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EFFECT OF ESSENTIAL OILS ON GERMINATION AND SEEDLING GROWTH OF TWO RAGWEED SPECIES

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ABSTRACT
Effect of essential oils extracted from *Anethum graveolens*, *Origanum vulgare*, *Juniperus communis*, *Salvia officinalis* and *Satureja montana* on seed germination and seedling growth of common ragweed (A*mbrosia artemisiifolia*) and giant ragweed (*Ambrosia trifida*) were studied. For germination studies seeds incubated one week in the Petri dishes containing 5 ml of diluted oil (500 µl oil + 500 µl 70% ethanol / 100 ml distilled water) in the dark at 25 ^{o}C. In order to test effect of diluted oils on seedling growth, seeds germinated on destined water and when germinated (at the moment of radicle emergence) were transferred into Petri dishes containing 5 ml of diluted oil. Our results confirmed that some tested essential oils possess inhibitory effect, while some stimulate germination of ragweed species. Also, inhibitory effect on seedling growth were confirmed, except for the oil of *J. communis,* which slightly stimulated growth of common ragweed seedlings.

Keywords: A*mbrosia artemisiifolia*, *Ambrosia trifida*, essential oils, germination, seedling length.

INTRODUCTION

Common ragweed (A*mbrosia artemisiifolia*) and giant ragweed (*Ambrosia trifida*) are invasive species that are widespread across the globe. Common ragweed is widely distributed in almost all parties of Serbia, especially on territory of Vojvodina, Macva and Sumadija (Vrbničanin *et al.*, 2008), while giant ragweed found sporadically (villages Kucura, Savino Selo, Ravno Selo and Despotovo) in the territory of Serbia (Malidža and Vrbničanin, 2006). Both species are annual broadleaf weeds which are propagated by seeds. The additional problem with these species is allergenic pollen, that can cause serious health problems in both human and animal populations (Ognjenovic *et al.*, 2013). The main way for their control is herbicide application. But, fast evolving weed populations resistant to herbicides and increasing concern about the environmental impact of herbicides, lead to the development of more ecological acceptable methods for weed control. Some of that methods are application of plant growth-promoting rhizobacteria, biocontrol agents like mites, insects or parasitic plants, vegetation management, flaming, allelochemicals etc.

A variety of allelochemicals have been identified, including essential oils, that inhibit seed germination and plant growth (Almeida *et al.*, 2010; Ismail *et al.*, 2011; Paudel and Gupta, 2008). Essential oils very large and diverse group of plant products, which are very suitable for harmful organisms control in agriculture, especially in organic farming systems (Tworkoski, 2002). Their positive traits are little or no mammalian toxicity, low persistence in the soil, absence of risk for underground water pollution etc. (Isman, 2000).

Essential oils have potential as biopesticides for different groups of harmful organisms control. Therefore, it is confirmed some essential oils have antifungal activity and can be used for postharvest diseases control (Vitoratos *et al.*, 2013). Also, some essential oils have larvicidal, ovicidal and repellent activities against insects (Pushpanathan *et al.*, 2006). Many essential oils can be used as bioherbicides thanks to its inhibitory effects on weed seed germination and seedling growth. Except that, some investigations consider essential oils as "natural product herbicides" and applied them on the same way as synthetic foliar herbicides. Tworkoski (2002) conducted whole-pant assays with 22 essential oils extracted from different (mainly crop) plant species with the aim to assess their effect on weed species *Chenopodium album*, *Ambrosia artemisiifolia* and *Sorghum halepense*. His work showed essential oils can injure or kill weed plants depending of weed species and oil concentration. Although many studies focused on effects of essential oils on weed species, screening of all potential bioherbicides is desirable for evaluation of real suitable oils for involvement in weed management practices. The objectives of the present study were to evaluate effect of essential oils extracted from five different plant species on germination and seedling growth of two ragweed species invasive in Serbia.

