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Biocontrol of strawberry pathogen *Botrytis cinerea* using plant extracts and essential oils

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Abstract

Essential oils and plant extracts of the rosemary (*Rosmarinus officinalis* L.), bay-laurel (*Laurus nobilis* L.) and clove (*Syzygium aromaticum* L.) contain various valuable compounds that provide antimicrobial and antifungal properties. As a result, they have the potential to be used in plant-pathogen control. *Botrytis cinerea* is one of the main strawberry pathogens, which infects plants during various stages of growth. The study aimed to evaluate the efficacy of plant extracts and essential oils of rosemary, bay-laurel and clove, and their mixtures against *B. cinerea in vitro*. Rosemary, bay-laurel and clove extracts and essential oils were separately and in combinations mixed with potato dextrose agar media at different concentrations. Fractional inhibition concentration (FIC) indexes were calculated for the mixtures to determine synergistic, additive or antagonistic effects of the combinations of the investigated plant extracts and essential oils.

Results of the experiment showed that clove extract had the highest inhibition (100%) of *B. cinerea*, and rosemary essential oil had the lowest inhibition (31.91%). The mixtures, which managed to fully inhibit the growth of *B. cinerea*, were clove extract + clove essential oil, bay-laurel extract + clove essential oil and rosemary extract + clove essential oil. Synergy was found between bay-laurel extract and clove essential oil, and rosemary extract and essential oil, the FIC indexes were equal to 0.75 for both mixtures. The mixture of bay-laurel extract and bay-laurel essential oil was antagonistic as the FIC index was 2.5.

Key words: antifungal effect, biocontrol, *Botrytis cinerea*, essential oil, plant extract, synergy.

Introduction

Plants are a natural source of bioactive compounds, which are responsible for their antifungal, aromatic, medicinal and other properties. Some of the essential oils are well known for their antifungal properties, which are expressed through various mechanisms: disruption of cell membrane or wall, mitochondria, reduction of cell growth, inhibition of biofilm development and other. Besides, by various extraction methods, essential oils and extracts can be obtained from plants (Nazzaro et al., 2017). Plant extracts and essential oils differ in chemical composition regarding the extraction method (Danh et al., 2013). Rosemary (*Rosmarinus officinalis* L.), bay-laurel (*Laurus nobilis* L.) leaves and clove (*Syzygium aromaticum* L.) buds are widely used in food, medicine and cosmetic industries (Al-Kashimi, Mahmood, 2016; Oliveira et al., 2016). Antimicrobial activity and antifungal potential of rosemary essential oil have already been observed against foodborne pathogens (Fakoor, Rasooli, 2008; Soylu et al., 2010; Caputo et al., 2018). Foodborne pathogen *Aspergillus niger* is partly controlled by rosemary essential oil (Baghloul et al., 2017), besides it reduces *A. flavus* mycotoxin production (Mahmoud et al., 2014). Derwich et al. (2009) determined the inhibitory effect of bay-laurel essential oil on human pathogenic bacteria. Clove bud essential oil consists mainly of eugenol and its derivative eugenyl acetate (Hatami et al., 2019). In a comparison of extraction methods, clove oil obtained by supercritical CO_2 extraction was found to have a higher concentration of eugenol and eugenyl acetate than that obtained by steam distillation (Yazdani et al., 2005). Olea et al. (2019) determined the antifungal activity of eugenol and its derivatives against *Botrytis cinerea*.

Antifungal and antimicrobial activity of plant ingredients is getting increasingly more attention in plant protection (Nikkhah et al., 2017), as alternative plant protection products are needed due to high toxicity of the chemical pesticides to humans and the environment (Choudhury et al., 2018; Rosero-Hernandez et al., 2019). Biodegrable, non-toxic chemicals from plants with selective activity against pathogens could become safer disease control agents (Soylu et al., 2010).

Biopesticides derived from plants are divided into extracts and essential oils depending on the method of extraction (Lengai, Muthomi, 2018). Due to the valuable composition and properties, rosemary, bay-laurel leaves

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and clove bud extracts and essential oils may be one of the options in this area. Higher efficacy of the biocontrol product could be achieved by combining several natural compounds. According to Mbunde et al. (2019), synergism occurring in herbal drugs improves efficacy, determines the faster therapeutic effect and also provides an alternative way to treat resistant microorganisms. However, a similar effect may be achieved in antifungal mixtures against plant pathogens. Several studies on the combinations of bioactive compounds can be found in the literature (Kocic-Tanackov et al., 2014; Milenkovic et al., 2018; Mbunde et al., 2019). Clove bud essential oil, together with eucalyptus essential oil, demonstrated synergistic antimicrobial activity against human pathogens *Staphylococcus aureus*, *Escherichia coli* and *Microsporum gypseum* (Kirui et al., 2014). Aguilar-Gonzalez et al. (2015) investigated clove bud essential oil for antifungal effect on *B. cinerea* together with mustard essential oil on strawberries in the vapour phase.

