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TARC Report

Prevention of Aflatoxin Contamination in Thai Maize

1. Infection of Thai maize with Aspergillus flavus

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Abstract
Shelling was found to be the most critical process for the infection of Thai maize with *Aspergillus flavus*. Infection was initiated from damaged maize kernels. In fully mature maize kernels, *A. flavus* infection was slower but a higher rate of aflatoxin contamination was detected compared to immature ones.

Discipline: Postharvest technology
Additional key words: carcinogenic, mature maize, shelling

Introduction

Thai maize is highly preferred especially in the egg-producing industry due to its bright yellowish color. In Thailand around 4 to 5 million t of maize are produced annually. Japan imported over 970 thousand t of Thai maize in 1971/72 but now the trading has practically stopped due to the aflatoxin problems.

Aflatoxin contamination in Thai maize[1, 4-6, 8, 9, 13] is a serious problem not only for overseas trading but for the nation's health because aflatoxin is known to be the strongest carcinogenic substance ever reported.

Materials and methods

1) Effect of maize maturity on Aspergillus flavus infection and aflatoxin contamination

Maize (Suwan-I) samples at five different stages of maturity were collected at Phraphutabat Field Crop Experiment Station (FCES). Shelled maize (inoculated or uninoculated with *A. flavus*) was stored for 15 days and the infection level of *A. flavus* and resulting aflatoxin contamination were investigated.

(1) Maize samples and treatments

Maize (Suwan-I) was harvested on 5 different dates in Phraphutabat FCES in 1988 as follows :

a) September 5 : 98 days after planting (DAP), moisture content (mc) 28.9%,
b) September 12 : 105 DAP, mc 25.7%,
c) September 19 : 112 DAP, mc 24.5%,
d) September 26 : 119 DAP, mc 20.4%,
e) October 3 : 126 DAP, mc 17.5%.

The maize field (ca. 80 × 40 m) was divided into 10 sections and at each harvest maize ears were collected from 2 sections (114-326 kg). The harvested maize was shelled immediately with a me-

The Tropical Agriculture Research Center, Japan, and the Department of Agriculture, Ministry of Agriculture and Agricultural Cooperatives, Thailand, implemented a collaborative research project on aflatoxin contamination in Thai maize from 1985 to 1990. This report is a part of the results of the project.
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chanical sheller installed in Phraphutabat FECS, and after mixing, kernels with a sound external appearance were packed in 2 or 4 jute bags (10 kg per bag) and then sent to the Department of Agriculture, Bangkok on the same day for further treatments. Kernels were divided into 4 groups as follows :

A) undried and not inoculated with *A. flavus* : maize from a) b) c) d) e),
B) undried and inoculated with *A. flavus* : maize from a) b) c) d) e),
C) slightly dried and inoculated with *A. flavus* : maize from a) b) c),
D) dried and inoculated with *A. flavus* : maize from a) b).

To prepare maize with a low moisture content, the kernels were dried (mc 18-22%) or slightly dried (23-24%) with a forced air circulation dryer at ca. 70°C.

For all the treatments, the kernels (10 kg each in jute bag) were kept at ambient temperatures (about 25-30°C) and after 0, 2, 5, 10 and 15 days, about 1.5 kg each of maize sample was sampled to check the moisture content, infection level and aflatoxin contamination.

(2) Inoculation

A. flavus was incubated on milled rice (60 g milled rice, 30 m*l* distilled water, autoclaved) for 10 days. Maize powder (ca. 800 g, sterilized by ethylene oxide) was mixed to the rice culture and homogenized with a blender. To every 10 kg of maize, 0.5% equivalent of inoculum (50 g) was mixed (population of *A. flavus* in inoculum : 6.8×10^6/g).

(3) Moisture content

Moisture content was determined gravimetrically by drying 10 to 15 g of whole kernels at 135°C for 16 hr. This procedure was repeated three times.

(4) Detection of infected kernels with *A. flavus*

Two hundred kernels were sterilized with a 3% NaOCl solution for 1 min and rinsed with sterilized water five times afterwards. Kernels were then incubated for 5 days at ambient temperature (25-30°C) on PDA (potato dextrose agar) including 30 mg of chloramphenicol and 30 mg of rose bengal per liter.

