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Botryosphaeriaceae fungi on apple fruit – identification and sensitivity to fungicides and essential oils *in vitro*

Milica Milošević*, Jelena Stepanović, Emil Rekanović and Miloš Stepanović

Institute of Pesticides and Environmental Protection, Banatska 31b, Belgrade, Serbia

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*Corresponding author: milica.milosevic@pesting.org.rs

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SUMMARY

Apple production suffers significant economic losses and fruit quality reduction due to fungal pathogens, particularly ones that cause postharvest fruit rot, such as Botryosphaeriaceae fungi. Isolates used in this study were obtained from symptomatic apples and, based on morphological characteristics and sequence analysis of two genes (EF 1-α and β-tubulin), they were identified as *Diplodia seriata* and *Botryosphaeria dothidea*. Pathogenicity tests on healthy apple fruits revealed that *D. seriata* was more aggressive than *B. dothidea,* with significantly higher average values of lesion diameter and depth. Fungicide sensitivity tests showed that *D. seriata* was more sensitive to the combination fluopyram + tebuconazole (EC_{50}=0.00023 µg a.i. ml^{-1}), while *B. dothidea* exhibited higher sensitivity to pyraclostrobin (EC_{50}=0.025 µg a.i. ml^{-1}). With 98.44% and 97.56% percent growth inhibition (PGI) rate of *D. seriata* and *B. dothidea* (respectively) at 10 µg a.i. ml^{-1}, the tested combination of fungicides surpassed pyraclostrobin in inhibition potential. Four essential oils (thyme, rosemary, lavender and lemongrass) were also tested for antifungal activity using the fumigant macrodilution method. Thyme oil demonstrated the highest antifungal potential, completely inhibiting the mycelial growth of both species at 0.05 µl ml^{-1} of air. Strong inhibition potential was also shown by lemongrass oil with 100% inhibition of *D. seriata* and *B. dothidea* mycelial growth at 0.07 and 0.09 µl ml^{-1} of air, respectively. Rosemary oil showed a moderate inhibition potential, while lavender oil was the least effective. These findings highlight the inhibiting potential of fungicides against *D. seriata* and *B. dothidea*, but they also indicate that thyme and lemongrass essential oils could be used as viable alternatives. Further research is needed to determine their effectiveness in *in vivo* assays and potential impact on fruit quality and the environment.

Keywords: apple, fungal pathogens, postharvest fruit rot, fungicides, essential oils, antifungal activity

INTRODUCTION

Fruit production accounts for 5.7% of Serbia's overall agricultural production, and apple was the leading fruit species in 2023. Serbia is a major apple producer in South East Europe with a total apple output of 379,690 tons, and 27,412 ha of harvested area (FAOSTAT, 2023; Statistical Office of the Republic of Serbia, 2024*).* Apple production is threatened by numerous postharvest phytopathogenic fungi, such as *Penicillium expansum*, *Botrytis cinerea*, *Fusarium avenaceum*, *Botryosphaeria* spp., *Diplodia* spp., *Alternaria* spp., *Monilinia* spp., *Neofabrea* spp., *Diaporthe* spp. and some other, which cause severe fruit quality and yield losses (Konstantinou et al., 2011; Di Francesco et al., 2019; Vučković et al., 2022). The family Botryosphaeriaceae (Botryosphaeriales, Ascomycetes) includes a large and diverse group of fungi known for their global distribution in a wide variety of woody hosts (Batista et al., 2021). These fungi are described as aggressive pathogens, endophytes or opportunistic pathogens associated with host stress (Slippers et al., 2013). Many members of the family cause stem canker, die-back and fruit rot in apple-growing areas around the world (Tang et al., 2012). Several species of Botryosphaeriaceae have been reported as apple pathogens, but the most frequent ones are *Botryosphaeria dothidea* and *Diplodia seriata* (Delgado-Cerrone et al., 2016). The presence of these two species was previously confirmed on apple fruit in Serbia (Stojanović et al., 2003; Vasić et al., 2013; Vučković et al., 2022).

