Influence of the essential oils on invasive species seed germination

Mária Pluchtová[1] – Vito Pelizzeri[2] – Daniela Gruľová[1]

Abstract

Solidago canadensis is a dangerous invasive weed which spread after transfering from North America as ornamental and melliferous plant to Europe. In last decades, its expansion threat and suppress narutal flora. As strategy for surviving, weeds develop thousands of seeds and S. canadensis do as well. The aim of presented experiment was to evaluate the viability of Solidago canadensis seeds after 2 years of collection and provide test of biological potencial of various essential oil to slow down even stop goldenrod seeds germination. Three essential oils of family Lamiaceae *representants* (Salvia officinalis *L.*, Mentha piperita *L.* and Origanum vulgare *L.*) *and three of family* Apiaceae *(*Foeniculum vulgare Mill., Anethum graveolens *L.*, Pimpinella anisum *L.) were used in bioassays. Highlighted point by our study was that the relation between essential oil concentration and inhibition of seeds germination is not always dose-dependent.*

Keywords

goldenrod, monoterpenes, phytotoxicity, secondary metabolites, sesquiterpenes

Introduction

Solidago canadensis L. (*Asteraceae*) is indigenous in american continent and approximately before four centuries was introduced into Europe as an ornamental and melliferous plant (Amtmann, 2010; Weber, 2001). Generally, introduced species in new environment have no natural enemies and its competitive ability comparing to native populations often has been found higher (Jakobs, 2004; Abhilasha et al., 2008; Meyer & Hull-Sanders, 2008). *S. canadensis* L. is considered to be one of the top five wide spread dangerous plant in Europe (Weber, 2001, Bochenek et al., 2016). Comparable to another weeds, their strategy of colonisation is by production tens of thousands achenes which are 1–1.8 mm long in single ramet (Werner & Platt, 1976), so its removal is very difficult.

S. canadensis forms large clonal colonies that tend to suppresses the indigenous flora (Amtmann, 2010; Weber, 1998) and than participate in reduction of native plant species richness or diversity (Levine et al., 2003; Huang et al.,2005; De Groot et al., 2007). Germination presents the first phase of the successful establishment of plant species in environment, although, there has been very little published research into the factors that affect the germination of invasive goldenrod seeds (Walck et al., 1997a, b; Bochenek et al., 2016). Generally there are three broad categories cover most invasive plant control: mechanical, chemical, and biological. Mechanical control means physically removing plants from the environment through cutting or pulling. Chemical control uses herbicides to kill plants and inhibit regrowth. Techniques and chemicals used will vary depending on the species. Biological controls use plant

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diseases or insect predators, typically from the targeted species' home range. Several techniques may be effective in controlling a single species, but there is usually one preferred method, the one that is most resource efficient with minimal impact on nontarget species and the environment.

New strategies to prevent invasive plant species spreading are based on the use of natural compounds such as eugenol, benzyl benzoate, isoeugenol, carvacrol, carvone, thymol, trans-anethole, linalool etc. which were identified in differen EO (Dudai et al., 1999; Reigosa et al., 1999; Tworkoski, 2002; Angelini et al., 2003; Campiglia et al., 2007; Vasilakoglou et al., 2007). Essential oils (EOs) were characterised as allelochemicals and are able to exerts their phytotoxic effects to limit the growth of different plants (Anaya, 1999).

Aim of the present research was to evaluate the viability af two years old seeds from invasive species and evaluate the effect of six essential oils extracted from sage (*Salvia officinalis* L.), peppermint *(Mentha×piperita* L.), oregano (*Origanum vulgare* L.), fennel *(Foeniculum vulgare* Miller), anise (*Pimpinella anisum* L.) and dill (*Anethum graveolens* L.), which belongs to two families *Lamiaceae* and *Apiaceae* on *S. canadensis* seeds germination.

Materials and Methods

Seeds collection

Seeds were collected on September 2014, when the flowers finalise their ontogeny. There was selected locality Chminianska Nová Ves, where is the lower distribution of mentions species and it is possible to monitor also spreading of Goldenrod within years. The locality Chminianska Nová Ves (N49.000419 E021.079893) (Slovakia) is situated 11.7 km westward from the city of Prešov. The altitude of the collecting area is 362 m above sea level and inclination is 11°. Flowers were left on filter paper for few hours at room temperature and later were slightly shaked to release the seeds. Seeds were transfered to paper envelope and kept in dry place with room temperature until were use for experiment. Seeds were used for germination availability and evaluation phytotoxic activity of EOs.

