Chemical composition and *in vitro* herbicidal activity of five essential oils on seeds of Johnson grass (*Sorghum halepense* [L.] Pers.)

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The evaluation of the inhibition effect achieved by essential oils of dill (*Anethum graveolens* L.), oregano (*Origanum vulgare* L.), juniper (*Juniperus communis* L.), sage (*Salvia officinalis* L.) and winter savory (*Satureja montana* L.) on seed germination and shoot growth of Johnson grass (*Sorghum halepense* L.) was tested in laboratory. The chemical composition of essential oils was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The major constituents were carvon (40.5%) and limonene (32.2%) for *A. graveolens* essential oil, carvacrol (73.7%) for *O. vulgare* essential oil, α-pinene (43.5%) for *J. communis* essential oil, β-thujone (32.7%) and camphor (17.2%) for *S. officinalis* essential oil, thymol (44.6%) and *p*-cimene (13.4%) for *S. montana* essential oil. The *in vitro* study on herbicidal activity was carried out on seed germination and shoots length of *S. halepense*. Essential oils of *A. graveolens*, *O. vulgare* and *S. montana* significantly inhibited the germination and shoot length and their herbicidal activity could be attributed mainly to their high content of carvone, carvacrol and thymol. Their essential oils reduced seed germination by 61.5%, 52.7% and 47.3%, respectively, while *J. communis* and *S. officinalis* essential oils stimulated germination (7.7% and 2.2%, respectively). The shoot growth reduction for almost all essential oils, except *J. communis* essential oil, was more than 30%. The solution of *A. graveolens*, *O. vulgare* and *S. montana* essential oils exhibited more powerful bio-herbicidal effect compared to *J. communis* and *S. officinalis* essential oils on the germination and shoot growth of *S. halepense*.

Key words: *essential oils, seed germination, shoot length, bioherbicides, Johnson grass*

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1. INTRODUCTION

Modern agriculture is associated with numerous challenges in weed management which occur due to a raising development of weed resistance to herbicides, as well as due to an increased environmental concern and pressure to reduce pesticide use. To solve this problem researchers discovered alternative protection strategies that could be applied in weed control. Among all approaches the most prominent ones are the application of essential oils (Fouad et al., 2015; Ramezani et al., 2008; Synowiec et al., 2016), plant growth promoting rhizobacteria (Bozic et al., 2014; Vrbnicanin et al., 2011), mulching (Franczuk et al., 2010; Jodaugien, 2006) and flaming (Knezevic et al., 2014; Rajković et al., 2011). The alternative weed control methods would be beneficial for both, human health and the environment (Anaya, 1999). Some of the approaches are directed in seed germination inhibition, plant growth suppression or destruction of plant tissue, while the other, like essential oil or plant growth promoting rhizobacteria application, might stimulate weed seed germination and promoting its uniform emergence, enabling their defeat in the next step, which actually facilitates the reduction of seed bank in the soil.

Johnson grass (*S. halepense*) is monocot perennial belonging to family Poaceae. It is a quite noxious perennial cosmopolitan weed, with strong underground stems (rhizomes), presenting significant competitive force in cultivated crops. Since the reproduction of the plant can be both, generative (seeds) and vegetative (rhizomes), the *S. halepense* can produce a large number of off springs in each year. Therefore, it is widespread all over the Central Europe, while in Serbia, it is the most

