INCIDENCE OF AFLATOXIN CONTAMINATION IN CASSAVA (*Manihot esculenta* Crantz)

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ABSTRACT

The incidence of aflatoxin contamination in cassava roots at different stages of maturity and during processing and storage was determined. No aflatoxin was obtained from 7- to 14-month old cassava roots. During storage, however, trace amounts of aflatoxin B_1 were observed in stored cubes but not in stored chips and unprocessed roots. Blanching generally inhibited the growth of other fungi but not *A. flavus*. In the absence of competing fungi, fresh cassava roots may be a good substrate for the growth of *A. flavus* and *A. parasiticus* but not for aflatoxin production. A fluorescent compound behaving like aflatoxin B_1 was observed in samples which were not dried after 48 hours as well as in unprocessed stored cassava roots showing vascular discoloration. Confirmatory test showed that it was not aflatoxin.

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KEY WORDS: Cassava (*Manihot esculenta* Crantz). Aflatoxin. Contamination. *Aspergillus flavus*. Blanching. Storage.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the world's most important staple food crops. It plays a prominent role in the daily subsistence and culture of many indigenous populations of South and Central America, Africa and Asia. In the tropics, cassava constitutes the largest portion of the total perishable agricultural commodities production of around 210 million tons (Ingram and Humphries, 1972).

The form in which cassava is consumed varies with country and region. It may be utilized either as fresh vegetable or in some simple processed form (Phillips, 1974). Cassava is also used as animal feed. More than one million tons of dried cassava are imported annually by the European Economic Com-

munity (EEC) countries for this purpose (Phillips, 1974).

One problem that confronts cassava consumers and producers is the ability of this commodity to support aflatoxin formation. Ilag (1977) observed that cassava is one agricultural commodity which produces the highest amount of aflatoxin B_1.

Improper commodity storage has been theoretically and experimentally implicated as a cause of aflatoxin contamination (Stoloff, 1976). However, it is not the sole cause. Accumulated evidence shows that contamination most likely occurs prior to harvest (Anderson et al., 1975; Fennel et al., 1975; Knake and Deyoe, 1973; Rambo and Caldwell, 1974).

This study investigated the incidence of aflatoxin in cassava roots at different stages of maturity and during processing and storage.

MATERIALS AND METHODS

Harvesting and Preparation of Samples

Eight-month old cassava (var. Golden Yellow) roots were harvested manually by directly pulling the plant from the ground. The harvested cassava roots were washed to remove soil and debris.

Fungal Invasion and Aflatoxin Formation in Freshly Harvested Cassava Roots at Different Ages

A farmer's field approximately 5 x 47 meters and located in barrio Bubon, Baybay, Leyte was utilized in this study. Monthly field sampling of cassava roots was done for 8 consecutive months starting from the seventh until the fourteenth month after planting by taking 5-10-kg root samples at random. A one-kg composite sample was then grated and mixed thoroughly until a homogeneous sample was obtained. Mold count, fungal species identification and aflatoxin content were determined at each sampling time. The actual sample for mold count was taken using the quartering procedure (Jones, 1972) with three counts made per sampling time. Fungal species was identified using the slide culture technique while aflatoxin content was determined by TLC chromatographic technique described by Jones (1972).

Fungal Invasion and Aflatoxin Formation in Unprocessed Cassava Roots

Thirty pieces of fresh unprocessed roots were placed in wooden crates and stored at ambient temperature for 6 days. Sampling was done every 2 days until the 6th day. Mold count, fungal species identification and aflatoxin content were determined at each sampling time using the methods previously described.

Fungal Inoculation. There were two fungal species tested in this experiment, namely *Aspergillus flavus* and *A. parasiticus*. Five-day old cultures of each fungal species were carefully scraped in sterile distilled water. The spore suspension

was sprayed on whole tubers which were previously surface sterilized with 70% ethanol and artificially wounded by slicing. There were two treatments as follows: a) unprocessed, inoculated cassava roots; and b) unprocessed, uninoculated cassava roots.

A follow-up comparative study using sterilized/unsterilized corn grits and cassava roots as substrates of *A. flavus* and *A. parasiticus* was conducted. After 2 weeks of incubation under ambient temperature, aflatoxin content was determined.

