J. Agric. Sci. Mansoura Univ., 28(4): 2747 - 2756, 2003

INHIBITORY EFFECT OF ESSENTIAL OILS TOWARDS SOME PLANT PATHOGENIC FUNGI

El-Ghadban,E.A.E.; Faten R. Moussa and Nawal G. Ghally
Medicinal and Aromatic Plant Research Department, Horticulture Research Institute, Agriculture Research Center, Dokki, Giza, Egypt

ABSTRACT

This study was done during (2000) to study the anti-fungal activity of some essential oils namely thyme, rosemary, lemongrass and geranium essential oils against four pathogenic plants fungi namely *Alternaria solani, Alternaria alternata, Marcophomina phaseolina* and *Sclerotium cepivorum.*

The physico-chemical properties of these oils were determined. Also, the chemical composition and the main constituents were identified.

The results revealed that, all the tested essential oils had different anti-fungal activity against the fungi under study.

Thyme oil showed the highest anti-fungal efficacy, when its inhibitory rate ranged between 125 and 500 ppm. Followed by lemongrass oil (250 to 1000 ppm.), however, geranium oil showed a moderate anti-fungal efficacy varied from 500 to 1000 ppm.

Rosemary oil was the lowest one (1000 To 1200 ppm.), so that, it had no anti-fungal effect against *Sclerotium cepivorum* fungus. On the other hand, *Alternaria solani* fungus was sensitive towards most of the oils under test.

Keyword: Thyme, rosemary, lemongrass, geranium, essential oil, inhibitory, pathogenic fungi.

INTRODUCTION

The presence of highly toxic and carcinogenic mycotoxins excreted by certain types of fungi in plant and foodstuffs have led to extensive research involving methods for inhibiting the synthesis of mycotoxins.

Several workers studied the effect of various essential oils on the fungal growth and aflatoxin production. For example, Maruzzella and Liquri (1958) reported that, many volatile oils such as those of cinnamon, caraway, geranium and peppermint had pronounced activities on the growth of many fungal genera.

Chakravarty and Baruah (1970) tested 9 phenolic compounds for growth inhibitory effects on the pathogenic fungi, *Helminthosporium oryza.* Anethole had some inhibitory effect.

Monohydric phenols such as menthol, thymol, and o.cresol were inhibitory to spore formation than dihydric phenols such as, hydroquinone pyrocatecol or trihydric phenols such as phlorogluccinol. Most of the phenolic compounds produced toxic effect to the fungus at concentration less than 0.1%

Rao and Joseph (1971) reported that the essential oil of lemongrass and other essential oils had antifungal activity against *physalospora tucamanesis, caratocytic paradoxa, carvularia* and *fusarium moniliforme.*

Study of some anti-fungal terpenoids was preformed by Agarwal and Mathela (1979). The essential oil of *Thymus serpyllum* was highly active against 6 penicillium and fusarium species. The activity was attributed mainly to carvacol and thymol present in the oil.

Among other terpeniod, geraniol was most effective followed by â-pinene, p-cymene, cinole and –Jterpineol, carvacrol and thymol, showed higher fungicidal activities than those of standard anitfungal.

Effect of volatile compounds of carrot seed oil on growth and aflatoxin production by *Aspergillus parasiticus* was studied by Batt *et al.*, (1983).Geraiol, citral and terpeneol prevented growth and no aflatoxin produced.

Limonene and terpinene did not affect the fungal growth but inhibited aflatoxin production.

Pouli and Knobioch (1987) studied the anti-fungal properties of equiamolar amounts of 16 components, constituents of essential oils against 5 *Asperagillus spp.* 4 *penicillium spp.*, and *Fusarium spp.* Anti-fungal activity was compared to that of phenol and the results showed that substituted phenols had the strongest anti-fungal activity.

Most potent components being iso-eugenol, cinamaldhyde, carvacrol, eugonal and thymol.

El-Baroty (1988) mentioned that anti-fungal potentiality was determined in-vitro by adding various concentrations of cumin, thyme, clove, caraway and sage essential oils to *Aspergillus parasiticus* culture medium.

The oils caused complete inhibition in both mycelia growth and aflatoxin production at certain levels depending on the oil type.

According to the minimum inhibitory concentration (MIC), the inhibitory effect of the oils followed descending sequence, thyme > cumin > clove, caraway > rosemary > sage.

The (MIC) towards fungus metabolism for the oil and their basic components were exactly by the same.