abundant in Vojvodina (Follak and Essl, 2013; Vrbničanin et al., 2009). Sometimes, it is the major weed causing serious yield decrease of the cultivated crop (Uludag et al., 2007) due to its strong competing capability and allelopathic properties (Stef et al., 2013), but also because of hosting many crop pests. However, as its abundance generally threatens the development of cultivated crops and its resistance to several known herbicides already has been confirmed (Heap, 2018), it is evident why *S. halepense* attracts huge research interest in discovering alternative herbicides that will keep it under control. As a part of the weed control strategy, natural products, particularly the essential oils, appear to be in the focus (Balah, 2013; Isman, 2000; Nishida et al., 2005; Ramezani et al., 2008). In favor of its herbicidal application Isman (2000) stated that essential oils do not persist in soil nor contaminate ground water, and they could cause little or no mammalian toxicity. Numerous essential oils or their individual constituents have already been proved to have a delay/inhibit germination property and that they could impair the growth of various weeds, though they sometimes also impact cultivated crop (Angelini et al., 2003; Synowiec et al., 2016; Đorđević et al., 2013). However, a fundamental understanding of interactions between the seed germination of weeds and essential oils is required for its possible implication a weed management system. Several studies have already reported potential of essential oils for this purpose that is quite promising (Campiglia et al., 2007; Fouad et al., 2015; Ramezani et al., 2008; Đorđević et al., 2013). The objectives of this study were to examine *in vitro* potential of five essential oils on seed germination and seedlings growth of weed species *S. halepense*.

2. MATERIALS AND METHODS

2.1. Weed seeds source

The mature seeds of the weed species *S. halepense* were collected in Belgrade, locality Nova Galenika (44°51'29"N, 20°22'09"E). Prior to the experiment, the seeds were stored in laboratory conditions, in the dark place, at room temperature.

2.2. Gas chromatography and Mass spectroscopy

2.2.1. Essential oils source

Five commercial essential oils were used in this experiment. Essential oil from the above-ground part of *Satureja montana* L. was purchased from "Elmar d.o.o.", Trebinje, Bosnia and Herzegovina, the essential oil from the aboveground part of *Salvia officinalis* L. from "MP Ljekobilje", Trebinje, Bosnia and Herzegovina, while the essential oil from the aboveground part of *Origanum vulgare* L. was purchased from „Jacob Hooy", Limmen, Holland. The essential oil from berries of *Juniperus commmunis* L. was purchased from „Prirodno bilje d.o.o.", Banja Luka, Bosnia and Herzegovina, while the essential oil from seeds of *Anethum graveolens* L. from Herbal Pharmacy of the Institute for Medicinal Plants Research „Dr Josif Pančić" Belgrade, Serbia.

2.2.2. GC-FID analysis

The gas chromatography-flame ionization detector (GC-FID) analysis was performed on GC Agilent Technologies 7890A apparatus, equipped with the split-splitless injector and automatic liquid sampler, attached to HP-5 column (30×0.32 mm, film thickness 0.25 µm) and fitted to FID. Operating conditions were as follows: carrier gas was H_2 (1 mL/min/210 °C); temperatures of injector and detector were set at 250 and 280 °C, respectively, while the column temperature was linearly programmed 40–260 °C at 4 °C/min. Solutions of essential oils' samples in ethanol (ca. 1%) were consecutively injected by automatic liquid sampler (1 µL, split-mode). The percentile presence of components in essential oils' samples were calculated from the peak areas obtained in the area percent reports (obtained as a result of standard processing of chromatograms) without correction factors, using normalization method.

2.2.3. GC/MS analysis

The GC/MS analysis was performed on HP G 1800C Series II GCD analytical system equipped with HP-5MS column (30 m×0.25 mm, film thickness 0.25 µm). Carrier gas was He (1 mL/min). Other chromatographic conditions were as those for GC-FID. Transfer line was heated at 260 °C. Mass spectra were recorded in EI mode (70 eV), in a range of *m/z* 40–450. Solutions of essential oil samples in ethanol (ca. 1%) were consecutively injected by automatic liquid sampler (0.2 µL, split mode). The identification of essential oils' components was based on the matching of their mass spectra peaks with those from Wiley 275 and NIST/NBS libraries. The experimental values for Kovats' retention indices (RI) were determined by using calibrated AMDIS v. 2.1. software, compared to those from available literature (Adams, 2007), and they were used as an additional tool to support MS findings.

2.3. *In vitro* testing of essential oils herbicidal activity

2.3.1. Preparing testing and control solutions

For the essential oil solution, the 500 /*micro*L of an essential oil and 500 µL of 70% ethanol were dissolved in 100 mL of distilled water and a drop of detergent was added to improve essential oil solubility. The control solution was prepared with 500 /*micro*L EtOH dissolved in 100 mL of distilled water.

