

## Impact of some essential oils on the growth of toxigenic fungi and their toxin production

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### Abstract

The impact of twelve essential oils (ginger, black pepper, black cumin, turmeric, baladi mint, peppery mint, cumin, marjoram lupine, cinnamon, thyme and cloves) on the growth of 11 toxigenic fungi and their ability for producing toxins were examined. Thyme, clove, baladi mint, peppery mint and cumin completely inhibited the growth of all tested fungi at two tested concentrations (10 and 50 µl/ 20 ml medium). Marjoram essential oil completely inhibited the fungal growth at 50 µl. Cinnamon essential oil exhibited moderate inhibitory effect on the growth of all tested fungi at 50 µl. Ginger oil generally stimulated the growth of most the tested fungi. Black pepper, turmeric and lupine were recorded as low active oils. Whereas, Black cumin essential oil did not display any inhibitory effect on the growth of the toxigenic fungi at 50 µl. Thyme, clove and mint essential oils (50 µl/ 50 ml medium) completely inhibited toxin production by all the tested toxigenic fungi. Black pepper and ginger essential oils reduced mycotoxin formation.

**Key words:** toxigenic fungi, mycotoxins, essential oils.

### Introduction

Spoilage and poisoning of foods by fungi is a major problem, especially in developing countries. *Aspergillus*, *Fusarium* and *Penicillium* species are the most important fungi causing spoilage of foodstuffs. Growth of these fungi in food crops are also responsible for off-flavour formation and production of allergenic compounds and mycotoxins, which lead to qualitative losses (Nielsen and Rios, 2000; Bennett and Klich, 2003). Aflatoxin B<sub>1</sub>, Ochratoxin A and fumonisin B<sub>1</sub> produced by these fungi display carcinogenic properties in humans and in laboratory animals, leading to the appearance of hepatocarcinoma (IARC, 1993; Pfohl-Leszkowicz and Manderville, 2007). However, in most countries, chemical and physical preservation are not permitted in foods. The need thus arises for natural preservatives that could be used for semi processed and processed foods. Currently the global trend is turned to safer and eco-friendly

alternative approaches (Mari *et al.*, 2007; Sharma *et al.*, 2009). One of these possibilities is the use of essential oils to control mycotoxigenic fungi.

The antimicrobial properties of essential oils have been recognized and experimentally evaluated for many years. The essential oils have been involved in several applications such as natural antimicrobial agents in the field of pharmacology, phytopathology, clinical microbiology and food preservation. The essential oil preparations that possess antimicrobial activities have been the subject of many investigations resulted in screening of a wide variety of plant species, and have revealed structural and biological unique active compounds (Yoon *et al.*, 1994; Vukovic *et al.*, 2007). The general antifungal activity of essential oils is well documented (Alankararao *et al.*, 1991; Gogoi *et al.*, 1997; Meepagala *et al.*, 2002). The advantage of essential oils is their bioactivity in the vapour phase, a

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characteristic that makes them attractive as possible fumigants for stored product protection. Also, these essential oils are thought to play a role in plant defense mechanisms against phytopathogenic microorganisms (Mihaliak *et al.*, 1991). Most of the essential oils have been reported to inhibit post harvest fungi *in vitro* conditions (Bellerbeck *et al.*, 2001; Hidalgo *et al.*, 2002).

Some of the essential oils have been reported to protect stored commodities from bio-deterioration. There are also some reports on essential oils in enhancing storage life of fruit and vegetables by controlling their fungal rotting. In this respect, Dubey and Kishore (1988) found that the essential oils from leaves of *Melaleuca leucadendron*, *Ocimum canum* and *Citrus medica* were able to protect several stored food commodities from bio-deterioration caused by *Aspergillus flavus* and *Aspergillus versicolor*. The potential of using essential oils by spraying or dipping to control post harvest decay has been examined in fruits and vegetables (Tripathi and Dubey, 2004).

