**Annals of the Institute of Biology - University of Sarajevo**
ISSN- ISSN 2831-0705 (Online) http://aibus.pmf.unsa.ba/
AIBUS 45: 21-28
DOI: 10.35666/2831-0705.2023.45.21
Original Article UDC [581.135.5:581.524.13]:582.929.4
14 December 2023

# Allelopathic Effect of *Lavandula angustifolia* Mill. and *Thymus serpyllum* L. Essential Oils on Five Selected Plant Species

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## Abstract

In this study allelopathic effects of essential oils from English lavender (*Lavandula angustifolia*) and creeping thyme (*Thymus serpyllum*) on seed germination and seedling growth of mint (*Mentha spicata*), lemon balm (*Melissa officinalis*), tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*), and black locust seeds (*Robinia pseudoacacia*) was evaluated. The assessment of the allelopathic effect of essential oils was examined in a laboratory biological test. Working solutions of essential oils were prepared in three concentrations (10, 20 and 30 μg/mL). Both oils exhibited allelopathic effects, however, the concentrations that exhibited suppressing effects were different among the plants. It was noticed that English lavender and creeping thyme oils reduced the germination and growth of mint and black locust seeds and significantly suppressed tomato seed germination at higher concentrations (20 and 30 μg/mL), while it increased the lemon balm seed germination.

Keywords: alleopathy, creeping thyme, English lavender, essential oils, *Lavandula angustifolia*, *Thymus serpyllum*.

Received 1 June 2023; revision received 31 October 2023; accepted 1 November 2023 Editor in Chief: prof. dr. Samir Đug.

## 1. Introduction

The allelopathic effect of essential oils and their chemical components is very well known. Their herbicidal, insecticidal, nematicidal, fungicidal, and bactericidal effects have been described in previous works (Isman et al., 2011; Pavela and Banelli, 2016; Martinez et al., 2017). On the other hand, they have low toxicity for mammals and they decompose faster, and therefore represent a "green" alternative to the synthetic agents that are used in agricultural production today.

The Lamiaceae family contains highly active components such as α-pinene, limonene, camphor, carvacrol, thymol, etc. Citronella oil and D-limonene are components of some registered herbicides such as Barrier H®, Avenger Organic Weed Killer®, and Organic Interceptor® (Giepen et al., 2014). The Lamiaceae family also contains thymol and carvacrol, which, in addition to having a strong antimicrobial, antiviral, antifungal, and antioxidant effect, have also been shown to have a strong effect on the inhibition of plant germination (Edris, 2007; Synowiec et al., 2017; Synowiec et al., 2019), for controlling plant diseases, and life extending of fruits and vegetables (Solgi and Ghorbanpour, 2014).

Essential oils of *Thymus vulgaris* L. and *Lavandula angustifolia* Mill. (Lamiaceae) have long been known for their medicinal properties, i.e. antibacterial, antimicrobial, antifungal, and insecticidal effects (Verma et al., 2014). Previous research has indicated the promising potential of these essential oils for aphid control (Yousefzadi et al., 2009). There are also reports on the allelopathic effect of extracts and essential oils of these plants on various other species (Cavalieri and Caporali, 2010; Zheljazkov et al., 2021; Zhou et al., 2021).

The main objective of this study was to determine the allelopathic effects of essential oils of English lavender (*Lavandula angustifolia*) and creeping thyme (*Thymus serpyllum*) on seed germination and growth of mint (*Mentha spicata* L.), lemon balm (*Melissa officinalis* L.), tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), and black locust seeds (*Robinia pseudoacacia* L.).

## 2. Material and Methods

### *2.1. Plant material and essential oils*

Commercially produced seeds [S.i.p.a.s, Via Emilia 1810/A, Longiano (FC), Italia] of mint (*Mentha spicata* L.), lemon balm (*Melissa officinalis* L.), tomato (*Lycopersicon esculentum* Mill.) and cucumber (*Cucumis sativus* L.) were used. Black locust seeds (*Robinia pseudoacacia* L.), in their reproductive phase, were collected in Dariva areas of Sarajevo (Bosnia and Herzegovina; 43°51'25'' N, 18°26'42'' E). The collected seeds were cleaned and stored at 4°C until use. Essential oils of English lavender (*Lavandula angustifolia* Mill.) and creeping thyme (*Thymus serpyllum* L.), commercially available (Cydonia d.o.o., Lipa 73, Gračanica, BiH), were used. Stock solutions of essential oils were prepared using DMSO (dimethyl sulfoxide; Sigma-Aldrich, Deinheim, Germany) and serial dilutions were prepared with distilled water to give the concentrations of 10, 20 and 30 µg/mL.

