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MYCOTOXIN RESEARCH IN BRAZIL: THE LAST DECADE IN REVIEW

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REVIEW

ABSTRACT

The number of research papers (128 papers) on mycotoxins published by Brazilian researchers in 1991-2000 surpassed the total number (85 papers) published in the preceding three decades (1961-1990). Thirty percent of the papers surveyed mycotoxins in foods and feeds. AFs in peanut and peanut products continued to be alarming, and high incidence and levels of FBs in corn and corn products also appeared as a serious problem. Contamination with other toxins, such as ZEA, OTA and trichothecenes, was low. Occurrence of AFM_1 in milk and dairy products and patulin in apple juice needs to be verified as the results are somewhat diverging. Work on analytical methods, mycological examination and toxic effects constituted 16, 13 and 13%, respectively, of the published papers in the decade assessed. Attempts to find means of preventing/controlling fungal growth and mycotoxin production notably increased, making up 27% of the papers, including investigations on influencing factors (e.g. genotype resistance, water content/a_w, relative humidity, temperature, presence of metals, type of soil, mite infestation) and antagonistic potential of other microorganisms against mycotoxin-producing fungi. Effects of plant extract, flavonoids, fungicides and other chemicals, storage bag material, adsorbents, cooking and processing of food were also studied. Thus, notwithstanding constraints on resources, Brazilian research responds to the needs of the country, reflects international concerns and recent developments in the area.

Key words: mycotoxins, occurrence, influencing factors, research, Brazil

INTRODUCTION

As a continuation of a review article published in 1993 (109), which integrated published research on mycotoxins in Brazil from 1961 to 1990, the present review examines papers published by Brazilian researchers in national and international journals in one decade, 1991-2000. Investigations in this area intensified in this decade, the number of papers (128 research papers) surpassing the total number published in the preceding three decades (85 research papers). The studies also showed an evident widening of scope and depth. In 1961-1990, most of the investigations involved only aflatoxins and were conducted in the state of São Paulo. In the last decade, other mycotoxins were also studied and researchers from other states gave significant contribution.

There was also a shift in focus. In 1961-1990, the distribution of the papers according to topic was: occurrence of mycotoxins, 38 papers (45%); analytical methods, 11 papers (13%); microbiological studies, 12 papers (14%); prevention, control and effects of food processing, 12 papers (14%); mycotoxicoses, toxic effects, 12 papers (14%). In the decade 1991-2000, the papers were distributed as follows: occurrence of mycotoxins, 39 papers (30%); analytical and mycological methods, 20 papers (16%); mycological surveys and mycotoxin-producing potential, 17 papers (13%); prevention, control and effects of food processing, 35 papers (27%); toxic effects and mode of action, 17 papers (13%). Although the incidence of mycotoxins continued to receive the most attention, its percentage in relation to the total number of papers declined. There was a considerable increase in

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research on prevention, control and processing effects. These changes indicate that Brazilian investigators are shifting from verifying the existence and magnitude of mycotoxin problems to finding ways and means of diminishing the problem.

OCCURRENCE OF MYCOTOXINS

Considering year-to-year variations and differing climate, agricultural practices, post-harvest handling, processing and storage conditions in different parts of a country, especially a huge country like Brazil, the information given in Table 1 should not be taken as absolute but as indicative of the existing situation. Nevertheless, some trends can be discerned.

Aflatoxins (AFs) in peanuts and peanut products continued to be an alarming problem, although the extent and levels were generally lower than reported previously (109). AF occurrence in corn, the commodity internationally considered to be most susceptible to AF contamination, was much lower, occurring only occasionally. As in other parts of the world, including other Latin American countries, there was widespread, high contamination of corn and corn-based products with fumonisins (FBs). Incidence of other mycotoxins, such as zearalenone (ZEA), ochratoxin A (OTA) and trichothecenes, was low. Occurrence of aflatoxin M_1 (AFM_1) and patulin (PAT) needs verification because the results diverge somewhat.

Concern about ochratoxin contamination of coffee was recently raised, especially in Europe. Three surveys in Brazil (50,97,124) (Table 1) showed that ochratoxin A, although detected, was present at very low levels in Brazilian coffee. Thus, contamination with this toxin does not appear to be a public health problem.

Aside from the surveys cited in Table 1, the incidence of macrocyclic trichothecenes in Brazilian *Baccharis* species (shrub) was also investigated, only *B. cardifolia* and *B. megapotamica* being found to contain these toxins (49).

ANALYTICAL AND MYCOLOGICAL METHODS

In the surveys presented in Table 1, aflatoxins B (AFBs) and G (AFGs) were generally determined by thin layer chromatography (TLC); FBs and PAT by high performance liquid chromatography (HPLC); AFM_1 by TLC, HPLC and enzyme-linked immunosorbent assay (ELISA); trichothecenes by gas chromatography (GC) and TLC; ZEA and OTA by TLC, in various papers together with AFs and esterigmatocystin by a multi-toxin TLC method (125). OTA specifically in coffee was determined by HPLC.

