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BIOCIDAL ACTIVITY OF *ORIGANUM VULGARE SUBSP. HIRTUM* ESSENTIAL OIL

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Abstract

Toxic effect of *Origanum vulgare* subsp. *hirtum* (Link) Ietsw. essential oil was assayed on weeds (*Lolium perenne* L. *Trifolium pratense* L. and *Trifolium repens* L.), potato plants (*Solanum tuberosum* L.) and *Myzus persicae* Sulz. The GC–MS analysis of the essential oil revealed carvacrol as the main component. The essential oil solutions completely inhibited the seed germination of the target weeds at concentration 2 µL/mL in Petri dishes. In contrast, in soil, this effect was achieved at a concentration of 5 µL/mL for studied dicot and at 10 µL/mL for the monocot species. Total destruction of target weeds at post-emergence stage after spraying with solution of the essential oil in concentrate 10 µL/mL was found. Mature potato plants were poorly affected by the essential oil. A completely toxic effect on *M. persicae* was established by applying a solution with a concentration of 3 µL/mL of the essential oil. The experimental data indicate that the essential oil of the species has great potential, as an alternative to synthetic biocides, for the production of eco-friendly formulations for pest control.

Keywords: phytotoxicity, insecticidal, carvacrol, Greek oregano, *Myzus persicae*, weeds

INTRODUCTION

The extensive use of synthetic biocides in the modern agriculture results in grave environmental and health problems. The negative consequences of their use provoke intensive research of natural products as an alternative for pest control. Natural biocides have advantages over the synthetic, such as rapid biodegradation, low toxicity to non-target organisms, and unique modes of action with little mammalian toxicity (Mann and Kaufman, 2012).

Plants are an important source of bioactive compounds. They produce secondary metabolites as defence compounds against abiotic (*ca.* UV radiation) and biotic (*ca.* herbivores and microorganisms) factors or allelochemicals that provide them advantages in the competition for resources within the plant communities (Berkov *et al.*, 2014). The knowledge about the chemoeclogical interactions of the plants with other organisms is a starting point for the discovery of new natural biocide molecules with a potential for application in agriculture. Many compounds belonging to the major groups of natural compounds, such as alkaloids, terpenes, sterols, flavones, coumarins, iridoids, phenylpropanoids, have been active against different organisms, including pests (González-Coloma *et al.*, 2013). The monoterpenes are the wealthiest group of compounds with biocidal activities (Vokou *et al.*, 2003; Dayan *et al.*, 2009).

Hydrocarbon and oxygenated monoterpenes and sesquiterpenes are the main components in the essential oils, which also may contain phenylpropanoids, hydrocarbons and compounds from other chemical classes. Particularly abundant in the aromatic plants, the essential oils have been intensively studied for their herbicidal and insecticidal potential during the last decades (De Almeida *et al.*, 2010; Amri *et al.*, 2013a; Ibañez

et al., 2017; Synowiec *et al*., 2017). The screening for herbicidal activity initially includes assessment of the effects of the tested compound(s), extract(s) or product(s) on seed germination and seedling growth (Scognamiglio *et al*., 2013; Amri *et al*., 2013; Dayan *et al*., 2009; Dhifi *et al*., 2016).

Origanum vulgare L., also known as oregano, is an important medicinal and aromatic plant with application in culinary and folk medicine. Recent studies on allelopathic effects of *O. vulgare* essential oils and extracts on other plants, insects and microorganisms indicate interesting biocidal activities (Dragoeva *et al.*, 2014; García-Beltrán and Esteban, 2016). Green manure and water extracts from *O. vulgare* have shown phytotoxic effects on barnyard grass (*Echinochloa crus-galli*), bristly foxtail (*Setaria verticillata*), common purslane (*Portulaca oleracea*), cotton (*Gossypium hirsutum*), and corn (*Zea mays*) (Vasilakoglou *et al.*, 2011). Thymol type essential oil from *O. vulgare* has shown significant phytotoxic effects on the monocot *Triticum aestivum* and *Hordeum vulgare* as well as on dicot *Lepidium sativum* and *Sinapis alba*. However, it does not influence these species' germination (Grul'ová *et al.*, 2020). Carvacrol type oregano essential oils inhibited seed germination and seedling growth of *Portulaca oleracea* L., *Lolium multiflorum* Lam. and *Echinochloa crus-galli* (L.) Beauv., at all concentrations assayed (0.125–1 µL/mL) (Ibáñez and Blázquez, 2017).

