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Natural preservation techniques: using essential oils to extend the shelf life of cherry tomatoes

Prirodne tehnike očuvanja: primjena eteričnih ulja za produljenje trajanja svježine cherry rajčica

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ABSTRACT

Tomatoes are among the most widely consumed plant species globally, with cherry tomatoes often sold in plastic packaging to maintain freshness. *Botrytis cinerea* Pers. is a major post-harvest pathogen affecting tomatoes, commonly controlled by synthetic fungicides. Essential oils (EOs), natural volatile compounds produced by plants, play a crucial role in plant defence. This study aimed to evaluate the potential of essential oils for controlling *B. cinerea* in packaged cherry tomatoes. Accordingly, this study investigated the antifungal efficacy of essential oils in the vapour phase. *In vitro* tests were conducted using EOs from bay laurel (*Laurus nobilis* L.), fennel (*Anethum foeniculum* L.), holy basil (*Ocimum tenuiflorum* L.), lavender (*Lavandula angustifolia* Mill.), rosemary (*Salvia rosmarinus* (L.) Schleid), sage (*Salvia officinalis* L.), and sweet marjoram (*Origanum majorana* L.), at concentrations of 100, 50, 25, and 10%, corresponding to vapor phase concentrations of 585, 292.5, 146.25, and 58.5 µL/L. Except for sage, all the EOs fully inhibited mycelial growth at the lowest concentration tested. The six EOs that showed complete inhibition were further tested *in vivo* on cherry tomatoes, at 10% (58.5 µL/L). Holy basil, lavender, rosemary, and sweet marjoram completely inhibited fungal growth on the fifth day, while fennel showed weaker inhibition. Bay laurel slowed mycelial growth but was less effective. A sensory evaluation of cherry tomatoes treated with EOs at 10% was conducted with six participants, revealing differences in sensory characteristics. Holy basil received the highest ratings, followed by lavender. Results suggest that EOs may serve as a natural alternative for extending the shelf life of packaged cherry tomatoes, providing a useful substitute for chemical agents and reducing the risk of food contamination by mycotoxins.

Keywords: *Botrytis cinerea*, food safety, microbial contamination, natural fungicides, post-harvest pathogens

SAŽETAK

Rajčice su među najčešće konzumiranim biljnim vrstama u svijetu, pri čemu se cherry rajčice često prodaju u plastičnoj ambalaži radi očuvanja svježine. *Botrytis cinerea* Pers. je glavni uzročnik bolesti rajčica nakon berbe, a obično se suzbija kemijskim fungicidima. Eterična ulja (EU), prirodni hlapljivi spojevi koje proizvode biljke, imaju ključnu ulogu u obrani biljaka. Glavni cilj ovo istraživanja bio je ispitati potencijal eteričnih ulja za kontrolu *B. cinerea* kod pakiranih cherry rajčica. U skladu s time, u ovom istraživanju ispitan je protugljivični utjecaj eteričnih ulja u volatilnom stanju. *In vitro* testiranje provedeno je koristeći EU lovora (*Laurus nobilis* L.), komorača (*Anethum foeniculum* L.), svetog bosiljka (*Ocimum tenuiflorum* L.), lavande (*Lavandula angustifolia* Mill.), ružmarina (*Salvia rosmarinus* (L.) Schleid), kadulje (*Salvia officinalis* L.) i mažurana (*Origanum majorana* L.), u koncentracijama od 100, 50, 25 i 10%, što odgovara koncentracijama para EU u zraku od 585, 292,5, 146,25 i 58,5 µL/L. Osim kadulje, sva EU potpuno su inhibirala rast micelija pri najnižoj koncentraciji.

Šest EU koja su u potpunosti inhibirala rast micelija u uvjetima *in vitro* dodatno su testirana u uvjetima *in vivo* na cherry rajčicama pri koncentraciji od 10 % (58,5 µL/L). Sveti bosiljak, lavanda, ružmarin i mažuran potpuno su inhibirali rast micelija gljive nakon pet dana, dok je komorač pokazao slabiju inhibiciju. Lovor je usporio rast micelija, ali je bio manje učinkovit. Provedena je i senzorska evaluacija cherry rajčica tretiranih s EU pri koncentraciji od 10%, sa šest sudionika, koja je potvrdila razlike u senzorskim svojstvima. Sveti bosiljak dobio je najviše ocjene, a potom slijedi lavanda. Rezultati sugeriraju da EU mogu poslužiti kao prirodna alternativa za produljenje trajanja svježine pakiranih cherry rajčica.

Ključne riječi: *Botrytis cinerea*, sigurnost hrane, mikrobna kontaminacija, prirodni fungicidi, skladišni patogeni

INTRODUCTION

Tomatoes are one of the most widely cultivated and consumed crops globally. While they are often considered vegetables in culinary contexts, tomatoes are botanically considered fruits since they contain seeds and develop from the flowering part of the plant (EUFIC, 2020). In Croatia, tomatoes are produced on approximately 440 ha, yielding around 22.4 thousand tons annually (FAO, 2025). Globally, tomatoes are grown on approximately 5.4 million ha, producing an annual yield of about 192 million t (FAO, 2025).

Even after harvest, fruits and vegetables maintain their status as living organisms, actively respiring, which makes them susceptible to deterioration during transportation and storage (Sandhya, 2010). This is a significant challenge for fresh produce as it typically undergoes minimal quality control once it has been harvested, processed, and packaged (Alam et al., 2021). Fruits are generally classified as either climacteric or non-climacteric (Vendrell et al., 2001). Tomatoes belong to the climacteric group, undergoing a distinct phase of accelerated respiration and increased ethylene production after harvest. Ethylene plays a key role in ripening by promoting processes such as color change, fruit softening, and flavor development (Barry and Giovannoni, 2007). However, as ripening progresses, tomatoes become more susceptible to pathogen attack due to the increase in sugar content, moisture content and softening of the fruit. Improper handling or processing further increases the risk of contamination by harmful microorganisms and poses a major challenge to the safety of fresh produce (Alam et al., 2021).