Work on methodology continued during this period. TLC methods for the determination of trichotecenes and ZEA in grains (58) and of AFB_1, AFM_1, ZEA and OTA in animal tissues (126), an HPLC method for the simultaneous determination of tenuazonic acid (TEA) and cyclopiazonic acid (CPA) (71), a method for determining FB_1 in corn, using immunoaffinity column clean-up and TLC/densitometry (101), and an HPLC method involving fluorimetric quantification of OPA-derivatives of FB_1 and FB_2 in corn and *Fusarium moniliforme* culture extracts (23) were developed. A GC method for quantification and confirmation of trichothecenes in wheat (40), TLC systems for the confirmation of trichothecenes (59) and a commercial ELISA kit for AFM_1 in milk (74) were assessed. Comparisons were carried out with ELISA and TLC methods for AFB_1 in samples of corn, feed and peanut (106) and for AFs, OTA and ZEA in corn and corn meal (63); two TLC methods for OTA in green coffee (66); and TLC and HPLC methods for AFM_1 in milk (116) and for FB_S in corn (80). Screening methods for AFs in corn and peanut were also evaluated (44,83,119). A sampling scheme was proposed for AF analysis in grains (29) and anomalous recoveries of AF from peanut and Brazil nuts measured by ELISA was reported (26).

A method for inoculating peanuts with *A. flavus* was standardized (88) and a simple and rapid method for screening high numbers of soil microorganisms capable of producing antifungal substances against *F. moniliforme* was developed (67).

MYCOLOGICAL SURVEYS AND MYCOTOXIN-PRODUCING POTENTIAL

Compared to previous decades, mycological examination increased in number but decreased slightly in percentage of the total number of papers during the period assessed. Some of these studies accompanied mycotoxin surveys and/or evaluated correlation with abiotic conditions. Corn was the most investigated commodity, and mycotoxigenic molds were found at high frequency in this food. Thus, the potential for mycotoxin production exists although, except for FBs, the occurrence of other mycotoxins in corn has been low in Brazil.

Aflatoxins were not detected in 10 varieties of recently harvested corn from Minas Gerais (1992-93 season) but there was high fungal contamination, with massive infection of *Fusarium* (100%), followed by *Penicillium* (60%) and *Aspergillus* (35%) (72). The highest moisture content was 14%. Among 20 *Aspergillus* isolates, 35% belonged to the species *A. fumigatus*, 30% to *A. flavus*, 30% to *A. niger* and 5% to *A. ochraceus*. Three of 6 strains of *A. flavus* were able to produce AFB_1.

In 130 samples of postharvest and stored corn from São Paulo (1991 harvest), *Fusarium* spp. was also the dominant fungi (84%), followed by *Penicillium* spp (55%) and *Aspergillus* spp. (41%) (85). However, only one sample had AFB_1; OTA, ZEA and DON were not detected. The *Fusarium* genus (but not *Penicillium* and *Aspergillus*) had significant positive correlation with moisture content of the grains and significant negative correlation with minimum and medium temperatures, rainfall and relative humidity. Similarly, in 195 samples of three hybrids of corn, analyzed monthly during one year, *Fusarium* spp. predominated, followed by *Penicillium* spp. and *Aspergillus* spp. (82) *Fusarium moniliforme* was the most prevalent *Fusarium* species.

Table 1. Incidence and levels of mycotoxins in foods and feeds.

