Evaluating the efficiency of essential oils as fumigants in controlling *Penicillium digitatum* in citrus fruits

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Abstract. Citrus fruits are highly valued for their flavor, nutritional content, and versatility, but their perishable nature makes them susceptible to postharvest losses due to fungal infections. Conventional synthetic fungicides, while effective, raise concerns about food safety and environmental impact. Essential oils have emerged as promising alternatives due to their natural antifungal properties. This study investigates the antifungal activity of four plant essential oils as antifungal fumigants against *Penicillium digitatum*, the primary cause of green mould disease in citrus fruits. In vitro fumigant assays, *Satureja montana* essential oil exhibits the highest inhibition percentage against P. *digitatum*. In vivo experiments confirmed the efficacy of S. *montana* oil in reducing fungal growth on mandarins. Further analysis of different parameters revealed potential alterations in fruit quality attributes based on ripening, acidity, and firmness. Therefore, essential oils could be a potential alternative and eco-friendly approach for postharvest disease management in citrus fruits, contributing to efforts towards food safety, environmental sustainability, and public health.

Keywords: Antifungal activity, Citrus fruits, Post-harvest disease management, *Satureja montana*

1 Introduction

Citrus fruits (*Citrus* spp.*)*, such as oranges, lemons, limes, and grapefruits, are highly valued for their vivid flavors, high nutritional content, and versatility in both culinary and industrial applications [1]. However, because of their perishable nature, they are extremely vulnerable to several variables that can lead to losses after harvest. Such losses have greater consequences for market accessibility, food security, and environmental sustainability in addition to harming the profitability of citrus farming [2].

Citrus fruit postharvest loss during storage and transportation is mostly caused by fungal infections, among which green mould caused by *Penicillium digitatum* is found to be the most prominent pathogen worldwide followed by *Penicillium italicum* [3, 4]. P. *digitatum* is one of the most dangerous postharvest diseases, which seriously damages commercial and citricultural endeavors and has been found to be responsible for up to 90% of all postharvest losses in citrus fruit farming in arid zones and tropical subclimates [5, 6]. Conventional synthetic fungicides are the main treatment for post-harvest infections in citrus fruits, but their tendency to contaminate the environment and affect food quality raises concerns. Furthermore, the use of fungicides is becoming increasingly restricted due to severe regulations, carcinogenicity, high and acute residual toxicity, long degradation period, environmental impact, and increased public concern about chemical residues in fruits. [7, 8].

The use of essential oils could provide an economical and sustainable approach to controlling postharvest pathogens. Essential oils are natural substances derived from plants that can be added to a variety of foods as natural additions due to their antibacterial, antifungal, antioxidant, and anticarcinogenic qualities [9]. According to Regnier et al. [10], fruit coatings with plant essential oil proved to be an efficient in vivo management strategy against infections that cause spoiling after harvest. Essential oils are shown to have antifungal properties by disrupting the cell wall and membrane, causing the cytoplasm to coagulate. This mechanism leads to the destruction of cellular organelles and promotes macromolecule release [11-13].

Essential oils are composed of bioactive metabolites with antioxidant, allelopathic, bioregulatory, and anti-bacterial properties, which make them the ideal and safe alternatives to synthetic fungicides. Several research [14], have also shown that the use of essential oils on fruits has led to an increase in its shelf life and quality.

Reyes-Jurado et al. [15] reported that plant essential oils have a variety of biological effects, including significant antibacterial activity against a wide range of fungi that damage plants. Volatile plant essential oils

have been shown in previous studies to be effective in inhibiting fungal growth, especially when it comes to postharvest diseases such as *Botrytis cinerea* [16].

The objective of this research is to assess the antifungal activities of essential oils against fungal diseases on citrus fruits by evaluating their *in vitro* and *in vivo* activity against the salient fungal pathogen P. *digitatum* which attacks the citrus fruits in the post-harvest stage.

2 Materials and methods

2.1. Essential Oils

Four plant essential oils were used for the study. *Zingiber officinale* oil (EO1)*, Piper nigrum* oil (EO2), and *Cymbopogon citratus* oil (EO5) were obtained from Indian commercial market while *Satureja montana* oil (SM) was collected from Institute of Agricultural Sciences, CSIC, Serrano, Madrid, Spain.

2.2. Fruits

Mandarins (*Citrus reticulata* var. Tang Gold) of sizes 54-69 mm were purchased from supermarkets in Madrid, Spain, and used for the in vivo experiment.

