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# PLANT EXTRACTS AS BIOFUNGICIDES AGAINST SOIL-BORNE PATHOGEN *ALTERNARIA* SPP.

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In the presence of a warmer climate, increase the comparative abundance of fungal plant pathogens. Many of the most aggressive plant pathogens are soil-borne fungi. *Alternaria* species threaten food security as the chemical fungicides currently used against them are mostly ineffective because of the acquired resistance to active compounds. Broadleaf plantain (*Plantago major* L.) and rosemary (*Rosmarinus officinalis* L.) extracts, obtained by subcritical $CO_2$ extraction, were tested for their antifungal activity to investigate the possibility of their use for sustainable plant protection. The ability of the two plant extracts to inhibit mycelial growth was studied by *in vitro* assay on agar medium containing different concentrations of the tested extracts (200-1000 μL $L^{-1}$). *Alternaria* spp. isolate was transferred on the modified medium to test the extracts antifungal activity by calculating the average colony growth after 5 and 7 days. Results showed that rosemary extract had an apparent reducing effect on fungal growth that was dose-dependent. Because of this, it can be considered a potential alternative natural fungicide to the synthetic chemicals currently used to prevent and control soil-borne diseases.

*Keywords: Plantago major, Rosmarinus officinalis, fungal pathogen, biocontrol, mycelial growth.*

## INTRODUCTION

Microscopic fungi of the *Alternaria* genus have an extensive host range but are economically most important on carrots. These fungi cause carrot Alternaria leaf blight and black rot, with symptoms like black, necrotic lesions with yellow halos around them on leaves or spots on roots, leading to pre- and postharvest damage (Chan, Kumar, 2016). In addition, they reduce the nutritional and visual value, shelf life and disease resistance of carrots and cause significant losses to carrot growers worldwide. (Mamgain et al., 2013). *Alternaria* species threaten food security as the chemical fungicides currently used against them are mostly ineffective because of the acquired resistance to active compounds (Sharma et al., 2019). Moreover, residues of chemical pesticides can contaminate harvest and the environment (Carvalho, 2017). This leads to searching for alternative environmental-friendly horticultural disease management strategies.

An increasing number of studies have been conducted on the properties of active substances extracted from plants to influence the emergence and development of pathogenic fungi. Plant essential oils and extracts proved to have antimicrobial characteristics on *Alternaria*, *Botrytis*, *Colletotrichum*, *Fusarium*, *Penicillium* and other genera fungi (Zabka et al., 2009; Riccioni, Orzali, 2011; Dėnė, Valiuškaitė, 2021; Morkeliūnė et al., 2021).

Rosemary (*Rosmarinus officinalis* L.) belongs to the family *Lamiaceae* and has multiple food applications with the head role as a naturally sourced antioxidant (Xie et al., 2017). However, other authors determined the antifungal effects of rosemary plant extracts obtained with various solvents and essential oil against *Alternaria* and *Phytophthora* species (Widmer, Laurent, 2006; Goussous et al., 2010; Sanit, 2016; El Alama et al., 2015; El-Naggar et al., 2017; Šernaitė et al., 2020b). *Plantaginaceae* family member broadleaf plantain (*Plantago major* L.) is more often considered a weed, so investigations on this plant is more on the topic of weed control (Bryson, De Felice, 2010; Patton et al., 2016). Although, in the literature, there are researches on antibacterial activity less on antifungal activity (Stanisavljević et al., 2008; Carvalho et al., 2011; Karima et al., 2015). Therefore, we aimed to investigate the antifungal effect of rosemary and broadleaf plantain $CO_2$ extracts on *Alternaria* spp. growth on PDA by the ability to control mycelium growth.

