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Herbicidal effects of *Satureja hortensis* L. and *Melissa officinalis* L. essential oils on germination and root length of *Lollium rigidum* L. and *Phalaris brachystachys* L. grass weeds

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Summary The herbicidal effect of *Satureja hortensis* L. and *Melissa officinalis* L. essential oils was tested on germination and root length of two grass weed species, *Lollium rigidum* L. and *Phalaris brachystachys* L., under laboratory conditions. Carvacrol and citral were the main constituents of the essential oil of *S. hortensis* and *M. officinalis*, respectively. *Melissa officinalis* essential oil showed higher inhibitory effect on germination and root length of *L. rigidum* and *Ph. brachystachys*, respectively. The phytotoxic effects were more pronounced on the latter weed species.

Additional keywords: allelopathy, carvacrol, geranial, neral

Introduction

The continuous use of the synthetic herbicides for weed control apart from their high efficacy, selectivity and relatively inexpensive cost to manufacture has raised concerns about their potential health and environmental impact (Dayan *et al.,* 2009) and the development of herbicide resistance among weed species (Batish *et al.,* 2004). Natural compounds with practical use as biocontrol agents are increasingly adopted in agriculture (Dayan *et al.,* 1999; Duke *et al.,* 2002; Singh *et al.,* 2003). Essential oils obtained from aromatic plants have been reported to exhibit herbicidal activity against seed germination (Muller *et al.,* 1964; Vaughn & Spencer, 1993; Dudai *et al.,* 1999). For example, it has been demonstrated that the essential oils of various aromatic plants, such as *Carum carvi* L. (Apiaceae), *Mentha spicata* L. (Lamiaceae), *Origanum vulgare* L. (Lamiaceae), *Thymbra spicata* L. (Lamiaceae), *Ocimum basilicum* L. (Lamiaceae), *Lavandula spp.* (Lamiaceae) and other members of *Lamiaceae* inhibited seed germination and/or root elongation of various weed species and crops (Vaughn & Spencer, 1993; Dudai *et al.,* 1999; Angelini *et al.,* 2003; Vasilakoglou *et al.,* 2007; Argyropoulos *et al.,* 2008; Azirak & Karaman, 2008).

Lemon balm (*Melissa officinalis* L.) is a perennial plant that belongs to *Lamiaceae* family, native to southern Europe and the Mediterranean region (Simon *et al.,* 1984). Lemon balm has been used in medicine, food, perfume and cosmetic industry (Simon *et al.,* 1984; Tagashira & Ohtake, 1998; Duke *et al.,* 2002; De Almeida *et al.,* 2010). De Almeida *et al.* (2010) found that the essential oil of *M. officinalis* was one of the four most active oils against both germination and radicle elongation of *Raphanus sativus* L. (Brassicaceae), *Lactuca sativa* L. (Asteraceae) and *Lepidium sativum* L. (Brassicaceae).

The genus *Satureja* L. (Lamiaceae) comprises numerous species growing wild in the Mediterranean area (Beziĉ *et al.,* 2005). In Europe, summer savory (*S. hortensis* L.) and winter savory (*S. montana* L.) are the most important species for cultivation (Askun *et al.,* 2012). Previous studies have revealed antioxidant, antibacterial, antifungidal activ-

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ity of summer savory (Gulluce *et al.,* 2003). Concerning the herbicidal activity of savory, Tworkoski (2002) reported that the essential oil of *S. hortensis* was phytotoxic and caused electrolyte leakage resulting in cell death when applied to detached leaves of dandelion *Taraxacum officinale* F. H. Wigg (Asteraceae) in the laboratory. Moreover, Angelini *et al*. (2003) showed that the essential oil of *S. montana* completely inhibited germination both of three crops [*Raphanus sativus*, *Capsicum annuum* L. (Solanaceae), *Lactuca sativa*] and three different annual weeds [*Chenopodium album* L. (Amaranthaceae), *Portulaca oleracea* L. (Portulacaceae), *Echinochloa crus-galli* L. Beauv. (Poaceae)]. Up to now there is limited information about the effect of the essential oil of *S. hortenis* and *M. officinalis* on grass weed species. The objective of this study was to evaluate in laboratory conditions the allelopathic activity of *S. hortensis* and *M. officinalis* essential oils on germination and root elongation of annual ryegrass [*Lollium rigidum* L. (Poaceae)] and short spiked canarygrass [*Phalaris brachystachys* L. (Poaceae)].