2.3.2. The seeds germination experiment

Thirty *S. halepense* seeds per treatment were selected for testing the efficacy of essential oils in preventing germination. The seeds were randomly placed in 9 cm diameter Petri dishes lined with a single layer of filter paper. Each Petri dish was treated with 5 ml of the essential oil solution, while the seeds in control treatment were treated just with 5 ml of control solution. The Petri dishes were wrapped with parafilm and left for seven days in an incubator (Binder CE), in the dark, at a constant temperature of 25 °C. Following this period, the germinated seeds were counted and the germination percentages were calculated. The seed was considered as "germinated" at the radicle appearance. Consequently, the germination reduction (%) was calculated from the obtained germination values relative to control.

2.3.3. The shoot length experiment

Thirty seeds of *S. halepense* were randomly scattered on filter paper in ten Petri dishes (9 cm in diameter), filled with 5 ml distilled water and left to germinate in the incubator, in the dark, at a constant temperature of 25 °C. After a one-week period, emerged seeds were transferred to another 15 Petri dishes with filter paper and prepared for transferring germinating seeds (the seeds with just emerged radicle), and filled with 5 ml of essential oils' solutions, while the seeds in control treatment were treated with 5 ml control solution. The Petri dishes were wrapped with parafilm and left for seven days in an incubator, in the dark, at a constant temperature of 25 °C, following this period, the *S. halepense* shoot length was measured. The reduction of shoots length (%) was calculated from the obtained shoots length values relative to control.

2.4. Statistical analysis

Statistical analysis of results obtained from experiment was performed estimating the statistical significance of differing treatments mean values against control treatment using Student's t-test at levels $P<0.01$ and $P<0.05$. All tests were done using statistical software package STATISTICA 5.0.

3. RESULTS AND DISCUSSION

3.1. The chemical composition of essential oils

A comparative presentation of the chemical composition of five essential oils used in this experiment is presented in Table 1. The least contribution of presented constituents to the essential oil was >0.5%, while the 'tr' stands for its presence in the essential oil but bellow this limit.

The analysis of the oils used in our experiment confirmed that *S. montana* essential oil is a thymol chemotype. Its chemical composition is in accordance to other reports (Damjanovic-Vratnica et al., 2011; Nikolić et al., 2014). Abundance in monoterpenoids (89.65%) with oxygenated monoterpenes being its major subclass (62.9%). Thymol, whose high content is characteristic for pre-flowering *S. montana* essential oil (Miladi et al., 2013), together with *p*-cymene and carvacrol contributed to total *S. montana* essential oil content with 64%. The abundance in monoterpenes is also confirmed in other essential oils used in this experiment, *S. officinalis* (96.06%), *O. vulgare* (98.83%), *J. communis* (86.93%) and *A. graveolens* (97.97%) essential oils. With regard to the ratio between the two monoterpenoid subclasses, monoterpene hydrocarbons and oxygenated monoterpenes, the most similar to *S. montana* essential oil was *S. officinalis* essentail oil (Table 1). *O. vulgare* essential oil was the richest in oxygenated monoterpenes (84.83%), *J. communis* essential oil in monoterpene hydrocarbons (85.81%), while *A. graveolens* essential oil has almost equal content of both monoterpenoid subclasses. According to Perry et al. (1999), *S. officinalis* essential oil could be classified as a *β*–thujone chemotype with high total thujone content (40.4%). The other major compounds present in this essential oil were camphor, 1,8-cineol and *α*–pinene, which together with the thujones account for 76.3% of the total *S. officinalis* essential oil. The most dominant constituents in *O. vulgare* essential oil were oxygenated monoterpenes, carvacrol, and thymol, which together with monoterpene hydrocarbons, *γ*–terpinene and *trans*-caryophyllene, account for 84.10% of the total essential oil content. Due to its high carvacrol content, as well as to the content of other characteristic constituents, our essential oil sample most probably originates from *O. vulgare* spp. *hirtum* (Padulosi, 1997), and it could be classified as carvacrol chemotype. *J. communis* essential oil belongs to *α*–pinene chemotype; its chemical composition is in agreement with compositions reported in other studies (Butkienė et al., 2015; Sela et al., 2011). Apart from *α*–pinene other major constituents of *J. communis* essential oil were sabinene, myrcene, and limonene, and they all belong to monoterpene hydrocarbons; together they account for 77.8% of the total essential oil. In comparison to other essential oils in this study, the *J. communis* essential oil prove to be the most different; it is the richest in monoterpene and sesquiterpene hydrocarbons and the poorest in oxygenated monoterpenes. The chemical composition of *A. graveolens* essential oil in this study was in consistence to other studies (Jirovetz et al., 2003), where carvon and limonene were the major constituents, and together with *α*–phellandrene they accounted for 86.8% of the total essential oil content.