MATERIALS AND METHODS

Source of essential oil plants.

The leaves of thyme *(Thymus vulgarius,L.)*, lemongrass *(Cymbogon citratus,L.)*, staple geranium *Pelarganium grveiens,L.* and rosemary *(Rosemarinus officinalis,L.)* belonging to Labiatae family were collected from the farm of Medicinal and Aromatic Plants Department at Dokki, Horticulture Research Institute, Agriculture Research Center,

All the essential oils samples were produced from crude parts by steam distillation as described by Guenther (1961) (during 2000) in laboratory of Medicinal and Aromatic Plants Department.

Source of microbial culture

Culture of *Alternaria solani, Alternaria alternata, Marcophomina phaseolina* and *Sclerotium cepivorum* were obtained from Cairo Mircen, Faculty of Agriculture, Ain Shams University, Egypt (during 2000).

Identification and determination of the essential oil composition

The G.L.C. analysis was carried out in the central laboratory of Fac. Agri., Cairo Univ. (during 2000).
The conditions used are described in:
PRO - Gc Pye Unicom
Column: PEGA 10 % on chromosorb

Temp. Programming:

Initial Temp.	70 °C
Rate	4 °C/mi
Final Temp.	190 °C
Final Time	20 min.
Detector Temp.	300 °C
Injection Temp.	250 °C
Chart, speed	2 min./cm.

Flow rate of gases

N2	30 ml./min.
H2	33 ml./'min.
Air	330 ml./min.

Determination of anti-microbial activity

This method was carried out to measure the anti-microbial activity according to Sleigh and Timburg (1981). The effect of different tested natural oils and their main components on the growth of tested fungi *(Alternaria solani, Alternaria alternata, Marcophomina phaseolina* and *Sclerotium cepivorum.)* was studied. The tested fungi were grown on water agar (WA) media to yield a low growth.

After an equal discs (5 ml. in diameter) from Whatman No. 1 filter paper were saturated with the different tested natural oils and their main components separately for 10 minutes, two saturated discs were used for each petridish and 4 dishes were used for each tested oil as replicates.

Dishes contained only WA media were used as control. The inhibition zones of fungal growth produced by different tested oils were measured in mm and tabulated.

Minimum inhibitory concentration (MIC)

Different concentrations 125, 250, 500, 1000 and 1250 ppm of oils under investigation were carried out, the filter discs were conducted to the above mentioned concentrations and the used in petridishes contained WA media as mentioned before. The inhibition zones were also measured as mentioned in disc diffusion method. The lowest quantities required to the determination of the fungi pathogenic fungi was designated as the MIC of the oil, Moussa (1998).

RESULTS AND DISCUSSION

A) sical and chemical properties of the essential oils :

The most important physical and chemical properties of thyme, rosemary, lemongrass and geranium essential oils are determined and the results are shown in Table (1). Most of the values were to be within the range mentioned by Guenther (1961).

Table (1): The physical and chemical properties of the tested Labiatae essential oils.

Oils	Specific Gravity at 15 °C	Refractive index	Optical rotation	Acid number	Ester number
Thyme Lemongrass	0.9538	1.4951	+6° 30'	1.5	1.52
Lemongrass	0.8871	1.4881	+11° 5'	3.75	20.27
Geranium	0.8985	1.4752	- 10° 8'	4.60	51.60
Rosemary	0.9153	1.4728	+11° 19'	1.20	18.80

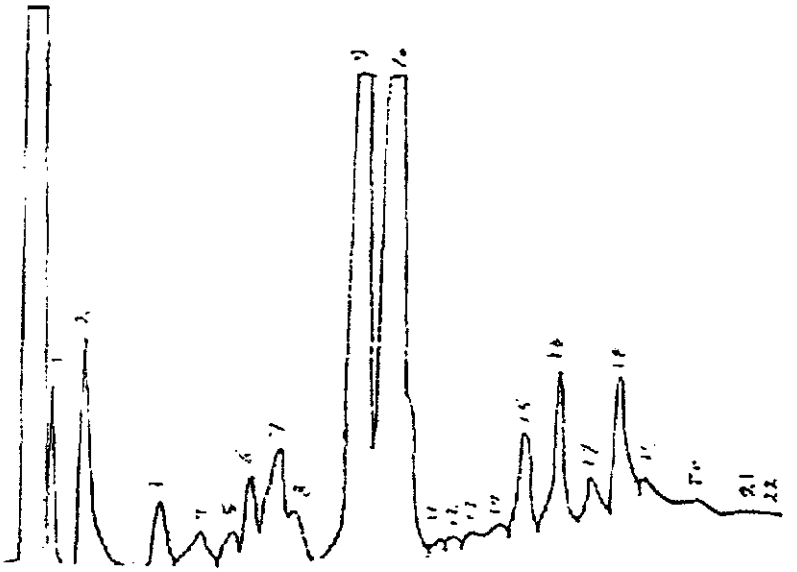
B) Chemical composition of the tested essential oils :

