
# The contribution of Mentha, Lavandula and Thymus genera to sustainable agriculture as antibacterial, antifungal and soil ameliorative agents

**Anastasios Alatzidis**

**SCHOOL OF ECONOMICS, BUSINESS ADMINISTRATION & LEGAL STUDIES**

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Student Name: Anastasios Alatzidis

SID: 4401180001
Supervisor: Associate Prof. Efimia Papatheodorou


I hereby declare that the work submitted is mine and that where I have made use of another's work, I have attributed the source(s) according to the Regulations set in the Student's Handbook.

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## Abstract

This dissertation was written as part of the MSc in Sustainable Agriculture and Business at the International Hellenic University.

The aim of this study was to evaluate the role of Medicinal Aromatic Plants (MAPs) as a multifunctional tool in sustainable agriculture, focusing on some of the most common MAPs in Greece which are considered the species from Mentha, Lavandula and Thymus genera. For this purpose two approaches were investigated through literature.

a) The evaluation of essential oils' (EOs) efficacy on agricultural application against bacterial and fungal plant pathogens, that cause great damages in crops and post harvested commodities. The antimicrobial properties of essential oils offer the prospect of using them as natural biodegradable pesticides with the advantage of social acceptance due to environmental friendly characteristics.

Furthermore it was attempted to assess the limitations followed their application. These involve the high essential oils' volatility and their short shelf life.

b) The interaction between MAPs and soil. It was investigated whether the MAPs can improve soil quality by enhancing the nutrient cycling. This involves mainly the effect of MAPs cultivation on total soil microbial biomass and soil enzymes activity.

The results showed that on both approaches the MAPs can provide significant advantages to sustainable agriculture. Despite the variance of the efficacy observed, the essential oils and extracts of Mentha, Thymus, and Lavandula species were proved strong antibacterial and antifungal agents against several crops and stored products pathogens.

Furthermore, their cultivation demonstrated that can improve the physical, chemical and biological properties of the soil. Thus, they have been revealed the beneficial effects of their using in intercropping systems or in the protection and exploitation process of degraded soils.

First and foremost I would like to express my deep gratitude to Professor Efimia Papatheodorou, my dissertation supervisor, for her patient guidance and generously willingness to offer her time.

Finally, I wish to thank my family for their support and encouragement throughout my study.

Keywords: Medicinal plants, soil properties, biopesticides, sustainable agriculture, intercropping

Anastasios Alatzidis
25-Nov-2019

## Preface

During recent decades there is a growing interest in medicinal and aromatic plants (MAPs) cultivation, following the increasing demand in pharmaceutical, cook and cosmetic industry.

Due to this trend and the high value of their products, the MAPs have become one of the most cost-effective crops. This group of plants can help small-scale farmers improve their livelihood, especially in barren and arid areas, leading in the socioeconomic development of the whole region.

Moreover their low demands on irrigation and soil fertility results in less environmental impacts making them absolute compatible to sustainable agriculture principles.

However their participation to the sustainability does not include only the above benefits. Equal important are considered those involving the control of pathogenic bacteria and fungi and the improvement of soil properties, which are comparatively less highlighted.

The emphasis of this specific aspect of MAPs profits, especially in Greece, is the aim of the present study.

The high MAPs diversity and endemism observed in Greece, combined with the favorable climatic and soil conditions of the country offer a quantitative and qualitative advantage in their products.

Three of the most common MAPs of Greek nature were selected and was investigated the probability of utilizing them in serving the sustainable agricultural development.

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## INTRODUCTION

### The problems statement and the objective of the study

The intensification that characterizes the conventional cultivation practices has caused the emergence of many serious ecological and agricultural issues. Among the main ones are considered the soil degradation and the health hazard in humans and animals due to incessant and increasing application of chemical pesticides.

Cultivation practices such as tillage result in bare soil which is vulnerable to erosion and runoff. In this case the raindrops damage the soil structure while the flow of water leads in erosion and runoff phenomena that deplete the soil nutrients and organic matter.

Furthermore agricultural ecosystems are threatened by decreased plant diversity as the dominant cultivation practice of monoculture is associated with reduced soil microbial biomass. The soil microbial communities play an important role in plant nutrient availability and absorption as well as in plant drought tolerance. Soil bacteria and mycorrhizal fungi can improve the activity of the crucial enzymes like cellulase, phosphatase, urease while the symbiotic relationships develop with the host plants enhance the nutrient and water uptake. Plant species diversity contributes to microbial diversity due to mixed plant litter added and diverse root exudates.

Meanwhile, the control of the various pathogenic bacteria and fungi, responsible for significant losses of crop production, has become a complex problem as many of them have acquired resistance to several of chemicals. The increased requirements in application rates and doses can be proved harmful to human health and cause deleterious side effects in beneficial microorganisms.

The above considerations have triggered the development of the sustainable agriculture principles that involve alternative management practices resulting in improved crop productivity with the less environmental impact. These principles could effectively be served by the medicinal and aromatics plants (MAPs) cultivation.

The vegetation cover that offer can protect soil surface from runoff and erosion, by intercepting raindrops and improving soil infiltration.

Moreover, many studies have demonstrated the not widely known ability of MAPs to stimulate the growth of soil microbial communities promoting important biological functions.

Finally, their essential oils usage against plant pathogenic microorganisms, provide a safety and ecofriendly antibacterial and antifungal control method.

Researchers have reported that some constituents of MAPs extracts and essential oils, such as the phenolic compounds, exhibit significant antibacterial and antifungal properties, providing a remarkable protection against pathogenic microorganisms.

The species from Mentha, Lavandula and Thymus genus are among the most common of Greek MAPs that can be used for the above purposes with excellent results.

**METHODS**

As it has already mentioned the present dissertation intended to highlight the contribution of Mentha, Lavandula and Thymus genus in sustainable agriculture through their action as natural bactericidal and fungicide and by improving soil properties.

For this purpose a research has been conducted using the "scopus" literature database. The key words and phrases have been used are "mentha genus", lavandula genus", thymus genus", "mentha and soil variables", "lavandula and soil variables", thymus and soil variables", "mentha as biopesticide", lavandula as biopesticide", "thymus as biopesticide".

Regarding to the subject area, the research is focused on agricultural and biological sciences while we collected articles published from 1999 to 2019.

The results of the search were evaluated so as to be excluded the studies referred to MAPs cultivation, to the use of EOs as insecticides as well as to the socioeconomic impacts of MAPs cultivation.

After that, 45 studies remained which then were categorized in the followed 3 groups titled as : "mentha, lavandula and thymus genus "mentha , lavandula and thymus genus as antibacterial and antifungal agents" , "mentha, lavandula and thymus genus and soil properties".

Of the total 45 studies, 12 of them deal with the antifungal properties, 4 with antibacterial properties, 2 with antifungal and antibacterial properties, 15 with the interaction with the soil and 12 with the general views of Mentha, Thymus and Lavandula species and the constituents of their essential oils.

## THE GENUS MENTHA – LAVANDULA - THYMUS

### *THE MENTHA GENUS*

The genus Mentha belongs to the Lamiaceae family and includes about 19 species and 13 natural hybrids widely grown worldwide *(*Akash Kedia et al, 2014).

They are perennial aromatic plant species, whose wide spread is justified by their ability to grow in different environments. However, they prefer rather wet environments. Regarding soil conditions they better growing in moist, clay-loam, fertile soils with pH between 6.0 and 8.5.

The Lamiaceae family comprises more than 232 genera and about 7200 species (Singh, P., Pandey, A.K., 2018), including some of the most widely known aromatics plants in Greece such as oregano, basil, rosemary, sage, thyme, peppermint, lavender etc.

The aromatics plants are characterized by the present of secondary metabolites volatile compounds, the terpenes / essential oils, in their leaves, stems and reproductive structures. These compounds have a variety of applications in medicine, culinary and cosmetics. Reports of the researchers on chemical analysis of essential oils of Mentha species revealed that most of the oils being rich in pulegone, menthon, menthol, carvone, 1, 8-cineole, limonene and b-caryophyllene. (Singh, P., Pandey, A.K., 2018). Menthol, pulegone and carvone are mainly the substances that give the characteristic aroma in mentha's essential oil.

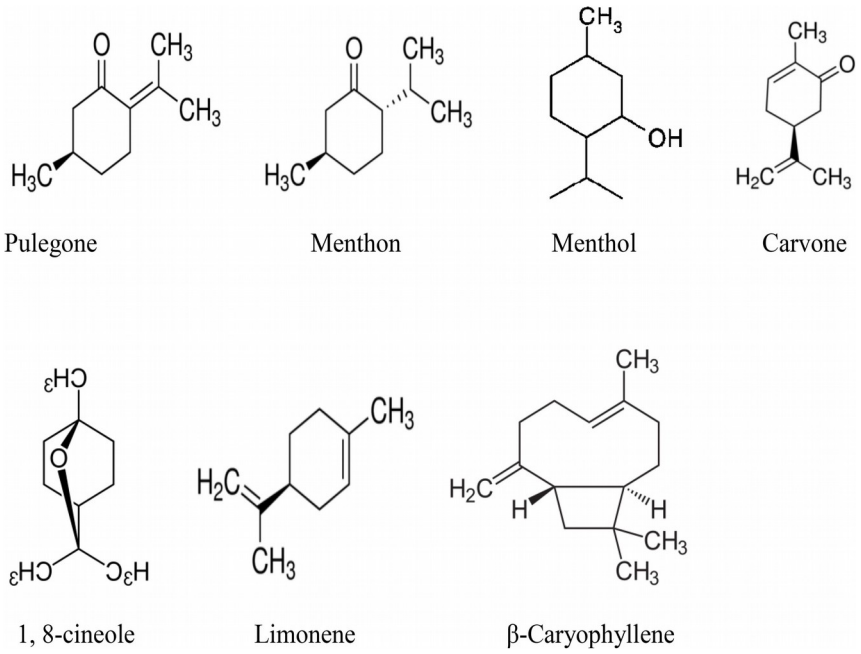


Fig. 1 Chemical structure of main compounds of Mentha species essential oils

(Singh, P., Pandey, A.K., 2018)
FIGURE 1 | Active compounds of different species of *Mentha* essential oil.

Among mentha species, the spearmint (*mentha spicata*) and peppermint (*Mentha piperita L.*), which is a natural hybrid between *Mentha spicata* and *Mentha aquatica* are considered the most widely cultivated.

The global production of Mentha essential oil (derived from peppermint and other Mentha species) set it second commercially important after the essential oil produced by citrus species (Amani Machiani et al, 2018).

***THE LAVANDULA GENUS***

The genus Lavandula comprises 39 species, numerous hybrids and approximately 400 registered cultivars (Herraiz-Pen ˜alver et al. 2013). It is another one commercially important group of aromatics plant of the Lamiaceae family. It includes three subgenus the Lavendula, Fabricia and the less defined Sabaudia .

The genus Lavandula includes annual herbaceous or shrubs species. They prefer sunny, dry, well drained rather alkaline soil (pH between 6 and 8). They can grow even in sandy or rocky soils, while their low requirements in nutrients allow exploiting poor and eroded areas. Lavandula species are tolerant to high drought and temperature conditions adapting well to Mediterranean environment and climate.

Lavenders are native to the Mediterranean regions but have widely distributed across Europe, northeast Africa, South and West Asia (Herraiz-Peñalver et al. 2013).

Their essential oils are accumulated mainly in flowers and are used widely in cosmetics and medicinal industry due to their pleasant fragrance and their several of antioxidant, antimicrobial effects.

Three Lavandula species are mostly cultivated for the commercial production of their essential oils, namely*: Lavandula angustifolia Mill. syn. L. officinalis Chaix ex Vill syn. L. vera DC syn. L. spica L.* (true lavender or fine lavender, English lavender), *Lavandula x intermedia Emeric ex Loisel syn. L hybrida L.* (lavandin), and *Lavandula latifolia Medicus* (spike lavender) (Aprotosoaie, A.C. et al., 2017).

Lavandin is a natural sterile hybrid originated from a cross of *L. latifolia × L. angustifolia.*

The essential oil's composition varies among species depending on genetic and environmental factors. Thus, the different chemical profile of species, allows their subgeneric and sectional classification. Monoterpenes and sespuiterpenes are the most dominant compounds. The mean absolute quantity of terpenes was 50-100 times higher in samples of the subgenus Lavendula than those of the subgenus Fabricia (YannGuitton et al. , 2018). The major components of the Lavendula group EO , that includes the three most commercially important species, are the monoterpenes, linalool, linalyl acetate, 1,8-cineole and camphor. Their concentration in the essential oil determines its quality and usage. High content in linalyl acetate and linalool favors

the fragrance quality and is desirable to perfume industry. In contrast camphor has a negative impact.

