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EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF *Origanum vulgare* L. ESSENTIAL OIL AND ITS POTENTIAL APPLICATIONS IN PLANT PROTECTION

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Abstract

In the current context of promoting sustainable agriculture, it is important to find alternative solutions to synthetic pesticides in the fight against phytopathogenic microorganisms. Volatile oils in general, and those of plants from the Lamiaceae family in particular, through their chemical profile, have proven their antifungal and bactericidal efficacy, offering an ecological solution for the protection of agricultural crops, without affecting the environment. The antimicrobial activity of oregano oil on plant pathogens is closely related to the oil concentration, chemotype, as well as the synergy of biologically active compounds. The scientific paper is a review and aims to present the comparative effectiveness of the essential oil obtained from species and subspecies of Origanum vulgare L. on the main pathogenic bacteria and fungi, in correlation with the chemical profile.

Key words*: antifungal effect, antibacterial effect, oregano.*

INTRODUCTION

Plant pathogens, such as fungi, bacteria, phytoplasmas and viruses, threaten agricultural and forestry production, causing diseases with significant economic and environmental impact (Camele et al., 2012).

Plant protection products, including herbicides, fungicides, and insecticides, are widely used to combat plant diseases, weeds, and pests. Excessive use of plant protection products can lead to microorganisms, weeds and insect resistance. Even though plant protection products are effective, they are not exclusive to target pathogens and can negatively affect soil, human and animal commensal and beneficial microorganisms, leaving toxic residues and contributing to environmental pollution (Onaran et al., 2014). According to the research of Ashraf & Zuhaib (2013), only 0.1% of synthetic chemicals reach the targeted pathogens, and the remaining 99.9% contaminate the environment. Globalisation and climate change have exacerbated these problems by facilitating emerging diseases' rapid emergence and spread. A trend exists towards reducing dependence on conventional pesticides and implementing integrated pest management (IPM) (Bonaterra et al., 2022). Modern agriculture is increasingly replacing synthetic chemicals with environmentally friendly biocontrol solutions, such as botanicals and industrial by-products used as organic fertilisers, which can inhibit pathogens and improve soil and crop quality (Greff et al., 2023).

Essential oils (EO), respectively, their chemical compounds, have become promising substitutes for traditional plant protection products due to their recognised antimicrobial properties, single or in combination duet synergistic reactions between constituents (Álvarez-García et al., 2023; Košćak et al., 2023). However, the composition of essential oils obtained from the same plant species can vary significantly depending on numerous factors, including chemotype**,** plant growing conditions, and genetic variability of plant species (Gruľová et al., 2020; Vasinauskienė et al., 2006; Gurita et al., 2019; Beicu et al., 2023). Fungi and bacteria cause various complex plant diseases. In the juvenile stage of plants, they can suffer from rot and wilting, and in adulthood, from moulds (Kosakowska et al., 2024).

Phytopathogenic fungi are responsible for about 30% of all diseases affecting crops and can significantly impact both during cultivation and, after harvesting, during the storage period (Raveau et al., 2020).

The antimicrobial activity of essential oils on bacteria is based on several effective and complex mechanisms. They degrade the bacterial cell wall and cytoplasmic membrane structure, increasing permeability by modifying fatty acids, polysaccharides, and phospholipid layers. These lead to reduced membrane potential, leakage of ions and cellular contents, reduction of ATP, and eventually cell lysis (Sotelo et al., 2023; Bălășoiu (Jigău) et al., 2024). The main mechanisms of action of essential oils on fungi are given by inhibition of cell wall formation, destruction of the cell membrane by inhibition of ergosterol synthesis, dysfunction of fungal mitochondria by inhibition of proton pumps and mitochondrial electron transport, inhibition of efflux pumps, cell division and protein or RNA/DNA synthesis (Raveau et al., 2020; Nazzaro et al., 2017; Taheri et al., 2023).

