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APPLICATION OF *SALVIA OFFICINALIS* AND *PICEA ABIES* ESSENTIAL OILS FOR CONTROLLING *COLLETOTRICHUM* SPP.

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Colletotrichum spp. is a significant strawberry fruit pathogen, causing yield losses of up to 80% - growing resistance to pesticides demands to new, environmentally-friendly plant protection. Essential oils (EO) are one of the biological plant protection products suitable for pathogens control. The antimicrobial and antifungal activity of EO, biodegradability and low toxicity, make it potential for use in plant protection against pathogens instead of chemical pesticides. The present study was carried out to investigate the antifungal effects of *Salvia officinalis* and *Picea abies* essential oils against strawberry *Colletotrichum* spp. isolate. The research carried out at the LAMMC Institute of Horticulture. The bio-fungicidal effect was assessed based on radial growth inhibitions. There were evaluated several EO concentrations from 1000 till 1800 µl/l. Single-spore isolate fragment placed in the centre of PDA with different concentrations. Plates were incubated at 25 º C in dark and evaluated after 2, 4, 7 days. *S. officinalis* and *P. abies* EO showed inhibitory effect on *Colletotrichum* spp. mycelial development. The *S. officinalis* inhibitory effect was more than 50% in all concentrations. The present study revealed that highest 1800 µl/l *S. officinalis* EO concentration *Colletotrichum* spp. colony diameter was significantly lower (1.84 cm) compared with control (5.75 cm). However, *P. abies* inhibition more than 50 % were only in concentrations from 1600 µl/l. According to our results, treatment with EO can reduce the growth of *Colletotrichum* spp. and EO could be an effective potential bio-fungicide to control strawberry anthracnose. EO as bio-fungicides characteristics is their natural origin and low risk for resistance development.

Keywords: *anthracnose, antifungal activity, essential oils, radial growth, inhibition.*

INTRODUCTION

Growing demand for food-crops increases crop production intensity. Intensive agriculture based on large consumption of chemical pesticides and fertilisers. However, it affects negatively the natural ecosystem species diversity, contaminates groundwater and induces soil quality degradation (Scotti et al., 2015). Food contamination and plant diseases are related because most of the plants are affected by pathogens in the field. Despite that control of plant diseases reduce food contamination (Fung et al., 2018). Fungal and bacterial pathogens may occur during all plant vegetation season (planting, harvesting, postharvest) (Kwon et al., 2001). The fruits contamination by pathogens reduce their self-life. Strawberry anthracnose caused by *Colletotrichum* spp. can infect up to 80% of nurseries plants and cause their death and reduce yield over 50% (Sreenivasaprasad and Talhinhas, 2005).

In agriculture are used significant amounts of pesticides for plant pathogens and insect's management all over the world. Recent studies showed that chemical pesticides have quite high side-effects, like affecting flora and fauna, water, soil and even air, besides cause resistance (Damos et al., 2015; Beckerman et al., 2015). Traditionally strawberry diseases management is based on chemical control. However, due high cost of pesticides and adverse environmental effects, it is essential to find new solutions (Rasiukevičiūtė et a., 2015; Valiuškaitė et al., 2017). In addition, natural and environmentally friendly products are investigated. The alternative plant protection means to extend shelf-life of strawberries are explored. Light-emitting diodes together with photoactive compound a perspective in the reduction of postharvest strawberry contamination (Rasiukevičiūtė et al., 2015). It's essential to develop environment-friendly plant protection products that could control plant diseases. Microbiological and natural plant products obtained from plants could effectively control plant pathogens and are environment-friendly. It's been reported by several researchers that plant extracts reduce plant diseases (Šernaitė, 2017; Köhl et al., 2017). Essential oils are one of the natural plant protection products suitable for plant pathogens control. Plants contain high amount of various organic compounds. The antimicrobial and antifungal activity of essential oils and plant extracts, biodegradability and low toxicity, make it potential for use in plant protection against pathogens instead of chemicals. In addition, they are environment-friendly and wider accepted by consumers. Plant extracts and essential oils are promising products of a natural compound for the development of bio-fungicides (Antunes and Cavaco, 2010; Šernaitė, 2017).

