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STRATEGIES FOR AFLATOXIN RISK REDUCTION IN ARABICA COFFEE: EXPERIMENTAL TRIALS ON ADDITIVE APPROACHES TO INHIBIT FUNGAL GROWTH

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Abstract

Aspergillus, a filamentous fungus, poses a significant threat to food and feed safety due to its ability to produce mycotoxins, particularly aflatoxins. These secondary metabolites, derived from polyketides, exhibit profound toxicity, mutagenicity, teratogenicity, and carcinogenicity in higher vertebrates and animals. Aflatoxins are primarily synthesized by several Aspergillus species, including Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius, Aspergillus toxicarius, and Aspergillus parvisclerotigens, commonly contaminating crops such as corn, peanuts, cotton seeds, and coffee beans.

The natural occurrence of toxigenic Aspergillus species and aflatoxin production in coffee beans has been extensively documented by researchers. Studies by Levi (1980), Daivasikamani and Kannan (1986), Abdel-Hafez and Elmaghraby (1992), Nakajima et al. (1997), and Batista et al. (2003) have provided compelling evidence of aflatoxin contamination in coffee beans. However, despite these findings, the prevalence and extent of aflatoxin contamination in coffee beans remain a concern for food safety and public health.

This paper aims to provide a comprehensive overview of the occurrence and implications of aflatoxin contamination in coffee beans. Drawing upon insights from existing literature and empirical studies, the paper examines the factors contributing to Aspergillus growth and aflatoxin production in coffee beans, including environmental conditions, post-harvest handling practices, and storage conditions. Furthermore, the paper discusses the potential health risks associated with aflatoxin consumption, emphasizing the need for stringent regulatory measures

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and quality control protocols to mitigate contamination risks and ensure consumer safety.

INTRODUCTION

Aspergillus is a filamentous fungus that produces mycotoxins in many food and feed crops. Aflatoxins are polyketide-derived, highly toxic, mutagenic, tetratogenic and carcinogenic secondary metabolites for higher vertebrates and other animals (Bokhari and Shaker, 2008). They are produced primarily by *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nomius, Aspergillus toxicarius* and *Aspergillus parvisclerotigens* (Holmes et al., 2008) on crops such as corn, peanuts, cotton seeds, and coffee beans. Furthermore, the natural occurrence of the toxigenic *Aspergillus* species and aflatoxin production in coffee beans was suggested by many authors (Levi, 1980; Daivasikamani and Kannan, 1986; Abdel- Hafez and Elmaghraby, 1992; Nakajima et al., 1997; Batista et al., 2003). Regulatory guidelines issued by the U.S. Food and Drug Administration (FDA) prevent sale of commodities if contamination by these fungi or mycotoxins exceeds certain levels. Aflatoxins B1 and B2 are produced by *A. flavus* and *A. parasiticus*, but the latter species also have been confirmed to form G1 and G2 toxins. On the other hand, ochratoxin was produced by *Aspergillus ochreceus, Aspergillus niger* and *Aspergillus carbonarius* (Bhatnagar et al., 2004). The fungal isolates were analyzed for toxins production in an attempt to differentiate aflatoxigenic from nonaflatoxigenic isolates. BoKhari (2007a) reported that coffee seeds were highly contaminated with toxigenic fungal isolates and toxins especially ochratoxin A. The occurrence of fungal contamination and mycotoxin production started at the beginning of har- vest due to high moisture content of the seeds and increased during transport, storage, or marketing.

