Bulgarian Journal of Agricultural Science, 32 (No 1) 2026, 130–136

Thymus longedentatus essential oil and methanolic extract: Chemical composition and activity against filamentous plant pathogens

Milena Nikolova[a], Aneta Lyubenova[b, c*] and Ina Aneva[a]

[a] *Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences 1113 Sofia, Bulgaria*
[b] *Agricultural Academy, AgroBioInstitute, Department of Agrobiotechnology, 1164 Sofia, Bulgaria*
[c] *Centre of Competence "Sustainable Utilization of Bio-resources and Waste of Medicinal and Aromatic Plants for Innovative Bioactive Products" (BIORESOURCES BG), Sofia 1000, Bulgaria*
[*] *Corresponding author:* anetalyubenova@abi.bg

Abstract

Nikolova, M., Lyubenova, A. & Aneva, I. (2026). *Thymus longedentatus* essential oil and methanolic extract: Chemical composition and activity against filamentous plant pathogens. *Bulg. J. Agric. Sci., 32*(1), 130–136

Thymus longedentatus (Degen & Urum.) Ronniger is a Balkan endemic species with a lemon-like odor that has a limited distribution in Bulgaria. The species has been studied phytochemically, and was successfully cultivated in the *ex situ* collection of IBER, BAS in the last years. The reported strong herbicidal properties for the species' essential oil (EO) led us to examine it for other pesticidal actions. The present study aimed to determine the chemical composition of *Thymus longedentatus* EO and methanolic extract (ME) and to evaluate their growth inhibitory activity against filamentous fungal and oomycete plant pathogens. The chemical composition of EO and ME was studied, using gas-chromatography/mass spectrometry (GC/MS). The antifungal activity was evaluated by conducting *in vitro* bioassay towards economically important plant pathogens: *Botrytis cinerea*, *Fusarium oxysporum* var. *fragariae, Fusarium oxysporum* var. *cyclaminis, Phytophthora cryptogea* and *Phytophtora nicotianae.* Citral isomers – neral (26.8%) and geranial (43.1%) were identified as the main components of the EO profile. Rosmarinic acid, chlorogenic acid, feruloylquinic acid, oleanolic acid, ursolic acid, micromeric acid, arbutin, geranic acid and hydroquinone were the main secondary metabolites in the methanolic extract. A strong inhibiting effect against *Botrytis cinerea, Phytophthora nicotianae and Phytophthora cryptogea* was established for the essential oil. Oppositely, the methanol extract, in which the Citral isomers were not found, did not affect the mycelial growth of the tested plant pathogens. The potency of the essential oil rich in Citral isomers, against oomycete and fungal plant pathogens is revealed.

Keywords: Citral isomers; *Phytophthora*; *Botrytis cinerea*; *Fusarium oxysporum;* bioassay