Fungal Invasion and Aflatoxin Formation in Processed Cassava Roots

Preparation of Cassava Chips. Clean cassava roots were individually peeled, cut into 10-15 cm slices and fed into the chipping machine to produce elongated, cylindrical chips 2-6 mm thick and 6 cm long.

Preparation of Cassava Cubes. Washed and peeled cassava roots were cut into cubes (1 cm^3) and divided into two lots. One lot was blanched for 5 minutes before sundrying, the other was unblanched and dried under the sun. Blanching was done to retard the growth of fungi particularly *A. flavus*.

Fungal Inoculation. An isolate of *A. flavus* obtained from the Culture Collection Section, Museum of Natural History, University of the Philippines at Los Baños was grown in potato dextrose agar (PDA) slant cultures. Five-day old culture of *A. flavus* was carefully scraped in sterile distilled water. Spore concentration of the suspension was determined with a microscope and haemacytometer just prior to inoculation by counting the total number of spores in two 25 square areas of the haemacytometer. Forty mL of the spore suspension which contained about 1.7×10^7 spores was thoroughly mixed by hand with 1 kg each of cassava chips and cubes. Inoculation was done right after processing and before sundrying both the chips and cubes. There were six treatments as follows: a) cassava chips, inoculated; b) cassava chips, uninoculated; c) cassava cubes, blanched, inoculated; d) cassava cubes, blanched, uninoculated; e) cassava cubes, unblanched, inoculated; and f) cassava cubes, unblanched, uninoculated.

Sun Drying. Cassava chips and cubes were spread out at 5-10 kg/m^2 on 0.90 m x 0.90 m wooden-framed trays with plastic mosquito screen, and placed 300 mm above the ground. Drying lasted until the safe level of 12.15% moisture content was attained.

Storage. Dried chips and cubes were placed in plastic sacks and stored at ambient temperature for 8 weeks. Mold count, fungal species identification, aflatoxin and moisture content determinations were done weekly throughout the course of storage.

RESULTS AND DISCUSSION

Fungal Invasion and Aflatoxin Formation in Freshly Harvested Cassava Roots at Different Ages

Results reveal that aflatoxin formation was absent in freshly harvested cassava roots at all ages tested. *A. flavus* was isolated from 7, 10, 12 and 14-month old cassava roots but not from 8, 9, 11 and 13-month old roots (Table 1). Its growth was scanty and commonly in combination with other field fungi. The wide variation in environmental conditions particularly relative humidity and temperature (Table 2) during the conduct of this experiment may have contributed to the observed differences in the counts of *A. flavus*. According to Galloway (1935) and Panassenko (1944), *A. flavus* is classified as a mesophyte on the basis of having a minimum moisture requirement for growth between 80 and 90% relative vapor pressure or RH. The minimum RH for spore germination, and sporulation of *A. flavus* are 80 and 85 percent, respectively.

Factors which could explain the absence of aflatoxin formation in the field were low inoculum levels of toxigenic strains of *A. flavus*, minimal mechanical damage, and microbial competition (Ashworth et al., 1965; Marsh and Taylor, 1958).

Fungal Invasion and Aflatoxin Formation in Unprocessed Cassava Roots

Aflatoxin was absent in unprocessed cassava roots even after 6 days of storage inspite of approximately 90% fungal invasion observed. As shown in Table 3, both inoculated and uninoculated tubers showed high *A. flavus* counts. Nevertheless, several contaminating fungi were found associated with *A. flavus* growth. Among the contaminating fungi, yeasts were the most prevalent and usually exceeded *A. flavus* and other contaminating fungi in quantity although not included in the mold count. The high moisture content of the tubers (52-54%) apparently satisfied the water requirements of most molds thus increasing competition between organisms and making competition the most likely limiting factor in the production of aflatoxin. However, other factors that may affect the development of aflatoxin contamination such as fungal strain, substrate temperature, and relative humidity should not be overlooked. According to Schroeder (1969), different species of *A. flavus* and *A. parasiticus* vary widely in the quantity of aflatoxin produced on the same natural substrate. In general, *A. parasiticus* produced large quantities of the toxin while *A. flavus*, large to moderate. Results using both organisms as inoculum, however, showed that aflatoxin formation was absent throughout the duration of storage (Tables 3 and 4).

