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Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi

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Abstract

Essential oils of 12 medicinal plants were tested for inhibitory activity against *Aspergillus flavus, A. parasiticus, A. ochraceus* and *Fusarium moniliforme*. The oils of thyme and cinnamon (⩽500 ppm), marigold (⩽2000 ppm), spearmint, basil, quyssum (3000 ppm) completely inhibit all the test fungi. Caraway was inhibitory at 2000 ppm against *A. flavus*, *A. parasiticus* and 3000 ppm against *A. ochraceaus* and *F. moniliforme*. *A. flavus, A. ochraceus, A. parasiticus* and *F. moniliforme* were completely inhibited by anise at ⩽500 ppm. However, chamomile and hazanbul at all concentrations were partially effective against the test toxigenic fungi. The results indicate that the test toxigenic fungi are sensitive to the 12 essential oils, and particularly sensitive to thyme and cinnamon. The results also showed that the essential oils of thyme, cinnamon, anise and spearmint have more effect on fungal development and subsequent mycotoxin production in wheat grains. The extent of inhibition of fungal growth and mycotoxin production was dependent on the concentration of essential oils used.

Keywords: Mycotoxins; Fungi; Essential oil; Medicinal

1. Introduction

The presence and growth of fungi in food may cause spoilage and result in a reduction in quality and quantity. Some *Aspergillus* species are xerophilic fungi and are responsible for many cases of food and feed contamination (Abarc et al., 1994; Katta et al., 1995). *A. flavus* and*A. parasiticus* are able to produce aflatoxins in food and feedstuffs (Guo et al., 1996).

Aflatoxins are known to be potent hepatocarcinogens in animals and humans (Dvorackova, 1990). Toxigenic strains of *Fusarium* are able to produce fumonosins (Chamberlain et al., 1993). *A. ochraceus* produces ochratoxin A (OTA), which is a mutagen and animal carcinogen (IARC, 1993). Furthermore, OTA has been linked to nephropathies in pigs and humans (Krogh et al., 1974). Therefore, the presence of toxigenic fungi and mycotoxins in foods and grains stored for long periods of time presents a potential hazard to human and animal health. Many investigators used essential oils such as cinnamon, peppermint, basil and thyme to protect maize kernels against *A. flavus* infection, without affecting germination and corn growth (Montes-Belmont and Carvajall, 1998).

Considerable interest has developed during recent years on the preservation of grains by the use of essential oils to effectively retard growth and mycotoxin production (Bullerman et al., 1977). Because of health and economic considerations, a search was made to find some essential oils that could safely be used as substitutes for fungicides to partially or completely inhibit the growth of fungi and mycotoxins.

2. Materials and methods

2.1. Preparation of essential oils

The essential oils used in this study were prepared by steam distillation according to the procedure of Gunther (1961). The extracted oils were dried with anhydrous sodium sulphate and stored in a sterilized vial at 4 °C until use. Commercially available supplies of the plant materials used throughout this study are presented in Table 1.

Abbreviations: OTA; ochratoxin A; PDA; potato dextrose agar

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Table 1
Family, species and common name of the plant

Family	Species	Common name
Umbellifereae	*Pimpinella anisum* L.	Anise
	Carum carvi L.	Caraway
	Foenicculum vulgare L.	Fennel
Labiateae	*Thymus vulgaris* L.	Thyme
	Mentha viridis	Spearmint
	Ocimum basilicum L	Basil
Compositeae	*Matricaria chamomillal* L.	Chamomile
	Calendula ofricinalis L.	Marigold
	Achillea millefalium	Hazanbul
	Achillea fragrantissima	Qyssum
Rosaceae	*Agrimonia eupatoria*	Ghafath
Lauraceae	*Cinnamomum zeylanicum* L.	Cinnamon

2.2. Determination of the fungistatic effect of the extracted oils on some mycotoxigenic fungi

2.2.1. Mycotoxigenic fungi tested

Aspergillus flavus, A. parasiticus, A. ochraceus and *Fusarium moniliforme* were used as test organisms. These fungi were obtained from the Phytopathology Department, National Research Center, Giza, Egypt.

