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# The Essential Oils of Thyme, Sage and Peppermint against Strawberry Anthracnose

**Amina Morkeliūnė *, Neringa Rasiukevičiūtė and Alma Valiuškaitė**

Laboratory of Plant Protection, Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, LT-54333 Babtai, Kaunas District, Lithuania; neringa.rasiukeviciute@lammc.lt (N.R.); alma.valiuskaite@lammc.lt (A.V.)

* Correspondence: armina.morkeliune@lammc.lt

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**Abstract:** Strawberry *Colletotrichum* spp. is a significant strawberry pathogen with yield losses of up to 50 %, due to agrometeorological conditions change its spread in a temperate climate is growing. The most convenient way for controlling diseases is the use of chemical fungicides. The demand for alternative measures for biological protection is growing because of the increasing pathogens resistance and pesticides harm for human and environment. Thyme, sage and peppermint are a source of natural antioxidants and biologically active compounds. The findings of antimicrobial activities of these plants, low toxicity and biodegradability of essential oils (EO) make them suitable for biological protection against pathogens. This study aims to evaluate the inhibition of *Colletotrichum* spp. by thyme, sage and peppermint EO in vitro and on detached strawberry leaves. The results revealed that thyme EO inhibited *Colletotrichum* spp. completely above 200 μL $L^{-1}$ concentration *in vitro*. Peppermint and sage EO reduced mycelial growth of *Colletotrichum* spp. However, *in vitro*, results are auspicious for biological control. The experiments on detached strawberry leaves showed that *Colletotrichum* spp. disease reduction 4 days after inoculation were 15.8% at 1000 μL $L^{-1}$ of peppermint EO and 5.3% at 800 μL $L^{-1}$ of thyme compared with control. Our findings could potentially help to manage *Colletotrichum* spp.; however, the detached strawberry leaves assay showed that EOs efficacy was relatively low on tested concentrations and need further investigations with higher concentrations.

**Keywords:** biocontrol; inhibition; *Mentha piperita*; *Salvia officinalis*; *Thymus vulgaris*

## 1. Introduction

The strawberry anthracnose is one of the important diseases, which caused by several species complex of *Colletotrichum* spp.: *C. acutatum* J. H. Simmonds, brooks and *C. gloeosporioides* (Penz.) Penz. & Sacc. [1,2]. It also infects and causes diseases in many economically important crops. Strawberry anthracnose caused yield losses of up to 50% and 80% plant death and was considered to be warmer climate zone pathogen, where the optimal temperature from 15 to 30 °C, with optimal 25 °C temperature [3–5]. Strawberry diseases controlled by several fungicide applications [6,7]. The growing resistance to pesticides and their adverse environmental effects leads to a new environment safer disease control strategy [8–10].

Essential oils (EO) demonstrated a distinct level of antimicrobial activity to various ranges of strawberry fungal and bacterial pathogens [6,9,11,12]. The EO includes terpenes, terpenoids, aromatic and aliphatic constituents are a source of natural antioxidants and biologically active compounds. The 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 [13–17].

Plant EO are developed commercially on a large scale, most of which are the members of the *Lamiaceae* family, including *T. vulgaris, S. officinalis, M. piperita* [18,19]. Thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.) and peppermint (*Mentha piperita* L.) EO, as products from plants, have a wide application in pharmacy, fragrance, food industries, however recent studies of essential oils

revealed their potential antimicrobial activity [20–22]. *Salvia officinalis* EO affects *Fusarium* spp. growth [15]. *Mentha piperita* EO inhibits spread of *Alternaria* spp. and *Fusarium* spp. pathogens [23]. *Thymus vulgaris* EO has antifungal activity against plant pathogens such as *M. fructicola*, *B. cinerea, A. flavus* [16,24,25]. *T. vulgaris*, EO can be used as a natural preservative in food against casual agents of food-borne diseases like *E. coli*, *Pseudomonas* spp. and others [26].

