Effects of Some Essential Oils on *Aspergillus flavus* Growth and Afflation Production

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ABSTRACT

The present study aimed at investigating the effects of some essential oils on inhibiting fungal growth of *A. flavus* and afflation production. Clove oil was found as the best among the six different oils tested against the radial growth at the concentration of 0.05. mL/100ml. However the other oils were also significantly better than the control except the Pumpkin oil. Different concentrations (0.00, 0.01, 0.03 and 0.05 ml/100ml) of only three of the oils were tested against the radial growth of *A. flavus*. Clove and Cumin oils were significantly effective than the control at all these concentrations. Although Pumpkin oil was slightly more effective than the control at its higher concentration (0.05) ml/100ml), it was not effective at its lower concentrations (0.01 and 0.03 ml/100ml). Clove oil was also the best in suppressing mycelial growth at the concentration of 0.05 ml/100ml. However, the other oils were also significantly better than the control, while, Pumpkin oil was non-effective. Spore germination was also affected by the oils tested. Clove oil gave complete inhibition at its higher concentration followed by Cumin, Rehan, Garlic and Desert date, while Pumpkin oil was the least one. Aflatoxin production was highly affected by the essential oils tested. Clove and Cumin exhibited a complete inhibition, followed by Rehan; Garlic and Desert date while Pumpkin oil was non-effective.

Key words: Essential oils, aflatoxins, clove, cumin, garlic, pumpkin.

INTRODUCTION

Aflatoxins are natural poisons produced by two common fungi, *Aspergillus flavus* and *A. parasiticus*. Aflatoxin contamination in a variety of field crops and agricultural commodities worldwide has a serious impact on the economies and food safety of these products. The short-term toxicity of aflatoxin exposure of human to these compounds in foods, leading to liver cancer, has been well established. Due to the food safety problems, aflatoxin contaminated commodities cannot be sold at level above 20 parts per billion (ppb) in food and feed and 0.5 ppb in milk and eggs (Abdel-Rahim, 2005). Aflatoxins were found to cause many other health hazards in both human beings and animals (Horst, 1996).

Many studies had been done to control aflatoxin poisoning and to prevent growth of the aflatoxin producing fungi. The most important of which was the use of essential oils and plant extracts (Abdel-Rahim *et al*., 1997). Essential oils are concentrated volatile aromatic compounds produced by plants (the easily evaporated essences that give plant their wonderful scents).

Essential oils are used by the plants as hormone-like compounds and to work as chemical defense against fungal, viral and animal foes.

Essential oils were considered to be more effective than plant extracts (Frazier and Westhoff, 1967). It was also shown that essential oils as well as the oils extracted from citrus fruits could inhibit *A. flavus* growth and aflatoxin production (Alderman and Marth, 1976). In a study done by Mishra *et al*. (1989) to detect the toxicity of the oils extracted from 11 species of higher plants against *A. flavus*, Mexican tea oil, Cinnamon oil, Citrus fruit oil, Rehan oil as well as the oil extract from the plant *Melaleve lencadendron* were found effective at the concentration 2000 ppm. The toxicity of these oils was considered to be more effective in comparison with the fungicides; Agrosan GN, Cersan copper oxychloride, Diathane and Thiovit (Abdel-Rahim *et al.*, 2002). The objective of the present study is to test the effect of essentisl oils on the growth of the fungus *A. flavus* and aflatoxin production.

MATERIALS AND METHODS

Samples of groundnut seeds, collected from Wad Medani local markets, were used for isolation of the fungus *Aspergillus flavus*. Samples of six different oils including; Pumpkin (*Cucurbita pepol*), Clove (*Eugenia caryophyllata*), Garlic (*Allium sativum*), Cumin (*Cuminum cyminum*), Rehan (*Ocimum basilium*), and Desert date *(Balanites aegyptiaca*), were also obtained from the local market and used in the following experiments.

For fungal radial growth each of the six oils was incorporated into a molten PDA medium to give different concentrations (0.00, 0.01, 0.03and 0.05 ml/ 100 ml). Before solidification the mixture was poured into sterile Petri dishes and each Petri dish was inoculated centrally with a fungal disc (0.5 cm diam.). Radial growth was then measured every two days over a period of eight days of incubation at 25^0C (Mishra and Batra, 1987).

The spore germination test was conducted using the oil concentrations as above but in the synthetic SMKY broth medium (Abdel-Rahim and Arbab, 1985). A drop of about 0.5 ml was transferred from each solution onto a clean sterile glass slide and inoculated with 0.1 ml of a spore suspension. Inoculated slides were kept into sterile Petri dishes lined with moistened sterile filter paper to increase relative humidity. Readings were taken periodically (6, 12, 18 and 24 h.) by examining 100 spores in a microscopic field. Germination was then calculated as percentage.

Samples of (100 ml) of the synthetic SMKY medium containing different concentrations of the oils were inoculated with 1.0 ml spore suspension as above and incubated at 25^0C. After a seven days period of incubation, the mycelial mats were collected and weight immediately and reweighed after drying at 80^0 C (Jones, 1972 ; and Chatterjee, 1989). The filtrate was then kept for the aflatoxin analysis.