2.3 Fungal Pathogen

The pathogen used for the study was *Penicillium digitatum,* responsible for green mould disease. The pathogen was isolated from a diseased lemon (*Citrus limon*) procured from Aldi supermarket in Madrid, Spain. The isolated fungus was identified by morphological and molecular biological methods at Institute of Agricultural Sciences, CSIC, Serrano, Madrid, Spain. They were cultured on potato dextrose agar (PDA) and incubated at 28ºC approximately for 5-7 days [17].

The spore solution for the pathogen was prepared by harvesting the fungal spores from the potato dextrose agar (PDA) using sterile distilled water and an inoculation loop. Glycerol was added to the spore suspension to reach a concentration of 15-20% glycerol [18] to maintain spore viability and then quantified using a hemacytometer and fluorescence microscope to determine their concentration within the suspension, ensuring precise quantification [19].

2.4 Fumigant Antifungal activity of essential oils in vitro

A stock solution (8 mg/mL) of the essential oils were prepared using ethanol (EtOH). A 12-well tissue culture plate was used in which Potato Dextrose Agar (PDA) was poured in 4 wells. 5 µl of spore solution of P. *digitatum* at a concentration of 1 x 10^7 spores/mL were inoculated into the PDA with a micropipette [20]. A total of 250 µL of essential oil from stock solution was taken on cellulose disks with a diameter of 2 cm for treatment and placed in the centre well of the plate (2 mg oil per disk) and 250 µL of ethanol (EtOH) was used as control. The plates were then covered and sealed with parafilm to prevent external contamination.

The plates were then incubated at 25 ºC for 3 days or until the control reaches 1.8-2cm. The growth of the fungus was measured and compared with the control disks' radial growth. The experiment was done in 2 replications. The growth of each fungus was measured by averaging two diameters for each disk, with ImageJ software utilized for precise measurements.
The inhibition percentage was calculated using the formula:

$$\text{Inhibition (\%)} = Gc - \frac{Gt}{Gc} \text{x100 [21]}$$

Where,
Gt: Diameter of fungal growth using essential oil (cm)
Gc: Diameter of fungal growth using ethanol (cm)

Fig. 1. Illustration of In vitro fungal assay using a 12-well tissue culture plate

2.5. Fumigant Antifungal activity of essential oils In-vivo

Winter Savoury (*Satureja montana*) essential oil was used for the In-vivo experiments, and cellulose disks with a diameter of 2 cm were prepared. Glass jars with a volume of 400 ml served as the experimental container. The stock solution of the essential oil was prepared at 50 mg/mL in ethanol (EtOH). For the treatments, 250 µL of essential oil from this stock solution, containing a total of 12.5 mg of oil, was added to a cellulose disk.

Mandarins (*Citrus reticulata* var. Tang Gold) obtained from Lidl supermarket were used for the experiments. The surface of the fruits was first washed thoroughly with tap water and surface sterilization was done using 1% (v/v) sodium hypochlorite solution, followed by hexane, ethyl acetate treatment, and a final rinse with sterile distilled water to ensure the elimination of external contaminants.

The fruits were punctured (approximately 0.2 cm) at 3 places to optimize spore dispersion and 10 µL of a spore solution, with a concentration of 1 x 10^7 spores/mL was inoculated on the punctured areas [22].

The fruits were then placed in jar (one fruit in one jar) and the disk containing essential oil was pasted on the lid of the jar. The jars were then incubated at 25ºC for 7 days and observations were taken at 24 hour interval. The experiment was performed in quadruplicates.

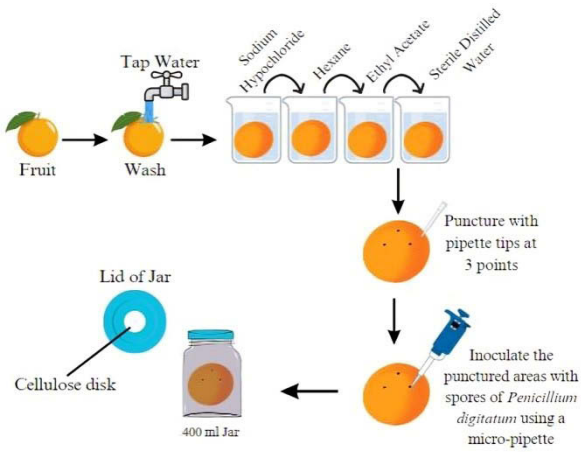

Fig. 2. Illustration of in vivo fungal assay

To ensure the management of the experiment and to compare against the treatment group, several controls were employed- Control samples with punctures and fungal inoculation, with 250µL of ethanol applied to the cellulose disk that served as the main control group, control samples of unaltered fruits with no inoculation, essential oil, or ethanol and control samples with only punctured wounds, no inoculation, and no essential oil or ethanol.