Introduction

Thymus longedentatus (Degen & Urum.) Ronniger is a Balkan endemic species, naturally distributed in Bulgaria, mainly in the southeastern part of the country. In the last few years, the species has been studied phytochemically, as well as the possibilities of its hydroponic cultivation (Aneva et al., 2019; Georgiev et al., 2022; Nikolova et al., 2021; Nikolova et al., 2023; Nikolova et al., 2024; Traykova et al., 2024). Plant material from a natural locality has been successfully grown in the *ex-situ* collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (IBER, BAS) for several years. The essential oil composition of the plant material collected from the natural population of the species in the Eastern Rhodopes Mts. Bulgaria has been reported and classified as a geranial chemotype (Aneva et al., 2019). The main components of *T. longedentatus* EO neral and geranial are known as citral isomers. These substances, in addition to a lemon-like odor, also exhibit important biological activities – antimicrobial, anti-inflammatory, analgesic, antioxidant, antiproliferative, spasmolytic, antigenotoxic, chemopreventive, and others (Aprotosoaie et al., 2019; Wang et al., 2019; Gutiérrez-Pacheco et al., 2023). Subsequent studies found that the EO of the species also showed significant herbicidal properties, evaluated using *in vitro* assays. Completely inhibitory activity on seed germination of *Lolium perenne* has been demonstrated by applying an aqueous solution of essential oil at 3 µL/mL. With the solution at the same concentration, inhibition over 50% was found against *Trifolium pratense* L. seed germination (Nikolova et al., 2021). Significant antiradical activity has been reported for the methanolic extract of the species (Nikolova et al., 2023). In addition, high acetylcholinesterase inhibitory activity (IC_{50} value of 0.72 mg/mL) has been found for the EO of the species (Georgiev et al., 2022). The latter activity also suggests the presence of insecticidal properties. The above shows that the extracts and fractions of the species have the potential to be used as pharmaceutical agents, food additives, and pesticide products. The above led us to study the inhibitory activity of the oil on the mycelium growth of phytopathogenic fungi. One of the most challenging for agriculture plant pathogens are represented by *Fungi* and the algae-like *Oomycetes*. Although distant phylogenetically, both groups have similar filamentous growth and plant colonization mode. One very important difference is the lack of sensitivity to conventional fungicides in *Oomycetes* (Gutiérrez-Pacheco et al., 2023; Latijnhouwers et al., 2003). For our study, we selected widespread fungal and oomycete species with wide host ranges. *Botrytis cinerea* is a common plant pathogen that can cause huge damage to an extremely wide range of bulb, vegetable, fruit, flower, fiber, and oil crops, not only in the field and in greenhouses, but also during storage and transport (Jackson, 2014). It is also a commonly used object of study in a variety of antifungal experiments with different plants' secondary metabolites (Bouchra et al., 2003; Gholamnezhad, 2019; Dėnė and Valiuškaitė, 2021). The other used in our study genus *Fusarium* is characterized with high genetic variability, broad host specificity and production of toxins (Perincherry et al., 2019). *Phytophthora cryptogea* is the causing agent of several important diseases on cultural plans: mainly crown, foot and root roots of vegetable, ornamental and woody plants (Erwin and Ribeiro, 1996). *Phytophthora nicotianae* has a host range exceeding 255 species and is considered a continuous challenge to chemical-based plant disease management programs (Panabieres et al., 2016).

The present study aimed to determine the chemical composition of *Thymus longedentatus* EO and ME, collected from cultivated areas, and to evaluate their growth inhibitory activity against filamentous fungal and oomycete plant pathogens. The relationship of the main components of the EO profile of *T. longidorus* and the growth response of the studied isolates is discussed.

Material and Methods

Plant material

Aerial parts of *Thymus longedentatus* were collected from an experimental field at IBER, BAS (http://www.iber.bas.bg/sites/default/files/projects/plantscollection/index.html).

Preparation of extracts

The essential oil was obtained using the Clevenger apparatus by water distillation, from a sample of 50 g DW, in a flask with 500 ml of water, for 2 h. The methanolic extract was prepared by classical maceration with methanol for 24 hours.

Derivatization of the methanol extract

50 mg of methanolic extract was derivatized with 50 µL of *N,O* bis-(trimethylsilyl) trifluoro-acetamide (BSTFA) in 50 µL of pyridine for 2 h at 60 °C. After cooling, 300 µL of chloroform was added to the derivatized fractions, and analyzed by gas chromatography-mass spectrometry.

Gas chromatography mass spectrometry (GC-MS)

The GC–MS analysis was performed on a Thermo Scientific Focus gas chromatograph coupled with a Thermo Scientific dual stage quadrupole (DSQ) mass detector operating in electron ionization (EI) mode at 70 eV. ADB-5MS column (30 m × 0.25 mm × 0.25 µm) was used. Chromatographic conditions were as described by (Traykova et al., 2019) and (Haist et al., 2024) for essential oil and extract, respectively. Helium as carrier gas at a flow rate of 1 mL/min; injection volume was 1 µl, and the split ratio was 1:50. The quantities of each compound were expressed as the percentage from total ion current (TIC). The compounds were identified by comparing their MS spectra and retention indices (RI) with known compounds from the National Institute of Standards and Technology (NIST), Golm Metabolome Database, and literature data (Adams, 2007).

