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Anti-aflatoxigenic effect of essential oils on *Aspergillus* Spp. isolated from pistachio in Saudi Arabia

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***Aspergillus* spp. associated with pistachio from the regions of Riyadh, Dammam and Abha were isolated. The most frequently isolated *Aspergillus* spp. came from Dammam (56.1%), followed by Riyadh (47.6%), and the least frequently isolated species were from Abha (26.2%). *Aspergillus flavus* showed the highest prevalence in the investigated samples. Three different culture media were used to qualitatively measure aflatoxin production by *Aspergillus* under UV radiation (365 nm), which was expressed as positive or negative. The obtained data showed that six isolates of *A. flavus* and four isolates of *A. parasiticus* were positive for aflatoxin production, while all isolates of *A. niger* were negative. Extraction of aflatoxin from pistachio was then performed through high-performance liquid chromatography (HPLC). Commercially available essential oils from thyme, garlic, cinnamon, mint and rosemary were tested to determine their influence on growth and aflatoxin production in *A. flavus* and *A. parasiticus.* The results showed that the tested essential oils caused highly significant inhibition of fungal growth and aflatoxin production in *A. flavus* and *A. parasiticus*. The extent of the inhibition of fungal growth and aflatoxin production was dependent on the type and concentration of essential oils applied. The results indicate that cinnamon and thyme oils show strong antimicrobial potential.**

Key words: Anti-aflatoxigenic, high-performance liquid chromatography (HPLC), essential oils, *Aspergillus* spp., pistachio.

INTRODUCTION

Mycotoxins are natural products generated by many fungi. These highly toxic secondary metabolites are produced by taxonomic groups consisting of filamentous fungi found in cereals, dried fruits and nuts (Sekar et al., 2008). Many genera produce mycotoxins, and nearly 50 species of *Aspergillus*, which is one of the largest and most important genera of fungus, containing more than 100 species (Pitt and Samson, 2007), are listed as being capable of producing aflatoxins (Cole and Cox, 1981). *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius* are the most well-known species that produce aflatoxins in human foods and animal feed. Nuts are among the crops that can be contaminated by aflatoxins (AFs), which are mycotoxins that are mainly produced by *A. flavus* and *A. parasiticus* (Kurtzman et al., 1987). Contamination of pistachio (*Pistacia vera L.*) nuts by

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Abbreviations: AFs, Aflatoxin; **PDA,** potato dextrose agar; **YES,** yeast extract sucrose; **HPLC,** high-performance liquid chromatography.

aflatoxin is considered a major problem in the US (Bhatnagar et al., 2004), Asia (Pitt and Hocking, 2004; Bonjar, 2004) and Africa (Bankole et al., 2006). Previous studies showed that 30.97 million tons of seed products, mainly peanuts and pistachios from Asian and African countries, were contaminated by *A. flavus* and *A. parasiticus* (Dekoe et al., 2000) during or after harvesting, storage and transition (Bruce et al., 2003).

The worldwide production of pistachio is 944,347 metric tons, and this crop yields 2.03 tons/hectare (FAO, 2011). Four of these aflatoxins, AFB1, AFB2, AFG1 and AFG2, can be detected in plant products. Aflatoxin B_1 is the most dangerous of these substances because of its acute and chronic effects, and it is classified by the International Agency for Research on Cancer (IARC) as belonging to rank 1 because of its demonstrated carcinogenicity in humans (Castegnaro and Wild, 1995). Furthermore, it was found that *A. flavus* and *A. parasiticus* produce all four principal aflatoxin (AFs) (Gabal et al. 1994). Essential oils obtained from higher plant parts have been shown to display anti-oxygenic properties, associated with compounds such as phenylpropanoids, terpenoids and alkaloids (Rath et al., 2005; Kumar et al., 2007; Holmes et al., 2008; and Rasooli et al., 2008; Adinarayana et al., 2012). In Saudi Arabia, accurate data regarding pistachio contamination by *Aspergillus spp.* are lacking, as few studies addressing *Aspergillus spp.* and aflatoxin production have been carried out (Nawar, 2008; Al-Wakeel and Nasser, 2011), and no major survey on this subject has been performed. The aims of the present study were therefore (1) to survey pistachio from three main regions in Saudi Arabia for the presence of *Aspergillus spp.*; (2) to detect and determine the levels of AFs using HPLC; and (3) to determine the effect of essential oils on the dry weight of and aflatoxin production by *Aspergillus spp.*

MATERIALS AND METHODS

Collection of samples

Fifteen samples of pistachio were collected randomly from different markets at three locations in Saudi Arabia (Table 1) (Riyadh, Dammam and Abha) during 2012 for this experimental work. The samples were stored at 2°C until use (Czerwiecki et al., 2002).