2.6. Analysis of fruit after in vivo assay

The quality of fruits post-treatment was evaluated based on weight loss, pH, firmness and Total Soluble Solids (TSS). The weight of each fruit was recorded both before and after the assay (the weight of the fruit along with their respective jars) and weight loss percentage was calculated using the formula:

$$\text{Weight loss (\%)} = \frac{W0-WT}{W0} * 100 \text{ [22]}$$

Where,
W_0: Initial weight (g)
W_T: Final weight (g)

The pH of fruit juices was measured using a pH meter (Edge ® Dedicated PH/ORP Meter – HI2002). Fruit firmness was evaluated using a penetrometer. Total soluble solids (TSS) were measured using a refractometer to assess sweetness and overall quality. Data analysis procedures included calculating average values across replicates, identifying, and eliminating outliers, and computing standard deviations to evaluate variability within the dataset.

2.7. Statistical analysis

Microsoft Excel (2010) and IBM SPSS version 29.0.2 were utilized for statistical analysis and data processing in the study. In vitro study was done in 2 replicates and in vivo study in 4 replicates.

3 Results and Discussion

3.1. In vitro antifungal efficiency of essential oil fumigation treatments

In vitro fumigant antifungal activity of four essential oils was studied against P. *digitatum*. The concentration of all four essential oils was 8mg/mL. S. *montana* oil demonstrated the highest antifungal activity of 41.02% among all the essential oils while P. *nigrum* oil (EO2) exhibited an inhibitory percentage of 27.92%.

The inhibition percentages of the other essential oils were notably low at the employed concentration.

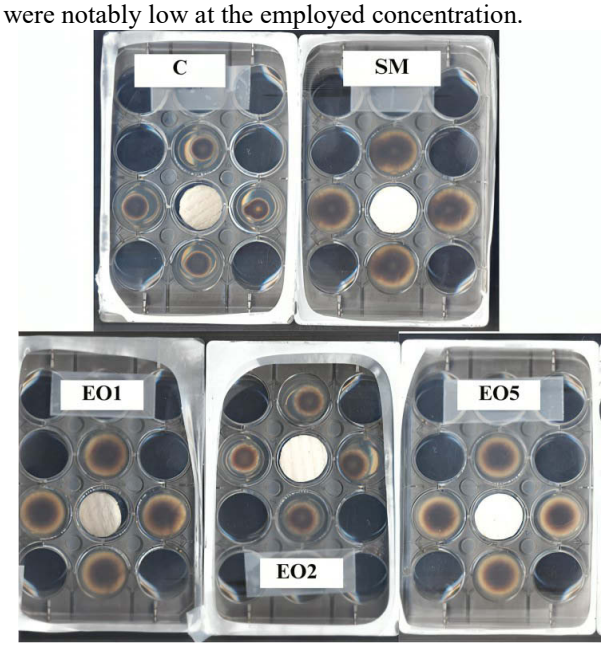

Fig. 3. In vitro antifungal efficacy of essential oil fumigation treatments at a concentration of 8 mg/mL. Measurement was taken after 3 days. C: Control; SM: S. *montana* oil; EO1: Z. *officinale* oil; EO2: P. *nigrum* oil; EO5: C. *citratus* oil.

Table 1. Fumigant antifungal activity of four essential oils against P. *digitatum* at a concentration of 8 mg/mL.

Treatment concentration (8 mg/mL)	Inhibition percentage (%)
SM	41.02±2.86[a]
EO1	2.09±2.95[b]
EO2	27.92±2.94[a]
EO5	2.47±2.78[b]

Note: SM: S. *montana* oil; EO1: Z. *officinale* oil; EO2: P. *nigrum* oil; EO5: C. *citratus* oil. The inhibition percentage (%) are presented in average of 2 replications and represented as mean ± SD. Different lowercase letter indicate significant difference between the treatments ($p<0.05$) in which a is highest.

Penicillium spp. has exhibited a relatively higher tolerance towards various essential oils (EOs) as it appears that they employ a distinct mechanism compared to other plant post-harvest pathogenic fungi, granting it a moderate resilience to adverse conditions, including exposure to antifungal compounds [23].