Observations suggest that cassava is not a good substrate for aflatoxin production. A follow-up comparative study using corn grits and cassava roots as substrates of *A. flavus* and *A. parasiticus* showed

Table 1. Moisture content, mold count and fungal species identified from cassava roots harvested at different ages.[1]

Age of Cassava Roots (months)	% Moisture	Fungal Species	Mold Count (colonies/g)
7	51.0	non-sporulating fungus with white septate mycelia	180
		Aspergillus flavus	20
8	54.6	*A. fumigatus*	660
		Penicillium sp.	420
9	52.7	*Fusarium* sp.	66
		Penicillium sp.	166
10	48.5	*A. niger*	7
		A. flavus	17
11	52.6	non-sporulating fungus with white septate mycelia	20
		Penicillium sp.	13
		Fusarium sp.	7
12	54.2	*A. flavus*	10
		Penicillium sp.	27
		A. niger	7
		Hyalodendron sp. (tentative identification)	3
		non-sporulating fungus with white septate mycelia	3
		Rhizopus sp.	
13	57.6	*Hyalodendron* sp. (tentative identification)	3
		A. fumigatus	3
		Penicillium sp.	7
		non-sporulating fungus with white septate mycelia	3
14	39.2	*Penicillium* sp.	3
		A. flavus	30
		Fusarium sp.	10
		Hyalodendron sp. (tentative identification)	170

[1] Aflatoxin content was determined by TLC chromatographic technique but no aflatoxin was detected at all ages of cassava roots.

Table 2. Monthly minimum and maximum relative humidity and temperature during the conduct of the experiment.

Month	Relative Humidity %		Temperature (°C)	
	Minimum	Maximum	Minimum	Maximum
April	62	97	23.3	32.3
May	55	97	23.3	33.7
June	73	93	22.7	33.2
July	63	95	22.4	33.0
August	56	92	23.3	32.5
September	58	98	22.2	32.5
October	63	95	23.2	32.3
November	59	98	22.4	32.0

that corn produced as much as 20-40 ppb aflatoxin B_1 (classified by its blue fluorescence under UV light) after 2 weeks of incubation under ambient temperature while only a trace amount was noted in cassava. Both substrates were fully invaded by the fungi although they differed in the amount of aflatoxin produced. This implies that the extent of mold infection is not a measure of the degree of aflatoxin production. Moreover in the absence of competing fungi, fresh cassava roots may be a good substrate for the growth of *A. flavus* and *A. parasiticus* but not for aflatoxin production.

Fungal Invasion and Aflatoxin Formation in Processed Cassava Roots

Cassava chips were easily processed and have good keeping qualities when adequately dried. It served as a good substrate for the growth of *A. flavus* and other storage fungi at 8.6-15% moisture content (Table 5). However, it did not promote aflatoxin formation.

On the other hand, cubing of cassava roots was laborious, required longer drying time hence, increased susceptibility to microbial contamination. Just like cassava chips, cassava cubes when stored

Table 3. Moisture content, mold count and fungal species identified from stored unprocessed cassava roots.[1]

Storage Period (Days)	Treatment[2]	Moisture Content (%)	Fungal Species	Mold Count (colonies/g)
2	Uninoculated	54.3	*Aspergillus flavus*	1,000
			A. niger	500
			A. wentii	130
			Penicillium sp.	160
			non-sporulating fungus with white septate mycelia	100
			Saccharomyces sp.	
	Inoculated	52.3	*A. flavus*	700
			Hyalodendron sp. (tentative identification)	160
			Penicillium sp.	200
			Saccharomyces sp.	
4	Uninoculated	53.2	*A. flavus*	1,200
			Fusarium sp.	1,033
			Saccharomyces sp.	
	Inoculated	54.4	*A. flavus*	5,200
			Saccharomyces sp.	
6	Uninoculated	52.5	*A. oryzae*	700
			Fusarium sp.	766
			Saccharomyces sp.	
	Inoculated	53.2	*A. flavus*	1,203
			Fusarium sp.	1,030
			Saccharomyces sp.	

[1]Aflatoxin content was determined by TLC chromatographic technique but no aflatoxin was detected throughout the duration of storage.