B. cinerea causes grey mould, a harmful disease with a wide range of hosts (Rasiukevičiūtė et al., 2018). It may infect plants at different growth stages and could result in high losses of the plants or whole yield (Rosero-Hernandez et al., 2019). One of the host plants is strawberry, which can get infected through various parts, like leaves, fruits, flowers, petioles and stems (Rasiukevičiūtė et al., 2018). Although *B. cinerea* is controlled using integrated disease management, the most efficient way to control this pathogen is synthetic fungicides (Abbey et al., 2019). *B. cinerea*, determined as a high risk pathogen, is able to develop resistance to widely used fungicides (Minova et al., 2015) as a result of new, resistant fungal strains (Yildirim, Yapici, 2007). As the negative effects of the chemical fungicides on humans and environment are already known, new ways to control pathogens are under investigation (Rasiukevičiūtė et al., 2015; 2019; Mirmajlessi et al., 2016; Valiuškaitė et al., 2017).

Based on the demand for alternatives to chemicals for controlling grey mould, which is a common strawberry disease, the aim of this study was to investigate the efficacy of essential oils and extracts of rosemary and bay-laurel leaves and clove buds against *B. cinerea in vitro* and to examine, whether the mixtures of these extracts have synergistic effect.

Materials and methods

The research was carried out in 2018–2019 at the Laboratory of Plant Protection, Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. Bay-laurel (*Laurus nobilis* L.), rosemary (*Rosmarinus officinalis* L.) and clove (*Syzygium aromaticum* L.) were selected for this research according to chemical composition of their bioactive compounds and antimicrobial and antifungal activities (Fakoor, Rasooli, 2008; Derwich et al., 2009; Soylu et al., 2010; Baghloul et al., 2017; Caputo et al., 2018; Olea et al., 2019), which have the potential to be effective against plant pathogens. Plant extracts and essential oils were produced using two methods; dried plant material was used in both of them to obtain higher yields of essential oil and to compare the efficacy of plant-based material from different extraction methods.

Isolation of plant extracts. High-pressure carbon dioxide (CO_2) extraction was used to obtain plant extracts. Dried bay-laurel and rosemary leaves and clove buds were purchased from the UAB "Sauda", Lithuania. CO_2 gas, intended for the food industry ("Gaschema", Lithuania), was used in the extraction process. Twenty-five kilograms of the plant material was placed in a high-pressure CO_2 extractor consisting of an extraction vessel, cooler, heater and liquid CO_2 collector. Extraction parameters were selected based on pressure-temperature diagram of CO_2, lower than critical point (Cunha et al., 2018). Extraction parameters were: 40 bar pressure, 10°C temperature and 6 hour extraction time.

Essential oil extraction. Dried bay-laurel leaves and clove buds were purchased from the Cash and Carry Ltd., Lithuania. One kilogram of the plant material was extracted using a Clevenger distillation system (Glassco, India). The time of the essential oil distillation was 2 hours (AOAC, 1990). Rosemary essential oil was obtained after transferring plant extract from CO_2 extraction to the Clevenger system and collecting essential oil.

Efficacy of individual plant extracts and essential oils. Rosemary, bay-laurel and clove extracts and essential oils were separately poured into the potato dextrose agar (PDA) at these concentrations: 0, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 µl L^{-1}. Discs (6 mm diameter) of 7-day old strawberry pathogen *Botrytis cinerea* single-spore isolate (from Laboratory of Plant Protection, Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry isolate collection) were cut and placed on each Petri dish with the PDA and the tested products. The fungus was used from the margin of the plate and put the mycelial side down in the centre of the Petri dish amended with PDA. Isolates were incubated for 7 days at 22 ± 2°C temperature in the dark. The radial growth (cm) of the *B. cinerea* colony (including the diameter of the disc) was measured after 2, 4 and 7 days. The efficacy of individual plant extracts and essential oils *in vitro* was expressed as mycelial growth inhibition (Cherkupally et al., 2017):

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

where C is radial growth of the pathogen colony in the control (cm), T – radial growth of the pathogen colony in the treatment (cm).

The lowest concentrations of each investigated plant extract or essential oil with no visible growth of the pathogen (mycelial growth inhibition equal to 100%) were considered to be minimal inhibition concentrations (MIC).

