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Mycoflora and mycotoxins of Brazilian cashew kernels

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Abstract

Kernel samples of common and dwarf Brazilian cashew nuts were highly contaminated with field and storage fungi in comparison to healthy ones. In general, dwarf cashews were more contaminated than common. A total of 37 fungal species were identified. *Aspergillus niger* was the dominant species with more colonies being isolated from dwarf kernels. *A. flavus* was the next most frequently isolated species. *Penicillium brevicompactum*, and *P. glabrum* were the most frequently isolated penicillia, with higher contamination recorded from dwarf kernels. *Chaetomium globosum* was recorded at a high level. Nine species were recorded from cashew kernels for the first time. Multimycotoxin analysis by tlc and hplc were positive for mycotoxins and other secondary metabolites particularly from the infected samples. Hplc was only carried out on dwarf cashews. Aflatoxins were not detected by quantitative high performance thin layer chromatography.

Key words: aflatoxins, cashew kernels, deterioration, fungi, mycotoxins

Introduction

Cashewnut (*Anacardium occidentale* L.) is one of the most important cash crops of the North-eastern region of Brazil. Approximately 700,000 hectares are planted with this crop giving employment to more than 100,000 people, and providing an annual turn-over of 150 million dollars. The processed kernels are the principal commodity exported to the USA, Europe and Japan [1].

Cashews are subject to microbial contamination pre- and postharvest, and during processing. Preliminary surveys conducted in Brazil during the last three years have shown that nearly 10% of the annual crop is unfit for human consumption and therefore cannot be exported, with an accompanying loss of revenue. Insects and, to a greater extent, fungi are responsible for these losses. Twenty-six fungal species have been identified previously from Brazilian cashew kernels [2]. In addition, there have been several studies on the mycoflora of cashew kernels in other countries. Potentially toxigenic fungi have been identified in all these surveys [3–8].

According to Miller [9], mycotoxin contamination of foods and feeds is not a particular problem to the developed world, although heavy economic costs are incurred in ensuring low concentrations of mycotoxins [10]. In poorer developing countries such contamination has more serious consequences, affecting agricultural economies, reducing annual production and good quality exports and seriously affecting the health of the population. Figures indicate that between 25% and 50% of world crops, in particular staple crops, are contaminated with mycotoxins [11]. In developing countries often the good quality products are exported while the substandard produce unacceptable to foreign buyers (because they exceed regulatory limits for mycotoxin content) is sold to the domestic market [12].

This paper presents a preliminary survey of the mycoflora in Brazilian cashews, and the first report of mycotoxins detected from cashews.

