

Natural Preservation of Horticultural Produce: Antimicrobial Efficacy of *Thymus hiemalis* Essential Oil in *in Vitro* and *in Situ* Models

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The present study evaluated the antimicrobial activity of *Thymus hiemalis* essential oil (THEO) against selected phytopathogenic bacteria and fungi using both *in vitro* and *in situ* approaches. Seven plant pathogens were tested: *Xanthomonas arboricola*, *Pectobacterium carotovorum*, *Pseudomonas syringae*, *Agrobacterium radiobacter*, *Fusarium solani*, *Monilia fructigena*, and *Botrytis cinerea*. *In vitro* antimicrobial activity was assessed using the disk diffusion method and determination of minimum inhibitory concentrations (MICs). THEO exhibited broad-spectrum antimicrobial effects, with the strongest inhibition observed in fungal isolates, particularly *B. cinerea* and *F. solani*. *In situ* assays were conducted on fresh-cut horticultural produce models – strawberry, apple, carrot, and parsley – to simulate natural microbial contamination. The antimicrobial efficacy of THEO in the vapour phase was tested at concentrations of 500, 250, 125, and 62.5 $\mu\text{L}\cdot\text{L}^{-1}$. Growth inhibition was quantified using stereological image analysis, and the percentage reduction in microbial volume density (*Vv*) was calculated. Statistically significant inhibitory effects were observed across all tested matrices, especially at higher concentrations. The highest biological growth inhibition (*BGI*) values were recorded for *F. solani* and *M. fructigena*, indicating strong fungistatic potential of THEO vapours in horticultural systems. These findings suggest that *Thymus hiemalis* essential oil possesses effective antimicrobial properties with practical relevance for horticultural applications, particularly in the control of postharvest spoilage and decay caused by phytopathogens.

Keywords: *Thymus hiemalis* essential oil, antimicrobial activity, phytopathogens, *in situ* analysis, postharvest decay, horticultural produce, minimum inhibitory concentration, stereology

1 Introduction

Postharvest microbial spoilage represents a critical challenge to the shelf life and quality of horticultural products, contributing to substantial economic losses worldwide (Bisht & Singh, 2024). Although synthetic fungicides and bactericides have long been used to control postharvest pathogens, increasing concerns about their environmental persistence, toxic residues, and the emergence of resistant microbial strains have

led to stricter regulations and a growing interest in safer, more sustainable alternatives (Fenta & Mekonnen, 2024). Among these alternatives, essential oils (EOs) have garnered considerable attention due to their natural origin, volatility, and broad-spectrum antimicrobial properties (Ben Miri, 2025). In particular, EOs from the genus *Thymus* have shown promising efficacy against a wide range of foodborne and phytopathogenic microorganisms. While *Thymus vulgaris* and *Thymus zygis* have been extensively studied (Salehi et al., 2019),

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the essential oil (EO) of *Thymus hiemalis* – a lesser-known species endemic to southeastern Spain – remains relatively unexplored. Its chemical profile is typically dominated by 1,8-cineole, camphor, and linalool, compounds well known for their potent antimicrobial activity (De Martino et al., 2009; Etri & Pluhár, 2024). Recent studies have highlighted the advantages of vapour-phase application of EOs, which not only enhances antimicrobial efficacy – particularly against airborne and surface-associated pathogens – but also minimizes direct contact with food matrices, thereby reducing sensory alterations and concerns about chemical residues (Laird & Phillips, 2012; Reyes-Jurado et al., 2020; Zubair et al., 2022). The aim of this study was to comprehensively evaluate the antimicrobial activity of *Thymus hiemalis* essential oil (THEO) against key phytopathogenic microorganisms, including *Agrobacterium radiobacter*, *Xanthomonas arboricola*, *Pseudomonas syringae*, *Pectobacterium carotovorum*, *Fusarium solani*, *Botrytis cinerea*, and *Monilia fructigena*. The antimicrobial efficacy of THEO was assessed using a dual approach, combining *in vitro* methods (disk diffusion and minimum inhibitory concentration) with *in situ* vapour-phase treatments on fresh horticultural substrates (strawberry, apple, carrot, and parsley). The study also aimed to explore the practical potential of THEO for bio-based preservation under simulated postharvest conditions.

2 Materials and Methods

2.1 Essential Oil

The essential oil (EO) of *Thymus hiemalis* was purchased from Hanus, s.r.o. (Nitra, Slovakia). It was obtained by steam distillation of the flowering aerial parts of the plant and stored in the dark at 4 °C until use. According to the manufacturer, the oil's chemical composition was as follows: 1,8-cineole (30%), camphor (10%), linalool (7%), α -pinene (5.7%), β -pinene (2.3%), limonene (2.5%), borneol (4%), neral (1.5%), and verbenone (0.13%).

2.2 Tested Microorganisms

The study involved the following phytopathogenic microorganisms: *Xanthomonas arboricola* (CCM 1441), *Pectobacterium carotovorum* (CCM 1008), *Pseudomonas syringae* (CCM 2868), *Agrobacterium radiobacter* (CCM 2926), *Monilia fructigena* (CCM F-300), *Fusarium solani* (Martius) Saccardo (CCM 8014), and *Botrytis cinerea* Pers.: Fr. (F-314). All pure cultures were obtained from the Czech Collection of Microorganisms (CCM, Brno, Czech Republic).

2.3 Disk Diffusion Method

The antimicrobial activity of *Thymus hiemalis* essential oil (THEO) was evaluated using the standard disk diffusion method. Bacterial strains were cultured on Mueller–Hinton agar (MHA; Oxoid, Basingstoke, UK) for 24 hours at 37 °C, while filamentous fungi were grown on Sabouraud dextrose agar (SDA; Oxoid, Basingstoke, UK) for 5 days at 20 °C. Microbial inocula were adjusted to a turbidity equivalent to the 0.5 McFarland standard (approximately 1.5×10^8 CFU.mL⁻¹), and 100 μ L of each suspension was spread evenly onto the surface of the respective agar medium. Sterile 6 mm paper disks were impregnated with 10 μ L of THEO and placed on the inoculated agar plates. Bacterial plates were incubated for 24 hours at 37 °C, and fungal plates for 5 days at 20 °C. Gentamicin (for bacteria) and fluconazole (for fungi) were used as positive controls (Oxoid, Basingstoke, UK), while disks treated with 0.1% dimethyl sulfoxide (DMSO; Centralchem, Bratislava, Slovakia) served as negative controls. All assays were performed in triplicate (Kačániová et al., 2021; Kluz & Vukovic, 2025).