To control fungal contamination there are two possibilities, heat or chemical treatments, but it is necessary to replace chemical fungicides by natural products to avoid health problems. So, the present investigation aimed to evaluate the potential of 12 essential oils for bio-control of the fungal growth and toxin production by 11 toxigenic fungi.

## Materials and methods

### Selection of toxigenic fungi

A total of 11 toxigenic fungal isolates were selected for this study. Five highly toxigenic local isolates from different food sources in Sohag Governorate, Egypt named *Aspergillus flavus* 30 (Aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> producer), *A. ochraceus* 76 (Ochratoxins A, B), *Aspergillus nidulans* 69 (Sterigmatocystin), *Penicillium digitatum* 131 (Patulin) and *Alternaria alternata* 5 (Alternariol). six highly toxigenic fungal isolates were purchased from CBS (Central Bureau voor Schimmelcultures), named *Aspergillus parasiticus* CBS 571.65 (Aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>), *A. ochraceus* CBS 589.68 (Ochratoxin A), *Penicillium griseofulvum* CBS 589.68 (Patulin), *P. scabrosum* CBS 530.97

(Fumagillin), *Fusarium equiseti* CBS 406.86 (Zearalenone) and *Phaeosphaeria nodorum* CBS 438.87 (Alternariol).

### Essential oils

Essential oils of ginger, black pepper, black cumin, turmeric, baladi mint, peppery mint, cumin and marjoram were extracted using hot water steam distillation method (Kawther Abed, 2007). On the other hand, other four essential oils (lupine, cinnamon, thyme and cloves) were purchased from different markets at Sohag city.

### The effect of essential oils on growth of toxigenic fungal isolates

The method described by Elena *et al.* (2009) was employed as follow: Fungi were grown in dishes on the potato dextrose agar medium. The oils were dripped on the covers of Petri dishes. Two different concentrations of each essential oil (10 and 50 µl) were tested after sowing the fungi and dripping oil. The dishes were sealed using the adhesive tape, turned over and put into a thermostat (temperature 28 °C). The inhibition effect of the oil was detected by measuring the diameter of fungal colonies after 7 days of incubation and by comparing them to the control sample (without oil).

### The effect of essential oils on mycotoxins production

To determine the effect of essential oils on mycotoxin formation, five oils were chosen as follow: baladi mint, thyme and cloves which completely inhibited all toxigenic fungal growth; black pepper which exhibited no antifungal activity and ginger which stimulated all toxigenic fungal growth. Each individual fungal isolate was cultivated on potato - dextrose liquid medium. Erlenmeyer flasks of 250 ml capacity were used. Each flask contained 50 ml medium.

The flasks were sterilized at 121 °C for 20 minutes and inoculated after cooling with two ml of the inoculum suspension of 10 days old culture of the pure organism. 50 µl of tested essential oil were added. The cultures were incubated at 28 ± 2°C as static cultivation for 10 days. At the end of incubation period, the content of each flask (medium + mycelium) were homogenized for five minutes in a high

speed blender (16000 rpm) with 100 ml chloroform. The extraction procedure was repeated three times. The combined chloroform extracts were washed with equal volume of distilled water, dried over anhydrous sodium sulphate, filtered then concentrated to near dryness. Mycotoxin levels were detected using thin layer chromatography (Scott *et al.*, 1970; Gimeno, 1979; El-kady and Moubasher, 1982).

### Results and Discussion

The antifungal activity of twelve essential oils on the growth of the eleven toxigenic fungal strains was examined and listed in tables (1 & 2). The results of the present study revealed that the essential oils at each of 10 and 50 µl/ 20 ml of thyme, clove, mint baladi, peppery mint and cumin completely inhibited the growth of all tested toxigenic fungi. Marjoram essential oil at 50 µl/ 20 ml medium (2500 ppm) completely inhibited the fungal growth and moderately inhibited them at 10 µl/ 20 ml medium (500 ppm).