[2]Unprocessed roots were inoculated with *A. flavus*.

served as good medium for microbial development. It supported the growth of *A. flavus* at a moisture content of 10-12.5% (Table 6). In addition, it promoted the formation of trace amounts of aflatoxin B_1.

Table 4. Moisture content, mold count and fungal species identified from stored unprocessed cassava roots.[1]

Storage Period (Days)	Treatment[2]	Moisture Content (%)	Fungal Species	Mold Count (colonies/g)
2	Uninoculated	57.0	*Saccharomyces* sp.	
	Inoculated	54.5	*Aspergillus parasiticus*	120
			Saccharomyces sp.	
4	Uninoculated	55.5	non-sporulating fungus with white septate mycelia	20
			Rhizopus sp.	
			Penicillium sp.	10
			A. flavus	10
			Saccharomyces sp.	
	Inoculated	54.5	*A. parasiticus*	50
			Rhizopus sp.	
			Saccharomyces sp.	
6	Uninoculated	50.7	*A. flavus*	20
			non-sporulating fungus with white septate mycelia	1
			Rhizopus sp.	
			Saccharomyces sp.	
	Inoculated	52.5	*A. parasiticus*	228
			Rhizopus sp.	
			non-sporulating fungus with white septate mycelia	11
			Saccharomyces sp.	

[1] Aflatoxin content was determined by TLC chromatographic technique but no aflatoxin was detected throughout the duration of storage.

[2] Unprocessed roots were inoculated with *A. parasiticus*.

Results show that despite the luxuriant growth of *A. flavus*, processed cassava roots contained either only traces of aflatoxin B_1 or none at all (Tables 5 and 6). This strongly supports the earlier

Table 5. Moisture content (dry basis), mold count and fungal species identified from stored cassava chips.[1]

Storage Period After Drying Chips (weeks)	Treatment	Moisture Content (%)	Fungal Species	Mold Count (colonies/g)
1	Inoculated	8.6	*A. flavus*	2,767
			A. niger	67
	Uninoculated	8.4	*A. wentii*	33
			non-sporulating fungus with whitish septate mycelia	33
2	Inoculated	10.0	*A. flavus*	40
			A. niger	3
			Penicillium sp.	3
	Uninoculated	7.5	*A. niger*	1
			A. fumigatus	1
			Penicillium sp.	1
3	Inoculated	10.0	*A. flavus*	40
			non-sporulating fungus with whitish septate mycelia	40
	Uninoculated	10.0	*A. niger*	15
			Penicillium sp.	1
			A. fumigatus	1
4	Inoculated	15.0	*A. flavus*	36
			A. fumigatus	33
			Penicillium sp.	8
			A. niger	16
	Uninoculated	12.5	*A. flavus*	1
			A. fumigatus	1
			A. niger	1
			Penicillium sp.	14
5	Inoculated	10.0	*A. wentii*	210
			A. niger	20
	Uninoculated	10.0	*Penicillium* sp.	5
			A. niger	1
			A. fumigatus	1
6	Inoculated	12.5	*A. fumigatus*	12
			A. oryzae	11
	Uninoculated	12.5	*A. candidus*	2
			A. oryzae	8
7	Inoculated	10.0	*A. oryzae*	12
			Penicillium sp.	1
	Uninoculated	7.5	*A. niger*	2
			A. fumigatus	1
8	Inoculated	10.0	*Rhizopus* sp.	
	Uninoculated	12.5	*A. fumigatus*	3

[1] Aflatoxin content was determined by TLC chromatographic technique but no aflatoxin was detected throughout the duration of storage.

Table 6. Moisture content, aflatoxin content, mold count and fungal species identified in stored cassava cubes.

Storage Period After Drying Cubes (weeks)	Treatment	Moisture Content (%)	Aflatoxin Content	Fungal Species	Mold Count (colonies/g)
1	Inoculated Blanched	12.5	not detectable	*A. flavus*	110
	Unblanched	12.5	not detectable	*A. flavus*	33
				A. glaucus	1
	Uninoculated Blanched	10.0	not detectable	*Penicillium* sp.	30
				A. fumigatus	3
				A. niger	7
	Unblanched	12.5	not detectable	*Penicillium* sp.	20
				non-sporulating fungus with whitish septate mycelia	1

Table 6. Continued . . .

