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ANTIFUNGAL PROPERTIES OF SOME ESSENTIAL OILS AGAINST PATHOGENIC FUNGI

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ABSTRACT

This study was done during 2006 to study the antifungal properties of some essential oils belonging to Lilliaceae family (garlic and onion), Labiatae family (spearmint) and Apiaceae family (cumin) against four pathogenic fungi namely *Alternaria alternata, Aspergillus niger, Aspergillus flavus* and *fusarium oxysporum*. The physico-chemical properties of these oils were determined. Also, the chemical composition was determined by GLC analysis. The main components were identified. The antifungal activity of these oils were investigated using disc-diffusion method Also, minimum inhibitory concentration (MIC) was determined to know the minimum concentration of each oil required to inhibit each fungus. The obtained results revealed that all the essential oils under study had antifungal against these pathogenic fungi. Garlic oil showed the highest antifungal efficacy, followed by onion oil, and cumin oil however, spearmint oil was the lowest. *Alternaria alternata* fungs showed sensitive behaviour to all essential oils species. On the other hand, *A.Flavus* was very resistant to different essential oils. *A.niger* showed sensivity towards onion oil. *F oxysporum* showed a moderate resistance. MIC of these essential oils ranged between 0.2 to 2.3 mg oil/ml medium.

Key words: Antifungal, minimum inhibitory concentration, Disk diffusion.

INTRODUCTION

In world crop production, pre harvest losses due to fungal disease may amount to 12% in developing countries. Chemicals used in the control of diseases pollute the atmosphere and affect the

properties of medicinal plants. To avoid the hazardous effect of chemicals, natural products of some plants have been used to control plant diseases (Rahber-Bhatti, 1986; Bowers, and Locke, 2000; Momin and Nair 2001). Fungal species of genera *Aspergillus, Fusarium, Alternaria* and *Drechslera* species have been considered to be major plant pathogens worldwide (Ghafoor, Khan, 1976; Mirza, Queshi, 1982). *Aspergillus* and *fusarium* species produce mycotoxins in food besides causing seedling blight, seed rot, kernel rot, stalk rot, with and stunt (Blat, 1969; Thiel *et al.*, 1991; Fandohan *et al.*, 2003).

Garlic (*Allium sativum L.*) is among the few herbs that have universal usage and recognition. 1st daily usage oils and supports the body in ways that no other herb does. It is one of the most effective anti-microbial plants available acting on bacteria, viruses and alimentary parasites. The volatile oil is an effective agent as it is largely excreted via the lungs. It is used in infections of this system such as chronic bronchitis, respiratory catarrh, recurrent colds and influenza. It may be helpful in the treatment of whooping cough and as part of broader approach to bronchial asthma. Volatile oil consisting of sulfur-containing compounds including allicin, allymelhyl sulphide allypropyldisulphide, diallyltrisulphide, Hoffmann and Hons (2001).

Onion (*Allium cepa L.*) is distributed worldwide, its blubs contains numerous organic sulfur compounds. Its volalile oil composed mainly of sulpher compounds including dipropyl sulphide (Leung and Foster, 1996). Onion is used for decreasing cancer tumor initiation, promote healing of stomach ulcers, inhibits the proliferation of cultured ovarian, breast and colon cancer cells, reduces the cholesterol, blood pressure and symptoms associated with diabetes mellitus, inhibit platelets aggregation (involved in thrombosis) and prevents inflammatory process associated with asthma (Dorsch and Wanger, 1991; Augusit, 1996).

Cumin is a strong aromatic of dried ripe fruit (seed) of cuminum cyminum L. (Apiaceae family). Cumin has about 2-4.5% of volalile oil. The chemical composition of cumin oil showed the presence of cuminic aldhyde (32.4%), D-terpinene (29.5%) and other phenolic terpens, Amin (1991). Cumin seed is an ancient spice with strong aromatic smell and warm. It is medley used in Tran and India both as a condiment and flavouring in many eastern dishes. Cumin is a common flavour in confectionery, meat sausage and breadmanu.