The antifungal activity of thyme, clove and spearmint on the toxigenic fungi: *A. flavus*, *A. parasiticus*, *A. ochraceus*, *A. fumigatus* and *Fusarium* spp was demonstrated by Montes-Belmont and Carvajall (1998) and Basilico and Basilico (1999). Montes-Belmont and Carvajall (1998) reported that the oils of clove and thymus caused a total inhibition of *A. flavus* on maize kernels. Nguéfac *et al.* (2004) found that the essential oil of thymus inhibits the growth of various fungi involved in food spoilage, mycotoxin producers, pathogenic and wood decay fungi. Enas Amer (2012) examined the antifungal activity of six types of plant essential oil against the growth of 31 isolates of eight toxigenic fungal species and found that the oil of thyme completely suppressed the growth of all fungal isolates. Gorran *et al.* (2013) reported that thyme essential oil completely inhibits the growth of *A. flavus* at the concentration of 500 mg/L. A concentration of 200-250 ppm of clove oil inhibited the growth of *A. parasiticus* (Bullerman *et al.*, 1977).

Clove oil has also been found to be an effective inhibitor of *Alternaria alternata*, *Fusarium oxysporum*, *F. culmorum*, *F. griseocyaneus*, *Mucor circinelloides*, *Rhizopus*

*stolonifer*, *Cladosporium cladosporioides*, *Penicillium citrinum*, *Saccharomyces cerevisiae* and *Aspergillus niger* (Schmitz *et al.*, 1993; Meena and Sethi, 1994). Soliman and Badeaa (2002) revealed that spearmint contained carvone as a main component of its essential oil, which may be responsible for their antifungal activity.

Cinnamon essential oil at 50 µl/ 20 ml medium moderately inhibited the growth of all tested toxigenic fungi (Tables 1 & 2). Sukatta *et al.* (2008) previously showed that mixing clove and cinnamon oils at the appropriate ratios result in an improvement of the efficacy against the post harvest decay fungi of grapes. Also cinnamon oil is a potential inhibitor of *Penicillium expansum* which is a cause of spoilage of apples (Ryu and Holt, 1993).

Ginger oil generally stimulated the growth of most toxigenic fungi under study except *A. flavus* 30 and *Penicillium griseofulvum* CBS 589.68 at 50 and 10 µl/ 20 ml medium (Tables, 1 & 2). Similar results were observed by Mabrouk and EL-Shayeb (1981) who reported that ginger stimulated fungal growth at all the concentration tested. Black cumin essential oil displayed no antifungal effect on the growth at both tested concentrations. Similar findings were reported by Maraqa *et al.* (2007) using black cumin essential oil.

Other three oils named black pepper, turmeric and lupine were recorded as low active oils at 50 µl/ 20 ml medium and had no inhibitory effect on the growth of the different toxigenic fungal strains using 10 µl/ 20 ml medium (Tables, 1 & 2). In contrast, Sindhu *et al.* (2011) evaluated the potential of turmeric on control of *A. flavus* growth and aflatoxin production. Bokhari (2007) reported that black pepper did not affect the growth of the toxigenic *A. versicolor*.

The results in tables 3 & 4 indicated the important role of essential oils (especially thyme, clove and mint) in inhibiting toxin production by all the 11 toxigenic fungi under examination. The inhibitory effects of some plants essential oils against aflatoxin biosynthesis by *A. flavus* and *A. parasiticus* were reported in previous studies (El-Kady *et al.*, 2000; Attanda *et al.*, 2007; Mohamed *et al.*, 2011). The effects of clove essential oil on

growth and mycotoxin production by some toxigenic fungal genera such as *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. have been reported (Velluti *et al.*, 2004; Nesci *et al.*, 2005, 2011). Patkar *et al.* (1993) reported that clove essential oil inhibited either aflatoxin or ochratoxin accumulation in different substrates. Reddy *et al.* (2010) reported the efficacy of certain plant extracts on mycelial growth of *A. ochraceus* and ochratoxin biosynthesis. The oils of thyme and cinnamon completely inhibit all the test fungi and ochratoxin production at 3000 ppm (Soliman and Badaea, 2002). Velluti *et al.* (2004) found that clove essential oil was able to inhibit zearalenone and deoxynevalenol synthesis under certain environmental conditions in sterile maize inoculated with *Fusarium* species.

