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STUDIES ON THE EFFECTS OF SOME MEDICINAL PLANTS ESSENTIAL OILS ON THE GROWTH AND AFLATOXINS SYNTHESIS OF *Aspergillus flavus*

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ABSTRACT

The antifungal activity of essential oils (EO) of peppermint (*Mentha piperita*), clove (*Syzygium aromaticum*), rosmary (*Rosmarinus officinalis*), anise (*Pinpinella anisum*), fenugreek (*Terigonella foenum-graecum*), Thymus (*Thymus vulgaris*), black mustard (*Brassica nigra*) and fennel (*Foeniculum vulgare*) was evaluated in vitro against *Aspergillus flavus* growth and aflatoxins production. The effect of different concentrations of essential oils on *A. flavus* and aflatoxin production was determined. At levels of plant Eo (0.4, 0.6 and 0.8%) , results indicated that there were no inhibition effect on the growth of *A. flavus*, except *metha piperita* oil. The Eo of mint at all assayed concentrations (0.6. 0.8 and 0.1%) showed strongly inhibition effect on the production of aflatoxins B_1, B_2, G_1 and G_2. GC/MS was used to identify the main components of the oils under investigation. High amount of menthol in the *Mentha piperita* Eo showed strongly antifungal activity. The level of aflatoxin B_1 production was 0.0 ppb for the samples treated with the mint oil and synthesis menthol at concentrations of 0.6 and 0.8%.

Keywords: Essential oil- Medicinal plant- Aspergillus flavus– Menthol - Mentha piperita.

INTRODUCTION

Aspergillus genus, which persents species inserted in the group of infesting living plants (e.g. *A. flavus*) and infesting stored food products fungi (e.g. *A.parasiticus A. ochraceus. A. fumigatus, A. chevalieri and A. clavatus*), is responsible for many causes of food contamination all over the world (Adam *et al*., 1998; Wangikan *et al.,* 2005 and Silva *et al*., 2010). The growth of *Asperigillus* in foodstuffs is toxicologically significant since some species are known to produce mycotoxins when exposed to suitable conditions (Mares *et al*., 2004 and Marina *et al.,* 2004).

Aflatoxins are toxic and hepatocarcinogenic polyketides produced by some *Aspergillus* species, especially, *A. flavus* and *A. parasiticus. A. flavus,* the most common causal fungus, produces aflatoxins B_1 and B_2. The occurrence of aflatoxins in food and feed commoditions has been a potential threat to consumer safety and to the world market due to the extremely low tolerance levels (Marina *et al.,* 2004; Hitokoto *et al.,* 2004; and Karapinar, 1985). The special risk posed by aflatoxins provokes studies on development of novel technologies for inhibiting the growth of aflatoxingenic moulds and/or the synthesis of aflatoxins in foods. Thus, the presence and growth of this fungus in food threatens human and animal health.

The use of chemical or synthetic agents with antifungal activity (as inhibitors, growth reducers or even in activators) is one of the oldest

techniques for controlling fungal growth and mycotoxins production in foods. The application of preservatives to foods is fundamental if their safety is to be maintained (Marina *et al*., 2004 and Karapinar , 1985).

The increased demand for safe and natural food, without chemical preservations, provokes many researchers to investigate the antimicrobial effects of natural compounds (Rasooli *et al.,* 2006). Numerous investigation have confirmed the antimicrobial action of essential oils in model food systems and in real foods (Koutsoumanis *et al.,* 1998 and Tsigard *et al.,* 2000).

Previous studies on medicinal plants have concentrated on the antibacterial activity and very few studies have targeted the antifungal activities of medicinal plants (Obi *et al.,* 2003; Tshikalange *et al.,* 2005 and ; McGaw *et al*., 2007). Plants are not only important to the millions of people to whom traditional medicine serves as the only opportunity for health care and to those who use plants for various purposes in their daily lives, but also as a source of new pharmaceuticals (Deboer *et al.,* 2005).