Origin of sample/ Reference	Crop year	Mycotoxin	Commodity	Positive/ Total no. of samples	Positive samples (µg/kg)		Analytical Tech
					Range	Mean	
Federal District (113)	85-95	AFB_1+AFG_1	Peanut, prods.	89/450	<10-600	NA	TLC
São Paulo (36)	1988	AFB_1+AFG_1	"Paçoca"	194/316	4-195	NA	TLC
São Paulo (31)	1988	AFB_1+AFG_1	Peanut	270/517	5-1150	134	TLC
São Paulo (104)	1989	AFB_1+AFG_1	Peanut	37/108	9-12999	415	TLC
	1994	AFB_1+AFG_1	Peanut, prods.	142/321	>5-2440	305	TLC
São Paulo (105)	95-97	AFB_1+AFG_1	Peanut, prods.	62/137	>5-536	NA	TLC
São Paulo (8)	1994	AFB_1	Peanut, prods.	32/66	28-997	133	TLC
	1994	AFG_1	Peanut, prods.		14-149		
São Paulo (37)	95-96	AFs	Peanut, prods.	41/80	43-1099	399	TLC
Goiás (79)	NA	AFB_1+AFG_1	Peanut	40/104	37-522	187	TLC
Pernambuco (6)	1993	AFB_1	Peanut	26/86	10-2000	420	TLC
	1993	AFB_2	Peanut	20/86	10-400	134	TLC
	1993	AFG_1	Peanut	6/86	20-800	207	TLC
	1993	AFG_2	Peanut	6/86	20-400	95	TLC
Paraná (61)	93-94	AFB_1	Peanut	32/72	1-679	94	TLC
	93-94	AFB_2	Peanut	30/72	1-192	51	TLC
	93-94	AFG_1	Peanut	23/72	1-680	110	TLC
	93-94	AFG_2	Peanut	22/72	1-320	51	TLC
Santa Catarina (111)	98-99	AFB_1+AFG_1	Peanut, prods.	16/131	127(max)	NA	TLC
Rio Grande do Sul,	1988	AFB_1+AFG_1	Corn	11/36	10-906	131	TLC
Mato Grosso (47)	1988	ZEA, OTA	Corn	0/36	-	-	TLC
Minas Gerais (72)	92-93	AFs	Corn	0/40	-	-	TLC
São Paulo (85)	1991	AFs	Corn	1/130	500	-	TLC
	1991	ZEA,OTA	Corn	0/130	-	-	TLC
	1991	DON	Corn	0/130	-	-	TLC
Minas Gerais (95)	1991	AFL, ZEA	Corn	0/40	-	-	TLC
	1991	OTA, DON	Corn	0/40	-	-	TLC
São Paulo, Paraná	93-94	AFB_1	Corn	97/292	2-89	NA	TLC
Mato Grosso (Sul)	93-94	AFB_2	Corn	33/292	1-17	NA	TLC
Mato Grosso	93-94	AFG	Corn	13/292	2-85	NA	TLC
Goiás (43)	93-94	AFG	Corn	7/292	1-6	NA	TLC
	93-94	ZEA, OTA	Corn	0/292	-	-	TLC
Brazil, Argentina	94-95	DON	Corn	7/115	102-542	NA	TLC
Paraguay (98)	94-95	T-2	Corn	1/115	104	-	ELISA
Rio Grande do Sul	96-97	AFB_1	Corn prods.	3/39	30-163	75	TLC
(39)	96-97	ZEA, OTA	Corn prods.	0/39	-	-	TLC
Mato Grosso (22)	1999	AFs	Corn	64/140	2-431	NA	ELISA
Paraná, Mato Grosso	90-91	FB_1	Corn	47/48	600-18500	5490	HPLC
do Sul, Goiás (48)	90-91	FB_2	Corn	46/48	1200-19130	4820	HPLC
Paraná(81)	95-96	FB_1	Corn	149/150	70-13460	NA	HPLC
	95-96	FB_2	Corn	138/150	80-6920	NA	HPLC
São Paulo (82)	NA	FB_1	Corn	176/195	870-49310	9730	HPLC
	NA	FB_2	Corn	190/195	1960-29160	7600	HPLC
São Paulo (55)	1999	FB_1	Corn prods.	40/81	30-4930	1180	HPLC
	1999	FB_2	Corn prods.	44/81	20-1380	290	HPLC
São Paulo (21)	92-93	AFM_1	Raw milk	0/144	-	-	TLC
São Paulo (118)	1989	AFM_1	Pasteurized, powdered milk	0/86	-	-	TLC
	1990	AFM_1	Cheese, yoghurt	0/66	-	-	HPLC
	1992	AFM_1	Pasteurized milk	4/52	0.07-.37[1]	156[1]	HPLC

Table 1 (continuação). Incidence and levels of mycotoxins in foods and feeds.

