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## Pathogenicity of *Colletotrichum acutatum* to different strawberry cultivars and anthracnose control with essential oils

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### Abstract

*Colletotrichum* spp. is a significant strawberry (*Fragaria* × *ananassa* Duch.) pathogen causing yield losses of up to 80%. Due to the changing agrometeorological conditions, pathogens are able to spread in cooler climate countries. Essential oils (EO), as one of the environmentally friendly plant protection products, can be used to control plant pathogens. They possess antibacterial and antifungal properties. Because of the low toxicity and biodegradability, EO can be used in plant protection against pathogens instead of chemical products.

This study aimed to determine the pathogenicity of strawberry *C. acutatum* and evaluate five essential oils at different concentrations as potential bio-fungicides against strawberry anthracnose (*Colletotrichum acutatum*). The *C. acutatum* pathogenicity was evaluated on detached strawberry leaves. The inhibitory effect of five EO at 200, 400, 600, 800 and 1000 µl L<sup>-1</sup> was evaluated *in vitro*, and at 800 and 1000 µl L<sup>-1</sup> – on detached strawberry leaves. The cultivar ‘Deluxe’ was found to be the most susceptible to strawberry anthracnose, while ‘Rumba’ and ‘Elegance’ exhibited resistance. The results of *Mentha piperita* EO revealed its antifungal activity against *C. acutatum*. Meanwhile, *Salvia officinalis* EO had no influence. The *Coriandrum sativum* and *Hyssopus officinalis* EO slightly inhibited *C. acutatum* growth. The results of *Thymus vulgaris* EO assay *in vitro* demonstrated that it could be an effective biocontrol agent against strawberry anthracnose.

EO inhibited the mycelial growth of *C. acutatum* *in vitro*; however, the results of the assay on detached strawberry leaves showed that the efficacy of EO was not sufficient and needed further research.

Key words: antifungal activity, common sage, common thyme, coriander, hyssop, peppermint.

### Introduction

The extreme temperature changes favour the spread of plant diseases and evolution of new strains. New or invasive species with possible higher dispersal capability could quickly adapt to variable conditions (Freeman et al., 2001; Aguado et al., 2014). Due to the agrometeorological conditions, pathogenicity, diversity and taxonomy of *Colletotrichum* species complex change the distribution of the pathogen and disease severity across the growing areas (Freeman et al., 2001; Avila-Quezada et al., 2018).

The *Colletotrichum* species complex infects and causes diseases on many economically important crops. *Colletotrichum* spp. causes fruit and crown rots, infections of stolons, petioles and leaves by *C. acutatum*, *C. fragariae* and *C. gloeosporioides* (Debode et al., 2015; Wagner, Hetman, 2016). The *Colletotrichum* spp. is dangerous to fruit plants and propagation material in nurseries, as it infects not only young or senescent tissues but also the whole plant at all growth stages (Peres et al., 2005; Wagner, Hetman, 2016). Warm temperatures

promote the incidence of the pathogen and its severity during the growing season (Miller-Butler et al., 2019). The favourable conditions for fungus development are 25°C up to 40°C temperature and high humidity (Peres et al., 2005; Moral et al., 2012). *C. acutatum* can survive in the soil or on its surface in Nordic conditions (Parikka et al., 2016). The yield losses depend on the resistance of strawberry (*Fragaria* × *ananassa* Duch.) cultivars to *Colletotrichum* spp. (Wagner, Hetman, 2016); however, it can cause up to 80% of plant death in nurseries and more than 50% yield losses (Sreenivasaprasad, Talhinhas, 2005).

Screening of *Colletotrichum* spp. pathogenicity on the detached strawberry leaves helps assess symptom development and reduce the overall time (Miller-Butler et al., 2018). Information about strawberry pathogenicity helps choose cultivars and design effective disease management systems (Freeman et al., 2001; MacKenzie et al., 2009; Wagner, Hetman, 2016; Miller-Butler et al., 2018).

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Growing of strawberry cultivars resistant to the *Colletotrichum* spp. is more environmentally friendly and cost-effective than chemical control (Wagner, Hetman, 2016). However, overuse of fungicides could lead to pathogen resistance (Miller-Butler et al., 2019). The control of *Colletotrichum* spp. is based on several strategies, including chemical and biological control, resistant cultivars, etc. (Aguado et al., 2014).

Natural plant protection agents that have a less adverse effect on crops such as plant extracts and essential oils showed a different level of antimicrobial efficacy to various ranges of fungal and bacterial pathogens. The essential oils (EO) and extracts can be applied as one of the eco-friendly plant protection products controlling plant pathogens or as plant defence inducers (Çetin et al., 2011; Aguado et al., 2014; Aćimović et al., 2016; Bajpai et al., 2019). Research studies have found that antibacterial and antifungal properties, biodegradability and low toxicity of EO make them a potential source of natural plant protection (Çetin et al., 2011; Aćimović et al., 2016). The EO include terpenes, esters, aldehydes and phenols are a source of natural antioxidants and biologically active compounds. EO mostly are secondary metabolites, which play an essential role in plant defence, as they often possess antimicrobial properties and are non-toxic and biodegradable (Girish, Fathima, 2019). As products from plants, EO have a wide application in pharmacy, fragrance and food industries; however, recent EO studies revealed their potential antimicrobial activity (Starović et al., 2016; Kumar, Kudachikar, 2018).