Within the last decade, significant advances have been made in mycotoxin detection methods and control strategies as well as in studying the effect of environ-mental factors on toxin synthesis (Patterson, 1984; Nakajima et al., 1997). The most significant environmental factors that influence toxin synthesis are media used especially carbon and nitrogen sources, pH, temperature, water activity, and plant metabolites (Bhatnagar,et al., 2003; Calvo, et al., 2002). Natural plant metabolites affect toxin production and fungal development. Earlier research efforts in this, as well as other labs, have shown that plant components or volatile extracts can alter either *Aspergillus* growth or aflatoxin production, i.e. volatile aldehydes, vitamin C (Clevstrom et al., 2004), caffeine (Buchanan et al., 1983b) and other compounds from neem leaf (Zeringue and Bhatnagar, 1999), cotton leaf (Zeringue and McCormick, 1990) and corn-leaf (Wilson et al., 1981). Anthocyanins and related flavonoids also affect aflatoxin biosynthesis (Norton, 1999). In some cases, growth was not significantly aff-ected by various metabolites, while aflatoxin biosynthesis and fungal development were significantly decreased (Juglal et al., 2002; Basilico and Basilico, 1999). The plant linoleic acid, its derivatives and their precursor affect sexual and asexual sporulation in *Aspergillus nidulans*, sclerotial development, and toxin synthesis (Champe and Zayat, 1989). The conversion of oleic acid (18:1) to linoleic acid (18:2) is a critical biosynthetic step in the generation of sporogenic psi factors. Similarly, Hitokoto et al. (1980) reported that addition of cloves or cinnamon inhibited *Aspergillus* growth but *Aillium cepa* or green tea leaves inhibited toxin production.

The objectives of the present study were to identify the fungal populations and their toxins associated with coffee bean seeds, collected from Jeddah, Saudi Arabia and to evaluate the potential of the toxigenic isolate (*A. flavus*) to produce aflatoxins G1 which was more dominant mycotoxin compared with the other toxins detected. No studies were found dealing with the occurrence of Afl. G1 in coffee bean seeds or the factors affecting its production. Different factors witch affect aflatoxin G1 production and the effect of different traditional additive (saffron, ginger, cinnamon, cloves and cardamom) of coffee used in Saudi Arabia were also studied.

MATERIALS AND METHODS Collection of samples

Thirty coffee bean samples with different varieties of *Coffee Arabica* L. (about 1-1.5 kg each) were collected from different markets of Jeddah governorate, where coffee beans were sold in open bags. Each sample consisted of three replicates. The samples were collected in a sterile polyethylene bags to minimize the loss of water content and provide sufficient aeration, sealed, transferred immediately to the laboratory, kept at 4ºC until mycological and mycotoxins analysis.

Moisture content of the green coffee bean samples

The moisture content of the samples was directly determined by dry weight method (Aziz, 1987). About 10 g of each sample was transferred to an oven at ° under vacuum for 12- 24 h and until a con-stant weight. Percentage of water content was calculated.

Fungal isolation and identification

Isolation was carried out by the method described by Batista et al. (2003). To isolate fungi associated with the coffee bean seeds, the seeds were placed directly on filter paper, moistened with sterile dist. water. Then, thirty beans were collected randomly from each coffee bean sample. A total of 15 seeds were plated directly on agar plates (3 seeds/plate) and the other were disinfected with 1% sodium hypochloride for 2 min in order to permit isolation of fungi present in the interior of the beans. The media used are either potato dextrose agar or Malt extract agar (Merck, Germany) for isolation of different types of fungi. The compositions of malt agar were as the following: malt extract, 20 (g/l); glucose, 20 (g/l); peptone, 15 (g/l); agar, 20 (g/l). Ten plates for each sample (five plates for each medium) were used. The plates were incubated at 28°C for 7-10 days and the developing fungi were counted and identified according to macro and microscopic characteristics as described in Raper and Fennell (1965), Moubasher (1993) and Samson et al. (1995). The total counts (T.C.) of each species of fungi were calculated and were divided by the weight of the coffee bean seeds used in milligram (TC/mg).

Extraction of mycotoxins from coffee bean seed samples

A one hundred grams of each sample was extracted with 100 ml chloroform twice times and following the procedures described by Sorenson et al. (1967). The chloroform extract was dried over anhydrous sodium sulfate, filtered, then concentrated under vacuum and the dry material was transferred to a dark vial with a small amount of chloroform, which was evaporated to near dryness.

Detection of mycotoxins

Thin layer chromatographic technique of the clean extract was done on percolated silica gel plates (Merck, Silica Gel 60, 25 mm, 20 x 20). Detection of the different mycotoxins was carried out according to standard procedures described by Roberts and Patterson (1984) and El-Shanawany et al. (2005). A mixture of toluene-ethyl acetate90% formic acid (50:40:10) was used as a mobile phase. Mycotoxins were visualized under ultraviolet light at 366 nm in a chromatovisor. Aflatoxin, ochratoxin A, patulin and sterigmatocystin, isolated from fungi contaminated the coffee beans, had a retention time and fluorescent spot similar to the standard mycotoxins being tested (Sigma).

