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ANTAGONISTIC ANTIFUNGAL ACTIVITIES OF *MENTHA SUAVEOLENS* AND *ARTEMISIA ABSINTHIUM* ESSENTIAL OILS FROM MOROCCO

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Abstract

Natural antimicrobial agents have received much interest in recent years, so these combinations can be used to control food-borne bacteria and other pathogenic microorganisms. In this study, the antifungal potential of *Mentha suaveolens* and *Artemisia absinthium*, both alone and in combination, was studied in vitro against the food-borne pathogens *Fusarium oxysporum*, *Fusarium solani*, and *Botrytis cineria*. Possible interactions between essential oils were examined using the well diffusion method. There are four types of effects that might result from the interaction of essential oil compounds: antagonistic, synergistic, indifferent, or additive effects. After The gas chromatography-mass spectrometry (GC-MS) analysis of these oils, seventeen components characterized the essential oil of *M. suaveolens* representing 90.6% of the total oil. Quantitatively, the most abundant were Piperitenone oxide (52.4%) and Piperitenone (12.1%). And a total of nineteen compounds were identified in the essential oil of *A. absinthium* representing 95.3 % of the total oil. The major compound was β-Thujone (85.1%). There was a clear antifungal inhibition by both *Mentha suaveolens* and *Artemisia absinthium* essential oils on the studied organisms, after the microdilution and well diffusion assays. In combination though, the essential oils have shown antagonistic activity against all the studied fungi strains.

Keywords: *Mentha suaveolens, Artemisia absinthium, essential oils , composition, antifungal activity.*

Introduction

Contamination of agro-food items with mycotoxins produced by various classes of phytopathogenic fungi is a long-standing issue that has piqued interest in recent decades. Natural chemicals have attracted interest in recent years, and concerns about the safety of synthetic compounds have prompted further in-depth research into plant resources [1-3]. Essential oils are complex natural combinations of volatile secondary metabolites, extracted from plants by hydro or steamdistillation. The fragrance and biological capabilities of aromatic and therapeutic plants are due to the major components of essential oils, mono- and sesquiterpenes, including ethers, aldehydes , carbohydrates, alcohols, and ketones [4]. Spices and herbs have been used in food as preservatives and flavoring agents since ancient times because of these qualities. Essential oils have been extracted from various parts of plants for ages and are used for similar purposes. Essential oils cover a broad spectrum of activities. Pharmacological effects of essential oils include anti-inflammatory [5], antioxidant [6], and anticancerogenic activities [7]. Others are biocides against viruses [8], protozoa [9], bacteria [10], fungi [11], insects [12], and plants [13].

Aroma therapists combine (or blend) essential oils for use in a variety of applications. It is a widely held belief among aroma therapists [14]. When essential oils are 'mixed' or 'blended' in this way, they have a more beneficial effect; this is known as 'synergy' [11]. There are four sorts of impacts that might result from the interaction of essential oil compounds: antagonistic, synergistic, indifferent, or additive, effects. When the total effect equals the sum of the individual effects, this is known as an additive effect. When one or both substances are treated together, their effect is reduced compared to when they are applied separately. Synergism occurs when the combined effect of the substances is higher than the sum of their separate effects, whereas indifference occurs when there is no interaction [2].

The oxygenated terpenoids in essential oils have the most antibacterial action, but certain hydrocarbons also have antimicrobial properties. These components' interactions may have antagonistic, additive, or synergistic effects. Some studies have shown that complete essential oils have higher antibacterial activity than mixes of their major components, implying that minor components are important for synergistic activity, however antagonistic and additive effects have also been documented [2] The majority of research linked phenolic and alcohol components to additive and synergistic effects. Compounds with similar structures, on the whole, have an additive rather than synergistic effect. The primary phenolic components in various essential oils have been linked to the occurrence of additive interactions [15].

Wormwood (*Artemisia absinthium*) is a medicinal and aromatic bitter herb that has been used as an antibacterial agent in traditional medicine since ancient times [16]. Several researchers and producers throughout the world are interested in the major active components, essential oil, and bitter compounds [17]. Wormwood is native to Europe and may be found all the way north to Lapland, Karelia, and Southern Siberia in temperate Asia, but it has also been naturalized in North and South America, as well as New Zealand [18]. *Artemisia absinthium* has produced a number of secondary metabolites and other compounds, the most notable of which is the essential oil extracted from glands on the aerial portions [19]. The essential oil of this plant has a strong aromatic smell due to large quantities of volatile terpenes, notably in the leaves and flowers [17]. The bicyclic monoterpene thujone is commonly known and stated to be abundant in the essential oil of *A. absinthium*, and thus may be regarded the most distinctive ingredient of wormwood oil [20].

