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Mycotoxin Contamination in Grains

Papers presented at the 17th ASEAN Technical Seminar on Grain Postharvest Technology, Lumut, Malaysia, 25–27 July 1995

Editors: **E. Highley and G.I. Johnson**

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Contents

Preface

THE annual ASEAN Technical Seminars on Grain Postharvest Technology, held since 1978, are the premier regional forum for the presentation of the results of postproduction research, development, and extension studies on grains and related durable commodities.

This technical report publishes the papers presented during a session on mycotoxin contamination in grain that was part of the 17th ASEAN postharvest seminar, held in Lumut, Malaysia in July 1995. The seminar was organised by the ASEAN Food Handling Bureau (AFHB) and supported by the National Paddy and Rice Company of Malaysia (BERNAS) and ACIAR.

ACIAR deemed the publication of these papers as important, given the growing concern in ASEAN and elsewhere of the regular occurrence of unacceptable levels of mycotoxins in a number of food and feedstuffs, most importantly maize and peanuts. High levels of mycotoxins are hazardous to human and animal health, and have wider economic implications through loss or devaluation of commodity and by impeding international commodity trade.

The papers in this report constitute a detailed account of the status of food-grain fungi and mycotoxin problems and research in the region. There are country papers from all the ASEAN grain-producing countries, covering all staple grains, and more general papers on grain drying and other means of preventing or reducing fungal infection and mycotoxin contamination. There is also a paper giving a detailed economic assessment of the costs and potential benefits from two ACIAR-supported research projects which gathered information about fungal infection and mycotoxin contamination in staple food and feedstuffs in Southeast Asia. The assessment focuses on aflatoxin contamination of peanuts and maize and its impact on human health and animal production. It suggests that substantial benefits will accrue from technological advances in pre- and postharvest practices likely to be stimulated by the research.

Mycotoxins are a real problem in ASEAN and the wider Asian region, with profound implications for human and animal health, national economies, and international trade. ACIAR will continue to support well-founded collaborative research to further characterise the problem and develop cost-effective solutions.

G.I. Johnson
Coordinator
ACIAR Postharvest Technology Program

Current Knowledge of Fungi and Mycotoxins Associated with Food Commodities in Southeast Asia

J.I. Pitt and Ailsa D. Hocking*

Abstract

A five-year study was carried out on the occurrence of fungi and mycotoxins in all major food commodities traded in Thailand, Indonesia, and the Philippines. More than 1700 samples were examined, including maize, peanuts, rice, soybeans, mung beans, sorghum, cashews, spices, and herbs. Samples were collected randomly from farm storage, middlemen, and retail shops, to follow the time course of infection. Fungi were detected and enumerated by direct plating on Dichloran Rose Bengal Chloramphenicol agar and Dichloran 18% Glycerol agar after surface disinfection in 10% chlorine solution. By this means only fungi actually growing in the foodstuffs were detected. More than 35 000 fungi were isolated and identified. The major fungi found in maize were *Aspergillus flavus* and *Fusarium moniliforme*. In peanuts, the predominant fungi were *A. flavus* and *A. niger*. The most common *Fusarium* species were *F. semitectum* and *F. equiseti*, while *Penicillium citrinum* and *Eurotium* species were isolated frequently also. Infections in rice and beans were dominated by field fungi. In paddy rice, *Trichoconiella padwickii* (= *Alternaria padwickii*) was most commonly encountered. The dominant *Fusarium* species was *F. semitectum*. *Aspergillus* species, including *A. flavus*, were less common. Polished rice was practically free of fungi, presumably due to the heat generated during the dehulling process. Infection levels in most bean samples were low. *F. semitectum* was the species most commonly isolated from mung beans, while higher levels of *A. flavus* were found in soybeans than other beans. Selected samples were assayed for aflatoxins. High levels were often found in maize and peanuts. The potential for production of fumonisins in maize due to the high presence of *F. moniliforme* is also important.

FUNGAL invasion of commodities before and after harvest, and during distribution and storage, is a well recognised problem throughout the world. This is especially true in tropical countries, where mycotoxin contamination of food supplies remains a major threat. Over a period of five years, we studied the occurrence of fungi and mycotoxins in all major food commodities traded in Thailand, Indonesia, and the Philippines. The work was confined to good grades and commercial channels. No attempt was made to study lower grade commodities, both because lower grade materials are highly variable in quality, even within a lot, and because such commodities are difficult to obtain in any systematic way. Most samples studied were of acceptable visual quality, free of visible mould and insect damage.

More than 1700 samples were examined. Major commodities studied in each country were maize, peanuts, soybeans, mung beans, and rice. This paper provides an overview of the results obtained, and of over 700 aflatoxin assays in maize and peanuts.

Results for commodities examined in less detail, or in only one or two countries, including cashews, sorghum, black and red beans, copra, black and white sesame seeds, cassava, black rice, copra, wheat, spices, and herbs, have been reported elsewhere (Pitt et al. 1993, 1994) or will be covered in papers in preparation.

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Materials and Methods

Samples

Samples of major commodities were obtained as far as possible from throughout the distribution chain, from farmer storage, middlemen, and the retail system. Retail samples were chosen randomly, avoiding selection by the retailer. Sample sizes were 400–800 g. Samples were divided into two equal portions: one was examined by local collaborators in each country, and the other was sent to the CSIRO Food Research Laboratory, North Ryde, Sydney, Australia. For insect control, samples for examination in Australia were usually held in quarantine at –20°C for 6 weeks before delivery to the laboratory. In some cases, where insects emerged during transit to Australia, samples were alternately frozen and thawed, sometimes repeatedly, before release. Results reported here are for samples examined at North Ryde.

Mycological examination

At North Ryde, commodities were examined by standard techniques (Pitt et al. 1992). Grains or particles were surface disinfected in 10% household chlorine bleach (i.e. approximately 0.5% active chlorine) for 2 minutes, then 50 grains or particles were plated directly (6–10 particles per plate) onto each of several suitable gelled agar media. General purpose enumeration media used were Dichloran Rose Bengal Chloramphenicol agar [DRBC (King et al. 1979), but with chloramphenicol substituted for chlortetracycline (Pitt and Hocking 1985)], and Dichloran 18% Glycerol agar [DG18 (Hocking and Pitt 1980)], the latter being especially useful for xerophilic fungi. For detection of *Fusarium* and dematiaceous Hyphomycetes including *Alternaria*, *Bipolaris*, *Curvularia*, and *Nigrospora*, Dichloran Chloramphenicol Peptone Agar [DCPA (Andrews and Pitt 1986)] was used, and for fungi producing aflatoxins, Aspergillus Flavus and Parasiticus Agar [AFPA (Pitt et al. 1983)].

Plates were incubated at 25°C for 5–7 days, and then examined both with the unaided eye and under the stereo microscope. Where possible, species were recognised and differentially enumerated at that time. However, all fungi which appeared to be different were subcultured for positive identification, and differential counts adjusted in the light of these results.

Aflatoxin assays

Aflatoxins were assayed essentially by the method of Beebe (1978). Samples were ground, subsampled, extracted with methanol: 0.1N HCl (4: 1), washed with hexane, then extracted with chloroform. If necessary, further clean up was achieved with a silica Sep-Pak (Millipore Waters, Sydney). After concentration, samples were derivatised with trifluoracetic acid (Beebe 1978) and assayed by HPLC using a Shimadzu LC-10A system (Shimadzu, Sydney). Methanol: acetonitrile: water (10: 23: 67) was the solvent system used.

Results and Discussion

Mycology

More than 35 000 fungi were isolated and identified during the course of this study. An overview is presented here.

Maize. The major field fungi found in maize were *Fusarium moniliforme*, present in 323 (84%) of samples and *Lasiodiplodia theobromae*, in 131 (34%) of samples (Table 1). Up to 90% of kernels in an individual sample were infected with *F. moniliforme*, with an overall infection rate of 20%. For *L. theobromae*, infection rates were lower: this species was isolated from up to 46% of kernels in infected samples, but only in 3% of kernels overall. Infection rates with *Aspergillus flavus*, perhaps also a field fungus, were especially high, as it was found in 338 (88%) of the 384 samples examined, up to 100% of kernels in some samples, and overall 31% of the nearly 20 000 kernels examined were infected with this fungus. *Fusarium semitectum*, *Aspergillus niger*, and *Penicillium citrinum* were the other common species (Table 1).

Peanuts. Nearly 500 samples of peanuts were examined. The most common fungi found were similar to those from maize, except that *Fusarium moniliforme* was absent (Table 2). Again, *Lasiodiplodia theobromae* was a common field fungus, but slightly less frequently found than *Macrophomina phaseolina*, reflecting the importance of the latter in legumes. The predominant fungus was again *A. flavus*: this species was present in 482 (97%) of the 497 samples examined, in up to 100% of nuts in infected samples, and in a very high 48% of the almost 25 000 kernels examined overall. *A. niger* was also common, in 85% of samples and 32% of all kernels. *F. semitectum* was the most common *Fusarium* species, found in 15% of

samples, with up to 36% infection rates in infected samples, but in only 1% of kernels examined overall. *Penicillium citrinum* and *Eurotium* species were isolated frequently as well, indicating growth of some fungi during storage (Table 2).

Rice. The species of fungi infecting rice differed from those in maize and peanuts. In paddy rice, the dominant species was *Trichoconiella padwickii* [commonly known as *Alternaria padwickii*, and reported under that name by Pitt et al. (1994)]. This species was found in 104 (70%) of the 148 samples examined, at up to 90% infection rates in infected samples, and in 18% of grains examined overall (Table 3). Field fungi, especially *Alternaria* and *Curvularia* species, were commonly encountered. *Alternaria longissima* was found in 16% of samples, at up to 56% infection rate, but only in 4% of grains overall. The most common *Fusarium* species was *F. semitectum*, which was present in 72% of samples, and 13% of all grains examined. *Aspergillus* species, including *A. flavus*, were less common than in maize and peanuts, being present in 33% of samples, at up to 100% of grains in particular samples, but only at an 8% infection rate overall. Although the storage fungus *Eurotium rubrum* was present in 87% of samples, only 1% of grains were infected overall (Table 3). Polished rice samples examined were practically free of fungi. This was presumably the result of the dehulling process, in which considerable heat is generated in the grains. Samples from Indonesia were mostly from retail stores, but how many had come from the long-term storage used for rice in Indonesia is not known.

Table 1. The field and storage fungi most frequently isolated from Southeast Asian maize (384 samples).

	Infection (%)		
	Samples infected	Range of infection	Particles infected (all samples)
Aspergillus flavus	88	2–100	31
A. niger	66	2–100	11
Eurotium chevalieri	44	2–100	11
Fusarium moniliforme	84	2–90	20
F. semitectum	31	2–36	3
Lasiodiplodia theobromae	34	2–46	3
Penicillium citrinum	49	2–65	5

Table 2. The field and storage fungi most frequently isolated from Southeast Asian peanuts (497 samples).

	Infection (%)		
	Samples infected	Range of infection	Particles infected (all samples)
Aspergillus flavus	97	2–100	48
A. niger	85	2–100	32
Eurotium chevalieri	52	2–100	19
E. rubrum	57	2–100	22
Fusarium semitectum	15	2–36	1
Lasiodiplodia theobromae	34	2–64	5
Macrophomina phaseolina	42	2–60	7
Penicillium citrinum	47	2–100	8

Table 3. The field and storage fungi most frequently isolated from Southeast Asian paddy rice (148 samples).

	Infection (%)		
	Samples infected	Range of infection	Particles infected (all samples)
Alternaria longissima	16	2–56	4
Aspergillus flavus	33	2–100	8
Eurotium rubrum	87	2–60	1
Fusarium semitectum	72	2–65	13
Trichoconiella padwickii	70	2–90	18

Beans. Infection levels in most bean samples were low. *Fusarium semitectum* was the field fungus most commonly isolated from mung beans, and appeared to have a specific association with that crop (Table 4). It was present in 50% of samples, at up to 76% infection in infected samples, and in 7% of beans examined overall. *Aspergillus flavus* and *Eurotium rubrum* were also present in 7% of kernels overall: *A. flavus* in 59% of samples, but *E. rubrum* in only 24%. *Macrophomina phaseolina*, *Aspergillus niger*, and *Penicillium citrinum* were also commonly found, in 17–31% of samples, but at only 1–2% infection overall.

Higher levels of *A. flavus* were found in soybeans than other beans, as it was present in 79% of samples, and 11% of beans were infected overall (Table 5). The storage fungi *Eurotium chevalieri* and *E. rubrum* were also present in 11% of beans overall, perhaps reflecting longer storage periods for soybeans than most other commodities examined.

Mycotoxins

Aflatoxins. Nearly 1000 samples were examined for aflatoxins, including 740 of maize and peanuts, the commodities of most interest. Data are summarised in Table 6, adapted from Lubulwa and Davis (1994), but updated substantially with previously unpublished data.

A simplified version of these data is also given (Table 7). If 50 µg/kg is a level for total aflatoxins considered acceptable and attainable by tropical countries, it is clear that such a target is not being met currently, even in the more highly developed Southeast Asian nations. Samples exceeding 50 µg/kg ranged from 30–50% of those examined. Aflatoxin levels exceeding 300 µg/kg must be considered unsatisfactory by any criterion: about 5% of maize samples from Indonesia and the Philippines exceeded this figure. The 15% of maize samples exceeding 300 µg/kg in Thailand is actually less important, because maize is used almost entirely for animal feeds in

Table 4. The field and storage fungi most frequently isolated from Southeast Asian mung beans (92 samples).

	Infection (%)		
	Samples infected	Range of infection	Particles infected (all samples)
Aspergillus flavus	59	2–100	7
A. niger	31	2–60	2
Eurotium chevalieri	20	2–84	3
E. rubrum	24	2–100	7
Fusarium semitectum	50	2–76	7
Macrophomina phaseolina	17	2–40	1
Penicillium citrinum	20	2–30	2

Thailand. Figures for peanuts were less satisfactory: 33% of Indonesian peanut samples examined exceeded this level, and peanuts are used almost entirely for human food throughout Southeast Asia.

Levels of aflatoxin exceeding 1000 µg/kg must be considered totally unsatisfactory, capable of inducing acute toxic effects in both humans and animals. Few maize samples exceeded 1000 µg/kg aflatoxin, but an

Table 5. The field and storage fungi most frequently isolated from Southeast Asian soybeans (92 samples).

		Infection (%)	
	Samples infected	Range of infection	Particles infected (all samples)
Aspergillus flavus	79	2–100	11
Eurotium chevalieri	44	2–60	11
E. rubrum	54	2–70	11
Fusarium semitectum	39	2–45	4
Lasiodiplodia theobromae	19	2–26	1
Macrophomina phaseolina	19	2–26	2
Penicillium citrinum	26	2–26	2

Table 6. Levels of aflatoxin found in peanut and maize samples from Indonesia, the Philippines and Thailand (µg/kg).[a]

Total aflatoxin (range)	Indonesia Maize %	Indonesia Peanuts %	Philippines Maize %	Philippines Peanuts %	Thailand Maize %	Thailand Peanuts %
≥ 5	68	44	44	67	53	63
> 5 ≤ 10	2	1	9	5	0	4
> 10 ≤ 50	8	10	27	6	17	8
> 50 ≤ 300	18	12	14	6	15	14
> 300 ≤ 1000	3	11	5	9	11	8
> 1000 ≤ 5000	1	17	1	4	4	3
> 5000	0	5	0	3	0	0
Total no. of samples	96	215	146	81	108	94
Total production (t, 1991)	6409	920	4655	34	3990	164

[a] Updated from Lubulwa and Davis (1994).

Table 7. Aflatoxin levels in maize and peanut samples from Indonesia, Thailand and the Philippines.

	Per cent of samples with levels		
	> 50 µg/kg	> 300 µg/kg	>1000 µg/kg
Maize			
Indonesia	22	4	1
Thailand	30	15	4
Philippines	20	6	1
Peanuts			
Indonesia	45	33	22
Thailand	25	11	3
Philippines	22	16	7

alarming 22% of peanut samples examined from Indonesian sources exceeded this figure. Studies to determine why peanuts in Indonesia contain so much more aflatoxin than in the other countries, and the status of peanuts in other Southeast Asian countries, are urgent.

The high level of *Fusarium moniliforme* in Southeast Asian maize is of concern because of the potential for fumonisin production. The ability of Southeast Asian isolates of *F. moniliforme* to produce fumonisins has already been demonstrated (Miller et al. 1993).

As reported above, ***Fusarium semitectum*** is widespread and abundant in Southeast Asian commodities. However, available information indicates only a low potential to produce significant mycotoxins. Even heavily infected commodities appear to carry a low health risk.

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Controlling Aflatoxin Contamination in Maize Stored under Tropical Conditions

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Abstract

A study was made of how well a prototype in-store drying system would control aflatoxin contamination and how much energy the system would consume. Tests were done using air with a defined relative humidity and temperature. To maintain high quality maize by limiting aflatoxin production, high moisture maize should be dried to 18 or 19% moisture content (wet basis) within 2 days and continuously dried to 14% within 14 days. To decrease moisture content from 19 to 12–13% using an airflow rate of 3.6–4.6 $m^3/min/m^3$ of maize, required an electrical energy consumption of 0.46–0.9 MJ/kg water evaporated or 16–27 baht/t of maize.

IN Thailand, maize is usually harvested at 18–30% moisture content (unless otherwise noted, wet basis moisture contents are used throughout this paper) and then shelled by a machine. To prevent fungi growing and producing toxins, maize should be dried to 14%. This may be achieved by sun drying the maize on a concrete pad or by mechanically drying it. Drying on a concrete pad may not be successful, especially during the rainy season because of either insufficient sunshine or occurrence of rain. When high moisture maize is not dried immediately, aflatoxins are produced and the maize becomes rancid. Kositcharoenkul et al. (1992) found that a large amount of aflatoxin production occurred in maize held by merchants in silos or warehouses.

Kalchick et al. (1981) conducted maize drying experiments at a farm in Michigan state, in the northern part of the USA, for three years. It was found that in-store maize drying using ambient air and a low airflow rate, consumed energy at approximately 3.2 MJ/kg water evaporated. When drying air was heated, the energy consumption increased to 6.6 MJ/kg water evaporated.

Brooker and Duggal (1982) used the mathematical model of Thompson et al. (1968) to study parameters, such as initial moisture content and initial temperature of maize, that affected safe storage time of maize (defined as the period before there is a loss of 0.5% of dry matter). Simulations were of two types: no ventilation and airflow rate of 0.111 $m^3/min/t$. The weather data of Missouri state were used in this model. With no ventilation, the initial moisture content and temperature had the greatest influence on safe storage time. Thus it was shown that at a temperature of 15.6°C and an initial moisture content of 16%, the safe storage time was 180 days. However, the safe storage time decreased markedly to 25 days if the initial moisture level was 18%. The safe storage time was reduced to 55 days if the initial moisture content was 16% and the temperature 21°C. When ventilation was used, the rate of airflow rather than initial moisture content was most important. At a flow rate of 0.111 $m^3/min/t$, and an initial moisture content of 20%, the period of safe storage was 60 days, but when the airflow rate was doubled this was increased to 120 days.

Eckhoff et al. (1984) investigated the inhibition of microorganism growth by sulfur dioxide gas mixed with ambient air. The initial moisture content of maize was 29.8% wet-basis. It was found that the number of microorganisms was very small when the gas mixture flowed from the top to the bottom of the bin. However, if the gas mixture flowed in the opposite direction, the microorganisms were widely spread at the top of the bin where there was high moisture maize. Microor-

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ganisms were distributed throughout the bin when sulfur dioxide gas was mixed with maize before loading of the bin.

Wongvirojtana et al. (1992) researched the feasibility of in-store maize drying in a tropical climate. The results showed that the in-store drying technique was feasible for maize drying in a hot and humid climate. The aflatoxin B_1 produced after drying was quite low at conditions of airflow rate 2–2.5 $m^3/min/m^3$ and initial moisture content not higher than 19%.

El-Gazzar and Marth (1988) slowed the growth of *Aspergillus parasiticus* and prevented aflatoxin production by using hydrogen peroxide at various concentrations on 10^6 spores of *Aspergillus parasiticus* inoculated on a glucose–yeast–salts medium. The results showed that during 90 days of incubation at temperatures of 14 and 28°C, the growth rate of *Aspergillus parasiticus* was slowed at concentrations of 0.3 and 0.5% hydrogen peroxide.

Wongurai et al. (1992) studied the effect of water activity on *Aspergillus flavus* growth in maize. It could be concluded that *Aspergillus flavus* severely affected maize at a water activity of 0.94–1 and a temperature of 28°C, corresponding to equilibrium moisture contents of 22–41%. With a water activity of 0.85–0.93 and a temperature of 28°C (equivalent to an equilibrium moisture content of 18–20%), the *Aspergillus flavus* grew moderately well. When water activity was less than 0.85 (corresponding to equilibrium moisture content lower than 18%), *Aspergillus flavus* growth was completely inhibited.

Nilrattanakoon et al. (1993) studied the optimum rate of carbon dioxide fumigation used to control aflatoxin rapidly produced in high moisture maize. The maize bulk previously covered by plastic sheets was fumigated with different rates of carbon dioxide: 0.5, 0.75, 1 and 2 kg/t of maize. The results indicated that the optimum rate of carbon dioxide fumigation to efficiently control aflatoxin and growth of *Aspergillus flavus* was 0.5 kg/t of maize.

From several years of research as outlined above, in-store drying was found to be feasible for maize drying under hot and humid climates at initial moisture contents lower than 20% without aflatoxin problems. The objective of this research was to study the prevention of aflatoxin production and energy consumption of in-store maize drying. A prototype was designed, installed and tested at Pra Buddha Bath Settlement Agricultural Cooperatives.

Materials and Methods

The duct system for maize drying shown in Figure 1 consists of main ducts (Nos 1, 2, 3, 4, 5 and 6) and branching ducts (Nos 7–9, 10–12 and 13–15). The 0.4 × 0.4 m cross-sectional main duct was made from galvanised steel sheets of 1.5 mm thickness. For the branching ducts, the distributor plate was of 1.5 mm thickness with perforations of 1.5 mm diameter. It was designed with a 55 cm diameter and 1.20 long semicircular duct so that the distribution of stress would be equally stable throughout thickness of the material. The backward curved blade centrifugal fan was driven by a 3.7 kW motor. The temperature was measured by a type K thermocouple (accuracy of ± 1°C) from which measurements were transferred to a data logger. The positions at which pressure and temperature were monitored are shown in Figure 1. Three trials of in-store maize drying were performed. In the first two trials, 30 t of maize with initial moisture contents of 23.4 and 18.9%, respectively, were stored in a shed of 5 × 5 m. The depth of the maize bulk was 1.5 m. Ambient air at temperature of 29.2 and 31°C and relative humidity of 58 and 78%, respectively, was periodically ventilated through the maize bulk until the grain was dried to 13–14% moisture content. In the third trial, 40 t of maize 1.7 m deep and with an initial moisture content of 18.7% was stored in the same shed. The air ventilated through the maize bulk had a temperature of 30°C and a relative humidity of 57.4%.

Maize samples were taken to test moisture content, aflatoxin B_1 production and percentage of maize kernel infected with *Aspergillus flavus*. To determine moisture content, maize was dried in an air oven at a temperature of 103°C for 72 hours. Aflatoxin B_1 production was detected by thin-layer chromatography. In the first experiment at 23.4% moisture content, maize was collected at depths of 30, 60, 90 and 150 cm from the surface of maize bulk before and after drying and at depths of 50 and 120 cm while maize was being dried. For each depth, maize samples were collected at three positions. In the second and third experiments, at 18.9 and 18.7% moistute content, respectively, before and after drying, maize was collected at 0, 50, 100 and 150 cm depths from the surface of maize bulk and at 0 and 30 cm depths during drying. For each depth, maize was collected at two positions. To analyse the amount of aflatoxin B_1 and percent of maize kernel infected with *Aspergillus flavus*, maize samples must be dried to 14% and kept at a temperature lower than 10°C to inhibit growth of microorganisms.

Results and Discussion

Distribution of maize moisture at each depth

Figures 2 and 3 show the changes in local moisture content at 50 and 30 cm depths from the surface. It was found that maize moisture, at different positions at the same depth, was slightly different because of uneven flow of air through the maize bulk. As mentioned above, the average moisture content shown in Figures 4 and 5, and calculated by arithmetic mean, represented the local moisture content. The moisture content at a depth of 50 cm from the surface was higher than the moisture content at 120 cm. For an initial maize moisture of 18.7%, the results were similar to those shown in Figures 2–5.

Energy consumption

Figure 6 shows the profile of moisture content of maize after drying. For an initial moisture content of 23.4%, the moisture content of maize was slightly higher at the surface of the bulk, but when the initial moisture content of maize was 18.9 or 18.7%, the moisture content throughout the maize bulk was almost uniform. In calculating the energy consumption of in-store maize drying, it was assumed that the moisture content of maize after drying was the same at all depths.

Energy consumption measurements are shown in Table 1. When maize was dried from an initial moisture content of 19 to 12–13% using an airflow rate of 3.6–4.6 $m^3/min/m^3$ of maize, the energy consumption was 0.46–0.9 MJ/kg water evaporated or 16–27 baht/t of maize for electricity (electricity price was 1.65 baht/kWh).

Temperature in maize bulk

Figure 7 shows the changes in temperature in the maize bulk and for ambient air during the experiment. At the early stage of maize drying, the moisture

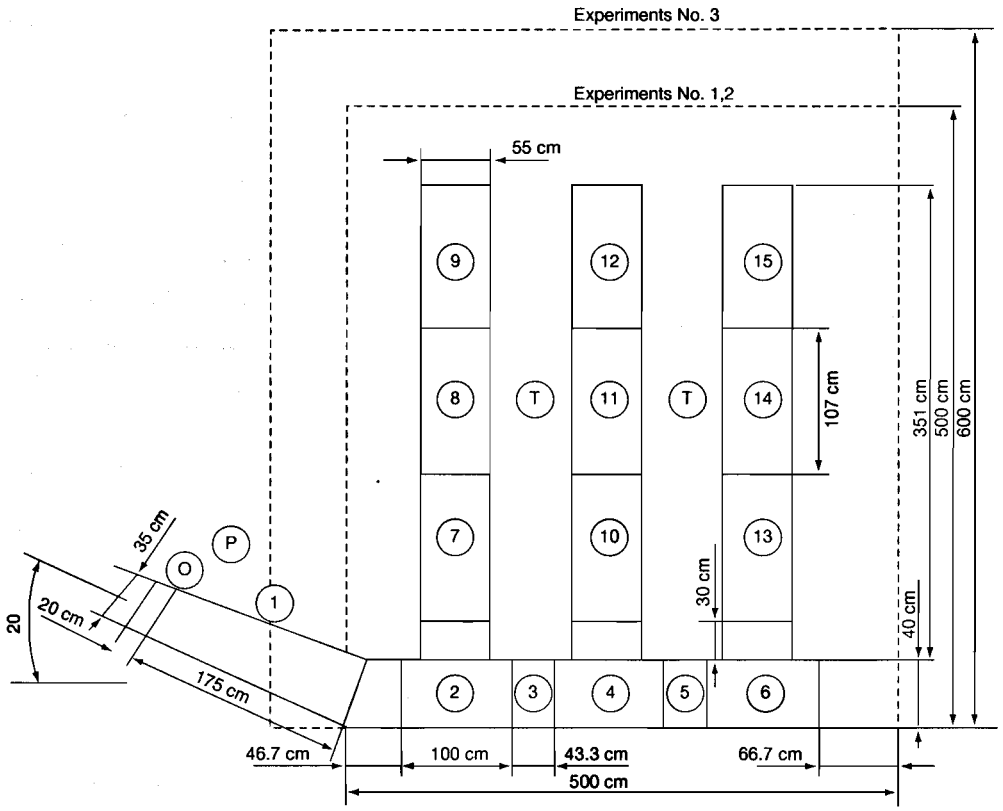

Figure 1. Plan of ducts on floor showing position of temperature (T) and pressure (P) sensors. See text for further details.

content of maize at 80 cm depth in the bulk was higher than that at 150 cm depth. Therefore, the temperature in maize bulk at 150 cm depth was closer to ambient air temperature than that at 80 cm depth. At the later stages of drying, the temperature throughout the maize bulk was in thermal equilibrium with the ambient air ventilated through the bulk.

Maize quality

The measurement of maize qualities such as the amount of aflatoxin B_1 and percentage of maize infected with *Aspergillus flavus* was an important aspect of this research.

Figure 8 shows the changes in average moisture content of maize and the amount of aflatoxin B_1 produced at an initial moisture content of 23.4%. Before drying, aflatoxin B_1 could not be detected but on the second day of drying, the amount of aflatoxin B_1 increased sharply.

Figure 9 shows the changes in average moisture content of maize and the percent of maize infected with *Aspergillus flavus* at an initial moisture content of 23.4%. There was a small percentage of maize infected with *Aspergillus flavus* at the beginning of drying.

The percentage infection rapidly increased during the second day of drying. After that, the percentage of infected maize was stable. When interpreting Figures 8 and 9, it should be noted that *Aspergillus flavus* in its growth phase does not readily produce aflatoxin B_1. However, after the second day of drying, *Aspergillus flavus* entered its stationary phase and then produced aflatoxin B_1.

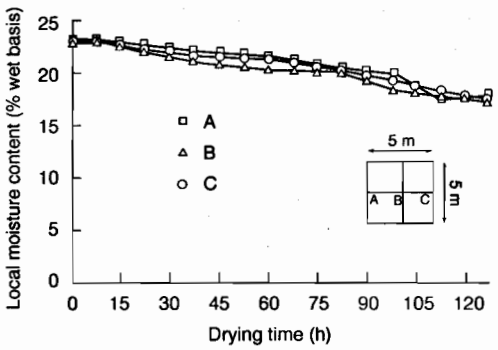

Figure 2. Plot of moisture content versus time at 50 cm depth in bulk maize (initial moisture content = 23.4% wet basis).

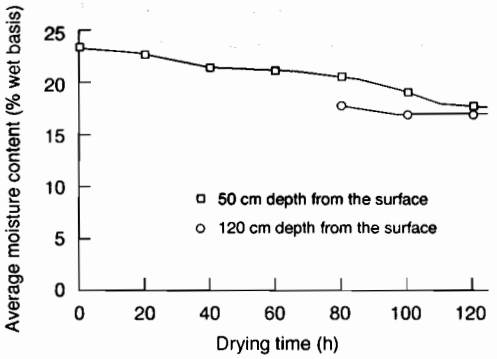

Figure 4. Plot of average moisture content versus drying time at 50 and 120 cm depths in bulk maize (initial moisture content = 23.4% wet basis).

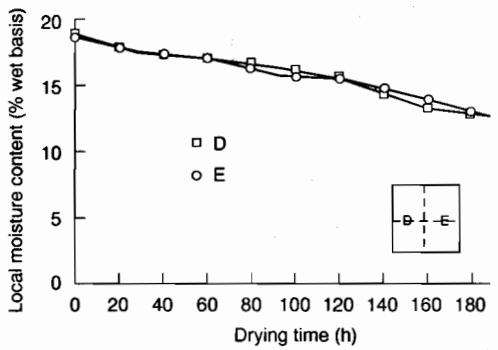

Figure 3. Plot of moisture content versus time at 30 cm depth in bulk maize (initial moisture content = 18.9% wet basis).

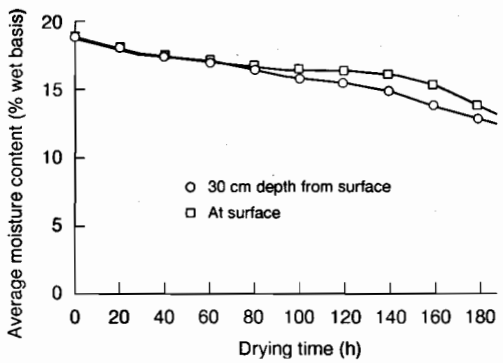

Figure 5. Plot of average moisture content versus drying time at 0 and 30 cm depths in bulk maize (initial moisture content = 18.9% wet basis).

Figure 10 shows the changes in average moisture content of maize and the amount of aflatoxin B_1 produced at an initial moisture level of 18.9% wet-basis. Aflatoxin B_1 could not be detected during the early phase of maize drying. During the seventh day of the drying, there was a marked increase in the amount of aflatoxin B_1 produced. After that, the amount of aflatoxin B_1 being produced rapidly decreased but the percentage of maize infected with *Aspergillus flavus* was constant. When the initial moisture content was 18.7% (see Figs 11 and 12), the results were similar to those at other moisture contents.

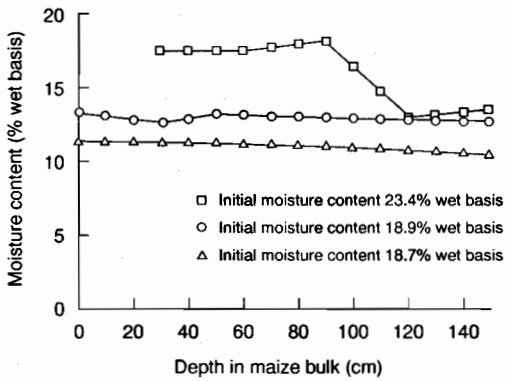

Figure 6. Moisture content profiles in bulk maize after drying from various initial moisture contents.

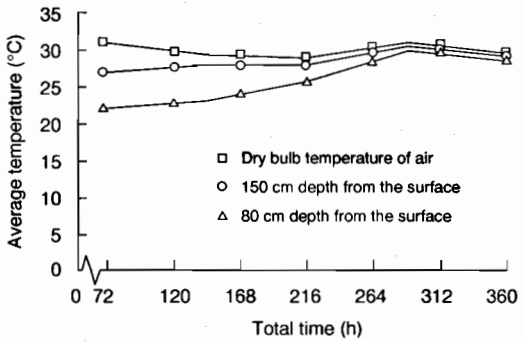

Figure 7. Changes in temperature at 80 and 150 cm depths in bulk maize with drying time, compared with drying air temnperature (initial moisture content = 18.7% wet basis).

Table 1. Experimental drying results.

Descriptions	Experiment		
	No. 1	No. 2	No. 3
Average drying air conditions			
Temperature (°C)	29.2	31.0	29.7
Specific airflow rate (m^3/min/m^3 of maize)	4.6	4.5	3.6
Ambient air conditions			
Average temperature (°C)	29.2	31.0	29.7
Average relative humidity (%)	58.0	78.0	57.4
Conditions of maize			
Initial moisture content (% wet basis)	23.4	18.9	18.7
Initial Aflatoxin B_1 (ppb)	0.0	0.0	53.5
Final moisture content (% wet basis)	15.2	13.0	11.8
Final Aflatoxin B_1 (ppb)	250	40	32.7
Initial weight (t)	31	30	40
Energy Consumption			
Total time (h)	360	504	360
Drying time (h)	126	186	134
Electricity (kWh)	449	498	396
Specific energy consumption (MJ/kg water evaporated)	0.54	0.88	0.46
Electricity cost (baht/t)	24	27	16.33

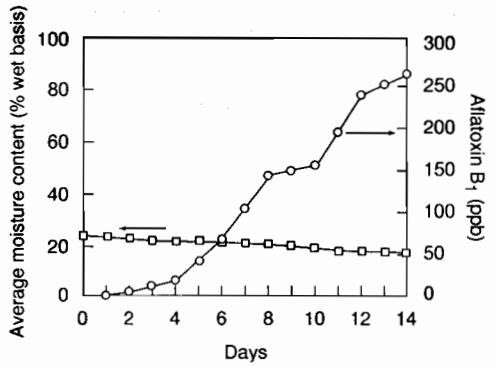

Figure 8. Changes in average moisture content and aflatoxin B_1 concentration at 50 cm depth in bulk maize versus drying time (initial moisture content = 23.4% wet basis).

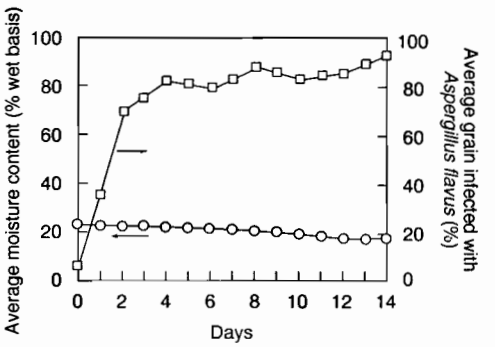

Figure 9. Changes in average moisture content and average grain infected with *Aspergillus flavus* at 50 cm depth in bulk maize versus drying time (initial moisture content = 23.4% wet basis).

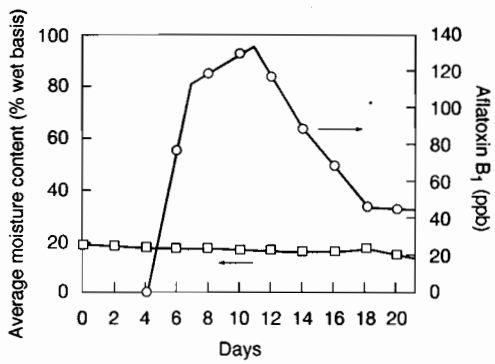

Figure 10. Changes in average moisture content and aflatoxin B_1 concentration at 30 cm depth in bulk maize versus drying time (initial moisture content = 18.9% wet basis).

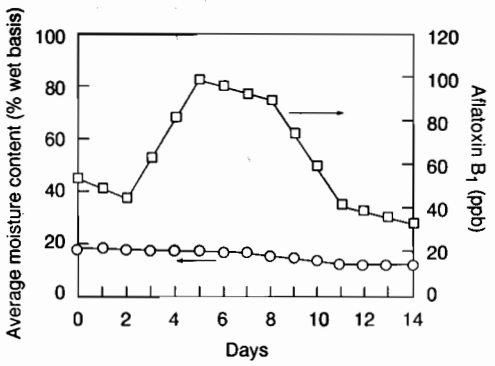

Figure 11. Changes in average moisture content and aflatoxin B_1 concentration at 30 cm depth in bulk maize versus drying time (initial moisture content = 18.7% wet basis).

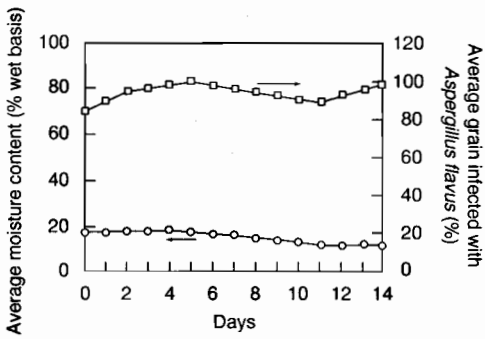

Figure 12. Changes in average moisture content and average grain infected with *Aspergillus flavus* at 30 cm in bulk maize versus drying time (initial moisture content = 18.7% wet basis).

Conclusion

The prototype for in-store drying was reasonably efficient in terms of quality and energy consumption when the initial moisture content of maize was not more than 19% wet-basis. The air ventilated through the maize bulk was uniform. High moisture maize should be dried to a moisture content of 18–19% within two days in order to control aflatoxin B_1, and continuously dried to 14% within 14 days. To decrease moisture content from 19 to 12–13% wet-basis using an airflow rate of 3.6–4.6 $m^3/min/m^3$ of maize, energy consumption was 0.46–0.9 MJ/kg water evaporated. The electricity cost was 16–27 baht/t of maize.

Acknowledgment

The authors would like to express their sincere thanks to the Australian Centre for International Agricultural Research (ACIAR) and the Department of Technology Promotion, Ministry of Science, Technology and Environment, Thailand, for financial support to this project, and to Pra Buddha Baht Settlement Agricultural Cooperatives for their assistance in installation and test of in-store drying system.

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Occurrence and Significance of Storage Fungi and Associated Mycotoxins in Rice and Cereal Grains

Zofia Kozakiewicz*

Abstract

Members of the genus *Aspergillus* and its close relative *Penicillium* are the dominant fungal contaminants of stored grain. Species of both genera are responsible for the production of harmful mycotoxins.

This paper reviews the distribution and occurrence of the two genera, the conditions common to Asian storage parameters that favour their growth, and the importance of correct identification at the species level. Criteria for safe storage practice are also discussed.

The mycotoxins that they produce are considered in depth, relative to the conditions responsible for their development and their detrimental effects on the health of humans and livestock.

POSTHARVEST losses of durable crops are estimated at 10% worldwide, reaching an unacceptable level of over 20% in developing countries, particularly in the tropics (FAO 1991). Humid tropical conditions, resulting in fungal growth on stored food and feedstuffs, often strongly favour the production of mycotoxins.

Many of the factors that cause postharvest losses are avoidable. For example, in rice, intensified production programs cause stress in new varieties leading to shattering of the grain and resultant microbial growth. Multiple cropping forces rice to be harvested during the wet season. The flow of labour from rural to urban areas means that stacks or sacks of rice are held longer for threshing and drying. These delays result in yellowing and other discolouration of the grain, a sign of fungal deterioration. Mechanisation reduces holding time at high moisture, but if not used correctly, can itself cause damage and so promote fungal growth.

Members of the genus *Aspergillus* and its close relative *Penicillium* are the dominant fungal contaminants of stored products, foods, and feedstuffs. Both genera are major producers of mycotoxins. Some aspergilli are human and animal pathogens. But others are also beneficial to humans, notably as producers of penicillin (*P. chrysogenum*), and in the food industry for cheese-making (*P. camembertii*, and *P. roquefortii*), and in soy sauce production (*A. oryzae*). Therefore, because of their dual role, both harmful and beneficial, correct identification at the species level is of paramount importance (Kozakiewicz et al. 1994).

Important *Aspergillus* Species, and Their Mycotoxins, Significance, and Occurrence

Many *Aspergillus* species are found on cereals, nuts, coffee beans, cocoa pods and palm kernels. Only those species of significance in the tropics and their associated mycotoxins will be discussed here.

Most *Aspergillus* toxins can be placed in one of three groups: carcinogens (aflatoxin), nephrotoxins (ochratoxin A) and neurotoxins (territrems). Only a few are currently considered to pose a serious threat to human and animal health. Table 1 lists important *Aspergillus* species and their toxins.

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Table 1. Important mycotoxins produced by *Aspergillus* species and their teleomorphs.

Species	Mycotoxins
A. clavatus	cytochalasin E, patulin, tryptoquivalins
A. fumigatus	fumigaclavines, fumigatin, fumitoxins, fumitremorgins, gliotoxin, tryptoquivalins, verruculogen
A. restrictus	mitogillin
Eurotium amstelodami	physions
E. chevalieri	physions
E. repens	physions
E. rubrum	physions
A. terreus	citreoviridin, citrinin, patulin, territrems
A. versicolor	nidulotoxin, sterigmatocystin
Emericella nidulans	nidulotoxin, sterigmatocystin
A. candidus	terphenyllin, xanthoascin
A. flavus	aflatoxin B_1, B_2, aflatrem, kojic acid, maltoryzin, 3-nitropropionic acid
A. nomius	aflatoxin B_1, B_2, G_1, G_2, kojic acid, nominine
A. parasiticus	aflatoxin B_1, B_2, G_1, G_2, kojic acid, 3-nitropropionic acid
A. tamarii	cyclopiazonic acid, fumigaclavine A, kojic acid
A. niger	malformins, naphthopyrones
A. ochraceus	ochratoxin, penicillic acid, secalonic acid A, xanthomegnin, viomellein

Aflatoxin producers

Aspergillus flavus, A. parasiticus, and A. nomius

These three species produce aflatoxins and are probably the most important of all mycotoxigenic species, because aflatoxin is carcinogenic. Differentiation between these species is important since *A. flavus* produces only aflatoxin B_1 and B_2, whilst *A. parasiticus* produces aflatoxin B_1, B_2, G_1, and G_2, and *A. nomius* produces aflatoxin B_1, B_2, G_1, G_2 and a unique metabolite, nominine (Kozakiewicz 1994). In addition, only 50% of *A. flavus* isolates are toxigenic, whilst all isolates of *A. parasiticus* and *A. nomius* are toxigenic. All three species grow rapidly on most media.

Description. With experience one can distinguish *A. flavus* morphologically from *A. parasiticus*. Conidial heads of *A. flavus* are both uniseriate and biseriate, whilst those of *A. parasiticus* are strictly uniseriate. When viewed under a ×100 objective, conidia of *A. flavus* are delicately roughened and those of *A. parasiticus* are echinulate. A definitive separation, using conidial ornamentation can be achieved using scanning electron microscopy (Kozakiewicz 1982). *A. nomius* is morphologically similar to *A. flavus* but produces smaller more elongated sclerotia than the more globose ones of *A. flavus* (Kozakiewicz et al. 1994).

Occurrence. Aflatoxins are common on produce high in oils. This includes: peanuts, walnuts and other edible nuts; cotton seed; palm kernels; spices; and cereals such as maize, parboiled rice, rice bran, sorghum and millet from warmer climates (Moss 1991; Scudamore 1993). Aflatoxins are not found in significant quantities on soybeans (Moss 1991).

Aflatoxin production appears to vary depending on the region. In the USA, storage systems are of high quality and problems appear to be confined to pre-harvest contamination of maize and peanuts (Miller 1995). In tropical countries, such as Thailand and the Philippines, postharvest crop storage is an additional problem (Siriacha et al. 1991; Quitco 1991).

During the past ten years, research in the USA on the field ecology of aflatoxin-producing fungi has shown that *A. flavus* infects field maize in two ways. Airborne or insect-transmitted conidia contaminate the silks and grow in the ear, and insect or bird damaged kernels become colonised with fungus and accumulate aflatoxin

(Miller 1995). In both cases, drought-, temperature-, or nutrient-stressed plants are more susceptible to colonisation by *A. flavus* (Diener et al. 1987). Conidia of *A. flavus* do not overwinter in soil in the USA. However, *A. flavus* sclerotia and maize debris left in the field after harvesting combine to act as a source of inoculum for the subsequent crop (Wicklow and Wilson 1986).