MATERIAL AND METHODS

Mature seeds of common ragweed (A*mbrosia artemisiifolia*) were collected during autumn 2014 at ruderal area in Belgrade (Serbia), while seeds of giant ragweed (*Ambrosia trifida*) were collected in the field in village Kosancic (Serbia). Collected seeds storage at room temperature (approximately 20-25^{o}C) in a dark place. Essential oils extracted from *Anethum graveolens* (EO1), *Origanum vulgare* (EO2), *Juniperus communis* (EO3), *Salvia officinalis* (EO4) and *Satureja montana* (EO5) were used for study. All oils diluted in distilled water (500 µl oil / 100 ml distilled water) and 500 µl of 70% ethanol were added. Germination was evaluated in 9 cm diameter Petri dishes containing 5 ml of diluted oil and 30 randomly selected seeds. Control with distilled water was included. In order to test effect of essential oils on seedling growth seeds germinated on distilled water and when germinate were transferred into Petri

dishes containing 5 ml of diluted oil or distilled in control. Three dishes were used for each treatment and experiment repeated three times. Petri dishes took place in an incubator (Binder CE), in the dark at 25 ^{o}C. The seeds were considered to be germinating at the moment of radicle emergence. The number of germinated seeds/seedlings length was recorded 7 days after set up of experiment. Obtained data were analyzed using t-test in statistical software STATISTICA 5.0

RESULTS AND DISCUSSION

Effect of essential oils on seed germination

The germination results obtained with seeds of two ragweed species at solutions of different essential oils are presented in Figure 1. Generally, germination of the both species was similar and pure (slightly more than 5%) in control, while germination between treatments with essential oils differs. Namely, some oils (EO1, EO2, EO5) had very significant inhibitory effect on seed germination ($P<0.01$; Tab. 1). Germination reduction for common ragweed was about 93, 80 and 46%, respectively, while seed germination of giant ragweed was reduced about 46, 100 and 100%, respectively. Obtained results are in accordance with results published by Almeida *et al.* (2010) who confirmed that oils from some Mediterranean aromatic plants are very active against germination and radicle elongation of *Raphanus sativus*, *Lactuca sativa* and *Lepidium sativum.* Similar, Ismail *et al.* (2011) found oils extracted from *Juniperus oxycedrus* completely inhibited seed germination and seedling growth of weed species *Phalaris paradoxa*, *Trifolium campestre* and *Lolium rigidum.* Contrary to that findings, EO3 and EO4 more or less promoted germination of both species. The highest promotional effect was detected for EO3 which was increased germination of common ragweed more than 3 times in comparison with control (P=0,00075), while promotion of giant ragweed germination was less significant (P=0,03964). Opposite results were found for EO4, which increased germination of common ragweed slightly more than 30% (P=0,011479), while enhancement of giant ragweed germination was more than 50% (P=0,006197).

