Most biological activities of essential oils are mediated through direct interactions with the lipid layers of biological membranes. Essential oils as herbicides disrupt both the germination process and the growth of seedlings of treated plants. In seedlings, essential oils, or their main compounds, cause oxidative damage mediated by reactive oxygen species (Amri et al. 2013; Ahuja et al. 2015). Foliar-applied essential oils cause visible leaf wilting within only a few hours of their application, as a result of membrane disruption (Poonpaiboonpipat et al. 2013), a decrease in the chlorophyll content and a decline in cellular respiration (Kaur et al. 2010).

Although their herbicidal utilization seems promising (Duke et al. 2002), only a few essential oils have been commercialized as natural herbicides, e.g., lemongrass oil (Dayan et al. 2012).

Difficulties in testing the agro-biological effects of essential oils of a particular species for pesticidal properties are associated with the variability in oil composition, which results mainly from the genetic and geographical variability of essential oil-bearing plants (Thompson et al. 2003; Belhattab et al. 2014; Mancini et al. 2014; Martínez-Natarén et al. 2014). Other difficulties are caused by the low water solubility and high volatility of essential oils, which can be corrected by the addition of organic solvents or emulsifiers (Isman 2000).

Based on bioassays, a few authors have summarized the phytotoxic potential of different essential oils or their main compounds from different geographic localities. For example, De Martino et al. (2012) reviewed their research of 33 essential oils isolated from Mediterranean aromatic plants and the activity of 27 components against the germination and seedling growth of two weeds: *Lepidium sativum* and *Raphanus sativus*. The essential oils of *Carum carvi* and *Verbena officinalis* appeared to be the most toxic. Azirak and Karaman (2008) tested ten essential oils from plants of Turkish origin against seven weeds and found that the most toxic were the essential oils of *C. carvi*, *Mentha spicata*, *Origanum onites* and *Thymbra spicata*. The main components of these oils, i.e., thymol, carvacrol and carvone, showed strong inhibition of plant germination. The same compounds plus *trans*-anethol and linalool are listed in the work conducted by Vasilakoglou et al. (2013), who tested 19 major components of essential oils against the germination of rigid ryegrass (*Lolium rigidum*). Previous research showed that phytotoxicity varied from one essential oil to another and from one treated plant to another but it was always dose-dependent.

In Poland, most of the aromatic species belong to the Apiaceae, Lamiaceae and Asteraceae botanical families. Different species from these families have been tested for phytotoxic effects in other climatic regions of the world, mostly the Mediterranean (Dudai et al. 1999; Angelini et al. 2003; De Martino et al. 2012), but there is no information on the phytotoxic potential of essential oils from oil-bearing species grown under the temperate climate of Poland against weeds and crops grown in the same climate. As other authors proved, the climatic and environmental conditions change both the amount as well as chemical composition of essential oils in plants (Mancini et al. 2014; Boz et al. 2014).

In this work, we aimed to fill this gap by evaluating the phytotoxic effect of essential oils that were distilled from plants grown in Poland against the germination of four weed and three crop species in laboratory conditions. The essential oils were selected with respect to both their availability and possible wide chemodiversity. We tested these oils against the germination of four of the most common weeds in Poland and three crops: *Avena sativa* (representing cereals), *Brassica napus* (representing industrial crops) and *Zea mays* (the most popular fodder crop in Poland). We included crops in our research because it is important that essential oils used as herbicides do not inhibit crop growth, and only in a few previous reports was the phytotoxic activity of essential oils tested against both weeds and crops, e.g., *Z. mays* (Kpoviessi et al. 2009), *Z. mays* and *Gossypium* sp. (Tursun et al. 2006), *Avena sativa* (Tzakou et al. 2011), and *Raphanus sativus*, *Capsicum annuum* and *Lactuca sativa* (Angelini et al. 2003).

Materials and methods

Characteristics of the tested essential oil-bearing plants and oil isolation method

All of the essential oils (EOs) used in the experiments were obtained by hydrodistillation from 12 of the most common essential oil-bearing species grown in a temperate climate, which were collected from different locations in Poland in June–August 2011–2013. The essential oils were classified into three groups according to their availability. The first group represents commercially available products: calamus oil (Cal), caraway oil (Car), German chamomile oil (Cha) and fennel oil (Fen). The second group comprises essential oils from plants that are a common source of essential oils and are commonly cultivated in Poland: lavender oil (Lav), lemon balm oil (Lem), peppermint oil (Min), sage oil (Sag) and thyme oil (Thy). The essential oils derived of wild plants common in Poland, i.e., yarrow oil (Yar), tansy oil (Tan) and goldenrod oil (Gol), belong to the third group. The EOs of calamus (*Acorus calamus* L.) rhizomes and caraway (*Carum carvi* L.) fruits (grown near Skibice 52°54′N 18°99′E), German chamomile (*Chamomilla recutita* L. (Rauschert)) flowers and fennel (*Foeniculum vulgare* Mill.) fruits (grown near Kruszynek, 52°56′N 18°98′E) were purchased from the herbal company HerbaNordPol (Poland), whereas the other EOs were hydrodistilled in a laboratory from cultivated herbs: flowers of lavender (*Lavandula angustifolia* Mill.) (grown near Gołcza, 50°19′N 19°55′E); herbs of lemon balm (*Melissa officinalis* L.) and peppermint (*Mentha* × *piperita* L.) (grown near Michałów, 50°29′N 20°27′E); herbs of sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) (grown near Sandomierz, 50°44′N 21°51′E); and those collected from natural habitats: herb of yarrow (*Achillea millefolium* L.) and umbels of tansy (*Tanacetum vulgare* L.) (collected near Skawina 49°58′N 19°45′E) and herb of goldenrod (*Solidago canadensis* L.) (collected near Lodz, 51°49′N 19°35′E). The herbs were harvested in the full vegetative growth or blooming stage. For the collected species, herbarium vouchers were deposited in the Department of Agrotechnology and Agricultural Ecology, University of Agriculture in Krakow, Poland. The EOs were isolated in laboratory conditions by hydrodistillation in Clevenger type apparatus for 4 h and stored in dark glass bottles at 4 ± 2 °C.

