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Allelopathic and toxicological effects of *Origanum vulgare* L. essential oil

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Abstract. *Origanum vulgare* L. has been proven to be the strongest herbal antiseptic in the world, native to the Mediterranean region, but is widely naturalized elsewhere in the temperate Northern Hemisphere. This study aimed to estimate the phytotoxic effect of three different concentrations of oregano essential oil (*O. vulgare*) on three selected plant species namely, wheat, tomato and mint using biotest germination and effects on seedling growth, as well as its toxicological properties using *Allium* test. Our results revealed that oregano essential oil exhibits allelopathic effect on selected species. All three tested concentrations of oregano essential oil caused a significant inhibition of *Allium cepa* L. root growth, as well as a reduction in the mitotic index values in *A. cepa* meristem cells. *O. vulgare* essential oil demonstrated phytotoxic and antiproliferative effects. Further research is needed to confirm our results.

Keywords: allelopathy, phytotoxicity, essential oil, *Origanum vulgare*, toxicological effects, *Allium* test.

INTRODUCTION

The environmental constraints of plant production have sparked many interests in alternative weed control and control strategies. In fact, continued use of synthetic herbicides can endangered sustainable agricultural production, resulting in serious environmental problems such as, increased frequency of weed resistance to important herbicides and increased environmental pollution and health hazards (Owen and Zelaya 2005). The production and accumulation of secondary metabolites (allelochemicals), which inhibit and/or stimulate the germination and development of other plants, is an important mechanism in the interaction between plants. Allelopathy offers the potential for selective biological weeds through the production and release of allelochemicals from leaves, flowers, seeds, stems and roots, living or decomposing plant material (Weston 1996). Together with the investigation of the herbicidal activity of essential oils on weeds, there are some studies on agricultural plants in order do evaluate their effect on adjacent plants (Ibáñez and Blázquez 2020). Under appropriate conditions, allelochemicals can be released in quantities that suppress the development of weed seedlings (Wu

et al. 2001). Allelopathic inhibition is usually a result of the combined action of a group of allelochemicals that interfere with several biochemical interactions between plants, including those mediated by soil microorganisms. Aromatic plants can play an important role in plant-plant interactions and form the primary source of potential allelochemicals. Various allelochemicals have been identified, including essential oils that inhibit seed germination and plant growth (Aliotta et al. 1994).

Essential oils (EOs) are natural, complex compounds characterized by strong odors and formed by aromatic plants as products of secondary metabolism, generally localized in temperate to warm regions where they represent an important part of traditional pharmacopoeia. They are liquid, volatile, clear and rarely colored, soluble in lipids and in organic solvents, have lower density than water, can be synthesized by all plant organs, and are stored in secretory cells, cavities, ducts, epidermal cells or glandular trichomas (Masotti et al. 2003).

O. vulgare belongs to the *Lamiaceae* family, native to Europe, the north of the African continent and most of temperate Asia, but the focus of diversity is found in the Mediterranean region, predominantly in Türkiye. The composition of oregano essential oil varies depending on environmental conditions, geographical area, harvest time and stage of maturity of the plant. Essential oil from plants in different countries has been evaluated in terms of chemical composition and the main components have been identified depending on the area of origin. *O. vulgare* oil contains a large amount of volatile phenylpropanoids, thymol and carvacrol (carvacrol (53.2%) and p-cymen (12.7%), which are known to have a highly herbicidal effect on germination and growth of weed seedlings and cultivated crops (Karalija et al. 2020; Lombrea et al. 2020). According to the available literature, several monoterpenes, such as α and β-pinene, limonene, 1-8-cineol, myrcene, camphor, thymol and carvacrol inhibit seed germination and the growth of primary plant roots, mainly weeds (Singh et al. 2009; De Martino et al. 2010). These studies suggest that monoterpenes, as highly lipophilic compounds, interact with the constituents of biological membranes, altering their densities and fluidity. It has been described that the presence of monoterpenes increases level of malondialdehyde, proline and hydrogen peroxide, which indicates lipid peroxidation and induction of oxidative stress (Witzke et al. 2010).