Origanum vulgare is represented by six subspecies worldwide *O. vulgare* subsp. *glandulosum* (Desf.) Ietsw., *O. vulgare* subsp. *gracile* (K.Koch) Ietsw., *O.vulgare* subsp. *hirtum* (Link) Ietsw., *O. vulgare* subsp. *virens* (Hoffm. et Link) Ietsw., *O. vulgare* subsp. *viridulum* (Martrin-Donos) Nyman (http://www.theplantlist.org/). As opposed to *O. vulgare*, the biocidal effects of *O. vulgare* subsp. *hirtum* (Link) A. Terracc., (Greek oregano) distributed in Southeast Europe and Turkey, are poorly studied. Carvacrol type essential oils from this subspecies influence the germination and radicle elongation of *Sinapis arvensis* L., *Phalaris canariensis* L., *Lepidium sativum* L., and *Raphanus sativus* L. They also show antifungal activity against the phytopathogens *Monilinia laxa, M. fructigena,* and *M. fructicola* (Mancini *et al.*, 2014). Antibacterial and antifungal activities of the essential oil of *Origanum vulgare* subsp. *hirtum* have also been reported (Adam *et al.*, 1998; Schillaci *et al.*, 2013). La Pergola *et al.* (2017) have found that carvacrol type commercial oregano oil has more potent repellent activity against *Sitophilus oryzae* (rice weevil) and *Tribolium confusum* (confused flour beetle) than the tymol type oil from *O. vulgare* subsp. *hirtum.*

Aphids are among the most important pests and virus vectors on crops. *Myzus persicae* Sulz. is a polyphagus that infests plant species from more than 40 families. It is a vector of more than 100 plant viruses, and it has a cosmopolitan distribution. The species is one of the main pests on potato (Blackman and Eastop, 2000; Petrović-Obradović *et al.*, 2011). On the other hand, potato is essential crop in Europe and the control of some of its pests with bio-based products, in accordance with the Council Directive 2002/56/EC; Commission Implementing Directive 2014/21/EU for healthy, no diseases, potato seeds. In our preliminary study on herbicide potential of plant extracts and essential oils, *Origanum vulgare* subsp. *hirtum* was selected as perspective for more detailed study (Yankova-Tsvetkova *et al.*, 2020). In the present work the biocidal potential of an essential oil (EO) from *O. vulgare* subsp. *hirtum* was evaluated by several experimenthal approaches:

i) assessment of the inhibitory activity of the EO on the germination of the seeds of the target species (*Lolium perenne*., *Trifolium repens* and *T. pratense*) in Petri dishes and soil;
ii) evaluation of phytotoxic effect of the EO on target weeds in the post-emergence stage and on mature potato plants;
iii) determination of insecticidal effect of the EO on *Myzus persicae*.

MATERIALS AND METHODS

Plant Material

Aerial parts of *O. vulgare* subsp. *hirtum* were collected during the flowering stage from one of its natural habitats at the Struma valley, Bulgaria. The plant material was air-dried at room temperature in the shade. Seeds of *Lolium perenne*, *Trifolium repens* and *Trifolium pratense* were purchased from Florian Company, Bulgaria (http://www.florianbg.com). Potato cultivar Soraya was purchased from the Potato Experimental Station, Samokov, Bulgaria.

Isolation of the Essential Oil

The essential oil was extracted on a Clevenger apparatus by water distillation from 50 g dry plant material in a flask with 500 ml water for 2 h. The extraction was repeated several times to obtain the necessary amount of essential oil for the experiments.