2.4 Minimum Inhibitory Concentration (MIC)

Microbial inocula were cultured for 24 hours in Mueller–Hinton broth (MHB; Oxoid, Basingstoke, UK) for bacteria at 37 °C, and in Sabouraud dextrose broth (SDB; Oxoid, Basingstoke, UK) for filamentous fungi at 25 °C. Each inoculum was adjusted to a turbidity equivalent to the 0.5 McFarland standard, and 50 μ L was added to the wells of a 96-well microtiter plate. *Thymus hiemalis* essential oil (THEO) was serially diluted in MHB and SDB to yield concentrations ranging from 400 mg.mL⁻¹ to 0.2 mg.mL⁻¹. Then, 100 μ L of each diluted oil solution was mixed thoroughly with the inoculum in the respective wells. Bacterial samples were incubated for 24 hours at 37 °C, while fungal samples were incubated at 25 °C for the same duration. Negative controls consisted of wells containing MHB or SDB with THEO but without inoculum, and positive controls consisted of wells with broth and inoculum but no essential oil. After incubation, microbial growth was assessed by measuring absorbance at 570 nanometers (nm) using a GloMax[®] spectrophotometer (Promega Inc., Madison, WI, USA) for non-adherent organisms (Kačániová et al., 2021; Kluz & Vukovic, 2025).

2.5 In situ Analyses

The antimicrobial activity of the vapour phase of *Thymus hiemalis* essential oil (THEO) was evaluated *in situ* against all tested bacterial and fungal phytopathogens using a model system composed of strawberry, apple, carrot, and parsley slices. Heated Mueller–Hinton agar (MHA) and

Sabouraud dextrose agar (SDA) were poured into 60 mm Petri dishes and their lids. Slices of fruit and vegetable tissue (approximately 0.5 mm thick) were placed on the agar surface. Microbial inocula were prepared as previously described. THEO was diluted in ethyl acetate to concentrations of 500, 250, 125, and 62.5 $\mu\text{L}\cdot\text{L}^{-1}$ and applied to sterile filter paper discs. After one minute of evaporation of residual ethyl acetate, the Petri dishes were sealed and incubated for seven days at 37 °C for bacterial strains and 25 °C for fungal strains. Microbial growth inhibition *in situ* was assessed using stereological methods. Specifically, the volume density (V_v) of microbial colonies was estimated by superimposing a stereological grid over captured images in ImageJ software and counting the number of points intersecting the colonies (P) and the reference substrate (p). Volume density was calculated according to the formula (1):

$$V_v (\%) = \frac{P}{p} \cdot 100 \quad (1)$$

The antimicrobial activity of the essential oil was expressed as the bacterial growth inhibition percentage (BGI), calculated as (2):

$$BGI = \frac{(C - T)}{C} \cdot 100 \quad (2)$$

where: C – represents microbial growth in the control group, T – denotes microbial growth in the treatment group, both expressed as volume density (V_v). Negative BGI values indicated stimulation of microbial growth (Kačániová et al., 2021; Kluz & Vukovic, 2025).

2.6 Statistical Analysis

All experiments were performed in triplicate, and results are presented as means with corresponding standard deviations (SD). In the agar disc diffusion assay, inhibition zone diameters were analysed using descriptive statistics to determine the average antimicrobial activity of *Thymus hiemalis* essential oil (THEO) against the tested phytopathogens. For the minimum inhibitory concentration (MIC) assay, absorbance data collected at 570 nm were used to determine the lowest concentration of THEO capable of completely inhibiting microbial growth. Mean absorbance values were calculated for each concentration, and MIC values were established based on both visual assessment and spectrophotometric thresholds. Where applicable, one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was used to compare each treatment group against the control ($p < 0.05$).

One-way ANOVA followed by Tukey's honest significance test (HSD) test ($p < 0.05$) confirmed statistically significant differences between all tested concentrations. *In situ* antimicrobial activity was evaluated on sliced strawberry, apple, carrot, and parsley substrates using a stereological approach. Microbial volume density (V_v) was quantified using ImageJ software by superimposing a stereological grid over the image and counting the number of points intersecting microbial colonies. Biological growth inhibition (BGI) was calculated as the percentage reduction in V_v relative to the untreated control. The resulting BGI matrix, representing all microbial species, concentrations, and substrates, was visualised as a clustered heatmap. All statistical analyses and data visualisations were performed using Microsoft Excel (Microsoft, USA) and GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA).

3 Results and Discussion

3.1 Disc diffusion Method of THEO

The disk diffusion assay revealed that *Thymus hiemalis* essential oil (THEO) exhibited clear antimicrobial activity against all tested phytopathogenic microorganisms (Fig. 1).

The relatively moderate inhibition zones observed in the disc diffusion assay are consistent with previous findings on essential oils with high contents of oxygenated monoterpenes such as 1,8-cineole, linalool, and borneol – compounds known for their antimicrobial activity but typically exhibiting reduced efficacy in solid media due to limited diffusion capacity (Burt, 2004; Rota et al., 2008; Hossain, 2024). The lower diffusion rate of essential oil constituents in agar can lead to an underestimation of their true antimicrobial potential, particularly in comparison with low-molecular-weight antibiotics such as gentamicin or fluconazole, which diffuse more uniformly (Rios et al., 1988). The higher susceptibility of filamentous fungi such as *Botrytis cinerea* and *Monilia fructigena* compared to bacteria may reflect differences in cell wall composition and membrane permeability, with fungal hyphae being more prone to structural disruption by lipophilic compounds (Semeniuc et al., 2017). The relatively high resistance of *Agrobacterium radiobacter* aligns with previous reports describing the intrinsic tolerance of Gram-negative bacteria with robust outer membranes and efflux mechanisms (Nazzaro et al., 2017). Although the inhibition zones of THEO were smaller than those of standard antibiotics, the essential oil's activity remains relevant, especially in light of its natural origin, volatility, and safety profile. It is also important to consider that the disc diffusion method