Isolates

The fungal and oomycete isolates used originate from agricultural ecosystems in Bulgaria. They were identified based on morphology and ITS sequences, except *Phytophthora cryptogea*, for which enzyme restriction was used (unpublished study). More details, including NCBI GenBank accession numbers of the sequences, are presented in Table 1. The basal media were V8 Agar Media (16 g agar, 100 mL

V8 Juice, and 900 mL distilled water) for the two *Phytophthora* spp, and PDA (BD Difco™) for the fungal isolates.

Bioassay

The antifungal activity of the essential oil (EO) and methanolic extract (ME) obtained from *T. longedentatus* was evaluated by using the Agar disk-diffusion method with modifications (Balouiri et al., 2016). The dry extract was dissolved in 100% methanol to a concentration of 100mg/ml. The experiment consisted of four variants for each isolate: treatment with essential oil, treatment with methanolic extract, control treatment with the solvent (100% methanol), and control without treatment. Three biological replications were set for each variant. Prior to the application of *T. longedentatus* EO and ME, Petri dishes (9 mm) with corresponding basal agar media were inoculated with fresh mycelium agar blocks (≈2×2 mm). The isolates were incubated overnight for the initiation of simultaneous growth. The next day, two drops (15 µl each) of the methanol extracts were dripped directly on the agar media into the Petri dish with the corresponding isolate, at equal distances from its center. In the same way, the essential oil was applied with a drop volume of 2 µl. The Petri dishes were cultivated in a climatic chamber in darkness at 25 °C. The results were documented after 6 days. Photographs (Canon EOS 4000D) of all mycelial colonies were taken, and their mycelial growth areas were measured, using the image analysis program ImageJ (Schneider et al., 2012). On the basis of the obtained data (average mycelial growth area for each treatment/isolate variant) was calculated the percentage of inhibition (Zygadlo et al., 1994) by the equation: $\%IMG = 100(C-T)\,C^{-1}$, where %IMG is percentage of inhibition of the mycelial growth, C is the area of the fungal colony without treatment (control), and T is the area of the fungal colony with treatment.

Results and Discussion

Phytochemical analysis

The essential oil profile of the *T. longedentatus* sample from cultivated areas is presented in the GC/MS chromatogram in Figure 1. Citral isomers – neral (26.8%) and geranial (43.1%) were identified as the main components of the EO profile that determined the lemon-like odor of the oil. Geranyl acetate (5.8%), 1,8-Cineole (eucalyptol) (2.9%), linalool (3.8%), and camphor (2.4%) were also detected in significant amounts.

The established essential oil profile agrees with those reported for the species from the natural population (Aneva et al., 2019). In the sample from the cultivated area, the citral isomer content is slightly higher than in the sample from the natural population. The difference in the quantity can be attributed to local environmental conditions and seasonal variability because many scientific reports show these factors influence essential oil composition (Rana et al., 2009, 2016). There are many reports that citral isomers exhibited a broad spectrum of biological activity, including antifungal (Leite et al., 2014; Aprotosoaie et al., 2019; Wang et al., 2019; Gutiérrez-Pacheco et al., 2023; Zheng et al., 2021). Due to their high content in the studied essential oil, we assume that, to a large extent, they determine the established antipathogenic activity.

In the methanolic extract, mono- and di-saccharides were found in large amounts. Rosmarinic acid, chlorogenic acid, feruloylquinic acid, oleanolic acid, ursolic acid, micromeric acid, arbutin, geranic acid, and hydroquinone were determined as the main secondary metabolites. Chlorogenic acid and rosmarinic acid were found to be the predominant phenolic compounds. Bioactive compounds – 4-thujanol, linalool, carvacrol, and hydroquinone are poorly represented quantitatively in the methanolic extract. The detailed results are presented in Table 2.

Antifungal Activity

The results from the bioassay are visualized in Figure 2 and in the chart in Figure 3.