GC/MS Analysis of the Essential Oil

Oil sample analyses were performed on a Thermo GC equipped with a Focus DSQ II mass detector coupled with a HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thicknesses). Chromatographic conditions were as follows: helium as carrier gas at a flow rate of 1 mL/min; injection volume was 1 µl, and the split ratio was 1 : 50. Column temperature was 60 °C for 10 min, and programmed at the rate of 3 °C/min to 200 °C, and finally, held isothermally for 10 min. The injection port was set at 220 °C. Significant quadrupole MS operating parameters: interface temperature 240 °C; electron impact ionization

at 70 eV with scan mass range of 40 to 400 m/z at a sampling rate of 1.0 scan/s (Traykova *et al.*, 2019). The components were identified by comparing their mass spectra and retentions indexes (RI) with the retention indexes of authentic standards, mass spectra from the National Institute of Standards and Technology (NIST) and literature data (Adams, 2007). The amounts of the identified compounds are relatively expressed as a percentage of the area of all the chromatogram peaks.

Phytotoxic and Insecticidal Activity of the Essential Oil

Inhibition on Seed Germination in Petri Dishes

Aqueous solutions of the essential oil, at concentration 0.5, 0.75, 1, 1.5, 2 and 3 µL/mL, were prepared using 0.1% of Tween 40 (Sigma) as an emulsifier. A hundred seeds of *Lolium perenne, Trifolium repens* and *Trifolium pratense* were placed in Petri dishes on filter papers moistened with the tested solutions and incubated at room temperature for 7 days. The rate of germination inhibition was calculated by using formula after Atak *et al.* (2016).

Inhibition on Seed Germination in Soil

Ten seeds from the tested species *Lolium perenne, Trifolium repens* and *Trifolium pratense*, were placed in pots (8 cm in diameter) filled 2/3 with soil and 1/3 with soil and superabsorbent Terawet (R), USA (https://www.agriculture-xprt.com/companies/terawet-green-technologies-inc-tgt-90016/) in a ratio of 1 : 1. In advance the superabsorbent was pre-moistened with distilled water for the controls and essential oil solutions at concentrations of 3, 5 and 10 µL/mL of the essential oil. The absorbance ratio of the solution was 5 g dry crystals per liter of liquid. The pots were stored at room temperature for 30 days and periodically moistened with distilled water from the bottom of the pots. The rate of germination inhibition was calculated by using a formula after Atak *et al.* (2016).

Phytotoxicity Test

The phytotoxicity test was performed on seedlings of target plants grown in pots (15 plants per pot, 8 cm in diameter) and potato plants, cultivar Soraya (one plant per pot, 15 cm in diameter). The pots were placed in a growth chamber with temperature 23 °C and 30% humidity. Potato at vegetative stage and seedlings at cotyledone stage were sprayed with aqueous solutions of 5 µL/mL and 10 µL/mL of the essential oil prepared with 0.1% Tween 40 (Sigma) as an emulsifier. The spraying rate was 50 mL/m^2 for the weeds and 100 mL/m^2 for the potato plants. Seven days after spraying, the treated plants were checked for visible chlorotic or necrotic areas and mortality. The test was repeated three times for each species. The results are reported as the lethality percentage (LP) using the following formula:

$$LP = [(N - n)/N] \times 100, \quad (1)$$

where:

N ...the number of healthy individuals before treatment;

n ....the number of healthy individuals after treatment.

Leaf Dip Bioassay

„The dip leaf test method“ (FAO) (Anonymous, 1979) with some modifications was applied to evaluate the bioactivity of *O. vulgare* subsp. *hirtum* essential oil on *Myzus persicae* on potato plant leaves. *Myzus persicae* was initially collected from infested plants in the greenhouse of the Institute of Ornamental and Medicinal Plants – Sofia, Bulgarian Agricultural Academy. Some aphid specimens were used for precise identification, which was conducted using permanent microscope slides, after the traditional method of Hille Ris Lambers (1950). The aphid' identification was performed following Blackman and Eastop (2000) identification kay. The aphids were reared in cages in laboratory conditions in a Growth Chamber GC 400 in optimal for their development conditions – 16/8 h light/dark, temperature 23 °C ± 2 °C and 70% ± 5% relative humidity. *M. persicae* was reared for more than 30 generations without any pesticide application until we got a standard population to conduct the studies of biocidal effect.

Potatoes were grown as hydroponic in perlite substrate and KNOP medium in a laboratory at the same conditions as the aphids in the growth chamber.