For aflatoxin determination, the resultant filtrates were analyzed using the methods described by Dienner and Davis (1966) and Jones (1972), TLC chromatographic plates of silica gel were employed. The loaded plates were developed in diethyl ether first and redeveloped in a mixture of chloroform and methanol (95:5), before being examined under an ultraviolet lamp (Jones, 1972). The results were statistically analyzed using the randomized block design.

RESULTS AND DISCUSSION

Due to the deleterious effects of the aflatoxins many studies have been done to control their production and to prevent growth of fungi producing them (Alderman and Marth, 1976; Misra and Batra, 1987 Singh and Upadhyay, 1991). However, the problem was more compounded by the fact that the toxins cannot be eliminated from foodstuffs or animal feeds, by ordinary processing practices (McDonald and Harkness, 1963, El_Nur and Ibrahim, 1970). Since fungicides cannot be applied to foodstuff and animal feeds, the use of essential oils and plant extracts was the most attractive procedure (Abdel-Rahim *et. al*., 1989).

The present study investigated the inhibitory effects of some essential oils (Clove, Rehan, Garlic, Pumpkin, Desert date and Cumin) on growth of the fungus *A. flavus* as well as on its aflatoxin production capacity. The results (Table, 1) demonstrated that Clove oil was the best among the six oils tested in reducing mycelial radial growth of the fungus. However, it gave an almost complete inhibition of mycelial radial growth at its two higher concentrations (Table, 2).

Table.1. Effect of some essential oils (at a concentration of 0.05) on *A. flavus* radial growth, during different incubation periods at 25^0 C.

Essential oils	Radial growth (cm)			
	Incubation period (days)			
	2	4	6	8
Clove	0.5	0.7	0.7	0.8
Cummin	1.5	1.8	2.3	3.0
Rehan	2.0	2.0	2.5	3.0
Garlic	2.0	2.2	4.0	4.0
Desert date	2.0	3.0	4.0	5.0
Pumpkin	2.0	4.0	6.0	9.0
Control	2.5	4.0	6.0	7.0

LSD =O. 48

Table (2): Effect of different concentrations (mL/100mL) of some essential oils on *A. flavus*, radial growth % from the control (0.00 conc.) and after 8 days of incubation.

Essential oils	radial growth (cm) Concentrations ml / ml			
	0.00	0.01	0.03	0.05
Clove	8.0	2.50	0.80	0.00
Cumin	8.0	3.5	340	3.00
Pumpkin	8.0	4.0	5.50	9.00

LSD = 0.51

The effects of Clove, Cumin and Pumbkin oils on mycelial dry weight was almost similar to their effects on radial growth (Table, 3). In a similar work Abdel-Rahim *et al.* (1997) reported that Clove and Rehan oils were able to inhibit mycelial radial growth and mycelial dry weight of both *A. flavus* and *A. parasitic us*... Moreover, Bullerman *et al*. (1977) have already mentioned that Clove oil at its different concentrations had an inhibitory effect on *A. flavus* growth.

Table (3): Effect of different concentrations (mL/100mL) of some essential on *A. flavus* mycelial dry weight, % from the control (0.00 conc.) and after 8 days of incubation.

Essential oils	% Mycelial dry weight Concentrations ml /100 ml			
	0.00	0.01	0.03	0.05
Clove	100.0	60.84	48.36	21.17
Cumin	100.0	86	72.04	60.04
Pumpkin	100.0	95.72	91.36	90.72

LSD = 21.14.

Although Cumin oil was not inhibiting mycelial growth completely, it was the second best to Clove oil. It gave a significantly better inhibition than the control at all of its tested concentrations (Table, 3). Singh and Upadhyay (1991) stated that, Cumin oil could only inhibit mycelial growth of the aflatoxin producing fungi at very high concentration (3000ppm). According to Chatterjee (1989) both Clove and Cumin oils were effective because they both contain Eugenol, the active ingredient against fungi, although Clove oil contains far higher amounts of Eugenol (95%) than Cumin oil.

In an elaborated investigation Mishra *et al*. (1989) studied the inhibitory effects of eleven essential oils on *A. flavus* and found that the oils of Mexcan tea, Cinnamon, Rehan, *Citrus*

Medica and *Melaleuce Lencadendron* were effective at the concentration 2000ppm while the rest oils were non-effective. They also added that the effectiveness of those oils was even better than the fungicides; Agrosan GN, Cerean, Copper oxychlorate, Diathane and Thiovit in inhibiting growth of these fungi. On the other hand Garlic and Desert date were less effective while Pumpkin oil was found the least one in inhibiting mycelial growth (both radial and dry weight) of the fungus *A. flavus*. However, it was even enhanced mycelial growth at its lower concentrations (Tables 1, 2 and 3).