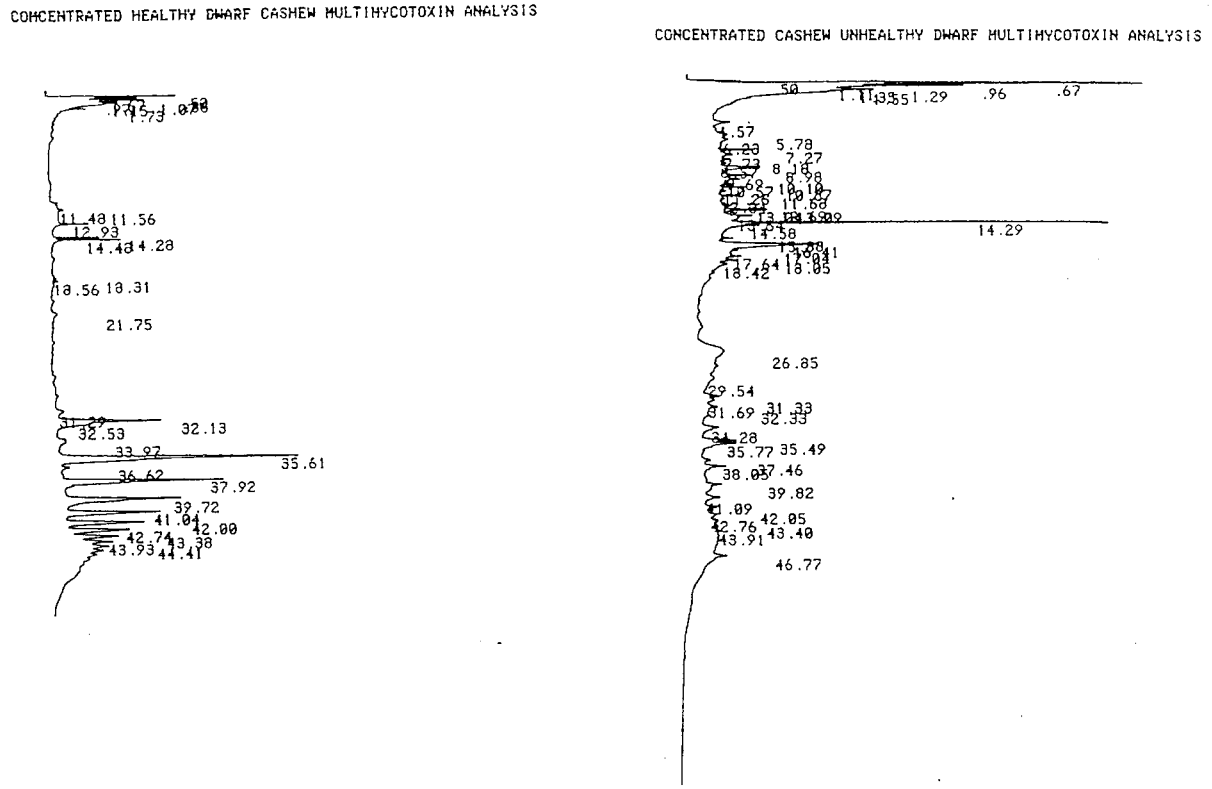

Figure 1. Hplc chromatograms of healthy and infected dwarf cashews. (Waters 600, Waters 486 UV/VIS detector set at 254 nm, Waters 746 Data Module. Column was a reversed phase Nova Pak C18 (3.9 × 150 mm). A gradient solvent of initially 10% acetonitrile/90% (v/v) hplc water was used changing to 50/50%; 90/10%; 90/10, 10/90% in 23, 40, 43, 49 min respectively; and finishing at 10/90% in 50 min by elution curve "6" of the HPLC controller. Injection volume 10 μl.

Materials and methods

Sampling

One hundred kilogram samples were taken from stores of raw common and dwarf cashews which were harvested in 1996 from the plantations of Pacajus county, Ceara State, Brazil. These were further sub-sampled into 10 kg lots. Nuts were mechanically opened and 1 kg lots of visually healthy and infected kernels were taken. Healthy kernels are defined as those without blemishes, and infected as those which were shrivelled and/or with dark spots.

Fungal isolations

Direct plating

Kernels were soaked for 2 mins in a 0.5% solution of sodium hypochlorite for surface sterilisation and rinsed twice in sterile distilled water. They were then cut into 5 × 5 mm pieces under sterile conditions, and 10 pieces per 9 cm petri-dish placed directly onto the agar surface. The following media were used: tap water agar [TWA] (Oxoid Agar No. 3 15 g, 1 litre tap water; sterilise for 15 mins at 121 °C), Czapek yeast autolysate agar [CYA] (K_2HPO_4, 1.0 g, Czapek concentrate 10 mls, Oxoid Yeast extract or autolysate 5.0 g, sucrose analar 30 g, Oxoid agar No. 3 15 g, distilled water 1 litre; sterilise for 15 mins at 121 °C; Czapek concentrate: $NaNO_3$ 30 g, KCl analar 5.0 g, $MgSO_4$ $7H_2O$ analar 0.5 g, $FeSO_4.7H_2O$ 0.1 g, distilled water 100 mls; sterilise for 15 mins at 121 °C) and malt plus 20% sucrose agar [M20] (Malt extract 20 g, sucrose 200 g, agar 20 g, tap water 1 litre; sterilise for 15 mins at 121 °C) and malt plus 40% sucrose agar [M40] (malt extract 20 g, sucrose 400 g, agar 20 g, tap water 1 litre; stenlise for 15 mins at 121 °C) respectively. Plates were incubated at 25 °C for one week to detect anamorphs or 2 weeks to detect teleomorphs. Frequencies of occurrence were calculated as a percentage of all cashew particles plated on the four media.