Traditional cultivation practices, such as pruning of infected branches, apple bagging technology or girdling (Dai et al., 2017), are labour intensive, so that preventive fungicide treatments are still an effective strategy for managing Botryosphaeriaceae pathogens. Their management is complicated due to their latent infection potential and wide host range. There are a number of fungicides from different chemical groups that have been registered against postharvest pathogens on apple, whose efficacy has already been evaluated and confirmed (Song et al., 2018; Fan et al., 2019; Thomidis & Prodromou, 2020; Fan et al., 2022). However, currently there are no fungicides registered in Serbia for the control of Botryosphaeriaceae in apple, although some active ingredients from the chemical groups of Quinone outside Inhibitors (QoI), DeMethylation Inhibitors (DMI) and Succinate-dehydrogenase Inhibitors (SDHI) have been registered against other apple postharvest pathogens (*Gleosporium* spp., *Monilinia* spp., *Penicillium* spp.) (Team of Editors, 2024). Regarding QoI fungicides, pyraclostrobin with its preventive, curative and eradicative effects has demonstrated exceptional degrees of efficacy against a broad spectrum of fungal pathogens (Yuan et al., 2015), including Botryosphaeriaceae species on apples, although it is not registered for its control (Fan et al., 2016; Fan et al., 2019). Fluopyram (SDHI fungicide) in combination with tebuconazole (DMI fungicide) is commercially available and registered for the management of rot-causing pathogens in apple orchards. Based on the FRAC classification, pyraclostrobin is categorized as high-risk, fluopyram as medium to high risk, and tebuconazole as medium risk active ingredient for fungicide resistance development (FRAC, 2024).

However, control measures against Botryosphaeriaceae pathogens need to be carefully addressed, considering the limitations inherent to fruit exportation, such as low fruit residue levels and environmental regulatory requirements restricting the selection of agrochemicals. The requirements for minimal pesticide residues in plant products, but also for a healthier environment, has led to the development of environmentally-friendly alternatives to synthetic fungicides, such as biological control agents or naturally derived compounds with antifungal potential (essential oils - EOs or plant extracts). EOs can have antifungal, antibacterial, antiviral, herbicidal or insecticidal effects (Sayed-Ahmad et al., 2017; Verdeguer et al., 2020). Many studies have explored the use of essential oils to control postharvest losses in fruit and vegetable crops (Abd-Alla et al., 2011; Grahovac et al., 2011; Lopez-Reyes et al., 2010, 2013; Antonioli et al., 2020; Kontaxakis et al., 2020; Di Francesco et al., 2022; Soppelsa et al., 2023). Therefore, EOs may be considered as an alternative resource for pest and disease control, since they constitute a rich source of bioactive compounds that are potentially suitable for use in integrated management programs (Merah et al., 2020; Verdeguer et al., 2020).

Hence, the aim of this study was to: 1) isolate and identify the pathogens that caused postharvest decay of apples; 2) evaluate *in vitro* effects of fungicides from three chemical groups (QoI, SDHI and DMI); and 3) evaluate the *in vitro* inhibitory activity of four EOs in the control of apple diseases caused by Botryosphaeriales fungi.

MATERIAL AND METHODS

Fungal isolation and morphology

Apple fruits with symptoms of rot were collected from a non-commercial orchard (with no history of fungicide application) in Rakari (Kolubara district, Serbia) in 2023. The fruits were surface-sterilized with 70% ethanol and isolation was performed by aseptically cutting small fragments from the margins of infected tissue and placing them on Potato Dextrose Agar (PDA). Fungal colonies showing characteristics of Botryosphaeriaceae were transferred to new PDA plates and incubated at 25°C for 14 days in darkness. Morphological characterization was performed based on macroscopic colony morphology (colour and mycelial type). Based on those characteristics, the isolates were divided into distinct groups.

DNA extraction and molecular identification of isolates

DNA was extracted from 7-day old mycelium grown on PDA following the 3% CTAB DNA isolation protocol described by Doyle and Doyle (1990) with some modifications. The quality of DNA was tested by amplification of the ITS genomic region, while fungal identification to species level was performed by sequencing of two genes: translation elongation factor 1-α (EF1-α) and β-tubulin. These genomic regions were amplified employing ITS1/4 (White et al., 1990), EF1-728F/EF1-986R (Carbone & Kohn, 1999) and Bt2a/Bt2b (Glass & Donaldson, 1995) primer pairs, respectively. The PCR reactions were performed in Thermal Cycler (Biometra) at the final volume of 25 µl containing 1x PCR MasterMix (Thermo Fischer Scientific, Vilnius, Lithuania), 0.4 µM of each primer and 10x diluted sample DNA. Samples lacking the template DNA were considered as negative controls. The thermocycling pattern for amplification of the ITS region consisted of an initial denaturation at 94°C for 90 s; 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, with final elongation at 72°C for 9 min and 30 s. PCR conditions for the EF1-α and β-tubulin genes included initial denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 53°C for 1 min and 72°C for 1 min; and final extension at 72°C for 10 min. PCR products were separated by 1% agarose gel electrophoresis and commercially sequenced with forward primers employed for their amplification (Macrogen, Netherland). Sequences were analyzed with the Gap4 program from Stadden Package (Staden et al., 2000), manually inspected and compared to sequences publicly available in NCBI's GenBank through BLAST search analysis.

