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Inhibition of Aflatoxin Formation by Some Spices

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Hemmung der Aflatoxinbildung durch einige Gewürze

Zusammenfassung. Der Einfluß von Pfeffer, Zimt, Pfefferminz, Kumin (römischer Kümmel), Ingwer und Nelken auf das Wachstum von *Aspergillus flavus* und dessen Aflatoxinproduktion wurde auf Reismehl als Nährboden studiert. Die Hemmung der ersten fünf Gewürze konnte bei der Aflatoxinproduktion früher als beim Mycelwachstum nachgewiesen werden. Andrerseits hemmt Nelkenpulver das Mycelwachstum und die Aflatoxinproduktion bei einer Konzentration über 0,1% vollständig. Kein Alfatoxin konnte bei Konzentration von 5–10% Kumin- oder Pfefferminzpulver, bei Pfeffer und Ingwer erst ab 10% festgestellt werden. Konzentrationen bis zu 10% stimulieren bei Pfefferminz, Kumin und Ingwer das Mycelwachstum.

Summary. The effects of black pepper, cinnamon, peppermint, cumin, ginger and clove on growth and aflatoxin formation of *Aspergillus flavus* were studied in rice powdercorn steep (RC) medium. The effects of the first five spices were judged to be inhibition of aflatoxin formation rather than of mycelial growth. Clove completely inhibited both mycelial growth and aflatoxin formation at a concentration above 0.1%. No aflatoxin was produced when cumin and mint levels of 5% and 10% were used. Black pepper and ginger levels of 10% decreased aflatoxin formation by 100%. Higher concentrations of cinnamon, mint, cumin and ginger stimulated mycelial growth.

The possible presence of highly toxic and carcinogenic mycotoxins in foods and foodstuffs have led to extensive research involving methods for inhibiting the synthesis of mycotoxins and of aflatoxin in particular [1]. There has been increasing interest in identifying naturally occurring compounds that limit growth and/or toxin production by the aflatoxigenic fungi [2]. It has been known for sometime that essential oils of certain spices posses antimicrobial activities [3]. Cinnamon has been reported to inhibit the growth and aflatoxin production by *A. parasiticus* in yeast extract-sucrose medium and on raisin bread, with toxin production being inhibited to a greater extent than mycelial growth [4].

The object of the present paper was to examine the effects of the six spices; black pepper, ginger *(Zingiber officinale)*, cumin, mint, cinnamon and clove, on the growth and aflatoxin formation in rice powdercorn steep medium by *Aspergillus flavus* M 93, in relation to different concentrations at 25 °C.

Materials and Methods

1. Microorganism. Aspergillus flavus M 93 was obtained from the Food and Drug Administration, Washington D.C. In a previous study, this organism proved to be a good aflatoxin producer [5]. Stock cultures were maintaned on Dox's agar medium. Transfers were made to potato-dextrose-agar (PDA) [6] plates for 7 days at 28 °C.

2. Medium. 5% rice powder + 4% corn steep liquor (RC) medium was prepared according to Bullermann [7], but without adding agar. This medium was found in a previous work [5], to be more suitable for aflatoxin formation than yeast extract-sucrose (YES) medium.

3. Culture Technique. Four concentrations (0.1%, 0.5%, 5% and 10%, *w/v*) of each of the six spices, black pepper, ginger, cumin, mint, cinnamon and clove were added in a ground form to 250-ml Erlemeyer flask, each containig 50-ml aliquots of RC medium. Control flasks of RC medium only were also included. Sterilization was carried out at 121 °C for 15 min. Each flask was then inoculated with two discs, each of 1 cm diam · cut from the 7 days old culture plates of PDA medium. After incubating for 6 days at 25 °C (static culture), the culture medium from each flask was filtered off and the pH was determined. The above-mentioned temperature (25 °C) was used throughout this work, as it was found to be optimum for aflatoxin formation [8].