## RESEARCH METHODS

Experiments were carried out at the LAMMC Institute of Horticulture, Lithuania, in 2020. For this study, extracts of rosemary (RE) (*Rosmarinus officinalis* L.) and broadleaf plantain (BPE) (*Plantago major* L.) were used. Both dried materials were extracted with liquid $CO_2$ (“Gaschema”, Lithuania) for 6 hours at 40 bar pressure and 10°C temperature. Mycelial growth was determined on solid potato dextrose agar (PDA) medium amended with RE and BPE at the following concentrations: 0 (control), 200, 600, and 1000 µL $L^{-1}$.

Experiments were conducted four in repetitions with four replications (Raudonius, 2008) in Petri plates containing the modified medium and inoculated with 6 mm plugs of single-spore *Alternaria* spp. isolates that were isolated from rotten carrot roots. Inoculation plates were kept in the dark at 25°C for seven days. The diameter of colonies in centimetres was measured after 5 and 7 days at two perpendicular points along the diameter of the plate, and the mean of these two readings was used to calculate the average colony growth (Feng, Zheng, 2007; Šernaitė et al., 2020a). Concentrations of the extracts, which demonstrated reduced pathogen growth, were considered to have antifungal effects. The mycelial growth inhibition was calculated using the formula by Yanar et al. (2011):

$$Mycelial\ growth\ inhibition\ (\%) = \frac{C-T}{C} \times 100\ , \qquad (1)$$

*C*—lesion diametre in the control (cm); *T*—lesion diametre in the treated samples (cm).

Experimental data were analysed by a one-way ANOVA with the SAS Enterprise Guide 7.1 program (SAS Inc., USA). Duncan’s multiple range test ($p < 0.05$) was used for the comparison of obtained means.

## RESEARCH RESULTS

The antifungal effect of RE and BPE on *Alternaria* spp. growth was investigated on PDA by the ability to control mycelium growth. Results at five days after inoculation are presented in Figure 1.

RE treatment had a strong antifungal effect, which revealed as reduced growth of the pathogen with increasing concentration of this extract. For example, at 1000 µL $L^{-1}$ of RE, mycelial growth was 2.7 cm, almost 50% lower than the control treatment (5.3 cm). Evaluating BPE did not significantly reduce the growth of *Alternaria* spp. at all tested concentrations and had low inhibition (6%, 8%, and 4%) compared to control. The average colony growth at 200 µL $L^{-1}$ of BPE was 5 cm, at 600 µL $L^{-1}$–4.9 cm, and at 1000 µL $L^{-1}$–5.1 cm. When in the control plates, the growth reached 5.3 cm indicating 600 µL $L^{-1}$ as the most effective concentration.