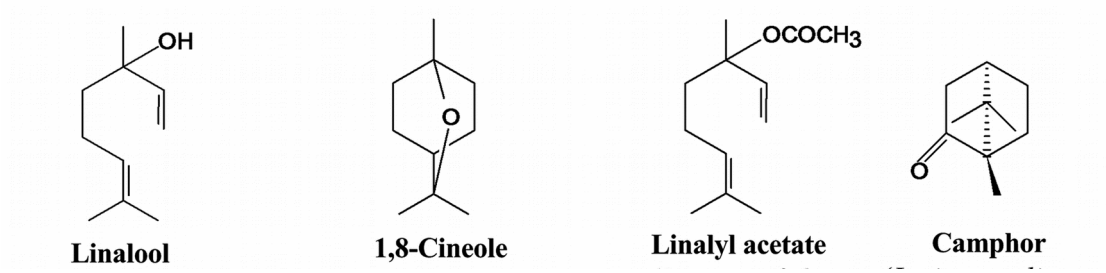


Fig. Chemical structure of main compounds of Lavandula species essential oils (Aprotosoaie, A.C. et al., 2017).

As the essential oil of *Lavandula angustifolia* combines high levels of both linalool / linalyl and low levels of camphor, it is mainly used in cosmetic. On the other hand the essential oils derived from lavandin are rich in camphor and thus are mostly used in low-value hygiene and household products, utilizing their antiseptic, antifungal and insecticidal properties.

However, lavandin is clearly a more productive species. Its essential oil yield is approximately three-fold higher than the than the oil produced by L. angustifolia one.

***THE THYMUS GENUS***

*Thymus*, which includes around 215 species, is a commercially important genus of the Lamiaceae family due to its production of secondary compounds. It comprises several species which are native to the Mediterranean region and can serve as valuable medicinal plants, because of their biological and pharmacological properties. Many studies have depicted the antibacterial, antifungal, antiviral and antiparasitic action of Thymus essential oils. (Tohidi et al., 2017).

The monoterpene phenolsThymol (2-isopropyl-5-methylphenol) and Carvacrol (5-isopropyl-2-methylphenol) are the dominant compounds of the most essential oils. Many studies revealed an inverse relationship between thymol and carvacrol concentrations (when thymol is high the carvacrol is low and vice versa). (Salehi B. et al. , 2019)

The biosynthetic relationship between thymol and carvacrol is reflected to the differences observed in the ingredients of the thyme essential oil. (Tohidi B. et al., 2019). These two substances are also responsible for the characteristic aroma and flavor of the thymus EO as well as for its significant biological properties.

Other active compounds frequently found at various concentrations in thyme EO are p-cymene, γ-terpinene, borneol, geraniol, and linalool (Tohidi B. et al., 2019).

The variation in essential oils' chemical composition is a common phenomenon of thymus genera. It is depended on the genetic background of the species as well as on environmental factors and cultivations practices (Tohidi, B. et al., 2019).

However, thymol and carvacrol are always contained in all species EO with different proportion.

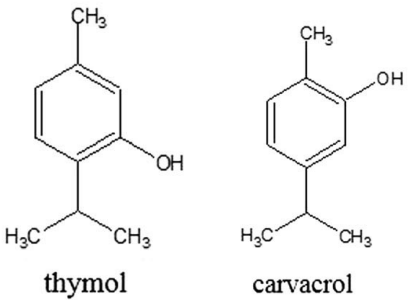


Fig. 2 Chemical structure of main compounds of Thymus species essential oils (Muchembled, J., Deweer, C., Sahmer, K., Halama, P., 2018)

Among thymus species, *Thumus vulgaris* (thyme) is considered as one of the most commercially important with several applications in food and medicinal industry.

Thyme is a perennial shrub which growing better in hot climate with plenty sun and well-drained soil. However mainly due to its remarkable resistance to drought and freezing, can adapt in a wide range of environmental conditions.

In addition, as it has a dense root system can prevent soil erosion in mountainous areas with slope.

## THE MEDICINAL AND AROMATICS PLANTS AS ANTIBACTERIAL AND ANTIFUNGAL AGENTS

## THE ESSENTIAL OILS AND EXTRACTS AS ANTIBACTERIAL AGENTS

Bacteria can infect plant crops as well as postharvest commodities causing serious diseases with enormous economic losses throughout the world (Singh, P., Pandey, A.K., 2018).

The bacterial genera of *Xanthomonas, Pseudomonas*, and *Erwinia* are responsible for approximately 30–40% of yield loss in pre and postharvest crop produced per year (Singh, P., Pandey, A.K., 2018).

In response to this serious threat, the use of chemicals had been widely adopted in previous years. However their increasing use has caused many negative side effects. The high residual toxicity of pesticides has negative impact on human health, on non-target organisms and crops as well.

Furthermore many pathogenic bacteria have acquired resistance against some of the synthetic pesticides.

The above considerations have triggered the interest in developing of biological origin bactericides which can combine the traits of high efficacy with low toxicity and easy biodegradation.

In this direction there are reports available showing the antibacterial action of plant secondary metabolites which are the main compounds of MAPs essential oils.

To evaluate in vitro the essential's oils antibacterial action, the MIC and ZOI indicators are mainly used. The MIC (Minimum Inhibitory Concentration) indicates the lowest concentration of the essential oil which is required to inhibit the visible growth of a bacterium while the ZOI (Zone Of Inhibition) is the diameter of a circular area around the essential oil spot in which the bacterium cannot grow.

### *Antibacterial activity of Mentha species*

Many investigations have shown the efficacy of Mentha EO against some of the most destructive bacteria strains.

Singh, P., Pandey, A.K., (2018) reported the strong antibacterial activity of *Mentha piperita* EO against *P. syringae pv. syringae, Pseudomonas syringae pv. tomato, P. sy-*

*ringae pv. phaseolicola, Xanthomonas campestris pv. campestris, and X. campestris pv. phaseoli* at 0.07–1.25 mg/ml range of MIC values by broth dilution bioassay (Singh, P., Pandey, A.K., 2018).

Similar investigation conducted by Soltani and Aliabadi (2012), it revealed that the essential oil from the two most common Mentha species, *M. piperita* and *M. spicata* showed the same level of bactericidal activity onto *X. campestris pv. juglandis*. (Singh, P., Pandey, A.K., 2018).

Vasinauskienë et al. (2006) evaluated the efficacy of M. piperita EO against some bacteria strains of Xanthomonas, Pseudomonas, and Erwinia genera. The results clearly showed its antibacterial action. The zone of inhibition (ZOI) was 2–6 cm against *P. syringae pv. syringae, P. syringae pv. tomato, Erwinia carotovora subsp. Carotovora* and 6–12 cm against *X. vesicatoria* in disk diffusion bioassay. It was also revealed the bactericidal properties of *M. spicata* essential oils against *Acidovorax avenae subsp. citrulli (Aac)* causing an important seed borne disease called the Bacterial fruit blotch (BFB). The Minimum bactericidal concentration was ranged from 6 to 40 mg/ml. (Singh, P., Pandey, A.K., 2018).

Another research by Kiran S. Chudasama & Vrinda S. Thaker, (2012) evaluated in vitro the efficacy of 100 essential oils against *Xanthomonas campestris pv. Citri* isolated from infected *Citrus limon*, shown that *Mentha piperita* essential oils exhibited 7 mm zone of inhibition. *Xanthomonas campestris pv. Citri* is the pathogen of citrus canker an important disease causes significant economic losses.

Although the antibacterial activity demonstrated by all the above investigations, there was observed a variation of efficacy among of Mentha essential oils against the same pathogen. This may be due to differences in chemical structure of EO, variant sensibility among bacterial strain as well as the different test method. (Singh, P., Pandey, A.K., 2018).

Other investigations were focused on the antibacterial activity of some of the major constituents of Mentha essential oils. Thus, Menthol showed 0.625 mg/ml MIC value against *X. campestris pv. phaseoli*, 1.25 against *Pssyringae pv. phaseolicola*, 0.07 against *Ps. syringae pv. Tomato* and 0.156 against *Ps. syringae pv. syringae and X. campestris pv. campestris*. Similarly, menthone inhibited the growth of *X. campestris pv. phaseoli, P. syringae pv. phaseolicola, P. syringae pv. syringae* at 2.5 mg/ml MIC

value, while of *X. campestris pv. campestris and P. syringae pv. tomato* at 1.25 mg/ml (Singh, P., Pandey, A.K., 2018).

The mode of action of EO was related to the presence of phenolic compounds, mainly menthol and carvacrol. These compounds interact with bacterial enzymes and proteins forming complexes which inhibit bacterial growth. As the phenols are dissolved in cell membrane cause the disruption of plasma membrane which leads in cell death. (Singh, P., Pandey, A.K., 2018)

Table 1: Antibacterial effects of Mentha species EO and extracts

| Mentha species / active compounds | Extracts type / Application type | Dose - Concentration | Pathogenic bacterium | Effect | Study type | Reference |
|---|---|---|---|---|---|---|
| *Mentha piperita* | EO | 0.07–1.25 mg/ml | *P. syringae pv. syringae, Pseudomonas syringae pv. tomato, P. syringae pv. phaseolicola, Xanthomonas campestris pv. campestris, and X. campestris pv. phaseoli* | 100% Growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha spicata* | EO | 6 to 40 mg/ml. | *Acidovorax avenae subsp. citrulli (Aac)* | 100% bactericidal | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha piperita* | EO | | *Pseudomonas* syringae pv. syringae, P. syringae pv. tomato, Erwinia carotovora subsp. Carotovora | 2–6 cm zone of inhibition (ZOI) | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha piperita* | EO | | *Xanthomonas* vesicatoria | 6–12 cm zone of inhibition (ZOI) | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha piperita* | EO | | *Xanthomonas campestris pv.* | 7 cm zone of inhibition (ZOI) | In vitro | Kiran S. Chudasama & Vrinda S. Thaker, 2012 |
| *Menthol* | Menthol | 0.625 mg/ml | *Xanthomonas* campestris pv. phaseoli | 100% Growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Menthol* | Menthol | 1.25 mg/ml | *Pseudomonas syringae pv. phaseolicola* | 100% Growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Menthol* | Menthol | 0.07 mg/ml | *Pseudomonas syringae pv. Tomato* | 100% Growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Menthol* | Menthol | 0.156 mg/ml | *Pseudomonas syringae pv. syringae and* Xanthomonas *campestris pv. campestris* | 100% Growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |

Table 1 Continued

| Mentha species / active compounds | Extracts type / Application | Dose – Concentration | Pathogenic bacterium | Effect | Study type | Reference |
|---|---|---|---|---|---|---|

| | type | | | | | |
|---|---|---|---|---|---|---|
| Menthone | Menthone | 2.5 mg/ml | *Xanthomonas* campestris pv. phaseoli, Pseudomonas syringae pv. phaseolicola, P. syringae pv. syringae | 100% Growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| Menthone | Menthone | 1.25 mg/ml | *Xanthomonas* campestris pv. campestris and Pseudomonas syringae pv. tomato | 100% Growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |

**Antibacterial activity of Thymus species**

The antibacterial action of thymus vulgaris was evaluated by the previously mentioned study of Kiran S. Chudasama & Vrinda S. Thaker, (2012). The pathogen *X. campestris* was found greatly sensitive to *T. vulgaris* essential oil as the zone of inhibition measured in vitro was 23 mm.

The results place the T. vulgaris essential oil between the nine strongest inhibitors among the 100 EO which were evaluated, against the specific pathogen. The higher ZOI was observed by *C. cassia* (59 mm), followed by *C. zeylanicum* (45 mm), *S. aromaticum* (24 mm), *T. vulgaris* (23 mm), *L. nobilis* (21 mm), *S. sclarea* (20 mm) , *B. carterii* (19), *R. officinalis* (17 mm) and *O. basilicum* (16 mm) essential oils.

The study attempts to correlate the chemical composition of the EO and their antibacterial activity as the chemical structures of the dominant components determine their action.

The essential oil of *Thymus vulgaris* used in the investigation was subjected to gas chromatography–mass spectra (GC–MS) to identify its chemical composition. The main compound based on their retention time, were camphene (87.29%), a-pinene (0.29%), b-pinene (0.75%), p-cymol (6.50%), limonene (1.01%) and terpinene (4.16%).

Similary to Mentha, the antimicrobial activity of *T. vulgaris* is evidently related to terpenes type components of its essential oil which are mainly monoterpenoids.

These volatile phenolic compounds have hydrophobic nature exhibiting low solubility in the hydrophobic domain of cytoplasmic membrane of bacterial cells between the lipid layers. The mechanism, in which these lipophilic compounds act against bacteria, involves their cell membrane disruption. Monoterpenoids interact with phospholipids bilayer of the bacterial cell membrane, causing increased permeability, leading at first in loss of cell components and finally in cell death.

Furthermore they inhibit the function of proteins and enzymes existing in the bacterial cell membrane (Kiran S. Chudasama & Vrinda S. Thaker, 2012).