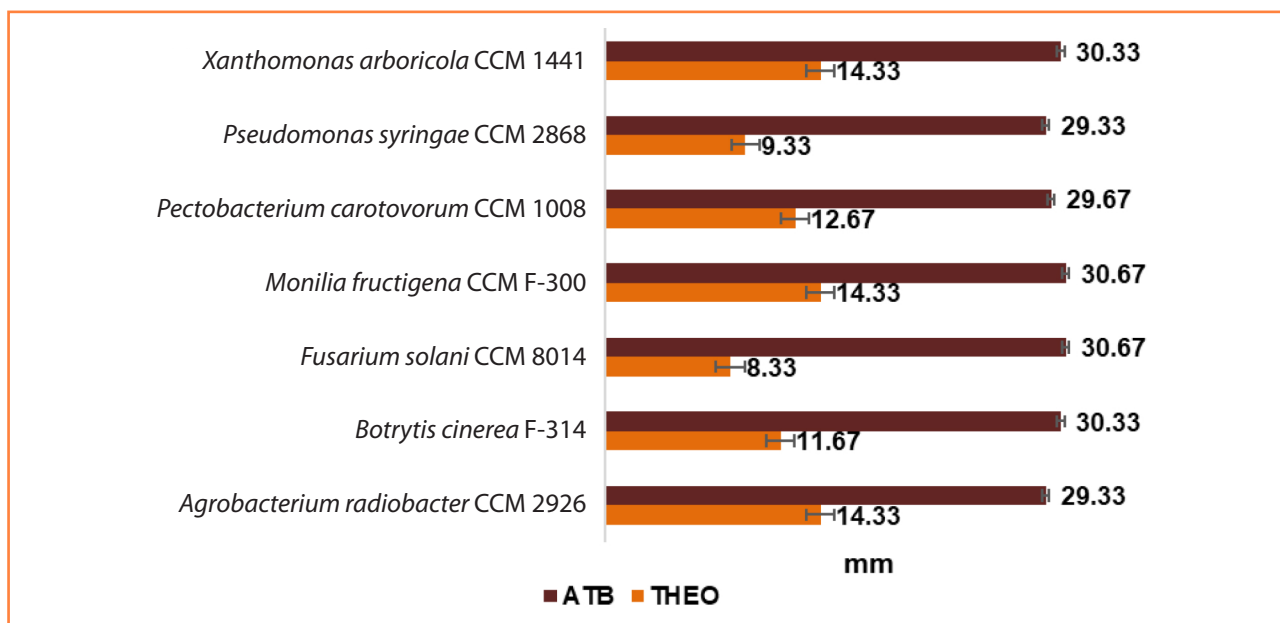


Figure 1 Antimicrobial analyses with disc diffusion method

evaluates contact-based inhibition, whereas essential oil vapours may exert stronger effects in closed systems, as discussed in vapour-phase assays (Opara & Ogra, 2024). These results thus reinforce the importance of selecting appropriate methodologies for EO evaluation and highlight the potential role of THEO as a complementary agent in sustainable plant protection or postharvest disease management.

3.2 Minimal Inhibitory Concentration of THEO

These findings are in line with the known antimicrobial mechanisms of oxygenated monoterpenes such as

thymol, borneol, and linalool – key constituents of THEO – which act primarily by disrupting microbial membrane integrity, leading to cell leakage and impaired metabolic functions (Burt, 2004; Dorman & Deans, 2000). The relatively lower MIC values observed for *Pectobacterium carotovorum* and *Monilia fructigena* may reflect the greater membrane permeability or metabolic vulnerability of these species to EO components (Fig. 2). Interestingly, although *Pseudomonas syringae* exhibited reduced sensitivity in MIC assays, it was still strongly inhibited in vapour-phase experiments. This discrepancy suggests that contact-independent mechanisms – such

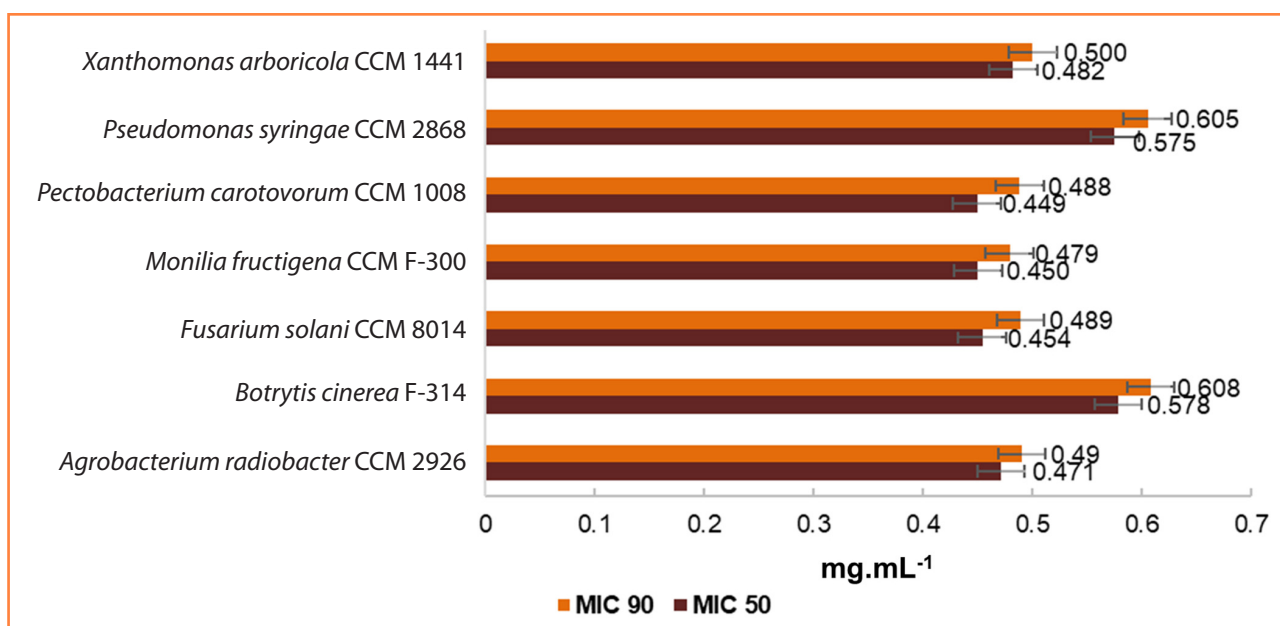


Figure 2 Minimal inhibitory concentration

as vapour pressure-driven diffusion or respiratory interference – may enhance EO activity *in situ*, beyond what is reflected in broth microdilution tests. A similar phenomenon has been described for thyme and oregano oils, whose volatile fractions showed increased antimicrobial action under closed system conditions (Bukovská et al., 2007). Taken together, these MIC data support the use of THEO as a natural antimicrobial agent with flexible application potential – either through direct formulation or as part of vapour-phase delivery systems targeting postharvest pathogens.