It has been reported in the literature, that thyme, coriander and peppermint EO had antifungal activity against plant diseases, including *Colletotrichum* spp. (Çetin et al., 2011; Duduk et al., 2015; Aćimović et al., 2016; Kumar, Kudachikar, 2018). The antifungal effect of *Coriandrum sativum* EO on the growth rate of *Colletotrichum* pathogens has been highlighted (Aćimović et al., 2016). *C. sativum* EO has antimicrobial activity against various plant and human pathogens (Laribi et al., 2015) as *Aspergillus* spp. and *Fusarium* spp. (Singh et al., 2006). The *Salvia officinalis* is rich in essential oils, flavonoids, phenolic and terpenoid compounds (Yılar et al., 2018). *S. officinalis* EO affects *Fusarium* spp. growth (Starović et al., 2016). *Mentha piperita* EO shows significant antifungal activity against *Alternaria alternaria*, *Fusarium tabacinum*, *F. oxysporum* and other pathogens (Desam et al., 2017). According to Çetin et al. (2011), *T. sipyleus* subsp. *sipyleus* var. *rosulans* and *Origanum acutidens* EO can be used as a natural preservative in food against casual agents of food-borne diseases like *Escherichia coli*, *Pseudomonas* spp., *Geotrichum candidum* and others. However, there is a lack of literature on the antifungal activity of *Hyssopus officinalis* EO. Some of the EO have been evaluated for controlling *Colletotrichum* spp. (Mandal, Mandal, 2015; Aćimović et al., 2016). However, there is a lack of investigations on the effects of *T. vulgaris*, *M. piperita*, *S. officinalis* and *H. officinalis* EO on strawberry *Colletotrichum* spp.

Due to the increasing cultivation area of new strawberry cultivars from warmer climate zones and growing disease incidence, it is essential to evaluate the susceptibility of various strawberry cultivars to *Colletotrichum* spp. Alternative products for biological plant protection are under investigation due to the increasing resistance of pathogens and the harm of pesticides to humans and the environment. As EO have antifungal properties, they could be one of the environment-friendly plant protection products.

This study aimed to determine the pathogenicity of strawberry *Colletotrichum acutatum* and evaluate different concentrations of five essential oils as potential bio-fungicides against strawberry anthracnose.

## Materials and methods

The research was carried out at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry in 2017–2020.

*Extraction of essential oils (EO) and plant material.* EO were obtained from naturally dried seeds of common thyme (*Thymus vulgaris* L.), common sage (*Salvia officinalis* L.), peppermint (*Mentha piperita* L.), hyssop (*Hyssopus officinalis* L.) and coriander (*Coriandrum sativum* L.). The EO were extracted by the Clevenger-type (Glassco, India) hydro-distillation for 2 hours.

*Colletotrichum* spp. isolates used in this research were collected in 2018 from the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry's experimental field from the infected strawberry (*Fragaria × ananassa* Duch.) cultivar 'Deluxe' fruits at BBCH 87 growth stage. The *Colletotrichum* sp. mycelium was maintained by sub-culturing on a potato-dextrose agar (PDA) at 25°C temperature for 7 days. This step was repeated twice to purify the culture, then single-spore isolates were extracted. Single spore *Colletotrichum* sp. isolates No. Fo5 (44, 61 and 65) were stored on the PDA at 4°C temperature at the Laboratory of Plant Protection, Institute of Horticulture's isolate collection.

The isolates were initially identified by morphological attributes and confirmed by PCR as *C. acutatum* by ITS4 (TCCTCCGCTTATTGATATGC-3) and CaInt2 (GGGGAAGCCTCTCGCGG) primers (Xie et al., 2010). The concentration of *Colletotrichum* sp. DNA was determined by measuring absorbance by a spectrometer NanoDrop 1000 (Thermo Fisher Scientific, USA). The PCR was performed in 25 µL volume: 5 µL 10 × Tag buffer, 0.5 µL 10 mM dNTP Mix, 1.5 µL 25 mM MgCl<sub>2</sub>, 0.5 µL of each (CaInt2 and ITS4) primer, 1 µL of DNA and 0.2 µL 5 U µL<sup>-1</sup> Taq DNA polymerase (Thermo Fisher Scientific), and 15.8 µL DNase/RNase-free water. The PCR was performed using a thermal cycler UNO 96 (VWR®), and the conditions were 35 cycles, 1 min at 95°C, 30 s at 54°C and 1 min at 72°C. PCR products were separated in 1.5% agarose gels with Midori Green Direct (Nippon Genetics Europe GmbH). The PCR reactions were repeated twice.