Isolation, purification and identification of *Aspergillus* associated with pistachio from Riyadh, Dammam and Abha

The samples were surface-sterilized with 5% sodium hypochlorite solution for one minute before being rinsed three times with sterilized, distilled water. Five pieces of each sample were placed on the surface of Petri dishes (9 cm diameter) containing potato dextrose agar (PDA), and each entry was replicated three times. The Petri dishes were incubated for 7 days at 25°C and observed daily for the emergence of colonies, which were then counted. Isolates were purified through single-spore methods and then transferred to PDA slants. All of the obtained isolates of mycotoxigenic fungal species were identified to the species level using keys and manuals (Raper and Fennell, 1973; Gilman, 1966; Domsch and Gams, 1993). The identification of isolates was confirmed by the Regional Center of Fungi and their Applications, Al-Azhar University, Cairo, Egypt. The frequency of fungi of particular species within a genus was calculated using the formula described by Ghiasian et al. (2004).

$$\text{Frequency} = \frac{\text{Number of fungal species isolated}}{\text{Total Number of fungi isolated}} \times 100$$

Extraction of aflatoxin from pistachio

For aflatoxin extraction, 20 g of each tested nut was mixed with 100 ml of a 4% acetonitrile aqueous solution of potassium chloride (9:1), followed by shaking for 20 min and filtration through Whatman No. 4 filter paper under vacuum. For purification, 100 ml of n-hexane was added to the filtrate, and the solution was shaken for 10 min. After separation, the upper phase (n-hexane) was discarded, and 50 ml of deionized water and 50 ml of chloroform were added to the lower phase. This solution was then shaken for 10 min. The upper phase was subsequently extracted twice more with 25 ml of chloroform, and the chloroform was evaporated at 40°C in a water bath at low speed. Subsequently, 2 ml of methanol was added, and the solution was filtered through a 0.45-µl filter (Zaboli et al, 2011).

Detection of aflatoxins via fluorescence (UV)

Czapek's agar, yeast extract sucrose (YES) agar and potato dextrose agar (supplemented with sodium chloride) were used as culture media. The cultures at 25°C for 4 days, and the presence or absence of fluorescence in the agar surrounding the assayed colonies was determined under UV radiation (365 nm) and expressed as positive or negative according to Franco et al. (1998).

Effect of essential oils on aflatoxin production by *Aspergillus* spp.

Commercially available essential oils from thyme, garlic, cinnamon, mint and rosemary were purchased from Al-ahlam for seed-oil production (Jeddah, Saudi Arabia). Three different concentrations (1, 2 and 4%) of the five different oils that were used in this experiment were produced as follows: 4 ml of essential oil and 0.4 mL of Tween 80 were placed in sterile tubes, and the volume was increased to 5 mL using distilled, sterile water. This mixture was shaken for 5 min using a vortexer, and serial dilutions were performed to obtain solutions with final concentrations of 1, 2 and 4%. Tween 80 was included as control (Souza et al., 2005).

The anti-aflatoxigenic efficacy of each tested essential oil was determined using SMKY liquid medium (sucrose, 20 g; magnesium sulfate, 0.5 g; potassium nitrate, 3 g; yeast extract, 7 g; and distilled water, 1,000 ml) (Abd El-Aziz et al., 2012). Three different concentrations of the essential oils (1, 2 and 4%) were prepared and added to flasks, followed by inoculation with 6-mm diameter discs of the toxigenic *Aspergillus spp*. at 25 ± 2°C for 7 days (Paranagama et al., 2003). A control group was run in parallel to the treatment group, but without the essential oils. After incubation, the content of each flask was filtered (Whatman, No. 1), and biomass of filtered mycelium was dried at 70°C for 4 days till their weights remains constant. Mycelial dry weights of treatments and control was determined and the filtrates were each treated three times with 50 ml of chloroform in a separatory funnel. The chloroform extracts were separated and dehydrated with anhydrous sodium sulfate and evaporated until dryness in a water bath at 50°C under vacuum. The residues were dissolved in 10 ml of methanol (Mostafa et al., 2011).