Many natural plant extracts contain primarily phenolic-compounds, which are potent antioxidants (Wong *et al.,* 1995). Some phenolie comounds such as sage, rosemary, thyme, mint hops, coriander, tea, cloves and basil are known to possess antimicrobial effects against food-borne pathogens (Davidson and Naidu, 2000 and Elgayyar *et al*., 2001). Phenols are one of most important groups of natural antioxidants. They occur only in material of plant origin and they are known to easily protect oxidizable constituents of food from oxidation. Especially worthy of notice are spices and herbs which for many year have been used as additives to enhance the sensory features of food (Wang *et al.,* 1996).

Peppermint (*Mentha piperita L.*) belongs to *Labiateae* family and originated from Mediterranean Regions . It is widely cultivated in the world and is a hybrid mint, a cross between water mint and spearmint (Frampton, 2009). The plant, indigenous to Europe, is now widespread in cultivation throughout all regions of the world. It is found wild occasionally with its parent species (Harley,1975).

M. piperita has been shown to possess strong antifungal activity, even when compared to synthetic fungicides. Peppermint oil showed antifungal activity against *A. niger, Alternaria alternate* and *Fusarium* sp. by agar well diffusion method (Aqil *et al* 2000). The chemical responsible for this action was menthone (Solovic *et al .,* 2009). Peppermint has a high menthol content, and is often used in tea and for flavouring ice cream, confectionery, chewing gum and toothpaste. The oil also contains menthone and menthyl esters, particularly menthyl acetate. Dried peppermint typically has 0.3-4% of volatile oil containing menthol (7-48%), menthone (20-46%), menthyl acetate (3-10%), menthofuran (1-17%) and 1.8-cineol (3-6%). Peppermint oil also contains small amounts of many additional compounds including limonene, pulegone,eucalyptol, caryophyllene and pinene (Leung, 1980). It is the oldest and most popular flavour of mint-flavoured confectionery . Peppermint can also be found in some shampoos, soaps and skin care products. Menthol activates cold-sensitive TRPM8 receptors in the skin and mucosal tissues, and is the primary source of the cooling sensation that follows the topical

application of peppermint oil (Eccles, 1994).Peppermint oil has a high concentration of natural pesticides, mainly polygone and menthone (Krieger, 2001). Mint essential oils are generally used externally for antipruritic, astringent, rubefacien, antiseptic, and antimicrobial purposes, and for treating neuralgia, myalgia, headaches, and migrains (Hendriks, 1998).

Natural plant extracts are of interest as a source of safer or more effective substitutes for synthetically produced antimicrobial agents and may provide and alternative way to prevent food or feed from fungal contamination (Chin yin and Wen Shen, 1998; Mahmoud, 1999; Thanaboripat, 2002 and Thandborinpat, 2003). Powders and extracts of various herbs, spices and essential oils have been reported to have antimicrobial activity and some also to inhibit aflatoxin formation (Masood *et al* 1994; Prasad *et al* 1994; Thanaboripat *et al.,* 1989,2000,2004 and 2005 and Bankole and Joda, 2004).

In the present study some selected medicinal plants oils were investigated for their antifungal activities against *A. flavus* and the potential of the *A. flavus* to produce aflatoxins B_1, B_2, G_1 and G_2. The oils were analyzed by GC/MS to identily the major components of the selected plants oils.

MATERIALS AND METHODS

Plant essential oils:

Peppermint (*Mentha piperita*), clove (*Syzygium aromaticum*), rosmary (*Rosmarinus afficinalis*), anis (*Pin pinellaanisum*), fenugreek (*Trigonella foenum- grae cum*),Thymus (*Thymus vulgaris*), black mustard (*Brasica nigra*) and fennel (*Foeniculum vulgare*) essential oils were purchase from Health shop, Cairo, Egypt, and stored in hermetically sealed flasks at 4ºC in dark condition.

Microorganism and media:

Aspergilus flavus (NRRL 3145) was obtained from Plant Pathology Institute, Agricultural Research Center, Giza, Egypt. The fungal strain cultures were maintained on a potato dextrose agar (PDA) slant at 4ºC in a refrigerator. The old cultures were transferred to fresh slopr every two months to avoid a decline in strain viability.