Chemical confirmation of mycotoxins

Chemical confirmation of mycotoxins was performed directly on the developed TLC plate. Two spraying reagents were used to visualization and increase the fluorescence intensity of the mycotoxins (Grabarkiewicz-Saczesna et al., 1985). The plates were then sprayed with either 20% $AlCl_3$ solution or 20% sulfuric acid, heated to 110ºC and examined under UV light (365 nm) as described by Bokhari (1993).

Cultivation of *A. flavus* isolated from coffee bean seeds in liquid media

A. flavus was cultured in liquid media to study different factors affecting growth and toxin production. Each treatment had three replicates. For inoculum preparation, *A. flavus* was grown on Czapex Dox agar containing rose bengal and chloramphenicol (25 mg/ml) in Petri dishes at 28°C until extensive formation of conidia was observed. The use of a combination of rose bengal at a concentration of about 1- 30,000 and chloramphenicol in fungus plating media to prevent growth of bacteria and restrict size of colonies was found to be far superior to the older standard procedure. Thus, a disc of 10 days old culture was used as a source of conidia for inoculating of the different flasks.

Extraction of *Aspergillus* toxins

After incubation, the content of each flask (medium + mycelium) was homogenized for 5 min in a high-speed blender with 100 ml chloroform. The extracts procedure was repeated three times. The chloroform extracts were combined, washed, dried, filtered, and concentrated near to dryness, cleaned and mycotoxins detected as previously described (Dos Santos et al., 2003).

Effect of different temperature on growth and Aflatoxin G1 production

Erlenmeyer flasks (250 ml) containing 50 ml of sterile Malt extract medium were inoculated with about 3 ml of heavy spore suspension containing 2 x 10^4 spore/ml (optical density, 0.65). The flasks were incubated at different temperatures (4, 10, 16, 22, 28, 30, 34°C). After 7 days of incubation, the contents of each flask were mixed with 120 ml of chloroform: water (100:10, v/v) and were shaken vigorously by a rotary shaker (200 rpm) overnight. The extract was sequentially filtered through anhydrous sodium sulfate. The chloroform extract was collected, dried and Afl. G1 was detected as a fluorescent green color under the UV light.

Effect of different media on growth and aflatoxin G1 production by *A. flavus*

A. flavus was cultured in different media. The media used were coffee broth, coffee dextrose, potato dextrose or malt extract. After incubation at 28°C for 7 days, the fungal growth and the quantity of Afl. G1 were determined as described above.

Effect of different concentrations of caffeine or vitamin C on growth and aflatoxin G1 production by *A. flavus*

Different concentrations of either vitamin C or caffeine were added to the growth media of *A. flavus* in 250 ml Erlenmeyer flasks containing 50 ml of sterile malt extract medium. The vitamin C concentrations were 0, 1.1, 2.2, 3.3 and 4.4 g/l as well as the caffeine concentrations ranged from 0 to 1 g/l. The growth and the quantity of Afl G1 were determined and compared with the results of control (containing no caffeine or vitamin C).

The effect of different medicinal plants on *A. flavus* growth and aflatoxin G1 production

The ability of *A. flavus* to grow and produce Afl G1 in a medium containing different types of medicinal plants or spices was investigated. The medium used was malt extract; and pH of the medium was adjusted to 5.5. The plants used were cardamom, cinnamon, cloves, saffron, and ginger. They were collected, washed, cut into pieces, dried at 60ºC, milled and sieved with 1 mm mesh and added to the fungal medium (1 g/l). After 10 days of incubation at the appropriate temperature, the growth and Afl G1 were quantified..