Further, experiments were conducted at a lower concentration of essential oil at 4mg/mL to determine the lowest inhibitory concentration against P. *digitatum,* using the essential oils S. *montana* and P. *nigrum*. In this study S. *montana* essential oil demonstrated a 25.26% inhibition rate while P. *nigrum* essential oil achieved a lower inhibition rate of 12.72%.

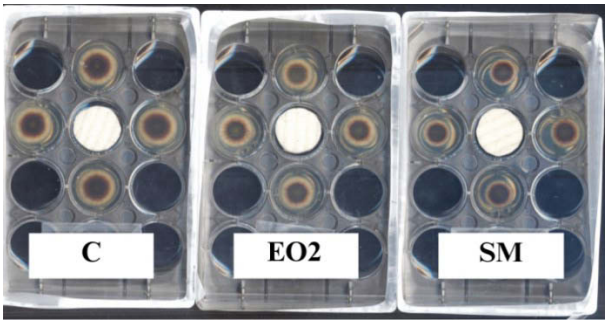

Fig. 4. In vitro antifungal efficacy of essential oil fumigation treatments at a concentration of 4 mg/mL. Measurement was taken after 3 days. C: Control; SM: S. *montana* oil; EO2: P. *nigrum* oil.

Table 2. Fumigant antifungal activity of S. *montana* and P. *nigrum* oil against P. *digitatum* at a concentration of 4 mg/mL.

Treatment concentration (4mg/mL)	Inhibition percentage (%)
SM	25.26±1.31[a]
EO2	12.72±2.38[b]

Note: SM: S. *montana* oil; EO2: P. *nigrum* oil. The inhibition percentage (%) are taken in average of 2 replications and represented as mean ± SD. Different lowercase letter indicate significant difference between the treatments ($p<0.05$) in which a is highest.

The primary compounds in S. *montana* oil are carvacrol (44.5%), p-cymene (16.9%), γ-terpinene (8.7%), borneol (5.1%), and thymol (3.5%) [24, 25].

Previous research [26] reported that essential oils with notable carvacrol content have higher antifungal activity than other essential oils, in line with earlier studies that have demonstrated the compound's strong antifungal properties [27-29].

Carvacrol-rich essential oil was notably more effective and demonstrated the highest reduction in fungal decay. Because of the way it interacts with cell membranes, it is known as a phenolic chemical with fungicidal properties. Its antibacterial action is explained by the hydroxyl group's ability to destabilise the cytoplasmic membrane and even cause cell death [30-33].

The essential oil of *Piper nigrum* contains limonene (18.8%), sabinene (16.5%), β-caryophyllene (15.6%), and β-pinene (10%) as the major components [34, 35].

Akthar et al. [36] reported that *Piper nigrum* oil has antifungal activity against *Candida albicans, Trichophyton terrestre,* T. *tonsurans,* A. *nidulans,* A. *fumigatus, Monosporium apiospermum, Histoplasma capsulatum* and *Sporotrichum schencki,*
Castellanos et al. [37] also mentioned that essential oils/extracts from black pepper (*Piper nigrum* L.) could be a safer alternative to toxic chemical fungicides for treating tomato fruit during transit and storage and might also aid in lowering postharvest infections.

3.2. In-vivo Fumigant Antifungal Activity of Plant Essential Oils

The essential oil demonstrating the highest antifungal activity in the in vitro assay, S. *montana* oil, was selected for the in vivo study. In vivo results revealed that after 48 hours of fungal inoculation, white spots emerged on the punctured areas. Additionally, dark patches were also observed on fruit surfaces presumably due to hexane treatment utilised for surface sterilization of the fruits.

After 72 hours, the white spots increased in size, becoming more prominent.

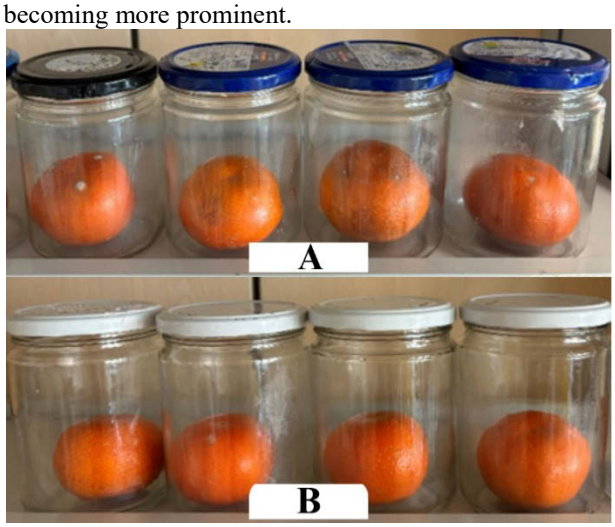

Fig. 5. In vivo fumigation assay after 72 hours. A: Control (fruit with ethanol); B: Treatments (fruit with essential oil).