### *2.2. Cultivation of the seeds*

The effect of the essential oils of *L. angustifolia* and *T. serpyllum* was evaluated by cultivating the seeds in a growth chamber. In Petri dishes, with two layers of filter paper, 25 seeds of each species were placed. Five mL of essential oils at three different concentrations (10, 20 and 30 µg/mL) were added to Petri dishes, and distilled water was used as the control. The Petri dishes were then placed in a growth chamber at 23°C and 16 hours photoperiod. Seeds were considered as germinated when radicles were longer than 1 mm and with a visible root (Kolb et al. 2016). The test was performed in four replications in completely randomized design. After five, seven and ten days, the results were recorded. We analyzed index of germination (AOSA, 1983), germinationpercentage (Scott et al., 1984); germination vigor index (Ranal and Santana, 2006), percentage of germination inhibition (Ali et al., 2015), and phytotoxicity index (Mekki et al., 2007). Phytotoxicity index ranges between (0) and (1), where a higher value indicates a negative (toxic) effect and a lower value a positive (stimulating) effect.

### *2.3. Statistical analysis*

All results were expressed as the mean of four replicates (± standard deviation). The one-way ANOVA, followed by Duncan's multiple range test and Pearson's correlation coefficient were applied. The significance level of $p<0.01$ is considered as very significant.

## 3. Results

Essential oils of English lavender and creeping thyme significantlly decreased germinatin index of *M. spicata*, *L. esculentum* and *C. sativum*, especially at higher concentrations (Table 1). Seeds of *M. officinalis* were not affected by English lavender and creeping thyme oils while *R. pseudoacacia* seeds were not affected only by English lavender oil. Germination index in all cases decreased with the increasing oils concentrations

***Table 1.*** *Effect of different concentrations of* L. angustifolia *and* T. serpyllum *essential oils on germination index (GI), percentage of germination (G%), germination vigor index (GVI), percentage of germination inhibition (GI%), and index of phytotoxicity (IPH)*