Table 1. Plant species, family and common name.

Plant specie	Family	Common name
Cinnamomum zeylanicum	Lauraceae	Cinnamon
Allium sativum	Alliaceae	Garlic
Mentha arvensis	Lamiaceae	Mint
Rosmarinus officinalis	Salicaceae	Rosemary
Thymus vulgaris	Lamiaceae	Thyme

Table 2. Frequency (%) of fungi isolated from pistachio specimens from Riyadh, Dammam and Abha.

Isolated fungi	Riyadh	Dammam	Abha
Aspergillus flavus	30.1	33.7	12.7
Aspergillus niger	12.4	14.6	7.6
Aspergillus parasiticus	5.1	7.8	5.9

Table 3. Determination of aflatoxin concentrations (µg/kg) from pistachio from Riyadh, Dammam and Abha.

Sample	Riyadh				Dammam				Abha			
	G1	G2	B1	B2	G1	G2	B1	B2	G1	G2	B1	B2
1	26.9	18	54.7	48.8	45.7	20.3	81.4	52	7.3	14.0	43.76	4
2	10.1	16.4	118	67.3	51.5	25.0	272	70	0.0	0.0	94.8	35.5
3	0.0	0.0	95.8	55.6	0.0	0.0	117.0	87.8	9.0	8.4	37	0.0
4	17.7	28.0	74	88.0	22.0	24.0	81.0	92.3	11.7	15.0	40	23
5	12.0	16.6	56.7	63.7	14.4	18.7	67.5	78.5	0.0	0.0	32	7

High -performance liquid chromatography (HPLC)

Aflatoxin levels were measured using high-performance liquid chromatography (HPLC) (model: PerkinElmer series 200 UV/VIS) with a C18 column that had an internal diameter of 300 x 3.9 mm. The HPLC apparatus was equipped with a UV detector, and fluorescence was measured using 365-nm excitation and 430-nm emission wavelengths. The mobile phase consisted of methanol:acetic:acid:water (20:20:60 v/v/v). The total run time for the separation was approximately 30 min, and the flow rate was 1 ml/min (Christian, 1990). Aflatoxin inhibition was calculated according to Mostafa et al. (2011) as follows:

Percentage of toxin inhibition = [A – a / A] x 100

Where, A is the concentration of aflatoxin in the treated sample, and a is the concentration of aflatoxin in the control.

Statistical analysis

All of the data from three independent replicate trials were subjected to analysis using Statistical Package for the Social Sciences (SPSS) 10.0 statistical software (Chicago, USA). The data are reported as the mean ± standard deviations, and significant differences between mean values were determined with Duncan's Multiple Range test ($p<0.05$), followed by one-way ANOVA.

RESULTS AND DISCUSSION

Isolation, purification and identification of *Aspergillus spp*. associated with pistachio from Riyadh, Dammam and Abha

The data presented in Table 2 show that *Aspergillus spp*. were most frequently isolated from Dammam (56.1%), followed by Riyadh (47.6%) and, finally, Abha (26.2%). *A. flavus* showed the highest prevalence in the investigated samples. This result was in agreement with the findings of Shahidi (2004), Yu et al. (2004), Rahimi et al. (2008), Kabirian et al. (2011) and Khodavaisy et al. (2012).

Determination of aflatoxin levels in pistachio nuts from Riyadh, Dammam and Abha

The data presented in Table 3 indicate that the highest percentage of aflatoxins was found in the regions of Riyadh and Dammam, whereas the lowest percentage was found in the region of Abha. The highest levels of aflatoxin contamination were detected in sample No. 2, which was contaminated with aflatoxins B1, B2, G1 and

G2 (272, 70, 51.5 and 25 µg/kg, respectively). Four samples produced only aflatoxins B1 and B2: samples No. 2 and 5, collected from the Abha region, and sample No. 3, collected from the Riyadh and Dammam regions. Eleven samples produced all four aflatoxins (B1, B2, G1 and G2) in varying amounts. The regional differences in the aflatoxin contamination of crops might be attributable to climatic conditions and agricultural practices that increase the susceptibility of plants to invasion by *A. flavus*. Moreover, the relative humidity (Nawar, 2008), pre-harvest temperature and humidity conditions in the field and improper postharvest handling and storage (Nakai et al*.,* 2008) play a vital role in the development and spread of fungal contamination.