Gas liquid chromatography was used to determine the chemical composition of four essential oils of Labiatae family (thyme, lemongrass, geranium and rosemary).

The chemical composition of these oils is listed in Table (2) and their chromatograms in Figures (1 - 4).

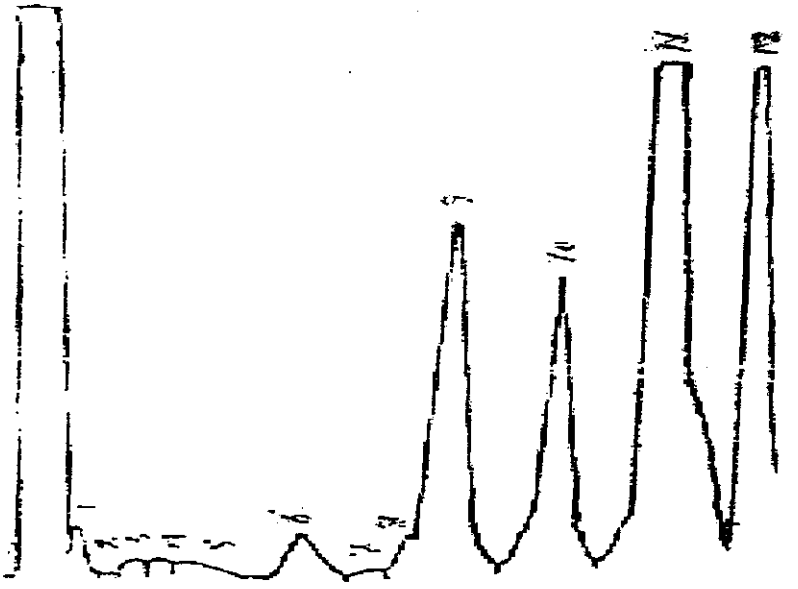
Table (2): Chemical components of the tested essential oils

Compounds %	Essential oils			
	Thyme	Lemongrass	Geranium	Rosemary
α. Pinene	1.1	1.3	0.6	12.7
β. Pinene	0.3	-	0.2	2.5
Camphene	-	-	-	0.1
Limonene	0.3	4.7	0.3	6.0
γ. Terpinene	0.3	-	-	15.6
Phellandrene	1.5	-	-	1.2
Myrecene	-	-	-	0.2
P-Cymene	36.0	1.1	-	3.0
Caryophyllene	-	-	-	9.6
Eugenol	-	-	-	-
Thymol	42.7	4.8	-	0.4
Citronelloi	-	-	13.9	-
Thujone	-	-	-	0.2
Linalooi	-	2.08	-	0.6
Geraniol	-	24.8	25.9	0.3
Citral	-	33.7	-	-
Cineole	-	1.2	-	-
Borneol	0.7	-	10.4	26.5
Linalyl Acetate	1.0	3.8	-	0.3
Terpinyl Acetate	0.1	-	-	0.1
Geranyl Acetate	-	1.1	-	-
Geranyl	-	1.0	13.5	-



1. α. Pinene
2. Limonene
3. Unknown
4. Unknown
5. Menthone
6. Linalool
7. Linalyl acetate
8. Cineole
9. Geraniol
10 Citral
11Unknown
12.Unknown
13.Geranyle acetate
14.Anucic
15.Thymol

Fig.(1) Gas chromatogram of lemongrass oil



1. α. Pinene
2. Unknown
3. β. Pinene
4. limonene
5. α. Terpnine
6. Unknown
7. Unknown
8. Methone
9. Borneol
10.Unknown
11. Geraniol
12.Geranyl formate
13. Unknown
14. Unknown
15.Anicic
16.Citronellol