Efficacy of the mixtures. After evaluation of the efficacy of extracts and oils, MIC of the investigated plant extracts (PE) and essential oils (EO) were determined: clove EO MIC = 1200 µl L^{-1}, clove PE MIC = 600 µl L^{-1}, rosemary EO MIC = 2000 µl L^{-1}, rosemary PE MIC = 1600 µl L^{-1}, bay-laurel EO MIC = 2000 µl L^{-1} and bay-laurel PE MIC = 1200 µl L^{-1}. The efficacy of 600 µl L^{-1} concentration of rosemary PE differed from other investigated concentrations and was not chosen as MIC. The prepared mixtures were binary and contained plant extract and essential oil in five different ratios of the determined MIC of each component: ¼ MIC_{PE} + ¼ MIC_{EO}, ½ MIC_{PE} + ½ MIC_{EO}, MIC_{PE} + MIC_{EO}, 1¼ MIC_{PE} + 1¼ MIC_{EO} and 1½ MIC_{PE} + 1½ MIC_{EO}. The calculated amounts of plant extracts and essential oils were mixed with the PDA medium and further investigation of the efficacy of the mixtures was carried out in the same way as described for individual plant extracts and essential oils.

Determination of fractional inhibition concentration (FIC) indexes. A few steps were made to investigate, whether individual plant extracts and essential oils act synergistically in the mixtures. Firstly, the minimal inhibition concentrations (MIC) of the plant extract (PE) and essential oil (EO) in the mixture were determined. Then, fractional inhibitory concentration index (FIC_{index}) was calculated using the formula, adapted by Stevic et al. (2014):

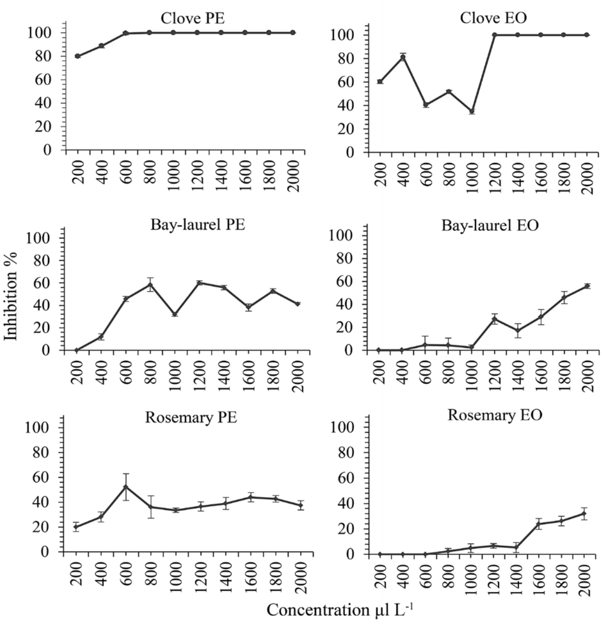
$$FIC_{index} = \frac{MIC_{EO} \, in \, mixture}{MIC_{EO}} + \frac{MIC_{PE} \, in \, mixture}{MIC_{PE}}$$

The mixture had a synergistic effect, meaning that it had higher efficacy than individual components: when evaluated $FIC_{index} < 1$ the mixture had additive effect, when the efficacy of the mixture was equal to that of its components and $1 \leq FIC_{index} \leq 2$; when efficacy of the mixture was lower than that of individual components, the mixture had an antagonistic effect and $FIC_{index} > 2$ (Kocic-Tanackov et al., 2014).

Statistical analysis. The program *SAS Enterprise Guide*, version 7.1 (SAS Institute Inc., USA) was applied for the analysis of the results with the ANOVA procedure. The experiments were carried out in four replicates. Duncan's multiple range test was used to determine significant differences between treatments ($P < 0.05$). Results in the figures are represented as the mean, vertical bars indicate standard deviation. Results in the table are represented as the mean ± standard error.

Results and discussion

Efficacy of the plant extracts and essential oils. The inhibition of mycelial growth of strawberry pathogen *B. cinerea* was determined by separately mixing plant extracts and essential oils with PDA at different concentrations. The results are presented in Figure 1. Clove extract, whose lowest concentration (200 µl L^{-1}) showed more than 80% inhibition, had the highest efficacy against the pathogen. This extract inhibited the pathogen growth 100% at 600–2000 µl L^{-1} concentration. However, clove essential oil had versatile inhibition at 200–1000 µl L^{-1} and fully (100%) inhibited growth of *B. cinerea* at 1200–2000 µl L^{-1} concentration. The differences in the efficacy of these two plant-derived products may depend on the different extraction methods.



Note. Results are presented as the mean, vertical bars indicate standard deviation.

Figure 1. Inhibition (%) of *Botrytis cinerea* on PDA: clove essential oil (EO) and plant extract (PE), bay-laurel EO and PE and rosemary EO and PE

Clove plant extract, as a more concentrated product of CO_2 extraction, resulted in a consistent growth of the efficacy. Meanwhile, clove essential oil exhibited high efficacy at 200–1000 µl L^{-1} concentration, though the composition of essential oil was not strong enough to fully suppress the pathogen growth.

Although clove products were not widely investigated on plant pathogenic fungi, there is evidence that ethanolic extract of clove 100% inhibited the growth of *Sclerotium rolfsii*, *Alternaria alternata*, *Fusarium moniliformae*, *Rhizoctonia solani* and *Aspergillus niger* (Singh et al., 2019). Additionally, clove essential oil was effective against phytopathogenic bacteria (Huang, Lakshman, 2010; Bozik et al., 2017).