Visible fungal growth was observed in the punctured areas after 96 hours of inoculation. However, fungus growth was notably slower and may even be negligible in the presence of essential oils compared to the controls.

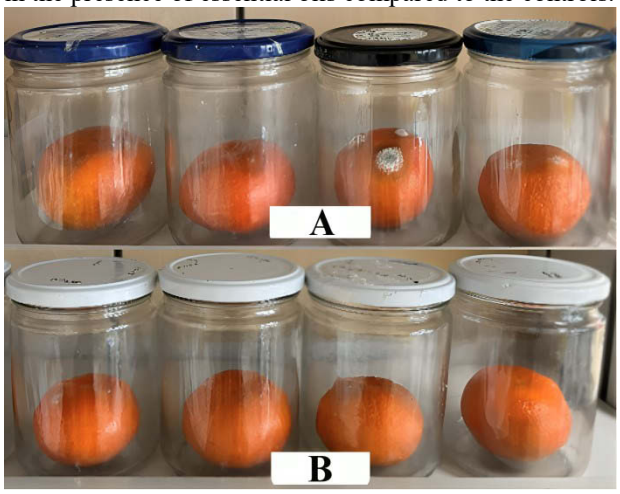

Fig. 6. In vivo fumigation assay after 96 hours. A: Control (fruit with ethanol); B: Treatments (fruit with essential oil).

The in vivo assay was concluded on the 7th day from its initiation. Daily observation of the samples revealed that treatments of the fruits with essential oil exhibited noticeably slower fungal growth as compared to the

control (fruit treated with ethanol) and image records suggest that the essential oils ceased emitting volatile compounds by the 5th day of the assay. From day 5 to day 7, fungal growth increased, suggesting a correlation between the cessation of volatile emissions and the resurgence of fungal growth.

Fig. 7. In vivo assays after the 7th day from its initiation. A: Ethanol control (inoculated fruit with EtOH); B: Treatments (inoculated fruit with essential oil).

Previous literature [38] had also highlighted the efficacy of S. *montana* essential oil in controlling various post-harvest pathogens including *Botrytis cinerea*, Monilinia brown rots, and *Penicillium expansum* in vivo. However, there have been reports of phytotoxic effects at specific levels. Therefore, for practical applications, it is crucial to carefully assess dosage levels to optimize the efficacy of essential oils with potential phytotoxicity.

3.3. Analysis of Citrus fruit post in vivo assay

The analysis of fruit was evaluated in terms of Total Soluble Solids (TSS), pH, and firmness, on the first and seventh day of the experiment. Results (Table 3) revealed that treatment of tangerines with essential oils exhibited closer TSS and pH values to the no inoculation controls compared to the ethanol controls (fruit with ethanol), indicating potential alterations in sugar content and acidity during storage. Moreover, the treatment of tangerines with essential oils demonstrated higher firmness values, suggesting potential preservation effects or reduced softening. These findings suggest that the essential oil aids in maintaining fruit quality, acidity, and firmness.

Table 3. Effect of S. *montana* oil on different parameters of citrus fruit post in vivo study (after 7 days) alongside a non-experimented fruit.

Sample	TSS (°Brix)	pH	Firmness
Treatment	16.2	3.86	12.13
Control 1 (EtOH)	17.2	3.73	7.35
Control 2	10.4	4.4	17.94
Control 3	11	4.36	17.54

Note: Treatment: inoculated fruit treated with essential oil; Control 1 (EtOH): Inoculated fruit with ethanol; Control 2: Control without any treatment i.e., fruit without punctures, no inoculation, no essential oil or ethanol; Control 3: Control with only wound i.e., fruit with only punctures, no inoculation, no essential oil or ethanol. Results are presented in average of all replicates.

Table 4. Weight loss percentage of citrus fruit after 7 days of in vivo study.