The potato leaves were cut off from the plant and treated through direct dipping into essential oil solutions for 10 sec. The solutions were prepared by dissolving in dH_2O essential oil at eight concentrations 0.1, 0.5, 1, 1.5, 2, 3, 4 and 5 µL/mL. After that leaves were placed on a filter paper for about 30 min (till solution's evaporation) and in consequence they were placed in Petri dishes with their abaxial surface facing skywards. Thirty adult apterous females were transferred using paint brush in a fine sifter and were dipped into the tested substances for 5 sec after that they were placed onto previously treated leaves in Petri dishes. The tests were conducted in a controlled regime as for the aphids in the growth chamber. The mortality assessment was made under stereo microscope Zeiss 24 hours after the treatment. Aphids' mortality was calculated using Abbott's formula (Abbott, 1925).

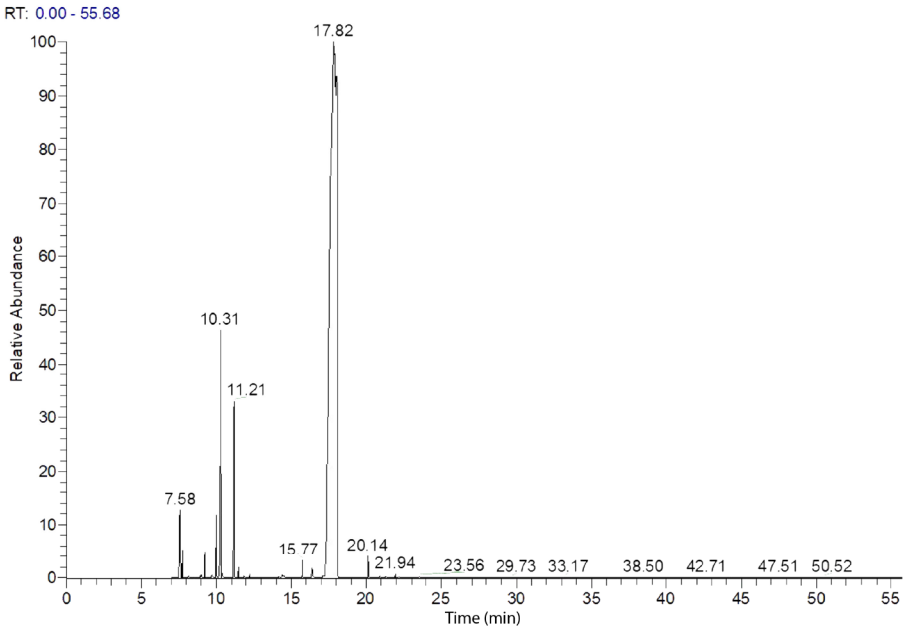
$$\textit{Corrected mortality}\ (\%) = (1 - Y/X) \times 100,$$

where:

X ....the number/percent living aphids in control;

Y ....the number/percent living aphids after the treatment.

The obtained results were compared with a positive control Nurelle D® – a commercial insecticide at a concentration of 0.05% (as it is recommended for other aphid species), and negative control – dH_2O.



1: *GC/MS chromatogram of O. vulgare subsp. hirtum essential oil*

Statistical Analysis

All assays were performed in three independent analyses. The results are presented as mean with standard division (SD). Statistical analyzes were calculated using Microsofte Excel. The IC_{50} values were calculated by Software Prizm 3.00.

RESULTS AND DISCUSSION

In the present study the biocidal potential of the essential oil from *O. vulgare* subsp. *hirtum* on target species: *Lolium perenne*, *Trifolium pratense*, *Trifolium repens* and *Myzus persicae* was evaluated.

Essential Oil Composition

Chemical profile of the essential oil from *O. vulgare* subsp. *hirtum* was determined by GC/MS. The main components were identified as carvacrol (86.4%), *p*-cymene (5.98%), *γ*-terpinene (4.24%), and *β*-pinene (1.58%). The other components are presented in quantities of less than 1%. The GC/MS chromatogram of essential oil is presented in Fig. 1.

A few chemotypes within the species have been reported in the literature, the dominant being those containing as a major component the phenolic compounds carvacrol/thymol (Martino *et al.*, 2009; Mancini *et al.*, 2014). The carvacrol-rich essential oil composition, reported in the present study, coincides with the most commonly reported for samples from Greece, Italy, Bulgaria, Turkey, and Hungary (Baser *et al.*, 1994; Veres *et al.*, 2003; Konakchiev *et al.*, 2004; Martino *et al.*, 2009; Mancini *et al.*, 2014).