Essential oils

Pure essential oils from salvia (*Salvia officinalis* L.), pepermint (*Mentha×piperita* L.), origanum (*Origanum vulgare* L.), fennel (*Foeniculum vulgare* Mill.), anise (*Pimpinella anisum* L.), dill (*Anethum graveolens* L.) were obtained from company Calendula a.s. Nová Ľubovňa (Slovakia). Each EO was analysed by GC-MS for quantitative and qualitative properties at Laboratory of plant and animal ecophyphysiology as described in Gruľová et al. (2016).

Bioassay

Each of the six EOs were diluted in double distilled water (DDW) with acetone in range 99.5: 0.5 (v/v) at different concentrations (2.5; 1.25; 0.625; 0.25; 0.125; 0.062 $\mu g.ml^{-1}$). Plastic Petri dishes (PD) (100 mm x 20 mm; Sigma-Aldrich) were filled with filter paper (Wattmann) on which was drawn grid with 100 places for exact placement of *S.canadensis* seeds (Figure 1). Because of the very small size of seeds (1-2 mm), each seed was controlled and placed into the position on filter paper under the binocular magnifier SZM-168 (Motic, China) (Figure 2). In one Petri dish were

placed 100 seeds. Into each PD was added 7 mL of solution (diluted EO). Negative control was prepared DDW/acetone (99.5/0.5). The Petri dishes were sealed with parafilm to reduce loss of moisture and essential oil to the atmosphere. Each sample was prepared in triplicates. The PDs were kept in growth chamber (Sanyo, MLR-351H) under the temperature 22±2 °C and photoperiod (14 hours daylight and 10 hours dark). The number of germinated seeds were checked after 8 days (8d) and 16 days (16d).

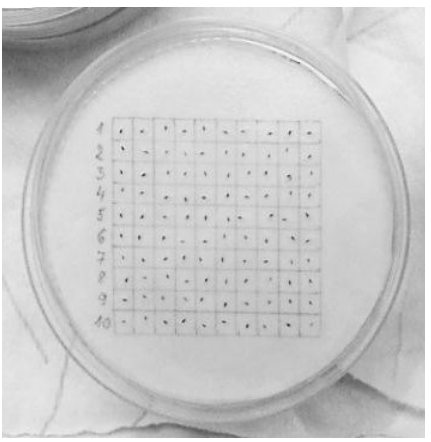

Figure 1. Filter paper with grid and placed *S. canadensis* seeds. (Photo: Mária Pľuchtová.)

Figure 2. About 2 mm long *S. canadensis* seed, photo was made by using binocular magnifier SZM-168 (Motic). (Photo: Mária Pľuchtová.)

Statistical Analysis

Excell was used for simple statistical analysis using *student T-test*. Data were expressed as means and their standard deviations (AVG±SD) and percentage (%) of stimulation or inhibition effect of EOs.

Results

Evaluation of seed germination of S.canadensis in natural conditions

It is widely reported, that in natural conditions *S. canadensis* spread very fast. Each year we can observe occurence of new individuals or populations of mentioned species in new areas. Before the experiment started, we tested seed germination of *S. canadensis* in laboratory conditions. Seeds collected in two different years placed in PD on filter paper with DDV were stored in growing chambers in simulated natural conditions and observed for their germination.

We have been tested seeds collected in year 2014 and seeds from (that time) last vegetation season in 2016. After 8 days in growing chambers only 30 % were germinated, while seeds collected in the year 2016 reached 73 % of germination. After 16 days, seeds 2014 did not reached 50 % of germinated seeds (see Table 1), while younger seeds collected in 2016 reached almost 90 % of germinated seeds. Older seeds collected in 2014 required longer time for seed germination. Before the experiment

were seeds stored in paper envelope in dry place in laboratory contiditons. There are significant differences between germination of the seeds collected in different years. According results, seeds of *S. canadensis* lost almost 50 % viability after two years.

Table 1. Comparison of germination of *S. canadensis* seeds from different year collection.

Year of seed collection	Number of germination days	AVG ± SD
2014	8d	30.00 ± 14.61
	16d	48.50 ± 20.12
2016	8d	73.06 ± 10.75
	16d	88.89 ± 4.51

*Average are means from the triplicate repetition of evaluated germinated seeds from total number 100 seeds in each repetition.