Plant protection measures against plant diseases are necessary to avoid yield and crop losses. However, pesticides have an adverse effect on plants and humans, as they leave residues in it. The European Green Deal provides a plan to increase environmentally friendly technologies by supporting strategy to reduce the usage of pesticides and make agriculture more sustainable. Growing pathogens resistance is because of the extensive use of chemical pesticides for plant protection. The new sources of natural active ingredients for plant protection may solve pesticides resistance problem and reduce environment and food contamination [7,11,12,27].

This study aims to evaluate the inhibition of *Colletotrichum* spp. by thyme, sage and peppermint EO in vitro and on detached strawberry leaves.

## 2. Experiments

### *2.1. Essential Oil Extraction.*

The common thyme (*Thymus vulgaris* L.), common sage (*Salvia officinalis* L.), peppermint (*Mentha piperita* L.) essential oils (EO) extracted from dry herbs by Clevenger-type hydro-distillation. Volatile compounds of essential oils were determined by gas chromatography/mass spectrometry (GC-MS).

### *2.2. Antifungal Activity In Vitro*

The research was carried out at the Laboratory of Plant Protection, Lithuanian Research Centre for Agriculture and Forestry Institute of Horticulture (LAMMC) in 2017–2020. To evaluate antifungal activity against *Colletotrichum* spp. 50–1800 μl $L^{-1}$ concentrations of EO were used. Isolates were obtained from infected strawberry fruits and had previously been identified as *Colletotrichum* spp. The 5 mm mycelial plugs of 7-day old fungus cut and placed in the centre of Petri plates containing PDA. Different concentrations of pure essential oil added to cooled at 45 °C PDA.

The mycelium put upside down (mycelia side) in the centre of Petri. The Petri plates incubated at 25 ± 2 °C in the dark. The control treatments were essential oil-free but inoculated with pathogen. The diameter (mm) of *Colletotrichum* spp. colony growth in two directions measured 4 days after inoculation (DAI). Four replications were carried out.

The mean of colony growth diameter used for mycelial growth inhibition (MGI) calculations. The MGI (%) was determined using the formula:

$$\text{MGI (\%)} = (C - T)/C \times 100,$$

where C is mycelium diameter of the pathogen colony in control Petri dish, mm; T—mycelium diameter of the pathogen colony in the essential oil-treated Petri dish, mm [11,28,29].

### *2.3. EO antifungal Activity on Detached Strawberry Leaves*

The essential oils inhibitory effect evaluated on detached strawberry cultivar 'Deluxe' leaves. Healthy strawberry leaves without any visible disease symptoms were soaked in 70% ethanol solution for 3 min and rinsed 4–5 times with sterile distilled water (SDW). Each leaf placed in a Petri dish with 5 mL of SDW. Detached strawberry leaves were sprayed with essential oils, then wounded with a sterile needle and a 9-mm plug of 7-day-old *Colletotrichum* spp. was placed on the wound. Incubation was carried out at 25 ± 2 °C in the dark for 7 days. The disease severity and reduction evaluated 4 DAI. The control treatments were not sprayed with EO but inoculated with pathogen. Four replications were carried out.

Disease severity (DS) of each inoculated plant leaf assessed at 4 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 [30,31].

$$DS(\%) = \frac{(0 \times P0) + (1 \times P1) + (2 \times P2) + (3 \times P3) + (4 \times P4) + (5 \times P5)}{N \times G} \times 100$$

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 scale [32].

$$DR(\%) = \frac{Xc - Xt}{Xc} \times 100,$$

where Xt is the mean of DS per treatment, and Xc is the mean of DS in the untreated inoculated control [33].

### *2.4. 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. The standard error (SE) in the figures marked as an error bar estimated for growth rates of isolates.

## 3. Results

### *3.1. Antifungal Activity In Vitro*

The antifungal activity of EO was investigated on PDA (potato dextrose agar) under different concentrations. The inhibition of *Colletotrichum* spp. by thyme EO is presented in Figure 1. Thyme EO showed 100% MGI at 100 μL $L^{-1}$ at 4 days after inoculation (4 DAI) but did not demonstrate highest antifungal effect at 150 μL $L^{-1}$ at 4 (87.96%) DAI. However, mycelial pathogen growth was inhibited above 200 μL $L^{-1}$.