Bioassay of Phytotoxic Effects of the Essential Oil from *O. vulgare subsp. hirtum*

Inhibition on Seed Germination in Petri Dishes

The inhibitory activity of the essential oil on seed germination of *L. perenne*, *T. pretense* and *T. repens* was studied in the concentration range of 0.5–3 µL/mL. The results expressed as germination inhibition rate are presented in Tab. I.

Inhibitory activity of essential oil was found to be species-dependent and increases with elevation

I: *Germination inhibitory activity of essential oil from O. vulgare subsp. hirtum in vitro*

Concentraction [µL/mL]	Germination inhibition [%]		
	Lolium perenne	*Trifolium repens*	*Trifolium pratense*
0.5	84 ± 7.5	16.3 ± 4.7	24 ± 5.3
0.75	96 ± 0.5	73 ± 3.1	32.7 ± 2.1
1	100 ± 0	96 ± 0.5	59 ± 5.7
1.5	100 ± 0	97 ± 0.3	93.7 ± 5.4
2	100 ± 0	100 ± 0	100 ± 0
3	100 ± 0	100 ± 0	100 ± 0

concentration. *Lolium perenne* seeds were found to be the most sensitive to the treatment with essential oil whereas T. *pratense* was the least sensitive. Total inhibition of seed germination of the tested species was achieved with a solution at a concentration of 2 µL/mL of essential oil. Such an inhibitory concentration is comparable with those reported for essential oils from plants considered as strong bio-herbicides as *Satureja hortensis*, *S. montana*, *Mentha piperita*, boldo and lemon grass (Blázquez and Carbó, 2015; Amri *et al.*, 2013b; Ibáñez and Blázquez, 2017).

Total inhibition in concentration range 4–5 µL/mL of essential oil has been considered as potent (Amri *et al.*, 2017). Our results are also similar to those of Mancini *et al.*, (2014) and Ibáñez and Blázquez (2017) who reported inhibitory activity of *O. vulgare* subsp. *hirtum* essential oil on seed germination of other weed species.