Therefore, the present study aimed to assess the allelopathic effects of *O. vulgare* essential oil against wheat, tomato and mint, as well as its toxicological properties in *A. cepa* root apical meristem.

MATERIALS AND METHODS

O. vulgare essential oil and chemicals

For testing herbicidal properties against wheat (*Triticum aestivum* L.), tomato (*Lycopersicon esculentum* Mill.) and mint (*Mentha spicata* L.) as well as toxicological effects on *A. cepa*, commercially available essential oil of *O. vulgare* (Biohalilovic, B&H) was used. Stock solution of *O. vulgare* essential oil was made using dimethyl sulfoxide ≥99% (DMSO) (Sigma-Aldrich, St. Louis, MO). The stock solution was diluted with distilled water to the final concentrations of essential oil: 0.2 µg/ml, 0.4 µg/ml and 0.8 µg/ml.

Seed germination and phytotoxicity bioassay

The allelopathic effect of oregano essential oil (EO) on wheat, tomato and mint seeds was carried out by cultivating the seeds of these species in the air chamber. In 36 Petri dishes, with two layers of filter paper, 25 seeds were arranged which were then treated with 5 ml of oregano EO solution in three different test concentrations (0.2 µg/ml, 0.4 µg/ml and 0.8 µg/ml). The test was performed in four replications for each tested oil concentration. Parallel with treatments, four control replications in the form of distilled water, were also performed. Then, the Petri dishes were closed, sealed with parafilm and stored in the air chamber (constant 22°C temperature and 16 h photoperiod) for 4 to 7 days. The following parameters were determined: germination index (Abdul-Baki and Anderson 1973), percentage of seed germination (Scott et al. 1984), percentage of inhibition of germination (Cayuela et al. 2007), and phytotoxicity (Rusan et al. 2015).

Allium test

For the purposes of the *Allium* test, healthy onion (*A. cepa*) seed bulbs were placed onto glass specimens filled with fresh tap water for 72 hours in the dark at room temperature in order to grow. The water was renewed every 24 hours. After 72 hours, prior to the treatment of the *A. cepa* bulbs with the test concentrations of *O. vulgare* essential oil, the length of the roots was measured as suggested by Fiskesjö (1985). The roots were then treated with three different test concentrations of *O. vulgare* eseential oil (0.2 µg/ml, 0.4 µg/ml and 0.8 µg/ml) for 24 hours. Simultaneously, a control (untreated) group was established. For each test concentration and control group, series of three bulbs were used. After

the exposure period, roots length from the experimental sets and control was also measured. The roots were then excised from each bulb and placed in methanol/glacial acetic acid (3:1, v/v) fixative for 24 hours at +4°C. Afterwards, the roots were hydrolyzed in 1 N HCl for 15 minutes at room temperature. After hydrolysis, the root apical 2 mm were cut and placed onto clean glass slide in a drop of 2% acetorcein, and then squashed. For each tested concentration of the *O. vulgare* EO and control group, one slide per bulb was prepared and analyzed.

For cytogenetic analysis, three microscope slides were analyzed for each test concentration, as well as for the control. In this sence, the mitotic index (MI) values were determined, as the measure of cell proliferation. The MI was calculated as the quotient between the number of cells in mitosis (cell division) and the total number of cells analyzed, scoring 1000 cells per slide (3000 cells per each test concentration of essential oil and control group).

Statistical analysis

The obtained results were statistically processed using Microsoft Excel 2019 (Microsoft Corporation, Redmond, USA) and IBM SPSS Statistics for Windows, version 20.0 (Armonk, NY, USA). Mean values and standard deviations for each of the test concentrations and control group for all analyzed parameters, were calculated. In order to determine significant differences between the test substance (*O. vulgare* EO) and control group for all analyzed parameters, one-way ANOVA with post-hoc multiple comparison test (LSD) was carried out. The differences were considered statistically significant at the value of $P \leq 0.05$.

RESULTS

Allelopathic effects of O. vulgare essential oil

The highest value of the germination index (Table 1) was recorded for wheat seeds when treated with the lowest concentration of oregano EO, but with an increase in the tested concentrations, decrease in the germination index was observed. Contrary, in tomato seeds the highest recorded value was in control treatment. Also, the highest value of the germination index in mint seeds was at the lowest concentration of oregano EO.