Microbial pathogens account for approximately 15% of crop losses due to field damage, with postharvest diseases further reducing yields by 20–25% (Oerke, 2005; Sharma et al., 2009). Among these, *Botrytis cinerea* Pers. is a significant necrotrophic plant pathogen, affecting over 500 plant species, especially fresh fruits and vegetables (Hua et al., 2018). Grey mould, a disease caused by this fungus, is among the most widespread threats to tomato crops, both pre- and post-harvest, leading to substantial economic damage (Alkilayh et al., 2024). This airborne pathogen can destroy tomato cells and release harmful toxins, leading to severe yield reductions throughout all stages of crop development, including after harvest. Yearly economic losses attributed to grey mould are estimated to exceed 80% (Rhouma et al., 2023). Global yearly losses attributed to *B. cinerea* are estimated between $10 and $100 billion (Hua et al., 2018).

Cherry tomatoes are often packaged in plastic containers or wraps for additional protection, which helps maintain freshness, provides protection during transport and handling, and extends shelf life. Given their natural origin and potential to prolong the freshness of fruit, essential oils represent a healthier alternative to synthetic compounds commonly used in active packaging (Phothisarattana et al., 2022).

The primary method for controlling postharvest diseases caused by *B. cinerea* is the application of synthetic fungicides. Almost 10% of the global fungicide market is used for the control of *B. cinerea* (Abbey et al., 2018). *B. cinerea* is considered a high-risk pathogen for the development of fungicide resistance, as its genomic plasticity allows for the accumulation of multiple mutations in various target genes, resulting in multiple

resistance, as well as mutations that cause overexpression of efflux transporters, contributing to multidrug resistance (Sofianos et al., 2023). Additionally, the use of fungicides poses risks to both human health and the environment (Zhou et al., 2025). As a result, recent research has increasingly focused on the development of biological methods for controlling fungal pathogens in both pre- and postharvest scenarios.

Plant-derived compounds, such as essential oils (EOs), have emerged as one of the most promising alternatives due to their minimal negative impact on human health and the environment (Švitek et al., 2024). EOs are aromatic, volatile liquids obtained from plant materials through steam distillation and are named after the plant from which they are derived. They are defined either as products or mixtures of fragrant substances or as blends of fragrant and non-fragrant substances (Ríos, 2016). EOs have demonstrated antimicrobial activity against a variety of microorganisms (Puškárová et al., 2017). They have been shown to inhibit fungal mycelial growth (Ammad et al., 2018), reduce mycotoxin production (Rodrigues et al., 2019), and induce cell death through the destruction of the fungal cell membrane (Harris, 2002). Many EOs have been granted GRAS (Generally Recognized As Safe) status by the United States Food and Drug Administration (FDA), indicating that they are considered safe for use in food under specific conditions (FDA, 2025). This designation is based on a long history of use in food or on the results of scientific research reviewed by qualified experts. The GRAS list includes a variety of essential oils such as basil (*Ocimum basilicum* L.), bay (*Laurus nobilis* L.) and fennel (*Foeniculum vulgare* Mill.), among others. However, the safety of EOs depends on their concentration and application method, and they should be used in accordance with regulatory guidelines to ensure efficacy and consumer safety (FDA, 2025).

Mediterranean coastal countries have long relied on wild-growing plants and herbs for dietary uses. The distinctive Mediterranean climate, alongside the low productivity of rocky terrains, particularly in the Dalmatian karst region, has fostered the growth of various native herbs. Many of these plants have since been cultivated in several regions across the world due to their adaptability and beneficial properties (Dolina and Łuczaj, 2014). Mediterranean herbs are well-known for their potent antimicrobial properties, primarily due to their high concentrations of bioactive compounds such as phenolic acids, flavonoids, and EO. Research has identified several herbs, including oregano, thyme, rosemary, and sage, as particularly rich in compounds like thymol and carvacrol. These substances have demonstrated strong antimicrobial activity, effectively inhibiting a variety of pathogens (Gonelimali et al., 2018, Oppedisano et al., 2023), highlighting their importance not only in traditional uses but also in modern medicinal and preservation applications

This study aimed to investigate the antifungal activity of various EOs under both *in vitro* and *in vivo* conditions against the growth of the pathogenic fungus *B. cinerea*, with particular emphasis on EOs derived from Mediterranean herbs that complement the flavor profile of cherry tomatoes, as a natural alternative to chemical agents. In addition, a sensory analysis was performed to assess consumer preferences and the potential applicability of these EOs in the packaging of cherry tomatoes.

MATERIALS AND METHODS

Fungal isolate

The antifungal efficacy of EOs in the vapour phase was tested on an isolate of *B. cinerea*, obtained from tomato fruit harvested from family farm in Zagreb in 2019, and provided by the Department of Phytopathology at the Faculty of Agrobiotechnical Sciences Osijek. The species was confirmed through molecular analysis using PCR and sequencing. The isolate was cultivated on Potato Dextrose Agar (PDA) for 18 days at 22 °C in the dark. Total genomic DNA was extracted using the Extract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, Merck, Saint Louis, MO, USA), and the internal transcribed spacer (ITS) regions were amplified using the primer pair ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4