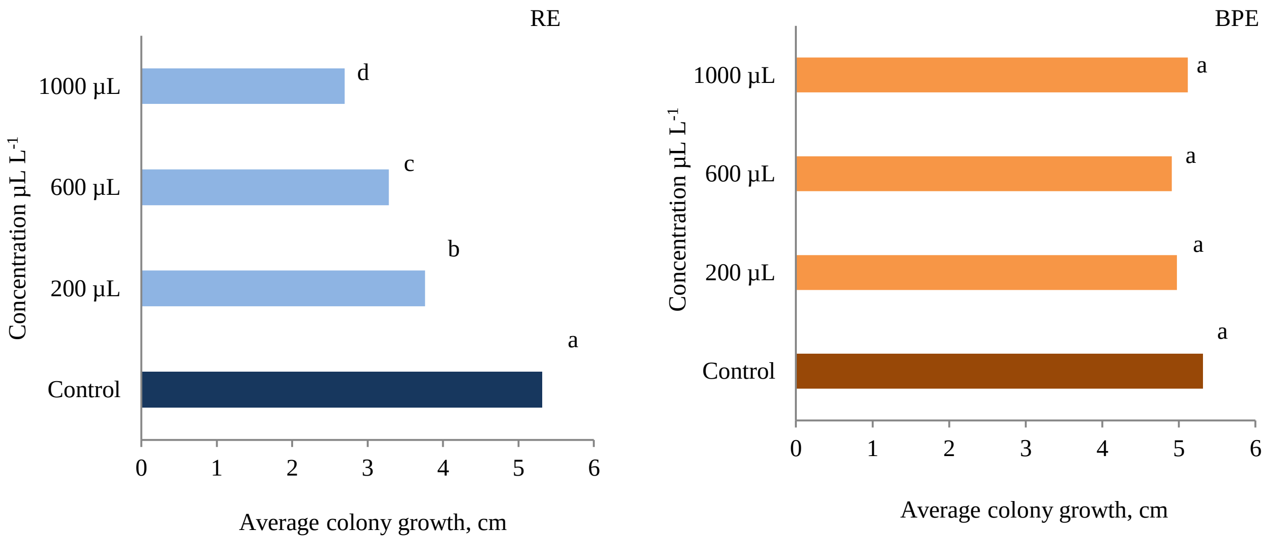


Figure 1. The antifungal effect of rosemary (RE) and broadleaf plantain (BPE) extracts on *Alternaria* spp. colony growth at different concentrations, five days after inoculation.

The average growth of the *Alternaria* spp. colony was also observed seven days after inoculation to evaluate the possibility of maintaining the inhibitory effect on the fungus under-investigated concentrations (Figure 2).

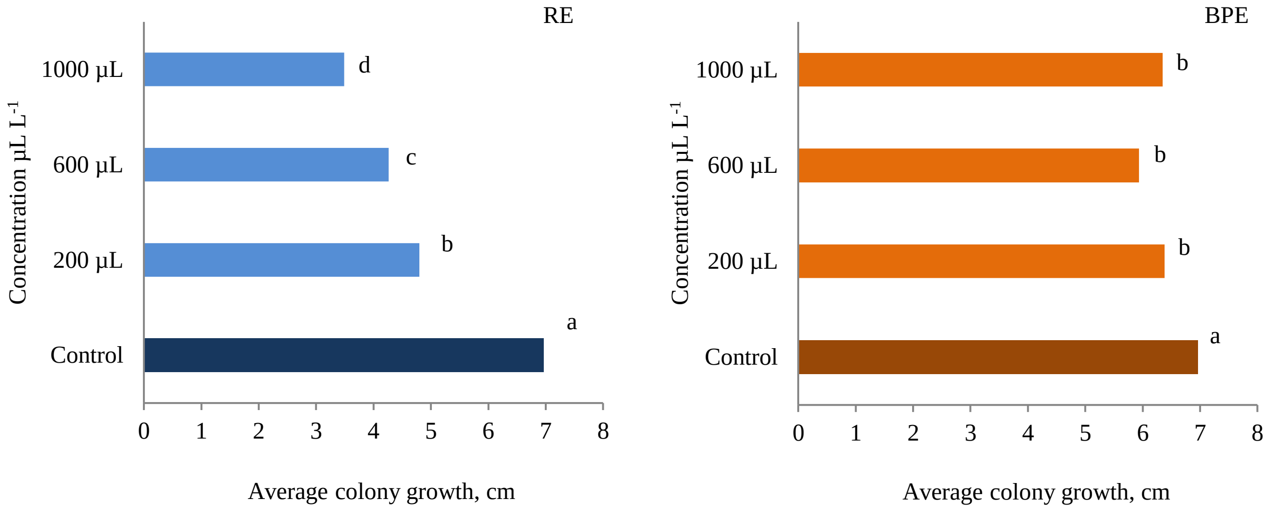


Figure 2. The antifungal effect of rosemary (RE) and broadleaf plantain (BPE) extracts on *Alternaria* spp. colony growth at different concentrations, seven days after inoculation.