Despite adverse effect on chemical pesticides used for plant disease control and growing interest in integrated and alternative plant protection researchers are involved to find environment friendly plant protection (Rasiukevičiūtė et al., 2015; Minova et al., 2015; Acimovic et al., 2016; Desam et al., 2017; Yilar et al., 2018). The *Colletotrichum* spp. could be inhibited by *Coriandrum sativum* essential oil (Acimovic et al., 2016). The *Mentha piperita* EO has an antimicrobial effect on *Alternaria* spp. and *Fusarium* spp. (Desam et al., 2017). The antifungal activity of plant phenolic compounds could destroy fungal cells (Freiesleben and Jager, 2014). Lamiaceae family plants such as *Salvia officinalis* are rich in terpenoid compounds, essential oils, flavonoids, phenolic compounds. It's been reported that *Salvia* species has antibacterial, antifungal and antimicrobial effect (Yilar et al., 2018). Research on antifungal activity of pine and spruce essential oils and extracts against *Fusarium* spp*., Heterobasidion* spp*., Penicillium funiculum, Colletotrichum acutatum, Botrytis cinerea* and other pathogens available (Minova et al., 2015).

In this paper, we have used two essential oils, as potential bio-fungicides from *Salvia officinalis* and *Picea abies.* The aim of the present study was to evaluate the antifungal effect of *Salvia officinalis* and *Picea abies* essential oils against strawberry *Colletotrichum* spp.

MATERIALS AND METHODS

The experiments carried out at the LAMMC Institute of Horticulture at the Laboratory of Plant Protection in Lithuania, in 2018-2019. Essential oil (EO) of dried *Salvia officinalis leaves* were extracted by hydro-distillation in a Clevenger-type system by 2 hours (AOAC, 1990). The EO of *Picea abies* leaf were obtained (UAB Naujoji Barmune).

To evaluate the effectiveness of *P. abies* and *S. officinalis* EO on *Colletotrichum* spp. different concentrations of EO evaluated. The essential oils were mixed separately with cooled at 45 ºC temperature potato dextrose agar (PDA) in concentrations from 1000 µl/l, 1200 µl/l, 1400 µl/l, 1600 µl/l up to 1800 µl/l. Each concentration homogenised with PDA and distributed in Petri dishes. The isolate (~10 mm diameter) of *Colletotrichum* spp. placed in the centre of PDA with different EO concentrations. The single spore isolates from *Colletotrichum* spp. infected strawberry obtained from LAMMC IH Laboratory of plant protection isolate collection. There were four replicates of individual plates per treatment. The mycelium of 7-day old fungus was put the mycelia side in the centre of Petri. Plates were incubated at 25 ± 2°C ºC in dark. The diameter (cm) of *Colletotrichum* spp. width of the disc was measured after 2, 4 and 7 days after inoculation (DAI). To determine the recovery of the isolates after 7 DAI done reinoculations and growth measured after 48 h. The percentage of mycelial growth inhibition calculated according to the formula by Ouoba et al. (2018). The data were analysed with ANOVA of SAS Enterprise Guide 7.1 program (SAS Inc., USA). The Duncan's Multiple Range Test used to determine differences among treatments.

RESEARCH RESULTS

The present study was designed to evaluate in vitro antifungal activity of *Salvia officinalis,* and *Picea abies* essential oils against *Colletotrichum* spp. growth. The results of *P. abies* EO at 4, 7 DAI and reinoculation are presented in Figure 1. *P. abies* EO inhibition more than 50 % were in concentrations 1800 µl/l 2 DAI and from 1600 µl/l 7 DAI. In addition, *Colletotrichum* spp. colony diameter at 1000 µl/l *P. abies* EO was lower (1.69 cm/day) compared with control (2.96 cm/day) 4 DAI. The growth in 1000 µl/l concentration 4 DAI were 1.69 cm/day, 1200 µl/l – 1.50 cm/day, 1400 µl/l – 1.68 cm/day, 1600 µl/l – 1.55 cm/day, 1800 µl/l – 1.65 cm/day and in control 2.96 cm/day. It was still reduced by 43-49% compared to control. The 7 DAI data show that the highest inhibition was at 1600 µl/l (53.70%) and 1800 µl/l (52.61%) concentrations. The results 7 DAI show that concentrations at 1000 µl/l (3.04 cm/day), 1200 µl/l (3.00 cm/day), 1400 µl/l (2.98 cm/day), 1600 µl/l (2.66 cm/day) and 1800 µl/l (2.73 cm/day) had an effect on pathogen growth compared with control (5.75 cm/day). Reinoculation data present fungal recovery after the treatment with EO. The data revealed that the lowest growth of *Colletotrichum* spp. was in 1000-1200 µl/l concentrations compared with control. Besides slight lower in 1400-1800 µl/l concentrations compared with control.