Pathogenicity and aggressiveness tests

Apple fruits (cv. Golden delicious) without physical injuries were used in pathogenicity and aggressiveness tests. The fruits were surface disinfected with 2% sodium hypochlorite solution for 2 min, rinsed twice with sterile distilled water, and air dried in laminar flow. Three wounds (3x3 mm) were made aseptically on the equator of each apple prior to inoculation. PDA plugs of 3 mm in diameter containing mycelium were taken from a 5-day-old colony and used for artificial inoculation. For each isolate, two apple fruits with three inoculation sites were used. The fruits were placed in plastic boxes and incubated at room temperature for seven days. The lesion diameter was measured in two mutually perpendicular directions, and the average of two values was defined as the lesion diameter. Lesion depth was also recorded. Lesion diameters and depths in the pathogenicity test were subjected to an analysis of variance (ANOVA) and the mean values were separated by Tukey's test ($p = 0.05$).

In vitro fungicide sensitivity assay

A single isolate per each of the two identified species was chosen for a sensitivity assay. Isolates JR13/II, identified as *Diplodia seriata,* and JR57, identified as *Bothryosphaeria dothidea,* were tested for pyraclostrobin and fluopyram + tebuconazole sensitivity. The commercial fungicides Luna experience (200 g/L fluopyram + 200 g/L tebuconazole, Bayer CropScience LP, MO, USA) and Retengo (200 g/L pyraclostrobin, BASF SE, Ludwigshafen, Germany) were dissolved in sterile distilled water to prepare different fungicide concentrations. Adjusted fungicide concentrations were incorporated into autoclaved PDA medium cooled to 50°C to obtain final concentrations of active ingredients (a.i.) at: 0.0001, 0.001, 0.01, 0.1, 1, 10 and 100 µg a.i. ml^{-1} for Luna experience, and 0.01, 0.1, 1, 10 and 100 µg a.i. ml^{-1} for Retengo. The fungicide-amended medium (20 ml) was dispensed in 90 mm diameter Petri plates. Control Petri plates were not amended with fungicides. Both fungicide-amended and control Petri plates were inoculated with 6 mm mycelial plugs cut from the margin of actively growing cultures of both fungal isolates. All Petri plates were incubated in darkness at 25°C for 5 days. All assays, including controls, were performed in triplicates and the whole experiment was repeated twice. When mycelial mat of the controls reached ¾ of Petri plates, colony radial growth was measured in three directions. The percentage of inhibition was calculated for both fungicides using the formula: $PGI = (a–b)/a \times 100$, where *a* was the colony diameter of control plates and *b* was the colony diameter of fungicide-amended plates. The concentration of each fungicide inhibiting mycelial growth by 50% (EC_{50}) was estimated using Probit analysis.

To determine antifungal properties (fungicidal or fungistatic) of the fungicides against the test pathogens, transfer experiments were performed. Mycelial plugs that failed to grow were transferred to fresh PDA medium (15 ml/plate) and incubated at 25°C. After 5 days, the activity of each fungicide was classified either as fungicidal if the pathogen failed to grow or as fungistatic if pathogen growth did occur.

Antifungal effect of essential oils *in vitro*

Antifungal activity of four commercially available EOs (Herba, Belgrade): thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), lavender (*Lavandula* spp.) and lemongrass (*Cymbopogon citratus*), was tested using the fumigant macrodilution method against the same two isolates used in fungicide sensitivity assays (JR13/II and JR57). The experiment was conducted in glass Petri plates (diameter: 90 mm) on PDA medium with mycelial fragments (diameter: 6 mm) placed at the center of each plate. Five different volumes of selected EOs were pipetted onto filter paper cuttings attached to the inner side of plate covers to achieve the final air phase concentrations: 0.02, 0.05, 0.07, 0.09 and 0.12 μl ml^{-1} of air. The plates were inverted, sealed with parafilm to prevent oil evaporation and incubated at 25°C for 5 days. Distilled water was used as negative control. The percentage growth inhibition (PGI) was calculated as previously described. The experiment was repeated twice.

