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Herbicide Effect of Greek Oregano Essential Oil on Metabolite Profiles of Target Plants

Milena Nikolova[*]*, Boryanka Traykova, Strahil Berkov*

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 23, Acad. G. Bonchev str., 1113 Sofia, BULGARIA
[*]Corresponding author: mtihomirova@gmail.com

Abstract. *Origanum vulgare* ssp. *hirtum* (Link) Ietsw. essential oil has been found to possess a wide range of biological activity among which the biocidal properties are particularly valuable in view of the need to use natural products in organic farming. In the present study, attention is focused on the metabolic variations of weeds after treatment with Greek (white) oregano essential oil as a bioherbicide. *Dasypyrum villosum* (L.) Borbás, *Matricaria chamomilla* L., *Sinapis arvensis* L., *Lolium perenne* L., *Trifolium repens* L. and *Trifolium pratense* L. were used as target weeds. The essential oil was applied on weed seedling in the form of an aqueous solution at 5 and 10 µg/mL concentrations by spraying. The effect was reported on the seventh day after treatment and expressed as lethality percentage. The studied Poaceae species were found to be the most resistant, retaining almost 100% of their viability at the both tested concentrations, while the other species at the higher concentration were completely destroyed or significantly damaged. The aerial parts of the surviving individuals of each species were collected and examined by GC/MS for the content of main metabolites. Organic, phenolic, fatty and amino acids, sterols, polyols as well as mono- and disaccharides were identified. Variations in the content of metabolites after treatment with essential oil were observed. The response of treated plants appears to be specific to each species. The results obtained provide data on the use of Greek oregano essential oil as an herbicide in post-emergence stage and complement knowledge of metabolic response of plant to stress factors.

Key words: *Origanum vulgare ssp. hirtum,* phytotoxicity, eco-metabolomics, abiotic stress.

Introduction

Eco-Metabolomics (or "Ecometabolomics") is a new field of study of metabolic response and accumulation to environmental changes or allelopathic interactions (Sardans et al., 2011; Peters et al., 2018; Sardans et al., 2020). Accepting the broader meaning of this concept, metabolic changes arising under the influence of pesticides can be included also. Although Lydon & Duke (1989) summarized the data concerning to effects of pesticides on secondary metabolites of higher plants this type of researchers is limited. In the last decades essential oils are examined as a promising alternative to synthetic herbicides (Campiglia et al., 2007; Cai & Gu, 2016; Nikolova & Berkov, 2018; Synowiec et al., 2019; Frabboni et al., 2019; Verdeguer et al., 2020). The most of the research have been directed to the establishment of inhibitory activity of essential oils against seed germination in *in vitro* assays (Ibáñez & Blázquez, 2017; Hazrati et al., 2018; Grulová

et al., 2020). Much less studies have been conducted *in vivo* in greenhouse or in the field conditions, as well as those related to the application of essential oils at post-emergence stage (Frabboni et al., 2019; Verdeguer et al., 2020). Despite the demonstrated toxicity of essential oils against weeds, Jouini et al. (2020) have established their safety for soil organisms which confirms their potential such as environmentally friendly herbicide. Essential oils of *Origanum vulgare* and other Lamiaceae species have been proved to possess significantly phytotoxic potential (de Almeida et al., 2010; Atakm et al., 2016; Ibáñez & Blázquez 2017). In a our previous screening study of plant extracts and fractions as inhibitors of seed germination, the essential oil of *Origanum vulgare* ssp. *hirtum* was identified as the most active (Yankova-Tsvetkova et al., 2020). In the present study herbicidal potential of Greek oregano essential oil was assessed by *in vivo* experiment treating seedlings of six plant species with an aqueous solution of the essential oil. In addition, aerial parts of surviving individuals were analyzed for content of main metabolites by GC/MS. Data on metabolic changes complement the assessment of the phytotoxic potential of the essential oil.

Material and methods

Plant materials. Aerial parts of donor plant (*Origanum vulgare* ssp. *hirtum*) were collected from the *ex situ* collection of Institute of Biodiversity and Ecosystem Research (IBER), http://www.iber.bas.bg/sites/default/files/projects/plantscollection/index.html.
Seeds of target species were collected from natural population (*Dasypyrum villosum*) and *ex situ* collection of IBER (*Matricaria chamomilla, Sinapis arvensis*) as well as were purchased from Florian Company - https://brcci.eu/en/florian-ood (*Lolium perenne, Trifolium repens* and *Trifolium pretense*).