It has been observed that the *S. officinalis* EO inhibitory effect was more than 50% in all concentrations. The results of *S. officinalis* at 4, 7 DAI and reinoculation are presented in Figure 2. After 2 DAI the mycelium diameters in *S. officinalis* were 100 % suppressed in 1000 µl/l and 1400-1800 µl/l concentrations. The results demonstrated that *Colletotrichum* spp. at 1200 µl/l concentration were suppressed by 86.2 % 2 DAI. The 1800 µl/l concentration showed the highest (87.3%) inhibition to *Colletotrichum* spp. growth after 4 DAI (Fig. 2). The growth in 1000 µl/l concentration 4 DAI were 1.18 cm/day, 1200 µl/l – 1.30 cm/day, 1400 µl/l – 1.16 cm/day, 1600 µl/l – 0.99 cm/day, 1800 µl/l – 0.38 cm/day and in control 2.96 cm/day. The results 7 DAI show that concentrations at 1000 µl/l (2.89 cm/day), 1200 µl/l (2.53 cm/day), 1400 µl/l (2.48 cm/day), 1600 µl/l (2.30 cm/day) and 1800 µl/l (1.84 cm/day) had an effect on pathogen compared with control (5.75 cm/day). The highest concentration of *S. officinalis* EO inhibited *Colletotrichum* spp. more than 68 % 7 DAI. However, in 1000-1600 µl/l inhibition were from 49.8 % up to 60.0%. Results showed that in reinoculation in all concentrations, isolates recovered slower than in control. The research data showed that *S. officinalis* essential oil was more effective against *Colletotrichum* spp. compared with *P. abies* EO.