Storage Period After Drying Cubes (weeks)	Treatment	Moisture Content (%)	Aflatoxin Content	Fungal Species	Mold Count (colonies/g)
2	Inoculated Blanched	10.0	trace	*A. flavus*	137
				Penicillium sp.	1
	Unblanched	10.0	not detectable	*A. flavus*	81
				A. fumigatus	1
				non-sporulating fungus with whitish septate mycelia	1
	Uninoculated Blanched	10.0	not detectable	*A. flavus*	16
				A. niger	5
				A. fumigatus	5
				Penicillium sp.	67
	Unblanched	12.5	trace	*A. flavus*	11
				Penicillium sp.	50

Table 6. Continued . . .

Storage Period After Drying Cubes (weeks)	Treatment	Moisture Content (%)	Aflatoxin Content	Fungal Species	Mold Count (colonies/g)
3	Inoculated Blanched	12.5	trace	*A. flavus*	90
	Unblanched	10.0	trace	*A. flavus*	67
	Uninoculated Blanched	10.0	not detectable	*Penicillium* sp.	20
				A. fumigatus	7
	Unblanched	10.0	not detectable	*Monocillium* sp.	2
				A. fumigatus	2
				A. flavus	2
				Penicillium sp.	2
				A. wentii	2
4	Inoculated Blanched	10.0	not detectable	*A. flavus*	209
				Rhizopus sp.	

Table 6. Continued . . .

Storage Period After Drying Cubes (weeks)	Treatment	Moisture Content (%)	Aflatoxin Content	Fungal Species	Mold Count (colonies/g)
	Unblanched	10.0	not detectable	*A. flavus*	2
				A. niger	1
	Uninoculated Blanched	10.0	not detectable	*Penicillium* sp.	3
				A. fumigatus	1
	Unblanched	10.0	not detectable	*A. restrictus*	3
5	Inoculated Blanched	10.0	trace	*A. flavus*	127
	Unblanched	10.0	not detectable	*A. flavus*	1
				A. candidus	1
				Penicillium sp.	1
	Uninoculated Blanched	12.5	not detectable	*A. fumigatus*	28

Table 6. *Continued . . .*

Storage Period After Drying Cubes (weeks)	Treatment	Moisture Content (%)	Aflatoxin Content	Fungal Species	Mold Count (colonies/g)
	Unblanched	12.5	not detectable	*Penicillium* sp.	31
				A. niger	3
				A. restrictus	3
6	Inoculated Blanched	12.5	trace	*A. flavus*	10
				Penicillium sp.	11
				A. niger	10
	Unblanched	10.0	not detectable	*Monocillium* sp.	63
				A. fumigatus	13
				A. niger	14
				Rhizopus sp.	
	Uninoculated Blanched	10.0	not detectable	*Monocillium* sp.	77
				A. fumigatus	50

Table 6. Continued . . .

Storage Period After Drying Cubes (weeks)	Treatment	Moisture Content (%)	Aflatoxin Content	Fungal Species	Mold Count (colonies/g)
	Unblanched	10.0	not detectable	*Penicillium* sp. *A. flavus*	60 14
7	Inoculated Blanched	10.0	not detectable	*A. flavus*	190
	Unblanched	10.0	not detectable	*Monocillium* sp. *A. fumigatus*	40 10
	Uninoculated Blanched	12.5	not detectable	*Penicillium* sp. *A. flavus*	4 1
	Unblanched	10.0	not detectable	*A. flavus*	1

Table 6. Continued . . .

Storage Period After Drying Cubes (weeks)	Treatment	Moisture Content (%)	Aflatoxin Content	Fungal Species	Mold Count (colonies/g)
	Unblanched	10.0	not detectable	*Penicillium* sp. *A. flavus*	60 14
7	Inoculated Blanched	10.0	not detectable	*A. flavus*	190
	Unblanched	10.0	not detectable	*Monocillium* sp. *A. fumigatus*	40 10
	Uninoculated Blanched	12.5	not detectable	*Penicillium* sp. *A. flavus*	4 1
	Unblanched	10.0	not detectable	*A. flavus*	1

Table 6 Continued .....