Statistical analysis

Each experiment has three replicates and three determinations were conducted. The numerical data were presented as mean ± standard deviation. The Student t-test was used to compare between numerical data of control and treated. P-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The results in Table 1 clearly show that coffee beans samples, collected from Jeddah, were highly contaminated with fungi which were represented by seven genera and twenty six species. More fungal species were counted by Abdel-Hafez and Maghraby (1992) in Egypt and Bucheli et al. (1998) in Thailand. Abdel-Hafez and Maghraby (1992) could isolate 26 fungal species belonging to 16 genera from coffee beans samples. The total viable counts of mold in all coffee beans samples were 419 and 346 colonies/mg dry coffee seeds on each malt extract agar and potato dextrose agar, respectively. The levels or occurrences of the different fungal population encountered in the current study ranged from high, moderate to rare and were almost in agreement to those observed in coffee beans studied by Batista et al. (2003). Examination of coffee bean samples for the presence of toxigenic and non toxigenic fungi was carried out. It has been found that *Aspergillus* and *Penicillium* followed by *Fusarium* and *Mucor* were the most prevalent genera on the two used isolation media. These results were similar to a great extent to results obtained by Panneerselvam et al. (2001). They studied microflora of coffee beans and reported that the genera *Aspergillus*, *Penicillium, Cladosporium, Trichoderma* and *Mucor* were the most dominant genera. The same findings were obtained by many other (Nunnes et al., 2001; Bokhari, 2007a). *Aspergillus* was the commonest genus in all samples examined. It was represented by 9 species and appeared in 73 and 100% of the samples tested on the two used media. It was represented by 63-65 % of the total fungi examined. *A. flavus* was the commonest species. It was represented by 24 and 14% of the total *Aspergillus* species recovered on Malt extract and Potato dextrose agar, respectively. *A. candidus, A. fumigatus, A. niger, A. ochraeceous, A. sydowii, A. terreus,* and *A. versicolor* were less dominant compared to *A. Flavus*. Abdel-Hafez (1984) reported that coffee seeds were highly contaminated by the genus *Aspergillus,* followed by *Penicillium* and *Rhizopus*. The second highest incidence rate was represented by genus *Penicillium*. It was recovered from 86 to 100% of coffee beans seeds samples. Most of *Penicillium* species were prevalent on Malt extract agar medium and with moderate incidence or completely absent on PDA medium. From the genus *Penicillium,* 8 species were identified of which *Penicillium chrysogenum; Penicillium duclauxii* and *Penicillium janczweskii* were the most prevalent. *Fusarium* and *Mucor* were also common genera, recovered at the average of 63 and 50% of all samples examined, constituting, 5.5 and 5% of total fungi on Malt extract agar medium and at the average of 37 and 50% of all samples, constituting 3.4 and 6% of the total fungi examined on PDA. The remaining genera and species were remarked in low or rare frequencies of occurrence on one or the two isolation media (Table 1). As shown in Table 2, thirty samples of coffee beans were collected from different places and % of water content was determined. The contamination of seeds with different mycotoxins was determined qualitatively. It was found that mycotoxin production capability by fungi can be limited or impeded by water activity. Increasing water content of the sample increased the number of toxins detected. Four samples (No. 1, 5, 10, and 26) had moisture contents ranged from 13.6–14% and contaminated by at least two mycotoxins.

The % of moisture content of samples 18

Table 2. Moisture content, number of toxigenic isolates and natural occurrence of mycotoxins recorded in the different coffee bean samples, collected from various sources.

Sam NO.	% Moisture content	No. of fungal isolates	No. of toxigenic isolates	% of toxigenic isolates	Quality of the toxin detected B1	B2	G1	G2	Och. A	Patulin	Streg.
1	13.6	22	3	13.6	+	+					
2	12.9	9	1	11.1			+			+	
3	13.4	6	0	0.0							

Table 1: Total counts (TC per mg), number of cases of isolation out of 30 samples and occurrence remarks of fungal genera and species recovered from coffee bean samples at 28ºC for 10 days at two types of media (Malt dextrose agar and Potato dextrose agar).

