

## ANTIFUNGAL AND ANTI-MYCOTIC PROPERTIES OF ESSENTIAL OILS EXTRACTED FROM DIFFERENT PLANTS ON PATHOGENIC FUNGI THAT BIOSYNTHESIZE MYCOTOXINS

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### Abstract

*The current scientific paper presents a review of essential oils with antifungal effect, the mechanism of action of the main components of essential oils and the possible synergistic actions between them on pathogens. Essential oils of oregano, cinnamon, fennel, mint and dill in various concentrations have demonstrated effects on ergosterol biosynthesis, specifically reducing the amount of ergosterol. Thus, a significant change in ergosterol biosynthesis will inhibit the growth of fungi and cause their death. An advantage of using essential oils over synthetic ones is the use of small amounts of essential oils to achieve strong fungistatic and fungicidal effects. In conclusion, through this work we tried to make: an enumeration / revision of volatile oils with bio pesticidal potential, highlighting the effects produced on fungi from the Aspergillus, Fusarium and Penicillium families, but also on the mycotoxins biosynthesized by them.*

**Key words:** agricultural products, antifungal effect, antimycotoxin effect, essential oil.

### INTRODUCTION

Losses of agricultural products due to fungal contamination create big problems in both underdeveloped and developed countries. The problems created by fungal contamination occur throughout the food chain. These problems resulting from the contamination are of an economic nature (quantitative and qualitative losses of the obtained products), ecological (pollution of the environment with toxic residues following the use of synthetic fungicides) and sanitary one (biosynthesis of numerous mycotoxins such as aflatoxins, fumonisines, ochratoxins, zearalenones, etc.) (El Khoury et al., 2016; Lai et al., 2021).

About 25-40% of the cereals consumed worldwide are contaminated with mycotoxins. Of the mycotoxins, the aflatoxins produced mainly by *Aspergillus flavus* are the most dangerous, and about 4.5 billion people in underdeveloped countries are exposed to aflatoxicosis (Štřelková et al., 2021).

Direct exposure of consumers to secondary metabolites/mycotoxins leads to serious health problems due to their carcinogenic, immunosuppressive, nephrotoxic, teratogenic

and mutagenic attributes; Aflatoxin B1, among the most common mycotoxins, is classified in group 1 carcinogen by the International Agency for Research on Cancer. The major classes of mycotoxins of the highest agro-economic importance are aflatoxins, ochratoxins, fumonisins, trichothecenes, fusarium emerging mycotoxins, enniatins, ergot alkaloids, alternaria toxins and patulin (Agriopoulou et al., 2020; Bennett & Klich, 2003; Omotayo et al., 2019; Ponzilacqua et al., 2018).

Due to its effectiveness and ease of application, the main approach currently used to control pathogenic fungi, harmful insects, etc., in agriculture is the use of synthetic chemicals (based on: carbamates and dithiocarbonates, benzimidazoles, imidazole and triazoles, morpholines, phenylpyrrole, etc.). They have negative effects on the environment (soil, water and air pollution) but also on consumers, due to the chemical residues resulting from their use; chemical residues that will bioaccumulate in soil, water and in living organisms (organs, muscle tissue, adipose tissue, milk) and that cause toxic, carcinogenic, allergenic effects, negative effects on the endocrine system, reproductive, gastrointestinal, respiratory and

neurological systems of consumers (Aimad et al., 2022; Aktar et al., 2009; Li et al., 2022; Nicolopoulou-Stamati et al., 2016; Regulation (EC) No 1185/2009 of the European Parliament and of the Council of 25 November 2009 on statistics on pesticides, n.d.).

Annually, about 2 million tons of pesticides are used worldwide. Of the 2 million tons of pesticides, 17.5% are fungicides. China is the largest contributing country, followed by the USA, Argentina, Thailand, Brazil, Italy, France, Canada, Japan and India. However, by 2020, global pesticide use has been estimated to increase by up to 3.5 million tons per year (Kumar et al., 2021; Sharma et al., 2019). The share of global pesticide consumption from 2015 to 2018 was 52.2% for Asia, 32.4% for the US, 11.8% for Europe and 2% for Asia (Kumar et al., 2021). In Romania, between 2007 and 2020, the surfaces on which chemicals were applied increased: by 42.9% in the case of insecticides; by 52.2% in the case of fungicides and by 31.1% in the case of herbicides (Popescu et al., 2021).

Substances of natural origin, such as essential oils, obtained from various medicinal plants can represent, nowadays, an important ecological reservoir. The use of volatile oils, as biopesticides, in agriculture, especially in post-harvest practices, can be a more sustainable solution for protecting crops, the environment and last but not least protecting the health of people and animals/consumers. Many studies demonstrate the antifungal and antimicrobial properties of the main bioactive compounds of essential oils. Biopesticides, such as volatile oils, are highly effective in small quantities and decompose quickly without leaving potentially toxic residues in the environment (Achimón et al., 2021; Agriopoulou et al., 2020; Bota et al., 2022; El Khoury et al., 2016; Esper et al., 2014; Gwiazdowska et al., 2022; Han et al., 2022; Hua et al., 2014; Kalagatur et al., 2020; Kumar et al., 2021; Oliveira et al., 2020; Ozcakmak et al., 2017; Perczak et al., 2019; Střelková et al., 2021; Sumalan et al., 2013; Y. Wang et al., 2018).

The purpose of this review is to present the mode of action of essential oils with effects on pathogenic fungi in agriculture. It also highlights the necessity and importance of using essential oils as future biopesticides on

pathogenic fungi that cause economic, ecological and sanitary damage in agriculture.

## CHEMICAL COMPOSITION AND PHYSICO-CHEMICAL PROPERTIES OF ESSENTIAL OILS

Essential oils are mixtures of volatile compounds (sometimes with over 300 different compounds), which are secondary metabolites of plants. These secondary metabolites, together with the mineral elements, play an important role in the defence system of higher plants (Beicu et al., 2021; Dhifi et al., 2016; Ebani & Mancianti, 2020; Imbrea et al., 2016; Karpiński, 2020).