Facturing and as preservative in food processing, Jackson (1968). Essential oil of *Mentha spicata L.* had already been found by the outhors to exhibit strong fungitoxicity against some storage fungi (Yadav, 2002). The plant belongs to the family Labiateae commonly called spearmint.

MATERIALS AND METHODS

Materials:

Source of essential oils:

Four plants garlic (*Allium sativum* L.), Onion (*Allium cepal.*), cumin (*Cuminum cyminum* L.) and spearmint (*Menlha spicata* L.) belonging to lilliaceae family for garlic and onion, Apiaceae family for cumin and labiatae family for spearmint were contacted from the experimental farm of Horticulture Research Station in Kassassen, Ismailia governorate, Egypt, during 2006 year. All essential oils samples were extracted by steam distillation in Clevenger apparatus for three hours from crude parts (bulbs in garlic and onion, fruits in cumin and herb in spearmint).

Source of microbial culture:

Culture of Aspergillus niger, Aspergillus flavus, Alternaria alternate and Fusarium oxysporum were obtained from Cairo Mircen, Faculty of Agriculture, Ain Shams University, Egypt.

Methods:

Determination of physical and chemical properties of essential oils:

Specific gravity, refractive index-optical rotation- acid value, and ester number were determined according to the method described by A.O.A.C. (1995).

Identification and determination of the essential oil composition:

Samples of essential oils stored under refrigeration, protected from light in glass flasks with screwed cups and scatted with parfilm. They were analyzed in chromatography in gas phase. A chromatograph equipped with fire ionization detector (FID) and capillary tubes BPx.5 (30 ml length and 0.25 mm of internal diameter (ID) was used. The plunging gas was nitrogen. The initial temperature of the colum was 70-80°C, at rate of 5°C/min, from 80-120 °C. Programmed to raise 10 °C/min until the maximum temperature 19 °C.

The temperature of the injection and detector were fixed at 250 and 300, receptivity.

Determination of antifungal activity:

Disc diffusion method was used as antimicrobial method (Yin and Tsao, 1999). Sterile nutrient agar at 43-45 °C and poured into Petri plates (9 cm diameter). Then the agar was allowed to solidify at +4 °C for 1hr, 0.2 ml of each organism (Alternaria alternata, Aspergillus niger, Aspergilus flavus and fusarium oxysporum) inoculum applied to each plate. Inoculm was evently spread on agar using a glass rod spreader. The Petri were left at 4 °C for 1 hr to allow agar surface to dry. Sterile filter papers 16 mm diameter were placed on the culture medium and were impregnated with different essential oils, placed on the culture medium and placed on the inoculated plates. Plates were turned upside down and incubated at 30 °C for 24 hr. At the end period, inhibition zones formed in the medium were measured in millimeters (mm). All experiments were done in three replicates.

Determination of minimum inhibitory concentration (MIC):

5 ml of sterile essential oils at different concentration (0.2-2.3 mg/ml) were taken into the sterile empty tubes and 1 ml of each organism was added to the tube. After that lml of (essential oil + organism). Culture was added into 5ml of sterile nutrient agar both in the tubes. Then, all the tubes were incubated at 30 °C for 15 days. Observation were made for visible growth of fungi. The highest dilution (lowest concentration) is detected whose represents MIC, Abbasglu, 1996; Yin and Tsao, 1999; Rasooli and Abyaneh, 2004).

RESULTS AND DISCUSSION

A) Physical and chemical properties of tested essential oils:

The most important physical and chemical properties of garlic, onion, spearmint and cumin were determined and the results are shown in Table (1). Most of the values were found to be within the range by Guenther (1982).

B) Chemical composition of tested essential oils:

Gas liquid chromatography was used to determine the chemical composition of four essential oils of lilliaceae family (onion and garlic), Labiatae family for spearmint and Apiaceous family for cumin

oil. The chemical composition is listed in Table (2) and their chromatograms in Figs. (1-4).

