

THE EFFECT OF SPICE EXTRACTS ON STRAWBERRY PATHOGEN *BOTRYTIS CINEREA*

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Botrytis cinerea, the causal agent of strawberry grey mould, has become a severe problem of horticultural farms due to its genetic variability and growing resistance to frequently used fungicides. Plant disease could induce significant losses of yields and plants thus it is necessary to discover and apply novel approaches to control strawberry pathogens. In addition, chemical plant protection products can leave undesirable residues in fruits and the environment. Plant protection should be more friendly to the environment and human than already used chemical measures. One of the alternatives could be plant-based extracts, produced from aromatic and medicinal herbs, spices, fruits. Therefore, the aim of this study was to determine the *in vitro* effect of cinnamon bark (*Cinnamomum cassia* L.) and clove bud (*Syzygium aromaticum* L.) extracts on *B. cinerea* colony growth. Experiments were carried out at LAMMC Institute of Horticulture, Lithuania. Both extracts were obtained from dried material by extraction with CO₂. For *in vitro* assay, extracts were separately mixed with potato dextrose agar in concentrations from 200 to 1000 µl/l, poured into Petri dishes, then each dish was inoculated with 6 mm diameter *B. cinerea* disc and incubated at 22±2°C in the dark for 7 days. The diameter of the pathogen colony was measured 2, 4 and 7 days after inoculation. Concentrations of the extracts, which demonstrated reduced growth of the pathogen, were considered to have an antifungal effect. The results showed that extracts of cinnamon bark and clove bud had a similar antifungal effect on *B. cinerea* and suppressed the growth of 100% from 600 µl/l concentrations 4 days after inoculation. At 600-1000 µl/l, no growth of the pathogen was detected for both extracts 7 days after inoculation. However, clove bud extract considered to be more effective, as the diameter of *B. cinerea* colonies was smaller compared to cinnamon bark extract at 200-400 µl/l concentrations. To conclude, spice extracts of cinnamon bark and clove bud demonstrated antifungal effect on *B. cinerea* *in vitro* and could possibly be applied for control of strawberry grey mould.

Keywords: antifungal, *Cinnamomum cassia*, grey mould, *Syzygium aromaticum*.

INTRODUCTION

Botrytis cinerea can infect a variety of plant hosts, including strawberry, tomato, onion. Grey mould, caused by this pathogen, is responsible for high losses of plants and fruit yield (Neri et al., 2016; Rasiukeviciute et al., 2018). Commonly, grey mould is controlled by the application of fungicides (Mahdy et al., 2014; Rasiukeviciute et al., 2019). However, due to the spread of the pathogens from different geographical areas and growing resistance, it is becoming harder to avoid plant disease (Nazir et al., 2018). *B. cinerea* has high genetic variability, and the use of chemical pesticides leads to the development of new strains of this fungus (Fernandez et al., 2014). Additionally, chemical plant protection products can leave undesirable residues in fruits and environment, and adverse effect leads to the trend of applying eco-friendly measures for control of pre- and postharvest diseases (Antunes, Cavaco, 2010). New ways of biocontrol, such as disease forecasting, application of antagonistic microorganisms, essential oils and extracts are investigated (Mahdy et al., 2014; Mirmajlessi et al., 2016; Valiuskaite et al., 2017; Zatla et al., 2017; Pretschner et al., 2018; Rasiukeviciute et al., 2019). Plants are a comprehensive source of natural compounds for the discovery of botanical products for biocontrol (Szekacs, Komives, 2017). Secondary metabolites of plants provide extracts and

essential oils with antifungal, antiviral, antibacterial and various other properties. Therefore, they may become active substances for alternative plant disease management (Yami, Shukla, 2016).

Cinnamon and clove are spices used for food, aromatherapy, and medicine. Overall, spices might have anti-inflammatory properties (Mueller et al., 2010). In the literature, research on various effects of spices could be found. Clove extract, obtained by hydrodistillation and extraction with dichloromethane, showed antimicrobial activity against some foodborne bacteria strains (Das et al., 2018). Other authors determined the insecticidal properties of clove essential oil and its derivative (Cansian et al., 2017). Research on spices extracts against plant pathogens could be found. Cinnamon and clove essential oils together with other oils were tested against bacterial postharvest pathogens, and results showed that cinnamon oil was found to be one of the most effective (Bozik et al., 2017). The essential oil of clove inhibited the growth of *Phytophthora infestans* (Mazakova et al., 2018) and emulsion and nanoemulsion of cinnamon (*Cinnamomum zeylanicum* L.) essential oil demonstrated antifungal effect against *B. cinerea* and *Rhizopus stolonifera* (Naserzadeh et al., 2019). Regarding all mentioned properties, extracts of cinnamon and clove potentially have active substances against plant pathogens.