From a structural point of view, the chemical constituents identified in the composition of essential oils extracted from different parts of plants or even the whole plant, can be classified into four groups: terpenes, terpenoids, phenylpropanoids and other constituents (amino acids, lipids, sulphur derivatives, etc. (Masyita et al., 2022).

**Terpenes (isoprenesides)** - are the major constituents found in the composition of essential oils. From the chemical point of view terpenes are molecular structures that contain 2-methylbuta-1,3-dienial carbon skeletons (isoprene units) that can be rearranged in cyclic structures, depending on the number of isoprene units that make up the terpenes, there are: hemiterpenes - are formed by a unit of isoprene (C<sub>5</sub>), monoterpenes (C<sub>10</sub>), sesquiterpenes (C<sub>15</sub>), diterpenes (C<sub>20</sub>), triterpenes (C<sub>30</sub>) and tetraterpenes (C<sub>40</sub>) (Agriopoulou et al., 2020; Aktar et al., 2009; El Khoury et al., 2016; Lai et al., 2021; Li et al., 2022; Ponzilacqua et al., 2018).

**Terpenoids** - these are another type of terpenes that contain oxygen molecules. Terpenoids can be divided into alcohols, aldehydes, esters, ethers, epoxides, ketones and phenols. Examples of terpenoids most often identified in the composition of essential oils are: carvacrol, citronellal, geraniol, linalool, linalool acetate, piperitone, menthol and thymol. The compounds listed above are those that provide the important biological property of essential oils (Beicu et al., 2021; Bhavaniramy et al., 2019; Chouhan et al., 2017; Dhifi et al., 2016; Masyita et al., 2022; Nazzaro et al., 2013;

Rassem et al., 2016; Sánchez-González et al., 2011).

In the group of **phenylpropanoids** there are aromatic compounds such as: anethole, cinnamaldehyde, eugenol, isoeugenol, myristicin, safrole and vanillin. According to recent research on these components, their antibacterial, antitumor, antifungal, antiproliferative, antidiabetic, analgesic, anti-inflammatory, etc. effect has been highlighted (Chouhan et al., 2017; Masyita et al., 2022; Nazzaro et al., 2013; Sánchez-González et al., 2011). As a percentage, essential oils contain 85-99% volatile components and 1-15% non-volatile components. Volatile constituents are a mixture of terpenes, terpenoids and other aromatic and aliphatic constituents (Ciobotaru et al., 2017; Sadgrove et al., 2021; Sánchez-González et al., 2011).

In terms of solubility, essential oils are soluble in alcohol, ether and other oils, but insoluble in water (Bhavaniramy et al., 2019; Dhifi et al., 2016; Rassem et al., 2016).

## ESSENTIAL OILS' ANTIFUNGAL AND ANTIMICOTOXINIC MECHANISM OF ACTION

The effect of essential oils on pathogenic fungi can be observed both at the macromorphological level (inhibition of the mycelium) and at the cellular level. Some of the macromorphological changes are: lack of sporulation or pigmentation, change in the number of conidia, increased branching of hyphae or change in their size. These changes are the consequence of the activities of the components of oils on the enzymatic reaction of the synthesis of the cell wall, which affects the growth of mold and morphogenesis; also, as a result of the pulling back of the cytoplasm from the hyphae, the death of the mycelium occurs (Carmo et al., 2008; Cotuna et al., 2016;

Kiraly et al., 2017; Mătășărean et al., 2017; Plavsic et al., 2017).

The actions of essential oils and their components list the following effects at the fungal cell level (Bennett & Klich, 2003; Chouhan et al., 2017; Dhifi et al., 2016; Karpiński, 2020; Maurya et al., 2021; Mothana, 2012; Nazzaro et al., 2013; Popescu et al., 2021; Ramsey et al., n.d.; Rezende et al., 2021; Tian et al., 2012; Y. Wang et al., 2018): cell wall degradation; reduction of ergosterol production, see Table 1; damage to cytoplasmic membranes; impairment of membrane ionic channels - > decreased pH - > apoptosis and necrosis of fungal cells; deterioration and disturbance of the activity of some cellular organelles with a vital role for the cell-mitochondria; coagulation of the cytoplasm; changes in the amounts of membrane fatty acids; accumulation in the fungal cell of free radicals; decreases in membrane potential; damage to membrane proteins; increasing the permeability of the cell membrane that leads to the outflow of the cellular content; decrease in the synthesis of ATP; actions on enzymes such as ATPase, histidine carboxylase, amylase and protease; changes in the expression of genes involved in mycotoxin biosynthesis, see Table 2. Given the effects produced by the synergistic action of essential oil components on fungal cells, but also on bacterial cells, there are studies that evaluate the toxic potential of essential oils (Bota et al., 2022; Gurita et al., n.d.). For example, the antifungal activity of cinnamon oil at the concentration of 0.25 mg L<sup>-1</sup> on *Penicillium expansum* (CGMDD3.3703) caused a decrease in the germination rate of *P. expansum* spores. After 12 hours of treatment, the spores germination rate was below 20% compared to the control, where the spores germination rate was above 75% (Lai et al., 2021).