El Alama et al. (2015) found that clove and rosemary essential oil is effective against *Candida albicans*. Methanol, ethanol and water extracts of rosemary demonstrated antimicrobial activity (El-Naggar et al., 2017). In our study, rosemary extract was moderately effective against *B. cinerea* with the highest inhibition of 52.21% at 600 µl L^{-1} and 43.97% at 1600 µl L^{-1}. Meanwhile, rosemary essential oil was least effective of all the tested plant-derived products: it demonstrated 31.91% of inhibition at the highest (2000 µl L^{-1}) concentration. It can be assumed that rosemary essential oil contained the least amount of antifungal compounds or they were not entirely extracted using hydrodistillation method, because CO_2-extracted rosemary exhibited higher efficacy. Bay-laurel leaf extract did not demonstrate the high inhibition (60.15% at 1200 µl L^{-1}). *B. cinerea* growth was reduced with increased concentrations of bay-laurel leaf essential oil at 1400–2000 µl L^{-1}, and the highest inhibition reached was 55.88%. The active components of bay-laurel plant extract have also been extracted better with CO_2 extraction than hydrodistillation, because higher efficacy was observed at lower concentrations compared to bay-laurel essential oil. Investigation of higher concentrations of both bay-laurel plant extract and essential oil is needed, as they demonstrated potential to suppress the pathogen. Other studies determined that microemulsion with bay-laurel essential oil inhibited growth and conidia germination of *A. alternata* (Xu et al., 2017) and expressed bactericidal activity (Derwich et al., 2009). The determined minimal inhibition concentrations (MIC) of each tested product: clove EO MIC = 1200 µl L^{-1}, clove PE MIC = 600 µl L^{-1}, rosemary EO MIC = 2000 µl L^{-1}, rosemary PE MIC = 1600 µl L^{-1}, bay-laurel EO MIC = 2000 µl L^{-1}, bay-laurel PE MIC = 1200 µl L^{-1}, were used in the further assay with mixtures.

Overall, plant extracts were more effective against *B. cinerea* compared to essential oils. It may depend on the method of the extraction of antifungal compounds.

Efficacy of the mixtures. Mixtures of two components with each tested product were formed after evaluation of individual plant extracts *in vitro*. The efficacy of the mixtures with the different ratios of extracts and essential oils' MIC from the previous experiment is presented in Table.

The most effective mixtures were clove bud extract and essential oil at all investigated ratios, also bay-laurel PE + clove EO and rosemary PE + clove EO, which gave 100% inhibition of pathogen growth at four mixture combinations. Clove PE + bay-laurel EO and clove PE + rosemary EO showed 100% inhibition at three tested combinations. However, other investigated mixtures increased inhibition of *B. cinerea* with higher ratio of the components in the mixture. The lowest

Table. Mycelial growth inhibition (%) of *Botrytis cinerea* by plant extracts (PE) and essential oil (EO) mixtures

Ratio of $MIC_{PE} + MIC_{EO}$ in the mixture	clove PE + clove EO	clove PE + bay-laurel EO	clove PE + rosemary EO	bay-laurel PE + clove EO	bay-laurel PE + bay-laurel EO
¼ + ¼	93.28 ± 7.15 ba	56.62 ± 3.67 k	53.53 ± 4.16 lk	73.97 ± 0.74 ih	7.50 ± 5.77 o
½ + ½	100.00 ± 0.00 a	82.50 ± 3.34 dfe	79.41 ± 3.81 gf	100.00 ± 0.00 a	43.68 ± 2.69 m
1+1	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	72.50 ± 8.21 ihj
1¼ + 1¼	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	82.35 ± 2.84 dfe
1½ + 1½	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	85.15 ± 9.95 dce
Ratio of $MIC_{PE} + MIC_{EO}$ in the mixture	bay-laurel PE + rosemary EO	rosemary PE + clove EO	rosemary PE + bay-laurel EO	rosemary PE + rosemary EO	
¼ + ¼	30.88 ± 4.74 n	41.91 ± 1.55 m	42.94 ± 2.76 m	33.09 ± 4.41 n	
½ + ½	45.15 ± 3.48 m	100.00 ± 0.00 a	50.88 ± 2.98 l	54.12 ± 4.38 lk	
1+1	67.06 ± 7.22 j	100.00 ± 0.00 a	68.68 ± 3.27 ij	67.35 ± 7.65 j	
1¼ + 1¼	82.50 ± 1.30 dfe	100.00 ± 0.00 a	87.50 ± 3.34 dc	74.41 ± 3.48 gh	
1½ + 1½	90.44 ± 0.56 bc	100.00 ± 0.00 a	100.00 ± 0.00 a	80.15 ± 5.53 fe	
Control 0.00 ± 0.00 *p*					

Note. MIC – minimal inhibition concentrations; results are presented as the mean ± standard error; the same letter indicates no significant differences between treatments ($P < 0.05$).

efficacy (7.50%) was shown by the mixture of ¼ MIC_{PE} + ¼ MIC_{EO} bay-laurel PE and EO. There are no other studies where mixtures of plant extracts and essential oils were investigated. However, studies on the combinations of essential oils can be found in the literature. The mixtures of clove and mustard (*Brassica nigra*) essential oils fully inhibited strawberry pathogen *B. cinerea* at various compositions (Aguilar-Gonzalez et al., 2015).