Detection of aflatoxins produced by *Aspergillus spp*. isolates using UV radiation

Three types of culture media (Czapek's agar, potato dextrose agar (PDA) and yeast extract sucrose agar (YES agar)) were used to screen colonies for aflatoxin production. The presence or absence of fluorescence in the agar surrounding the assayed colonies was determined using UV radiation (365 nm) and was expressed as positive or negative. The data presented in Table 4 and Figure 1 show that non-aflatoxigenic aspergilli did not display fluorescence, whereas the aflatoxigenic strains were positive for fluorescence. This table also shows that six isolates of *A. flavus* and four isolates of *A. parasiticus* isolated from pistachio from three different regions were positive for aflatoxin production, while seven isolates of *A. flavus* and two isolates of *A. parasiticus* were negative for fluorescence. In addition, the table shows that all isolates of *A. niger* were negative for fluorescence. These results agree with those of Fente et al*.* (2001).

Effects of the five essential oils at three different concentrations on the dry weight of the mycelia (g) of *A. flavus and A. parasiticus*.

Tables 5 and 6 show the effects of different concentrations of essential oils on the dry weights of *A. flavus* and *A. parasiticus* mycelia after incubation at 25°C for 7 days. All of the tested essential oils were more effective in inhibiting mycelium growth at the three tested concentrations than the control, and the percentage (%) inhibition of dry weight decreased with increasing concentrations of all of the essential oils. The highest growth rate inhibition for the tested fungi was observed with the cinnamon and thyme oils at a concentration of 4%: for *A. flavus* and *A. parasiticus*, these values ranged from 61.8 to 86.6% and 68.5 to 83.2%, respectively. Mint oil at 4% was the most effective treatment for inhibiting two isolates (No. DP4 and RP2), whereas the rosemary and garlic oils showed lower inhibitory effects. The growth of *Aspergillus* was slightly inhibited at lower oil concentrations, and the inhibitory effects of the oils increased in proportion to their concentrations. Statistical analyses showed that the type and concentration of essential oil applied had a significant influence on dry weight $p<0.05$.

Effects of the five essential oils at a 4% concentration on aflatoxin B (µg/ml) production by *A. flavus* and *A. parasiticus*

The data presented in Table 7 indicate that nine isolates were capable of producing detectable levels of aflatoxin B, but one isolate (AP8) failed to produce any detectable amount of this toxin. The anti-aflatoxigenic effects of the five essential oils were examined, and cinnamon and thyme oils at 4% resulted in the highest levels of inhibition, ranging from 60.3 to 75.6% and 54.2 to 81.1%, respectively. In addition, treatment with cinnamon or thyme oil was most effective against *A. parasiticus* (DP9 and DP10), followed by the inhibitory effects of garlic and mint oils, which ranged from 47.8 to 73.0% and 70.2 to 51.8%, respectively. Rosemary oil resulted in the lowest rate of inhibition (45.8 to 54.8%).

Effects of the five essential oils at a 4% concentration on aflatoxin G (µg/ml) production by *A. flavus* and *A. parasiticus*

The data shown in Table 8 indicate that seven isolates were capable of producing detectable levels of aflatoxin G. Cinnamon and thyme oils at a concentration of 4% led to the highest levels of inhibition, which ranged from 59.7 to 85.7% and 61.0 to 78.7%, respectively. Cinnamon oil treatment was more inhibitory against *A. parasiticus*, as aflatoxin G was not produced on three isolates of *A. parasiticus* (RP1, DP9 and DP10), and this treatment led to the complete inhibition of aflatoxin G2 production in isolate AP3. Mint oil resulted in the next highest rates of inhibition, ranging from 58.7 to 72.8%, followed by rosemary oil. These results indicate that the examined toxigenic fungi are sensitive to the five tested essential oils, and particularly to thyme and cinnamon oils. These results have been confirmed by the findings of many researchers (Rasooli and Razzaghi, 2004; Nicola et al., 2005; Omidbeygi et al. 2006; Rad et al., 2011; Eweis et al*.,* 2012; Abd El-Aziz et al., 2012).

The extent of the inhibition of fungal growth and mycotoxin production is dependent on the concentration of the essential oils that were used (Soliman and Badeaa, 2002), and a direct correlation has been found between fungal growth and aflatoxin production (El-Habib, 2012). The antimicrobial properties of essential oils are mostly due to the presence of phenols and other compounds (Toda et al., 1989; Ebana et al*.,* 1991). Treatment with essential oils regulates conidiogenesis, and aflatoxin

Table 4. Detection of aflatoxins produced by *Aspergillus spp*. isolates under UV irradiation.