GLC analysis of the fresh essential oils showed that garlic and onion were characterized by the presence of sulphide compounds such as diallydisulphide, diallyl trisulphide in garlic oil, whowever, onion oil contains fistulosin and dipropyl compound. Spearmint oil was characterized by the presence of carvone and linalool alcohol and Eugenol. As for cumin oil, the main components of it were cumin aldhyde and D-terpinene.

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

	Specific gravity (15°C)	**Refractive index (20°C)**	**Optical rotation**	**Acid number**	**Ester number**
Cumin	0.925	1.506	+ 3°	-	-
Spearmint	0.9126	1.4825	+ 22°	1.5	13.3
Onion	1.098	1.537	+ 1°	-	-
Garlic	1.050	1.580	+ 1°	-	-

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

Component %	Essential oils			
	Spearmint	**Cumin**	**Garlic**	**Onion**
α- Pinene	1.4	0.5	-	-
β-Pinene	4.01	19.0	-	-
Limonene	16.6	-	-	-
1,8 cineole	1.02	0.2	-	-
α- Terpinene	1.09	-	-	-
Linolool	5.05	-	-	-
Carvone	60.9	-	-	-
Caryophyllenc	3.3	-	-	-
Eugenol	1.61	-	-	-
Myrcene	-	0.3	-	-
P- cymene	-	8.5	-	-
D-terpincne	-	13.0	-	-
Cuminic aldehyde	-	32.4	-	-
Firstulosin	-	-	-	45.9
Dipropyl disulphide	-	-	-	6.8
Dipropyltrisulphide	-	-	-	1.1
Methyl propyldisulphide	-	-	-	0.8
Mctlylpropyl trisulpnide	-	-	-	0.5
Diallyldisulphide	-	-	22.2	-
Diallyltrisulphide	-	-	20.6	-
Allymcthyl disulphide	-	-	5.8	-
Allymcthyl trisulphid	-	-	4.5	22.2
Dimellyl disulphide	-	-	2.4	-
Dimelly trisulphide	-	-	4.3	-