Studying the antifungal effect of essential oils:

A suspension o f 10^6 cfu/ml of *Aspergillus flavus* (NRRL 3145) spores in yeast extract broth was prepared according to (Davis *et al* 1966). Ten folded serial dilution of this suspension was done using sterile buffer solution which then inoculated in 7 sets of sterile Petri dishes. Each set was poured by about 15 ml of sterile rose Bengal chloramphenicol agar contained 7 different concentrations of each essential oil type (0.0, 0.05, 0.1, 0.2 , 0.4, 0.6, and 0.8%) . The controls did not contain tested essential oils. After solidification, the poured plates were incubated at 25ºC for 7days then counted and recorded (NMKL, 2005).

Studying the aflatoxins production :

Aflatoxins were produced by inoculating liquid yeast extract with one ml of fungal suspension of spores contained 10^6 colony forming units (cfu)/ml of *Aspergillus flavus* (NRRL 3145) followed by incubation at 30ºC for10 days (Davis *et al* 1966). Mycelium mat was broken using glass rod and collected by filtration through filter paper. The filtrate, containing total aflatoxins (will be called in the present work, mother solution) was stored at 4ºC for later use. Such technique yielded great amounts of aflatoxins, specially B_1 and G_1.

The broth medium (yeast extract sucrose) was prepared for the experiments by adding the essential oil of each plants (0.0, 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8%) to the broth medium before incubation at 27ºC for 10 days. The broth medium without oil used as a control after inoculated by 10^6 of *A. flavus spores.*

Aflatoxins determination by HPLC:

The solutions were filtrated through filter paper. A volume of the filtrate was transferred to a separator containing chloroform. The contents of the separator was shaken 30-60 sec. The bottom layer (chloroform) was separated and concentrated to dryness under a slow stream of nitrogen and then redissolved in acetone/water (15:85v/v) . Aflatoxins were determined using HPLC technique (Agillent 1200 Series U.S.A) with column C18 (Lichrospher 100 RP-18, 5 umx25cm) according to the following technique: The mobile phase consisted of water: methanol: acetonitrile (54:29:17, v/v/v) at flow rate of 1ml/min. The excitation and emission wavelengths for all aflatoxins were 362 and 460 nm (fluoresces detector), respectively (Roos *et al*., 1997).

Chemical components of plant essential oils:

The concentrated essential oils were dissolved in ethanol and analyzed by GC/MS technique. The analysis of the oil was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000 Triple Quad) equipped with an apolar Agilent HP-5ms (5% phenyl methyl poly siloxane) capillary column (30 mx0.25 mm i.d. and 0.25 µm film thickness). The carrier gas was helium with the linear velocity of 1 ml/min. The oven temperature was set at 50ºC for 2min. then programmed until 100ºC at a rate of 10ºC C/min with a hold-time of 3 min, once more heated to 150ºC at a 5ºC /min rate with a 2 min hold-time, then to 200ºC at the 10ºC/min rate and finally increased at the rate 20ºC/min to 280ºC, isothermal at the temperature for 2 min. the injector and detector temperatures were 300ºC and 200ºC, respectively. Injection mode, split: split ratio: 100 , volume injected 1µl of sample. The MS operating parameters were as follows: ionization potential 70eV, interface temperature 200ºC, and acquisition mass range 50-800.

Identification and quantification of constituents: The relative percentage of the volatile oil components was evaluated from the total peak area (TIC) by apparatus software.

The identification of volatile oil components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature (Adams, 2004).

RESULTS AND DISCUSSION

There has been an increasing consumer demand for foods free or with low added synthetic preservatives because it could be toxic to human and animal. Concomitantly, consumers have also demanded for wholesome and safe food with long shelf lives. These requirements are often contradictory and have pressure on the food industry for progressive removal of chemical preservatives and adoption of natural alternatives to obtain its efforts concerning safe food with long shelf lives (Parekh and Chanda, 2007).