Sample	Weight loss percentage (%)
Treatment	0.24 ± 0.12^{NS}
Control 1 (EtOH)	0.40 ± 0.11^{NS}
Control 2	0.18 ± 0.01^{NS}
Control 3	0.17 ± 0.01^{NS}

Note: Treatment: inoculated fruit treated with essential oil; Control 1: Inoculated fruit with ethanol; Control 2: Control without any treatment i.e., fruit without punctures, no inoculation, no essential oil or ethanol; Control 3: Control with only wound i.e., fruit with only punctures, no inoculation, no essential oil or ethanol. The weight loss percentage (%) are taken in average of their replicates and represented as mean ± SD.
NS indicates no significant difference between the treatments ($p>0.05$)

Both the treatment (fruit with essential oil) and the ethanol control (fruit with EtOH) showed variations in weight before and after the in vivo assay. The treated fruits exhibited a relatively lower average weight loss (0.24%) compared to the controls (0.40%), indicating potentially better preservation of fruit weight throughout the study. However, it is important to note that there is variability in weight loss within each group, as indicated by the standard deviation values as shown in Table 4. Individual data points reveal variations in weight loss for both the treatments and the controls. These fluctuations indicate possible variations in fruit responses, influenced by factors like maturity, initial weight, and environmental conditions.

4 Conclusion

The study emphasizes the potential of essential oils as effective alternatives to synthetic fungicides for controlling postharvest fungal pathogens in citrus fruits, particularly against *Penicillium digitatum*. The in vitro experiments revealed notable antifungal activity of *Satureja montana* oil, with promising inhibition percentages against P. *digitatum* and in vivo assays further supported its efficacy in inhibiting fungal growth on citrus fruits, highlighting its practical applicability. These findings support the use of essential oils as eco-friendly and sustainable alternatives for managing postharvest diseases in citrus fruits, which may aid in eradicating the problems related to food safety, environmental contamination, and public health.

Conflict of interest

The authors declare no conflict of interest.