Contamination by *A. parasiticus* is relatively uncommon in the USA (Payne 1992). Likewise, in Asia *A. parasiticus* does not appear to occur in maize.

It is important to note that the ecology of *A. flavus* in the tropics is different from that described for the USA (Miller 1995). There is a rapid rise in aflatoxin levels immediately postharvest (Kawashima et al. 1993; Quitco 1991). Conidia of *A. flavus* appear to be common in the soil throughout the year in maize-growing areas of Thailand (Siriacha et al. 1991), which may account for the high toxin levels found there.

Poor storage facilities in Asia are an added problem. Studies in Thailand have shown that both mechanically-dried and sun-dried maize can be stored for at least two months with low aflatoxin levels, provided that the drying period does not exceed 48 hours (Flach 1991). Farmers prefer to sell their maize as quickly as possible, but market forces sometimes dictate that maize has to be stored on-farm. Storage facilities are usually inadequate and maize reaching the primary merchant often contains high levels of aflatoxin (Flach 1991). Indeed, until there are quality control methods in place, and cash premiums for supply of better quality maize, the situation will not improve.

Aflatoxin levels in peanuts in the USA are high only when plants have been stressed, as in drought conditions (Cole and Dorner 1993). Further increases in such levels can be minimised with good storage management. This includes using well-ventilated stores with sound roof, side walls and floor to prevent rain re-wetting the peanuts, uniformly loading the store to allow excessive heat and moisture to escape, and reducing areas favourable for insect infestation. All these measures reduce mould growth and aflatoxin contamination (Cole and Dorner 1993).

Monitoring programs in Malaysia have identified aflatoxins in peanuts and groundnut products. Unshelled peanuts showed low aflatoxin levels, but some shelled peanuts and peanut butter (locally made rather than imported), peanut candy, peanut cake and peanut gravy showed high aflatoxin levels (Mat and Siong 1984).

Milled rice usually contains no or very low levels of aflatoxin, but parboiled rice often has high contamination levels. A recent study in Sri Lanka showed that commercially parboiled rice had higher levels of aflatoxin than domestic parboiled rice (Flach 1991). Domestic parboiled rice is prepared under clean, controlled conditions, whereas commercial parboiling can mean soaking for 2 to 4 days in cold water before steaming. Quite often the same water is reused, resulting in mouldy paddy with a strong fermentative odour.

Many other foodstuffs have been shown to be contaminated with aflatoxins. Dietary aflatoxin contamination is widespread in hot and humid climates, but since many cooler climate countries now import foods from these areas aflatoxins are of worldwide concern. Recent food surveys in the U.K. have shown that imported spices such as black pepper, chilli powder, and ground ginger contain high levels of aflatoxin (Garner and Dingley 1993).

Significance. Recent evidence suggests that aflatoxin may co-occur with fumonisin (Miller et al. 1993; Pitt et al. 1993), a toxin produced by *Fusarium moniliforme* and responsible for leucoencephalomalacia in horses (Ross et al. 1990). Surveys on mycotoxin levels in maize in Southeast Asia have found fumonisins in some samples (Ueno et al. 1993). The combined effects of field-produced fumonisin and storage-produced aflatoxin are a new and worrying development for the tropics. Field-produced toxins in a commodity going into store can affect the growth of toxigenic storage fungi. Laboratory tests on the tricothecene T-2, another field-produced mycotoxin, have shown it promotes the production of aflatoxin by *A. flavus* (Fabbri et al. 1984). There are no data available on the effect of fumonisin on aflatoxin production (Miller 1995).

Aflatoxins are both acutely and chronically toxic to animals and humans. Their effects include acute liver damage, liver cirrhosis, tumour induction and teratogenic effects. Aflatoxin B_1 is the most toxic liver carcinogen, causing disease and death in both humans and animals. Human liver cancer appears most prevalent in central Africa and parts of Southeast Asia.

Kwashikor is widely believed to be a manifestation of protein malnutrition. However, some of the symptoms of kwashikor (fatty liver, immunosuppression, hypoalbuminaemia) are also symptomatic of aflatoxin poisoning in animals. Autopsy samples of children with kwashikor from Nigeria and South Africa have shown a high incidence of aflatoxin (Hendrickse 1984).

Recent studies in the U.K. have shown that aflatoxin combines with DNA to form afladducts. If such DNA adducts are formed in particular sequences of DNA corresponding to tumour suppressor genes or proto-oncogenes, it is believed the alterations may lead to mutations that are capable of initiating carcinogenic transformations (Harris 1991).

Regulatory limits. Improvements in analytical methods have made it possible to screen foods and feedstuffs for toxin levels. Various governments have now set regulatory limits for mycotoxins in food and animal feedstuffs (Table 2). U.K. limits for foods include 4 μg/kg for aflatoxin B_1 in nuts, dried figs, and their products used for direct consumption. The limit is 10 μg/kg for these products if they are to be processed further, provided that the processing will reduce the level to 4 μg/kg. In animal feedstuffs the limit varies depending on the animal, with 5 μg/kg being set for young animals and dairy cattle (MAFF 1993).

Ochratoxin producers

Aspergillus ochraceus

A. ochraceus and other closely related species—*A. alliaceus, A. melleus, A. sclerotiorum* and *A. sulphureus*—have all been recorded as producing ochratoxins.

Description. A. ochraceus is the most commonly isolated species in the Section Circumdati (formerly known as the *A. ochraceus* species group). Colonies are relatively slow growing on most media, producing dense, pale yellow, globose shaped biseriate conidial heads. *A. ochraceus* produces several toxic metabolites including penicillic acid, xanthomegnin, viomellein, vioxanthin and, most commonly, ochratoxin A (OA).

Occurrence. In Sweden, high levels of OA have been recorded in rye, bran products, and brown kidney beans (Olsen et al. 1993), whilst in the U.K., OA has been found in soya beans, soya flour, maize, cornflour, nuts, and cocoa beans (MAFF 1980). In Germany it has been frequently isolated from meat products (Pitt and Hocking 1985).

However, in more tropical countries OA is associated with mouldy green coffee beans. Indeed, the literature contains many reports of OA in both raw and roasted coffee beans as well as the coffee brew (Studer-Rohr et al. 1993), although much of this evidence is conflicting. Some workers have reported that roasting coffee beans does not remove OA contamination (Tsubouchi et al. 1987), whilst others report that it does (Levi 1980). More work needs to be done to clarify whether coffee at source is yet another food posing a threat in the human diet.

Significance. OA is a nephrotoxin, and has been an important cause of disease of pigs in Denmark, where it was considered to be associated with mouldy barley. Being fat soluble it is not readily excreted, and therefore accumulates in fatty tissue. It is possible that OA may pose a serious threat to human health, particularly in other large pork-consuming countries such as Germany and eastern Europe. In addition, OA has recently been isolated from cow's milk in Sweden (Breitholtz-Emanuelsson et al. 1993), and pig products in the UK (MAFF 1993). It has been implicated in Balkan endemic nephropathy, a kidney disease prevalent in Rumania, Bulgaria, and the former Yugoslavia.

Sterigmatocystin producers

Aspergillus versicolor

Sterigmatocystin is produced by several species of fungi, but *A. versicolor* is the most important. *A. versicolor* is classified in the Section Versicolores (formerly the *A. versicolor* species group).

Table 2. Range of regulatory limits for mycotoxins.

Mycotoxin	Reg. limit (μg/kg)	Number of countries
Aflatoxin in foods	0-50	53
Aflatoxin M1 in milk	0-0.5	15
Ochratoxin A in foods	1-300	6
Deoxynivalenone in wheat	1000-4000	5
T2 Toxin	20-50	2
Zearalenone	30-1000	4

Source: Moss (1995).

Description. A. versicolor is a slow-growing species on most media, producing low, green colonies with biseriate heads and small, echinulate conidia. Reddish drops of exudate and a red-brown reverse colour for the colonies are useful additional diagnostic characters (Kozakiewicz 1989).

Occurrence. A. versicolor is a storage species of worldwide distribution, and has been isolated from: small grains such as wheat, barley, and rice; animal feedstuffs; wheat and oat-based breakfast cereals (Yoshizawa 1991); spices; and acid-treated bread (Frisvad 1988). It has been reported infrequently in the tropics. Its slow growth rate requires special techniques such as dilution plating and has undoubtedly contributed to poor recovery in tropical surveys.

Significance. Sterigmatocystin is a precursor of aflatoxin, but because it has very low solubility in water and gastric juices it is not as acutely toxic (Cole and Cox 1981). However, it is a liver carcinogen causing hepatocellular carcinomas in test animals and a wide range of tumour growths at the sites of application (Smith and Ross 1991).

Important *Penicillium* Species, and Their Mycotoxins, Significance, and Occurrence

Penicillium species produce a vast range of secondary metabolites, of which twenty-seven are considered to be toxic (Pitt 1991). Many of the toxin-producing penicillia grow best under temperate climates and are therefore not found in the tropics. Only those penicillia commonly associated with tropical products will be discussed here.

Most *Penicillium* toxins can be placed in one of two groups: those which are nephrotoxins and those which are neurotoxins. A list of important mycotoxins produced by *Penicillium* species is given in Table 3.

Table 3. Important mycotoxins produced by *Penicillium* species.

Species	Mycotoxin
P. aurantiogriseum	penicillic acid, terrestric acids, toxic glycopeptides, verrucosidin
P. cyclopium	penicillic acid, viomellein, vioxanthin, xanthomegnin
P. brevicompactum	brevianamide A and B, mycophenolic acids, Raistrick phenols
P. camembertii	cyclopiazonic acid
P. chrysogenum	meleagrin, penicillic acid, roquefortine C
P. citreonigrum	citreoviridin
P. citrinum	citrinin
P. commune	cyclopiazonic acid
P. crustosum	penitrems, roquefortine C
P. expansum	citrinin, patulin, roquefortine C
P. glandicola	patulin, penitrem A
P. griseofulvum	cyclopiazonic acid, griseofulvins, patulin, roquefortine C
P. hordei	roquefortine C, terrestric acid
P. islandicum	erythroskyrin, islanditoxin, luteoskyrin, skyrin
P. janczewski	griseofulvin, penicillic acid, penitrem A
P. neoechinulatum	penicillic acid
P. oxalicum	oxaline, roquefortine C, secalonic acid
P. roquefortii	roquefortine A, B, C, and D mycophenolic acid, PR-toxin
P. simplicissimum	xanthomegnin
P. solitum	viridicatins
P. verrucosum	citrinin, ochratoxin A and B, oxalic acid
P. viridicatum	viridic acid, viomellein, vioxanthin, xanthomegnin

Yellow rice toxins: citreoviridin producers

Penicillium citreonigrum

One of two *Penicillium* species producing yellow rice toxins, *P. citreonigrum* (= *P. citreoviride, P. toxicarium*) produces the mycotoxin, citreoviridin.

Description. P. citreonigrum grows relatively slowly on most media, producing grey–green colonies, and yellow mycelium, soluble pigment, and reverse. Conidial heads are monoverticillate, producing conidia that are small and smooth-walled.

Occurrence. P. citreonigrum is the causal agent of the human disease cardiac beri-beri, or 'yellow rice' disease. Since 1910, when the sale of yellow rice was banned, the disease has virtually disappeared in Japan. This was a direct result of the pioneering work of Sakaki in the 1890s (Ueno and Ueno 1972) who implicated mouldy 'yellow rice' as a probable causal agent in fatalities resulting from consumption of this rice. Various Japanese workers later proved that acute

cardiac beri-beri was a mycotoxicosis resulting from the growth of *P. citreonigrum* in rice, which produced citreoviridin (Uraguchi 1969; Ueno and Ueno 1972). Although the disease has disappeared in Japan, it may well still exist in other parts of Asia.

Fortunately, apart from rice, the fungus is uncommon on cereals and rare in other foods. The moisture content band for growth of *P. citreonigrum* in rice is very narrow. It will grow in rice after harvest when the moisture content reaches 14.6% (Uraguchi 1969). However, at 1% higher, other fungi will overgrow it. In addition, *P. citreonigrum* growth in rice appears to occur at low temperatures and short day lengths (Pitt 1991).

Significance. Citreoviridin is a neurotoxin and symptoms of citreoviridin poisoning include vomiting, convulsions, respiratory arrest, and increasing paralysis. These symptoms are similar in both animals and humans. To my knowledge there are no known studies to determine the incidence of citreoviridin in developing world rice crops, or its implications in human or animal health (Kozakiewicz 1994).

Citrinin producers

Penicillium citrinum

Together with *P. verrucosum* and *P. expansum*, *P. citrinum* produces the renal toxin, citrinin. *P. viridicatum* was also reported as a major producer of citrinin (Friis et al. 1969; Krogh et al. 1973), but these reports were based on misidentifications of *P. verrucosum* (Pitt 1987).

Description. P. citrinum is a ubiquitous species and readily recognised. It produces restricted, blue–green colonies on most media, with a yellow reverse. Conidial heads are biverticillate, producing globose to subglobose, delicately roughened conidia.

Occurrence. P. citrinum is readily isolated from numerous foods, including milled grains and flour (Frisvad 1988) and whole cereals, in particular rice, wheat and maize (Pitt and Hocking 1985). However, for reasons unknown, in cereals it is *P. verrucosum* which is the main producer of citrinin. Indeed, citrinin and OA often co-occur, but it is OA that is isolated more frequently.

Significance. Citrinin affects domestic animals, including dogs (Carlton et al. 1974) and pigs, in which it causes porcine nephropathy (Rosa et al. 1985). Kidney degeneration is the cause, with similar kidney damage implicated in humans. To my knowledge, there are no scientific references to the in situ detection of citrinin in Asian regions.

Yellow rice toxins: skyrins and islanditoxin producers

Penicillium islandicum

Together with *P. citreonigrum*, *P. islandicum* causes 'yellow rice' toxin. It produces at least four mycotoxins (unique to the species), two of which, cyclochlortine and islanditoxin, are highly poisonous.

Description. P. islandicum is readily recognisable by its slow growing blue–green colonies, with brilliant orange mycelium and reverse. It has a biverticillate penicillus producing conidia which are elliptical and smooth-walled.

Occurrence. Reports of the occurrence of *P. islandicum* in nature are infrequent (Pitt and Hocking 1985). Considering its very striking appearance this seems unusual. But it is of rare occurrence in temperate regions, and it is here that most of the studies into the occurrence of penicillia in foods and feedstuffs have been undertaken.

However, recent studies in Indonesia on the yellowing of rice during postharvest storage have indicated that several penicillia and aspergilli may be involved (Phillips et al. 1988). Yellowing increased from 0.5–5.5% when the grain was dry, at moisture contents of less than 14%. This was related to earlier mould growth before and during the drying period, particularly in the upper layers of grain which had taken longer to dry. *Penicillium* species isolated included *P. miczynski* and *P. purpurogenum*. Aspergilli included *A. flavus* and *A. candidus*. However, observations on their growth in relation to rice discolouration could not pinpoint the cause of yellowing. More work needs to be done.

Significance. Because it causes toxic 'yellow rice' syndrome, Japanese scientists have studied the toxicology of *P. islandicum* very closely (Saito et al. 1971) (see also section on *P. citreonigrum*).

Both cyclochlorotine and islanditoxin cause liver dysfunctions (Scott 1977), but there is scant information on animal diseases caused by ingestion of contaminated feed.

Erythroskyrin and luteoskyrin are two further mycotoxins produced by *P. islandicum*. Although less toxic than cyclochloritine, they are liver and kidney toxins (Kozakiewicz 1994). Luteoskyrin is also carcinogenic. There are few reports of animal diseases (Pitt and Leistner 1991). They are usually contaminants of rice,

but there are few data to determine their real importance to animal health.

Secalonic acid producers

Penicillium oxalicum

Secalonic acid is produced by *P. oxalicum.*

Description. This is a fast growing species producing grey–green colonies, the conidial heads of which, when examined under the stereoscopic microscope, appear as shiny, silken threads. It has a biverticillate penicillus producing large, elliptical, smooth-walled conidia.

Occurrence. Secalonic acid is found in cereals stored in subtropical and tropical areas (Frisvad and Lund 1993). It has also been found in the southern USA on maize (Reddy and Reddy 1991), and in maize dust (Ehrlich et al. 1982) at levels of up to 4.5 mg/kg. Currently, there are no data on its toxigenic properties.

Conclusions

Contamination of food and feedstuffs by *Aspergillus* and *Penicillium* species and their toxic metabolites is a serious problem worldwide. They have adverse effects on animal and human health and cause economic problems for international trade, in particular that of more tropical countries.

A recent paper has tried to quantify the direct cost of aflatoxin contamination in Southeast Asia (Lubulwa and Davis 1994). Aflatoxin contamination of maize and peanuts in Thailand, Indonesia, and the Philippines was assessed using published literature. The authors concluded that nearly US$361 million per annum was the cost of contamination to these two commodities alone. Maize was the more important commodity, costing 66% of the total, with Indonesia being the worst affected, bearing 48% of the losses.

However, despite the wealth of literature on this topic, much more basic survey and research work is needed. Information is required on the true extent of fungal and mycotoxin contamination, the parameters that cause mycotoxins to be produced whether preharvest or postharvest, and the true cost of mycotoxin contamination—both in direct (consumption by animals and man) and indirect terms (testing, monitoring, detoxification etc. of commodities).

Therein lies the problem. Such surveys and research programs require adequately trained personnel, who are in short supply. A few specialists in taxonomy, systematics, and toxicology are to be found in Europe, North America, and Australia, but not in developing countries where many of the mycotoxin problems occur.

The loss of nearly 20% of some crops in tropical countries that can ill-afford such large financial losses is also untenable. Clearly, more training at the farmer level is required. Farmers need to understand the relationships between environmental conditions and mycotoxin development, and how mechanical damage to the kernels through rough handling provides sites of infection for fungal growth. This can be achieved only through local expertise.

Many of these problems are already being addressed in the ASEAN countries through government agencies, often in collaboration with outside agencies such as ACIAR. But much work still needs to be done. There must be an urgent and significant exchange of information between mycologists and crop specialists of developed and developing countries, so that the developing countries can benefit from the former's accumulated knowledge and expertise. Such exchange is best achieved through formal cooperative links.

To reach this goal for developing countries, the following proposals are suggested:

- strong, official links should be set up between developing and developed country agencies;
- personnel running taxonomic and mycotoxin research laboratories should be given expert assistance and advice in taxonomy, identification, and mycotoxin analysis methodology;
- in parallel, surveys need to be conducted to determine fungal flora of food after harvest in order to identify cause and effect and propose solutions;
- the methodology for identification and enumeration of mycotoxigenic fungi in foods and feedstuffs needs to be standardised;
- procedures should be developed so that fungal isolates used in taxonomic, biochemical, and toxicological studies can be deposited in recognised culture collections where they can be kept under optimal conditions;
- funding should be provided to permit the development and establishment of subnational and national culture collections;
- training of taxonomic personnel in database storage and the safe deposit of commercially valuable isolates.

The proposal for an ASEAN Grain Postharvest Technology Centre in Jakarta will provide an encouraging beginning into what hopefully will be a new era of research in postharvest storage problems within the ASEAN countries.

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Effect of Drying Control on Mycotoxin Production

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Abstract

The presence of mycotoxins is one of the major factors affecting the quality of grains. The production of mycotoxins is affected by preharvest conditions and postharvest conditions such as moisture, temperature, drying rate, substrate, modified atmosphere, and milling.

This paper reviews the literature on the relationships between production of mycotoxins and postharvest grain handling practices. The most important factors affecting the production of mycotoxins are moisture, temperature, and drying rate. If moisture content, temperature, and drying rate can be controlled then the production of mycotoxins can be minimised.

Dantec's DryerMaster automatic moisture and temperature control system is an effective tool for controlling moisture, temperature, and drying rate. The DryerMaster has been used extensively to control moisture and temperature in grain dryers (maize, rice, soybeans). It has been shown that the DryerMaster can: reduce energy consumption by up to 30%; reduce moisture standard deviation by 50%; reduce electrical consumption by up to 12%; increase moisture removal per pass by 18%; eliminate one drying pass; and maintain head yield.

Results of recent studies will be presented.

FUNGI can lead to degradation of stored grain and production of mycotoxins. Many species of fungi will grow on rice. Dharmaputra et al. (1993) identified the following eleven species in one study:

- *Aspergillus candidus*
- *A. flavus*
- *A. pencililloides*
- *A. sydowii*
- *A. versicolor*
- *Cladosporium cladosporioides*
- *Eurotium chevalieri*
- *E. rubrum*
- *Penicillium citrinum*
- *P. fellutanum*
- *P. apxilli*

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Since fungi live on the nutrients in the grain, their growth will reduce the weight of dry matter and so cause a loss in production. Fungi have been found to grow under many conditions; some fungi have even been found to grow at temperatures approaching freezing point.

As well as reducing dry matter, some fungi are known to produce toxic chemicals known as mycotoxins. Different grain fungi produce different mycotoxins. For example, the fungus *Aspergillus flavus* is well known to produce a mycotoxin called aflatoxin. Aflatoxin is very toxic and is believed to be carcinogenic to humans, causing liver cancer. The World Health Organization has acknowledged the significance of mycotoxins for our food supply and has more than 100 researchers in 27 countries working on the mycotoxin problem. Therefore, it is of paramount importance to minimise and control the growth of fungi and the production of mycotoxins in stored grains.

Fungi, once grown, can be destroyed only by chemical or biochemical fumigation; low temperatures and moisture levels can do no more than render the

fungi dormant. However, fumigants are expensive and there is a health concern about their use. The growth of fungi, once commenced, can be controlled by several factors. These factors will not destroy the fungus but will keep it in check and minimise its growth. Figure 1 illustrates the growth of a typical fungus as a function of time (Dharmaputra et al. 1993). Note that the fungus population, measured in colonies/g, tends to increase with time. Any reduction should be viewed as an unrepresentative sample.

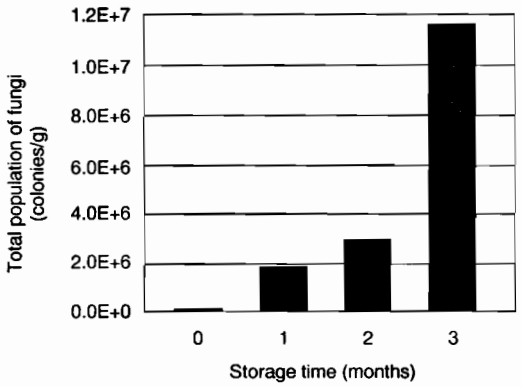

Figure 1. Growth of fungus on rice, plotted against storage time.

There have been many studies on the factors that affect the growth of grain fungi and the production of mycotoxins. Several of the key references will be identified here. Brooker et al. (1992) state that the most important factors affecting the growth of moulds on stored grains are moisture content and grain temperature. Other factors include grain history, grain genotype, grain damage level, foreign material content, degree of blending, and extraneous organism development.

Wongvirojtana et al. (1993) state that when sun drying is used the drying time is long and fungal growth is high. Fungal growth will always increase with time, under normal conditions (everything else being equal). They suggest that mechanical drying be used.

Paz et al. (1993) compared aflatoxin levels in sun-dried maize with those in mechanically-dried maize. They found that mechanical drying resulted in a dramatic reduction in the production of aflatoxin. It is important to note that the amount of aflatoxin does not decrease with mechanical drying but that the mechanical drying slows the growth rate of aflatoxin producing fungi.

As shown in Figure 1, Dharmaputra et al. (1993) found that an increase in storage time resulted in an increase in the population of fungi on rice. This is understandable since the amount of fungi will stay constant or continue to increase with time unless it is killed. Our goal therefore is to minimise the growth rate of fungi. Dharmaputra et al. (1993) also showed that an increase in the degree of milling decreased the amount of fungi on rice.

Legge (R. Legge, University of Waterloo, Canada, pers. comm. 1995) states that fungi tend to increase the production rate of mycotoxins when put under stress; this is thought to be a type of defence mechanism. Stresses that would affect the fungi are extremes in temperature, especially high temperatures, and high drying rates.

How to Reduce Mycotoxins

The literature suggests five key techniques to reduce the production of mycotoxins. They are:

1. Minimise the storage time for wet grain.
2. Minimise the handling of grain after harvest.
3. Minimise the stress on grain after harvest.
4. Maintain low temperatures in stored grain.
5. Ensure good control of moisture and temperature.

1. Minimise the storage time for wet grain

Field moulds will continue to grow after harvest if the grain is not dried. This will result in continued degradation of the grain. Therefore it is important to dry the grain as soon as possible after it has been harvested. If one can reduce or eliminate the fungi from the grain in the first place, then a significant reduction will appear in the final product. To ensure this, adequate drying facilities must be made available. It has been estimated that 50% of all grain losses are from inadequate drying and storage facilities.

It has been suggested that the first drying pass be completed within 18 hours of receiving the grain at the dryer facility. The grain can then be put into temporary storage without significant fungal growth.

2. Minimise the handling of grain after harvest

The more the grain is handled or moved the greater the chance of it becoming contaminated by other

fungus-contaminated grain. In addition, increased handling will cause increased damage and cracking. Therefore, from the point of view of fungal control, it is important to minimise grain handling. This can be achieved by drying the paddy at the highest drying rate possible (without damage to the paddy and reduced head yield). Drying at a higher rate will reduce the number of dryer passes required and therefore the amount of handling will correspondingly be reduced. On the other hand, drying at an excessively high rate may result in the creation of stress cracks, reducing the head yield and providing a opportunity for moulds to develop and penetrate the kernel.

3. Minimise the stress on grain after harvest

Stress can be caused by too much handling, excessive drying rates, and high temperatures. Legge (R. Legge, University of Waterloo, Canada, pers. comm. 1995) states that high temperatures or drying rates put existing moulds under stress. The response of the moulds to this stress is the production of mycotoxins. Stress has also been shown to lead to cracking. Cracks are ideal locations for fungi to develop. Stress cracks can be prevented by drying the grain slowly and at low temperatures. This, however, will result in long drying times, more dryer passes than necessary and consequently increased handling. This then will lead to the development of more moulds. Therefore, a trade-off or optimum exists and the dryer operator must balance the positive and negative effects of increased drying rates.

4. Maintain low temperatures and moisture contents in stored grain

Many studies have identified temperature and moisture level as significant factors for the development and growth of moulds and the production of their associated mycotoxins (Brooker et al. 1992). Low temperatures and moisture levels retard biological activity. Low temperatures can be achieved through chilling of the stored grains. This has been shown to be an effective method for controlling mould development. Low moisture levels can be achieved through mechanical drying.

5. Ensure good control of moisture and temperature

Control of the moisture and temperature levels in storage and dryers is important because it maintains a uniform product and helps to eliminate pockets of 'off-spec' material, e.g. high temperature or high moisture. Without good monitoring and control, the dryer operator does not know if pockets of wet grain are passing out of the dryer. The pockets of wet grain will continue to support the development of moulds and the production of mycotoxins. Often 'off-spec' grain is blended after drying in an attempt to create a final mixture that is up to required standard. Although this may seem like an acceptable way to correct poor dryer control, Brooker et al. (1992) shows that blending does not yield an equilibrium moisture. In other words, you will have high moisture and low moisture regions in the resultant mixture. The high moisture regions will, of course, continue to promote mould development and mycotoxin production.

In addition, good moisture control results in reduced energy consumption, increased throughput, and a reduction in the number of drying passes.

An attractive control solution is provided by the DryerMaster automatic moisture and temperature control system.

DryerMaster Results

Dantec (1995) has, over the past 10 years, developed a grain drying technology that is currently used in many of the cereal-grain producing countries of the world including Argentina, Brazil, Canada, China, France, Malaysia, Spain and the USA. The benefits include improved quality, increased yield and throughput, and reduced operating costs. This is especially true for the drying of paddy since paddy is temperature sensitive.

BERNAS (National Paddy and Rice Company of Malaysia)—(formerly LPN), GRT Industries SDN BHD, and Dantec Electronics Limited have cooperated in implementing the DryerMaster paddy drying technology in Malaysia. Implementation occurred in two stages: first, Dantec developed a paddy dryer and collected data to determine the specific requirements of BERNAS; second, BERNAS installed a DryerMaster system at the Selcinchan Kompleks, Selangor which has proven to be most acceptable to the site manager and easily used by the dryer operators.

Figure 2 is a schematic diagram of a typical installation. The dryer was equipped as follows so that the

process would be properly controlled and accurate information collected.

- inlet moisture sensor chute with rotary feeder;
- outlet moisture sensor chute with rotary feeder;
- inlet paddy temperature;
- outlet paddy temperature;
- ambient temperature/humidity sensor;
- exhaust air humidity sensor;
- exhaust temperature sensor; and
- drying air temperature sensor.

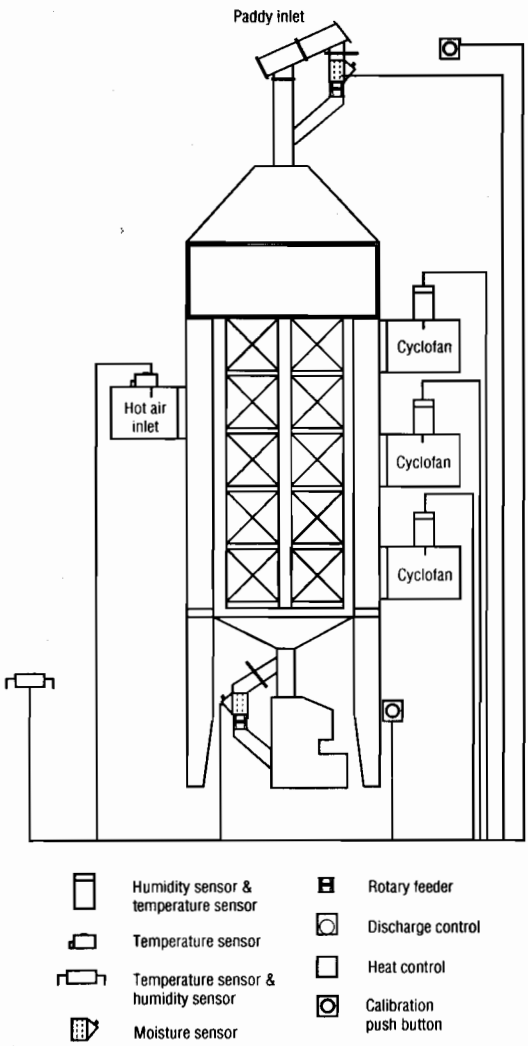

Figure 2. Schematic diagram of a typical rice dryer.

The DryerMaster system then adjusts the drying air temperature and the discharge rate or frequency to maintain operator-specified paddy moisture and temperature set points. The DryerMaster automatic control system has three significant features:

- the DryerMaster moisture meter directly measures a large representative sample of the paddy passing through the dryer;
- the moisture meter calibration is updated on-line by the operator using both on-line data and off-line laboratory measurements;
- the DryerMaster uses model-based control algorithm in which a model of the dryer is embedded in the control algorithm for better control of the drying process.

Figure 3 shows outlet paddy moisture content measured by the DryerMaster and using off-line laboratory techniques. It is clear that the DryerMaster moisture measurement system is accurate. This is very important because without accurate sensors good control is impossible.

Figure 4 is a typical plot of outlet paddy moisture versus time under manual control. Notice that the temperature is essentially constant because the operator is making infrequent changes to the temperature. Also notice the outlet moisture is varying by ±5% and the standard deviation is about 2.7%. Figure 5 is a plot of outlet paddy moisture versus time for the same dryer under automatic control. In this case, the DryerMaster is adjusting the temperature to maintain the paddy at about 16% moisture. The variation of the moisture has been reduced significantly.

Figure 6 shows clearly the reduction in variation of moisture between the manual and automatic control. In quantitative terms the standard deviation has been reduced from 2.7 under manual control to 1.3 under automatic control; this represents a reduction of more than 50%! This reduction in standard deviation results in a more uniform product which is very important when trying to maintain high quality grain.

Another benefit that results from the use of the DryerMaster is a reduction in energy costs. For example, at one particular site savings of 4.1 litre of oil/tonne of paddy and 3.2 kWh/tonne of paddy were identified. The use of the DryerMaster allowed the operators to increase the moisture removal per pass by 18% and reduce the handling of the paddy by 21% The quality of the rice, measured in terms of head yield, *increased* by 0.8%.

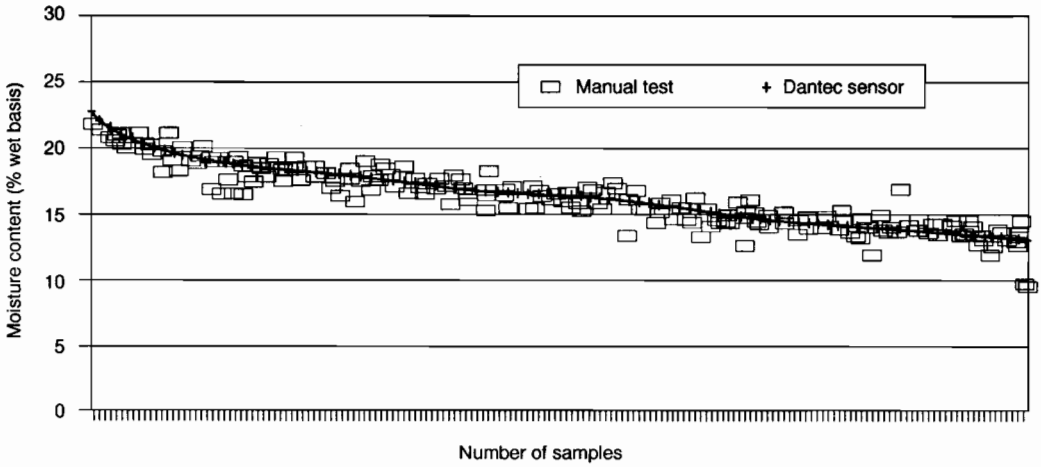

Figure 3. Moisture comparison: manual test versus Dantec on-line moisture meter.

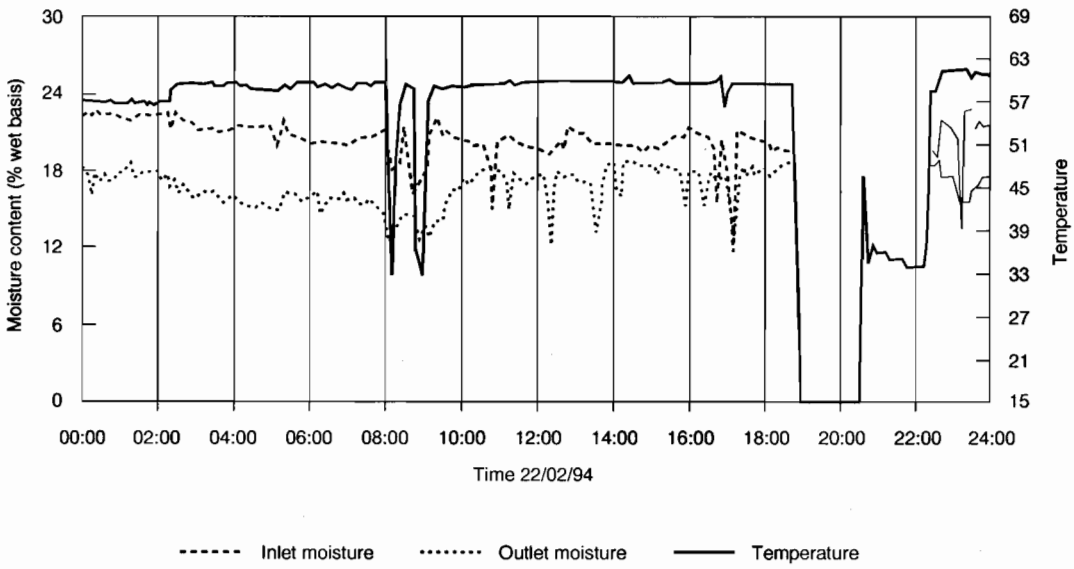

Figure 4. Moisture and temperature measurements from a dryer under manual control.

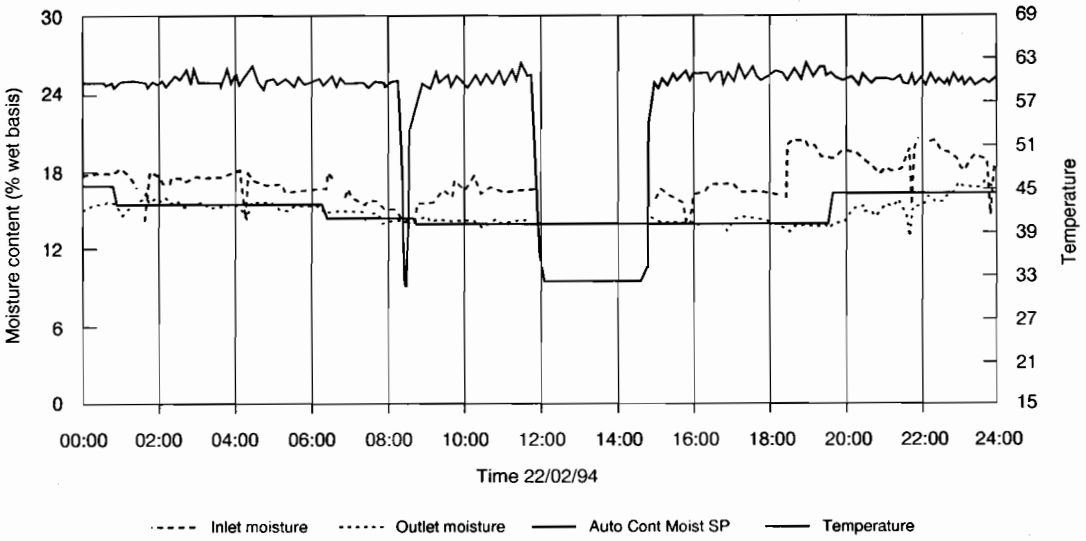

Figure 5. Moisture and temperature measurements from a dryer under automatic control.

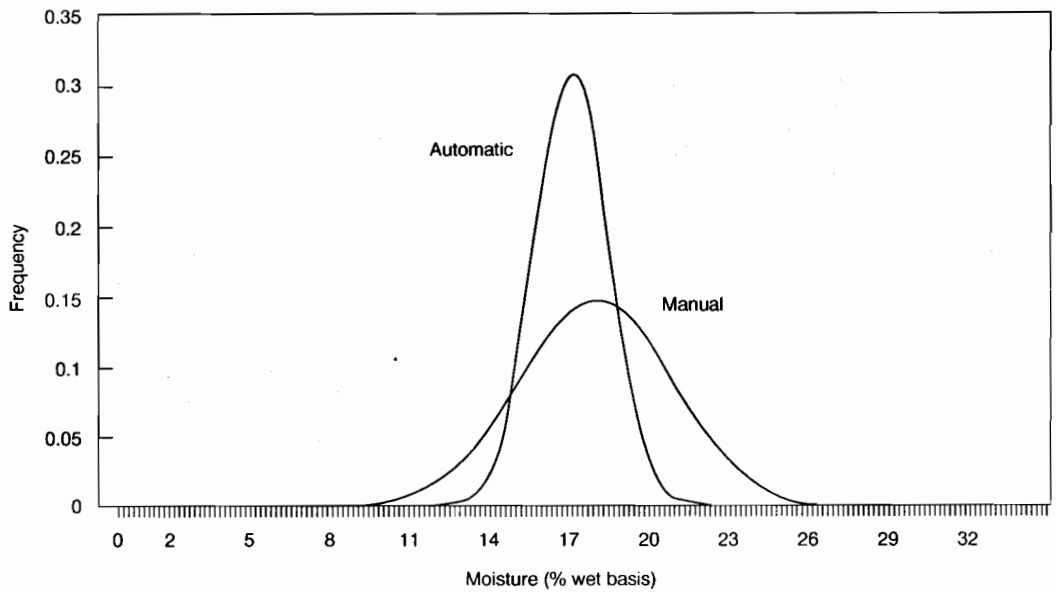

Figure 6. Normalised moisture distributions: manual versus automatic control.

Conclusions

The major factors contributing to the production of mycotoxins are high moisture content, high temperature, and long storage times before drying.

To reduce the production of mycotoxins one should:

- adopt good postharvest grain practices;
- provide adequate grain storage and drying facilities; and
- provide systems for good control of moisture and temperature.

The DryerMaster automatic moisture control system reduces the variability of outlet moisture content, energy consumption, and number of dryer passes, while maintaining head yield.

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The Aflatoxicosis Problem and Management of Aflatoxin in Feed in Vietnam

Tran Van An and Duong Thanh Liem*

Abstract

The warm, humid climate of Vietnam is conducive to the rapid development of fungi in stored fodder, especially during the rainy season. Surveys conducted by the Food and Commodities Control Centre (HCMC) have shown aflatoxicosis to be the cause of serious production losses in swine and poultry.

Diagnosis of aflatoxicosis was based chiefly on aflatoxin levels in ingested food and histopathological changes in liver. Practical ways to prevent aflatoxicosis include monitoring fodder moisture and aflatoxin levels, and timely drying. Simple, cheap, and rapid analysis methods such as BGYF and minicolumn are suitable for the current level of development of Vietnamese agriculture.

An active campaign to disseminate information through the mass media and via agricultural extension services has been used to promote aflatoxin prevention measures among feed mills and livestock farms.

A network of services for aflatoxin monitoring is being established throughout the country.

THE hot and humid climate of Vietnam is very conducive to mould contamination in fodder, especially during the rainy season. The southern lowland of Vietnam, including the Mekong Delta, has two distinct seasons: dry and rainy. The rainy season extends from mid-May to mid-November, but the highest rainfall is in June, July, August, September and October when it rains more than 15 days a month. The rainfall varies from 200–700 mm. The rainfall pattern is intermittent rather than consistent. The relative humidity is very high (greater than 84%) from June to October but can vary greatly within a 24-hour period. For example, it may be lower than 70% from 0100–1700 hours and then higher than 85% from 2200 hours to 0500–0600 hours the next day.

There are a number of types of cereals harvested in the rainy season in the southern provinces. They include rice (4 Mt), maize (280 kt), soybean (30 kt), and peanuts (72 kt).

Eighty percent of the maize annual production is harvested in August and fifty percent of peanuts in July—times when the rains are heaviest.

Farmers generally cannot afford dryers, and dry their cereals at random in the sunshine. However, it is difficult to adequately dry harvested grain in the rainy season using sunlight. On the other hand, we have counted about 450 small flatbed dryers and some large ones, such as in the Tra Noc-Cai Rang (Can Tha Province) Satake factory (IICMC). These satisfy only a small part of the need for cereal drying in the southern lowlands. In view of these drying conditions, it is not surprising that mould contamination of feed is a serious problem, and that aflatoxin levels in feed are high (Table 1).

Table 1. Levels of aflatoxin found in animal feeds in Vietnam.

No.	Product	Number of samples	Average level (ppb)	Maximum level (ppb)
01	Peanut cake[a]	35	1140	5000
02	Yellow maize for feed	31	225	750
03	Mixed feed	28	105	500

Source: Le Van To and Tran Van An (1994)
[a]Obtained after extraction of oil.

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Aflatoxin levels in material for animal feed varies greatly with the season. The rainy season provides conditions that favour mould development and accordingly the aflatoxin content increases greatly (Table 2).

Table 2. Variation between rainy and dry seasons in aflatoxin levels in feeds.

	Product	Number of samples	Average level (ppb)	Maximum level (ppb)
	Rainy season			
01	Peanut cake	17	1520	5000
02	Yellow maize for feed	18	240	750
	Dry season			
03	Peanut cake	18	525	1160
04	Yellow maize for feed	13	120	450

Source: Le Van To and Tran Van An (1995).

These results show that efforts to prevent an increase in aflatoxin should be concentrated on the rainy season and, as the aflatoxin level of peanut cake is always high, the risk of using it as a component for mixed feed is quite great.

Incidence of Aflatoxicosis in South Vietnam

In recent years, there have been many incidents in which it was reported that swine and poultry died after being given fodder contaminated by mould. From 1990 to 1994, there were at least 100 declared and confirmed outbreaks of aflatoxicosis at swine, poultry, and duckling farms Such outbreaks have caused great losses.

We cite here several notable cases:

In swine: aflatoxicosis with apparent symptoms affected more than 32% of pigs on the U.A. swine farm (Ho Mong Hai 1992).

In laying hens: hatching rate was only 2% in comparison with the normal 80% in the B.A. poultry farm (Duong Thanh Liem et al. 1993).

In chicken broilers: mortality was more than 30% after 70 days in D. poultry farm (Nguyen Thi Thuan 1994).

In ducklings given feed containing 500 ppb aflatoxin, mortality was nearly 100% (Ho Mong Hai and Le Anh Phung 1992–1994).

There were no characteristic symptoms of aflatoxicosis (stunting, icteria, bruising, reduced rate of gain, higher rate of mortality) but histopathological changes in liver tissue were very characteristic. These changes include: fatty degeneration of cells, fibrosis, and proliferation of the bile duct epithelium. Thus, diagnosis was based on both aflatoxin analysis in feed and examination of histopathological changes in liver.

Prevention of Aflatoxicosis in Vietnam

From the foregoing it is clear that aflatoxicosis is a serious problem in Vietnam. Aflatoxicosis in the provinces of southern Vietnam was discussed in a symposium on animal feed in 1992. All participants recognised the threat of aflatoxin accumulating in fodder such as maize and peanut cake. Many specialists considered that, for the current level of development of Vietnamese agriculture, the safe limit of aflatoxin in feed should not exceed 100 ppb. Since the aflatoxin level in peanut cake is always found to be high, the Department of Agriculture of Ho Chi Minh City has prohibited the use of peanut cake in animal feed. Since prohibition of its use was introduced, most livestock and poultry farms have not used peanut cake in fodder and there have been many fewer outbreaks of aflatoxicosis.