Mentha suaveolens, often known as mint timija or 'timijja' in Arabic, is a strictly Moroccan native perennial plant with several therapeutic benefits. Moroccan traditional medicine has utilized the leaves and flowering section of the species as a powder or infusion to treat coughs, bronchitis, ulcerative colitis, as an antispasmodic, and as an excellent carminative [21-22]. Mint timija is a fragrant shrub that is often used in herbal tea for its tonic and stimulating effects. Furthermore, the species is utilized as an aroma and flavor enhancer

in a variety of cuisines. [21-22] Previous pharmacological research have shown that mint timija extract has antibacterial and antiseptic properties [23].

The aim of this study was to determine the susceptibility of three food-borne fungi species (*Fusarium oxysporum, Fusarium solani*, and *Botrytis cineria*) to single and paired combinations of *Mentha suaveolens* and *Artemisia absinthium* essential oils.

Materiel and methods

Plant material

The leaves of *Mentha suaveolens* and *Artemisia absinthium* were collected from the city of Khenifra, Beni-Mellal Khenifra region, center of Morocco, in November 2020. The morphological traits and the literature data base were used to identify the two plants.

Extraction of the essential oils

To produce the essential oil, the leaves of *Mentha suaveolens* and *Artemisia absinthium* were oven dried at 60°C, then hydrodistilated for 4 hours using a Clevenger-type apparatus. The essential oil was extracted and dried over anhydrous sodium sulphate Na_2SO_4 before being kept in sealed vials at 4 degrees Celsius.

Analysis and chemical compound identification

A gas chromatographic-mass spectral analysis was performed on the essential oils of *A. absinthium* and *M. suaveolens* using an Agilent 6890 GC with Agilent 5973 mass selective detector (scan range = 45-400 amu, and scan rate = 3.99 scans/s ,EIMS, electron energy = 70 eV), and a fused silica capillary column (HP-5ms, 30 m × 0.25 mm). The carrier gas was helium, with a flow rate of 1 mL/min and a temperature of 200°C for injection [24]. By comparing their retention indices to a homologous sequence of n-alkanes and matching their mass spectral fragmentation patterns to those published in the literature, the oil components were determined.

Antifungal activity

The antifungal activity was tested against food-borne pathogens *Fusarium oxysporum, Fusarium solani* and *Botrytis cineria,* the strains were isolated from an experimental station

The presence or absence of mycelial growth inhibition zones and MIC values were used to test the antifungal activity of *A. absinthium* and *M. suaveolens* essential oils alone against the examined fungi strains in vitro and for the combination of the two essential oils we only used the mycelial growth inhibition values.

The broth microdilution test was used to determine the MIC. The two essential oils were first serially diluted in PDB (potato dextrose broth) to get final concentrations of 100, 200, 400, 800, 1600, and 3200 g/ml (v/v). Then, in each well, 10 µL of fungal inoculum was added. As a bacterial growth indicator, 5 µL of resazurin was added to each well after one week of incubation at 28°C. The bacterial growth was demonstrated by the color change from purple to pink after a second incubation at 37°C for 2 hours. The MIC was calculated as the lowest concentration that prevented resazurin from changing color [25].

The mycelial growth inhibition of the essential oils alone and in combination was determined using the well diffusion method. The broth cultures of Fusarium oxysporum, Fusarium solani, and Botrytis cineria were swabbed onto PDA culture medium (Potato Dextrose Agar) plates and left for 15 minutes for absorption. The broad end of a sterile Pasteur pipette (6 mm diameter) was used to make wells in agar plates, and 5 µl of oil was added to each well. The plates were incubated for one week at 25°C, following which the sizes of the inhibitory zones were measured in millimeters.

The same procedure was taken for the combination of the two studied essential oils, using a mix of decreasing concentrations of *M. suaveolens* and increasing concentrations of *A. absinthium* : 50% + 50 % (1 :1) as demonstrated in table 1.

The absence of growth was reflected by the formation of a transparent circular zone around the well, indicating the action of the essential oils mixture. The inhibitory zone's diameter was measured in millimeters. The more vulnerable the strain is to the tested essential oil, the greater the diameter of the area.