GC-MS analysis of selected EOs

Chemical composition of peppermint, oregano and sage EOs from the *Lamiaceae* family and fennel, anise and dill EOs from *Apiaceae* family were evaluated by GC/MS analysis and results were displayed in Table 2. From the total amount of components 98.6% were identified in pepermint EO, 95.9% in oregano EO, 90.2% in sage EO, 99.7% in fennel EO, 97.6% in anise EO and 98.8% in dill EO. Oxygenated monoterpenes (78.8% in peppermint and 57.5% in sage EO) and phenolic compounds (80.1% in oregano EO) were dominated in *Lamiaceae* family while other compounds (78.2% in fennel EO and 93.0% in anise EO) as well as oxygenated monoterpenes (61.6% in dill EO) were identified as dominant in *Apiaceae* family. Within the evaluation of individual EO, the highest number of component in peppermint EO were identified menthol (49.3%) and menthone (22.4%) followed by limonene (9.4%) and menthyl acetate (6.4%). Carvacrol (78.2%) was identified as most abundant in oregano EO followed by m-cymene (4.4%) and γ-terpinene (3.2%). In sage EO were identified in highest content of thujone (34.2%), camphor (19.8%) and limonene (15.0%) followed by camphene (6.3%) and α-pinene (3.5%). Almost the same content of estragole (40.1%) and anethole (38.1%) were identified in fennel EO followed by fenchone (16.5 %). Anethole (88.6%) was determined as dominant component of anise EO and anisole (4.4%). In dill EO were identified carvone (58.4%) and limonene (35.8%) as dominant components. Other minor components were lower of 2%.

Phytotoxic effect of EOs on S. canadensis seeds germination

Similarly, as was evaluated the different *S. canadensis* seeds germination rate under the „natural" condition, there were evaluated significant differences in germination rate after influence of all six EOs in different concentration on seeds collected in 2014. Seeds collected in 2014 – two years old seeds, reached one third or maximum about 50 % of germinated seeds (average number of germinated seeds evaluated after 8 and 16 days) (Table 3, Figure 3).

Decreasing number of germinated seeds with the increasing concentration of EOs were evaluated after influence of sage EO and anise EO. The opposite effect was noted in the rest of EOs – peppermint, oregano, fennel and dill, where with decreasing EOs concentration, the number of germinated seeds decreased.

Table 2. GC-MS analysis of selected EOs.

			Content of components (%)						
			Lamiaceae			*Apiaceae*			Identif.[b)]
No.	**Name of component**	**Rt**	**p.mint**	**oregano**	**salvia**	**fennel**	**anise**	**dill**	
1.	α-Pinene	11.085	0.4	0.2	3.5	2.2	0.7		1,2,3
2.	Camphene	12.185		0.2	6.3	0.2			1,2
3.	β-Pinene	14.117	0.9	0.4	1.7	0.1	0.1	0.2	1,2,3
4.	β-Myrcene	15.336			1.0				1,2,3
5.	α-Phellandrene	16.285			0.1	t	0.5	1.2	1,2,3
6.	α-Terpinene	17.129	0.1	0.8	0.3		0.1	t	1,2
7.	m-Cymene	17.322	0.1	4.4	0.2	0.6	0.1	0.1	1,2
8.	Limonene	17.634	9.4	0.5	15.0	1.4	0.8	35.8	1,2,3
9.	γ-Terpinene	19.480	0.2	3.2	0.4	t	0.1		1,2
10.	Fenchone	20.003				16.5			1,2,3
11.	Linalool	22.070					1.4		1,2
12.	Thujone	22.336			34.2				1,2,3
13.	Camphor	24.445		0.9	19.8	0.4			1,2,3
14.	Menthone	25.035	22.4						1,2,3
15.	Isomenthone	25.417	5.7						1,2
16.	Borneol	25.781		0.8	2.2				1,2,3
17.	1-Terpinen-4-ol	26.265		0.7	0.4	0.1	0.2	t	1,2
18.	Menthol	26.445	49.3						1,2,3
19.	α-Terpineol	27.087	0.5	0.2					1,2
20.	Myrtenol	27.090			0.9				1,2
21.	Estragole/Anisole	27.258				40.1	4.4		2
22.	Dihydrocarvone	27.514						3.2	1,2
23.	Pulegone	29.577	0.7						1,2,3
24.	Carvone	30.244		0.1				58.4	1,2,3
25.	Piperitone	30.634	0.3						1,2,3
26.	Bornyl acetate	32.782			0.5			t	1,2,3
27.	Anethole	33.002				38.1	88.6		1,2,3
28.	Menthyl acetate	33.158	6.4		0.2				1,2,3
29.	Thymol	33.375		1.9	0.1				1,2,3
30.	Carvacrol	34.073		78.2		0.1			1,2,3
31.	β-Caryophyllene	37.019	1.4	2.3	0.8		0.3		1,2,3
32.	γ-Muurolene	37.278	0.1	0.2					1,2
33.	Germacrene D	38.636	0.2						1,2,3
34.	Valencene	38.944	0.1	0.1	2.4		0.1		1,2
35.	α-Gurjunene	39.107	0.1						1,2
36.	α-Muurolene	39.181		0.1	0.1				1,2
37.	β-Bisabolene	39.456		0.2			0.1		1,2
38.	γ-Cadinene	39.608	0.2	0.1					1,2
39.	β-Cadinene	39.769	0.1	0.5	0.1		0.1		1,2
40.	Caryophyllene oxide	42.708		0.1	0.1				1,2
	Total identified		**98.6**	**95.9**	**90.2**	**99.7**	**97.6**	**98.8**	