Table 1. Essential oils that reduce ergosterol production

Essential oils	Reduction of ergosterol to:	Percentage of reduction [%]:	References:
<i>Thymus vulgaris</i>	<i>Aspergillus flavus</i>	-	(Karpiński, 2020)
<i>Anethum graveolens</i>		79.4%	
Oregano	<i>Fusarium graminearum</i>	99.97%	(Perczak et al., 2019)
	<i>F. culmorum</i>	99.98%	
Cinnamon	<i>F. graminearum</i>	99.89%	(Perczak et al., 2019)
	<i>F. culmorum</i>	99.81%	
Orange	<i>F. culmorum</i>	68.13%	(Perczak et al., 2019)
Mixture of cinnamaldehyde and citral	<i>Penicillium expansum</i>	39.40%	(Y. Wang et al., 2018)

Table 2. Essential oils that modulate gene expression

Essential oils:	Effects on genes responsible for mycotoxin biosynthesis:	References:
Rosemary, Fennel, Anise, Chamomile, Cardamom, Celery	The concentration of 1.0 µl/ml essential oil reduces the expression of genes: acOTApks, laeA, acOTAnrps, acpks and veA involved in the biosynthesis of ochratoxin A in <i>A. carbonarius</i>	(El Khoury et al., 2016; Maurya et al., 2021)
Turmeric	Dose-dependent reduction of aflR transcription factor expression (5 days of treatment at 2, 4 and 8 µl/ml) Downregulates the relative expression of the genes: aflO, aflP, aflM, aflD and aflQ, determining the inhibition of aflatoxin B1 production	(Maurya et al., 2021)
Mixture of 3 essential oils (cinnamon, oregano and lemongrass in a ratio of 1:5:48)	At 0.6 µL/disc essential oil, it modulates the gene expression of the aflT, aflD, aflP, aflM and aflS genes, thus causing a disruption of aflatoxin biosynthesis in the case of <i>Aspergillus flavus</i> At 1.0 µL/disc (58.04%) there was a significant reduction of the aflR gene.	(Xiang et al., 2020)

Table 3. Essential oils with antifungal effects on *Penicillium* spp.

Fungal species:	Essential oil of:	The major components of the essential oil:	Result:	References:
<i>Penicillium ochrochloron</i>	<i>Thymus vulgaris</i> -vapor <i>Origanum vulgare</i> -vapor phase <i>Cymbopogon citratus</i> -vapor phase	thymol (58%), p-cymene (22%), and linalool (3%) carvacrol (70%), followed by p-cymene (11%) and thymol (3%) geraniol (42%) and neral (28%), and in smaller quantities geraniol (5%) and geranyl acetate (4%)	MIC 62.5 µL/L MIC 62.5 µL/L MIC 62.5 µL/L	(Střelková et al., 2021)
<i>Penicillium verrucosum</i>	<i>Syzygium aromaticum</i> -vapor phase <i>Salvia officinalis</i>	eugenol (80%), eugenol acetate (7%), and caryophyllene (7%) borneol (15.67%), 1,8 cineole (21.12%), tyrantol (13.30%)	MIC 62.5 µL/L MIC 65 µL/ml	(Ozcakmak et al., 2017)
Dierckx (D-99756)	<i>Mentha x piperita</i> <i>Allium sativum</i> <i>Origanum onites</i>	menthol (42.55%), menthone (30.51%), neo-menthol (13.19%) allyl disulfide (60.23%), allyl sulfide (19.68%) carvacrol (78.10%), borneol (2.85%), cymene (7.2%)	MIC 65 µL/ml MIC 65 µL/ml MIC 65 µL/ml	
<i>Penicillium verrucosum</i>	<i>Curcuma longa</i> <i>Ocimum basilicum</i> <i>Zingiber officinale</i>	ar-turmerone (46.13%) and ar- curcumene (8.33%) eugenol (36.58%) and linalool (10.83%) geraniol isobutanoate (10.41%), geranyl acetone (11.05%), geranyl acetate (14.59%), geranyl propionate (18.93%), thymol (10.86%) and limonene (10.88%)	MIC 1329 µl/ml MFC 1771 µl/ml MIC 1006 µl/ml MFC 1512 µl/ml MIC 1255 µl/ml MFC 1442 µl/ml	(Kalagatur et al., 2020)
<i>Penicillium aurantiogriseum</i>	<i>Cymbopogon martini</i> <i>Cinnamomum zeylanicum</i> <i>Origanum vulgare</i>	terpinen-4-ol (11.52%) and geranyl acetate (14.88%) limonene (10.54%), (E)-cinamaldehyde (35.81%) and eugenol (12.41%) trans-caryophyllene 30.729%, sabinene-18.16%, caryophyllene oxide-8.635% and germacrene-D-8.159%	MIC 964 µl/ml MFC 1221 µl/ml MIC 837 µl/ml MFC 1441 µl/ml MIC 0.5 mg·L <sup>-1</sup> MFC 5 mg·L <sup>-1</sup>	(Rus et al., n.d.)

MIC- minimum inhibitory concentration, MFC-minimal fungicidal concentration

## EFFECTS OF ESSENTIAL OILS ON FUNGI AND THEIR MYCOTOXINS

According to reports in the specialized literature, it seems that essential oils containing monoterpenes: α-pinene, β-pinene, p-cimena, γ-terpinene, linalool and citral (neral and geraniol); compounds with phenolic

characteristics (carvacrol and thymol); and phenylpropanoids (eugenol) are considered by some authors, the main compounds responsible for the antifungal and antimycotoxin actions (see Table 3) of essential oils (Beicu et al., 2021; Chouhan et al., 2017; Maurya et al., 2021; Rezende et al., 2021; L. Wang et al., 2019).

Citral (3,7-dimethyl-2-6-octadienal) is the name given to a mixture of two geometric isomers: geranial (trans-citral, citral A) and neral (cis-citral, citral B). These unsaturated monoterpene  $\beta$ -aldehydes are found naturally in many citrus essential oils and other herbs or spice (Leite et al., 2014). It has broad-spectrum inhibitory effects against various pathogens such as: *Alternaria alternata*, *A. solani*, *Penicillium italicum*, *P. expansum*, *A. flavus*, *Fusarium moniliform*, *Candida albicans*. The main effects of citrate on fungi are: destroying the integrity of the cell membrane, releasing cellular components, inhibiting mycelium growth through a mechanism of cell membrane damage, compromising its integrity and permeability, negatively affecting the germination of spores, inhibiting the formation of pseudo hives and chlamydia, inducing disruption of oxidative balance, resulting in disruption of cell integrity. It also reduces the expression of genes involved in the biosynthesis of alternariol and its alternariol derivative monomethyl ether, including *pkfI* and *omfI* (Leite et al., 2014; L. Wang et al., 2019).