The determined fractional inhibitory indexes (FIC_{index}) are shown in Figure 2. Only bay-laurel PE + clove EO and rosemary PE + rosemary EO demonstrated the synergy between the components, and the inhibition was higher than that of individual components. A similar result was obtained by Aguilar-Gonzalez et al. (2015); they found a synergy between clove and mustard essential oils. Besides, clove essential oil acted synergistically with eucalyptus (*Eucalyptus citriodora*) essential oil against several bacteria strains (Kirui et al., 2014). A mixture of bay-laurel PE + bay-laurel EO was found to be antagonistic.

Regarding this mixture, bay-laurel EO may significantly reduce the efficacy of the bay-laurel PE, as its inhibition was higher at many concentrations according to the results *in vitro* (Fig. 1). Rosemary PE + clove EO and clove PE + clove EO mixtures had the same effect as their components as determined FIC index was equal to 1 and was considered as additive.

The rest of the mixtures also had additive effect as their FIC index did not exceed 1.5. The results are similar to those of Mbunde et al. (2019), who reported that most of the investigated crude plant extract mixtures had additive effect. A few synergistic interactions between crude plant extracts against human pathogenic fungi were documented by Mbunde et al. (2019). The author emphasised that despite the low efficacy as an individual component, natural components may be useful when used in mixtures because of the synergy. For example, Milenkovic et al. (2018) examined essential oils and did not find them to have antimicrobial properties

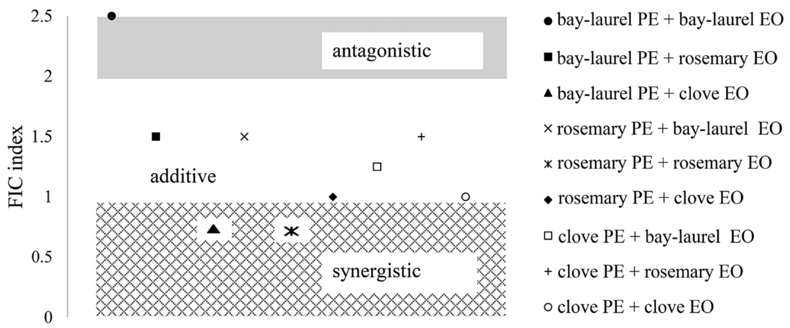


Figure 2. Fractional inhibition concentration (FIC) indexes of the plant extracts (PE) and essential oil (EO) mixtures and demonstration of the synergistic, additive and antagonistic effect

against several bacteria. However, in combination with antibiotics, the effect of mixtures varied from antagonistic to synergistic. We can partly agree with this statement, because rosemary PE + rosemary EO, not as effective as individual components, managed to have higher inhibition due to the synergy. However, further results are in contrast to those of other authors. In the case of mixture with clove PE + bay-laurel EO, again bay-laurel EO might be responsible for the lower efficacy of this extract, as clove PE was the most effective as an individual plant component. Overall, the efficacy of clove EO and PE was reduced by the second component of the mixture in many cases.

Conclusions

1. Individual plant extracts (PE) and essential oils (EO) of rosemary, bay-laurel and clove and their mixtures were investigated against strawberry pathogen *Botrytis cinerea*. Results revealed that the most effective was clove plant extract, and the lowest inhibition was exhibited by rosemary essential oil.

2. Binary mixtures of clove bud extract and essential oil, bay-laurel leaf extract and clove essential oil, and rosemary leaf extract and clove essential oil demonstrated the highest inhibition against *B. cinerea* and reduced the pathogen growth by up to 100%.

3. Mixtures of bay-laurel leaf extract and clove bud essential oil, and rosemary leaf extract and essential oil showed synergistic effect on the inhibition of *B. cinerea*, the fractional inhibition concentration (FIC) index was 0.75. The mixture of bay-laurel plant extract and essential oil had an antagonistic effect – FIC_{index} = 2.5.