(5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). Each polymerase chain reaction (PCR) mixture was prepared with a final volume of 25 µL, consisting of 12.5 µL of EmeraldAmp® GT PCR Master Mix (Agilent Technologies, Santa Clara, CA, USA), 0.5 µL of each primer (10 µM), 6.5 µL of nuclease-free water (Agilent Technologies, Santa Clara, CA, USA), and 5 µL of template DNA at a concentration of 5 ng/µL. PCR amplification was performed following the protocol outlined by Aktaruzzaman et al. (2018). The PCR protocol consisted of an initial denaturation at 94 °C for 4 minutes, followed by 35 cycles of denaturation at 94 °C for 35 seconds, primer annealing at 52 °C for 55 seconds, and elongation at 72 °C for 1 minute. The reaction was completed with a final elongation step at 72 °C for 10 minutes. Gel electrophoresis was performed using a 1% agarose gel at 110 V for 25 minutes in a 1× TAE buffer to verify the success of the PCR reaction by visualizing the amplified DNA fragments. The PCR product was subsequently sent for sequencing to Macrogen Europe (Amsterdam, the Netherlands), using both ITS1 and ITS4 primers. The obtained nucleotide sequences were processed and refined using Sequencher® software (Gene Codes Corporation, Ann Arbor, MI, USA). To determine species identity, a comparison was carried out with reference sequences retrieved from the NCBI GenBank database (National Library of Medicine, National Center for Biotechnology Information, Bethesda, MD, USA), using BLAST. Final consensus sequence generated during this research was deposited in GenBank database (National Library of Medicine, National Center for Biotechnology Information, Bethesda, MD, USA) under the name BC_ZG_TOMATO, with an accession number for the ITS gene region PQ327539.

Essential oils

Seven commercially available EOs were used in the *in vitro* study using vapour-phase diffusion method. The list of EOs and the Latin names of the plant species from which they originate are presented in Table 1. For the *in vivo* study on cherry tomatoes and sensory analysis, six EOs were used — all of the previously mentioned EOs except for sage, which was found to be the least effective in the *in vitro* study. The EOs were obtained from Pranarōm International Ltd. (Ath, Belgium).

Table 1. List of used EOs and Latin names of plant species

EO source plant	Latin name
Bay laurel	*Laurus nobilis* L.
Fennel	*Anethum foeniculum* L. (syn. *Foeniculum vulgare* Mill.)
Holy basil	*Ocimum tenuiflorum* L. (syn. *Ocimum sanctum* L.)
Lavender	*Lavandula angustifolia* Mill. (*Lavandula officinalis* Chaix ex Vill)
Rosemary	*Salvia rosmarinus* (L.) Schleid (syn. *Rosmarinus officinalis* L.)
Sage	*Salvia officinalis* L.
Sweet marjoram	*Origanum majorana* L. (syn. *Majorana hortensis* Moench)

Fungal growth inhibition assay

In vitro testing

The vapour-phase diffusion method was used to assess the inhibitory effect of EOs on the growth of *B. cinerea*. The isolate was cultivated on PDA for 7 days at 22 °C. Petri dishes with a 90 mm diameter were filled with 10 mL of PDA medium. A 4 mm diameter *B. cinerea* mycelium plug was placed in the centre of the medium. A Whatman filter paper (Cytiva, USA), measuring 6 mm, was placed in the centre of the Petri dish lid and infused with 50 µL of the EO solution, with EO concentrations of 100%, 50%, 25%, and 10%, corresponding to vapor-phase concentrations of 585 µL/L, 292.5 µL/L, 146.25 µL/L, and 58.5 µL/L, respectively. A 9.9 % ethanol solution (Gram-Mol d.o.o., Zagreb, Croatia) was used to dilute the EO to the desired concentrations. The Petri dishes were sealed with parafilm and incubated at 22 °C in the dark. The experiment was conducted in triplicate. To determine whether ethanol influenced the experimental results, specifically the inhibition effect, 25 µL of a 9.9 % ethanol solution (the highest concentration used to

dilute the EOs) was applied instead of EO. Untreated pure PDA inoculated with a mycelium plug served as the control. Colony growth was monitored on the 5th day of incubation, after which the diameter of fungal mycelium was measured in two perpendicular directions (length and width), and the average value was used for further analysis. To assess whether the treatment exhibited fungistatic or fungicidal activity, a small mycelial plug (2 mm in diameter) was transferred onto fresh PDA medium. If growth resumed, the treatment was considered fungistatic; if no growth was observed, it was classified as fungicidal. In this context, the minimum inhibitory concentration (MIC) refers to the lowest concentration of an antifungal agent that visibly inhibits the growth of the tested fungal strain, indicating fungistatic activity. Conversely, the minimum fungicidal concentration (MFC) is defined as the lowest concentration that results in fungal death, confirmed by the absence of growth after subculturing, thus reflecting fungicidal activity.

In vivo testing

For the *in vivo* testing of the antifungal effectiveness of EOs, untreated cherry tomatoes of the Cherry roma variety (syn. Baby roma) were used. The fruits were selected to be approximately uniform in colour and shape, maturity, without any signs of mechanical damage or infection, and equally ripe. The fruits were washed under tap water, immersed in a 1% bleach solution (Cekina, Zagreb, Croatia), for 5 min, and then rinsed in sterile distilled water for 5 min (Tančinová et al., 2022). They were placed on paper in a laminar flow cabinet to air-dry. After drying, a hole was made in each fruit using a cork borer with a diameter of 4 mm. The isolate of *B. cinerea* was cultivated on PDA for 7 days at 22 °C. A *B. cinerea* mycelial plug of the same diameter (made with a cork borer) was placed in the hole, and the fruit was placed at the bottom of a 50 mL Falcon tube. A 6 mm diameter Whatman filter paper was placed on the Falcon tube cap. Given that the fruit occupied one-fifth of the Falcon tube's volume, 23.4 µL of a 10% EO solution was pipetted onto the filter paper, resulting in an EO vapor concentration of 58.5 µL/L in the air. For the preparation of the 10% EO solution, a 9.9 % ethanol solution was used, as previously described. The Falcon tubes were incubated at 22 °C for five days, after which the diameter of the fungal mycelium was measured in two perpendicular directions, and the average value was used for further analysis, as previously done in the *in vitro* assay. To assess the impact of ethanol on the experimental results under *in vivo* conditions, the same amount of ethanol used for EO dilution was applied (21.06 µL of a 9.9 % ethanol solution). As a control, the fruits inoculated with *B. cinerea* without ethanol and EOs were used. All experiments were performed in triplicate, using three replicates for each treatment.