Phenolics, such as eugenol, chavicol and 4-allyl-2-6-dimethoxyphenol, have higher antifungal properties, compared to cinnamic and hydro cinnamic acids; antifungal activity decreases depending on the type of chemical groups as follows: phenols  $\rightarrow$  alcohols cinnamic aldehydes  $\rightarrow$  aldehydes  $\rightarrow$  ketones  $\rightarrow$  ethers  $\rightarrow$  hydrocarbs. Thus, the antifungal efficacy of volatile oils extracted by different methods from various plants is influenced by the presence of various active constituents, such as: monoterpenes, sesquiterpenes, phenols, aldehydes and ketones. Constituents such as: terpenoids, alcohols and phenolic terpenes in an oxygenated form increase the antifungal activity of volatile oils (Maurya et al., 2021; Saad et al., 2013).

There are studies that have shown the possibility of using essential oils in the form of

vapors, in various concentrations to control the contamination of stored wheat with deposit fungi and also the prevention of contamination with mycotoxins such as mycotoxin deoxynivalenol, a secondary metabolite of *Fusarium*, see Tables 3, 4, 5, 6 (Bota et al., 2022).

The essential oil extracted from the leaves of *Origanum compactum* (oregano), composed mainly of Carvacrol (38%) and Timol (31.46%), has been tested on *Aspergillus flavus*, *A. niger* and *Fusarium oxysporum*. Inhibition of *A. niger* at a minimum concentration of 3,125  $\mu\text{g/ml}$  was observed. In the case of *A. flavus* and *F. oxysporum*, concentrations of 6.25 and 12.5  $\mu\text{g/mL}$  were required for inhibition, respectively, compared to the controls. Also in this study, a comparison was made between the fungal cultures subjected to the essential oil of oregano and those subjected to fluconazole treatment. The obtained results indicate lower values of the minimum inhibitory concentration following the application of the essential oil compared to the tested fungal strains, compared to the Fluconazole standard (Aimad et al., 2022).

Another study that aimed to highlight the antifungal potential, antimicrobial and phytotoxic effect of essential oils of: *Origanum vulgare*, *Thymus vulgaris* and *Coriandrum sativum*, confirmed that the treatment of cereals with the 3 essential oils listed above, leads to a significant decrease in mycotoxin levels, namely the level of deoxynivalenol in wheat samples, depending on the type of volatile oil, concentration and time of fumigation. The maximum inhibition percentage was obtained 21 days after the application of volatile oils in the form of vapors at a concentration of 0.2%. Inhibition of the development of mycotoxin deoxynivalenol also occurred after 7 days of application of the essential oils of *Origanum vulgare* and *Thymus vulgaris* as vapors (Bota et al., 2022).

Table 4. Essential oils with antifungal effects on *Fusarium* spp.

Fungal species:	Essential oil of:	The major components of the essential oil:	Result:	References:
<i>Fusarium sporotrichioides</i> and <i>Fusarium solani</i>	<i>Thymus vulgaris</i> - vapor	thymol (58%), p-cymene (22%) and linalool (3%)	MIC 62.5 µL/L MFC 125 µL/L	(Střelková et al., 2021)
	<i>Origanum vulgare</i> - vapor phase	carvacrol (70%), p-cymene (11%) and thymol (3%).	MIC 62.5 µL/L MFC 125 µL/L	
	<i>Cymbopogon citratus</i> - vapor phase	geranial (42%) and neral (28%), geraniol (5%) and geranyl acetate (4%)	MIC 62.5 µL/L MFC 125 µL/L	
	<i>Syzygium aromaticum</i> - vapor phase	eugenol (80%), eugenol acetate (7%), and caryophyllene (7%)	MIC 62.5 µL/L MFC 125 µL/L	
<i>Fusarium graminearum</i> KZF-1	<i>Cinnamomum zeylanicum</i>	cinnamic aldehyde ≤ 70%, eugenol ≤ 4.4%, linalool ≤ 2.6%, limonene ≤ 1.1%, benzyl benzoate ≤ 1.1%, benzaldehyde 0.5%, cinnamic alcohol ≤ 0.4%, and cuminaldehyde ≤ 0.2%	5% essential oil concentration reduces micelle growth by 90%	(Gwiazdowska et al., 2022)
	<i>Cymbopogon martini</i>	geraniol 85%, linalool 2-3%, limonene 1% and citral 1%;	5% essential oil concentration reduces micelle growth by 1%	
	<i>Thymus hiemalis</i>	citral 42% and limonene 40%	5% essential oil concentration reduces micelle growth by 68%	
<i>Fusarium graminearum</i>	<i>Coriandrum sativum</i>	linalool	CMFs 0.5%	(Alexa et al., 2022)
	<i>Thymus vulgaris</i>	thymol and o-cymene	CMFs 0.1% CMFg 0.6%	
	<i>Origanum vulgare</i>	carvacrol and o-cymene	CMFs 0.06% CMFg 0.2%	
	A mixture of thyme and oregano essential oil	o-cymene (33.25%), thymol (21.04%) and carvacrol (30.33%)	complete inhibition of the micelle after 28 days	
	A mixture of thyme and coriander	o-cymene (29.33%), β-linalool (28.87%) and thymol (26.18%)	complete inhibition of the micelle after 28 days	
<i>Fusarium verticillioides</i> M3125	A mixture of oregano and coriander	o-cymene (24.35%), β-linalool (28.22%) and carvacrol (33.72%)	complete inhibition of the micelle after 28 days	(Achimón et al., 2021)
	A mixture of thyme, oregano and coriander	o-cymene (14.59%), β-linalool (21.68%) and thymol (17.22%)	complete inhibition of the micelle after 28 days	
	<i>Curcuma longa</i>	α-turmerone (44.70%), β-turmerone (20.67%), and Ar-turmerone (17.27%)	125 ppm, inhibition of radial growth was 38.8%	
	<i>Pimenta dioica</i>	methyl eugenol (53.09%), eugenol (16.70%), and β-myrcene (12.80%)	125 ppm, inhibition of radial growth was 20.8%	
	<i>Syzygium aromaticum</i>	eugenol (88.70%), and β-caryophyllene (6.55%)	125 ppm, inhibition of radial growth was 57.4%	
<i>Fusarium avenaceum</i>	<i>Rosmarinus officinalis</i>	1,8-cineole (53.48%), α-pinene (15.65%), and (-)-camphor (9.57%)	125 ppm, inhibition of radial growth was 13.3%	(Chakroun et al., 2021)
	<i>Ammoides pusilla</i> (lot 1)	Thymol (34.70%), γ-terpinen (27.03%), p-cymene (19.89%) and thymol methyl ether (9.18%)	MIC 0.5 µL·mL <sup>-1</sup> produces a 99.2% inhibition of fungal growth after 7 days of treatment	
	<i>Ammoides pusilla</i> (lot 2)	Thymol (53.55%), γ-terpinen (16.82%), p-cymene (14.59%) and thymol methyl ether (8.07%)	MIC 0.25 µL·mL <sup>-1</sup>	
<i>Fusarium oxysporum</i> (MTCC 9913)	<i>Nigella sativa</i>	O-cymene (37.82%), carvacrol (17.68%), α-pinene (10.09%), trans-sabinene hydrat (9.90%) and terpinen-4-ol (7.15%)	MIC 2.69 µg/mL	(Zouirech et al., 2022)