Storage Period After Drying Cubes (weeks)	Treatment	Moisture Content (%)	Aflatoxin Content	Fungal Species	Mold Count (colonies/g)
				A. fumigatus	1
				Monocillium sp.	1
				Penicillium sp.	1
8	Inoculated Blanched	12.5	not detectable	*A. flavus*	108
	Unblanched	12.5	not detectable	*Monocillium* sp.	73
				A. fumigatus	44
	Uninoculated Blanched	10.0	not detectable	*A. niger*	20
				A. flavus	3
	Unblanched	10.0	not detectable	*Monocillium* sp.	10
				A. funigatus	27

contention that the extent of mold infection is not a good measure of the degree of aflatoxin production.

Stored Cassava Chips

Due to generally low moisture levels in uninoculated samples (Table 5), reduced mold counts were obtained. Among the most prevalent fungi were *Aspergillus wentii, A. niger,* and *Penicillium* sp. Other species of fungi identified included *A. fumigatus, A. flavus, A. candidus* and *A. oryzae*. Aflatoxin formation was not observed in uninoculated samples throughout the course of storage.

For cassava chips inoculated with *A. flavus,* plating revealed contamination with other storage fungi like *A. niger, Penicillium* sp., *A. fumigatus, A. wentii* and *A. oryzae* but *A. flavus* was the most dominant mold attacking the samples. Despite the aseptic conditions during inoculation, this contamination occurred possibly due to the invasion of other organisms into the inoculated samples during drying in the open and prior to inoculation.

Observations further revealed a sudden reduction in mold count of inoculated samples from 2,767 colonies/g after 1 week of storage to 40 colonies/g after 2 weeks. This indicates that the host tissue could not furnish adequate quantities of nutrients to the microorganisms so as to sustain their growth during storage.

Stored Cassava Cubes

In general, uninoculated cassava cubes showed lower mycofloral count (Table 6). Blanching of cassava cubes before drying generally inhibited the growth of other fungi but not *A. flavus*. Higher counts of this species was more often obtained in blanched cassava cubes than in unblanched ones. This may be due to the impairment of enzyme activity and subsequent biosynthesis of secondary metabolites like phenols, terpenes and coumarines which inhibit the growth of fungi.

The predominant fungi observed were *Penicillium* sp., *A. fumigatus,* and *Monocillium* sp. During storage, trace amount of aflatoxin was detected in the different treatments but apparently aflatoxin production is not affected by storage duration, blanching and inoculation. These observations could possibly be explained as follows. First, samples might not have been thoroughly mixed, hence, the representative 50 g sample used for toxin extraction contained no aflatoxin. Second, absence of aflatoxin formation may be associated with the degradation process caused by some agents. Majunder et al. (1965) reported that *A. flavus* produces other metabolites aside from aflatoxin such as glucosone, β -nitropropionic acid, kojic acid, a pyran, penicillin F, and flavacol which may cause alteration of the structure of aflatoxin. Moreover, environmental conditions such as moisture and temperature greatly influence aflatoxin production.

Thus, aflatoxin can only be formed under certain moisture levels and temperature.

A. flavus was the dominant mold attacking blanched cassava cubes inoculated with the same fungus. Contaminating organisms occurred less frequently. Oftentimes, a homogeneous population of the fungi was noted. However in unblanched cassava cubes, plating revealed contamination by other storage fungi like *A. glaucus, A. fumigatus, Penicillium* sp., *A. niger, A. candidus* and *Monocillium* sp. Although yeasts were not counted, they usually outnumbered the fungal contaminants mentioned.

Aflatoxin-Like Compound in Cassava

An aflatoxin-like compound which also exhibited blue flourescence was present in both processed and unprocessed cassava samples infected by *A. flavus*. Samples which were not dried after 48 hours, as well as unprocessed stored cassava roots showing vascular discoloration exhibited this blue fluorescence but not the freshly harvested roots. This fluorescent compound was mistaken as aflatoxin B_1 but confirmatory test showed it is not. Similar observation was noted by Nagarajan et al. (1973) as well as Nartey (1966) using cassava samples. This is perhaps due to the production of coumarine components (e.g. scopoletin, scopolin and esculin) in cassava with vascular discoloration resulting from stress or the biosynthesis of a complex U.V. fluorescent material associated with the growth of *A. flavus*.