Continue of the Table 2

Monoterpene hydrocarbons	11.2	9.7	28.5	4.5	2.4	37.3
Oxygenated monoterpenes	78.8	2.7	57.5	17.0	1.7	61.6
Sesquiterpene hydrocarbons	2.2	3.3	3.3	0.0	0.5	0.0
Oxygenated sesquiterpenes	0.0	0.1	0.1	0.0	0.0	0.0
Phenolic compounds	0.0	80.0	0.1	0.1	0.0	0.0
Other compounds	6.4	0.0	0.7	78.2	92.9	t

blank space - not identified; t-traces; Identification: 1, comparison of Kovats retention indices with published data; 2, comparison of mass spectra with those listed in the NIST 02 libraries and with published data; 3, coinjection with authentic compound.

Table 3. *S. canadensis* seeds germination after influence of different concentrations of EOs.

concentration [µg.ml-1]	peppermint	oregano	sage	fennel	anise	dill
	Average number of germinated seeds ± SD					
2.500	55 ± 12	43 ± 9	**23 ± 11***	38 ± 19	**23 ± 15*****	**54 ± 13****
1.250	54 ± 17	33 ± 10	**32 ± 14***	36 ± 23	**29 ± 8****	38 ± 11
0.625	44 ± 11	34 ± 9	32 ± 16	**21 ± 15***	**35 ± 9***	34 ± 12
0.250	51 ± 19	34 ± 9	**26 ± 11***	37 ± 18	**34 ± 9***	30 ± 9
0.125	**30 ± 6***	34 ± 10	33 ± 9	31 ± 13	36 ± 9	26 ± 8
0.062	**25 ± 9***	40 ± 11	48 ± 16	31 ± 10	44 ± 10	32 ± 13
control	43 ± 9	44 ± 6	41 ± 10	47 ± 17	48 ± 11	34 ± 9

The numbers presents the average number of germinated seeds evaluated after 8 and 16 days with their standard deviation. Asterix means the significant influence in $p<0.5$ (*); $p<0.01$(**); $p<0.001$(***) after statistical analysis student T-test.

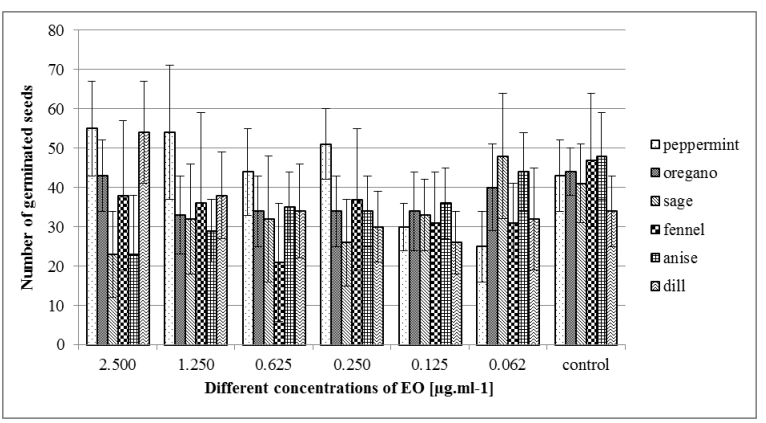

Figure 3. Comparison of influence of different EO concentration on seed germination.

Comparison with the controls were statistically processed by using *student T-test*. Significant inhibition effect was evaluated after using peppermint EO in two lowest concentrations (0.0625 and 0.125 μg.ml^{-1}), after using sage EO in three higher concentrations (2.5, 1.25 and 0.25 μg.ml^{-1}), after using fennel EO in one concetration (0.625 μg.ml^{-1}) and the most significant effect was noted after using anise EO in four concentrations (2.5 – 0.25 μg.ml^{-1}).

No significant inhibition effect was evaluated after using oregano EO and the opposite effect – stimulation of seed germination was noted after using highest concetration(2.5 μg.ml^{-1}) of dill EO.

When were numbers of germinated seeds compared with the control samples, we could note variable effect of EOs in different concentrations. EOs extracted from the plants belonging to *Lamiaceae* and *Apiaceae* family expressed different effects.