Chemical composition of the essential oils

The chemical composition of the EOs was analyzed using gas chromatography-mass spectrometry (GC–MS). After dilution in diethyl ether (10 μL in 1 ml), the EOs were analyzed using a Trace GC Ultra apparatus (Thermo Electron Corporation, Milan, Italy) with an FID and MS DSQ II detector. A simultaneous GC-FID and MS analysis was performed using an MS-FID splitter (SGE, Analytical Science). The operating conditions were as follows: apolar polydimethylsiloxane capillary column Rtx-1ms (Restek), 60 m × 0.25 mm i.d., film thickness 0.25 μm; temperature program, 50–310 °C at 4 °C/min; SSL injector temperature 280 °C; FID detector temperature 300 °C; split ratio 1:20; helium carrier gas at regular pressure 300 kPa. Mass spectra were acquired over the mass range 30–400 Da, ionization voltage 70 eV; ion source temperature 200 °C. Identification of the components was based on a comparison of their MS data and retention indices (RIs) with data stored in computer libraries NIST 98.1, Wiley 275.1 and MassFinder 4.1. The retention indices (RI, apolar column) were determined with relation to a homologous series of alkanes (C_8–C_{26}) under the same condition with linear interpolation. Percentages were obtained from the FID response without the use of correction factors.

Seed germination bioassay

Four weeds and three crops were tested in a seed germination bioassay. The tested species represent the common groups of weeds and crops in Poland. The weed species included two grasses, *Avena fatua* (AV) and *Bromus secalinus* (BR), and two forb species, *Amaranthus retroflexus* (AM) and *Centaurea cyanus* (CE). The crops included *Zea mays* (MA) cv. 'Lokata,' *Avena sativa* (OA) cv. 'Borowiak' and *Brassica napus* (RA) cv. 'Huzar.' The seeds of the weeds were collected from the arable weed flora in the south of Poland in their maturity phase in June–August 2013. The collected weeds were air-dried in a barn after which the seeds were hand cleaned. Certified seeds of crops were purchased from the breeding companies (MHR Sp z o.o., PL and HR Strzelce Sp z o.o., PL) in Spring 2013. Prior to the experiments, all of the seeds were maintained at 4 ± 2 °C.

Oil-in-water solutions of each EO were prepared using distilled water with the addition of 5 % acetone as a solvent. The control treatment contained only water and acetone. Five concentrations of each oil, i.e., 0.2, 0.6, 1.2, 2.4 and 7.2 g l^{-1} (w/w), were used. The solutions were prepared prior to the experiment. Two layers of filter paper were placed on the bottom of 11-cm-diameter glass petri dishes. Then, 6 ml of the oil solution was added and 30 seeds were immediately placed in each dish. The petri dishes were sealed with parafilm to reduce loss of moisture and essential oil to the atmosphere. All petri dishes were randomly placed in a growth cabinet at a constant temperature of 18 °C, in darkness, for 6 days. After this time, the number of germinated seedlings was counted, and their coleoptile and root lengths were measured. Seedlings with a coleoptile at

least 1 mm long and a visible root were recognized as germinated (Kolb et al. 2016). Each dose of EO was replicated three times. The whole experiment was repeated twice. The effectiveness of the EOs was presented as the ED50 value, which is the concentration of EO that causes a 50 % reduction in seed germination (Moreno et al. 2001).

Weed germination was tested in the presence of all 12 EOs. Crop germination was tested against the six EOs that showed the most variable effect in the weed germination test, i.e., caraway (Car), goldenrod (Gol), peppermint (Min), sage (Sag), tansy (Tan) and thyme oils (Thy), to test similarities/differences in the crop responses to the EOs compared with the weeds.

Statistical analysis

The results of the percentage of germinated seeds were analyzed using dose-response non-linear analysis (*drc*), with the statistical software R, ver. 3.0.2 (Ritz and Streibig 2005). Three parameters were used to fit the log-logistic curve (Y) according to Knezevic et al. (2007), where the lower limit is equal to zero:

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

where e is the ED50 value, d is the upper limit, b denotes the relative slope around e, and x is the percentage germination. The ED50 value was calculated in the *drc* package and further used to compare the phytotoxic effect of the EOs against the tested plants.

The relationships between all traits were estimated on the basis of Pearson correlation coefficients (Kozak et al. 2010) and were presented in a scatter-plot matrix. The analyzed traits included the mean values of the germination percentage, ED50, and the lengths of the seedling roots and coleoptiles. Each treatment (five concentrations of essential oils × plant species) was replicated six times because there were two series of seed germination bioassays with three replicates each.

The Mahalanobis distance (MD) has been suggested as a measure of multi-trait object (essential oils) similarity, the significance of which is verified by means of a critical value D_α, termed the least significant distance (Penny 1996). The Mahalanobis distance is a way to measure the distance that accounts for the correlation between variables: the lower the MD value is, the higher the correlation between each pair of EOs.

A graphic distribution of the essential oils, described by all traits, was obtained using an analysis of canonical varieties (Morrison 1976). Principal component analysis (PCA) of 66 objects (12 essential oils and 4 weeds, and 6 essential oils and 3 crops) was then performed on all traits. Data analysis was performed using the statistical package GenStat v. 17 (VSN Int. Ltd., UK).

Results

Chemical composition of the tested essential oils

The major constituents of 12 EOs are reported in Table 1, and the sums of the main groups of the constituents are included at the bottom of the table. The more detailed composition of EOs, based on compounds that were present in amounts higher than 0.3 % in at least one EO, is presented as Online Resource 1. The qualitative composition of all EOs was the same as previously reported, and the quantitative composition of the EOs was well within the range of percentages presented in the literature.

Caraway oil (Car) contained mainly carvone (63.2 %) and limonene (34.8 %); lavender oil (Lav) contained linalool (36.8 %) and linalyl acetate (36.0 %); lemon balm oil (Lem) contained geranial (40.2 %) and neral (28.2 %); sage oil (Sag) contained α-(27.6 %) and β-thujone (12.8 %), camphor (24.0 %), 1,8-cineole (10.3 %) and borneol (7.2 %); these compositions are very common for these oils. The main constituents of tansy oil (Tan) were β-thujone (46.8 %), α-thujone (11.2 %) and camphor (12.6 %). Thyme oil (Thy), which contained high amounts of thymol (72.9 %) and low amounts of p-cymene (7.2 %), γ-terpinene (4.5 %) and carvacrol (3.1 %), belongs to the thymol chemotype.

The main constituents of fennel oil (Fen) were fenchone (33.4 %), *trans*-anethol (39.2 %) and p-anisaldehyde (7.4 %). Peppermint oil (Min) contained the main constituents that are common for this oil, such as menthone (36.8 %), menthol (24.0 %), menthyl acetate (7.7 %) and 1,8-cineole (3.7 %) as well as an unusually high amount of piperitenone oxide (9.6 %).