Origin of sample/ Reference	Crop year	Mycotoxin	Commodity	Positive/ Total no. of samples	Positive samples (µg/kg)		Analytical Tech
					Range	Mean	
São Paulo (75)	92-93	AFM_1	Reconstituted milk power	33/300	.10-1.00[1]	270[1]	ELISA
Minas Gerais (100)	96-98	AFM_1	cheese	56/75	0.02-6.92	0.45	HPLC
Rio de Janeiro (32)	NA	AFB_1	egg	2/120	2, 5	3	TLC
	NA	AFM_1	egg	0/120	-	-	TLC
Santa Catarina, Rio	NA	AFB_1	Swine liver and	1/43	27	-	TLC
Grande do Sul (108)		AFM_1	kidney	0/43	-	-	TLC
		AFB_1	Poultry liver and	0/40	-	-	TLC
		AFM_1	kidney	1/40	trace	-	TLC
Rio de Janeiro (25)	NA	AFB_1	Chicken liver	3/6	1.2-3.2	2.1	TLC,HPLC
	88-90	AFs, ZEA	Wheat	0/18	-	-	TLC
	88-90	OTA	Wheat	1/18	40	-	TLC
	88-90	DON	Wheat	1/18	400	-	GC
Brasil, Argentina,	88-90	DAS	Wheat	1/18	300	-	GC
Uruguay (41)	88-90	T-2	Wheat	2/18	350, 360	355	GC
	88-90	HT-2, NIV	Wheat	0/18	-	-	GC
	88-90	T-2 tetraol	Wheat	1/18	1680	-	GC
	88-90	T-2 triol	Wheat	0/18	-	-	GC
São Paulo (42)	1990	AFs, OTA	Wheat	0/20	-	-	TLC
	1990	ZEA	Wheat	3/20	130-400	250	TLC
	1990	DON	Wheat	4/20	470-590	550	GC
	1990	NIV	Wheat	3/20	160-400	250	GC
	1990	T-2	Wheat	2/20	400, 800	600	GC
	1990	DAS	Wheat	1/20	600	-	GC
São Paulo (122)	1991	AFs, ZEA, OTA,	Wheat, prods.	0/38	-	-	TLC
	1991	DON,	Wheat, prods.	0/38	-	-	GC
	1991	NIV, DAS, T -2,	Wheat, prods.	0/38	-	-	GC
	1991	HT-2,	Wheat, prods.	0/38	-	-	GC
	1991	T-2 triol,	Wheat, prods.	0/38	-	-	GC
	1991	T-2 tetraol	Wheat, prods.	0/38	-	-	GC
Rio Grande do Sul (39)	96-97	AFB_1	Wheat prods.	0/79	-	-	GC
	96-97	ZEA	Wheat prods.	2/79	97, 105	101	TLC
	96-97	OTA	Wheat prods.	2/79	18, 26	22	TLC
Rio Grande do Sul (127)	95-96	AFs	Flour	0/54	-	-	TLC
	95-96	ZEA	Flour	1/54	53	-	TLC
	95-96	OTA	Flour	3/54	2-23	12	TLC
Rio Grande do Sul (39)	96-97	AFB_1	Rice prods.	1/47	48	-	TLC
	96-97	ZEA	Rice prods.	0/47	-	-	TLC
	96-97	OTA	Rice prods.	2/47	19, 35	27	TLC
São Paulo (123)	1991	AFs,ZEA,	Health foods,	0/103	-	-	TLC
		OTA	Break. cereals				TLC
Federal District (113)	85-95	AFB_1+AFG_1	Nuts	1/117	1200	-	TLC
São Paulo (38)	1991	AFL,ZEA,	Tree nuts	0/56	-	-	TLC
	1991	OTA	Tree nuts	0/56	-	-	TLC
	1995	AFB_1	Tree nuts	2/54	10-26	18	TLC
	1995	AFG_1	Tree nuts	1/54	15	-	TLC
	1995	ZEA, OTA	Tree nuts	0/54	-	-	TLC
São Paulo	NA	OTA	*C. arabica*	3/24	1.7-13	6.9	HPLC
Minas Gerais	NA	OTA	*C. arabica*	8/37	2.7-10	5.9	HPLC
Paraná	NA	OTA	*C. arabica*	0/7	-	-	HPLC

Table 1 (continuação). Incidence and levels of mycotoxins in foods and feeds.

Origin of sample/ Reference	Crop year	Mycotoxin	Commodity	Positive/ Total no. of samples	Positive samples (µg/kg)		Analytical Tech
					Range	Mean	
Espirito Santo	NA	OTA	*C. canephora*	0/4	-	-	HPLC
Rondônia (124)	NA	OTA	*C. canephora*	2/5	5.5, 114	60	HPLC
Minas Gerais (97)	98-99	OTA	Coffee,roasted, ground	33/47	0.99-5.87	1.75	HPLC
	98-99	OTA	Soluble coffee	31/37	0.25-2.00	0.72	HPLC
São Paulo (50)	NA	OTA	Coffee,roasted, ground	23/34	0.3-6.5		HPLC
			Instant coffee	14/14	0.5-5.1	2.2	HPLC
Paraná (54)	92-93	PAT	Apple juice	15/76	6-77	15	HPLC
São Paulo (117)	92-93	PAT	Fruit, juice	1/149	17	-	HPLC
	95	PAT	Fruit, juice	0/36	-	-	HPLC
Federal District (113)	85-95	AFB_1+AFG_1	Various foods	0/114	-	-	
São Paulo (21)	92-93	AFB_1	Feed	14/96	11-287	NA	TLC
Rio Grande do Sul (73)	93-94	AFB_1	Corn for feed	1/115	10	-	TLC
	93-94	ZEA	Corn for feed	15/115	413-4130	1430	TLC
Amazonas (78)	1995	AFL	Feed	0/60	-	-	TLC
São Paulo, Paraná (45)	1986	AFB_1	Cottonseed meal	114/169	<10-40	NA	TLC

[1]mg/l; NA - data not available.

In 66 samples of three hybrids of freshly harvested corn from three regions of the state of São Paulo (1995 crop), the fungal population was also mainly composed of *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp. and two other filamentous fungal genera, which were isolated from corn with a_w of 0.30 to 0.99 and moisture content of 5 to 20% (1). The most frequent species of the genera *Fusarium* and *Aspergillus* were *F. moniliforme* and *A. flavus.* All of forty isolated strains of *F. moniliforme* produced FB_1 and/or FB_2. Of 10 *A. flavus* isolates, six strains produced AFB_1 and/or AFB_2. In 17 samples of freshly harvested corn from 16 different sites in the state of São Paulo in 1992, *Fusarium* and *Penicillium* incidence was also high; the genus *Aspergillus* was isolated but at a lower frequency (15). Four of 17 *A. flavus* isolates were found to be AFB_1 or AFG_1 and AFB_2 producers.