Sensory analysis

For the sensory analysis, Cherry Roma tomatoes were selected based on the same criteria as in the *in vivo* assay. The fruits were then washed under tap water and left to air-dry. The tomatoes were then placed in sterile glass jars with a volume of 720 mL. A 60 mm diameter filter paper was placed on the lid of each glass jar. Given that the fruits occupied one-seventh of the jar's volume, 361 µL of a 10% EO solution was pipetted onto the filter paper, resulting in an EO vapor concentration of 58.5 µL/L in the air. For the preparation of the 10% EO solution, a 9.9 % ethanol solution (=10% solution of absolute (99%) ethanol) was used, as previously described. The jars were stored in a refrigerator at 4 °C for five days. The storage conditions applied for the sensory analysis were adapted according to the protocol outlined by Tančinová et al. (2022). A temperature of 4 °C was selected as it represents a standard postharvest storage condition for climacteric fruits such as tomatoes. While it is recognized that the volatility of EOs is reduced at lower temperatures, conducting the sensory evaluation under these conditions ensures alignment with realistic storage scenarios and enables a more practical evaluation of the effects of EO vapors on sensory attributes. After incubation, the tomato fruits were randomly selected from the glass jar and presented whole to the evaluators, who consumed the fruits in their entirety. The panel consisted of six previously trained participants (four females and two males), aged between 25 and 65 years.

Sensory quality was assessed using a 10-point scale (1 = dislike, 10 = excellent) to evaluate aroma, taste, texture, juiciness, and overall impression.

Statistical analysis

Data from the fungal inhibition analysis were evaluated using a one-way analysis of variance (ANOVA), followed by a post hoc Tukey HSD test, using the SAS Enterprise Guide 8.4 (SAS Institute, Cary, NC, USA). Data from the sensory evaluation were processed using Microsoft Excel. For each treatment, individual scores for five sensory parameters were recorded by six panellists. Mean values were calculated for each parameter per treatment. A graphical representation of the results was also created using Microsoft Excel.

RESULTS

In vitro evaluation

Except for sage (Figure 1), all EOs completely inhibited mycelial growth at a concentration of 10% (vapor phase concentration of 58.5 µL/L) on the fifth day of incubation. Sage inhibited mycelial growth at concentrations ≥ 25%. However, although no mycelial growth occurred beyond the inoculation plug, thickening and the appearance of fluffy mycelium above the plug were observed in treatments with the EOs of bay laurel, fennel, and lavender. Upon re-inoculation of the treatments onto fresh PDA, where no growth was initially observed, only sweet marjoram and rosemary EOs at a concentration of 10% completely inhibited mycelial growth (Table 2).

(a)

(b)

Figure 1. *In vitro* testing of the antifungal effectiveness of EOs: a) holy basil EO: left – mycelial disc at 10% concentration showing no growth, middle – control, right – treatment with ethanol; b) sage EO: far left – growth observed with 10% EO, middle left – no growth with 25% EO treatment, middle right – control, far right – treatment with ethanol

Table 2. MIC and MFC values determined for the treatments in this study

Essential oil	MIC (%)	MFC (%)
Bay laurel	10	50
Fennel	10	50
Holy basil	10	25
Lavender	10	25
Rosemary	/	10
Sage	25	/
Sweet marjoram	/	10

*/ = not determined

Therefore, these two EOs were classified as fungicidal at concentrations ≥ 10%, while the other EOs at 10% had a fungistatic effect. Lavender and holy basil EOs exhibited a fungicidal effect on mycelial growth at concentrations ≥ 25%, while fennel and bay laurel showed a fungicidal effect at concentrations ≥ 50%. Even at the highest concentration of 100%, sage demonstrated only fungistatic effects. The 9.9% ethanol solution did not affect mycelial growth inhibition, as the mycelium developed similarly to the control treatment.

In vivo evaluation

In vivo testing of the antifungal effectiveness of EOs on mycelial growth showed different results compared to *in vitro* testing. On the fifth day, no mycelial growth was observed beyond the inoculation point on cherry tomatoes in treatments with holy basil, lavender, rosemary, and sweet marjoram EO (Figure 2). The fruit did not soften, and no changes in colour were recorded. Fennel EO also demonstrated high efficacy, with minimal residual mycelial growth (1.33 ± 0.29 mm), which was significantly lower compared to the control. Bay laurel EO showed a weaker effect (9.67 ± 1.56 mm), but still significantly reduced growth in comparison to the untreated control (14.83 ± 3.21 mm). Also, in treatments with fennel and bay laurel EOs, the fruit did not soften as in the control treatment and the treatment with ethanol. Ethanol did not show a significant difference compared to the control (14.17 ± 3.40 mm), confirming that the inhibitory effect resulted solely from the active components of the EO (Figure 3).