Region	Number of isolates	*Aspergillus spp.*	Culture Media		
			Yeast extract	Czapek – Dox-agar	PDA+ NaCl
Riyadh	RP1	*A. flavus*	+	-	-
Riyadh	RP2	*A. flavus*	+	-	-
Riyadh	RP3	*A. flavus*	-	-	-
Riyadh	RP4	*A. flavus*	-	-	-
Riyadh	RP5	*A. niger*	-	-	-
Riyadh	RP6	*A. niger*	-	-	-
Riyadh	RP7	*A. niger*	-	-	-
Riyadh	RP8	*A. parasiticus*	+	-	+
Riyadh	RP9	*A. parasiticus*	-	-	-
Dammam	DP1	*A. flavus*	+	-	-
Dammam	DP2	*A. flavus*	-	-	-
Dammam	DP3	*A. flavus*	-	-	-
Dammam	DP4	*A. flavus*	+	-	+
Dammam	DP5	*A. flavus*	-	-	-
Dammam	DP6	*A. niger*	-	-	-
Dammam	DP7	*A. niger*	-	-	-
Dammam	DP8	*A. niger*	-	-	-
Dammam	DP9	*A. parasiticus*	+	+	-
Dammam	DP10	*A. parasiticus*	+	-	-
Abha	AP1	*A. flavus*	-	-	-
Abha	AP2	*A. flavus*	-	-	-
Abha	AP3	*A. flavus*	+	-	-
Abha	AP4	*A. flavus*	+	-	-
Abha	AP5	*A. niger*	-	-	-
Abha	AP6	*A. niger*	-	-	-
Abha	AP7	*A. niger*	-	-	-
Abha	AP8	*A. parasiticus*	+	+	-
Abha	AP9	*A. parasiticus*	-	-	-

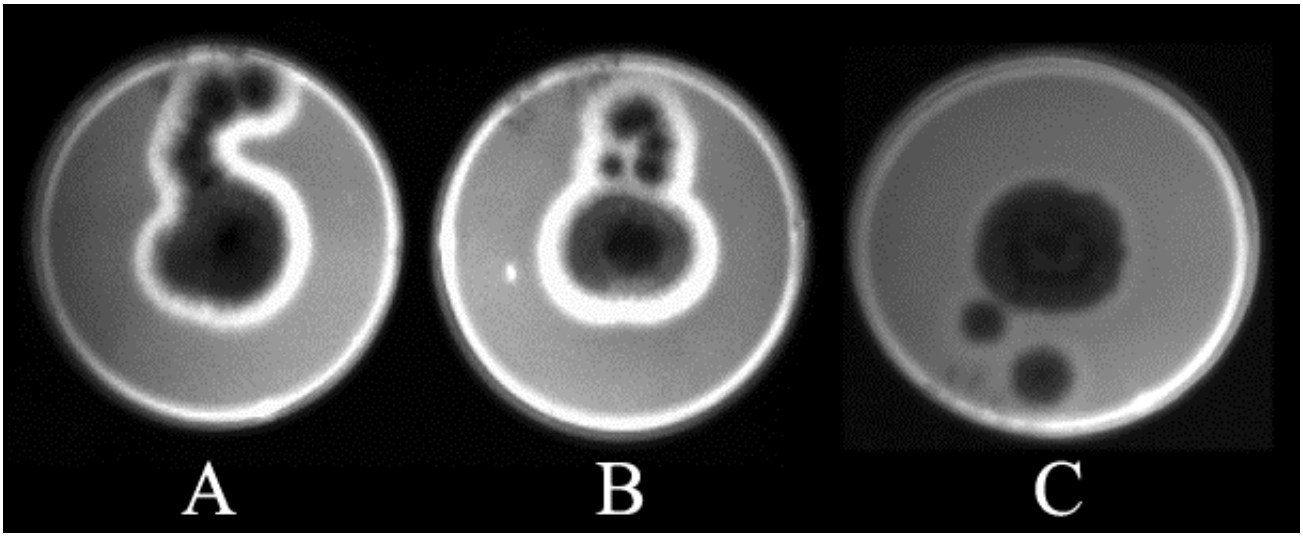

Figure 1. Aflatoxigenic strains of *A. flavus* (A), *A. parasiticus* (B) and *A. niger* (C) visualized under visible light and under 365-nm UV light. The white ring around colonies of aflatoxigenic strains displays faint blue fluorescence.