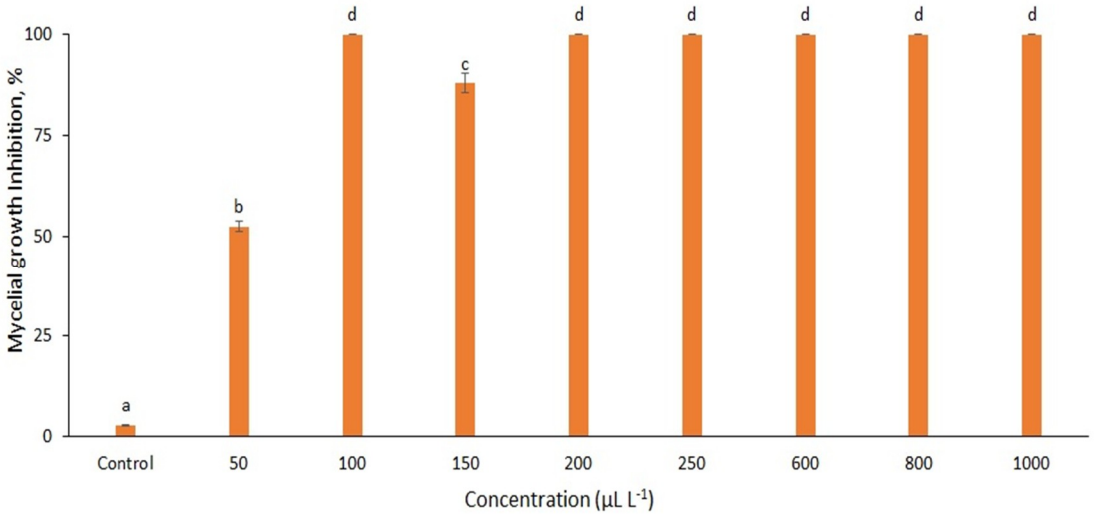


**Figure 1.** Inhibition (%) of *Colletotrichum* spp. mycelial growth by thyme (*T. vulgaris*) EO at 4 days after inoculation (4 DAI). Results are presented as means (n = 4). The same letter indicates no significant differences between treatments ($p < 0.05$).

The inhibition of *Colletotrichum* spp. by sage EO is presented in Figure 2. Data indicate that this EO was not as effective as thyme. Sage EO showed antifungal activity up to 1000 μL $L^{-1}$ at 4 DAI and achieved the highest effect of 88.14% at 1800 μL $L^{-1}$.

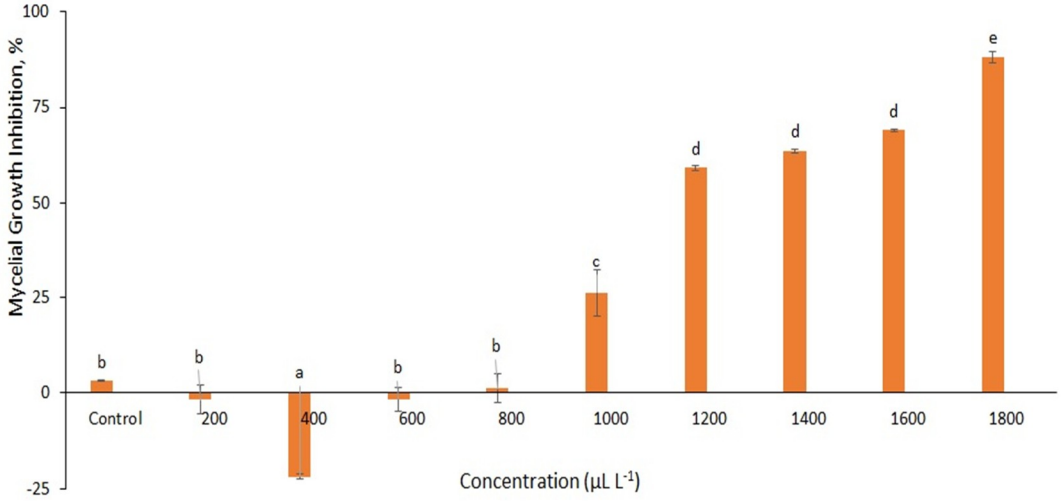


**Figure 2.** Inhibition (%) of *Colletotrichum* spp. mycelial growth by sage (*S. officinalis*) EO at 4 days after inoculation (4 DAI). Results are presented as means (n = 4). The same letter indicates no significant differences between treatments ($p < 0.05$).