The Lamiaceae family comprises about 7200 plants organised into 236 genera, most of which are aromatic species containing essential oils, which can be easily cultivated and multiplied. The genus *Origanum* includes 10 sections, comprising 43 plant species, six subspecies, three botanical varieties, and 18 natural hybrids (Ietswaart, 1980; Kokkini, 1997; Mutu, 2020; Maithani et al., 2023). Due to its biological activities, *Origanum vulgare* L. (elderberry, oregano) is a medicinal species among the most commercially important plants (Morshedloo et al., 2017; Lukas et al., 2015). From the taxonomy point of view, Ietswaart described six subspecies of *Origanum vulgare*, such as ssp. *vulgare* L., ssp. *glandulosum* (Desf.) Ietsw., ssp. *gracile* (K. Koch) Ietsw., ssp. *hirtum* (Link) Ietsw., ssp. *virens* (Hoffmanns. & Link) Ietsw., ssp. *viridulum* (Martin-Donos) Nyman (Ietswaart, 1980; Kokkini, 1997; Lotti et al., 2019; Lukas et al., 2015; Mutu, 2020; Kaouther et al., 2017; Mechergui et al., 2016). Oregano (*Origanum vulgare* L.) grows wild in Mediterranean areas and is widely distributed naturally in Europe and North Africa (Kokkini, 1997; Lotti et al., 2019; Skoufogianni et al., 2019; Kaouther et al., 2017; Marrelli et al., 2018; Mechergui et al., 2016).

This paper aims to highlight the mode of oregano essential oils action (O.E.O.s) on phytopathogenic bacteria and fungi affecting cereals. The paper will also highlight the benefits of using oregano essential oil in grain protection, highlighting their potential in sustainable agriculture.

CHEMICAL COMPOSITION OF *Origanum vulgare* L. ESSENTIAL OIL

Essential oils are oily liquids extracted by hydrodistillation or solvent extraction from different parts of plants (leaves, stems, roots, seeds, fruit, flowers, resins/bark), containing over 300 compounds (Dhifi et al., 2016; Raveau et al., 2020; Taheri et al., 2023). Essential oils are soluble in organic solvents (alcohol, ether) and insoluble in inorganic solvents (water) (Dhifi et al., 2016), contain 90-95% volatile components and 1-10% non-volatile components (Malik, 2019). Oils are vital components of many plants, located in secretory trichomes or mucous canals, and protect plants from insects, bacteria, and fungi attack (Harčárová et al., 2021; Horablaga et al., 2023).

The quantitative and qualitative profile of essential oils is influenced by environmental factors, soil pollution, water stress, salinity (Morshedloo et al., 2017), genotype, cultivation conditions and geographical locations (Lotti et al., 2019; Gurita et al., 2019; Skoufogianni et al., 2019; Raveau et al., 2020), leading to notable variation between populations of the genus *Origanum*.

Skoufogianni et al. (2019), observed significant seasonal variation in the quantitative profile of essential oil from the Crete and Amorogos Islands (Greece). In the autumn, the plant essential oil content varied between 1.0-3.1%, while in the summer it varied between 4.8-8.2%.

From a chemical point of view, the main constituents of essential oils are terpenes, terpenoids, phenylpropanoids and other constituents (lipids, sulphur derivatives, amino acids: alanine, isoleucine, leucine, valine and methionine) (Masyita et al., 2022; Butta et al., 2023).

Terpenes (terpenoids, isoprenoids) are the main compounds of essential oils and constitute the most diverse class of chemical compounds among the plant's secondary metabolites. They are classified according to the number of isoprene contain: hemiterpenes (C_5H_8), monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), norisoprenoids (C_{13}); depending on the biochemical structure: acyclic, cyclic, phenolic monoterpenes (Mutu, 2020; Marrelli et al., 2018; Morshedloo et al., 2017; Taheri et al., 2023; Butta et al., 2023; Oliva et al., 2015; Nazzaro et al., 2017). Terpenoids are terpenes with oxygen molecules added or with modified methyl groups, the most known being: thymol, carvacrol, linalool and menthol (Nazzaro et al., 2013). Moreover, essential oils also contain terpene-free compounds, such as eugenol, cinnamaldehyde, and safrole, produced via the phenylpropanoid pathway. Biogenetically, terpenoids and phenylpropanoids have different precursors and biosynthesis pathways: terpenoids are formed via the mevalonate and the deoxyxylulose phosphate pathway, and phenylpropanoids are generated via the shikimate pathway (Dhifi et al., 2016).