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References

1. Abbey J. A., Percival D., Abbey L., Asiedu S. K., Prithiviraj B., Schilder A. 2019. Biofungicides as alternative to synthetic fungicide control of grey mould (*Botrytis cinerea*) – prospects and challenges. Biocontrol Science and Technology, 29 (3): 207–228. https://doi.org/10.1080/09583157.2018.1548574
2. Aguilar-Gonzalez A. E., Palou E., Lopez-Malo A. 2015. Antifungal activity of essential oils of clove (*Syzygium aromaticum*) and/or mustard (*Brassica nigra*) in vapor phase against gray mold (*Botrytis cinerea*) in strawberries. Innovative Food Science and Emerging Technologies, 32: 181–185. https://doi.org/10.1016/j.ifset.2015.09.003
3. Al-Kashimi A. G., Mahmood S. A. 2016. The Nutritional value and antioxidant activity of bay-laurel leaves (*Laurus nobilis* L.). Basrah Journal of Veterinary Research, 15 (2): 246–259. https://doi.org/10.33762/bvetr.2016.124310
4. AOAC. 1990. Volatile oil in spices. Official methods of analysis (15th ed.) AOAC International, p. 1001.
5. Baghloul F., Mansori R., Djahoudi A. 2017. *In vitro* antifungal effect of *Rosmarinus officinalis* essential oil on *Aspergillus niger*. National Journal of Physiology Pharmacy and Pharmacology, 7 (3): 285–289. https://doi.org/10.5455/njppp.2017.7.7021513102016
6. Bozik M., Novy P., Kloucek P. 2017. Chemical composition and antimicrobial activity of cinnamon, thyme, oregano and clove essential oils against plant pathogenic bacteria. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 65 (4): 1129–1134. https://doi.org/10.11118/actaun201765041129
7. Caputo L., Trotta M., Romaniello A., De Feo V. 2018. Chemical composition and phytotoxic activity of *Rosmarinus officinalis* essential oil. Natural Product Communications, 13 (10): 1367–1370. https://doi.org/10.1177/1934578X1801301033
8. Cherkupally R., Kota R., Amballa H., Reddy B. N. 2017. *In vitro* antifungal potential of plant extracts against *Fusarium oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina*. Annals of Plant Sciences 6 (9): 1676–1680. https://doi.org/10.21746/aps.2017.9.2
9. Choudhury D., Dobhal P., Srivastava S., Saha S., Kundu S. 2018. Role of botanical plant extracts to control plant pathogens – a review. Indian Journal of Agricultural Research, 52 (4): 341–346.
10. Cunha V. M. B., Silva M. P., Costa W. A., Oliveira M. S., Bezerra F. W. F., melo A. C., Pinto R. H. H., Machado N. T., Araujo M. E., Carvalho Junior R. N. 2018. Carbon dioxide use in high-presuure extraction processes. Karame I. et al. (eds). Carbon dioxide chemistry, capture and oil recovery. IntechOpen, p. 211–240. https://doi.org/10.5772/intechopen.71151
11. Danh L. T., Han L. N., Triet N. D. A., Zhao J., Mammucari R., Foster N. 2013. Comparison of chemical composition, antioxidant and antimicrobial activity of lavender (*Lavandula angustifolia* L.) essential oils extracted by supercritical CO_2, hexane and hydrodistillation. Food and Bioprocess Technology, 6 (12): 3481–3489. https://doi.org/10.1007/s11947-012-1026-z
12. Derwich E., Benziane Z., Boukir A. 2009. Chemical composition and antibacterial activity of leaves essential oil of *Laurus nobilis* from Morocco. Australian Journal of Basic and Applied Sciences, 3 (4): 3818–3824.
13. El Alama H., El Aissami A., Ait Haj Said A., El Alaoui-Faris F. E. 2015. Evaluation of anti-candida albicans activity of essential oils of six medicinal plants: Synergy concept. Journal of Chemical and Pharmaceutical Research, 7 (10): 281–284.
14. El-Naggar M. N., Abdulla G., El-Shourbagy G. A., El-Badawa A. A., El Sohaimy S. A. 2017. Antimicrobial and antioxidant activities of some plant extracts. Zagazig Journal of Agricultural Research, 44 (3): 11. https://doi.org/10.21608/zjar.2017.52305
15. Fakoor M. H., Rasooli I. 2008. Pathogen control by antioxidative characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Acta Horticulturae, 786: 125–136. https://doi.org/10.17660/ActaHortic.2008.786.13
16. Hatami T., Johner J. C. F., Zabot G. L., Meireles M. A. 2019. Supercritical fluid extraction assisted by cold pressing from clove buds: extraction performance, volatile oil composition, and economic evaluation. The Journal of Supercritical Fluids, 144: 39–47. https://doi.org/10.1016/j.supflu.2018.10.003
17. Huang Q., Lakshman D. K. 2010. Effect of clove oil on plant pathogenic bacteria and bacterial wilt of tomato and geranium. Journal of Plant Pathology, 92 (3): 701–707.
18. Kirui J. K., Ngure R., Bii C., Karimi P. N., Mutai C., Amugune B. K. 2014. Combined antibacterial and antifungal activities of *Eucalyptus citriodora* and *Syzygium aromaticum* essential oils. African Journal of Pharmacology and Therapeutics, 3 (1): 29–37.
19. Kocic-Tanackov S. D., Dimic G. R., Mojovic L. V., Pejin J. D., Tanackov I. J. 2014. Effect of caraway, basil, and oregano extracts and their binary mixtures on fungi in growth medium and on shredded cabbage. LWT – Food Science and Technology, 59 (1): 426–432. https://doi.org/10.1016/j.lwt.2014.05.023
20. Lengai G. M. W., Muthomi J. W. 2018. Biopesticides and their role in sustainable agricultural production. Journal of Biosciences and Medicines, 6: 7–41. https://doi.org/10.4236/jbm.2018.66002
21. Mahmoud M. A., El-Zaidy M., Al-Othman M. R., Abd El-Aziz A. R. M. Al-Gahtani M. F. 2014. Efficacy of *Rosmarinus officinalis* essential oil on *Aspergillus flavus* and *parasiticus*. Journal of Pure and Applied Microbiology, 8 (2): 185–190.
22. Mbunde M. V. N., Mabiki F., Innocent E., Anderccon P. 2019. Antifungal activity of single and combined extracts of medicinal plants from Southern Highlands of Tanzania. Journal of Pharmacognosy and Phytochemistry, 8 (1): 181–187.
23. Milenkovic M., Stosovic J., Slavkovska V. 2018. Synergy between essential oils of *Calamintha* species (Lamiaceae) and antibiotics. Natural Product Communications, 13 (3): 371–374. https://doi.org/10.1177/1934578X1801300325
24. Minova S., Seskena R., Voitkane S., Metla Z., Daugavietis M., Jankevica L. 2015. Impact of pine (*Pinus sylvestris* L.) and spruce (*Picea abies* (L.) Karst.) bark extracts on important strawberry pathogens. Proceedings of the Latvian Academy of Sciences, 69, Section B, p. 62–37. https://doi.org/10.1515/prolas-2015-0008
25. Mirmajlessi S. M., Mand M., Najdabbasi N., Larena I., Loit E. 2016. Screening of native *Trichoderma harzianum* isolates for their ability to control *Verticillium* wilt of strawberry. Zemdirbyste-Agriculture, 103 (4): 397–404. https://doi.org/10.13080/z-a.2016.103.051
26. Nazzaro F., Fratianni F., Coppola R., De Feo V. 2017. Essential oils and antifungal activity. Pharmaceuticals, 10 (4): 86. https://doi.org/10.3390/ph10040086
27. Nikkhah M., Hashemi M., Najafi M. B. H., Farhoosh R. 2017. Synergistic effects of some essential oils against fungal spoilage on pear fruit. International Journal of Food Microbiology, 257: 285–294. https://doi.org/10.1016/j.ijfoodmicro.2017.06.021
28. Olea A. F., Bravo A., Martinez R., Thomas M., Sedan C., Espinoza L., Zambrano E., Carvajal D., Silva-Moreno E., Carrasco H. 2019. Antifungal activity of eugenol derivatives against *Botrytis cinerea*. Molecules, 24 (7): 1239. https://doi.org/10.3390/molecules24071239