EO decreased germination in tomatoes, when the highest concentration was applied in comparison with control, while in wheat and mint the percentage of germination increased. Accordingly, the highest percentage of germination inhibition was recorded in tomato seeds when treated with the highest concentration of oregano EO (17.72%). No effects of oregano EO were observed for wheat and mint, when treated with the lowest concentration (0.2 μg/ml) (Table 1). The treatment with the highest concentration of oregano EO (0.8 μg/ml) resulted in the highest phytotoxicity index (0.56%) in tomato seeds.

Table 1. Effect of different concentrations of *O. vulgare* essential oil on the germination index, germination, germination inhibition, phytotoxic index of the tested plant species.

Plant species	Essential oil concentration (μg/ml)	Index of germination	Germination (%)	Germination inhibition **(%)**	Phytotoxic index
T. aestivum	0	94.91±1.52	93.00±3.81	-	-
	0.2	96.08±11.05	93.00±3.82	0.00	-0.07±0.04
	0.4	86.50±2.69*	90.00±2.30*	3.22±0.50*	0.39±0.06
	0.8	86.75±4.64*	97.00±2.00*	-4.30±0.50*	0.29±0.01
L. esculentum	0	47.21±5.00	79.00±7.57	-	-
	0.2	39.08±5.97	75.00±8.24	5.06±1.78	0.03±0.03
	0.4	39.35±6.96	78.00±14.78	1.26±3.20	0.27±0.02
	0.8	25.41±7.13	65.00±6.83	17.72±1.47	0.56±0.01
M. spicata	0	3.20±5.12	45.00±3.82	-	-
	0.2	12.20±5.38	45.00±8.86	0.00	0.15±0.04
	0.4	12.06±6.00	49.00±16.12	-8.89±4.03	0.001±0.006
	0.8	11.53±6.01	50.00±5.16	-11.11±1.29	0.18±0.05

The results represent the mean values of four independent replications ± standard deviation.
* Statistically significant differences compared to the control group ($P \leq 0.05$).

Table 2. Results of the roots length increment and mitotic index values of *A. cepa* after treatment with different concentrations of *O. vulgare* essential oil.

Essential oil concentration (μg/ml)	Roots length increment (cm)	Mitotic index (%)
Control group	0.55±0.05	2.96±0.94
0.2	0.20±0.10*	0.12±0.05*
0.4	0.16±0.15*	0.20±0.10*
0.8	0.13±0.15*	0.23±0.05*

The results represent the mean values ± standard deviation.
* Statistically significant differences compared to the control group ($P \leq 0.05$).

Toxicological effects of O. vulgare essential oil

After the *Allium* test, a significant effect of test concentrations of *O. vulgare* EO on the growth of *A. cepa* roots was observed, as well as on the values of the mitotic index (Table 2). All three tested concentrations of oregano EO caused a significant inhibition of root growth, which was followed by a statistically significant decrease in the mitotic index (MI) value, as compared to the control group. It is important to hightlight that with the increase in the tested concentration of oregano EO, an increase in the inhibitory effect on the growth of roots was detected. As for the MI, the lowest value of the MI was recorded at the concentration of 0.2 μg/ml, while at the concentrations of 0.4 μg/ml and 0.8 μg/ml there was a slight increase in MI values. However, for all three tested concentrations of oregano EO, a statistically significant reduction in the value of the MI when compared to the control group, was demonstrated.

DISCUSSION

It has been observed that essential oils (EOs) from different plant species belonging to the *Lamiaceae* family have allelopathic properties (Verdeguer et al. 2009). The allelopathic effects of EOs are associated with the essential oil itself, its composition, the concentration applied, and the species to which they are applied. It has been recorded that oregano oil contains a large amount of volatile phenylpropanoids, thymol, linalool as well as p-cymene, which are known to have a high herbicidal effect on the germination and growth of weed seedlings and cultivated crops, as well as carvacrol, which is considered the main component of oregano oil that has a strong inhibitory effect on the growth of the stem, its thickness as well as the rate of photosynthesis (Dudai et al. 2004; Argyropoulos et al. 2008; Kadoglidou et al. 2020; Karalija et al. 2020; Lombrea et al. 2020).