3.3 *In situ* Antimicrobial Analyses of THEO

3.3.1 *In situ* Analyses of Strawberry Model

The *in situ* antimicrobial efficacy of *Thymus hiemalis* essential oil (THEO) in vapour phase was evaluated against seven phytopathogenic microorganisms using a fresh strawberry model (Fig. 3). The tested concentrations were 500, 250, 125, and 62.5 $\mu\text{L.L}^{-1}$. Mean growth inhibition ranged from 27.3% (*Pseudomonas syringae*, 500 $\mu\text{L.L}^{-1}$) to 70.5% (*Agrobacterium radiobacter*, 62.5 $\mu\text{L.L}^{-1}$). At the lowest tested dose (62.5 $\mu\text{L.L}^{-1}$), the inhibition percentages were as follows: *A. radiobacter* 70.5%, *Botrytis cinerea* 68.9%, *Fusarium solani* 66.7%, *Monilia fructigena* 69.2%, *Pectobacterium carotovorum* 65.9%, *P. syringae* 63.4%, and *Xanthomonas arboricola*

67.8%. These results confirm a strong and consistent antimicrobial effect even at the lowest concentration tested. One-way ANOVA followed by Tukey's HSD test ($p < 0.05$) revealed statistically significant differences among all tested concentrations. However, no significant differences were observed between microbial species at the same concentration level, suggesting a broad and uniform antimicrobial spectrum of THEO in the vapour phase. These findings align with previous studies demonstrating the high efficacy of essential oil vapours from various *Thymus* species against postharvest pathogens and spoilage organisms. For instance, Lopez-Reyes et al. (2013) reported significant inhibitory effects of *Thymus vulgaris* EO vapour on *Botrytis cinerea* and *Penicillium expansum* in apples, while Sharma et al. (2017) confirmed the antifungal activity of thyme volatiles under *in situ* conditions. Similarly, Sokolić-Mihalak et al. (2012) demonstrated the biofumigant potential of thyme EO in reducing *Aspergillus* growth on soft fruits. Standard deviations in the present study ranged from 0.7% to 3.2%, indicating high reproducibility. The porous and soft surface of strawberries, combined with their high respiration rate and thin cuticle, may have enhanced the absorption of volatile compounds, which supports the application of THEO vapour for biofumigation of soft fruits. This is consistent with the matrix-dependent efficacy of EO vapours reported by Passone et al. (2012),

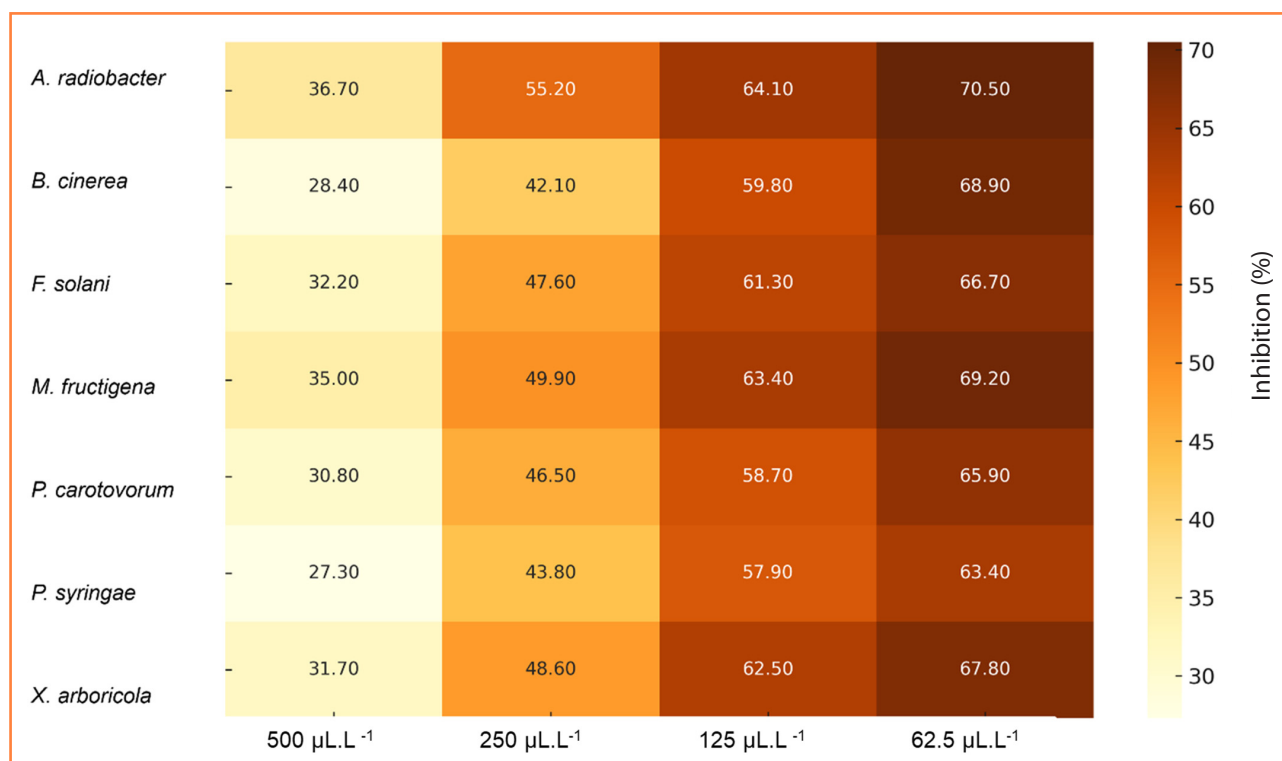


Figure 3 Heatmap of growth inhibition (%) of phytopathogenic microorganisms exposed to *T. hiemalis* vapour in the strawberry model

who noted that surface hydrophobicity and tissue porosity influence EO diffusion and antimicrobial impact. Overall, the uniform response across microbial species in the strawberry model confirms the suitability of THEO as a broad-spectrum, contactless antimicrobial treatment in fresh produce systems, particularly for berries and other high-moisture commodities.