Isolation and identification of the essential oil composition. Essential oil of *O. vulgare* ssp. *hirtum* was extracted on Clevenger apparatus by water distillation. The conditions of isolation and identification of the essential oil composition was described by Traykova et al., (2019).

In vivo toxicity test. Number of fifteen seeds per plant species were planted in plastic pot (8 cm diameter) filled with substrate. Pots were placed in a growth chamber with average temperature 23°C and 30% humidity. The studied plants at second true leaf stage were sprayed with an aqueous solution of essential oils at concentration 5 and 10 µl/mL by hand sprayer. The spraying rate was 50 mL/m^2. Seven days after spraying, the treated plants were checked for visible injury and the vital individuals were counted. The results are reported as the lethality percentage (LP) using the following formula:

$$LP=[N-n/N] \times 100,$$

where: N is number of healthy individuals before treatment; n is number of alive individuals after treatment.

The experiment was repeated three times for each plant species. Aerial parts of the surviving individuals were collected for subsequent analysis by GC/MS.

GC/MS analysis. Air dried, ground plant material of target plants (50 mg) was placed in Eppendorf and was extracted with 1mL methanol for 24 hours at room temperature with added internal standard 3,4 dichloro-4-hydroxybenzoic acid (50 µg/mL) at the beginning of the extraction. The amounts of metabolites were estimated against this standard. For GC/MS analysis 300 µL of each extract was transferred to a vial and evaporated to dryness, then silylated with 50 µL of N, O-Bis-(trimethylsilyl) trifluoroacetamide (BSTFA) in 50 µL of pyridine for 2 h at 50°C. The spectra were recorded on a Thermo Scientific Focus GC combined with a Termo Scientific DSQ mass

detector as described previously (Nikolova et al., 2019).

Statistical analysis. Statistical analyzes were performed using Microsoft Excel software. The results are presented as mean with standard deviation (SD).

Results and Discussion

Essential oil composition. Chemical composition of *Origanum vulgare* ssp. *hirtum* essential oils was analyzed by GC/MS. The main components were identified as carvacrol (74,34%), *p*-cymene (9,46%), *γ*-terpinene (4,24%) and *β*-pinene (1,73%). The other components are presented in quantities of less than 1%.

The established composition of the essential oil is in accordance with the previously reported profiles of samples collected from Bulgaria and Hungary (Veres et al., 2003; Konakchiev et al., 2004).

In vivo toxicity test. Seeds of five target species were grown in plastic plots to the second true leaf stage. At this stage seedling were treated with essential oil and after 7 days the results were assessed by the number of surviving individuals and their morphological status. It was established that the treatments had the phytotoxic effect on the target plants. The plant response to essential oil treatment was found to be dose-dependent and specific to each species. Poaceae species – *D. villosum* and *L. perenne* had kept their vitality at 100% at the both tested concentrations, while the other species – *S. arvensis*, *M. chamomilla*, *T. repens T. pratense* at the higher tested concentration were completely destroyed or significantly reduced (Table 1). The treatment of weeds with 5 μg/mL essential oil solution had caused 64% mortality of *T. repens*, 41% of *T. pretense,* 33% of *S. arvensis* and 15 % of *M. chamomilla*. No lethal effect was found for Poaceae species. Application of a solution with concentration at 10 μg/mL resulted to lethality rates 100% of *T. repens,* 94% of *M. chamomilla,* 89% of *T. pretense,* 42% of *S. arvensis* and 33% of *L. perrene.* No lethal effect was found on *D. villosum*. The state of untreated (controls) and the treated *T. pratense* plants with the both concentrations of essential oil, seven days after treatment, are presented at Fig.1

Morphological changes on the treated plants were examined also. On Poaceae species only a slight burn at the top of single leaves was found while in more sensitive species spots of different size and color on the leaves were observed.

Table 1. Lethality of target plant species after treatment with essential oil solution.