29. Oliveira M. S., Costa W. A., Pereira D. S., Botelho J. R. S., Alencar Menezes T. O., Aguiar Andrade E. H., Silva S. H. M., Silva Sousa Filho A. P., Carvalho R. N. 2016. Chemical composition and phytotoxic activity of clove (*Syzygium aromaticum*) essential oil obtained with supercritical CO_2. The Journal of Supercritical Fluids, 118: 185–193. https://doi.org/10.1016/j.supflu.2016.08.010
30. Rasiukevičiūtė N., Valiuškaitė A., Uselis N., Buskienė L., Viškelis J., Lukšienė Ž. 2015. New non-chemical postharvest technologies reducing berry contamination. Zemdirbyste-Agriculture, 102 (4): 411–416. https://doi.org/10.13080/z-a.2015.102.052
31. Rasiukevičiūtė N., Rugienius R., Šikšnianienė J. B. 2018. Genetic diversity of *Botrytis cinerea* from strawberry in Lithuania. Zemdirbyste-Agriculture, 105 (3): 265–270. https://doi.org/10.13080/z-a.2018.105.034
32. Rasiukevičiūtė N., Uselis N., Valiuškaitė A. 2019. The use of forecasting model iMETOS® for strawberry grey mould management. Zemdirbyste-Agriculture, 106 (2): 143–150. https://doi.org/10.13080/z-a.2019.106.019
33. Rosero-Hernandez E. D., Moraga J., Collado I. G., Echeverri F. 2019. Natural compounds that modulate the development of the fungus *Botrytis cinerea* and protect *Solanum lycopersicum*. Plants, 8 (5): 111. https://doi.org/10.3390/plants8050111
34. Singh J., Bhatnagar S. K., Tomar A. 2019. Study of fungicidal effect of plant extracts on plant pathogenic fungi and the economy of extract preparation and efficacy in comparison to synthetic/chemical fungicides. Journal of Applied and Natural Science, 11 (2): 333–337. https://doi.org/10.31018/jans.v11i2.2053
35. Soylu E. M., Kurt S., Soylu S. 2010. *In vitro* and *in vivo* antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. International Journal of Food Microbiology, 143: 183–189. https://doi.org/10.1016/j.ijfoodmicro.2010.08.015
36. Stevic T., Beric T., Šavikin K., Sokovic M., Godevac D., Dimkic I., Stankovic S. 2014. Antifungal activity of selected essential oils against fungi isolated from medicinal plant. Industrial Crops and Products, 55: 116–122. https://doi.org/10.1016/j.indcrop.2014.02.011
37. Valiuškaitė A., Uselis N., Kviklys D., Lanauskas J., Rasiukevičiūtė N. 2017. The effect of sustainable plant protection and apple tree management on fruit quality and yield. Zemdirbyste-Agriculture, 104 (4): 353–358. https://doi.org/10.13080/z-a.2017.104.045
38. Xu S., Ni Z., Ma L., Zheng X. 2017. Control of *Alternaria* rot of cherry tomatoes by food-grade *Laurus nobilis* essential oil microemulsion. Journal of Food Safety, 37 (1): e12286. https://doi.org/10.1111/jfs.12286
39. Yazdani F., Mafi M., Farhadi F., Tabar-Heidar K., Aghapoor K., Mohsenzadeh F., Darabi H. R. 2005. Supercritical CO_2 extraction of essential oil from clove bud: effect of operation conditions on the selective isolation of eugenol and eugenyl acetate. Zeitschrift fur Naturforschung B, 60 (11): 1197–1201. https://doi.org/10.1515/znb-2005-1113
40. Yildirim I., Yapici B. M. 2007. Inhibition of conidia germination and mycelial growth of *Botrytis cinerea* by some alternative chemicals. Pakistan Journal of Biological Sciences, 10 (8): 1294–1300. https://doi.org/10.3923/pjbs.2007.1294.1300