Of 39 corn samples from Parana and nine samples from Mato Grosso do Sul and Goias (1990-91 harvest), *F. moniliforme* and *Aspergillus* spp. section *Flavi* were detected in 41 and 33 samples, respectively (48). In a recent survey involving 150 samples of freshly harvested corn from the Central-Southern, Central-Western and Northern regions of the state of Parana, the samples were frequently contaminated with *Fusarium* spp. (99-100%) and *Penicillium* spp. (93-100%), *Aspergillus* spp. showing lower frequency (not detected to 28%) (81). The highest contamination of potentially mycotoxigenic fungi occurred in corn from the Central-Western region. However, although FBs were found in all samples from the Central-Western and Northern regions, FB levels were higher in the North, the difference in FB contamination being attributed to the difference in rainfall levels.

In 90 samples of corn from various regions of Brazil (crop year not specified), *Aspergillus, Penicillium, Fusarium, Rhizopus, Acremonium, Cladosporium, Neurospora* and *Pacilllomyces* were the genera isolated (7). *A. flavus* was the most frequently isolated among the *Aspergillus* species from samples with moisture content between 14 and 18%; 39% of the isolates were toxigenic and produced only B aflatoxins. *A. parasiticus* was the third most frequent species; all cultures were toxigenic and produced AFB_1, AFB_2, AFG_1 and AFG_2.

Five recently harvested corn hybrids produced in Rio Grande do Sul were examined in relation to macroscopic appearance, fungal contamination, AF production by *Aspergillus parasiticus*, NRRL 2999 and consumption of dry matter in fungal culture (24). The hybrids had macroscopic damage and showed fungal contamination by *Penicillium* spp. (14%), *Aspergillus* spp. (24%) and *Fusarium* spp. (57%). AF production by the hybrids cultured for 5 and 10 days showed difference only in relation to AFG_2 in cultures of 5 days.

F. graminearum was found in both local (12 samples) and imported (6 samples) wheat stored in elevators in Rio Grande do Sul, southern Brazil, in 1990 but not in the 1988-89 season (41). It was the main *F.* species in Argentinean and Uruguayan wheat, while *F. dimerum* predominated in Brazilian wheat. Twenty samples of two wheat cultivars from São Paulo (1990 harvest) had *Alternaria, Drechslera, Epicoccum* and *Cladosporum* as prevailing genera (42). Among the *F.* spp., *F. semitectum* was present in 19 samples and *F. moniliforme* in 18 samples; but *F. graminearum* was not found.

The mycoflora of 140 samples of freshly harvested and stored sorghum was monitored for one year (112). There was a predominance of the genera *Phoma* (57%), *Aspergillus* (43%), *Fusarium* (25%) and *Rhizopus* (21%), along with the presence of nine other filamentous fungi. The species most frequently found were *Aspergillus flavus* and *Fusarium moniliforme*.

In 90 samples of milled rice negative to AFs from different regions in Brazil in 1987-88, *Aspergillus, Penicillium, Cladosporium, Rhizopus and Rhodotorula* were isolated (4). *A. parasiticus* was the most frequent of the *Aspergillus* genus, isolated from samples with moisture content of 14-17%. All cultures were toxigenic, producing AFB_1, AFB_2, AFG_1 and AFG_2. *A. flavus* was the second most frequent, 22% of which was toxigenic, with the production of AFB_1 or AFB_1 and AFG_1.

After milling of 30 samples of rough rice stored for 6, 12 or 24 months, samples of polished rice, rice bran and rice hull (30 samples each) were evaluated and the following fungi, in decreasing order of frequency, were found: *Aspergillus* spp., *Nigrospora* spp., *Penicillium* spp., *Fusarium* spp., *Mucor* spp., *Cladosporium* spp., *Trichosporon* spp. and non-sporulated fungi (52). Fungal contamination was lowest in polished rice, increasing progressively in samples of rice bran and rice hull. Of the *Aspergillus species, A. flavus* and *A. candidus* were the most frequently isolated and 53% strains of the *A. flavus* isolates were found to be toxigenic, producing only the group B aflatoxins.

A total of 37 fungal species were identified in common and dwarf cashew nuts (34). *A. niger* was the dominant species followed by *A. flavus. P. brevicompactum* and *P. glabrum* were the most frequently isolated penicillia. Higher contamination was found in dwarf kernels.

Forty-two species of field and storage fungi were isolated in black and white pepper (35). *A. flavus* and *A. niger* were encountered most frequently, prevalence being greater in the black pepper. Other potential mycotoxigenic species isolated were: *A. ochraceus, A. tamarii, A. versicolor, Emericella nidulans, Chaetomium globosum, P. brevicompactum, P. citrinum, P. islandicum and P. glabrum.*

Fusarium (68%; main species, *F. moniliforme*), *Aspergillus* (58%; main species, *A.flavus*) and *Penicillium* were the principal genera in 96 samples of cattle feedstuffs from São Paulo (21). Twenty of 26 *A. flavus* isolates were AFB producers. AFB_1 and AFB_2 were found in 14 samples. In 60 samples of poultry feedstuffs from Amazonas, northern Brazil, however, the major genera were *Aspergillus* (72%), *Rhizopus* (28%), *Absidia* (27%), *Penicillium* (12%), *Mucor* (12%) and *Fusarium* (10%). *A. flavus* was the most frequently isolated *A.* species, 44% of the strains being toxigenic, but AFs were not detected in the 60 samples.