Numerous studies have been carried out on the *Origanum vulgare* L. essential oil, highlighting the biochemical variability of the six subspecies: ssp. *vulgaris* contains carvacrol and thymol; ssp. *hirtum* is rich in carvacrol, thymol, *p*-cymene and γ-terpinene; ssp. *glandulosum* is rich in monoterpene compounds (carvacrol, thymol and their derivatives); ssp. *gracile* contains acyclic compounds, carvacrol or sesquiterpenoids; ssp. *virens* is rich in acyclic compounds and sesquiterpenes; ssp. *viridulum* contains a high amount of sabinene (Mutu, 2020).

On the other hand, the results obtained by studying essential oils from Lithuania (Vilnius) have shown that the main constituents in ssp. *glandulosum* is carvacrol, ssp. *gracile* contains thymol and sabinen-germacren D., ssp. *virens* has increased amounts of germacrene D-sabinene, γ-terpinene, and ssp. *viride* is rich in linalyl acetate, β-caryophyllene and sabinene (Mockute et al., 2001).

The oils also have remarkable antimicrobial properties and can be found in all parts of aromatic plants (Imbrea et al., 2016; Álvarez-García et al., 2023; Maithani et al., 2023). Volatile oils allow plants to regulate the environment, acting as a chemical signal to attract pollinators, inhibit seed germination, communicate between plants, and repel predators (Taheri et al., 2023).

ANTIBACTERIAL ACTIVITY OF OREGANO ESSENTIAL OIL AGAINST PHYTOPATHOGENIC BACTERIA

The soil may contain important bacterial pathogens, including *Agrobacterium*, *Pectobacterium*, *Pseudomonas*, *Ralstonia* and *Xanthomonas*. However, these microorganisms are conditionally pathogenic; the disease occurrence is influenced by the presence of an open wound or natural holes to invade the host plant. The bacteria determine morphological changes in the host plant's root system, invade the vessels of the xylem and cause wilting symptoms, eventually leading to the death of the plants (Greff et al., 2023).

Chemical composition of *Origanum vulgare* ssp. *hirtum* essential oil (Greek oregano), is characterised by the predominance of monoterpenes, respectively of phenolic monoterpenes such as carvacrol (35.79%), of monoterpene hydrocarbons (*p*-cymene - 17.01% and γ-terpinene - 13.76%), and oxygenated monoterpenes (6.65%). On the other hand, the essential oil of *Origanum vulgare* ssp. *vulgare* (common oregano) is dominated by oxygenated sesquiterpenes (35.80%), mainly caryophyllene oxide (18.89%), but it also contains sesquiterpene hydrocarbons and oxygenated monoterpenes in considerable quantities. Due to these compounds, Greek oregano essential oil presents antimicrobial activity against *Pseudomonas syringae* (see Table 1) and *Xanthomonas hortorum.* According to the study, Greek oregano essential oil has more antimicrobial activity against *Xanthomonas hortorum* than common oregano essential oil (Kosakowska et al., 2024).

Similarly, Gruľová et al. (2020), demonstrated significant antibacterial activity of thymol (76%) chemotype oregano essential oil against *Pseudomonas savastanoi* and *Xanthomonas campestris* (see Table 2). Moreover, Sotelo et al. (2023), observed that oregano essential oils

inhibited all phytopathogenic *Pseudomonas syringae* strains, with MIC values between 11.56 mg·mL^{-1} to 92.5 mg·mL^{-1}.

Other studies demonstrated the effect of oregano essential oils against pathogenic factors of bacteria. Carezzano et al. (2017), investigated the impact of *Origanum vulgare* L. essential oils on some pathogenic factors of *Pseudomonas syringae*, including anti-toxin and anti-biofilm activities. The results showed that oregano essential oil strongly inhibited biofilm formation and the production of coronatin, syringomycin, and tabtoxin at MIC values between 5.8 and 11.6 mg·mL^{-1}. Moreover, the essential oil also demonstrated complete inhibition (100%) of syringomycin production in all *Pseudomonas syringae* strains at concentrations between 0.003 and 0.11 mg·mL^{-1}.