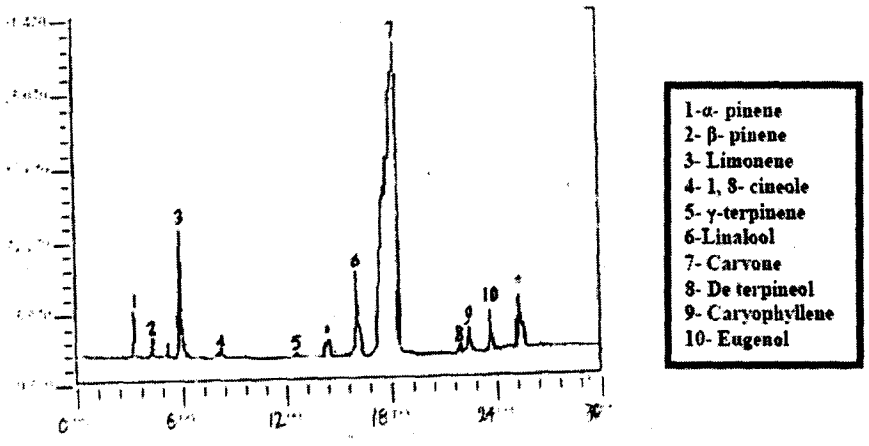


Fig. (1) Gas Chromatogram of Spearmint Oil

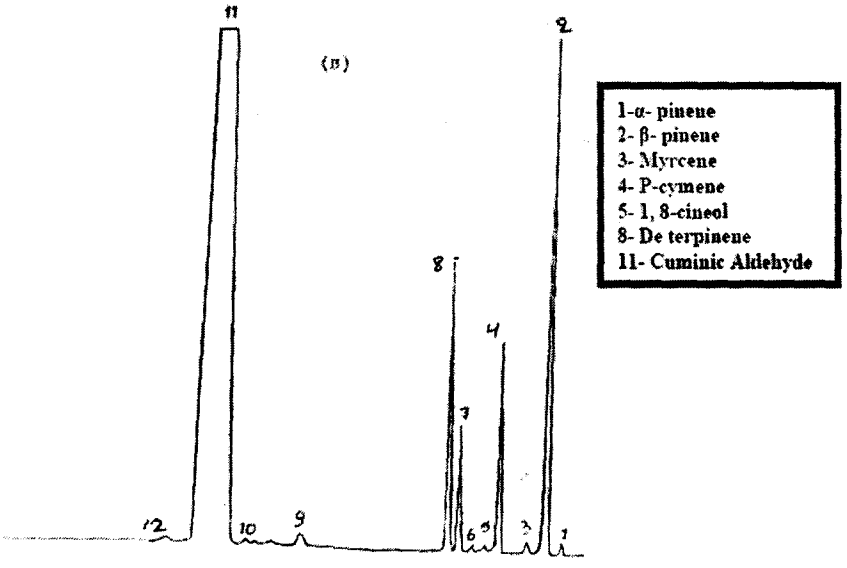


Fig. (2) Gas Chromatogram of Cumin Oil

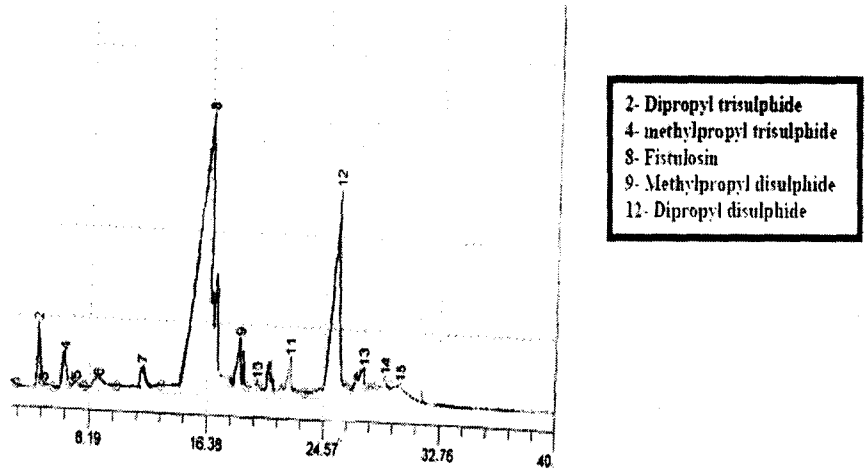


Fig. (3) Gas Chromatogram of Onion Oil

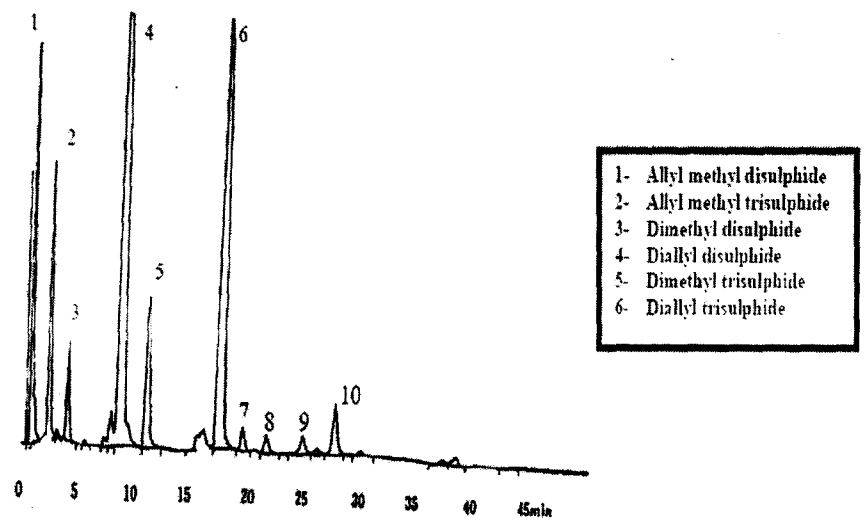


Fig. (4) Gas Chromatogram of Garlic Oil

C) Antifungal effect of the essential oils under study:

Molds and fungi are very common in our environment, and there occurrence in plant diseases and food showed be particularly, concerned. The efforts could be used to control the growth of mold and fungi. Some of these agents have offensive odors and others can not be added to plants and food for there hazardous effects.