Table 2: Antibacterial effects of Thymus species EO and extracts

| Thymus species / active compounds | Extracts type / Application type | Dose –Con-centration | Pathogenic bacterium | Effect | Study type | Reference |
|---|---|---|---|---|---|---|
| *Thymus vulgaris* | EO | | *Xanthomonas campestris* | 23 mm zone of in-hibition (ZOI) | In vitro | Kiran S. Chudasama & Vrinda S. Thaker, 2012 |

## THE ESSENTIAL OILS AND EXTRACTS AS ANTIFUNGAL AGENTS

Pathogenic fungi are considered the major threat to crops as well as to stored agricultural products (Sharifi-Rad et al., 2018).

The damages they can cause are not only limited to plant harms. In many cases, due to toxic metabolites they produce, they negatively affect the nutritional value of products which become dangerous to human health. (Paranagama et al., 2003; Sonker et al., 2015). The fungi mainly infect the crops and stored commodities include the genera of Aspergillus, Cladosporium, Fusarium, Penicillium, Alternaria, Macrophomina, Rhizoctonia, Colletotrichum, and Botrytis (Pandey et al., 2017). These are responsible for a 40–50% loss in agricultural production (Singh, P., Pandey, A.K., 2018).

To deal with this problem mainly two methods have been widely applied. The use of synthetic fungicides and the development of resistant cultivars.

However both methods involve significant drawbacks. The continuous applications of especially single-site fungicides, definitely lead in resistance development while quite often synthetic chemicals have toxic action to crops as well as to beneficial organisms. Furthermore, fungicides because of the residual toxicity on the crop products and their retention in agricultural soils they are considered a serious hazard to human health and environment.

Regarding the development of resistant cultivars, some complex issues arise. These cultivars should combine in addition to resistance and some other desirable quality characteristics, which is not always feasible. Moreover, cultivars are resistant only against to specific pathogens and for limited time. New pathogenic races can be created with the ability to overcome host resistance (Fravel et al. 2003).

All these facts have caused an increasing demand for alternative, safer and effective control measures. Nowadays the essential oils are considered the promising method to satisfy this demand. The application of essential oils as fungicide concentrates the advantages of broad range fungicidal action, less toxicity to non-target organisms and quick degradation.

The methods widely used to evaluate the EOs efficacy against pathogenic fungi are mainly the poison food method, the inverted Petri plate method and the agar dilution methods (Singh, P., Pandey, A.K., 2018).

Poison food method was used to develop contact fungicides and involves the measuring of decrease in mycelial growth caused after poisoning the nutrient medium by an antifungal agent. The inverted Petri plate method was used to develop fumigant fungicides and involves the inoculation of a nutrient medium by the pathogen in Petri dishes which then are remained inverted so that to avoid the disturbance from airborne particles and water condensations. Finally according to the agar dilution methods, different concentrations of the antifungal agent are used in order to determine the Minimum Inhibitory Concentration (MIC) (Singh, P., Pandey, A.K., 2018).

**Antifungal activity of Mentha species**

During recent years many studies have proved the efficacy of Mentha species essential oils against several fungi pathogen which infect crop in the field or cause damages in storage commodities (Singh, P., Pandey, A.K., 2018).

A study by Kedia A. et al., (2014) was performed to investigate the antifungal potency of *Mentha spicata* essential oil, against toxigenic strain of *Aspergillus flavus [LHP(C)-D6].*

The genus Aspergillus includes species that are responsible for deterioration of stored food by causing molds and secreting mycotoxins. The food-borne molds and their toxic metabolites cause a significant quantitative and qualitative deterioration of agricultural food products that reaches at 25% looses, worldwide. The qualitative deterioration involves changes in color and texture, increase in free fatty acids, and reduced nutritional value and germination ability. Aspergillus flavus is one of the most deleterious species for the stored agricultural products mostly because of the aflatoxins that it can secrete.

The chemical composition of Mentha spicata essential oil used in study, was identified by GC/GCeMS analysis. Carvone was found to be the major ingredient (59.6%) followed by Limonene (25.59%).

These detections are in agreement with the findings of Sokovic et al. (2009) and Sertkaya et al. (2010), who also mention the carvone as the dominant component of *M. spicata* essential oil (>50%) (Kedia A. et al., 2014).

The fungal toxicity was evaluated by testing different concentrations of Mentha spicata EO. The MIC was measured at 1.0 µl $ml^{-1}$ whereas a 0.9 µl $ml^{-1}$ concentration was sufficient to completely inhibit the aflatoxin production.

The study claims that the essential oil acts on fungus cell membrane by affecting the ergosterol content which plays a critical role in its function. It is found that the ergosterol content in cell membrane is proportionally reduced as the concentration of EO is increased.

Concerning the aflatoxin production the EO proved even more effective as it was required a lower concentration than the MIC. In this case the study suggests a different mode of action. According to this, the EO affects the function of some key enzymes that results in carbohydrate catabolism.

The efficacy of EO also tested against 19 food deteriorating fungus. The results shown that at 1.0 µl $ml^{-1}$ concentration the oil was able to inhibit by 100 % the mycelial growth in 17 of them, acting as fungicidal or fungistatic agent. These fungus species are *Absidia ramosa, Alternaria alternata, Aspergillus fumigatus , Aspergillus glaucus, Aspergillus niger, Aspergillus unguis, Cladosporium cladosporioides, Curvularia lunata, Fusarium oxysporum, Mucor sp., Mycelia sterilia, Penicillium citrinum, Penicillium italicum, Penicillium luteum, Penicillium purpurogenum, Rhizopus stolonifer, Absidia ramosa. Against the other two species Aspergillus luchuensis and Aspergillus terreus* that tested, the oil inhibited the mycelial growth by 91.72 % and 75.95 % respectively.

A seed germination test was also conducted to assess the potential phytotoxicity effects of the essential oil. For this, the germination of chickpea seeds treated with M. spicata EO, at 0.0125, 0.025, 0.050, and 0.1 µl $ml^{-1}$ air concentrations was tested. The seeds tested showed 100% germination, which means that there is not any phytotoxic effect from the EO's application.

The MIC measured in the current study is lower than those have been reported by others studies that evaluated different EOs as well as synthetic fungicides. In particular Shukla et al., (2009) for the EO of *Lippia alba*, Prakash et al., (2013) for EO of *Cinnamomum glaucescens*, Tian et al., (2012) for the EO of *Cinnamomum jensenianum*, Prakash et al., (2012b) for the EOs of *Origanum majorana, Coriandrum sativum, Hedychium spicatum, Commiphora myrrha, and Cananga odorata* Prakash et al., (2010) for the synthetic fungicides Nystatin and Wettasul-80.

This finding is of great importance for the commercial utilization of *M. spitata* EO as it allows lower and more cost-effective doses (Kedia A. et al., 2014).

Another study by MimicaDukic et al., (2003) that tested the EO of *Mentha longifolia* demostated that the oil was fungistatic against *Aspergillus niger, A. versicolor, Cladosporium fulvum, Fusarium tricinctum, F. sporotrichioides, Penicillium funiculosum, P. ochrochloron* at 2.5 µl/ml and against *C. cladosporioides* at 12.5 µl/ml (Singh, P., Pandey, A.K., 2018) while a similar by Džamic et al. ´ (2010) reported that 10 ml/ml of *M. longifolia* essential oil showed fungicidal activity against Aspergillus and Fusarium species, *P. funiculosum, Trichoderma* viride and 2.5 ml/ml to *C. fulvum, C. cladosporioides and P. ochrochloron*. (Singh, P., Pandey, A.K., 2018).

A research by Tyagi and Malik, (2011) found the MIC of *M. piperita* EO to vary from 1.13 to 2.25 mg/ml and 2.25 to 4.5 mg/ml for *P. digitatum, A. flavus, A. niger, Mucor spp*, and *F. oxysporum* (Singh, P., Pandey, A.K., 2018).

The M. piperita EO was also tested by Djordjevic et al., (2013) against the *Fusarium oxysporum f.sp. lycopersici* infect tomato plants. The MIC found to be 0.3 µl/ml while the Minimum Fungicidal Concentrations (MFC) was >0.6 µl/ml (Singh, P., Pandey, A.K., 2018).

The *M. spicata* and *M. piperita* EOs were also tested against major pathogens of button mushroom, i.e., *Verticillium fungicola* and *T. harzianum*, by Sokovic and van Griensven, (2006). Both oils exhibited a remarkable antifungal activity. However, the M. spicata EO was proved more effective than *M. piperita* EO as it was able to act fungistatic at 0.5–2.5 µl/ml and fungicidal at 1.5–2.5 µl/ml concentrations, while the corresponding concentrations of *M. piperita* oil were 2.5–3.5 µl/ml and 3.0–4.0 µl/ml respectively (Singh, P., Pandey, A.K., 2018).

The efficacy of *M. longifolia*, *M. piperita*, and *M. spicata* EOs was assessed by Hussain et al., (2010a) in terms of inhibition zones. The researchers found that these varied from 11 - 32 mm for *M. longifolia*, 19 - 30 mm for *M. piperita* and 16–29 mm *M. spicata* against *Rhizopus solani, A. niger*, and *Alternaria alternata* which are fungus species that infect stored food. The same study measured the MIC of *M. longifolia*, *M. piperita*, and *M. spicata* EOs at 44.1–157.8, 52.9–130.1, and 53.2–133.1 mg/mL respectively against these fungus species. (Singh, P., Pandey, A.K., 2018).

For the control of *Aspergillus strains*, a study by Sahearkhiz et al., (2012) tested the *M. piperita* EO. It was found that at 0.5–4 µl/ml concentration the EO was able to effectively control the pathogen. (Singh, P., Pandey, A.K., 2018).

The results of Moghaddam et al., (2013) research, have further broadened the pathogenic targets range of Metha piperita. In particular, this research has shown that the EO of *M. piperita* at 1600 ppm concentration act effectively even against soil-borne pathogens such *as Drechslera spicifera, F. oxysporum f.sp. ciceris*, and *Macrophomina phaseolina*. (Singh, P., Pandey, A.K., 2018).

In addition, Regnier et al., (2014) investigated the antifungal potency of *M. spicata* EO against *Geotrichum citri-aurantii, P. digitatum*, and *P. italicum* which are Fungal pathogens causing diseases in post-harvest citrus. The findings shown that citrus fruit treated with a dose of 750 µl/l of M. spicata EO was effectively protected from these infections. (Singh, P., Pandey, A.K., 2018).

The observed variation in MICs may be due to the differences in essential oils ingredients as well as in the different pathogenic targets and also depends on the methodology used.

There is also another interesting group of studies which tested formulation of essential oils. In many cases, due to adjuvant ingredients, they have shown better results. Thus, Beyki et al., (2014) found that for the *A. flavus* controlling was needed 800 ppm of encapsulated oil of *M. piperita* while a much higher concentration of its pure oil up to 3000 ppm, was proved ineffective (Singh, P., Pandey, A.K., 2018).

The Sokovic et al. (2009), found that the MIC of *Mentha spicata* EO in ethanol against the plant pathogens *A. niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, A. alternata, P. ochrochloron, P. funiculosum, C. cladosporioides, T. viride, F. tricinctum*, and *Phomopsis helianthi* ranged between 1.0–2.5 µl/ml. Even more effective was the essential oil in Tween, an emulsifying agent used in oil-in- water emulsions, as it resulted in a lower MIC value between 0.5–1.5 µl/ml against the same pathogens. Slightly less effective was proved the M. piperita EO exhibiting MICs range between 1.5– 3.0 µl/ml in ethanol and 1.0–2.5 µl/ml in Tween. (Singh, P., Pandey, A.K., 2018).

Researchers suggest that the antifungal properties of Mentha EO are due to the presence of Monoterpenoids compounds mainly Menthol and Carvone. This explains the reason for the high efficacy of the essential oils rich in these compounds. The pre-

viously mentioned research by Sokovic et al. (2009) reported that Menthol extracted from *M. spicata* showed MICs of 0.25–1.5 µl/ml in ethanol and 0.05–1.0 µl/ml in Tween, while carvone from *M. piperita* exhibited higher antifungal activity with MICs value 0.25–1.0 µl/ml in ethanol and 0.05–0.5 µl/ml in Tween. Limonene, the other major compound of EO, showed significantly lower fungistatic potency against the tested pathogens as the MICs were 6.0–11.0 µl/ml in ethanol and 5.0–9.0 µl/ml in Tween (Singh, P., Pandey, A.K., 2018).

Trombetta et al., (2005), reported, that the mode of action of Monoterpenoids which mainly involves damages on the fungi cell membrane, caused by their action on the lipid fraction of plasma membrane resulting in loss of intracellular content.

Aside from this, Cox et al., (2000), also supports that Monoterpenoids can inhibit the oxygen uptake and consequently oxidative phosphorylation by affecting the respiratory enzyme's function.

