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The effect of *Lamiaceae* plants essential oils on fungal plant pathogens *in vitro*

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Abstract. Fungal pathogens *Alternaria* spp., *Botrytis* spp. and *Colletotrichum* spp. cause a significant loss of horticultural crops and their yield annually. The most convenient way for controlling diseases caused by these pathogens is the use of chemical fungicides. However, current practices still result in soil, water and air pollution, contribute to the loss of biodiversity and climate change, also are harmful to human health. Therefore, there is a growing demand for environmentally friendly plant protection methods. Herbs, especially, volatile oils, are a natural source of active ingredients. The findings of antimicrobial and antifungal activities, low toxicity, and biodegradability of essential oils make them potential for use in plant protection against pathogens instead of chemicals. This research aimed to evaluate the ability of *Lamiaceae* plants essential oils to suppress the growth of *Alternaria* spp., *Botrytis* spp., and *Colletotrichum* spp. *in vitro*. The study was carried out at the LAMMC Institute of Horticulture, Lithuania. Essential oils from lavender (*Lavandula angustifolia*) and thyme (*Thymus vulgaris*) were obtained by hydrodistillation and poured to potato dextrose agar medium at 200–1,000 μL L^{-1} concentrations. The radial colony growth of each pathogen measured after placing mycelial plugs of each fungus on Petri dishes. Results demonstrated that thyme essential oil significantly suppressed the growth of all three investigated fungal pathogens at concentrations starting from 400 μL L^{-1} 7 days after inoculation as no growth of the pathogens observed. Meanwhile, lavender essential oil had lower antifungal activity than thyme. The most significant concentration of lavender essential oil was 1,000 μL L^{-1}. To conclude, thyme essential oil showed high antifungal activity, and lavender essential oil showed moderate antifungal activity for our tested horticultural crop fungal pathogens. Both oils can be applied as one of the eco-friendly ways to control plant pathogens.

Key words: antifungal, inhibition, *Lavandula angustifolia*, lavender, *Thymus vulgaris*, thyme.

INTRODUCTION

Nowadays about 80% of foods are of plant origin due to new consumer habits. The safety of agricultural products means much more than the quantitative and qualitative safety of the foods produced (Carvalho, 2017). There are many factors affecting plant health, and fungal pathogens are ones of them. Diseases caused by pathogens have a significant economic impact on plant production for different crops (Survilienė et al., 2010; Singh et al., 2015; Boddy, 2016; He et al., 2019). It may infect plants though miscellaneous parts at diverse growth stages, and resulting in high losses of the plants or whole yield (Patriarca et al., 2014; Neri et al., 2016; He et al., 2019; Rosero-Hernandez et al., 2019). Anthracnose, caused by *Colletotrichum* species, and grey mould, caused by *Botrytis cinerea*, are primary diseases of strawberry, nevertheless, could be found among other hosts as well (Boddy, 2016; Rasiukevičiūtė et al., 2018; He et al., 2019; Jakobija et al., 2020). A common fungal genus *Alternaria* spp. causes Alternaria leaf spot or Alternaria leaf blight that leads to pre- and postharvest damage to agricultural products, including vegetables, fruits, and cereal (Patriarca et al., 2014). In addition to spoiling a wide variety of foods, some *Alternaria* species can produce secondary metabolites considered as mycotoxins, which can be harmful to humans and animals, and phytotoxins, which play an essential role in the pathogenesis of plants (Lõiveke et al., 2004; Kütt et al., 2010; Patriarca et al., 2014).

Plant protection products, such as chemical fungicides, are used to protect crops from fungal diseases (Survilienė & Dambrauskienė, 2006; Abbey et al., 2019). Based on research in recent years, it can be confirmed that pesticide residues are present in about half of food products, and several of them can be detected quite often in various products (Carvalho, 2017). The combined effects may be complementary, but there are also cases where active substances in different chemical plant protection products enforce each other`s adverse effects. It is known that several plant protection products have a more substantial overall impact on each other`s presence. The current legislation does not address the overall effects of active substances (Sharma et al., 2019).