References

1. Z. Wang, Y. Sui, J. Li, X. Tian, Q. Wang. Biological control of postharvest fungal decays in citrus: a review. Crit Rev Food Sci Nutr, **62**(4), 861–870 (2022). DOI:10.1080/10408398.2020.1829542
2. M. S. Ladaniya. Citrus Fruit: Biology, Technology and Evaluation. London, UK: Elsevier Inc. (2008).
3. A. R. Ballester, M. T. Lafuente, L. González-Candelas, Spatial study of antioxidant enzymes, peroxidase, and phenylalanine ammonia-lyase in the citrus fruit–*Penicillium digitatum* interaction, Postharvest Biol Technol, **39**(2), 115-124 (2006). DOI: 10.1016/j.postharvbio.2005.10.002
4. L. Poppe, S. Vanhoutte, M. Höfte. Modes of action of *Pantoea agglomerans* CPA-2, an antagonist of postharvest pathogens on fruits. Eur. J. Plant Pathol., **109**, 963-973 (2003). DOI:10.1023/B:EJPP.0000003747.41051.9f
5. N.G. Ghooshkhaneh, M.R. Golzarian, M. Mamarabadi. Detection and classification of citrus green mold caused by *Penicillium digitatum* using multispectral imaging. J. Sci. Food Agric., **98** (9), 3542-3550 (2018). DOI: 10.1002/jsfa.8865
6. D. Macarisin, L. Cohen, A. Eick, G. Rafael, E. Belausov, M. Wisniewski, S. Droby. *Penicillium digitatum* suppresses production of hydrogen peroxide in host tissue during infection of citrus fruit. Phytopathology, **97**, 1491-1500 (2007). DOI: 10.1094/PHYTO-97-11-1491
7. P. Tripathi & N. Dubey. Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Postharvest Biol Technol, **32**, 235-245 (2003). DOI: 10.1016/j.postharvbio.2003.11.005
8. W. Hao, H. Li, M. Hu, L. Yang, M. Rizwan-ul-Haq. Integrated control of citrus green and blue mold and sour rot by *Bacillus amyloliquefaciens* in combination with tea saponin, Postharvest Biology and Technology, **59** (3), 316-323 (2011), ISSN 0925-5214, DOI:10.1016/j.postharvbio.2010.10.002
9. W. du Plooy, T. Regnier, S. Combrinck. Essential oil amended coatings as alternatives to synthetic fungicides in citrus postharvest management. Postharvest Biol Technol, **53**(3), 117-122 (2009). DOI: 10.1016/j.postharvbio.2009.04.005
10. T. Regnier, W. du Plooy, S. Combrinck, B. Botha. Fungitoxicity of *Lippia scaberrima* essential oil and selected terpenoid components on two mango postharvest spoilage pathogens. Postharvest Biol Technol, **48**, 254-258 (2008). DOI: 10.1016/j.postharvbio.2007.10.011
11. S. S. Mahmoud, R. B. Croteau. Strategies for transgenic manipulation of monoterpene biosynthesis in plants. Trends Plant Sci, **7**(8), 366–373 (2002). DOI: 10.1016/S1360-1385(02)02303-8
12. S. Burt. Essential oils: their antibacterial properties and potential applications in foods-a review. Int J Food Microbiol, **94**(3), 223–253 (2004). DOI: 10.1016/j.ijfoodmicro.2004.03.022
13. M. Hyldgaard, T. Mygind, R. L. Meyer. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology, **3**, 1–24 (2012). DOI: 10.3389/fmicb.2012.00012
14. I. Camele, V. De Feo, L. Altieri, E. Mancini, L. De Martino, G. Lu-iqi Rana. An attempt of post-harvest orange fruit rot control using essential oils from Mediterranean plants. J Med Food, **13**, 1515-1523 (2010). DOI: 10.1089/jmf.2009.0285
15. F. Reyes-Jurado, A. R. Navarro-Cruz, C. E. Ochoa-Velasco, E. Palou, A. Lopez-Malo, R. Avila-Sosa. Essential oils in vapor phase as alternative antimicrobials: Crit Rev Food Sci Nutr, **60**(10), 1641-1650 (2020). DOI: 10.1080/10408398.2019.1586641
16. A. Servili, E. Feliziani, G. Romanazzi. Exposure to volatiles of essential oils alone or under hypobaric treatment to control postharvest gray mold of table grapes. Postharvest Biol Technol, **133**, 36–40 (2017). DOI: 10.1016/j.postharvbio.2017.06.007
17. A. Al-Jaradi, I. Al-Mahmooli, R. Janke, S. Maharachchikumbura, N. Al-Saady, and A. M Al-Sadi. Isolation and identification of pathogenic fungi and oomycetes associated with beans and cowpea root diseases in Oman. PeerJ, **6**:e6064. (2018). DOI: 10.7717/peerj.6064
18. K. K Nakasone, S. W Peterson, S. C Jong. Preservation and distribution of fungal cultures. Biodiversity of Fungi: Inventory and Monitoring Methods (Bills G. Muller G.M. Foster M.S., eds), 37–47. Elsevier, Amsterdam (2004).
19. A. Vitoratos, D. Bilalis, A. Karkanis, & A. Efthimiadou. Antifungal Activity of Plant Essential Oils Against *Botrytis cinerea, Penicillium italicum* and *Penicillium digitatum*. Not Bot Horti Agrobo, **41**(1), 86-92 (2013). DOI: 10.15835/nbha4118931
20. S. A. M. Alamri, M. Hashem, M. S. A. Alqahtani, A. M. A. Alshehri, Z. A. Mohamed, & E. S. H. Ziedan. Formulation of mint and thyme essential oils with Arabic gum and Tween to enhance their