However there are studies showed the poor antifungal efficacy of some Mentha species and furthermore their phytotoxic action. Thus, the research by Lopez-Reyes et al. (2013) found that the essential oil of *M. arvensis* at 10% concentration was proved inefficient in controlling *Botrytis cinera* and *Monilinia laxa* on apricots while it was phytotoxic on them. The same researchers had reported earlier (Lopez-Reyes et al., 2010), the poor antifungal results of 1 and 10% essential oil emulsion of *M. arvensis* against Botrytis cinera and *Penicillium expansum* on apples (Singh, P., Pandey, A.K., 2018).

The low fungicidal activity of *M. arvensis* EO was also reported by Gupta et al., 2011 when it was tested against *Fusarium oxysporum* at 10 and 20% concentration (Singh, P., Pandey, A.K., 2018).

In addition to *M. arvensis* the study of Bouchra et al. (2003) showed that also the *M. pulegium* had a moderate antifungal action. *M. pulegium* oil inhibited only by 58.5% the mycelial growth when it was tested against *Botrytis cinera* at 250 ppm concentration (Singh, P., Pandey, A.K., 2018).

In conclusion many studies have revealed in vitro the antifungal properties of Mentha species essential oils against a broad spectrum of fungi species which either infect crops in the field or deteriorate stored commodities.

However further large-scale investigations needed to evaluate their efficacy in the field and in real storage conditions taking into account potential drawbacks such as the phytotoxic effects.

The evaluation should include not only the essential oils but also their formulations as well as their isolated chemical components.

Furthermore commercial production requires to evaluate their cost-efficacy in specific fungi pathogens compared to others plants' essential oils and synthetic fungicides.

Table 3: Antifungal effects of Mentha species EOs and extracts

| Mentha species / active compounds | Extracts type / Application type | Dose – Concentration | Pathogenic fungus | Effect | Study type | Reference |
|---|---|---|---|---|---|---|
| *Mentha spicata* | EO | 1.0 µl.ml$^{-1}$ | *Aspergillus flavus* | 100% growth inhibition | In vitro | Kedia A. et al., (2014) |
| *Mentha spicata* | EO | 0.9 ml ml$^{-1}$ | *Aspergillus flavus* | 100% aflatoxin production inhibition | In vitro | Kedia A. et al., (2014) |
| *Mentha spicata* | EO | 1.0 µl.ml$^{-1}$ | *Absidia ramosa, Alternaria alternate, Aspergillus fumigatus, Aspergillus glaucus, Aspergillus niger, Aspergillus unguis, Cladosporium cladosporioides, Curvularia lunata, Fusarium oxysporum, Mucor sp., Mycelia sterilia, Penicillium citrinum, Penicillium italicum, Penicillium luteum, Penicillium purpurogenum, Rhizopus stolonifera, Absidia ramosa* | 100% growth inhibition | In vitro | Kedia A. et al., (2014) |
| *Mentha spicata* | EO | 1.0 µl.ml$^{-1}$ | *Aspergillus luchuensis* | 91.72 % growth inhibition | In vitro | Kedia A. et al., (2014) |
| *Mentha spicata* | EO | 1.0 µl.ml$^{-1}$ | *Aspergillus terreus* | 75.95 % growth inhibition | In vitro | Kedia A. et al., (2014) |
| *Mentha longifolia* | EO | 2.5 µl/ml | *Aspergillus niger, A. versicolor, Cladosporium fulvum, Fusarium tricinctum, F. sporotrichioides, Penicillium funiculosum, P. ochrochloron* | 100% growth inhibition | In vitro | MimicaDukic et al., (2003) |
| *Mentha longifolia* | EO | 12.5 µl/ml | *Cladosporium cladosporioides* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., (2018) |

Table 3 Continued

| Mentha spe cis / active compounds | Extracts type / Application type | Dose – Concentration | Pathogenic fungus | Effect | Study type | Reference |
|---|---|---|---|---|---|---|
| *Mentha longifolia* | EO | 10 ml/ml | *Aspergillus and Fusarium species, Penicillium funiculosum, Trichoderma viride* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha longifolia* | EO | 2.5 ml/ml | *Cladosporium fulvum, C. cladosporioides and Penicillium ochrochloron* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha piperita* | EO | 1.13 - 2.25 mg/ml and 2.25 - 4.5 mg/ml | *Penicillium digitatum, Aspergillus flavus, A. niger, Mucor spp, and Fusarium oxysporum* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha piperita* | EO | 0.3 µl/ml | *Fusarium oxysporum f.sp. lycopersici* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha piperita* | EO | >0.6 µl/ml | *Fusarium oxysporum f.sp. lycopersici* | 100% Fungicidal | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha spicata* | EO | 0.5–2.5 µl/ml | *Verticillium fungicola, Trichoderma harzianum* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 (Sokovic and van Griensven, 2006) |
| *Mentha spicata* | EO | 1.5–2.5 µl/ml | *Verticillium fungicola, Trichoderma harzianum* | 100% Fungicidal | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha piperita* | EO | 2.5–3.5 µl/ml | *Verticillium fungicola, Trichoderma harzianum* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |

Table 3 Continued

| **Mentha species / active compounds** | **Extracts type / Application type** | **Dose – Concentration** | **Pathogenic fungus** | **Effect** | **Study type** | **Reference** |
|---|---|---|---|---|---|---|
| *Mentha piperita* | EO | 3.0–4.0 µl/ml | *Verticillium fungicola, Trichoderma harzianum* | 100% Fungicidal | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha longifolia* | EO | 44.1–157.8 mg/ml | *Rhizopus solani, Aspergillus niger, Alternaria alternata* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha piperita* | EO | 52.9–130.1 mg/ml | *Rhizopus solani, Aspergillus niger, Alternaria alternata* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha spicata* | EO | 53.2–133.1 mg/ml | *Rhizopus solani, Aspergillus niger, Alternaria alternata* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha piperita* | EO | 0.5–4 µl/ml | *Aspergillus strains* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha piperita* | EO | 1600 ppm | *Drechslera spicifera, Fusarium oxysporum f.sp. ciceris, Macrophomina phaseolina* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha spicata* | EO | 750 µl/l | *Geotrichum citri-aurantii, Penicillium digitatum, and Penicillium italicum* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha piperita* | *Encapsulated oil* | *800 ppm* | *Aspergillus* flavus | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha spicata* | *EO in ethanol* | *1.0–2.5 µl/ml* | *Aspergillus* niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, A. alternata, *Penicillium* ochrochloron, P. funiculosum, *Cladosporium* cladosporioides, *Trichoderma* viride, *Fusarium* tricinctum, and Phomopsis helianthi | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |

Table 3 Continued

| Mentha species / active compounds | Extracts type / Application type | Dose – Concentration | Pathogenic fungus | Effect | Study type | Reference |
|---|---|---|---|---|---|---|
| *Mentha spicata* | *EO in Tween* | *0.5–1.5 µl/ml* | *Aspergillus* niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, A. alternata, *Penicillium* ochrochloron, P. funiculosum, *Cladosporium* cladosporioides, *Trichoderma* viride, *Fusarium* tricinctum, and Phomopsis helianthi | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha piperita* | *EO in ethanol* | *1.5– 3.0 µl/ml* | *Aspergillus* niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, A. alternata, *Penicillium* ochrochloron, P. funiculosum, *Cladosporium* cladosporioides, *Trichoderma* viride, *Fusarium* tricinctum, and Phomopsis helianthi | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha piperita* | *EO in Tween* | *1.0–2.5 µl/ml* | *Aspergillus* niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, A. alternata, *Penicillium* ochrochloron, P. funiculosum, *Cladosporium* cladosporioides, *Trichoderma* viride, *Fusarium* tricinctum, and Phomopsis helianthi | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Menthol extracted from Mentha spicata* | Menthol in ethanol | 0.25–1.5 µl/ml | *Aspergillus* niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, A. alternata, *Penicillium* ochrochloron, P. funiculosum, *Cladosporium* cladosporioides, *Trichoderma* viride, *Fusarium* tricinctum, and Phomopsis helianthi | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |

Table 3 Continued

| **Mentha species / active compounds** | **Extracts type / Application type** | **Dose – Concentration** | **Pathogenic fungus** | **Effect** | **Study type** | **Reference** |
|---|---|---|---|---|---|---|
| *Menthol extracted from Mentha spicata* | Menthol in *Tween* | 0.05–1.0 µl/ml | *Aspergillus* niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, A. alternata, *Penicillium* ochrochloron, P. funiculosum, *Cladosporium* cladosporioides, *Trichoderma* viride, *Fusarium* tricinctum, and Phomopsis helianthi | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Carvone from Mentha piperita* | Carvone in ethanol | 0.25–1.0 µl/ml | *Aspergillus* niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, A. alternata, *Penicillium* ochrochloron, P. funiculosum, *Cladosporium* cladosporioides, *Trichoderma* viride, *Fusarium* tricinctum, and Phomopsis helianthi | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Carvone from Mentha piperita* | Carvone in Tween | 0.05–0.5 µl/ml | *Aspergillus* niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, A. alternata, *Penicillium* ochrochloron, P. funiculosum, *Cladosporium* cladosporioides, *Trichoderma* viride, *Fusarium* tricinctum, and Phomopsis helianthi | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Limonene* | Limonene in ethanol | 6.0–11.0 µl/ml | Aspergillus *niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, A. alternata,* Penicillium *ochrochloron, P. funiculosum,* Cladosporium *cladosporioides,* Trichoderma *viride,* Fusarium *tricinctum, and Phomopsis helianthi* | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |

Table 3 Continued

| **Mentha species / active compounds** | **Extracts type / Application type** | **Dose – Concentration** | **Pathogenic fungus** | **Effect** | **Study type** | **Reference** |
|---|---|---|---|---|---|---|
| *Limonene* | Limonene in Tween | 5.0–9.0 µl/ml | *Aspergillus* niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, A. alternata, *Penicillium* ochrochloron, P. funiculosum, *Cladosporium* cladosporioides, *Trichoderma* viride, *Fusarium* tricinctum, and Phomopsis helianthi | 100% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha arvensis* | EO | 10% | *Botrytis cinera, Monilinia laxa* | inefficient | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha arvensis* | 1 and 10% EO emulsion | 10% | *Botrytis cinera, Penicillium expansum* | inefficient | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha arvensis* | EO | 10 and 20% | *Fusarium oxysporum* | inefficient | In vitro | Singh, P., Pandey, A.K., 2018 |
| *Mentha pulegium* | EO | 250 ppm | *Botrytis cinera* | 58.5% growth inhibition | In vitro | Singh, P., Pandey, A.K., 2018 |

**Antifungal activity of Thymus species**

The antifungal properties of thymus products have also been assessed by many researchers.

Such an interesting study has conducted by Ben Jabeur, M. et al., 2017 to evaluate in vitro the antifungal activity of *Thymus vulgaris* EO and its pure major component thymol against the pathogenic fungus *Mycosphaerella graminicola*.

*Mycosphaerella graminicola* is one of most important wheat plant pathogen causing septoria leaf blotch leading in great yield loses. Its control has become quite difficult due to resistance development to several commercially available fungicides.

The essential oil used, was derived by the aerial parts of *Thymus vulgaris* after isolated by hydrodistillation. Gas chromatographies coupled to mass spectrometry (GC-MS) were used to identify its chemical composition. The results showed that the dominant ingredients were thymol (76.96%), ρ-cymene (9.89%), γ-terpinene (1.92%) and caryophyllene oxide (1.69%) (Ben Jabeur, M. et al., 2017).

They were used increasing doses of thyme essential oil (25, 50 and 100 $\mu L.L^{-1}$) and thymol (50, 100 and 200 $\mu L.L^{-1}$) (Ben Jabeur, M. et al., 2017).

The fungicidal activity of both products was tested against the strain M.graminicola St-08-46, derived from infected durum wheat. The researchers evaluated their efficacy by calculating the half-maximal inhibitory concentration (IC50) on pathogen spore germination. (Ben Jabeur, M. et al., 2017).

The Thyme oil was proved more effective as its $IC_{50}$ measured at 31.04 $\mu L.L^{-1}$ while $IC_{50}$ of thymol was much higher reaching 123.6 $\mu L.L^{-1}$ (Ben Jabeur, M. et al., 2017). The increased efficacy is might due to the synergetic action of thyme oil's different ingredients against pathogens. (Ben Jabeur, M. et al., 2017).

In agreement with other studies, the research suggests that the fungicidal action of both products might due to lipophilic properties of monoterpenes compounds as these can harm the fungus by acting on the lipid part of its cell membrane.

Another study by Gill T.A. et al., (2016) attempted to evaluate thymol-based emulsions as antifungal agents against the fungal plant pathogen *Fusarium graminearum* which causes the Fusarium head blight (FHB) disease in cereal crops. (Gill, T.A. et al., 2016)

Because of the yield losses and the remediation cost, the economic damage to growers is enormous.