3.2. Herbicidal activity of essential oils

Knowledge about the effect of some natural and environment-friendly materials would be very helpful for understanding the potential of alternative method to suppress weed. Recent studies are frequently interested in the biologically active compounds isolated from medicinal plant species for a weed management (Fouad et al., 2015; Ramezani et al., 2008; Synowiec et al., 2016). A crucial step in the life cycle of the most plant species is seed germination. Several environmental factors, such as temperature, light, pH and soil moisture, are known to affect it (Chauhan et al., 2006; Nandula et al., 2006). Chemical components present in the soil may also affect seed germination and initial seedling establishment (Ishii-Iwamoto et al., 2012).

3.2.1. The seed germination

Results of germination of *S. halepense* following the application of essential oils are presented in Figure 1.

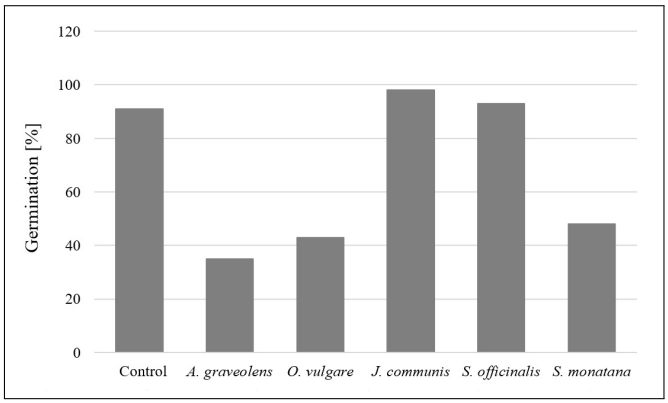

Fig. 1. *In vitro* efficacy of essential oils on *S. halepense* seed germination

In general, some of the essential oils (*A. graveolens*, *O. vulgare*, *S. montana*) had a herbicidal impact on germination, while the others (*J. communis* and *S. officinalis* essential oils) was stimulating. *A. graveolens*, *O. vulgare*, *S. montana* essential oils significantly (P<0.05, Figure 1) reduced germination by 61.5%, 52.7% and 47.3%, respectively. *J. communis* essential oil had very significant (P=0.003776) stimulative effect on germination in comparison to control, while the stimulative effect of *S. officinalis* essential oil was not statistically significant. It is well known that inhibition of germination by essential oils increases together with an increase of essential oil concentration (Barton et al., 2010; Hanana et al., 2017; Uremis et al., 2009). However, herbicidal activity is well recognized as toxicity-dependent, on dosage and exposure time, particularly in the case of chemical compounds. In our study, dosage and exposure time of essential oils were the constant values, and the emphasis was placed on essential oil chemical constituents and their combined reactions, weather synergistic or antagonistic, on seed germination (Vokou et al., 2003). Several studies conducted on weed seeds germination reported various "synergies" composed of different essential oil compounds all of them being effective to a certain extent (Angelini et al., 2003; Atak et al., 2016; Azirak and Karaman, 2008; Campiglia et al., 2007; Romagni et al., 2000). Study of the chemical composition of many essential oils with herbicidal activity revealed that among the most frequent constituents in such essential oils were 1,8-cineol, *α*-pinene, carvacrol, carvone, limonene, thymol, etc. According to Romagni et al. (2000), 1,8-cineol completely inhibited germination of *Echinochloa crus-galli* essential oil while in the experiment of Atak et al. (2016) and Hanana et al. (2017) it also expressed certain inhibitory effects on *Sinapis arvensis*. Angelini et al. (2003) tested two types of 1,8-cineol rich *R. officinalis* essential oil on weeds germination; the essential oil with 47% cineol inhibited germination of annual weeds *E. crus-galli*, *Chenopodium album*, *Portulaca oleracea* more effectively than the essential oil with 23% cineol, which also had high content of *α*-pinene (37%). This is in disagreement with our findings; in this study *S. officinalis* essential oil contained 10.1% of 1,8-cineol but it showed stimulating effect on germination of *S. halepense* seeds, though it also contained camphor (17.2%), which was, together with cineol, already reported as very effective in inhibiting germination but of *S.*

