***Aspergillus flavus* and aflatoxin contaminations of maize ears and kernels in Thailand**

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Aspergillus flavus およびアフラトキシン汚染

Summary

The population of *Aspergillus flavus* and the amounts of aflatoxins in maize ears and kernels collected from 4 areas in Thailand in 1987 were studied. Eleven maize ear samples from the randomly selected fields showed no infection of *A. flavus* and very low amount (9 ppb) of aflatoxin B₁ was found in only one sample. Similarly, the infection of *A. flavus* in 6 maize ear samples collected from farmers' and middlemen's storages was very low (0-3% of kernels from the ear); two samples of them were positive for aflatoxins (aflatoxin B₁ 9 and 58 ppb). However, all 15 maize kernel samples collected from the middlemen's storages were found to be highly contaminated with *A. flavus* and aflatoxins. There were clearly less contaminations of *A. flavus* and aflatoxins in the maize ear than those in the kernels.

Aflatoxin contamination of maize at the pre-harvest period of its growth has been reported in the Southern States of the U.S.A., where it is generally associated with drought stress during the growing period^{1,2)}. Recent reports in Thailand³⁻⁷⁾ and Indonesia^{8,9)}, however, strongly indicate that the pre-harvest contamination is not a serious problem in these countries. Surveys of *Aspergillus flavus* and aflatoxin contaminations in maize were carried out to confirm that aflatoxin contamination is low at harvest in all of the main crop areas in Thailand. The objective of this study is to assure the above conclusion and to study the difference in the levels of contaminations of *A. flavus* and aflatoxins between maize ears and kernels.

Materials and Methods

1. **Samples:** In 1987, from August to October, maize samples were collected from fields, and farmers' and middlemen's storages in four maize crop areas, viz. the Central area (Lop Buri, Phetchabun, Phitsanulok, Nakon Sawan), the Northern area (Chiang Rai, Chiang

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Mai), the Eastern area (Prachinburi, Chantaburi) and the Northeastern area (Nakon Ratchasima) in Thailand. In each sample about 5 kg of kernels (shelled grain) and/or 50 maize ears were used for the survey of *A. flavus* and aflatoxin contaminations.

2. *Number of kernels infected by A. flavus*: In each sample 200 kernels were surface sterilized with 3% NaOCl for 1 min and then rinsed 4 times with sterile distilled water. They were plated in Petri dishes on PDA (Difco) amended with 30 mg of chloramphenicol and 30 mg of rose bengal per L. The number of kernels from which *A. flavus* grew was then counted after 5 days incubation at room temperature (28–30°C).

3. *Population of A. flavus on surface of kernels*: Maize kernels (20 g) were randomly taken from each sample and washed with sterile water (100 ml) for 3 min. This washed-out water was diluted to 2–3 dilutions. One ml each diluent was mixed with melted PDA and solidified. The number of *A. flavus* colonies was counted after incubation under the same condition as mentioned above.

4. *Analysis of aflatoxins*: Five kg of maize sample was ground and divided sequentially by a sample splitter until around 500 g portions were obtained for the analysis. The extrac-

Fig. 1. Extraction and purification method of aflatoxins in maize sample.

tion of aflatoxin and subsequent purification was carried out by the modified CB method as shown in Fig. 1. The purified extracts were applied on silica gel TLC plates (Merck 5721) and developed in a chamber using toluene: ethyl acetate: 90% formic acid (60:30:10, v/v/v) as the mobile phase. Then the amounts of aflatoxins of each sample were determined by a TLC scanner.

Results and Discussion

As shown in Tables 1 and 2, eleven maize ear samples from the randomly selected fields

Table 1. *Aspergillus flavus* and aflatoxin contamination in maize ears and kernels collected from fields and storages in Thailand, 1987