Table 1. Comparison of the fungal flora obtained from healthy and infected sterilized cashew kernels[1]

	Dwarf cashew (%)		Common cashew (%)	
	Infected	Healthy	Infected	Healthy
Acremonium roseolum	2.0	–	–	–
Acremonium sp.	–	–	3.0	–
Alternaria alternata	3.5	1.0	2.0	–
Aspergillus candidus	1.0	–	–	–
A. clavatus	1.0	–	1.5	–
A. flavus	25.5	–	17.5	–
A. niger	51.0	2.0	38.5	3.5
A. tamarii	13.5	–	4.5	–
A. terreus	6.5	–	5.0	–
A. versicolor	5.0	–	8.0	–
Chaetomium funicola	7.5	–	2.5	–
C. globosum	16.5	–	1.5	–
Cladosporium herbarum	11.0	2.0	5.5	1.0
C. sphaerospermum	6.5	2.0	3.0	–
Cunninghamella elegans	2.5	1.0	6.5	–
Curvularia senegalensis	12.5	–	5.0	–
Emericella nidulans	9.5	–	–	–
E. rugulosa	6.0	–	–	–
Eurotium amstelodami	15.5	–	8.5	–
E. chevalieri	21.0	1.0	16.5	–
Fusarium pallidoroseum	2.0	–	–	–
Geotrichum candidum	2.5	–	1.0	–
Microascus cinereus	4.5	–	9.5	1.0
Nigrospora oryzae	18.5	–	6.0	–
Ophiostoma sp.	1.0	–	–	–
Penicillium brevicompactum	13.5	–	8.5	–
P. glabrum	14.0	2.0	3.5	–
Pestalotiopsis guepinii	3.0	–	2.0	–
Phellinus sp.	1.0	–	–	–
Poitrasia circinans	2.5	–	–	–
Rhizopus oryzae	15.5	–	12.0	–
R. stolonifer	12.0	1.5	2.5	–
Scopulariopsis gracilis	3.0	–	1.0	–
Spegazzinia tessarthra	1.5	–	3.5	–
Spiniger sp.	1.0	–	–	–
Thielavia terricola	3.5	–	1.0	–
Trichoderma atroviride	1.5	–	4.0	–

[1] Mean of infected particles on the four media.

Dilution plating

Fifty gram samples were placed in 450 mls of 0.1% peptone water with 0.05% Tween (bacteriological peptone 2 g, distilled water 2 litres, Tween 80 1 mls, NaCl 17.8 g, adjust pH to 7.2–7.4, sterilise for 15 min at 121 °C) and macerated in a Waring blender using the high setting for 2 min. Serial dilutions to 10^3 with 4 replicates per dilution, were made.

Spread plates

For each of the above dilutions, 0.2 mls of diluent (0.1% peptone with 0.05% Tween) was pipetted onto the surface agar of the four media used (4 plates each) and spread using sterile plastic loops. Plates were

Table 2. Numbers of fungal colonies/g Brazilian cashew kernels[1]

Media	Poured plates				Spread plates			
	Common healthy cashew	Common infected cashew	Dwarf healthy cashew	Dwarf infected cashew	Common healthy cashew	Common infected cashew	Dwarf healthy cashew	Dwarf infected cashew
TWA	<10	10^2–2.0×10^2 (1.3×10^2)	<10	10^2–3.0×10^2 (1.7×10^2)	<10	10^2–10^3 (3.1×10^2)	<10	2.0×10^2–10^3 (4.6×10^2)
CYA	<10	0.8×10^2–10^3 (2.7×10^2)	<10	0.7×10^2–2.0×10^3 (5.9×10^2)	<10	1.3×10^2–2.0×10^3 (9.6×10^2)	<10	2.3×10^2–3.0×10^3 (1.2×10^3)
M20	<10	10^2–2.1×10^2 (1.3×10^2)	<10	1.7×10^2–3.0×10^2 (2.0×10^2)	<10	1.5×10^3 (4.7×10^2)	<10	2.6×10^2–10^3 (5.7×10^2)
M40	<10	10^2–10^3 (3.8×10^2)	<10	1.6×10^2–10^3 (4.1×10^2)	<10	0.9×10^2–10^3 (4.2×10^2)	<10	3.0×10^2–1.7×10^3 (7.3×10^2)

[1] Mean of colony numbers on the four media.

inverted and incubated at for 7 days in the dark. Numbers of colony forming units per gram (cfu/g) were calculated in those plates with 10–150 colonies.