Extraction with carbon dioxide (CO₂) is an advanced method for obtaining oil from plants (Haddi et al., 2017). CO₂ as a solvent is environment-friendly, non-toxic. The method itself allows to obtain a product without solvent residue and to keep compounds, sensitive to high temperature (Sernaite et al., 2019). CO₂ extraction is suitable to be applied in the investigation of alternatives to chemical fungicides, for obtaining active substances. Therefore, the aim of this study was to determine the *in vitro* effect of cinnamon bark (*Cinnamomum cassia* L.) and clove bud (*Syzygium aromaticum* L.) extracts on *B. cinerea* colony growth.

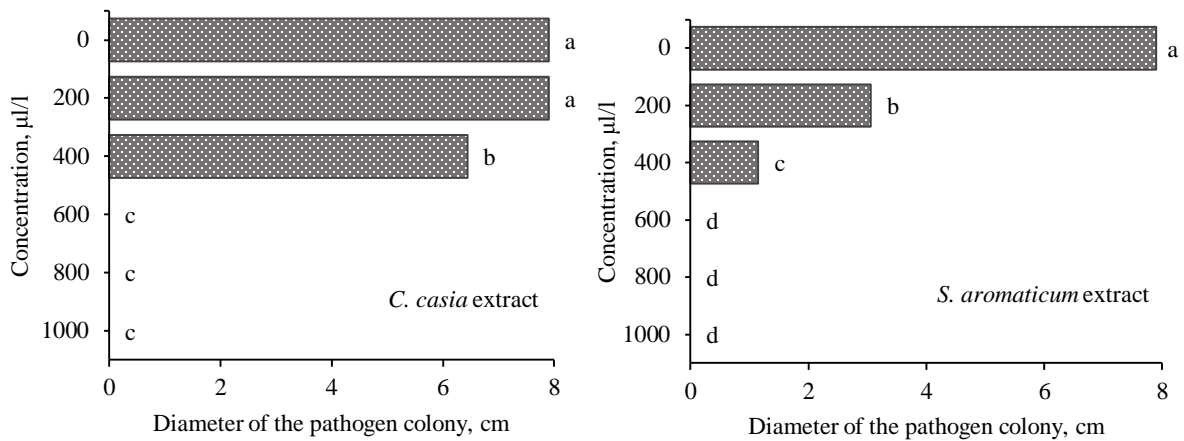
RESEARCH METHODS

Experiments were carried out at the LAMMC Institute of Horticulture, Lithuania. For this study cinnamon (*Cinnamomum cassia* L.) and clove (*Syzygium aromaticum* L.), extracts were used. Dried cinnamon bark and clove bud were obtained (Saldva, Lithuania). The extraction of both materials was done with liquid CO₂ at the following parameters: pressure 40 bar, temperature 10°C, and time 6 hours. The CO₂ gas for the food industry (“Gaschema”, Lithuania) was used. The antifungal effect was investigated by mixing extracts separately with potato dextrose agar (PDA) in concentrations 0 (control), 200, 400, 600, 800 and 1000 µl/l. The different concentrations of both extracts were homogenized with the PDA medium. The 6 mm diameter single spore isolate of *B. cinerea* (strawberry) disc was inoculated in the centre of the Petri dish. Petri dishes were incubated at 22±2°C in the dark for 7 days. Mycelial growth of the pathogen colony was measured 2, 4 and 7 days after inoculation (DAI) in centimetres (pathogen disc excluded). Concentrations of the extracts, which demonstrated reduced growth of the pathogen, were considered to have antifungal effects. Experimental data 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 comparison of obtained means of four replicates. Results in the figures are presented as means.