In this study black pepper essential oil completely inhibited toxin production of each of *F. equiseti* CBS 406.86, *P. nodorum* CBS 438.87, *A. alternata* 5 and *A. nidulans* 69. Also, it reduced fumagillin production by *P. scabrosum* CBS 530.97 and Patulin production by *P. digitatum* 131 to 40 and 20%,

respectively. Black pepper essential oil did not inhibit the production of aflatoxin and ochratoxin using both standard and local fungal strains. Ito *et al.* (1994) reported that pepper extracts have the ability to reduce aflatoxin production in *A. parasiticus* IFO 30179 and *A. flavus* var. *columnaris* S46.

Ginger oil completely inhibited the production of zearalenone and alternariol and reduced the patulin formed by standard and local fungal strains to 40 and 80%, respectively. Fumagillin production was reduced by 20% using ginger essential oil, while the production of sterigmatocystin was stimulated by 20% using this oil. Aflatoxin and ochratoxin production by the standard and local strains were not affected by the presence of ginger essential oil. Ginger has been listed in Generally Recognized as Safe (GRAS) and has antimicrobial and antimycotoxigenic effects (Tatsadjieu *et al.*, 2009) and also because of its aroma and taste, it has been used for culinary purposes from ages. Ginger essential oil is indeed effective against several mycotoxins in stored commodities (Sharma *et al.*, 2013).

Toxigenic fungal strain		Conc. of essential oil (µl/20 ml median)	<i>A. parasiticus</i> CBS 571.65		<i>A. ochraceus</i> CBS 589.68		<i>F. equiseti</i> CBS 406.86		<i>P. griseofulvum</i> CBS 312.63		<i>P. scabrosum</i> CBS 530.97		<i>Patulinogenic nodorum</i> CBS 438.87	
			Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %
Essential oils	control	0	7.0	0	5.0	0	7.0	0	2.5	0	2.0	0	3.0	0
	Black	10	7.0	0	5.0	0	7.0	0	2.5	0	2.0	0	3.0	0
		50	7.0	0	5.0	0	7.0	0	2.5	0	2.0	0	3.0	0
	Black pepper	10	7.0	0	5.0	0	7.0	0	2.5	0	2.0	0	3.0	0
		50	6.8	3	4.3	14	6.5	7	2.5	0	1.5	25	2.5	17
	Cumin	10	0.8	89	1.0	80	-	100	-	100	-	100	-	100
		50	-	100	-	100	-	100	-	100	-	100	-	100
	Ginger	10	7.2	+2	5.3	+6	7.5	+7	2.5	0	2.2	+10	3.2	+6
		50	7.4	+5	5.5	+10	7.8	+11	2.5	0	2.4	+20	3.2	+6
	Marjoram	10	6.5	7	2.8	44	5.4	23	1.8	28	1.4	30	-	100
		50	-	100	-	100	-	100	-	100	-	100	-	100
	Baladi mint	10	5.8	17	2.0	60	2.5	65	1.2	52	0.8	60	-	100
		50	-	100	-	100	-	100	-	100	-	100	-	100

**Table (1):** Impact of some extracted and purchased essential oils (10 & 50 µl/ 20 ml medium) on the growth of standard toxigenic fungi.