The resistant wheat and barley varieties which have already developed provide only partial resistance to FHB. Therefore an effective control method also requires the application of a fungicide. The frequent applications at large quantities of Triazoles and other demethylation inhibitor (DMI) fungicides have led to resistance development. Thus, there is a great demand for an alternative fungicide that could overcome these issues.

The research investigates the chance of being the thymol the effective antifungal agent that could address these problems.

It has been reported that thymol can affect ergosterol biosynthesis causing cell membrane disruption. In addition, as it can be diffused in the soil within a few days, the risk of resistance development by pathogens is eliminated (Gill, T.A. et al., 2016).

However, some of the thymol characteristics limit its use. These are mainly the hydrophobic nature and the high volatility. Due to poor water solubility thymol often dissolved with organic solvents such as dimethyl sulfoxide. Alternatives methods such as polymeric encapsulation or emulsification which often used, involve some significant drawbacks. Emulsifying agents interact with thymol and reduce its antifungal potency (Gill, T.A. et al., 2016).

In the present study researchers tested several emulsion variants of thymol emulsions with a low level of surfactant using an anionic, the sodium laureth sulfate (SLS) or a non-ionic surfactant the polysorbate (Tween 20). The aim was to produce highly stable emulsions that would retain the antimicrobial properties of thymol. Additionally, sunflower oil was added so that to reduce the volatility and enhance the adhesion on plant surfaces resulting in increased contact time (Gill, T.A. et al., 2016). The results show that both emulsifying agents can provide negatively charged emulsions at both the micro and nanoscale. The nanoemulsions behaved better exhibiting more resistance to coalescence, flocculation and settling effects. The thymol concentration influences the size of droplets, determining the micro or nano scale of the emulsions (Gill, T.A. et al., 2016).

The emulsion's antifungal activity evaluated in terms of Minimum Fungicidal Concentration $MFC_{100}$ value which found to be 200 μg/ml against *F. graminearum*. This

value is comparable to pure thymol MFC values at 115 µg/mL and 108 µg/mL against *Fusarium oxysporum* and *Fusarium verticilliodes* reported by Zabka and Pavela (2013) (Gill, T.A. et al., 2016).

Another similar study by Abbaszadeh et al. (2014) found that at 100ug/mL to 500ug/mL concentration range pure thymol were able to control effectively fungus pathogens from genus Aspgergillus, Alternaria, Botrytis, Cladosporium, Penicillium and Rhizopus (Gill, T.A. et al., 2016).

The study also investigated the effects of adding sunflower oil. Although it certainly decreases emulsion's volatility, there are previous studies such as Gaysinsky et al. (2007) and Ziani et al. (2011) reported lipids' negative impacts in thymol antifungal strength.

However, the results showed that adding oil, slightly reduced thymol efficacy.

An active thymol concentration 0.06% was needed to control *Fusarium graminearum*, while a slightly increased concentration of thymol 0.10% with oil, was required to provide equal efficacy.

In addition to in vitro investigations, both thymol emulsions with Sodium Lauryl Sulfate (SLS) and Tween 20, were tested in field application. It was found that at 0.06%, and 0.1% thymol concentration, the emulsions effectively suppressed the pathogen when the wheat heads were sprayed by them, after *F. graminearum* inoculation.

At these thymol concentrations, the emulsions applications proved to be safe also for the plants without any visible phytotoxic effect. However, at the higher thymol concentration of 0.5% that was tested, the emulsions were phytotoxic.
Thymol at high concentrations can damage also plant cell membrane as it does on pathogenic cells.

The study also investigated the efficacy of thymol emulsions as a preventive antifungal agent. It was found that emulsions without adding oil, did not provide any protection to wheat against *F. graminearum*. On the other hand, when oil was added, the infections were less, due to reduced thymol volatility that increases its contact time with the pathogen. However, it is clear that post-infection sprays were far more effective (Gill, T.A. et al., 2016).

In conclusion, the paper shows that the thymol sub-micron emulsions can be a safe and reliable alternative fungicide. Emulsions were able to effectively control the Fusarium graminearum when were applied to wheat after the onset of the disease at thymol concentrations up to 0.1 % without any phytotoxic effects. Nevertheless theoretically, higher concentrations could be used in field post-harvest applications aiming in reducing the pathogen threat to next crop. Therefore, relevant studies are needed to investigate this hypothesis.

Another study by Mohammadi, Nazari, Imani, & Amrollahi, (2014) tested the antifungal properties of the essential oils from the *speciesT. kotschyanus and T. daenensieds.* The results showed that EO from *T. kotschyanus* at 0.5 to 1 mg/mL concentration, inhibited the mycelial growth of the plant fungal pathogens *Fusarium oxysporum, Aspergillus flavus* and *Alternaria alternate* while EO from *T. daenensis* showed relatively lower antifungal effect as it was required higher concentration ranged from 1 to 4 mg/ml to inhibit the same pathogens (Salehi, B. et al., 2019).

In vivo research by Nikkhah, Hashemi, Habibi Najafi, & Farhoosh, (2017) found that the EO of T. vulgaris has a synergistic effect when combined with cinnamon EO. In particular the EO of Thymus vulgaris exhibited 78 μg/mL MIC in the presence of cinnamon EO against *Botrytis cinerea* (Salehi, B. et al., 2019).

The efficacy of thyme EO against Alternaria alternate, in cherry tomatoes, was evaluated by Feng, Chen, Zheng, and Liu (2011) using a fumigant method, testing the vapor and direct contact application. Although in both cases, the EO effectively controlled the pathogen, the vapor application method provided better results (Salehi, B. et al., 2019).

The control of fungal diseases that infect postharvest grains was the subject of Anžlovar, Likar, and Koce, (2017) study. The researchers tested the antifungal effect of the direct and indirect application of thyme EO on stored wheat grains. It was revealed that although the direct application was more effective to control the pathogens, it negatively affected the seeds' germination (Salehi, B. et al., 2019).

Similarly, another study by Božik et al. (2017), showed the antifungal activity of thyme EO in vapor application against *Aspergillus spp*. (*A. clavatus, A. flavus, and A. parasiticus*) on stored oat seeds (Salehi, B. et al., 2019).

These reports reinforce the concept of using thyme EO as biofungicide against stored seeds' pathogens.

However extensive investigations should be conducted for the most appropriate application method in relation to seed germination.

As in Mentha case, most of the researchers tend to support the theory that the antifungal mode of action of thyme EO, involves the disruption of the fungal cell membrane and the inhibitory of cell enzyme activity. Potentially, the same effects will be caused also on seeds' cells, inhibiting their germination. Geransayeh et al., (2012) also suggest that pathogen cells die as a consequence of the disorder of anion proportion as cell membrane permeability is changing.

Nevertheless, Bill, Sivakumar, Beukes, & Korsten, (2016), raise a different approach. They claim that the fumigation with thyme EO, on fruits, can induce their defense response. At first, the thyme EO stimulates the expression of the glucanase and chitinase genes of fruits which then cause the hydrolytic split of the fungal cell membrane (Salehi, B. et al., 2019).

Another study by La Torre, A. et al., (2016) evaluated the efficacy of thyme oil, derived by *thymus vulgaris*, and its major component thymol, against *Fusarium oxysporum f. sp. lycopersici* which causes the fusarium wilt in tomato plants. As a soil-borne pathogen it is difficult to be controlled. The control methods often require soil fumigation with environmentally hazardous chemical fungicides. The study also includes others EOs such as clove oil, rosemary oil and some of their components, as well as two of commercial fungicides based on essential oils. The $MIC_{50}$ of thyme oil was found to be among the lowest, measured at 152 μg/mL, slightly higher than eugenol the major component of clove oil, which was found to be 128 μg/mL. In contrast, thymol showed much lower efficacy in inhibiting the mycelial growth as its $MIC_{50}$ value reached the 295 μg/mL.

However, the opposite results were observed regarding the conidial germination inhibition. In this case, thymol proved more effective than thyme oil. Thus, thymol even at the lowest tested concentration of 50 μg /mL completely prevented the conidial germination after 24 and 48 hours, while at the same thyme oil concentration 54% and 68% of the conidial eventually germinated after 24 and 48 hours respectively.

Table 4: Antifungal effects of Thymus species EOs and extracts

| Thymus species / active compounds | Extracts type | Dose – Concentration | Pathogenic fungus | Effect | Study type | Reference |
|---|---|---|---|---|---|---|
| *Thymus vulgaris* | EO | 31.04 µL.L$^{-1}$ | *Mycosphaerella graminicola* | 50% inhibition on spore germination | In vitro | Ben Jabeur, M. et al., 2017 |
| Thymol | Thymol | 123.6 µL.L$^{-1}$ | *Mycosphaerella graminicola* | 50% inhibition on spore germination | In vitro | Ben Jabeur, M. et al., 2017 |
| Thymol | Thymol-based emulsion (with SLS or Tween 20) | 200 µg/ml | *Fusarium graminearum* | 100% Fungicidal | In vitro | Gill, T.A. et al., 2016 |
| Thymol | Thymol | 115 µg/mL | *Fusarium oxysporum* | 100% Fungicidal | In vitro | Gill, T.A. et al., 2016 |
| Thymol | Thymol | 108 µg/mL | *Fusarium verticilliodes* | 100% Fungicidal | In vitro | Gill, T.A. et al., 2016 |
| Thymol | Thymol | 100ug/mL - 500ug/mL | *Genus Aspgergillus, Alternaria, Botrytis, Cladosporium, Penicillium and Rhizopus* | 100% Fungicidal | In vitro | Gill, T.A. et al., 2016 |
| Thymol | Thymol | 0.06% | *Fusarium graminearum* | 100% Fungicidal | In vitro | Gill, T.A. et al., 2016 |
| Thymol | Thymol with sunflower | 0.10% | *Fusarium graminearum* | 100% Fungicidal | In vitro | Gill, T.A. et al., 2016 |

Table 4 Continued

| **Thymus species / active compounds** | **Extracts type** | **Dose – Concentration** | **Pathogenic fungus** | **Effect** | **Study type** | **Reference** |
|---|---|---|---|---|---|---|
| Thymol | Thymol-based emulsion (with SLS or Tween 20) | 0.06%, and 0.1% | *Fusarium graminearum* | 100% Fungicidal | In vivo | Gill, T.A. et al., 2016 |
| *Thymus kotschyanus* | EO | 0.5 - 1 mg/mL | *Fusarium oxysporum, Aspergillus flavus and Alternaria alternate* | 100% Growth inhibition | In vitro | Salehi, B. et al., 2019 |
| *Thymus daenensieds* | EO | 1 - 4 mg/ml | *Fusarium oxysporum, Aspergillus flavus and Alternaria alternate* | 100% Growth inhibition | In vitro | Salehi, B. et al., 2019 |
| *Thymus vulgaris* | EO of Thymus vulgaris combined with cinnamon EO | 78 µg/mL | *Botrytis cinerea* | 100% Growth inhibition | In vino | Salehi, B. et al., 2019 |
| *Thymus vulgaris* | EO | 152 µg/mL | *Fusarium oxysporum f. sp. lycopersici* | 50% Growth inhibition | In vitro | La Torre, A. et al., 2016 |
| *Thymol* | Thymol | 295 µg/mL | *Fusarium oxysporum f. sp. lycopersici* | 50% Growth inhibition | In vitro | La Torre, A. et al., 2016 |

Table 4 Continued

| Thymus species / active compounds | Extracts type | Dose – Concentration | Pathogenic fungus | Effect | Study type | Reference |
|---|---|---|---|---|---|---|
| *Thymus vulgaris* | EO | 50 µg/mL | *Fusarium oxysporum f. sp. lycopersici* | 54% inhibition of conidial germination after 24 hours<br><br>68% inhibition of conidial germination after 48 hours | In vitro | La Torre, A. et al., 2016 |
| Thymol | Thymol | 50 µg/mL | *Fusarium oxysporum f. sp. lycopersici* | 100% inhibition of conidial germination after 24 and 48 hours | In vitro | La Torre, A. et al., 2016 |

**Antifungal activity of Thymus and Mentha species**

There are also studies that have tested both Mentha and Thymus EOs. An interesting one by Khaledi et al. (2015), evaluated the antifungal activity of EOs of *Mentha piperita* and *Thymus vulgaris* against soil-borne pathogens *Rhizoctonia solani* and *Macrophomina phaseolina*, on bean plants. Both of EOs had toxic effects on the pathogens and effectively inhibited their growth. The MICs of *M. piperita* and *T. vulgaris* oils against *R. solani* were measured at 850 ppm and 1100 ppm and against *M. phaseolina* 950 ppm and 1150 ppm respectively. These values indicated a high antifungal efficacy compared to the results of two synthetic fungicides which also tested. Thus, Carboxin and Thiabendazole exhibited MICs at 4000 ppm and 2000 ppm respectively against both *R. solani* and *M. phaseolina*.