Statistical Analysis

Tests were performed in triplicates to compare the mycelial growth inhibition zone and MIC values. Analysis of variance was performed. Significant differences between means were determined by the risk of 0.05%.

Results and discussion

Extraction and chemical composition of the essential oils

The hydrodistillation of the leaves of *Mentha suaveolens* and *Artemisia absinthium* provided two essential oils characterized with typical odors, and yields of 1.14% and 0.92% respectively.

The gas chromatographic-mass spectral analysis of the essential oils results are presented in table 2 and table 3. Seventeen components characterized the essential oil of *M. suaveolens* representing 90.6% of the total oil. Quantitatively, the most abundant were Piperitenone oxide (52.4%) and Piperitenone (12.1%). Minor components were Camphene (0.7%) and α-Humulene (0.6%). Previous studies of this essential oil had also proven that Piperitenone oxide and Piperitenone are the major compounds [26-28]. A total of nineteen compounds were identified in the essential oil of *A. absinthium*, representing 95.3 % of the total oil. The major compound was β-Thujone (85.1%). The minor compounds were α-Terpinene (0.1%), γ-Terpinenecis-Sabinene (0.1%) and hydrate Germacrene D (0.1%). Several studies of this essential oil have shown that its major compound is β-Thujone [17,20,24].

Antifungal activity

The single antifungal effects of *M. suaveolens* and *A. absinthium* essential oils were tested using the well diffusion method and the microdilution assay. The mycelial growth inhibition zones and the MIC values of each essential oil are represented in table 4. The two essential oils inhibited mycelial development in the species tested. However, it is obvious that the three strains investigated are more resistant to the essential oil of Mentha suaveolens. The *A. absinthium* essential oil had more mycelial growth inhibition since the inhibition zones were wider than those caused by the introduction of the *M. suaveolens* essential oil on every studied strain. The maximum inhibition zone was encountered on the *F. solani* (22.8 mm) and it was caused by the *A. absinthium*. The minimal inhibition zone (13.5 mm) was caused by the *M. suaveolens* on the *B. cineria* strain.

The results from the microdilution assay showed that *M. suaveolens* and *A. absinthium* essential oils possessed antifungal activities against all the tested fungi strains with MIC values ranging from 145.54 to 475.32µg/mL. For *M. suaveolens* essential oil, the minimum inhibitory concentrations (MICs) of *Fusarium oxysporum, Fusarium solani and Botrytis cineria* were found to be 475.32 µg/ml , 401.85 µg/ml and 145.54 µg/ml ,respectively.The *Botrytis cineria* strain was the most sensible to this essential oil because it had the lowest MIC value. Also, the *A. absinthium* displayed significant antifungal effect as minimum inhibitory concentrations against all tested pathogens, *Fusarium oxysporum , Fusarium solani and Botrytis cineria* with their respective MIC values being 388.71 µg/ml, 408.14 µg/ml and 391.02 µg/ml. The *Fusarium oxysporum* strain was the most sensible organism to this essential oil. The *A. absinthium* essential oil has generally shown more antifungal effect on the tested strains. Except for the *Botrytis cineria* strain, this has shown resistance to the essential oil of *A. absinthium*.

The results of the well diffusion assay of the combinations of the *Mentha suaveolens* and *Artemisia absinthium* essential oils is shown in table 5. The 100 % concentration of *Artemisia absinthium* essential oil produced the largest inhibitory zones. The values were 22.3 mm, 22.8 mm and 20.1 mm for the *Fusarium oxysporum*, *Fusarium solani* and *Botrytis cineria* strains respectively. The combinations of the two essential oils in all concentrations were generally lower than the combined effect of the essential oils tested individually. In addition, the mixture of oils showed a significant decrease in the antifungal activity against all three fungi species, with the increase of the concentration of the *Mentha suaveolens* essential oil. Which directly apply that the antifungal activity of the Artemisia *absinthium* essential oil is inhibited by the *Mentha suaveolens* essential oil. There are four sorts of impacts that might result from the combination of essential oil compounds:

indifferent, additive, antagonistic, or synergetic effects. When the total effect equals the sum of the individual effects, this is known as an additive effect. When one or both substances are treated together, their effect is reduced compared to when they are applied separately. Synergism occurs when the combined effect of the substances is higher than the sum of their separate effects, whereas indifference occurs when there is no interaction. [2] And seeing that our *in vitro* investigations have resulted in: a combined effect lower than that of the individual essential oil. We can say that the interaction between *Mentha suaveolens* and *Artemisia absinthium* essential oils has a predominantly antagonistic profile against all the studied pathogens.