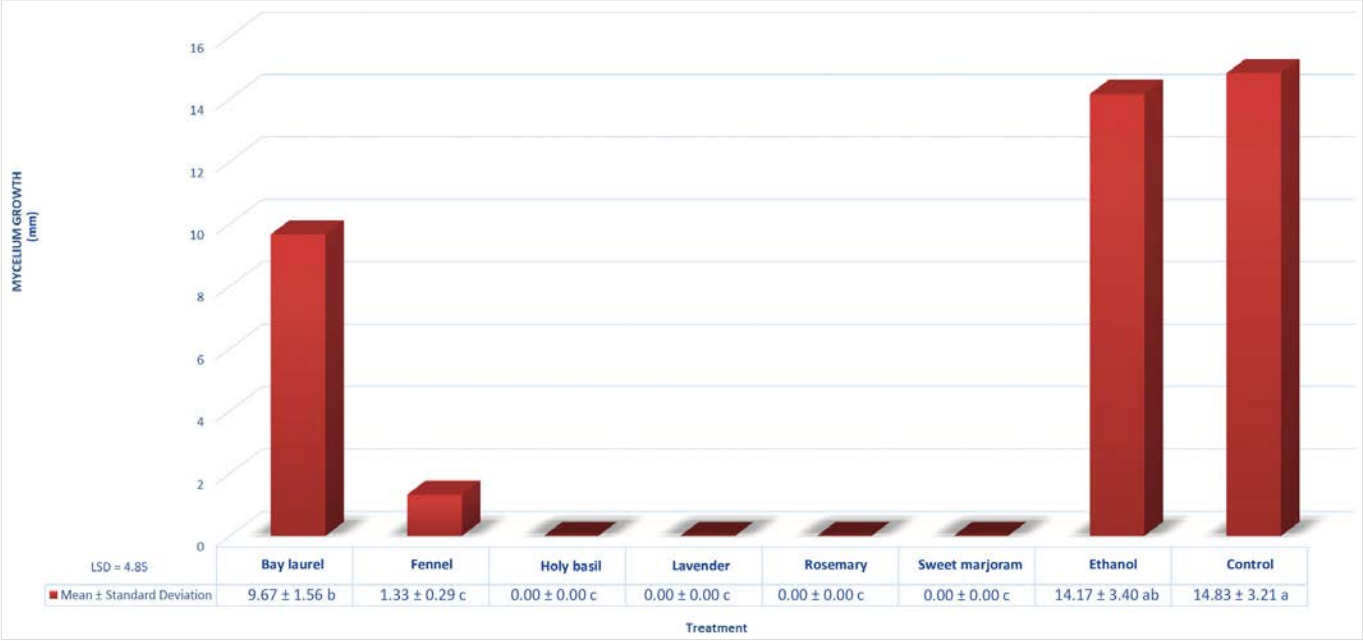

Figure 2. Graphical representation of one-way ANOVA results from *in vivo* testing of the antifungal efficacy of Eos (mycelium growth is presented in mm, and statistically significant differences between treatments are indicated by letters (a, b, c); LSD refers to the least significant difference)