RESEARCH RESULTS

The antifungal effect of cinnamon and clove extract on *B. cinerea* growth was investigated on PDA by the absence or the presence of mycelium growth. Results at 4 DAI are presented in Figure 1. The growth of the pathogen was reducing respectively, with increasing concentration of investigated extract. Both extracts had a strong antifungal effect from 600 µl/l concentration, as no pathogen colony growth was observed at 600-1000 µl/l. However, the cinnamon extract did not manage to reduce any growth of *B. cinerea* at 200 µl/l and had low inhibition at 400 µl/l compared to control (0 µl/l). The diameter of the pathogen colony at 200 µl/l was 7.9 cm, which is equal to the diameter of the control plate, and diameter at 400 µl/l was 6.44 cm (Figure 2). In contrast to cinnamon extract, clove extract in present research demonstrated more than half reduced diameter of the *B. cinerea* colony at 200 µl/l and low pathogen growth at 400 µl/l compared to control. The measured diameter of the pathogen colony was 3.06 cm at 200 µl/l and 1.15 cm at 400 µl/l, which is more than 50-70% lower than in control (7.9 cm).

The diameter of *B. cinerea* colony growth was also observed 7 DAI to evaluate the duration of the ability to suppress pathogen under-investigated concentrations. The results are presented in Figure 3. The cinnamon extract did not maintain the reduced pathogen growth at 200 µl/l and 400 µl/l. The diameter of the colonies was 7.9 cm in both cases and became the same as in control. Whereas, clove extract was still able to suppress the growth of *B. cinerea* at lowest investigated concentrations. The colony diameter of 5.05 cm was observed at 200 µl/l and 2.50 cm 400 µl. It was still reduced by 20-60% compared to control. 600-1000 µl/l concentration range, which had a high antifungal activity 4 DAI, had the same effect 7 DAI. *B. cinerea* remained inhibited at these concentrations.

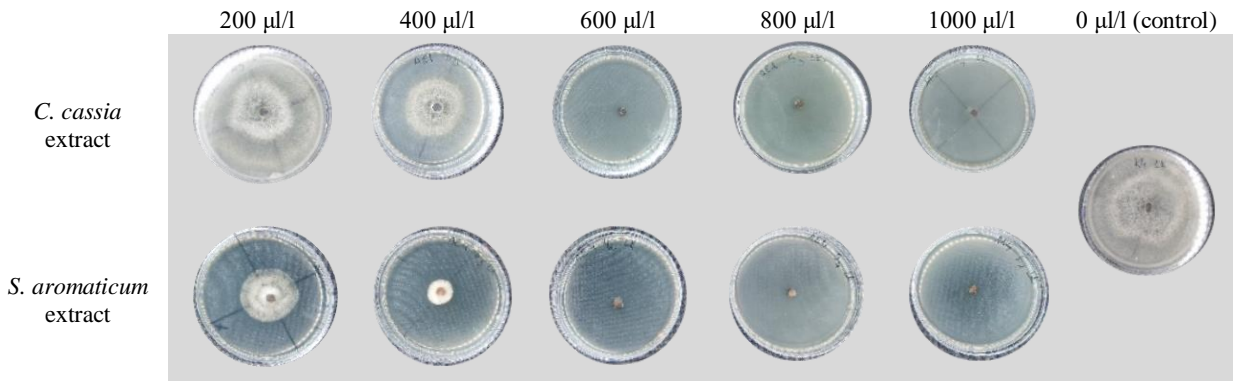


The same letter indicates no significant differences between treatments ($P < 0.05$).

Figure 1. The antifungal effect of cinnamon (*C. cassia*) and clove (*S. aromaticum*) extracts on *B. cinerea* colony growth at different concentrations, four days after inoculation