Despite the fact that the mechanisms of interaction that cause antagonistic effects have received less attention than those that produce synergetic activity , The interaction between non-oxygenated and oxygenated monoterpene hydrocarbons has been linked to an antagonistic effect in recent studies [15]. Antagonism occurs when the biological activity of a mixture of components is reduced as compared to the individual activity of each component [11]. In the case of antagonistic effects, researchers have focused on the occurrence of decreases in the antibacterial effects of combination treatments to help: (1) avoid overestimating the Essential oil's efficacy and (2) develop countermeasures against these unexpected combined effects before they are used [29]. However, the combined effects of essential oils can differ depending on the target species, emphasizing the significance of evaluating antimicrobial complexes for each target species.

Conclusion

Because of their potential use as natural additives, the antimicrobial characteristics of essential oils and different extracts from numerous plants have recently piqued the interest of both academics and the food industry. This stems from a growing trend to replace synthetic antimicrobials with natural ones. The focal point of this study was the antifungal effect of the combining of plants essential oils against some food-borne pathogens. Owing to strong antifungal features exhibited in antifungal activity tests, the essential oil of *Mentha suaveolens* and *Artemisia absinthium* could be considered great antifungal agents. But according to our results; in vitro testing demonstrated that the combination of these essential oils is antagonistic against the studied fungi species. But we should stress that the combined effects of essential oils can differ dependent on the target pathogenic species, emphasizing the need of the evaluation of antimicrobial complexes for each target.

References

1. Ainane, A., Khammour, F., Charaf, S., Elabboubi, M., Elkouali, M., Talbi, M., Ainane, T. (2019). Chemical composition and insecticidal activity of five essential oils: Cedrus atlantica, Citrus limonum, Rosmarinus officinalis, Syzygium aromaticum and Eucalyptus globules. Materials Today: Proceedings, 13, 474-485.
2. Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. International journal of food microbiology, 94(3), 223-253.
3. Elabboubi, M., Bennani, L., Ainane, A., Charaf, S., Bouhadi, M., Elkouali, M. H., Ainane, T. (2019). Treatment of mycoses by essential oils: Mini Review. Journal of Analytical Sciences and Applied Biotechnology, 1(2), 35-40.
4. Schwab, W., Davidovich-Rikanati, R., & Lewinsohn, E. (2008). Biosynthesis of plant-derived flavor compounds. The plant journal, 54(4), 712-732.
5. García-Salinas, S., Evangelopoulos, M., Gámez-Herrera, E., Arruebo, M., Irusta, S., Taraballi, F., Tasciotti, E. (2020). Electrospun anti-inflammatory patch loaded with essential oils for wound healing. International journal of pharmaceutics, 577, 119067, 1-6.
6. Amor, N. B., Nava, V., Albergamo, A., Potortì, A. G., Turco, V. L., Mansour, H. B., & Di Bella, G. (2021). Tunisian essential oils as potential food antimicrobials and antioxidants and screening of their element profile. European Food Research and Technology, 247(5), 1221-1234.