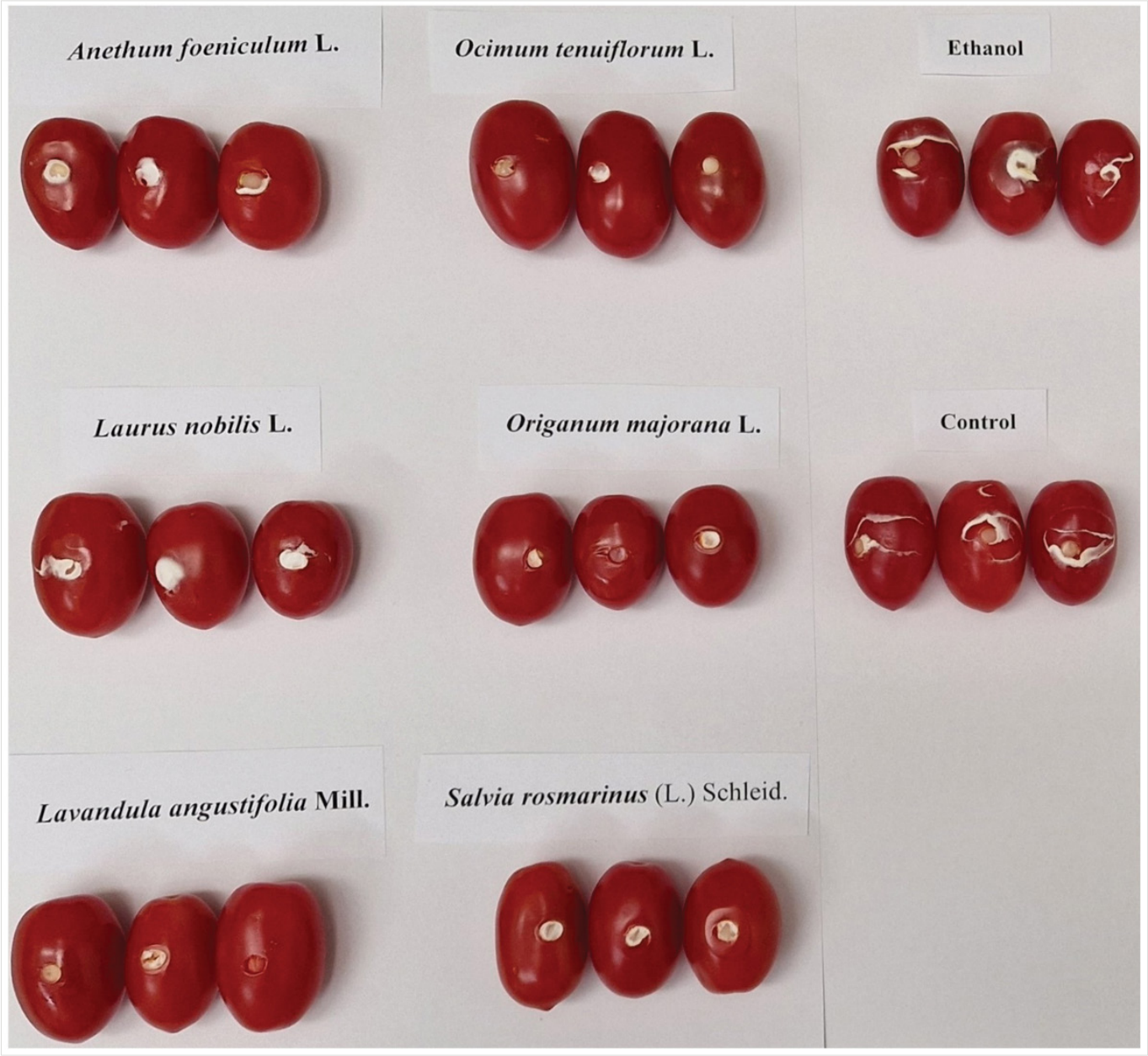

Figure 3. Results from *in vivo* testing of the antifungal effectiveness of EOs: three replicates per treatment

Sensory analysis

The sensory evaluation of Cherry Roma tomatoes treated with EOs revealed significant variations across the sensory characteristics assessed (Figure 4). Cherry tomatoes treated with holy basil emerged as the most appealing, achieving an overall score of 8.6. It scored particularly well in texture (9.1) and juiciness (9.1), indicating that the fruit treated with holy basil retained a pleasant mouthfeel and moisture level. Additionally, its aroma (8.1) and taste (8.3) were well-received, making holy basil the most favourable treatment among the tested EOs. Lavender followed behind with an overall score of 7.9. Lavender received high scores for its aroma (8.1) and juiciness (8.5), although its taste (7.1) and overall impression (7.6) were slightly lower compared to holy basil. Rosemary achieved a moderate overall score of 7.3, performing best in juiciness (8.3) and texture (7.8).