Previous studies have shown that monoterpenes act on seeds in very low concentrations (Dudai et al. 2004), and that they possess very powerful inhibitors of wheat seed germination (Dragoeva et al. 2014). The results of our study demonstrated inhibitory effect of *O. vulgare*. EO at the lowest concentration, but stimulated at the highest concentration on the germination of wheat seeds. A similar trend can be noticed for mint, opposite to tomato. Argyropoulos et al. (2008) observed that oregano EO inhibits germination of tomato seeds (88.3%), even when used in the lowest concentration (1 μl/ml), which is attributed to the higher concentration of carvacrol (Ibáñez and Blázquez 2020). Our results indicate that the highest percentage of tomato seeds inhibition was recorded in treatments with the highest concentration of oregano EO (17.72%). Previously was reported that monoterpene compounds of EO are responsible for germination inhibition (Dudai et al. 2004), and that oregano essential oil had the strongest phytotoxic effect on seed germination and growth of tomato seedlings, in comparison with other applied oils (Ibáñez and Blázquez 2020).

The phytotoxic effect of *O. vulgare* EO was significant in monocotyledons (*T. aestivum* and *Hordeum vulgare*), while in dicotyledons the stimulating effect was observed (Grulova et al. 2020). Our results revealed that *O. vulgare* essential oil showed different effects on monocotyledons and dicotyledons, respecting their biological activity. The lowest value of the phytotoxicity index in *T. aestivum* (-0.07%) was recorded at the lowest concentration of oregano oil, while the value of the phytotoxicity index in *L. esculentum* increased with an increase in the oregano EO concentration.

The beneficial effect of oregano (*O. vulgare* L. subsp. *hirtum*) and green mint (*M. spicata* L.) on agronomic characteristics (taller plants with thicker stems), improvement of physiological characteristics of tomato (higher index of chlorophyll content and speed of photosynthesis), was also observed as an increased yield and improved quality characteristics of tomato fruits (Kadoglidou et al. 2020). This study revealed that direct incorporation of oregano or spearmint plant material into the soil can improve tomato resistance to soil-borne fungi, soil fertility, and consequently increase yield and product quality.

It has been proven that root growth is regulated by a cell division in the zone of active meristem and subsequent cell elongation, which represents independent events (Shishkova et al. 2007). In fact, the rate of root growth is directly affected by the disruption of either of

these two processes (Obroucheva 2008). Therefore, it is reasonable to assume that the decrease in *A. cepa* root growth caused by oregano essential oil (*O. vulgare*) is a consequence of a reduced mitotic index (cell division) in *A. cepa* root apical meristem.

The results of the present study are supportive to the results of a recent study which demonstrated that oregano *O. vulgare* EO led to a decrease in the value of the mitotic index (MI) in onion meristem cells (*A. cepa*) (Grondona et al. 2014). Similarly, one study evidenced that a significant decrease in *Vicia faba* L. mitotic activity caused by oregano oil was more pronounced than that caused by rosemary oil. According to cytotoxicity limit values, both oils showed subletal cytotoxic effects (Hamedo and Abdelmigid 2009). Furthermore, our results are consistent with the results of an investigation in which the antiproliferative potential of ethanol extract of oregano on Caco-2 cells of adenocarcinoma of the colon, through an increased proapoptotic activity, was reported (Savini et al. 2009). Begnini et al. (2014) observed that EOs from *O. vulgare* inhibited cell proliferation in breast adenocarcinoma cells (MCF-7) and colon adenocarcinoma (HT-29). Similarly, Marrelli et al. (2016) showed antiproliferative effect of deer grass essential oil (*O. dictamnus*) on the cell line of colon cancer (LoVo), as well as on the hepatocarcinoma (HepG2) cell line.

In conclusion, the essential oil of oregano showed a phytotoxic effect on selected species. In addition, it exhibited antiproliferative effects in *A. cepa*. Studies like this can contribute to a better understanding of the allelopathic and toxicological potential of essential oils, but further research is needed to evaluate their potential use for natural weed control.

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