2.2.2. The fungitoxic activity

The tested fungi were grown on potato dextrose agar (PDA) medium, on petri dishes, for 5–7 days. Each of the tested oils was used at different concentrations: 500, 1000, 2000 and 3000 ppm. Anise and thyme were used at 125, 250, 500 and 1000 ppm. Each concentration was mixed with sterilized semi-solidified PDA medium and then poured into sterilized Petri dishes (10 ml in each plate). A 5-mm diameter disk was placed on the surface of the agar and inoculated with a suspension of the test

Table 2
Minimum inhibitory concentration (ppm) and fungistatic and fungicidal activity of oil extracted from some medicinal plants belonging to the family *Umbellifereae* on *A. flavus*, *A. parasiticus, A. ochraceus* and *F. moniliforme*

Essential oil (ppm)	Fungus											
	A. flavus			*A. parasiticus*			*A. ochraceus*			*F. moniliforme*		
	S.a	R%	F-s-c	S.a	R%	F-s-c	S.a	R%	F-s-c	S.a	R%	F-s-c
Caraway												
0.0	59.4			60.2			59.5			62.2		
500	43.0	28.0	+	26.0	57.0	+	18.0	70.0	+	13.0	79.0	+
1000	17.0	71.0	+	23.0	62.0	+	9.0	85.0	+	5.0	92.0	+
2000	0.0	100.0	–	0.0	100.0	–	6.0	90.0	+	3.0	95.0	+
3000	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
a		15.80			24.40			35.04			40.54	
b		0.03384			0.03031			0.02612			0.02512	
r		0.91444			0.88910			0.78511			0.72629	
Fennel												
0.0	59.4			60.2			59.5			62.2		
500	15.0	75.0	+	23.0	62.0	+	10.0	83.0	+	9.0	86.0	+
1000	10.0	83.0	+	6.0	90.0	+	3.0	95.0	+	7.0	89.0	+
2000	5.0	92.0	+	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
3000	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
a		36.49			0.078			43.78			42.61	
b		0.02588			0.02817			0.02502			0.02491	
r		0.77130			0.80162			0.70335			0.72153	
Anise												
0.0	59.4			60.2			59.5			62.2		
125	55.4	6.7	+	31.9	47.0	+	20.1	91.4	+	44.4	28.6	+
250	46.4	21.9	+	10.1	83.2	+	8.9	85.0	+	37.1	40.3	+
500	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
1000	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
a		−5.40			26.27			43.59			7.83	
b		0.03932			0.03059			0.02438			0.03535	
r		0.94356			0.86087			0.68978			0.95300	

S.a: surface area; R%: reduction%; F-s-c: fungistatic and fungicidal activity; *a*: intercept; *b*: slope; *r*: correlation coefficient.

Table 3
Minimum inhibitory concentration (ppm) and fungistatic and fungicidal activity of oil extracted from some medicinal plants belonging to the family *Labiateae* on *A. flavus*, *A. parasiticus*, *A. ochraceus* and *F. moniliforme*

Essential oil (ppm)	Fungus											
	A. flavus			*A. parasiticus*			*A. ochraceus*			*F. moniliforme*		
	S.a	R%	F-s-c	S.a	R%	F-s-c	S.a	R%	F-s-c	S.a	R%	F-s-c
Thyme												
0.0	62.4			58.1			56.2			56.7		
125	7.3	88.0	+	15.0	74.0	+	8.2	85.0	+	3.4	94.0	+
250	0.0	100.0	–	8.6	85.0	+	3.0	95.0	+	0.0	100.0	–
500	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
1000	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
a		46.31			36.99			43.81			48.59	
b		0.02407			0.02678			0.02474			0.02324	
r		0.66340			0.77492			0.69405			0.63419	
Spearmint												
0.0	62.4			58.1			56.2			56.7		
500	30.0	52.0	+	14.0	76.0	+	9.0	84.0	+	19.0	67.0	+
1000	28.0	55.0	+	7.0	88.0	+	3.0	94.0	+	13.0	77.0	+
2000	3.0	95.0	+	4.6	92.0	+	0.0	100.0	–	4.0	93.0	+
3000	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
a		20.41			38.21			43.19			31.90	
b		0.03076			0.02538			0.02493			0.02731	
r		0.91788			0.75027			0.70200			0.82517	
Basil												
0.0	62.4			58.1			56.2			56.7		
500	44.0	30.0	+	17.0	71.0	+	11.0	80.0	+	24.0	58.0	+
1000	17.0	73.0	+	9.0	85.0	+	5.0	91.0	+	19.0	67.0	+
2000	8.0	87.0	+	7.0	88.0	+	3.0	95.0	+	7.0	88.0	+
3000	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
a		16.53			35.34			40.66			25.59	
b		0.03190			0.02574			0.02503			0.02847	
r		0.91957			0.77840			0.72505			0.88482	