RESULTS

Fungal identification

After the isolation protocol, 17 isolates were obtained from apple fruits and, based on their colony morphological traits, they were divided into two groups. The cultures of both groups were initially white, turning pale olive-brown after three days, and becoming dark olive brown to grey brown after seven days. Finally, the cultures became dark grey on the surface and black on the reverse after 14 days. Isolates in the first group (7 isolates) formed moderately dense to dense mycelium, with woolly, cottony and fluffy aerial appearance, while isolates in the second group (10 isolates) formed abundant fluffy aerial mycelium occasionally grouped in tufts reaching the lid of Petri plates. Based on colony morphological characteristics, the isolates from the first and second group were identified as *Diplodia seriata* and *Bothryosphaeria dothidea*, respectively.

One representative isolate from each group (JR 13/II and JR 57) was subjected to molecular identification. For both isolates ITS, EF1-α and β-tubulin genomic regions were successfully amplified. The obtained sequences of EF1-α and β-tubulin genes were 212 and 396 nt long for isolate JR13/II, and 229 and 377 nt long for isolate JR 57, respectively. BLAST analysis of both genes of isolate JR 13/II showed that they shared 100% identity with all 100 strains of *D. seriata* listed (such as strain Bot-2018-S7 from an apple tree from Chile - MH745087/MH908101, culture strain CBS:121110 from *Prunus armeniaca* from South Africa - MT592554, and CBS:134700 from *Prunus laurocerasus* from Italy - KC869998) while isolate JR 57 was identified as *B. dothidea,* also with 100% identity with all isolates listed. The listed strains included strains SHP6H3I9_2020 from walnut from France (PQ303808), as well as voucher MFLU 22-0097 strain from *Prunus serrulata* from Taiwan (ON677459), and voucher CGMCC 3.19257 from *Vaccinium uliginosum* L. from China (MK085916).

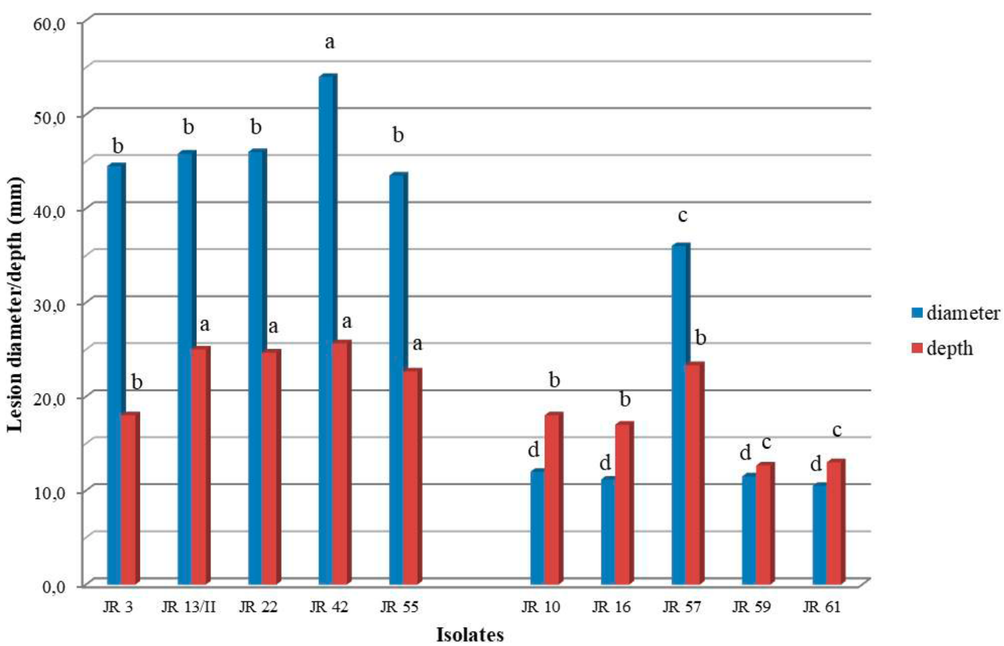

Figure 1. Lesion diameter and depth on apples inoculated with *D. seriata* (JR 3, JR 13/II, JR 22, JR 42, and JR 55) and *B. dothidea* (JR 10, JR 16, JR 57, JR 59, and JR 61)

Pathogenicity and aggressiveness tests

Five isolates from each of the two morphological groups were selected for pathogenicity and aggressiveness tests. The *D. seriata* isolates (JR 3, JR 13/II, JR 22, JR 42 and JR 55) showed significantly higher aggressiveness than *B. dothidea* (JR 10, JR 16, JR 57, JR 59 and JR 61) based on the average values of lesion diameter and depth (Figure 1), which were 46.8±4.17 mm and 23.2±3.11 mm, respectively. The average lesion diameter was 16.2±11.06 mm and depth 16.8±4.35 mm on apple fruits inoculated with B. *dothidea*.