In two cultivars of Brazilian apples, Gala and Fiji, inoculated with *P. expansum* NRRL 1172 or toxigenic *P. variabile* isolated from apples, and stored for 15 to 90 days at 0 to 25ºC, patulin production was negative until the 30th day of storage at 0ºC (103). Thereafter and under the other storage conditions, patulin was produced with both *Penicillium* strains.

Strains of *Aspergillus* and *Penicillium* were isolated from several samples of Brazilian cheese and their toxin producing potential was evaluated (120). Two of the isolated *Penicillium* species produced citrinin, while another produced patulin. None of the mycotoxins (AFs, OTA, PAT, penicillic acid, citrinin) analyzed, however, was detected in the samples.

Zearalenone production by *Fusarium graminearum* induced by the mutagenic agent nitrosoguanidine was also investigated in 40 samples differing from the control in morphological aspects, growth rates and pigmentation (60). Ten variants showed an increase in yield of 2 to 16 times, compared to the control.

PREVENTION, CONTROL AND EFFECTS OF PROCESSING

Efforts to find means of preventing or controlling fungal growth and mycotoxin production had received much greater attention in the decade focalized.

In the peanut variety "Tatu Vermelho", optimum production of aflatoxin occurred at a_w of 0.93; at a_w 0.86 there was no formation of AF in 120 days (92). In beans inoculated with *A. alutaceus* Berk & Curt, an ochratoxigenic strain, production of OTA and fungal growth were not observed even after 30 days of incubation at a_w of 0.75; at a_w 0.80, OTA was detected after 20 days and at a_w 0.84, after 10 days (64). Maximum OTA production occurred at a_w 0.90.

The influence of pH and acids on the level of glucose needed to induce aflatoxin production was studied by Luchese and Harrigan (53). The results indicated that the major role of pH is related to the initial events of the synthesis. Hydrochloric acid and lactic acid had little effect. Glucose was found not to be a limiting factor for AF synthesis to occur.

Three varieties of corn inoculated with *A. flavus* NRRL 6513 were shown to be equally susceptible to fungal infection and AFB_1 production (94). AFB_1 production of four genotypes of peanuts (including the most planted in Brazil), inoculated with *A. flavus* IMI 190443, was also investigated by Prado *et al*. (89,99). The genotype 2117, originating from India, presented the lowest AFB_1 levels, indicating varietal resistance as a possible means of control.

AFs were not detected in five samples of recently harvested peanut produced in places with sandy soil, while both AFB_1 and AFG_1 were produced in six of seven samples of the same peanut cultivar cultivated in places with clay soil, suggesting some influence of the type of soil on aflatoxin production (93).

In both the rainy seasons of 1990 and 1991, after storage for 80 and 30 days, respectively, the AF level was considerably lower in moist in-shell peanuts stored in jute bags than in those stored in polypropylene bags (30). There was a slightly better moisture loss in jute bags compared to polyethylene bags. Electronic color sorting was efficient in eliminating highly contaminated lots, directing them to rejected portions, although there was no obvious improvement in the overall initial contamination (130). The presence of *A. flavus* in corn enhanced growth of mites

which, in turn, efficiently dispersed viable fungal spores from the inoculated to the uncontaminated compartments (33).

The antagonistic potential of some microorganisms against mycotoxin-producing fungi was also studied. Screening of 80 soil and corn samples yielded 51 microorganisms antagonistic to *F. moniliforme* 113 F, of which 3 sporulated gram positive bacilli and 2 gram positive cocci showed the best antifungal activity (68,69). Soil-isolated bacteria, with proven *in vitro* activity against *F. moniliforme*, were found more effective than four chemical fungicides (benomyl, triflumizole, perfurazoate, prochloraz) in controlling a fungal disease in rice plants (70). Of 150 isolates, attention was drawn to *Bacillus* spp. and a yeast, obtained from silage and corn, respectively, which showed intense proliferation and antifungal activity which impeded the growth of *F. moniliforme* (11). These three sporulated bacilli and yeast were subsequently shown to degrade 43-83% and 57%, respectively, of the initial FB_1 concentration in a corn culture inoculated with *F. moniliforme* (12).

Twenty-one isolates of yeasts from apple, with antagonistic activity against *Penicillium* spp., were assessed in terms of a detoxifying action against patulin (51). The best results were obtained with two isolates which reduced the patulin concentration by 85 and 75%.