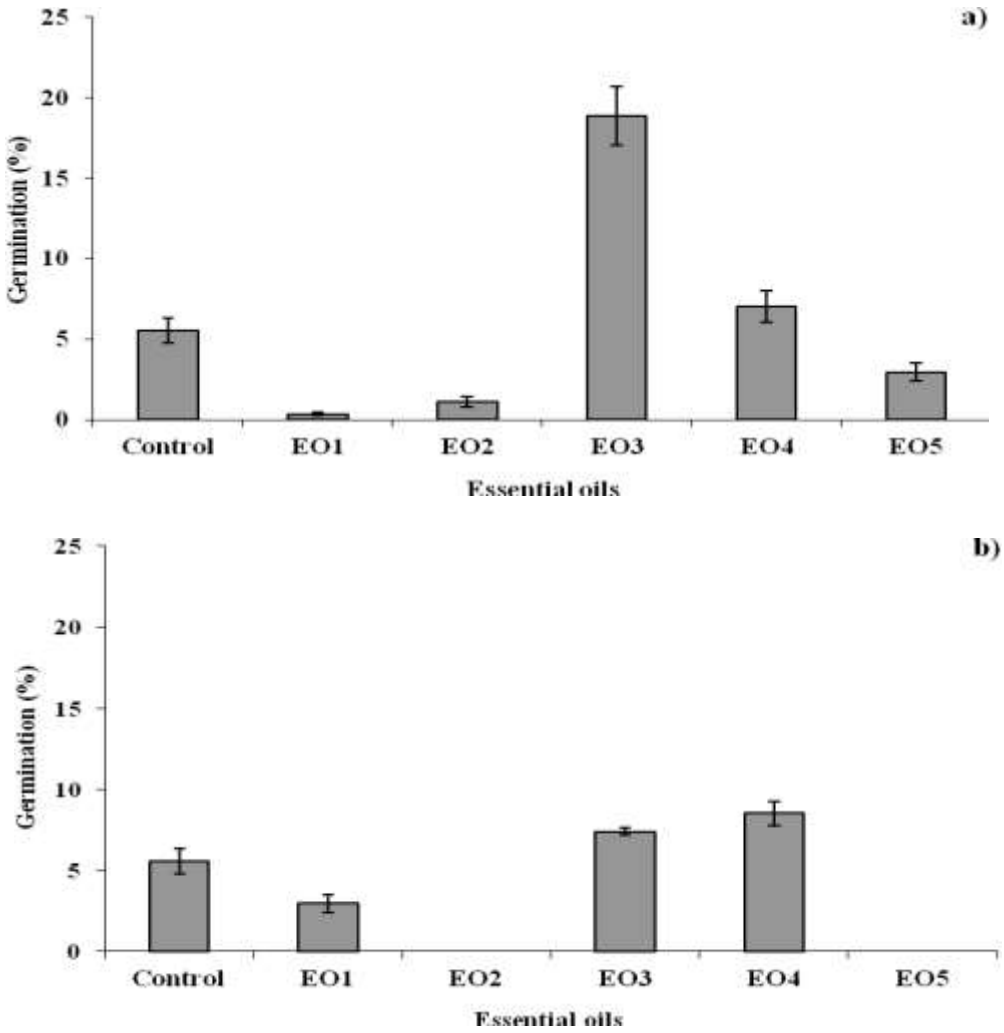


Figure 1. Effect of different essential oils on common (a) and giant (b) ragweed germination.

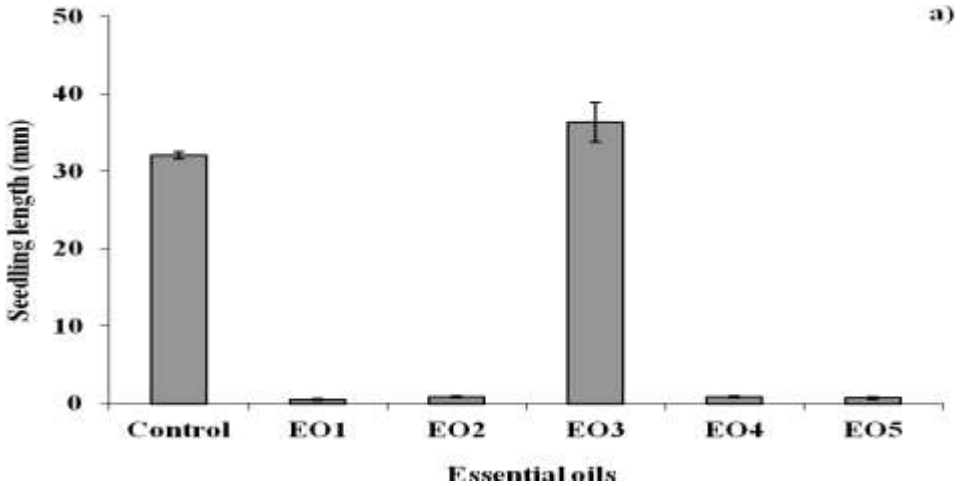
Table 1. Significance of essential oils effects on common and giant ragweed germination (t-test).

		Control	EO1	EO2	EO3	EO4
common ragweed	EO1	0,002779**				
	EO2	0,001475**	0,259382ns			
	EO3	0,00075**	0,007803**	0,004513**		
	EO4	0,011479*	0,048574*	1,000000ns	0,004519**	
	EO5	0,003260**	0,460856ns	0,900026ns	0,008770**	0,911303ns
giant ragweed	EO1	0,003387**				
	EO2	0,002748**	0,406098ns			
	EO3	0,039640*	0,036827*	0,013353*		
	EO4	0,016185*	0,178944ns	0,267035ns	0,014752*	
	EO5	0,006197**	0,622648ns	0,257373ns	0,151702ns	0,161980ns

P> 0.05 ns (not significant difference), 0.01<P<0.05 * (statistically significant difference), P<0.01 ** (statistically highly significant difference).