Table 1. Antibacterial effect of oregano essential oil against *Pseudomonas* ssp.

O.E.O.	Bacterial strain	Methods	Results	Major essential oil compounds identified by GC-MS / GC-FID	References
Origanum vulgare	*Pseudomonas savastanoi* pv. *glycinea*	Disk-diffusion	MIC = 1600 ppm MBC = 3200 ppm Inhibition of bacteria growth by 2.0±0.8 mm	Carvacrol 62.37± 0.01%, γ-terpynen 4.85± 0.002%, thymol 3.15± 0.055%	Tarakanov & Dzhalilov, 2022
	Pseudomonas savastanoi	Disk-diffusion	Inhibited bacterial growth at 10.000 ppm	Thymol 76.0%, *p*-cymene 5.7%, carvacrol 3.2%, linalool 2.6%	Gruľová et al., 2020
	Pseudomonas syringae	Broth microdilution method	MIC = 5.8 - 46.3 mg·mL^{-1} MBC = 0.022 - 0.36 mg·mL^{-1} O.E.O. inhibited the production of coronatine at concentrations between 0.045 mg·mL^{-1} to 0.09 mg·mL^{-1}	Carvacrol, *p*-cymene, c-terpinene	Carezzano et al., 2017
	Pseudomonas syringae pv. *atropurpurea*		MIC = 11.9 mg·mL^{-1} MBC = 0.012 mg·mL^{-1} O.E.O. inhibited tatoxin production at 0.006 mg·mL^{-1}		
	Pseudomonas savastanoi pv. *glycinea*		MIC = 5.8 mg·mL^{-1} MBC = 0.22 mg·mL^{-1}		
	Pseudomonas syringae pv *syringae*	Broth microdilution method	MIC = 23.1 - 46.3 mg·mL^{-1}	γ-terpinene 22.7%, carvacrol 19.7%, cis-sabinene hydrate 19.7%, *p*- cymene 11.5%	Oliva et al., 2015
	Pseudomonas syringae	Broth microdilution method	MIC = 11.56 - 46.26 mg·mL^{-1}	Cis-sabinene hydrate 20.99%, thymol 12.03%, carvacrol 11.39%	Sotelo et al., 2023
Origanum vulgare* spp. *vulgare	*Pseudomonas syringae*	Broth microdilution method	MIC = 4 µL·mL^{-1} MBC = 4 µL·mL^{-1}	Caryophyllene oxide 18.89%, β-caryophyllene 5.64%, β-cubebene 5.51%, carvacrol 3.97%	Kosakowska et al., 2024
Origanum vulgare* spp. *Hirtum			MIC = 0.125 µL·mL^{-1} MBC = 0.250 µL·mL^{-1}	Carvacrol 35.79%, *p*-cymene 17.01%, γ-terpinene 13.76%	

*O.E.O. (Oregano Essential Oil), MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration), GC-MS (Gas Chromatography-Mass Spectrometry), GC-FID (Flame Isomerism Detector for Gas Chromatography.

Table 2. Antibacterial effect of oregano essential oil against *Xanthomonas* ssp.

O.E.O.	Bacterial strain	Methods	Results	Major Essential Oil Components determined by GC-MS / GC-FID	References
Origanum vulgare	*Xanthomonas campestris*	Disk - diffusion	Inhibited growth at 10.000 ppm	Thymol 76.0%, *p*-cymene 5.7%, carvacrol 3.2%, linalool 2.6%	Gruľová et al., 2020
Origanum vulgare* spp. *vulgare	*Xanthomonas hortorum*	Broth microdilution	MIC = 2 μL·mL^{-1} MBC = 2 μL·mL^{-1}	Caryophyllene oxide 18.89%, β-caryophyllene 5.64%, β-cubebene 5.51%, carvacrol 3.97%	Kosakowska et al., 2024
Origanum vulgare* spp. *hirtum			MIC = 0.125 μL·mL^{-1} MBC = 0.250 μL·mL^{-1}	Carvacrol 35.79%, *p*-cymene 17.01%, γ-terpinene 13.76%	

*O.E.O. (Oregano Essential Oil), MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration), GC-MS (Gas Chromatography-Mass Spectrometry), GC-FID (Flame Isomerism Detector for Gas Chromatography).