Toxicogenic fungal strain		Conc. of essential oil (µl/ 20 ml medium)	<i>A. niger</i> CBS 571.65		<i>A. ochraceus</i> CBS 589.68		<i>F. equiseti</i> CBS 406.86		<i>P. griseofulvum</i> CBS 315.63		<i>P. saccharinum</i> CBS 430.97		<i>Phaeoannellaria odoratum</i> CBS 438.87	
			Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %
Extracted essential oils	control	0	7.0	0	5.0	0	7.0	0	2.5	0	2.0	0	3.0	0
	Peppery mint	10	5.8	17	-	100	-	100	1.5	40	0.7	65	-	100
		50	-	100	-	100	-	100	-	100	-	100	-	100
	Turmeric	10	7.0	0	5.0	0	7.0	0	2.5	0	2.0	0	3.0	0
		50	6.8	3	3.5	30	5.3	10	1.8	28	1.8	10	1.0	67
	Purchased essential oils	Cinnamon	10	7.0	0	5.0	0	7.0	0	2.5	0	2.0	0	3.0
50			5.5	21	3.5	30	4.0	43	2.2	12	0.6	70	1.0	67
Cloves		10	-	100	-	100	-	100	-	100	-	100	-	100
		50	-	100	-	100	-	100	-	100	-	100	-	100
Lupine		10	7.0	0	5.0	0	7.0	0	2.5	0	2.0	0	3.0	0
		50	6.2	11	4.6	8	5.5	7	1.0	60	1.2	40	1.3	57
Thyrsae		10	-	100	-	100	-	100	-	100	-	100	-	100
		50	-	100	-	100	-	100	-	100	-	100	-	100

Table (1): Continued

Toxicogenic fungal strain		Conc. of essential oil (µl/ 20 ml medium)	<i>Alternaria alternata</i> 5		<i>A. flavus</i> 30		<i>A. nidulans</i> 69		<i>A. ochraceus</i> 76		<i>P. digitatum</i> 131	
			Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %
Extracted essential oils	control	0	6.8	0	6.0	0	5.5	0	3.8	0	4.5	0
	Black	10	6.8	0	6.0	0	5.5	0	3.8	0	4.5	0
		50	6.8	0	6.0	0	5.5	0	3.8	0	4.5	0
	Black pepper	10	6.8	0	6.0	0	5.5	0	3.8	0	4.5	0
		50	5.8	15	5.6	7	5.4	2	3.7	3	3.5	22
	Cumin	10	2.7	60	0.8	87	0	100	0	100	0	100
		50	0	100	0	100	0	100	0	100	0	100
	Ginger	10	7.0	2	6.0	0	5.6	1	4.0	5	4.7	4
		50	7.0	2	6.0	0	5.8	5	4.0	5	4.8	7
	Marjoram	10	4.5	34	5.8	4	5.3	4	3.2	15	2.2	51
		50	0	100	0	100	0	100	0	100	0	100
	Baladi mint	10	3.8	44	4.0	33	4.0	27	2.5	34	1.2	73
		50	0	100	0	100	0	100	0	100	0	100

Table (2): Impact of some extracted and purchased essential oils (10 & 50 µl/ 20 ml medium) on the growth of local toxicogenic fungi (growth diameter measured by cm after 7 days of incubation on PDA medium at 28 °C).

Toxicogenic fungal strain		Conc. of essential oil ( $\mu$ l/20 ml medium)	<i>A. alternata</i> 5		<i>A. flavus</i> 30		<i>A. nidulans</i> 69		<i>A. ochraceus</i> 76		<i>P. digitatum</i> 131	
			Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %	Fungal growth (cm)	Inhibition %
Extracted essential oils	control	0	6.8	0	6.0	0	5.5	0	3.8	0	4.5	0
	Peppery mint	10	4.2	38	4.5	25	4.3	22	1.5	61	1.2	73
		50	0	100	0	100	0	100	0	100	0	100
	Turmeric	10	6.8	0	6.0	0	5.5	0	3.8	0	4.5	0
		50	5.0	27	5.3	3	5.4	2	3.5	8	3.6	20
	Purchased essential oils	Cinnamon	10	6.8	0	6.0	0	5.5	0	3.8	0	4.5
50			6.2	9	5.3	3	3.5	37	3.6	5	3.5	22
Cloves		10	0	100	1.2	80	2.8	49	0	100	0	100
		50	0	100	0	100	0	100	0	100	0	100
Lupine		10	6.8	0	6.0	0	5.5	0	3.8	0	4.5	0
		50	5.8	15	5.7	5	5.4	2	3.3	13	3.2	29
Thyme		10	0	100	0	100	0	100	0	100	0	100
		50	0	100	0	100	0	100	0	100	0	100