In vitro fungicide sensitivity assay

The radial growth of *D. seriata* and *B. dothidea* isolates was significantly inhibited by both tested fungicides. The combination of fluopyram and tebuconazole showed higher efficacy against the isolates of both tested species, compared to pyraclostrobin. However, some differences in sensitivity were noted between the species. Calculated EC_{50} values showed that *D. seriata* was more sensitive to fluopyram + tebuconazole (EC_{50}=0.00023 µg a.i. ml^{-1}) in comparison with *B. dothidea* isolate (EC_{50}=0.00108 µg a.i. ml^{-1}). The concentration of 10 µg a.i. ml^{-1} of the fungicide combination highly inhibited mycelial growth of both isolates tested, with PGI values of 98.44% and 97.56%, respectively. The maximal concentration (100 µg a.i. ml^{-1}) totally inhibited mycelial growth of both isolates, and showed fungicidal effect on the *D. seriata* isolate, while the same concentration demonstrated fungistatic activity against the *B. dothidea* isolate.

The EC_{50} values obtained for radial mycelial growth suggested that pyraclostrobin was less effective than the combination fluopyram + tebuconazole. The calculated EC_{50} values for pyraclostrobin showed that *D. seriata* was less sensitive to that fungicide (EC_{50}=3.895 µg a.i. ml^{-1}) than the *B. dothidea* isolate (EC_{50}=0.025 µg a.i. ml^{-1}). The mycelial growth of *D. seriata* and *B. dothidea* at the maximum tested concentration (100 µg a.i. ml^{-1}) was inhibited by 81.88% and 88.60%, respectively.

Antifungal effect of essential oils *in vitro*

Thyme essential oil demonstrated the highest antifungal activity against both fungi, achieving complete inhibition of mycelial growth at oil concentration of 0.05 µl ml^{-1} of air phase. Lemongrass essential oil also revealed a strong inhibition potential with PGI ranging from 85.1% at 0.02 µl ml^{-1} of air phase to 100% at 0.07 µl ml^{-1} of air phase against *D. seriata*, while *B. dothidea* was inhibited from 73.6% at 0.02 µl ml^{-1} of air phase to 100% at 0.09 µl ml^{-1} of air phase.

Moderate antifungal activity was demonstrated by rosemary EO with PGI values ranging from 34.2% against *D. seriata* and from 20.6% against *B. dothidea* at the lowest concentration, up to 53.8% against *D. seriata* and 59.7% against *B. dothidea* at the highest concentration tested.

Lavender EO showed the lowest inhibition potential against both isolates with PGI values ranging from 0% to 13.8% and 5.4% to 13.2% for *D. seriata* and *B. dothidea*, respectively (Figures 2 and 3).