(5) Toxin analysis

Kernels (ca. 1 kg) were sterilized by dipping in 70% ethanol for 30 sec. After sun-drying for 1-2 hr, the kernels were mechanically dried at 75°C (mc less than 12%), sieved off and milled with an ultra-centrifugal mill (Retsch Co. Ltd., West Germany). Fifty gram of the powder was used for toxin analysis by TLC following the modified CB method[10].

2) Detection of post-harvest infection with A. flavus

About 1.5 t each of shelled maize was purchased from three Thai farmers and the distribution steps after harvest were traced in order to determine how and where the maize had been infected with *A. flavus*. Farmers were asked to treat their maize as usual, including processes such as harvesting, grading, shelling, sun-drying, packaging, storage, etc. At every step, the moisture content, presence of *A. flavus* infection and aflatoxin contamination were recorded.

Results and discussion

1) Effect of maize maturity on A. flavus infection and aflatoxin contamination

(1) Infection of kernels

The moisture content of the maize kernels was markedly related to the infection with *A. flavus*[11]. It was indicated that mature maize kernels (about 110 days after planting) were most heavily contaminated with aflatoxin compared to immature ones even though the growth of inoculated *A. flavus* was faster in immature maize kernels due to the higher moisture content. The moisture content of the maize kernels decreased from 28.9 to 17.5% (Fig. 1). Immature maize kernels (89 days after planting, initial mc 28.9%) were dried to reach 23.8 or to 20.7% mc and inoculated with *A. flavus*. Infection with *A. flavus* spread rapidly irrespective of the inoculation in maize kernels with a high moisture content (mc 28.9 or 23.8%) while in those with a low mc (mc 20.7%) it remained at a low rate (Fig. 2). In 105 days old kernels, the rate of infection increased rapidly in samples with 25.7 and 23.6 mc, but not in those with a moisture content of 21.6%.

In 112 days old kernels, the infection occurred at the mc level of 24.5% but the rate of infection was low in kernels with an mc of 18.2%. In 119 days and 126 days old kernels, the moisture content was 20.4 and 17.5%, respectively and it was too low to induce infection with *A. flavus* during the experimental period.

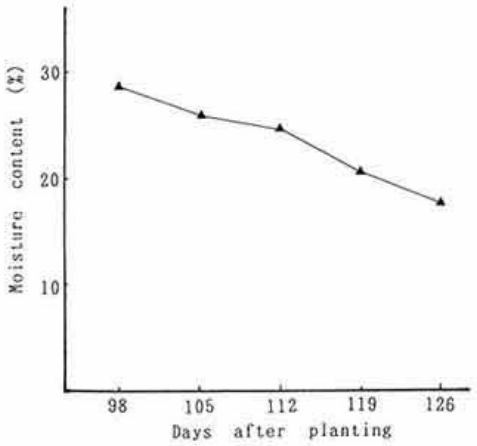

Fig. 1. Relationship between moisture content* of maize and harvesting time
* moisture/wet matter.

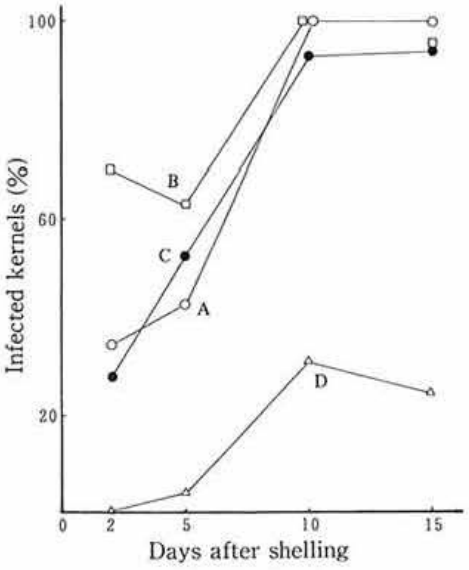

Fig. 2. Relationship between moisture content and infection of maize kernels with *A. flavus*
Maize : 98 days after planting.
Initial mc: A; 28.9%, B; 28.9%, C; 23.8%, D; 20.7%.
All maize inoculated with *A. flavus* except A immediately after shelling.