Essential oil treatment

Among the fungal isolates, the development of *Botritys cinerea* was constrained to the highest extend with 73 % in-

Table 1. Detailed information about the plant pathogens used in this study

Name of the isolate	Host plant	Date of isolation and origin of the sampled plant	Species	NCBI GenBank accession number
B. c.	*Fragaria* sp. plant	2020, Razgrad, Bulgaria	*Botrytis cinerea*	PQ345538
F. o. Rd	*Fragaria* sp. plant	2020, Razgrad, Bulgaria	*Fusarium oxysporum* var. *fragariae*	PQ345539
Ciklama1	*Cyclamen* sp. plant	2022, Sofia, Bulgaria	*Fusarium oxysporum* var. *cyclaminis*	PQ345541
Troyan 2	*Rubus* sp. rhizosphere soil	2016, Troyan, Bulgaria	*Phytophthora cryptogea*	na*
Roza 1	*Rosa* sp. rhizosphere soil	2023, Sofia, Bulgaria	*Phytophtora nicotianae*	PQ460002

*na – not applicable

Source: Authors' own elaboration

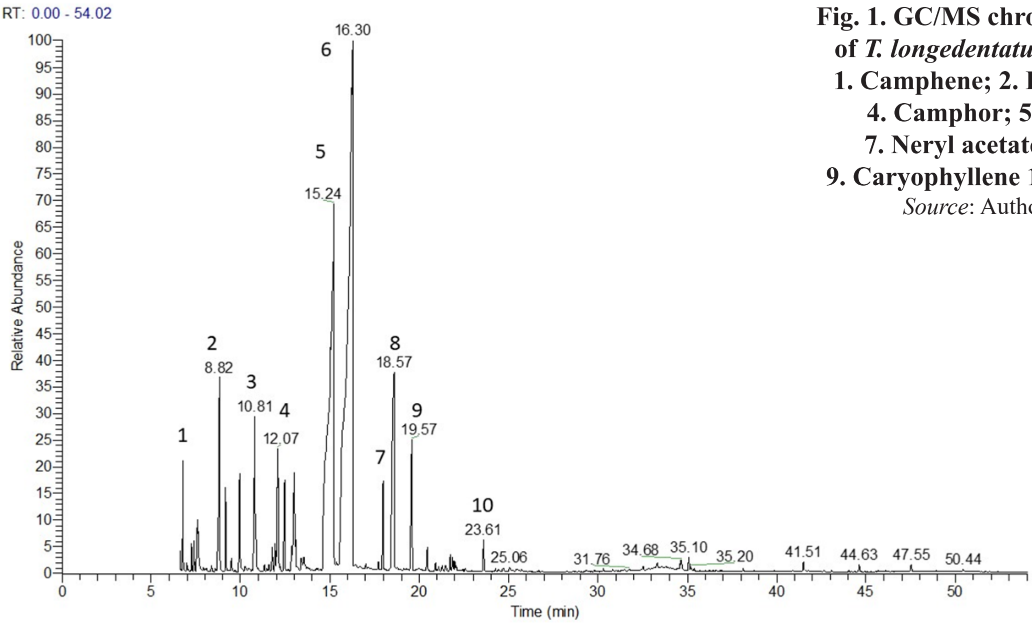

Fig. 1. GC/MS chromatogram of EO profile of *T. longedentatus* from cultivated areas 1. Camphene; 2. Eucalyptol; 3. Linalool; 4. Camphor; 5. Neral; 6. Geranial; 7. Neryl acetate; 8. Geranyl acetate 9. Caryophyllene 10. Caryophyllene oxide
Source: Authors' own elaboration

Table 2. Compounds identified in the methanolic extract of the studied sample by GC/MS

RI	Compouds	Area %
1202	4-Thujanol	0.25
1227	Linalool	0.32
1286	Phosphoric acid	0.21
1289	Glycerol	3.66
1321	Succinic acid	0.67
1339	Carvacrol	0.08
1345	Fumaric acid	0.12
1396	Hydroquinone	0.36
1448	Geranic acid	0.75
1488	Malic acid	1.13
1515	Pyroglutamic acid	0.36
1637	4-Hydroxybenzoic acid	0.01
1800	Fructose 1	3.97
1823	Fructose 2	6.99
1835	Protocatechuic acid	0.02
1843	Quinic acid	4.75
1882	Glucose	3.53
1939	Hydroxycinnamic acid	0.04
1945	Glucose	4.35
2041	Hexadecanoic acid (C16:0), *palmitic acid*	1.45
2080	myo Inositol	3.16
2104	Ferulic acid	0.01
2141	Caffeic acid	0.04
2212	Octadecadienoic acid (C18:2)	0.35
2218	Octadecatrienoic acid, (C18:3), *α-linolenic acid*	0.37
2238	Octadecanoic acid (C18:0), *stearic acid*	0.25
2561	Arbutin	1.44
2628	Sucrose	25.51
3074	5-O-Feruloylquinic acid	1.02
3091	Chlorogenic acid	2.72
3138	4-O-Feruloylquinic acid	0.98
3455	Rosmarinic acid	2.22
3525	Oleanolic acid	4.28
3530	Ursolic acid	4.76
3642	Micromeric acid	0.71