MIC- minimum inhibitory concentration, MFC-minimal fungicidal concentration, CMFs- minimum concentration with fungistatic effect

Table 5. Essential oils with antifungal effects on *Aspergillus* spp.

Fungal species:	Essential oil of:	The major components of the essential oil:	Result:	References:
<i>Aspergillus niger</i>	<i>Thymus vulgaris</i> - vapors	thymol (58%), p-cymene (22%), and linalool (3%)	MIC 62.5 µL/L	(Štřelková et al., 2021)
	<i>Origanum vulgare</i> - vapor phase	carvacrol (70%), p-cymene (11%) and thymol (3%).	MIC 62.5 µL/L	
	<i>Cymbopogon citratus</i> -vapor phase	geranial (42%), neral (28%), geraniol (5%) and geranyl acetate (4%)	MIC 62.5 µL/L	(El-Soud et al., 2012; Štřelková et al., 2021; Zhao et al., 2016)
	<i>Syzygium aromaticum</i> -vapor phase	eugenol (80%), eugenol acetate (7%), and caryophyllene (7%)	MIC 62.5 µL/L	
	<i>Origanum vulgare</i> - vapor phase	carvacrol (70%), p-cimen (11%) și timol (3%).	MIC 62.5 µL/L	
	<i>Origanum vulgare</i>	4-terpineol (44.11%), Linalool (15.22%), α-terpineol (5.96%)	5.0 µL of essential oil produces a 40.93% inhibition of fungal growth	
	<i>Ageratum conyzoides</i>	Dimetoxi ageratocromene (96.53%)	5.0 µL of essential oil produces a 88.37% inhibition of fungal growth	
	<i>Cymbopogon citratus</i> - vapors	geranial (42%) and neral (28%), geraniol (5%) and geranyl acetate (4%)	MIC 62.5 µL/L	
	<i>Syzygium aromaticum</i> -vapors	eugenol (80%), eugenol acetate (7%), and caryophyllene (7%)	MIC 62.5 µL/L	
	<i>Carum carvi</i>	carvone (70.1%); γ-terpinene (12.6%); limonene (5.1%)	At 1000 ppm complete inhibition of fungal growth	
<i>Coriandrum sativum</i>	γ-terpinene (10.6%); linalool (40.9%); geranyl acetate (12.8%)	At 1000 ppm complete inhibition of fungal growth		
<i>Foeniculum vulgare</i>	estragole (50.1%); limonene (20.2%); fenchone (10.6%) eugenol (82.52%)	At 1000 ppm, 50% inhibition of fungal growth		
<i>Aspergillus flavus tulpina MC11</i>	<i>Eugenia caryophyllata</i>		0.1 and 0.25 µL mL <sup>-1</sup> suppress mycelial growth by 75 and 85%, respectively	(Oliveira et al., 2020)
	<i>Rosmarinus officinalis</i> chemotip camfor	α-pinen (22.65%)	0.1 and 0.25 µL mL <sup>-1</sup> do not suppress mycelial growth	
<i>Aspergillus flavus</i> NRRL 3357	<i>Cinnamomum verum</i>	cinnamaldehyde (89.33%), (E)-2-methoxycinnamaldehyde (4.66%) and carveol (2.20%)	0.25µl/disc completely inhibits fungal growth	(Xiang et al., 2020)
	<i>Origanum vulgare</i>	carvacrol (84.96%) and thymol (13.26%)	2.50 µl/disc completely inhibits fungal growth	
	<i>Cymbopogon citratus</i>	(Z)-citral (43.66%) and (E)-citral (43.55%)	6.00 µl/disc completely inhibits fungal growth	
<i>Aspergillus ochraceus</i> (CCDCA 10506), <i>Aspergillus flavus</i> (CCDCA 10508)	<i>Satureja montana</i>	borneol (36.18%), carvacrol (11.07%), camphene (5.35%), γ-terpineol (12.66%) and p-cymene (9.57%)	MFC for <i>A. ochraceus</i> 3.91 µl/ml, and for <i>A. flavus</i> is 0.98 µl/ml	(Rezende et al., 2021)
	<i>Myristica fragrans</i>	limonene (10.15%), sabinene (49.23%), terpinen-4-ol (4.99%), α-pinen (13.81%) and β-pinen (10.75%)	MFC for <i>A. ochraceus</i> and <i>A. flavus</i> is 15.62 µl/ml	
	<i>Cymbopogon flexuosus</i>	geranial (59.66%) and general (38.98%)	MFC for <i>A. ochraceus</i> and <i>A. flavus</i> 0.98 µl/ml	
<i>Aspergillus parasiticus tulpina</i> CECT 2682	Lavandin Grosso	linalool (31.65%), linalyl acetate (24.98%), 1,8-cineole (8.69%), camphor (6.96%), and terpinen-4-ol (3.10%)	MIC and MFC 3µl/ml essential oil	(Lorán et al., 2022)
	Lavandin Abrial	linalool (31.04%), linalyl acetate (19.57%), 1,8-cineole (10.46%), camphor (8.86%), and (E)-β-ocimene (3.50%)	MIC 3µl/ml essential oils, MFC 5µl/ml	
	<i>Origanum virens</i>	carvacrolul (28.71%), p-cimen (9.55%), Y-terpinen (5.22%), α-terpinen (3.00%), mircen (2.05%) and thymol (1.78%)	MIC and MFC 0.6 µl/ml essential oil	
<i>Aspergillus parasiticus tulpina</i> CGC34 (Ap)	chemotip linalool - <i>Tymus zygis</i> - vapor	p-Cymen (39.10%), linalool (32%), thymol (12.11%) and α-pinen (2.5%)	Ap fungal inhibition: 47.78% Ao fungal inhibition: 48.33%	(Hlebová et al., 2021)
	chemotip thymol - <i>Tymus vulgaris</i> - vapor phase	p-Cymen (18.36%), thymol (53.40%), γ-terpinene (6.44%), linalool (5.10%) și carvacrol (2.56%)	Ap fungal inhibition: 48.33% Ao fungal inhibition: 37.22%	
<i>Aspergillus ochraceus tulpina</i> CGC87 (Ao)	<i>Eucalyptus globulus</i> - vapor phase	Cineol (80.01%) α-pinen (2.88%), p-cimen (6.30%), limonene (6%), γ-terpinene (2.45%)	Ap fungal inhibition: 43.33% Ao fungal inhibition: 33.33%	
	<i>Lavandula angustifolia</i> - vapor phase	Linalool (40.59%), Linalyl anthranilate (10.15%), cineol (12.01%), borneol (7.43%)	Ap fungal inhibition: 65.00% Ao fungal inhibition: 37.78%	
	<i>Mentha piperita</i> - vapori	Menthol (41.84%), citronellal (22.09%), borneol (8.40%), p-Cimen (6.3%), 2-undecanone (5.72%) and β-cariofilene (3.44%)	Ap fungal inhibition: 40.00% Ao fungal inhibition: 26.67%	
	<i>Prunus dulcis</i> - vapor	Benzaldehyde (98.20%)	Ap fungal inhibition: 86.67% Ao fungal inhibition: 88.33%	
	<i>Cinnamomum zeylanicum</i> - vapors	Cinnamaldehyde (65.30%), eugenol (21.03%), β-cariofilene (4.16%)	Ap fungal inhibition: 88.33% Ao fungal inhibition: 83.89%	
<i>Litsea cubeba</i> - vapor phase	Limonene (11.50%), β-citral (29.27%), α-citral (37.15%)	Ap fungal inhibition: 88.89% Ao fungal inhibition: 90.00%		