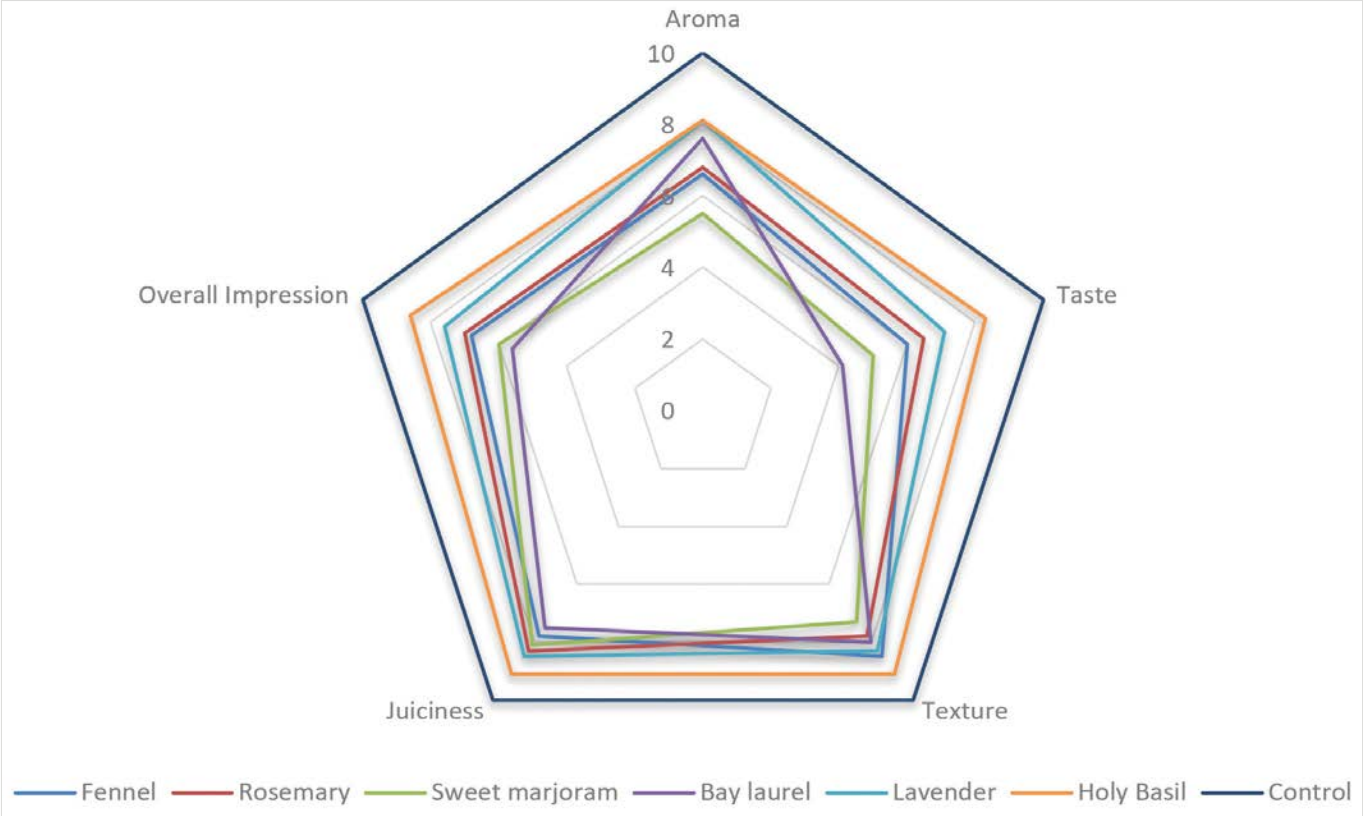

Figure 4. Radar plot of the sensory evaluation of cherry tomatoes treated by EOs

However, its lower scores in aroma (6.8) and taste (6.5) suggest that rosemary was less appealing in terms of flavour profile, despite maintaining a favourable texture and moisture level. Fennel received an overall score of 7.1, performing similarly to rosemary. Its highest ratings were for texture (8.5) and juiciness (7.8). However, the relatively low scores in aroma (6.6) and taste (6) indicate that fennel-treated fruits were less attractive in terms of flavour, potentially limiting their overall sensory appeal. Bay laurel scored 6.6 overall, receiving high scores for aroma (7.6) and texture (8). However, its taste (4.1) was rated significantly lower, which greatly impacted its overall impression (5.6). The contrast between its relatively high aroma and low taste scores suggests that bay laurel may have contributed positively to the fragrance of the fruit but negatively affected its flavour, making it a less favourable treatment overall. Lastly, sweet marjoram was rated the lowest, with an overall score of 6.4. It received the lowest scores in aroma (5.5) and taste (5), which had a substantial impact on its overall acceptability. Despite moderate ratings for juiciness (8.1) and texture (7.3), the poor aromatic and flavour profile of sweet marjoram-treated fruits made it the least preferred treatment in this analysis.

DISCUSSION

Ensuring food quality and preventing spoilage are critical components of food preservation. Oxidation and microbial activity are the primary contributors to food spoilage. Microbial spoilage, in particular, not only compromises food safety but also detracts from the sensory appeal of food, altering its flavor, appearance, and freshness (Generalić Mekinić et al., 2019).

Soylu et al. (2010) demonstrated that the EO of rosemary (*S. rosmarinus*), among the others tested, can inhibit the growth of *B. cinerea*. The vapor-phase effect of these EOs proved to be more effective in inhibiting fungal growth compared to their direct contact phase effect. Rosemary inhibited fungal growth, but higher concentrations were required (1.6 µg/ml in the air and 25.6 µg/ml). The EOs caused significant structural changes in fungal hyphae, such as hyphal shrinkage, protoplast leakage, and vacuolization, ultimately leading to the inhibition of growth and spore germination. The inhibitory potential of EOs against microorganisms is influenced by multiple factors, including the type of EO, its chemical composition, the targeted pathogen, host plant species, applied concentrations or volumes, and agricultural practices, as reported by Ćosić et al. (2014) and Karimi et al. (2016). EOs derived from the same botanical species can exhibit significant variation in chemical composition, even when cloned from genetically identical plants (Bowles, 2012). These variations can substantially impact their antimicrobial efficacy. In our study, with the exception of sage, all EOs completely inhibited mycelial growth at the lowest concentration under *in vitro* conditions. In the *in vivo* test, holy basil, lavender, rosemary, and sweet marjoram completely inhibited fungal growth on the fifth day. Regarding holy basil EO, although no studies have been found specifically examining the antifungal activity of *O. tenuiflorum* EO against *B. cinerea*, some data exist regarding its effects on other Botrytis species. For example, *B. fabae* exhibited notably reduced mycelial growth when exposed to two chemotypes of *O. tenuiflorum* EO (methyl chavicol and linalool types) (Oxenham et al., 2005). Šernaitė et al. (2020) investigated the antifungal effects of EO from rosemary (*S. officinalis*), bay laurel (*L. nobilis*), and a combination with clove (*Syzygium aromaticum*) against *B. cinerea* isolated from strawberries. Among the tested EO, rosemary exhibited the weakest inhibitory effect, with a maximum reduction in fungal growth of 31.91%. In contrast, the antifungal activity of bay laurel oil increased with concentration, achieving up to 55.88% inhibition at 1400–2000 µL/L.

Fincheira et al. (2023) investigated the antifungal effects of five EOs, including lavender (*L. angustifolia*) Fluorescence microscopy shows that the EOs caused structural alterations in the fungal cell walls, reducing hyphal width. Additionally, propidium iodide and Calcein-AM staining indicated a loss of membrane integrity and reduced fungal cell viability following treatment with the EOs. The treated fungi also exhibited decreased mitochondrial activity and impaired respiratory processes, further contributing to the inhibition of fungal growth. In the study by Allagui et al. (2024), the EO of lavender (*L. angustifolia*), bay laurel (*L. nobilis*), and fennel (*F. vulgare*) demonstrated strong antifungal activity against *B. cinerea*. Among these, *F. vulgare* essential oil exhibited the highest efficacy in inhibiting the pathogen's growth.

Aminifard and Mohammadi (2013) reported that, *in vitro*, the growth of *B. cinerea* was entirely suppressed by black caraway (*Carum carvi* L.) and fennel EOs at concentrations of 400 and 600 µL/L, respectively. *In vivo* experiments demonstrated that fennel, among others, effectively inhibited *B. cinerea* growth on plum fruits at all tested concentrations. Additionally, these three EOs had a beneficial impact on fruit quality parameters, including titratable acidity, total soluble solids, carbohydrate content, pH, and weight loss, thereby extending the storage life of the plums. Although the *in vitro* testing with fennel EO in our study showed 100% growth inhibition at the lowest concentration, the *in vivo* testing revealed an increase in mycelial growth. According to Abdolahi et al. (2009), the EO of fennel was able to completely inhibit the growth of *B. cinerea* when applied in the vapour phase at concentrations ≥ 10 µL.

Lopez-Reyes et al. (2013) evaluated the antifungal potential of EO as postharvest treatments for stone fruits affected by *B. cinerea*. Among the EOs tested, *O. majorana* EO showed promising results by effectively suppressing grey mould development on apricots and plums. However, its application on nectarines proved problematic due to phytotoxic effects on the fruit surface. Notably, treatments with EO concentrations of 10% (v/v), although effective in controlling the pathogen, were associated with significant phytotoxicity. In our study, the EO of *O. majorana* also demonstrated strong antifungal activity, confirming its potential as a natural alternative for managing *B. cinerea*.