Source: Authors' own elaboration

hibition of the mycelial growth (%IMG). *F. oxysporum* f. sp. *fragariae* and *F. oxysporum* f. sp. *cyclaminis* were also affected, expressed in 46% IMG and 36% IMG, respectively. *Alternaria alternata* growth was not significantly disturbed. The growth of both *Phytophthora* species was also hindered, resulting in 75% IMG of *P. nicotianae* and 64% IMG of *P. cryptogea*.

Methanolic extract treatment

The controls to which 100% methanol was applied did not differ from the controls without treatment. As shown in Figures 2 and 3, the *T. longedentatus* extracts did not inhibited the mycelial growth of the tested isolates, as opposed to the essential oil. The results from the phytochemical analysis revealed that in the methanol extract, the content of terpenes,

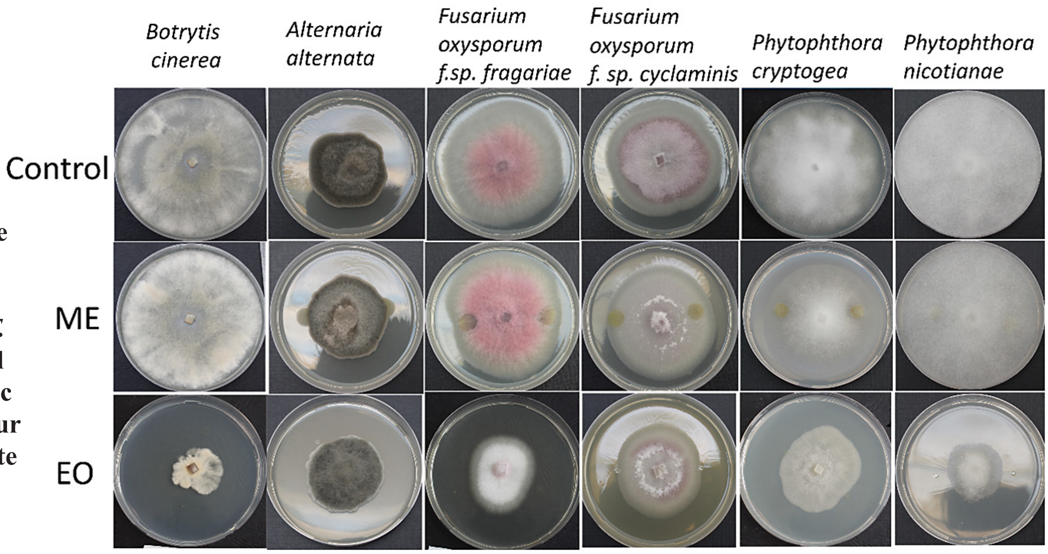

Fig. 2. Results from the *in vitro* evaluation of the growth inhibitory activity of cultivated *T. longedentatus* essential oil (EO) and methanolic extract (ME) against four fungal and two oomycete isolates
Source: Authors' own elaboration

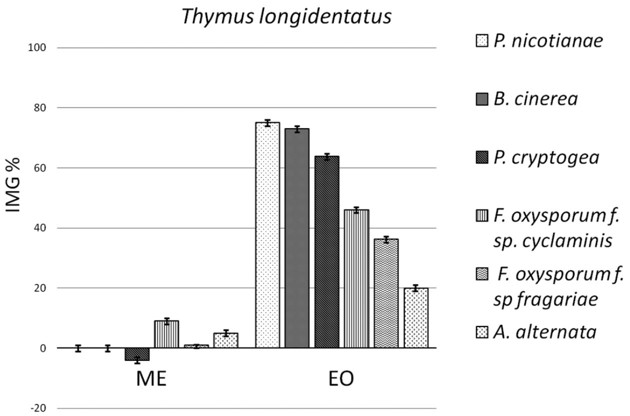

Fig. 3. Inhibition of the mycelial growth (IMG%) of the plant pathogens investigated in this study, based on the average mycelial colony area (cm^2) in the treated with EO and ME variants compared to the untreated controls, according to Zygadlo et al., 1994 equation. Bars represent the standard error of the mean.
Source: Authors' own elaboration