Table 5. Essential oils with antifungal effects on *Aspergillus* spp. - continuation

Fungal species:	Essential oil of:	The major components of the essential oil:	Result:	References:
<i>Aspergillus parasiticus</i> tulpina CGC34 (Ap)	<i>Cymbopogon citrati</i> - vapor phase	Limonene (14.50%), $\beta$ -citril (33.37%), $\alpha$ -citril (39%)	Ap fungal inhibition: 87.78% Ao fungal inhibition: 87.78%	(Hlebová et al., 2021)
<i>Aspergillus ochraceus</i> tulpina CGC87 (Ao)	<i>Zingiber officinalis</i> - vapor phase	Camphene (3.49%), limonene (3.76%), $\alpha$ -curcumene (14.20%), Zingiberen (44.36%), $\alpha$ -farnesen (12.40%) and Sesquiphellandren (11.41%)	Ap fungal inhibition: 46.67% Ao fungal inhibition: 33.44%	
<i>Aspergillus ochraceus</i>	<i>Cinnamomum zeylanicum</i>	limonene (10.54 %), (E)-cinnamaldehyde (35.81 %), and eugenol (12.41 %)	MIC 1106 $\mu$ l/ml MFC 1430 $\mu$ l/ml	(Kalagatur et al., 2020)
	<i>Curcuma longa</i>	ar-turmerone (46.13%) and ar- curcumene (8.33%)	MIC 1608 $\mu$ l/ml MFC 2140 $\mu$ l/ml	
	<i>Cymbopogon martini</i>	terpinen-4-ol (11.52 %) and geranyl acetate (14.88 %)	MIC 1308 $\mu$ l/ml MFC 1430 $\mu$ l/ml	
	<i>Zingiber officinale</i>	geraniol isobutanoate (10.41 %), geranyl acetone (11.05 %), geranyl acetate (14.59 %), geranyl propionate (18.93 %), thymol (10.86 %), and limonene (10.88 %)	MIC 1898 $\mu$ l/ml MFC 1756 $\mu$ l/ml	
	<i>Ocimum basilicum</i>	eugenol (36.58%) and linalool (10.83%)	MIC 1791 $\mu$ l/ml and MFC 2255 $\mu$ l/ml	