The study found that Menthol, the main component of *M. piperita* EO, was even more effective as its MICs at 700 ppm against *S. solani* and 500 ppm against *M. phaseolina*, were lower than those of *M. piperita* EO.

The study also conducted a novel investigation by assessing the EO's effects on the activity of cell wall degrading enzymes (CWDEs), produced by fungi. These enzymes, mainly pectinase and cellulase, play an important role in the process of plant infection by pathogens. The fungi use the cellulolytic and pectolytic properties of those enzymes to penetrate the plant cell walls which are consisted of the polysaccharides cellulose and pectin.

The results revealed that both the EO of *M. piperita* and *T. vulgaris* at very low concentrations (0.01× $IC_{50}$), they were able to reduce the enzyme's activity in vitro. The essential oils at these concentrations were not capable to cause any effect in mycelial growth.

The current study also conducted Greenhouse experiments in vivo to evaluate the application methods of Mentha EO and Menthol against the same pathogens. Thus the efficacy of, seed treatment, soil treatment and foliar sprays were tested. It was found that both seed treatment and foliar spray were effective methods.

*M. piperita* EO when applied by foliar spray, at $IC_{50}$ concentration, suppressed the *R. solani* by 68.3% ± 2.3 and the *M. phaseolina* by 72.6 % ± 9.4. Again Menthol, at the

same concentration was proved more effective resulting in 74.6% ±1.9 and 90.8% ± 10.5 suppression respectively.

On the other hand, seed treatment showed comparatively lower efficacy. The *M. piperita* EO, at the same concentration showed 40.5% ± 5.3 suppression efficacy against R. solani and 5.4% ± 3.6 against *M. phaseolina* while Menthol 45.1% ± 1.9 and 38.9% ± 10.5 respectively.

The study claims that the observed efficacy by both seed treatment and foliar spray applications against soil-borne pathogens should also be partially attributed in the stimulation of plant defense mechanisms. The study in agreement with Kagale et al. (2011) suggested that the observed efficacy by both seed treatment and foliar spray applications against soil-borne pathogens might be due to the stimulation of plant defense mechanisms.

In reference to soil treatment, provided poorer results than seed treatment, probably due to the degradation of essential oil and menthol in the soil (Khaledi, et al., 2015).

Another study by Muchembled, J., et al., (2018), evaluated in vitro the antifungal efficacy of Mentha spicata and Thymus vulgaris EOs as well as their major compounds against the fungus Venturia inaequalis responsible for the apple scab disease. Doubtless, one of the most common and serious diseases in apple trees resulting in enormous economic losses caused by yield reduction and degrading fruit quality. Two fungus strains were tested. Specifically, the S755 which is sensitive to chemical fungicide tebuconazole and the rs552 which is less sensitive. The concentration of the dominant compounds of the EOs was evaluated as 60% carvone for the *Mentha spicata* EO and 51% thymol for the *Thymus vulgaris* EO.

Thus, the $IC_{50}$ of *M. spicata* EO was 83.1 mg/L against S755 strain and 24 mg/L against rs552 strain, while the D-carvone's 137.3 mg/L and 55.9 mg/L and the L-Carvone's 119.6 mg/L and 68.7 mg/L. These findings, in agreement with the study of Van Vuuren, (2008) support the hypothesis that in some cases, the compounds of the EOs, even in minor concentrations, act in compination, increasing the antifungal efficacy.

However, the same view does not stand in the case of thyme oil and thymol. The $IC_{50}$ of *T. vulgaris* EO was 141.9 mg/L against S755 strain and 69.6 mg/L against rs552

strain, while the $IC_{50}$ values of thymol were found to be similar to those of the EO, at 132.2 mg/L and 107.4 mg/L, respectively.

Explaining the antifungal activity of the EOs as a whole, the study argues that these compounds as secondary metabolites are produced by the plants for protective purposes as a response to several external factors, playing a critical role in the plant's defense mechanisms. Thus, their properties could be utilized against pathogenic fungi.

Within this frame, the study suggests that the tested EO, as well as their predominant compounds, could be included in an integrated antifungal management system to contribute in reducing chemical applications.

Another diversified study by Ameziane, N. et al., (2007) tested, in vitro, the antifungal activity of not only EOs but also powders and methanolic and chloroformic extracts of various MAPs.

The pathogenic targets were the fungi species *Penicillium digitatum, Penicillium italicum* and *Geotrichum candidum* which are the major sources of threats for postharvest citrus. The commonly used control practice involves spraying with chemicals fungicides during the waxing process which comes with the inevitable issues of resistant development and health safety (Amueziane, N. et al., 2007).

Among 21 tested MAPs the *Thymus leptobotrys* was found to be the most effective in all three use forms. The application of powder resulted in 100% inhibition of mycelial growth in all fungi. Equally, the EO at 1.2 g/L concentration, managed to completely inhibit the pathogens, as the corresponding powder. The same efficacy was observed by the chloroformic extracts which at 0.3% (w/v) concentration caused 100% pathogens suppression, while the methanolic extracts at a concentration of 1.5% (w/v) showed a bit lower efficacy by restricted the mycelial growth to 71–76%.

However, another Thymus species that tested was found by far less effective. Thus, the powder of thymus pallidus inhibited the mycelial growth of *P. digitatum* by 38%, of *P. italicum* by 58% and of *G. candidum* by 45%.

Suchlike, poor results were also obtained by the two evaluated Mentha species. The powders from *Mentha pulegium L.* and *Mentha rotundifolia L.* inhibited the growth of *P. digitatum* by 59% and P. italicum by 21% and 22% respectively. Furthermore, in the case of *G. candidum* the powders even supported the fungus growth by 15% and 12% respectively.

The results of the research demonstrate the antifungal properties of some of the MAPs even in powder and solvent extracts forms. Nevertheless, as the effectiveness among species varies, extended research needed to identify the most potent combinations of the MAP and application form against specific or range of pathogens.

The subject of another research by Tancinová, D. et al., (2018) was the essential oils' efficacy, of some of Lamiaceae family plants, against six isolates, KMi 383, KMi 392, KMi 510, KMi 512, KMi 524 of the genus Rhizopus spp. It is a fungus species responsible for soft rot disease. One of the most common and devastating diseases damaging several horticultural commodities during transport or storage process (Tancinová D. et al., 2018).

Among others they were tested the EOs, in vapor phase, of some of Mentha, Thymus, and Lavandula genus species. The results displayed that the EOs of *Mentha piperita L., Thymus vulgaris L., Thymus serpyllum L.*, and *Lavandula angustifolia MILLER*. at concentration 0.625 $\mu L.cm^{-3}$ of air, completely inhibited all six isolates growth by 100%.

The effective control of fungal pathogens infect postharvest by the EO use is also the subject of a study by Combrinck S. et al., (2011). The five selected pathogens *Lasiodiplodia theobromae, Colletotrichum gloeosporioides, Alternaria citrii, Botrytis cinerea* and *Penicillium digitatum* are among the most common source of diseases of postharvest fruits.

Within the tested EOs and compounds were included the *Mentha piperita, Mentha spicata*, and *Thymus vulgaris* oils and their major compounds R-carvone and thymol.

The best results provided by *T. vulgaris* oil which at a concentration of 1000 µl/l and lower, totally inhibited the growth of all pathogens, except of *P. digitatum*. A higher concentration at 3000 µl/l required for the less sensitive *P. digitatum*. However, even this concentration is considered quite promising and commercially applicable as the amount of the required EO is not too high. For example, it is feasible to be applied during the waxing process in citrus fruits. The Thymol which is the predominant compound of *T. vulgaris* EO, showed similar or even higher efficacy to pure oil, except in the case of *C. gloeosporioides*.

Generally, both Mentha species EOs exhibited sufficient, but lower than to Thyme oil efficacy, except against *P. digitatum*. In this case the *M. spicata* EO at 1000 µl/l concentration completely inhibited the pathogen. The R-carvone at 1000 µl/l concentration managed to inhibit all the pathogens demonstrating higher efficacy than Mentha oils, with the exception of A. citrii. The complete inhibition of the A. citrii required 3000 µl/l concentration of R-carvone or 2000 µl/l concentration of *M. spicata* EO.

The occurrence of higher efficacy of EOs than this of pure compounds has been also reported by Van Vuuren, (2008) that associated it to synergistic action of the EO's components.

Nevertheless, it is clear, that an effective pathogen control method using EOs, requires their constant chemical composition. However, this is not always the case. Changes in EO's chemical composition affect their efficacy.

In addition, the study suggests the potential of EO combination, with the aim of broadening the target range and enhances their antifungal activity. For this purpose, expanded researches should be conducted to investigate the chemical compatibility among the EOs, in order to find the appropriate combination against specific pathogens.

Thymus and Mentha species are also included in research by Varo A. et al., (2017). The aim of this study was to investigate the effects of not only the EOs, but also the plant extracts (PEs) against fungus pathogen *Verticillium dahlia* in olive. For this purpose 20 EOs and 44 PEs, obtained by steam distillation, were tested.

*Verticillium dahlia* causes the Verticillium wilt one of the most threatening diseases in olive trees. The pathogen in the form of microsclerotia (MS) is very resistant and can survive for many years in the soil. Their germination is induced by root exudates with favorable environmental conditions. The pathogen affects plant's vascular system as the fungal hyphae growing within the xylem leading in water stress (Varo A. et al., 2017).

Thus, the study is not limited to mycelial growth inhibition but also focuses on the equal important issue of microsclerotia germination by conducted experiments, on naturally infested soil. In addition, in vivo tests were also included to evaluate the effects of PEs and EOs on the disease development in olive trees.

The results showed that the EOs exhibited stronger antifungal activity than PEs.

Especially in the case of MS, none of the PEs was able to affect their viability.

Thymus treatment was demonstrated among the most effective. Its essential oil at 500, 2500 and 5000 mg/L concentrations, inhibited the viability of MS by 77.3, 99.3 and 100% respectively. Furthermore, the inhibition in mycelial growth caused by the high dose of 5000 mg/L reached 100 %, while in vivo test showed that can reduce the disease development by 65%. However, the application of thymus EO in olive trees at doses higher than 1000 mg/L was proved phytotoxic.

With regard to Mentha oil, this showed a significant but lower to thymus antifungal activity by inhibiting mycelial growth by 60 - 65% at the higher dose. The study also reports the findings of Lopez-Escudero et al. (2007) research that showed the antifungal effect on MS of *V. dahliae* caused by dried plant residues of *Thymus mastichina* and *Lavandula stoechas* when these incorporated into the soil. However, in some cases this treatment leads in increasing number of viable MS. This unexpected development might be due to stimulation on MS by the residues extracts or their suppressing effects on beneficial soil microorganisms which act against the pathogen (Varo A. et al., 2017).

The study in agreement with others relevant, suggests that antifungal properties of Thymus EO are due to the present of monoterpentanes and phenolic compounds. These compounds have shown their efficacy even against MS which are durable structures. Thus, they are expected to be more effective on the weaker fungal forms such as conidia and mycelium (Varo A. et al., 2017).

However, the risk of unfavorable phytotoxic effects makes vital the necessity for further research in the real field conditions.