Table 1. Chemical composition of essential oils used in the experiment

Compounds	RI[a]	Contribution to essential oil [%m/m][b]				
		S. montana	*S. officinalis*	*O. vulgare*	*J. communis*	*A. graveolens*
cis-Salvene	861	-	0.6	-	-	-
α-Thujene	923	0.6	-	-	1.8	0.6
α-Pinene	927	1.9	8.6	tr	43.5	-
Camphene	942	0.9	7.3	tr	tr	-
Sabinene	968	-	-	-	15.5	-
β-Pinene	969	1.4	1.2	1.3	2.6	-
Myrcene	985	1.3	0.8	tr	14	tr
α-Phellandrene	1001	tr	-	tr	tr	14.1
p-Mentha-1(7),8-diene	1004	0.9	-	-	-	-
α-Terpinene	1013	1.8	tr	0.5	0.6	-
p-Cimene	1019	13.4	1.1	3.3	0.9	1.8
Limonene	1023	-	2.1	0.8	4.8	32.2
1,8-Cineol	1025	0.5	10.1	1.2	tr	-
γ-Terpinene	1053	4.7	tr	3.1	1.1	-
α-Terpinolene	1086	-	tr	-	1.1	-
Linalool	1097	2.5	-	2.16	-	-
β-Thujone	1103	-	32.7	-	-	-
α-Thujone	1115	-	7.7	-	-	-
Camphor	1140	-	17.2	0.8	tr	-
Borneol	1165	2.8	2.4	1.3	-	-
Terpinene-4-ol	1176	2.1	tr	0.8	1.1	-
Dill ether	1176	-	-	-	-	6.6
α-Terpineol	1187	0.6	-	0.6	-	-
cis-Dihydro carvone	1189	-	-	-	-	1.2
trans-Dihydro carvone	1196	-	-	-	-	1.1
Thymol, methyl ether	1233	1.9	-	-	-	-
Carvone	1238	-	-	-	-	40.5
Carvacrol, methyl ether	1242	1.1	-	-	-	-
Bornyl acetate	1285	-	1.2	-	tr	-
Thymol	1293	44.6	-	4.3	-	-
Carvacrol	1299	6.2	-	73.7	-	-
α-Cubebene	1337	-	-	-	0.6	-
Thymol acetate	1352	0.6	-	-	-	-
β-Elemene	1386	-	-	-	1.3	-
trans-Caryophyllene	1412	3.7	0.9	3	1.8	-
γ-Elemene	1430	-	-	-	1.7	-
α-Humulene	1446	tr	3.5	0.8	1.5	-
Germacrene D	1480	-	-	-	2.6	-
β-Bisabolene	1502	3.7	-	0.17	-	-
δ-Cadinene	1516	0.4	-	tr	0.7	-
Caryophyllene oxide	1574	0.7	-	0.5	-	-
Globulol	1583	-	0.6	-	-	-
Monoterpene hydrocarbons		26.8	21.7	9.0	85.8	48.7
Oxygenated monoterpenes		62.9	71.4	84.8	1.1	49.5
Sesquiterpene hydrocarbons		7.8	4.5	4.0	10.3	0.0
Oxygenated sesquiterpenes		0.7	0.6	0.5	0.0	0.0
Total identified compounds		98.1	98.1	98.3	97.2	98.2
Number of identified compounds		23	16	17	18	8