Effect of peppermint EO

Evaluating the seeds germination after influence of peppermint EO after 8 days resulted in stimulation effect at EO concentrations 2.5, 1.25 and 0.625 μg.ml^{-1}, while inhibition effect was evaluated in lower concentrations of EO (from 2.25 – 0.0625 μg.ml^{-1}). Significant inhibition effect was evaluated after influence of peppermint EO in concentration 0.125 μg.ml^{-1}($p<0.05$) and 0.0625 μg.ml^{-1} ($p<0.01$).

After 16 days of seeds germination the difference comparing to observation after 8 days was in concentration 0.25 μg.ml^{-1} where weak inhibition effect changed into significant stimulating effect ($p<0.05$) and inhibition effect in concentrations 0.125 μg.ml^{-1} and 0.0625 μg.ml^{-1} became more stronger $p<0.01$ and $p<0.001$, respectivelly (Figure 4).

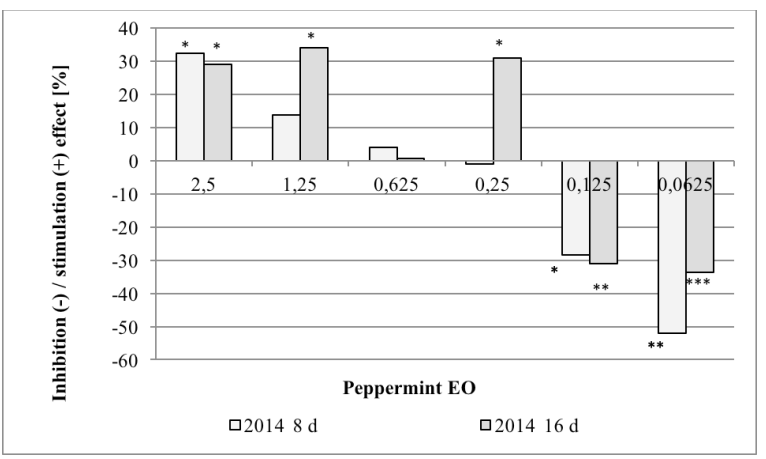

Figure 4. Evaluation of phytotoxic effect of peppermint EO on seeds after 8 and 16 days (8d and 16 d) of germination. Asterix means the significant influence in $p<0.5$ (*); $p<0.01$(**); $p<0.001$(***). The values express the percentage over or below the control value.

Effect of oregano EO

Except two cases, where stimulation effect on seeds after 16d was observed in concentration 2.5 and 0.0625 μg.ml^{-1}, oregano EO influenced phytotoxically on seeds. Significant phytotoxic effect (p<0.05) was evaluated on seeds after 8 days in concentration 1.25 μg.ml^{-1} and after 16 days in the same concentration as well as in concentration 0.125 μg.ml^{-1}. Stronger significant phytotoxic (p<0.01) effect was evaluated only after 8 days in four concentrations, decreasing from 0.625 until 0.0625 μg.ml^{-1} (Figure 5).

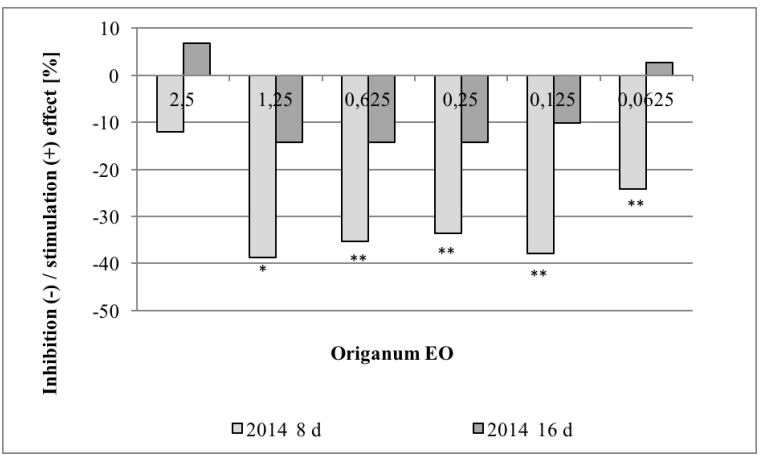

Figure 5. Evaluation of phytotoxic effect of oregano EO on seeds after 8 and 16 days (8d and 16 d) of germination. Asterix means the significant influence in p<0.5 (*); p<0.01(**); p<0.001(***). The values express the percentage over or below the control value.

Effect of salvia EO

Influence of the salvia EO on seeds is graphically presented in Figure 6. Inhibiton effect on seeds germination was evaluated in seeds in each concentration except one, the lowest concentration (0.0625 μg.ml^{-1}) where stimulation of seeds germination was noted. Signifficant (p<0.05 and p<0.01) inhibition effect was evaluated in concentrations 2.5 and 0.25 μg.ml^{-1} after 8 and 16 days and in concentrations 1.25 and 0.625 μg.ml^{-1} only after 8 days.