Identification of species

Isolates of *Aspergillus* and *Penicillium* were transferred onto Czapek Dox agar [CZ] (Stock solution A 50 mls, Stock Soln. B 50 mls, distilled water (DW) 900 mls, sucrose analar 30 g, Oxoid agar No. 3 20 g, $ZnSO_4.7H_2O$ analar solution 1 mls (1.0 g in 100 mls DW), $CuSO_4.5H_2O$ analar solution 1 mls (0.5 g in 100 mls DW); Stock Soln. A: $NaNO_3$ 40 g, KCl analar 10g, $MgSO_4.7H_2O$ analar 10 g, $FeSO_4.7H_2O$ analar 0.2 g; DW 1 litre; Stock Soln. B: K_2HPO_4 20 g, DW 1 litre), and all other genera onto potato carrot agar [PCA] (grated potato 20 g, grated carrot 20 g, Oxoid Agar No. 3 20 g, tap water 1 litre).

Multi-mycotoxin analysis

Samples of cashews (25 g) were blended in a Virtisheer blender at the lowest speed setting for 1 min in 100 ml chloroform/methanol (1/1; v/v). The mixture was filtered and the filtrate was evaporated to dryness by rotary evaporation. The residue was partitioned between 50 ml n-hexane and 50 ml 90% methanol (1/1; v/v), the n-hexane layer was discarded and the methanol layer evaporated to dryness as above. The residue was resuspended in 1 ml chloroform and filtered with a millipore filter (25 μm) for hplc analysis. Peak identity was achieved by calculating retention indices with alkylphenones retention times which had been run previously in triplicate [13], and comparing to the data base in [14]. Hplc conditions are provided in Figure 1.

Cashews for tlc analysis were treated as above and, after the methanol was evaporated, the solid was partitioned between 50 ml chloroform and 50 ml deionised water. The chloroform layer was extracted with saturated sodium hydrogen carbonate solution (3×50 ml). The chloroform layer was concentrated to dryness and contains any neutral toxins. The aqueous layer was acidified to pH 2.0 with 0.5 M HCl and extracted with chloroform (3×50 ml) and evaporated to obtain any acidic mycotoxins [15]. The samples were resuspended in 5 ml of chloroform and aliquots (10 μl) were spotted onto a tlc plate (20×20) Merck aluminium sheets, silica gel 60, layer thickness 0.2 mm) and developed in toluene, ethyl acetate, 90% formic acid (5/4/1, v/v/v). Metabolites on the tlc plate were visualised as described in Table 3, and identified from

Table 3. Retention times (and relative percent area) of dwarf cashews, together with retention indices (RI) of the peaks which are unique to each sample. Possible identities of the peaks are provided. The fungi known to produce these compounds are listed in [14], and the toxicity of most compounds are listed in [18].

Healthy	Infected	RI	Identity
0.5(2.7)	0.5(0.7)		
0.7(3.0)	0.7(17.2)		
0.9(3.3)		587	
1.0(1.2)	1.0(9.2)		
1.1(0.9)	1.1(2.3)		
1.2(4.1)	1.3(3.8)		
	1.4(7.2)	596	
	1.5(12.3)	598	
1.7(0.3)	602		
	4.6(0.8)	654	
	5.8(0.7)	676	Nivalenol
	6.2(0.5)	684	Deoxynivalenol
	7.3(0.5)	704	Austdiol (see tlc)
	7.7(0.6)	711	Penicillic acid
	8.2(0.3)	720	Orsellinic acid
	8.6(0.2)	723	Neosolaniol
	9.0(2.2)	734	Gibberellic acid
	9.7(0.8)	747	Ferulic acid
	10.1(0.5)	755	Aurantioclavine
	10.6(0.1)	764	Festuclavine
	10.9(0.3)	769	Aflatoxin G_2
11.5(0.7)	11.3(0.1)		
11.6(0.4)	11.7(0.3)		
	12.7(0.2)	802	"PR-1635"
12.9(0.6)	13.0(0.6)		
	13.1(0.4)	809	2,4-dihydroxy-6-(2-oxopropyl) benzoic acid, lactol
	13.6(0.3)	818	Aflatoxin M_1
14.3(0.8)	14.3(6.3)		
		833	Cyclopaldic acid
		834	Aflatoxin G_2
14.5(1.2)	14.6(0.4)		
	15.9(0.1)	860	Aflatoxin G_1
	16.4(6.3)	869	Aflatoxin B_2
	17.0(0.3)	880	Isochromantoxin
	17.6(0.1)	891	Prechinulin
	18.0(0.3)	898	Lapidosin
18.3(0.1)	18.4(0.3)		
		906	Desacetylpebrolide
18.6(0.1)		912	Brefeldin A
21.7(0.3)		984	Cladosporin
	26.8(11.0)	1111	Desertorin C
	29.5(2.4)	1186	Fumitremorgen B
31.3(0.6)	31.3(0.9)		
	31.7(0.5)	1263	Luteoskyrin
32.1(6.7)	32.3(0.7)		
32.5(2.1)		1293	Paxilline
34.0(0.9)	34.3(1.2)		
35.6(18.9)	35.5(1.3)		
36.6(0.3)		1449	Trichorzianines B VII
37.9(11.3)	37.5(0.3)		
	38.0(0.6)	1506	Aflatrem
39.7(9.8)	39.8(0.4)		
41.0(6.5)	41.1(0.8)		
42.0(5.5)	42.0(0.5)		
42.7(4.4)	42.8(0.4)		
43.4(3.5)	43.4(0.3)		
43.9(3.0)	43.9(0.3)		
44.4(6.7)		1816	
	46.77(0.7)	1940	Stigmasterol