Effect of essential oils on seedling growth

The results obtained for seedling growth of two ragweed species indicated that seedling length mainly decreased under effects of essential oils. These results are consistent with results of many studies which confirmed inhibitory effect of essential oils on seedling growth. For example, Rassaeifar *et al.* (2013) found essential oils of *Eucalyputs globulus* significantly decreased germination and seedling growth of *Amaranthus blitoides* and *Cynodon dactylon.* Similarly, essential oils from *Cymbopogon citratus, Eucalyptus citrodora* and *Cinnamomum camphora* significantly affected seedling lengths of *Parthenium hysterophorus* (Paudel and Gupta, 2008). Although almost all oils (exception is EO3) in our study reduced seedling length of common and giant ragweed, effects differ between species. Namely, effect of four oils (EO1, EO2, EO4, EO5) were stronger against common than against giant ragweed seedlings, while effect of EO3 was opposite. Therefore, applied oils reduced of common ragweed seedling length more than 97%, except EO3 which increased seedling length 6%. Statistical analysis using t-test revealed that oils EO1, EO2, EO4 and EO5 very significantly ($P<0.01$) reduced seedling length of common ragweed, while simulative effect under treatment with EO3 was insignificant ($P=0.586320$). Contrary to that, all applied oils significantly reduced seedling length of giant ragweed ($P<0.05$) and reduction caused by different oils was similar and between 61 and 75%. Effect of EO3 and EO4 on seedling growth was opposite in comparison to effect on seed germination. Similar to that Angelini *et al.* (2003) found that essential oils from some Mediterranean Lamiaceae did not inhibited germination of *Lactuca sativa* and *Echinochloa crus-galli*, but slowing the germination process and reduced seedling and radicle length.



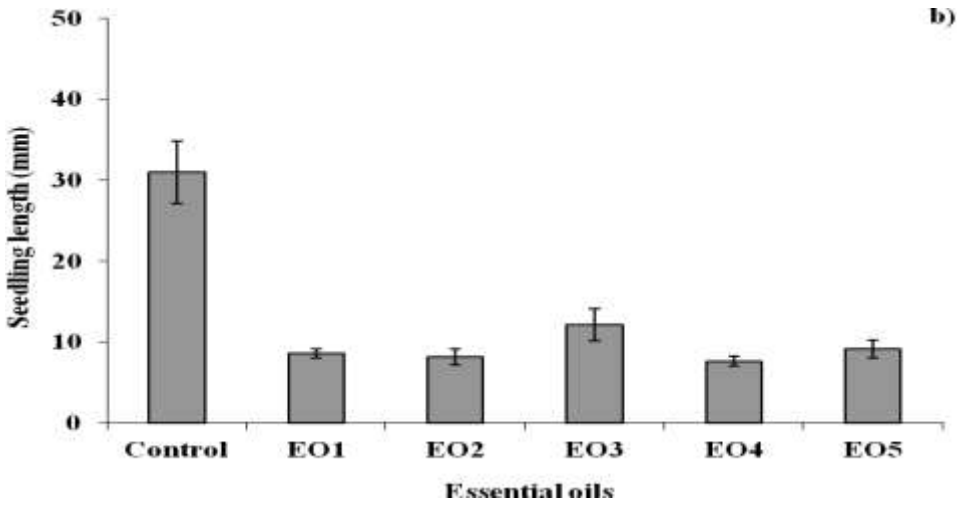


Figure 2. Effect of different essential oils on common (a) and giant (b) ragweed seedling growth.

Table 2. Significance of essential oils effects on common and giant ragweed seedling length (t-test).

		Control	EO1	EO2	EO3	EO4
common ragweed	EO1	0,001058**				
	EO2	0,001794**	0,952068ns			
	EO3	0.586320ns	0,002469**	0,002477**		
	EO4	0,007137**	0,191875ns	0,195333ns	0,005419**	
	EO5	0,004493**	0,568378ns	0,589740ns	0,000979**	0,452878ns
giant ragweed	EO1	0,007926**				
	EO2	0,006166**	0,249705ns			
	EO3	0,164270*	0,031760*	0,022637*		
	EO4	0,008283**	0,200767ns	0,222731ns	0,015708*	
	EO5	0,003365**	0,257016ns	0,871563ns	0,351667ns	0,136315ns

P> 0.05 ns (not significant difference), 0.01<P<0.05 * (statistically significant difference), P<0.01 ** (statistically highly significant difference).

In conclusion, essential oils from *Anethum graveolens*, *Origanum vulgare*, and *Satureja montana* have inhibitory effect on seed germination of both ragweed species, while oils from *Juniperus communis* and *Salvia officinalis* had contrary effect. On the other hand, almost all applied oils decreased seedling growth of ragweed species, with exception of oil from *Juniperus communis*, which had slightly promotional effect on common ragweed seedlings growth. Therefore, obtained results indicate selected essential oils have potential as bioherbicides, but additional studies in the field conditions are necessary, because field conditions can affect oils activity.

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