MIC- mimimum inhibitory concentration, MFC-minimal fungicidal concentration

Tabel 6. Essential oils with an effect on the biosynthesis of mycotoxins

Mycotoxins:	The effects of essential oils:	References:
<b>Aflatoxin B1</b> , synthesized by <i>Aspergillus flavus</i>	<i>Carum carvi</i> - completely suppresses the production of aflatoxin B1 at the concentration of 1000 ppm essential oil; <i>Coriandrum sativum</i> - completely suppresses the production of aflatoxin B1 at concentrations of 500, 750 and 1000 ppm essential oil; <i>Ageratum conyzoides</i> - the volumes of 50, 30 and 15 $\mu$ L of the essential oil inhibited the production of aflatoxin B1 in maize by 93.70, 34.15 and 15.45%, respectively, and in the case of soybeans, the same volumes of oil essentially inhibited mycotoxin production over 75%; <i>Oreganum vulgare</i> - volumes of 10, 50, 100 and 200 $\mu$ L showed inhibitory effect on aflatoxin production in soybeans at 54.4; 88.68; 86.94 and 88.16%; <i>Eugenia caryophyllata</i> - after 7 days of incubation at a concentration of 0.5 $\mu$ L mL <sup>-1</sup> essential oil, a 100% inhibition of aflatoxin B1 was observed; <i>Rosmarinus officinalis</i> camphor chemotype – produced an inhibition of 81.4% after 7 days of incubation at a concentration of 5 $\mu$ l ml-1 essential oil; Mixture of essential oil of cinnamon, oregano and lemongrass in a ratio of 1:5:48 - at 0.6 $\mu$ l/disc of essential oil, a 67.53% inhibition of aflatoxin B1 is obtained, and at a concentration of 1.0 $\mu$ l/disc 72.68%. <i>Litsea cubeba</i> (vapors) significantly affected mycotoxin production at a concentration of 15.625 $\mu$ l/l of air; <i>Cymbopogon citrati</i> (vapors) significantly affected mycotoxin production at a concentration of 15.625 $\mu$ l/l of air;	(El-Soud et al., 2012; Esper et al., 2014; Oliveira et al., 2020; Xiang et al., 2020)
<b>Aflatoxin B1 and G1</b> , synthesized by <i>A. parasiticus</i>	<i>Satureja montana</i> - very low level of aflatoxins at the concentration of 0.015 $\mu$ l/ml essential oil, and at a higher concentration of essential oil the inhibition of aflatoxins is 100%; <i>Myristica fragrans</i> - very low level of aflatoxins at the concentration of 0.015 $\mu$ l/ml essential oil, and at a higher concentration of essential oil the inhibition of aflatoxins is 100%; <i>Cymbopogon flexuosus</i> - very low level of aflatoxins at a concentration of 0.015 $\mu$ l/ml essential oil, and at a higher concentration of essential oil the inhibition of aflatoxins is 100%; <i>Rosmarinus officinalis</i> - almost completely inhibits aflatoxin production as follows: over 89% for AFG2 and over 99% for the other aflatoxins (B1, B2 and G1); Lavandin Grosso – significantly inhibited the synthesis of aflatoxins (B1, B2, G1 and G2) in a concentration-dependent manner; <i>Origanum virens</i> - significantly inhibited the synthesis of aflatoxins (B1, B2, G1 and G2) in a concentration-dependent manner;	(Rezende et al., 2021)
<b>Aflatoxins B1, B2, G1 and G2</b> synthesized by <i>Aspergillus parasiticus</i> tulpina CECT 2682	Lavandin Abrial – stimulated the production of aflatoxins B1 and G1 at concentrations of 0.8 $\mu$ l/ml and 1 $\mu$ l/ml essential oil, also at these concentrations it significantly reduced the percentage of aflatoxins B2 and G2; <i>Melissa officinalis</i> - 2000ppm essential oil produced a 79.67% inhibition of the mycotoxin; <i>Salvia officinalis</i> - 2000ppm essential oil produced a 96.6% inhibition of the mycotoxin; <i>Coriandrum sativum</i> - 2000ppm essential oil produced a 94.64% inhibition of the mycotoxin; <i>Thymus vulgaris</i> - 500ppm essential oil produced a 97.32% inhibition of the mycotoxin; <i>Mentha x piperita</i> - 2000ppm essential oil produced a 95.77% inhibition of the mycotoxin; <i>Cinnamomum zeylanicum</i> - 500 ppm essential oil produced a 97.32% inhibition of the mycotoxin;	(Lorán et al., 2022)
<b>Fumonisin</b> synthesized by <i>Fusarium</i> spp.	<i>Origanum vulgare</i> (vapors) - at the concentration of 0.2% essential oil, after 28 days of treatment, DON was inhibited by a percentage of 55.35%, and at a concentration of 0.4%, also under the same conditions, the inhibition was at a percentage of 43.88%; <i>Thymus vulgaris</i> (vapors) - at the concentration of 0.2% essential oil, after 28 days of treatment, DON was inhibited by a percentage of 64.65%, and at a concentration of 0.4%, also under the same conditions, the inhibition was at a percentage of 39.84%;	(Sumalan et al., 2013)
<b>Deoxynivalenol (DON)</b> , synthesized by <i>Fusarium</i> spp.		(Bota et al., 2022; Gwiazdowska et al., 2022; Perczak et al., 2019)



Table 6. Essential oils with an effect on the biosynthesis of mycotoxins - continuation