*Colletotrichum acutatum* pathogenicity experiments. To evaluate the pathogenicity of *C. acutatum*, the detached strawberry leaf assay was developed. Leaves were obtained from the Institute of Horticulture. Twelve strawberry cultivars: 'Sonata', 'Elkat', 'Rumba', 'Pandora', 'Syria', 'Elegance', 'Pegasus', 'Darselect', 'Asia', 'Furore', 'Deluxe' and 'Malvina', were chosen. The visually healthy strawberry leaves composed of three leaflets on a petiole without any visible disease symptoms were sterilized in a 70% ethanol solution for 3 min, rinsed 4–5 times with sterile distilled water and surface-dried for 5 min on sterile filter paper. The upper surface of leaves was wounded with a sterile needle and inoculated with 5 mm mycelial plugs (mycelial side down) in the centre of each multiple leaf.

The control treatment was not inoculated with the *C. acutatum* (control-1) to reject infection with other pathogens. A total of 16 leaves were used per treatment. The experiment was repeated four times,

with four replicates. The plates were incubated at 25°C temperature in the dark. *C. acutatum* pathogenicity on strawberry cultivars was assessed at 14 days after inoculation (DAI). At the end of the experiment, the *C. acutatum* was reisolated from infected leaves and plated on PDA to recover the fungi. The pathogenicity ratings were based on a rating score (1–5): 1) no visible infection, 2) 1–5% leaf area infected, 3) 5–10% leaf area infected, 4) 20% leaf area infected and 5) 50% or more leaf area infected. The infected leaves were categorized (with some modifications) into four rating classes: 1) highly susceptible (3.5–5 rating), 2) susceptible (2.6–3.49 rating), 3) moderately resistant (1.6–2.59 rating) and 4) resistant (0–1.59 rating) (Prom et al., 2012).

**Inhibitory effect of EO in vitro.** To evaluate the inhibitory effect of different EO concentrations on *C. acutatum*, the 5 mm mycelial plugs of 7-day old fungus (No. Fo5) were cut and placed in the centre of PDA containing Petri plates. The 200, 400, 600, 800 and 1000 µl L<sup>-1</sup> of pure EO were added to 45°C PDA medium. The plates were incubated at 25 ± 2°C temperature in the dark. To compare growth without EO, the control treatment was not sprayed with EO (control-2). The experiments were repeated twice. The diameter (cm) of *C. acutatum* colony (including the width of the disc) growth in two directions was measured after 2, 4 and 6 DAI. Calculation of the mean of colony growth diameter used for mycelial growth inhibition (MGI). The MGI was determined using the formula:

$$\text{MGI (\%)} = \frac{(\text{dc} - \text{dt})}{\text{dc}} \times 100,$$

where dc is mycelium diameter of the pathogen colony in the control treatment in Petri dish, cm; dt – mycelium diameter of the pathogen colony in the EO-treated Petri dish, cm (Idris et al., 2015; Šernaitė et al., 2020).

**EO inhibitory effect on detached strawberry leaves.** The leaves of the strawberry cultivar ‘Deluxe’ were prepared as described in pathogenicity experiments. The sterilized rinsed and dried leaves were put on filter paper in a sterile tray, and 50 ml of sterile distilled water was added. The upper surface of leaves was inoculated with 5 mm mycelial plugs (mycelial side down) in the centre of each multiple leaf. A total of 16 leaves were used per treatment; the experiment was repeated three times with four replicates. The leaves were sprayed with 5 ml of 800 or 1000 µl L<sup>-1</sup> of EO. To evaluate fungal growth without EO, the control treatment was inoculated with *C. acutatum* but not sprayed with EO (control-3). The trays with detached leaves were incubated at 25°C temperature in the dark. The inhibitory effect of the treatments was evaluated by the infection area (cm) and leaf area infected (%) after 4 and 6 DAI. At the end of the experiment, the *C. acutatum* was reisolated from infected leaves and plated on PDA to recover the fungi.

The disease incidence was estimated as the number of symptomatic leaves over the total leaves by the rating score: 1) 0% – no visible infection, 2) 5%, 3) 10%, 4) 20% and 5) 50% or more area of leaf infected (Oliveira et al., 2019).

Disease severity index (DSI) of each inoculated plant leaf was assessed at 5 and 7 DAI by calculating the percentage of leaf area affected: 1) 0% – no visible infection, 2) 5%, 3) 10%, 4) 20% and 5) 50% or more area of leaf infected using the formula:

$$\text{DSI (\%)} = \frac{(0 \times P_0) + (1 \times P_1) + (2 \times P_2) + (3 \times P_3) + (4 \times P_4) + (5 \times P_5)}{N \times G} \times 100,$$

where P0 to P5 is the total number of observed leaves in each corresponding scale, N – total number of leaves, G – number of maximum grades observed in the scale (Kone et al., 2017; Bajpai et al., 2019).

**Statistical analysis.** The experimental data were analysed using the analysis of variance (ANOVA) from the software *SAS Enterprise Guide*, version 7.1 (SAS Inc., USA). The standard error (SE) in the figures is marked as an error bar estimated for isolates growth rates. Duncan’s multiple range test ( $P < 0.05$ ) was used to determine differences among the treatments. Disease severity index expressed as mean ± standard deviation.