The relation between the infection with *A. flavus* and initial moisture content is shown in Fig. 3. These figures indicate that the minimum initial moisture content of maize kernels to prevent *A.*

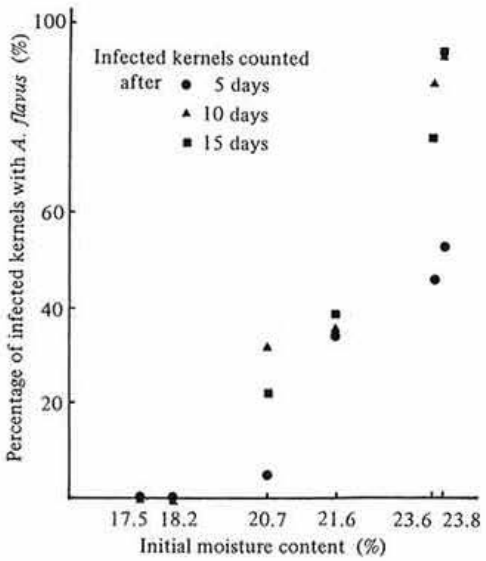

Fig. 3. Relationship between moisture content and changes in the percentage of infected kernels during storage
Maize: 10 kg each in a gunny bag. Maize samples were taken 98, 105, 112, 119 and 126 days after planting. Some of them were dried to various mc levels. Five, 10 and 15 days after the inoculation with *A. flavus* the number of infected kernels was counted.

flavus infection ranged between 17.5 and 18.2%. For safety, a 17% mc is recommended. Maize kernels with an 18.5% moisture content are likely to be heavily infected with storage fungi at 25-30°C within a week to two[8]. Lopetz concluded that *A. flavus* growth will not increase appreciably when the moisture content of the kernels is lower than 17.5% at 35°C. JICA researchers also reported that, at less than 17% mc inoculated *A. flavus* failed to infect damaged maize kernels[6]. As a limit of the moisture level for contamination, Trenk et al. reported that when the moisture content was 18%, aflatoxin was not detected even in rewetted maize[12]. The minimum moisture content to prevent infection with *A. flavus* may vary depending on the humidity level, temperature and storage period.

(2) Aflatoxin contamination

Aflatoxin contamination in immature maize kernels with a high moisture content remained at a low level during the 15-day experimental period compared to mature kernels with a lower moisture content.

Within 2 days in every treatment aflatoxin was not detected. However Winn and Lane[14] inoculated *A. flavus* to autoclaved grain and after 48 hr they detected a 15 ppb level of aflatoxin B_1 at 25°C while at 30°C the level was 40 ppb. Fig. 4 shows that the difference in the aflatoxin content of immature maize kernels (98 days after planting) with different mc values (28.9 and 23.8%) was not appreciable. The sample dried to an mc value of 20.7% did not contain aflatoxins at all even though there was some infection with *A. flavus* (Fig. 2).

In mature maize kernels with an initial mc of 25.7 % the highest level of toxin contamination was recorded and even after drying to an mc of 23.6% a high toxin value was detected (Fig. 5). There was a considerable difference in the toxin content between the maize kernels with almost the same mc level but different degrees of maturity: mc 23.8% for 98 days old maize kernels, toxin ca. 500 ppm (Fig. 4) and mc 23.6% for 105 days old kernels toxin ca. 1,200 ppm

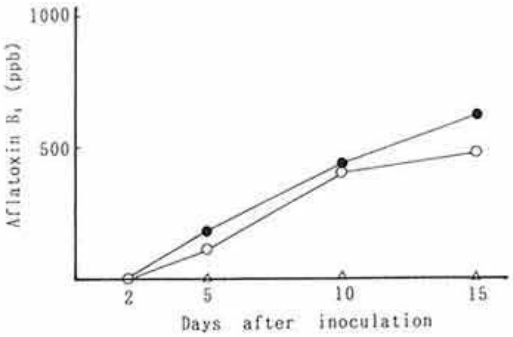

Fig. 4. Relationship between moisture content and aflatoxin contamination of immature maize kernels
Maize: 98 days after planting.
Mc: ●; 28.9%, ○; 23.8%, △; 20.7%.

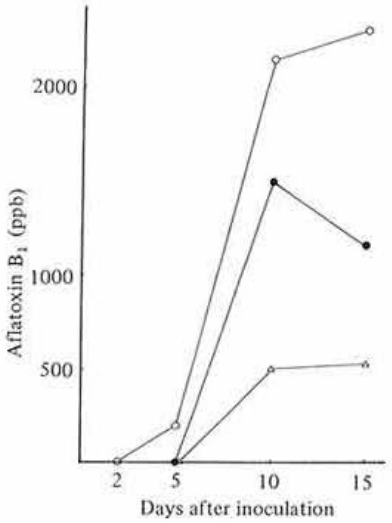

Fig. 5. Relationship between moisture content and aflatoxin contamination of fully mature maize kernels
Maize : 105 days after planting.
Mc : ○; 25.7%, ●; 23.6%, △; 21.6%.