Moreover, surface waters and soil are contaminated with fungicides remains too, and the global warming increases toxicity of pesticides at higher temperatures (Op de Beeck et al., 2017; Sharma et al., 2019). Additionally, a significant impact on pathogen resistance is observed, which causes difficulties in controlling diseases and imbalance the microbial community in soil. According to the requirements of EU Regulation 2009/128/EC, reducing chemical products residues is one of the primary purposes of engaging in crop production. It is essential to use those active substances in disease control that address specific plant protection problems and pose the least risk to the environment and human health (OJEC, 2009).

Therefore, to solve pesticides contamination problems, several research groups have explored a natural source of effective ingredients to control soil-borne pathogens. The findings of low toxicity, biodegradability, antifungal and antimicrobial activities of plants essential oils allow us to use eco-friendly plant protection instead of chemical (Abdolahi et al., 2010; Hussein & Joo, 2017; Reang et al., 2020; Šernaitė et al., 2020). Essential oils have been widely studied and applied in the food, medicine and cosmetic industries (Zabka et al., 2014; Carvalho, Estevinho & Santos, 2016; Karpiński, 2020).

Lamiaceae family plants are found all over the world, and many are familiar garden herbs such as rosemary, oregano, mint, basil, lavender and thyme. As a result, it becomes a cheap raw material and the application of these herbs essential oils for horticultural crop fungal pathogens control as a biofungicides represents an alternative disease management strategy due to its ability to provide environmentally friendly disease control (Feng et al., 2011; Mamgain et al., 2013). Various essential oils for the management of *Alternaria* spp., *Botrytis* spp., *Colletotrichum* spp. and other fungal pathogens in field and greenhouse conditions have been investigated (Abdolahi et al., 2010; Sarkhosh et al., 2018a; Awais et al., 2020). However, in reviewing the available literature, it was observed that there is a lack of studies on the antifungal activity of thyme and lavender essential oils against horticultural crop fungal pathogens.

This research aimed to evaluate the ability of *Lamiaceae* plants essential oils to suppress the growth of *Alternaria* spp., *Botrytis* spp., and *Colletotrichum* spp. *in vitro*.

MATERIALS AND METHODS

The research was carried out at the LAMMC Institute of Horticulture (LAMMC IH) Laboratory of Plant Protection in 2018–2019. Essential oils (EOs) of lavender (*Lavandula angustifolia* Mill.) and thyme (*Thymus vulgaris* L.) herbs were selected for this study according to their antifungal and antimicrobial activities, which have the potential to be effective against plant pathogens such as *Alternaria* spp., *Botrytis* spp., and *Colletotrichum* spp.

Essential Oils Extraction

Thymus and lavender herbs were collected from the experimental fields of LAMMC IH and naturally dried. According to the Association of Official Analytical Chemists (AOAC, 1990) methods, one kilogram of each plant material was extracted using a Clevengerdistillation system (Glassco, India). The time of each materials extraction was 2 hours under normal atmospheric pressure.

Efficacy of Essential oils

The lavender and thyme essential oils separately poured into the potato dextrose agar (PDA) medium at concentrations of 200, 400, 600, 800, and 1,000 $\mu L\ L^{-1}$, homogenised and distributed in sterilised Petri dishes at a temperature of 45 °C. Mycelium plugs (6-mm-diameter) of 7-day old single spore isolates of *Alternaria* spp., *Botrytis cinerea*, *Colletotrichum* spp. (from LAMMC IH Laboratory of Plant Protection isolate collection)were cut and placed fungal side down in the centre on each Petri dish with the PDA and the tested EOs option. Pathogens obtained from infected carrot (*Alternaria* spp.) and strawberry fruits (*B. cinerea*, *Colletotrichum* spp.) were identified by using of 10x and 40x magnifications on the microscope evaluating their sporangiophores, sporangia, hyphae, conidiophores, conidia, colony texture and growth pattern (Simmons, 2007; Kumar & Kudachikar, 2018; Rasiukevičiūtė et al., 2018). There were four replications of the same treatment. Plates incubated at 22 ± 2 °C temperature in the dark for 7 days. After 2, 4, and 7 days inoculation (DAI), radial growth (cm) of mycelium (including the diameter of the disc) was measured and compared with the results of the control.