Fungi Isolated	Potato dextrose agar			Malt extract agar		
	Occurrence remarks	No. of appearance /30 sample	T.C. /mg Dry seed	Occurance remarks	No. of appearance /30 sample	T.C. /mg Dry seed
Aspergillus	**H**	**30**	**234**	**H**	**22**	**275**
A. candidus	M	9	21	M	6	11
A. flavus	H	22	33	H	19	67
A. fumigatus	H	19	22	H	14	33
A. melles	R	3	11	R	1	5
A. niger	H	25	7	R	2	22
A. ochraeceous	H	18	33	H	17	45
A. sydowii	M	10	36	R	3	31
A. terreus	H	19	40	H	19	32
A. versicolor	M	11	31	M	9	29
Penicillium	**H**	**30**	**50**	**H**	**26**	**70**
P. variable	H	15	13	H	19	34
P. canescens	R	0	0	R	5	7
P. chrysogenum	H	30	7	R	3	7
P. citrinum	R	4	11	M	6	11
P. glabrum	R	0	0	R	1	4
P. rubrum	M	12	16	R	1	2
P. oxalicum	R	0	0	R	2	5
P. italicum	R	5	3	R	0	0
Fusarium	**H**	**18**	**8**	**H**	**19**	**23**
F. oxisporium	M	7	5	R	5	3
F. moniliforme	M	11	3	H	14	20
Mucor	**M**	**21**	**14**	**M**	**15**	**21**
M. racemosus	M	9	10	M	7	3
M. circinelloides	0	0	0	H	7	11
M. hiemalis	M	12	4	R	1	7
Rhizopus	**M**	**8**	**5**	**R**	**4**	**9**
R. stolonifer	M	8	5	R	4	9
Alterneria	**M**	**9**	**25**	**M**	**11**	**11**
A. chlamydospora	M	6	9	M	11	11
A. .solani	M	8	5	R	4	9

4	12.8	12	2	15	+
5	13.8	21	2	9.5	
6	12.4	12	0	0.0	
7	14.1	19	1	5.2	+
8	13.0	20	0	0.0	
9	13.9	24	4	16.0	
10	13.6	14	4	29	+ +
11	12.8	10	0	0.0	
12	12.9	7	0	0.0	
13	12.4	13	0	0.0	
14	13.8	3	1	33.5	
15	12.8	5	0	0.0	
16	12.8	14	0	0.0	
17	12.8	9	0	0.0	
18	12.0	27	3	11.1	+ + +
19	11.8	13	0	0.0	
20	12.9	19	0	0.0	
21	11.7	9	0	0.0	
22	12.8	11	0	0.0	
23	12.3	12	0	0.0	
24	12.0	12	2	11.1	+
25	12.1	5	1	20.6	
26	14.0	4	2	50	+ +
27	12.8	8	0	0.0	
28	11.2	4	0	0.0	
29	12.0	14	0	0.0	
30	12.6	6	2	33.5	+

Sam: Sample number, B1, B2, G1 and G2: Aflatoxin B1, B2, G1 and G 2, Och.A :Ochratoxin A, Strg. : Stergmatocystin, +: Detected.

A. alternata	M	9	25	M	9	9

H, High occurrence (more than 15); M, Moderate (less than 15 and more than 5); R, Rare (less than 5).

From 12-12.6% and found to be contaminated with four different toxins. All the 30 coffee bean samples were tested qualitatively for naturally occurring mycotoxin contaminations and showed that 57% of the samples were mycotoxin-free (Table 2). It was found that five samples were contaminated by aflatoxins B1 and/or B2 while, Afl. G1 was recovered in the extract of seven coffee bean samples, thus, it was selected for more studies. Only samples (No. 4, 18) as in Table 2 were contaminated with Afl. G2. Also, the results revealed that 17% of the tested samples (5 out of 30) were contaminated by aflatoxins B1 and/or B2; 30% contains aflatoxins G1 or G2; 10%, ochrotoxin; 17%, patulin; but only 7%, sterigmatocystin. Bucheli et al. (1998) recorded that *A. flavus, A.*

niger, A. fumigatus were isolated from the coffee seeds contaminated by fungi. On contrast, *Fusarium solani* was the commonest fungus in coffee seeds (Muthappa, 1984) but *A. flavus, A. niger, A. funiculosum and Cladosporium* were the commonest in coffee bean samples collected from Egypt. Nunes et al. (2001) reported that coffee seeds were contaminated by the genus *Aspergillus*, especially, *A. ochraceus* (10.9%) and *A. niger* (22.9%). Out of 79 fungal isolated belonging to the genus *Aspergillus* and *Penicillium*, only 8.9% were toxigenic isolates, 7,6% belonging to genus *Aspergillus*, especially, to *A. flavus* which was represented by 38% of the total toxigenic isolates of the genus *Aspergillus*. Similar results were obtained by Sinha and Sinha (1991), who found that out of 48 strains isolated in India, 33 belonged to toxigenic isolates of the genus *A. flavus*. the total toxigenic isolates of the genus *Aspergillus*. Similar results were obtained by Sinha and Sinha (1991), who found that out of 48 strains isolated in