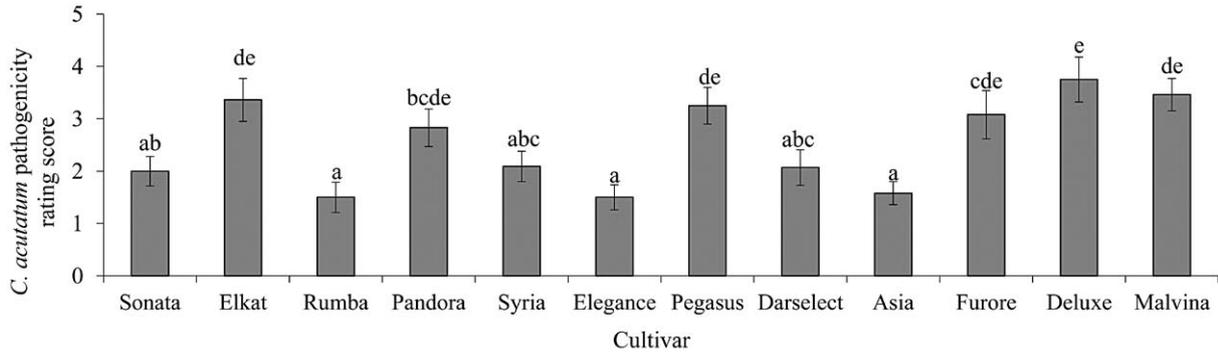
## Results

***Colletotrichum acutatum* pathogenicity.** The detached leaf assay was developed to determine the pathogenicity of *C. acutatum* on various strawberry cultivars. The results revealed that *C. acutatum* isolate from strawberry was able to cause anthracnose to all cultivars and was pathogenic in this study. The clear *C. acutatum* necrotic (black) areas developed on inoculated leaves. The cultivar ‘Deluxe’ showed pathogenicity (3.75) and was attributed to the group of cultivars highly susceptible to anthracnose. Cultivars ‘Malvina’, ‘Elkat’, ‘Pegasus’, ‘Furore’ and ‘Pandora’ were attributed to the group of cultivars susceptible to anthracnose and had lower pathogenicity rating (3.46, 3.36, 3.25, 3.08 and 2.83, respectively) compared with the ‘Deluxe’. Additionally, ‘Syria’, ‘Darselect’ and ‘Sonata’ were attributed to the group of moderately resistant cultivars. They were not significantly different from one another with 2.09, 2.07 and 2.0 pathogenicity rating, respectively. Compared with other cultivars, ‘Asia’, ‘Elegance’ and ‘Rumba’ had significantly lower disease incidence and showed the highest resistance. The least susceptible to *C. acutatum* was ‘Rumba’ (1.5) and ‘Elegance’ (1.5) (Figure 1).

**Inhibitory effect of EO in vitro.** The differences of *C. acutatum* growth among the five EO were assessed after 2, 4 and 6 DAI (Figure 2). The results showed that *Thymus vulgaris* EO completely suppressed *C. acutatum* at concentrations > 200 µl L<sup>-1</sup>: at 6 DAI, there were no visible fungal growth symptoms. The *C. acutatum* growth in the control-2 treatment at 2 DAI was 0.13 cm day<sup>-1</sup> and at 6 DAI – 2.65 cm day<sup>-1</sup>, as at all thymus concentrations growth was 0 cm day<sup>-1</sup>. *T. vulgaris* EO achieved the highest mycelial growth inhibition of 100% at all tested concentrations.