Sampling location*1	Maize sample	% kernels infected with <i>A. flavus</i>	<i>A. flavus</i> population on kernel surface (log)	Aflatoxins (ppb)*2			
				B ₁	B ₂	G ₁	G ₂
Farmers' fields							
Khon Buri (NE)	ear	0.5	0	9	ND	ND	ND
Phetchabun (C)	ear with husk	0	0	ND	ND	ND	ND
Phetchabun (C)	ear with husk	0	0	ND	ND	ND	ND
Takhli (C)	ear with husk	0	0	ND	ND	ND	ND
Chiang Mai (N)	ear with husk	0	0	ND	ND	ND	ND
Chiang Mai (N)	ear with husk	0	0	ND	ND	ND	ND
Chiang Rai (N)	ear with husk	0	0	ND	ND	ND	ND
Chiang Rai (N)	ear with husk	0	0	ND	ND	ND	ND
Wang Num Yen (E)	ear	0	1.95	ND	ND	ND	ND
Wang Num Yen (E)	ear	0	2.48	ND	ND	ND	ND
Sa Kaeo (E)	ear with husk	0	2.17	ND	ND	ND	ND
Farmers' storages							
Phetchabun (C)	ear	0	0	ND	ND	ND	ND
Chiang Rai (N)	ear	3	3	ND	ND	ND	ND
Sa Kaeo (E)	ear	1.3	4.45	9	ND	ND	ND
Middlemen's storages							
Khon Buri (NE)	ear	0	—	ND	ND	ND	ND
Chiang Saen (N)	ear	1.5	2.84	ND	ND	ND	ND
Pong Num Ron (E)	ear	3	4.3	58	trace	ND	ND
Khon Buri (NE)	kernel	23.5	—	35	ND	ND	ND
Sikhiu (NE)	kernel	68.5	—	174	9	ND	ND
Sikhiu (NE)	kernel	29.0	—	69	4	ND	ND
Sikhiu (NE)	kernel	11.5	—	22	ND	ND	ND
Pak Chong (NE)	kernel	98.0	—	124	7	ND	ND
Chai Badan (C)	kernel	68.0	4.25	425	29	ND	6
Lop Buri (C)	kernel	25.5	2.42	112	11	trace	ND
Bung Sam Phan (C)	kernel	38.0	3.05	434	35	trace	7
Wang Chomphoo (C)	kernel	60.5	4.30	309	41	ND	4
Chiang Saen (N)	kernel	20.5	3.9	90	3	ND	ND
Chiang Saen (N)	kernel	100	4.78	547	40	ND	9
Wang Num Yen (E)	kernel	32	5.23	383	19	ND	5
Pong Num Ron (E)	kernel	100	6.74	1334	76	ND	20
Pong Num Ron (E)	kernel	27	3.34	87	3	ND	ND
Pong Num Ron (E)	kernel	100	4.47	209	10	52	5

*1 C=Central area; N=Northern area; E=Eastern area; NE=Northeastern area. ** ND=Not detected; Determination: about 8 ppb for aflatoxins B₁ and G₁, about 3 ppb for aflatoxins B₂ and G₂.

Table 2. Incidence of aflatoxin contamination in maize ears and kernels

Sample	Number of total samples	Number of aflatoxin positive samples	Number of samples at the specified aflatoxin B ₁ levels (ppb)						
			ND	<10	10-49	50-99	100-300	>300	>500
Maize ears-1* ¹	11	1	10	1	—	—	—	—	—
Maize ears-2* ²	6	2	4	1	—	1	—	—	—
Kernels* ³	15	15	—	—	2	4	3	4	2

*¹ Farmers' fields; *² Farmers' and middlemen's storages; *³ Middlemen's storage.

showed no infection of *A. flavus* inside kernel and very low amount (9 ppb) of aflatoxin B₁ was found in only one sample, even though small numbers of *A. flavus* were isolated from the surface of maize ears in the Eastern area (Figs. 2-5).

The infection of *A. flavus* in 6 maize ear samples collected from farmers' and middlemen's storages was also found to be very low (0-3% of kernels from the ears). There were some contaminations of *A. flavus* on the ears surface, and two aflatoxin positive samples with the level of aflatoxin B₁ 9 and 58 ppb were detected (Table 1).

On the other hand, all 15 maize kernel samples from middlemen's storage were found to be contaminated with *A. flavus* both on the surface and inside of kernels. These kernel samples also showed rather high levels of aflatoxin contamination; most of them were in a range of aflatoxin B₁ 50 to 300 ppb and less than 500 ppb as shown in Table 2.

Fig. 2. Samples collected from Central area. Number (%) of maize kernels infected with *A. flavus* (○) and population (log) of *A. flavus* on surface of maize ears and kernels (△) collected from 4 areas of maize plantations. EF=ears collected from fields. ES=ears collected from farmers' and middlemen's storages. K=kernels collected from middlemen's storage.

Fig. 3. Samples collected from Northern area. (See foot-note of Fig. 2.)

Fig. 4. Samples collected from Eastern area.
(See foot-note of Fig. 2.)

Fig. 5. Samples collected from Northeastern area.
(See foot-note of Fig. 2.)

From Figs. 2-5, the results also clearly showed that *A. flavus* contamination was obviously low on the maize ears in contrast to the kernels. This reflected on the difference of aflatoxin contamination between the maize ears and the kernels collected from four main crop areas: aflatoxin detection in the maize ears was remarkably lower than that in the kernels. A comparison of the results of our survey with those of similar previous studies^{4-6,10} in Thailand reveals no significant differences. Although there were some isolation of *A. flavus* from the surface of the ear samples collected from the fields in the Eastern area, these ears had neither infection of *A. flavus* nor aflatoxin contamination in the kernels.

In conclusion, more external and internal contamination of *A. flavus* was found in the kernels rather than in the maize ears, and thus the postharvest aflatoxin contamination of maize assumed to be considerably important in all of the main crop areas in Thailand. The results suggest the need for further detailed investigation of factors on kernel infection with aflatoxigenic *A. flavus* especially in shelling process.

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