Fig.(2) Gas chromatogram of Geranium oil

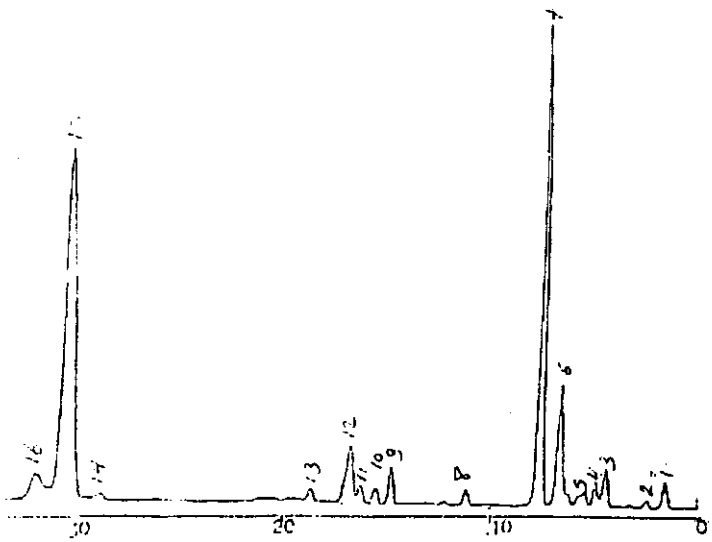


Fig.(3) Gas chromatogram of Thyme oil

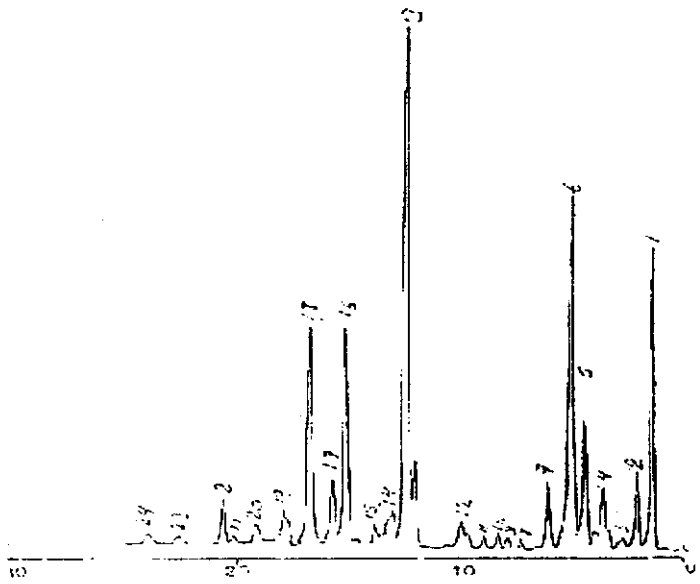


Fig.(4) Gas chromatogram of Rosemary oil

GLC analysis of the fresh essential oils in table (2) and their chromatograms in Fig.(1 - 4) showed that, thyme oil was characterized by the presence of thymol (42.7), beside a great amount of myrcene (36.0%).
However, the major components of lemongrass oil were citral (33.7%), and geraniol (24.8 %) beside thymol (4.8%).
Geranium oil was characterized by the presence of two compounds, geraniol and citronellol (25.9% and 13.9%) respectively.
As for rosemary oil, the main compounds were borneol (26.5%) α- Terpinene (15.6%) and α-Pinene (12.7%).

C) Anti-fungal effect of the essential oils under study

Molds and fungi are very common in our environment, and their occurrence in plant diseases should be particularly concerned.
The obtained data in Table (3) of the inhibition zone (mm) on various fungal growth with different essential oils components indicated that, thymol alcohol in thyme oil had the highest anti-fungal activity followed by citral in lemongrass oil.
Citronellol was slightly powerful than geraniol in geranium oil but borneol in rosemary oil was the lowest one.

Table (3): Diameter of inhibition zone (mm) of the main components of the tested essential oils

Number	Main compounds	Microorganisms			
		Alternaria solani	*Alternaria altrenata*	*Marcophomina phaseoline*	*Sclerotium Cepivorum*
1	**Thymol (thyme)**	30	28	27	25
2	**Citral (lemongrass)**	22	20	21	18
3	**Geraniol (geranium)**	18	17	16	13
4	**Citronellol (geranium)**	20	18	17	12
5	**Borneol (rosemary)**	15	12	12	No effect

Also the obtained data in table (4) indicated that, all the tested essential oils (without dilution) had different effects against the four fungi under study in most cases, with an exception in case of *sclerotium cepivorum* fungus whose was resistance towards rosemary essential oil.
This anti-microbial activity of essential oils against microorganism might be due to that essential oils cause damage to biological membrane due to lipophilic properties interfering with membrane integrated enzyme, El. Baroty (1988).