Data in Table (1) showed the effect of different concentrations (0.0, 0.05, 0.1, 0.2, 0.4. 0.6 and 0.8%) of plants oil (po) on total counts of *A. flavus* (cfu/g) after incubation period. Mint oils (Mo) was found to be highly active against *A. flavus,* this activity may be due to the presence of menthol substances. Data obtained illustrated that, increasing the inclusion rate of MO into the used culture media gradually decreased the total number of *A. flavus* (cfu). This reduction reach to the firth at indicated levels 0.05 and 0.1% of Mo. Results showed that the incidence of *A .flavus* was negative at levels of 0.2, 0.4, 0.6 and 0.8% of Mo. Results indicated that the Mo showed the greatest effects on reducing total count of *A. flavus* (100%) followed by Thymus oil and fennel oil (34%),fenugreek oil (28%) cloves oil (15%), black mustard oil (6%) and rosemary oil (zero %).

At high levels of Po (0.4, 0.6, and 0.8) ,results indicated that the Po did not inhibit the growth of *A. flavus*, exept Mo. Though, results revealed that out of 8 extracts, 7 extract not possess clear antifungal activity against *A. flavus*. There may be several reasons for the lack of antimicrobial activity in these plants, either the plant part used or the type of extraction might have resulted in the nil activity, or the time of collection of herbal material and climate, which might turn affect the amount of active constituents in the plant (Pundir and Jain, 2010).

Results showed no inhibitory effect on fungal growth by fenugreek, anise and rosemary oils. Burt (2004) suggested that there is some evidence that minor components have a critical part in antimicrobial activity, possibly by producing a synergistic effect between other components.

Similar findings were reported by Pundir and Jain, (2010) , who found no. antifungal affects on *A. flavus* growth by fenugreek, anis, tea, Cinnamon and Black pepper oils . Datat in this study point to no antifungal effectives of thyme oil. On the other hand, different findings were observed by Nguefack *et al.*,(2004),who reported that thyme oil at a concentration of 200 ppm reduced the radial growth of *A. flavus* by 81%. The same effect was resulted by Soliman and Badea (2002), who reported that thyme Eo (≤500 ppm) completely inhibited the growth of *A. flavus* and *A. parasiticus*.

Same results were obtained by Kazemi *et al.*, 2012 who reported that mint (*M. piperita)* showed strong antibacterial and antifungal activities. Farshbaf *et al.*, (2004) revealed that *M. piperita* oil exhibited a significant antifungal activity.

Mentha piperita was found to have a wide spectrum of activity against all filmentous fungi examined by Mousavi and Raftos, (2012).

Table (1):The effect of different concentrations of plant oils on total counts of *A. flavus* (cfu/gm) after incubation period.

Concentration of plant oils %	Thymus	Fenugreek	Anis	Rosemary	Fennel	Mint	Clove	Black mustard
0.0	32x10	32x10	32x10	32x10	32x10	32x10	32x10	32x10
0.05	17x10	26x10	25x10	32x10	30x10	15x10	20x10	33x10
0.1	2.1x10	29x10	23x10	28x10	25x10	10x10	19x10	31x10
0.2	21x10	23x10	27x10	35x10	21x10	-ve	24x10	30x10
0.4	15x10	30x10	31x10	17x10	18x10	-ve	25x10	22x10
0.6	25x10	16x10	31x10	24x10	26x10	-ve	19x10	28x10
0.8	25x10	26x10	23x10	28x10	26x10	-ve	23x10	30x10

Results of the inhibitors effect of different concentrations of plant oils (PO) on the aflatoxin production by *A. flavus* are shown in Table (2). The essential oil of mint at all assayed concentrations (0.6, 0.8 and 1%) strongly inhibited the production of aflatoxins B_1, B_2, G_1 and G_2. At 1% mint oil assay, the aflatoxins quantities of B_1, B_2, G_1, and G_2 were 3.4, 27.8, 5.1, and 0.9 ppb respectively , while the corresponding figures for control assay (without the essential oil) were 622.7, 93.4, 2791.2 and 216.2 ppb, respectively. On the other hand, the essential oils of clove, anise, fenugreek, black mustard, thymus, fennel and rosemary did not show any effects against aflatoxins production, where the production of aflatoxins B_1, B_2, G_1 and G_2 increased at all levels under investigation. Aflatoxins increscent may be due to presence of starch and other nutrients in the tested essential oils which encourage aflatoxins productions..

Quantity aflatoxin B_1 (AFB1) was 7045.9, 3621.6, 3531.6, 1947.3, 4180.9, 2571.5, 3188.4 and 3.4 ppb for treatments with the essential oil of cloves, anise, fenugreek, black mustard, thymus, rosmary, fennel and mint at 1.0%, respectively.