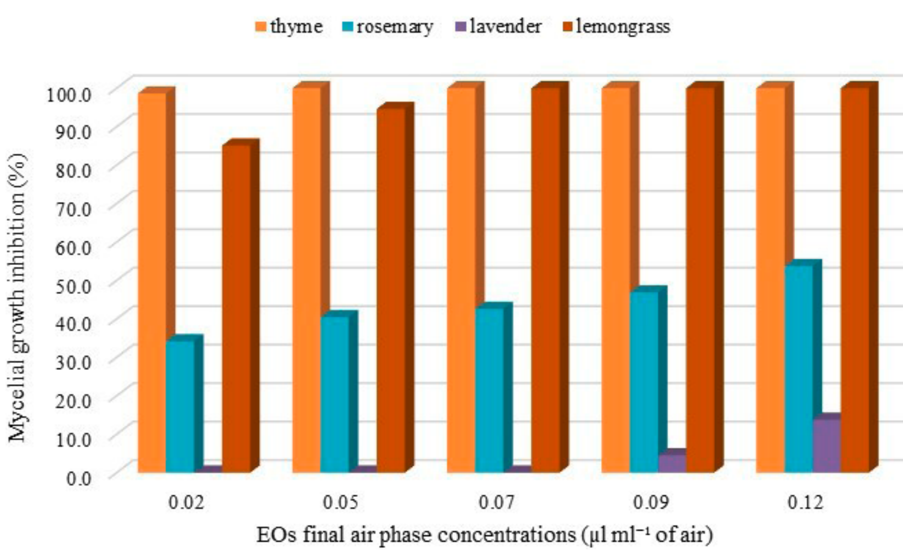

Figure 2. Effects of different EOs to mycelial growth of *D. seriata*

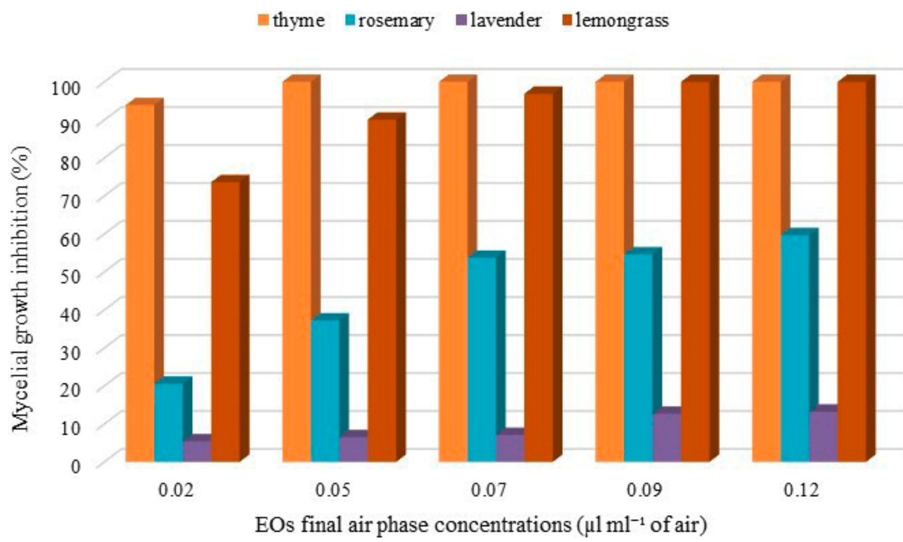

Figure 3. Effects of different EOs to mycelial growth of *B. dothidea*

DISCUSSION

Botryosphaeriaceae is a fungal taxon within Ascomycota representing a serious threat to several perennial species, including fruit crops (Billones-Baaijens & Savocchia, 2019; Batista et al., 2021; Bezerra et al., 2021; Martino et al., 2024) that can be colonized and damaged by species in this family. Several species of the family Botryosphaeriaceae can cause postharvest fruit rot that can adversely affect apple yield in major growing regions around the world, and the most frequent are *Diplodia seriata* and *Botryosphaeria dothidea* (Delgado-Cerrone et al., 2016).

Identification of Botryosphaeriaceae species is mainly based on colony morphology and molecular analyses of genomic regions (Phillips et al., 2013; Zhou et al., 2015; Delgado-Cerrone et al., 2016). Based on morphological characteristics and comparisons of DNA sequences of the translation elongation factor 1-α (EF1-α) and β-tubulin genomic regions, 17 isolates of Botryosphaeriaceae in this study were identified either as *D. seriata* (7 isolates) or *B. dothidea* (10 isolates). The presence of both species as dominant pathogens causing fruit rot had been previously reported on apple fruit in Serbia (Stojanović et al., 2003; Vasić et al., 2013; Vučković et al., 2022). Both species were found to cause postharvest apple fruit rot. Pathogenicity tests showed statistically significant higher aggressiveness of *D. seriata* in comparison with *B. dothidea* isolates.