Chamomile oil (Cham) was characterized by a high amount of (*E*)-β-farnesene (32.9 %) and also contained α-bisabolol oxide B (15.5 %), bisabolone oxide A (11.1 %), α-bisabolol oxide A (7.3 %) and chamazulene (3.7 %). The main constituents of calamus oil (Cal) were camphene (13.6 %), camphor (6.6 %), shyobunone isomers (5.7 and 4.4 %) and acorenone (5.5 %). Yarrow oil (Yar) contained 1,8-cineole (13.3 %), β-pinene (11.2 %), sabinene (7.6 %), borneol (4.1 %), α-terpineol (3.5 %) and terpinen-4-ol (3.0 %). Goldenrod oil (Gol) contained germacrene D (27.5 %), α-pinene (26.0 %) and limonene (11.5 %).

Phytotoxicity of the tested EOs

All of the tested EOs used in the bioassays influenced the germination of the tested seeds, but to different extents. The log-logistic germination curves for the crops and weeds are displayed in Online Resource 2 and Online Resource 3, respectively. The application of a higher dose of essential oil caused a consistent decrease in the number

Table 1 Major constituents (%) of 12 essential oils distilled from plants grown in the temperate climate

RI_{exp}	RI_{lit}	Constituent	Cal	Car	Cha	Fen	Gol	Lav	Lem	Min	Sag	Tan	Thy	Yar
933	936	α-Pinene	2.3			2.8	**26.0**		0.3	0.2	0. 7	0.3	0.1	2.8
946	950	Camphene	**13.6**			0.2	0.5		0.1		1.0	0.5	0.1	0.4
975	978	β-Pinene	0.5			0.2	2.1		0.4	0.3	0.9	0.2		**11.2**
1020	1025	1,8-Cineole	0.1					0.4	1.4	3.7	**10.3**	2.5	0.4	**13.3**
1025	1027	Limonene	0.9	**34.8**	0.2	2.1	**11.5**	0.1	0.2	0.4	0.1	0.1	0.1	0.9
1072	1069	Fenchone				**33.4**								
1090	1087	Linalool	2.4	0.1				**36.8**	1.3	0.2			2.3	0.2
1088	1089	α-Thujone								0.2	**27.6**	**6.3**		
1100	1103	β-Thujone									**12.8**	**46.8**		0.2
1117	1123	Camphor	**6.6**			0.6		0.3	0.1		**24.0**	**12.6**	0.2	**4.0**
1139	1136	Menthone							**4.7**	**36.8**				
1152	1152	Umbellulone										**11.2**		
1163	1163	Menthol							**4.1**	**24.0**				
1215	1214	Carvone	0.1	**63.2**	0.2			0.2						
1217	1215	Neral						1.1	**28.2**					
1239	1239	Linalyl acetate	0.1					**36.0**						
1247	1244	Geranial							**40.2**					
1266	1262	*trans*-Anethol				**39.2**								
1270	1267	Thymol						0.1	0.2				**72.9**	
1449	1447	(*E*)-β-Farnesene	0.4	0.1	**32.9**			0.3		0.1				
1480	1480	Germacrene D			1.9		**27.5**			0.7				2.0
1655	1654	α-Bisabolol oxide B			**15.5**									
1675	1675	Bisabolone oxide A			**11.1**									
		Monoterpene hydrocarbons	22.5	35.2	0.8	7.2	46.5	1.2	3.5	2.2	3.5	3.3	13.6	34.7
		Oxygenated monoterpenes	10.5	64.1	2.1	34.1	2.1	91.3	89.3	93.3	86.1	95.2	83.7	21.6
		Sesquiterpene hydrocarbons	26.1	0.4	42.4	0	45.8	1.4	0.5	2.1	5.3	0.1	0.4	9.4
		Oxygenated sesquiterpenes	37.1	0.1	43.0	0	5.6	1.0	3.0	0.3	4.2	0.5	0.7	30.7
		Phenylpropanoids				48.6								

Bold values represent the main compounds of essential oils

Cal, calamus oil (*Acorus calamus*); Car, caraway oil (*Carum carvi*); Cha, German chamomile oil (*Chamomilla recutita*); Fen, fennel oil (*Foeniculum vulgare*); Gol, goldenrod (*Solidago canadensis*); Lav, lavender oil (*Lavandula angustifolia*); Lem, lemon balm oil (*Melissa officinalis*); Min, peppermint oil (*Mentha* × *piperita*); Sag, sage oil (*Salvia officinalis*); Tan, tansy oil (*Tanacetum vulgare*); Thy, thyme oil (*Thymus vulgaris*); Yar, yarrow oil (*Achillea millefolium*)

RI_{exp}—experimental retention index on Rtx-1 ms column; RI_{lit}—literature retention index on apolar column

of germinating seeds. Based on the log-logistic germination curves, the doses of EOs that caused a 50 % reduction in germination (ED50) were calculated and are presented in Table 2.

Based on the ED50 values, three oils, namely caraway, thyme and peppermint, were classified as the most phytotoxic (Table 2). The ED50 values were in the range of 0.04–0.51 g l^{-1} for caraway oil, 0.09–1.15 g l^{-1} for thyme oil and 0.06–1.03 g l^{-1} for peppermint oil. The least phytotoxic was goldenrod oil, with an ED50 value in the range of 0.33–13.2 g l^{-1} (Table 2).

Analysis of the ED50 values also revealed large differences in the overall susceptibility of the tested species to the EOs. Three weeds, i.e., *B. secalinus*, *A. retroflexus* and *C. cyanus*, were the most susceptible and exhibited responses to the lowest ED50 values for the caraway, thyme and peppermint oils, i.e., in the range of 0.04–0.28 g l^{-1} (Table 2). The germination of *B. secalinus* was most strongly inhibited by the sage, thyme, lavender, peppermint, lemon balm and tansy EOs. *A. retroflexus* had the lowest germination rate in the presence of the lemon balm, caraway, sage and thyme EOs. The germination of *C. cyanus* was most strongly inhibited by the sage, caraway, fennel and peppermint EOs (Table 2). The weed that was most tolerant to the tested EOs was *A. fatua*, which was tolerant even in the presence of the three most phytotoxic EOs, with ED50 values in the range of 0.17–0.72 g l^{-1} (Table 2).