In testing the antifungal activity of EOs, the choice of solvent for dilution is crucial to avoid influencing experimental results. One of the most commonly used solvents in such studies is DMSO (dimethyl sulfoxide) (Tančinová et al., 2022); however, DMSO can also affect the inhibition of the fungus *B. cinerea*, particularly at higher concentrations (Petruccelli et al., 2020). Therefore, in our study, ethanol was used as the solvent, as it is more suitable for studies involving sensory characteristics. No antifungal effect of ethanol was observed under either *in vitro* or *in vivo* conditions, compared to the control.

The sensory analysis evaluates a product's attributes, such as texture, flavour, taste, appearance, and aroma, using the senses of participants. For centuries, this method has been employed to determine whether a food product is acceptable or not. Initially seen as a complement to technological and microbiological assessments of food quality, sensory analysis has since evolved into a crucial tool. Over the past few decades, it has gained recognition as one of the most important methodologies, driving innovation and ensuring that new products meet consumer preferences and expectations (Ruiz-Capillas and Herrero, 2021).

In the research by Chrysargyris et al. (2021), tomatoes at both the breaker and red ripening stages were treated with sage EO (50 µL/L or 500 µL/L). In breaker-stage fruits, the application of EO at a lower concentration contributed to preserving fruit firmness, regulating respiration rates, and lowering ethylene production. Conversely, higher EO levels accelerated metabolic activity, leading to reduced firmness, increased respiration and ethylene emission, and alterations in antioxidant processes. These effects were more noticeable over the 14-day storage period compared to the standard storage conditions. EO treatment also effectively reduced weight loss in both breaker and red fruits. Quality attributes were more significantly affected in green fruits, while red ones showed fewer changes. Organoleptic assessments, based on factors like appearance, colour, and texture, demonstrated a clear preference for red fruits treated with EO. The use of natural volatile compounds not only maintained fruit quality but also provided antimicrobial protection during storage and transport, with some effects continuing even after EO removal from the storage environment. Sage EO in our study was effective at a concentration of 25% *in vitro*, and exhibited only fungistatic effects even at the highest concentration.

In the study by Tančinová et al. (2022), EO concentrations above 125 µL/L (specifically 250 and 500 µL/L) were found unacceptable in the preliminary sensory analysis of EO-treated strawberries. Similarly, Mrvová et al. (2024) reported that the vapour phase of EOs at 125 µL/L influenced sensory characteristics; however, thyme and oregano EOs did not negatively impact the sensory profile, likely due to their common culinary association with tomatoes. In our study, sweet marjoram EO received the lowest sensory ratings, even at a lower concentration than that used in previously mentioned research. This is notable, given that sweet marjoram is characterized by a milder and sweeter flavor compared to the more pungent and robust profile of oregano.

EOs can be utilized in tomato-based products as well, offering both preservative and functional benefits. For example, Espina et al. (2014) demonstrated that the addition of up to 20 µL/L of pennyroyal mint (*Mentha pulegium* L.) or lemon (*Citrus × limon*) EO to tomato juice did not significantly affect taste acceptance.

Considering all the above, there is a genuine potential for the use of EOs as agents for preserving the quality

and freshness of products. However, several limitations complicate their application. Studies have shown discrepancies in the effectiveness of EOs, both *in vitro* and *in vivo* conditions, and sensory evaluation. These differences can largely be attributed to the chemical composition of the EO. Notably, EOs derived from the same botanical species, even when cloned from identical plants, may produce different chemical compositions when cultivated under varying climatic conditions or in different geographic regions (Bowles, 2012). Moreover, variations also arise due to the fungal isolates used. As indicated by Kaliterna (2013), there is intraspecific variability among isolates, such as differences in cardinal temperatures for mycelial growth and growth rates, which remain not fully understood. This was similarly observed in our earlier research (Petrović et al., 2024). Additionally, there are differences in pathogenicity among the isolates. Differences also arise due to the preferences of the participants, among other factors. The use of EO with well-defined chemical compositions, combined with studies on a broad range of fungal isolates and plant varieties of the same species, as well as investigating the precise mechanism of EO action on both the pathogen and the plant, increases the likelihood of practical EO application. Additionally, it is important to include a larger pool of participants in the sensory analysis, comprising both professional tasters and laypersons, to ensure a more comprehensive evaluation. Furthermore, it is essential to determine the minimum concentrations that are both effective in *in vitro* and *in vivo* conditions, while also being acceptable in terms of sensory properties for consumers.

CONCLUSION

In an era where consumer demand for fresh, high-quality produce is increasing, maintaining food safety and quality during storage and transport poses significant challenges. Synthetic fungicides, commonly used to control *B. cinerea* are becoming less effective due to the pathogen's adaptability and resistance mechanisms, raising concerns about their long-term viability. This research highlights the antimicrobial strength of EOs like holy basil, lavender, rosemary, and sweet marjoram, which completely inhibited fungal growth at low concentrations. In addition to microbial challenges, maintaining the sensory quality of fresh produce—flavour, aroma, and texture—while extending shelf life through natural means remains a delicate balance. The sensory analysis revealed differences in consumer preferences, with holy basil emerging as the most acceptable EO, indicating that while EOs are effective, their influence on consumer perception needs careful consideration. In conclusion, EOs offer a natural, eco-friendly alternative for extending the shelf life of cherry tomatoes, but further optimization is needed to balance antimicrobial efficacy with sensory quality. As food safety concerns grow, natural preservation techniques such as EOs could play a crucial role in reducing reliance on synthetic chemicals.

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