Practical measures for preventing aflatoxicosis include monitoring fodder moisture, monitoring aflatoxin in feed and fodder before use, and promoting timely drying of agricultural commodities.

Monitoring fodder moisture

To be free of harmful levels of fungi, the water activity (a_w) of fodder must be lower than 0.7, which corresponds to a moisture level of 13.5% for maize and 7–8% for oilseeds. Therefore, high moisture levels in fodder need to be detected in time to prevent mould developing. Moisture meters are, however, relatively expensive and not readily available in country areas. Therefore, the Food Control Centre (FCC) of Ho Chi Minh City has recommended that farmers use the salt liquefaction test (FAO 1995).

This quite simple and very cheap technique is as follows: put 40–80 g of sample into a wide-mouthed flask (about 500 mL), seal the flask with a stopper and allow at least 2 hours for it to equilibrate. Then open

the flask and spread a very thin layer of vaseline on the inner surface of the stopper. With a spatula put several dozen copper chloride ($CuCl_2$) crystals on the vaseline layer. Close the flask and let it stand for 4 hours to equilibrate. Then open the flask and observe the salt crystals to see whether they are liquefied or not. If 50% or more of the crystals are liquefied, the test is judged positive. That is, the a_w of the fodder is above 0.7 and it needs to be dried promptly.

Monitoring aflatoxin in maize

Since 1994, with the aid of the ACIAR grain drying project (ACIAR PN9008), the FCC has promoted the use of the BGYF test by feed mills and livestock farms in southern Vietnam for monitoring aflatoxin in maize. The test requires neither analytical skill nor chemicals and can therefore be used by staff with very little training. Though it has some limitations (being a 'yes' or 'no' method only), the BGYF test has the potential to monitor aflatoxin throughout Vietnam more effectively and greatly improve the quality of maize.

Grading fodder by aflatoxin content

Since 1989, with the aid of the Department of Agriculture in Thailand, the FCC has started to use the minicolumn test to analyse aflatoxin semiquantitatively. The accuracy of the analysis is enhanced by using aflatoxin minicolumn standards (50, 100, 200 ppb). These minicolumn standards are calibrated by thin-layer chromatography (TLC). The FCC has also promoted the minicolumn test for 20 feed mills, livestock, and poultry farms in the south of Vietnam. This test is simple (it can be used by staff with two days of training), cheap (it costs only US$0.80 per sample), rapid (30 min/test), and needs little equipment (see Box), and has therefore been welcomed by all feed mills and farms throughout the country.

Timely drying of newly harvested grain

Timely drying of recently harvested grain is essential for preventing mould contamination. It is difficult to dry newly harvested maize and peanut effectively in July and August, and therefore dryers are particularly useful in the rainy season. Results obtained by the University of Agriculture and Forestry (UAF), Ho Chi Minh City shows clearly that maize dried timely has very low aflatoxin content in comparison with maize not timely dried (Nguyen Le Hung et al. 1995).

Equipment needed for minicolumn test for aflatoxin

I. Glassware

1. Test tube rack	1
2. Large graduated test tube with stopper	2
3. Test tube	1
4. Small funnel	2
5. Glass rod	1
6. 1 mL glass syringe	2
7. Filter paper	1 box
8. Rubber bulb	1
9. Minicolumn	10
10. High speed blender	1
11. Small balance 100 ± 1 g	1
12. UV box (365 nm)	1

II. Chemicals

1. Salt solution 1% (NaCl, 10 g; H_2O, 1 L)
2. Burning alcohol
3. Salt solution (NaCl, 150 g; $Zn(OAc)_e$, 150 g; HOAc, 4 mL; H_2O, 1 L)
4. Chloroform acetone ($CHCl_3$, 90 mL; Acetone, 10 mL)
5. Benzene (reagent grade)

III. Cost for one sample

1. Minicolumn	US$0.50
2. Chemicals	US$0.20
3. Labour cost (for 30 min of labour)	US$0.10
Total	US$0.80

Campaign to Promote Aflatoxin Prevention Measures among Feed Mills and Farms

The FCC, UAF, Service of Science and Technology and Environment, Department of Agriculture, and the Agricultural Extension Service of Ho Chi Minh City have introduced an information and agricultural extension program on aflatoxin prevention.

Information program using the mass media

There are programs about aflatoxicosis and prevention measures on radio and TV, as well as articles in magazines and at seminars, and exhibitions. Whereas five years ago, very few people were aware

of aflatoxin, nowadays almost all feed mills, livestock farm managers, and technicians understand the danger of aflatoxicosis and how to prevent it.

The Service of Science, Technology and Environment of Ho Chi Minh City has supported research by FCC, UAF, and the Biotechnology Center of HCMC on how to prevent aflatoxin accumulating in maize. Detoxification of aflatoxin in peanut cake using alcohol and ammonia have been tested and the results are very promising.

Agricultural extension

With the aid of the ACIAR drying project, the FCC has begun training courses for moisture and aflatoxin monitoring in fodder. Participants are managers and technicians from feed mills, and livestock and poultry farms. The length of these training courses has been only two days. Of this time, one half-day is used to explain conditions of mould contamination in grain, and how to prevent it. The remaining time is used to practice testing. Participants are trained in the crystal liquefaction test, the BGYF, and the minicolumn test. If a person is not successful, they are asked to repeat the test until it is completed satisfactorily.

After the training course, participants are supplied with kits for the BGYF and minicolumn tests. So far, 20 feed mills and farms are able to monitor aflatoxin in fodder. In the near future, a short training course will be initiated for veterinarians in provinces of southern Vietnam. We hope that these measures will greatly reduce the loss and damage caused to our grain industry by aflatoxin .

Network of Services for Aflatoxin Monitoring in Vietnam

In Hanoi, there are several laboratories for aflatoxin analysis with advanced techniques such as HPLC, HPTLC, and TLC.

In Ho Chi Minh City there are three main laboratories for aflatoxin analysis (FCC laboratory, Vinacontrol and the Service of Standardisation) with techniques such as HPLC, HPTLC and TLC.

In the Mekong Delta, there is one laboratory for commodity analysis (including aflatoxin analysis) with HPLC and TLC.

In central Vietnam there are two laboratories for aflatoxin analysis in Binh Dinh and Khanh Hoa.

A network of services for aflatoxin analysis is being established for the whole country with the aim of providing each province with its own laboratory for aflatoxin monitoring.

We would like to thank the ACIAR Drying Project for its help to our agricultural research and extension work.

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Surveys on Postharvest Handling, *Aspergillus flavus* Infection, and Aflatoxin Contamination of Maize Collected from Farmers and Traders

O.S. Dharmaputra*, I. Retnowati†, H.K. Purwadaria§, and M. Sidik**

Abstract

Postharvest handling, *Aspergillus flavus* infection, aflatoxin contamination, moisture contents, and percentages of damaged kernels of maize were surveyed in Central Lampung (Lampung province) and Kediri (East Java province) regencies. Farmers and traders were surveyed during the dry and wet seasons in 1993–1994.

The results showed that, in both regencies, most of the farmers sun dried their maize, either shelled or on the cob, on paved floors. They generally used a mechanical sheller and stored maize in jute or polypropylene bags.

The moisture levels in maize collected from farmers and traders during the wet season in both regencies were higher than those in maize collected during the dry season. The moisture levels in maize collected from farmers during the two seasons were higher than those in maize from traders.

The percentage of damaged kernels collected from farmers was higher in the dry season than in the wet season. However, season did not affect the percentage of damaged kernels in maize collected from traders.

The location of farmers did not have a significant effect on the percentage of damaged kernels but, in maize collected from traders, there was a higher percentage of damaged kernels from Central Lampung regency.

In kernels collected from farmers, there was a higher percentage infection by *A. flavus* in maize from Central Lampung regency than from Kediri regency. During the dry season, the percentage of kernels infected by *A. flavus* in maize collected from both farmers and traders was higher than during the wet season. In maize collected from farmers, the percentage of kernels infected by the fungus during both seasons in the two regencies was lower than in that collected from traders.

Total aflatoxin B_1 in maize collected from farmers was not significantly different from that in maize from traders.

MAIZE can be infected with *A. flavus*, a fungus that can produce aflatoxin (Butler 1974; Lillehoj and Hesseltine 1977), both before and after harvest. Aflatoxin is an extremely potent carcinogen that affects several animal species and is often found on maize (Cole et al. 1982).

Dharmaputra et al. (1993) reported that 35 maize samples collected from farmers and traders in Lampung Province contained 23–367 ppb of aflatoxin. Thirty samples contained more than 30 ppb.

Postharvest handling (including drying and shelling) can influence the degree of fungal infection. Freshly harvested maize is generally moist and therefore is a good substrate for fungal growth. Shelling can cause mechanical damage, which allows fungal spores to infect the kernels. Consequently, the kernels should be shelled using a proper tool at appropriate moisture levels to reduce damage.

The objective of this study was to evaluate:

- maize drying, shelling, and storage methods used by farmers and traders; and
- relationships between *A. flavus* infection, aflatoxin contamination, moisture content, and damage to kernels of maize collected from farmers and traders in some maize-growing areas.

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Materials and Methods

Time and location of surveys

Surveys were conducted during the dry and wet seasons in Central Lampung (Lampung province) and Kediri (East Java province) regencies in 1993–1994 (Table 1). These districts were selected because they produce large quantities of maize.

The surveys comprised:

- interviews, using questionnaires, with farmer groups, farmers, and traders, and direct observation at survey locations; and
- random sampling of maize derived from farmers and traders in selected areas. The traders bought the maize directly from the farmers. The samples were analysed for moisture content, damaged kernels, fungus (*A. flavus*), and aflatoxin.

Obtaining samples

The numbers of villages (sample locations) and districts sampled in each regency during the dry and wet season are shown in Table 2. Twenty-seven samples were taken from the farmers and eight from the traders during each season in each location (regency). Maize sampling was conducted randomly according at five stages of postharvest handling: 1) freshly harvested maize cobs; 2) dry maize cob; 3) shelled maize before drying; 4) dry shelled maize; and 5) dry shelled maize stored by traders. For stages 1–4, maize samples were collected from farmers.

The primary samples (about 500 g each) were divided several times using a sample divider to obtain samples for analysing moisture content, percentage of damaged kernels, *A. flavus*, and aflatoxin.

Moisture content, damaged kernels, *A. flavus*, and aflatoxin analyses

A Cera tester was used to analyse moisture levels in maize at the time of sampling. The percentage of damaged kernels was determined by weighing the damaged kernels and the total weight of kernels analysed. Kernels classified as damaged included those that were cracked, broken, discoloured, and damaged by insects and fungi.

A. flavus was isolated by plating on *Aspergillus flavus* and *A. parasiticus* agar (AFPA) (Pitt and Hocking 1985). Aflatoxin content was determined using high performance liquid chromatography (Rodriquez and Mahoney 1994).

The data were analysed using split block with 2 factors: location (Central Lampung and Kediri regencies), and season (dry and wet seasons).

Results and Discussion

Interviews with farmer groups

Twenty farmers groups were interviewed in Central Lampung and 14 in Kediri. The mean sizes of farmer groups were 60 and 51, respectively.

Maize is dried in four different ways by farmers in Central Lampung regency. It may be spread on the soil, on a mat, on a plastic sheet, or on a paved floor. In Kediri regency drying on soil was not used (Table 3).

Four methods were used for shelling the maize by farmers in Central Lampung regency: by hand, using a wooden stick, using a nail-down wood, and mechanical shelling. In Kediri regency all farmers used a mechanical sheller (Table 3).

Table 1. Sampling time for reach regency.

Season	Central Lampung Regency	Kediri Regency
Dry	18–24 August 1993	26 September–2 October 1993
Wet	5–11 December 1993	11–17 January 1994

Table 2. Number of villages (sampling place) and districts sampled for each regency and season.

Season	Central Lampung Regency				Kediri Regency			
	Farmer		Trader		Farmer		Trader	
	No. districts	No. villages	No. districts	No. villages	No. districts	No. villages	No. districts	No. villages
Dry	5	10	4	8	6	13	4	5
Wet	2	4	2	4	2	4	2	5

Table 3. Results of questionnaire of farmer groups on maize postharvest handling in Central Lampung Regency, Lampung province (December 1993), and Kediri Regency, East Java province (January 1994).

Detail	Central Lampung	Kediri
Number of respondents (farmer groups)	22	14
Average members/group of farmers	60	51
Average area (ha)	31	31
Average production (t/ha)	3.5	6.3
Postharvest process		
Drying facilities		
above ground	9.3	0
a woven bamboo mat	7.7	1.2
plastic sheet	13.9	2.4
a paved floor	69.1	96.4
Shelling		
using hand	4.6	0
using stick	0.9	0
using nail-down wood	21.1	0
using mechanical sheller	73.4	100

Interviews with farmers

The number of respondents (sample numbers) during each season in each location (regency) was 27.

Three methods of drying were used by the farmers: drying maize cobs after harvest, drying of shelled maize, and using two phases of drying (when the maize was still on the cob and after shelling). Also, some farmers delayed the time of drying, or they did not dry the maize at all (Table 3).

Drying maize cobs after harvest was the method used most often (Table 4) However, most farmers in the Kediri regency used two drying phases during the wet season (Table 4).

Maize was generally stored for a short period (2–3 days) before being sold to traders. However, in Kediri regency 14.8 and 40.7% of the respondents sold the maize directly after harvest during the dry and wet seasons, respectively (Table 4).

In Central Lampung regency the usual storage method during the dry season was to spread the maize on paved floors. In the wet season the maize was generally packed in plastic (polypropylene) bags inside the farmer's house or warehouse (81.5% of respondents).

In Kediri regency maize was stored during the dry and wet seasons inside the farmer's house or warehouse. During the dry season maize was generally packed in gunny sacks (59.3% of respondents), while during the wet season plastic bags were also used (44.4% of respondents) (Table 4).

Interviews with traders

The number of respondents (sample numbers) during each season in each location (regency) was eight.

In Central Lampung regency most (87.5%) of the traders dried the maize on paved floors during the dry season and during the wet season all used this method (Table 5).

In Kediri regency 87.5% of traders surveyed dried the maize on paved floors during the dry season. During the wet season 62.5% of the respondents did not dry the maize (Table 5).

In both regencies maize was stored during the dry season for 1–30 days in gunny sacks and plastic bags in a standing position, whereas during the wet season, it was stored for 1–7 days.

Moisture content and damaged kernels

The effect of location and season on moisture content and damaged kernels at farmer level

From the analysis of variance, the location of farms did not significantly affect moisture content and the percentage of damaged kernels. Season had a very significant effect and the interaction between the location and the season affected moisture content significantly, but not the percentage of damaged kernels (Tables 6 and 7).

Table 4. Results of questionnaire of farmers on maize postharvest handling during the dry and wet seasons in Central Lampung regency, Lampung province, and Kediri regency, East Java province.

Detail	Central Lampung		Kediri	
	Dry season	Wet season	Dry season	Wet season
Number of respondents	27	27	27	27
Method of drying				
Ears of maize were dried immediately after harvest	55.6	85.2	51.9	7.4
Maize was dried after shelling	18.5	0	11.1	0
Two-phase drying	3.7	7.4	37.0	92.6
Postponement of drying time	3.7	7.4	0	0
Without drying	18.5	0	0	0
Storing				
Without storing (sold directly)	0	0	14.8	40.7
Spreading on a paved floor	55.6	0	0	4.0
In the house/warehouse using jute bag	14.8	22.2	59.3	18.5
In the house/warehouse using plastic (polypropylene) bag	33.3	81.5	29.6	44.4

Table 5. Results of questionnaire of traders on maize postharvest handling during the dry and wet seasons in Central Lampung regency, Lampung province, and Kediri regency, East Java province.

Detail	Central Lampung		Kediri	
	Dry season	Wet season	Dry season	Wet season
Number of respondents	8	8	8	8
Drying				
Without drying	12.5	0	12.5	62.5
With drying	87.5	100	87.5	37.5
Duration of storage in jute and plastic bags (day)	1–30	1–7	1–30	1–7

Table 6. Analysis of variance on the effect of location, season, and interaction between location and season on moisture content of maize collected from farmers.

Source of variance	df	SS	MS	F Value
A	1	22.687500	22.687500	1.37
Error A	26	430.270000	16.548846	
B	1	503.971204	503.971204	20.88**
Error B	26	627.516296	24.135242	
AB	1	169.500833	169.500833	7.64*
Error AB	26	577.206667	22.200256	

A = location.
B = season.
AB = interaction between location and season.
* = significantly different at 95% confidence level.
** = significantly different at 99% confidence level.

Table 7. Analysis of variance on the effect of location, season, and interaction between location and season on damaged kernels in maize collected from farmers.

Source of variance	df	SS	MS	F Value
A	1	244.201482	244.201482	4.17
Error A	26	1524.298519	58.626866	
B	1	1093.157037	1093.157037	21.42**
Error B	26	1326.652963	51.025114	
AB	1	183.561481	183.561481	3.73
Error AB	26	1279.638519	49.216866	

A = location.
B = season.
AB = interaction between location and season.
** = significantly different at 99% confidence level.

During the wet season the moisture content of maize collected in Central Lampung and Kediri regencies was higher than that of maize collected during the dry season (Table 8). Differences in rainfall, number of rainy days, and relative humidity between the wet and dry seasons are shown in Table 9.

More kernels were damaged during the dry season than during the wet season (Table 10). It was assumed that the damage was caused by the mechanical sheller. Kernels with higher moisture content can be cracked and broken more easily than kernels with lower moisture content.

The effect of location and season on moisture content and damaged kernels at trader level

From the analysis of variance, location did not significantly affect moisture content of maize collected from traders. However, both season and the interaction between location and season were significant (Table 11).

Location significantly affected the number of damaged kernels. However, season and interaction between location and season had no significant effect (Table 12).

The moisture content of maize at trade level in both Central Lampung and Kediri regencies was higher during the wet season than the dry season (Table 13).

More damaged kernels were collected from traders in Central Lampung regency than in Kediri regency (Table 14). It was assumed that the difference in the amount of damage could be attributed to the age of harvested maize and the facilities used for shelling it. In Kediri regency, farmers generally harvested maize at an appropriate age and used mechanical shellers.

Table 8. Moisture content of maize collected from farmers during the dry and wet seasons in Central Lampung and Kediri regencies.

Season	Moisture content (%)	
	Central Lampung	Kediri
Dry	21.9 a	19.3 b
Wet	23.8 a	25.4 c

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

Table 9. Rainfall and the number of rainy days during the dry and wet seasons when sampling in Central Lampung and Kediri regencies.

Location	Rainfall (mm) in season		Rainy days in season	
	Dry	Wet	Dry	Wet
Central Lampung	73	343	6	16
Kediri	7	308	1	16

Table 10. Degree of kernel damage in maize collected from farmers during the dry and wet seasons.

Season	Damaged kernels (%)
Dry	10.7 a
Wet	4.4 b

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

Table 11. Analysis of variance on the effect of location, season, and interaction between location and season on moisture content in maize collected from traders.

Source of variance	df	SS	MS	F Value
A	1	0.427813	0.427813	0.21
Error A	7	14.579688	2.082813	
B	1	233.820313	233.820313	100.00**
Error B	7	16.367188	2.338170	
AB	1	33.825313	33.825313	12.02*
Error AB	7	19.702188	2.814598	

A = location.
B = season.
AB = interaction between location and season.
* = significantly different at 95% confidence level. ** = significantly different at 99% confidence level.

Table 12. Analysis of variance on the effect of location, season, and interaction between location and season on damaged kernels in maize collected from traders.

Source of variance	df	SS	MS	F Value
A	1	147.490313	147.490313	12.86**
Error A	7	80.287188	11.469598	
B	1	346.502813	346.502813	4.49
Error B	7	540.074688	77.153527	
AB	1	77.190313	77.190313	1.43
Error AB	7	377.487187	53.926741	

A = location.
B = season.
AB = interaction between location and season.
** = significantly different at 99% confidence level.

Table 13. Moisture content of maize collected from traders during the dry and wet seasons in Central Lampung and Kediri regencies.

Season	Moisture content (%)	
	Central Lampung	Kediri
Dry	14.4 a	12.1 c
Wet	17.7 b	19.5 d

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

Table 14. Percentages of damaged kernels in maize collected from traders in Central Lampung and Kediri regencies.

Location	Damaged kernels (%)
Central Lampung	12.7 a
Kediri	8.4 b

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

Table 15. Analysis of variance on the effect of respondent type (farmer or trader) on moisture content during the dry season in Central Lampung and Kediri regencies.

Source of variance	df	SS	MS	F Value
Respondents	1	620.460080	620.460080	16.23**
Error	68	2599.628634	38.229833	

** = significantly different at 99% confidence level.

Relationship between type of respondent (farmer or trader) and moisture content and damage to kernels during the dry and wet seasons in Central Lampung and Kediri regencies

From the analysis of variance, location did not have a significant effect on moisture content of maize collected from farmers and traders, therefore only the effect of respondents on moisture content during the dry and wet seasons was analysed. The result showed that type of respondent significantly affected moisture content during the two seasons (Tables 15 and 16).

The moisture content of maize from farmers during the dry and wet seasons was higher than that in maize collected from traders (Table 17). This was because traders purchased dried and shelled maize from the farmers.

The percentages of damaged kernels collected from traders during the dry and wet seasons in Central Lampung and Kediri regencies were higher than those in maize from farmers. The percentage of damaged kernels in maize collected from farmers in Central Lampung regency during the dry and wet seasons were 13.5 and 4.6%, respectively, while in Kediri regency they were 7.9 and 4.2%, respectively (Table 22). Nevertheless, from statistical analysis, the percentages of damaged kernels in maize collected from traders during the two seasons were not significantly different from those in maize from farmers (Tables 18, 19, 20 and 21).

The effect of postharvest handling on moisture content and damaged kernels during the dry and wet seasons in Central Lampung and Kediri regencies

As stated earlier, in this study postharvest handling was differentiated into five stages: 1) freshly harvested maize cobs; 2) dry maize cobs; 3) shelled maize before drying; 4) dry shelled maize; and 5) dry shelled maize stored by traders. For the 1st and 2nd stage, we shelled cobs by hand.

Postharvest handling significantly affected moisture content during the dry and wet seasons (Tables 23 and 24). During the dry season, the moisture content of maize fell between the 1st to the 5th stages, and the difference was statistically significant. Nevertheless, the moisture content between the 2nd and 3rd stages, and between the 4th and 5th stages were not significantly different (Table 25).

Table 17. Moisture content of maize collected from farmers and traders during the dry and wet seasons.

Respondents	Moisture content (%) in season	
	Dry	Wet
Farmer	20.6 a	24.6 c
Trader	13.2 b	18.6 d

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

The moisture content of maize fell also during the wet season between postharvest stages 1 and 5. The difference was statistically significant. Differences in moisture content between stages 3, 4, and 5 were not significant (Table 25).

During the wet season, postharvest handling significantly affected percentages of damaged kernels in Central Lampung. In Kediri regency, the effect was rated highly significant (Tables 26 and 27). During the dry season, however, postharvest handling did not affect percentages of damaged kernels significantly (Tables 28 and 29).

During the wet season, the highest percentage of damaged kernels was recorded at stage 5 in Central Lampung and at stage 4 in Kediri (Table 30). At both locations, damage at stage 3 was higher than at stages 1 and 2. Maize at stage 3 was shelled using nail-down wood and a mechanical sheller. Stage 1 and 2 maize was shelled by hand.

The percentages of damaged kernels in freshly harvested maize cobs (1st stage) in Central Lampung and Kediri regencies were higher than those of dried cobs (2nd stage) (Table 30).

Table 16. Analysis of variance on the effect of respondent type (farmer or trader) on moisture content during the wet season in Central Lampung and Kediri regencies.

Source of variance	df	SS	MS	F Value
Respondents	1	444.960214	444.960214	11.33**
Error	68	2670.012500	39.264890	

** = significantly different at 99% confidence level.

Table 18. Analysis of variance on the effect of respondent type (farmer or trader) on the percentage of damaged kernels in maize during the dry season in Central Lampung regency.

Source of variance	df	SS	MS	F Value
Respondents	1	98.331857	98.331857	0.82
Error	33	3979.315000	120.585303	

Table 19. Analysis of variance on the effect of respondent type (farmer or trader) on the percentage of damaged kernels in maize during the dry season in Kediri regency.

Source of variance	df	SS	MS	F Value
Respondents	1	30.045974	30.045974	1.15
Error	33	859.055741	26.031992	

Table 20. Analysis of variance on the effect of respondent type (farmer or trader) on the percentage of damaged kernels in maize during the wet season in Central Lampung regency.

Source of variance	df	SS	MS	F Value
Respondents	1	66.173716	66.173716	2.17
Error	33	1008.081713	30.547931	

Table 21. Analysis of variance on the effect of respondent type (farmer or trader) on the percentage of damaged kernels in maize during the wet season in Kediri regency.

Source of variance	df	SS	MS	F Value
Respondents	1	38.172466	38.172466	2.87
Error	33	438.302963	13.281908	

Table 22. Percentage of damaged kernels in maize collected from farmers and traders during the dry and wet seasons in Central Lampung and Kediri regencies.

Respondents	Central Lampung		Kediri	
	Dry	Wet	Dry	Wet
Farmer	13.5 a	4.6 b	7.9 c	4.2 d
Trader	17.5 a	7.8 b	10.1 c	6.7 d

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

Table 23. Analysis of variance on the effects of postharvest handling on moisture content during the dry season in Central Lampung and Kediri regencies.

Source of variance	df	SS	MS	F Value
Criteria	4	1732.505657	433.126414	18.93**
Error	65	1487.583058	22.885893	

** = significantly different at 99% confidence level.

Table 24. Analysis of variance on the effects of postharvest handling on moisture content during the wet season in Central Lampung and Kediri regencies.

Source of variance	df	SS	MS	F Value
Handling stage	4	2587.475960	646.868990	79.71**
Error	65	527.496754	8.115335	

** = significantly different at 99% confidence level.

Table 25. Relationships between moisture content of maize and postharvest handling stage during the dry and wet seasons.

Handling stage	Moisture content (%) in season	
	Dry	Wet
1	25.2 a	32.9 e
2	20.2 b	24.4 f
3	21.1 b	20.7 g
4	13.4 c	17.5 gh
5	13.2 c	18.6 h

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

Table 26. Analysis of variance on the effects of postharvest handling on the percentage of damaged kernels in maize during the wet season in Central Lampung regency.

Source of variance	df	SS	MS	F Value
Handling stage	4	286.799393	71.699848	2.73*
Error	30	787.456036	26.248535	

* = significantly different at 95% confidence level.

Table 27. Analysis of variance on the effects of postharvest handling on the percentage of damaged kernels in maize during the wet season in Kediri regency.

Source of variance	df	SS	MS	F Value
Handling stage	4	190.141397	47.535349	4.98**
Error	30	286.334032	9.544468	

** = significantly different at 99% confidence level.

The higher percentage of damaged kernels from freshly harvested cobs rather than dried can be attributed to the higher moisture content in the fresh cobs during the dry and wet seasons. (Table 25). According to BULOG, IPB and UGM (1988) shelling of freshly harvested maize (ca 37% m.c.) damaged a higher percentage of kernels than shelling of maize after the 1st phase of drying (ca 25% m.c.). SFCDP (1990) reported that, in general, if moisture content of maize collected from East Java and South Sulawesi (Indonesia) was high, the percentage of damaged kernels was also high.

A. flavus and aflatoxin content

The effect of location and season on infection by A. flavus *and aflatoxin levels in kernels collected from farmers*

From analysis of variance: location significantly affected the percentage of kernels infected by *A. flavus* at farmer level; the season gave a very significant difference; and the interaction between location and season was not significant (Table 31).

In this study, aflatoxin B_1, B_2, G_1, and G_2 were obtained and the latter three converted into aflatoxin B_1.

Table 28. Analysis of variance on the effects of postharvest handling on the percentage of damaged kernels in maize during the dry season in Central Lampung regency.

Source of variance	df	SS	MS	F Value
Handling stage	4	565.906714	141.476679	1.21
Error	30	3511.740143	117.058005	

Table 29. Analysis of variance on the effects of postharvest handling on the percentage of damaged kernels in maize during the dry season in Kediri regency.

Source of variance	df	SS	MS	F Value
Handling stage	4	102.574254	25.643564	0.98
Error	30	786.527460	26.217582	

The location, season, and interaction between location and season on total aflatoxin B_1 content did not yield significant differences (Table 32).

The percentage of kernels infected by *A. flavus* in Central Lampung regency was higher than in Kediri regency, while the total aflatoxin B_1 content in Central Lampung regency was lower than that in the Kediri regency. However, from the analysis, the differences were not statistically significant (Table 33). According to Wilson et al. (1983) the growth of *A. flavus* and aflatoxin production could be affected by the metabolites produced by maize. The kind of metabolites depended on maize variety. In this study, maize samples were obtained from different maize varieties. Widstrom et al. (1990) reported that environmental conditions, especially temperature and relative humidity, during maize cultivation could cause aflatoxin contamination of maize.

Aflatoxin production has also been reported to depend on the strains of *A. flavus* (Diener and Davis 1969) and the interaction between the fungus and other fungal species (Widstrom et al. 1990).

The percentage of kernels infected by *A. flavus* during the dry season was higher than that of kernels collected during the wet season. On the other hand, aflatoxin content of maize collected during the dry season was higher than that during the wet season (Table 33). Nevertheless, these differences were not statistically significant (Table 32). It was assumed that other fungal species that compete with *A. flavus* become more significant in the wet season.

In another study results showed that the percentage of kernels infected by *Fusarium* spp. (*F. moniliforme* and *F. semitectum*) was higher during the wet season than during the dry season. According to Zummo and Scott (1992) *F. moniliforme* could inhibit *A. flavus* growth and aflatoxin production on maize. Horn and Wicklow (1983) and Choudhary (1992) reported that *F. moniliforme, Penicillium citrinum,* and *A. ochraceus* inhibited aflatoxin production.

The effect of location and season on A. flavus *and aflatoxin levels in kernels collected by traders*

From analysis of variance, season significantly affected the percentage of kernels infected by *A. flavus* in maize collected by traders. However, location and the interaction between location and season did not have a significant effect (Table 34). The percentage of kernels infected by *A. flavus* during the dry season was higher than during the wet season (Table 36). It was assumed that during the wet season other fungal species competed with *A. flavus*. Total aflatoxin B_1 content during the dry season was higher than during the wet season (Table 36), but analysis indicated the difference was not statistically significant (Table 35). According to Choudhary and Sinha (1993) fungi that competed with *A. flavus* and inhibited aflatoxin B_1 production by more than 85% were *A. niger*, *A. niger* + *Aspergillus* spp., *A. niger* + *Penicillium* spp., *Rhizopus nigricans* + *Aspergillus* spp., *Curvularia lunata*, *Chaetomium globosum*, and *Cladosporium herbarum*.

Relationship between type of respondent, the percentage of kernels infected by A. flavus and total aflatoxin B_1 content

The percentages of *A. flavus*-infected maize kernels collected from farmers during the dry and wet seasons in Central Lampung and Kediri regencies were lower than those of kernels collected from traders (Table 41).

Table 30. Percentage of damaged kernels in maize f rom several types of postharvest handling during the wet season in Central Lampung and Kediri regencies.

Postharvest handling stage	Damaged kernels (%)	
	Central Lampung	Kediri
1	2.6 ab	2.7 cd
2	0.6 a	1.0 c
3	7.2 b	5.2 de
4	7.7 b	7.4 e
5	7.8 b	6.7 e

Table 31. Analysis of variance on the effect of location, season, and interaction between location and season on the percentage of kernels infected by *A. flavus* collected from farmers.

Source of variance	df	SS	MS	F Value
A	1	2296.333333	2296.333333	5.97*
Error A	26	10006.666667	384.871790	
B	1	61442.370370	61442.370370	56.51**
Error B	26	28267.629630	1087.216520	
AB	1	202.814810	202.814810	0.31
Error AB	26	17227.185200	662.584000	

A = location.
B = season.
AB = interaction between location and season.
* = significantly different at 95% confidence level.
** = significantly different at 99% confidence level.

Table 32. Analysis of variance on the effect of location, season, and interaction between location and season on total aflatoxin B_1 content in maize collected from farmers.

Source of variance	df	SS	MS	F Value
A	1	6247.659675	6247.659675	2.50
Error A	26	65068.171250	2502.621970	
B	1	51.515445	51.515445	0.02
Error B	26	86431.549280	3324.290360	
AB	1	3939.892000	3939.892000	1.47
Error AB	26	69760.587100	2683.099500	

Table 33. The percentage of kernels infected by *A. flavus* and total aflatoxin B_1 content in maize collected from farmers during the dry and wet seasons in Central Lampung and Kediri regencies.

Effects	Kernels infected by *A. flavus* (%)	Total aflatoxin B_1 content (ppb)
Location		
Central Lampung	66.3 a	61.4 e
Kediri	57.1 b	76.7 e
Season		
Dry	85.6 c	69.7 f
Wet	37.9 d	68.4 f

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

Table 34. Analysis of variance on the effect of location, season, and interaction between location and season on the percentage of kernels infected by *A. flavus* in maize collected from traders.

Source of variance	df	SS	MS	F Value
A	1	116.281250	116.281250	0.26
Error A	7	3182.468750	454.638390	
B	1	11590.031250	11590.031250	16.49**
Error B	7	4920.718750	702.959820	
AB	1	1212.781250	1212.781250	3.20
Error AB	7	2652.968750	378.995540	

A = location; B = season.
AB = interaction between location and season.
** = significantly different at 99% confidence level.

Table 35. Analysis of variance on the effect of location, season, and interaction between location and season on total aflatoxin B_1 content in maize collected from traders.

Source of variance	df	SS	MS	F Value
A	1	1768.637813	1768.637813	2.43
Error A	7	5092.944537	727.563505	
B	1	504.825313	504.825313	0.49
Error B	7	7192.731137	1027.533020	
AB	1	854.497800	854.497800	3.01
Error AB	7	1984.239850	283.462840	

A = location; B = season.
AB = interaction between location and season.

Nevertheless, from analysis of variance, the differences between the results for farmer- and trader-sourced were not statistically significant for the percentages of infected kernels during the two seasons in the two regencies (Tables 37, 38, 39, and 40).

Total aflatoxin B_1 content in kernels collected at farmer level was higher that in kernels from traders (Table 43). Nevertheless, based on the analysis of variance, they were not significantly different (Table 42).

The effect of postharvest handling on the kernels infected by A. flavus *and total aflatoxin B_1 content during the dry and wet seasons in Central Lampung and Kediri regencies*

The type of postharvest handling resulted in very significant differences between the percentage of kernels infected by *A. flavus* during the wet season in Central Lampung and Kediri regencies (Tables 46 and 47), but did not result in significant differences during the dry season (Tables 44, 45, 46, and 47).

The highest percentages of kernels infected by *A. flavus* during the wet season in Central Lampung and Kediri regencies were from those at postharvest stages 3 and 4. The lowest were from those at stage 1 (Table 48). It was assumed that for the 3rd and 4th stages of postharvest handling, maize was shelled using tools (a nail-down wood and a mechanical sheller), while for the 1st stage, it was shelled by hand. Shelling with tools caused more damage (cracked or broken) to the kernels and made them more susceptible to *A. flavus*.

According to Siriacha et al. (1988) *A. flavus* infection of shelled maize was higher than that of maize cob. Siriacha et al. (1989) reported that the infection was promoted by damage to kernels during shelling and by the effects of poor drying.

Postharvest handling did not significantly affect total aflatoxin B_1 content (Table 49). The highest total aflatoxin B_1 content was in kernels that were at the 3rd stage, while the lowest was in those at the 5th stage. Nevertheless, the levels were not significantly different (Table 50).

Conclusions

1. Most of the farmers and traders used paved floors for sun drying shelled and cob maize.
2. Farmers and traders generally used a mechanical sheller, and jute or polypropylene bags to store the maize.
3. The moisture contents of maize collected from farmers and traders during the wet season in both regencies were higher than those during the dry season. In both seasons, the moisture contents of maize collected from farmers were higher than those of maize collected from traders.
4. The percentage of damaged kernels from farmers during the dry season was higher than those during the wet season, while for those from traders there was no significant difference between seasons.
5. At the farmer level, the effect of location did not result in any significant difference to the percentage of damaged kernels, whereas at trader level, a higher percentage of kernels from Central Lampung regency were damaged than from Kediri regency.
6. The percentage of kernels collected from farmers in Central Lampung regency and infected by *A. flavus* was higher than for kernels collected from farmers of Kediri regency. At both farmer and trader levels the percentage of fungus-infected kernels was higher during the dry season. In both seasons in both regencies, levels of kernel infection were lower at farmer level.
7. Total aflatoxin B_1 content of maize collected at farmer level was not significantly different from that at trader level.

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Table 36. The percentage of kernels infected by *A. flavus* and total aflatoxin B_1 content in maize collected from traders during the dry and wet seasons.

Season	Kernels infected by *A. flavus* (%)	Aflatoxin B_1 total content (ppb)
Dry	91.8 a	57.4 c
Wet	53.8 b	49.5 c

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

Table 37. Analysis of variance on the effect of respondent type (farmer or trader) on the percentage of kernels infected by *A. flavus* during the dry season in Central Lampung regency.

Source of variance	df	SS	MS	F Value
Respondents	1	427.144048	427.144048	2.03
Error	33	6927.541667	209.925505	

Table 38. Analysis of variance on the effect of respondent type (farmer or trader) on the percentage of kernels infected by *A. flavus* during the dry season in Kediri regency.

Source of variance	df	SS	MS	F Value
Respondents	1	106.667196	106.667196	0.29
Error	33	12012.018519	364.000561	

Table 39. Analysis of variance on the effect of respondents (farmer and trader) on the percentage of kernels infected by *A. flavus* during the wet season in Central Lampung regency.

Source of variance	df	SS	MS	F Value
Respondents	1	437.304762	437.304762	0.39
Error	33	37192.666667	1127.050505	

Table 40. Analysis of variance on the effect of respondent type (farmer or trader) on the percentage of kernels infected by *A. flavus* during the wet season in Kediri regency.

Source of variance	df	SS	MS	F Value
Respondents	1	3370.675132	3370.675132	2.58
Error	33	43106.296296	1306.251403	

Table 41. The percentage of kernels infected by *A. flavus* at farmer and trader levels during the dry and wet seasons in Central Lampung and Kediri regencies.

Effect	Kernels infected by *A. flavus* (%)
Central Lampung	
Dry season	
Farmer	91.6 a
Trader	99.9 a
Wet season	
Farmer	41.1 b
Trader	49.5 b
Kediri	
Dry season	
Farmer	79.6 c
Trader	83.8 c
Wet season	
Farmer	34.6 d
Trader	58.0 d

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

Table 42. Analysis of variance on the effect of respondent type (farmer or trader) on the total aflatoxin B_1 content.

Source of variance	df	SS	MS	F Value
Respondents	1	6008.620851	6008.620851	2.60
Error	138	318539.313471	2308.255895	

Table 43. Total aflatoxin B_1 content at farmer and trader levels.

Effect	Total aflatoxin B_1 content (ppb)
Farmer	69.1 a
Trader	53.5 a

Table 44. Analysis of variance on the effects of postharvest handling on the percentage of kernels infected by *A. flavus* during the dry season in Central Lampung regency.

Source of variance	df	SS	MS	F Value
Handling stage	4	1020.953571	255.238393	1.21
Error	30	6333.732143	211.124405	

Table 45. Analysis of variance on the effects of postharvest handling on the percentage of kernels infected by *A. flavus* during the dry season in Kediri regency.

Source of variance	df	SS	MS	F Value
Handling stage	4	1978.938095	494.734524	1.46
Error	30	10139.747619	337.991587	

Table 46. Analysis of variance on the effects of postharvest handling on the percentage of kernels infected by *A. flavus* during the wet season in Central Lampung regency.

Source of variance	df	SS	MS	F Value
Handling stage	4	14137.020630	3534.255160	4.51**
Error	30	23489.950797	782.998360	

** = significantly different at 99% confidence level.

Table 47. Analysis of variance on the effects of postharvest handling on the percentage of kernels infected by *A. flavus* during the wet season in Kediri regency.

Source of variance	df	SS	MS	F Value
Handling stage	4	24410.258730	6102.564680	8.30**
Error	30	22066.712700	735.557090	

** = significantly different at 99% confidence level.

Table 48. The kernels infected by *A. flavus* in several postharvest handling criteria during the wet season in Central Lampung and Kediri regencies.

Handling stage	Kernels infected by *A. flavus* (%)	
	Central Lampung	Kediri
1	14.4 a	7.4 d
2	30.2 ab	15.2 de
3	65.5 c	43.2 ef
4	62.3 bc	76.1 g
5	49.5 bc	58.0 fg

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

Table 49. Analysis of variance on the effects of postharvest handling on aflatoxin B_1 total content.

Source of variance	df	SS	MS	F Value
Handling stage	4	12336.817160	3084.204290	1.33
Error	135	312211.117160	2312.674940	

Table 50. Total aflatoxin B_1 content in maize treated in several ways after harvesting.

Handling stage	Total aflatoxin B_1 content (ppb)
1	63.1 a
2	74.8 a
3	80.7 a
4	62.7 a
5	53.5 a

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

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Relationship between Soil Populations of *Aspergillus flavus* and Aflatoxin Contamination under Field Conditions in the Philippines

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Abstract

Field plot studies were conducted during the wet and dry seasons of 1993 in Ilagan, Isabela, Philippines to determine the relationship between populations of *Aspergillus flavus* in the soil and the extent of infection and aflatoxin contamination of preharvest maize. The experimental set-up was a randomised-complete-block-design (RCBD) with seven treatments and five replications. Treatments consisted of two types of inoculation and different levels of inocula.

Initial sampling of soil showed great variability in *Aspergillus* propagule counts within the field. Highest infection and contamination were observed in the treatment which was basally inoculated with the highest level of inoculum. This indicates a direct relationship between soil population and extent of infection and aflatoxin contamination occurring in preharvest maize. The uninoculated control also became infected and contaminated, suggesting that there is sufficient indigenous inoculum level in the soil to cause infection. The extent of infection and aflatoxin contamination were generally lower during the wet than the dry season trial. Results from the vegetative compatibility groupings (VCG) analyses revealed the majority of the *Aspergillus* isolates from the infected grains came from the strain used as inoculum. The marked strain has indeed been transmitted into the developing maize causing infection and aflatoxin contamination.

Although field infection and contamination are complex phenomena, results from VCG complementation tests of *Aspergillus flavus* isolated from the maize grains suggest and support previous literature reports that the soil is the principal source of primary inoculum.

AFLATOXIN contamination is one of the major problems in maize production, especially in the tropics where environmental conditions are conducive to *Aspergillus* growth. This fungus can infect seeds and plant debris of crops in the field, during harvest, in storage, and during processing (Lillehoj et al. 1975, 1976). Ordinarily, *Aspergillus* is not a problem in the field but develops on seeds after harvest. However, the detection of aflatoxin in preharvest maize (Anderson et al. 1975) has prompted investigations of the factors influencing infection in the field.

Diener et al. (1987) considered the potential sources of inoculum to be the spores and sclerotia of *A. flavus* in the soil, and mycelia in plant debris and litter. Disruption of soil and vegetation during cultivation causes redistribution of these propagules and, when nutrients are available and environmental conditions favourable, the fungal population could increase rapidly in association with the growing crop (Cotty et al. 1994).

Aflatoxin-forming fungi like *A. flavus* could colonise plant debris and other cover crops (Griffin and Garren 1976) and eventually increase soil population density. In addition, soil properties (Diener et al. 1987) and cultural practices could also affect the number of propagules in the soil. A greater number was observed in finer textured soils and no isolates were observed from virgin prairie soil (Angle et al. 1982).

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The population of *A. flavus* in the soil is important since it is presumed to be the primary source of inoculum for field infection. The spores may be carried by insects (Fennel et al. 1977) or may be readily dispersed by air movements. Lee et al. (1986) observed that wind-driven soil inoculated with *A. flavus* propagules was dispersed to developing cotton bolls and caused infection when followed by rain. Extent of infection and contamination are affected by the inoculum level and the accessibility of the plant to fungal conidia (Jones et al. 1981).

Several factors influencing invasion and infection by *A. flavus* have been reviewed by Diener et al. (1987). Studies of preharvest infection have been conducted in maize-growing areas in the United States but current knowledge of this aspect is still very limited in the Philippines.

Monitoring the movement of *A. flavus* from the soil to the developing crop could be useful for determining the importance and relationship between soil populations and the occurrence of aflatoxins in field crops. Vegetative compatibility groups (VCG) which have been useful in population studies of fungal plant pathogens (Glass and Kaldau 1992) are used to monitor the movement of *A. flavus*. By introducing into soil a marked strain of known VCG, the movement of *A. flavus* isolated from the infected maize grain could be characterised and classified to determine its degree of complementation with testers of the introduced strain. This, however, requires a preliminary survey of existing VCG in the field to ascertain that the inoculum used is not indigenous to the area.

The objective of the study reported here was therefore to determine the relationship between soil populations of *A. flavus* and aflatoxin contamination of maize grown under field conditions in the Philippines.

Materials And Methods

Land preparation, planting, and layout

The experimental area was prepared in accordance with the conventional method of land preparation in upland areas. This consisted of ploughing three times, with harrowing after each ploughing. Furrows were then set 75 cm apart with a plot size of 6 × 6 m. Only the inner rows of each plot, or a total area of 3.5 × 3.0 m (10.5 m^2), were inoculated.