Again, the RE was able to keep the inhibitory effect after seven days, and as in the previous assessment, 1000 µL $L^{-1}$ was the most effective. The calculated average diameter of the pathogen colony was 4.8 cm at 200 µL $L^{-1}$, 4.3 cm at 600 µL $L^{-1}$, and 3.5 cm at 1000 µL $L^{-1}$, respectively mycelial growth inhibition reached 31%, 38%, and 50%. The diameter in the control treatment was 7 cm. With the BPE, the growth suppression differences became more pronounced. The value of 600 µL $L^{-1}$ concentration (5.9 cm) differed by one centimetre from the control results (6.9 cm) and inhibited the growth of *Alternaria* spp. up to 15%. In contrast, values of 200 and 1000 µL $L^{-1}$ differed by about 0.6 cm (6.4 cm) with up to 9% inhibition.

## CONCLUSIONS AND DISCUSSION

This study tested rosemary and broadleaf plantain $CO_2$ extracts for their antifungal activity against *Alternaria* spp. *in vitro*. The data presented here show that rosemary extract suppresses mycelium growth. Under the influence of different amounts of rosemary extract, the lowest average colony growth was reached with 1000 µL $L^{-1}$.

Unfortunately, the lack of research on $CO_2$ extracts of rosemary and broadleaf plantain against *Alternaria* micromycetes was noticed. Meanwhile, the antifungal properties exhibited by the essential oil, aqueous and ethanolic extracts obtained from *R. officinalis* were reported by several researchers (Widmer, Laurent, 2006; Goussous et al., 2010; Sanit, 2016; El Alama et al., 2015; El-Naggar et al., 2017; Šernaitė et al., 2020b).

In Goussous et al. (2010) research, complete *A. solani* development inhibition was achieved after doubling ethanolic treatment concentration to 20%, containing 100 mg plant powder in 1 mL PDA medium. Additionally, ethanolic crude extract of rosemary was tested by poisoned food technique against *Alternaria* spp. isolated from rice by Sanit (2016). Sanit investigation revealed that, mycelial growth and spore germination inhibitions were highest (100%) at 5000 µL $L^{-1}$. Widmer and Laurent (2006) found that aqueous extract of this plant at 25% dilution also fully repressed zoospore germination of *Phytophthora* spp.

Evaluating the essential oil efficacy, it appears to be least effective against *Botrytis cinerea* with 31.91% of inhibition at the highest (2000 µl $L^{-1}$) tested concentration (Šernaitė et al., 2020b). However, water, ethanol, and methanol extracts of rosemary proved antibacterial activity against *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* spp. (El Alama et al. 2015; El-Naggar et al., 2017).

Our results revealed broadleaf plantain is less effective in controlling fungi of the genus *Alternaria* indicating 600 µL $L^{-1}$ as the most effective concentration. Our findings agree with Carvalho et al. (2011); the effects of 20 plant extracts, including broadleaf plantain, on *A. alternata* showed no statistically significant results and had no potential to be used as a biofungicide. Although, there are reports about antimicrobial properties of *P. major* extracts. For example, water-ethanolic extracts had better antimicrobial activity against yeasts than bacteria (Stanisavljević et al., 2008), and ethyl acetate fraction was the most active *in vitro* against Gram-positive bacteria strains: highest inhibition zone of 16.7±1 mm and 14.3±0.6 on *S. aureus* and *Bacillus cereus* (Karima et al., 2015).

To conclude, rosemary extract was determined to have better dose-depend antifungal activity against *Alternaria* spp. than broadleaf plantain. The best results were reached with 1000 µL $L^{-1}$ of rosemary extract at 5, and 7 days after inoculation. The effect of broadleaf plantain extract had no significant difference from the control at 200-1000 µL $L^{-1}$ after 5 days. Although, later, some differences from the control after 7 inoculation days could be seen, and the suppression remained stronger under 600 µL $L^{-1}$ concentration of broadleaf plantain extract. Despite these changes, the examination of rosemary extract in this study was considered more effective against carrot *Alternaria* spp. and showed promising prospects for using natural plant extract as a potential source of sustainable, eco-friendly botanical fungicides. Therefore, it would be worthwhile to try higher concentrations of its extract.

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