Mycotoxins:	The effects of essential oils:	References:
<b>Deoxynivalenol (DON)</b> , synthesized by <i>Fusarium</i> spp	<i>Coriandrum sativum</i> (vapors) - at the concentration of 0.2% essential oil, after 28 days of treatment, DON was inhibited in percentage of 44.03%, and at a concentration of 0.4%, after 14 days of fumigation, the inhibition was in percentage of 57.05%; Concentrations of 20% essential oil of: <i>Cinnamomum zeylanicum</i> , <i>Origanum vulgare</i> , <i>Cymbopogon martini</i> , <i>Thymus hiemalis</i> , <i>Mentha viridis</i> , <i>Foeniculum vulgare dulce</i> , <i>Aniba rosaeodora</i> and <i>Citrus aurantium</i> produced over 99% inhibition of mycotoxin secretion in the process; Concentrations of 1% essential oil of: <i>Cinnamomum zeylanicum</i> , <i>Cymbopogon martini</i> and <i>Thymus hiemalis</i> , reduce mycotoxin levels by 100%;	(Bota et al., 2022; Gwiazdowska et al., 2022; Perczak et al., 2019)
<b>Ochratoxin A (OTA)</b> synthesized by <i>Aspergillus carbonarius</i> S402	Chamomile - OTA reduction by 67.5% at a concentration of 1µl/ml; Celery- reduction of OTA by 68.5% at a concentration of 1µl/ml essential oil; Rosemary- reduction of OTA by 57.3% at a concentration of 1µl/ml; Anise- reduction of OTA by 76.6% at a concentration of 1µl/ml; Cardamon- reduction of OTA by 74.2% at a concentration of 1µl/ml; Fennel - reduction of OTA by 88.9% at a concentration of 5µl/ml essential oil;	(El Khoury et al., 2016)
<b>Ochratoxin (OTA)</b> synthesized by <i>Aspergillus ochraceus</i>	<i>Satureja montana</i> - at the concentration of 0.015µl/ml essential oil, the mycotoxin was inhibited in a percentage of 34.35%, and at the concentration of 0.24µl/ml essential oil, the complete inhibition of the mycotoxin was obtained (100%); <i>Myristica fragrans</i> - at the concentration of 0.015µl/ml essential oil, the mycotoxin was inhibited by a percentage of 31.24%, and at the concentration of 0.24µl/ml essential oil, the complete inhibition of the mycotoxin was obtained (100%); <i>Cymbopogon flexuosus</i> - at the concentration of 0.015µl/ml essential oil, mycotoxin inhibition was achieved in a percentage of 24.52%, and at the concentration of 0.24µl/ml essential oil, the inhibition was achieved in a percentage of 93.72%; <i>Prunus dulcis</i> – vapors- at the concentration of 15.625µl/l of essential oil air, mycotoxin inhibition is over 50%;	(Hlebová et al., 2021; Rezende et al., 2021)
<b>Ochratoxin A</b> synthesized by <i>Penicillium verucosum</i>	<i>Salvia officinalis</i> , <i>Mentha x piperita</i> - in the treatments with concentrations of 500, 250, 125 and 65 µL/ml essential oil, the mycotoxin level was reduced; <i>Allium sativum</i> , <i>Origanum onites</i> - in treatments with concentrations of 250 and 500 µL/ml essential oil, the mycotoxin was no longer detected;	(Ozcakmak et al., 2017)
<b>Zearalenone (ZEA)</b> , synthesized by <i>Fusarium graminearum</i>	<i>Cinnamomum zeylanicum</i> , <i>Origanum vulgare</i> , <i>Cymbopogon martini</i> , <i>Thymus hiemalis</i> <i>Mentha viridis</i> , <i>Foeniculum vulgare dulce</i> and <i>Aniba rosaeodora</i> (20% essential oil concentration) reduces the amount of ZEA by 100%; <i>Citrus aurantium</i> (20% essential oil concentration) reduces the amount of ZEA by 99.99%; Concentrations of 1% essential oil of <i>Cinnamomum zeylanicum</i> , <i>Cymbopogon martini</i> and <i>Thymus hiemalis</i> reduce mycotoxin levels by over 84%;	(Gwiazdowska et al., 2022; Perczak et al., 2019)
<b>Zearalenone</b> , synthesized by <i>Fusarium culmorum</i>	<i>Cinnamomum zeylanicum</i> , <i>Origanum vulgare</i> , <i>Cymbopogon martini</i> , <i>Thymus hiemalis</i> , <i>Mentha viridis</i> , <i>Foeniculum vulgare dulce</i> and <i>Aniba rosaeodora</i> (in a concentration of 20% essential oil) reduce the amount of mycotoxin in a percentage between 99.08-99.99%; <i>Citrus aurantium</i> (20% essential oil concentration) reduces the amount of mycotoxin by 68.33%	(Perczak et al., 2019)
<b>Ennantine (ENN)</b> synthesized by <i>F. avenaceum</i>	<i>Ammoides pusilla</i> (batch 1)-ENN accumulation was inhibited by 65.76 and 100% at concentrations of 0.1, 0.25 and 0.5 LµmL <sup>-1</sup> essential oil, respectively, and <i>Ammoides pusilla</i> (batch 2) - causes a 92% reduction of ENN at the concentration of 0.15 L µmL <sup>-1</sup> .	(Chakroun et al., 2021)

## CONCLUSIONS

The use of essential oils in agriculture as biopesticides can be a sustainable alternative to protecting stored crops, agricultural products, including the environment and the health of the consumer. In addition to the biopesticide wheel, volatile oils can be used to reduce the level of mycotoxins of cereals in the deposits, so these natural products reduce the economic losses caused by contamination and most

importantly the risk of poisoning and other pathologies resulting from the consumption of contaminated products. Also, volatile oils, due to the synergistic mode of action between the components, can successfully replace the synthetic preservatives used in the preservation of vegetables and fruits. The preservative effect of oils extracted from plants is due to chemical compounds that have proven antibacterial, antifungal and antioxidant effects.

Most of the pathogens of agricultural crops become resistant to the chemical treatments used. Thus, most of the times, the dose of chemicals is used to be increased in order to succeed in combating them. The increase in the quantity of synthetic substances leads to a direct proportional increase in the negative effects created by the substances used and their residues on the environment but also on the health of consumers, be they of human or animal nature.

The replacement of synthetic substances with eco-friendly substances, such as essential oils extracted from various plants, can be a durable solution in combating the resistance of phytopathogens, due to their vast chemical composition and synergistic effects between the main constituents.

A plus of the use of essential oils at the expense of synthetic substances is the use of small amounts of ethereal oils to achieve strong fungistatic and fungicidal effects. They also decomposes quickly without leaving toxic residues in the environment.

In conclusion, through this scientific research we tried to make: an enumeration of volatile oils with the potential of biopesticides, a highlighting of the effects and the antifungal mode of action of the major components identified in their composition, on pathogenic fungi and biosynthesized mycotoxins.

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