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Braškių patogeno *Botrytis cinerea* biokontrolė naudojant augalinius ekstraktus ir eterinius aliejus

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Santrauka

Rozmarinų, lauramedžių ir gvazdikmedžių eteriniai aliejai bei ekstraktai turi vertingų junginių, suteikiančių jiems antimikrobinių ir antigrybinių savybių, taigi šios priemonės turi potencialą būti panaudotos augalų patogenų kontrolei. *Botrytis cinerea* yra vienas pagrindinių braškių patogenų, augalus pažeidžiantis įvairiais augimo tarpsniais. Tyrimo metu siekta įvertinti kvapiojo rozmarino (*Rosmarinus officinalis* L.), kilniojo lauramedžio (*Laurus nobilis* L.) ir kvapniojo gvazdikmedžio (*Syzygium aromaticum* L.) ekstraktų bei eterinių aliejų efektyvumą braškių patogeno *B. cinerea* kontrolei *in vitro*. Rozmarinų, lauramedžių ir gvazdikmedžių ekstraktai bei eteriniai aliejai buvo tirti vienkomponenčiai ir mišiniuose, skirtingomis koncentracijomis sumaišyti su bulvių dekstrozės agaro terpe. Siekiant nustatyti tirtų ekstraktų ir eterinių aliejų sinerginį, papildantį arba antagonistinį poveikį, apskaičiuoti mišinių patogeno frakcinės slopinimo koncentracijos indeksai.

Tyrimo rezultatai parodė, kad braškių patogeną *B. cinerea* kaip vienkomponentė medžiaga labiausiai (100 %) slopino gvazdikmedžių ekstraktas, mažiausiai (31,91 %) – rozmarinų eterinis aliejus. Mišiniai, visiškai nuslopinę patogeno augimą, buvo gvazdikmedžių ekstraktas + gvazdikmedžių eterinis aliejus, lauramedžių ekstraktas + gvazdikmedžių eterinis aliejus ir rozmarinų ekstraktas + gvazdikmedžių eterinis aliejus. Sinergija buvo nustatyta tarp lauramedžių ekstrakto bei gvazdikmedžių eterinio aliejaus ir rozmarinų ekstrakto bei eterinio aliejaus; abiem atvejais patogeno slopinimo frakcinės koncentracijos indeksas buvo 0,75. Lauramedžių ekstrakto ir eterinio aliejaus mišinys buvo antagonistinis, o patogeno frakcinės slopinimo koncentracijos indekso reikšmė – 2,5.

Reikšminiai žodžiai: antigrybinis poveikis, augalinis ekstraktas, biologinė kontrolė, *Botrytis cinerea*, eterinis aliejus, sinergija.