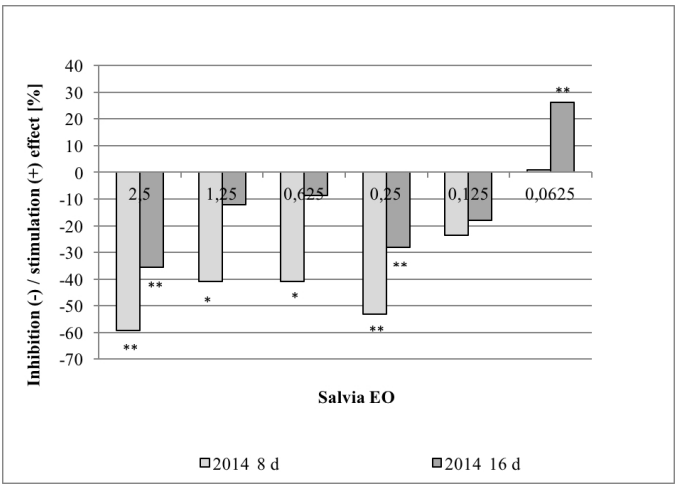

Figure 6. Evaluation of phytotoxic effect of salvia EO on seeds after 8 and 16 days (8d and 16 d) of germination. Asterix means the significant influence in $p<0.5$ (*); $p<0.01$(**); $p<0.001$(***). The values express the percentage over or below the control value.

More effective phytotoxic effect on seeds germination seems to be using EO from the species belonging to *Apiaceae* family.

Effect of fennel EO

Fennel EO expressed its activity on seeds germination mostly as phytotoxic. In all concetrations (2.5 – 0.0625 μg.ml^{-1}) was evaluated inhibition effect on seeds after 8 and 16 days, although significance was confirmed only in two cases in concentrations 1.25 and 0.625 μg.ml^{-1} after 8 days ($p<0.05$) (Figure 7).

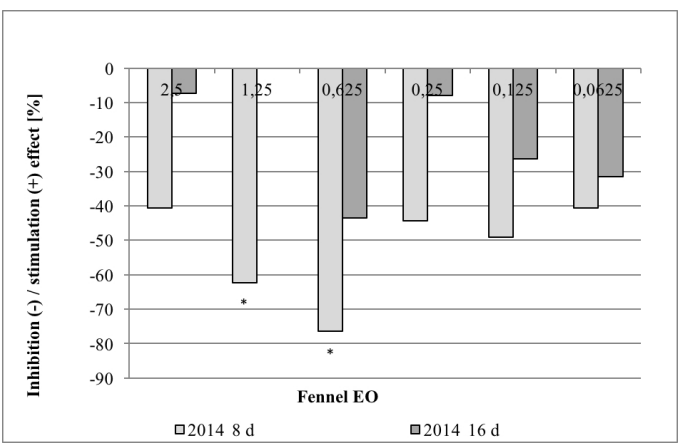

Figure 7. Evaluation of phytotoxic effect of fennel EO on seeds after 8 and 16 days (8d and 16 d) of germination. Asterix means the significant influence in $p<0.5$ (*);

p<0.01(**); p<0.001(***). The values express the percentage over or below the control value.

Effect of anise EO

Similar effect as in fennel EO was evaluated by influence of anise EO. Inhibition effect on seeds germination was noted after application of all concetrations. Significant (p<0.05) in concentrations 2.5 and 0.125 μg.ml^{-1} after 8 days and stronger significance (p<0.01) in concentrations 2.5 and 1.25 μg.ml^{-1} after 16 days (Figure 8).

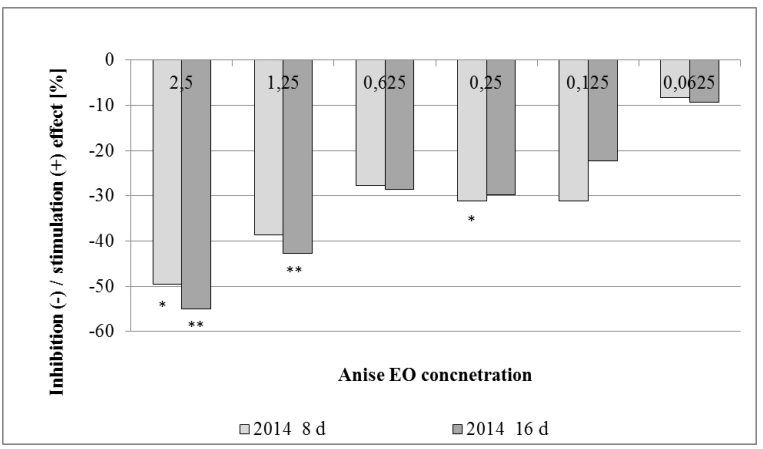

Figure 8. Evaluation of phytotoxic effect of anise EO on seeds after 8 and 16 days (8d and 16 d) of germination. Asterix means the significant influence in p<0.5 (*); p<0.01(**); p<0.001(***). The values express the percentage over or below the control value.