The present study was concerned to study the effect of some essential oils (garlic, onion, cumin and spearmint) against four fungi causing disease to the plants except one only causes spoilage in food. These fungi namely *Allernaria alternata, Aspergillus niger, Aspergillus flavus* and *fusarium oxysporum.*

The data in Table (3) indicated that all the tested essential oils (without dilution) had antifungal activity against the four fungi and it differs from oil to oil. This antifungal activity of essential oils against micro-organisms might be due to the presence of phenolic terpenes that cause damage to biological membrane due to lipophilic properties interfering with membrane-intergrated enzyme, El-Baroty (1988). Volatile oil plants have been generally recognized as safe (GRAS) (Newberne et al., 2002). Systematic screening for biological interactions between microorganisms and plant products has been valuable source of new and effective antimicrobial substances, which could have different action ways on microbial cell, when compared to other conventional antimicrobials plant synthesized by a secondary metabolism. These compounds with complex molecular structures and some of them have been related with antimicrobial properties found in plant, and their derivatives. Among these secondary metabolites are found trepenes and phcnolic compounds, (Simoes et al., 1999).

The data in Table (3) revealed that garlic essential oil showed very powerful antifungal activity towards all fungal strains comparable with other essential oils followed by onion oil and cumin and spearmint oil respectively.

Aternaria alternata fungus was sensitive to various essential oils. However, *A. flavus* was very resistance one. *F. oxysporum* had a moderate degree. *A. Niger* had a surprising behavior. Although it is known to infect onion bulb, it showed high senstivity to onion essential oil as it is clear from Table (3).

The minimum inhibitory concentration (MIC) in Table (4) indicated that garlic oil had the smallest (MIC) compared with other essential oil followed by onion followed by cumin and spearmint,

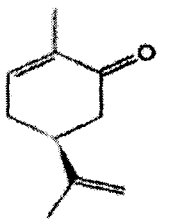
respectively. This indicated that garlic and onion essential oils had a powerful antimicrobial activity against all fungi strains.

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

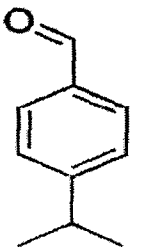
Oils	Microorganism			
	Alternaria alternate	***Aspergillus niger***	***Aspergillus flavns***	***Fusarium oxysporum***
Garlic	26	23	20	22
Onion	24	25	18	19
Cumin	23	22	13	15
Spearmint	20	18	13	14

Table (4): Minimum inhibitory concentration of the test essential oils on the growth of different fungi:

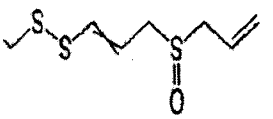
Fungi	Garlic	Onion	Cumin	Spearmint
	MIC (mg/ml)			
Alternaria alternate	0.5	0.7	1.2	2.3
Aspergillus niger	0.7	0.6	1.0	1.5
Aspergillus flavns	0.9	1.1	1.8	2.0
Fusarium oxysporum	0.8	1.0	2.0	2.1



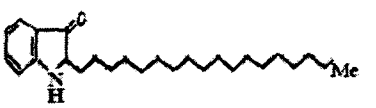
Carvone
In Spearmint Oil



Cuminaldehyde
In Cumin Oil



Ajoene
In Grlic Oil



Fistulosin
In Onion

Fig. (5): Some chemical compounds in the tested essential oils

It appears that, there is a relationship between the chemical composition of the oil ands its powerful inhibitory against microorganisms. 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 organism enzyme, El-Baroty (1988). Garlic oil had the highest antifungal activity due to ajoene compound which is a derivative of allicin. It is very inhibitory against A.niger (Naganawa *et al.*, 1996) Yoshida *et al.*, (1987) reported that ajoene compound from

garlic have stronger antifungal activity than allicin. They reported that ajoene damages the cell walls of fungi.