Table 5. Effects of the five essential oils at three different concentrations on the dry weight of the mycelia (g) of *A. flavus* and *A. parasiticus.*

Isolate	Control	Cinnamon			Garlic			Mint			Rosemary			Thyme		
		1%	2%	4%	1%	2%	4%	1%	2%	4%	1%	2%	4%	1%	2%	4%
RP1	3.84^{bc}±0.22	3.11^{c}±0.02	2.11^{c}±0.11	1.01^{abcd}±0.04	3.75^{bc}±0.16	2.62^{de}±0.05	1.80^{de}±0.05	2.66^{ef}±0.12	2.11^{ef}±0.02	1.25^{a}±0.04	3.30^{bc}±0.10	2.42^{e}±0.06	1.35^{de}±0.03	3.56^{bc}±0.07	1.77^{de}±0.07	1.09^{cde}±0.02
RP2	5.17^{a}±0.27	4.94^{a}±0.08	3.15^{ab}±0.13	1.36^{cd}±0.06	4.68^{a}±0.06	4.19^{a}±0.25	3.27^{ab}±0.20	4.43^{a}±0.14	2.37^{b}±0.14	0.99^{cd}±0.50	4.90^{a}±0.31	4.09^{a}±0.22	1.92^{b}±0.18	5.00^{a}±0.18	3.11^{ab}±0.12	1.35^{abc}±0.12
RP8	3.40^{C}±0.15	2.81^{c}±0.16	1.22^{e}±0.02	0.77^{de}±0.08	3.00^{c}±0.12	2.28^{e}±0.08	1.74^{ef}±0.06	2.82^{def}±0.13	1.96^{cd}±0.05	1.27^{a}±0.08	3.32^{c}±0.09	1.99^{cd}±0.11	1.59^{cd}±0.05	3.20^{bc}±0.12	1.08^{d}±0.04	0.97^{bcd}±0.07
DP1	3.92^{bc}±0.22	3.85^{b}±0.16	2.08^{cd}±0.04	1.02^{bcd}±0.07	3.71^{b}±0.04	3.12^{b}±0.11	2.18^{cd}±0.04	3.17^{c}±0.09	1.90^{cde}±0.07	1.41^{a}±0.03	3.83^{bc}±0.09	2.77^{b}±0.14	1.73^{bcd}±0.05	2.71^{f}±0.07	1.51^{d}±0.03	0.91^{cd}±0.08
DP4	3.72^{bc}±0.25	3.26^{c}±0.08	1.83^{cd}±0.14	1.42^{a}±0.09	3.50^{c}±0.29	2.72^{cde}±0.27	1.49^{f}±0.06	2.25^{g}±0.10	1.61^{e}±0.02	1.01^{a}±0.10	3.21^{cb}±0.08	2.05^{c}±0.07	1.15^{e}±0.04	3.27^{bc}±0.07	1.85^{bcd}±0.10	1.17^{ab}±0.02
DP9	4.05^{b}±0.07	2.97^{c}±0.12	1.74^{d}±0.14	0.75^{de}±0.06	3.89^{bc}±0.13	2.55^{de}±0.05	1.20^{g}±0.05	2.58^{f}±0.04	1.80^{cde}±0.03	1.27^{a}±0.05	3.52^{bc}±0.17	2.48^{bc}±0.29	1.63^{cd}±0.10	2.92^{ef}±0.12	1.49^{c}±0.03	0.68^{e}±0.05
DP10	4.09^{b}±0.23	3.06^{c}±0.27	2.46^{abc}±0.10	0.62^{e}±0.03	3.85^{bc}±0.15	2.41^{de}±0.12	1.82^{de}±0.09	2.97^{cde}±0.05	1.73^{cd}±0.15	1.51^{d}±0.02	3.83^{b}±0.21	2.53^{abc}±0.27	1.75^{cd}±0.07	2.68^{f}±0.08	1.66^{cd}±0.10	0.83^{de}±0.10
AP3	4.99^{a}±0.18	4.33^{a}±0.30	2.85^{b}±0.15	0.67^{de}±0.34	4.94^{a}±0.10	3.41^{b}±0.16	2.97^{b}±0.13	4.07^{a}±0.15	3.02^{b}±0.12	1.15^{ed}±0.04	4.70^{a}±0.21	3.92^{a}±0.13	2.90^{b}±0.13	3.90^{b}±0.11	1.89^{b}±0.09	0.90^{cd}±0.06
AP4	3.93^{bc}±0.10	2.80^{c}±0.14	1.93^{cd}±0.04	0.86^{cde}±0.03	3.80^{bc}±0.14	2.86^{cd}±0.05	1.68^{f}±0.05	2.50^{fg}±0.05	2.02^{cd}±0.09	1.18^{a}±0.03	3.70^{bc}±0.11	2.55^{d}±0.06	1.57^{cd}±0.04	2.79^{f}±0.12	1.64^{f}±0.06	0.96^{bcd}±0.04
AP8	4.16^{b}±0.10	2.95^{c}±0.08	2.81^{a}±0.16	1.22^{abc}±0.05	3.90^{bc}±0.11	3.39^{b}±0.25	2.06^{cd}±0.0+4	3.00^{cd}±0.07	1.87^{cde}±0.14	1.34^{a}±0.09	3.84^{b}±0.15	2.68^{d}±0.16	1.47^{cd}±0.04	2.65^{f}±0.08	1.60^{e}±0.30	1.05^{bcd}±0.07
LSD0.05	0.563	0.483	0.341	0.355	0.426	0.423	0.263	0.300	0.281	0.492	0.499	0.506	0.258	0.320	0.350	0.209