Materials and methods

Essential oils isolation

The plant material, aerial parts of *M. officinalis* and *S. hortensis,* was collected at bloom stage from experimental plots of Medicinal and Aromatic Plants Department of the National Agricultural Research Foundation (NAGREF) in Thessaloniki, Greece (40° 32΄ 16.32΄΄ B, 23° 00΄ 00.65΄΄ E).

Dried upper leaves, 100g and 30 g of *M. officinalis* and *S. hortensis,* respectively, were hydrodistilled for two hours with a distillation rate of 3 to 3.5 mL/min by using a Clevenger–type apparatus (Chatzopoulou *et al.,* 2006).

Essential oil analyses

The essential oil samples were analyzed by Gas Chromatograph Hewlett Packard 5890 Series II connected to a chromatographic integrator (Hewlett Packard 3396 Series II Dual Channel). Two fused silica columns of different polarity were used: Durabond-DB 1 and DB-Wax. Temperature program: 45 to 220°C at 3.5°C/min, carrier gas nitrogen: 140Kpa, injection temperature: 220°C, detector temperature 300°C. Sample injection: 0.2–0.3μl of a 10% essential oil solution in pentane; split 1:20. The percentage compositions were computed after 3 GC runs of each sample from the peak areas without correction factors. The GC/MS analysis was performed on a fused silica column DB-5, using a Gas Chromatograph 17A Ver. 3 interfaced with a Mass Spectrometer Shimadzu QP-5050A supported by the Class 5000 software. Injection temperature: 260°C, interface heating: 300°C, ion source heating: 200°C, EI mode: 70eV, scan range: 41-450 amu, and scan time 0.50s. Oven temperature programs: a) 55-120°C (3°C/min), 120-200°C (4°C/min), 200-220°C (6°C/min) and 220°C for 5min and b) 60-240°C at 3°C/min, carrier gas He, 54.8kPa, split ratio 1:30.

Identification of the essential oils components

The identification of the constituents was based on comparison of their Kovats indices (RI) relative to n-alkanes with corresponding literature data, as far as by matching a) their spectra with those from MS libraries (NIST 98) (Adams, 1995) and b) the RT of co-eluting reference compounds – peak enrichment technique (authentic samples by Roth and Sigma Aldrich).

Petri dish bioassay

Petri dish bioassay was performed to compare the germination and the radical length of annual ryegrass and shortspiked canarygrass treated with different concentrations of essential oils obtained from *S. hortensis* and *M. officinalis*. Twenty seeds of each species were placed separately at the bottom of 10cm glass Petri dishes and were covered with 5g of perlite. Deionized water (12ml) was added in each Petri dish. Each essential oil was loaded on a piece of filter paper, which was attached to the inner side of a small aluminum cup placed in the centre of each Petri dish, at 0, 8, 16, 32

and 64μl. Taking into account that the volume of the Petri dishes was approximately 100 cm^3, the above quantities were equal to concentrations (v/v) of 0, 0.08, 0.16, 0.32 and 0.64μl of essential oil per ml of Petri dish, respectively. Each Petri dish was sealed by film so as to prevent escape of the volatile compounds and all the Petri dishes were placed in a growth chamber in the dark at 22°C. The Petri dishes were arranged in a completely randomized design. After seven days of incubation, the germinated seeds were counted and their radical length was recorded. Each treatment was replicated three times and the bioassay trial was repeated twice and data were averaged. Bioassay data were analyzed over repetition time by using a factorial approach (essential oil x essential oil concentration). Data was expressed in % of control and was arcsine square root transformed before the ANOVA to reduce the heterogeneity, but means presented are back-transformed. LSD (5%) values were employed for the mean comparison. The ANOVA was performed separately for each weed species and oil.