7. Kasapoğlu, K. N., Altin, G., Farooqi, A. A., Salehi, B., Özçelik, B., Setzer, W. N., & Sharifi-Rad, J. (2020). Anti-proliferative, genotoxic and cytotoxic effects of phytochemicals isolated from Anatolian medicinal plants. Cellular and Molecular Biology, 66(4), 145-159.
8. Antonelli, F., Bartolini, M., Plissonnier, M. L., Esposito, A., Galotta, G., Ricci, S., Romagnoli, M. (2020). Essential Oils as Alternative Biocides for the Preservation of Waterlogged Archaeological Wood. Microorganisms, 8(12), 2015, 1-25.
9. Belanche, A., Newbold, C. J., Morgavi, D. P., Bach, A., Zweifel, B., & Yáñez-Ruiz, D. R. (2020). A meta-analysis describing the effects of the essential oils blend agolin ruminant on performance, rumen fermentation and methane emissions in dairy cows. Animals, 10(4), 620, 1-15.
10. Atif, M., Ilavenil, S., Devanesan, S., AlSalhi, M. S., Choi, K. C., Vijayaraghavan, P., ... & Alanazi, N. F. (2020). Essential oils of two medicinal plants and protective properties of jack fruits against the spoilage bacteria and fungi. Industrial Crops and Products, 147, 112239.
11. Othman, M., Saada, H., & Matsuda, Y. (2020). Antifungal activity of some plant extracts and essential oils against fungi-infested organic archaeological artefacts. Archaeometry, 62(1), 187-199.
12. Ainane, A., Abdoul-Latif, F. M., Mohamed, J., Attahar, W., Ouassil, M., Shybat, Z. L., Ainane, T. (2021). Behaviour desorption study of the essential oil of Cedrus atlantica in a porous clay versus insecticidal activity against Sitophilus granarius: explanation of the phenomenon by statistical studies. International Journal of Metrology and Quality Engineering, 12, 12, 1-11.
13. Gruľová, D., Caputo, L., Elshafie, H. S., Baranová, B., De Martino, L., Sedlák, V., De Feo, V. (2020). Thymol chemotype Origanum vulgare L. essential oil as a potential selective bio-based herbicide on monocot plant species. Molecules, 25(3), 595.
14. Price, S., Price, L., & Price, P. (Eds.). (2021). Aromatherapy for Health Professionals Revised Reprint E-Book. Elsevier.
15. Chagas, E. C., Majolo, C., Monteiro, P. C., Oliveira, M. R. D., Gama, P. E., Bizzo, H. R., & Chaves, F. C. M. (2020). Composition of essential oils of Mentha species and their antimicrobial activity against Aeromonas spp. Journal of Essential Oil Research, 32(3), 209-215.
16. Benkhaled, A., Boudjelal, A., Napoli, E., Baali, F., & Ruberto, G. (2020). Phytochemical profile, antioxidant activity and wound healing properties of Artemisia absinthium essential oil. Asian Pacific Journal of Tropical Biomedicine, 10(11), 496.
17. Blagojević, P., Radulović, N., Palić, R., & Stojanović, G. (2006). Chemical composition of the essential oils of Serbian wild-growing Artemisia absinthium and Artemisia vulgaris. Journal of Agricultural and Food Chemistry, 54(13), 4780-4789.
18. Maw, M. G., Thomas, A. G., & Stahevitch, A. (1985). THE BIOLOGY OF CANADIAN WEEDS.: 66. Artemisia absinthium L. Canadian journal of plant science, 65(2), 389-400.
19. Riahi, L., Chograni, H., Elferchichi, M., Zaouali, Y., Zoghlami, N., & Mliki, A. (2013). Variations in Tunisian wormwood essential oil profiles and phenolic contents between leaves and flowers and their effects on antioxidant activities. Industrial Crops and Products, 46, 290-296.
20. Juteau, F., Jerkovic, I., Masotti, V., Milos, M., Mastelic, J., Bessière, J. M., & Viano, J. (2003). Composition and antimicrobial activity of the essential oil of Artemisia absinthium from Croatia and France. Planta medica, 69(02), 158-161.
21. Bellakhdar, J. (1996). Medicinal plants in North Africa and basic care in. Hand book of Modern Herbal Medicine. Le Fennec. Medicinal plants in North Africa and basic care in. Hand book of Modern Herbal Medicine. Le Fennec, 385.
22. Kasrati, A., Jamali, C. A., Bekkouche, K., Lahcen, H., Markouk, M., Wohlmuth, H., ... & Abbad, A. (2013). Essential oil composition

and antimicrobial activity of wild and cultivated mint timija (Mentha suaveolens subsp. timija (Briq.) Harley), an endemic and threatened medicinal species in Morocco. Natural product research, 27(12), 1119-1122.
23. Bayoub, K., Baibai, T., Mountassif, D., Retmane, A., & Soukri, A. (2010). Antibacterial activities of the crude ethanol extracts of medicinal plants against Listeria monocytogenes and some other pathogenic strains. African Journal of Biotechnology, 9(27), 4251-4258.
24. Sharopov, F. S., Sulaimonova, V. A., & Setzer, W. N. (2012). Composition of the Essential oil of Artemisia absinthium from Tajikistan. Records of Natural Products, 6(2).
25. Puškárová, A., Bučková, M., Kraková, L., Pangallo, D., & Kozics, K. (2017). The antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells. Scientific reports, 7(1), 1-11.
26. Benayad, N., Ebrahim, W., Hakiki, A., & Mosaddak, M. (2012). Chemical characterization and insecticidal evaluation of the essential oil of Mentha suaveolens L. and Mentha pulegium L. growing in Morocco. Scientific Study & Research. Chemistry & Chemical Engineering, Biotechnology, Food Industry, 13(1), 27.
27. Bouyahya, A., Belmehdi, O., Abrini, J., Dakka, N., & Bakri, Y. (2019). Chemical composition of Mentha suaveolens and Pinus halepensis essential oils and their antibacterial and antioxidant activities. Asian Pacific Journal of Tropical Medicine, 12(3), 117.
28. Civitelli, L., Panella, S., Marcocci, M. E., De Petris, A., Garzoli, S., Pepi, F., Angiolella, L. (2014). In vitro inhibition of herpes simplex virus type 1 replication by Mentha suaveolens essential oil and its main component piperitenone oxide. Phytomedicine, 21(6), 857-865.
29. Cho, T. J., Park, S. M., Yu, H., Seo, G. H., Kim, H. W., Kim, S., & Rhee, M. S. (2020). Recent Advances in the Application of Antibacterial Complexes Using Essential Oils. Molecules, 25(7), 1752.