S.a: surface area; R%: reduction%; F-s-c: fungistatic and fungicidal activity; *a*: intercept; *b*: slope; *r*: correlation coefficient.

fungi. Plates were incubated for 7–14 days at 28±1 °C (five replicates were used for each treatment). Two perpendicular diameters of the growth zone were measured from which the average growth area was calculated. Disks showing no growth were transferred to dishes containing media without oil to determine the fungistatic and fungicidal effects of the oil used.

2.2.3. Wheat grain protection

Essential oils of thyme, cinnamon, anise and spearmint were tested at concentrations of 0.1, 0.5, 1 and 2%. At each of these concentrations 100 wheat grains were immersed in the oil in the neat state for 30 min, dried for 30 min, and distributed between five Petri dishes with wet cotton. They were then sprayed with the fungal spore suspension and incubated for 7–14 days; the percentage of grains showing fungal growth was then calculated. The same procedure was followed for mycotoxin production with inoculated wheat grains (100 g into flasks) and incubated for 2–8 weeks.

The aflatoxins and ochratoxin A were extracted and purified according to the methods of AOAC (1995). The extraction and purification of FB_1 from samples was carried out according to Rottinghaus et al. (1992). The identification of toxins was performed using HPLC.

2.2.4. Statistical analysis

Results were analysed statistically as described by Steel and Torrie (1960).

3. Results and discussion

The effect of essential oils of 12 plants belonging to five families as well as statistical analysis (linear model, $y = a + b\ x$, where *a*: intercept; *b*: slope; *r*: correlation coefficient) is presented in the tables as follows: Fam. *Umbellifereae* (anise, caraway and fennel)—Table 2; Fam. *Labiateae* (thyme, spearmint and basil)—Table 3; Fam. *Compositeae* (chamomile, marigold, hazanbul and quyssum)—Table 4; Fam. *Rosaceae* (Ghafath) Table 5; Fam. *Lauraceae* (cinnamon)—Table 6.

The essential oils of anise, fennel and caraway (Table 2) showed inhibitory effects on the four tested

Table 4
Minimum inhibitory concentration (ppm) and fungistatic and fungicidal activity of oil extracted from some medicinal plants belonging to the family *Compositeae* on *A. flavus*, *A. parasiticus*, *A. ochraceus* and *F. moniliforme*

Essential oil (ppm)	Fungus											
	A. flavus			*A. parasiticus*			*A. ochraceus*			*F. monliforme*		
	S.a	R%	F-s-c	S.a	R%	F-s-c	S.a	R%	F-s-c	S.a	R%	F-s-c
Chamomile												
0.0	62.4			58.1			56.2			54.7		
500	15.0	76.0	+	30.0	48.0	+	24.0	57.0	+	17.0	69.0	+
1000	11.0	82.0	+	10.0	83.0	+	11.0	80.0	+	13.0	76.0	+
2000	9.0	86.0	+	8.0	86.0	+	8.0	86.0	+	8.0	85.0	+
3000	6.0	90.0	+	3.0	95.0	+	4.0	93.0	+	5.0	91.0	+
a		38.16			26.90			29.87			33.67	
b		0.02203			0.02731			0.02564			0.02348	
r		0.70385			0.83905			0.81621			0.76714	
Marigold												
0.0	61.5			56.1			54.1			56.7		
500	40.0	35.0	+	18.0	68.0	+	16.0	70.0	+	30.0	47.0	+
1000	20.0	68.0	+	10.0	82.0	+	13.0	76.0	+	8.0	89.0	+
2000	6.0	90.0	+	4.0	93.0	+	0.0	100.0	–	0.0	100.0	–
3000	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
a		17.22			33.61			33.07			27.82	
b		0.03183			0.02691			0.02779			0.03029	
r		0.93097			0.80598			0.81572			0.83958	
Hazanbul												
0.0	62.2			58.1			52.1			54.7		
500	36.0	42.0	+	46.0	21.0	+	37.0	29.0	+	25.0	54.0	+
1000	33.0	50.0	+	38.0	35.0	+	27.0	48.0	+	18.0	67.0	+ +
2000	23.0	63.0	+	30.0	48.0	+	20.0	62.0	+	20.0	63.0	+
3000	21.0	66.0	+	18.0	69.0	+	15.0	71.0	+	14.0	74.0	+
a		20.06			6.90			13.65			26.81	
b		0.01857			0.02131			0.02181			0.01907	
r		0.84202			0.97929			0.92709			0.77214	
Quyssum												
0.0	62.2			58.1			54.1			56.7		
500	52.0	16.0	+	26.0	55.0	+	15.0	72.0	+	10.0	82.0	+
1000	19.0	70.0	+	12.0	79.0	+	9.0	83.0	+	6.0	89.0	+
2000	10.0	84.0	+	6.0	90.0	+	0.0	100.0	–	3.0	95.0	+
3000	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
a		10.29			27.75			35.70			40.88	
b		0.03362			0.02850			0.02716			0.02486	
r		0.92682			0.85994			0.78711			0.72194	