Values in the same column followed by (±) are significantly different (P = 0.05). The presented data are the mean (n = 3) ± standard error of three replicates. Data followed by the same letter are not significantly different at P≤0.05, whereas those followed by different letters are significantly different at P≤0.05).

Table 6. Effects of the five essential oils at three different concentrations on the % inhibition of the dry weight of *A. flavus* and *A. parasiticus.*

Isolate	Cinnamon			Garlic			Mint			Rosemary			Thyme		
	1%	2%	4%	1%	2%	4%	1%	2%	4%	1%	2%	4%	1%	2%	4%
RP1	19.0	45	73.7	2.3	31.9	53.1	30.7	45.1	67.4	14.1	36.9	64.8	7.3	35.9	71.6
RP2	23.0	39.1	73.7	9.4	18.9	36.8	14.3	54.2	80.8	5.2	20.8	62.9	3.3	39.8	73.9
RP8	17.4	64.1	77.4	12.8	32.9	48.8	17.1	42.4	62.6	2.3	41.5	35.2	5.9	68.2	71.5
DP1	17.8	46.9	73.9	5.4	20.4	44.4	19.1	51.5	46.0	2.2	29.3	55.9	30.9	61.5	76.8
DP4	12.3	50.8	61.8	5.9	26.9	59.9	39.5	56.7	72.8	13.7	44.9	69.1	12.1	50.3	68.5
DP9	26.7	57	81.5	3.9	37.0	70.3	36.3	55.5	68.6	13.1	38.8	59.8	27.9	63.2	83.2
DP10	25.2	39.9	84.8	6.2	41.1	55.5	27.4	57.7	63.1	6.4	38.1	64.3	45.3	59.4	79.7
AP3	13.2	42.9	86.6	1.0	31.7	40.4	18.4	39.5	76.9	5.8	21.4	41.9	21.8	62.1	81.9
AP4	28.8	50.8	78.1	3.3	27.2	57.2	36.4	48.6	69.9	8.8	35.1	60.1	29.1	58.3	75.6
AP8	29.1	32.5	70.7	6.3	18.5	50.5	27.9	55.0	67.8	7.7	35.6	64.7	36.3	61.5	74.8

Table 7. Effects of the five essential oils at 4% on aflatoxin B (µg/ml) production by *A. flavus* and *A. parasiticus*.