In line with natural control of phytopathogens, using amylase inhibitors, the amilases of *Fusarium moniliforme* and *Aspergillus flavus* were produced and characterized in terms of pH and temperature of maximum activity and stability (28). A culture medium containing starch, glycerol, wheat bran or corn and the respective starch or supernatant fraction was also evaluated (27). The medium with milky stage corn supernatant promoted the best mycelial growth while that with 2% ground corn in milky stage corn supernatant gave the highest amylase production.

Aluminum, iron and zinc added at 40-160 µg/g inhibited AFB_1 production in autoclaved peanuts, inoculated with spores of *A. flavus* NRRL 6513 (87). Nickel at 4.0 µg/g stimulated AFB_1 production but inhibited it at 1.0 and 2.0 µg/g. However, iron applied as ferrous sulfate solution on the leaves (91) and in the soil (90) did not appear effective in lowering the AFB_1 content of peanuts inoculated with *A. flavus* NRRL 5940 and IMI 190443, respectively.

Propionic acid (as ammonium propionate) sprayed on rehydrated (16-18% moisture) inshell peanuts at 5g/kg was effective in controlling total and aflatoxigenic fungal growth and aflatoxin production during the entire evaluation period (28 days) (9,10,102). At 3 g/kg, it was efficient only until 14 days. All other treatments (grapefruit seed extract at 5 and 10 g/kg, sodium orthophenylphenate at 2.5 and 5 g/kg, thiabendazole at 1 and 5 g/kg) were inefficient.

An initial study indicated that phosphine fumigation might affect the growth of *A. flavus/A. parasiticus* and AF production in peanuts stored in warehouses with moisture content above the recommended level (13). In a subsequent study, fumigation with phosphine (3 applications in 7 days) controlled fungal development and maintained AF levels in high-moisture (18-21%) unshelled peanuts, while the untreated stacks showed staggering increase (14). After a month, however, no difference was observed in AF contamination and infection by *A. flavus* and *A. parasiticus* between the untreated and treated stacks.

Application of the fungicide iprodione in aqueous or oily solution reduced AF levels in corn stored in a ventilated atmosphere (86). Without ventilation, reduction of AF was not significant, especially at elevated moisture levels.

Sodium bentonite (110) and the synthetic zeolite NaA (62) were also shown to counteract some of the toxic effects of AF in growing broiler chicks.

Roasting of artificially contaminated peanuts in a microwave oven for 6 min decreased AFB_1 and AFG_1 by 41 to 70% (96). In Brazilian beans, previously inoculated with the ochratoxigenic strain *A. alutaceus*, cooking under pressure at 115ºC for 45 min decreased OTA substantially (up to 84%), especially when soaked in water for 12 h before cooking (65).

A patulin-producing *Penicillium expansum* strain, isolated from apples, was inoculated in sound apples and migration of patulin from the point of inoculation was studied (121). Trimming just the rotten tissue was not enough to exclude all patulin, but removal of 1 cm around the rotten tissue would be satisfactory, the toxin not being detected at this distance.

The effect of parboiling in rice (with bran), artificially contaminated with AF and naturally contaminated with OTA, was also assessed (20). Migration of 32% AFB_1, 44% AFB_2, 36% AFG_1, 21% AFB_2 and 66% OTA was observed.

Roasting with oil at 195ºC and frying yielded nearly total destruction of Afs in naturally contaminated peanuts (115). Laboratory or industrial dry roasting at 130ºC resulted in practically no destruction of AFs. Boiling in water or water with 5% salt caused approximately 80% AF destruction. Thus, thermal treatment was found to be an adequate means of detoxifying naturally contaminated peanuts provided the temperature was kept at 195 ± 5ºC.

Filtered juice of *Agave sisalana* leaves inhibited the growth of *A. flavus, A. parasiticus* and *A.* sp. in corn (84). The flavonoids quercetin, kaempherol, kaempheritrin and naringinin at 300, 100, 300 and 125 ppm showed reductions of 36, 40, 49 and 60% of *A. flavus* growth in culture media (57). The greatest inhibition of AFB_1 production (90%) was seen with kaempheritrin at 100 ppm.

TOXIC EFFECTS AND MODE OF ACTION

The acute effects of a single intraperitoneal dose of AFB_1 (60 mg/kg animal weight) on different inbred mouse strains were evaluated (2,3). Histopathologic lesions and biochemical changes differed with the different strains, probably reflecting the strains' ability to biotransform and eliminate AFB_1 and its metabolites.

Citrinin inhibited the growth of three renal cells, i.e. LLC-MK_2, PK-15 and MDBK, especially in the first hours (129). The LLC-MK_2 cells decreased the most and the MDBK cells showed distinct

morphological alterations. Studies on the mechanism of citrinin-induced dysfunction of the rat mitochondria were undertaken (16-19). Citrinin depressed the phosphorylation efficiency of renal cortical mitochondria and inhibited most of the enzymes of the respiratory chain of the rat renal cortical and liver mitochondria. This mycotoxin also decreased the rate of mitochondrial swelling induced by the valinomycin-K^+ complex, suggesting its interference on the inner mitochondrial membrane fluidity.