[a]Kovats retention index relative to C7-C40 *n*-alkanes on the HP-5MS capillary column
[b]tr - stands for compounds represented in lower amount than 0.5%m/m

arvensis seeds (Atak et al., 2016). Therefore, the synergistic activity of some compounds might also be the weed seed-specific. Further, limonene, thymol, carvacrol, and carvon were reported to express high herbicidal activity on germination of various weed species (Angelini et al., 2003; Azirak and Karaman, 2008) which was in agreement with results obtained in this study. The considerably high inhibitory effect on germination of *S. halepense* achieved by *A. graveolens* essential oil, 61.5%, might be explained by its major components, carvon (40.5%) and limonene (32.2%) which already proved herbicidal efficiency. According to Azirak and Karaman (2008), *Carum carvi* essential oil, also contained mentioned two components, and inhibited germination of *Sonchus oleraceus* 100%, and other six weeds 90%. Similarly, the essential oils with high carvacrol content, were also effective in the reduction of weed seed germination (Atak et al., 2016; Hanana et al., 2017). Angelini et al. (2003) reported that essential oil from *S. montana* of carvacrol chemotype (57% carvacrol) completely inhibited germination of seeds of *Ch. album*, *P. oleracea* and *E. crus-galli*. However, the *S. montana* essential oil used in our study was thymol chemotype (44.6% thymol) and had no effects on germination of *S. halepnese* though it contained *p*-cimene (13.4%), which was suggested as component that may add to overall herbicidal effect (Atak et al., 2016; Hanana et al., 2017). Further, our carvacrol rich (73.7%) *O. vulgare* essential oil inhibited germination of seeds of *S. halepense* 52.7%, similarly to 97-100% germination of *S. arvensis* with the application of 57% carvacrol - *Origanum onites* (Atak et al., 2016). We assume that the application of carvacrol-rich-essential oils is quite efficient in the inhibition of germination of various weed seeds, but again, we confirm our hypothesis that the efficacy is a weed seed-dependent. Generally, the exact mechanism of essential oil activity on germination was not enough clear, although some authors already tried to suggest some. For 1,8-cineole, it is suggested that it is a mitotic inhibitor (Baum et al., 1998; Flamini, 2012; Vaughn and Spencer, 1993) while for camphor, limonene and α-pinene, that they are capable to interfere with respiratory activity of plant living cells (Flamini, 2012). Some of these compounds may act by inhibiting seed germination and affecting plant vigour. The potential for using allelopathy in weed management has been well documented (Neori et al., 2000; Wu et al., 2002).

3.2.2. The shoots length

Results of *S. halepense* shoots length following the application of essential oils are presented in Figure 2.

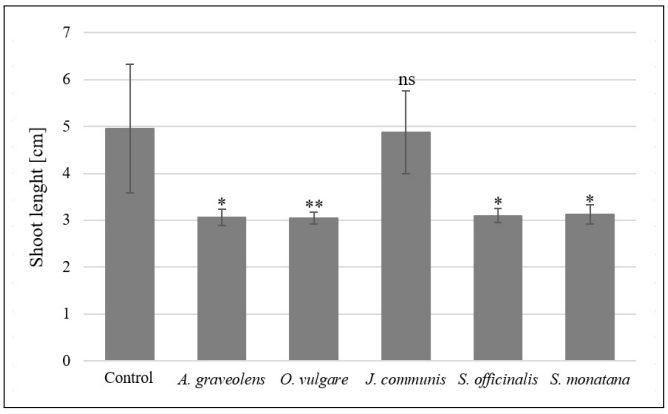

Fig. 2. *In vitro* efficacy of essential oils on *S. halepense* shoot length; Error bars denote two-fold standard deviation; **, * and ns – stands for Student's t-test statistical significance of mean difference against control at levels P<0.01, P<0.05 and not significant, respectively