LITERATURE CITED

ANDERSON, H.W., HEHRING, E.E. and WICHSER, W.R. 1975. Aflatoxin contamination of corn in the field. J. Agr. Food Chem. 23:775-782.

ASHWORTH, L.J., SCHROEDER, H.W. and LANGLEY, B.C. 1965. Aflatoxin environmental factors governing occurrence in Spanish peanuts. Science 148:1228-1229.

CAGAMPANG, G.B. and RODRIGUEZ, F.M. 1980. Methods of analysis for screening crops of appropriate qualities. IPB Bulletin No. 2. Analytical Service Lab. I.P.B., U.P. at Los Baños, p. 33.

CALDERWOOD, D.L. and SCHROEDER, H.W. 1968. Aflatoxin development and grade of undried rough rice following prolonged storage in aerated bins. U.S. Dept. Agr. Res. Serv. Rep. 52-56. 32p.

DIENER, U.L. 1976. Environmental factors influencing mycotoxin formation in the contamination of foods. Proc. Amer. Phytopath. Soc. St. Paul, Minnesota. 3:126-139.

FENNEL, D.I., LILLEHOJ, E.B. and KWOLEK, W.F. 1975. *Aspergillus flavus* and other fungi associated with insect-damaged field corn. Cereal Chem. 52:314-321.

GALLOWAY, L.D. 1935. The moisture requirements of mold fungi with special reference to mildew in textiles. J. Textile Inst. Trans. 26:123-129.

ILAG, L.L. 1977. Comparative ability of various agricultural commodities to support aflatoxin formation. Abstract of paper presented at the 14th annual meeting of the Phil. Phytopathological Society Inc., Bacolod City. 18-20 May 1977.

INGRAM, J.S. and HUMPHRIES, J.R.O. 1972. Cassava storage: A review. Trop. Sci. 14:131-148.

JONES, B.D. 1972. Methods of Aflatoxin Analyses. London, Tropical Products Institute Report G70. p. 32.

KNAKE, R.P. and DEYOE, C.W. 1973. Production of aflatoxin by *Aspergillus parasiticus* in maturing white corn. Poultry Sci. 52:20-49.

MAJUNDER, S.K., NARASIMHAN, K.S. and PARPIA, H.A.B. 1965. Microecological factors of microbial spoilage and the occurrence of mycotoxins on stored grains. *In* Mycotoxins in Foodstuffs. G.N. Wogan (ed.). MIT Press, Cambridge. pp. 27-47.

MARSH, P.B. and TAYLOR, E.E. 1958. The geographic distribution of fiber containing fluorescent spots associated with *Aspergillus flavus* in in U.S. cotton crop. Plant Dis. Reptr. 42:1368-1371.

NAGARAJAN, V., BHAT, R.V. and TULPEELE, P.G. 1973. Aflatoxin-like factor in cassava (*Manihot utillissima*). Environ., Physio. and Biochem. 3:13-18.

NARTEY, F. 1966. Aflatoxin of *Aspergillus flavus* grown on cassava. Physiol. Plant. 19:818-822.

PANASSENKO, V.T. 1941. Mould fungi of confectionery goods and their control. Microbiology (USSR) 10:470-479.

PHILLIPS, T.P. 1974. Cassava Utilization and Potential Markets. International Development Research Centre. Ottawa, Canada. 1982 p.

RAMBO, G.W. and CALDWELL, R.W. 1974. *Aspergillus flavus* and aflatoxin in preharvest corn from Indiana in 1971 and 1972. Cereal Chem. 51:595-604.

SCHROEDER, H.W. 1969. Factors influencing the development of aflatoxin in some field crops. J. Stored Prod. Res. 5:187-192.

STOLOFF, I. 1976. Incidence, distribution, and disposition of products containing aflatoxins. Proc. Amer. Phytopath. Soc. (Minnesota) 3:156-171.

TUASON, M.A. and MADAMBA, L.S.P. 1980. Aflatoxin production in copra by *Aspergillus flavus*. Phil. Agric. 63:189-196.

WILDMAN, J.D., STOLOFF, L. and JACOBS, R. 1967. Aflatoxin production by a potent *Aspergillus flavus* Link. isolate. Biotechnology and Bio'eng. 9:420-437.

WOGAN, G.M. 1968. Aflatoxin risks and control measures. Fed. Proc. 27:932-938.