Aflatoxins analysis showed that the mint essential oil exhibited a higher antiaflatoxigenic activity compared to other oils under investigation. Similar results were obtained by Mossini *et al*..(2004) and Arroteia *et al*.. (2007) who demonstrated the antifungal activity of same plant extracts and their ability to inhibit mycotoxin production. In addition , they have attempted to elucidate the effect of bioactive chemicals on growth and morphological features and on primary and secondary fungal metabolism.

The mechanism of inhibition effect of the essential oil of *C. longa* L. and curcumin for aflatoxins production may be related to inhibition of the early steps of aflatoxins biosynthesis involving lipid peroxidation and oxygenation. Hua *et al*., (1999) showed that phenolic compounds exhibit inhibitory activity on AFB_1 biosynthesis by *A .flavus*. It is clear that phenolic compounds have inhibited one or more early, rather than late, steps in the aflatoxins biosynthesis pathway. According to Farag *et al*., (1989), the presence of phenolic groups to form hydrogen bonds with the active sites of target enzymes was capable of increasing antimycotoxigenic activity. Jayashree and Subramanyam (1999) reported that phenolic compounds inhibited aflatoxin production without any significant effect on growth of the organism.

Table (2):The effect of different concentrations of plant oils on aflatoxins production by *A. flavus* (ppb).

Concentrations of plant oils %	Quantity of Aflatoxins Produced (ppb)				
	B_1	B_2	G_1	G_2	Total aflatoxins
Control	**622.7**	**93.4**	**2791.2**	**216.2**	**3723.5**
Clove					
0.6	7514.5	532.6	3210.2	403.1	11661.4
0.8	6915.2	291.6	3611.2	315.5	11143.5
1.0	7045.9	558.7	2484.6	215.0	10304.2
Anis					
0.6	3423.6	567.8	3290.3	441.8	7723.5
0.8	1514.6	237.2	1950.9	229.7	3932.4
1.0	3621.6	517.25	4773.2	523.8	9435.9
Fenugreek					
0.6	3669.9	631.7	6122.5	899.7	11323.8
0.8	3104.7	569.9	4969.8	787.6	9432.0
1.0	3531.6	476.5	7132.8	730.43	11871.3
Black Mustard					
0.6	2191.2	384.7	4164.7	620.2	7360.8
0.8	2976.5	484.2	5791.6	817.9	10070.2
1.0	1947.3	293.9	4423.1	533.57	7197.9
Thymus					
0.6	4752.1	823.3	816.9	1216.7	14961.0
0.8	2759.9	492.8	5380.5	872.2	9509.4
1.0	4180.9	708.3	5811.6	835.5	11536.3
Rosemary					
0.6	3171.3	573.8	3938.9	591.6	8275.6
0.8	2722.5	461.7	4956.3	696.7	8837.2
1.0	2571.5	444.9	3626.2	510.9	7153.5
Fennel					
0.6	4899.3	711.1	4608.2	582.0	10800.6
0.8	4592.7	703.2	5448.8	739.6	11484.3
1.0	3188.4	459.5	3995.9	516.8	8160.6
Mint					
0.6	13.1	27.8	15.2	1.9	58.0
0.8	6.5	20.6	15.4	1.9	44.4
1.0	3.4	27.8	5.1	0.9	37.2

The results of oils extracted analysis from eight different plants by GC/MS are shown in Tables (3-10). Obviously, extractions contain essential oils, organic acids and fatty acids components. The results show that the more content in oils are: menthol in peppermint 43.3%, eugenol in clove 68.4%, anethol in fennel 53.7%, eucalyptol & heptenal in rosemary 26 and 26.8% anethole in anis 42.2%, octadecanoic acid 27% in fenugreek, thymol in thyme 38.8% and 1- monolinoleoyl glycerol 82% in mustard.

Comparing the major constituent of the peppermint (*Mentha piperita*) oil (menthol ,(43.3%) with that observed by Sahakhiz *et al.*, (2012).They reported that the main constituent of the mint oil was menthol (53.28%). On

the other hand, the major compounds was carvone (49.52%) in *Mintha spicata* samples analyzed by Sokovic *et al*., (2007).