S.a: surface area; R%: reduction%; F-s-c: fungistatic and fungicidal activity; *a*: intercept; *b*: slope; *r*: correlation coefficient.

fungi, *A. flavus, A. parasiticus, A. ochraceus* and*F. moniliforme*, at all concentrations. It could be seen that as the oil concentration increases the inhibitory effect increases. In other words, the inhibitory effect of the oil is proportional to its concentration. Anise essential oil has more inhibitory effect than the other two members of the *Umbellifereae* family, caraway and fennel. It completely inhibited the four fungi at 500 ppm, while caraway and fennel had the same effect at 2000 ppm. Anise, fennel and caraway essential oils contain anithole. Its concentration is about 90% in anise and 60% in both fennel and caraway. The difference in concentration may explain the antifungal effect seen, since the effect of anise as a fungicide is much greater than fennel and caraway. Also, many investigators have demonstrated the fungistatic and fungicidal effects of anise, caraway and fennel essential oils against *A. flavus* and *A. parasiticus* (Farag et al., 1989; Hasan, 1994; Soher, 1999).

In addition, Dwividi and Dubey (1993) found antifungal activities of oils extracted from plants belonging to the *Umbellifereae* family against *A. flavus*. Caraway and spearmint belong to different families but they contain carfone as a main component of their essential oils, which may be responsible for their antifungal activity.

Table 5
Minimum inhibitory concentration (ppm) and fungistatic and fungicidal activity of oil extracted from some medicinal plants belonging to the family *Rosaceae* on *A. flavus*, *A. parasiticus, A. ochraceus* and *F. moniliforme*

Essential oil (ppm)	Fungus											
	A. flavus			*A. parasiticus*			*A. ochraceus*			*F. moniliforme*		
	S.a	R%	F-s-c	S.a	R%	F-s-c	S.a	R%	F-s-c	S.a	R%	F-s-c
Ghafath												
0.0	61.5			60.2			59.4			62.2		
500	34.0	45.0	+	20.0	67.0	+	17.0	71.0	+	20.0	68.0	+
1000	28.0	55.0	+	18.0	70.0	+	13.0	78.0	+	14.0	75.0	+
2000	11.0	82.0	+	13.0	78.0	+	4.0	93.0	+	7.0	89.0	+
3000	4.0	94.0	+	2.0	97.0	+	3.0	95.0	+	0.0	100.0	–
a		18.28			29.92			34.59			31.57	
b		0.02840			0.24980			0.02424			0.26790	
r		0.93258			0.81774			0.77930			0.82450	

S.a: surface area; R%: reduction%; F-s-c: fungistatic and fungicidal activity; *a*: intercept; *b*: slope; *r*: correlation coefficient.

Table 6
Minimum inhibitory concentration (ppm) and fungistatic and fungicidal activity of oil extracted from some medicinal plants belonging to the family *Lauraceae* on *A. flavus*, *A. parasiticus, A. ochraceus* and *F. moniliforme*

Essential oil (ppm)	Fungus											
	A. flavus			*A. parasiticus*			*A. ochraceus*			*F. moniliforme*		
	S.a	R%	F-s-c	S.a	R%	F-s-c	S.a	R%	F-s-c	S.a	R%	F-s-c
Cinamone												
0.0	62.2			58.1			54.1			56.7		
500	4.0	94.0	+	11.0	81.0	+	6.0	89.0	+	3.0	95.0	+
1000	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
2000	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
3000	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–	0.0	100.0	–
a		48.59			43.66			46.69			48.97	
b		0.02324			0.02503			0.02393			0.02310	
r		0.63419			0.69484			0.65864			0.62921	

S.a: surface area; R%: reduction%; F-s-c: fungistatic and fungicidal activity; *a*: intercept; *b*: slope; *r*: correlation coefficient.