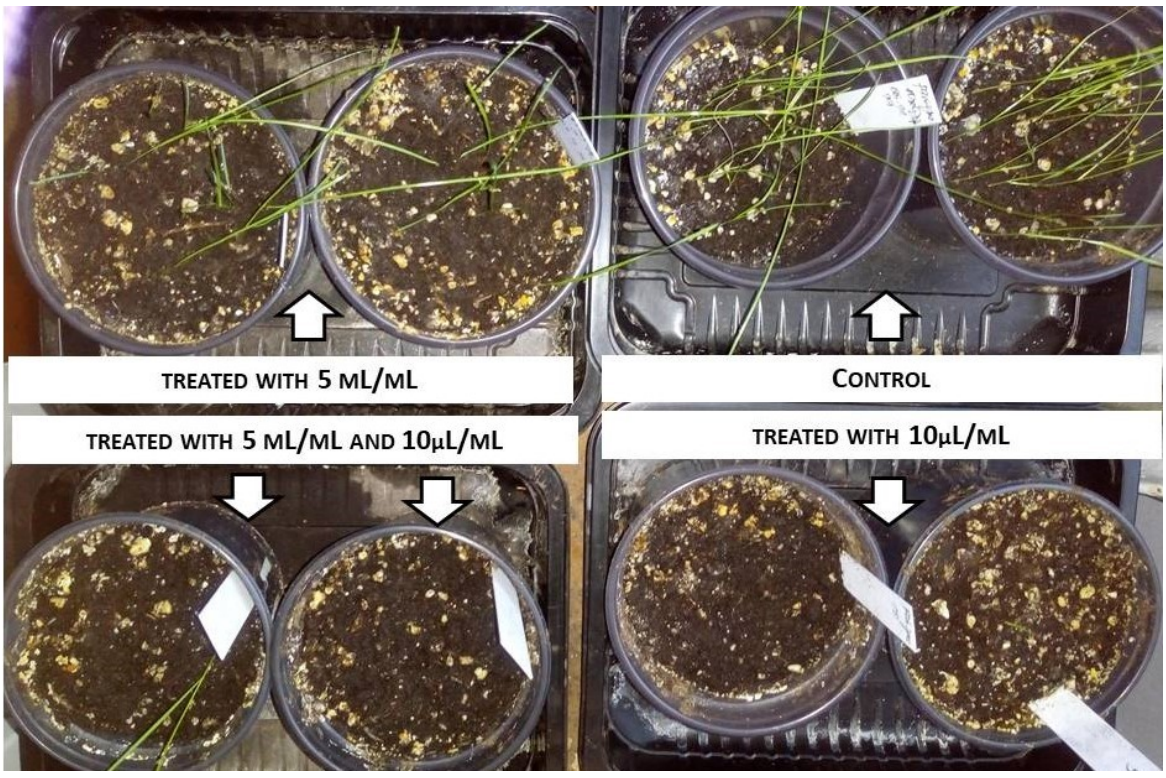
Inhibition on Seed Germination in Soil

Most of the phytotoxicity experiments with essential oils are performed *in vitro* in Petri dishes while data from *in vivo* experiments are limited. The inhibitory effect of *Origanum* essential oil on seed germination of target species was examined with solutions at three concentrations, 3, 5 and 10 µL/mL in soil. The use of the superabsorbent Terawet[R] in the experiment prevented the rapid evaporation of the essential oil that prolonged the effectiveness of treatment. The results expressed as germination inhibition rate are presented in Tab. II and Fig. 2. Similarly to the results from the in vitro assay, the inhibitory effect of the essential oil on seed germination was found to be species-dependent and increases in a concentration-dependent manner.

However, the effective concentrations for soil treatment are considerably higher as compared to the *in vitro* trials. In contrast to *in vitro* germination assay, *Lolium perrene* is less sensitive than the *Trifolium* species which germination was inhibited

II: *Germination inhibitory activity of essential oil from O. vulgare subsp. hirtum in soil*

Concentraction [µL/mL]	Germination inhibition [%]		
	Lolium perenne	*Trifolium repens*	*Trifolium pratense*
3	47 ± 7.1	92 ± 0.5	89 ± 3.3
5	64 ± 9.8	100 ± 0	97 ± 5.7
10	100 ± 0	100 ± 0	97 ± 5.7



2: *Effect of application of O. vulgare subsp. hirtum essential oil at a concentration of 5 and 10 µL/mL in soil with L. perrene seeds*

47% and 67% with solutions at a concentration of 3 and 5 µL/mL. Treatment with solutions at concentration of 10 µL/mL of essential oil resulted in 97% inhibition of germination of *L. perenne* seeds and complete germination inhibition in the *Trifolium* species. The latter species were inhibited over 80% even with a solution of essential oil with a concentration of 3 µL/mL. Frabboni *et al.*, (2019) have been reported that soil treatment with 50% diluted and undiluted oil *O. vulgare* essential oil inhibited weed seed germination. Application of *Origanum* green manure in corn and cotton fields suppressed the germination of weeds significantly and increased the yields greatly compared to the controls (Vasilakoglou *et al.*, 2011). The high volatility of essential oils is a major problem when applied in field conditions. Recent studies have been published that this problem is solved through the application of essential oils by microencapsulation and nanoemulsion (Hazrati *et al.*, 2017). In the present study, a solution of essential oil is included in a superaborbent, which ensures the gradual release of the oil into the soil, which prolongs its action.

