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Bożena Denisow; University of Life Sciences in Lublin, Poland; https://orcid.org/0000-0001-6718-7496

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MTN conceived and designed the experiments, analyzed the data, and wrote the manuscript; EYT and TS conducted the in vitro seed germination experiment; BT performed the in vivo seed germination experiment and isolated the essential oils; IA and AD contributed to the identification and collection of the plant material; SB contributed to the analysis of the essential oils and writing the manuscript

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ORIGINAL RESEARCH PAPER in HERBOLOGY

Herbicide Potential of Selected Essential Oils From Plants of Lamiaceae and Asteraceae Families

Milena Nikolova[1*], Boryanka Traykova[1], Elina Yankova-Tsvetkova[1], Tatyana Stefanova[1], Anatoli Dzhurmanski[2], Ina Aneva[1], Strahil Berkov[1]

[1]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Bulgaria
[2]Institute of Roses, Essential and Medicinal Cultures, Kazanluk, Bulgaria

[*] To whom correspondence should be addressed. Email: mtihomirova@gmail.com

Abstract

Essential oils from *Monarda fistulosa* L., *Satureja pilosa* Vel., *Origanum vulgare* subsp. *hirtum* Ietswaart. (Greek oregano), *Micromeria dalmatica* Benth., *Thymus longedentatus* (Degen & Urum.) Ronniger, and *Artemisa campestris* L. were evaluated as inhibitors of seed germination in target plants *Lolium perenne* L. and *Trifolium pratense* L. using in vitro assays. The essential oils were applied on the seeds as aqueous solutions at concentrations ranging from 0.5–3.0 µL/mL. Complete inhibition was established at a concentration of 1.5 µL/mL with the most effective essential oils. Oregano oil was evaluated for its inhibitory activity on seed germination under field conditions; the essential oil was applied as an aqueous solution at concentrations of 3, 5, and 10 µL/mL on superabsorbent Terawet. The mass obtained was mixed with the seeds of the target plants and planted in a field. After 1 month, the results were evaluated based on the weight of the aerial parts of the plants from control and experimental areas. At the highest concentration studied, the weight of the plants was 77% lower in the experimental areas than in the controls. The chemical composition of the essential oils was analyzed using gas chromatography–mass spectrometry. The results showed that carvacrol-rich essential oils had a strong inhibitory effect on seed germination. The inclusion of the essential oil on a superabsorbent was a good way to preserve its herbicidal activity under field conditions as this prevented its rapid evaporation.

Keywords

inhibition of seed germination; *Micromeria*; *Artemisia*; *Satureja*; *Monarda*; *Origanum*; *Thymus*

1. Introduction

The use of natural products for controlling crop pests is a priority in modern organic farming. Recently, many studies have focused on assessing the herbicidal potential of plant extracts and essential oils (Bari & Kato-Noguchi, 2017; Campiglia et al., 2007; Chovancova et al., 2019; Frabboni et al., 2019; Haliniarz et al., 2020; Nikolova & Berkov, 2018; Synowiec et al., 2017; Synowiec & Nowicka-Połeć, 2016; Werrie et al., 2020). Some components of essential oils – such as carvacrol (a monoterpene phenol), thymol, α- and β-pinene, 1,8-cineole, borneol, limonene, and camphor – have a strong inhibitory activity on seed germination (Amri et al., 2013; Bendre et al., 2018; Dayan et al., 2012). Essential oils isolated from some species of Lamiaceae, Asteraceae, Mirtaceae, and Pinaceae could be promising sources of herbicides (Grul′ová et al., 2020; Hazrati et al., 2018; Ibáñez & Blázquez, 2017). The present study examines previously unexplored species that are taxonomically close to species with proven herbicidal potential. For example, *Satureja pilosa* was

selected for this study because *Satureja hortensis* essential oil has been examined as an inhibitor of seed germination for a long time and commercial products have been developed based on it (Gitsopoulos et al., 2013; Hazrati et al., 2017). Similarly, *Artemisia campestris* was selected because there are data for the phytotoxic potential of other *Artemisia* species (Benvenuti et al., 2017; Kaur et al., 2010). *Monarda fistulosa* has been reported to contain carvacrol (Ghosh et al., 2020). Citral isomers (geranial and neral) have been found to be substances with strong herbicidal effects (Dayan et al., 2012). *Thymus longedentatus*, a plant endemic to the Balkans, was also selected for this study, because a phytochemical analysis had showed that its essential oil profile is rich in geranial and neral (Aneva et al., 2019).

Finally, in our preliminary study *Origanum vulgare* subsp. *hirtum* and *Micromeria dalmatica* have been selected as promising sources of herbicidal activity for more detailed study (Yankova-Tsvetkova et al., 2020).