a data base of tlc characteristic for pure compounds [13, 16].

Quantitative aflatoxin analysis

Cashew kernels (300 g) of each sample were ground using a coffee grinder, thoroughly mixed and 50 g (5 $\times$ 10 g) of each sample was drawn using a small sterile scoop from throughout the mixed sample. Sample extraction was conducted by shaking in an acetone:water (70:30) mixture for 45 min, followed by a clean-up using a phenyl-bonded solid phase extraction column (Varian). Aflatoxins B_1, B_2, G_1 and G_2, were quantified twice by high performance thin layer chromatography using a CAMAG Scanner II fluorodensiometer controlled by software (CATS 3, CAMAG) [17]. Required precision of standards of less than or equal to CV% of 5% (actually 3.5%) were achieved, as were the required precision of replicate samples of less than or equal to CV% of 10%. Required precision of recoveries from extracts spiked with aflatoxin were greater than 70%.

Results

More taxa were isolated from infected kernels than the healthy ones, and where the same species were isolated the highest percentages of contamination were from the infected samples (Table 1). The number of taxa identified was 37. *Aspergillus niger* was the dominant species, with counts for dwarf infected cashews

(DI), common infected cashews (CI), dwarf healthy cashews (DH), and common healthy cashews (CH) of 51%, 38.5%, 3.5% and 2.0% respectively. *A. flavus* was the second most frequently isolated species from DI and CI (25.5% and 17.5% respectively), but it was not isolated from DH or CH. All isolates of *A. flavus* were non-sclerotial forms. *Penicillium brevicompactum* (13.5% (DI), 8.5% (HI), 0% (DH), and 0% (CH)) and *P. glabrum*, (14.0% (DI), 3.5% (HI), 2% (DH), and 0% (CH)) were the dominant penicillia. The levels of the other fungi can be obtained from Table 1. *Acremonium roseolum*, *Fusarium pallidoroseum*, *Microascus cinereus*, *Ophiostoma* sp., *Phellinus* sp., *Poitrasia circinans*, *Spegazzinia tessarthra*, *Spiniger* sp., and *Thielavia terricola* were recorded for the first time from cashews. *Spiniger* sp. is an uncommon fungus which was isolated at 1% from all TWA spread plates.

The number of cfu's/g of kernels were lower for poured than spread plates, with highest numbers again recorded from dwarf kernels (Table 2). Healthy kernels gave fewer colonies (<10/g) than infected ones. Values from CI and DI were similar for poured plates except for colonies on CYA where almost twice as many colonies were isolated on CYA from DI than CI. The counts from CI and DI on spread plates were also similar with the possible exception of those from M40 where 7.3×10^2 cfu's were isolated from DI and 4.2×10^2 cfu's were isolated from CI. The taxa isolated were similar to those from direct platings.

Figure 1 demonstrates the hplc chromatograms for DH and DI, and the retention times of peaks data are presented in Table 3, which includes an indication of the identity of the metabolites. More peaks were obtained from the infected kernels. The retention indices of the metabolites detected from tlc (Table 4) are included in Table 3 for reference. The hplc analysis indicated that DI was contaminated with a wide range of fungal metabolites which may be toxic.