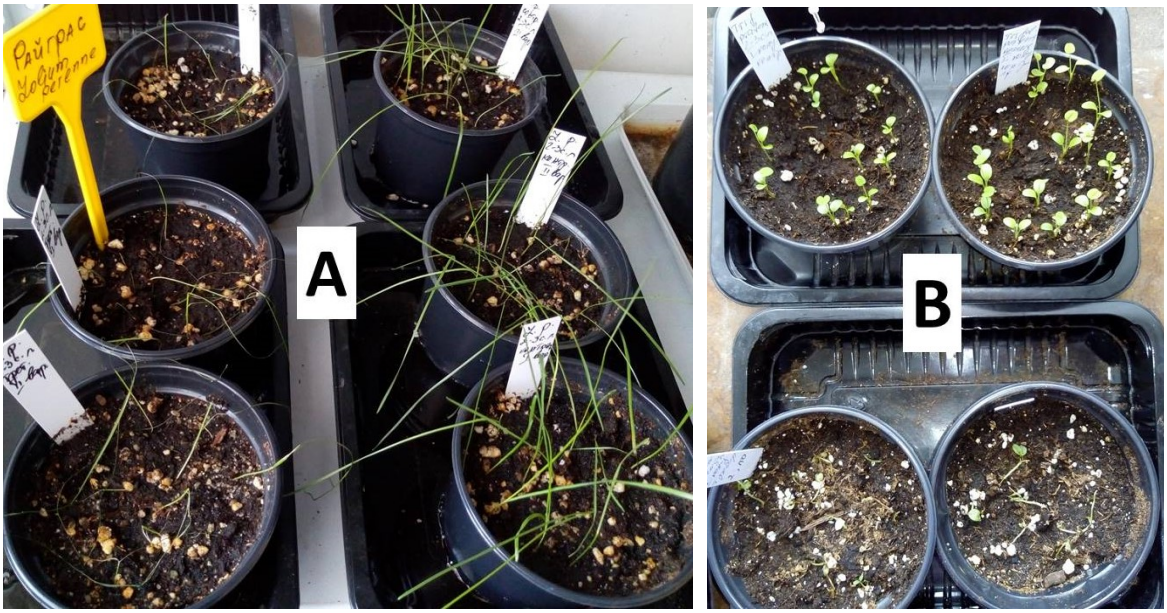
Phytotoxicity Assay

The phytotoxicity effect of Origanum essential oil was evaluated on seedlings of target species and potato plants by spraying on solutions with concentration 5 and 10 µL/mL. Seven days after the seedling treatment with 5 µL/mL essential oil solution, the lethality rates were 13% for the *T. pratense* individuals and 39% for the *T. repens* individuals. The *L. perenne* individuals were very poorly affected, which is manifested by yellowing some individuals' top part. A solution with concentration at 10 µL/mL resulted in lethality rates of 100%, 97% and 66% for individuals of *T. repens, T. pratense* and *L. perenne*, respectively. The results are presented in Tab. III and Fig. 3.

The received results determined the phytotoxicity of the essential oil from *O. vulgare* ssp. *hirtum* is much stronger than essential oils from other plants such as *Artemisia annua* and *Xanthium strumarium*, which have shown activity at a concentration of 1 000 µL/mL (Benvemti *et al.*, 2017). Hazrati *et al.* (2017) reported complete lethality on *Amaranthus retroflexus* and *Chenopodium album* 10 days post-treatments with

III: *Lethality of weed individuals seven days after treatment with O. vulgare subsp. hirtum essential oil*

Weed species	Concentration [µL/mL]	Lethality of individuals [%]
Loliun perenne	5	0 ± 0
	10	66 ± 19
Trifollium pratense	5	92 ± 8
	10	97 ± 5
Trifollium repens	5	39 ± 9
	10	100 ± 0



3: *Effect of application of O. vulgare subsp. hirtum essential oil at a concentration of 10 µL/mL on the survival of L. perrene (A) and T. pratense (B)*

nanoemulsion of essential oil of *Satureja hortensis* at a concentration 4 µL/mL.

Complete weed control has been achieved with 5 to 10% solutions of cinnamon and clove essential oils (Tworkoski, 2002). Post-emergence application of Artemisia scoparia oil (2%, 4%, and 6%, v/v) on 6-week-old weed plants has been reported to cause visible injury ranging from chlorosis to complete wilting of target plants (Cassia occidentalis, Parthenium hysterophorus, Echinochloa crus-galli and Ageratum conyzoides) (Kaur *et al.*, 2010). On the other hand, our results are in accordance with Dayan *et al.* (2009) and Amri *et al.* (2012) who have established that dicot weeds are more sensitive than monocot ones.

The treatment of potato plants with the Origanum essential oil solutions resulted in slight phytotoxic effects. Small spots were observed on the surface of single leaves. Similarly, Synowiec *et al.* (2017) have observed that the crops are more resistant than weeds to the toxicity effects of essential oils. Moreover, the authors suggest that seed size is related to its resistance to essential oils. In their experience, this has been observed with Zea mays seeds. The results of the present study with potato tubers confirm these conclusions.