Statistical Analysis

SAS Enterprise Guide 7.1 program (SAS Institute Inc., Cary, NC, USA) was applied for the analysis of experimental data. Analysis of variance (ANOVA) procedure was processed, and Duncan`s multiple range test ($p < 0.05$) was used for the comparison of obtained means.

RESULTS AND DISCUSSION

The ability of EOs from *Lamiaceae* plants to suppress the growth of *Alternaria* spp., *B. cinerea*, *Colletotrichum* spp. was investigated on PDA under different concentrations.

The antifungal effect of thyme and lavender EOs on *B. cinerea* colony growth 2, 4 and 7 DAI is presented in Fig. 1. Different EOs exhibited various antifungal activities. The thyme EO concentrations of 400, 600, 800, and 1,000 μL L^{-1} significantly inhibited mycelium growth ($p < 0.05$) through the whole experimental period. However, the effect of 200 μL L^{-1} concentration was weaker at 7 DAI. The measured radial colony growth of the pathogen was 1.23 cm, while no radial colony growth was observed at other concentrations. Similarly to our results, Abdolahi et al. (2010) found that thyme EO strongly suppressed *B. cinerea* and *Mucor piriformis* growth when added at concentrations from 800 μL L^{-1}. However, thyme oil at 200 μL L^{-1} only presented a fungistatic effect against *B. cinerea* (Abdel-Rahim & Abo-Elyousr, 2017). In other research, Reang et al. (2020) evaluated five essential oils (*Syzygium aromaticum* L., *T. vulgaris* L., *L. angustifolia* L., *Cymbopogon citratus* and *Mentha piperita* L.) against grey mould at 0.5%, 1% and 1.5% concentration under *in vitro* conditions. Obtained results agree with our study: among the five essential oils, thyme oil showed maximum growth inhibition of *B. cinerea* at all concentrations. Meanwhile, *L. angustifolia* EO had a moderate effect on the radial growth of this fungus.

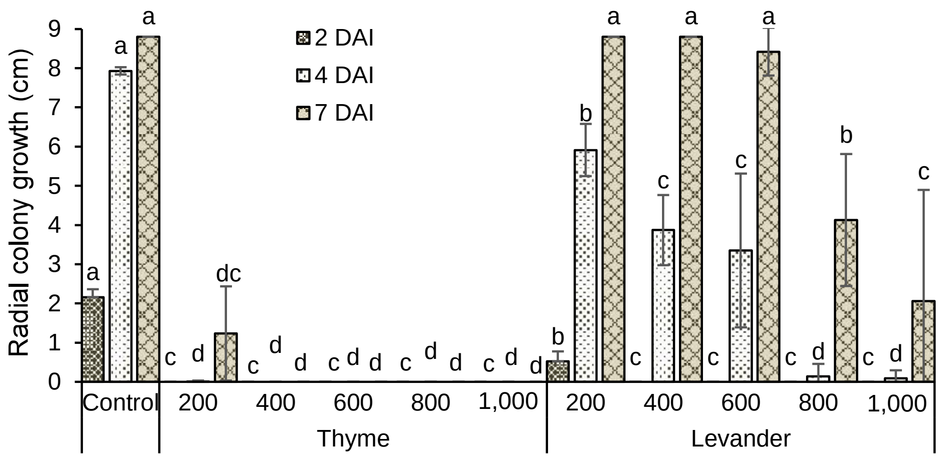

Figure 1. The antifungal effect of thyme and lavender essential oils on *B. cinerea* colony growth at different concentrations 2, 4, and 7 days after inoculation (DAI). The same letter indicates no significant differences between treatments ($p < 0.05$).

In our study, concentrations from 400 μL L^{-1} to 1,000 μL L^{-1} of lavender EO showed pathogen suppression ($p < 0.05$) 2 DAI ultimately. However, inhibition of the mycelium growth decreased 4 and 7 DAI. This EO demonstrated minimal inhibition at 200 μL L^{-1} at 2 DAI (0.52 cm), 4 DAI (5.91 cm), and 7 DAI (8.80 cm) compared to control (2.16, 7.93 and 8.80 cm). The radial growth of the *B. cinerea* was reducing with the increasing concentration of lavender oil. Soylu et al. (2010) described a 25.6 μg mL^{-1} concentration as having the best fungicidal properties while investigating *in vitro* and *in vivo* antifungal activities of volatile oils. Inhibition of spore germination was also observed, but at a sufficiently higher concentration (51.2 μg mL^{-1}).