For the aforementioned reasons, therefore, the essential oils of selected species from the families Lamiaceae and Asteraceae – i.e., *Satureja pilosa*, *Origanum vulgare* subsp. *hirtum*, *Monarda fistulosa*, *Thymus longedentatus*, *Micromeria dalmatica*, and *Artemisia campestris* – were evaluated for the inhibitory effect they had on seed germination under laboratory conditions.

Although there has been extensive laboratory research on the herbicidal potential of essential oils, the same work in field conditions has been limited (Verdeguer et al., 2020). The main problem that must be solved when applying essential oils in the field is their volatility. Various approaches have been applied to overcome this disadvantage, including the encapsulation of the essential oil, its inclusion in nanoemulsions (Alipour et al., 2019; Hazrati et al., 2017). Thus, the second aim of the present study was to assay the inhibitory activity of essential oils on seed germination in the field by applying the most effective essential oil onto superabsorbent Terawet (https://terawet.eu/) used in the soil.

2. Material and Methods

2.1. Plant Material

Material was collected from *Monarda fistulosa* L. (flower heads), *Satureja pilosa* Vel. (aerial parts), and *Origanum vulgare* subsp. *hirtum* Ietswaart. (aerial parts) at an experimental field at the Institute for Roses and Aromatic Plants, Kazanluk, Bulgaria. The field was in the Kazanlak Valley at an altitude of 372 m a.s.l. The soil type was the Cinnamonic forest and the climate was transitional continental. Aerial parts of *Micromeria dalmatica* Benth., *Thymus longedentatus* (Degen & Urum.) Ronniger, and *Artemisia campestris* L. were collected from the natural populations. All samples were collected in 2019 while the plants were in the flowering stage. The plant material gathered was dried at room temperature (23–26 °C) without direct sunlight.

Seeds from the target plants – *Lolium perenne*, *Trifolium repens*, and *Trifolium pratense* – were purchased from Florian Company, Bulgaria (http://www.florianbg.com/).

2.2. Isolation and Identification of Essential Oils

The essential oil was extracted from *O. vulgare* subsp. *hirtum* via water distillation performed using a Clevenger apparatus. Oil sample analyses were performed by a Thermo Scientific Focus gas chromatograph equipped with a Thermo Scientific DSQ II mass detector coupled with an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness of 0.25 μm). The detailed chromatographic conditions were described by Traykova et al. (2019). The components were identified by comparing their mass spectra and retention indices (RI) with the retention indices of authentic standards, mass spectra from the National Institute of Standards and Technology (NIST), and data from the literature (Adams, 2007).

2.3. Inhibition of In Vitro Seed Germination

Aqueous solutions of the essential oils were prepared at concentrations of 0.5, 1.0, 1.5, 2.0, and 3.0 µL/mL using 0.1% Tween 40 (Sigma-Aldrich) as an emulsifier. One hundred *L. perenne* and *T. pratense* seeds were placed in Petri dishes on filter papers moistened with the test solutions and incubated at room temperature for 7 days. The rate of germination inhibition was calculated using the formula in Atak et al. (2016). The IC_{50} values were calculated using Prizm 3.00.

2.4. Inhibition of Field-Based Seed Germination

The experiment was conducted at an experimental field at the Institute of Biodiversity and Ecosystem Research, Sofia, located at an altitude of 595 m a.s.l.; the soils were resinous. A dry superabsorbent (5 g) was placed in 1 L of the different concentrations of the aqueous essential oil solution (3, 5, and 10 µg/mL). Essential oil solutions were prepared using 0.1% Tween 40 as an emulsifier. After the superabsorbent took up the aqueous solution, the amount of each superabsorbent that was moistened was divided into three to produce three replicates. Each part (1/3) of the superabsorbent was mixed with 15 g of the seeds being tested (5 g of *L. perenne*, *T. repens*, and *T. pratense*) and spread out in surface soil over the entire plot area (40 × 60 cm). In the control area, the superabsorbent was treated only with water. After 30 days, the aboveground parts of the plants that developed in each experimental area were collected and their fresh and dry weights were determined. The samples were collected during the vegetative stage. Inhibition of seed germination and growth was expressed as a percentage of the reduction in the weight of the plant material in the experimental area relative to that in the control. The reduction rate was calculated using the following equation:

$$Reduction\ fresh/dry\ plant\ material\ (\%) = \frac{W_{exp} - W_{cont}}{W_{cont}} \times 100,$$

where W_{exp} is the weight of the aerial parts of weeds in the experimental area and W_{cont} is the weight of the aerial parts of weeds in the control area.