In summary, we suppose that the potent phytotoxicity of the *O. vulgare* ssp. *hirtum* essential oil is due to its major (over 80%) component, carvacrol. Several articles point to carvacrol as a potent inhibitor of seed germination (De Almeida *et al.*, 2010; Synowiec *et al.*, 2016; Ulukanli *et al.*, 2018; Bendre *et al.*, 2018). Araniti *et al.*, (2018) show 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.). All these processes lead to destruction of the plant.

Bioassay of Insecticidal Activity of the Essential Oil from *O. vulgare* subsp. *hirtum*

The results of the insecticidal test showed that the essential oil had completely toxic effects on *Myzus persicae* at a concentration 3 µL/mL. Dose-dependent increase of the biocidal activity was obtained after the treatment with the essential oil on *M. persicae.* Immediately after the 5 sec treatment with concentrations of 3 µL/mL or higher (obtained from dry and fresh leaves) a knock out effect (i.e. 100% aphid mortality) was observed. At the same concentrations, potato leaves revealed a slight phytotoxic effect after 10 sec. direct dip application. Lack of phytotoxic effect on leaves and insignificant mortality of aphids were reported at concentrations lower than 2 µL/mL (Tab. IV). The IC_{50} value – extract concentration providing 50% aphid mortality was calculated as 2.2 µL/mL. As a comparison, the positive control Nurelle D® resulted in 100% mortality in aphids and no phytotoxic effect was observed on the leaves. Unlike the above-reported phytotoxicity on the treated cut off potato leaves, the laboratory tests on whole potato plants, sprayed with 5 µL/mL essential oil, resulted in a less visible effect. That gives us a suggestion that the whole plants are more resistant to the essential oil application than the cut off leaves directly dipped into the tested essential oil solutions.

IV: *Aphid mortality after treatment with O. vulgare subsp. hirtum essential oil*

Concentration [µL/mL]	Aphid mortality after treatment [%]
0.1	0 ± 0.8
0.5	5 ± 1.6
1	10 ± 1.4
1.5	15 ± 0.8
2	30 ± 1.4
3	100 ± 0.8
4	100 ± 0
5	100 ± 0

Our results confirm an earlier conducted laboratory experiments with *O. vulgare* essential oil, showing a strong insecticidal activity against the cotton aphid – *Aphis gossypii* Glover, which is very close to the activity of another standard insecticide Karate Zeon® (Atanasova *et al.*, 2018). According to Dancewicz *et al.* (2012) it was found that the essential oil of *O. vulgare* also posses a strong and long-lasting deterrent effect on the pea aphid – *Acyrthosiphon pisum* Harris, but it has a weak and transitory deterrent effect on *M. persicae*. It is also revealed that the oligophagous *A. pisum* is more sensitive to this essential oil comparing to *M. persicae.* As a suggestion further experiments might prove that the essential oil of *O. vulgare* ssp. *hirtum* could also be used as an insect repellent against certain pest species. On the other hand, the polyphagous insects have the ability to avoid/reduce the toxic effect of many xenobiotics and pesticides by expressing a wide range of enzymes (Li *et al.*, 2000). We suppose that the results obtained by us might show even better insecticidal activity of *O. vulgare* ssp. *hirtum* essential oil on some other pests, especially oligophagous and monophagous aphids. This assumption is confirmed by Digilio *et al.*, (2008) for *O. vulgare* essential oil, proving that *A. pisum* is more susceptible to the treatment than *M. persicae.*

CONCLUSION

The results have shown that the essential oil of *O. vulgare* subsp. *hirtum* exhibits a potent inhibitory activity on seed germination of *L. perenne*, *T. pretense* and *T. repens* at concentration of 2 µL/mL, it destroys the target weeds at a concentration of 10 µL/mL, and posssess inhibitory activity against the development of aphids on potatoes at a concentration of 3 µL/mL. By the inclusion of the oil in the superabsorbent Terawet^{R} was overcome its rapid evaporation upon application to the soil. Complete inhibition of seed germination in soil was achieved at a concentration of 5 µL/mL of essential oil for studied dicot and at 10 µL/mL for the monocot species. Further field experiments are necessary to prove the effectiveness of *Origanum vulgare* subsp. *hirtum* essential oil for control of pests in field experiments.

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