3.3.2 In situ Analyses of Apple Model

The *in situ* antimicrobial efficacy of *Thymus hiemalis* essential oil (THEO) was evaluated using an apple model inoculated with seven phytopathogens. The tested concentrations (500, 250, 125, and 62.5 $\mu\text{L.L}^{-1}$) revealed a clear dose-dependent inhibition of microbial growth. At the highest concentration (500 $\mu\text{L.L}^{-1}$), mean inhibition ranged from 61.2% for *Pseudomonas syringae* to 68.0% for *Agrobacterium radiobacter*. Even the lowest tested concentration (62.5 $\mu\text{L.L}^{-1}$) showed appreciable inhibitory effects, ranging from 28.0% (*P. syringae*) to 34.1% (*Monilia fructigena*), confirming consistent bioactivity of THEO at low doses (Fig. 4). These findings align with previous studies demonstrating the antimicrobial activity of thyme essential oils. For instance, thyme essential oil has been shown to reduce gray mold rot development in apples, indicating its effectiveness against *Botrytis cinerea* and other pathogens. Statistical analysis (one-way ANOVA followed by Tukey's HSD test, $p < 0.05$) confirmed

significant differences among all concentration levels, supporting a dose-dependent inhibition trend. However, no significant differences were observed between microbial species at the same concentration, suggesting that THEO exerts a uniform antimicrobial action across different phytopathogens. The relatively homogeneous response of the apple surface to EO vapours also supports the potential of vapour-phase applications for controlling postharvest diseases in fruits. Studies have shown that essential oils, including thyme, can effectively reduce microbial growth on various fruit matrices when applied in vapour form. Standard deviations ranged from 0.3% to 4.2%, indicating good reproducibility. Overall, the results underscore the potential of THEO vapour as a natural preservative for semi-firm fruits such as apples.

3.3.3 In situ Analyses of Carrot Model

In the carrot model, the vapour-phase application of *Thymus hiemalis* essential oil (THEO) exhibited a clear concentration-dependent antimicrobial effect against all seven tested phytopathogens (Fig. 5).

At the lowest concentration (62.5 $\mu\text{L.L}^{-1}$), inhibition rates ranged from 62.9% (*Pseudomonas syringae*) to 71.3% (*Agrobacterium radiobacter*), confirming good efficacy even at low doses. At the highest concentration (500 $\mu\text{L.L}^{-1}$), the mean inhibition values were as follows: *A. radiobacter* 35.9%, *Botrytis cinerea* 30.1%, *Fusarium*

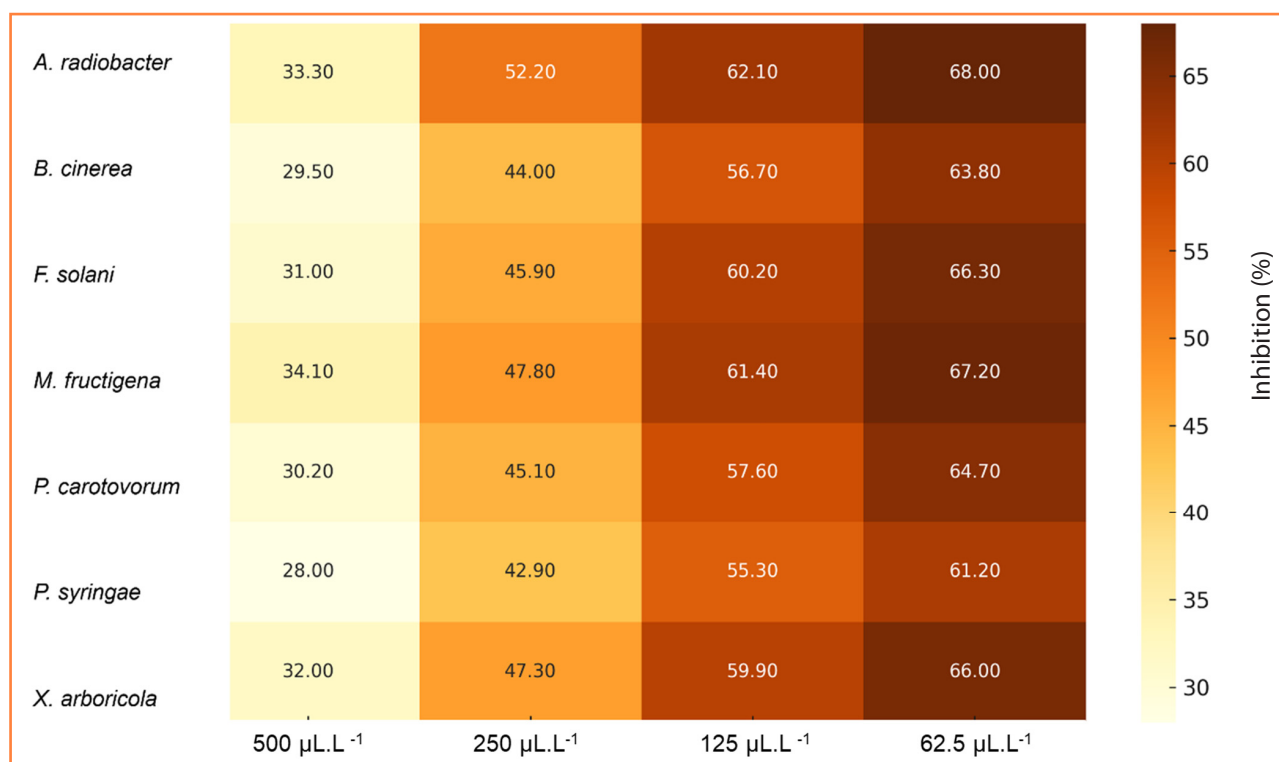


Figure 4 Heatmap of growth inhibition (%) of phytopathogenic microorganisms exposed to *T. hiemalis* vapour in the apple model