Effect of dill EO

Stimulation and inhibition effect was evaluated by application of dill EO in different concentrations. Stimulation of seeds germination was noted in concentrations 2.5, 1.25, 0.625 and 0.0625 μg.ml^{-1} after 8 and 16 days, and in concentration 0.25 μg.ml^{-1} only after 16 days. Inhibition effect on seeds germination was evaluated in concetration 0.25 μg.ml^{-1} after 8 days and 0.125 μg.ml^{-1} after 8 and 16 days. Strong significant effect was evaluated only in highest concentration (2.5 μg.ml^{-1}) after 8 days (p<0.01) and after 16 days (p<0.001) (Figure 9).

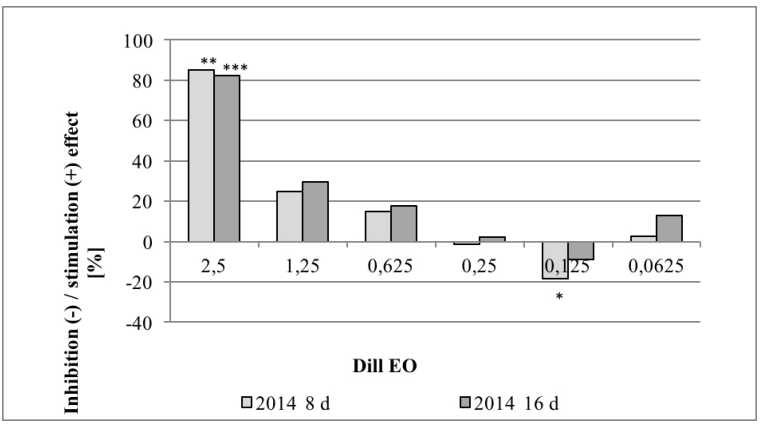

Figure 9. Evaluation of phytotoxic effect of dill EO on seeds after 8 and 16 days (8d and 16 d) of germination. Asterix means the significant influence in $p<0.5$ (*); $p<0.01$(**); $p<0.001$(***). The values express the percentage over or below the control value.

After calculation average number of germinated seeds by influence of EOs in all concentrations, the results were as follows: after influence of oregano EO was 42 ± 10 germinated seeds, after influence of peppermint EO there were 43 ± 11 germinated seeds, after influence of salvia EO 32 ±13 germinated seeds; after influence of fennel 32 ± 16 germinated seeds; after influence of dill EO 36 ± 11 germinated seeds and after influence of anise EO up to 34 ±10 germinated seeds.

Growth inhibition effect of EOs on *Solidago canadensis* seeds with decreasing biological activity were ordered: salvia (68 % of seed germination inhibition) > fennel (68 %) > anise (66 %) > dill (64 %) > origanum (58 %) > peppermint (57%) (Figure 10).

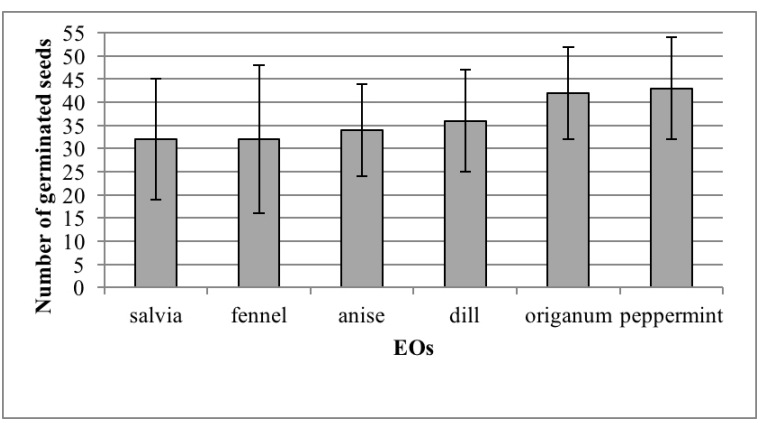

Figure 10. Average number of germinated seeds of *Solidago canadensis* in all concentrations after influence of different EOs.