Maruzzella (1962) arranged the active part of volatile oil according to their anti-microbial activities in the decreasing order as follows: aldehyde, phenols, alcohols, ketones, hydrocarbons.

The obtained data in Table (4) revealed that, thyme oil showed higher anti-fungal activity against all selected strains of fungi than comparable essential oils followed by lemongrass oil and geranium, respectively, while rosemary oil had the lowest one.

Also, the same data indicated that, every essential oil showed more powerful anti-fungal activity than its main component, Generally, it can be concluded that, essential oil of thyme followed by lemongrass had the most anti-fungal effect against the tested fungi.

Table(4): Diameter of inhibition zone (mm) of the tested essential oils.

No.	Oils	Microorganisms			
		Alternaria solani	*Alternaria altrenata*	*Marcophomina phaseoline*	*Sclerotium cepivorum*
1	Thyme	35	30	28	26
2	Lemongrass	24	22	22	19
3	Geranium	19	19	18	14
4	Rosemary	16	14	13	No effect

The minimum inhibitory concentration (MIC) in Table (5) indicated that, *Alternaria solani* fungus was more sensitive to all essential oils where their (MIC) for this fungus were 125, 250,500 and 1000 ppm for thyme, lemongrass, geranium and rosemary oils respectively.

On the other hand, *Sclerotium cepivorum* was tolerance towards the tested fungi as descending order, thyme, lemongrass, geranium, and rosemary while *Alternaria alternata* fungus was more sensitive to thyme and lemongrass oil, where their (MIC) 250 and 500 ppm, respectively, but it was resistant to geranium and rosemary oils.

Generally, the same sequence of tolerance towards the tested fungi as a descending order was in the same trend of their oils.

Table (5): Minimum inhibitory concentration of the tested essential oils on the growth of different fungi.

Number	Fungi	Thyme	Lemongrass	Geranium	Rosemary
		(MIC ppm)			
1	Alternaria solani	125	250	500	1000
2	Alternaria alternata	250	500	1000	1000
3	Marcophomina phaseoiina	250	500	1000	1250
4	Sclerotium cepivorum	500	1000	1000	No effect

Fig. (5) shows the chemical structure of the most compounds in the tested essential oils. It appears that, there is a relationship between the chemical composition of the oil and its inhibitory activity, this inhibitory effect might be due to the presence of group which is more active and easily forms bonds with the active sites of the fungal enzyme, El-Baroty (1988).