Results and Discussion

Essential oil composition

The main essential oils' constituents and their percentage yield are presented in Table 1. In *S. hortnesis* oil, carvacrol (46.94%) and *γ*-terpinene (29.14%) were the main components, followed by *a*-terpinene (5.16%) and *p*-cymene (4.62%). In *M. officinalis* oil the dominant components were geranial (47.16%) and neral (36.10%).

Inhibitory effects

Both germination and root length of each species were significantly affected by essential oil concentration ($P<0.001$), however not by the interaction of essential oil x oil concentration. Statistically significant difference in the effect of the two essential oils was recorded. In particular, increased inhibition of *L. rigidum* germination and *Ph. brachystachys* root length were recorded when treated with *M. officinalis* essential oil (Table 2). Since germination and root length was significantly affected by oil concentration, ANOVA was performed separately for each weed species and oil. More specifically, the germination of *L. rigidum* ranged from 70 to 99% and 57 to 91% of control after the application of *S. hortensis* and *M. officinalis* essential oils, respectively, while, the root length of this weed species ranged from 28 to 54% and 25 to 66% of control, respectively (Table 3). Inhibition of *Ph. brachystachys* germination was more pronounced and ranged from 28 to 75% and from 21 to 85% of the control when treated with *S. hortensis* and *M. officinalis* oil, respectively (Table 4). The root length of this weed species ranged from 24 to 71% and from 8 to 74% of control, respectively (Table 4).

The main components of the essential oil of *M. officinalis* are citral (neral and geranial), citronellal, linalool, geraniol and *β*-caryophyllene-oxide (Adzet *et al.,* 1992). Citral is a mixture of two monoterpenes: geranial (citral A) and neral (citral B). It is a volatile essential oil component of lemongrass [*Cymbopogon citrate* (Poaceae)] and other aromatic plants and shows allelopathic traits (Dudai *et al.,* 1999). In a previous study, citral exhibited low phytotoxicity to soybeans and high phytotoxicity to velvetleaf [*Abutilon theophrasti*, Medik. (Malvaceae)], large crabgrass [*Digitaria sanguinalis*, L. Scop. (Poaceae)], redroot pigweed [*Amaranthus retroflexus*, L. (Amaranthaceae)] and italian ryegrass [*Lollium multiflorum*, Lam. (Poaceae)] (Vaughn & Spencer, 1993). Citral was found to be a strong inhibitor of wheat, *Amaranthus palmeri* S. Wats. (Amaranthaceae) and *Brassica nigra* L. (Brassicaceae) seed germination (Dudai *et al.,* 1999). Dudai (2007) reported that citral is absorbed by wheat seed through the abscission layer, and that it reaches highest concentration in the embryo, where it accumulates in the aleurone, scutellum and parts of the endosperm. As reported by Chaimovitsh *et al.* (2012) citral, as an allelochemical or a potential herbicide interferes with cell division cytokinesis and cell elongation. More specifically, they reported that at lower concentrations citral in-

Table 1. Chemical composition of *Satureja hortensis* and *Melissa officinalis* essential oils.

S. hortensis essential oil constituents	% yield	*M. officinalis* essential oil constituents	% yield
a-thujene	2.10	2-hexenal	0.27
α-pinene	1.90	1-octen-3-ol	0.59
β-pinene	1.05	5-hepten-2one-6-methyl	1.10
β-myrcene	3.20	*β*-myrcene	0.17
a-phellandrene	0.58	trans *b*-ocimene	0.13
a-terpinene	5.16	linalool	0.59
p-cymene	4.62	citronellal	0.32
sylvestrene	0.90	neral	36.10
γ-terpinene	29.14	geranial	47.16
carvacrol	46.94	methylgeranate	0.31
β-caryophyllene	1.29	geranyl acetate	2.98
b-bisabolene	0.70	*β*-caryophyllene	2.62
		caryophyllene oxide	1.57

Table 2. Effect of essential oil (% of control) of *Satureja hortensis* and *Melissa officinalis* on germination and root length of *Lollium rigidum* and *Phalaris brachystachis.*

Essential oil	*L. rigidum*		*Ph. brachystachys*	
	germination	root length	germination	root length
	% of control			
S. hortensis	82[1] a[2]	38 a	54 a	47a
M. officinalis	72 b	41 a	47 a	35 b
	**P<0.05	NS[3]	NS	***P<0.001

[1] Data were arcsine square root transformed before statistical analyses, De-transformed data are presented.
[2] Means within each column followed by the same letter are not significantly different at P = 5% level of significance
[3] NS= not significant

terferes with cell division by disrupting mitotic microtubules and cell plates while at higher concentrations it inhibits cell elongation by disrupting cortical microtubules. Carvacrol has also been reported to exhibit potential for use in weed control (Azirak & Karaman, 2008).