The present study also investigated the effect of the essential oils on spore germination, because for fungi in general, the spore germination is a crucial event in the probation of the species, and for pathogenic fungi in particular, it is a deleting factor in the onset of host colonization (Allen, 1976). The slide test method which was recommended by the American Phytopathological Society, have been used throughout the spore germina-tion experiments, due to its high preference and quick results. The results (Tables 4) showed that Clove oil is also the best among the other oils tested. It allowed only 3.7% of the spores to germinate at the concentration tested (0.05). However, Cumin oil was the second best giving similar results to that in the mycelial growth followed by Rehan and Garlic oils. Pumpkin oil was not effective in suppressing spore germination of the fungus (Tables 4).

Table (4): Effect of some essential oils on *A. flavus* % spore germination at a concentration of 0.05 ml/100ml.

Essential oil	Spore germination% Incubation period (hrs.)			
	6	12	18	24
Clove	3.33	3.44	3.57	3.70
Cummin	6.66	7.01	10.00	11.59
Rehan	9.09	36.66	37.14	41.02
Garlic	43.40	50.00	55.58	72.88
Desert date	44.00	59.45	69.49	65.71
Pumpkin	61.90	92.23	86.88	64.55

LSD = 3.275.

Results in Table (5) represented the effects of different concentrations of Clove, Cumin and Pumpkin oils on spore germination of *A. flavus*, respectively. Clove oil was highly effective in reducing spore germination. It allowed only 3.7% of the spores to germinate at its higher concentration (Table, 5). Cumin oil was also highly effective at its higher concentration, but was less effective at its lower concentrations. Pumpkin oil, on the other hand, was the least effective even at its higher concentration. However, at its lower concentration, pumpkin oil was encouraging spore germination of the fungus (Table, 5).

Table (5): Effect of different concentrations (mL/100mL) of some essential oils on *A. flavus* spore germination, % from the control (0.00 conc.) and after 24 hours of incubation.

Essential oils	% spore germination			
	Concentrations ml / 100 ml			
	0.00	0.01	0.03	0.05
Clove	100.0	5.5	4.7	3.7
Cumin	100.0	48.0	45.2	11.6
Pumpkin	100.0	76.7	66.7	49.1

LSD = 21.14.

It is interesting to point out that aflatoxin production was also inhibited by the essential oils that inhibit fungal mycelial growth and spore germination (Table6). The same conclusions were also made by Abdel-Rahim *et.al*. (1997). However, Abdel-Rahim (2005) added that both essential oils and plant extracts were more effective when tested on aflatoxin production. In the present study Clove oil was proved to be the best among the other oils tested. These findings were also in agreement with Bullerman *et al*. (1977) who showed that, lower concentrations of Clove oil had an inhibitory effect on aflatoxin production.

Table (6): Effect of some essential oils on aflatoxin production (ml/100 ml).

Essential oils	0.01	0.03	0.05
Clove	+	-	-
Cummin	+	-	-
Rehan	++	++	+
Garlic	++	++	+
Desert date	++	++	+
Pumpkin	+++	+++	+++

- = Negative, + = Presence low, ++ = Moderate, +++ =High.

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التأثير المثبط لبعض الزيوت العطرية على الفطر *Aspergillus flavus* وإنتاجة للسموم الفطرية (الأفلاتكسينات)

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هدفت هذه الدراسة الى اختبار التأثير المثبط لبعض الزيوت العطرية على نمو الفطر *A. Flavus* وإنتاجه لسموم الأفلاتكسينات. وجد أن زيت القرنفل هو الأفضل من بين الستة زيوث التي تم إختبارها على النمو القطري للفطر *A. Flavus* وعلى التركيز 0.05 %. ومع ذلك فقد أعطت الزيوت الأخرى فروق معنوية مع تجربة المقارنة، ما عدى زيت القرع. وتم إختبار تراكيز مختلفة (0.00، 0.0%، 0.03% و0.05%) لثلاثة من الزيوت فقط، على النمو القطري للفطر. وكانت زيوت القرنفل والحبة السوداء أكثر فعالية في كل تلك التركتيزات مقارنة مع تجربة المقارنة. وعلى الرغم من أن زيت القرع كان اكثر فعالية كلى التركيز العالي (0.05)، إلا أنه كان غير فعالاً كلى التاكيز المنخفضة. وكان زيت القرنفل الأكثر فعالية بين الزيوت الأخرى في تثبيط النمو الميسليومي، في حين أعطت الزيوت الأخرى فروق معنوية مع تجربة المقارنة، وكان زيت القرع غير فعال في تثبيط النمو الميسليومي.

نموالجراثيم الفطرية تأثر أيضاً بالزيوت المستخدمة، حيث أعلى زيث القرنفل ثبيطاً كاملاً للنمو، تلاه زيت الحبة السوداء، ثم اليحان، الثوم واللالوب، بينما كان زيت القرع الأقل تأثيراً. وتأثر كثيراًً إنتاج السموم الفطرية (الأفلاتكسينات) بالزيوت المستخدمةً، حيث أوقفت زيوت القرنفل والحبة السوداء إنتاج تلك السموم بالكامل، تلتها زيوت الريحان، الثوم ثم اللالوب. هذا وكان زيت القرع غير فعال.