medium up to 4.4 g/l enhanced the fungal growth and mycotoxin production by A. flavus. Similar results were obtained by Clevstrom et al. (2004). They found that aflatoxins produced by A. flavus which was either isolated from cereal, barley, or coffee were enhanced up to 800 times with the presence of vitamin C in the culture medium. The increase in growth and patulin production got to 30% by the addition of Vit. C to the Czapex Dox broth medium (Podgorska, 1992).

Similarly, the effect of additions of five different plant materials to the growth medium of A. flavus was studied as can be seen in Table 7. It was found that cinnamon, cloves or both inhibited both growth (50%) and Afl. G1 production (100%) of A. flavus. On contrast, the presence of either saffron or ginger did not significantly affect the Aspergillus's growth or toxin production, but cardamom decreased fungal growth with no significant effect on the quantity of Afl.G1 production. Similar results were obtained by Hitokoto et al. (1980) who reported that addition of some spices or medical plants or some plant extracts to the fungal growth media may inhibit or enhance fungal growth and toxin production. Cinnamon, cloves inhibited growth of A. flavus, A. ochraceus and A. versicolor. In contrast, Allium cepa, green tea and other additive completely prevented toxin production by toxigenic fungi. Likewise, inhibitions in mycotoxins biosynthesis were noticed by Juglal et al. (2002) using clove and by Bokhari (2007b) using cloves, black pepper, peppermint, cardamom, cumin and marjoram. She attributed the inhibition of fungal growth and mycotoxin production to the presence of the antitoxic eugenol, found in both cinnamon and cloves (Bokhari, 2007b).

Table 3. The effect of different media on growth and aflatoxin production by *Aspergillus flavus*, grown for 10 days at 28ºC.

Media used	**Fungal growth (mg/ml)**	**Aflatoxin G1 production**	**Quantity of Aflatoxin produced ng/g**
Coffee dextrose	7.3 ± 1.3	++	9.6 ± 1.3
Potato dextrose	8.0 ± 2.4		11 ±0 .9
Malt extract	13.0± 1.0*	+++	16± 1.3*
Sabouraud	3.4± 0.6	+	6.4± 0.3
Czapek's- Glucose	5.0± 1.3	+	6.0± 0.9

*:significant results at $p<0.05$, +++: high production, ++: moderate production, +: low production

Table 4. The effect of different temperature on growth and aflatoxin production by *Aspergillus flavus* grown on Malt extract for 10 days.

India, 33 belonged to toxigenic isolates of the genus *A. flavus*.

A. flavus, which was isolated from coffee beans was the best producer for aflatoxins especially G1. The factors affecting the growth and production of Afl. G1 were studied. The effect of five different media on fungal growth

Temperature	Fungal growth (mg/ml)	Aflatoxin G1 production	Quantity of aflatoxin produced ng/g
4 °C	1.4 ± 1.3*	ND	ND
10 °C	6.8 ± 2.4*	ND	ND
16°C	8.2± 1.0*	+	11.5*
22 °C	11.4± 0.6*	++	12.0
25 °C	12.0± 1.3		13.0
28 °C	13.3 ± 1.3	+++	16.0
30°C	8.0 ± 2.4*	++	12.0*
35 °C	7.0± 1.0*	+	4.8*
38 °C	5.4± 0.6*	ND	ND

*:significant results at $p<0.05$, +++: high production, ++: moderate production, +: low production. ND: not detected

of *A. flavus* and Afl. G1 production was detected (Table 3). It was found that the semi synthetic malt extract broth was the best for both fungal growth and Afl. G1 production, followed by potato dextrose and coffee dextrose. On the other hand, inoculation of *A. flavus* in Sabouraud broth medium decreased the growth and Afl. G1 by 30 and 40% respectively compared to inoculation in malt dextrose broth. Natural media, especially PDA increased mycotoxin production compared with the synthetic media (Abramson et al., 1990; Cortes-Esposa et al., 2006; AlArjani, 2008).