ANTIFUNGAL EFFICACY OF OREGANO ESSENTIAL OIL AGAINST PHYTOPATHOGENIC FUNGI

Infections caused by fungal pathogens can be transmitted through agricultural and horticultural products, affecting the soil. Species such as *Fusarium*, *Verticillium*, *Rhizoctonia*, *Sclerotinia*, *Phytophthora* and *Pythium* can survive in soil and plant debris for long periods, forming resistant structures (sclerotia, microsclerotia, oospores, chlamydospores or hyphae). Some species can invade the host plant through roots and stems or spread rapidly among seedlings, causing root necrosis, vascular disease, rot, gall, and root and tuber proliferation (Greff et al., 2023).

The antifungal components of plant-derived essential oils have been intensively studied and can be classified in the following order: phenols-alcohols-aldehydes-ketones-ethers-hydrocarbons (Xiang et al., 2020; López et al., 2004). The active antifungal compounds are monoterpenes, sesquiterpenes, phenols, aldehydes and ketones. Constituents such as terpenoids, phenolic terpenes, and alcohols significantly increase the antifungal activity of volatile oils (Butta et al., 2023).

The inhibitory activity of oregano essential oil was demonstrated against *Aspergillus* spp., *Fusarium* spp. and *Penicillium ochrochloron*. After 13 and 10 days of incubation, in the presence of essential oil, *Aspergillus niger* and *Aspergillus flavus* reached the complete growth of the mycelium (4.75 cm). Instead, *Aspergillus* spp., *Penicillium ochrochloron* did not reach maximum growth, highlighting that this strain is less invasive than those studied (Střelková et al., 2021).

Similarly, Duan et al. (2024), highlighted that the carvacrol (66.01%) chemotype of the oregano essential oil inhibited the proliferation of *Aspergillus flavus* in wheat grain. The carvacrol disrupts the cell membrane and cell wall structure, causing mitochondrial dysfunction and preventing DNA replication. Studies conducted by Ji et al. (2022), demonstrated the efficacy of fumigation treatment with oregano essential oil (carvacrol ≥88.3%) on *Aspergillus flavus*, by inducing oxidative stress. The treatment resulted in a significant increase in the production of superoxide anions (from 1.57 to 9.14 times) and the degree of lipid peroxidation (from 2.89 to 11.06 times) in the fumigation interval of 2 to 8 hours at 45°C, resulting in decreased mitochondrial activity (by 50.17%, 64.21%, and 89.41%) and ATP production (by 21.55%, 60.34%, and 70.30%) over the 4 to 8-hour fumigation interval. In addition, oregano essential oil vapour inhibited metabolic activities related to protein and methylglyoxal synthesis and the production of aflatoxin AFB1 (AFB1) during 5 days of incubation.

Numerous studies have shown that the antifungal activity of volatile oils can be amplified by combining different essential oils to achieve a synergistic effect. For example,

Xiang et al. (2020), demonstrated the synergistic activity of cinnamon, oregano and lemon oils (COL-CEO) against the aflatoxin B1 production of (AFB1) *Aspergillus flavus*. The production of this toxin is reduced by 67.53% at a concentration of 0.6 µL/disc and by 72.68% at a concentration of 1.0 µL/disc (see Table 3). COL-CEOs also have synergistic effects when the three oils are combined in a ratio of 1:5:48.