2.5. Statistical Analysis

Statistical analyses were performed using Microsoft Excel. The results are presented as the mean values ± standard division (*SD*) of three replicates. Significant levels were set at $p < 0.05$ as determined by a *t* test.

3. Results

3.1. Composition of Essential Oils

The composition of the essential oils in the species studied is presented in Table 1. Carvacrol was identified as the main component of the essential oils in *M. fistulosa*, *S. pilosa*, and *O. vulgare* subsp. *hirtum*. In *M. dalmatica* essential oil, monoterpene ketone-piperitone oxide, β-pinene, and limonene were the most abundant components along with carvacrol. Capillene (phenyl alkyne), β-pinene, and ocimene were found in large amounts in the essential oil profile of *A. campestris*. Citral isomers – geranial and neral – were established as the main compounds in the essential oil from *T. longedentatus*.

3.2. Inhibition of In Vitro Seed Germination

The results of the analysis of the inhibitory activity demonstrated by the essential oils against germination of *L. perenne* and *T. pratense* seeds are presented in Table 2. All the oils studied showed significant inhibitory activity, with IC_{50} (the concentration at which 50% inhibition occurred) ranging from 0.52 to 1.6 µL/mL against *L. perenne* seeds and 0.97 to 3 µL/mL against *T. pratense* seeds. The action of the essential oils on seeds of the target plants was found to be selective; *L. perenne* seeds were more affected than *T. pratense* seeds.

Table 1 Identified compounds in the essential oils of studied species.

Compounds	RI*	Content of the studied essential oils (%)					
		Ovh**	Sp	Mf	Ac	Md	Tl
α-Thujene	930		0.8	2.6			
α-Pinene	932	0.7			1.9	2.2	0.7
Camphene	946	0.8					0.8
Sabinene	971						0.3
β-Pinene	978	4.9		2.1	11.9	14.1	0.4
β-Myrcene	988	1.9					3.7
p-Cymene	1,025	12.6					
1,8-Cineole	1,026						7.8
Sylvestrene	1,031					10.5	
cis-β-Ocimene	1,032	0.3					0.4
trans-β-Ocimene	1,044	8.2	10.5	20.9	13.1		7.5
γ-Terpinene	1,059	2.9		0.5	4.1		
Linalool	1,094	0.3	8.7				1.1
Camphor	1,141						2.1
Isocitral	1,160						2.2
Terpinen-4-ol	1,175	0.9	0.5		0.3	0.2	0.2
Terpineol	1,186						0.8
Neral	1,227						27.5
Pulegone	1,237					0.2	
Carvacrol methyl ether	1,245	0.3	0.9				1.6
Geraniol	1,249						0.5
Piperitone	1,253					3.6	
Geranial	1,264						30.3
Thymol	1,290	0.2	21.2	21.5			
Carvacrol	1,299	60.5	54.8	50.5		10.8	
Piperitone oxide	1,369					34.7	
Eugenol	1,359				4.1		
Neryl acetate	1,359						1.8
Geranyl acetate	1,379						1.9
β-Bourbonene	1,387					0.9	0.3
cis-β-Farnesene	1,440						0.6
Germacrene	1,480					2.9	4.4
β-Caryophyllene E	1,466	2.8					
Capillene (phenyl alkynes)	1,490				37.2		
Germacrene D-4-ol	1,576						0.8
Spathulenol	1,578				0.6		
Caryophylene oxide	1,590	0.6				0.3	
α-Cadinol	1,652						0.4

* RI – retention index; ** Ovh – *Origanum vulgare* ssp. *hirtum*; Sp – *Satureja pilosa*; Mf – *Monarda fistulosa*; Ac – *Artemisia campestris*; Md – *Micromeria dalmatica*; Tl – *Thymus longedentatus*.

3.3. Inhibition of In Field Seed Germination

Oregano essential oil was tested for use as an inhibitor of seed germination on target plants in the field. To achieve this, the essential oil was included in a superabsorbent, thereby allowing its gradual release and relatively long-lasting effect. The inhibitory effect of the essential oil was found to be dose-dependent and resulted in a decrease in weight of the aerial parts of plants that developed from the experimental areas compared to those that developed in the control. The fresh weight of the aerial parts of the target plants dropped by 11%, 32%, and 77% when the mixture of superabsorbent and essential oil was applied at concentrations of 3, 5, and 10 µL/mL,

Table 2 Inhibition on seed germination of target weeds by essential oil of selected species.