| | | GI | | G% | | GVI | | GI% | | IPH | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **English lavander** | **Creeping thyme** | **English lavander** | **Creeping thyme** | **English lavander** | **Creeping thyme** | **English lavander** | **Creeping thyme** | **English avander** | **Creeping thyme** |
| *Mentha spicata* | Control | 18.74±2.84$^{c}$ | 18.74±2.84$^{c}$ | 87.00±6.00$^{a}$ | 87.00±6.00$^{a}$ | 11.92±2.28$^{c}$ | 11.92±2.28$^{c}$ | - | - | - | - |
| | 10 µg/mL | 18.36±0.64$^{c}$ | 15.08±1.63$^{b}$ | 86.00±9.52$^{a}$ | 86.00±2.31$^{a}$ | 11.72±1.38$^{c}$ | 10.21±1.06$^{b.c}$ | 6.07 | 6.79 | -0.10 | 0.07 |
| | 20 µg/mL | 13.75±1.49$^{b}$ | 13.12±1.37$^{b}$ | 81.00±3.83$^{a}$ | 88.00±7.30$^{a}$ | 8.48±1.73$^{b}$ | 8.19±1.29$^{b}$ | 6.73 | 5.81 | 0.21 | 0.25 |
| | 30 µg/mL | 10.22±1.65$^{a}$ | 10.00±1.09$^{a}$ | 73.00±12.38$^{a}$ | 82.00±2.31$^{a}$ | 4.38±1.31$^{a}$ | 5.41±0.41$^{a}$ | 15.5 | 13.21 | 0.54 | 0.50 |
| *Melissa officinalis* | Control | 21.56±3.49$^{a}$ | 21.56±3.49$^{a}$ | 75.00±8.25$^{a}$ | 75.00±8.25$^{a}$ | 29.47±7.60$^{b}$ | 29.47±7.60$^{b}$ | - | - | - | - |
| | 10 µg/mL | 21.19±3.18$^{a}$ | 20.45±4.15$^{a}$ | 75.00±6.83$^{a}$ | 78.00±15.49$^{a}$ | 26.17±4.55$^{b}$ | 33.03±6.51$^{b}$ | 2.75 | 22.25 | 0.10 | -0.13 |
| | 20 µg/mL | 20.46±3.53$^{a}$ | 19.59±2.24$^{a}$ | 83.00±11.49$^{a}$ | 83.00±7.57$^{a}$ | 22.81±5.96$^{a.b}$ | 24.80±3.03$^{a.b}$ | 10.50 | 14.25 | 0.32 | 0.22 |
| | 30 µg/mL | 17.52±2.86$^{a}$ | 17.65±0.64$^{a}$ | 81.00±8.87$^{a}$ | 85.00±3.83$^{a}$ | 16.90±2.40$^{a}$ | 17.90±1.56$^{a}$ | 11.25 | 14.50 | 0.50 | 0.48 |
| *Lycopersicon esculantum* | Control | 26.05±2.50$^{c}$ | 26.05±2.50$^{c}$ | 84.00±8.64$^{c}$ | 84.00±8.64$^{c}$ | 80.83±15.10$^{b}$ | 80.83±15.10$^{c}$ | - | - | - | - |
| | 10 µg/mL | 14.61±1.44$^{b}$ | 16.45±1.48$^{b}$ | 72.00±5.66$^{c}$ | 81.00±5.03$^{c}$ | 57.75±3.49$^{b}$ | 73.87±2.39$^{c}$ | 16.00 | 13.50 | 0.20 | 0.00 |
| | 20 µg/mL | 3.07±2.39$^{a}$ | 4.43±1.32$^{a}$ | 25.00±13.22$^{b}$ | 38.00±9.52$^{b}$ | 8.27±6.68$^{a}$ | 17.83±5.37$^{b}$ | 70.50 | 53.75 | 0.73 | 0.51 |
| | 30 µg/mL | 0.61±0.60$^{a}$ | 2.41±1.11$^{a}$ | 8.00±7.90$^{a}$ | 17.00±5.03$^{a}$ | 0.79±0.77$^{a}$ | 3.29±1.03$^{a}$ | 89.25 | 79.00 | 0.92 | 0.81 |
| *Cucumis sativus* | Control | 44.07±4.21$^{c}$ | 44.07±4.21$^{c}$ | 98.00±2.31$^{a}$ | 98.00±2.31$^{a}$ | 38.88±3.85$^{b}$ | 38.88±3.85$^{b}$ | - | - | - | - |
| | 10 µg/mL | 33.12±0.82$^{b}$ | 44.64±4.82$^{c}$ | 100.00±0.00$^{a}$ | 99.00±2.00$^{a}$ | 48.16±3.43$^{c}$ | 42.50±9.49$^{b}$ | 2.00 | 1.00 | -0.27 | -0.15 |
| | 20 µg/mL | 31.37±0.58$^{b}$ | 30.62±1.19$^{b}$ | 100.00±0.00$^{a}$ | 97.00±3.83$^{a}$ | 43.73±2.31$^{c}$ | 45.75±4.50$^{b}$ | 2.00 | 3.00 | -0.18 | -0.33 |
| | 30 µg/mL | 20.39±1.01$^{a}$ | 20.23±1.79$^{a}$ | 92.00±5.66$^{a}$ | 95.00±5.03$^{a}$ | 23.28±1.08$^{a}$ | 18.51±2.91$^{a}$ | 8.00 | 3.00 | 0.30 | 0.48 |
| *Robinia pseudoacacia* | Control | 29.68±7.87$^{a}$ | 29.68±7.87$^{b}$ | 87.00±18.29$^{a}$ | 87.00±18.29$^{b}$ | 41.87±5.83$^{c}$ | 41.87±5.83$^{c}$ | - | - | - | - |
| | 10 µg/mL | 26.33±5.81$^{a}$ | 29.39±4.96$^{b}$ | 85.00±11.94$^{a}$ | 90.00±7.66$^{b}$ | 29.51±8.61$^{b}$ | 36.41±9.34$^{b.c}$ | 28.75 | 21.00 | 0.26 | 0.21 |
| | 20 µg/mL | 23.90±2.92$^{a}$ | 21.55±3.19$^{a}$ | 83.00±5.03$^{a}$ | 77.00±5.03$^{a.b}$ | 30.54±3.34$^{b}$ | 30.02±5.18$^{b}$ | 20.25 | 18.50 | 0.20 | 0.22 |
| | 30 µg/mL | 21.48±7.51$^{a}$ | 14.81±1.60$^{a}$ | 76.00±4.62$^{a}$ | 68.00±6.53$^{a}$ | 20.58±1.55$^{a}$ | 18.52±2.74$^{a}$ | 24.75 | 24.75 | 0.40 | 0.41 |

**Note**: Values are expressed as means ± standard deviations of four replications; Means in the same column with different letters in superscript are significantly different at $p<0.01$