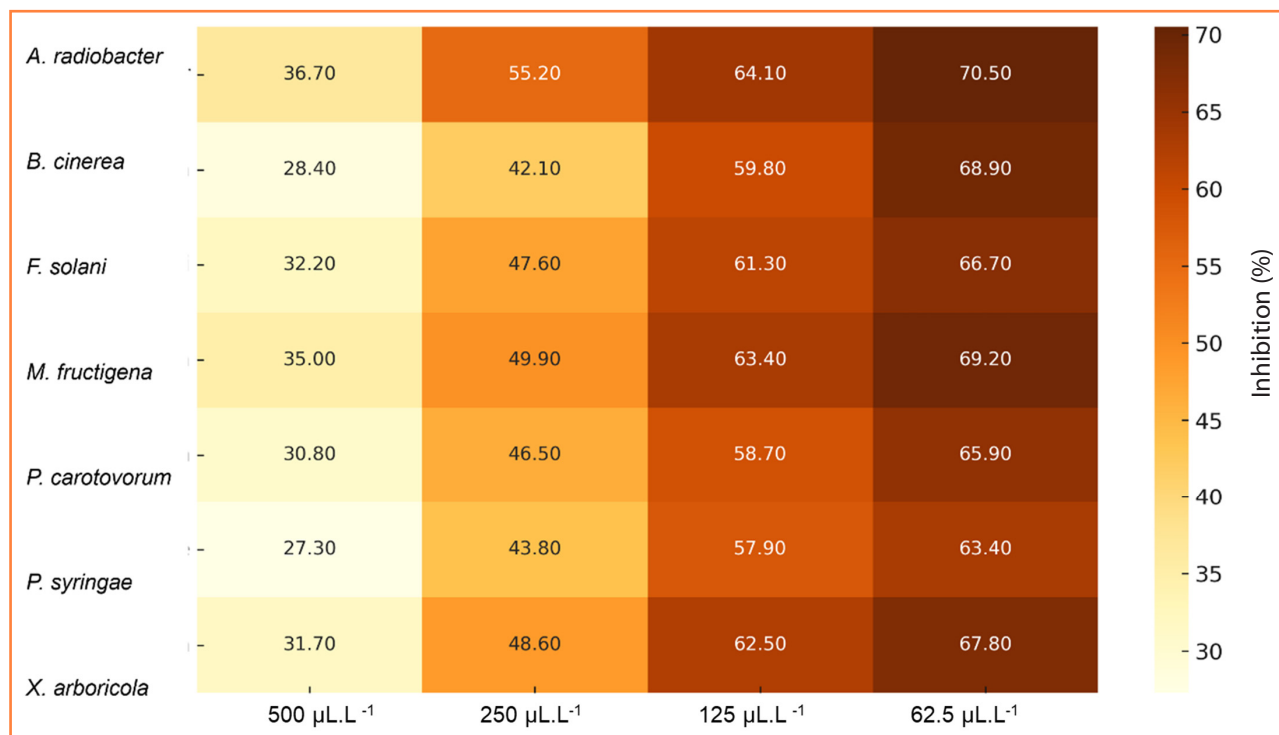


Figure 5 Heatmap of growth inhibition (%) of phytopathogenic microorganisms exposed to *T. hiemalis* vapour in the carrot model

solani 33.0%, *Monilia fructigena* 36.0%, *Pectobacterium carotovorum* 31.1%, *P. syringae* 28.4%, and *Xanthomonas arboricola* 32.9%. One-way ANOVA followed by Tukey's HSD test ($p < 0.05$) confirmed statistically significant differences between all tested concentrations. As in the previous fruit models, no significant differences were observed among microbial species at a given concentration, reinforcing the broad-spectrum activity of THEO. Standard deviations in this model ranged from 1.2% to 3.9%, indicating satisfactory reproducibility. Compared to strawberries and apples, the carrot model showed slightly lower inhibitory values at higher concentrations. This may be attributed to the firmer texture and lower moisture content of carrot tissue, which could limit the absorption and distribution of essential oil vapours. These findings are consistent with earlier reports by Burt (2004) and Tzortzakis & Economakis (2007), highlighting the influence of matrix properties on EO efficacy. Notably, *F. solani* and *M. fructigena* remained the most susceptible species, supporting the established sensitivity of filamentous fungi to oxygenated monoterpenes such as thymol and borneol – key components of THEO (Bozin et al., 2006).

3.3.4 In situ Analyses of Parsley Model

Among all *in situ* tested matrices, parsley demonstrated the highest overall antimicrobial response to *Thymus hiemalis* essential oil (THEO) vapour (Fig. 6). At 62.5 $\mu\text{L.L}^{-1}$,

inhibition rates ranged from 64.3% (*Pseudomonas syringae*) to 72.0% (*Agrobacterium radiobacter*), confirming high efficacy even at the lowest tested dose. At the highest concentration (500 $\mu\text{L.L}^{-1}$), the inhibition values were as follows: *A. radiobacter* 37.1%, *Botrytis cinerea* 31.0%, *Fusarium solani* 34.4%, *Monilia fructigena* 37.2%, *Pectobacterium carotovorum* 32.6%, *P. syringae* 29.2%, and *Xanthomonas arboricola* 33.5% (Fig. 6). One-way ANOVA confirmed a statistically significant inhibition effect across all tested concentrations ($p < 0.05$), with post hoc testing revealing clear separation, particularly between 500 and 125 $\mu\text{L.L}^{-1}$. Standard deviations ranged from 1.0% to 3.2%, indicating high reproducibility and stable EO distribution within the model. Compared to carrot and fruit models, parsley likely benefits from its relatively dry and hydrophobic leaf surface, which may enhance the volatility and atmospheric stability of EO components, thereby improving antimicrobial efficacy. The stronger inhibitory effect observed in fungi such as *F. solani* and *M. fructigena* is consistent with previous studies describing the fungistatic and fungicidal properties of thymol, linalool, and borneol – dominant terpenes present in THEO (Dorman & Deans, 2000). The pronounced vapour activity in this model confirms the suitability of THEO for use on leafy vegetables, where surface contamination is common and vapour-based delivery systems could help reduce postharvest spoilage.