which are usually carriers of high antimicrobial activity is low. This probably determines the lower activity of the methanol extract, compared to the EO,whose main components, Citral isomers – neral (26.8%) and geranial (43.1%), have been previously recognized as a promising agents for the clinical control of the human skin fungal pathogen *Trichophyton rubrum* (Zheng et al., 2021).

The monoterpene aldehyde Citral is present in the majority of lemongrass essential oils. It is also found in a wide diversity of plant leaves and fruits, and is accepted as safe and is commonly used as a citrus base flavoring in different products (Gutiérrez-Pacheco et al., 2023). Most of the studies on Citral antimicrobial activity are focused on bacteria, human pathogens, and problematic microbes for the food industry. It was found that the essential oil of the wild fragrant plant *Litsea cubeba*, distributed in southern China, is rich in citral and can induce systemic acquired resistance against tobacco mosaic virus and plant fungal diseases (Jiang et al., 2022). Another study on the inhibitory activity of both Thyme essential oil and citral demonstrates their promising potential if used as alternative control measures against the growth of tomato root rot pathogens (El-Mohamedy et al., 2013). The antifungal and antibacterial properties of rich in citral essential oils, as well as their mechanisms of action, continue to be of great interest (Cai et al., 2019; Gutiérrez-Pacheco et al., 2023). More studies on their possible applications in agriculture will complete the knowledge about these plant secondary metabolites.

Conclusions

The essential oil of *Thymus longedentatus* is rich on Citral isomers – neral and geranial, for which have been previously found to possess strong antimicrobial properties. The species is naturally distributed in Bulgaria, and its high breeding and cultivation potential is acknowledged. The conducted bioassays revealed that the essential oil from *T. longedentatus* exhibits a strong inhibiting effect against the important plant pathogens *Botrytis cinerea, Phytophthora nicotiane, and Phytophthora cryptogea*. Oppositely, the methanol extract, in which the Citral isomers were not found, did not affect the mycelial growth of the tested plant pathogens. The potency of the essential oil rich on Citral isomers, against oomycete and fungal plant pathogens has been demonstrated.

Acknowledgments

This work was supported by the Centre of Competence "Sustainable Utilization of Bio-resources and Waste of Medicinal and Aromatic Plants for Innovative Bioactive Products" (BIORESOURCES BG) project BG16RFPR002-1.014-0001, funded by the Program "Research, Innovation and Digitization for Smart Transformation" 2021-2027, co-funded by the EU.

Conflict of interest

No potential conflict of interest was reported by the authors.

References

Adams, R. (2007). Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. *Carol Stream*, *16*, 65 – 120.

Aneva, I., Trendafilova, A., Nikolova, M., Todorova, M. & Georgieva, K. (2019). Composition of the essential oil of Thymus longedentatus (Degen & Urum.) Ronniger endemic of the Balkans. (Composición del aceite esencial de Thymus longedentatus (Degen & Urum.)) Ronniger endemic de los Balcanes. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, *18*(2), 197 – 203 Article 2. https://doi.org/10.37360/blacpma.19.18.2.13. *Latin American and Caribbean Bulletin of Medicinal and Aromatic Plants.*

Aprotosoaie, A. C., Luca, V. S., Trifan, A. & Miron, A. (2019). Chapter 7 – Antigenotoxic Potential of Some Dietary Non-phenolic Phytochemicals. In Atta-ur-Rahman (Ed.). *Studies in Natural Products Chemistry*, *60*, 223 – 297. Elsevier. https://doi.org/10.1016/B978-0-444-64181-6.00007-3.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71 – 79. https://doi.org/10.1016/j.jpha.2015.11.005.