Table 5: Antifungal effects of Thymus and Mentha species EOs and extracts

| Thymus - Mentha species / active compounds | Extracts type / Application type | Dose – Concentration | Pathogenic fungus | Effect | Study type | Reference |
|---|---|---|---|---|---|---|
| *Mentha piperita* | EO | 850 ppm | *Rhizoctonia* solani | 100% Growth inhibition | In vitro | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| *Thymus vulgaris* | EO | 1100 ppm | *Rhizoctonia* solani | 100% Growth inhibition | In vitro | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| *Mentha piperita* | EO | 950 ppm | *Macrophomina phaseolina* | 100% Growth inhibition | In vitro | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| *Thymus vulgaris* | EO | 1150 ppm | *Macrophomina phaseolina* | 100% Growth inhibition | In vitro | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| Menthol | Menthol | 700 ppm | *Rhizoctonia* solani | 100% Growth inhibition | In vitro | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| Menthol | Menthol | 500 ppm | *Macrophomina phaseolina* | 100% Growth inhibition | In vitro | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| *Mentha piperita* | EO | 0.01× $IC_{50}$ | *CWDEs* | reduce the enzyme's activity | In vitro | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| *Thymus vulgaris* | EO | 0.01× $IC_{50}$ | *CWDEs* | reduce the enzyme's activity | In vitro | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| *Mentha piperita* | EO /foliar spray | $IC_{50}$ | *Rhizoctonia* solani | 68.3% ± 2.3 Growth inhibition | In vivo | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |

Table 5 Continued

| Thymus - Mentha species / active compounds | Extracts type / Application type | Dose – Concentration | Pathogenic fungus | Effect | Study type | Reference |
|---|---|---|---|---|---|---|
| *Mentha piperita* | EO /foliar spray | $IC_{50}$ | *Macrophomina phaseolina* | 72.6 % ± 9.4. Growth inhibition | In vivo | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| Menthol | Menthol /foliar spray | $IC_{50}$ | *Rhizoctonia* solani | 74.6% ±1.9 Growth inhibition | In vivo | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| Menthol | Menthol /foliar spray | $IC_{50}$ | *Macrophomina phaseolina* | 90.8% ± 10.5 Growth inhibition | In vivo | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| *Mentha piperita* | EO /seed treatment | $IC_{50}$ | *Rhizoctonia* solani | 40.5% ± 5.3 Growth inhibition | In vivo | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| *Mentha piperita* | EO /seed treatment | $IC_{50}$ | *Macrophomina phaseolina* | 5.4% ± 3.6 Growth inhibition | In vivo | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| Menthol | Menthol / seed treatment | $IC_{50}$ | *Rhizoctonia* solani | 45.1% ± 1.9 Growth inhibition | In vivo | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| Menthol | Menthol / seed treatment | $IC_{50}$ | *Macrophomina phaseolina* | 38.9% ± 10.5 Growth inhibition | In vivo | Khaledi, N., Taheri, P., & Tarighi, S. , 2015 |
| *Mentha spicata* | EO | 83.1 mg/L | *Venturia inaequalis S755* | 50% Growth inhibition | In vitro | Muchembled, J., Deweer, C., Sahmer, K., Halama, P., 2018 |

Table 5 Continued

| Thymus - Mentha species / active compounds | Extracts type / Application type | Dose – Concentration | Pathogenic fungus | Effect | Study type | Reference |
|---|---|---|---|---|---|---|
| *Mentha spicata* | EO | 24 mg/L | *Venturia inaequalis rs552* | 50% Growth inhibition | In vitro | Muchembled, J., Deweer, C., Sahmer, K., Halama, P., 2018 |
| D-carvone | D-carvone | 137.3 mg/L | *Venturia inaequalis S755* | 50% Growth inhibition | In vitro | Muchembled, J., Deweer, C., Sahmer, K., Halama, P., 2018 |
| D-carvone | D-carvone | 55.9 mg/L | *Venturia inaequalis rs552* | 50% Growth inhibition | In vitro | Muchembled, J., Deweer, C., Sahmer, K., Halama, P., 2018 |
| L-carvone | L-carvone | 119.6 mg/L | *Venturia inaequalis S755* | 50% Growth inhibition | In vitro | Muchembled, J., Deweer, C., Sahmer, K., Halama, P., 2018 |
| L-carvone | L-carvone | 68.7 mg/L | *Venturia inaequalis rs552* | 50% Growth inhibition | In vitro | Muchembled, J., Deweer, C., Sahmer, K., Halama, P., 2018 |
| *Thymus vulgaris* | EO | 141.9 mg/L | *Venturia inaequalis S755* | 50% Growth inhibition | In vitro | Muchembled, J., Deweer, C., Sahmer, K., Halama, P., 2018 |
| *Thymus vulgaris* | EO | 69.6 mg/L | *Venturia inaequalis rs552* | 50% Growth inhibition | In vitro | Muchembled, J., Deweer, C., Sahmer, K., Halama, P., 2018 |

Table 5 Continued

| **Thymus - Mentha species / active compounds** | **Extracts type / Application type** | **Dose – Concentration** | **Pathogenic fungus** | **Effect** | **Study type** | **Reference** |
|---|---|---|---|---|---|---|
| Thymol | Thymol | 132.2 mg/L | *Venturia inaequalis S755* | 50% Growth inhibition | In vitro | Muchembled, J., Deweer, C., Sahmer, K., Halama, P., 2018 |
| Thymol | Thymol | 107.4 mg/L | *Venturia inaequalis rs552* | 50% Growth inhibition | In vitro | Muchembled, J., Deweer, C., Sahmer, K., Halama, P., 2018 |
| *Thymus leptobotrys* | Powder | | *Penicillium digitatum, Penicillium italicum, Geotrichum candidum* | 100% Growth inhibition | In vitro | Ameziane, N. et al., 2007 |
| *Thymus leptobotrys* | EO | 1.2 g/L | *Penicillium digitatum, Penicillium italicum, Geotrichum candidum* | 100% Growth inhibition | In vitro | Ameziane, N. et al., 2007 |
| *Thymus leptobotrys* | Chloroformic extracts | 0.3% (w/v) | *Penicillium digitatum, Penicillium italicum, Geotrichum candidum* | 100% Growth inhibition | In vitro | Ameziane, N. et al., 2007 |
| *Thymus leptobotrys* | Methanolic extracts | 1.5% (w/v) | *Penicillium digitatum, Penicillium italicum, Geotrichum candidum* | 71–76%. Growth inhibition | In vitro | Ameziane, N. et al., 2007 |
| *Thymus pallidus* | Powder | | *Penicillium digitatum* | 38% Growth inhibition | In vitro | Ameziane, N. et al., 2007 |
| *Thymus pallidus* | Powder | | *Penicillium italicum* | 58% Growth inhibition | In vitro | Ameziane, N. et al., 2007 |
| *Thymus pallidus* | Powder | | *Geotrichum candidum* | 45% Growth inhibition | In vitro | Ameziane, N. et al., 2007 |

Table 5 Continued

| Thymus - Mentha species / active compounds | Extracts type / Application type | Dose – Concentration | Pathogenic fungus | Effect | Study type | Reference |
|---|---|---|---|---|---|---|
| *Mentha pulegium L.* | Powder | | *Penicillium digitatum* | 59% Growth inhibition | In vitro | Ameziane, N. et al., 2007 |
| *Mentha rotundifolia L.* | Powder | | *Penicillium digitatum* | 59% Growth inhibition | In vitro | Ameziane, N. et al., 2007 |
| *Mentha pulegium L.* | Powder | | *Penicillium italicum* | 21% Growth inhibition | In vitro | Ameziane, N. et al., 2007 |
| *Mentha rotundifolia L.* | Powder | | *Penicillium italicum* | 22% Growth inhibition | In vitro | Ameziane, N. et al., 2007 |
| *Mentha pulegium L.* | Powder | | *Geotrichum candidum* | 15% Growth support | In vitro | Ameziane, N. et al., 2007 |
| *Mentha rotundifolia L.* | Powder | | *Geotrichum candidum* | 12% Growth support | In vitro | Ameziane, N. et al., 2007 |
| *Mentha piperita L., Thymus vulgaris L., Thymus serpyllum L.* | EO / vapor phase | 0.625 µL.cm$^{-3}$ of air | *Rhizopus spp KMi 383, KMi 392, KMi 510, KMi 512, KMi 524 isolates* | 100% Growth inhibition | In vitro | Tancinová, D. et al., 2018 |
| *Thymus vulgaris* | EO | 1000 µl/l | *Lasiodiplodia theobromae, Colletotrichum gloeosporioides, Alternaria citrii, Botrytis cinerea* | 100% Growth inhibition | In vitro | Combrinck S. et al., 2011 |
| *Thymus vulgaris* | EO | 3000 µl/l | *Penicillium digitatum* | 100% Growth inhibition | In vitro | Combrinck S. et al., 2011 |

Table 5 Continued

| Thymus - Mentha species / active compounds | Extracts type / Application type | Dose – Concentration | Pathogenic fungus | Effect | Study type | Reference |
|---|---|---|---|---|---|---|
| *Mentha spicata* | EO | 1000 µl/l | *Penicillium digitatum* | 100% Growth inhibition | In vitro | Combrinck S. et al., 2011 |
| R-carvone | R-carvone | 1000 µl/l | *Lasiodiplodia theobromae, Colletotrichum gloeosporioides, Botrytis cinerea, Penicillium digitatum* | 100% Growth inhibition | In vitro | Combrinck S. et al., 2011 |
| R-carvone | R-carvone | 3000 µl/l | *Alternaria citrii* | 100% Growth inhibition | In vitro | Combrinck S. et al., 2011 |
| *Mentha spicata* | EO | 2000 µl/l | *Alternaria citrii* | 100% Growth inhibition | In vitro | Combrinck S. et al., 2011 |
| *Thymus vulgaris* | EO | 500 mg/L | *Verticillium dahlia* | 77.3% microsclerotia viability inhibition | In vitro | Varo A. et al., 2017 |
| *Thymus vulgaris* | EO | 2500 mg/L | *Verticillium dahlia* | 99.3 % microsclerotia viability inhibition | In vitro | Varo A. et al., 2017 |
| *Thymus vulgaris* | EO | 5000 mg/L | *Verticillium dahlia* | 100 % microsclerotia viability inhibition | In vitro | Varo A. et al., 2017 |
| *Thymus vulgaris* | EO | 5000 mg/L | *Verticillium dahlia* | 100 % Growth inhibition | In vitro | Varo A. et al., 2017 |
| *Thymus vulgaris* | EO | 5000 mg/L | *Verticillium dahlia* | 65 % reduce the disease development | In vivo | Varo A. et al., 2017 |
| *Menth asp.* | EO | 5000 mg/L | *Verticillium dahlia* | 60-65 % Growth inhibition | In vitro | Varo A. et al., 2017 |

**Antifungal activity of Lavandula species**

Relatively fewer studies have been conducted to investigate the EOs of Lavandula species as antifungal agents.

Apart from that of Tancinová, D. et al., (2018) already mentioned, there is another one by Císarová, et al., (2016) which attempted to evaluate, in vitro, the antifungal activity of the vapor phase of Lavandula angustifolia MILLER against the fungal pathogen Aspergillus niger and Aspergillus tubingensis isolated from grapes. The aim was to find how effective it could be a fumigant method using the EOs to protect stored grapes from the black mold disease caused by the two pathogens. The EO of Lavandula *angustifolia MILLER*, mainly consisted of linalool and linalyl acetate, demonstrated significant antifungal activity.

The minimum inhibitory dose (MID) of EO, was calculated at 0.313 $\mu L.cm^{-3}$.

Although, the results clearly depicted its antifungal activity, the efficacy was not the best recorded by the study. The EOs of *Thymus vulgaris* and *Origanum vulgare* which also have been tested resulted in MIDs 0.125 $\mu L.cm^{-3}$.

Despite the fact that lavender EOs proved less effective than others in both studies, their antifungal properties remain undoubted. Thus, extended researches should be conducted testing their EOs against different pathogens or in combination with others EOs and active compounds.

In addition, the study reports the results of Soylu et al., (2010) research, which measured the MID of levender EO at comparatively higher concentration of 25.6 $\mu g.mL^{-1}$ against *Botrytis cinerea.*

Table 6: Antifungal effects of Lavandula species EOs and extracts

| Lavandula species / active compounds | Extracts type / Application type | Dose – Concentration | Pathogenic fungus | Crop species | Effect | Study type | Reference |
|---|---|---|---|---|---|---|---|
| *Lavandula angustifolia MILLER* | EO | 0.313 µL.cm$^{-3}$ | *Aspergillus niger, Aspergillus tubingensis* | grapes | 100% Growth inhibition | In vitro | Císarová, M., Tančinová, D., Medo, J., 2016 |
| *Lavandula angustifolia MILLER* | EO | 0.625 µL.cm$^{-3}$ | *Rhizopus spp. , KMi 383, KMi 392, KMi 510, KMi 512, KMi 524 isolates* | | 100% Growth inhibition | In vitro | Tancinová, D. et al., 2018 |
| *Lavender* | EO | 25.6 µg.mL$^{-1}$ | *Botrytis cinerea* | | 100% Growth inhibition | In vitro | Císarová, M., Tančinová, D., Medo, J., 2016 |

## THE INTERACTION OF MEDICINAL AND AROMATICS PLANTS AND SOIL.

The International Soil Science Society has aptly characterized the soil as a “limited and irreplaceable resource”. (Zuazo, V.H.D. et al., 2011)

Obviously, for agriculture, it is one of the principal resources for its existence.

However, some anthropogenic activities such as cultivation practices accompanied by unfavorable climate conditions have led in soil degradation.

The conventional agricultural practices disturb microbial structure resulting in the reduction of soil population diversity and activity (Ouahmane et al., 2016). This in turn negatively affects the nutrient and water availability to plants as well as the formation and stability of soil aggregates. Furthermore, the continuous cultivation of a field with the same crop species could cause an increase in the populations of pathogenic micro-organisms.

In addition, extreme climate conditions with irregular and intense unpredictable rainfalls, often observed in the semiarid Mediterranean areas, highly increase the risk of erosion and runoff phenomena. The raindrops detach the soil particles, while the flowing water transfers cultivable soil and deposit it in streams and water bodies. Thus, soil structure is destroyed and the nutrients and organic matter are removed from the rhizosphere. The soil nutrients depletion leads to increased demands for large quanti-ties of chemical fertilizers with deleterious environmental impacts (V.H. Durán Zuazo et al., 2011).