So, it can be figured that menthol is the individual aroma constituent responsible for the antifungal properties of peppermint oil.

The compositions of the plant may reflect variations due to geographical location from which the plant was collected. Abbaszadeh *et al*., (2009) , studying the compounds variations of essential oil in leaves of Mentha species, indicated that significant difference between essential oil yields in leaves of mint species was recorded. Moghtader (2013) showed that the main components in mint (*M. piperita*) oil were menthol (38.33%), limonene (5.33) menthone (21.45%), 1.8-cineole (3.27%), sabinene (2.32%) and menthyl acetate (12.49%).

Table (3):Major compounds of peppermint (*Mintha piperita*) essential oil determined using GC/MS.

Component	Content (%)
Limonene	4.16
3-octanol	2.06
1-p-Menthene	1
B-pinene	5.2
Menthol	43.3
Isopulegol	21
Cineole	6.4
Terpineol	6
Acetaldehyde	9.06
Menthyl acetate	1.61

Table (4): Major compounds of clove (*Syzygiam aromaticum*) essential oil determined using GC/MS.

Component	Content (%)
Eugenol	68.4
Eugenol acetate	5.79
Palmitin	4.1
Oleic acid	8.6
Linoleic acid	13

Table (5): Major compounds of fennel (*Foeniculum valgaris*) essential oil determined using GC/MS.

Component	Content (%)
Anethole	53.7
Palmitic acid	8.29
10 Undecenal	8
Oleic acid	6.5
Linoleic acid	17.8
Tetradecenal	2.5
6-Octadecenoic acid	3.1

Table(6):Major compounds of rosemary (*Rosmarinus officinalis*) essential oil determined using GC/MS.

Component	Content (%)
Eucalyptol	26
A-Pinene	8.5
2.4. Decadoema;	16
2- Heptenal	26.8
2- Decenal (E)	12.5
Camphor	9.2

Table(7):Major compounds of anise (*Pnpinella anisum*) essential oil determined using GC/MS.

Component	Content (%)
Anethole	42.2
Linoleic acid	30
Oleic acid	7.3
Isoeugenol	5.8
Anisaldehyde	6.1
Linolenin	3.9
2,4- Decadienal	4

Table(8):Major compounds of fenugreek (*Terigonella foenum-graecum*) essential oil determined using GC/MS.

Component	Content (%)
Linoleic	22.6
Tetradecane	1.41
Octadecanoic acid	27
Undecane	10.5
3- Decenoicacid	19.4
Oleic acid	1
Pamitic acid	16.6
Diplamitin	1.16

Table (9): Major compounds of thyme (*Thymus vulgaris*) essential oil determined using GC/MS.

Component	Content (%)
Thymol	38.8
Oleic acid	26
Oleoylglycerol	1.48
2-Undecenal	15.15
Palmitic acid	13
Octadecanoic acid	5.4

Table (10): Major compounds of black mustard (*Brassica nigra*) essential oil determined using GC/MS.

Component	Content (%)
Linoleic acid	9.1
Diplamitoylglycerol	4.3
Oleic acid	3.8
1-Monolinoleoylglycerol	82

The effect of different concentrations of synthetic menthol on total counts of *A. flavus* are listed in Table (11). The levels 0.4, 0.6, and 0.8% of menthol exhibited strong antifungal activity (zero cfu/ml) compared with the control (>10^2 cfu/ml). These results agree with those of Ferdes and Ungureanu (2012) who suggested that the most effective against all tested fungal strains was the menthol. Moghtader (2013) reported that 5 ppm concentration of menthol completely inhibited the mycelial growth of *A. niger*. In a study, menthol was found to be the active responsible for the antifungal effect (Edris and farrag, 2003).

Table (11): The effect of different concentrations of menthol on total counts of *A . flavus* (cfu/ml) after incubation period .