Table 1. Percentage mixtures of *M. suaveolens* and *A. absinthium* for the well diffusion assay.

Percentage of essential oil %	
Mentha suaveolens	***Artemisia absinthium***
100	0
90	10
80	20
70	30
60	40
50	50
40	60
30	70
20	80
10	90
0	100

Table 2. Chemical composition of the *Mentha suaveolens* essential oil.

Peak	**Constituents**	**RI**	**Content (%)**
1	α-Pinene	938	1.6
2	Camphene	945	0.7
3	Sabinene	971	1.1
4	β-pinene	976	1.8
5	Myrcene	980	3.1
6	Limonene	1022	1.8
7	1.8-Cineole	1030	0.8
8	Menthone	1211	2.5
9	Pulegone	1244	5.7
10	cis-Piperitone	1295	1.8
11	Piperitenone	1321	12.1
12	Piperitenone oxide	1349	52.4
13	α-Caryophyllene	1398	1.2
14	β-Caryophyllene	1412	0.9
15	α-Humulene	1448	0.6
16	Germacrene D	1483	1.1
17	Spathulenol	1541	1.4
Total identified compounds (%)			90.6

Table 3. Chemical composition of the Artemisia absinthium essential oil.

Peak	Constituents	RI	Content (%)
1	α-Pinene	938	0.2
2	Sabinene	971	1.1
3	β-Pinene	978	0.5
4	α-Terpinene	1013	0.1
5	1.8-Cineole	1030	0.6
6	γ-Terpinene	1062	0.1
7	cis-Sabinene hydrate	1072	0.1
8	Linalool	1095	2.1
9	α-Thujone	1105	1.4
10	β-Thujone	1130	85.1
11	Pulegone	1235	0.6
12	cis-Piperitone epoxide	1252	0.2
13	cis-Chrysanthenyl acetate	1260	0.3
14	Thymol	1289	0.9
15	(E)-Caryophyllene	1416	1.1
16	Germacrene D	1483	0.1
17	Neryl 2-methylbutanoate	1571	0.2
18	Pogostol	1650	0.2
19	Chamazulene	1730	0.4
Total identified compounds (%)			95.3

Table 4. Antifungal activity of the essential oils of *Mentha suaveolens* and *Artemisia absinthium.*

	Mentha suaveolens		***Artemisia absinthium***	
	Mycelial growth inhibition (mm)	**MIC (µg/ml)**	**Mycelial growth inhibition (mm)**	**MIC (µg/ml)**
Fusarium oxysporum	18.5	475.32	22.3	408.14
Fusarium solani	21.2	401.85	22.8	388.71
Botrytis cineria	13.5	145.54	20.4	391.02

Table 5. Synergetic antifungal activity of the two essential oils in combination.

Percentage of essential oil		**Mycelial growth inhibition (mm)**		
Mentha suaveolens	***Artemisia absinthium***	***F. oxysporum***	***F. solani***	***B. cineria***
100	0	18.5	21.2	13.5
90	10	17.4	21.1	13.4
80	20	17.4	21.1	13.4
70	30	17.2	20.8	14.8
60	40	17.2	19.5	16.4
50	50	17.1	19.5	16.5
40	60	16.8	19.4	16.4
30	70	17.5	19.4	17.8
20	80	18.4	20.6	18.5
10	90	20.1	22.6	19.8
0	100	22.3	22.8	20.1