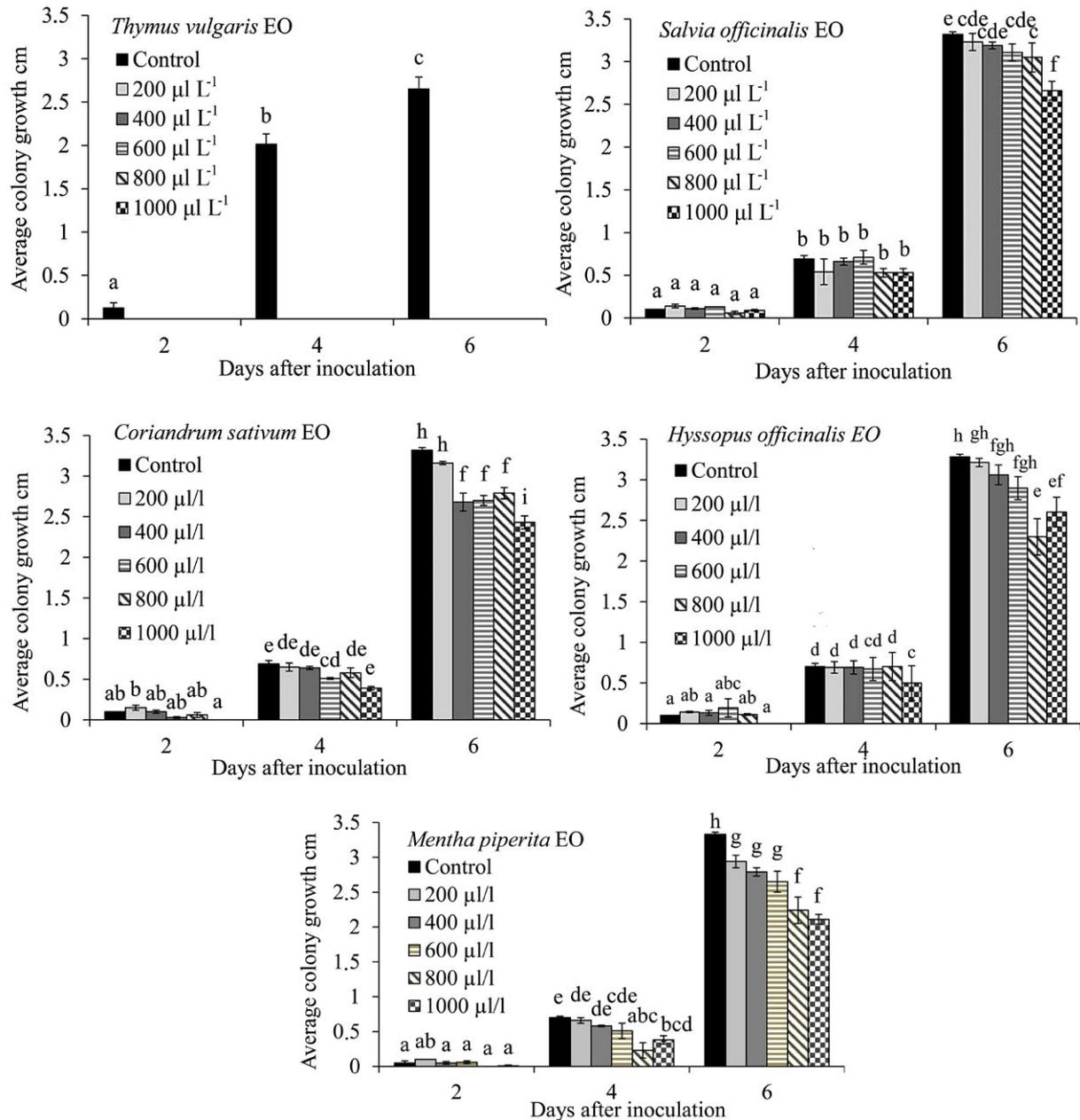
The data of our experiment indicate that the concentrations of *Salvia officinalis* EO against *C. acutatum* were not as effective as those of *T. vulgaris*. However, at the 800 µl L<sup>-1</sup>, slight mycelial growth inhibition (0.06 cm day<sup>-1</sup>) can be seen. The results of 4 DAI show that the 200 µl L<sup>-1</sup> (0.54 cm day<sup>-1</sup>), 800 µl L<sup>-1</sup> (0.53 cm day<sup>-1</sup>) and 1000 µl L<sup>-1</sup> (0.53 cm day<sup>-1</sup>) had a slight effect on pathogen compared with the control-2 treatment. Besides, it was observed that the 400, 600 and 800 µl L<sup>-1</sup> colony growth suppressed from 3.19 to 3.05 cm day<sup>-1</sup>. However, at 6 DAI *S. officinalis* EO suppressed pathogen growth (2.66 cm day<sup>-1</sup>) at the 1000 µl L<sup>-1</sup> with the highest mycelial growth inhibition of 19.88%, while in the control-2 treatment, the average colony growth of *C. acutatum* was 3.32 cm<sup>1</sup> (Figure 2).

The data of *Coriandrum sativum* EO at 2 DAI demonstrated a slight mycelial growth inhibition from 0.15 to 0 cm day<sup>-1</sup>; meanwhile, in the control-2 treatment, mycelium growth was 0.1 cm day<sup>-1</sup>. At 4 DAI, the highest concentration of *C. sativum* EO showed the best result.



Note. Means followed by the same letter did not differ significantly ( $P < 0.05$ ).

Figure 1. Pathogenicity of *Colletotrichum acutatum* to the strawberry cultivars at 14<sup>th</sup> day after inoculation



Note. Means followed by the same letter did not differ significantly ( $P < 0.05$ ).

Figure 2. The inhibitory effect of five essential oils (EO) against *Colletotrichum acutatum* on strawberry after 2, 4 and 6 days after inoculation