The occurrence of five cases of equine leukoencephalomalacia associated with the ingestion of moldy corn during the winter of 1990 in São Paulo was reported, with detailed description of clinical signs and the results of histopathological examination (128). The clinical, etiologic and pathological diagnosis of an outbreak of equine leukoencephalomacia in Rio Grande do Sul was also described (56). Two samples of corn consumed by the affected horses contained FB_1 (46 and 53 µg/g) and *F. moniliforme*. FB_1 (0.2-38.5 µg/g) and FB_2 (0.1-12.0 µg/g)were found in 20 and 18 samples, respectively, of 21 *F. moniliforme*-contaminated feed samples from Paraná associated with outbreaks of confirmed and suspected mycotoxicoses in various animal species (114). With the exception of one, all 26 *F. moniliforme* isolates from the feed samples were acutely toxic to ducklings and contained FB_1 and FB_2 at 65-4420 and 5-1380 µg/g, respectively.

AFB_1, AFM_1 and aflatoxicol were not found in the liver, kidney or urine of calves intoxicated chronically, but detectable levels of AFB_1 were found in tissues and urine of two calves that received single doses of 0.8 and 1.8 mg AFB_1/kg animal weight (107). The livers of laying hens exposed to AFB_1 (100, 300, 500 µg/kg feed, for 60 days, 4 hens per group) appeared congested and showed signs of degeneration (77). Hepatic cell vacuolation with fatty infiltration were observed in all groups, including the control, but was more marked with increasing mycotoxin dose, being maximum in hens that received rations with 500 µg/kg of the toxin. Bile duct proliferation and trabecular disorder were seen in the livers of hens exposed to AFB_1 at levels above 300 µg/kg

AFB_1 residues were determined in eggs of young laying hens fed with rations containing different levels of the mycotoxin (0, 100, 300 or 500 µg/kg feed, for eight weeks, 24 birds for each group) and detected only in the eggs of hens given 500 µg/kg feed, at levels ranging from 0.05 to 0.16 µg/kg (76). The results indicated that the feed to egg AFB_1 transmission ratio was about 5000:1.

In a preliminary study carried out to evaluate the incidence of hepatic diseases, especially hepatocellular carcinoma, in children and adults from the State of Santa Catarina from 1980 to 1998, mycotoxin contamination of food was cited as one of the possible factors that could lead to these diseases (46).

The prominent signs of aflatoxicoses in several species, including mammals, are hypolipidaemia, hypocholesterolaemia and hypocarotenaemia, associated with severe hepatic steatosis and weight loss. It is suggested that these signs of acute imbalance of lipid metabolism can be the result of chemical modification of the LDL apoprotein by the activated AFB_1 (5). Modified LDLs are not recognized by their specific receptors, and bind to liver cells. Lipid starvation of peripheral tissues takes place while fat accumulates in the liver. This abnormal state is maintained and reinforced by further modification of nascent apoproteins for as long as AF continues to be available to the liver.

CONCLUDING REMARKS

In spite of constraints in human and material resources, Brazilian researchers are responding to the needs of the country in confronting the mycotoxin problem. The research activities undertaken reflect current international concerns and recent developments in the area. Recognition of this work by government authorities involved in policy making is imperative to transform research results into practical applications.

RESUMO

Pesquisa em micotoxinas no Brasil: a última década em foco

O número de artigos de pesquisa (128 artigos) sobre micotoxinas publicados por pesquisadores brasileiros em 1991-2000 superou a soma de artigos (85 artigos) publicados nas três décadas anteriores (1961-1990). Trinta por cento das publicações foi levantamento da ocorrência de micotoxinas em alimentos e rações. Aflatoxinas em amendoim e produtos de amendoim continua sendo um problema alarmante, e a alta incidência e níveis elevados de fumonisinas em milho e produtos de milho também parecem ser um problema sério. A contaminação com outras micotoxinas, como zearalenona, ocratoxina A e tricotecenos, foi baixo. A ocorrência de aflatoxina M_1 em leite e laticínios e de patulina em suco de maçã precisa ser verificada, pois, há uma certa divergência nos resultados. Trabalhos sobre os métodos analíticos, estudos micológicos e efeitos tóxicos constituíram 16, 13 e 13%, respectivamente, dos artigos publicados na década avaliada. A busca de meios de prevenção/controle da contaminação fúngica e produção de micotoxinas aumentou notadamente, perfazendo 27% dos artigos, incluindo investigações sobre fatores influentes (por exemplo, resistência de genótipos, conteúdo/atividade de água, umidade relativa, temperatura, presença de metais, tipo de solo, infestação com inseto) e o potencial antagônico de outros microrganismos contra os fungos produtores de micotoxinas. Os efeitos de extrato de planta, flavonóides, fungicidas e outros químicos, sacos utilizados para estocagem, adsorventes e processamento de alimentos foram também estudados. Portanto, apesar das limitações de recursos, a pesquisa brasileira responde as necessidades do país, reflete as preocupações internacionais e os desenvolvimentos recentes na área.

Palavras-chave: micotoxinas, ocorrência, fatores influentes, pesquisa, Brasil

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