The MAPs cultivation could play an important role in inhibiting the physico-chemi-cal and biological soil degradation process.

Plants exchange chemical compounds with the soil in the rhizosphere where also microbial communities live. Their interaction can be considered as a unified ecological system adapted and beneficial for both (Shi, S. et al., 2018). The MAPs roots exudates could enhance microbial population and stimulate their activity.

### Mentha, Thymus and Lavandula species and soil properties

Although there are numerous studies on insecticidal and antimicrobial properties of MAPs, relatively few have been carried out so far, on the effect of MAPs on soil properties.

One of them conducted by Shi, et al., (2018), involved a three-year field experiment to evaluate the interaction between soil and four MAPs including Mentha haplocalyx.

The results showed that the three-year growth of MAP caused significant changes in soil properties. The enzymatic activities of solid cellulase (S-CL), soil catalase (S-CAT), Solid-urease (S-UE) and Solid-alkaline phosphatase (S-ALP) of rhizospheric soil of Mentha haplocalyx were enhanced. Especially the urease activity, which is related to the availability of Nitrogen and Phosphorous and the presence of Arbuscular Mycorrhiza Fungi (AMF), recorded by far higher than that of the other tested plants (Shi, S. et al., 2018). These activities are linked to MAPs growth, as they were not found any changes in the non-rhizosphere enzymes.

In agreement with these findings, Meng et al., (2012) also reported that urease, soil phosphatase, catalase, and other enzymes activity significantly increased in the rhizosphere of MAPs.

The Shi, S. et al., (2018), assumes that MAPs stimulate the gathering of bacteria and fungi communities. External factors could trigger the release of the enzymes by the soil microorganisms associated with plants. The correlation among enzymes activity and rhizosphere microorganisms is demonstrated by Mantel test and confirmed by RDA analysis. The bacteria found in rhizosphere improve nutrient exchange and plant metabolic activity (Shi, S. et al., 2018). In *Mentha haplocalyx* rhizosphere were found *Bacillus sp., Pseudoxanthomonas sp* and *Sulfitobacter sp. Bacillus sp.* is beneficial bacteria for plant growth whereas sulfitobacter can protect plants from harms caused by SO2 (Shi, S. et al., 2018) .

Regarding fungi soil communities, the AMF, included *Glomus sp*., were appeared in the MAPs rhizosphere. The symbiotic relationship being developed has multiple benefits for the host plants. It can improve soil water relations and nutrient absorption by the host plants, while enhances their tolerance in pathogens and adverse environmental conditions. In addition, AMF contribute to the soil aggregates formation, resulting in a better soil structure.

The study also reveals that the kinds of bacteria and fungi soil communities are determined by the host plant species. Thus, among MAPs of the study the *Mentha haplocalyx* and the *Perilla frutescens* exhibited similar bacteria and AMF communities in their rhizosphere as both belong to the Lamiaceae family (Shi, S. et al., 2018).

In conclude taking into account the results of the investigation, the study suggests that MAPs could be successfully integrated into an intercropping system aiming at improving and maintaining a healthy soil ecosystem (Shi, S. et al., 2018).

A different approach to the interaction between soil and MAPs attempts to investigate another work by K. Karamanoli et al., (2018). The study examined the changes on essential oils caused by adding MAPs as soil amendments, during the decomposition process. For this scope, the shoots of peppermint and spearmint are added in the soil at a concentration of 4% (w/w). The soil mixture was then distilled and identified the quantitative and qualitative composition of the essential oils.

The results showed that the essential oil content of soil mixture reduced dramatically with time. The decrease in EO's concentration reaches 90% after 30 days. In parallel to quantitative, also other, equally important, qualitative changes occurred. The initial content of monoterpenoids of the spearmint and peppermint EOs reduced from 90% to 45% and 20% respectively, after 60 days.

In particular, the menthol contribution in peppermint EO decreased from 40% to 3.2% while the carvone in spearmint from 28% to 0.4%, after 60 days.

The monoterpenoids are reduced in the favor of sesquiterpenoids. Thus, in the same period, β-caryophyllene was found to increase from 1.54% to 27% and 15% in the peppermint and spearmint EOs respectively. Simultaneously, oxygenated sesquiterpenes also increase due to oxidative reactions occurring in the soil. Under laboratory conditions, the oxygenated sesquiterpenes are proved more active in affecting plant growth than non-oxygenated (K. Karamanoli et al., 2018).

Although the antimicrobial properties of EO have been demonstrated by many studies there were also recorded stimulating effects on soil properties. The more confirmed ones involve the enhancement of soil metabolism and the promoting of microbial communities' development (K. Karamanoli et al., 2018). Apart from these, Chalkos et al., (2010) and Kadoglidou et al., (2011) reported that *Mentha spicata* is able to stimulate the growth of tomato seedlings. In contrast, another study by Bouzoukla,

(2017), found that rosemary EO caused the opposite effects by inhibiting the tomato plant growth. However, all these activities, to a great extent, are depended on the ability of the EOs and their components maintained in the soil environment (K. Karamanoli et al., 2018).

The study by K. Karamanoli et al., (2018), states the potential of using the MAPs in the form of soil amendments as a plant growth-stimulating agent. Although there is lots of evidence support this aspect, further experiments are required to clarify the interaction process with the soil, to explain the contrasting effects as well as to estimate the losses of the various EO due to volatilization and leaching (K. Karamanoli et al., 2018).

Another study by N. Monokrousos et al., (2004) investigated, among other plants, the temporal and spatial variability of soil chemical and biological variables of Thymus capitatus rhizosphere compared to the grass-covered ones.

In agreement with other similar, the study established that each plant species affect its own rhizosphere variables, creating a distinct soil environment.

The total amount of C and N of Thymus capitata rhizosphere was found to be higher than that of grassland ones, while the fungal biomass at the same level as that has recorded in the literature (N. Monokrousos et al., 2004).

Similar results are also provided by another study by Nikolaos Monokrousos et al., (2014) which attempts to investigate the effects of vegetation on Mediterranean serpentine soil.

The variables of bare serpentine soil were compared with those derived from vegetation-covered serpentine soil. Thymus sibthorpii was among the vegetation plants that tested. The bare soil exhibited the typical features of serpentine ones, which have described above. Thus, the abundance of phytoparasites, that play an important role in the function of the soil food web, was found extremely low, might due to a lack of plant litters and root exudates (Monokrousos et al., 2014).

On the contrary, the presence of *Thymus sibthorpii* resulted in increased soil nutrients amounts and increased phytoparasites population, such as the nematode genera *Chiloplacus, Malenchus* and *Ditylenchus.* (Monokrousos et al., 2014).

The ability of Lavender and Thyme plants to develop symbiotic relationships with arbuscular mycorrhizal fungi (AMF), especially in drought conditions, was the subject of a study conducted by Pirzad, A., Mohammadzadeh, S., (2018).

Although many studies have depicted the Lamiaceae family plants as sufficient mycorrhizal plants, it would be interesting to investigate their behavior in water-stress conditions. The results of the study showed that the colonization of lavender's and thyme's roots by AMF was increased in water stress conditions. The extreme drought conditions might stimulate the secretion of root exudates which then reinforce the microorganism growth (Pirzad, A., Mohammadzadeh, S., 2018). The arbuscular mycorrhizal fungi (AMF) symbiosis improves the water absorption by the host plant, due to extended mycorrhizal hyphae, enhancing their tolerance to drought (Pirzad, A., Mohammadzadeh, S., 2018).

However, the AMF benefits are not limited to promote host plant growth. The whole soil ecosystem is positively affected by the formation of soil aggregates and increased microbial biomass (Pirzad, A., Mohammadzadeh, S., 2018). From the research findings, we assume that lavender and thyme in a drought region could result in improved soil quality through the enhancement of AMF colonization.

In agreement with the above results, another research by L. Ouahmane et al., (2005), also found that lavandula and thymus species increase significantly the number of mycorrhizal propagules in their rhizospheres. More specific, the Most Probable Number (MPN) of mycorrhizal propagules per 100 g of dry soil which was found to be 7.82 in bare soil reached a value of 179.7 in the rhizosphere of *lavandula dentata* and 244.5 in the rhizosphere of *lavandula stoechas* and thymus satureioides (L. Ouahmane et al., 2005). The current results along with other similar from previous studies classified the lavandula spp. and thymus satureioides species as highly mycorrhizal dependent plants.

In contrast with AMF abundance, the soluble phosphorus was found to be at lower concentrations in the rhizosphere than in bare soil. This could be explained by the high absorption rate by the plants and should not be account as phosphorus deficiency. It has been found that lavender plants store high amounts of P for using when the demands increased. (L. Ouahmane et al., 2005).

In conclude, the study suggests that Lavandula spp. and *Thymus satureioides*, as highly mycotrophic plants could be used in a self-sustaining vegetation cover scheme in degraded soils to improve the physico-chemical and biological soil properties and inhibit the desertification process.

The suggestion of using Thymus and Lavandula species as soil cover plants is also supported by V.H. Durán Zuazo et al., (2010) research. *Thymus mastichina* and *Lavandula dentata* were evaluated, along with other plants, for their ability to minimize the risk from soil erosion and heavy metal pollution. The results showed that when *Thymus mastichina* and *Lavandula dentata* were used as soil cover plants, they reduced runoff by 88 and 71% and erosion by 79 and 70% respectively, in relation to bare soil (V.H. Durán Zuazo et al., 2010). The protective action of soil cover plants involves the interception of rainfall drops by the plants' canopy and the improvement of soil water infiltration (V.H. Durán Zuazo et al., 2010). The low runoff and erosion levels of vegetation-covered soils were reflected also in reduced nutrients and soil organic matter losses. Thus, the highest nutrient losses of the study were recorded in bare soil, while significantly lower were found, in the vegetation-covered soils.

In particular, the *Thymus mastichina* and *Lavandula dentata* resulted in lowest Nitrogen and Phosphorous losses among the tested cover plants (V.H. Durán Zuazo et al., 2010).

Similarly, the carbon losses were reduced in the second year of the experiment, by 89 and 85% in soils under *Thymus mastichina* and *Lavandula dentata* respectively, with regard to bare soil (V.H. Durán Zuazo et al., 2010).

Furthermore, the rhizosphere of *Thymus mastichina* and *Lavandula dentata* showed the lowest heavy metal losses. Covering the soil with these plants could be an effective management practice in moderating the heavy metal pollution. Heavy metals such as Mn, Cr, Co, Ni, Cu, Zn, Mo, Cd, and Pb, might become serious pollutants, due to their transport and accumulation by runoff (V.H. Durán Zuazo et al., 2010).

The results of the current research clearly demonstrated that the cultivation of *Thymus mastichina* and *Lavandula dentata* can effectively protect agricultural soil from runoff and erosion, providing multiple benefits in its physicochemical properties.

## Conclusions

The current study clearly showed the promising potential of using the MAPs as an effective multifunctional tool of sustainable agriculture practices.

Reviewing the literature we found several in vitro and in vivo investigations recording the efficacy of MAPs EOs and extracts, against a wide spectrum of plant pathogens.

In particular, the EOs of Mentha and Thymus species showed a significant antibacterial and antifungal activity while the genus Lavandula seems to have been poorly studied, especially for its antibacterial activity. Despite there are lots of studies dealing with the antibacterial activity of the EOs of Lavandula species on humans and animals, there is no relevant study to their effects on crops pathogenic bacteria.

Taking into account the environmental issues caused by the intensive use of chemical pesticides as well as the major problem of the resistance development by the pathogens, we could assume that the integration of MAPs EOs in the pathogen control system, provides a variety of profits. Their exclusive use or combined with chemicals could improve the efficacy of the control methods and reduces the doses of synthetic pesticides.

However, their commercial exploitation requires dealing with some limiting factors. These mainly include the high volatility and degradation rate, as well as the possibility of phytotoxic effects. In addition, commercial utilization requires a detailed cost-effective study that has not yet been done.

Further investigations should be conducted firstly focusing on clarifying the mode of action the EOs. Apart from the effects of phenolic compounds on the bacteria and fungi growth, the hypothesis of plants' defense mechanisms stimulation, which has been reported by some studies, is worth thoroughly investigated.

Another important finding arising from the study is the synergistic action of EOs ingredients. Extended in vitro and vivo experiments are needed to be discovered the most effective combinations among EOs or among their compounds against specific pathogens.

Furthermore the potential of formulation development offers the prospective to overcome the drawbacks of volatility by extending the EOs active period.

Regarding the interaction between the MAPs and the soil, the subject is of great importance, especially in the cases of degraded soils as well as on the application of intercropping practices. In addition to the improvement of soil physical properties, the findings of the positive effects on microbial communities are considered of high interest. The ability of each species creating its own distinct rhizosphere environment should be further studied and utilized in order to identify the proper crop combination in an intercropping cultivation system.

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