*Lavandula angustifolia* and *T. serpyllum* essential oils exhibited a dose-dependent phytotoxic activity against the seed germination *of L. esculentum*, with significantly decreased germination at the lower dose (20 µg/mL) tested (25%), and almost total inhibition at the highest dose (30 µg/mL) applied (8% with English lavender and 17% with creeping thyme oil) (Table 1). Germination percentage of *R. pseudoacacia* was slightly affected only by creeping thyme essential oils at higher concentrations (20 and 30 µg/mL). All other species did not show significantly changes in germination percentages after application these two essential oils (Table 1). The percentage of inhibition of germination follows these results with the greatest effect on tomatoes, where the percentage of inhibition of germination was 89.25 (at 30 µg/mL of English lavender oil, and 79% at the same concentration of creeping thyme oil).
Furthermore, both oils at the highest concentration (Table 1) completely suppressed vigor index in tomato (0.79 and 3.29 µg/mL respectively), moderate suppressed in mint (4.38 and 5.41 µg/mL respectively), and slightly in lemon balm (16.90 and 17.90 µg/mL respectively), black locust (20.58 and 18.52 µg/mL respectively), and cucumber (23.28 and 18.51 µg/mL respectively). The opposite effect was noticed only in cucumber at 10 and 20 µg/mL concentrations of both oils (Table 1).
Negative phytotoxic index was noticed at the highest concentration of both oils in mint, lemon balm and tomato (Table 1), and at 20 µg/mL concentration in tomato. In all other cases the phytotoxic indexes were positive.

### 3. Discussion

The essential oils of English lavender and creeping thyme showed different effects on seed germination and early growth of the investigated plant species (Table 1). The percentage of germination decreased with the increase in the concentration of essential oils with the exception of *M. officinalis*. Higher concentrations (20 and 30 µg/mL) of both essential oils showed a high percentage of inhibition in tomato seeds germination, with almost complete inhibition of seed germination at a concentration of 30 µg/mL. A similar observation of the inhibitory effect of *L. angustifolia* essential oil on the percentage of tomato germination was observed by Ibáñez and Blázquez (2019). It is well documented that some allelochemicals suppress the mitotic activity of initially developed cells, which leads to inhibition of seed germination (Rice, 1987).
In most cases, growth parameters varied due to different percentage concentrations of essential oils with increasing concentrations, the inhibitory effects progressively increased. The root length of the germinated seeds was more sensitive to allelochemicals compared to the shoot, which was reflected in the percentage of inhibition of germination and the phytotoxicity index. Such effect could be expected, considering that roots first absorb allelochemicals and then transfer them to shoots that is confirmed in previous studies (Turk and Tawaha, 2003; Sarkar et al., 2012).
Seed vigor is an important parameter for testing seed quality. Essential oils of English lavender and creeping thyme at application rates of 20 and 30 µg/mL completely suppressed germination vigor index (Table 1). In addition, when applied at 10 µL both essential oils slightly decreased the vigor index compared to the control or, in the case of the creeping thyme essential oil, increased it in *M. officinalis*. The results were similar to

those of Zheljazkov et al. (2021) in vigor index of wheat and barley seeds, especially at higher concentrations of *L. angustifolia* and *T. vulgaris* essential oils.

The percentage of inhibition of germination is dose-dependent and varies in different species. Both essential oils have an inhibitory effect on tomato seedlings with relatively high rates of inhibition, which was not observed in the other tested species. The inhibition of germination under the influence of essential oils, mainly terponoids, monoterpenes and sesquiterpenes, has already been recorded in different crops and weeds (Batis et al., 2004; Uremis et al., 2009; Gitsopoulus et al., 2013). Thus, it was observed that carvacrol and thymol showed complete inhibition of seed germination and seedling growth of numerous plant species (Kordali et al., 2008; de Almeida et al., 2010; Ibáñez and Blázquez, 2017). Ibáñez and Blázquez (2017) observed that oregano essential oil with 60.42% carvacrol exhibited complete inhibition of *Portulaca oleracea* L.*, Lolium multiflorum* Lam., and *Echinochloa crus-galli* (L.) P. Beauv., while de Almeida et al. (2010) observed a complete inhibition of *Lepidium sativum* L., *Raphanus sativus* L., and *Lactuca sativa* L. under the influence of, among others, oils of oregano and thyme. These results show that the applied oils could be both effective and an alternative to synthetic herbicides and cause less problems for the environment and human health.

**5. Conclusions**

Our results show that essential oils of *Lavandula angustifolia* and *Thymus serpyllum* stimulated the germination of *Melissa officinalis* seeds; negatively affected the germination of *Lycopersicon esculentum* and *Robinia pseudoacacia* seeds, and had a moderate inhibitory effect on Mentha spicata seed germination. Both essential oils can be considered as potential allelopathic agents that, due to their organic origin, easy degradability in nature, without known negative effects on humans and nature, could significantly improve agricultural practices.

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