A table of the tlc characteristics and identities of metabolites from the cashew nuts is provided in Table 4, and the metabolite profiles of the samples are given in Figure 2. Desacetylpebrolide was identified only from CI in the neutral and acid fractions. Austdiol was identified only from CI in the acid fraction. Aflatoxin G_2 was identified in the neutral fraction of CH and in the acid fraction of DI. The quantitative aflatoxin analyses were negative.

Samples	CHN	CIN	CHA	CIA	DHN	DIN	DHA	DIA	
A	O	O	O	O	O	O	O	O	
B		O		O				O	Desacetylpebrolide
C	O							O	Aflatoxin G_2
D	O	O	O	O	O	O	O	O	
E							O	O	
F	O	O	O	O	O	O	O	O	
G				O					Austdiol
H	O	O	O	O	O	O	O	O	
I	O	O	O	O	O	O	O	O	
J	O	O	O	O	O	O	O	O	
K	O	O	O	O	O	O	O	O	
L						O			
M							O	O	
N							O		Cyclopaldic acid
O	O	O	O	O	O	O	O	O	
P							O		
Q							O		
R	O	O	O	O	O	O	O	O	
S	O	O	O	O	O	O	O	O	
T							O	O	
U	O	O	O	O	O	O	O	O	
V	O	O	O	O	O	O	O	O	

Figure 2. Metabolite profiles from the samples. CHN = common healthy cashews neutral fraction, CIN = common infected cashews neutral fraction, CHA = common healthy cashews acid fraction, CIA = common infected cashews acid fraction, DHN = dwarf healthy cashews neutral fraction, DIN = dwarf infected cashews fraction, DHA = dwarf healthy cashews acid fraction, DIA = dwarf infected cashews acid fraction. O = metabolite detected. Letters A–V are arbitrary and are used to facilitate reference to the metabolites listed in Table 4.

Discussion

New records for fungi from Brazilian cashew kernels are reported here. Most of the fungi isolated are considered to be field species. Namely, *Acremonium roseolum*, *Cladosporium herbarum*, *C. sphaerospermum*, *Curvularia senegalensis*, *Fusarium pallidoroseum*, *Geotrichum candidum*, *Nigrospora oryzae*, *Pestalotiopsis guepinii*, *Poitrasia circinans*, *Rhizopus oryzae* and *R. stolonifer.* Others are considered to be post-harvest spoilage fungi such as *Aspergillis candidius*, *A. clavatus*, *A. niger*, *A. tamarii*, *A. terreus*, *A. versicolor*, *Chaetomium funicola*, *C. globosum*, *Emericella nidulans*, *E. rugulosa*, *Eurotium amstelodami*, *E. chevalieri*, *Penicillium brevicompactum* and *P. glabrum. Aspergillus flavus* and *Alternaria alternata* are considered to be the more well known my-

Table 4. Metabolites detected from cashews

Letter	Rf × 100	Visualisation methods 1	2	3	4	5	6	Identity
A	7		b		p	y		
B	12			p				Desacetylpebrolide
C	12		b					Aflatoxin G_2
D	12					y		
E	13			y				nut component
F	21		b	y	p	yr		
G	22				p			Austdiol
H	25				p	y		
I	30		y	y	p			
J	36					y		
K	38					y		
L	38	gy						unidentified
M	41	y	y				y	nut component
N	44						gy	cyclopaldic acid
O	52		b		b	y	y	
P*	54				br	y		
Q	55					bk	bk	
R	60				br	br (d)		
S	65				p	y	y	
T	66	y		y				nut component
U	70		b		b	br	br	
V	82				p	y	y	

Visualisation methods are 1 = UV light (365 nm), 2 = UV light (254 nm), 3 = Spray TLC plate with (0.5%, v/v) p-anisaldehyde in methanol-acetic acid-concentrated sulphuric acid (17/2/1; v/v/v), heat for 8 min at 105 °C, and view with white light, 4 = u.v. light (365 nm), 5 = u.v. light (254 nm). Metabolite colours are g = green, b = blue, y = yellow, br = brown, bk = black, gy = grey, or = orange, p = purple, r = red, (d) = dark.
NB it is thought that P may be sterigmatocystin but the spot co-eluted with Q making an identification more difficult.

cotoxin producers. Several other species listed in Table 1 have the potential to produce toxic metabolites. A list of metabolites produced by most of these fungi is provided in [14], and toxicity data can be obtained in [18]. *Chaetomium globosum* is of particular interest as it is known to produce a wide range of toxic metabolites with unusual properties, and it was isolated at a high level. The wide range of field and storage fungi isolated here indicates that storage conditions for cashew nuts in north-eastern Brazil may be inadequate. The high humidity and storage temperatures may have contributed to continued growth of the field fungi and possibly mycotoxin production.