In the present study, the essential oil of *M. officinalis*, with citral as main constituent, showed greater phytotoxicity on *L. rigidum* germination and root length of *Ph. brachystachys* compared to *S. hortensis* essential oil, with carvacrol being its main component. The greater effect of *M. officinalis* essential oil could be attributed to higher citral concentration (83%), as compared with carvacrol concentration (47%) in *S. hortensis* essential oil. Our findings come in agreement with the results of Dudai *et al.* (2000) which revealed that inhibition of wheat germination was more pronounced by citral compared to carvacrol. Apart from the higher activity of *M. officinalis*, different response of the two grass weed species was evident, with the *Ph. brachystachys* germination more affected by both essential oils. However, previous studies reported that whatever activity has been found against a certain target species will not necessarily be maintained against another target species, even at the same family or genus (Vokou *et al.,* 2003). Summarizing, the results of this study indicated that both essential oils exhibited herbicidal activity on both grass weed species, with the essential oil of *M. officinalis* to be more active and *Ph. brachystachys* to be more suscepti-

Table 3. Effect of *Satureja hortensis* and *Melissa officinalis* essential oil concentration on *Lolium rigidum* germination and root length (% of control).

Concentration (µl/ml)	Essential oil			
	S. hortensis	*M. officinalis*	*S. hortensis*	*M. officinalis*
	germination		root length	
	% of control			
0.08	99 b	91 c	54 b	66 c
0.16	83 ab	78 b	38 a	43 b
0.32	74 a	64 ab	33 a	30 ab
0.64	70 a	57 a	28 a	25 a

Same letters within each column indicate no significant difference between means at P= 5%.

Table 4. Effect of *Satureja hortensis* and *Melissa officinalis* essential oil concentration on *Phalaris brachystachis* germination and root length (% of control)

Concentration (µl/ml)	Essential oil			
	S. hortensis	*M. officinalis*	*S. hortensis*	*M. officinalis*
	germination		root length	
	% of control			
0.08	75 c	85 c	71 c	74 d
0.16	64 bc	52 b	53 bc	37 c
0.32	50 b	30 a	37 ab	19 b
0.64	28 a	21 a	24 a	8 a

Same letters within each column indicate no significant difference between means at P= 5%.

ble. Further studies could provide more information concerning the concentration of each oil applied in order to achieve high efficacy against weeds.

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Ζιζανιοκτόνος δράση των αιθερίων ελαίων των *Satureja hortensis* L. και *Melissa officinalis* L. στη βλάστηση και το μήκος ριζιδίου των αγρωστωδών ζιζανίων *Lollium rigidum* L. και *Phalaris brachystachys* L.

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Περίληψη Σε συνθήκες εργαστηρίου μελετήθηκε η ζιζανιοκτόνος δράση των αιθερίων ελαίων των *Satureja hortensis* L. και *Melissa officinalis* L. στη βλάστηση και το μήκος ριζιδίου των αγρωστωδών ζιζανίων *Lollium rigidum* L. and *Phalaris brachystachys* L. Η καρβακρόλη και η κιτράλη ήταν τα κύρια συστατικά των αιθερίων ελαίων των *S. hortensis* και *M. officinalis*, αντίστοιχα. Το αιθέριο έλαιο του *M. officinalis* επηρέασε περισσότερο τη βλάστηση του ζιζανίου *L. rigidum* και το μήκος ριζιδίου του ζιζανίου *Ph. brachystachys*. Η ζιζανιοκτόνος δράση των αιθερίων ελαίων ήταν περισσότερο έντονη στο ζιζάνιο *Ph. brachystachys*.

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