Onion essential oil show strong inhibitory effects against A.niger (Benkeblia, 2004). The powerful antifungal activity of onion oil due to fistulosin, an antifungal compound isolated from onion, this compound shows antifungal activities against several fungal species (Phary *et al.*, 1999).

These results were in agreement with results reported by Benkeblia (2004) who observed inhibitory effect of onion essential oil on A.niger and F.oxysporum. As for spearmint oil its antifungal activity due to carvone and limonene. Many authors have emphasized that the antimicrobial effect of essential oil constituents has been dependent on their hydrphobicity and partition in the microbial plasmatic membrane. Effect of specific ions due to their addition in/on plasmatic membrane had great effect on the protons motive force, intracellular AIP content and overall activity of microbial cells, including tug or pressure control, solutes transport and metabolism regulation Lanciotti *et al.*, (2004). Antifungal activity of cumin essential oil which could be attributed to the presence of cumin aldehyde beside other terpene compound which do synergistic effect against fungi.

These results were in agreement with the results reported by El-Baroty (1988), Mousa (1998) who observed that there is a relationship between the antifungal activity of the oil and its chemical composition, therefore, we may know its future as antifungal activity when we know its chemical composition.

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النشاط المضاد للفطريات الممرضة لبعض الزيوت العطرية

فاتن رمزي موسى -حسام رمضان محيسن-إبراهيم أحمد شلبي
قسم بحوث النباتات الطبية والعطرية – معهد بحوث البساتين - مركز البحوث الزراعية - الجيزة

أجري هذا البحث خلال سنة 2006 وذلك لمعرفة تأثير الزيوت الطبية والعطرية على تثبيط بعض الفطريات المسببة لأمراض النبات والتسمم الغذائي. حيث تم الحصول على الزيوت العطرية لنباتات الثوم والبصل والنعناع البلدي والكمون بطريقة الاستخلاص بالبخار من مصادرها الطبيعية (سيقان نباتات البصل والثوم وأوراق النعناع وبذور الكمون) وكذلك أجري تقدير الصفات الطبيعية والكيماوية لهذه الزيوت والتركيب الكيماوي لها بطريقة التحليل الكروماتوجرافي والتعرف على المركبات الرئيسية بها كما تم اختبار هذه الزيوت كمضادات فطرية واختبار أقل تركيز ممكن لتثبيط أربعة أنواع من الفطريات المرضية وهي الترناريا الترناتا, اسبرجلس نيجر واسبرجلس فلافس وفيوزاريم أوكسيسبورم.

ولقد أسفرت هذه الدراسة على النتائج الآتيـــة:

1- أن لكل هذه الزيوت المختبرة نشاط مضاد للفطريات.
2- أن زيت الثوم من أقوى هذه الزيوت الأربعة كمضاد فطري يليه زيت البصل ثم زيت الكمون وأقلهم زيت النعناع البلدي.
3- أن فطر اسبرجلس نيجر كان حساسًا جداً لزيت البصل.
4- أن فطر اسبرجلس فلافس وهو فطر يسبب تسمم الأغذية كان شديد المقاومة لهذه الزيوت.
5- فطر فيوزاريم أوكسيسبورم كان متوسط المقاومة لهذه الزيوت.
6- عند تقدير أقل تركيز مثبط لهذه الزيوت وجد أن أقلهم قيمة (أكثرهم تأثيراً) كان زيت الثوم يليه زيت البصل ثم الكمون والنعناع البلدي.
7- وجد أن المركبات الكيماوية الموجودة في زيت البصل والثوم من أكثر المركبات المثبطة لنشاط الفطريات.
8- هناك علاقة بين التركيب الكيماوي للزيت ونشاطه كمضاد فطري.