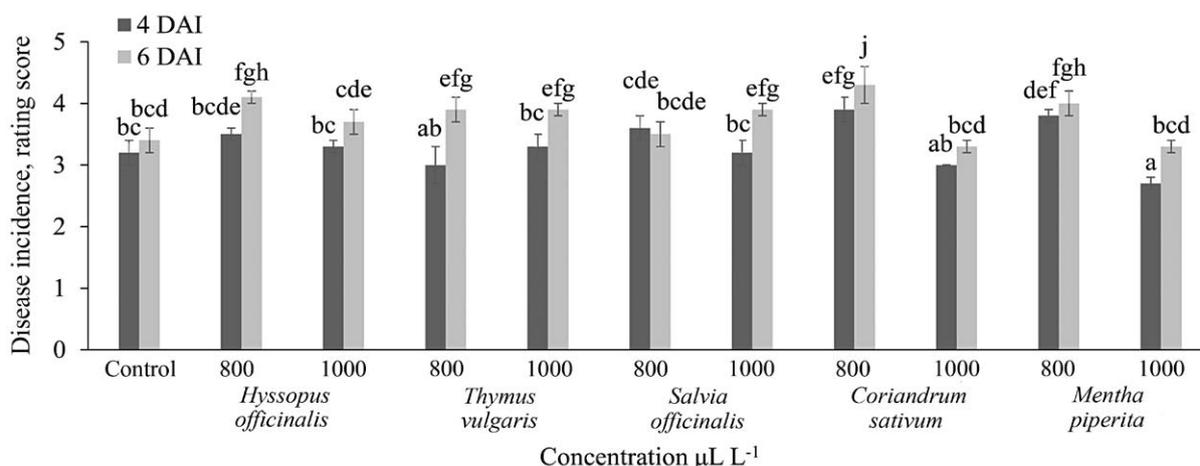
It reduced *C. acutatum* growth to 0.39 cm day<sup>-1</sup> compared with the control-2 treatment (0.69 cm day<sup>-1</sup>). According to the *C. sativum* data, there was observed a noticeable antifungal activity at 6 DAI compared EO concentrations with the control-2 treatment. The lowest colony growth was observed at 1000 µl L<sup>-1</sup>: colony diameter was significantly lower (2.43 cm day<sup>-1</sup>) than in the control-2 treatment (3.32 cm day<sup>-1</sup>). The highest mycelial growth inhibition was 26.81%, when the 400, 600 and 800 µl L<sup>-1</sup> suppressed colony growth from 2.79 to 2.68 cm day<sup>-1</sup> (Figure 2).

*Hyssopus officinalis* EO at 1000 µl L<sup>-1</sup> completely inhibited the growth of *C. acutatum* at 2 DAI. However, the growth of pathogen was 0.1 cm day<sup>-1</sup> in the control-2 treatment. The *H. officinalis* EO at 1000 µl L<sup>-1</sup> reduced colony growth (0.49 cm day<sup>-1</sup>) compared with the control-2 treatment (0.69 cm day<sup>-1</sup>) at 4 DAI. The 800 µl L<sup>-1</sup> was most effective and showed the best result compared with the other concentrations at 6 DAI (2.26 cm day<sup>-1</sup>) and reached the highest mycelial growth inhibition rate of 30.03% (Figure 2).

The data of our experiment show that 4 DAI at the 800 µl L<sup>-1</sup> of *Mentha piperita* EO colony growth decreased (0.23 cm day<sup>-1</sup>) compared with the control-2 treatment (0.70 cm day<sup>-1</sup>). The lowest colony growth at 6 DAI was recorded at the highest concentration of 1000 µl L<sup>-1</sup> (2.11 cm day<sup>-1</sup>) compared with the control-2 treatment (3.33 cm day<sup>-1</sup>). The highest concentration demonstrated 36.64% MGI. *M. piperita* EO had better antifungal activity than *S. officinalis*, *H. officinalis* and *C. sativum* EO 6 DAI against *C. acutatum* (Figure 2).

**EO inhibitory effect on detached strawberry leaves.** Evaluation of the highest efficient concentrations (800 and 1000 µl L<sup>-1</sup>) of EO on detached strawberry leaves is presented in Figure 3.

The results of the experiment revealed that the highest disease incidence score was recorded at 6 DAI of the treatment with 800 µl L<sup>-1</sup> of the *C. sativum* EO. However, the results of 1000 µl L<sup>-1</sup> were significantly different. *C. sativum* EO reduced *C. acutatum* growth at 4 DAI, and disease incidence rating was equal compared with the control-3 treatment at 6 DAI. *T. vulgaris* EO



Note. Means followed by the same letter did not differ significantly ( $P < 0.05$ ).

**Figure 3.** The inhibitory effect of essential oils (EO) against *Colletotrichum acutatum* on detached strawberry leaves at 4 and 6 days after inoculation (DAI)

showed inhibition of *C. acutatum* at 800 µl L<sup>-1</sup> at 4 DAI. *T. vulgaris* EO was found to be the most effective against strawberry anthracnose and gave the highest inhibition of *C. acutatum* growth. *M. piperita* EO at 1000 µl L<sup>-1</sup> reduced disease severity compared with control-3 treatment and other concentrations. However, in *H. officinalis* EO treatment at 800 µl L<sup>-1</sup>, the suppression of *C. acutatum* was not efficient (Figure 3).

The disease severity index ranged from 66.67 to 100 at 4 DAI. The lowest disease severity index value was observed of *M. piperita* EO at 800 µl L<sup>-1</sup> at 4 DAI. Reduction of disease severity by *M. piperita* EO was significantly higher than control-3 and other treatments at 4 DAI (Table).