The essential oils of members of the *Labiateae* family (Table 3) have inhibitory effects on the four toxigenic fungi tested. Basil and spearmint caused complete growth inhibition of the four fungi at 3000 ppm. The fungus *A. ochraceus* was affected severely by spearmint and basil oils, while *A. parasiticus* and *F. moniliforme* were moderately affected. *A. flavus* was more resistant to basil and spearmint oils. Thyme oil was more toxic to the four pathogenic fungi than the other two members of the *Labiateae* family. Its effect was drastic at 250 ppm on *A. flavus* and *F. moniliforme,* and at 500 ppm on *A. parasiticus and A. ochraceus*. Spearmint and basil oils acted as fungistatic agents at 2000 ppm and as fungicidal agents at 3000 ppm. Thyme oil was fungistatic at 250 ppm and had fungicidal activity at 500 ppm.

These antifungal activity of thyme, spearmint and basil was also demonstrated by Montes-Belmont and Carvajall (1998) and Basilico and Basilico (1999) on the toxigenic fungi *A. flavus, A. parasiticus, A. ochraceus, A. fumigatus* and *Fusarium* spp. Ela et al. (1996), Inouye et al. (1998, 2000), El-Maraghy (1995) and Dube et al. (1989) studied the antifungal activity of mint, basil and thyme on some pathogenic fungi, including *A. flavus* and *A. parasiticus,* and proved their inhibitory effect. This effect could be related to several components known to have biological activities, such as α-pinene (2.9, 1.32, 1%), β-pinene (0.4, 0.8, 1%) and (3.8, 37.6, 29.9%) in thyme, basil and spearmint, respectively. In addition, thyme oil contained thymol (40%) and P-cymene (25.2%) as the most prevalent components. The major substances for basil oil were ocimene (11.2%) and methyl chavecol (50%). Spearmint was characterized by the presence of menthone (39.3%) and menthol (29.9%) as a major component.

Data presented in Table 4 show that the essential oils extracted from some medicinal plants belonging to the family *Compositeae* have fungistatic activity against all toxigenic fungi. The highest fungistatic effect of chamomile and hazanbul essential oils (95 and 74% reduction, respectively) was recorded at a concentration of 3000

ppm, while marigold and quyssum completely inhibited the growth of four fungi (100% reduction) at 3000 ppm.

Table 5 presents the results of the effect of ghafath essential oil on the growth of *A. flavus, A. parasiticus, A. ochraceus* and *F. moniliforme*. It showed fungicidal activity only in *F. moniliforme* at 3000 ppm (100% reduction), while the same concentration (3000 ppm) has fungistatic activity on *A. flavus, A. ochraceus* and *A. parasiticus* (94, 95 and 97% reduction, respectively).

Regarding cinnamon oil, its effect on the different tested fungi is presented in Table 6. The essential oil of cinnamon at concentrations of 1000 ppm and above caused complete inhibition of the growth of the test fungi. The three components of cinnamon that have been identified as the agents active against moulds are cinnamic aldehyde (Bullerman, 1974), *O*-methoxycinnamaldehyde (Morozumi, 1978) and carfone (Dwividi and Dubey, 1993). Many previous studies had verified cinnamon oil as a fungistatic agent against many toxigenic fungi and proved its highly fungicidal activity (Salmeran and Pozo, 1991; Patkar et al., 1993; Ryu and Holt, 1993; Sinha et al., 1993; Mukherjee and Nandi, 1994).

It is interesting to mention that thyme, cinnamon, anise and spearmint were found to induce complete inhibition of fungal growth. Consequently, we tried to evaluate the role of these essential oils in the prevention of toxin production in wheat inoculated with toxigenic fungi during storage. The data presented in Table 7 indicate that the essential oils under study inhibited fungal growth. The inhibition of growth was dose-dependent with the concentration of essential oils. Moreover, thyme and anise oils were more effective than others.