Diagnosing the pathogens of apple fruit rot is crucial for successful control of this disease. The availability of control strategies against diverse Botryosphaeriales pathogens is essential for integrated management of fruit rot of apple. Although some cultivation practices have been used to protect apple orchards from species in Botryosphaeriaceae family, preventive applications of fungicides from different chemical groups are still a practical and effective strategy for managing many pathogens, including those responsible for apple fruit rot. Even though some active ingredients from QoI, DMI and SDHI chemical groups are registered against apple postharvest pathogens (*Gleosporium* spp., *Monilinia* spp., *Penicillium* spp.), no fungicides are currently approved in Serbia for the control of Botryosphaeriaceae in apple (Team of Editors, 2024). Among the commercial chemical formulations registered for use against other fungal diseases of apple, two products with three active ingredients and different modes of action were selected in this study to examine their inhibition activity against two causal agents of apple fruit rot: *D. seriata* and *B. dothidea*. The study considered their inhibitory effects on mycelium growth of pathogens. The tested products (fluopyram + tebuconazole and pyraclostrobin) strongly inhibited mycelial growth of both fungi and some differences in sensitivity between the species were noted. Variation in fungicide inhibitory effects had been previously confirmed between Botryosphaeriaceae species, even within the same region and crop (Bester et al., 2007; Amponsah et al., 2012; Pitt et al., 2012). The calculated fluopyram + tebuconazole EC_{50} values for *D. seriata* (EC_{50}=0.00023 µg a.i. ml^{-1}) and *B. dothidea* (EC_{50}=0.00108 µg a.i. ml^{-1}), as well as pyraclostrobin EC_{50} values for *D. seriata* (EC_{50}=3.895 µg a.i. ml^{-1}) and *B. dothidea* (EC_{50}=0.025 µg a.i. ml^{-1}) demonstrated high inhibition potential of these active ingredients. Previously, Fan et al. (2019) tested 97 isolates of *B. dothidea* from apple for their sensitivity to pyraclostrobin and the EC_{50} values ranged from 0.7010 to 7.1378 µg ml^{-1}, with the mean EC_{50} of 3.0870±0.1560 µg ml^{-1}, confirming strong inhibition effects of pyraclostrobin. Also, EC_{50}s between 0.004 and 2.15 mg a.i. l^{-1} showed that pyraclostrobin, fluazinam, tebuconazole, fludioxonil and prochloraz were highly effective and similar in their inhibitory effects on mycelium growth and conidium germination of *D. seriata* (Antony et al., 2024). *In vitro* results obtained in a study by Torres et al. (2013) demonstrate strong effectiveness of tebuconazole in the control of conidial germination and radial growth of *D. seriata* and *D. mutila*. Fluopyram, tebuconazole and pyraclostrobin were also among the tested fungicides and endorsed for their efficacy against species of the Botryosphaeriaceae family in apple (Song et al., 2018), almond (Olmo et al., 2017), and grapevine (Bester et al., 2007; Amponsah et al., 2012; Pitt et al., 2012).

Despite their high efficacy, synthetic chemical fungicide treatments pose many risks, including mounting health concerns voiced by consumers and health authorities, which has led to demands to reduce human and environmental exposure to chemicals, increased restrictions imposed by regulatory agencies on specific agro-chemicals and/or their allowable residues, especially after harvest (Romanazzi & Droby, 2016). The need for reduced pesticide residues in plant products has led to the development of alternatives to synthetic fungicides, such as naturally derived compounds with antifungal potential. In recent years, as a strategy to control apple fruit fungal pathogens, the application of plant essential oils has been considered a natural alternative to synthetic fungicides (Lopez-Reyes et al., 2010; Di Francesco et al., 2022; Soppelsa et al., 2023). Each of the four essential oils (thyme, rosemary, lavender, and lemongrass) tested in this study had some reducing effect on the mycelial growth of *D. seriata* and *B. dothidea*. The results of the present study indicated that the volatile phases of thyme and lemongrass essential oils possess high antifungal activity against both fungi, achieving a complete inhibition of mycelial growth at oil concentrations of 0.05 and 0.07 µl ml^{-1} of air phase, respectively. Moreover, the phenolic monoterpenoid compound thymol (major constituent of thyme EO) has an important activity against fungal plant pathogens, attributing to its antioxidant activity (Elshafie et al., 2015) and stimulating plant defences (Lopez-Reyes et al., 2013; Sivakumar & Bautista-Baños, 2014). Moderate antifungal activity was demonstrated by rosemary EO, while lavender EO showed the lowest inhibition potential against both tested isolates. A large variety of volatile compounds have been shown to have strong antifungal effect when tested under laboratory and small-scale *in vivo* conditions. Strong effects of EOs against *Botryosphaeria* species was confirmed in a study by Sarkhosh et al. (2018). The results of their *in vitro* testing of essential oils extracted from eight plant species showed a 100% reduction in the mycelium growth of *Botryosphaeria*, *Colletotrichum*, *Fusarium* and *Phytophthora* species after applying thyme EO at a concentration of 100 µg l^{-1}. Also, in a study by Wang et al. (2023), cinnamon and clove EOs exhibited high inhibitory activity against the mycelial growth of *B. dothidea*, both in vapor and contact phases under *in vitro* conditions. *In vivo* testing showed that the EO vapor treatments also alleviated the severity of fruit rot in artificially infected apples. Several studies have also reported efficient activities of thyme, lemongrass and rosemary essential oils (Lopez-Reyes et al., 2010; 2013; Grahovac et al., 2011; Ali et al., 2015; Císarová et al., 2016; Servili et al., 2017; Di Francesco et al., 2022).