The fungicidal activity of peppermint EO against *Colletotrichum* spp. is shown in Figure 3. This EO had a similar effect on *Colletotrichum* spp. comparing with sage. Meanwhile, peppermint EO reduced the mycelial growth at 600–1800 µL $L^{-1}$ from 20% to 88%. However, the highest antifungal activity was reached at 1600 µL $L^{-1}$ at 4 DAI. 1800 µL $L^{-1}$ EO efficiency decreased to 62.54%.

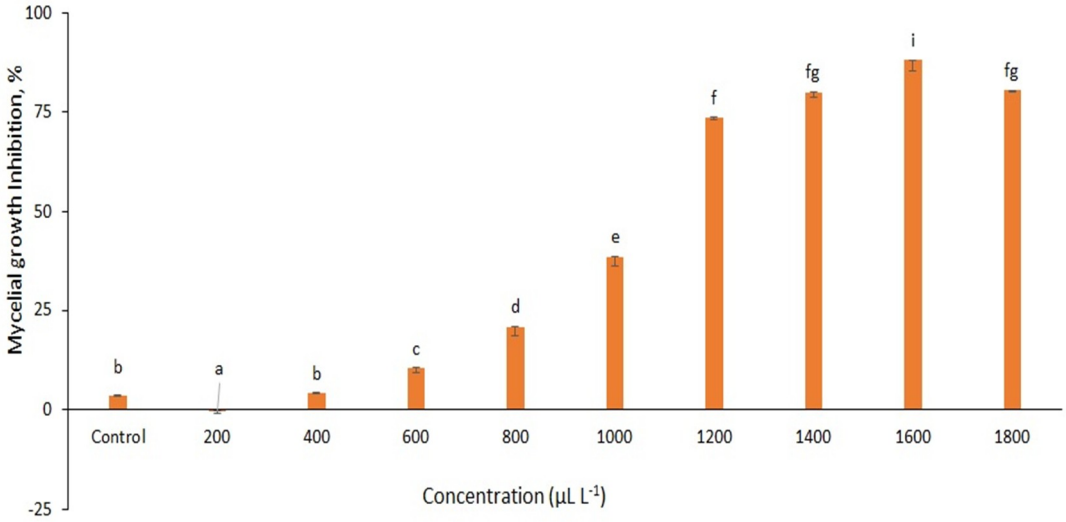


**Figure 3.** Inhibition (%) of *Colletotrichum* spp. mycelial growth by peppermint (*M. piperita*) EO at 4 days after inoculation (4 DAI). Results are presented as means (n = 4). The same letter indicates no significant differences between treatments ($p < 0.05$).

*T. vulgaris* EO suppressed the mycelial growth at 200–1000 µL $L^{-1}$. The determined MIC (minimal inhibitory concentration) was equal to 200 µL $L^{-1}$. *S. officinalis* and *M. piperita* EO reduced mycelial growth compared to control. However, the antifungal effect was insufficient to inhibit the spread of anthracnose infection.

### *3.2. Antifungal Activity on Detached Strawberry Leaves*

The detached strawberry leaf assay developed to determine the efficiency of the essential oil against *Colletotrichum* spp. on detached strawberry leaves. The results revealed that among all the investigation treatments, only 1000 μL $L^{-1}$ concentration of peppermint EO (5.3%) and 800 μL $L^{-1}$ concentration of thyme (15.8%) reduced the infection on strawberry leaves compared to inoculated control 4 DAI (Table 1). Sage EO had no positive influence on infection spread.