The maize variety used was IES Cnl, the most popular open-pollinated variety used by farmers in the area. Seeds were drilled at a distance of 25 cm between hills and thinned to one plant per hill two weeks after planting.

The experimental design was a randomised-complete-block-design (RCBD) with seven treatments and five replications with blocks set 1.5 m apart. Treatments consisted of two types of inoculation (basal and spray) at varying levels of inoculum, and uninoculated control.

Inoculum preparation and inoculation

Basal inoculum was prepared by placing whole maize grains (50 g, 200 g, 400 g) in heat-resistant plastic bags, adjusting moisture to 30% before autoclaving at 15 psi for 15 minutes. The grains were then inoculated with 1 mL each of 10^{-6} spores/mL suspension of *Aspergillus flavus* (Min1A_2), and incubated stationary at room temperature (30±2°C) for 7 days. The bags were occasionally shaken by hand to prevent clamping of kernels and to reduce sporulation on the maize surface.

For basal inoculation, colonised maize at varying levels (i.e. different weights of colonised maize were used) was spread evenly on the soil surface along the rows at 20 days after emergence (20 DAE).

For spray inoculation, 10^6, 10^8, and 10^{10} spores/mL were prepared by growing the *Aspergillus flavus* isolate (Min1A_2) in V-8 juice agar for 7 days. Spores were harvested and adjusted to the required concentration. Tween 80 (0.01%) was added to the suspension as a surfactant before inoculation by spraying onto the soil surface at a rate of 100 mL/plot using an atomiser.

Wounding simulation of developing ears

To facilitate infection, ears were wounded by piercing the developing kernels through the husks with improvised nailboards (20 × 8 cm) containing eight number-two nails spaced 2 cm apart. All ears in the inoculated area were wounded, including the uninoculated control.

Harvesting, drying, and shelling

At maturity, 20 ears per plot were selected and harvested (total of 700 ears) and sun dried with husks for 24 hours. Husks were then removed and ear damage rated visually. During shelling, kernels adjoining the damaged area (wounded) were separated from sound kernels further away (sound) and analysed separately.

Assessment of kernel infection

In each replicate, 400 kernels from each subgroup, or 800 kernels per plot (total of 28 000 kernels), were assessed for kernel infection. Kernels were surface sterilised with 1% NaOCl (Zonrox brand) for 5 minutes, washed three times with sterile distilled water, blotted dry, and plated on Malt Salt Agar (MSA) at 25 seeds per plate. The plates were incubated at room temperature for 10 days and visually assessed for *A. flavus* growth.

Isolation and characterisation of *A. flavus* from maize grains

Distinct *A. flavus* growth on maize kernels plated on MSA were isolated, purified, and paired with testers of the inoculated strain to determine vegetative compatibility. Initially, 25 µL spore suspensions of the purified culture were seeded into wells in a plate containing 35 g Czapek-Dox agar (CD), 35 g potassium chlorate, and 10 mg rose bengal. Cultures were incubated at room temperature for 1–4 weeks until chlorate-resistant mutants were generated. Phenotypes of the purified mutants were determined by growing on CD agar containing different nitrogen sources (i.e. nitrate, nitrite, and hypoxanthine).

Complementation between the mutants and the testers of the inoculated strain was determined by seeding conidial suspension in three wells, 2 cm apart in CD agar for 7 days at room temperature. Each isolate mutant was paired with two complementary testers and complementation was indicated by the formation of a zone of dense, wild type growth.

Aflatoxin analysis of maize

Aflatoxin B_1 content of maize was determined using previous modification to methods of the Association of Official Analytical Chemists (Cotty 1989; Stoloff and Scott 1984).

All kernels not used to assess kernel infection (200 g) were pooled and ground using a manual grinder. After mixing, a 50 g subsample was taken and added to 200 mL aqueous acetone. The mixture was shaken for 15 seconds; allowed to settle over night; and filtered through Whatman No. 4 filter paper. The filtrate (100 mL) was collected and mixed with 220 mL solution of zinc acetate and aluminium chloride [1.1M Zn $(CH_3COO)_2$ and 0.04M $AlCl_3$], together with 80 mL water and 5 g celite (Fluka 545). The mixture was shaken, settled for 30 min–1 hour, and filtered as above. The filtrate (100 mL) was extracted twice with 25 mL methylene chloride. Extracts were combined, passed through 3 g anhydrous sodium sulfate, and evaporated to dryness. Residues were transferred quantitatively to a vial using methylene chloride, before being evaporated to dryness and stored at 0°C and assayed by TLC.

Thin layer chromatography (TLC)

TLC plates (20 × 20 cm) coated with 250 microns silica gel (Analtech Uniplates No. 7020111) were reactivated at 105°C for 1 hour and cooled in a desiccator before use. Initially, residues were solubilised in 1.0 mL methylene chloride and shaken uniformly with a vortex mixer. Plates were spotted with both 5 and 10 microliters (µL) each of the sample extract, internal standard (sample spotted over the standard), and standard. Spotted plates were developed in diethyl ether: methanol: water (97:2:1 v/v) until the solvent front had traversed about 8–10 cm from the origin. Plates were then removed from the developing tank and air dried for 2 min. Sample fluorescences were compared to the standards under long-wave ultraviolet light viewer. Estimates of the amount of aflatoxin B_1 were based on relative fluorescence. If sample fluorescence exceeded that of the standard, the sample was diluted appropriately. For each sample containing aflatoxin B_1, aflatoxin identification was confirmed by spraying the plate with 25% sulfuric acid (Scott 1990).

Results and Discussion

Estimates of the soil population of *Aspergillus flavus* before planting and at harvest during the dry season are shown in Table 1. Variability in the fungal population was present in uninoculated plots. Inoculation of *A. flavus* into the soil at 20 DAE, whether by basally applying colonised maize grains or by spraying with spore suspension, generally increased fungal population in the soil until harvest. This increase in the population of *A. flavus* was also associated with higher infection in the harvested grain. Highest infection of kernels directly adjacent to the damaged area (wounded) resulted from basal inoculation with the highest level of inoculum, but differences among the varying inoculum levels were not significant.

Colonised maize grain provides a readily available substrate in the soil for fungal propagation and spore production. The soilborne and aerial conidia of *A. flavus* can be dispersed during cultivation and disseminated by air currents and possibly by insects into the standing crop where entry and infection could begin (Widstrom 1979). Successful establishment and

Table 1. Relationship between soil populations of *Aspergillus flavus* and aflatoxin contamination of maize, 1993 dry season.[a]

Treatment	Mean *A. flavus* propagules/gm soil[b]		Kernel infection, %[c]		Aflatoxin content, mg/kg[d]	
	Before planting[e]	After harvest[f]	Wounded[g]	Sound[h]	Wounded	Sound
10^6 spores/mL (spray)	1720b[i]	1740c	7.5b	7.7g	50d	48c
50 g colonised maize (basal)	1762b	3520c	16.5ab	16.2d	30c	20f
10^8 spores/mL (spray)	2500ac	2220c	18.0ab	9.3f	54c	28e
200 g colonised maize (basal)	1350c	5070b	16.6ab	12.2e	83b	128b
10^{10} spores/mL (spray)	1025d	2740c	15.2ab	15.4d	28f	36d
400 g colonised maize (basal)	612e	9940a	26.7a	22.3a	176a	148a
Control (uninoculated)	1612bc	1880c	10.7ab	17.0b	28f	16g

[a] Dry season extended from December 1992 to March 1993.
[b] Each value is an average propagule count of 4 plate counts per replication.
[c] Each value is an average of 5 replications with 20 ears per replicate. Average of 400 kernels per replicate.
[d] Each value is an average of 5 replications with 20 ears per replicate. Kernels were pooled and ground using a manual grinder. After mixing, 50 g subsamples were analysed for aflatoxin.
[e] Field not yet inoculated (indigenous *A. flavus*)
[f] Soil samples were collected during or immediately after maize harvest.
[g] Wounded=damaged kernels and kernels directly adjacent to damaged kernels.
[h] Sound=sound kernels separated form the damaged kernels by at least 2 kernels.
[i] Means in a column followed by a common letter are not significantly different at the 5% probability level by Duncan's Multiple Range Test (DMRT).

spread of the fungus within the ear has been widely reported to be associated with insect damage and insect activity (Fennell at al. 1975; Widstrom et al. 1975, 1976). High aerial spore load associated with high soil population may have brought about the high infection observed in the grain.

Sound kernels away from the damaged area or point of entry also showed some degree of infection. This illustrates the ability of the fungus to spread within the ear. However, factors other than insect activity could not be discounted, as Wicklow et al. (1988) observed infection in kernels from ears diagnosed as insect-free.

Aflatoxin contamination in kernels directly adjacent to the damaged area and in kernels further away was also observed to be highest in the treatment corresponding to the highest inoculum level and infection. This indicates a direct relationship between soil population, extent of infection, and aflatoxin contamination.

In the wet season trial, estimates of soil population before planting and during harvest were generally lower than those observed during the dry season (Table 2). Variability in propagule count both before inoculation and at harvest was also observed. Similarly, there was an apparent increase in soil population, except for the treatment sprayed with 10^8 spores/mL suspension and the uninoculated control, for which a slight population decrease was observed. Obviously, this observation is not consistent with the one occurring during the dry season trial. This difference could be attributed to the effects of environmental conditions prevailing during the cropping season. Cultural practices and management employed during the two cropping seasons also varied and this may influence the soil population of *A. flavus* (Angle et al. 1982).

Extent of infection and aflatoxin contamination were generally lower during the wet season trial. This suggests that environmental conditions were not conducive to the propagation of *A. flavus*. Frequent rain associated with the wet season may have washed away the inoculum in the soil thus reducing inoculum level. Weather conditions could also have reduced the dispersal and dissemination of the fungal spores into the developing crop. Varying levels of contamination associated with cropping seasons of cotton were also observed by Ashworth and McMeans (1966) and Russel et al. (1987).

Another factor that may have contributed to the lower infection and contamination is competition between *A. flavus* and other fungal pathogens of maize. Invasion by other fungal genera may have excluded and inhibited *A. flavus* colonisation. Zummo and Scott (1990, 1992) observed an apparent inhibition of *A. flavus* by *Fusarium moniliforme*.

Table 2. Relationship between soil population of *Aspergillus flavus* and aflatoxin contamination of maize, 1993 wet season.[a]

Treatment	Mean *A. flavus* propagules/gm soil[b]		Kernel infection, %[c]		Aflatoxin content, mg/kg[d]	
	Before planting[e]	After harvest[f]	Wounded[g]	Sound[h]	Wounded	Sound
10^6 spores/mL (spray)	420g[i]	152g	6.3d	5.7a	7.1bc	7.5ab
50 g colonised maize (basal)	487f	1078d	5.0e	3.0e	10.4ab	10.1a
10^8 spores/mL (spray)	920d	280c	14.0a	5.1b	8.5abc	10.1a
200 g colonised maize (basal)	1566b	2652b	8.8c	3.6c	12.3a	6.9ab
10^{10} spores/mL (spray)	1480c	1413c	4.0f	5.0b	9.6ab	10.7a
400 g colonised maize (basal)	2360a	3167a	10.8b	5.4a	5.6bc	4.7ab
Control (uninoculated)	760e	405e	4.0f	3.3d	3.6c	1.6b

a Dry season extended from June 1993 to September 1993.
b Each value is an average propagule count of 4 plate counts per replication.
c Each value is an average of 5 replications with 20 ears per replicate. Average of 400 kernels per replicate.
d Each value is an average of 5 replications with 20 ears per replicate. Kernels were pooled and ground using a manual grinder. After mixing, 50 g subsamples were analysed for aflatoxin.
e Field not yet inoculated (indigenous *A. flavus*)
f Soil samples were collected during or immediately after maize harvest.
g Wounded = damaged kernels and kernels directly adjacent to damaged kernels.
h Sound = sound kernels separated from the damaged kernels by at least 2 kernels.
i Means in a column followed by a common letter are not significantly different at the 5% probability level by Duncan's Multiple Range Test (DMRT).

Maize kernels harvested from the uninoculated control for both trials showed some degree of infection and contamination. This implies the presence of sufficient indigenous fungi. However, in comparison with the inoculated treatments, the extent of infection and contamination were lower. Inoculating with non-indigenous strain increased the level of infection and contamination.

The extent of transmission of the inoculated strain into harvested maize grain was illustrated by the degree of complementation obtained between mutants generated from the maize surface and grain isolates and the testers of inoculum. Percent complementation during the dry season ranged from 0–2.5% for the mutants isolated from the maize surface (data not shown) and 10–52% for the mutants isolated from the maize grains (Table 3). In tests on maize harvested during the wet season, percent complementation ranged from 0–12% for the mutants isolated on the surface and 0–44% for the mutants isolated on the maize grains. Furthermore, the following results were obtained when Min1A2 inoculum (250 g/plot) was basally applied and 10^6 spores/mL sprayed at the silking stage of maize. For both unwounded (control), data showed that 0–32% and 0–24% mutant complementation, respectively, during the dry season while during the wet season it was 0–12% and 0–4% respectively. These data show that the non-indigenous strain added to the soil has been transmitted into the growing crop and was able to penetrate and colonise the developing maize kernels.

The relatively low transmittance of the propagules in the soil to the maize kernels could perhaps be attributed to the varying effect of factors associated with spore dispersal and dissemination. Once fungal spores are deposited on the proper infection sites, successful establishment and colonisation are still affected by competition, especially from indigenous strains of *Aspergillus flavus* in the area. Competition among *A. flavus* strains has been reported by Cotty (1991). It may be possible that the inoculated strain was not as competitive as the indigenous strains, which have already adapted to their natural habitat.

Variability in the relationships observed during the two cropping seasons further illustrates the complexity of the mechanism involved in preharvest aflatoxin contamination. Infection and contamination could result from an interplay of several environmental factors and the specific effect of each is quite difficult to quantify under field conditions.

Although *A. flavus* infection and aflatoxin contamination are complex phenomena occurring in the field, results from VCG complementation tests of *Aspergillus flavus* isolated from the maize grains suggest and support previous conclusions in the literature that soil is the principal source of primary inoculum.

Table 3. Percent mutant complementation between the isolated mutant from the grains and the testers, dry season and wet seasons.

Treatment	Mean mutant complementation[a]	
	Dry Season[b]	Wet Season[c]
10^6 spores/mL (spray)	42b[d]	0c
50 g colonised maize (basal)	14e	34ab
10^8 spores/mL (spray)	18d	14ab
200 g colonised maize (basal)	10f	18bc
10^{10} spores/mL (spray)	52a	22b
400 g colonised maize (basal)	20c	44a
Control (uninoculated)	20c	30ab

[a] Each value is an average of 5 replications with 10 isolates per replications with 5 mutants per isolate. Average of 250 isolate mutants per replicate.
[b] Dry season extended from Dec. 1992 to March 1993.
[c] Wet season extended from June 1993 to Sept. 1993.
[d] Means in a column followed by a common letter are not significantly different at the 5% probability level by Duncan's Multiple Range Test (DMRT).

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Inhibition of Aflatoxin Contamination in Rice by *Cladosporium fulvum*

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Abstract

Cultural and morphological studies indicated that a fungal isolate with characteristics typical of *Cladosporium fulvum* possesses inhibitory potential against *A. parasiticus*.

Antagonistic activity of *C. fulvum* against *A. parasiticus* was temporary for the first few days. Inhibition of growth of *A. parasiticus* was observed when *C. fulvum* was allowed to grow five days ahead and complete suppression of growth was possible when seven days headstart was given to *C. fulvum*.

Pigment produced by *C. fulvum* is believed to possess an inhibitory compound and its effect on *A. parasiticus* includes modifying the morphology and altering the germination capability of the organism. The pigment produced by *C. fulvum* was fungistatic to *A. parasiticus* but non-toxic to chick embryos and white mice.

THE Philippines is one of the rice producing countries in Asia and rice is the staple food of most Filipinos. With improved cultural practices, and development of better varieties, production yield is expected to increase. However, it is sad to note that in spite of these factors, there have been times when the Philippines has needed to import rice from other rice producing countries. This means that the supply of grain was not sufficient to feed the country. The shortage of grain can be attributed to the amount wasted through poor post-harvest handling and improper storage. The result of poor storage is reduced grain quality because of pests. These include rodents and insects as well as undesirable microorganisms that result in poor milling yield and inferior sensory qualities.

Majunder et al. (1965) reported that an estimated 1–2% of world grain production is lost because of microbial activities that cause caking of dry matter, development of off-flavour, discolouration, and physical breakdown of grain kernels.

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In the Philippines, rice is usually harvested manually, and rice panicles stacked in the open field until the grains are collected and properly stored. The practice of stacking the rice panicles in an open field, allows contaminants to invade, grow, multiply, and cause undesirable qualities to develop in the rice.

IDRC (1976) reported that in the Philippines the predominant microorganisms usually associated with stored products are fungi belonging to the *Aspergillus* group. The presence of this group in a food crop not only destroys the quality of the crop but renders the crop hazardous for human consumption as some species of *Aspergillus* produce aflatoxins.

Aflatoxin is the generic term for the metabolite produced by *Aspergillus parasiticus* and *Aspergillus flavus* which is highly toxic and carcinogenic. It is toxic not only to bacteria but also to humans. Production of aflatoxin is favoured by warmth and high humidity, conditions typical of tropical regions like the Philippines (CAST 1979). The discovery of mycotoxins, particularly aflatoxin, in stored cereals has challenged researchers to look for ways to prevent, if not completely destroy, toxin-producing organisms.

Several researchers have explored the possibility of inhibiting toxin-producing microorganisms by chemical means. Although there have been some reports of

success, the chemicals reduce food crop quality and are also expensive. Furthermore, chemical treatments may leave undesirable, or even toxic, residues.

The biological approach has been considered recently as an alternative solution. However, there are many questions on its efficiency and safety which need to be investigated and fully understood.

The research reported here was carried out to partially resolve some issues related to microbial control of aflatoxin-producing fungi in cereals. More specifically, the object was to assess the inhibitory potential of *Cladosporium fulvum* against *A. parasiticus* in cereals and to determine the possible mechanism of inhibition.

Methodology

Screening and identification of potential microorganisms

Various yeasts and moulds were screened for their potential to inhibit *A. parasiticus*. The modified flask assay method of Ciegler (1966) was used to activate both organisms and determine their inhibitory potential. A loop full of the test organism was transferred to an Erlenmeyer flask containing 10 mL malt extract broth (MEB). The same procedure was applied to *A. parasiticus*. Flasks containing *A. parasiticus* and test organisms were incubated in a rotary shaker (100 rpm) at 30–32° for 5–7 days. Growth of both organisms and formation of zones of inhibition were noted.

The agar block technique employed in the morphological examination and identification of the isolates was based on the descriptions of Raper and Fennel (1965) and de Vries (1967).

Fungi that resembled *C. fulvum* were further plated with *A. parasiticus* on potato dextrose agar plates at varying inoculation periods. One set of plates was inoculated with both organisms at the same time and in subsequent studies *C. fulvum* was given a headstart of 3, 5, and 7 days before inoculation of *A. parasiticus*.

Determination of inhibitory potential in rice dextrose agar and rice grains

To test the inhibitory potential of *C. fulvum* against *A. parasiticus* in rice, potato dextrose agar was modified by substituting rice infusion for potato. The medium was poured into petri plates and allowed to solidify. Four set of plates were prepared. One set was inoculated with both organisms at the same time. The second, third, and fourth sets of plates, were inoculated with test organisms at 3, 5, and 7 days ahead of *A. parasiticus*. Plates were incubated at 32°C for 3–5 days and reactions monitored daily.

The ability of fungal isolates to inhibit aflatoxin production by *A. parasiticus* was determined using the method of Wicklow et al. (1990). A cell and conidial suspension of *C. fulvum* was prepared from a 14-day-old culture. Nine sets of test plates were prepared by placing undamaged and aflatoxin-free rice grains on the media. Five millilitres of distilled water was added and the grains allowed to stand for 2 hours before being sterilisation. One set of plates with grains was then inoculated with *C. fulvum* and *A. parasiticus*. The other sets were inoculated with the test organisms and given a 3, 5, 7, and 9 day headstart before being inoculated with *A. parasiticus*. The plates were incubated at 32°C for 3–5 days and growth of the organisms monitored daily. After the incubation period, the inoculated grains were separately dried and aflatoxin contamination was carefully determined.

Determination of biological activity

Biological activity of *C. fulvum* against *A. parasiticus* was determined by the modified streak method of Walkman and Relly (1945). Freshly collected culture filtrate, water-soluble extract, and oil-soluble extract of *C. fulvum* were serially diluted from 10^1 to 10^6. One millilitre from each dilution was thoroughly mixed with 9 mL of melted rice dextrose agar and potato dextrose agar.

The agar was allowed to solidify and carefully streaked with *A. parasiticus*. Plates were incubated at 32°C for 3–5 days, and monitored daily for growth of organisms. The highest dilution at which the organisms failed to grow was taken as the end point. Biological activity was expressed in units using the ratio between the volume of the medium and the end point of growth or dilution at which growth was totally inhibited.

Studies on possible mode of inhibition

Serial dilutions from week-old culture filtrate, water extracts, and oil-soluble extracts from *C. fulvum* and pigment extracted from culture filtrates were carefully prepared. One millilitre of each dilution was thoroughly mixed with 10 mL melted sterile culture medium and allowed to solidify. Conidial suspension from 5-day-old culture of *A. parasiticus* was streaked on the solidified medium and allowed to stand for 1–2 minutes to allow penetration of conidial suspension on the culture

medium. The agar block was cut into pieces and transferred into sterile incisions inside the plates. Observations were carried out as soon as growth began. Morphological changes were carefully observed and recorded.

Toxicity evaluation

Water soluble extracts obtained from dried and powdered *C. fulvum*, and from the medium used for mass production of the organisms, were used for toxicity evaluation.

Chick embryo assay was used to detect the presence of toxic substance in *C. fulvum*. A total of 210 day-old chicken eggs was incubated in an automated incubator for 5 days. The eggs were candled to distinguish live from dead embryos and non-embryonated eggs were discarded. Eggs with live embryos were surface sterilised with 95% ethanol. A flame-sterilised dissecting needle was used to penetrate the area of the air sac and 0.1 mL of the extract was injected before the hole was sealed using sterilised melted paraffin. The eggs were labelled and incubated in the automated incubator for 4 days. Candling was done after the incubation to determine the number of live embryos.

To further check whether *C. fulvum* is non-toxic, another toxicity test was carried out on white mice. Seven groups (10 each group) of one-month-old, strong, female, albino mice were used to check the toxic effect of *C. fulvum*.

One millilitre of extract previously prepared (used in chick embryo assay) was injected intraperitoneally into the different groups of white mice. The assay was carried out for 7 days and symptoms of toxicity monitored daily. The animals were sacrificed and sections of the liver tissue collected and examined histologically for toxic effects.

Results and Discussion

Screening and identification of microorganisms with inhibitory potential

Results revealed that, of the several microorganisms evaluated, only one was capable of completely inhibiting the growth of *A. parasiticus*. There are other organisms that showed inhibitory potential against *A. parasiticus* but it was noted that recovery was possible after a few days of incubation. *A. fumigatus*, an isolate from air, was found to inhibit *A. parasiticus* but potential toxicity limits its application, particularly in commodities for human consumption.

A fungal isolate whose cultural and morphological characteristics were typical of *C. fulvum* showed excellent antagonistic potential by inhibiting the growth of *A. parasiticus* in potato dextrose agar. Complete suppression of growth was possible when *C. fulvum* was allowed to grow ahead of *A. parasiticus*.

Inhibitory potential of *C. fulvum*

C. fulvum possesses inhibitory potential against *A. parasiticus* as shown by the production of a clear and a pigmented zone around the *A. parasiticus* colonies. The growth of *A. parasiticus* was found to be limited to the non-pigmented area of the culture media.

Antagonistic potential of *C. fulvum* against *A. parasiticus* was found to be temporary for the first few days, but if given a headstart of seven days or more, complete suppression of growth was possible. The inhibitory potential of *C. fulvum* against *A. parasiticus* can be associated with the production of a pigmented substance which is believed to restrict the growth of *A. parasiticus*. Walkman and Relly (1947) reported that some microorganisms produce pigmented compounds for their survival and that these substances were often inhibitory to other microorganisms.

Production of pigment by *C. fulvum* was influenced by the maturity of the organisms. The longer the incubation period, the wider the area of pigmented zone (Table 1) and the deeper the shade of the pigment produced by the organism.

Table 1. Average (n = 5) width (mm) of pigmented zone around the colonies of *C. fulvum* as influenced by period of incubation.

Incubation period (days)	Area (mm)
7	345.50 a
5	66.50 b
3	21.27 c
1	0.00

Values followed by same letter are significantly different from each other.

The ability of *C. fulvum* to inhibit *A. parasiticus* in rice grains was found to be temporary if both organisms were simultaneously plated together. When *C. fulvum* was given a 3–5-day headstart, suppression of growth was possible. Although *A. parasiticus* was able to recover after a few days of incubation, the growth was relatively slow compared with that when both were plated at the same time. A headstart of either

7 or 9 days suppresses the growth of *A. parasiticus* in rice grains. The complete suppression can be attributed to the fact that, during this period, production of pigment by *C. fulvum* is intense, as indicated by the very dark pigment produced in culture media.

The above result can be substantiated by the aflatoxin analysis of the grains. Seven and nine days headstart stopped growth of *A. parasiticus* and no aflatoxin was produced (Table 2). When *C. fulvum* was given 3–5 days headstart, *A. parasiticus* was able to recover after a few days of incubation. However, the level of aflatoxin in the sample was found to be significantly lower than the control when both organisms were plated together.

Table 2. Aflatoxin content of rice grains as influenced by the headstart given to *C. fulvum*.

Headstart (days)	Aflatoxin content µg/kg
Control 1	10 500
1	10 500
3	600
5	18
7	–
9	–
Control 2	–

Control 1 = samples inoculated with *A. parasiticus* alone.
Control 2 = samples inoculated with *C. fulvum* alone.

Possible mechanisms of inhibition

When *A. parasiticus* was allowed to grow on potato dextrose agar with culture filtrate, the growth was affected. The effect of *C. fulvum* on *A. parasiticus* includes deformation and size reduction of hypha. The same results were noted when water-soluble and oil-soluble extracts from *C. fulvum* were used. If pigment extracted under neutral conditions was used, the same findings were observed in addition to enlargement of some parts of the mycelium of *A. parasiticus*. When pigment extracted under acidic and alkaline conditions was used, different morphological deformities were observed. Some hyphae were shortened and contracted while others had disintegrated. Fewer spores and smaller spores were produced by *A. parasiticus* (Table 3).

The overall effect of antagonistic substances produced by *C. fulvum* comprise the changes in form, size, direction of growth, and abbreviation of hyphal structure.

Biological activity

The extracted pigment from *C. fulvum* was found active in suppressing the growth of *A. parasiticus*. When the substance was extracted under acidic conditions, higher biological activity was noted. However, under alkaline conditions (pH 8.5), the biological activity of the pigment was found to be 1:1000. When extracted under neutral conditions the biological activity was 1:10 000. Although this effect of pH has not been investigated thoroughly, it is believed that more pigment can be extracted under acidic conditions as indicated by a deep purple colour in culture filtrate.

Toxicity test

The toxicological test using the chick embryo bioassay shows 100% survival with both water- and oil-soluble extracts from culture medium and oil-soluble extract from *C. fulvum*. This finding indicates that there are no toxic components present in the extracts. However, 97% survival was observed from distilled water-injected controls, water-soluble extract from *C. fulvum* and egg controls whereas there was only an 80% survival of embryo injected with oil control (Table 4). Death of chick embryos in the above treatments was believed to be the result of the physical condition of the chicks rather than toxic substances in the extracts since no mortality occurred in the water- and oil-soluble extracts from culture medium or from oil-soluble extract from *C. fulvum*. The occurrence of 3% mortality in the control treatment (uninjected egg) supports the view that some chick embryos were weak. The mortality in the oil control can be attributed to substances such as antioxidants in oil that might be detrimental to sensitive embryos. This result is in agreement with the findings of Villaralbo (1988) (wherein chick embryos injected with high grade cooking oil yielded only 90.45% survival). Also, 80% survival falls within normal levels of the fertility rate of hatching eggs of the Alabang Experimental Station (Santiago 1991) where the experiment was conducted.

When the extracts were used to inject albino mice, no mortality occurred among the experimental animals and there was no sign of toxicity in the liver of the animals. This implies that *C. fulvum* is a non-toxic fungus (Table 5).

Table 3. Effect of *C. fulvum* on the morphology of *A. parasiticus*.

Source of active material	Morphological changes
Culture filtrate	Thin, elongated and twisted mycelium spores
Water soluble extract	Same result as for culture filtrate
Oil soluble extract	Same result as for culture filtrate; also few spores developed
Pigment at neutral condition	Same effect, except that some mycelium parts are bulging
Pigment at alkaline conditions	Same as for acidic conditions

Table 4. Percentage survival of chick embryos four days after inoculation with water soluble and oil soluble extract of *C. fulvum*.

Type of Treatments	Number of inoculated eggs	Number of eggs with viable embryos 4 days after inoculation	Embryo Survival %
Distilled water control	30	29	97
Oil Control	30	24	80
Water soluble extract from culture medium	30	30	100
Oil soluble extract from culture medium	30	30	100
Water soluble extract from *C. fulvum*	30	29	97
Oil soluble extract from *C. fulvum*	30	30	97
Uninjected egg control	30	29	97

Table 5. Percent survival and toxic effect of extracts in white mice.

Treatments used	No. of animals injected	Survival *after 7 days*		Presence of toxic effect (in lever)
		No. of survivor	%	
Distilled water control	25	25	100	NAS
Oil Control	25	25	100	NAS
Water soluble extract from culture medium	25	25	100	NAS
Oil soluble extract from culture medium	25	25	100	NAS
Water soluble extract from *C. fulvum*	25	25	100	NAS
Oil soluble extract from *C. fulvum*	25	25	100	NAS

NAS—no apparent symptoms

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Completed-project Economic Assessment of Two ACIAR Projects on Fungi and Aflatoxins:

a Discussion of Methodology Issues and Some Estimates of Potential Benefits

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Abstract

This paper presents a detailed economic assessment of the costs and potential benefits from two research projects which gathered information about fungal infection and mycotoxin contamination in staple food and feedstuffs in Southeast Asia. The assessment focuses on aflatoxin contamination of peanuts and maize and its impact on human health and animal production. It suggests that substantial benefits will accrue from technological advances in pre- and postharvest practices likely to be stimulated by the research.

THIS paper describes a completed-project assessment of two ACIAR funded projects:

- PN8806: Fungi and mycotoxins in Asian food and feedstuffs; and
- PN9104: Factors affecting invasion by *Aspergillus flavus* and aflatoxin formation in Asian peanuts.

ACIAR Project Number (PN) 8806 involved collaborative research between the following organisations:

- CSIRO Division of Food Science and Technology, Food Research Laboratory, in Australia;
- National Postharvest Institute for Research and Extension in the Philippines;
- Research Institute for Veterinary Science, Bogor, Indonesia;
- South-East Asian Centre for Tropical Biology (SEAMEO-BIOTROP), Bogor, Indonesia;
- Faculty of Agricultural Science and Technology, Gadjah Mada University, Yogyakarta, Indonesia; and
- Division of Plant Pathology and Microbiology, Department of Agriculture, Bangkhen, Bangkok, Thailand.

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ACIAR PN9104 involved collaborative research between CSIRO Division of Food Science and Technology, Food Research Laboratory in Australia; and the Fields Crops Division, Department of Agriculture, Bangkhen, Bangkok, Thailand.

The projects and their objectives

The objectives of the two projects are given in Appendix A. ACIAR PN8806 studied the fungi on a wide variety of commodities collected in Indonesia, the Philippines, and Thailand. These studies demonstrated the widespread occurrence of the aflatoxin-producing fungus *Aspergillus flavus* in the edible portion of most crops grown in the region (Miller 1993). PN9104 addressed the issue of whether invasion of peanuts by *Aspergillus flavus*, and the production of aflatoxins in peanuts, occurs before or after harvest. This issue is important because vastly different processes for the control of aflatoxin production in grains are required depending on whether invasion and production of aflatoxins occurs before or after harvest.

The following table indicates the extent to which the two projects achieved their objectives as perceived by their reviewers. King (1991) reviewed PN8806 and Miller (1993) reviewed PN9104.

A reviewer of PN9104 during its proposal development phases, noted that:

> The project will not result in any tangible, possible commercialisable outputs, but rather will open the way for the development of appropriate control measures, some of which might be commercialisable. (Dr K.J. Middleton, pers. comm., June 1991)

A similar comment can be made about the other ACIAR project, PN8806, in that its objective was not to develop any particular technology. Rather, its aim was to provide data on the quality of food and fodder, by establishing the level and frequency of fungi and aflatoxin contamination of foods and fodder in the Southeast Asian region.

The welfare impacts of a research project depend on the adoption of an idea or a technology developed in the project, or developed as a result of the project. A project that generates information that is catalytic in the future development of as yet undeveloped technology is judged as having had no welfare impacts until someone develops the technology, and it is adopted and put to use in some economic activity.

The word 'technology' is used in this paper to mean a wide variety of responses to the aflatoxin problem including:

- the use of information to change decision-making or resource-use practices;
- aflatoxin regulations to limit exposure of human and livestock populations to aflatoxins in grains;
- improved grain-pricing regimes which reward production of aflatoxin-free grains;
- selection of cultivars of maize and peanuts that are aflatoxin resistant;
- use of non-toxigenic fungi to competitively exclude the toxigenic fungi;
- better drying methods for grains; and
- chemical methods to detoxify or decontaminate aflatoxin-contaminated grains.

This paper estimates potential benefits, as opposed to realised benefits from a technology developed in response to information generated under the two projects, PN8806 and PN9104. To estimate the potential impacts of the two projects, two scenarios are constructed in this paper as follows:

- an optimistic 'free lunch' scenario, and
- a scenario involving a farm-level aflatoxin control method that leads to an increase in farm-level costs.

An optimistic 'free lunch' scenario assumes that a solution to the aflatoxin problem in Indonesia, Philippines, and Thailand is found that reduces aflatoxin contamination in maize and peanuts but does not require additional inputs at the farm level or in the postharvest sector. It is very unlikely that such a technology is feasible. However, this scenario is used to indicate the potential benefits from PN8806 and PN9104 under optimistic circumstances.

The other scenario asks the following question: How would the net present value of benefits and the associated internal rate of return change if an aflatoxin control method increased costs at the farm level by 10%?

The scope of the paper

Section 2 of the paper describes the factors that are likely to influence the impact of the two research projects. Section 3 describes an approach for estimating the social costs of these impacts. Section 4 applies the above approach to estimate potential impacts of two collaborative research projects:

- PN8806: Fungi and mycotoxins in Asian food and feedstuffs; and
- PN9104: Factors affecting invasion by *Aspergillus flavus* and aflatoxin formation in Asian peanuts.

Section 5 discusses the impact on scientific knowledge of the projects, while Section 6 discusses project impacts on human and institutional capacity to carry out research on mycotoxins in grains in the Southeast Asian region. Section 7 makes concluding remarks.

Factors Likely to Influence the Impact of Aflatoxin Research

Lubulwa and Davis (1994) identified from the scientific literature five potential impacts of fungi and aflatoxins, namely:

- quality deterioration in agricultural products;
- spoilage of agricultural products;
- mutagenic and carcinogenic effects on humans who consume aflatoxin-contaminated food over a long period;
- livestock health and productivity effects arising from the use of aflatoxin-contaminated fodder—the emphasis is on increases in mortality rates and reductions in feed-to-weight conversion ratios for chickens, ducks, egg layers, and pigs; and
- the loss of export markets due to aflatoxin regulations restricting international trade in aflatoxin-contaminated grains.

Research under the two ACIAR projects provided information that made it possible to assess the magnitude of these impacts in the Southeast Asian context. In addition to these factors, the impact of research projects like PN8806 and PN9104 is likely to be dependent on the level of production, prices, and demand and supply elasticities of the commodities susceptible to the aflatoxin contamination and of those commodities that are susceptible to aflatoxicosis. In addition, the impact of research is likely to depend on the extent to which the human and livestock populations are exposed to aflatoxins in the different countries.

The production and consumption of commodities susceptible to aflatoxins in maize and peanuts

The production levels of maize and peanuts are important factors which are likely to determine the impacts of projects on aflatoxin contamination of the grains. Maize and peanuts are internationally traded commodities. These grains are used as feed for livestock and thus they are important on the supply side of pig meat, poultry meat, and hen eggs which are also internationally traded commodities. If a research project affects the quality, the quantity, or cost of producing an internationally traded commodity, the impact of research extends beyond the country where the research is undertaken. It affects all countries that trade in the commodity, through the research project's impact on the world price. To capture these world price spillover effects, this paper employs a model that recognises 70 regions of the world. Tables B1–B5 in Appendix B give the production, consumption, and trade status of the commodities that are susceptible to the impacts of fungi and aflatoxins. These commodities are:

- maize (Table B1);
- peanuts (Table B2);
- pig meat (Table B3);
- poultry meat (Table B4); and
- hen eggs (Table B5).

The economic and social impacts of the two projects are likely to depend on how maize and peanuts are used in the three countries where the research was undertaken: Indonesia, the Philippines, and Thailand. Table 1 summarises the usage of the commodities in the three countries

Let Q_i be the output of livestock sector i which is vulnerable to aflatoxicosis because some of the feed in sector i is derived from aflatoxin-contaminated maize or peanut products. Let S_i be defined as in equation 1:

$$S_i = Q_i / \Sigma Q_i \quad (1)$$

Table 1. Usage of maize and peanuts in ACIAR mycotoxin project countries.

	Indonesia		Philippines		Thailand	
	Maize (%)	Peanuts (%)	Maize (%)	Peanuts (%)	Maize (%)	Peanuts (%)
Seeds	2	na	2	na	0.4	na
Exports	0	0.19	0	0	30	6
Staple food	74	84.00	18	80	1	63
Feeds						
Pig meat	5	1.23	44	11	15	9.9
Poultry meat	10	2.08	19.2	4.8	33	6.5
Hen eggs	8	1.70	16.8	4.2	22	4.6
Other industrial uses	1	10.80	0	0	0	10
Total	100	100	100	100	100	100

Source: Lubulwa and Davis (1994).

In equation 1, Q_i is the total output, in tonnes, of pigmeat, poultry meat, and hen eggs produced in a country. The shares S_i are used in the estimation of the quantity of aflatoxin contaminated feed used by livestock sector i in a country. The values of S_i used in the evaluation of the two ACIAR projects are as follows:

	Share of livestock sector i in aflatoxin contaminated feed by country		
Livestock sector	Indonesia	Philippines	Thailand
Pig meat	0.24	0.55	0.22
Poultry meat	0.42	0.24	0.47
Hen eggs	0.34	0.21	0.31
Total	1	1	1

Source: Lubulwa and Davis (1994).

Product quality impacts of fungi and aflatoxins before research

Grades of maize and peanuts

Total aflatoxins (B_1, B_2, G_1, and G_2) in micrograms per kilogram of product can give an indication of some of the quality attributes of the product. Using data from ACIAR PN8806 (see ACIAR 1989, 1990, 1991, 1992, 1993) on the levels of aflatoxin contamination in peanuts and maize in Southeast Asia, Lubulwa and Davis (1994) identified three distinct quality grades of produce:

- high quality produce—this is produce that contains no more than 50 micrograms of total aflatoxins (B_1, B_2, G_1, and G_2) per kilogram of product;
- medium quality produce—this is produce containing more than 50 micrograms of aflatoxins but the level of aflatoxin contamination is less than, or equal to, 300 micrograms of total aflatoxins (B_1, B_2, G_1, and G_2) per one kilogram of product; and
- low quality produce—this is produce which contains more than 300 micrograms of total aflatoxins (B_1, B_2, G_1, and G_2) per kilogram of product.

The category of high quality produce includes almost aflatoxin-free produce containing no more than 5 micrograms of aflatoxins per kilogram of product. In many countries the limit of 5 micrograms per kilogram of product is applicable to baby food products [(see Lubulwa and Davis (1994)].

The upper limit of 50 micrograms of total aflatoxins (B_1, B_2, G_1 and G_2) per kilogram of product for high quality produce is consistent with the literature on aflatoxin regulations specifying maximum acceptable levels of aflatoxin contamination in foods and fodder. Lubulwa and Davis (1994) list these limits for selected countries. Different countries have different limits. In 1991, for peanuts, maize, and maize products, the maximum acceptable level of aflatoxin contamination was 50 micrograms per kilogram of product [(see Lubulwa and Davis (1994), Tables B.1 and B.2].

The upper limit of 300 micrograms of total aflatoxins (B_1, B_2, G_1, and G_2) per kilogram of product for the medium quality product is based on the United States limit of 300 micrograms of total aflatoxins (B_1, B_2, G_1, and G_2) per kilogram of product for fodder for adult beef cattle, sheep, and goats.

Contaminated products that contain more than 300 micrograms of total aflatoxins (B_1, B_2, G_1, and G_2) per kilogram of product are considered to be low quality products. Such products contain more than 10 times the levels of aflatoxins acceptable in some western countries and more than 60 times the levels acceptable in western countries with the lowest aflatoxin tolerance levels.

Tiongson and Gacilos (1990) give some support for the approach of using postharvest aflatoxin contamination levels to define grades of farm-level output when they conclude that:

> No definite pattern of increase in the incidence of aflatoxin was observed among different stages of operation. This suggests that the grain may reach substantial level of aflatoxin contamination even at the start of off-farm operation depending on the degree by which the grains were earlier predisposed to *Aspergillus flavus* infection and to on-farm conditions that favour aflatoxin formation during the preharvest stages of the crop.

Table 2 summarises the relevant data on the quality of maize and peanuts in Indonesia, Philippines, and Thailand. The data in Table 2 may be conservative compared with results from other studies. For example, in the case of the Philippines, Agaceta et al. (1993) collected 200 poultry feeds, 300 pig feeds, and 100 prawn feeds from different feed mills and farms in Luzon, Visayas, and Mindanao and found that 63% of poultry feeds, 61% of swine feeds, and 52% of prawn feeds contained more than 50 micrograms of aflatoxins per kilogram of feed.

Table 2. The aflatoxin content of maize and peanuts in Indonesia, Philippines, and Thailand. (Percentage of samples tested which had the level of aflatoxin contamination in column 2 of the table.)

Commodity grades	Micrograms of aflatoxin $B_1 + B_2 + G_1 + G_2$ per kilogram of product	Indonesia		Philippines		Thailand	
		maize[a]	peanuts[a]	maize[a]	peanuts[a]	maize[a]	peanuts[a]
High quality (1) (almost aflatoxin free)	$\mu g/kg \leq 5$	68	44	44	67	53	64
High quality (2)	$5 < \mu g/kg \leq 10$	2	1	9	5	0	4
High quality (3)	$10 < \mu g/kg \leq 50$	8	10	27	6	18	7
High quality—total	$\mu g/kg \leq 50$	78	55	80	78	71	75
Medium quality	$50 < \mu g/kg \leq 300$	18	12	14	6	15	14
Low quality (1)	$300 < \mu g/kg \leq 1000$	3	11	5	9	11	7
Low quality (2)	$1000 < \mu g/kg \leq 5000$	1	17	1	4	4	3
Low quality (3)	$5000 < \mu g/kg \leq 10000$	0	4	0	2	0	0
Low quality (4)	$\mu g/kg$ exceed 10000	0	1	0	1	0	0
Low quality—total	$\mu g/kg$ exceed 300	4	33	6	16	14	11
Total percentage	Not applicable	100	100	100	100	100	100
Total number of samples	Not applicable	96	215	146	81	108	94
Total production	'000 t (1991)	6445[b]	1056[c]	4677[b]	35[d]	4035[b]	163[c]

Sources: [a]ACIAR (1989, 1990, 1991, 1992 and 1993); [b]CIMMYT (1992); [c]Food and Agriculture Organization of the United Nations (1992); [d]Bureau of Agricultural Statistics (1993)

Price versus quality before research

Tiongson and Gacilos (1990) observed an inverse relationship between the price of corn grits and aflatoxin content in the Philippines—that is the lower the level of aflatoxin content, the higher was the price of corn grits.

Cardino-Bermundo et al. (1991) concluded that moisture content and colour of the commodity determines the price of maize grain in the Philippines. Bottema and Altemeier (1990) and Wattanutchariya et al. (1991) indicate that these two factors (moisture content and colour) are the most important two determinants of grain price in Indonesia and Thailand. In these countries the grain trader (middleman) measures the two factors through sensory evaluation and visual observation[1]. Generally, local grain traders and processors do not use laboratory equipment, like moisture testers, to measure grain attributes. The trader discounts wet or discoloured grain by deducting a certain percentage off the gross weight of grain. Alternatively, the trader deducts a percentage off the market price to calculate the price per unit weight of wet or discoloured grain. The discounts increase with the wetness of grain. Cardino-Bermundo et al. (1991) observed the following discounts in the Philippines:

- for skin-dry produce, traders reduced the gross weight or the per unit weight price by a factor ranging from 5 to 10% depending on the level of dryness;
- for wet grain, traders reduced the weight or price of produce by a factor ranging from 15 to 20%; and
- for damaged grain, traders reduced the gross weight or the unit price of the produce by a factor ranging from 30 to 50%.