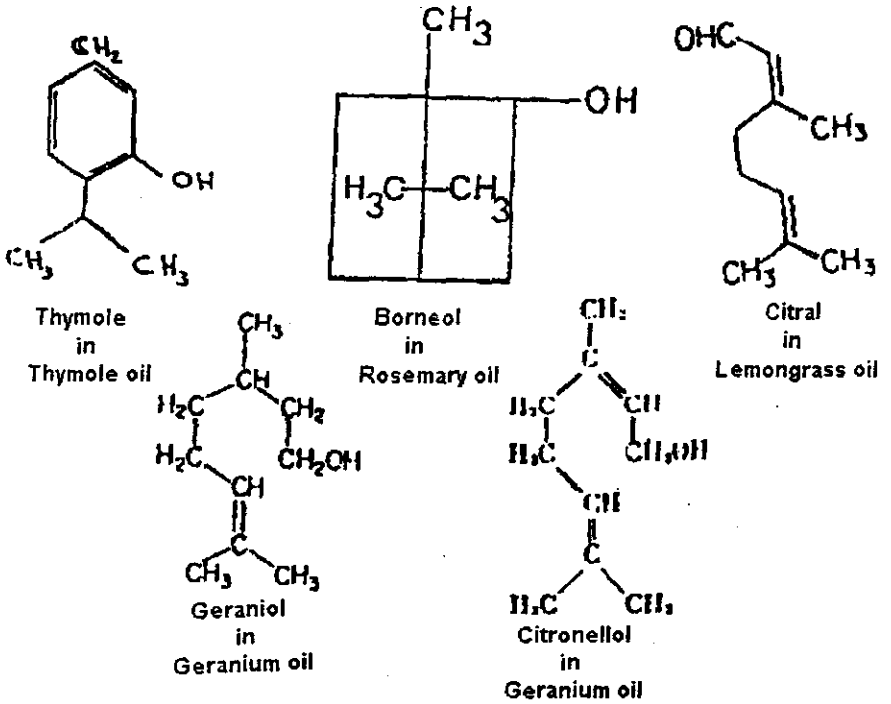


Fig.(5) Chemical structure of the basic comounds of some essential oils

Thyme oil had the highest anti-fungal effect due to the presence of the phenolic group of thymol compound besides the aromatic hydrocarbon p-cymene (36.0%).

Lemongrass oil was also a strong anti-fungal agent due to its containing a great amount of cetral aldhyde and geraniol alcohol (24.8%) beside thymol in small quantities.

As for geranium oil, it behaved a moderate anti-fungal agent because of it's containing alcoholic terpenes such as geraniol (25.9%) and citronellol (13.9%)

On the other hand, rosemary oil considered as the lowest anti-fungal agent in comparison to the other tested oils and its anti-fungal activity that attributed to the presence of borneol alcohol (26.5%), α -Terpinene (15.6%) and α- Pinene (12.7%)

These compounds together act as synergistic effect in its anti-fungal activity. El-Baroty and Moussa who indicated that there is a relationship between the chemical composition of the oil and its antimicrobial activity were in agreement with these results.

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التأثير المثبط لبعض الزيوت العطرية على بعض الفطريات الممرضة للنبات

الموافى عبده الموافى الغضبان - فاتن رمزى موسى - نوال جورج غالى

قسم النباتات الطبية والعطرية - معهد بحوث البساتين - مركز البحوث الزراعية - جيزة

أجرى هذا البحث خلال سنة ٢٠٠٠ وذلك لمعرفة تأثير الزيوت العطريه على تثبيط نمو الفطريات المرضية حيث تم الحصول على الزيوت العطرية (الزعتر- الحصالبان - حشيشة الليمون - العتر) المستخدمة في هذا البحث بطريقة التقطير بالبخار. وكذلك أجرى تقدير الصفات الطبيعة والكيميائية لهذه الزيوت المختبرة و تركيبها الكيماوى بطريقة التحليل الكروماتوجرافى ، والتعرف على المركبات الرئيسية بها . كما تم إختبار هذه الزيوت كمضادات فطرية ضد نشاط ونمو اربعة أنواع هامة من الفطريات المرضية للنبات (الترناريا سولانى - الترناريا الترناتا - ماركوفومينسا فازولينا - اسكليروتيم سيبيفورم) .

وقد أوضحت الدراسة النتائج التالية:

وجد أن جميع هذه الزيوت المختبرة لها تأثير مثبط قوى على هذه الفطريات المختبرة باستثناء فطر اسكليروتيم سيبيفوم والذى كان مقاوم لزيت الحصالبان، بينما كان فطر الترناريا سولانى من أكثر الفطريات حساسية لهذه الزيوت.

وتم تقدير اقل تركيز مثبط لهذه الزيوت على الفطريات المختبرة وكانت النتائج المتحصل عليها كالآتي :

- زيت الزعتر كان من أقوى الزيوت المثبطة لهذه الفطريات حيث كان اقل تركيز مثبط له يتراوح بين ١٢٥ - ٥٠٠ جزء فى المليون
- زيت حشيش الليمون جاء بعد زيت الزعتر فى الترتيب حيث كان له تأثير مثبط قوى أيضا على هذه الفطريات وكان أقل تركيز مثبط له يتراوح ما بين ٢٥٠ - ١٠٠٠ جزء فى المليون.
- زيت العتر كان له تأثير متوسط من حيث تثبيط نمو هذه الفطريات وكان اقل تركيز مثبط له يتراوح ما بين ٥٠٠ - ١٠٠٠ جزء فى المليون.

أما زيت الحصالبان فكان اقل هذه الزيوت تأثيرا على هذه الفطريات حيث كان اقل تركيز مثبط له يتراوح ما بين ١٠٠٠ - ١٢٠٠ جزء فى المليون.

من هذه الدراسة يتبين أن هناك علاقة قوية بين التركيب الكيماوى للزيت ومدى احتوائه على تربينات ومجاميع فينوليه وبين نشاطه كمضاد فطرى.