Table 2 Value of ED50 ±SE (g l^{-1}) for the tested species germinating in the presence of essential oils distilled from plants grown in the temperate climate

Essential oil	Crops			Monocotyledonous weeds		Dicotyledonous weeds	
	MA	RA	OA	AV	BR	AM	CE
Cal	n.t.	n.t.	n.t.	3.63 ± 0.10	0.49 ± 0.14	0.08 ± 0.07	2.35 ± 0.11
Car	0.51 ± 0.19	0.14 ± 0.05	0.49 ± 0.18	0.20 ± 0.03	0.17 ± 0.11	0.04 ± 0.01	0.04 ± 0.01
Cha	n.t.	n.t.	n.t.	4.04 ± 0.79	0.28 ± 0.11	0.33 ± 0.09	0.36 ± 0.26
Fen	n.t.	n.t.	n.t.	1.78 ± 0.31	0.29 ± 0.05	0.12 ± 0.03	0.04 ± 0.01
Gol	13.2 ± 6.98	5.85 ± 2.85	2.51 ± 0.30	7.27 ± 0.83	0.33 ± 0.20	3.21 ± 1.52	0.36 ± 0.12
Lav	n.t.	n.t.	n.t.	1.83 ± 0.26	0.16 ± 0.02	0.28 ± 0.05	0.35 ± 0.09
Lem	n.t.	n.t.	n.t.	2.41 ± 0.25	0.16 ± 0.02	0.002 ± 0.0004	0.21 ± 0.11
Min	1.03 ± 0.56	0.63 ± 0.15	0.53 ± 0.05	0.72 ± 0.15	0.16 ± 0.10	0.21 ± 0.02	0.06 ± 0.04
Sag	0.73 ± 0.13	0.35 ± 0.08	1.06 ± 0.09	2.30 ± 0.10	0.02 ± 0.01	0.04 ± 0.02	0.01 ± 0.01
Tan	2.21 ± 0.36	0.67 ± 0.08	1.06 ± 0.65	2.92 ± 0.59	0.16 ± 0.03	0.04 ± 0.02	0.13 ± 0.11
Thy	1.15 ± 0.25	0.34 ± 0.11	0.15 ± 0.02	0.17 ± 0.10	0.09 ± 0.14	0.16 ± 0.07	0.28 ± 0.11
Yar	n.t.	n.t.	n.t.	2.35 ± 0.25	0.17 ± 0.05	1.23 ± 0.17	0.23 ± 0.09

n.t.—not tested

MA, *Zea mays*; RA, *Brassica napus*; OA, *Avena sativa*; AV, *Avena fatua*; BR, *Bromus secalinus*; AM, *Amaranthus retroflexus*; CE, *Centaurea cyanus*

Essential oils: Cal, calamus oil (*Acorus calamus*); Car, caraway oil (*Carum carvi*); Cha, German chamomile oil (*Chamomilla recutita*); Fen, fennel oil (*Foeniculum vulgare*); Gol, goldenrod (*Solidago canadensis*); Lav, lavender oil (*Lavandula angustifolia*); Lem, lemon balm oil (*Melissa officinalis*); Min, peppermint oil (*Mentha* × *piperita*); Sag, sage oil (*Salvia officinalis*); Tan, tansy oil (*Tanacetum vulgare*); Thy, thyme oil (*Thymus vulgaris*); Yar, yarrow oil (*Achillea millefolium*)

On the other hand, the crops appeared to be more tolerant than the weeds to all six tested EOs; the *Z. mays* kernels were the least susceptible. *Z. mays* was the only species that was able to germinate, even at the highest doses of EOs, i.e., the ED50 values were 0.50 g l^{-1} for caraway oil and 13.2 g l^{-1} for goldenrod oil (Table 2; Fig. 1). Interestingly, in the case of crops, the most efficient oils were the same as those for weeds. *A. sativa* had the lowest germination rate in the presence of the caraway, peppermint and thyme oils, and *B. napus* had the lowest germination rate in the presence of the caraway, thyme and sage oils (Table 2).

The analysis of a single parameter—the ED50 value—revealed a large variation in the phytotoxicity of the tested EOs. For this reason, multivariate analysis, i.e., canonical analysis, was used, based on the biological features of the germinating seeds and seedlings.

The first two canonical variables accounted for 91.48 % of the total variability between the EOs, which is graphically presented in Fig. 1. On the graph, the coordinates of the points for particular objects are the values of the first and second canonical variables. Significant linear relationships with the first canonical variable were observed for the original variables: length of roots ($r = -0.975, p < 0.001$), ED50 ($r = -0.961$, $p < 0.001$), mean percentage of germinated seeds ($r = -0.941$, $p < 0.001$) and coleoptile length ($r = -0.808$, $p = 0.002$). Significant linear relationships with the second canonical variable were observed for coleoptile length ($r = 0.911$, $p = 0.0075$), mean percentage of germinated seeds ($r = 0.987, p < 0.001$) and the ED50 value ($r = 0.978$, $p < 0.01$). The multivariate cross-linking of biological data revealed significant differences in the phytotoxic potential of the tested EOs, and the upper right quadrant of the graph (Fig. 1) represents EOs with low values of the tested variables.

The canonical correlation analysis (Fig. 1) was strongly supported by the Mahalanobis distances between the EOs (MD, Table 3). Based on these two analyses, the 12 EOs were divided into four groups of contrasting phytotoxicity. The first group, which had the strongest phytotoxic potential, consisted of four EOs: caraway, mint, thyme and sage (the MD for each EO pair in this group ranged from 0.46 to 0.76). The second group (medium phytotoxicity) included the lemon balm, fennel and lavender EOs (MD of 0.53–0.90). The third group (low phytotoxicity, but rather homogeneous) consisted of tansy, calamus, yarrow and chamomile EOs with MDs of 0.24–0.88. Goldenrod oil was classified in the fourth group, i.e., the least phytotoxic; the MDs for this oil were consistently higher than the value of the least significant distance ($D_\alpha = 2.57$).

Chemically, three of the four most phytotoxic oils (thyme, peppermint and sage) contained monoterpene alcohols, esters and ketones as major constituents (83.7–93.3 %) (Table 1). In the most efficient EO, i.e., caraway oil, oxygenated monoterpenes constituted 64.1 %, with carvone (63.2 %) as the main constituent followed by monoterpene hydrocarbon limonene (34.8 %).