**Table 1.** The disease severity and reduction of anthracnose regarding strawberry cultivar 'Deluxe' by different essential oils concentrations at 4 days after inoculation. Means n = 18.

| Treatments | Disease Severity (%) | Disease Reduction (%) |
|---|---|---|
| Inoculated control | 79.2 ± 0.2 | n.a. * |
| *Thymus vulgaris* 800 μL/L | 75 ± 0.3 | 5.3 |
| *Salvia officinalis* 1000 μL/L | 80.6 ± 0.2 | 0 |
| *Mentha piperita* 1000 μL/L | 66.7 ± 0.2 | 15.8 |

## 4. Discussion

The growing interest in essential oils and their components, due to their volatility, relatively safe condition and wide acceptance by consumers, ecologically and biodegradable properties. For our study, we selected *T. vulgaris*, *S. officinalis* and *M. piperita* EOs. We analyzed antifungal activities also their effect on detached strawberry leaves, to assess the feasibility of using EOs as biocontrol agents in disease control measure. EO from thyme, sage, peppermint presented noticeable antifungal activity against *Colletotrichum* spp. *in vitro*.

In an investigation conducted by Duduk et al. [34], thyme EO showed good antifungal efficacy against *C. acutatum* on strawberry fruit. In our study, thyme EO inhibited *Colletotrichum* spp. mycelial growth in vitro above 200 μL $L^{-1}$. This suggests that the antifungal effect presence of the dominant components of EO, as main activity carriers. Palfi et al. [17] reported that thyme, sage and peppermint. EOs completely inhibited the mycelial growth of *F. oxysporum in vitro. H*owever, sage EO had a low inhibitory effect against *B. cinerea*. Oliveira et al. [35] reported that 5 μL/mL peppermint EO resulted in 100% MGI on all tested *Colletotrichum* stains. In our research peppermint, EO highest antifungal activity reached 1600 μL $L^{-1}$ (88%).

In our research, the antifungal activity of sage EO against *Colletotrichum* spp. achieved the highest effect of 88.14% at 1800 μL $L^{-1}$. In comparison, Yilmaz et al. [36] studied, that the application of sage EO resulted in slight inhibition on mycelial growth of *C. gloeosporioides* in fumigation bioassay and contact bioassay in vitro (solid media) and in vivo (apple) conditions. These results supported our findings, shows antifungal effect against *Colletotrichum* spp., but did not suppress totally.

However, to the best of our knowledge, no investigations have been previously performed on the antifungal effect of thyme, sage and peppermint EOs on detached strawberry leaves against *Colletotrichum* spp. In the present research, investigated EO result on detached strawberry leaves assay shows a less positive effect of reducing the spread of anthracnose infection. 1000 μL $L^{-1}$ concentration of peppermint EO (15.8%) and 800 μL $L^{-1}$ concentration of thyme (5.3%) reduced the infection on strawberry leaves. In the future research, a higher concentration of this EO should be investigated for higher effectiveness. The effect of EOs from plants as volatile compounds on the surface of strawberry leaves may induce a stressful environment [11,33].

In summary, examination with various EO and their concentrations in vitro exhibited promising prospects against strawberry anthracnose. However, the antifungal effect on detached strawberry leaves was relatively low.

## 5. Conclusions

Essential oil thyme showed total inhibition against *Colletotrichum* spp. *in vitro*. Peppermint and sage EOs showed significant antifungal activity at the highest concentrations. Infection of anthracnose on detached strawberry leaves was slightly reduced by the application of thyme EO and

more suppressed by peppermint EO at tested concentration. Sage EO did not influence the spread of *Colletotrichum* spp. on detached strawberry leaves. The detached strawberry leaves assay showed that the investigated essential oils were not equally effective and need further investigations with higher concentrations.

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## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| EO | essential oil |
| DAI | days after inoculation |
| LAMMC | Lithuanian Research Centre for Agriculture and Forestry |
| PDA | potato dextrose agar |
| MGI | mycelial growth inhibition |
| DS | diseases severity |
| DR | disease reduction |
| SDW | sterile distilled water |
| GC-MS | gas chromatography/mass spectrometry |

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