[1] Dr John Pitt and Dr Ailsa Hocking CSIRO, North Ryde, Sydney (personal communication, 14 January 1994) noted that (a) visual observation is a very poor and unreliable way to tell whether a product contains aflatoxins or not, (b) current pricing regimes do not capture aflatoxin content of products, (c) traders may have price differentials for other attributes of grains but those price differentials are not likely to reflect aflatoxin content. On the basis of these expert observations the rest of the paper, while differentiating grains by aflatoxin content, does not introduce aflatoxin-related, grain-price differentials. The paper uses the average price of maize and the average price of peanuts.

The pricing regime for grains that Cardino-Bermundo et al. (1991) observed[2] does not take into account the level of aflatoxin contamination of the grains.

However, the pricing regimes on the world market do take into account the aflatoxin content of grains. For example, Tangthirasunan (1991) notes that:

> Levels of aflatoxin acceptable to Japan are less than 20 ppb. Other countries required levels of less than 50 or 100 ppb. Thai maize frequently exceeds these levels in the rainy season. Because of the high incidence of aflatoxin, Thai maize regularly trades $15–$20 per tonne below comparable maize from other sources

A technology that makes it possible to produce aflatoxin-free produce leads to a quality change and to a shift to the right of the demand curve for grain.

The prices, own price supply, and own price demand elasticities of commodities

The farmgate prices for maize were obtained from CIMMYT (1992). The prices for peanuts used in the analysis were obtained from Rao (1993, Table 5.3). National prices for peanuts are not available and, where they are, it is often not clear whether they refer to peanuts in shell or to peanuts after they are shelled. In this paper, the price of maize and peanuts is the same irrespective of the level of aflatoxin contamination of the grain.

The prices of pig meat, poultry meat, and hen eggs were obtained from FAO (1992). The demand and supply elasticities used in the analysis are given in Table 3.

Product-spoilage effects of fungi and aflatoxins before and after research

It is possible for fungi to so adversely affect the sensory characteristics (such as taste, odour, texture, colour), the nutritional value and functional properties of grains that the grains become unacceptable as food or fodder. In such cases, the farmer or the grain handler has to discard the grain as waste. Therefore, some of the farm-level production of food or fodder does not reach the retail market. Spoilage of food and fodder between the farm sector and the retail sector affects the retail prices of these products. This paper explicitly takes into account these product-spoilage effects in estimating the impact of fungi and aflatoxins.

FAO (1983) uses the term 'damage' to indicate the physical or mechanical spoilage of a food grain; it may reflect partial deterioration of a food on the basis of a subjective judgment but not necessarily the loss in weight. Fungi and aflatoxins lead to product damage or spoilage in three different ways:

- fungi lead to discoloration and to deterioration in the physical appearance of grains which not only lowers product quality but often makes the product unacceptable for consumption as food or fodder and thus of no commercial value;
- storage fungi change the fat acidity of grains—fatty acids contribute to characteristic off-odours and rancidity (unpleasantly stale smell or taste) of stored commodities; and
- invasion of seeds by storage fungi drastically reduces germinability of the seed (FAO 1983).

Spoilage rates due to fungi and aflatoxins are described by probability functions. The probability that the spoilage rate takes a particular value is a function of various factors including: the variety of the product (e.g. yellow maize versus white maize), the time and method of harvest, the period and method of storage, the storage temperature, the moisture content, the drying method before storage, and so on (see Maize Quality Improvement Research Centre 1992). Thus, estimates of spoilage rates, in a mathematical statistics sense, are expected spoilage rates.

Current estimates[3] suggest that traders and users of maize and peanuts in Indonesia, Philippines, and Thailand throw away about 5% of the grain because of fungi and aflatoxin contamination. This estimate is consistent with that by Ren-Yong et al. (1992) who used systems analysis to estimate various postharvest losses in the grains sector and concluded that in China the postharvest spoilage rate due to aflatoxins in the grains sector was about 3.6%.

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[2] Cardino-Bermundo et al. (1991) note that this scheme does not provide adequate incentives for dried maize; the price differential between dried and wet maize is not enough to cover the cost of mechanical drying.

[3] Dr John Pitt and Dr Ailsa Hocking, CSIRO, North Ryde, Sydney (personal communication, 14 January 1994).

Table 3. The own price demand and own price supply elasticities for selected commodities.

	Maize		Peanut		Pigmeat		Poultry		Hen eggs	
Country/region	supply	demand	supply	demand	supply	demand	supply	demand	supply	demand
Bangladesh	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Bhutan	0.1	–0.2	0	0	0.7	–0.85	0.7	–0.8	0.7	–0.8
India	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Nepal	0.1	–0.2	0	0	0.7	–0.85	0.7	–0.8	0.7	–0.8
Pakistan	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Sri Lanka	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Burma	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Indonesia	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Kampuchea (Cambodia)	0.1	–0.3	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Laos PDR	0.1	–0.3	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Malaysia	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Philippines	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Thailand	0.1	–0.3	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Vietnam, Socialist Republic	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
China, People's Republic	0.3	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Mongolia	0.3	–0.2	0	–0	0.7	–0.85	0.7	–0.8	0.7	–0.8
Fiji	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Papua New Guinea	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Samoa (Western)	0.1	–0.2	0	0	0.7	–0.85	0.7	–0.8	0.7	–0.8
Solomon Is.	0.1	–0.2	0	0	0.7	–0.85	0.7	–0.8	0.7	–0.8
Tonga	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Vanuatu	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Other South Pacific	0.1	–0.2	0	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Ethiopia	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Kenya	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Malawi	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Mozambique	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Tanzania	0.1	–0.1	0.3	—0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Uganda	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Zambia	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Zimbabwe	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Zaire	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Ivory Coast	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Ghana	0.1	–0.1	1.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Nigeria	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Cameroon United Republic	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	0.8	0.7	0.8

Table 3. (Continued) The own price demand and own price supply elasticities for selected commodities.

	Maize		Peanut		Pigmeat		Poultry		Hen eggs	
Country/region	supply	demand	supply	demand	supply	demand	supply	demand	supply	demand
Angola	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Madagascar	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Sudan	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Africa-2	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Africa-3	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Africa-4	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Africa-5	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Africa-6	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Africa-7	0.1	–0.1	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Turkey	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Egypt, Arab Republic	0.2	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Africa-1	0.2	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Other West and North Africa	0.2	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Brazil	0.3	–0.3	0.3	–0.41	0.7	–0.85	0.7	0.8	0.7	0.8
Colombia	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Peru	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Venezuela	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Bolivia	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Ecuador	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Mexico	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Argentina	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Chile	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Paraguay	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Uruguay	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Latin-America 1	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Latin-America 2	0.1	–0.2	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Asia-Developed	0.3	–0.3	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Australia	0.4	–0.5	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Canada	0.4	–0.5	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
USA	0.4	–0.5	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
USSR	0.3	–0.3	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Japan	0.3	–0.3	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Developed 1–2	0.4	–0.5	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8
Developed 3–4	0.3	–0.3	0.3	–0.41	0.7	–0.85	0.7	–0.8	0.7	–0.8

Source: ACIAR's Economic Evaluation Unit database.

Human health effects of aflatoxins

When people ingest food containing aflatoxins they may suffer two major types of effects.

- The acute effects of high, short-term exposure to aflatoxins in humans may lead to fatal aflatoxicosis, with jaundice for example, and may play a role in kwashiorkor, and Reye's syndrome (Bhat 1989, 1991). Such acute outbreaks of disease are preventable if countries introduce and adhere to tolerance levels for aflatoxins in foods (Kuiper-Goodman 1991).
- There may be chronic mutagenic, carcinogenic effects which have long latency periods. They include primary liver cancer, Indian childhood cirrhosis—a liver disorder in India correlated with breast milk and baby food contaminated with aflatoxin, and chronic gastritis (Bhat 1989, 1991).

The weight of evidence favours the view that aflatoxins are carcinogenic. An FAO/WHO Expert Committee (WHO 1987) urged reduction of the intake of aflatoxin B_1 to the lowest practical level so as to reduce the potential for harm. The International Agency for Research on Cancer (IARC 1976, 1987) reviewed aflatoxin B_1 and concluded that it is a human carcinogen.

A number of studies[4] have established a strong correlation between ingestion of aflatoxins and the incidence of primary liver cancer. Most of these have been population-based correlation studies[5]. Since data in these studies are collected on populations rather than individuals, it is not possible to determine the exposure to aflatoxins of individuals who have the disease (Kuiper-Goodman 1991). Furthermore, it appears that primary liver cancer can have a multi-factorial origin. Factors like alcohol (Bulatao-Jayme et al. 1982) and hepatitis B virus (Croy and Crouch 1991) appear to have a synergistic effect on the incidence of primary liver cancer. As well, genetic differences, social economic status, sex, and age of the individual may play a role. However, Kuiper-Goodman (1991) has argued that hepatitis B virus is not a confounding factor unless its distribution in the various study populations is uneven. She concludes that it cannot be presumed *a priori* that all the older studies in which hepatitis B virus status of individuals was not measured are invalid.

Lubulwa and Davis (1994) estimated the numbers of primary liver cancer cases attributable to aflatoxins in maize and peanuts consumed in Indonesia, Philippines, and Thailand to give an indication of the human health effect of maize- and peanut- related aflatoxicosis in these three countries. They used a population-based correlation approach with the aim of providing indicative estimates of the human health effects of aflatoxins measured in terms of the number of primary liver cancer cases attributable to aflatoxins in maize and peanut. More accurate estimates need to take into account the confounding factors in the discussion above and must be individually based.

The estimates by Lubulwa and Davis are given in Table 4.

Livestock health and productivity impacts of aflatoxins

Using feed that contains aflatoxins leads to a number of negative effects on susceptible livestock and poultry. CAST (1989) notes that:

> The impact of fungal toxins upon animals extends beyond their obvious effect in producing death in the wide variety of animals that are likely to consume mycotoxin-contaminated grains or feeds. The economic impact of lowered productivity, reduced weight gain, reduced feed efficiency, less meat and egg production, greater disease incidence because of immune system suppression, subtle damage to vital body organs, and interferences with reproduction is many times greater than that of immediate morbidity and death.

A typical field case of aflatoxicosis is marked not by mortality but by a decline in productivity with no visible disease symptoms (Hamilton 1987).

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[4] See Shank et al. (1971, 1972a,b,c,d,e) on aflatoxicosis and primary liver cancer in Thailand. CAST (1989) discusses studies of aflatoxin poisoning in Western India, Uganda, Taiwan, Thailand, and Kenya. Peers et al. (1976, 1987) studied aflatoxicosis in Swaziland. Yeh et al. (1989) deals with hepatitis B virus and primary liver cancer in China while Bulatao-Jayme et al. (1982) correlates exposure to aflatoxin and the incidence of primary liver cancer in the Philippines.

[5] Exceptions include Bulatao-Jayme et al. (1982) and Yeh et al. (1989). Yeh et al. (1989) collected data on 7917 men residing in 5 different areas for a period of 3.8 years. However, the study estimated, at the population level, dietary aflatoxin levels for 4 out of 5 areas on the basis of market sample analyses.

Table 4. The incidence of liver cancer and estimates of the number of primary liver cancer deaths due to aflatoxins in maize and peanuts, and related data: Indonesia, Philippines, and Thailand.

	Maize			Peanut			Total
	high quality	medium quality	low quality	high quality	medium quality	low quality	
Indonesia							
Per capita consumption of aflatoxins per day by source in nanograms ($Z_{ji}W$)	108	1739	1436	14	213	2173	5683
Aflatoxin dosage in nanograms per kg body weight per day (Z_{ji})	2	35	29	0.28	4	43	114
Incidence of liver cancer/100 000 of population by source of aflatoxin (C_{ji})	0.23	3.69	3.04	0.03	0.45	4.61	12.05
Primary liver cancer deaths (D_{ji})[a]	426	6889	5686	55	843	8609	22509
Philippines							
Per capita consumption of aflatoxins per day by source in nanograms ($Z_{ji}W$)	57	700	1114	2	10	99	1982
Aflatoxin dosage in nanograms per kg body weight per day (Z_{ji})	1	14	22	0.04	0.20	2	40
Incidence of liver cancer/100 000 of population by source of aflatoxin (C_{ji})	0.12	1.48	2.36	0.004	0.02	0.21	4.20
Primary liver cancer deaths (D_{ji})[b]	76	933	1486	2	13	132	2642
Thailand							
Per capita consumption of aflatoxins per day by source in nanograms ($Z_{ji}W$)	3	40	137	7	94	274	554
Aflatoxin dosage in nanograms per kg body weight per day (Z_{ji})	0.05	0.79	2.74	0.14	1.88	5.48	11.08
Incidence of liver cancer/100 000 of population by source of aflatoxin (C_{ji})	0.01	0.08	0.29	0.02	0.20	0.58	1.17
Primary liver cancer deaths (D_{ji})[c]	3	48	166	9	114	332	672

[a] Estimated using the equation by Kuiper-Goodman (1991) and assuming that Indonesia's population, in 1991, was about 187 million.

[b] Estimated using the equation by Kuiper-Goodman (1991) and assuming that Philippine's population, in 1991, was about 63 million. The total incidence per 100 000 population of malignant neoplasm in the Philippines is 35.5 (National Statistical Coordination Board, The Republic of Philippines, 1991). Thus the estimated incidence of primary liver cancer due to aflatoxin in maize and peanuts is about 12% of the total incidence of malignant neoplasm in Philippines.

[c] Estimated using the equation by Kuiper-Goodman (1991) and assuming that Thailand's population, in 1991, was about 57 million. The total incidence per 100 000 population of malignant neoplasm in the Thailand is 20.2 (National Statistical Office, Thailand 1992) Thus the estimated incidence of primary liver cancer due to aflatoxin in maize and peanuts is about 6% of the incidence of malignant neoplasm in Thailand.

Source: Lubulwa and Davis (1994).

Losses that result from using contaminated grain as feed are difficult to measure for various reasons including the following:

- The consequences of aflatoxicosis depend on the dose of aflatoxin, the length of feeding toxic diets, and the age when exposure to the toxin first occurred (Rao and Reddy 1989).
- Subtle effects due to using aflatoxin-contaminated feed do not produce clinical symptoms of toxicity (Nichols 1987). These effects include reduced growth rate, reduced feed efficiency, the infertility syndrome in pigs and cattle, and loss of quality in animal products—examples include milk with aflatoxin M_1 because dairy cattle are fed on aflatoxin-contaminated feed, and chicken carcasses condemned or downgraded because of the broiler bruising syndrome[6] or the pale bird syndrome[7]. Since aflatoxicosis often occurs in these subtle ways, proper diagnosis is dependent on keen observation and good production records. Unfortunately, proper diagnosis is often not made.
- The effects of aflatoxins change when there are other mycotoxins in the feed. Feed mixtures may include mycotoxins other than aflatoxins and some of these have additive or synergistic effects with the aflatoxin (Pier 1987).
- Aflatoxins do not occur uniformly in feed. While the presence of moulds can be an indication that aflatoxins may be present, the degree of visible mould infestation is not necessarily an indication of the level of toxin production in fodder or food. Moreover, mouldiness may not be apparent after milling or processing.

The rest of this section discusses the impacts of aflatoxin-contaminated feed on each livestock group that is susceptible to aflatoxicosis.

Poultry meat and egg production

Smith et al. (1971) point out that aflatoxicosis in chicken is characterised by poor growth rates, inefficient feed conversion, and increased mortality rates. Aflatoxicosis seems to almost halve the chicken's growth rate, to reduce feed conversion efficiency by about 30%, and to increase mortality rates. Hamilton and Garlich (1971) and Huff at al. (1975) demonstrated that aflatoxicosis in laying hens causes an enlarged fatty liver and a decrease in egg production—fewer and smaller eggs are produced. The decrease in egg production does not occur immediately after aflatoxin is introduced in the diet but rather after a lag of 10 to 14 days.

There are other effects of aflatoxicosis in the poultry and egg production sector not taken into account in this paper because, in the literature, there is inadequate quantification of their magnitude. For example, Boulton et al. (1979) conclude that layers exposed to dietary aflatoxins at the time of Newcastle disease vaccination may not be adequately vaccinated and that more frequent vaccination may be required. Wyatt (1979) discusses the following additional effects of aflatoxicosis in the poultry and egg production sector: increased condemnation or downgrading of carcasses, poor pigmentation of poultry products which reduces their sale value, altered immunity which increases susceptibility to disease, and interference with the birds' normal processes of absorption, digestion, and utilisation of nutrients.

Pig production

The toxicity of aflatoxins has been reported in suckling piglets, growing and finishing pigs and breeder stock (CAST 1989). The most important three impacts of aflatoxicosis in the pig sector are: increased mortality rates, decreased weight gain, and decreased feed conversion efficiency. The effects of aflatoxins in pigs are varied, and may be more or less pronounced, depending upon the age of the animal, diet, concentration of aflatoxins, and length of exposure. Pigs appear to be resistant to dietary levels of aflatoxins up to 300 ppb fed from time of weaning to marketing (CAST 1989; Buhatel and Salajan 1977). Wilson et al. (1984) reported mortality rates of 10% in herds of 200 or more pigs and 28% in herds with 20 to 50 pigs. Wilson et al. (1984) report that 30 to 45% of the pigs in sampled herds were visibly ill from consuming grain with aflatoxin levels greater than 350 ppb.

Table 5 summarises conservative estimates reported in the literature on aflatoxicosis in livestock. The estimates of the benefits of aflatoxin research in the livestock sector will depend on the parameter values in Table 5.

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[6] Apparently healthy birds exhibit bruises and haemorrhaging at slaughter. Experiments revealed that aflatoxins increase capillary fragility and reduce the ability of supporting tissues to cushion the blood vessels against blows (Hamilton 1987).

[7] Chickens fed on aflatoxin-contaminated feed fail to realise their colour potential. The yellow colour of chicken skins and egg yolk is attributable to carotenoids. Aflatoxins interfere with the birds capacity to absorb, transport, and metabolise carotenoids (see Hamilton 1987).

Table 5. Livestock health and productivity impacts of aflatoxins.

Livestock	Type of impact	Impact with high quality feed Aflatoxin $B_1+B_2+G_1+G_2$ in the following range $0 \leq \mu g/kg \leq 50$	Impact with medium quality feed Aflatoxin $B_1+B_2+G_1+G_2$ in the following range $50 < \mu g/kg \leq 300$	Impact with low quality feed Aflatoxin $B_1+B_2+G_1+G_2$ in the following range $\mu g/kg > 300$
Poultry and egg production	Deaths per year: chickens (%)	9.0[a]	12.0[a]	14.0[a]
	Deaths per year: ducks (%)	12.0[b]	28.0[b]	no data
	Average weight of a bird (kg)	4.4[e]	3.3[d]	2.2[c]
	Feed/weight gain ratio	2.9[h]	3.4[g]	3.8[f]
	Egg weight/bird feed ratio	2.9	3.0[j]	3.1[i]
Pigs	Deaths per year (%)	1.5[l]	1.5[l]	28.0[k]
	Average weight of a pig (kg)	75.0[n]	75.0[n]	54.0[m]
	Feed consumed to weight gain ratio	2.4[n]	2.4[n]	6.0[n]

Notes

[a] From Shane (1991). The values for high quality feed correspond to Shane's standard values for these parameters. This figure includes condemned carcasses. A 3 and 5% increase in mortality rates is associated with medium quality feed and low quality feed respectively.
[b] Hetzel et al. (1984).
[c] Wu et al. (1991). This is the average weight for Thailand and Philippine chickens.
[d] This is an estimate of body weight of chicken fed on medium quality feedstuff. It is based on estimates in notes (c) and (e).
[e] Based on Smith et al. (1971) where presence of aflatoxins halves the growth rate of chicken.
[f] Wu et al. (1991) feed/gain ratio for Thai native chickens.
[g] Estimated from notes (f) and (h).
[h] Based on Smith et al. (1971).
[i] CAST (1989) estimates that aflatoxicosis could lead to a reduction of 5% in egg production in laying hens.
[j] By interpolation between the results for the high quality and low quality feed.
[k] Estimate from Wilson et al. (1984). This is the mortality rate for smaller herds in Georgia, USA and is used here on the assumption that Southeast Asian pig herds tend to be small.
[l] From CAST (1989). This is the overall mortality rate for pig producers in the southeastern United States and may be low in the case of Southeast Asia.
[m] Average of pig carcasses in Indonesia, Philippines, and Thailand from data in FAO (1992).
[n] Based on Buhatel and Salajan (1977) and CAST (1989).

Source: Lubulwa and Davis (1994).

Quantification of the Potential Social Welfare Impacts of ACIAR Projects PN8806 and PN9104

Lubulwa and Davis (1994) explored a range of possible approaches that could be used in the quantification of the social welfare impacts of aflatoxin-related research. They then used a set of separate single sector or single industry partial equilibrium models. The social costs of aflatoxins are estimated for each industry or sector separately, these are summed to give the total social cost of aflatoxins. The estimates of social costs under this approach approximate the estimates under the general equilibrium approach according to Just et al. (1982) who conclude that:

> Rather comprehensive applied welfare analysis is possible. Depending on empirical conditions, all of the private social welfare effects of a proposed new or altered government policy can be measured completely, at least in an approximate sense, in a single market, which is thereby distorted or in which a distortion is altered. If the policy introduces or alters several distortions, approximate measurement of all private effects is possible by considering the changes sequentially in the respective markets they affect directly.

Lubulwa and Davis (1994) used this approach and estimated the social cost of aflatoxins in the following major segments.

- To estimate the social costs of the product-spoilage effects of aflatoxins in the maize and peanuts food

sectors, a product wastage economic model is used. Fungi and aflatoxins affect both the food and feed sectors and so the total output of maize and peanuts is the basis for estimating the cost of product-spoilage effects.

- The social costs of quality changes in products due to aflatoxins are reflected in the costs of human health effects and the livestock productivity impacts of aflatoxins.
- The cost of the human health effects of consuming aflatoxin-contaminated maize and peanuts is equal to the monetary value of productive capacity lost due to premature death and increased morbidity from primary liver cancer attributable to the ingestion of aflatoxin-contaminated maize and peanuts. Only that part of maize and peanut output used as food is relevant in estimating costs of human health effects of aflatoxins.
- The social cost of aflatoxins in the livestock sectors is equal to the increase in the cost of producing livestock as a result of using aflatoxin-contaminated feed. Only that part of maize and peanut output used as feed is relevant in estimating the livestock productivity impacts of aflatoxins.
- The costs due to restrictions on trade in aflatoxin-contaminated products were not estimated.

The remainder of this section provides an outline of how the social costs were estimated.

Evaluating the social costs of the product-spoilage effects of fungi and aflatoxins

The annual cost of the product wastage effects of fungi and aflatoxins is equal to the annual economic surplus that households and producers forego in Indonesia, Philippines, and Thailand as a result of product-spoilage effects of fungi and aflatoxins. This estimate depends on the values of the own price demand and supply elasticities, the postharvest costs with and without aflatoxins, and the reduction in spoilage rates assumed.

A derivation of the equations of a model developed by Davis (1993), and used in the estimation of product-spoilage effects is in Davis and Lubulwa (1994). The product wastage economic model distinguishes between farm-level output and retail output for maize and peanuts. This model recognises that some of the farm-level output of maize and peanuts does not reach the retail market because of the spoilage effects of fungi and aflatoxins. The product-spoilage effects of fungi and aflatoxins mean that retail supply is lower and retail prices may be higher than they would be without the spoilage effects.

Pitt and Hocking (personal communication, January 1994) estimated that about 5% of the farm supply of maize and peanuts in Indonesia, Philippines, and Thailand is spoilt as a result of fungal attack and aflatoxin contamination.

The welfare benefits of a research project that reduces product-spoilage effects due to aflatoxin contamination in a grain are approximated by the changes in producer and consumer surplus as a result of the reduction in product wastage.

Evaluating the costs of the human health effects of aflatoxins

Disease leads to the following categories of cost (see Crowley et al. 1992):

- the cost of mortality which relates to the cost of productive capacity lost when people die before reaching the end of their productive life;
- the cost of morbidity which relates to value of production loss resulting from hospitalisation and the cost of health care services consumed when an individual is sick;
- the costs incurred by governments and hospitals in the provision of medical services for individuals suffering from primary liver cancer; and
- the cost of intangibles—pain, suffering, anxiety, and reduction in quality of life.

In this paper, the cost of the human health effects of fungi and aflatoxins include only the first two categories of the cost of primary liver cancer. The estimation of the costs in the third category requires data on the number and lengths of visits made by primary liver cancer patients to hospitals, medical centres, and medical facilities, the type of medical personnel that attended them, the drugs and other pharmaceutical products prescribed, and whether they were hospitalised or not. This category was excluded mainly because the data needed to enable their estimation are not available. The last category was excluded because at this time, there are no satisfactory monetary measures of the intangible cost of disease.

There are two main methods for determining the finite value of life (Crowley et al. 1992):

- the human capital approach; and
- the willingness-to-pay.

The human capital method equates the value of life with the present value of expected future earnings. The willingness-to-pay method uses contingency valuation surveys to ask people how much they would be willing to pay to avoid different levels and types of risks. The willingness-to-pay approach is inappropriate when people surveyed cannot perceive the risk whose cost they are asked to assess. In the case of aflatoxin-related, primary liver cancer deaths in the Southeast Asian region, it is not clear that people consuming aflatoxin-contaminated maize and peanuts realise the risk they face from aflatoxin-related primary liver cancer. This paper uses the human capital approach to estimate the cost of life. The details of the equations used are given in Lubulwa and Davis (1994).

Evaluating the livestock health and productivity impacts of aflatoxins

The livestock health and productivity cost of aflatoxins is equal to the welfare gains to producers and consumers of livestock as a result of removing aflatoxins in maize and peanut feed. For each grade *i*, of maize or peanut feed, estimating the social cost of aflatoxins in a livestock sector *h* requires the following:

- an estimate of the absolute change in the unit cost of livestock *h* fed on grain feed *j* of quality *i* as a result of using aflatoxin-free feed instead of aflatoxin-contaminated feed;
- the own price elasticity of supply of a livestock product;
- the own price elasticity of demand of a livestock product;
- the price of the livestock product *h*;
- the cost of aflatoxin-contaminated grain feed *j* of grade *i* used to produce livestock products before research; and
- the cost of feed per tonne of livestock *h* when feed is contaminated with aflatoxins and the cost of feed per tonne livestock without aflatoxins.

In this paper, the cost reduction for livestock sector i is calculated from the cost reductions by quality of feed grain in Lubulwa and Davis (1994), using equation 1 in which:

K_{wj} is the cost reduction in livestock sector *j*

M_j is the total amount of maize used as feed in livestock sector *j*;

G_j is the total amount of peanuts used as feed in livestock sector *j*;

M_1 is the proportion of maize grain which is of high quality;

M_2 is the proportion of maize grain which is of medium quality;

M_3 is the proportion of maize grain which is of low quality;

G_1 is the proportion of peanut grain which is of high quality;

G_2 is the proportion of peanut grain which is of medium quality;

G_3 is the proportion of peanut grain which is of low quality;

k_{1vj} is the cost reduction in livestock sector *j* due to a reduction in aflatoxin content of grain type *v* (where $v = m$ for maize and $v = g$ for peanuts) of quality 1 (high quality) as estimated by Lubulwa and Davis (1994);

k_{2vj} is the cost reduction in livestock sector *j* due to a reduction in aflatoxin content of grain type *v* (where $v = m$ for maize and $v = g$ for peanuts) of quality 2 (medium quality) as estimated by Lubulwa and Davis (1994); and

k_{3vj} is the cost reduction in livestock sector *j* due to a reduction in aflatoxin content of grain type *v* (where $v = m$ for maize and $v = g$ for peanuts) of quality 3 (low quality) as estimated by Lubulwa and Davis (1994).

The estimated weighted cost reductions in Indonesia, Philippines, and Thailand for the different livestock sectors are summarised in Table 6.

$$K_{wj} = \frac{M_j}{M_j + G_j}\left[\frac{M_1}{M}k_{1mj} + \frac{M_2}{M}k_{2mj} + \frac{M_3}{M}k_{3mj}\right] + \frac{G_j}{M_j + G_j}\left[\frac{G_1}{G}k_{1gj} + \frac{G_2}{G}k_{2gj} + \frac{G_3}{G}k_{3gj}\right] \quad (2)$$

Table 6. Estimates of the cost reduction in the livestock sectors in Indonesia, Philippines, and Thailand due to the reduction in aflatoxin contamination of maize and peanuts used as livestock feed.

	Pig meat			Poultry meat			Hen eggs		
	Indonesia	Philippines	Thailand	Indonesia	Philippines	Thailand	Indonesia	Philippines	Thailand
Quantity of maize used as feed ('000 t 1991)	371	2057	605	650	898	1328	516	786	887
Quantity of peanut used as feed ('000 t 1991)	13	3.8	7.5	22	1.7	16	18	1.5	11
Total feed from maize and peanuts ('000 t 1991)	384	2060.8	612.5	672	899.7	1344	534	787.5	898
Proportion of maize of medium quality grade	0.18	0.14	0.15	0.18	0.14	0.15	0.18	0.14	0.15
Proportion of maize of low quality grade	0.04	0.06	0.14	0.04	0.06	0.14	0.04	0.06	0.14
Cost reduction with respect to maize feed of medium quality grade	$0	$0	$0	($131)	($182)	($111)	($32)	($45)	($27)
Cost reduction with respect to maize feed of low quality grade	($777)	($1278)	($777)	($233)	($323)	($196)	($65)	($90)	($55)
Proportion of peanuts of medium quality grade	0.12	0.06	0.14	0.12	0.06	0.14	0.12	0.06	0.14
Proportion of peanuts of low quality grade	0.33	0.16	0.11	0.33	0.16	0.11	0.33	0.16	0.11
Cost reduction with respect to peanut feed of medium quality grade	$0	$0	$0	($437)	($437)	($437)	($108)	($108)	($108)
Cost reduction with respect to peanut feed of low quality grade	($3066)	($3066)	($3066)	($776)	($776)	($776)	($215)	($215)	($215)

Source: Lubulwa and Davis (1994).

The adoption of the results from the project

The benefits from the development of methods to control aflatoxin in maize and peanuts depend on the extent to which these methods are adopted by producers of maize and peanuts. In this paper it is assumed that the adoption levels of technologies to control aflatoxins are not likely to be different from observed levels of adoption for other technologies. Thus, the ceiling levels of adoption used in the analysis are listed in Table 7.

At this stage these adoption rates are hypothetical and are used for illustrative purposes to demonstrate the potential social welfare gains from technologies to reduce the aflatoxin content of maize and peanuts. Those for the livestock sectors are identical to the ones for the maize sector because the flow-on benefits to the livestock sector, from better aflatoxin control methods, are likely to depend on the extent to which the maize sector does adopt these methods.

It is important to note again at this point the comments made by Middleton (1991) when reviewing PN9104 during its proposal development phases:

> The project will not result in any tangible, possible commercialisable outputs, but rather will open the way for the development of appropriate control measures, some of which might be commercialisable.

A similar comment can be made about PN8806, in that its objective was not to develop any particular technology. Rather, its aim was to provide data on the quality of feed and food, by establishing the level and frequency of fungi and aflatoxin contamination of foods and feeds in the region.

Thus, the adoption patterns used do not relate to a particular technology developed under PN8806 or PN9104. There is a wide range of technologies that could be developed in response to data generated under the two ACIAR projects. Some of these are canvassed in GASGA (1993).

In the next section results are presented for three scenarios to indicate the potential benefits from research on better methods to control aflatoxins in maize and peanuts.

Results

Summary of results for the base case

The results on the potential benefits from technologies that are developed in response to ACIAR PN8806 and PN9104 are summarised under four main headings:

- the potential benefits to the maize and peanuts sectors from the fact that a technology that leads to better control of fungi and aflatoxins in grains reduces commodity wastage effects attributable to fungi and aflatoxins;
- the potential benefits to the pig meat, poultry meat, and hen eggs livestock sectors from the fact that a technology that leads to better control of fungi and aflatoxins in grains used as feedstuffs increases livestock productivity and reduces livestock mortality and morbidity; and
- the potential human health benefits to households in Indonesia, Philippines, and Thailand from the fact that a technology that leads to better control of fungi and aflatoxins in grains used for human consumption

Table 7. Ceiling levels of aflatoxin used in the quantification of the potential social welfare impacts of fungi and aflatoxin projects.

Country	Time in years along the adoption path	Maize	Peanuts	Pig meat	Poultry meat	Hen eggs
Indonesia	Year 1 to 13	0	0	0	0	0
	Year 13	0.3	0.3	0.3	0.3	0.3
	Year 20 to 30	0.47	0.65	0.47	0.47	0.47
Philippines	Year 1 to 13	0	0	0	0	0
	Year 13	0.3	0.3	0.3	0.3	0.3
	Year 20 to 30	0.45	0.65	0.45	0.45	0.45
Thailand	Year 1 to 13	0	0	0	0	0
	Year 13	0.3	0.3	0.3	0.3	0.3
	Year 20 to 30	0.42	0.65	0.42	0.42	0.42

reduces human mortality and morbidity rates due to primary liver cancer.

The results in this section are based on the assumption that a better method of controlling aflatoxins is developed as a result of PN8806 and PN9104, but that the nature of that technology is such that it does not lead to increases in farm level or postharvest costs.

An example of such a technology would be the discovery of maize and peanut cultivars that are resistant to *Aspergillus flavus* and the formation of aflatoxins in the maize and peanut grains. Extensive research has been carried out in this direction. For example GASGA (1993) notes that:

- A total of 133 seed entries from USA, India and the Philippines were evaluated under existing local conditions. IGS (E)-11, IGS-50, JL-24, BPI-P9, and ISU-PN-1 were found to have high yields under the Philippine conditions. In addition they were found to be aflatoxin resistant.

Potential benefits from the reduction in product-spoilage effects

Tables 8 and 9 summarise the potential benefits from the reduction in product-spoilage effects in the maize and peanuts sectors, respectively.

The results in the two tables are based on the assumption that the technology discovered reduces product-spoilage effects from their current estimates of 5% of maize and peanuts farm-level outputs to zero in Indonesia, Philippines, and Thailand.

The results in Tables 8 and 9 take into account the production and consumption of maize and peanuts worldwide and the effects of a change in the world price of maize and peanuts on consumers and producers in Indonesia, Philippines, and Thailand and the other countries in the world that trade maize and peanuts.

The benefits in Tables 8 and 9 are, for each year, the sum of the benefits to producers and to consumers in the different countries of the world as a result of better fungi and aflatoxin control methods introduced in Indonesia, Philippines, and Thailand.

In Tables 8 and 9 the benefits are zero in the first 13 years. The first 7 years in the analysis is the period when research on PN8806 and PN9104 was being conducted. The next 6 years are assumed to comprise the period when the technologies to make use of the information generated under PN8806 and PN9104 are developed.

Table 8. Flow of benefits accruing to Australia, Indonesia, Philippines, Thailand and the rest of the world due to research to reduce aflatoxin-related product-wastage in maize in $AU million, 1990.

	Year no.		Australia	Indonesia	Philippines	Thailand	Rest of the world	Global total
Start PN8806	1	1988	$0.000	$0.00	$0.00	$0.00	$0.00	$0
	2	1989	$0.000	$0.00	$0.00	$0.00	$0.00	$0
	3	1990	$0.000	$0.00	$0.00	$0.00	$0.00	$0
Start PN9104	4	1991	$0.000	$0.00	$0.00	$0.00	$0.00	$0
	5	1992	$0.000	$0.00	$0.00	$0.00	$0.00	$0
End PN8806	6	1993	$0.000	$0.00	$0.00	$0.00	$0.00	$0
End PN9104	7	1994	$0.000	$0.00	$0.00	$0.00	$0.00	$0
	8	1995	$0.000	$0.00	$0.00	$0.00	$0.00	$0
	9	1996	$0.000	$0.00	$0.00	$0.00	$0.00	$0
	10	1997	$0.000	$0.00	$0.00	$0.00	$0.00	$0
	11	1998	$0.000	$0.00	$0.00	$0.00	$0.00	$0
	12	1999	$0.000	$0.00	$0.00	$0.00	$0.00	$0
	13	2000	$0.000	$0.00	$0.00	$0.00	$0.00	$0
	14	2001	$0.004	$19.23	$13.82	$10.60	$9.82	$53
	15	2002	$0.004	$22.44	$16.12	$12.36	$11.44	$62

Table 8. (Continued) Flow of benefits accruing to Australia, Indonesia, Philippines, Thailand and the rest of the world due to research to reduce aflatoxin-related product-wastage in maize in $AU million, 1990.

	Year no.		Australia	Indonesia	Philippines	Thailand	Rest of the world	Global total
	16	2003	$0.005	$25.64	$18.42	$14.13	$13.05	$71
	17	2004	$0.006	$30.13	$20.72	$14.83	$14.67	$80
	18	2005	$0.006	$30.13	$20.72	$14.83	$16.19	$82
	19	2006	$0.007	$30.13	$20.72	$14.83	$17.60	$83
	20	2007	$0.007	$30.13	$20.72	$14.83	$18.97	$85
	21	2008	$0.008	$30.13	$20.72	$14.83	$20.33	$86
	22	2009	$0.008	$30.13	$20.72	$14.83	$21.40	$87
	23	2010	$0.008	$30.13	$20.72	$14.83	$21.44	$87
	24	2011	$0.008	$30.13	$20.72	$14.83	$21.44	$87
	25	2012	$0.008	$30.13	$20.72	$14.83	$21.44	$87
	26	2013	$0.008	$30.13	$20.72	$14.83	$21.44	$87
	27	2014	$0.008	$30.13	$20.72	$14.83	$21.44	$87
	28	2015	$0.008	$30.13	$20.72	$14.83	$21.44	$87
	29	2016	$0.008	$30.13	$20.72	$14.83	$21.44	$87
End of planning horizon	30	2017	$0.008	$30.13	$20.72	$14.83	$21.44	$87
Discount rate			$0.080	0.08	0.08	0.08	0.08	
Discounted benefits			$0.022	$94	$65	$47	$58	$264

Table 9. Flow of benefits accruing to Australia, Indonesia, Philippines, Thailand, and the rest of the world due to research to reduce aflatoxin-related product-wastage in peanuts $AU millions, 1990.

	Year no		Australia	Indonesia	Philippines	Thailand	Rest of the world	Global total
Start PN8806	1	1988	$0	$0	$0	$0	$0	$0.00
	2	1989	$0	$0	$0	$0	$0	$0.00
	3	1990	$0	$0	$0	$0	$0	$0.00
Start PN9104	4	1991	$0	$0	$0	$0	$0	$0.00
	5	1992	$0	$0	$0	$0	$0	$0.00
End PN8806	6	1993	$0	$0	$0	$0	$0	$0.00
End PN9104	7	1994	$0	$0	$0	$0	$0	$0.00
	8	1995	$0	$0	$0	$0	$0	$0.00
	9	1996	$0.00	$0.00	$0.00	$0.00	$0	$0.00
	10	1997	$0.00	$0.00	$0.00	$0.00	$0	$0.00
	11	1998	$0.00	$0.00	$0.00	$0.00	$0	$0.00
	12	1999	$0.00	$0.00	$0.00	$0.00	$0	$0.00
	13	2000	$0.00	$0.00	$0.00	$0.00	$0	$0.00

Table 9. (Continued) Flow of benefits accruing to Australia, Indonesia, Philippines, Thailand, and the rest of the world due to research to reduce aflatoxin-related product-wastage in peanuts $AU millions, 1990.

	Year no		Australia	Indonesia	Philippines	Thailand	Rest of the world	Global total
	14	2001	$0.02	$8.92	$1.25	$1.11	$4.84	$16.14
	15	2002	$0.02	$10.40	$1.46	$1.30	$5.65	$18.83
	16	2003	$0.03	$11.89	$1.67	$1.48	$6.45	$21.52
	17	2004	$0.03	$13.38	$1.88	$1.67	$7.26	$24.21
	18	2005	$0.03	$14.86	$2.08	$1.85	$8.07	$26.90
	19	2006	$0.04	$16.35	$2.29	$2.04	$8.88	$29.59
	20	2007	$0.04	$17.83	$2.50	$2.22	$9.68	$32.28
	21	2008	$0.04	$19.32	$2.71	$2.41	$10.49	$34.97
	22	2009	$0.04	$19.32	$2.71	$2.41	$10.49	$34.97
	23	2010	$0.04	$19.32	$2.71	$2.41	$10.49	$34.97
	24	2011	$0.04	$19.32	$2.71	$2.41	$10.49	$34.97
	25	2012	$0.04	$19.32	$2.71	$2.41	$10.49	$34.97
	26	2013	$0.04	$19.32	$2.71	$2.41	$10.49	$34.97
	27	2014	$0.04	$19.32	$2.71	$2.41	$10.49	$34.97
	28	2015	$0.04	$19.32	$2.71	$2.41	$10.49	$34.97
	29	2016	$0.04	$19.32	$2.71	$2.41	$10.49	$34.97
End of planning horizon	30	2017	$0.04	$19.32	$2.71	$2.41	$10.49	
Discount rate			0.08	0.08	0.08	0.08	0.08	
Discounted benefits			$0.12	$50.63	$7.10	$6.31	$27.49	$91.64

The total discounted benefits from a technology that reduces product-spoilage effects in Indonesia, Philippines, and Thailand are estimated to be about $AU358 million with the following distribution of these benefits:

- Indonesia's welfare increases by $AU145 million with $AU94 millions from a reduction of product-spoilage effects in the maize industry and about $AU51 million from a reduction in the product-spoilage effects in the peanut industry;
- Philippine's welfare increases by $AU72 millions with $AU65 millions from a reduction of product-spoilage effects in the maize industry and $AU7 millions from a reduction in the product-spoilage effects in the peanut industry;
- Thailand's welfare increases by $AU54 millions with $AU47 millions from a reduction of product-spoilage effects in the maize industry and about $AU7 millions from a reduction in the product-spoilage effects in the peanut industry; and
- The rest of the world's welfare increases by $AU86 millions with $AU58 millions from a reduction of product-spoilage effects in the maize industry and by about $AU28 millions from a reduction in the product-spoilage effects in the peanut industry.

Table 10 shows the details of how the estimated benefits from a technology that reduces product spoilage in maize and peanuts are distributed across the 70 regions in the research evaluation model used in this analysis. Consumers in all countries that trade in either maize or peanuts gain from a technology that reduces product wastage because such a technology increases retail supply of maize and peanuts and reduces the world price of these commodities. However, only producers in Indonesia, Philippines, and Thailand gain from this technology. This result is due to the assumption that only those countries that collaborated in either project PN8806 or PN9104 would develop the technology to control aflatoxins.

Table 10. The distribution of potential benefits from a technology to control fungi and aflatoxins which reduces product wastage effects in maize and peanuts.

Country or region	Maize discounted producer benefit	Maize discounted consumer benefit	Peanut discounted producer benefit	Peanut discounted consumer benefit	Total for maize & peanuts
Bangladesh	$0.00	$0.00	$0.00	$0.00	$0.00
Bhutan	$0.00	$0.00	$0.00	$0.00	$0.00
India	($9.40)	$9.68	($24.81)	$33.91	$9.39
Nepal	($1.05)	$1.08	$0.00	$0.00	$0.03
Pakistan	($1.06)	$1.09	($0.66)	$1.01	$0.38
Sri Lanka	($0.06)	$0.06	($0.23)	$0.04	($0.19)
Burma	($0.14)	$0.15	$0.00	$0.00	$0.00
Indonesia	$87.15	$6.46	$45.20	$7.35	$146.15
Kampuchea (Cambodia)	($0.04)	$0.04	$0.00	$0.00	$0.00
Laos PDR	($0.04)	$0.04	$0.00	$0.00	$0.00
Malaysia	($0.03)	$0.06	($0.38)	$0.41	$0.05
Philippines	$60.46	$4.58	$6.67	$0.70	$72.40
Thailand	$43.99	$3.33	$5.75	$0.80	$53.87
Vietnam, Socialist Republic	($0.46)	$0.48	($0.97)	$1.04	$0.09
China, People's Republic	($103.37)	$109.73	($6.29)	$8.58	$8.65
Mongolia	$0.00	$0.00	$0.00	$0.00	$0.00
Fiji	$0.00	$0.00	($0.37)	$0.37	$0.00
Papua New Guinea	$0.00	$0.00	($0.37)	$0.37	$0.00
Samoa (Western)	$0.00	$0.00	$0.00	$0.00	$0.00
Solomon Is.	$0.00	$0.00	$0.00	$0.00	$0.00
Tonga	$0.00	$0.00	($0.37)	$0.38	$0.01
Vanuatu	$0.00	$0.00	$0.00	$0.00	$0.00
Other South Pacific	$0.00	$0.00	$0.00	$0.00	$0.00
Ethiopia	($0.70)	$0.71	($0.39)	$0.26	($0.12)
Kenya	($2.36)	$2.41	($0.24)	$0.04	($0.14)
Malawi	($1.10)	$1.13	($0.48)	$0.38	($0.08)
Mozambique	($0.10)	$0.10	($0.60)	$0.55	($0.06)
Tanzania	($1.30)	$1.33	$0.00	$0.00	$0.03
Uganda	($0.19)	$0.19	$0.00	$0.00	$0.00
Zambia	($0.84)	$0.86	($0.47)	$0.60	$0.14
Zimbabwe	($1.84)	$1.88	($0.62)	$0.57	($0.01)
Zaire	($0.31)	$0.32	$0.00	$0.00	$0.01
Ivory Coast	($0.28)	$0.28	($0.82)	$1.36	$0.54
Ghana	($0.38)	$0.38	$0.00	$0.00	$0.01
Nigeria	($1.36)	$1.39	($3.94)	$8.17	$4.26
Cameroon United Republic	($0.20)	$0.21	($0.72)	$1.14	$0.42

Table 10. (Continued) The distribution of potential benefits from a technology to control fungi and aflatoxins which reduces product wastage effects in maize and peanuts.