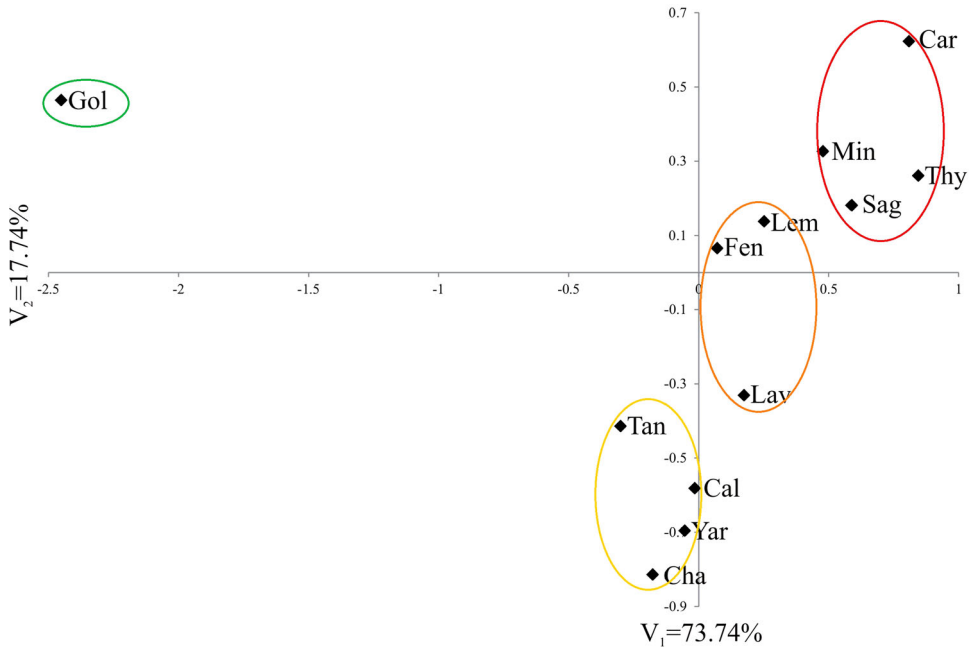

Fig. 1 Canonical correlation among the phytotoxic potentials of 12 essential oils of temperate climate origin. Essential oils localized on the *right side* of the y-axis are of higher phytotoxic potential due to their negative correlation with the measured variables—germination percentage, coleoptile and root growth, and ED50 value. Essential oils: Cal, calamus oil (*Acorus calamus*); Car, caraway oil (*Carum carvi*); Cha, German chamomile oil (*Chamomilla recutita*); Fen, fennel oil (*Foeniculum vulgare*); Gol, goldenrod (*Solidago canadensis*); Lav, lavender oil (*Lavandula angustifolia*); Lem, lemon balm oil (*Melissa officinalis*); Min, peppermint oil (*Mentha* × *piperita*); Sag, sage oil (*Salvia officinalis*); Tan, tansy oil (*Tanacetum vulgare*); Thy, thyme oil (*Thymus vulgaris*); Yar, yarrow oil (*Achillea millefolium*)

Table 3 Mahalanobis distances between all pairs of the studied essential oils

	Cal	Car	Cha	Fen	Gol	Lav	Lem	Min	Sag	Tan	Thy	Yar
Cal	0											
Car	1.54	0										
Cha	0.40	1.76	0									
Fen	1.09	1.01	1.11	0								
Gol	2.67	3.27	2.61	2.63	0							
Lav	0.63	1.15	0.66	0.53	2.76	0						
Lem	0.78	0.90	1.10	0.90	2.75	0.73	0					
Min	1.22	0.58	1.37	0.75	2.95	0.83	0.78	0				
Sag	1.11	0.76	1.33	1.04	3.08	0.91	0.74	0.40	0			
Tan	0.88	1.58	0.68	0.76	2.38	0.64	1.17	1.10	1.21	0		
Thy	1.22	0.46	1.49	1.04	3.31	0.96	0.65	0.63	0.59	1.48	0	
Yar	0.45	1.59	0.24	0.95	2.67	0.51	1.02	1.17	1.15	0.53	1.34	0

$D_{\alpha} = 2.57$

Essential oils: Cal, calamus oil (*Acorus calamus*); Car, caraway oil (*Carum carvi*); Cha, German chamomile oil (*Chamomilla recutita*); Fen, fennel oil (*Foeniculum vulgare*); Gol, goldenrod (*Solidago canadensis*); Lav, lavender oil (*Lavandula angustifolia*); Lem, lemon balm oil (*Melissa officinalis*); Min, peppermint oil (*Mentha* × *piperita*); Sag, sage oil (*Salvia officinalis*); Tan, tansy oil (*Tanacetum vulgare*); Thy, thyme oil (*Thymus vulgaris*); Yar, yarrow oil (*Achillea millefolium*)

D_{α}—the least significant distance

Two of the three medium phytotoxic EOs (lemon balm and lavender) also contained mainly oxygenated monoterpenes in the range similar to the more effective EOs (91.3 and 89.3 %, respectively). However, the main constituents belonged to different classes. The aldehydes neral and geranial (69 %) were dominant in lemon balm oil, and linalool (36.8 %) and linalyl acetate (36.0 %) were present in lavender oil. The fourth oil of this group, fennel oil,

contained only 34.1 % oxygenated monoterpenes, 48.6 % phenylpropanoids (*trans*- and *cis*-anethol, estragol and anethol epoxide) and 7.4 % p-anisaldehyde.

The main common features of the three EOs of the third group (calamus, yarrow and chamomile) were the rather high contents of oxygenated sesquiterpenes (30.7–40.3 %) and mono- and sesquiterpene hydrocarbons (43.2–48.6 %). Although the detailed composition of these three oils was totally different, the Mahalanobis distances outlined the linkages between them. Tansy oil contained high amounts of oxygenated monoterpenes (95.2 %) with the ketones α- and β-thujones (53.1 %) as the main compounds. It is worth mentioning that the composition of tansy oil is similar to the more effective sage oil (Table 1). In both of these EOs, the main components were α- and β-thujones (40.4 and 53.1 %) and camphor (24.0 and 12.6 % in sage and tansy oil, respectively), but sage oil contained more borneol, 1,8-cineole and oxygenated sesquiterpenes compared with tansy oil.

The least effective goldenrod oil was clearly distant from the other EOs, based on both MD values, which were higher than the least significant distance (D_α) and canonical correlation, all of which was in accordance with the lower level of its phytotoxicity (Fig. 1; Table 3). Chemically, this oil contained mainly mono- and sesquiterpene hydrocarbons (92.3 %) compared with the more phytotoxic EOs and consequently had a very low content of oxygenated monoterpenes (Table 1).