4. Preparation of Corn Stepp Liquor. This was done according to the method of Ligette and Koffler [9].

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Table 1. Effect of different concentrations of six spices on mycelial growth and aflatoxin formation by *A. flavus* M 93

Spice	% of spice g/100 ml	Final[b] pH	Mycelium Dry wt. g/50 ml	Aflatoxin						Total Aflatoxin[a]
				In culture filtrate μg/50 ml			In mycelium μg			
				B	G	Total	B	G	Total	
Clove	0	5.0	2.01	93.0	27.9	120.9	1016.0	101.1	1117.1	100
	0.1	4.7	1.01	45.1	7.7	52.8	75.0	38.8	113.8	13.5
	0.5	4.2	No growth	—	—	—	—	—	—	0
	5.0	4.2	No growth	—	—	—	—	—	—	0
	10.0	4.2	No growth	—	—	—	—	—	—	0
Cinnamon	0	5.0	2.01	93.0	27.9	120.9	1016.0	101.1	1117.1	100
	0.1	5.2	0.83	80.2	17.8	98.0	418.4	98.1	516.5	49.6
	0.5	5.5	1.02	76.1	17.7	93.8	252.5	96.7	349.2	35.8
	5.0	5.5	1.83	49.4	11.9	61.3	176.7	88.6	265.3	26.4
	10.0	5.0	2.80	14.5	5.6	20.1	53.5	24.2	77.7	7.9
Mint	0	5.0	2.01	93.0	27.9	120.9	1016.0	101.1	1117.1	100
	0.1	7.5	2.03	69.8	23.2	93.0	434.9	48.7	483.6	46.6
	0.5	8.0	2.06	33.2	21.1	54.3	147.4	22.2	169.6	18.1
	5.0	7.5	2.28	—	—	—	—	—	—	0
	10.0	6.5	2.26	—	—	—	—	—	—	0
Cumin	0	5.0	2.01	93.0	27.9	120.9	1016.0	101.1	1117.1	100
	0.1	6.5	1.45	69.5	17.9	87.4	781.3	107.1	888.4	78.8
	0.5	6.0	1.56	62.7	12.4	75.1	395.8	29.4	425.2	40.4
	5.0	5.5	1.61	—	—	—	—	—	—	0
	10.0	5.2	2.48	—	—	—	—	—	—	0
Ginger	0	5.0	2.01	93.0	27.9	120.9	1016.0	101.1	1117.1	100
	0.1	6.5	2.13	89.4	25.9	115.3	563.1	58.7	621.8	59.5
	0.5	6.5	2.25	85.6	20.9	106.5	395.8	29.5	425.3	43.0
	5.0	6.0	2.82	69.2	20.0	89.2	174.9	20.5	195.4	23.0
	10.0	5.0	3.01	—	—	—	—	—	—	—
Black Pepper	0	5.0	2.01	93.0	27.9	120.9	1016.0	101.1	1117.1	100
	0.1	7.5	1.63	94.8	24.6	119.4	300.1	55.8	355.9	38.4
	0.5	7.3	1.64	41.7	23.2	64.9	217.9	11.8	229.7	23.8
	5.0	7.3	1.78	15.4	—	15.4	117.9	—	117.9	10.8
	10.0	7.1	1.92	—	—	—	—	—	—	0

[a] Expressed as percentage of control
[b] Initial pH = 4.2. – Each experiment was carried out in duplicate
— = No detection

5. *Aflatoxin Analysis.* Aflatoxins in mycelia and culture filtrates were detected separately according to the methods previously described [7, 10], respectively. Extracts were concentrated under vacuum and examined by thin-layer chromatography on Silica gel G with an initial development in ethyl ether and then in 2% methanol in chloroform [11]. The aflatoxin bands were extracted with methanol and analysed by spectrophotometry as described before [11]. Aflatoxin B_1 and B_2 were determined as aflatoxin B and aflatoxin G_1 and G_1 as aflatoxin G, using the extinction coefficients of aflatoxin B_1 and G_1, respectively.

6. *Determination of Mycelial Dry Weight.* This was determined according to Swaminathan and Koehler [12].

Results

The influence of the six spices investigated on both mycelial growth and aflatoxin formation by *A. flavus* M 93 is summarized in Table 1.

Clove was the most active spice used. It completely inhibited both mycelial growth and aflatoxin formation at levels above 0.1 gm/100 ml. Furthermore, this latter concentration reduced growth and aflatoxin formation to 50% and 13%, respectively.

Cinnamon concentrations of 0.1% and 0.5% caused a reduction in mycelial growth by 60% and 50%, respectively. However, when 10% of cinnamon was used, the mycelial growth increased to about 140% of that of the control.

Increasing levels of cinnamon (0.1%–10%) added to RC medium inhibited aflatoxin formation from 51% to 93%.

When cumin levels of 0.1%–5% were used, the mould growth decreased to 70%–80%. However, stimulation of mycelial growth occurred at the 10% level. The effect of cumin on aflatoxin formation at the two lower levels was much lower than that exerted at the

higher levels. Thus, cumin concentrations of 0.1% and 0.5% caused a reduction in aflatoxin formation by about 23% and 60%, respectively, whereas complete inhibition of aflatoxin occured at 5% and 10% levels.