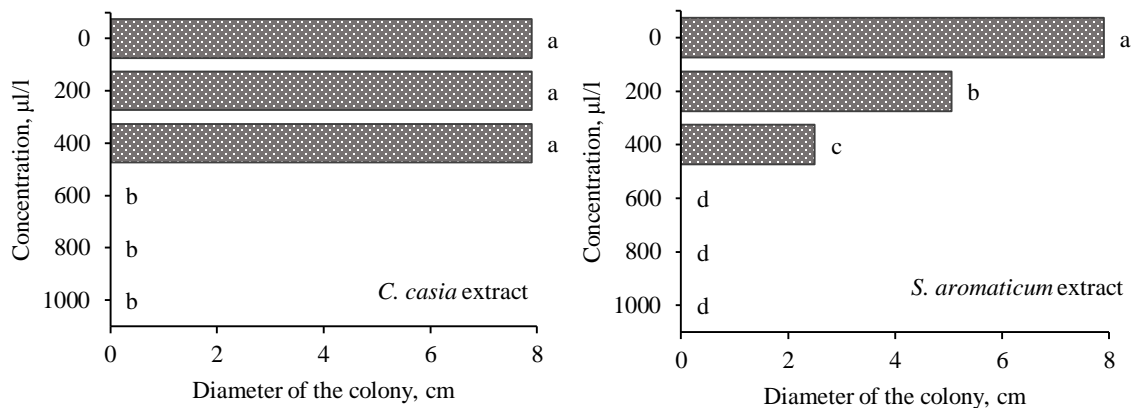
CONCLUSIONS AND DISCUSSION

During this study, CO₂ extracts of cinnamon and clove were examined for antifungal effects against *B. cinerea*. The data presented here show that clove and cinnamon extracts have an influence on *B. cinerea* growth. The cinnamon and clove extracts completely inhibited the growth of *B. cinerea* from 600 µl/l concentration.



Control plates contained no extracts in PDA.

Figure 2. The diameter of *B. cinerea* colonies after treating with different concentrations of cinnamon (*C. cassia*) and clove (*S. aromaticum*) extracts, four days after inoculation



The same letter indicates no significant differences between treatments ($P < 0.05$).

Figure 3. The antifungal effect of cinnamon (*C. cassia*) and clove (*S. aromaticum*) extracts on *B. cinerea* colony growth at different concentrations, seven days after inoculation

There is a lack of research on CO₂ extracts of cinnamon and clove. Meanwhile, the antifungal and antibacterial properties exhibited by essential oils extracted from *Cinnamomum* spp. and *S. aromaticum* were reported by several researchers (Bozik et al., 2017; Mazakova et al., 2018; Naserzadeh et al., 2019).

In the Naserzadeh et al. (2019) study, cinnamon (*C. zeylanicum*) essential oil as emulsion and nano-emulsion form was investigated against *B. cinerea*. The results agree with our study: a complete reduction of the pathogen growth was reached from 500 µl/l. However, emulsion and nano-emulsion of the cinnamon essential oil demonstrated more than 40% inhibition from 125 µl/l. It could be stated that additional substances may help to make new effective forms of antifungal compounds.

Comparing the antimicrobial properties of cinnamon and clove essential oil, investigated by Bozik et al. (2017) using the microdilution method, essential oils were efficacious against three postharvest bacteria strains. Cinnamon essential oil resulted in a stronger antimicrobial effect. We gained similar efficacy of both extracts against fungal pathogen *B. cinerea*, though clove extract reduced pathogen growth at lower concentrations. In addition, clove extract maintained the reduced pathogen growth for a more extended investigated period. For such a result, clove extract is considered to have a better antifungal effect against *B. cinerea* than cinnamon extract.

Clove essential oil also showed complete inhibition of *P. infestans* mycelial growth (Mazakova et al., 2018). This effect was achieved at lower than 1000 µl/l concentration. Antifungal activity at similar concentrations was obtained by Hamini-Kadar et al. (2014) in the study of suppression of *Fusarium* spp. growth by clove essential oil. The oil completely reduced the growth of *F. oxysporum* f. sp *radicis lycopersici* and *F. redolens* at 1000 µl/l and *F. commune* at 500 µl/l. Our study could agree with the concentration range of clove extract effectiveness from other studies, as complete inhibition of the pathogen growth started from 600 µl/l.

To conclude, clove (*Syzygium aromaticum* L.) and cinnamon (*Cinnamomum cassia* L.) extracts were determined to have a high antifungal effect against *B. cinerea* (obtained from a strawberry) at 600-1000 µl/l. Clove extract expressed higher inhibition at 200-400 µl/l than cinnamon extract 4 and 7 days after inoculation. The effect of cinnamon extract had no significant difference from control at 200-400 µl/l 7 days after inoculation. Therefore, a higher efficacy of clove extract was observed at lower concentration than cinnamon extract and the effect was longer, clove extract considered to be more effective against strawberry *B. cinerea*. The study of alternative plant protection measures is an essential strategy against occurring pesticide resistance, as well as developing environment-friendly products against plant diseases. Clove and cinnamon extracts have potential to be applied for control of strawberry grey mould.

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