A wider range of fungi were isolated from dwarf kernels. The reason for this is unclear but may relate to growing conditions such as the higher density of planting for the dwarf nuts (300 plants per hectare compared with 50 per hectare for common). The difference in size *per se* may affect the levels of contamination. In addition, the flowering and fruiting season for dwarf cashews is longer, allowing more insect damage [19], and hence the introduction of fungi to the flowering parts. There is evidence in the literature that *A. flavus* can develop in the flowering parts and the ovaries of certain crops [20]. In Brazil, bugs (Coreidae) have been seen feeding on young fruits [personal communication].

Although it is considered that direct plating exhibits a better representation of fungal counts within a commodity, fungi which do not sporulate profusely or may be slow growers, will tend to become overgrown by their more vigorous relatives. In this study we frequently encountered 2 or 3 different species growing from the same kernel fragment. But, despite its disadvantages direct plating continues to be the method of choice for most workers to assess fungal contamin-

Table 5. Fungi reported to produce the metabolites from cashews and whether the compounds are considered to be toxic [16].

Metabolite	Toxicity	Species
Aflatoxin G_2	Least acutely toxic of the 4 major naturally occurring aflatoxins	*Aspergillus nomius*, *A. parasiticus*
Austdiol	Acute gastrointestinal toxin	*A. ustus*
Cyclopaldic acid	Not listed	*A. duricaulis*, *A. puniceus*, *Penicillium commune* chemotype I & II, *P. mononematosum*, *Pestalotia palmarum*, *Neosartorya quadricincta*
Desacetylpebrolide	Not listed	*P. brevicompactum*

ation in cashew, cereals and other commodities [e.g., 3, 5, 7, 8]. It is well known that each of the methods used has the capacity to select for particular groups of fungi, and no technique is completely satisfactory.

It is noted that *Penicillium brevicompactum* was isolated in high amounts from infected samples and that desacetylpebrolide was detected by tlc which the fungus is known to produce. The generally recognised producers of aflatoxin G_2 (*A. parasiticus*, *A. nomius*) and austdiol (*A. ustus*) were not isolated although many other aspergilli were. However, it is well known that mycotoxins can be present in a commodity without the producing fungus also being present and vice versa. None of the generally recognised producers of cyclopaldic acid were isolated from the healthy dwarf kernels. Table 5 lists the fungi which are known to produce the identified toxins together with associated toxic effects. Much more work is required to identify the metabolites precisely, such as purification and uv and mass spectroscopy in the cases of the tlc and hplc analyses. It is stressed that in the case of the mycotoxin analyses which are reported here only limited inference can be made on the basis of the small sample weights, which are unlikely to be very representative of a consignment.

The identification of aflatoxin G_2 in the neutral fraction of the normal healthy and the acid fraction of the dwarf infected cashews is unusual. This compound would probably be expected to fractionate into the neutral fraction so the presence in the acid fraction is perhaps unlikely. Also, hptlc analysis did not reveal any aflatoxins although this could be explained by the fact that different samples were analysed. Multimycotoxin hplc of the infected samples did reveal some peaks which corresponded to aflatoxins (Table 3), however, the hptlc method is more accurate and sensitive than the other methods. It is noted that there were bigger peaks in the healthy samples at retention times of between 31–43 min (Figure 1). This may reflect the fungi metabolising the components which caused the peaks and hence lowering concentrations in the infected sample. Although there was a high incidence of *A. flavus* in infected kernels of common and dwarf cashew types, the different samples sent for aflatoxin analysis proved negative. However, a negative result from such an unrepresentative sample is meaningless in terms of the situation in the whole consignment. Had a positive result been obtained it could only have been considered to be a qualitative assessment.

The presence of mycotoxins in cashews could pose a health hazard to consumers particularly in rural areas of Brazil. Also, problems could arise for exported consignments if the nuts contain mycotoxins but which are not analysed routinely.

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