Country or region	Maize discounted producer benefit	Maize discounted consumer benefit	Peanut discounted producer benefit	Peanut discounted consumer benefit	Total for maize & peanuts
Angola	($0.12)	$0.13	($0.44)	$0.52	$0.09
Madagascar	($0.11)	$0.11	$0.00	$0.00	$0.00
Sudan	($0.01)	$0.01	($1.39)	$1.63	$0.24
Africa-2	($0.70)	$0.72	($5.08)	$10.65	$5.59
Africa-3	($0.66)	$0.68	($0.88)	$0.93	$0.06
Africa-4	($0.06)	$0.06	($0.87)	$1.47	$0.60
Africa-5	($0.14)	$0.14	($0.75)	$1.20	$0.46
Africa-6	($0.26)	$0.27	($0.21)	$0.01	($0.20)
Africa-7	($0.01)	$0.03	($0.37)	$0.38	$0.02
Turkey	($2.02)	$2.08	($0.41)	$0.28	($0.07)
Egypt, Arab Republic	($5.39)	$5.63	($0.32)	$0.15	$0.08
Africa-1	($0.43)	$0.86	($0.57)	$0.81	$0.67
Other West and North Africa	($0.37)	$0.39	($0.49)	$0.63	$0.16
Brazil	($21.62)	$23.12	($0.91)	$1.55	$2.14
Colombia	($0.99)	$1.02	($0.23)	$0.03	($0.17)
Peru	($0.40)	$0.41	($0.38)	$0.40	$0.03
Venezuela	($1.00)	$1.99	($0.43)	$0.51	$1.06
Bolivia	($0.10)	$0.11	($0.27)	$0.09	($0.18)
Ecuador	($0.36)	$0.37	$0.00	$0.00	$0.01
Mexico	($12.66)	$13.04	($0.75)	$1.21	$0.84
Argentina	($3.42)	$3.52	($1.39)	$1.63	$0.34
Chile	($0.76)	$0.78	$0.00	$0.00	$0.02
Paraguay	($0.53)	$0.55	($0.36)	$0.21	($0.13)
Uruguay	($0.08)	$0.08	($0.37)	$0.37	$0.01
Latin-America 1	($1.85)	$1.90	($0.74)	$1.18	$0.50
Latin-America 2	($0.02)	$0.04	($0.38)	$0.39	$0.04
Asia-Developed	($5.52)	$10.97	($0.85)	$1.41	$6.01
Australia	($0.21)	$0.24	($0.47)	$0.59	$0.14
Canada	($7.78)	$8.57	$0.00	$0.00	$0.79
USA	($223.48)	$246.25	($6.47)	$8.61	$24.93
USSR	($11.15)	$11.92	($0.38)	$0.40	$0.79
Japan	$0.00	$0.00	($0.50)	$0.65	$0.16
Developed 1–2	($37.12)	$40.90	($1.08)	$1.93	$4.63
Developed 3–4	($13.18)	$26.17	$0.00	$0.00	$13.00
World Total	($287.52)	$551.05	($12.89)	$107.88	$358.52

Potential benefits to the pig meat, poultry meat, and hen eggs livestock sectors

Tables 11–13 show the potential benefits from a technology that improves control of aflatoxins in maize and peanuts, and benefits that accrue to the pig meat, poultry meat, and hen eggs livestock sectors, respectively. The results in these tables are also based on production and consumption of pig meat, poultry meat, and hen eggs in 70 regions of the world. The key parameter in the generation of these estimates is the weighted cost reductions in the different livestock sectors (see Table 6) attributable to the reduction in aflatoxin contamination of maize and peanut-based feedstuffs for livestock.

The total discounted benefits from the three livestock sectors in the three countries are estimated to be as follows:

- Indonesia gains a total of about $AU34 million with $AU6 million estimated to accrue to the pig meat sector, $AU12 millions to the poultry meat sector, and $AU16 million to the hen eggs sector.
- The Philippines gains a total of about $AU49 million with $AU37 million estimated to accrue to the pig meat sector, $AU4 millions to the poultry meat sector, and $AU8 million to the hen eggs sector.
- Thailand gains a total of about $AU41 million with $AU11 million estimated to accrue to the pig meat sector, $AU18 millions to the poultry meat sector, and $AU12 million to the hen eggs sector.
- The estimated benefits to the livestock sectors in Australia and the rest of the world are negligible.

The potential human health benefits to Indonesia, Philippines, and Thailand

Tables 14 and 15 show the flow of human health benefits that could potentially flow from a technology developed fro m ACIAR PN8806 and PN9104 that reduces aflatoxin contamination of maize and peanuts used for human consumption.

The annual human health benefits were estimated in Lubulwa and Davis (1994). The flow of human health benefits is conditional on adoption of a technology in the maize and peanut production sectors. The human health benefits are assumed to accrue to countries that collaborated in ACIAR PN8806 and PN9104. There are no human health benefits to Australia because the current quality control regimes in the maize and peanut production sectors reduce the human exposure to aflatoxins to negligible, innocuous levels.

The human health benefits over a 30-year time horizon to the three collaborating countries are as follows:

- Indonesia's economic surplus is estimated to increase by a total of $AU364 million of human health benefits, with $AU193 million as benefits from the reduction in mortality rates and morbidity rates attributed to primary liver cancer from the consumption of aflatoxin-contaminated maize, and $AU171 million as benefits from the reduction in mortality rates and morbidity rates attributed to primary liver cancer from the consumption of aflatoxin contaminated peanuts.
- The Philippines' economic surplus is estimated to increase by a total of $AU56 million of human health benefits, with $AU52 million as benefits from the reduction in mortality rates and morbidity rates attributed to primary liver cancer from the consumption of aflatoxin contaminated maize, and $AU4 million as benefits from the reduction in mortality rates and morbidity rates attributed to primary liver cancer from the consumption of aflatoxin contaminated peanuts.
- Thailand's economic surplus is estimated to increase by a total of $AU38 million of human health benefits, with $AU10 million as benefits from the reduction in mortality rates and morbidity rates attributed to primary liver cancer from the consumption of aflatoxin contaminated maize, and $AU28 million as benefits from the reduction in mortality rates and morbidity rates attributed to primary liver cancer from the consumption of aflatoxin contaminated peanuts.

The potential net benefits of ACIAR PN8806 and PN9104

This section adds the benefits discussed at the beginning of the 'Results' section to give the total potential benefits of the ACIAR projects, PN8806 and PN9104. The research costs incurred by ACIAR, by the Australian commissioned organisation, and by the collaborating institutions in Indonesia, Philippines, and Thailand are taken into account to estimate the net present value of the two projects and the associated internal rate of return. The discounted total research costs are about $AU0.94 million.

The total discounted potential benefits from a technology that developed as a result of the two projects are estimated to be about $AU937 million over a 30-year time horizon. These are the sum of increases in

welfare to the producers and consumers of maize and peanuts, the producers and consumers of pig meat, poultry meat, and hen eggs, and the human health benefits from reduced incidence of primary liver cancer in Indonesia, Philippines, Thailand, and the rest of the world.

Table 11. Flow of benefits accruing to Australia, Indonesia, Philippines, Thailand, and the rest of the world due to research to reduce aflatoxins in feedstuffs in the pig meat sector in $AU million, 1990.

	Year no.		Australia	Indonesia	Philippines	Thailand	Rest of the world	Global total
Start PN8806	1	1988	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
	2	1989	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
	3	1990	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
Start PN9104	4	1991	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
	5	1992	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
End PN8806	6	1993	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
End PN9104	7	1994	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
	8	1995	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
	9	1996	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
	10	1997	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
	11	1998	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
	12	1999	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
	13	2000	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00
	14	2001	($0.0001)	$1.32	$7.85	$2.53	$0.00	$11.69
	15	2002	($0.0001)	$1.53	$9.15	$2.96	$0.00	$13.64
	16	2003	($0.0001)	$1.75	$10.46	$3.38	$0.00	$15.59
	17	2004	($0.0001)	$2.06	$11.77	$3.55	$0.00	$17.37
	18	2005	($0.0002)	$2.06	$11.77	$3.55	($0.01)	$17.37
	19	2006	($0.0002)	$2.06	$11.77	$3.55	($0.01)	$17.37
	20	2007	($0.0002)	$2.06	$11.77	$3.55	($0.01)	$17.37
	21	2008	($0.0002)	$2.06	$11.77	$3.55	($0.01)	$17.37
	22	2009	($0.0002)	$2.06	$11.77	$3.55	($0.01)	$17.37
	23	2010	($0.0002)	$2.06	$11.77	$3.55	($0.01)	$17.37
	24	2011	($0.0002)	$2.06	$11.77	$3.55	($0.01)	$17.37
	25	2012	($0.0002)	$2.06	$11.77	$3.55	($0.01)	$17.37
	26	2013	($0.0002)	$2.06	$11.77	$3.55	($0.01)	$17.37
	27	2014	($0.0002)	$2.06	$11.77	$3.55	($0.01)	$17.37
	28	2015	($0.0002)	$2.06	$11.77	$3.55	($0.01)	$17.37
	29	2016	($0.0002)	$2.06	$11.77	$3.55	($0.01)	$17.37
End of planning horizon	30	2017	($0.0002)	$2.06	$11.77	$3.55	($0.01)	$17.37
Discount rate			0.08	0.08	0.08	0.08	0.08	0.08
Discounted benefits			($0.0005)	$6	$37	$11	$0	$54.63

Table 12. Flow of benefits accruing to Australia, Indonesia, Philippines, Thailand, and the rest of the world due to research to reduce aflatoxins in feedstuffs in the poultry meat sector, in $AU million, 1990.

	Year no.		Australia	Indonesia	Philippines	Thailand	Rest of the world	Global total
Start PN8806	1	1988	$0.0000	$0.00	$0.00	$0.00	$0.000	$0.00
	2	1989	$0.0000	$0.00	$0.00	$0.00	$0.000	$0.00
	3	1990	$0.0000	$0.00	$0.00	$0.00	$0.000	$0.00
Start PN9104	4	1991	$0.0000	$0.00	$0.00	$0.00	$0.000	$0.00
	5	1992	$0.0000	$0.00	$0.00	$0.00	$0.000	$0.00
End PN8806	6	1993	$0.0000	$0.00	$0.00	$0.00	$0.000	$0.00
End PN9104	7	1994	$0.0000	$0.00	$0.00	$0.00	$0.000	$0.00
	8	1995	$0.0000	$0.00	$0.00	$0.00	$0.000	$0.00
	9	1996	$0.0000	$0.00	$0.00	$0.00	$0.000	$0.00
	10	1997	$0.0000	$0.00	$0.00	$0.00	$0.000	$0.00
	11	1998	$0.0000	$0.00	$0.00	$0.00	$0.000	$0.00
	12	1999	$0.0000	$0.00	$0.00	$0.00	$0.000	$0.00
	13	2000	$0.0000	$0.00	$0.00	$0.00	$0.000	$0.00
	14	2001	($0.0001)	$2.42	$0.87	$4.04	$0.000	$7.33
	15	2002	($0.0001)	$2.82	$1.01	$4.72	$0.000	$8.55
	16	2003	($0.0001)	$3.22	$1.15	$5.39	($0.001)	$9.77
	17	2004	($0.0001)	$3.79	$1.30	$5.66	($0.002)	$10.74
	18	2005	($0.0001)	$3.79	$1.30	$5.66	($0.002)	$10.74
	19	2006	($0.0001)	$3.79	$1.30	$5.66	($0.003)	$10.74
	20	2007	($0.0001)	$3.79	$1.30	$5.66	($0.003)	$10.74
	21	2008	($0.0001)	$3.79	$1.30	$5.66	($0.003)	$10.74
	22	2009	($0.0001)	$3.79	$1.30	$5.66	($0.004)	$10.74
	23	2010	($0.0001)	$3.79	$1.30	$5.66	($0.003)	$10.74
	24	2011	($0.0001)	$3.79	$1.30	$5.66	($0.003)	$10.74
	25	2012	($0.0001)	$3.79	$1.30	$5.66	($0.003)	$10.74
	26	2013	($0.0001)	$3.79	$1.30	$5.66	($0.003)	$10.74
	27	2014	($0.0001)	$3.79	$1.30	$5.66	($0.003)	$10.74
	28	2015	($0.0001)	$3.79	$1.30	$5.66	($0.003)	$10.74
	29	2016	($0.0001)	$3.79	$1.30	$5.66	($0.003)	$10.74
End of planning horizon	30	2017	($0.0001)	$3.79	$1.30	$5.66	($0.003)	$10.74
Discount rate			$0.0800	0.08	0.08	0.08	$0.080	
Discounted benefits			($0.0003)	$11.77	$4.07	$18.06	($0.007)	$33.89

Table 13. Flow of benefits accruing to Australia, Indonesia, Philippines, Thailand, and the rest of the world due to research to reduce aflatoxins in feedstuffs in the hen eggs sector, in $AU million, 1990.

	Year no.		Australia	Indonesia	Philippines	Thailand	Rest of the world	Global total
Start PN8806	1	1988	$0.00000	$0.00	$0.00	$0.00	$0.000	$0.00
	2	1989	$0.00000	$0.00	$0.00	$0.00	$0.000	$0.00
	3	1990	$0.00000	$0.00	$0.00	$0.00	$0.000	$0.00
Start PN9104	4	1991	$0.00000	$0.00	$0.00	$0.00	$0.000	$0.00
	5	1992	$0.00000	$0.00	$0.00	$0.00	$0.000	$0.00
End PN8806	6	1993	$0.00000	$0.00	$0.00	$0.00	$0.000	$0.00
End PN9104	7	1994	$0.00000	$0.00	$0.00	$0.00	$0.000	$0.00
	8	1995	$0.00000	$0.00	$0.00	$0.00	$0.000	$0.00
	9	1996	$0.00000	$0.00	$0.00	$0.00	$0.000	$0.00
	10	1997	$0.00000	$0.00	$0.00	$0.00	$0.000	$0.00
	11	1998	$0.00000	$0.00	$0.00	$0.00	$0.000	$0.00
	12	1999	$0.00000	$0.00	$0.00	$0.00	$0.000	$0.00
	13	2000	$0.00000	$0.00	$0.00	$0.00	$0.000	$0.00
	14	2001	($0.00002)	$3.26	$1.61	$2.66	$0.000	$7.53
	15	2002	($0.00002)	$3.80	$1.88	$3.10	$0.000	$8.79
	16	2003	($0.00002)	$4.35	$2.15	$3.55	$0.000	$10.04
	17	2004	($0.00002)	$5.11	$2.42	$3.72	$0.000	$11.25
	18	2005	($0.00003)	$5.11	$2.42	$3.72	($0.001)	$11.25
	19	2006	($0.00003)	$5.11	$2.42	$3.72	($0.001)	$11.25
	20	2007	($0.00003)	$5.11	$2.42	$3.72	($0.001)	$11.25
	21	2008	($0.00004)	$5.11	$2.42	$3.72	($0.001)	$11.25
	22	2009	($0.00004)	$5.11	$2.42	$3.72	($0.002)	$11.25
	23	2010	($0.00004)	$5.11	$2.42	$3.72	($0.002)	$11.25
	24	2011	($0.00004)	$5.11	$2.42	$3.72	($0.002)	$11.25
	25	2012	($0.00004)	$5.11	$2.42	$3.72	($0.002)	$11.25
	26	2013	($0.00004)	$5.11	$2.42	$3.72	($0.002)	$11.25
	27	2014	($0.00004)	$5.11	$2.42	$3.72	($0.002)	$11.25
	28	2015	($0.00004)	$5.11	$2.42	$3.72	($0.002)	$11.25
	29	2016	($0.00004)	$5.11	$2.42	$3.72	($0.002)	$11.25
End of planning horizon	30	2017	($0.00004)	$5.11	$2.42	$3.72	($0.002)	$11.25
Discount rate			$0.08000	0.08	0.08	0.08	$0.080	
Discounted benefits			($0.00010)	$15.87	$7.58	$11.88	($0.003)	$35.33

Table 14. Flow of human health benefits accruing to Australia, Indonesia, Philippines, Thailand, and the rest of the world due to research to reduce aflatoxins in maize used for human consumption, in$AU million, 1990

	Year no.		Australia	Indonesia	Philippines	Thailand	Rest of the world	Global total
Annual benefits from Lubulwa and Davis (1994)			$0	$132	$37	$8	$0	$177
Flow of benefits conditional on adoption of a technology which reduces aflatoxins in maize								
Start PN8806	1	1988	$0	$0	$0	$0	$0	$0
	2	1989	$0	$0	$0	$0	$0	$0
	3	1990	$0	$0	$0	$0	$0	$0
Start PN9104	4	1991	$0	$0	$0	$0	$0	$0
	5	1992	$0	$0	$0	$0	$0	$0
End PN8806	6	1993	$0	$0	$0	$0	$0	$0
End PN9104	7	1994	$0	$0	$0	$0	$0	$0
	8	1995	$0	$0	$0	$0	$0	$0
	9	1996	$0	$0	$0	$0	$0	$0
	10	1997	$0	$0	$0	$0	$0	$0
	11	1998	$0	$0	$0	$0	$0	$0
	12	1999	$0	$0	$0	$0	$0	$0
	13	2000	$0	$0	$0	$0	$0	$0
	14	2001	$0	$40	$11	$2	$0	$53
	15	2002	$0	$46	$13	$3	$0	$62
	16	2003	$0	$53	$15	$3	$0	$71
	17	2004	$0	$62	$17	$3	$0	$82
	18	2005	$0	$62	$17	$3	$0	$82
	19	2006	$0	$62	$17	$3	$0	$82
	20	2007	$0	$62	$17	$3	$0	$82
	21	2008	$0	$62	$17	$3	$0	$82
	22	2009	$0	$62	$17	$3	$0	$82
	23	2010	$0	$62	$17	$3	$0	$82
	24	2011	$0	$62	$17	$3	$0	$82
	25	2012	$0	$62	$17	$3	$0	$82
	26	2013	$0	$62	$17	$3	$0	$82
	27	2014	$0	$62	$17	$3	$0	$82
	28	2015	$0	$62	$17	$3	$0	$82
	29	2016	$0	$62	$17	$3	$0	$82
End of planning horizon	30	2017	$0	$62	$17	$3	$0	$82
Discount rate			0.08	0.08	0.08	0.08	0.08	
Discounted benefits			$0	$193	$52	$10	$0	$255

Table 15. Flow of human health benefits accruing to Australia, Indonesia, Philippines, Thailand, and the rest of the world due to research to reduce aflatoxins in peanuts used for human consumption, in $AU million, 1990

	Year no.		Australia	Indonesia	Philippines	Thailand	The rest of the world	Global total
Annual benefits from Lubulwa and Davis (1994)			$0.00	$96.50	$2.20	$16.00	$0.00	$114.70
Flow of benefits conditional on adoption of a technology which reduces aflatoxins in peanuts								
Start PN8806	1	1988	$0	$0	$0	$0	$0	$0.00
	2	1989	$0	$0	$0	$0	$0	$0.00
	3	1990	$0	$0	$0	$0	$0	$0.00
Start PN9104	4	1991	$0	$0	$0	$0	$0	$0.00
	5	1992	$0	$0	$0	$0	$0	$0.00
End PN8806	6	1993	$0	$0	$0	$0	$0	$0.00
End PN9104	7	1994	$0	$0	$0	$0	$0	$0.00
	8	1995	$0	$0	$0	$0	$0	$0.00
	9	1996	$0	$0	$0	$0	$0	$0.00
	10	1997	$0	$0	$0	$0	$0	$0.00
	11	1998	$0	$0	$0	$0	$0	$0.00
	12	1999	$0	$0	$0	$0	$0	$0.00
	13	2000	$0	$0	$0	$0	$0	$0.00
	14	2001	$0	$29	$1	$5	$0	$34.41
	15	2002	$0	$34	$1	$6	$0	$40.15
	16	2003	$0	$39	$1	$6	$0	$45.88
	17	2004	$0	$43	$1	$7	$0	$51.62
	18	2005	$0	$48	$1	$8	$0	$57.35
	19	2006	$0	$53	$1	$9	$0	$63.09
	20	2007	$0	$58	$1	$10	$0	$68.82
	21	2008	$0	$63	$1	$10	$0	$74.56
	22	2009	$0	$63	$1	$10	$0	$74.56
	23	2010	$0	$63	$1	$10	$0	$74.56
	24	2011	$0	$63	$1	$10	$0	$74.56
	25	2012	$0	$63	$1	$10	$0	$74.56
	26	2013	$0	$63	$1	$10	$0	$74.56
	27	2014	$0	$63	$1	$10	$0	$74.56
	28	2015	$0	$63	$1	$10	$0	$74.56
	29	2016	$0	$63	$1	$10	$0	$74.56
End of planning horizon	30	2017	$0	$63	$1	$10	$0	$74.56
Discount rate			0.08	0.08	0.08	0.08	0.08	
Discounted benefits			$0	$171	$4	$28	$0	$203

These benefits are distributed as follows: Indonesia gains by $AU542 million, Philippines by $AU177 million, Thailand by $AU133 million, and the rest of the world by $AU85 million.

The net present value of this flow of benefits after taking into account the research costs is estimated at $AU936 million, and the internal rate of return for the two projects is estimated at 69%.

These estimates are shown in Table 16. They need to be interpreted with caution. First of all, they are not benefits that directly flow from a technology developed under PN8806 and PN9104. As indicated earlier, these two projects did not develop any technology. However, they provided the basis, the justification for the development of a technology that could lead to the flow of benefits in Table 16. The results in Table 16 indicate the potential benefits from such a technology that leads to better control of aflatoxins in maize and peanuts.

Finally, other donor countries (for example, Japan and Canada) had projects in Southeast Asia that contributed to the awareness of aflatoxin contamination in maize and peanuts in Southeast Asia at the same time as when the ACIAR PN8806 and PN9104 were being undertaken. It would thus be an over-estimation to assume that all the benefits in Table 16 are from information generated by PN8806 and PN9104. Estimation of the proportion of the benefits from the two ACIAR projects is impossible without data on the funding of aflatoxin research in Southeast Asia from other sources and the quality of intellectual and other inputs from the different funding sources.

Sensitivity analyses

The results in Table 16 are based on a number of assumptions, including the following:

- the cost of research that generates the aflatoxin control technology are implicitly assumed to be zero; and
- the technology that leads to better control of aflatoxins in maize and peanuts does not change the farm-level costs.

This section summarises the sensitivity analyses which were used to explore the implications of changes in these assumptions.

Costs of research that generates better control of aflatoxins in maize and peanuts

Table 16 does not take into account the costs incurred in developing a technology that leads to better control of aflatoxins in maize and peanuts. One problem is that those costs are unknown. An interesting question to ask is how much would these research costs have to be to reduce the net benefits in Table 16 to zero, assuming that they were incurred annually from the end of ACIAR PN9104 in year 7 to the beginning of adoption of the technology in year 13?

In the case where total benefits include human health benefits, technology development research costs would have to be about $AU1273 million (equivalent to an annual flow of $AU248 000 for 7 years) in Table 16 to reduce the net benefits to zero. That is, the total research costs would have to increase by over 1100 times the total discounted research costs for ACIAR PN8806 and PN9104 for the net discounted benefits to fall to zero. Even then the internal rate of return would be about 10% over the 30-year time horizon. Excluding human health benefits, technology development research costs would have to be about $AU764 million (equivalent to an annual flow of $AU147 000 for 7 years) in Table 16 to reduce the net benefits to zero.

Changes in farm-level costs as a result of a better control of aflatoxins in maize and peanuts

One of the possible farm-level technologies that was explored under PN9104 was the use of non-toxigenic, competitive *Aspergillus flavus* and *Aspergillus parasiticus* as a solution to the aflatoxin problem in peanuts. Under this approach, strains of non-toxigenic competitive *Aspergillus flavus* and *Aspergillus parasiticus* are added to soils resulting in competitive exclusion of the toxigenic strains of these fungi. The research under PN9104 established this approach as potentially applicable in Thailand and probably in other countries in Southeast Asia, and may be the long term solution to the aflatoxin problem, at least in peanuts[8].

This section explores the implications of changes in farm-level costs for the net present value and the internal rate of return in Table 16.

The following question was posed: assuming that a technology to control aflatoxins in maize and peanuts leads to an increase in farm-level costs of producing maize and peanuts equal to 10% of farm-gate prices, how would the net present value of benefits and the corresponding internal rate of return in Table 16 be affected?

[8] Dr John Pitt, CSIRO, North Ryde, Sydney (personal communication, June 1995)

Table 16. ACIAR PN8806 and 9104: the flow of research costs and benefits accruing to Australia, Indonesia, Philippines, Thailand, and the rest of the world due to research to reduce aflatoxins in maize and peanuts used for human consumption and as feedstuffs, $AU million, 1990

	Year no		Australia	Indonesia	Philippines	Thailand	Rest of the world	Total benefits	Research costs	Net benefits
Start PN8806	1	1988	$0	$0	$0	$0	$0	$0	0.200825	($0.200825)
	2	1989	$0	$0	$0	$0	$0	$0	0.308093	($0.308093)
	3	1990	$0	$0	$0	$0	$0	$0	0.249498	($0.249498)
Start PN9104	4	1991	$0	$0	$0	$0	$0	$0	0.333957	($0.333957)
	5	1992	$0	$0	$0	$0	$0	$0	0.414347	($0.414347)
End PN8806	6	1993	$0	$0	$0	$0	$0	$0	0.130847	($0.130847)
End PN9104	7	1994	$0	$0	$0	$0	$0	$0		$0
	8	1995	$0	$0	$0	$0	$0	$0		$0
	9	1996	$0	$0	$0	$0	$0	$0		$0
	10	1997	$0	$0	$0	$0	$0	$0		$0
	11	1998	$0	$0	$0	$0	$0	$0		$0
	12	1999	$0	$0	$0	$0	$0	$0		$0
	13	2000	$0	$0	$0	$0	$0	$0		$0
	14	2001	$0	$104	$37	$28	$15	$184		$184
	15	2002	$0	$121	$43	$33	$17	$214		$214
	16	2003	$0	$138	$49	$37	$20	$245		$245
	17	2004	$0	$160	$56	$40	$22	$277		$277
	18	2005	$0	$166	$56	$41	$24	$287		$287
	19	2006	$0	$173	$56	$42	$26	$297		$297
	20	2007	$0	$179	$57	$43	$29	$307		$307
	21	2008	$0	$185	$57	$44	$31	$317		$317
	22	2009	$0	$185	$57	$44	$32	$318		$318
	23	2010	$0	$185	$57	$44	$32	$318		$318
	24	2011	$0	$185	$57	$44	$32	$318		$318
	25	2012	$0	$185	$57	$44	$32	$318		$318
	26	2013	$0	$185	$57	$44	$32	$318		$318
	27	2014	$0	$185	$57	$44	$32	$318		$318
	28	2015	$0	$185	$57	$44	$32	$318		$318
	29	2016	$0	$185	$57	$44	$32	$318		$318
End of planning horizon	30	2017	$0	$185	$57	$44	$32	$318		$318
Discount rate			0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Discounted benefits and costs			$0	$542	$177	$133	$85	$937	$0.94	$936
Internal rate of return									$0.94	69%

An increase in farm-level costs equal to 10% of the farm-gate price of maize and peanuts would reduce the net present value of benefits from $AU936 millions to $AU901 million and the internal rate of return would drop slightly from 69 to 68%. When human health benefits are excluded, an increase in farm-level costs equal to 10% of the farm-gate price of maize and peanuts would reduce the net present value of benefits from $AU479 million to $AU445 million and the internal rate of return would drop slightly from 60.86 to 60.06%

Impact on Scientific Knowledge

On the issue of the scientific impact of PN8806, King (1991) concluded that:

> This research has comprehensively defined the fungal flora and associated mycotoxins in major durable commodities in Thailand as an indicator of the status of commodities in Southeast Asia in general. This has never been done before, and is a significant achievement, adding greatly to the world knowledge of fungal spoilage of tropical stored commodities and the consequent potential for mycotoxin formation

Similar conclusions were made about the project by Miller (1993). Appendix C lists the papers published to date from the two research projects.

Human and Institutional Capacity Building Impacts of the Project[9]

Project 8806 involved an active training program for scientists from participating countries. During the project, eight scientists from collaborating laboratories in Indonesia, Philippines, and Thailand received training at the CSIRO Food Research Laboratory for approximately four weeks each. Four scientists were trained in techniques for isolation and identification of fungi from foods, and four in the techniques for extraction, detection, and quantification of mycotoxins.

Concluding Remarks

This paper has estimated the potential benefits from two ACIAR projects on fungi and aflatoxins. The assessment acknowledges that the two ACIAR projects were not designed to develop technologies to control aflatoxin contamination in maize and peanuts. However, the information the two projects generated could be catalytic in the development of technologies either at farm level or in the postharvest sector that could reduce or even eliminate aflatoxin in maize and peanuts and aflatoxicosis in the human population and in livestock (pigs, poultry, and egg laying chickens) in Indonesia, Philippines, and Thailand.

It is estimated that a technology that eliminates aflatoxins in maize and peanuts could generate up to $AU936 million in discounted net benefits over a 30-year time horizon and an associated internal rate of return of 69% (Table 16). If one excludes the human health benefits, such a project could generate a net present value of about $AU479 million over a 30-year horizon with an associated internal rate of return of 61%.

[9] This section draws heavily on King (1991).

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Appendix A: Objectives of ACIAR Projects PN8806 and PN9104

ACIAR project PN8806 had the following objectives:

(a) To isolate and identify fungi growing in specific commodities and to determine the mycotoxins they are capable of producing.

(b) To assess the significance of each species as a spoilage fungus or mycotoxin producer on the basis of their relative prevalence.

(c) To develop a computer database of fungi significant in Asian commodities, including incidence, mycotoxins, and factors influencing toxin production.

(d) To assess the mycotoxin status of each commodity by assaying the samples for specific mycotoxins, with samples of major commodities being assayed throughout their postharvest history.

(e) To attempt to correlate fungal invasion with visual deterioration, i.e. develop simple techniques for quality monitoring.

(f) To train Asian microbiologists and chemists in the standard methods being used, so that mycological and mycotoxin quality can be assessed locally but be comparable throughout the region.

(g) To explore techniques for simplified or quicker identification of important mycotoxigenic fungi, including development of computer-assisted keys.

(h) To assess, where appropriate, the impact of improved handling, drying, and storage procedures on the incidence of both fungi and mycotoxins.

(i) Where identifiable problems can be pinpointed, to seek solutions in collaboration with local authorities in terms of agricultural practice, handling, and storage.

(j) To undertake fundamental studies on the influence of environmental factors, especially water activity and temperature, on the growth of, and toxin production by, more significant fungi encountered. Such data will be of great value in assessing the efficacy of drying and storage treatments designed to inhibit fungal growth and toxin production.

ACIAR project PN9104 had the following objectives:

(a) To monitor peanut crops for the presence of *Aspergillus flavus* from the time of planting, through nut development, harvest, and storage. By this means, to clearly establish the main times of *Aspergillus flavus* entry into peanut plants and nuts.

(b) At the same time, to assess the development of aflatoxins in the nuts, again over the whole time span of nut development, harvest, and storage.

(c) To positively identify *Aspergillus flavus* and *Aspergillus parasiticus*, together with the related species *Aspergillus nomius*, and hence to determine the comparative distribution of these species in Thai peanuts and soils used for peanuts.

(d) Using this information, to assess the efficacy of techniques, currently under development at the CSIRO Food Research Laboratory, for reducing *Aspergillus flavus* invasion under Asian field conditions.

(e) If such experiments are successful, to plan field trials at one or more promising techniques.

(f) In glasshouse studies at the CSIRO Food Research Laboratory, to carry out preliminary studies on *Aspergillus flavus* and *Aspergillus parasiticus* invasion in maize.

(g) To study the influence of storage on *Aspergillus flavus* invasion, using peanuts obtained from commercial storage in Thailand.

(h) To assess the effect, if any, of such postharvest invasion on the efficacy of preharvest techniques designed to limit aflatoxin formation by introducing nontoxigenic competitive *Aspergillus flavus* strains into Thai peanuts.

Appendix B: Production, Consumption, and Trade Status of Commodities Susceptible to Fungi and Aflatoxicosis

Table B1. The production, consumption, and trade status of maize in 70 regions of the world, 1990.

Country/Region	Production 1988 to 1990 ('000 t)	Consumption 1988 to 1990 ('000 t)	Trade status
Bangladesh	3.04	3.04	Non trader
Bhutan	0.00	0.00	Non trader
India	9374.00	9375.79	Net importer
Nepal	1231.00	1301.00	Net importer
Pakistan	1184.50	1184.86	Net importer
Sri Lanka	57.50	104.46	Net importer
Burma	186.16	182.45	Net exporter
Indonesia	6766.26	6652.72	Net exporter
Kampuchea (Cambodia)	55.00	55.00	Non trader
Laos PDR	66.57	56.57	Net exporter
Malaysia	35.00	1343.87	Net importer
Philippines	4853.89	5218.37	Net importer
Thailand	3722.27	2489.32	Net exporter
Vietnam, Socialist Republic	728.00	694.00	Net exporter
China, People's Republic	95000.00	84963.36	Net exporter
Mongolia	0.00	0.00	Non trader
Fiji	1.95	2.80	Net importer
Papua New Guinea	1.45	3.95	Net importer
Samoa (Western)	0.00	0.05	Net importer
Solomon Is.	0.00	0.00	Non trader
Tonga	0.00	0.00	Non trader
Vanuatu	0.70	0.70	Non trader
Other South Pacific	0.55	5.89	Net importer
Ethiopia	1636.00	2216.00	Net importer
Kenya	2630.00	2641.50	Net importer
Malawi	1342.98	1527.98	Net importer
Mozambique	452.91	672.91	Net importer
Tanzania	2445.00	2765.00	Net importer
Uganda	584.00	609.00	Net importer
Zambia	1092.67	1672.23	Net importer
Zimbabwe	1993.30	1645.70	Net exporter
Zaire	870.00	970.00	Net importer

Table B1. (Continued) The production, consumption, and trade status of maize in 70 regions of the world, 1990.

Country/Region	Production 1988 to 1990 ('000 t)	Consumption 1988 to 1990 ('000 t)	Trade status
Ivory Coast	484.00	460.00	Net exporter
Ghana	552.60	611.10	Net importer
Nigeria	1831.50	1831.50	Non trader
Cameroon United Republic	380.00	406.69	Net importer
Angola	180.00	245.00	Net importer
Madagascar	155.00	140.90	Net exporter
Sudan	20.00	40.00	Net importer
Africa-2	964.82	1100.76	Net importer
Africa-3	829.64	805.49	Net exporter
Africa-4	107.90	134.70	Net importer
Africa-5	300.00	327.00	Net importer
Africa-6	314.00	702.72	Net importer
Africa-7	16.23	133.43	Net importer
Turkey	2100.00	2603.87	Net importer
Egypt, Arab Republic	4798.64	6505.78	Net importer
Africa-1	438.40	2447.00	Net importer
Other West and North Africa	890.37	2835.40	Net importer
Brazil	21339.44	24035.41	Net importer
Colombia	1213.30	1155.80	Net exporter
Peru	621.09	1067.43	Net importer
Venezuela	1002.40	1321.80	Net importer
Bolivia	406.68	403.23	Net exporter
Ecuador	465.40	465.46	Net importer
Mexico	14635.44	17221.14	Net importer
Argentina	5049.00	4042.46	Net exporter
Chile	823.15	950.22	Net importer
Paraguay	1138.94	888.94	Net exporter
Uruguay	112.31	153.72	Net importer
Latin-America 1	3210.15	5063.69	Net importer
Latin-America 2	30.04	361.30	Net importer
Asia-Developed	4870.10	17623.28	Net importer
Australia	200.00	199.92	Net exporter
Canada	7157.00	7095.01	Net exporter
USA	201508.00	153033.68	Net exporter
USSR	9900.00	26805.38	Net importer
Japan	0.80	15727.72	Net importer
Developed 1–2	42218.67	49890.84	Net importer
Developed 3–4	11359.43	15508.06	Net importer
World Total	477939.13	492704.32	

Source: FAO (1994).

Table B2. The 3-year average production, consumption and trade status of peanuts in 70 regions of the world

Country/Region	Production 1988 to 1990 (t)	Consumption 1988 to 1990 (t)	Tradestatus
Bangladesh	45.25	45.25	Net importer
Bhutan	0	0	Net importer
India	8,611.00	8,464.26	Net importer
Nepal	0	0.93	Net importer
Pakistan	82.88	82.89	Net importer
Sri Lanka	7.53	7.5	Net importer
Burma	472.19	472.19	Net importer
Indonesia	879.73	921.85	Net importer
Kampuchea (Cambodia)	2.67	2.67	Net importer
Laos PDR	5.61	5.61	Net importer
Malaysia	5.03	38.66	Net importer
Philippines	37.43	69.34	Net importer
Thailand	162.34	160.04	Net importer
Vietnam, Socialist Republic	212.06	110.58	Net importer
China, People's Republic	5,807.77	5,391.46	Net importer
Mongolia*	0	0	Non trader
Fiji	0.34	0.47	Non trader
Papua New Guinea	0.82	0.98	Non trader
Samoa (Western)	0	0	Non trader
Solomon Is.	0	0	Non trader
Tonga	1.63	1.63	Net importer
Vanuatu	1.82	1.82	Non trader
Other South Pacific	0	0	Non trader
Ethiopia	52.33	52.04	Non trader
Kenya	8.77	8.76	Net importer
Malawi	77	68.08	Net importer
Mozambique	111	110.25	Net importer
Tanzania	56.3	56.3	Net importer
Uganda	150.33	150.33	Non trader
Zambia	29.53	32.84	Net importer
Zimbabwe	116.21	108.21	Net importer
Zaire	423.33	423.33	Net importer
Ivory Coast	127	135.62	Non trader
Ghana	207.53	207.53	Non trader
Nigeria	999.07	1,018.50	Net importer
Cameroon	99.33	99.39	Net importer
Angola	20	24.86	Net exporter
Madagascar	30.9	30.9	Net importer
Sudan	332	298.34	Net importer
Africa-2	1,316.86	1,375.62	Net importer
Africa-3	189.05	186.71	Net importer

Table B2. (Continued) The 3-year average production, consumption and trade status of peanuts in 70 regions of the world

Country/Region	Production 1988 to 1990 (t)	Consumption 1988 to 1990 (t)	Tradestatus
Africa-4	141.54	143.95	Net importer
Africa-5	107.1	107.1	Net importer
Africa-6	2.17	2.1	Net importer
Africa-7	1.89	3.18	Net importer
Turkey	57.67	55.04	Net importer
Egypt, Arab Republic	30.75	28.36	Net importer
Africa-1	57.07	60.75	Net exporter
Other West and North Africa	33.8	36.68	Net exporter
Brazil	151.76	155.15	Net importer
Colombia	6.54	6.53	Net importer
Peru	4.5	4.57	Net importer
Venezuela	18.14	21.28	Net importer
Bolivia	17.82	17.81	Net importer
Ecuador	20.29	20.29	Net exporter
Mexico	108.16	123.02	Net importer
Argentina	330.81	171.39	Net importer
Chile	0	3.83	Non trader
Paraguay	42.39	26.88	Net importer
Uruguay	1	1.85	Non trader
Latin-America 1	104.22	104.45	Net importer
Latin-America 2	3.47	10.23	Non trader
Asia-Developed	133.77	191.05	Net importer
Australia	28.28	38.2	Net importer
Canada	0	97.6	Non trader
USA	1,749.97	1,387.39	Net exporter
USSR	4	86.27	Non trader
Japan	36.4	179.24	Non trader
Developed 1–2	199.68	372.27	Net exporter
Developed 3–4	0	469.5	Non trader
World total	24075.83	24075.83	

Source: FAO (1994).

Table B3. The 3-year average production, consumption, and trade status of pig meat in 70 regions of the world.

Country/Region	Production 1988 to 1990 ('000 t)	Consumption 1988 to 1990 ('000 t)	Trade Status
Bangladesh	0	0	Non trader
Bhutan	0	0	Non trader
India	358.75	358.75	Net importer
Nepal	9.34	9.34	Non trader
Pakistan	0	0	Net importer

Table B3. (Continued) The 3-year average production, consumption, and trade status of pig meat in 70 regions of the world.

Country/Region	Production 1988 to 1990 ('000 t)	Consumption 1988 to 1990 ('000 t)	Trade Status
Sri Lanka	1.46	1.47	Net importer
Burma	81.22	81.22	Non trader
Indonesia	279.58	280.54	Net importer
Kampuchea (Cambodia)	34.25	34.25	Non trader
Laos PDR	51.4	51.4	Non trader
Malaysia	184.64	192.53	Net importer
Philippines	603.22	605.41	Net importer
Thailand	335.17	334.49	Net exporter
Vietnam, Socialist Republic	725.33	725.25	Net exporter
China, People's Republic	21,405.00	21,209.45	Net exporter
Mongolia*	5.69	5.69	Non trader
Fiji	0.55	0.56	Net importer
Papua New Guinea	26.7	28.57	Net importer
Samoa (Western)	1.39	1.44	Net importer
Solomon Is.	1.71	1.75	Net importer
Tonga	1.51	1.54	Net importer
Vanuatu	3.88	3.88	Net importer
Other South Pacific	2.27	4.72	Net importer
Ethiopia	0.93	0.93	Non trader
Kenya	5.01	5.01	Net exporter
Malawi	10.4	10.41	Net importer
Mozambique	10.8	10.88	Net importer
Tanzania	8.06	8.08	Net importer
Uganda	29.89	29.89	Non trader
Zambia	7	7	Non trader
Zimbabwe	11.39	11.18	Net exporter
Zaire	29.43	30.7	Net importer
Ivory Coast	14.03	16.2	Net importer
Ghana	12.29	12.31	Net importer
Nigeria	40.04	40.05	Net importer
Cameroon	15.61	16.94	Net importer
Angola	17.2	25.17	Net importer
Madagascar	38.32	38.32	Net exporter
Sudan	0	0	Non trader
Africa-2	36	36.46	Net importer
Africa-3	29.22	31.06	Net importer
Africa-4	18.8	21.06	Net importer
Africa-5	8.05	8.05	Non trader
Africa-6	7.9	7.97	Net importer
Africa-7	8.92	18.49	Net importer

Table B3. (Continued) The 3-year average production, consumption, and trade status of pig meat in 70 regions of the world.

Country/Region	Production 1988 to 1990 ('000 t)	Consumption 1988 to 1990 ('000 t)	Trade Status
Turkey	0.32	0.32	Net importer
Egypt, Arab Republic	2.17	3.92	Net importer
Africa-1	0.98	1.02	Net importer
Other West and North Africa	0.69	2.99	Net importer
Brazil	1,100.00	1,102.83	Net importer
Colombia	122.13	122.66	Net importer
Peru	88.51	88.52	Net importer
Venezuela	126.58	126.35	Net exporter
Bolivia	63.79	64.01	Net importer
Ecuador	64.94	65.05	Net importer
Mexico	781.74	835.02	Net importer
Argentina	205.33	201.86	Net exporter
Chile	112.17	112.17	Net importer
Paraguay	120.54	120.65	Net importer
Uruguay	14.25	14.52	Net importer
Latin-America 1	205	210.11	Net importer
Latin-America 2	24.7	60.86	Net importer
Asia-Developed	1,842.27	1,815.00	Net exporter
Australia	307.34	299.29	Net exporter
Canada	1,169.09	927.94	Net exporter
USA	7,083.90	7,411.74	Net importer
USSR	6,631.67	6,847.68	Net importer
Japan	1,575.87	1,936.17	Net importer
Developed 1–2	8,362.70	8,761.09	Net importer
Developed 3–4	13,646.02	12,428.08	Net exporter
World total	68125.05	68125.05	

Source: FAO (1994).