Target weeds	C* (µL/mL)	Applied essential oils of the studied species					
		Ovh**	Mf	Sp	Tl	Md	Ac
Lolium perenne	0.5	81 ± 8	71 ± 9	33 ± 8	17 ± 1	15 ± 4	21 ± 4
	1.0	100 ± 0	100 ± 0	90 ± 9	28 ± 3	27 ± 5	36 ± 3
	1.5	100 ± 0	100 ± 0	100 ± 0	79 ± 2	55 ± 9	48 ± 9
	2.0	100 ± 0	100 ± 0	100 ± 0	98 ± 2	98 ± 2	96 ± 5
	3.0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	IC_{50}	0.52	0.53	0.83	1.3	1.5	1.6
Trifolium pratense	0.5	24 ± 5	27 ± 7	12 ± 0.1	7 ± 3	3 ± 2	11 ± 4
	1.0	59 ± 6	64 ± 5	46 ± 12	22 ± 4	3 ± 1	17 ± 7
	1.5	100 ± 0	100 ± 0	94 ± 7	27 ± 8	6 ± 3	19 ± 6
	2.0	100 ± 0	100 ± 0	100 ± 0	48 ± 9	10 ± 4	25 ± 8
	3.0	100 ± 0	100 ± 0	100 ± 0	63 ± 8	27 ± 6	67 ± 10
	IC_{50}	0.97	0.99	1.02	2.5	3.5	3

* Concentration; ** Ovh – *Origanum vulgare* ssp. *hirtum*; Sp – *Satureja pilosa*; Mf – *Monarda fistulosa*; Ac – *Artemisia campestris*; Md – *Micromeria dalmatica*; Tl – *Thymus longedentatus*.

respectively. The ratios expressed relative to the dry weight dropped by 19%, 41%, and 83% at essential oil concentrations of 3, 5, and 10 µg/mL, respectively. These reductions in the weights of the target plants reflect the smaller number of seeds that germinated. The differences in weight between the control and those treated with essential oil solutions at 5 and 10 µL/mL concentrations were significant ($p < 0.05$).

4. Discussion

4.1. Composition of Essential Oils

A monoterpene phenol – carvacrol – was identified as the main component in *S. pilosa*, *M. fistulosa*, and *O. vulgare* subsp. *hirtum* essential oils. These results agree with previously reported data (Ghosh et al., 2020; Mancini et al., 2014; Semerdjieva et al., 2020). The essential oil profile of *T. longedentatus* was reported in our previous study (Aneva et al., 2019).

4.2. Inhibition of In Vitro Seed Germination

All the oils studied showed significant inhibitory effects on seed germination. The established inhibitory activity was comparable to that reported for *Satureja hortensis*, *Thymus mastichina*, *Rosmarinus officinalis*, *Laurus nobilis*, and commercial samples of oregano and marjoram essential oils (Hazrati et al., 2017, 2018; Ibáñez & Blázquez, 2017). Essential oils obtained from the species *O. vulgare* subsp. *hirtum*, *M. fistulosa*, and *S. pilosa*, all of which were rich in carvacrol, showed the strongest activity. The good inhibitory effect exhibited by the above-mentioned essential oils on seed germination was likely due to their high carvacrol content. This compound is reported to have strong biocidal (phytotoxic, antifungal, and insecticidal) properties (Bendre et al., 2018; Liu et al., 2019; Muñoz et al., 2020). In addition, citral isomers and β-pinene have also been reported to have a high herbicide potential (Amri et al., 2013; Bendre et al., 2018). These compounds, as the main components of the other essential oils studied, are likely to determine their herbicidal properties. To the best of our knowledge, this was the first study to examine the essential oils from *S. pilosa*, *M. fruticosa*, and *A. campestris* as inhibitors of seed germination.

4.3. Inhibition of In Field Seed Germination

There are many fewer studies on the application of essential oils as herbicides in vivo than in vitro. The high volatility of essential oils is the reason why different approaches, such as nanoemulsions and encapsulation (Ibáñez et al., 2020;

Verdeguer et al., 2020), are used to apply them in field conditions. The present study proposes a new approach in which the essential oil was included as a solution with a superabsorbent. The essential oil was thus released gradually and had a longer effect.

5. Conclusion

The results of this study showed that essential oils from *M. fistulosa*, *S. pilosa*, *O. vulgare* subsp. *hirtum*, *M. dalmatica*, *T. longedentatus*, and *A. campestris* had significant inhibitory effects on the germination of seeds from the target plants studied; IC_{50} values 0.52–3 µL/mL were recorded, depending on the oil and target plant. Introducing the essential oil together with a superabsorbent prevented its rapid evaporation and was a good way to preserve its herbicidal activity in field conditions.

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