Analysis of the phytotoxic potential of combinations of EOs against plant species

Analysis of the ED50 values revealed considerable differences in the sensitivity of the tested species to the EOs. For this reason, a multivariate principal component analysis (PCA) was performed to highlight the response of the plants to the particular EOs. Sixty-six different combinations of EOs and species were included in the analysis, and the results are presented in Fig. 2 and Online Resource 4.

The first two variables accounted for 95.45 % of the total variability of the combinations of EOs and species (Fig. 2). Significant linear relationships with the first and second variables were observed for all traits ($p < 0.001$). The combinations of the most phytotoxic EOs and the most sensitive species are displayed in the bottom left quadrant of the graph (Fig. 2). A group composed of 11 combinations of oils and species clearly stands out in this quadrant, i.e., *B. secalinus* × thyme, caraway, sage or mint EOs; *A. retroflexus* × thyme, sage or lemon balm EOs; *C. cyanus* × sage or caraway EOs; *A. fatua* × thyme oil and *A. sativa* × thyme oil. The thyme and sage oils were dominant in this group. In the same quadrant, the EOs that were previously assigned as less phytotoxic, namely tansy, yarrow and calamus, were observed, but they were consistently combined with the most sensitive species, i.e., *C. cyanus* or *A. retroflexus*.

By contrast, goldenrod oil dominated in the group with the least phytotoxic combinations (upper right quadrant). In addition, two EOs with high and medium phytotoxicity, i.e., sage and lavender, were found in this quadrant, but each time they were combined with the least susceptible species, i.e., *A. fatua*. Combinations of *Z. mays* kernels with caraway, mint and sage EOs constituted a separate group with lower phytotoxicity (bottom right quadrant) (Fig. 2).

Discussion

The chemical composition of the common tested EOs (caraway, lavender, lemon balm, fennel, peppermint, sage, thyme) from the temperate climate of Poland was similar to that of the same species from a Mediterranean region (De Martino et al. 2012; Vasilakoglou et al. 2013; Rolli et al. 2014) and Asia (Gilani et al. 2010). However, differences in the percentages of some constituents were observed in each oil.

Based on previous work, three groups of laboratory methods used for the assessment of essential oil phytotoxicity, measured as disturbances in the process of seed germination, may be distinguished. According to the most frequently used method, an essential oil solution with water is applied to filter paper in a petri dish that contains seeds (De Martino et al. 2012; Araniti et al. 2013; Verdeguer et al. 2009). In other research, a pure essential oil is applied on the inner side of a petri dish cover, and the phytotoxicity of vapors is measured by fumigation (Dudai et al. 1999; Azirak and Karaman 2008; Kaur et al. 2010). In the third (rarely used) method, seeds are soaked in a solution of essential oil prior to germination (Angelini et al. 2003). We chose the first method, in which the action of the active agent on the tested seeds is bilateral; due to the high volatility of the essential oil constituents, an equilibrium is reached between the liquid and vapor phase of the oil during the time the seeds germinate inside the petri dish. As a result, the EO acts on the seeds and seedlings not only by direct contact, but also by a vapor phase. Essential oils are slightly soluble in water. Using a solvent (ethanol, acetone) or adjuvant (Tween), the solubility can be improved only to some extent. While the diluted solutions in our experiment were transparent, turbidity was observed at the highest (7.2 g l^{-1}) concentration. This effect should not hinder the surface contact between the oils and seeds.

Monoterpene hydrocarbons and oxygenated compounds differ significantly in their water solubility and vapor pressure. The water solubility of oxygenated compounds at