Treated weeds	**Applied concentration of essential oil [μl/mL]**	**Lethality of individuals [%]** mean±SD
Dasypyrum villosum	5	0±0
	10	0±0
Lolium perenne	5	0±0
	10	34±10
Sinapis arvensis	5	33±6
	10	42±11
Matricaria chamomilla	5	15±3
	10	94±9
Trifollium repens	5	64±11
	10	100±0
Trifollium pratense	5	41±15
	10	89±12

Metabolite analysis of treated plants. Methanolic extracts of aerial parts of surviving individuals were analyzed for metabolite profiles by GC/MS. Organic,

phenolic, fatty and amino acids, sterols, polyols (sugar alcohols) as well as mono- and disacharides were identified. Variations in the accumulation of studied metabolites after treatment with essential oil in comparison with the control were observed. A reduction in the amount of phenolic acids was found in *L. perrene* individuals (chlorogenic, quinic and protocatechuic acids) *S. arvensis* (ferulic and 3,4-dimethoxycinnamic acid), *D. villosum* (4(p)-hydroxybenzoic acid). Only in *M. chamomilla* increased quinic acid content was found after treatment. With regard to the content of organic acids, that of malic and succinic acid was found to decreased in the more resistant species (*D. villosum*, *L. perrene*, *S. arvensis*) while in the more sensitive species (*M. chamomilla*, *T. repens*, *T. pratense*) their amount was increased. A similar trend was observed with the accumulation of quinic acid. In the case of fatty acids it was found that the content was increased in *L. perenne*, *T. repens* and decreased in *T. pratense* and *D. villosum*. At both species - *M. chamomilla* and *S. arvensis* the content of octanoic acid decreased and that of hexadecanoic acid increased in treated individuals. The polyol - myoinositol was found to be increased in *D. villosum*, *L. perrene* and *M. chamomilla* but in other studied species its content decreased. The amount of disaccharide - sucrose decreased in all studied species with exception of *M. chamomilla*. Amino acid - prolin increased in *L. perene*, *M. chamomilla* and *T. pratense*. The content of amino acids - serine and threonine increased in *D. villosum*, *L. perrene*, *M. chamomilla*, *T. repens.* The sterol content of the studied species was not affected by treatment with exception of *S. arvensis* and *T. repens*.

Fig. 1. Herbicide effect of *O. vulgare* ssp *hirtum* on *T. pratense* seedling.
A - plants treated with 10 µg/mL essential oil solution;
B - plants treated with 5 µg/mL; C - controls, untreated individuals.

Table 2. Identified metabolites in methanolic extracts of treated weeds with essential oil of *Origanum vulgare* ssp *hirtum. Legend:* c – control; Metabolites quantification was based on internal standard, added in the beginning of the extraction, using the calculated areas for the both components.