efficiency in the control of postharvest rots of peach fruit. Can J Plant Pathol, **42**(3), 330–343 (2020). DOI: 10.1080/07060661.2019.1686654
21. J. Singh, N. Tripathi, Inhibition of storage fungi of blackgram (*Vigna mungo* L.) by some essential oils. Flavour and Fragrance Journal, **14,** 1-4 (1999). DOI:10.1002/(SICI)1099-1026(199901/02)14:1<1::AID-FFJ735>3.0.CO;2-R
22. N. Poovathumkadavil Thambi, P. Rani, M. Sharma, M. Katoch. A combinatorial approach of Monarda citriodora essential oil (MEO) and linalool vapors to control fruit rot of *Citrus limon* caused by a new pathogen, *Aspergillus foetidus*, and its underlying mode of action. J Appl Microbiol, **134**(12) (2023). DOI: 10.1093/jambio/lxad292
23. M. B Allagui, M. Moumni, G. Romanazzi. Antifungal Activity of Thirty Essential Oils to Control Pathogenic Fungi of Postharvest Decay. Antibiotics, **13**, 28 (2024). DOI: 10.3390/antibiotics13010028
24. M. Marin, M. Novakovic, V. Tesevic, et al. (2012). Antioxidative, antibacterial and antifungal activity of the essential oil of wild-growing *Satureja montana* L. from Dalmatia, Croatia. Flavour Fragr J, **27**, 216–23 (2012). DOI: 10.1002/ffj.3082
25. M. Milos, A. Radonic, N. Bezic, V. Dunkic. Localities and seasonal variations in the chemical composition of essential oils of *Satureja montana* L. and *S. cuneifolia* Ten. Flavour Fragr J, **16**, 157–160 (2001). DOI: 10.1002/ffj.965
26. H. S Elshafie, E. Mancini, S. Sakr, L. De Martino, C. A. Mattia, V. De Feo, et al. Antifungal activity of some constituents of *Origanum vulgare* L. essential oil against postharvest disease of peach fruit. J Medicinal Food, **18**(8), 929–934 (2015). DOI: 10.1089/jmf.2014.0167
27. L. Pinto, M. A. Bonifacio, E. De Giglio, S. Cometa, A. F. Logrieco, F. Baruzzi. Unravelling the antifungal effect of red thyme oil (*Thymus vulgaris* L.) compounds in vapor phase. Molecules, **25**, 1–16 (2020). DOI: 10.3390/molecules25204761
28. F. Buonsenso, G. Schiavon, D. Spadaro. Efficacy and mechanisms of action of essential oils' vapours against blue mould on apples caused by *Penicillium expansum*. Int J Mol Sci, **24**(3), 2900 (2023). DOI: 10.3390/ijms24032900
29. M. Kara, M. Türkmen, S. Soylu. Chemical compositions and in vitro antifungal activities of essential oils obtained from different Origanum species against postharvest gray mold rot of persimmon fruit. Acta Hortic, **1338**, 283–290 (2022). DOI: 10.17660/ActaHortic.2022.1338.41
30. A. Ultee, E. P. W. Kets, E. J. Smid. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. Appl Environ Microbiol, **65**, 4606–4610 (1999).
31. A. Ultee, M. H. J. Bennik, R. Moezelaar. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl Environ Microbiol, **68**, 1561–1568 (2002). DOI:10.1128/AEM.68.4.1561-1568.2002
32. R. J. W. Lambert, P. N. Skandamis, P. J. Coote, G. J. E. Nychas. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol, **91**, 453–462 (2001). DOI: 10.1046/j.1365-2672.2001.01428.x
33. F. J. Perina, D. C Amaral, R. S. Fernandes, C. R. G. Labory, G. A. Teixeira, E. Alves. *Thymus vulgaris* essential oil and thymol against *Alternaria alternata* (Fr.) Keissler: Effects on growth, viability, early infection and cellular mode of action. Pest Manag Sci, **71**, 1371–1378 (2015). DOI: 10.1002/ps.3933
34. G. Fichi, G. Flamini, F. Giovanelli, D. Otranto, and S. Perrucci, "Efficacy of an essential oil of *Eugenia caryophyllata* against *Psoroptes cuniculi*," Exp Parasitol, **115**(2), 168–172 (2007). DOI: 10.1016/j.exppara.2006.07.005
35. M. A. Abdulazeez, I. Sani, D. J. Bolanle, and S. A. Abdulmalik. Essential Oils in Food Preservation, Flavor and Safety: Black Pepper (*Piper nigrum* L.) Oil. Academic Press Elsevier, Cambridge, MA, USA, 277–285 (2016). DOI:10.1016/b978-0-12-416641-7.00031-6
36. M. Sayeed Akthar, G. Birhanu, and S. Demisse, Antimicrobial activity of *Piper nigrum* L. and *Cassia didymobotyra* L. leaf extract on selected food borne pathogens, Asian Pac J Trop Dis, **4**(2), 911–919 (2014). DOI: 10.1016/S2222-1808(14)60757-X
37. L. Muñoz Castellanos, N. Amaya Olivas, J. Ayala-Soto, C. M. De La O Contreras, M. Zermeño Ortega, F. Sandoval Salas, L. Hernández-Ochoa. 'In Vitro and In Vivo Antifungal Activity of Clove (*Eugenia caryophyllata*) and Pepper (*Piper nigrum* L.) Essential Oils and Functional Extracts Against *Fusarium oxysporum* and *Aspergillus niger* in Tomato (*Solanum lycopersicum* L.). Int J Microbiol, 1702037 (2020). DOI: 10.1155/2020/1702037
38. J. G. Lopez-Reyes, D. Spadaro, Ml. Gullinoa, A. Garibaldia. Efficacy of plant essential oils on postharvest control of rot caused by fungi on four cultivars of apples in vivo. Flavour Fragr J, **25**, 171-177 (2010). DOI: 10.4315/0362-028X.JFP-12-34