Most essential oils of the *Origanum* subspecies (*Origanum vulgare* spp. *virens*, *Origanum vulgare* spp. *vulgare*) have demonstrated significant antifungal activity against *Fusarium verticillioides* due to their high content of phenolic compounds (see Table 4). These phenolic compounds are essential for fighting pathogens, as they affect the permeability of the cell membrane of fungi, leading to the loss of cellular components and inhibition of cellular metabolism (Pizzolitto et al., 2020).

Also**,** oregano essential oil inhibited (100%) mycelial growth of *Fusarium graminearum***,** at concentrations of 1000 µg/mL and 500 µg/mL. A lower concentration of essential oil inhibited a lower percentage of mycelial growth. A concentration of 100 µg/mL inhibited the mycelium growth of *Fusarium graminearum* strain by 37.4% and 40.7% for the *Fusarium graminearum* (Harčárová et al., 2021).

Oregano essential oil composed of trans-caryophyllene 30.729%, sabinene 18.16%, caryophyllene oxide 8.635%, germacrene-D 8.59% (see Table 5), inhibited the growth of *Penicillium aurantiogriseum* by 2 mm in diameter, 9 days after inoculation on a medium with a concentration of 0.25 $mg \cdot L^{-1}$ (Rus et al., 2016).

Table 3. Antifungal effect of oregano essential oil against *Aspergillus* ssp.

O.E.O.	Fungal strain	Methods	Results	Major essential oil compounds identified by GC-MS / GC-FID	References
Origanum vulgare	*Aspergillus flavus*	Agar well diffusion	MIC of carvacrol = 0.18 µL/mL	Carvacrol 66.01%, linalool 5.17%, thymol 3.51%, o-cymene 3.03%, *γ*-terpinene 2.54%	Duan et al., 2024
	Aspergillus flavus	Disk volatilisation	MIC = 2.0 µl/L	Thymol 54%, carvacrol%	Paster et al., 1995
	Aspergillus flavus	Broth microdilution	MFC = 312.5 mg/L	Carvacrol ≥88.3%	Ji et al., 2022
	Aspergillus flavus	Disk - diffusion	5.0 µl O.E.O. produces a 40.93% inhibition of fungal growth; Volumes of 10 µl, 20 µl, 50 µl, and 100 µl showed inhibitory effects on the production of aflatoxin B1 in soybeans by 54.4%, 88.68%, 86.94% and 88.16%	4-terpineol 44.11%, linalool 15.22%, α-terpineol 5.96%, *γ*-terpinene 5.02%	Esper et al., 2014
***Origanum vulgare* in vapor phase**	*Aspergillus flavus*	Disk-diffusion	Inhibits growth at 2.50 µL/full disc	Carvacrol 84.96%, thymol 13.26%	Xiang et al., 2020
	Aspergillus flavus	Disk-diffusion	MIC = 62.5 µL/L	Carvacrol 70%, *p*-cymene 11%, thymol 3%	Střelková et al., 2021

Table 3. Antifungal effect of oregano essential oil against *Aspergillus* ssp. - continuation

O.E.O.	Fungal strain	Methods	Results	Major essential oil compounds identified by GC-MS / GC-FID	References
Origanum vulgare* ssp. *hirtum	*Aspergillus flavus*	Broth microdilution	MIC = 450 ppm MFC = 600 ppm	Z-sabinene hydrate 23.03%, thymol 18.66%, γ-terpinene 7.12 %, terpinen-4-ol 6.20%	Camiletti et al., 2014
	Aspergillus flavus		MIC = 650 ppm MFC = 800 ppm		
Origanum vulgare* ssp. *vulgare	*Aspergillus flavus*		MIC = 550 ppm MFC = 1100 ppm	o-cymene 14.33%, terpinen-4-ol 12.55%, E-*β*-terpineol 10.46%, thymol 9.44	
	Aspergillus flavus		MIC = 550 ppm MFC = 700 ppm		
Origanum vulgare	*Aspergillus niger*	Disk volatilisation	MIC = 62.5 µL/L	Carvacrol 70%, *p*-cymene 11%, thymol 3%	Střelková et al., 2021
	Aspergillus niger	Disk - diffusion	The highest inhibition activity was observed at 1000 and 500 ppm	Thymol 76.0%, *p*-cymene 5.7%, carvacrol 3.2%, linalool 2.6%	Gruľová et al., 2020
	Aspergillus niger	Disk volatilisation	MIC = 2.0 µl/L	Thymol 54%, carvacrol%	Paster et al., 1995
	Aspergillus ochraceus				