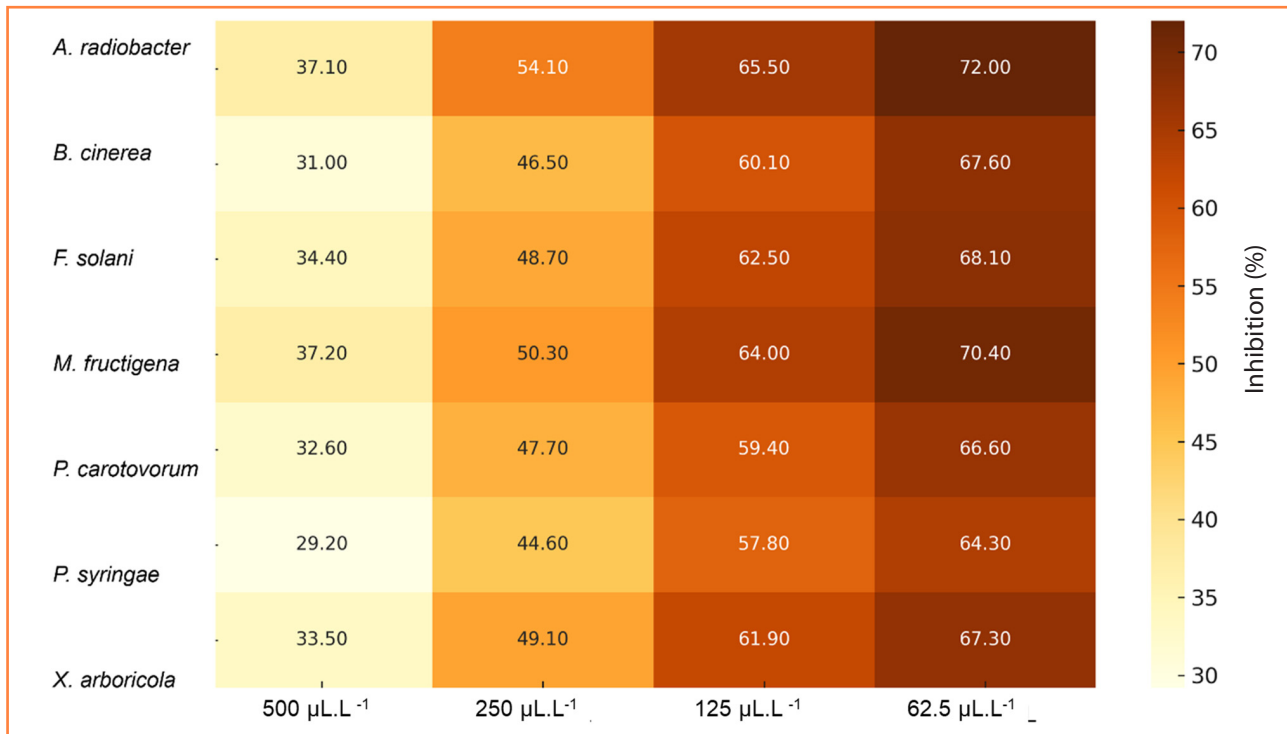


Figure 6 Heatmap of growth inhibition (%) of phytopathogenic microorganisms exposed to *T. hiemalis* vapour in the parsley model

4 Conclusions

The present study clearly demonstrated the antimicrobial activity of *Thymus hiemalis* essential oil (THEO) against a broad spectrum of phytopathogenic bacteria and fungi using both *in vitro* and *in situ* models. In disc diffusion assays, inhibition zones were generally more pronounced in fungal strains than in bacterial ones, with *Fusarium solani* and *Monilia fructigena* being the most consistently sensitive species – particularly in *in situ* models. The minimum inhibitory concentration (MIC) assays confirmed that fungal pathogens required lower concentrations of THEO for growth suppression compared to bacteria. Among the bacterial strains, *Xanthomonas arboricola* and *Agrobacterium radiobacter* showed relatively higher resistance. *In situ* tests using fresh horticultural substrates (strawberry, apple, carrot, and parsley) revealed that the antimicrobial efficacy of THEO vapour was influenced by both the microorganism and the matrix. Higher inhibition levels were consistently observed in the parsley model, followed by carrot, while strawberry and apple exhibited moderate responses. Although efficacy decreased with lower doses, measurable antimicrobial activity was retained even at 62.5 $\mu\text{L.L}^{-1}$. Statistical analysis confirmed significant differences ($p < 0.05$) among treatment groups, demonstrating the reproducibility and consistency of THEO's antimicrobial action across concentrations and commodity types. These findings support the potential

of THEO vapour as an effective non-contact antimicrobial treatment. Its application could be particularly valuable in postharvest handling of sensitive horticultural crops, where microbial spoilage poses a major limitation. THEO may thus be integrated into horticultural practice as a natural preservation strategy to reduce microbial contamination and extend the shelf life of fresh produce – without the need for synthetic additives. Considering its antimicrobial efficacy, the essential oil of THEO may hold potential for commercial application as a natural preservative in the fresh produce industry. However, further studies addressing formulation stability, sensory impact, and regulatory compliance are required to support its practical use.

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References

- Ben Miri, Y. (2025). Essential Oils: Chemical Composition and Diverse Biological Activities : A Comprehensive Review. *Natural Product Communications*, 20(1), 1934578X241311790. <https://doi.org/10.1177/1934578X241311790>