Table (2): Continued

Toxicogenic fungal strain		<i>A. parasiticus</i> CBS 571.65		<i>A. ochraceus</i> CBS 589.68		<i>F. equiseti</i> CBS 406.86		<i>P. griseofulvum</i> CBS 315.63		<i>P. scabrosum</i> CBS 530.97		<i>Phaeosphaeria nodorum</i> CBS 438.87	
		Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %
Extracted essential oils	Control	5	0	4	0	5	0	5	0	5	0	4	0
	Black pepper	4	0	3	0	3	100	4	-20	4	60	2	100
	Ginger	5	0	4	0	5	100	5	60	5	20	5	100
	Mint	-	100	-	100	-	100	-	100	-	100	-	100
Purchased essential oils	Cloves	-	100	-	100	-	100	-	100	-	100	-	100
	Thyme	-	100	-	100	-	100	-	100	-	100	-	100

Table (3): Impact of some extracted and purchased essential oils (50  $\mu$ l/ 50 ml medium) on fungal growth and mycotoxins formation by some standard toxicogenic fungal strains grown on PDA liquid medium supplemented with the essential oil at 28 °C for 10 days.

Toxicogenic fungal strain		<i>Alternaria alternata</i> 5		<i>A. flavus</i> 30		<i>A. nidulans</i> 69		<i>A. ochraceus</i> 76		<i>P. digitatum</i> 131	
		Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %	Visual growth	Inhibition of toxin production %
Essential oils	Control	5	0	5	0	5	0	5	0	5	0
	Black pepper	5	100	4	0	3	100	5	0	4	80
	Ginger	5	100	5	0	5	-20	5	0	5	20
	Mint	-	100	-	100	-	100	-	100	-	100
Purchased essential oils	Cloves	-	100	-	100	-	100	-	100	-	100
	Thyme	-	100	-	100	-	100	-	100	-	100

**Table (4):** The inhibitory effect (%) of some extracted and purchased essential oils (50 µl/ 50 ml medium) on fungal growth and mycotoxins formation by some local toxicogenic fungal strains grown on PDA liquid medium supplemented with the essential oil at 28 °C for 10 days.

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اهتم هذا البحث بدراسة تأثير نوع من الزيوت الطيارة (تركيز ميكرو لتر لكل ملى ميديا) على بعض العزلات الفطرية المحلية المنتجة للسموم والمعزولة من مواد غذائية مختلفة وايضا بعض الفطريات السامة القياسية والتي تم شرائها من مركز CBS الهولندي. أثبتت النتائج قدرة كل من زيوت الزعتر، القرنفل، النعناع البلدي، النعناع الفلفلي والكمون على منع نمو كل الفطريات السامة المختبرة باستخدام التركيزين المستخدمين ( ميكرو لتر لكل ملى ميديا). منع زيت البردقوش النمو الفطري بالكامل باستخدامه عند تركيز ( ميكرو لتر لكل ملى ميديا) بينما حفز زيت الزنجبيل نمو جميع الفطريات ماعدا أسبيرجليس يسليوم جريسيوفولفم . CBS عند استخدامه بجميع التركيزات. سجلت زيوت الفلفل الاسود تأثيرات ضعيفة على النمو عند استخدامها بتركيز ( ميكرو لتر ) بينما لم يؤثر زيت الكمون الاسود على نمو الفطريات السامة عند هذا التركيز . منعت زيوت الزعتر والقرنفل والنعناع انتاج جميع أنواع السموم محل الدراسة كما أظهر زيت الفلفل الاسود وزيت الزنجبيل قدرتهما على اختزال .