Discussion

Based on the plant genotype depends seeds germination, however wide range of physical environmental factors have important influence (Baskin & Baskin, 1998). Vitality is characterised as a seed tolerance to unfavourable conditions within the time of seed germination as well as the fitness of the seeds (Hosnedl, 1995). If the seeds germination is lower or not occured, it is possible that stress factors influenced the seeds within their development. Also as seeds get older, the amount of soluble sugars decreases, which limits seed vitality, decreases germination ability and compromises the structural integrity of membranes (Bernal-Lugo & Leopold 1992; Corbineau 2012; Obendorf & Gorecki, 2012). Experiments with *S. gigantea* and *Solidago shortii* (Buchele et al., 1991; Bochenek et al., 2016) conclude long-term maintenance of seed viability and vigor as well as the absence of dormancy and resistance to environmental stressors. Goldenrod seeds germinated rapidly and with nearly 100 % success regardless of temperature and storage time. Goldenrod seeds were highly resistant to environmental stressors in comparison with the seeds of other *Asteraceae* species (*Centaurea cyanus, Taraxacum officinale* or *Matricaria maritima*). Our results in "natural conitions" resulted in lower germination of older seeds. This findings shows decreasing vitability of *S. canadensis* seeds with their age. Resistance to changes in seed viability under the influence of various environmental factors may be attributed to different internal causes. The physiological mechanisms behind those traits have not yet been fully explored, but they could be associated with the relatively high sucrose-to-hexose ratio in seeds and significant sensitivity to ABA (Bochenek et al., 2016). Seeds vitality play important role in seed germination in stressed environmental conditions (Pazderů, 2013). Essential oils are known as sucessfull allelochemicals with inhibitory effect to seeds germination of various crops and weeds (Dudai et al., 1999). However, their selectivity on weed species has not been investigated. In our experiments with peppermint EO, the highest used concentrations (2.5 and 1.25 $\mu g.ml^{-1}$) caused inhibition of *S. canadensis* seed germination about 32 % while by using lower concentration (0.125 $\mu g.ml^{-1}$) there was noted inhibition about 51 %. According experiments with peppermint EO on seeds germination of different weeds were results as follow: for inhibition of 100 % of *Amaranthus retroflexus* L., *Portulaca oleracea* and *Vicia sat*iva seeds was enought 1.8 $mg.l^{-1}$ peppermint EO while for *Chenopodium album*, *Lolium* spp., *Sinapis arvensis* and *Solanum nigrum* was necessary concentration 5.4 $mg.l^{-1}$ for inhibition of 100 % of seed germination (Cavalieri & Caporali, 2010;). Rolim de almeida et al. (2010) used EOs from oregano, sage, fennel and anise to observe their phytotoxic activity on selected model plants (radish, and lettuce). Oregano EO inhibited seed germination from the doses of 1.25 µg/mL in garden cress, 0.125 µg/mL in radish and 0.25 *µg/mL* in lettuce. In our evaluation results presented significant inhibition of *S. canadensis* seeds in the lowest concetration 0.0625 *µg/mL*. Salvia EO inhibited seed germination of model plants from the doses of 2.5, 0.0625 and 0.125 *µg/mL*. In our experiment sage EO inhibited *S.canadensis* seeds germination at the minimum doses of 0.25 *µg/mL*. Fennel EO inhibited germination of garden cress seeds in two dosed 0.625 and 2.5 *µg/mL*; seeds of radish from the dose 0.25 *µg/mL* and no significant

inhibition was observed on seeds of lettuce. Significant inhibition of *S. canadensis* seeds in our experiment was only in two doses 0.625 and 1.25 μg/mL. Anise EO presented low phytotoxic activity (Rolim de Almeida, 2010).

Different concentrations of the same EO expressed inhibitory or stimulatory effects. This concept of a generalized "low-dose stimulation - high-dose inhibition" or "hormesis" was gradually supported by field observations (Stebbing, 1982). There is evidence that exposure to novel environments or a toxic substance increases the variance of phenotypic traits such as enzyme activity (Holloway et al. 1997), morphological features and growth (Forbes et al. 1996). However, the reasons for such increases and their adaptive implications remain unclear. The effect of an essential oil on seed germination is often explained in terms of the individual effects of some main constituents. However, an essential oil is a mixture of many compounds in different proportions, and it is often not known whether and how they might interact (Vokou et al., 2003). An important point highlighted by our study was that the relation between oil concentration and inhibitory ability was not always dose-dependent.

Acknowledgement

The research was supported by the Grant Agency for PhD. students and young researchers and pedagogical workers of University of Presov, Grant number: GaPU 22/2017 and project ITMS: 26220220182.

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