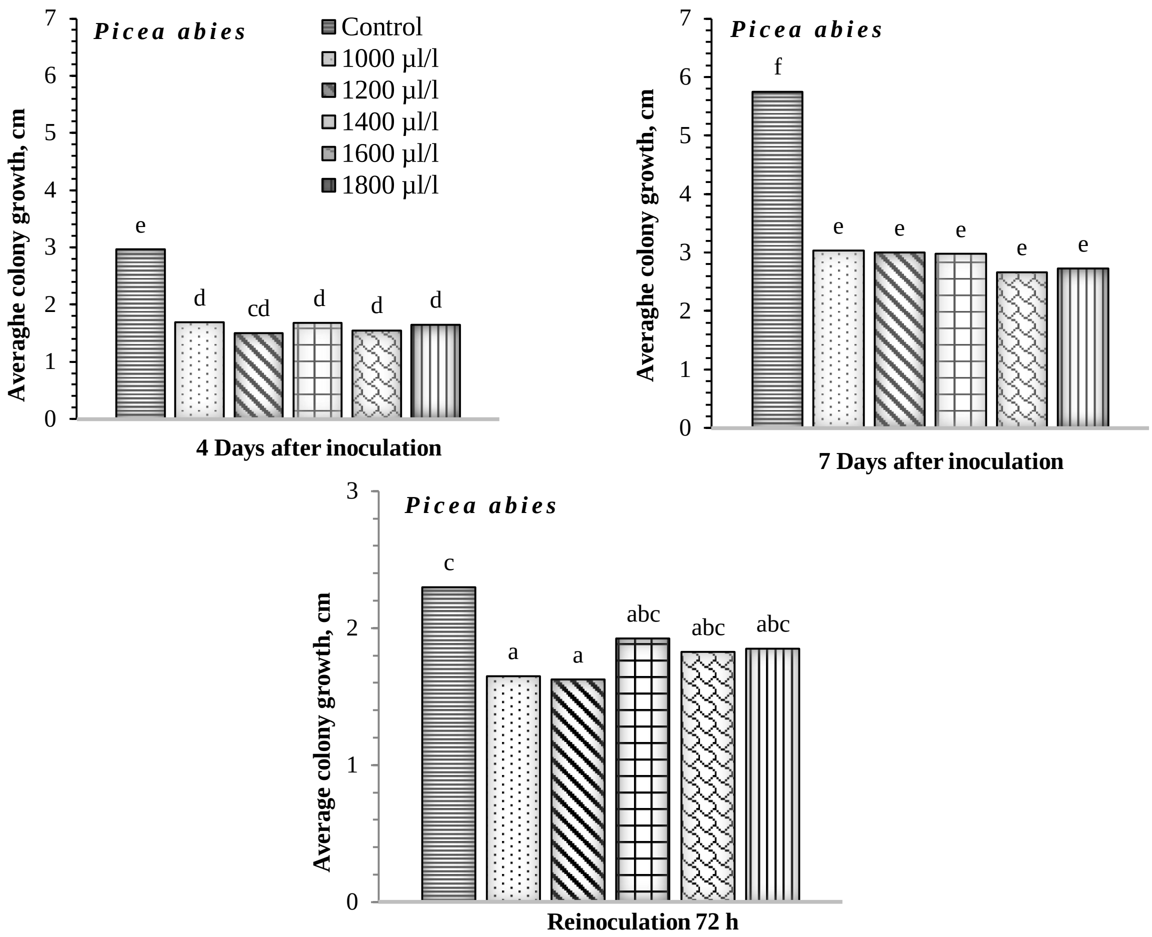

Figure 1. The antifungal effect of spruce essential oil on *Colletotrichum* spp. growth at different concentrations, 4 and 7 days after inoculation, and reinoculation