**Table.** Percentage of disease severity index on strawberry cultivar ‘Deluxe’ at 4 and 6 days after inoculation (DAI)

<i>Colletotrichum acutatum</i> disease severity index %	Control-3	<i>Hyssopus officinalis</i>		<i>Thymus vulgaris</i>		<i>Salvia officinalis</i>		<i>Coriandrum sativum</i>		<i>Mentha piperita</i>	
	Concentration µl L <sup>-1</sup>	-	800	1000	800	1000	800	1000	800	1000	800
4 DAI	79.2 ± 0.2 g	87.5 ± 0.1 i	83.3 ± 0.1 j	75.0 ± 0.3 e	81.9 ± 0.2 c	90.3 ± 0.2 n	81.9 ± 0.1 c	78.9 ± 0.2 f	100 ± 0 r	94.4 ± 0.1 p	66.7 ± 0.1 a
6 DAI	67.8 ± 0.2 d	82.2 ± 0.1 i	91.7 ± 0.2 o	77.8 ± 0.3 b	97.2 ± 0.1 q	88.9 ± 0.2 m	77.8 ± 0.1 b	85.6 ± 0.3 k	81.9 ± 0.1 c	80.0 ± 0.2 h	81.9 ± 0.2 c

Note. Means followed by the same letter did not differ significantly ( $P < 0.05$ ); results expressed as mean ± standard deviation.

## Discussion

According to Zhang et al. (2016), no strawberry cultivar has been found to show complete resistance to *Colletotrichum* spp. The pathogenicity tests showed significant differences among strawberry cultivars. Pathogenicity tests in the cultivar 'Deluxe' showed the highest pathogenicity while cultivars 'Rumba' and 'Elegance' had resistance to *C. acutatum*. The data of our experiment revealed that various cultivars are differently susceptible to *C. acutatum*. Wagner and Hetman (2016) reported that 'Florence' was more susceptible to *C. acutatum* than other cultivars, e.g., 'Darselect'. Leandro et al. (2001) reported that *C. acutatum* causing symptomless infection of strawberry is a source of inoculum for fruit infections. Garrido et al. (2008) found out that *C. acutatum* was more pathogenic than *C. gloeosporioides* to cultivars 'Camarosa' and 'Ventana'. El Kaissoumi et al. (2018) reported that there are significant differences in disease severity between cultivars. The most susceptible to *Colletotrichum* spp. was 'Fortuna'. Besides, due to *C. gloeosporioides* infection, pathogen develops brown spots and irregular blotches on strawberries. However, due to the *Colletotrichum* spp. tissues colonization, their pathogenicity and survival on different plant parts of various strawberry cultivars showed differences (El Kaissoumi et al., 2018). Seijo et al. (2008) found out different resistance levels in various strawberry cultivars.

The knowledge about various cultivars could help integrated plant protection. The effective EO as potential bio-fungicides could differently suppress *Colletotrichum* spp. on various cultivars. Pathogenicity results showed that *C. acutatum* is capable of infecting strawberry, and pathogenic variation could exist.

Results of our experiment indicate significant differences between all evaluated EO concentrations. Higher concentrations of EO show the inhibitory effect on strawberry pathogen *C. acutatum*.

Sarkhosh et al. (2018) found that *T. vulgaris* EO completely inhibited *Colletotrichum* spp. mycelial growth at a concentration of 100  $\mu\text{L}^{-1}$  in avocado, mango and papaya fruits. According to another study by Sarkhosh et al. (2017), *T. vulgaris* EO concentration of 125  $\mu\text{L}^{-1}$  completely inhibited *Colletotrichum* spp. in papaya fruits. Rodrigues et al. (2018) reported that different EO concentrations are suitable for *C. musae* control. Amini et al. (2012) observed antifungal activity of *T. vulgaris* EO against *Botrytis cinerea*, *Aspergillus* spp., *Rhizoctonia solani* and other pathogens. Foltinová et al. (2017) documented that *T. vulgaris* EO 100% inhibited the growth of tested isolates of *Aspergillus flavus*. The data of our experiment agree with those obtained by other researchers suggesting that *T. vulgaris* EO could be an effective biological agent to control *Colletotrichum* sp. until 6 DAI.

According to Scariot et al. (2016), *S. officinalis* EO has fungicidal activity against *Alternaria* spp. and *Phakopsora pachyrhizi* in beans. The data of our experiment indicate that *S. officinalis* EO did not demonstrate the high tendency of *C. acutatum* inhibition on strawberry. In comparison, the efficiency of *S. officinalis* and *C. sativum* EO against *C. acutatum* pathogen was very similar. Aćimović et al. (2016) reported that *C. sativum* EO could inhibit colony growth by 90%. However, the highest concentrations of EO should be used. Mandal and Mandal (2015) reported that *C. sativum* EO at the

1500–2000  $\mu\text{g mL}^{-1}$  could be useful against *Alternaria* spp., *Fusarium* spp. and other pathogens.

The data of our experiment indicate that *H. officinalis* EO had a better antifungal effect than *S. officinalis* and *C. sativum* EO. Letessier et al. (2001) observed that *H. officinalis* EO at 0.4% concentration completely inhibited mycelial growth of pathogenic fungi *Pyrenophora avenae* and *Pyricularia oryzae*.