The antifungal effect of thyme and lavender EOs on *Colletotrichum* spp. colony growth 2, 4, and 7 DAI is presented in Fig. 2. The graph shows that fungal growth was significantly inhibited ($p < 0.05$) by thyme EO, starting from minimal concentration (200 μL L^{-1}) during the research. A similar result was obtained by Sarkhosh et al. (2018b). Authors found that the minimum complete inhibitory concentration was determined to be 125 μL L^{-1}. In Vilaplana et al. (2018) study thyme oil demonstrated the best fungicidal effect against *Colletotrichum musae* at tested concentrations (100, 250, 500 and 1,000 μL L^{-1}). It exhibited significant mycelial growth inhibition ($p < 0.05$) compared to other essential oils tested 6 and 12 DAI. Another case revealed *in vitro* efficacy of the EO extracted from eight plant species (Sarkhosh et al., 2018a). The application rates were 100, 250, 500, 1,000, and 2000 μL L^{-1}. The results showed a 100% reduction of mycelium growth of *Colletotrichum*, *Botryosphaeria*, *Phytophthora*, and *Fusarium*, after applying thyme oil at all concentrations tested (Sarkhosh et al., 2018a).

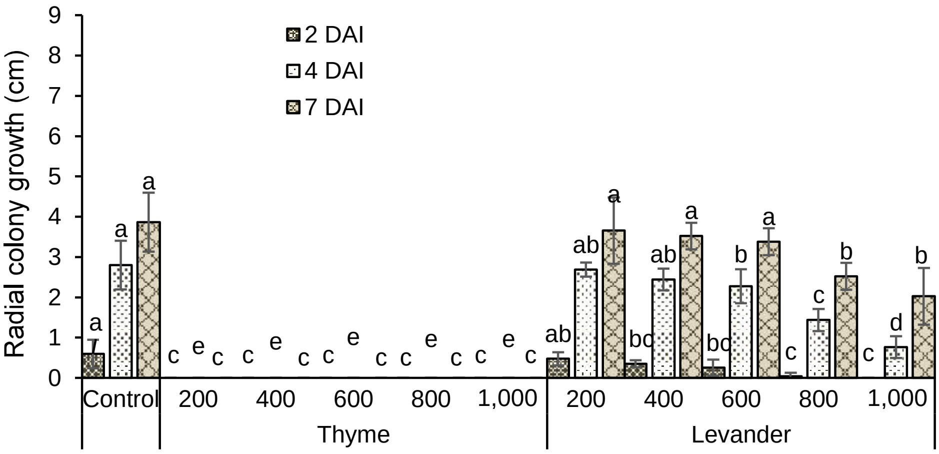

Figure 2. The antifungal effect of thyme and lavender essential oils on *Colletotrichum* spp. colony growth at different concentrations 2, 4, and 7 days after inoculation (DAI). The same letter indicates no significant differences between treatments ($p < 0.05$).

Unlike the thymus EO, the effect of lavender EO was not so high, and the growth of fungal colonies was very similar to that of the control. Only 1,000 μL L^{-1} of lavender EO showed significant growth repression 2 DAI. The same tendency was observed that with increasing concentration in the medium, radial colony growth decreased. Our results are similar to those of Sarkhosh et al. (2018b), who reported that mycelial growth of *C. gloeosporioides* at a higher application rate (1,000 μL L^{-1}) was completely inhibited. There was also a significant positive linear relationship between application rate and mycelial growth. Hoseini et al. (2019) study showed that *L. angustifolia* EO reached 84.5% mycelium growth inhibition at 1,000 μL L^{-1} 10 DAI.

The antifungal effect of thyme and lavender EOs on *Alternaria* spp. colony growth 2, 4, and 7 DAI is presented in Fig. 3. Thyme EO had a strong antifungal effect from 400 μL L^{-1} concentrations, as no *Alternaria* spp. colony growth was observed at 400–1,000 μL L^{-1} ($p < 0.05$). The concentration of 200 μL L^{-1} performed highly antifungal; however, the measured mycelium was 0.04 cm 7 DAI. Feng et al. (2011) study revealed that thyme EO at 500 μL L^{-1} showed a significant contact inhibition effect on *A. alternata* for 3 days both *in vitro* and *in vivo*. Either *A. citri* growth was completely prevented at 400 μL L^{-1} *in vitro* concentration of *T. vulgaris* EO. Moreover, the fungal growth steadily decreased with an increasing EO amount (Ramezanian et al., 2016).