The influence of mint on mycelial growth and aflatoxin formation was more or less similar to that of cumin. Mild stimulation of mycelial growth (12.5%) was noted at the highest concentrations of mint used (5%–10%). However, no aflatoxin was detected at mint levels above 0.5%. The pH of the fungal cultures was elevated to the alkaline range with the first three concentrations used.

At all of the reatment levels of ginger the weights of the moulds were higher than that of the controls, reaching about 150% at 10% ginger. With ginger levels of 0.1%–5%, a reduction in aflatoxin formation by 41%–77% occurred. A higher level of ginger (10%) caused complete inhibition of aflatoxin.

Black pepper at concentrations of 0.1%, 0.5%, 5% and 10% repressed aflatoxin formation by 62%, 76%, 89% and 100%, respectively. However, this inhibitory effect was not pronounced on mycelial growth, which was reduced only in the range of 5%–20% at the levels of black pepper used.

Discussion

It is generally accepted that the essential oils of certain spices have antimicrobial activities, but their actions against specific microbial processes have not been elucidated [4, 13]. Quinones found in essential oils seem to be highly inhibitory to catalases and other fungal enzymes [14].

Among the six spices in vestigated, clove was the only one that exhibited fungistatic activity. This result is in agreement with Bullerman et al. [15]. Based on the study of Merory [16] that clove contains 14%–20% volatile oil, 95% of which may be eugenol, it was concluded [15] that eugenol is the major antifungal ingredient of this essential oil. In addition, clove contains vanillin [17], which is closely related to eugenol [18] and hence may be involved in the fungistatic action.

The activities of the other five spices studied were found to be anti-aflatoxigenic rather than fungistatic.

Similar to our results, cinnamon has been reported to be an effective inhibitor of aflatoxin formation [4, 15, 19], even though mycelial growth may be permitted [15]. Cinnamon contains 0.5%–1.0% volatile oil, which is composed of approximately 8% eugenol, cinnamic acid and 75% cinamic aldehyde [16]. These three, and especially the last, are responsible for the inhibitory action [15]. However cinnamon was the only spice used that did not inhibit aflatoxin formation by 100%.

Mint oil contains 50%–78% free menthanol, 5%–20% combined in various esters, L-limonene, menthone, cineol and phellandrene [17]. These constituents, which are terpenes and their oxidised derivatives [18], may be responsible for the inhibition of aflatoxin formation by mint. In this respect, the inhibitory effect of D-limonene – the major constituent of citrus oils [20, 21] – on aflatoxin formation by *A. parasiticus* was reported [13]. It was also concluded that the oxidised derivatives of terpenes in citrus oils may be involved in this action [22]. Furthermore, the presence of valeric acid in mint [17] may also add to its inhibitory action [23] on aflatoxin formation.

The cumin aldehyde and paraldehyde present in cumin [17] may be responsible for its inhibitory action on aflatoxin formation.

The inhibitory action of ginger on aflatoxin formation may be accounted for by the presence of volatile oil (1%–3%) and gingerol (0.5%–1.5%) [17]. The latter consists of several homologous phenols. Similarly, Swaminathan and Koehler [12], isolated an inhibitor of *A. parasiticus* from white potatoes and it was inferred that this compound was phenolic in nature. Ginger also contains some terpenes [17], which like limonene [13] may also add to its inhibitory action. However, the stimulatory effect of ginger on mould growth at higher levels may be due to starch and mucilage, which are utilised as carbon source for mould growth.

The presence of essential oils (1%–2%) in black pepper, containing the terpenes, dipentene, phellandrene and chavicin, may be responsible for its inhibitory action on aflatoxin formation.

It is worth noting that both mint and cumin prevented aflatoxin formation above 0.5% which is a much lower concentration than the levels required for either ginger or black pepper to inhibit aflatoxin formation (10%).

According to Masimango et al [19] a compound is considered a positive inhibitor if it reduces aflatoxin formation to 50% of that of control. Thus, the six spices used may be considered as positive and effective inhibitors of aflatoxin formation, if used in sufficient amounts. These six spices may be feasible ingredients for the cheap, safe and effective control of aflatoxin contamination of food materials, since they are also used as flavouring agents [24]. Toxic fungicides such as mercuric chloride and thiourea cannot be used on food [25]. To our knowledge, this is the first investigation of the four spices, black Pepper, mint, cumin and ginger for controlling mycelial growth and/or aflatoxin formation by *A. flavus* moulds.

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