*O.E.O. (Oregano Essential Oil), MIC (Minimum Inhibitory Concentration), MFC (Minimum Fungicidal Concentration), GC-MS (Gas Chromatography-Mass Spectrometry), GC-FID (Flame Isomerism Detector for Gas Chromatography)

Table 4. Antifungal activity of oregano essential oil against *Fusarium* ssp.

O.E.O.	Fungal strain	Methods	Results	Major essential oil compounds identified by GC-MS / GC-FID	References
Origanum vulgare	*Fusarium avenaceum*	Disk-diffusion	Inhibition of fungal growth by 77.4%	Thymol 29.60%, *p*-cymene 29.40%, β-terpinene 26.80%	Hanana et al., 2017
	Fusarium culmorum		Inhibition of fungal growth by 90.3%		
	Fusarium culmorum	Disk-diffusion	MIC< 0.8 µL/cm3 At 20% O.E.O. concentration: ERG has been reduced by 99.98%, ZEA has been reduced by 99.08%, and DON has been reduced by 99.26%		Perczak et al., 2019
			The concentration of ERG was reduced by 90.31%, ZEA was reduced by 62.79% and DON by 100.00%	Carvacrol ≤ 80%, thymol 2%	Perczak et al., 2020
Origanum vulgare* spp. *vulgare	*Fusarium culmorum*	Broth microdilution	MIC = 1 µL/mL MFC = 1 µL/mL	Caryophyllene oxide 18.89%, β-caryophyllene 5.64%, β-cubebene 5.51%, carvacrol 3.97%	Kosakowska et al., 2024

Table 4. Antifungal activity of oregano essential oil against *Fusarium* ssp. - continuation

O.E.O.	Fungal strain	Methods	Results	Major essential oil compounds identified by GC-MS / GC-FID	References
Origanum vulgare* spp. *hirtum	*Fusarium culmorum*	Broth microdilution	MIC = 0.032 µL/mL MFC = 0.250 µL/mL	Carvacrol 35.79%, *p*- cymene 17.01%, γ-terpinene 13.76%	Kosakowska et al., 2024
Origanum vulgare	*Fusarium graminearum*	Disk-diffusion	MIC< 0.8 µL/cm3 At 20% O.E.O. concentration: ERG has been reduced by 99.97%, ZEA has been reduced by 100.00%, and DON has been reduced by 99.70%.		Perczak et al., 2019
	Fusarium graminearum		The concentration of ERG was reduced by 83.24%, ZEA was reduced by 26.05% and DON by 100.00%	Carvacrol ≤ 80%, thymol 2%	Perczak et al., 2020
	Fusarium graminearum	Broth microdilution	MIC = 0.4 mg·mL^{-1} IE = 47.8%	Carvacrol 85±3%	Harčárová et al., 2021
	Fusarium graminearum		MIC = 0.4 mg·mL^{-1}		
	Fusarium graminearum	Disk volatilisation	MIC = 0.02 µl/mL MFC = 0.02 µl/mL		Rekanović et al., 2012
	Fusarium graminearum		MIC = 0.08 µl/mL MFC = 0.08 µl/mL		
	Fusarium sporotrichioides	Disk volatilisation	MIC = 62.5 µL/L MFC = 125 µL/L	Carvacrol 70%, *p*-cymene 11%, thymol 3%	Střelková et al., 2021
	Fusarium solani				
	Fusarium subglutinans	Disk - diffusion	Inhibition of fungal growth by 94.4%	Thymol 29.60%, *p*-cymene 29.40%, β-terpinene 26.80%	Hanana et al., 2017
	Fusarium verticillioides		Inhibition of fungal growth by 67.3%		
	Fusarium verticillioides	Disk - diffusion	40, 50, 100, 200 µl/l O.E.O. reduced mycelium growth by 75%. On day 20, oregano essential oil (30 ppm) decreased the production of fumonizin B1 (FB1)	Thymol 21.2%, terpineol 21.1%, γ-terpinene 9.1%	López et al., 2004
Origanum vulgare* spp. *vulgare	*Fusarium verticillioides*	Mathematical model using arbitrary descriptors based on EO components	MIC = 250 µL/L	Thymol 29.950%, cis-sabinene hydrat 24.654%, γ-terpinene 12.045%, terpinen-4-ol 7.764%	Pizzolitto et al., 2020
Origanum vulgare* spp. *virens	*Fusarium verticillioides*		MIC = 250 µL/L	Thymol 34.045, cis-sabinene hydrat 19.094%, γ-terpinene 6.811%, terpinen-4-ol 6.660%	