- Bisht, A., & Singh, S. P. (2024). Postharvest Losses and Management of Horticultural Produce: A Review. *Journal of Scientific Research and Reports*, 30(3), 305–320. <https://doi.org/10.9734/jsrr/2024/v30i31881>
- Bozin, B., Mimica-Dukic, N., Simin, N., & Anackov, G. (2006). Characterization of the Volatile Composition of Essential Oils of Some Lamiaceae Spices and the Antimicrobial and Antioxidant Activities of the Entire Oils. *Journal of Agricultural and Food Chemistry*, 54(5), 1822–1828. <https://doi.org/10.1021/jf051922u>
- Bukovská, A., Cikoš, Š., Juhás, Š., Il'ková, G., Rehák, P., & Koppel, J. (2007). Effects of a Combination of Thyme and Oregano Essential Oils on TNBS-Induced Colitis in Mice. *Mediators of Inflammation*, 2007, 1–9. <https://doi.org/10.1155/2007/23296>
- Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods – a review. *International Journal of Food Microbiology*, 94(3), 223–253. <https://doi.org/10.1016/j.ijfoodmicro.2004.03.022>
- De Martino, L., Bruno, M., Formisano, C., De Feo, V., Napolitano, F., Rosselli, S., & Senatore, F. (2009). Chemical Composition and Antimicrobial Activity of the Essential Oils from Two Species of *Thymus* Growing Wild in Southern Italy. *Molecules*, 14(11), 4614–4624. <https://doi.org/10.3390/molecules14114614>
- Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 88(2), 308–316. <https://doi.org/10.1046/j.1365-2672.2000.00969.x>
- Etri, K., & Pluhár, Z. (2024). Exploring Chemical Variability in the Essential Oils of the *Thymus* Genus. *Plants*, 13(10), 1375. <https://doi.org/10.3390/plants13101375>
- Fenta, L., & Mekonnen, H. (2024). Microbial Biofungicides as a Substitute for Chemical Fungicides in the Control of Phytopathogens: Current Perspectives and Research Directions. *Scientifica*, 2024, 1–12. <https://doi.org/10.1155/2024/5322696>
- Hossain, T. J. (2024). Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. *European Journal of Microbiology and Immunology*, 14(2), 97–115. <https://doi.org/10.1556/1886.2024.00035>
- Káčániová, M., Galovičová, L., Valková, V., Ďuranová, H., Borotová, P., Štefániková, J., Vukovic, N. L., Vukic, M., Kunová, S., Felsöciiová, S., Miklášová, K., Savitskaya, T., & Grinshpan, D. (2021). Chemical composition and biological activity of *Salvia officinalis* essential oil. *Acta Horticulturae et Regiotecturae*, 24(2), 81–88. <https://doi.org/10.2478/ahr-2021-0028>
- Kluz, M. I., & Vukovic, N. (2025). Antimicrobial Activity of *Jasminum grandiflorum* Absolute *in vitro* and *in situ* Study against Phytopathogenic Bacteria. *Acta Horticulturae et Regiotecturae*, 28(1), 66–71. <https://doi.org/10.2478/ahr-2025-0008>
- Laird, K., & Phillips, C. (2012). Vapour phase: A potential future use for essential oils as antimicrobials? Essential oil vapours and their antimicrobial activity. *Letters in Applied Microbiology*, 54(3), 169–174. <https://doi.org/10.1111/j.1472-765X.2011.03190.x>
- Lopez-Reyes, J. G., Spadaro, D., Prella, A., Garibaldi, A., & Gullino, M. L. (2013). Efficacy of Plant Essential Oils on Postharvest Control of Rots Caused by Fungi on Different Stone Fruits *In Vivo*. *Journal of Food Protection*, 76(4), 631–639. <https://doi.org/10.4315/0362-028X.JFP-12-342>
- Nazzaro, F., Fratianni, F., Coppola, R., & Feo, V. D. (2017). Essential Oils and Antifungal Activity. *Pharmaceuticals*, 10(4), 86. <https://doi.org/10.3390/ph10040086>
- Opara, U. L., & Ogra, I. O. (2024). An Introduction to Postharvest Handling Technology of Fresh Fruits and Vegetables. S. Ali, S. A. Mir, B. N. Dar, & S. Ejaz. *Sustainable Postharvest Technologies for Fruits and Vegetables* (1st ed., pp. 3–41). <https://doi.org/10.1201/9781003370376-2>
- Passone, M. A., Girardi, N. S., Ferrand, C. A., & Etcheverry, M. (2012). *In vitro* evaluation of five essential oils as botanical fungitoxicants for the protection of stored peanuts from *Aspergillus flavus* and *A. parasiticus* contamination. *International Biodeterioration & Biodegradation*, 70, 82–88. <https://doi.org/10.1016/j.ibiod.2011.11.017>
- Reyes-Jurado, F., Navarro-Cruz, A. R., Ochoa-Velasco, C. E., Palou, E., López-Malo, A., & Ávila-Sosa, R. (2020). Essential oils in vapor phase as alternative antimicrobials: A review. *Critical Reviews in Food Science and Nutrition*, 60(10), 1641–1650. <https://doi.org/10.1080/10408398.2019.1586641>
- Rios, J. L., Recio, M. C., & Villar, A. (1988). Screening methods for natural products with antimicrobial activity: A review of the literature. *Journal of Ethnopharmacology*, 23(2–3), 127–149. [https://doi.org/10.1016/0378-8741\(88\)90001-3](https://doi.org/10.1016/0378-8741(88)90001-3)
- Rota, M. C., Herrera, A., Martínez, R. M., Sotomayor, J. A., & Jordán, M. J. (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hymnalis* essential oils. *Food Control*, 19(7), 681–687. <https://doi.org/10.1016/j.foodcont.2007.07.007>
- Salehi, B., Upadhyay, S., Erdogan Orhan, I., Kumar Jugran, A., L.D. Jayaweera, S., A. Dias, D., Sharopov, F., Taheri, Y., Martins, N., Baghalpour, N., C. Cho, W., & Sharifi-Rad, J. (2019). Therapeutic Potential of α - and β -Pinene: A Miracle Gift of Nature. *Biomolecules*, 9(11), 738. <https://doi.org/10.3390/biom9110738>
- Semeniuc, C. A., Pop, C. R., & Rotar, A. M. (2017). Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. *Journal of Food and Drug Analysis*, 25(2), 403–408. <https://doi.org/10.1016/j.jfda.2016.06.002>
- Sharma, A., Rajendran, S., Srivastava, A., Sharma, S., & Kundu, B. (2017). Antifungal activities of selected essential oils against *Fusarium oxysporum* f. Sp. *Lycopersici* 1322, with emphasis on *Syzygium aromaticum* essential oil. *Journal of Bioscience and Bioengineering*, 123(3), 308–313. <https://doi.org/10.1016/j.jbiosc.2016.09.011>
- Sokolić-Mihalak, D., Frece, J., Slavica, A., Delaš, F., Pavlović, H., & Markov, K. (2012). The Effects Of Wild Thyme (*Thymus serpyllum* L.) Essential Oil Components Against Ochratoxin-Producing *Aspergilli*/Majčina Dušica (*Thymus serpyllum* L.) I Njzine Komponente Protiv Okratoksikotvornih Vrsta *Aspergillus*. *Archives of Industrial Hygiene and Toxicology*, 63(4), 457–462. <https://doi.org/10.2478/10004-1254-63-2012-2309>
- Tzortzakakis, N. G., & Economakis, C. D. (2007). Antifungal activity of lemongrass (*Cymbopogon citratus* L.) essential oil against key postharvest pathogens. *Innovative Food Science & Emerging Technologies*, 8(2), 253–258. <https://doi.org/10.1016/j.ifset.2007.01.002>
- Zubair, M., Shahzad, S., Hussain, A., Pradhan, R. A., Arshad, M., & Ullah, A. (2022). Current Trends in the Utilization of Essential Oils for Polysaccharide- and Protein-Derived Food Packaging Materials. *Polymers*, 14(6), 1146. <https://doi.org/10.3390/polym14061146>