As can be seen in Table 4, temperature affects the growth and toxin production by *A. flavus*. Increasing the temperature enhanced the growth up to 28ºC where the growth was maximum and got to 13.3 g/l. Increasing temperature more than 28ºC decreased both fungal growth and Afl. G1 production. No toxin was recorded at 4, 10 and 38ºC. Davis and Diener (1978) recorded that the aflatoxins B1 and G1 were produced by *A. flavus* on a semi-synthetic medium and their quantities were affected by the media used and temperature of incubation. The ideal temperature for aflatoxin production is 29–30ºC (Schroeder, and Hein, 1967; Payne, 1998). Aflatoxin production is significantly decreased at temperatures below 25ºC, but is completely inhibited at 37ºC or above.

As shown in Table 5, addition of caffeine to the growth medium up to 1 g/l decreased the fungal growth and aflatoxin G1 production to about 67 and 23%, respectively. Similar results were obtained by Soliman (2002), who reported that the highest concentrations of the aflatoxins produced by *A. flavus* were detected in the decaffeinated green coffee bean compared with the normal green coffee bean samples. He added that addition of 1-2% caffeine reduced the growth of *A. flavus* in liquid medium by 50% and the level of aflatoxin in the medium was undetectable. Complete inhibition of ochratoxin A, produced by *A. ochraceus*, was carried out by addition of 1 g/l caffeine to YES growth medium (Nehad et al., 2005), but 2 g/l caffeine inhibited aflatoxins production by *A. parasiticus* in the same medium (Buchanan and Lewis, 1984). Similar reductions in Afl. B1, ochratoxins and zearalenone were observed by a number of authors (Tsubouchi et al., 1987; Buchanan et al., 1982, 1983a; Nunes et al., 2001).

Table 6 shows that addition of vitamin C to the growth

Table 5. The effect of different concentration of caffeine on growth and aflatoxin production by *Aspergillus flavus* grown on Malt extract for 10 days at 28ºC.

Caffeine concentration g/l	Fungal growth (mg/ml	Aflatoxin production	G1Quantity of aflatoxin produced ng/g
0.0 (control)	13.2±1.3	+++	16.5
0.2	11.3 ± 1.3*	++	11.0*
0.4	10.6± 0.3*		7.0*
0.6	8.3 ± 0.9*	+	4.8*
0.8	8.3 ± 1.1*	+	4.0*
1.0	8.2± 2.3*	ND	2.8*

*:significant results at $p<0.05$, +++: high production, ++: moderate production, +: low production. ND: not detected

Table 6. The effect of different concentrations of vitamin C on growth and aflatoxin production by *Aspergillus flavus* grown on Malt extract for 10 days at 28ºC.

Vitamine C concentration g/l	Fungal growth (mg/ml)	Aflatoxin production	G1Quantity of Aflatoxin produced ng/g
0.0 (control)	13.2± 2.3	+++	16.0
1.1	13.3 ± 1.3	++	13.7
2.2	14.6± 0.3		14.0
3.3	16.3 ± 0.9*	+++	15.8
4.4	17.3 ± 1.1*	+++	17.0

*:significant results at $p<0.05$, +++: high production, ++: moderate production, +: low production. ND: not detected

Table 7. The effect of different addition of medicinal plants or spices on growth and aflatoxin production by *Aspergillus flavus* grown on Malt extract for 10 days at 28ºC.

Type of Spices	Fungal growth (mg/ml)	Aflatoxin production	G1Quantity of flatoxin produced ng/g
Control	13.2+ 2.3	+++	16.0
Saffron	13.3 + 1.5	++	13.7*
Ginger	13.6+ 0.3		14.0*
Cinnamon	6.3 + 0.6*	ND	ND
Cloves	7.3 + 1.1*	ND	ND
Cardamom	11.2+ 2.3	+	10.0
Cloves+ Cinnamon	6.3 + 1.3*	ND	ND

*:significant results at $p<0.05$, +++: high production, ++: moderate production, +: low production. ND: not detected

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