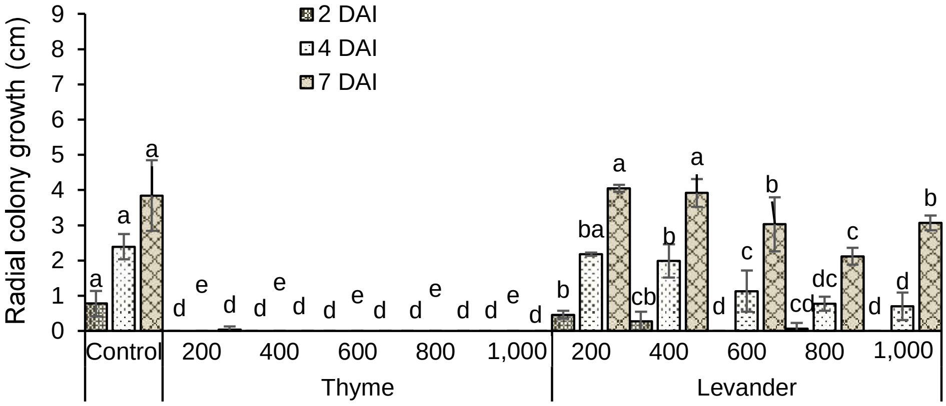

Figure 3. The antifungal effect of thyme and lavender essential oils on *Alternaria* spp. colony growth at different concentrations 2, 4, and 7 days after inoculation (DAI). The same letter indicates no significant differences between treatments ($p < 0.05$).

The lavender EO did not maintain the reduced *Alternaria* spp. growth at 200 μL L^{-1} and 400 μL L^{-1} 7 DAI and even promoted the spread of mycelium compared to control. The diameter of the colonies was 4.05 and 3.92 cm, respectively. The mycelium grew to 0.78 cm in the control treatment 2 DAI, 2.39 cm after 4 and 3.85 cm after 7. However, with increasing EO concentration, the antifungal effect intensified. The mycelium did not grow at a concentration range of 600–1,000 μL L^{-1} 2 DAI. Subsequently, mycelial growth of pathogen was not so effectively inhibited like after 2 days, although a smaller diameter was measured with increasing concentration at 4 and 7 DAI. According to Hussein & Joo (2017), distinctive antifungal activity against all fungi (*Alternaria*, *Fusarium*, *Sclerotinia*, C*ylindrocarpon* and *Botrytis* spp.) was showed under the influence of lavender EO 5 and 10% concentrations in Petri plates after 10 days of incubation. In Lu et al. (2019) work, lavender EO at 1,000 μL L^{-1} possessed high impact on radial colony growth among the 34 EOs at 3 DAI.

Analysing the antifungal effects of these EOs to radial growth of *Alternaria* spp., *B. cinerea*, and *Colletotrichum* spp., lavender EO was not so efficacy as thyme oil. In some cases discussed, radial colony growth was little different from the control treatment, which would mean investigating higher concentrations of the lavender EO in the future. Thyme EO can be stated as potential raw material for the development of eco-friendly plant protection products. The lowest concentration of thyme EO is 400 μL L^{-1} as no growth of the pathogens was observed 7 DAI. The effectiveness of thyme EO is perhaps due to its natural terpenoid thymol, which plays a vital role in inhibiting pathogens (Zabka et al., 2014).

CONCLUSIONS

To conclude, results demonstrated that thyme essential oil is an absolute inhibitor of all pathogenic fungi (*Alternaria* spp., *B. cinerea*, *Colletotrichum* spp.) growth during the whole experiment period. Meanwhile, the effect of lavender essential oil was short-lived and less powerful. The use of natural antifungal agents is increasingly encouraged nowadays. With more research on lavender essential oil in the future, both oils could be applied as environmentally friendly ways to control plant pathogens.

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