Compounds	***Dasypyrum villosum***			***Lolium perrene***			***Sinapis arvense***			***Matricaria chamomilla***		***Trifolium repens***		***Trifolim pratense***	
	***Control and applied concentrations of essential oil* [µg/mL]**														
	c	**5**	**10**	**c**	**5**	**10**	**c**	**5**	**10**	**c**	**5**	**c**	**5**	**c**	**5**
Succinic acid				82.1	23.2	11.4	56.8	19.4	5.8	17.8	74.5	9.3	10.3	8.9	10.3
Glyceric acid	13.0	33.5	28.7	4.6	15.4	11.4				72.7	7.3			7.4	5.1
Malic acid	21.5	10.5	9.7	46.5	44.6	39.4	278.6	118	58.7	68.2	133.7	20.6	47.4	10.9	27.1
Pyroglutamic acid	34.8	13.5	12.2	18.0	44.8	51.9	137.2	95.6	33.9			5.0	1		
4(p)-Hydroxy-benzoic acid	1.7	0.3	0.5	0.1	0.6	0.5		0.1	0.7			0.1			
Protocate-chuic acid	0.2			9.8	1.4		0.1		0.1					0.9	0.6
Quinic acid				48.3	33.1	28.6				1.3	6.7	0.1	0.2		
Ferulic acid							1.1	0.2	0.1						
3,4-Dimethoxy-cinnamic acid							13.1	4.3	4.4						
Chlorgenic acid				7.5	5.2	4.8									
Octanoic acid	64.6	35.2	32	34.8	91.2	111.8	137	83.9	44.9	313	228	10.6	17	47.3	17.4
Hexadecanoic acid	131	45	13.3	28.5	101.4	146.9	69.1	128.1	166.7	4.9	12.1	2.9	10.8	192.5	30.8
Octadecanoic acid										1.5	1.1	0.9	2. 1	42.4	30.9
Glycerol	70.5	41.6	38.2	210	120	144	149.3	141	141.2	203.2	227.9	21.8	30.6	225.8	148
Myo-Inositol	1.9	6.9	3.9	63.1	73.4	84.7	173.8	114	35.4	31.8	75.5	20.5	6.5	102.8	65.1
Fructose 1	52.2	37.8	39.5	71.2	38.4	44.6	158.9	138.5	153.5	0.5	0.6	16.1	13.1	20.8	22.4
Fructose 2	107.7	116.1	86.9	101.5	50.1	24.7	43.3	53.7	85.1	0.4	0.7	10.9	0.9	5.7	7.4
Glycose	101.9	122	99.2	42.6	45.1	50.8	6.6	6.1	12.5	7.7	4.2	41.3	50.9	17.6	21.9
Sucrose	321.8	380	255.9	445.3	193	176	564.2	251.9	177.8	43.5	121.3	34.8	11.9	430.3	82.5
Sterol	20.8	10.7	8.9												
Campesterol	0.4	0.2	0.3	0.9	0.5	0.8	16.8	5.7	1.7	6.2	3.5				
Stigmasterol										0.4	0.1				
ß-Sitosterol	6.9	6.5	5.8	10.9	9.2	9.8	83.7	24.2	7.4	1.3	1.2	10.5	2.2		
Proline	383.9	140	104	3.5	55.5	40.4	7.1	0.2		24.1	80.7	12.2	10.6	4.5	10.6
Glycine	7.0	5.5	7.8			1.3	38.7	5.4	8.1						
Serine	122.7	226.8	246.4	3.2	30.7	27.1	122.6	32.2	0.2	0.3	0.7	6.3	6.7	2.4	1.2
Threonine	48.7	55.1	142	7.4	13.1	52	214.1	74.6	12.7	12.1	23.5	2.6	3.4		1.1
Aspartic acid	9.0	9.8		2.9	6.2	17.7	12.6			2.3	2.7		3	0.5	0.1
Glutamic acid	10.0	6.6	6.8	0.1	5.8	1					1.8	0.8	1.4		
Phenylalanine	81.2	81.3	89.5		3.7	11.5	439.9	95.2	8.1	4.1	5.5	0.2	0.7		

The received results from the metabolic analysis showed that treatment with essential oil causes variations in the content of the several metabolites. The response appears to be dose-dependent and species-specific. The most common metabolic response of treated species is a decrease in sucrose levels which can be considered as an indicator of oxidative stress. Reduced sugar levels in treated plants are a sign also of impaired photosynthesis. Increased level of free amino acids often occurring after abiotic stress and probably this is the result of protein degradation. Decreased levels of malic acid are associated with increased activity of enzymes (NADP-depend) which

is also established as a plant response to various abiotic stressors (D'Abrosca et al., 2013). The accumulation of proline that is found in *L. perrene*, *M. chamomilla* and *T. pratense* is a non-specific reaction of plants to different types of abiotic stress (Singh et al., 2017). Araniti et al., (2018) shows in experiments with *Arabidopsis thaliana* seedlings that oregano essential oil disrupts the absorption of inorganic nitrogen in amino acids, which destroy the metabolism of glutamine and leads to an excess of ammonia in the leaves, that causes destructive chain processes (oxidative stress, disorders of photosynthesis and etc.). The metabolite variations observed in the present study showed indications for presence of oxidative stress, disorders of photosynthesis and etc. These are processes that occur under the influence of various abiotic stress factors.

Conclusion

The present study provides the data about herbicidal potential of *Origanum vulgare* ssp. *hirtum* essential oil applied on target plants at post-emergence state. Phytotoxic effect of the essential oil on all studied plants was established. Strong impact was found at the highest applied concentration (10 µg/mL) furthermore complete destruction was observed for *Matricaria chamomilla*, *Sinapis arvensis*, *Trifolim repens* and *Trifolium pratense*. The noted metabolic changes in the treated plants with oregano essential oil show that it has a phototoxic activity comparable to the effects of abiotic stress factors. The results obtained clearly characterize Greek oregano essential oil as a potent bio-herbicide for organic farming.

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