Isolate	Control		Cinnamon		% Inhibition	Garlic		% Inhibition	Mint		% Inhibition	Rosemary		% Inhibition	Thyme		% Inhibition
	B1	B2	B1	B2		B1	B2		B1	B2		B1	B2		B1	B2	
RP1	45.0	32.0	15.3	10.0	67.1	20.7	14.1	54.8	20.7	12.7	56.6	23.1	14.2	51.5	19.0	10.6	61.6
RP2	37.3	27.4	12.7	13.0	60.3	15.0	16.2	51.7	10.9	16.2	58.1	17.2	17.7	46.1	14.0	15.6	54.2
RP8	24.5	21.7	9.7	7.3	63.2	11.4	12.7	47.8	12.3	8.0	56.0	13.3	11.8	45.7	10.5	8.2	59.4
DP1	52.3	32.7	14.0	9.2	72.7	19.3	13.2	61.7	16.7	10.4	68.1	25.5	16.4	50.7	14.7	13.0	63.4
DP4	29.0	23.6	10.9	7.7	64.6	12.7	11.3	73.0	14.5	12.0	70.2	22.1	18.2	54.8	12.0	9.1	59.9
DP9	44.3	31.4	12.0	8.3	73.2	16.4	10.7	64.2	15.4	14.9	60.0	18.4	16.3	54.2	10.8	6.0	81.1
DP10	49.2	40.0	11.2	10.6	75.6	9.4	12.7	57.9	13.1	10.0	56.1	15.1	11.4	49.6	13.0	11.3	72.6
AP3	23.7	18.0	8.5	5.0	67.6	9.4	7.1	60.4	11.7	8.3	51.8	11.0	9.5	53.2	10.0	5.0	64.0
AP4	19.8	21.0	6.0	6.6	69.1	7.0	9.3	60.9	9.4	9.0	54.9	10.8	11.3	45.8	7.2	8.0	62.7
AP8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 8. Effects of the five essential oils at 4% on aflatoxin G (µg/ml) production by *A. flavus* and *A. parasiticus*.

Isolate	Control		Cinnamon		% Inhibition	Garlic		% Inhibition	Mint		% Inhibition	Rosemary		% Inhibition	Thyme		% Inhibition
	G1	G2	G1	G2		G1	G2		G1	G2		G1	G2		G1	G2	
RP1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
RP2	17.8	15.2	6.7	5.6	59.7	8.0	7.5	53.0	6.1	7.0	60.3	10.1	9.3	41.2	4.2	5.1	71.8
RP8	14.5	17.0	5.3	3.3	72.7	6.4	3.1	69.8	5.7	3.3	71.4	8.7	7.0	50.1	4.7	4.0	72.4
DP1	19.6	20.6	8.3	5.1	66.7	7.0	7.0	65.1	7.4	6.1	66.4	11.2	13.2	39.3	9.1	6.7	61.0
DP4	16.3	22.7	3.2	4.6	80.0	7.6	4.1	70.0	10.1	6.0	58.7	10.6	12.7	40.3	3.3	5.0	78.7
DP9	10.8	0.0	0.0	0.0	0.0	4.6	5.9	65.8	5.7	5.3	64.1	5.6	9.6	50.5	5.2	3.0	73.3
DP10	14.6	0.0	0.0	0.0	0.0	3.3	10.3	51.9	3.7	4.0	72.8	6.3	10.1	42.0	2.9	4.1	75.2
AP3	13.0	15.0	4.0	0.0	85.7	4.3	6.1	62.8	4.9	4.5	66.4	7.9	8.2	42.5	3.7	3.1	75.7
AP4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AP8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

synthesis may be interrelated with the loss of the aflatoxigenic capabilities of *A. parasiticus* that are correlated with alterations in conidial morphology (Kale et al., 1996). Moreover, lysis of hyphae and spores of the toxigenic fungi are characteristic of the aflatoxin deactivation process (Knobloch et al., 1988; Namazi et al., 2002).

Essential oils could act on the hyphae of the mycelium, leading to various components exiting the cytoplasm, potentially causing loss of the integrity and rigidity of the hyphal cell wall (Nicola et al., 2005; Sharma and Tripathi, 2008). Furthermore, antimicrobial activity might cause terpenes, which are present in essential oils, to cross cell membranes, thus penetrating the interior of the cell and interacting with critical intracellular sites (Cristani et al., 2007). Low concentrations of essential oils could lead to changes in the structure of the cell, inhibit respiration and alter the permeability of the microbial cell membrane, whereas high concentrations could damage the cell membrane

and lead to loss of homeostasis, which could affect enzymatic systems.

Conclusion

The results of the present study shows the possibility of using natural compounds as alternatives to pesticides for controlling fungal growth and aflatoxin production. According to the results, cinnamon and thyme oils at 4% resulted in the inhibition of growth and aflatoxin produced by *A. flavus* and *A. parasiticus* garlic and mint oils second in inhibitory effects, rosemary oil resulted in the lowest rate of inhibition.

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