In the current study, the results demonstrated that the main Botryosphaeriaceae pathogens causing postharvest decay of apple fruits were *D. seriata* and *B. dothidea*. *In vitro* fungicide sensitivity assays indicated that both pathogens were sensitive to the active ingredients pyraclostrobin, fluopyram and tebuconazole, which are registered in Serbia against some other apple postharvest pathogens, including *Gleosporium* spp., *Monilinia* spp., and *Penicilium* spp. The vapour phase of thyme and lemongrass essential oils effectively limited mycelial growth of both tested fungi, showing that these oils can be potential biocontrol agent candidates for preventing and controlling apple fruit rot. Despite their proven effectiveness under laboratory conditions, their efficacy still needs to be confirmed under large scale and commercial conditions, and potentially undesirable effects on postharvest fruit, human health and the environment require further detailed investigation.

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Botryosphaeriaceae kao patogeni jabuke – identifikacija i osetljivost na fungicide i etarska ulja *in vitro*

REZIME

Proizvodnja jabuke suočava se sa značajnim ekonomskim gubicima usled smanjenog kvaliteta plodova kao posledice prisustva velikog broja fitopatogenih gljiva, naročito prouzrokovača truleži plodova nakon berbe kao što su gljive iz familije Botryosphaeriaceae. Izolati korišćeni u ovom istraživanju prikupljeni su sa zaraženih plodova jabuka i na osnovu morfoloških karakteristika i analize sekvenci dva genska regiona (EF 1-α and β-tubulin) identifikovani kao pripadnici vrsta *Diplodia seriata* i *Botryosphaeria dothidea*. Na zdravim plodovima jabuka sproveden je test patogenosti kojim je na osnovu merenja prečnika i dubine truleži utvrđen značajno viši stepen agresivnosti izolata *D. seriata*. Veća osetljivost na kombinaciju fluopiram + tebukonazol uočena je kod izolata *D. seriata* (EC_{50}=0,00023 µg a.s./ml), dok je piraklostrobin jače inhibitorno delovanje ispoljio na izolat *B. dothidea* ($EC_{50=}$0,025 µg a. s./ml). Ispitivana kombinacija fluopiram + tebukonazol pokazala je veći inhibitorni potencijal u poređenju sa piraklostrobinom, sa inhibicijom porasta micelije od 98,44% za *D. seriata* i 97,56% za *B. dothidea* pri koncentraciji od 10 µg a.s./ml. Antifungalni efekat četiri etarska ulja (majčine dušice, ruzmarina, lavande i limunske trave) ispitan je primenom fumigantne makrodilucione metode. Ulje majčine dušice ispoljilo je najizraženije delovanje, potpuno inhibirajući porast micelije izolata obe vrste pri koncentraciji 0,05 µl/ml vazdušne faze. Jako inhibitorno delovanje pokazalo je i ulje limunske trave sa kompletnom inhibicijom porasta micelije pri koncentraciji 0,07 µl/ml vazdušne faze (*D. seriata*), odnosno 0,09 µl/ml vazdušne faze (*B. dothidea*). Umeren inhibitorni potencijal zabeležen je kod ulja ruzmarina, dok je ulje lavande ispoljilo najniži antifungalni efekat. Rezultati prikazani u ovom radu ukazuju na visok stepen osetljivosti izolata *D. seriata* i *B. dothidea* na ispitivane fungicide, ali i ulja majčine dušice i limunske trave kao njihove ekološki prihvatljivije alternative. Dalja istraživanja su potrebna kako bi se ispitala efikasnost ovih ulja u uslovima *in vivo*, kao i potencijalni uticaj na kvalitet plodova i životnu sredinu.

Ključne reči: jabuka, fitopatogene gljive, trulež plodova, fungicidi, etarska ulja, antifungalno delovanje