Table B4. The 3-year average production, consumption, and trade status of poultry meat in 70 regions of the world

Country/Region	Production 1988 to 1990 ('000 t)	Consumption 1988 to 1990 ('000 t)	Trade Status
Bangladesh	74.1	74.1	Non trader
Bhutan	0	0	Non trader
India	282.6	282.6	Net exporter
Nepal	7.3	7.3	Non trader
Pakistan	162.1	162.1	Non trader
Sri Lanka	12	12.1	Net importer
Burma	96.7	96.7	Non trader
Indonesia	450.1	450.2	Net importer

Table B4. (Continued) The 3-year average production, consumption, and trade status of poultry meat in 70 regions of the world

Country/Region	Production 1988 to 1990 ('000 t)	Consumption 1988 to 1990 ('000 t)	Trade Status
Kampuchea (Cambodia)	23.2	23.2	Non trader
Laos PDR	23.4	23.4	Non trader
Malaysia	333.7	335.6	Net importer
Philippines	261.3	261.5	Net importer
Thailand	622	503.8	Net exporter
Vietnam, Socialist Republic	160.5	160.5	Non trader
China, People's Republic	2,823.20	2,834.70	Net importer
Mongolia*	0.4	0.4	Non trader
Fiji	5	4.9	Net exporter
Papua New Guinea	4.3	6.3	Net importer
Samoa (Western)	0.4	2.3	Net importer
Solomon Is.	0.2	0.2	Net importer
Tonga	0.2	1.1	Net importer
Vanuatu	0.4	0.5	Net importer
Other South Pacific	1.4	11.5	Net importer
Ethiopia	75.6	75.7	Net importer
Kenya	43.5	43.5	Net exporter
Malawi	9.2	9.2	Net importer
Mozambique	20.6	21.2	Net importer
Tanzania	23	23	Non trader
Uganda	25.7	25.7	Non trader
Zambia	18.1	18.1	Net exporter
Zimbabwe	9.8	10.2	Net importer
Zaire	16	38.7	Net importer
Ivory Coast	43.6	49.3	Net importer
Ghana	11	14.5	Net importer
Nigeria	237.7	237.8	Net importer
Cameroon	17.1	17.8	Net importer
Angola	7.1	20.2	Net importer
Madagascar	81.6	81.6	Net exporter
Sudan	16.9	16.9	Non trader
Africa-2	106.5	108.3	Net importer
Africa-3	66.3	77.7	Net importer
Africa-4	10.1	23	Net importer
Africa-5	4.9	4.9	Non trader
Africa-6	4.5	8.5	Net importer
Africa-7	14.7	22.9	Net importer
Turkey	259.6	258.6	Net exporter
Egypt, Arab Republic	208.7	223.5	Net importer
Africa-1	299.6	300.1	Net importer

Table B4. (Continued) The 3-year average production, consumption, and trade status of poultry meat in 70 regions of the world

Country/Region	Production 1988 to 1990 ('000 t)	Consumption 1988 to 1990 ('000 t)	Trade Status
Other West and North Africa	720.2	775.1	Net importer
Brazil	2,187.50	1,922.20	Net exporter
Colombia	241.6	242.5	Net importer
Peru	249	249.2	Net importer
Venezuela	360.5	349.4	Net exporter
Bolivia	27.3	27.3	Net importer
Ecuador	61.4	61.4	Net importer
Mexico	705.8	767.9	Net importer
Argentina	371.6	371.5	Net exporter
Chile	115.6	110.9	Net exporter
Paraguay	28.2	28.2	Non trader
Uruguay	27.1	24.1	Net exporter
Latin-America 1	349.9	386.8	Net importer
Latin-America 2	107.1	192.2	Net importer
Asia-Developed	1,166.60	1,577.50	Net importer
Australia	396.6	393.9	Net exporter
Canada	716	753.6	Net importer
USA	10,182.80	9,692.10	Net exporter
USSR	3,271.70	3,463.20	Net importer
Japan	1,429.00	1,720.50	Net importer
Developed 1–2	4,270.70	4,188.40	Net exporter
Developed 3–4	4,340.40	3,945.50	Net exporter
World total	38302.5	38302.5	

Source: FAO (1994).

Table B5. The 3-year average production, consumption, and trade status of hen eggs in 70 regions of the world

Country/Region	Production 1988 to 1990 ('000 t)	Consumption 1988 to 1990 ('000 t)	Trade status
Bangladesh	75.2	75.2	Non trader
Bhutan	0	0	Non trader
India	1131	1130.5	Net exporter
Nepal	15.4	15.9	Non trader
Pakistan	208.1	208.1	Non trader
Sri Lanka	45.1	45.1	Net importer
Burma	47.2	47.2	Non trader
Indonesia	487	486.9	Net importer
Kampuchea (Cambodia)	14.7	14.7	Non trader
Laos PDR	31.7	31.7	Non trader
Malaysia	190.5	171.9	Net importer

Table B5. (Continued) The 3-year average production, consumption, and trade status of hen eggs in 70 regions of the world

Country/Region	Production 1988 to 1990 ('000 t)	Consumption 1988 to 1990 ('000 t)	Trade status
Philippines	321.7	322.2	Net importer
Thailand	247.7	240.7	Net exporter
Vietnam, Socialist Republic	162.5	159.1	Non trader
China, People's Republic	7366.3	7324.4	Net importer
Mongolia*	1.8	1.8	Non trader
Fiji	2.3	2.5	Net exporter
Papua New Guinea	2.9	2.9	Net importer
Samoa (Western)	0.2	0.2	Net importer
Solomon Is.	0.3	0.3	Net importer
Tonga	0.3	0.3	Net importer
Vanuatu	0.3	0.3	Net importer
Other South Pacific	3	3.1	Net importer
Ethiopia	78.7	78.7	Net importer
Kenya	40.3	40.3	Net exporter
Malawi	11.1	11.1	Net importer
Mozambique	12.8	12.8	Net importer
Tanzania	37.2	37.2	Non trader
Uganda	13.3	13.3	Non trader
Zambia	33.2	33.1	Net exporter
Zimbabwe	12.8	12.7	Net importer
Zaire	7.9	7.9	Net importer
Ivory Coast	15.2	15.2	Net importer
Ghana	10.9	10.9	Net importer
Nigeria	225	225	Net importer
Cameroon	11.6	12.1	Net importer
Angola	3.9	4.4	Net importer
Madagascar	21.9	21.9	Net exporter
Sudan	24.8	24.9	Non trader
Africa-2	57.6	58.2	Net importer
Africa-3	47.6	47.6	Net importer
Africa-4	3.8	4	Net importer
Africa-5	5.2	5.2	Non trader
Africa-6	2.1	3.2	Net importer
Africa-7	8.7	8.7	Net importer
Turkey	351.3	345.7	Net exporter
Egypt, Arab Republic	161.4	162.7	Net importer
Africa-1	342.7	349.5	Net importer
Other West and North Africa	505.8	522.4	Net importer
Brazil	1246.7	1244.8	Net exporter
Colombia	249.2	249	Net importer

Table B5. (Continued) The 3-year average production, consumption, and trade status of hen eggs in 70 regions of the world

Country/Region	Production 1988 to 1990 ('000 t)	Consumption 1988 to 1990 ('000 t)	Trade status
Peru	104.2	104.3	Net importer
Venezuela	131.2	130.7	Net exporter
Bolivia	31.2	31.2	Net importer
Ecuador	47.5	47.5	Net importer
Mexico	1049	1057.7	Net importer
Argentina	303	303.2	Net exporter
Chile	89.9	89.3	Net exporter
Paraguay	35.8	35.8	Non trader
Uruguay	21.7	19.4	Net exporter
Latin-America 1	320.4	323.7	Net importer
Latin-America 2	37.6	46.4	Net importer
Asia-Developed	1053.5	1189.4	Net importer
Australia	201.4	200.2	Net exporter
Canada	324.2	329.6	Net importer
USA	4036.7	3995.3	Net exporter
USSR	4713.7	4721	Net importer
Japan	2406.2	2433.4	Net importer
Developed 1–2	3223.1	3319.5	Net exporter
Developed 3–4	4325.5	4115.2	Net exporter
World total	36353.7	36353.7	

Source: FAO (1994).

Appendix C: Impact of Projects PN8806 and PN9104 on Scientific Knowledge

This appendix uses the scientific papers produced as a result of the two research projects to indicate the project's contribution to scientific knowledge. The publications resulting from the projects, either directly or peripherally, are listed below. There may be others from collaborators in participating countries of which we are not aware, particularly those in local language publications.

1988

Pitt, J.I. 1988. A laboratory guide to common *Penicillium* species. CSIRO Division of Food Processing, North Ryde, New South Wales.

Pitt, J.I. and Klich, M.A. 1988. A laboratory guide to Aspergillus species and their teleomorphs. CSIRO Division of Food Processing, North Ryde, New South Wales.

1990

Hocking, A.D. 1990. Responses of fungi to modified atmospheres. In: Champ, B.R, Highley, E. and Banks, H.J., ed., Fumigation and Controlled Atmosphere Storage of Grain. ACIAR Proceedings 25, 70–82.

1991

Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I., ed., 1991. Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand, 23–26 April 1991. ACIAR Proceedings No. 36, 270p.

Dharmaputra, O.S., Tjitrosomo, H.S.S., Sidik, M. and Umaly, R.C. 1991. The effects of phosphine on some biological aspects of *Aspergillus flavus*. In: Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I., ed., 1991. Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand, 23–26 April 1991. ACIAR Proceedings No. 36, pp. 244–248.

Hocking, A.D and Banks, H.J. 1991. Effects of phosphine fumigation on survival and growth of storage fungi in wheat. Journal of Stored Products Research, 27, 115–120.

Hocking, A.D. and Banks, H.J. 1991. Effects of phosphine on the development of storage mycoflora in paddy rice. Proceedings of the 5th International Working Conference on Stored Products Protection in Bordeaux, France, 1991. Fleurat-Lessard, F. and Ducom, P., ed., pp. 823–831.

Hocking, A.D. 1991. Effects of fumigation and modified atmosphere storage on growth of fungi and production of mycotoxins in stored grains. In: Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I., ed., 1991. Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand, 23–26 April 1991. ACIAR Proceedings No. 36, pp. 145–156.

Hocking, A.D. 1991. Isolation and identification of xerophilic fungi in stored commodities. In: Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I., ed., 1991. Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand, 23–26 April 1991. ACIAR Proceedings No. 36, pp. 65–72.

Pitt, J.I. 1991. Advances in the taxonomy of spoilage fungi. In: Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I., ed., 1991. Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand, 23–26 April 1991. ACIAR Proceedings No. 36, pp. 32–38.

Pitt, J.I. 1991. *Penicillium* toxins. In: Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I., ed., 1991. Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand, 23–26 April 1991. ACIAR Proceedings No. 36, pp. 99–103.

Pitt, J.I. and Hocking, A.D. 1991. Significance of fungi in stored products. In: Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I., ed., 1991. Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand, 23–26 April 1991. ACIAR Proceedings No. 36, pp. 16–21.

Quitco, R. 1991. Aflatoxin studies in the Philippines. In: Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I., ed., 1991. Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand, 23–26 April 1991. ACIAR Proceedings No. 36, pp. 180–187.

Sanimtong, A. and Tanboon-Ek. P. 1991. Detection of aflatoxin B_1 by enzyme-linked immunosorbent assay in Thailand. In: Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I., ed., 1991. Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand, 23–26 April 1991. ACIAR Proceedings No. 36, 227.

Siriacha, P., Tanboon-Ek, P. and Buangsuwon, D. 1991. Aflatoxin in maize in Thailand. In: Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I., ed., 1991. Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand, 23–26 April 1991. ACIAR Proceedings No. 36, pp. 187–193.

Wheeler, K.A. Hurdmann, B.F. and Pitt, J.I. 1991. Influence of pH on the growth of some toxigenic species of *Aspergillus, Penicillium* and *Fusarium*. International Journal of Food Microbiology, 12, 141–150.

Wheeler, K.A., Miscamble, B.F., NG, M., Hocking, A.D. and Bhudhasamai, K. 1991. Survey of fungi and mycotoxins associated with maize and other commodities in Thailand. In: Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I., ed., 1991. Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand, 23–26 April 1991. ACIAR Proceedings No. 36, pp. 214–216.

Zahari, P., Bahri, S. and Maryam, R. 1991. Mycotoxin contamination of peanuts after harvest in Sukabami. West Java, Indonesia. In: Champ, B.R., Highley, E., Hocking, A.D. and Pitt, J.I., ed., 1991. Fungi and mycotoxins in stored products. Proceedings of an international conference, Bangkok, Thailand, 23–26 April 1991. ACIAR Proceedings No. 36.

1992

Wheeler, K.A. and Hocking, A.D. 1992. Interactions between xerophilic fungi associated with dried salted fish. Journal of Applied Bacteriology, 74, 164–169.

Hocking, A.D. and Banks, H.J. The use of phosphine for inhibition of fungal growth in stored grains. International Conference on Controlled Atmosphere and Fumigation in Grain Storages, Winnipeg, June, 1992.

1993

Dyer, S.K. and McCammon, S. 1993. Detection of toxigenic isolates of *Aspergillus flavus* on coconut cream agar. Journal of Applied Bacteriology, 76, 75–78.

Miller, J.D., Savard, M.E., Sibilia, A., Rapior, S., Hocking, A.D. and Pitt, J.I. 1993. Production of fumonisins and fusarins by *Fusarium moniliforme* from Southeast Asia. Mycologia, 85, 385–391.

Pitt, J.I., Hocking, A.D., Bhudhasamai, K., Miscamble, B.F., Wheeler, K.A. and Tanboon-Ek, P. 1993. The normal mycoflora of commodities from Thailand. 1. Nuts, oilseeds. International Journal of Food Microbiology, 20, 211–226.

1994

Gibson, A. M., Baranyi, J. Pitt, J. I. Eyles, M. J. and Roberts, T. A. 1994. Predicting fungal growth: the effects of water activity on *Aspergillus flavus* and related species. International Journal of Food Microbiology, 23, 419–431.

Hocking, A.D., Miscamble, B.F. and Pitt, J.I. 1994. Water relations of *Alternaria alternata*, *Cladosporium cladosporioides*, *Cladosporium sphaerospermum*, *Curvularia lunata and Curv. pallescens*. Mycological Research, 98, 91–94.

Pitt, J.I. 1994. The current role of *Aspergillus flavus* and *Penicillium* in human and animal health. Journal of Medical and Veterinary Mycology, 32, Supplement 1, 17–32.

Pitt, J.I., Hocking, A.D., Bhudhasamai, K., Miscamble, B.F., Wheeler, K.A. and Tanboon-Ek, P. 1994. The normal mycoflora of commodities from Thailand. 2. Beans, rice small grains and other commodities. In preparation for International Journal of Food Microbiology. 23, 35–53.

Hocking, A.D., Miscamble, B.F. and Pitt, J.I. Influence of solute and substrate on the water relations of some *Chaetomium* and *Fusarium* species. (in preparation)

Hocking, A.D. and Miscamble, B.F. Water relations of some Zygomycetes isolated from food. (In preparation).

1995

Pitt, J.I. and Hocking, A.D. 1995. Fungi and food spoilage. Edition 2. Chapman and Hall, London.

Pitt, J.I. and Miscamble, B.F. 1995. Water relations of *Aspergillus flavus* and closely related species. Journal of Food Protection, 58: 86–90.

Pitt, J.I., Hocking, A.D., Miscamble, B.F., Dharmaputra, O.S., Sardjono, Kuswanto, K. and Noor, Z. 1995. The normal mycoflora of commodities from Indonesia. 1. Nuts and oilseeds (in preparation).

Pitt, J.I., Hocking, A.D., Miscamble, B.F., Dharmaputra, O.S., Sardjono, Kuswanto, K. and Noor, Z. 1995. The normal mycoflora of commodities from Indonesia. 2. Other commodities (in preparation).

Pitt, J.I., Hocking, A.D., Miscamble, B.F., Quitco, R.T. and Andeles, S.C. 1995. The normal mycoflora of commodities from Philippines. (in preparation).

Pitt, J.I., Hocking, A.D., Tobin, N.F., NG, M., Tanboon-Ek, V.M., Suhardi, P. and Esteves, L. 1995. Mycotoxin contamination in commodities from Southeast Asian sources (in preparation).

The Australian Mycotoxin Newsletter

During 1989–90, agreement was reached between ACIAR and the Australian Mycotoxin Data Centre at the CSIRO Food Research Laboratory on joint publication of the Australian Mycotoxin Data Centre Newsletter. A small injection of funds from Project PN8806 enabled commencement of the publication of the *Australian Mycotoxin Newsletter* as an insert in the *ACIAR Postharvest Newsletter*. The *Australian Mycotoxin Newsletter* consists of a comprehensive abstract service for world mycotoxin literature compiled by Mrs J. C. Eyles. Editorials, news on mycotoxin meetings, and book reviews are also published. The first issue appeared in March 1990, and since then, the *Australian Mycotoxin Newsletter* has been published quarterly and distributed with the *ACIAR Postharvest Newsletter*.

Mycotoxin Contamination of Grains—a Review of Research in Indonesia

Srikandi Fardiaz*

Abstract

Mycotoxins affect the agricultural economies of many countries, interfere with trade, reduce animal production, and affect human health. The five most agriculturally-important mycotoxins are aflatoxin, ochratoxin, deoxynivalenol, zearalenone, and fumonisin. Moulds that produce aflatoxin grow more frequently in warm climates, and grains originating from tropical countries such as Indonesia are frequently contaminated with aflatoxin. Food surveys in West Java, Indonesia, showed that many market foods, particularly peanut-based products, contained aflatoxin in high concentrations. The tropical climate and traditional processing methods favour contamination of peanuts by aflatoxin-producing moulds. Research has concentrated on the effects of various processing methods, including fermentation, oil extraction, peanut butter processing, and irradiation, on the aflatoxin contents of peanuts; and the effect of fumigation on the aflatoxin contents of cereal grains and nuts. All of these processing methods reduce the aflatoxin contents significantly. However, depending on the concentration of aflatoxin in the raw material, and the processing method, the final products may still contain aflatoxin in concentrations harmful to humans.

In Asia, mycotoxins have strong negative impacts on trade, particularly on markets in the European Economic Community (EEC). It has been estimated that between 25 and 50% of all commodities, especially staple crops, are contaminated by mycotoxins. Peanut meal imports into the EEC fell by 50% between 1980 and 1990, and imports of copra by 75%, primarily because of stringent regulations for aflatoxin (Miller and Beardall 1994). At the present time more than 300 different fungal species with variable toxic effects are known (Nassif 1992). However, there are five mycotoxins of agricultural significance: aflatoxin, ochratoxin, and three *Fusarium* toxins, deoxynivalenol, zearalenone, and fumonisin (ACIAR/CSIRO Team 1994). All of these mycotoxins cause animal disease and have major effects on animal productivity. Additionally, there is widespread human exposure to deoxynivalenol, fumonisin, and aflatoxin, with consequent effects on human health.

The growth of fungi and production of toxins are dependent upon environmental factors, particularly weather conditions such as warm temperatures and high humidity. Therefore, grains in tropical and subtropical regions are particularly susceptible to fungal infestation and consequently mycotoxin contamination. Also, moulds that produce aflatoxin are more frequent in warm climates. On the other hand, *Fusarium* toxins occur in regions with a moderate climate, sometimes at temperatures approaching 0°C (Nassif 1992).

In North America and Europe, mycotoxins in feedstuffs cause losses in animal production estimated at tens of billions of dollars per year (Miller and Beardall 1994). However, the mycotoxin-management strategies used in those countries are not universally applicable to Asia, including Indonesia. The application of existing technologies to manage postharvest contamination in staple crop production, plus education and extension packages directed at commercial farmers, will help to reduce the risk of mycotoxin contamination.

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Mycotoxin Limits in Food and Feed—Differences between Countries

Regulations defining mycotoxin tolerances for international trade comprise a major limiting factor for market access for products of some countries. Limits and regulations for aflatoxins and other mycotoxins in food and feedstuffs have expanded since 1981. Current U.S. Food and Drug Administration (FDA) policy for aflatoxins in human foods is based on the assumption that, until demonstrated otherwise, any animal carcinogen should be considered a human carcinogen—and that, as an unavoidable carcinogenic contaminant, aflatoxins should be present at the lowest practical levels. The FDA regulatory limits for aflatoxins in food and feed are presented in Table 1.

Table 1. Current total aflatoxin limits established by the FDA.

Food and feed	Limit (μg/kg, ppb)
Human foods (except milk)	20
Milk	0.5
Animal feeds (except as listed below)	20
Cottonseed meal (used for mature beef, pig, and poultry rations)	300
Maize for breeding beef cattle, breeding pigs, or mature poultry	100
Maize for finishing pigs	200
Maize for feedlot beef cattle	300

Source: Park (1993).

The differences between aflatoxin tolerances in various countries are sometimes quite large. Limits for aflatoxins in foods in different countries vary from 0 to 50 μg/kg, where zero is based on the capability of the analytical method (Table 2). Five μg/kg was the most common maximum limit used by regulators for aflatoxin B_1. The most widely used level for aflatoxin M_1 in milk was 0.5 μg/kg for North and South America and the Commonwealth of Independent States (formerly the USSR) and 0.05 μg/kg for European nations. For animal feeds, the limits varied from 10 to 300 μg/kg, depending on use (Park 1993). The Ministry of Health, Indonesia, proposed the limit of 20 μg/kg for total aflatoxins in certain foods such as peanuts and peanut products, maize and maize products, spices, and cereals and cereal products.

Mycotoxin Contamination in Cereal Grains and Peanuts—Case Studies in West Java

Contamination of food and feed with mycotoxin is well documented in most countries with developed economies, where thousands of analyses are conducted per year. However, only very limited analyses have been done in Indonesia. Various mycotoxins were qualitatively detected in cereals, legumes and coffee and cocoa beans as reported by Sukardi (1983) and presented in Table 3. Aflatoxin was detected in all samples, while zearalenone, ochratoxin, trichothecene, citrinin, penicillic acid, and sterigmatocystin were detected in some samples. This result indicates that aflatoxin is the most common mycotoxin in grains.

Food surveys in West Java showed that many market foods, particularly peanut-based products, contained aflatoxins in concentrations harmful to humans (Table 4). The tropical climate and traditional processing methods favour contamination of peanuts by aflatoxin-producing moulds. Some samples of ground peanuts in the forms of peanut sweets, peanut sauce, or peanut butter, contained aflatoxins at very high concentrations, while fried and boiled peanuts had relatively low concentrations of aflatoxin. The traditional fermented food such as fermented peanut presscake ('oncom') contained aflatoxin higher than the limit (20 ppb) established by FDA or proposed by the Indonesian Ministry of Health.

Dharmaputra et al. (1989) studied the contamination of *Aspergillus flavus* and aflatoxin in peanuts collected from markets in West Java, and found that 80% of the samples with moisture content ranging from 3.6 to 11.0% contained more than 30 ppb of aflatoxin B_1. They reported that first-grade peanuts did not always contain lower counts of *A. flavus*.

A study by Sutikno et al. (1993) on aflatoxin in duck feeds reported that 98% of the samples were contaminated. In maize-based mixed feeds, maize was the primary source of aflatoxin contamination. The incidence of aflatoxin contamination in maize was high, i.e. 97% of 138 samples tested contained aflatoxin with an average of 100±94 ppb. The percentage of samples contaminated with aflatoxin at levels less than 50 ppb was 30%, between 51 and 100 ppb it was 20%, and higher than 100 ppb 40%. The predominant aflatoxin found in maize was B_1 with B_2, G_1, and G being the next highest. All of these were also found in the mixed diets. Another study on the aflatoxin contamination in maize collected from Lampung,

Sumatra, reported that 100% of the maize samples contained aflatoxin B_1, while 31% contained aflatoxin B_2, and 86% of the samples contained aflatoxin at more than 30 ppb (Dharmaputra et al. 1993).

The results of these limited surveys indicate that peanuts and maize are the commodities most susceptible to aflatoxin contamination, while other cereal grains such as rice and soybean are less susceptible. Peanuts present a special problem because the fruit, after fertilisation, penetrates into the soil, where various fungi contaminate the shell, seed, and testa. Much of the damage sustained by peanuts occurs after the crop has been dug but before it is dry. Mechanical damage in harvesting, improper drying and storage, and insect infestation are important factors. In Indonesia, there are several practices that tend to raise moisture content in crops and thus favour mould growth. These include manual harvesting of peanuts from fields flooded with water to soften the earth, storing crops that are still wet, and harvesting and storing dry crops in humid weather.

The Effects of Processing and Fumigation on the Aflatoxin Contents in Peanuts and Cereal Grains

Traditional fermentation of peanuts

The effects of traditional fermentation processes on the aflatoxin contents in peanuts were studied by Edi et al. (1990) and Fardiaz et al. (1993). Peanuts were contaminated with aflatoxin-producing mould, i.e. *Aspergillus flavus*, and incubated at 30°C for 6 days. The oil was extracted by hydraulic pressure, and the peanut presscake processed to make a fermented product called 'oncom'. To make 'black oncom', the presscake was fermented with *Rhizopus oligosporus*, and to make 'red oncom' the presscake fermentation was with *Neurospora sitophila*. Aflatoxin contents were analysed after each step of processing by HPLC. Soaking of peanut presscake in water for 24 hours reduced the aflatoxin content to 48.6%, and it was

Table 2. Frequency distribution of (proposed) tolerated amounts of aflatoxin B_1 alone or the sum of aflatoxins B_1, B_2, and G_1 in food, in various countries.

Aflatoxin	Tolerated amount (µg/kg)										
	0	5	10	15	20	25	30	35	40	45	50
B_1 (n = 30)	5/2[a]	14	1	2	2	1	2	0	0	0	1
Total (n = 35)	3/1[a]	3/2[a]	8	5	8	0	3	1	0	0	1

Source: Park (1993).
[a]Countries had different tolerances for different products.

Table 3. Mycotoxins detected in various grains.

Grain	Mycotoxin[a]						
	a	b	c	d	e	f	g
Maize	+	+		+		+	
Rice	+		+	+			
Sorghum	+	+					
Groundnuts	+				+		
Cottonseed, soybean, coconut, and sunflower seed	+						
Peanut oil, coconut oil, and olive oil	+						
Other legumes	+		+			+	
Coffee beans	+	+					+
Cacao beans	+						

Source: Sukardi (1983).
[a]a — aflatoxin; b — zearalenone; c — ochratoxin: d — trichothecene; e — citrinin; f — penicillic acid; g — sterigmatocystin

reduced further to 42.9% after steaming at 95°C for 90 minutes. Fermentation of peanut presscake by *R. oligosporus* reduced the total aflatoxin content to 13.4%, while fermentation by *N. sitophila* reduced the total aflatoxin content to 41.1% of the original aflatoxin content in peanut presscake (Fig. 1). Aflatoxin B_1 appeared to be the most sensitive to soaking and steaming processes. The decrease in aflatoxin content during soaking and steaming might be due to leaching since aflatoxin is relatively resistant to heat.

Processing peanuts into peanut butter

Investigation was carried out by Fardiaz and Jenie (1992) on the effect of conventional peanut buffer processing on aflatoxin levels. Aflatoxin contaminated peanuts were roasted in a pan at 160°C for 20 minutes, cooled to room temperature, and sorted to remove epidermis and germs. A meat grinder was used to grind the roasted peanuts. Figure 2 shows the changes in aflatoxin content of peanuts during processing into peanut butter. The highest concentration of aflatoxin was B_1, with G_2 and B_2 being the next highest. Aflatoxin G_1 could not be detected. Roasting of peanuts reduced the total aflatoxin level to 78.9% of the original level, and sorting peanuts to remove epidermis and microorganisms also removed some aflatoxin, reducing the level to 59.5% of original. The final product still contained total aflatoxin at levels as high as 40.5% of the original. This result indicates that aflatoxin is relatively stable to roasting, even when heated to temperatures as high as 160°C for 20 minutes.

Table 4. Aflatoxin contents of some peanuts, peanut products and cereal grains collected from West Java.

Food sample	Aflatoxin content (µg/kg) range/average		Reference[a]
	B_1	G_1	
Raw peanuts	0–2000		d (n = 40)
	180	353	e (n = 20)
	927		f
Peanut sweets	0–566	0–700	a
	170	83	e (n = 5)
Fried peanuts	0–8	3–33	a
	0	0	e (n = 5)
Roasted peanuts	0–100	0–67	a
Boiled peanuts	10	0	a
Peanut sauce	66–600	34–267	a
	83	49	e (n = 5)
Peanut butter	30–134	7–135	a
Peanut oil	9	5	a
	61	82	e (n = 20)
Peanut presscake	126	174	e (n = 20)
	21–69	21–53	b (n = 3)
Fermented peanut presscake ('oncom')	67	120	e (n = 39)
Fried 'oncom'	41	83	e (n = 16)
Coconut presscake	0	0	b (n = 3)
Maize	0–69	0–11	b (n = 3)
	0–26	0–25	d (n = 20)
Rice	0–26	0–20	c n = 40
	0–25	0–20	d (n = 80)
Soybean	0	0	d (n = 14)

[a] a — Roedjito (1971); b — Rahayu and Ostroswki (1980); c — Kusbiantoro (1984); d — Muhilal 1986a; e — Muhilal 1986b; f — Purbasari and Fardiaz (1992).

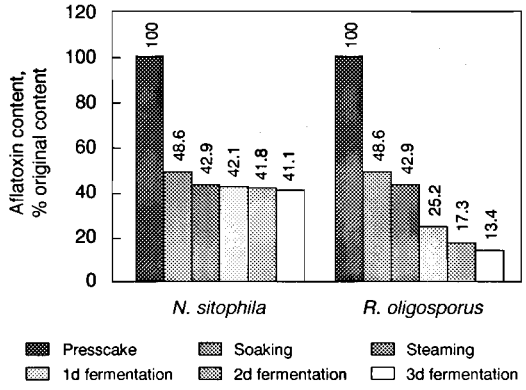

Figure 1. The effect of fermentation process on the aflatoxin content of peanuts (Edi et al. 1990; Fardiaz et al. 1993).

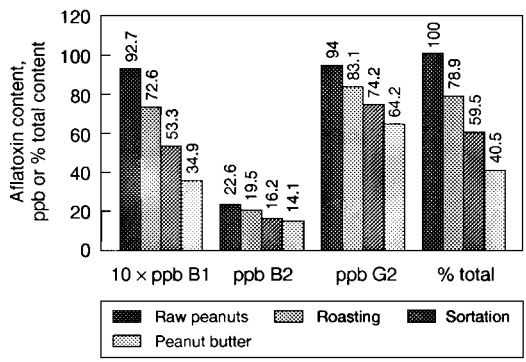

Figure 2. The effect of peanut butter processing on the aflatoxin content of peanuts (Fardiaz and Jenie 1992).

Extraction of peanut oil

The effects of different oil extraction methods on the aflatoxin content of peanut oil were studied by Fardiaz (1992). Oil was extracted from aflatoxin-contaminated peanuts by three different methods: wet rendering, hydraulic pressure, and solvent extraction. The changes in aflatoxin levels during extraction of peanut oil are presented in Figure 3. Wet rendering was the most effective method for reducing aflatoxin levels in peanut oil, while hydraulic pressure was the least effective. Heating peanuts at a minimum temperature of 60°C for several hours with hydraulic pressure and solvent extraction appeared to partially reduce aflatoxin levels, and removal of the water soluble component from peanuts markedly reduced levels. Solvent extraction effectively reduces aflatoxin levels in peanut oil. However, solvent extraction is the most expensive method to extract oil from agricultural products.

Irradiation of peanuts and nutmeg

The effect of irradiation on the aflatoxin content of peanuts was studied by Fardiaz and Irawati (1994). Aflatoxin-contaminated peanuts were irradiated in plastic bags at 10, 20, and 30 kGy. Aflatoxin B_1 appeared to be the most sensitive to irradiation and aflatoxin levels were reduced by 45.3% after irradiation with a dose of 30 kGy. Aflatoxin G_1 (36.2%) was the next most susceptible with G_2 (29.4%), and aflatoxin B_2 (26.63%) exhibiting greater resistance to irradiation (Fig. 4). A dose of 10 kGy did not significantly reduce the aflatoxin content of peanuts, particularly aflatoxin G_2, and at 30 kGy the reduction in all aflatoxin contents was less than 50%. The results indicate that the dose of irradiation permitted in grains in Indonesia, which is up to 1 kGy, is not enough to destroy aflatoxin that has already contaminated the grains.

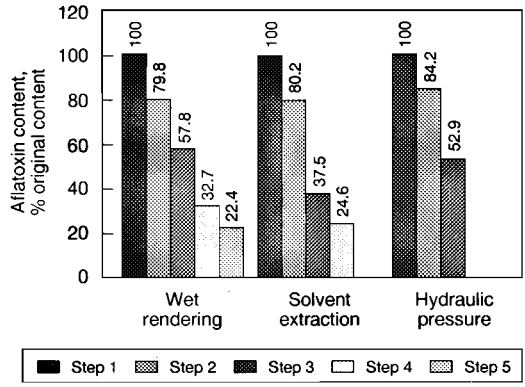

Figure 3. Effects of oil extraction methods on the aflatoxin content of peanut oil (Fardiaz 1992). Wet rendering: (1) Raw peanuts; (2) Epidermis removal; (3) Grinding, pressing, filtration, and separation of oil; (4) Heating 55–60°C, 45 min; (5) Crude oil. Solvent extraction: (1) Raw peanuts; (2) Drying 60°C, 18 hrs; (3) Extraction (hexane); (4) Crude oil. Hydraulic pressure: (1) Raw peanuts; (2) Grinding and heating 60°C, 16 hrs; (3) Crude oil.

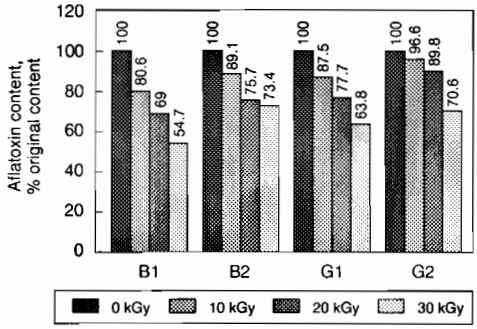

Figure 4. Effects of irradiation on the aflatoxin content in peanuts (Fardiaz and Irawati 1994).

Another study on the effect of irradiation on the growth of aflatoxin-producing mould and aflatoxin production in peanuts and nutmeg was carried out by Hilmy (1994). Growth of *Aspergillus flavus* in the samples was delayed for 1, 2, and 3 days after irradiation with 0.5, 1.0, and 1.5 kGy, respectively. Irradiation at 3 and 5 kGy completely inhibited the growth of the mould in all samples. Production of aflatoxin B_1 by *A. flavus* in peanuts was detected after 6 days of incubation, and doses of 0.5 and 1.0 kGy could not completely inhibit the production of aflatoxin B_1 by *A. flavus*, although the production decreased at higher dose of irradiation (Fig. 5). Production of aflatoxin B_1 in nutmeg was detected after 21 days of incubation, and irradiation at doses of 0.5 and 1.0 kGy significantly decreased the production of aflatoxin B_1 (Fig. 6). This result indicates that nutmeg is not as good a substrate as peanuts for production of aflatoxin by *A. flavus*.

Fumigation of cereal grains

In Indonesia, maize is the second most important crop after rice. Dharmaputra et al. (1990) studied the effect of CO_2 fumigation on storage fungi and aflatoxin production in maize. Stacks of maize were enclosed in PVC plastic sheets and treated with CO_2 for storage periods that ranged from 10 to 120 days. The concentration of CO_2 used was 2.4 kg/t. The control groups consisted of stacks of maize enclosed in plastic sheets without treatment with CO_2, and stacks that were not enclosed in plastic. Twelve species of mould were isolated from the stored maize, one of them being *A. flavus*. Fumigation with CO_2 had no significant effect on the total population of fungi and the population of most species of fungi. The total population of fungi increased significantly with the length of storage. The aflatoxin B_1 concentrations in maize enclosed in plastic sheets with and without fumigation with CO_2 were not significantly different, but were significantly lower than the control or untreated maize (Fig. 7). The aflatoxin content increased with length of storage time.

Another study by Dharmaputra et at. (1992a,b) used phosphine as a fumigant in maize storage. Phosphine is a fumigant effective for controlling insects during long-term storage of rice. Stacks of stored maize were treated with phosphine for 5 days at a concentration of 2 g/t. The control consisted of stacks that were not treated with phosphine. Eleven species of mould were isolated from the stored maize, one of them being *A. flavus*. There was no significant difference in aflatoxin B_1 content of maize in control stacks and in stacks treated with phosphine. It was concluded that aflatoxin-pro-

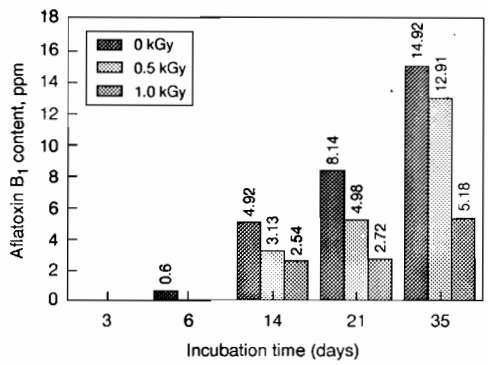

Figure 5. Effect of irradiation on the production of aflatoxin B_1 in peanuts at 97% relative humidity (Hilmy 1994).

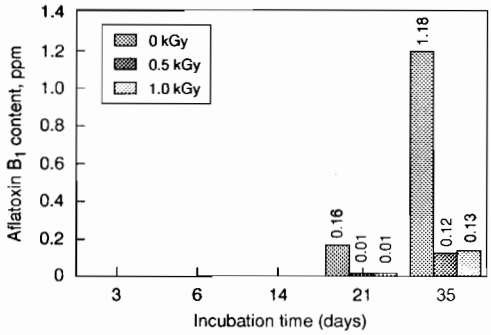

Figure 6. Effect of irradiation on the production of aflatoxin B_1 in nutmeg at 97% relative humidity (Hilmy 1994).

ducing mould was not killed by phosphine, so that aflatoxin was still produced during storage.

Dharmaputra et at. (1992a,b) also studied the effect of phosphine on the growth of fungi and aflatoxin production in soybean meal during storage. Stacks of soybean meal were fumigated with phosphine at a concentration of 2.1 g/t, and the control stacks were untreated. Fumigation was done at the beginning of the experiment and 95 days later, and samples were analysed periodically for mould and aflatoxin levels up to 190 days. Seventeen microbial species were isolated from the stored fumigated soybean meal, including *A. flavus*. Phosphine fumigation reduced the fungal population in soybean meal stacks, but the effect was not persistent. Treatment with phosphine reduced the production of aflatoxin B_1 in soybean meal, but prolonged storage of both treated and untreated soybean meal increased aflatoxin B_1 content (Fig. 8).

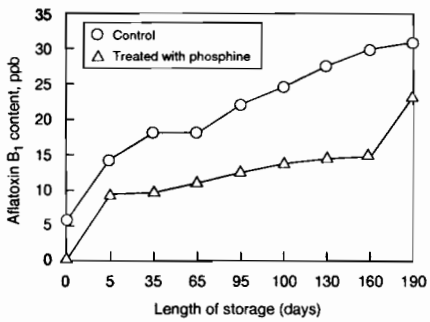

Figure 7. Effect of CO_2 fumigation on the production of aflatoxin B_1 in peanuts during storage (Dharmaputra et at. 1990).

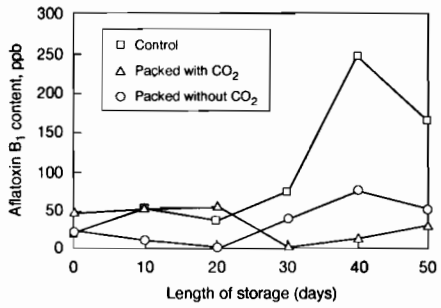

Figure 8. Effect of phosphine fumigation on aflatoxin B_1 production in soybean meal during storage (Dharmaputra et at. 1992a).

Conclusions

The differences between aflatoxin limits in foods and feeds in various countries are quite large, and in foods vary from 0 to 50 µg/kg. In Indonesia, peanuts and maize are the commodities most susceptible to aflatoxin contamination, and many peanut-based products collected from market contained aflatoxin in concentrations harmful to humans.

Various processing methods and treatment with fumigants during storage could partially, but not totally, reduce aflatoxin content in cereal grains. However, the final products, or stored products, may still contain aflatoxin at high concentrations.

Acknowledgments

The author wishes to thank Dr T. Purwadaria for her help in collecting information on aflatoxin research in Indonesia and Dr O.S. Dharmaputra for providing her publications on aflatoxin. Special thanks are due to ASEAN Food Handling Bureau for the financial assistance for presenting this paper at the 17th ASEAN Technical Seminar on Grain Postharvest Technology in Lumut, Perak, Malaysia.

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Overview of Aflatoxin Contamination of Selected Agricultural Commodities in Malaysia

A. Mat Isa and H. Abidin*

Abstract

The extent of total aflatoxin contaminations of selected agriculture commodities, as shown by screening studies begun in 1981, is discussed. Commodities screened were: raw, shelled peanuts and peanut products; black and white pepper; spices; dried cocoa beans; copra; and paddy and milled rice. Livestock produce, namely fresh milk, eggs and chicken liver, were also analysed.

Results of recent screening studies (1992–1995) on raw, shelled peanuts have generally shown a slightly lower level of contamination than those reported earlier (1981–1985). The 'Mengelembu' type, processed peanut was found to be free of aflatoxin contamination, whereas peanut butter and satay sauce were contaminated.

Pepper, spices, dried cocoa beans, and copra were found to be contaminated to some extent whereas paddy and rice were relatively free from aflatoxin contamination—even though *A. flavus* was isolated from some samples. One sample of fresh milk was found to be contaminated with aflatoxin M_1 at 0.24 ppb and some eggs and liver samples were also positive. Although there has been some screening since 1981, more extensive screening is needed to give a complete picture of the aflatoxin contamination problem.

ALTHOUGH Malaysia is becoming a manufacturing economy, like other ASEAN countries it is still dependent on agriculture for some economic activity. Malaysian commodities of economic importance are oil palm, rubber, rice, cocoa, and timber. Other commodities play a minor role but are important as industrial crops and also for the socioeconomic well-being of the people. These crops include coconut, pepper, tobacco, pineapple, coffee, tea, fruits, flowers, vegetables, legumes, and tubers.

Malaysia has a tropical climate with high temperatures around 28–31°C and heavy rainfall throughout most of the year. The average relative humidity ranges from 70–80% during the wet season and 50–60% during the dry season. Commodities stored under these conditions easily deteriorate and become very susceptible to contamination by moulds including mycotoxin-producing strains and species. As well as during storage, commodities can become contaminated by mycotoxin at any one of various points in the food chain from production through harvesting, storage, distribution, processing, and consumption.

The occurrence of aflatoxins

Aflatoxins are a class of mycotoxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* which grow on improperly stored foods and feedstuffs under high moisture and temperature conditions. Aflatoxins are among the most potent carcinogens known today (Anon. 1984a). They were initially discovered as contaminants of the peanut meal component of duck and turkey feed that caused a rapidly fatal liver destruction. Aflatoxins were subsequently shown to induce liver cancers in rats. In sub-Saharan Africa, where moulds contaminate foods, liver cancer is frequent. And in Japan, where moulds are frequently used for fermentation, the rate of stomach cancer is high. An impressive reminder of the hazard of aflatoxin to human health is an episode in India in 1974 in which there was acute poisoning of 400 people. The aflatoxins were in maize at concentrations ranging from 0.25–15.6 ppm and caused 106 deaths (Van Rensburg 1977).

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At least 18 types of aflatoxins have been reported, although only 13 of these are considered to occur naturally (Diener 1981). Of these, five are considered to be the major aflatoxins: B_1, B_2, G_1, G_2, and M_1. Aflatoxin B_1 occurs most frequently and in the largest quantities. It is also the most toxic of all aflatoxins. Aflatoxin M_1 is a derivative of aflatoxin B_1 and is found only in milk, egg, faeces, etc. of animals that have ingested aflatoxin B_1-contaminated feeds (Anon 1984a).

Conditions for aflatoxin production

The lower moisture limit for growth of fungi and aflatoxin production on a natural substrate is a water activity (a_w) of 0.85. This a_w level corresponds to a moisture content (m.c.) of 9–10% for nuts and oil cakes, and 16.5–18.5% for various cereals. The optimum temperature range for aflatoxin production is 25–30°C. Aflatoxin can be produced very rapidly within 48 hours of mould infestation and large quantities of aflatoxin produced within 7–10 days of infestation (Anon 1984a). The growth of *A. flavus* on a commodity does not necessarily imply that aflatoxin will be present. Also, it is possible to detect aflatoxin in a product when the mould is no longer growing in it.

Aflatoxin Problems in Selected Commodities

A report by Lim (1964) on an outbreak of a disease in 1960 on two pig farms in Melaka was probably the first indication of an aflatoxin problem in Malaysia. The disease caused gross liver damage and was considered to be associated with the introduction of diet containing imported peanut meal. Later, Lim and Yeap (1966) reported the detection of aflatoxins in various feed ingredients imported into the country, including several types of oil cakes and meals.

Surveying for aflatoxins in food and agricultural produce was first begun by IMR in 1965. Samples of groundnut and groundnut oil for cooking were examined (Chong and Beng 1965). Since then various studies and reviews have been published (Mat Isa and Tee 1984; Tee and Siti Mizura 1984). Since 1981, most surveys for aflatoxins in foods and agricultural produce have been undertaken by the Food Technology Research Centre of MARDI.

In this paper the extent of aflatoxin contamination of several commodities and some of their products, as detected by screening studies carried out since 1981, is discussed. The commodities screened have been groundnut and groundnut-based products, black and white pepper, spices, dried cocoa beans, copra, paddy and milled rice (Mat Isa and Abidin 1990), fresh milk, eggs, and chicken liver (Abidin and Mat Isa 1992).

Peanuts and peanut-based products

It is generally accepted that peanuts and maize are the commodities most susceptible to aflatoxin contamination. In Malaysia, raw shelled peanuts are available in almost all retailed outlets throughout the country. It is widely used as an ingredient or as a base material in a variety of popular foods and dishes.

In screening studies carried out from 1981–1984 (Mat Isa and Tee 1984), afltoxins were found in about 59% of raw shelled peanut samples. However, all Mengelembu-type peanuts were found to be free from aflatoxin contamination. This may be because of the stringent method of processing and drying of Mengelembu-type peanuts. Peanuts intended for processing into Mengelembu type are usually processed within 48 hours after harvest. About 80% of peanut butter samples were found to be contaminated by aflatoxins. Local peanut butter contained higher levels of aflatoxins than imported peanut butter. The two popular peanut products, satay sauce and rempeyek, were also found to contain higher levels of aflatoxin.

In another screening study (Anon. 1985), a total of 96 samples of raw shelled groundnut was randomly collected from retail outlets in most major towns throughout most of Peninsular Malaysia and analysed for total aflatoxin. The range of moisture content was from 5–11% with the majority of samples having a moisture content of around 7–8%. For safe, long-term storage of raw, shelled groundnuts, the moisture content should be below 7.5%. Of the 96 samples analysed, 88.5% were positive for aflatoxins. Samples that displayed visual evidence of mouldiness generally contained high levels of aflatoxin.