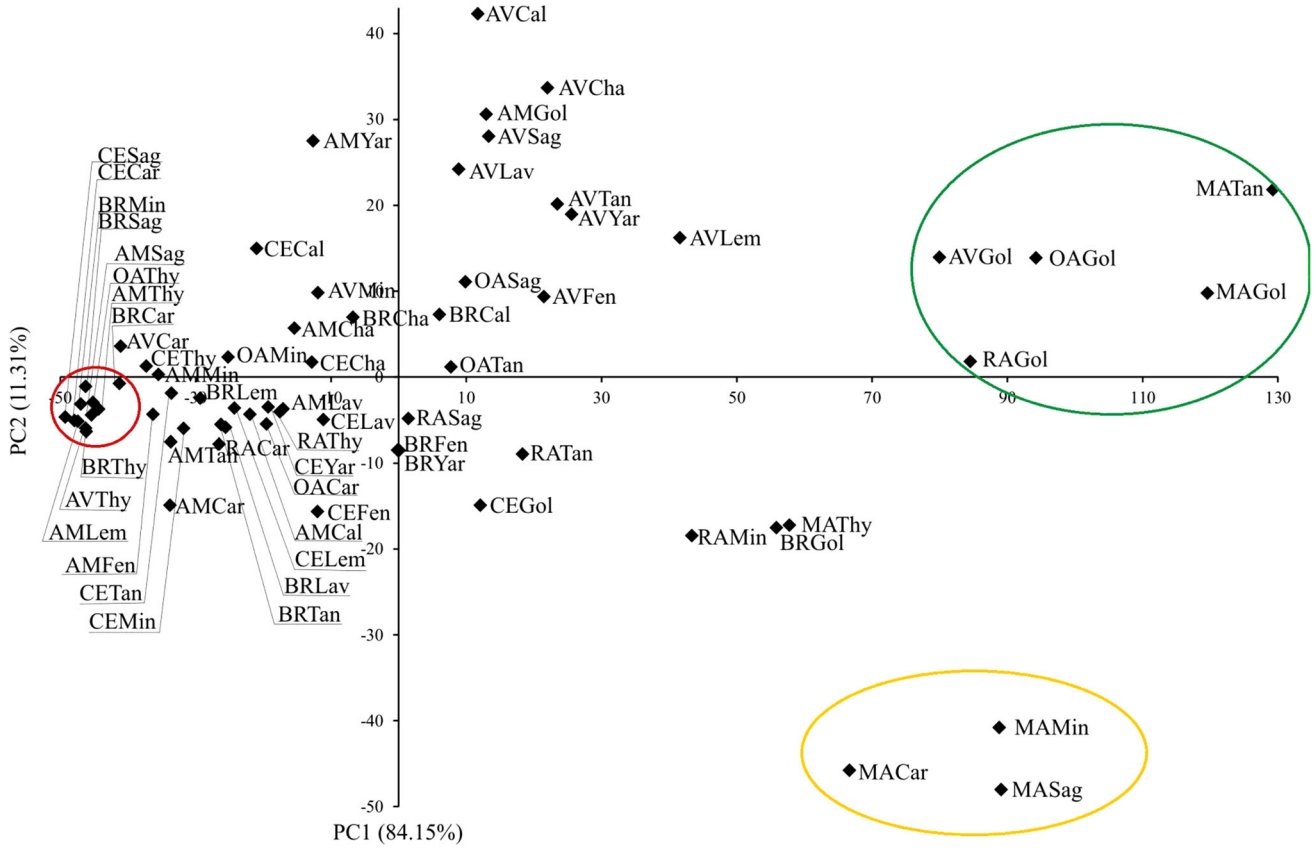

Fig. 2 Prinicipal component analysis for the 66 combinations of essential oil plus plant species. Combinations localized close to each other and on the same side of the x-axis (*bottom left quadrant*) are of higher phytotoxic potential. Combinations in the *upper* and *bottom* right quadrants of the graph are of lower phytotoxicity. MA, *Zea mays*; RA, *Brassica napus*; OA, *Avena sativa*; AV, *Avena fatua*; BR, *Bromus secalinus*; AM, *Amaranthus retroflexus*; CE, *Centaurea cyanus.* Essential oils: Cal, calamus oil (*Acorus calamus*); Car, caraway oil (*Carum carvi*); Cha, German chamomile oil (*Chamomilla recutita*); Fen, fennel oil (*Foeniculum vulgare*); Gol, goldenrod (*Solidago canadensis*); Lav, lavender oil (*Lavandula angustifolia*); Lem, lemon balm oil (*Melissa officinalis*); Min, peppermint oil (*Mentha* × *piperita*); Sag, sage oil (*Salvia officinalis*); Tan, tansy oil (*Tanacetum vulgare*); Thy, thyme oil (*Thymus vulgaris*); Yar, yarrow oil (*Achillea millefolium*)

25 °C amounts to 300–3000 mg l^{-1} and is much higher than that of hydrocarbons (5–30 mg l^{-1}). On the contrary, hydrocarbons have higher vapor pressure (100–550 Pa) than oxygenated monoterpenes (1–130 Pa) (Fichan et al. 1999). These values suggest that oxygenated monoterpenes act on seeds and the roots of seedlings by direct contact, and with respect to the action of hydrocarbons, vapor activity on the growth of seedling coleoptiles prevailed. Stolarska and Wieczorek (2015) examined the effects of the vapors of essentials oils of caraway, fennel and marjoram against the biochemical response, germination and seedling growth of caraway. They showed that the vapors of the tested EOs posed a stronger inhibiting effect against the seedlings than the seeds of caraway.

Based on the petri dish bioassays, we revealed large differences in the phytotoxicity of the essential oils extracted from plants grown under the temperate climate. The observed differences resulted from the chemical composition and the dose of the EOs as well as the sensitivity of the seeds.

We found the ED50 value to be a very useful tool in the initial assessment of the biological activity of the tested EOs. This value was successfully used by Araniti et al. (2013, 2014) and Saad et al. (2012) in the assessment of toxicity of various phytochemicals against the germination of weeds and crops. The ED50 value enabled a preliminary evaluation of the phytotoxicity of the essential oils, which was further developed by multivariate canonical analysis supported by Mahalanobis distances. On this basis, four EOs, namely caraway, thyme, peppermint and sage, were distinguished as the most phytotoxic oils against the germination of the tested species on filter paper within petri dishes, revealing ED50 values against weeds in the range of 0.01–0.28 g l^{-1}. The other three EOs, namely fennel, lemon balm and lavender, were classified as medium phytotoxic. Botanically, caraway and fennel belong to the

Apiaceae family, whereas the other five species are from the *Lamiaceae* family. Chemically, these EOs contained mostly oxygenated monoterpenes in a range of 64.1–93.3 %, except for fennel oil, which contained almost equal amounts of oxygenated monoterpenes and phenylpropanoids (34.1 and 48.6 %, respectively).

The phytotoxic effect of the tested EOs from this temperate climate was comparable to the same oils from other climatic regions, tested in similar petri dish bioassays. For example, Marichali et al. (2014) reported that caraway oil from Tunisia, which contained carvone (71.08 %) and limonene (25.42 %) as the main compounds, exhibited strong phytotoxic potential against seed germination and radicle elongation of maize, flax and wheat when 2 ml of water–methanol solution at an oil concentration of 100 µl ml^{-1} was applied to a petri dish and the germination of canary grass at a concentration of only 2 ml of 5 µl ml^{-1}. Caraway oil appeared to be the most effective among ten EOs tested in the vapor phase against the germination of seven weed seeds, whereas sage and fennel oils revealed lower activity (Azirak and Karaman 2008). Caraway and thyme oils displayed higher phytotoxic potential against the seed germination and seedling growth of *R. sativus*, *Lactuca sativa* and *L. sativum* compared with eight other essential oils from herbs of Mediterranean origin such as fennel, lemon balm, lavender and sage. The germination of the most susceptible species, *L. sativum*, was completely inhibited when 7 ml of a 2.5-µg ml^{-1} water–acetone solution of caraway or thyme oil was applied to a petri dish (De Almeida et al. 2010). Cavalieri and Caporali (2010) showed an inhibitory effect of peppermint and lavender oils, applied as water emulsions in a petri dish, against the germination of seven weeds. Lavender oil completely inhibited the germination of the most sensitive, i.e., *A. retroflexus*, at a dose of 5 ml of a 1.8-µg ml^{-1} solution. These reported doses of EOs were lower than in our research, where the germination of the most susceptible weed, *A. retroflexus*, was totally inhibited by 6 ml of a 0.6-mg ml^{-1} solution of caraway oil and a 1.2-mg ml^{-1} solution of thyme and lavender oils. Similar to our concentrations and amounts (4 ml of 1 g l^{-1} solutions) were those tested by Rolli et al. (2014), who assessed the inhibition of *Solanum lycopersicum* germination by 25 EOs in an in vitro test. They ranked peppermint oil as having medium activity (66.4 % inhibition). We have not found data on the phytotoxic activity of calamus, chamomile, goldenrod and tansy oils.