Data concerning the effects of essential oils against different mycotoxin production in inoculated wheat are presented in Table 8. Examination of samples indicates the important role of essential oils (thyme, cinnamon, anise and spearmint) at different concentrations in

Table 7
Determination of optimal wheat protection dosage in application of essential oil

Essential oils	0.0	0.1	0.5	1	2
A. flavus					
Thyme	100	42	30	0	0
Anise	100	35	19	0	0
Cinnamon	100	66	35	19	0
Spearmint	100	86	53	41	8
A. parasiticus					
Thyme	100	55	16	0	0
Anise	100	68	40	13	2
Cinnamon	100	100	60	41	22
Spearmint	100	100	80	66	51
A. ochraceus					
Thyme	100	47	19	5	0
Anise	100	42	16	7	0
Cinnamon	100	21	9	2	0
Spearmint	100	57	21	13	5
F. moniliforme					
Thyme	100	50	12	0	0
Anise	100	65	28	0	0
Cinnamon	100	72	28	6	0
Spearmint	100	100	65	10	0

Table 8
Effect of essential oils on mycotoxin production (ng/g) in inoculated wheat

Concn of oil	Week	Aflatoxins				Ochratoxin A				Fumonisin			
		Thyme	Anise	Cinnamon	Spearmint	Thyme	Anise	Cinnamon	Spearmint	Thyme	Anise	Cinnamon	Spearmint
0	2	27.73	27.73	27.73	27.73	21.46	21.46	21.46	21.46	115.75	115.75	115.75	115.75
	4	27.52	27.52	27.52	27.52	20.98	20.98	20.98	20.98	117.98	117.98	117.98	117.98
	8	26.98	26.98	26.98	26.98	21.35	21.35	21.35	21.35	115.55	115.55	115.55	115.55
0.1	2	N.D	N.D	2.67	3.71	N.D	N.D	1.91	2.97	N.D	N.D	22.68	31.22
	4	5.74	6.01	6.26	89.26	4.10	4.74	5.02	7.91	41.60	52.40	51.00	67.45
	8	5.92	6.91	7.51	9.74	5.49	4.82	9.97	11.93	47.84	53.35	62.60	90.52
0.5	2	N.D	N.D	Traces	1.38	N.D	N.D	Traces	1.26	N.D	N.D	Traces	24.81
	4	3.10	2.36	5.45	7.59	1.18	1.99	2.31	3.41	20.50	23.50	28.81	38.79
	8	3.24	3.51	2.57	7.99	3.39	4.59	4.63	3.84	29.73	25.38	31.70	44.50
1.0	2	N.D	N.D	Traces	0.96	N.D	N.D	N.D	0.18	N.D	N.D	Traces	13.22
	4	N.D	N.D	Traces	Traces	N.D	N.D	Traces	0.90	Traces	7.82	12.78	15.80
	8	N.D	N.D	1.87	1.17	N.D	N.D	Traces	1.03	Traces	8.03	17.00	20.03
2.0	2	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D.	N.D	N.D
	4	N.D	N.D	N.D	Traces	N.D	N.D	N.D	Traces	N.D	N.D	Traces	Traces
	8	N.D	N.D	N.D	0.75	N.D	N.D	N.D	N.D	N.D	N.D	Traces	13.77

inhibiting toxin production in inoculated wheat. It should be noted that there was a gradual increase in inhibition due to the increased concentration of essential oils. Nevertheless, the thyme and anise oils were more effective than cinnamon and spearmint. Although the highest concentrations were completely inhibitory, traces of mycotoxins were produced after storage especially in inoculated wheat treated with cinnamon and spearmint oils. This may be due to the loss of some of its protective activity and capacity to inhibit mould growth and mycotoxin production.

These findings clearly indicate that essential oils should find a practical application in the inhibition of mycotoxin production in stored grains. Essential oils could be safely used as preservative materials on some kinds of foods, such as thyme oil which completely stopped the growth of fungi at low concentrations, and could be added to grain in storage to protect it from fungal infection. Also, cinnamon powder could be used as an additive on some edible grains such as peanut and popcorn. Anise and spearmint also gave good results as protectants against fungi. These oils could be used as a substitute for chemical fungicides since they are natural, and non-toxic to humans. Further investigations are being conducted to evaluate the economics of the essentials in pilot and commercial applications.

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