In a more recent screening study carried out in 1992–1995, a total of 403 samples of raw shelled groundnut was randomly collected from retail shops in all major towns in the states of Selangor (227 samples), Negeri Sembilan (112 samples), and Melaka (64 samples). The modified minicolumn method (Anon. 1992) was used to analyse the samples (Table 1) for aflatoxin. Samples from

Selangor showed much lower levels of contamination than those from Negeri Sembilan and Melaka. Only 5.8% of samples from Selangor were unsuitable for human consumption because of levels of aflatoxin above 40 ppb. Most of these came from Hulu Langat district (Abidin and Mat Isa 1994). In general, results of these recent screening studies showed slightly lower levels of aflatoxin contamination in raw shelled peanuts than those reported earlier (Mat Isa and Tee 1984; Anon. 1985).

Black and white pepper

Pepper, both black and white, is a major export commodity, especially for Sarawak. A screening study was carried out in 1984 and 1985 (Anon. 1985; Mat Isa and Nazarifah 1986) on 51 samples of black pepper and 16 samples of white pepper received from the Pepper Marketing Board (PMB), Kuching, Sarawak. The moisture content range was from 7–16% with the majority of samples having a moisture content of around 9–11%. However, all samples were positive for aflatoxins. Aflatoxin contamination in pepper is thought to result from traditional processing and storage methods. However, pepper intended for export has been reprocessed (including recleaning) to reduce the extent of contamination and microbial loads.

Spices

A total of 155 samples from 19 different types of commonly-used home spices was analysed for aflatoxin contamination (Anon. 1987). The samples included dry as well as wet spices. The moisture content of dry spices is in the range of 3.5% for 'rempah kurma' to 13.1% for dried chillies, with the majority of samples in the range of 7–11%. Wet samples, such as 'cili boh', may have moisture levels as high as 72%. All samples were found to contain aflatoxins. This is probably because of storage conditions at retail outlets where these products are generally kept for long periods in open containers and subject to environmental changes, and possible contamination.

Dried cocoa beans

In a screening study of dried beans from smallholders, FAMA collecting and grading centres, and estates, in the states of Selangor and Perak, 36 representative samples were collected and analysed (Anon. 1986). Moisture content was found to be in the range 5.3–13.8% with half of the samples 11–13.8% m.c. About 31% of the samples were found to be contaminated by aflatoxins. Samples with the highest levels of aflatoxin had moisture contents in the higher range of 11–13.8%. Therefore, keeping dried beans below 7% m.c. as recommended is very important for preventing contamination by moulds and thus aflatoxin.

Copra

A total of 36 copra samples from smallholders, FAMA collecting centres, and private copra processors in the states of Selangor and Perak was collected and analysed in 1981 and 1984 (Anon. 1981, 1984b). About 69% of the samples were found to contain an appreciable amount of aflatoxin. Traditional methods of drying and storing of copra make it very susceptible to mould and aflatoxin contamination. However, contamination of aflatoxin in copra does not have a direct impact on human health because almost all of the toxins remain in the copra cake after oil

Table 1. Total aflatoxin content in raw shelled peanuts in the states of Selangor, Negeri Sembilan, and Melaka.

			Total aflatoxin levels (ppb)					
States	No.	+ve	5–10	10–20	20–40	40–80	80–160	>160
Selangor	227	54	26	8	7	5	4	4
	%	23.8	11.5	3.5	3.1	2.2	1.8	1.8
NegeriSembilail	112	65	28	14	9	14		
	%	58.0	25.0	12.5	8.0	12.5		
Melaka	64	43	14	12	6	9	2	
	%	67.2	21.9	18.8	9.4	14.1	3.1	

extraction. Studies on peanut oil also confirmed that refinement of crude oil removed the toxins and rendered the oil harmless.

Paddy and rice

In a screening study carried out in 1981 and 1982 (Anon. 1982), only 6 of 77 samples of stored paddy analysed were positive for aflatoxins. All 22 samples of stored rice of various grades from four LPN complexes in the MUDA area were found to be free from aflatoxin contamination. Only one of seven samples of rice flour analysed showed aflatoxin contamination at a level lower than 4 μg/kg. Rice and rice products are considered low risk commodities even though *A. flavus* was isolated from some of the negative aflatoxin samples.

Milk, eggs, and chicken liver

In screening studies during 1990 and 1991 (Abidin and Mat Isa 1992), 59 samples of locally produced fresh milk, 50 samples of eggs, and 24 samples of liver were collected from main wet markets in the major towns of Selangor, Negeri Sembilan, and Melaka, and analysed for aflatoxin M_1. Only one sample (1.7%) of fresh milk from Selangor was contaminated with aflatoxin M_1 at a level 0.24 ppb. A total of ten samples (20%) of eggs was found to have aflatoxins with levels ranging from 0.16–0.41 ppb. However, only three samples (12.5%) of liver were found to contain aflatoxin at levels between 0.17–0.67 ppb.

Existing Preventive Measures and Regulatory Control

There are no regulations governing mycotoxin contamination of stored commodities in Malaysia. However, it is generally accepted that commodities should be dried to a safe moisture content of about 13% for cereals and less than 8% for commodities containing high fats and oils, and that the relative humidity of the storage site should be below 60%. Malaysia's tropical climate, with a high rainfall and temperature throughout most of the year, means that ideal conditions for storage are difficult to achieve.

There are standards for some stored commodities. For example, the Standard Malaysian Cocoa (SMC) grade one (A, B, and C) specifies that mouldy beans should be less than or equal to 3%, the SMC grade two (A, B, and C) should be less than or equal to 4%, and the substandard may contain more than 4% mouldy beans. However, it is very difficult to enforce such control at the retail level, especially for a commodity like raw shelled peanuts.

Since the aflatoxin problem was not recognised until the 1960s, it was not covered by the *Sale of Food and Drug Ordinance, 1952*—which governed the food regulations in Malaysia until 1985. However, regulations governing aflatoxins in foods in other countries such as the U.K. and the USA were used as a reference. With the introduction and implementation of the *Food Regulations, 1985*, through the *Food Act 1983*, the permissible level of aflatoxin in foods is now specified. Table II of the Fifteenth Schedule (Regulation 39) states that the maximum permitted proportions of mycological contaminant (aflatoxin or any other mycotoxin) is 35 μg/kg. This regulation stipulated that there shall be no importation, preparation, advertisement for sale, or selling, of any food that contains mycological contaminants in a proportion greater than that specified in the table. This regulation has been enforced since then.

Conclusion

Of the commodities discussed, raw shelled peanuts and their products, except for 'Mengelembu' type peanuts, are the ones most susceptible to mould growth and subsequent aflatoxin contamination. Recent screening studies have generally shown a slightly improved percentage of contaminated samples over earlier ones. Since mould infection is considered a storage problem, a study should be made of methods for improved storage of peanuts, especially raw shelled groundnuts, in homes and shops.

Since pepper, both black and white, is an export commodity, further study on processing methods and drying to reduce or possibly eliminate moulds and aflatoxin contamination is warranted. Spices comprise another group of commodities for which a study of improved storage methods is urgently needed. Since cocoa is becoming more important as an export commodity, it also merits further studies on better methods of drying and storage, and possibly the effect of further processing on aflatoxin levels. Although copra seems to be highly contaminated, fortunately contaminants remain in the press cake and are not carried into the oil. Stored paddy and rice, Malaysia's staple food, were found to be safe, although some samples showed

positive results. Some samples of livestock products such as fresh milk, eggs, and chicken liver were positive for aflatoxins but only at a relatively low level of contamination.

From the scant literature on aflatoxins in Malaysia, it is clear that much more work needs to done on this very important subject. The screening work carried out so far has not provided a comprehensive and accurate picture of the mycotoxin contamination problem in Malaysia. There needs to be a greater awareness of the need for control measures. Thus there is a need for a more extensive and systematic screening program of susceptible commodities, local and imported, to understand and evaluate the extent of the mycotoxin problem. Efforts should be made to identify the stages of contamination by the toxin so that the problem can be effectively tackled and prevented. The need to prevent and control mycotoxin contamination should be continually emphasised at all levels, from production or import point right up to consumption. A consumer awareness program is also needed.

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The Challenges of Mycotoxin Research and Development Work on Foods and Feedstuffs in the Philippines

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Abstract

This paper summarises the outcomes of a national seminar–workshop focusing on means of solving fungi and mycotoxins problems in food and feedstuffs in the Philippines. Included are detailed assessments of actions needed, by sector and commodity. A framework is developed for mycotoxins research and development activities in the Philippines.

THE first research report published on mycotoxins in the Philippines was in 1964. Subsequently, mycotoxin studies were initiated by the (then) Food and Nutrition Research Center in 1967 (Dalmacio 1993). Since then, the agriculture, trade, and health sectors of the government have undertaken various studies on mycotoxins. The private sector has also been responsible for some mycotoxin research, but there is no accurate estimate of this research. It was not until early 1993 that the first assembly of scientists, researchers, planners, policymakers, and other interest groups in the private and public sectors, was convened by the government. The aim of the assembly was to develop an integrated program to control fungi and mycotoxins in foods and fodder. The assembly was convened as an offshoot of the International Conference on Fungi and Mycotoxin in Stored Products held in April 1991 in Thailand which recommended the design and implementation of a strategy and action plan on fungi and mycotoxin research and development work.

The First National Seminar–Workshop for an Integrated Control of Fungi and Mycotoxins in Philippines Foods and Feedstuff, held in 1993 examined: research and development (R&D) activities; research utilisation and problems; and constraints and gaps in the control of fungi and mycotoxins in food and fodder in the Philippines. The aim was to develop a national strategy and action plan for four commodity groups: grains, coconut, other foods, and feeds. After a comprehensive situation analysis, the workshop formulated the vision, mission, and objectives for each of the commodity groups, identified the components of the plan, and recommended appropriate mechanisms for implementing the plan. The summary of assessment and plan of action, and commodity group reports, are given in Annexes A and B, respectively. The target areas identified for implementing a plan of action for the control of fungi and mycotoxins are the R&D community, the public sector, and the various stakeholders in the food and fodder industries.

The R&D community is faced with problems of lack of a database and lack of coordination among the agencies concerned. There remains a research gap that needs to be narrowed using the limited resources for R&D available. The wealth of information available in the country needs to be translated into useable forms by way of policies and regulations. This information needs to be transferred not only to policymakers but also to important users such as farmers, traders, processors, and consumers.

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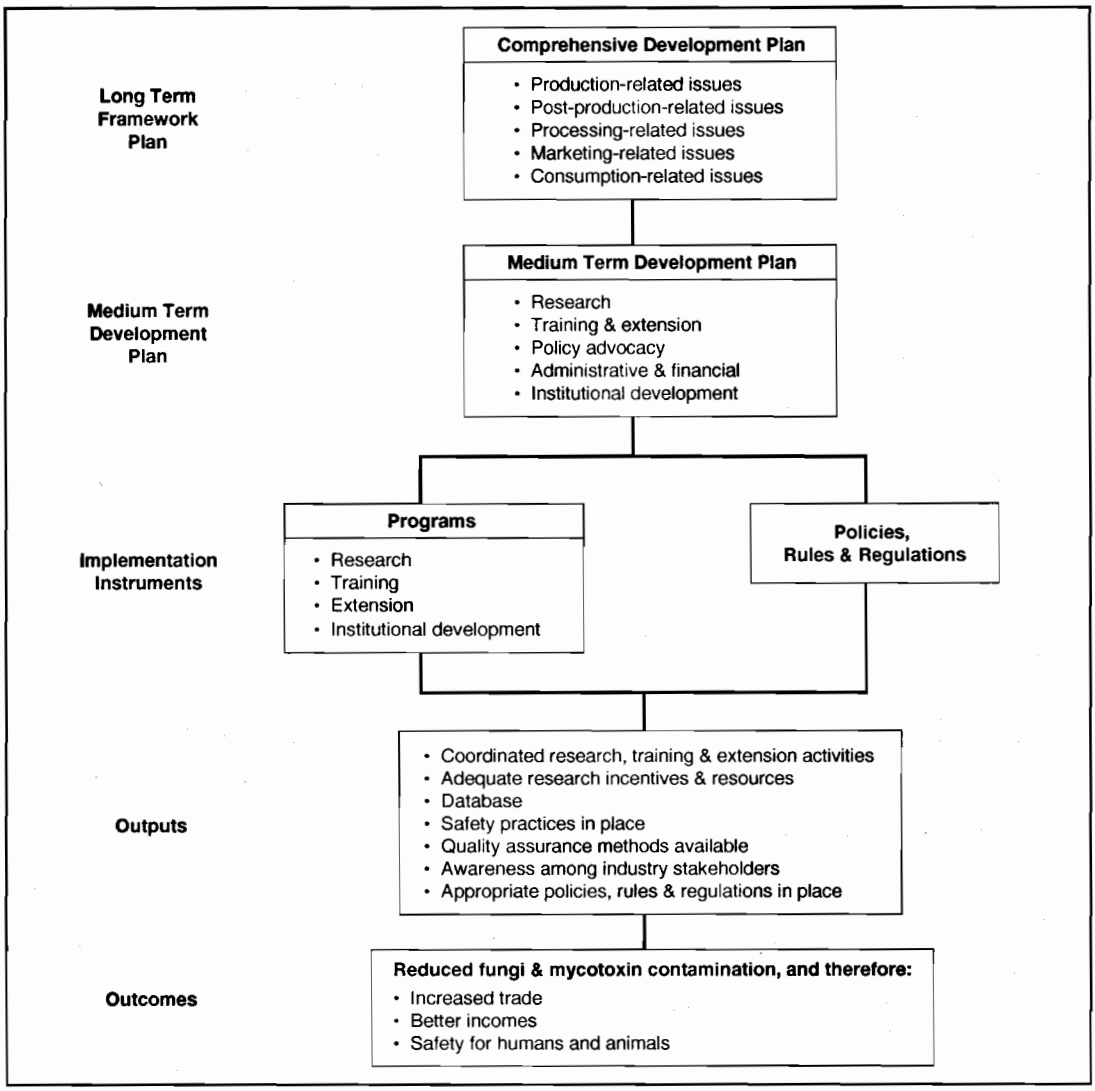

Figure 1. Suggested framework for fungi and mycotoxin research and development work in the Philippines.

Suggested Framework for Mycotoxin R&D Work

The challenge to the R & D community and all stakeholders in the food and fodder industries is how to achieve the desired outcome of the program based on the plan of action generated by the national seminar–workshop. Given this challenge, a framework for mycotoxin research and development work is suggested (Figure 1). This framework, which follows the planning model developed by Serote (1995), is one that could provide direction to achieve the vision or desired outcome of the proposed program.

First, there is the need to develop a long-term framework that could be documented in a comprehensive development plan covering a period of 20–30 years. This comprehensive development plan would address the production, postproduction, processing,

marketing, and trade-related issues bearing on the control of mycotoxins. The suggested time-frame is 20–30 years, since a long gestation period would be needed before the desired outcome of any R&D work could be achieved.

The second component of the suggested framework is a medium-term development plan covering a period of 6 years. Note that the reduced time-frame of 6 years takes political considerations into account, since this is the term of government in the Philippines. In addition, sectoral plans of government usually cover the same time-frame. This medium-term development plan would consist of five components: research, training and extension, policy advocacy, institutional development, and administrative and financial management. These components would, in turn, address the issues identified in the comprehensive development plan: production, postproduction, processing, marketing, and trade-related issues.

The medium-term development plan should detail the implementation instruments which could be divided into programs/projects/activities and policies/rules/regulations. The first set of implementation instruments—that is, the various programs—could cover research, training and extension, institutional development, and administrative and financial components. The second set (policies/rules/regulations) could cover the policy advocacy component. The outputs in the implementation of identified programs/projects/activities together with policies, rules, and regulations to guide the attainment of the desired outcome or vision of the overall program are expected to address the problems and constraints identified during the seminar–workshop.

Finally, the outcome of the desired outputs is the vision of the overall program; that is, a greatly reduced fungi and mycotoxin contamination, which should translate to higher incomes for farmers and other stakeholders, increased trade, and safe foods and fodder.

Model for an Integrated Mycotoxin R&D Work

While the suggested framework is meant to guide implementors towards achieving the desired outcome of the overall program, a model for integrating R&D activities becomes useful in an environment where numerous players are at work. In designing this model, there is a need to define the word *development.*

Development is used in various contexts including economic, political and social, and in almost all disciplines including agriculture, health, and trade. It has been defined in many ways depending on the discipline. In general, however, it is a process of transformation towards improvement or betterment. As in most disciplines, including agriculture, planners are of the view that to achieve development six elements are needed. These are: people, products, resources, means of production or technology, links, and organisations.

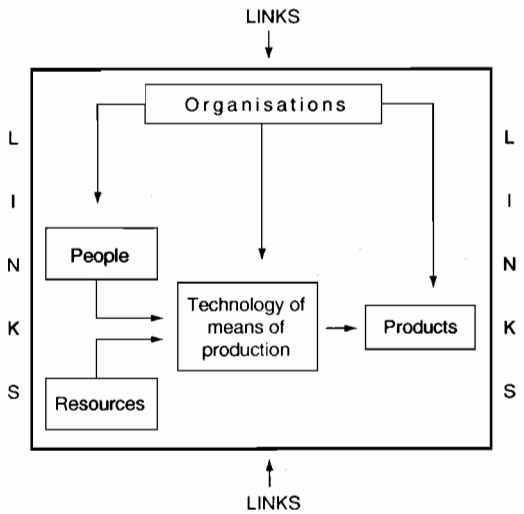

Figure 2. Suggested model for integrating mycotoxin R&D work in the Philippines.

Relationships between these elements are shown in Figure 2.

Again, the challenge to the R&D community is to orchestrate all projects and activities by identifying the specifics of the elements in the model and link these elements to ensure that the desired outcome of the overall program, that is reduced mycotoxin contamination in foods and fodder, is achieved. In the Philippines, steps are being taken towards this end. For example, the plan of action calls for an inter-agency committee to oversee in-country activities. An inventory of resources, including available laboratories, equipment and experts is being undertaken.

Much needs to be done, however, to create awareness among policymakers, industry stakeholders, and the general public—and to develop a database for use by the research community and other users. But, unless all elements of development are linked, it will be difficult to achieve the desired outcome of this development work.

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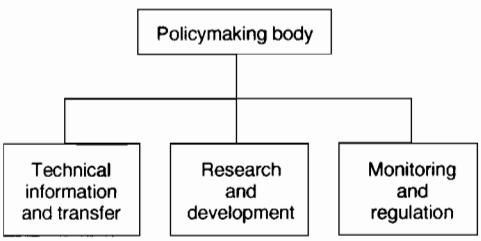
Annex A. Summary of Assessment and Plan of Action

Target	Problems/constraints	Plan of action
R&D community	Inadequate; no database/baseline information	Coordinative mechanism for R&D and technical transfer
	Unfilled research gap	Resource generation for research and incentives
	Inadequate; no R&D resources/incentives	National Testing/Monitoring Center
	Inadequate; no technology transfer	Training in quality and safety procedures and sampling
	Lack of coordination	
	Lack of quality assurance	
	Inadequate sampling methods	
	Inadequate safety practices	
Government regulatory and implementing agencies	Inadequate government practices	Coordinative mechanism for policy formulation, enforcement and surveillance
	Inadequate enforcement and control	National Testing/Monitoring Center
	Lack of coordination among agencies	Study/recommendation for improved policies (policy advocacy)
	Inadequate surveillance mechanism	Establish reporting system
	Inadequate postharvest facilities	Revitalisation of Codex Alimentarius Coordinating Committee
		Development of grading/pricing schemes
		Training in quality and safety procedures and sampling
Agriculture/ Industry/ Producers/ Processors/ Traders	Inadequate information and awareness	Training program for producers/manufacturers
	Lack of concern	Training in quality and safety procedures and sampling
		Self-monitoring
		Organisation/strengthening/sustenance of cooperatives/associations
Consumers	Inadequate information and awareness	Information campaign
		Mycotoxin Consciousness Week
		Use of mass media



Proposed structure of an inter-agency coordinating group

Annex B. Situation Analysis by Commodity Group

Constraint/problem area	Cause	Effect	Recommended solution	Strategic action plan	Proposed implementing guideline	Agencies involved
Socio-economic concerns						
1. Non-availability/ non-adoption of appropriate postharvest technologies	Financial constraints	Quality deterioration and loss to producers	Organise massive promotion, training & information dissemination of appropriate postharvest technologies	1. Linkage with media, training and extension organisations for massive promotion, training and information dissemination	Memorandum of Agreement among concerned government and private organisations, training and extension organisations	NAPHIRE and NFA
	Lack of price incentives	Undue influence of middlemen	Organise and strengthen farmers cooperative with adequate government and private sector support	2. Linkage among concerned government & private organisations & financial institutions for strengthening farmers, cooperatives	Coordination with Cooperative Development Authority & financial institutions	NAPHIRE and NFA
	Lack of effective information dissemination	Health risks to humans and animals				
2. Lack of general awareness on mycotoxin problems & appropriate control measures among producers, traders, and consumers	Ineffective/inefficient information dissemination	Health risks to humans and animals	Organise massive promotion, training & information dissemination of appropriate postharvest technologies			
3. Lack of concern from some sectors of the industry (e.g. traders)	No price incentive for quality grains	Health risks to humans and animals	Conduct effective general education campaign			
	Ineffective implementation of existing regulations					
Government policies/regulations						
1. Inconsistent implementation of government policies and regulations (e.g. monitoring/surveillance)	Budgetary constraints	Entry of mycotoxin-contaminated grains in the market	Seek appropriate executive and legislative action on the problem	1. Educational campaign the bad effects of aflatoxins	Multi-media approach	NAPHIRE and NFA
	Bureaucratic red tape	Health risks to humans and animals	Inform Food/Agriculture Committee (Congress) of the problem	2. Submission of workshop proceedings to Congress for appropriate executive and legislative action	Multi-media approach	NAPHIRE and NFA
	Ineffective enforcement of existing regulations					

Constraint/problem area	Cause	Effect	Recommended solution	Strategic action plan	Proposed implementing guideline	Agencies involved
2. Low priority for research & development	Lack of awareness among policymakers on the magnitude & severity of the problem	Slow work in research & development	Conduct information campaign for policymakers	Review existing policies on mycotoxins; conduct of seminars on mycotoxins for policymakers		
			Provide incentive & training for workers			
3. Poor coordination among agencies	Absence of network among concerned agencies	Fragmented/uncoordinated and overlapping work on mycotoxins	Seek creation of a network of mycotoxins	3. Provision of materials on mycotoxins; conduct of seminars on mycotoxins for policymakers	NAPHIRE and NFA	
				4. Training of workers on occupational safety	Source funds (local and International)	
				5. Creation of a body/council/committee to coordinate different agencies working on mycotoxins		
Technological/technical concerns						
1. Lack of rapid detection methods for early surveillance	Expensive methods	Non-enforcement of existing rules and regulations	Develop cheap, rapid, simple, and practical mycotoxin detection methods	1. Research to develop cheap, rapid, and simple methods for aflatoxin/mycotoxin detection	Research proposals for funding	Joint effort with FNRI as lead agency, BAI, BPI, NPF, NAPHIRE, UPLB, BFAD
2. Lack/absence of appropriate reference laboratory for detection and analysis	Absence of network of laboratories	Variability of results among laboratories	Seek creation of inter-laboratory testing centre	2. Creation of interlab testing centres — Identify laboratories — Establish a common method — Develop research materials		FNRI
3. Difficulty in carrying out sampling procedures	Lack of awareness/appreciation of critical importance of sampling	Non-representative sample which may lead to unreliable results	Reorient/train technical personnel	3. Reorientation of analysis on sampling		NAPHIRE
			Develop appropriate sampling devices	R&D on sampling devices		
			Evaluate existing sampling protocols	Research and evaluation on existing sampling tecnniques, standard procedures for adapting	Research proposals for funding	NFA

Constraint/problem area	Cause	Effect	Recommended solution	Strategic action plan	Proposed implementing guideline	Agencies involved
4. Prohibitive cost of mycotoxin analysis	Expensive chemicals/aflatoxin standards Lack of aflatoxin standards	Ineffective monitoring by agencies	Secure additional funding for mycotoxin analysis from government and private agencies	4. Reduction of cost of analysis tie-up with chemical company	Reference laboratory to work on the proposed tie-up	FNRI
5. Non-adoption of proper detoxification technologies	Lack of awareness of proper technologies High cost of detoxification methods	Health risks to humans and animals	Provide proper information on effective and cheap detoxification technologies	5. Proper information on cheap and effective detoxification through seminars/distribution of information materials		NAPHIRE

COCONUT

Problem/constraint	Cause	Effect	Recommended solution	Strategic action plan	Implementing guideline	Resource requirement	Duration	Agencies involved	Remarks
1. Lack of sustained funding for R&D programs	Low priority given to researchers	Termination/suspension of projects	Provide maximum regular budget allocation for mycotoxin R&D	Creation of a foundation for R&D to be provided by the private sector	Memorandum of Agreement	P5M to P50M per year	Continuing	PCOPA/CORA, CHAPCOM	Requires foreign assistance initially
	Change of management administration		Provide assistance/support from private industries						
2. Lack of massive information dissemination program	Lack of funds/manpower	Ignorance of farmers on proper field practices	Organise a massive information drive	Linkage/networking with media/farmers /traders /industries/feed manufacturers for information drive	Memorandum of Agreement with media	P10M per year	1993–1988	PCA (lead agency), PIA, UCAP, BAI, DECS	Requires foreign assistance
3. Lack of police power, PCA to enforce policies/orders	Lack of legislation	No enforcement of laws and regulations	Ask for additional powers for enforcement of orders/policies	Executive order for additional powers to PCA for enforcement of orders/policies	Creation of inter-agency task force	P0.5M per year	1993 until approved	PCA (lead agency), UCAP	
					Conduct of series of meetings/follow-ups				
4. Lack of incentive/low economic returns for farmers/producers	Low yield; not well organised farmers	Farmers resorting to loan sharks	Organise a strong farmers cooperative	Conversion of SFCO into a strong farmers cooperative	Conduct of series of meetings/seminars	P5M per year	1993–1998	PCA (lead agency), CDA	
		Poor quality of copra produced							
		Cutting of coconut trees							
5. Lack of coordinated R&D programs	No coordinating body	Disorganised R&D	Seek the creation of a centralised coordinating body for mycotoxin R&D	Linkage/networking with different research institutions/academies for centralised coordination	Memorandum of Agreement with foundations/agencies	P0.25M per year	Continuing	PCA (lead agency), FNRI-NOBT, UPLB, DA, PCRDF, UCAP, PCARRD, PCIERD	

Problem/constraint	Cause	Effect	Recommended solution	Strategic action plan	Implementing guideline	Resource requirement	Duration	Agencies involved	Remarks
					Conduct of series of meetings/seminars/workshops				
6. Lack of incentives for mycotoxin researchers /analysts	Lack of recognition/funding	Sickness, death and lack of mycotoxin analysts/researchers	Move for the revision of salary/compensation/benefits for mycotoxin analysts/researchers	Creation of an association for mycotoxin researchers/analysts for benefits/reforms	Conduct of series of meetings/follow-ups		1993 until implemented	All agencies/institution/academies involved in mycotoxin R&D	

FOOD

Problem/constraint	Cause	Effect	Recommended solution
Peanuts			
Postharvest handling — drying — storage facilities	Inadequate information, knowledge of farmers and warehouse personnel	Uncontrolled contamination	Provide technical assistance (information campaign, training)
	Lack of incentives to produce aflatoxin-free products		Include aflatoxin in grading/pricing schemes
	Farmers not organised		Organise farmers
			Enforce adherence to prescribed action levels for aflatoxins by processors
Maize and other susceptible products			
Lack of baseline information on maize-based and other susceptible products (e.g. milk, liver-based, dried fish)	Low priority	Unknown health risks	Conduct surveys (including methods for validation and sampling)
	Lack of resources and facilities		Provide adequate facilities
			Conduct training courses for laboratory analysts; organise
Imported goods/products			
Lack of control on imported peanuts and other susceptible products	Inadequate government resources	Unknown contamination	Implementation/enforcement of Consumer Act of 1990

Problem/constraint	Cause	Effect	Recommended solution	Strategic action plan	Implementing guideline	Resource requirement	Duration	Agencies involved	Remarks
1. Lack of awareness of livestock raisers and feedmillers	Lack of information campaign	Low priority given to problem	Set up a national mycotoxin monitoring and testing centre for livestock	1.1 Physical inventory of all existing laboratories	Conduct of series of meetings	P0.01M (network)	5 years; to be continued by local funding	BAI, BFAR, NFA, PCA, NAPHIRE, FNRI	Requires foreign assistance
	Lack of funds								
	Lack of manpower								
2. Lack/absence of government policies	Lack of awareness/lack of statistics/no incidence survey	Inadequate programs for postharvest	Set up a database network for mycotoxins in feeds and mycotoxicosis in animals	1.2 Identification of areas to be strengthened	Memorandum of Agreement	P500M (NTC)			
	Low priority for nutrition-related diseases	Improper handling and storage procedures	Declare a National Mycotoxin Consciousness Week	1.3 Formulation of a plan for a national testing centre/laboratory network					
			Conduct information campaign						
	Lack of capability to monitor such as equipment, laboratory, and manpower		Revitalise the Codex Alimentarius Standards Committee of DA	2.1 Physical inventory of all researches and studies	Conduct of series of meetings	P5M (continuing)	2 years PCARRD	BAI, UPLB	Short term project
	Prohibitive cost of analysis		Establish a research and training centre	2.2 Identification of areas	Creation of task force(s)				
	Lack of standards			2.3 Formulation of a plan including sources of information, methods of collection and evaluation					
	Lack of rapid tests								
	Lack of test protocols								

Problem/constraint	Cause	Effect	Recommended solution	Strategic action plan	Implementing guideline	Resource requirement	Duration	Agencies involved	Remarks
3. Incompatible production/consumption centres		Higher mycotoxicosis incidence	Reports of mycotoxin-related diseases	3. Tapping of NGO and professional groups, press, media for celebration of National Mycotoxin Consciousness Week, e.g. PHILSAN, PSAS, PSPVM	Executive order	P0.05M	Annual celebration	BAI, NGOs	
3.1 Lack of transportation facilities									
3.2 Warehousing/storage									
4. Lack of willingness to invest in higher quality feeds	Lack of premium for higher quality products			4.1 Physical inventory of all existing training facilities and modules	Conduct of series of meetings Memorandum of Agreement	P0.2M	Bi-annual	BAI, ATI	
				4.2 Identification of areas to be strengthened					
				4.3 Formulation of a plan for the training modules					
				5. Revival of the DA Codex Committee	Special Order		As need arises	DA	
				6. Inclusion of the mycotoxin problems in the animal health monitoring report	Administrative Order	P0.01M	Monthly	BAI	

Source: Report of the First National Seminar–Workshop for an Integrated Control Program on Fungi and Mycotoxins in Philippines Food and Feedstuff, 23–24 February 1993.

Control of Aflatoxin Contamination in Maize in Thailand

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Abstract

Thailand maize production has increased rapidly in the past decade and aflatoxin contamination has become the major problem for export maize and maize for local consumption. Postharvest technologies for controlling aflatoxin were studied and extended to components of the maize production system. These include field drying, mechanical drying, chemical and physical treatment, storage system, and management of handling. Field drying and mechanical drying are the most effective ways to control aflatoxin contamination. Many chemicals have been tested for their ability to control and detoxify aflatoxin but none has been applied commercially. Aflatoxin detection techniques such as ELISA and minicolumn have been developed. Carbon dioxide fumigation and storing moist maize in tightly-closed, plastic-lined, jute bags have been studied and are being applied to maize produced for local consumption.

Thailand is a tropical country with a hot and humid climate most of the year. Since the national social and economical development plans were instigated about 30 years ago maize production has been growing very rapidly. Production has increased from 1 Mt/year during late 1960s to 4.7 Mt in the 1988–989 crop year (Wattanutchariya et al. 1991) and more than 70% is exported. While the production of maize has remained quite stable in recent years, the development of the animal feed industry in the past decade has resulted in an increase in domestic demand for maize. This reduced the export share to only one-fifth of total production in 1991 and the government has tried to boost maize production to 6 Mt. With this need to increase maize production but without appropriate postharvest facilities and knowledge to maintain a high quality, aflatoxin has become a major factor affecting maize both for export and domestic consumption.

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Controlling Aflatoxin

In 1985 the Thai Government established the National Committee of Mycotoxin Control in Agricultural Commodities. Several national and international research projects (Thai–UK, Japan, USA, UNDP, FAO, etc.) were established and have succeeded in developing appropriate technology for controlling aflatoxin in maize. These technologies are being implemented and evaluated on a commercial scale. Measures to control aflatoxin contamination include field drying, mechanical drying, ensiling, and manipulation of cropping patterns.

Field Drying

Field drying is the process whereby maize is left standing unharvested in the field for a period after plant maturity. Studies in Thailand (Kawasugi et al. 1988; Kawashima et al. 1990; Nagler et al. 1989) have strongly suggested that field drying from 2–4 weeks may significantly reduce aflatoxin levels in maize. Field drying reduces the moisture content (m.c.) to 18–22%. This renders subsequent mechanical drying to achieve 14% m.c. more economical.

Disadvantages of field drying are: planting of a second crop may be delayed and may not be possible in some instances; there may be an increased risk of losses from storms or other natural hazards in some areas; and the farmer may have to bear the financial loss unless they are adequately compensated for weight loss from reduced moisture content.

Mechanical Drying

The UK–Thai Aflatoxin in Maize Project has identified a set of criteria, called the UK–Thai Project (UTP) System, shown to reliably lower aflatoxin content in maize during the rainy season. With the UTP system, maize is first field-dried on the stalk for 1–2 weeks before harvesting to reduce moisture content to 18–22%. It is then shelled within 24–48 hours of harvest, and loaded into a dryer within 12 hours of shelling. Thus, within 48 hours, it is dried to 14% m.c., with no part exceeding 15%. Aflatoxin content is monitored rapidly by a special adaptation of the bright greenish-yellow fluorescence (BGYF) test. Maize dried to 14% m.c. by the UTP system can safely be stored for a minimum of 2 months with no increase in aflatoxin content. Using this system, twenty-five 3 t batches of maize were successfully processed with a mean total aflatoxin content of 2.5 ppb and a range of 0–16 ppb at drying sites in two provinces. The system is now being used commercially for about 50 000 t of maize.

Chemical Treatment

Various chemical agents have been studied as inhibitors of *Aspergillus flavus* and aflatoxin formation in Thai maize. These include ethylene oxide, sulfur dioxide, theobromine, ethyl alcohol, methyl alcohol, acetic acid, propionic acid, sodium diacetate, sodium bisulfite, ammonia, and ammonium polypropionate (Chualprasit et al. 1985; Kawashima et al. 1990; Pupipat et al. 1986; Tanboon-Ek et al. 1987; Tanboon-Ek 1989).

While chemical treatments were reported to be effective in inhibiting *A. flavus* it should be noted that only one treatment, a mixture of propionic acid and ammonium bis propionate was tested on a commercial scale (Tanbook-Ek et al. 1987). Other experiments were generally conducted at the laboratory level. Application of propionic acid and ammonium bis propionate, at a rate of 6–7 L/t maize, could prevent the incidence of aflatoxin for more than 3 months. However, discolouration of maize kernels—and odour, and corrosive characteristics of the chemical—were reported.

The costs and benefits of alleviating aflatoxin problems by chemicals in Thai maize have been studied (Arunotog 1987). The results generally indicated that the possibility and feasibility of these chemicals being used in the marketing system are still unclear. Some chemical treatments appeared to be effective only under some conditions; for example, use of ammonia gas for high moisture content maize and ammonium propionate for maize with an initially low aflatoxin content.

Carbon dioxide fumigation has been used to control aflatoxin at local markets before maize can be mechanically dried. This method is economical and practical.

A simple control measure for A. *flavus* and aflatoxin in high moisture maize has been developed (Siriacha et al. 1991). Maize of high moisture content (30-35%) was tightly packed in high density polyethylene bags (45 μm). These bags were protected by outer jute bags. *A. flavus* and aflatoxin were not detected from maize stored in the plastic bags for 1–3 months. The dominant microorganism in the plastic bags was *Lactobacillus plantarum*. The toxigenic bacteria, *Staphylococcus aureus*, *Clostridium botulinum*, and *C. perfringen*, were not detected. Nutritional value of maize stored in the plastic bags was not significantly different from freshly harvested maize and commercial feed maize. Furthermore, it was proven that the stored maize could be used as feed for broiler chickens without any deleterious effect on consumers.

Cropping Pattern

There are two main growing seasons in Thailand. About 90% of the annual crop can be planted and harvested during the rainy season. The remaining 10% is planted during the middle to end of the rainy season, and harvested at the beginning of the dry season. The new cropping pattern suggested is aimed at harvesting the maize in the dry season to avoid conditions that favour high aflatoxin levels. This should be done by planting maize in July and harvesting in November. The results of many studies (Kawashima et al. 1990; Kawasugi et al. 1988; Nagler et al. 1989; Wirawat 1984) have indicated that maize harvested in the dry season should have lower moisture contents and produce low aflatoxin maize.

Detoxification

Approaches to detoxification of aflatoxin contaminated grain and feed in Thailand have included physical, chemical, and biological treatment.

The toxicity of aflatoxin B_1 contaminated maize and peanuts was reduced by a simple treatment with safe chemicals including ammonium salts (3–5%) in 20% moisture at room temperature 100°C for 1 hour. It was found the ammonium bicarbonate or ammonium carbonate generally reduces aflatoxin in maize and peanut to levels of 82–96% and 69–80% of the total toxin, respectively.

Chokethaworn et al. (1990) studied the effectiveness of ammonium bicarbonate, sunlight, short- and long-wavelength ultraviolet light, moisture, temperature and time period of treatment for reducing biological toxicity of aflatoxin in crops such as peanut, maize, and mungbean. Samples were well mixed with solid ammonium bicarbonate with 20% moisture and kept at room temperature for three days. Ammonium gas was then evaporated from the treated samples in the open air or a sunny place for one day. It was found that ammonium bicarbonate treatment could detoxify aflatoxin B_1 in the cereals by 81–100%. Exposure of crop samples to ultraviolet light or sunlight for 5 hours reduced aflatoxin levels by 25–30%. Combining ammonium bicarbonate and sunlight or ultraviolet light irradiations enhanced the detoxification effect to about 60%. The biological toxicity of the treated cereals was tested on experimental chickens. The decrease in toxicity from aflatoxin reduction was confirmed.

It was concluded that treatment by ammonium bicarbonate, heat, and sunlight significantly detoxified aflatoxin in cereals. Since these methods are simple and economical, they should be further introduced to farmers for detoxifying aflatoxin in their agricultural products.

Some chemisorbents (inorganic sorbent materials) with the capacity to tightly bind and immobilise mycotoxins in the gastrointestinal tract of animals have been tested. Natural zeolite, HSCAS (hydrated sodium calcium aluminosilicates), and bentonite have been added to broiler feeds (Charoon-Kiatkamcharn 1993) and to feed for growing pigs (Puminn 1993). Studies demonstrated that adding these three aluminosilicate products to the feed of broiler chicks and growing pigs reduced aflatoxin.

Detection

The detection procedure is necessary to monitor aflatoxin levels and control measures. The Department of Medical Science has developed a 'plastic minicolumn' for aflatoxin analysis in foods by using beverage-clear plastic tube as a robust minicolumn. Department of Agriculture has developed a simple and rapid 'DOA 1' and 'DOA 2' minicolumn method (Kositcharoenkul et al. 1995; Kositcharoenkul 1993, 1995) for aflatoxin detection in maize and peanuts, respectively, which can be used as a local quality control test by laboratories and people in rural areas.

New techniques such as ELISA are being developed. The Department of Agriculture has produced an antibody against aflatoxin B_1 and an ELISA test kit for aflatoxin detection in agricultural commodities (Tanboon-Ek et al. 1991). Chiang Mai University has also had some success in developing an ELISA method for aflatoxin B_1 in food.

Conclusion

There is a need to develop the essential components of an aflatoxin management system for preventing, detecting, and detoxifying aflatoxins. Optimal methods for food and feed production, harvesting, storage, and processing also need to be developed. This will foster an integrated approach to the control of aflatoxin, with broad implications for agriculture and the health of animals and humans.

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Aflatoxin in Animal Feeds and Effects on Poultry Production in Vietnam

Duong Thanh Liem and Le Anh Phung*

Abstract

Studies of incidences of aflatoxicosis in poultry in Vietnam have shown that feeding with contaminated peanut cake was the most common cause. Solutions to the problem lie in pre-treating contaminated feed or substituting rice or soybean based feeds for peanut and maize based feeds.

INDUSTRIALISATION of agriculture introduces problems absent from traditional systems. One of these is mycotoxicosis in poultry. The major causes of the increase in poultry mycotoxicosis in Vietnam likely include:

- The increasing number of crops which force farmers to use varieties with a short growing time, and to harvest in the rainy season when drying of harvested crops is slow.
- High-yield grains have high moisture content and take a long time to dry.
- In large-scale production, grains are stored in larger quantities and this promotes conditions favourable to fungal growth.
- The industrial high-yield breeds of poultry are very susceptible to aflatoxins.

Observations on Aflatoxicosis in Poultry

Aflatoxin in animal feed samples

Aflatoxin levels are highest in peanut cake, with maize grain the next highest (Table 1). The levels in maize harvested during the rainy season are higher than in maize harvested during the dry period of the year. Aflatoxin levels are lower in rice, by-products of rice, and soybean and soybean by-products than in peanut cake and maize.

Table 1. Aflatoxin levels in some animal feed samples.

Feeds	n	Average (ppb)	Maximum (ppb)
Maize grain	25	205	600
Broken rice	2	22	25
Soybean grain	1	50	50
Rice bran	3	29	55
Sesame oil cake	3	8	10
Coconut oil cake	7	17	50
Soybean oil cake	4	12	50
Peanut oil cake	29	1200	5000
Dry cassava powder	1		40

Source: Food and Commodities Control Center.

Observation in aflatoxicosis in poultry

Aflaxtoxicosis was first observed at Phuoc Long duck farm in exotic ducks (Cheery Valley) fed on concentrate (Table 2). When the manager changed the feed to a traditional one based on rice, the duck health improved and production levels recovered.

The second observation was on breeding chickens, in which embryonic mortality of eggs increased up to 80%. The toxicosis was attributed to peanut cake.

The third observation was on exotic Super-meat ducks fed on concentrate containing contaminated maize. Feed containing aflatoxin killed more than 20 000 ducklings.

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Table 2. Poultry production losses attributed to aflatoxicosis.

Year	Farm	Loss
1983	Phuoc Long duck farm	2000 ducks
1991	Binh An chicken farm	50 000 eggs
1992	Duck-meat farm	20 000 ducklings

Studies on prevention of aflatoxicosis

Avoiding feed likely to be contaminated

Results of an on-farm study of the effects of substituting soybean cake for peanut cake as feed for laying hens are shown in Tables 3 and 4.

The substitution of soybean for peanut cake improved egg quality as indicated by hatchability ratio. However, there was a lag of 1–2 months before the improvement.

It has been concluded that farmers should not substitute maize and peanut feeds for the more traditional rice-based feeds.

There were two observations of aflatoxicosis in Cheery Valley duck fed on concentrate. The first occurrence was in 1991 (2000 ducks lost) and the second in 1992 (20 000 ducklings lost). The farmers affected have reverted to using rice-based feed rather than maize and peanut products.

Pre-treatment of poultry feeds to eliminate aflatoxins

In an on-farm study of Super-meat duck it was shown that pretreatment of feeds could improve the health and food-conversion of ducks (Tables 5 and 6).

Hatchability of chicken eggs was also improved by feeding hens on feed pre-treated with ammonia (Table 6).

Table 3. Effects on egg hatchability of substituting soybean cake for peanut cake.

	Incubated eggs	Embryonic egg ratio (%)	Lethal embryonic ratio (%)	Hatching ratio (%)
Peanut cake	3591	91.1	84.5	5.9
Soybean cake	3391	95.2	34.7	56.4

Source: Duong Thanh Liem et al. 1993.

Table 4. Effects of the substitution of soybean cake for peanut cake on egg incubation in a breeding farm.

Periods	Number of eggs	Hatching ratio (%)
Initial period of peanut feeding (3 months)	100 000	63.6
Final period of peanut feeding (3 months)	39 135	16.0
Initial period of soybean substitution (2 months)	18 119	30.6
Final period of soybean substitution (3 months)	48 669	80.4

Source: Duong Thanh Liem et al. 1993

Table 5. Effect of pre-treatment of peanut cake on Super-meat duck.

	Diet[a]		
	Control	Lot 1	Lot 2
Live weigh of 8-week-old ducks (g)	2292	2671	2904
Feed conversion	3.5	3.1	2.8
Lethal ratio	36.7	0	0

[a] Control: Diet with 15% peanut cake (2000 ppb aflatoxin); Lot 1: Diet with peanut cake pre-treated with ammonia; Lot 2 : Diet with soybean.

Table 6. Effect of pretreatment of peanut cake with ammonia on hatchability of chicken eggs.

	Diet[a]		
Ratio (%)	Control	Lot 1	Lot 2
Embryonic egg ratio	76.9	86.2	89.2
Hatching ration	69.2	73.8	83.1

[a] Control: Diet with 15% peanut cake; Lot 1: Diet with 15% peanut cake pre-treated with ammonia; Lot 2: Diet with 15% soybean cake.

Conclusions

- Since poultry are very susceptible to aflatoxicosis, it is important to use feeds with low levels of aflatoxin such as rice and soybean, rather than feeds such as peanut and maize that are more likely to be contaminated.
- Contaminated poultry feeds can be used if they are pre-treated for aflatoxins.
- Farmers need to improve postharvest handling facilities to prevent crop products becoming contaminated.

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