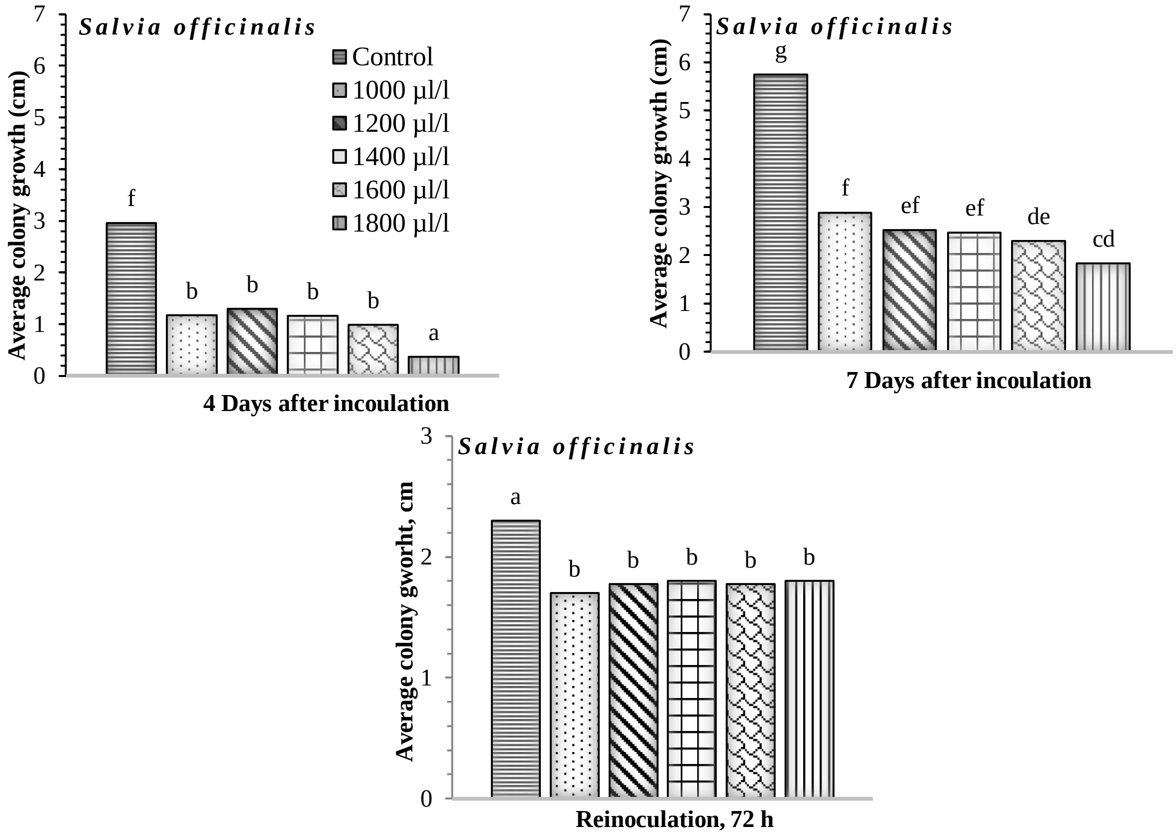

Figure 2. The antifungal effect of sage essential oil on *Colletotrichum* spp. growth at different concentrations, 4 and 7 days after inoculation, and reinoculation

CONCLUSIONS AND DISCUSSION

Plant pathogens could cause significant yield losses in strawberry production (Sreenivasaprasad and Talhinhas, 2005). Therefore, controlling plant pathogens has been of great importance. The environmental problems caused by extensive use of chemical pesticides and growing resistance increased the interest of environment safer control strategies (Damos et al., 2015; Beckerman et al., 2015; Rasiukevičiūtė et a., 2015; Valiuškaitė et al., 2017). Essentials oils as bio-fungicides play an essential role in the plant defence mechanism against phytopathogens (Antunes and Cavaco, 2010).

In recent years, the attention on environment-friendly plant protection pays the attention of many researchers. The essential oils and plant extract demonstrate antimicrobial and antifungal activity, biodegradability and low toxicity, make it potential for use in plant protection against pathogens instead of chemicals (Minova et al., 2015; Acimovic et al., 2016; Volkova et al., 2017; Desam et al., 2017; Nikolova et al., 2017; Yilar et al., 2018). For this reason, in the present study, we have investigated the effect of essential oils from *S. officinalis* and *P. abies* on primary strawberry pathogen *Colletotrichum* spp. as promising bio-fungicides. The results from our study demonstrated that essential oils from the *S. officinalis* were more effective than *P. abies* at evaluated concentrations. However, higher concentrations could give higher inhibition. Scariot et al. (2016) noticed that *S. officinalis* essential oil has antifungal activity against *Alternaria* spp. and *Phakopsora pachyrhizi* in beans. Yilar et al. (2018) find out that *Salvia tomentosa* water extract inhibits mycelium growth of *Botrytis cinerea* 100%.

In comparison, Nikolova et al. (2017) evaluated eighteen aqueous-methanolic extracts, and *S. officinalis* showed the highest activity against *Alternaria alternata*, *B. cinerea* and *Fusarium oxysporum.* There is a lack of research on *P. abies* EO against *Colletotrichum* spp. Comparing 20 g L^{-1} antifungal activity of pine and spruce bark extracts and essential oils mycelial growth inhibition of *B. cinerea*, *C. acutatum* and *Phytophthora cactorum* were 100 % (Minova et al., 2015). Spruce biomass extract for strawberries grey mould infield was not convincing (Volkova et al., 2017). Our data shows that *P. abies* EO inhibition of *Colletotrichum* spp. were highest more 50 % at 2 DPI in concentration 1800 µl/l. The EO of *S. officinalis* 2 DPI showed highest inhibition (100 %) at 1000 µl/l and 1400-1800 µl/l concentrations against *Colletotrichum* spp. However, our data indicate that that inhibition effect of EO on fungal growth varies during DPI. This data suggests that at tested concentrations we have shorter timing. Our data open perspective for environmentally-friendly bio-fungicides from *S. officinalis* and *P. abies* EO essential oils control strawberry anthracnose. The *S. officinalis* and *P. abies* EO have potential to be applied as bio-fungicides against strawberry anthracnose. In addition, bio-fungicides could be a solution for solving the pesticides resistance problem.

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