Bouchra, C., Achouri, M., Hassani, L. M. I. & Hmamouchi, M. (2003). Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against Botrytis cinerea Pers: Fr. *Journal of Ethnopharmacology*, *89*(1), 165 – 169. https://doi.org/10.1016/S0378-8741(03)00275-7.

Cai, R., Hu, M., Zhang, Y., Niu, C., Yue, T., Yuan, Y. & Wang, Z. (2019). Antifungal activity and mechanism of citral, limonene and eugenol against Zygosaccharomyces rouxii. *LWT*, *106*, 50 – 56. https://doi.org/10.1016/j.lwt.2019.02.059.

Dėnė, L. & Valiuškaitė, A. (2021). Sensitivity of Botrytis cinerea Isolates Complex to Plant Extracts. *Molecules*, *26*(15), Article 15. https://doi.org/10.3390/molecules26154595.

El-Mohamedy, R. S. R., Abdel-Kader, M. M. & Abd-El-Kareem, F. (2013). Essential oils, inorganic acids and potassium salts as control measures against the growth of tomato root rot pathogens in vitro. *International Journal of Agricultural Technology*, *9*(6), 1507 – 1520.

Erwin, D. C. & Ribeiro, O. K. (1996). Phytophthora diseases worldwide. *Phytophthora Diseases Worldwide.* https://www.cabdirect.org/cabdirect/abstract/19971001256.

Georgiev, B., Nikolova, M., Aneva, I., Dzhurmanski, A., Sidjimova, B. & Berkov, S. (2022). Plant products with acetylcholinesterase inhibitory activity for insect control. *BioRisk*, *17*, 309 – 315. https://doi.org/10.3897/biorisk.17.77052.

Gholamnezhad, J. (2019). Effect of plant extracts on activity of some defense enzymes of apple fruit in interaction with Botrytis cinerea. *Journal of Integrative Agriculture*, *18*(1), 115 – 123. https://doi.org/10.1016/S2095-3119(18)62104-5.

Gutiérrez-Pacheco, M. M., Torres-Moreno, H., Flores-Lopez, M. L., Velázquez Guadarrama, N., Ayala-Zavala, J. F., Ortega-Ramírez, L. A. & López-Romero, J. C. (2023). Mechanisms and Applications of Citral's Antimicrobial Properties in Food Preservation and Pharmaceuticals Formulations. *Antibiotics*, *12*(11), 1608. https://doi.org/10.3390/antibiotics12111608.

Haist, G., Sidjimova, B., Yankova-Tsvetkova, E., Nikolova, M., Denev, R., Semerdjieva, I., Bastida, J. & Berkov, S. (2024). Metabolite profiling and histochemical localization of alkaloids in *Hippeastrum papilio* (Ravena) van Scheepen. *Journal of Plant Physiology*, *296*, 154223. https://doi.org/10.1016/j.jplph.2024.154223.

Jackson, R. S. (2014). Botrytis. *In C. A. Batt & M. L. Tortorello* (Eds.), *Encyclopedia of Food Microbiology (Second Edition)* (Second Edition, 288 – 296). Academic Press. https://doi.org/10.1016/B978-0-12-384730-0.00042-2.

Jiang, Y., Ji, X., Zhang, Y., Pan, X., Yang, Y., Li, Y., Guo, W., Wang, Y., Ma, Z., Lei, B., Yan, H. & Liu, X. (2022). Citral induces plant systemic acquired resistance against tobacco mosaic virus and plant fungal diseases. *Industrial Crops and Products*, *183*, 114948. https://doi.org/10.1016/j.indcrop.2022.114948.

Latijnhouwers, M., de Wit, P. J. G. M. & Govers, F. (2003). Oomycetes and fungi: Similar weaponry to attack plants. *Trends in Microbiology*, *11*(10), 462 – 469. https://doi.org/10.1016/j.tim.2003.08.002.

Leite, M. C. A., Bezerra, A. P. de B., Sousa, J. P. de, Guerra, F. Q. S. & Lima, E. de O. (2014). Evaluation of Antifungal Activity and Mechanism of Action of Citral against Candida albicans. *Evidence-Based Complementary and Alternative Medicine*, (1), 378280. https://doi.org/10.1155/2014/378280.