Previous studies showed the effect of essential oils on different parameters of seedlings growth, including radicle and primary root length, as well as plumule and seedling height (Atak et al., 2016; Paudel and Gupta, 2009; Rassaeifar et al., 2013), etc. In our study, the shoots length of *S. halepense* was reduced by all essential oils to a certain extent (Figure 2); reduction achieved by all essential oils, except *J. communis* essential oil, was above 37%, representing considerable significant herbicidal effect of four out of tested five essential oils (Figure 2). In comparison to control, *O. vulgare* essential oil and *A. graveolens* essential oil were the best in the reduction of *S. halepense* shoot length, 38.4% and 38.2%, respectively, while *S. officinalis* essential oil and *S. montana* essential oil were slightly less effective, 37.4% and 37.0%, respectively. The mentioned four essential oil showed to be significantly different in comparison to control, but also to *J. communis* essential oil whose reduction was only 1.4%. The best results in this study achieved *O. vulgare* essential oil, which is characterized as carvacrol chemotype (73.7% carvacrol). Our results are in accordance to results of Dudai et al. (1999), where *Origanum syriacum* essential oil (60.1% carvacrol) totally inhibited radicals and coleoptiles growth of *Amaranthus* sp. The essential oil of *Salvia officinalis* of unknown chemical composition inhibited shoot length of *Rumex crispus* and *Cardaria araba* 100% (Onen et al., 2002). In our study essential oil from the same plant species, *S. officinalis* essential oil, achieved to inhibit *S. halepense* shoots length 37.4%, which might be explained by its major components, thujone (32.7%), camphor (17.2%) and 1,8-cineol (10.1%). These components were already reported as possessing herbicidal efficiency on shoot length by Barton et al. (2010); isolated 1,8-cineol and the 1,8-cineol rich essential oils of *Eucalyptus* sp. completely inhibited shoots growth of *Lolium rigidum*. In addition, Romagni et al. (2000) reported that inhibitory effect of 1,8-cineol on shoot length was stronger on monocot than on dicot weed, which is also in agreement with our results, as *S. halepense* is a monocot weed species. The same was confirmed by Angelini et al. (2003) who tested 1,8-cineol rich essential oil of *Rosmarinus officinalis* and showed significantly higher inhibition on shoot length of monocot *E. crus-galli* than on dicot weed *Chenopodium album*. The inhibition of shoots length by *S. montana* essential oil was 37%; this essential oil was rich in thymol (44.6%) with a second major component 13.4% *p*-cymene, which was reminiscent of *O. vulgare* essential oil reported by Hanana et al. (2017); the essential oil contained less thymol (30%) and higher *p*-cymene content (30%) but achieved to totally inhibit growth of *Sinapis arvensis*, *Phalaris paradoxa*, and *Lolium rigidum* seedlings.

CONCLUSION

The results obtained in this study revealed the following major insights, that all essential oils affect seed germination and shoots length of *S. halepense*, particularly *A. graveolens* seed essential oil. Also, all essential oils expressed certain potential on seeds of *S. halepense*, weather promoting or inhibiting, depending on essential oil. Almost all essential oils, except *J. communis* essential oil, reduced shoot length of *S. halepense* with success rate exceeding 37%. The efficacy of essential oils on weed seed germination and shoot elongation could be associated with essential oil chemistry, i.e. major essential oil constituents. Further research should be devoted to testing of various concentration of essential oils in order to precise minimal inhibitory concentration on seed germination and shoot elongation of *S. halepense* and to test all *in vivo* (field studies) as implementation of essential oils in weed management could be quite perspective in weed control in sustainable and organic agriculture.

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