*M. piperita* EO had better antifungal activity against *C. acutatum* than *S. officinalis*, *H. officinalis* and *C. sativum* EO at 6 DAI. The literature review has shown that *M. piperita* EO can be used as a biological control product against strawberry anthracnose. Results show that *M. piperita* EO at a concentration of 1500  $\mu\text{L}^{-1}$  gave complete mycelial growth inhibition (Sarkhosh et al., 2018). Based on the results of our experiment, it can be assumed that higher concentrations of *M. piperita* EO could effectively inhibit *C. acutatum*. Moghaddam et al. (2012) observed that *M. piperita* EO up to 1600  $\mu\text{L}^{-1}$  could control *Fusarium oxysporum* f. sp. *ciceris*, *Macrophomina phaseolina* and *Dreschlera spicifera*.

The micelial growth inhibition of *C. acutatum* by *T. vulgaris* EO was evident; it has the potential to control strawberry anthracnose and could be considered for use as a biocontrol agent against fungal pathogens. However, further research is needed to evaluate higher EO concentrations. The *T. vulgaris* EO inhibitory effect was evaluated on the detached leaves of strawberry cultivar 'Deluxe'.

In this study, five EO showed differing efficacy on *C. acutatum*. *T. vulgaris* EO was the most effective at the lowest concentration in *in vitro* experiments. However, in the assay of detached strawberry leaves, even at high concentrations *T. vulgaris* EO gave a slight disease severity reduction compared with the control-3 treatment at 4 DAI. *M. piperita* EO at higher concentration reduced disease severity compared with the control-3 and other EO treatments at 4 and 6 DAI. Oliveira et al. (2019) suggest that higher concentrations of EO are usually required in the experiments *in vivo*.

## Conclusions

1. According to the pathogenicity screening, 'Deluxe' was the most susceptible cultivar to strawberry anthracnose, while 'Rumba' and 'Elegance' exhibited resistance.

2. The growth of *Colletotrichum acutatum* was reduced with the tested essential oils (EO) applied at the highest concentrations *in vitro*.

3. *Thymus vulgaris* EO caused 100% micelial growth inhibition of *C. acutatum* at 200  $\mu\text{L}^{-1}$  and higher concentrations *in vitro*.

4. Essential oils inhibited the mycelial growth of *C. acutatum in vitro*; however, the assay of detached strawberry leaves showed that the efficacy of EO is not sufficient.

As a result, further investigation, including higher EO concentrations, is needed.

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## ***Colletotrichum acutatum* patogeniškas ir biokontrolė įvairių veislių braškėms panaudojus eterinius aliejus**

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### **Santrauka**

*Colletotrichum* spp. yra svarbus braškių patogenas, galintis iki 80 % sumažinti jų derlių. Dėl pakitusių agrometeorologinių sąlygų stebimas jo plitimas vėsesnio klimato šalyse. Kaip viena iš ekologiškų augalų patogenų kontrolės priemonių gali būti panaudoti eteriniai aliejai. Jie turi antibakterinių ir antigrybinių savybių; mažas toksiskumas ir biologinė sklaida aplinkoje juos leidžia naudoti augalų apsaugai nuo patogenų vietoj cheminių produktų.

Tyrimo metu siekta nustatyti braškių *C. acutatum* patogeniškumą ir įvertinti penkių koncentracijų eterinius aliejus kaip galimus biofungicidus apsaugai nuo braškių antraknozės. *C. acutatum* patogeniškumo tyrimas atliktas ant nuo augalo atskirtų braškių lapų. Eterinių aliejų slopinantis poveikis įvertintas panaudojus 200, 400, 600, 800 ir 1000 μl L<sup>-1</sup> koncentracijas *in vitro* ir 800 bei 1000 μl L<sup>-1</sup> koncentracijas ant atskirtų braškių lapų. Antraknozei jautriausias buvo veislės 'Deluxe' braškės, o veislių 'Rumba' ir 'Elegance' braškės buvo atsparios. Tyrimo duomenimis, *Mentha piperita* eterinis aliejus (EA) pasižymėjo antigrybiniu poveikiu nuo *C. acutatum*, o *Salvia officinalis* EA neturėjo jokio poveikio. *Coriandrum sativum* ir *Hyssopus officinalis* EA nežymiai slopino *C. acutatum* augimą. *Thymus vulgaris* EA *in vitro* tyrimo rezultatai atskleidė, kad tai gali būti veiksmingas braškių antraknozės biokontrolės agentas. *T. vulgaris* EA slopino *C. acutatum* micelio augimą *in vitro*, tačiau rezultatai ant atskirtų braškių lapų parodė, kad eterinių aliejų veiksmingumas buvo nepakankamas, todėl reikia tolesnių tyrimų.

Reikšminiai žodžiai: antigrybinis poveikis, blakinė kalendra, pipirmėtė, vaistinis čiobrelis, vaistinis isopas, vaistinis šalavijas.