Menthol %	(cfu/ml)
Control (0% Menthol)	> 10^2
0.05	11 x 10^2
0.1	53 x 10
0.2	80 x 10
0.4	Nil
0.6	Nil
0.8	Nil

The effect of different concentrations of synthetic menthol on aflatoxin production by *A. flavus* are shown in Table (12). The menthol showed high antiproduction of aflatoxins compared with the control results showed that the inhibitory property developed according to increases in concentration. Thus 0.2, 0.4, 0.6 and 0.8% of the menthol reached 100% inhibition for aflatoxin B_1. Similar results were obtained by Edris and farrag (2003) who reported that menthol was found to be the active responsible for the antifungal effect.

Table (12): The effect of different concentrations of menthol on aflatoxin production by *A. flavus* (ppb).

Menthol concentrations \ Aflatoxins type (ppb)	B_1	B_2	G_1	G_2	Total aflatoxin
Control (0.0)	5969	579	15942	2145	24635
0.05	17.7	1.5	195.5	9.3	194
0.1	1.2	0.0	13	0.6	15.37
0.2	0.4	0.2	6.2	0.0	6.93
0.4	0.4	0.0	5.5	0.0	5.9
0.6	0.47	0.0	3.6	0.0	4.0
0.8	0.0	0.0	14.87	0.0	1.87

Previous researchers have purposed that the inhibition of aflatoxins production by *A. flavus* caused by essential oils cannot by completely attributed to insufficient mycelium growth (Chang *et al*., 2001 and Tatsadjieu *et al*., 2009) Since aflatoxins are synthesized extramitochondrially from acetylcoenzyme A during a period of rapid glucose utilization, it has been suggested that the restriction of carbohydrates catabolism in *A. flavus* due to the interference of essential oils compounds on some key enzymes may result in decrease of its ability to synthesize aflatoxins. This hypothesis is reinforced for the findings of other studies where plant compounds were able to penetrate inside the fungal cell and react with active sites of key enzymes or act as H^+ carrier, depleting adenosine triphosphate pool and ultimately resulting in disturbance of the fungal metabolism.

Moreira *et al*., (2013) found that the essential oil from *H. suaveolens* cuased morphological changes in *A. flavus* including lack of sporulation, loss of cytoplasm content, loss of pigmentation and distorted development of hyphae. Regarding these findings the authors purposed that the anti-*Aspergillus* activity of the assayed essential oil probably includes attack on the cellwall and retraction of the cytoplasm in the hyphae resulting in death of mycelium (Moreira *et al*., 2013).

The peppermint (*Mentha piprita*) oil and thynsis menthol recommended for large scale application is based on its strong antifungal as well as anti *A. flavus* and aflatoxins production efficacy. The major components of peppermint oil is menthol. However, further studies need to be conducted to evaluate the efficacy of these essential oils for *Aspergillus flavus* control and aflatoxins production.

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دراسات علي تاثيرات الزيوت العطرية لبعض النباتات الطبية لمنع نمو فطر الاسبرجلس فلافس وانتاجه للافلاتوكسينات

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المركز الاقليمي للاغذية والاعلاف – مركز البحوث الزراعية – وزارة الزراعة الجيزة

تم استخدام الزيوت العطرية لبعض النباتات الطبية كالنعناع و القرنفل والروزماري واليانسون و الحلبة والخروع و بذور الخردل السوداء والشمر كمضادات فطرية لمنع نمو فطر الاسبرجلس فلافس وانتاجه للافلاتوكسينات وقد استخدم تركيزات مختلفة من تلك الزيوت السابقة , و اوضحت النتائج انه عند تركيزات 0.4 و 0.6 و 0.8 % لم يكن لهذه الزيوت اي تاثير علي منع نمو الفطر وانتاجه للافلاتوكسينات فيماعدا زيت نبات النعناع .

- اوضحت النتائج انه عند تركيزات 0.4 و 0.6 و 0.8% من زيت النعناع فان نمو الفطر توقف تماما وكذلك منع انتاج الافلاتوكسينات
- تم استخدام الجهاز الكروماتوجرافي المعتمد علي قياس الكتلة (جي سي ام اس) لتحديد اهم مكونات كل زيت علي حدة.
- عند استخدام تركيزات 0.6 و 0.8 % في زيت النعناع و كذلك في مادة المانيتول الصناعية فان تركيز افلاتوكسين ب1 كان صفر

قام بتحكيم البحث

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