Nikolova, M., Aneva, I., Zhelev, P. & Berkov, S. (2024). Analysis of exudate compounds of Thymus species. *Plant Biosystems – An International Journal Dealing with All Aspects of Plant Biology*, *158*(1), 142 – 146. https://doi.org/10.1080/11263504.2023.2293034.

Nikolova, M. T., Aneva, I. Y., Zhelev, P. & Berkov, S. H. (2023). Phenolic Acids and Free Radical Scavenging Activity of Endemic Thymus Species. *Biology Bulletin*, *50*(5), 895 – 900. https://doi.org/10.1134/S1062359023601969.

Nikolova, M., Traykova, B., Yankova-Tsvetkova, E., Stefanova, T., Dzhurmanski, A., Aneva, I. & Berkov, S. (2021). Herbicide Potential of Selected Essential Oils From Plants of Lamiaceae and Asteraceae Families. *Acta Agrobotanica*, *74*. https://doi.org/10.5586/aa.7411.

Panabieres, F., Ali, G. S., Allagui, M. B., Dalio, R. J. D., Gudmestad, N. C., Kuhn, M.-L., Roy, S. G., Schena, L. & Zampounis, A. (2016). Phytophthora nicotianae diseases worldwide: New knowledge of a long-recognised pathogen. *Phytopathologia Mediterranea*, *55*(1), Article 1. https://doi.org/10.14601/Phytopathol_Mediterr-16423.

Perincherry, L., Lalak-Kańczugowska, J. & Stępień, Ł. (2019). Fusarium-Produced Mycotoxins in Plant-Pathogen Interactions. *Toxins*, *11*(11), Article 11. https://doi.org/10.3390/toxins11110664.

Rana, V. S., Das, M. & Blazqeuz, M. A. (2016). Essential Oil Yield, Chemical Composition, and Total Citral Content of Nine Cultivars of Cymbopogon Species from Western India. *Journal of Herbs, Spices & Medicinal Plants*, *22*(4), 289 – 299. https://doi.org/10.1080/10496475.2016.1201718.

Rana, V. S., Devi, Ch. B., Verdeguer, M. & Blázquez, M. A. (2009). Variation of Terpenoids Constituents in Natural Population of Cinnamomum tamala (L.) Leaves. *Journal of Essential Oil Research*, *21*(6), 531 – 534. https://doi.org/10.1080/10412905.2009.9700237.

Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671 – 675. https://doi.org/10.1038/nmeth.2089.

Traykova, B., Nikolova, M., Stanilova, M. & Aneva, I. (2024). Growth and GC/MS based metabolic profile of the hydroponically propagated species of genus Thymus (Lamiaceae). *Phytologia Balcanica*, *30*. https://doi.org/10.7546/PhB.30.1.2024.8.

Traykova, B., Stanilova, M., Nikolova, M. & Berkov, S. (2019). Growth and Essential Oils of Salvia officinalis Plants Derived from Conventional or Aeroponic Produced Seedlings. *Agriculturae Conspectus Scientificus*, *84*(1), 77 – 81.

Wang, L., Jiang, N., Wang, D. & Wang, M. (2019). Effects of Essential Oil Citral on the Growth, Mycotoxin Biosynthesis and Transcriptomic Profile of Alternaria alternata. *Toxins*, *11*(10), 553. https://doi.org/10.3390/toxins11100553.

Zheng, Y., Shang, Y., Li, M., Li, Y. & Ouyang, W. (2021). Antifungal Activities of cis-trans Citral Isomers against Trichophyton rubrum with ERG6 as a Potential Target. *Molecules*, *26*(14), 4263. https://doi.org/10.3390/molecules26144263.

Zygadlo, J. A., Guzman, C. A. & Grosso, N. R. (1994). Antifungal Properties of the Leaf Oils of Tagetes minuta L. and T. filifolia Lag. *Journal of Essential Oil Research*, *6*(6), 617 – 621. https://doi.org/10.1080/10412905.1994.9699353.

Received: **December, 05, 2024**; *Approved*: **January, 05, 2025**; *Published*: **February, 2026**