Based on the chemical composition and the major components that usually play a principal role in the biological activity of mixtures such as essential oils, the efficacy of a mixture can, to a certain extent, be predicted (Kalemba and Kunicka 2003; Stokłosa et al. 2012). In comparative research on the phytotoxic activity of 47 monoterpenes in the vapor phase (Vokou et al. 2003) and 27 monoterpenes in a petri dish contact experiment (De Martino et al. 2010), it was shown that monoterpene alcohols and ketones were the most active, followed by aldehydes, ethers, alcohols and phenols. Acetates of monoterpene alcohols and hydrocarbons were the least inhibitory. Angelini et al. (2003) and Azirak and Karaman (2008) attributed the phytotoxic activity of different essential oils to their main constituents that belonged to oxygenated monoterpenes. Oxygenated monoterpenes, mainly alcohols and ketones, have been classified by other authors as predictors of the herbicidal activities of essential oils (López et al. 2009; Verdeguer et al. 2009; Mutlu et al. 2010). These authors pointed to monoterpene as the main phytotoxic compound of essential oils. Similarly, Rolli et al. (2014) revealed that higher contents of monoterpene alcohols, aldehydes and phenylopropanoids in an essential oil may be an indicator of higher phytotoxicity.

This was true in some cases in our research. Four EOs with the greatest phytotoxic potential contained oxygenated monoterpenes as the main constituents, e.g., caraway oil contained carvone, thyme oil contained thymol, peppermint oil contained menthone and menthol, and sage oil contained α-thujone and camphor. Carvone, menthol, camphor and limonene were classified among the 10 top phytotoxic monoterpenes out of 27 studied by De Martino et al. (2010). In other research, thymol and carvone revealed significantly higher phytotoxic activity than limonene (Azirak and Karaman 2008).

In the three EOs placed in the second group (medium activity), two main compounds constituted close to or more than 70 %. These EOs also supported the above rule because their main constituents (linalool and linalyl acetate in lavender oil, citral (a mixture of geranial and neral) in lemon balm oil and *trans*-anethol and fenchone in fennel) were previously reported as being highly or moderately effective against the germination of different weed seeds (De Martino et al. 2012; Vasilakoglou et al. 2013).

The rule of the fundamental role of the major constituents was also supported by the example of goldenrod. The main compounds of this oil were the hydrocarbons α-pinene (26.0 %) and germacrene-D (27.5 %), which is in accordance with earlier findings (Weyerstahl et al. 1993). According to previous reports, terpene hydrocarbons have poor phytotoxic properties (Vokou et al. 2003; De Martino et al. 2012; Vasilakoglou et al. 2013).

On the other hand, two oils, namely sage (highly phytotoxic) and tansy (poorly phytotoxic) oils, showed that both the major and minor constituents as well as their proportions play an important role in the final phytotoxicity of an EO. Tansy oil contained similar amounts of oxygenated monoterpenes as sage oil. However, compared with tansy oil, sage oil had a higher camphor content and

also contained borneol and 1,8-cineole, which were absent in tansy oil. These minor compounds most probably determined the higher phytotoxic activity of the sage oil.

In the case of antimicrobial activities of EOs, many authors agree that although the major components are very important for their biological activity, the minor components also play a significant role because of their additive and synergistic effects, although antagonistic effects have also been observed (Burt 2004; Perricone et al. 2015). This is the same in the case of phytotoxic activity. According to Vokou (1999), in the case of complex mixtures such as essential oils, their final phytotoxic effect may result from the interactions of particular compounds. As proved by Vokou et al. (2003), monoterpenoids tested in pairs on seed germination can act independently or show both synergistic and antagonistic characteristics. Allelopathic synergism among different constituents of essential oils has also been proved in the case of fenchyl acetate or γ-bisabolene and precocene. These two compounds had no inhibitory activity on the growth of *Ageratum conyzoides* seedlings when applied separately, but when mixed with precocene II, their inhibitory activity increased (Kong et al. 1999). Carvacrol, thymol, *trans*-anethol and linalool in combination with other compounds, e.g., carvone, thujone and fenchone, provided a greater inhibitory effect on rigid ryegrass germination and showed a statistically significant synergism (Vasilakoglou et al. 2013).

In our research, as well as in the research of other authors (Verdeguer et al. 2009; Cavalieri and Caporali 2010; De Martino et al. 2012), particular EOs displayed different phytotoxicity effects against plants, which were not only dose-dependent but also species-dependent. It is worth noticing that in our experiment, the crops were more tolerant to the tested EOs than the weeds. We found that seed size may be of importance with respect to susceptibility to an EO. Small-seeded species such as *A. retroflexus* and *C. cyanus* were more susceptible. With respect to the crops, the large kernels of *Z. mays* were the most tolerant to the EOs and were able to germinate even at the highest EO dose (7.2 g l^{-1}). Similar conclusions were drawn by Vaughn and Spencer (1993), who studied the phytotoxic effect of different monoterpenes, applied in the vapor phase, against the germination of four weed species and soybean, and Tursun et al. (2006), who examined the effect of thyme oil and carvacrol against the germination of weeds and crops. This means that the next indicator of EO phytotoxicity is a tested species. As a result, it may be expected that in the agrophytocenosis of a crop field, the application of EOs will have a selective effect that is species-specific. Moreover, the phytotoxic effects of EOs under field conditions will most probably be changed by weather and soil conditions (such as soil biological activity or soil sorption capacity) and may therefore differ from those observed under petri dish conditions. Undoubtedly, laboratory experiments are only preliminary research that allow the selection of essential oils of higher phytotoxic activity for further tests under field conditions.

Conclusion

In summary, among the tested EOs extracted from plants growing or cultivated in a temperate climate, the most phytotoxic against four common weeds under laboratory conditions were caraway, thyme, peppermint and sage oil. It is worth mentioning that three tested crops were significantly more tolerant to these oils. These EOs contained mainly oxygenated monoterpenes, with one or two main compounds, i.e., carvone, limonene, thymol, menthone, menthol, α-thujone or camphor. The least phytotoxic was the essential oil of goldenrod, which contained mainly mono- and sesquiterpene hydrocarbons. Although it seems that the major EO constituents contributed predominantly to their efficacy, it was proved that the minor components play a significant role in different biological activities. We proved statistically that the phytotoxic potential of an essential oil depends not only on the dose of the essential oil, but also on the tested plant species. In our experiment, the group with the most tolerant seeds and seedlings was represented by *Zea mays* and *A. fatua*. The species that were highly susceptible to most of the essential oils included *C. cyanus* and *A. retroflexus*.

Authors contribution A.S. conceived, designed and conducted the research. E.D. and D.K. hydrodistilled the essential oils, and D.K. analyzed the chemical composition of the essential oils. J.B. and A.S. analyzed the data. A.S. and D.K. wrote the manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest A. Synowiec declares that she has no conflict of interest. D. Kalemba declares that she has no conflict of interest. E. Drozdek declares that she has no conflict of interest. J. Bocianowski declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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