*O.E.O. (Oregano Essential Oil), MIC (Minimum Inhibitory Concentration), MFC (Minimum Fungicidal Concentration), IE = inhibitory effect, ERG (ergosterol), ZEA (zearalenone), DON (deoxynivalenol), GC-MS (Gas Chromatography-Mass Spectrometry), GC-FID (Flame Isomerism Detector for Gas Chromatography)

Table 5. Antifungal effect of oregano essential oil against *Penicillium* spp.

O.E.O.	Fungal strain	Methods	Results	Major essential oil compounds identified by GC-MS / GC-FID	References
Origanum vulgare	*Penicillium aurantiogriseum*	Poisoned medium method	MIC = 0.5 mg·L^{-1} MFC = 5 mg·L^{-1}	Trans-caryophyllene 30.729%, sabinene 18.16%, caryophyllene oxide 8.635%, germacrene-D 8.159%	Rus et al., 2016
	Penicillium ochrochloron	Disk volatilisation	MIC = 62.5 µL/L	Carvacrol 70%, *p*-cymene 11%, thymol 3%	Střelková et al., 2021
	Penicillium expansum	Disk - diffusion	The highest inhibition activity was in the case of 1000 and 500 ppm	Thymol 76.0%, *p*-cymene 5.7%, carvacrol 3.2%, linalool 2.6%	Gruľová et al., 2020
Origanum vulgare* ssp. *hirtum	*Penicillium oxalicum*	Poisoned medium method	MIC = 350 ppm MFC = 500 ppm	Z-sabinene hydrat 23.03%, thymol 18.66%, γ-terpinene 7.12 %, terpinen-4-ol 6.20%	Camiletti et al., 2014
	Penicillium minioluteum		MIC = 400 ppm MFC = 600 ppm		
Origanum vulgare* ssp. *vulgare	*Penicillium oxalicum*		MIC = 400 ppm MFC = 700 ppm	o-cymene 14.33%, terpinen-4-ol 12.55%, E-*β*-terpineol 10.46%, thymol 9.44%	
	Penicillium minioluteum		MIC = 500 ppm MFC = 600 ppm		

*O.E.O. (Oregano Essential Oil), MIC (Minimum Inhibitory Concentration), MFC (Minimum Fungicidal Concentration), GC-MS (Gas Chromatography-Mass Spectrometry), GC-FID (Flame Isomerism Detector for Gas Chromatography)

CONCLUSIONS

Plants and botanicals play a crucial role in promoting a sustainable and balanced agricultural system, which is important in environmental protection, biodiversity promotion, pest management, soil improvement, and reduction of dependence on synthetic products and toxicity. Sustainable agriculture aims to reduce the use of chemicals because most pathogens become resistant; thus, they are replaced by plant-derived products. Botanical substances, especially essential oils, are among the most widely used treatments against soil plant pathogens, having antibacterial and antifungal properties. Using less toxic essential oils than synthetic products represents an adequate alternative to controlling aflatoxins. Thus, oregano essential oil could protect against aflatoxin B1 in soybean and corn crops. This scientific research aimed to carry out an analysis of oregano essential oil, highlighting the effects and mode of antibacterial and antifungal action, as well as the mode of action of the chemical components identified in their composition, on the bacteria and fungi present in cereals.

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