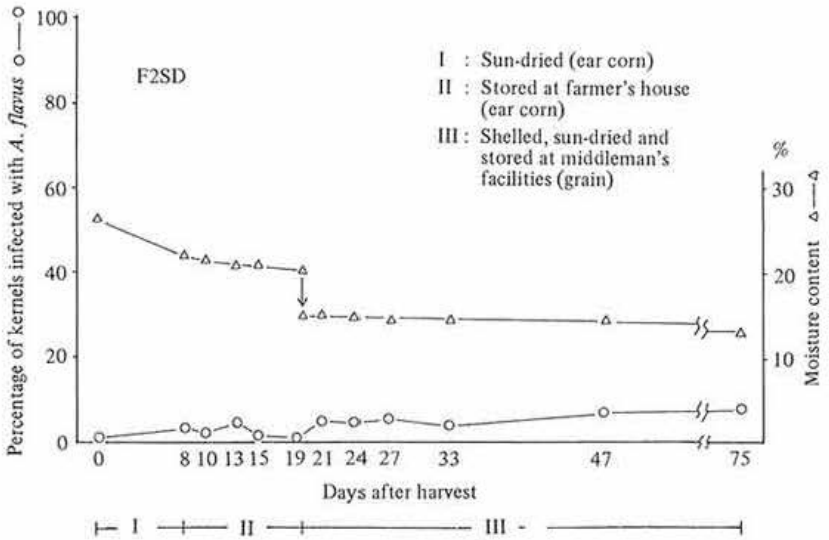

Fig. 6. Relationship between changes in moisture content and *A. flavus* infection during post-harvest treatment

(Fig. 5). This observation suggested that the maturity of maize somewhat influenced the aflatoxin production of *A. flavus* in maize kernels. Similar results were reported in a previous paper[14] where mature maize kernels (113 days after planting, initial mc 23.7%) showed a much higher level of aflatoxin contamination than immature wetter kernels (99 days, mc 30.7%). Ashworth et al.[2] who reported that aflatoxin production was low in peanuts with a high mc suggested the presence of microbial competition and microbial breakdown of toxins. In more mature maize kernels (112 days old) with an initial mc of 24.5%, the maximum aflatoxin content was approximately 500 ppb, which was far less compared to the value shown in Fig. 5.

2) Detection of post-harvest infection process with A. flavus

In order to identify the processes whereby *A. flavus* infection and aflatoxin contamination occurred, a total of 4.5 t of shelled maize from three local farmers was examined in every step throughout post-harvest operations. The following results were obtained.

(1) Infection rate of *A. flavus* in the field was usually very low (less than 5%), although the infection tended to increase during a dry spell.

(2) After harvest, wet maize was stored in the farmer's warehouse as ear corns. If the aeration was good, mold infection was not serious.

(3) After shelling, the grains were sun-dried on a concrete floor. If the weather was favorable, the mc of the grains decreased to about 14 to 15% within 1 to 2 days. Dried grains (mc 15.0%) were then stored in jute bags for another 55 days. This process did not cause any serious infection (Fig. 6).

(4) When mechanically shelled maize was traded in jute bags without any drying process, maize kernels (initial mc 23.0%) were completely infected

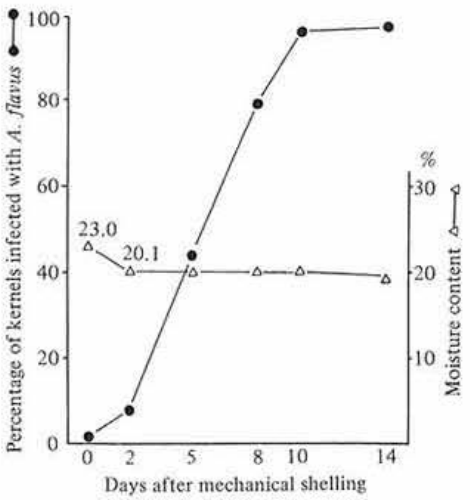

Fig. 7. Relationship between moisture content and *A. flavus* infection of undried shelled kernels

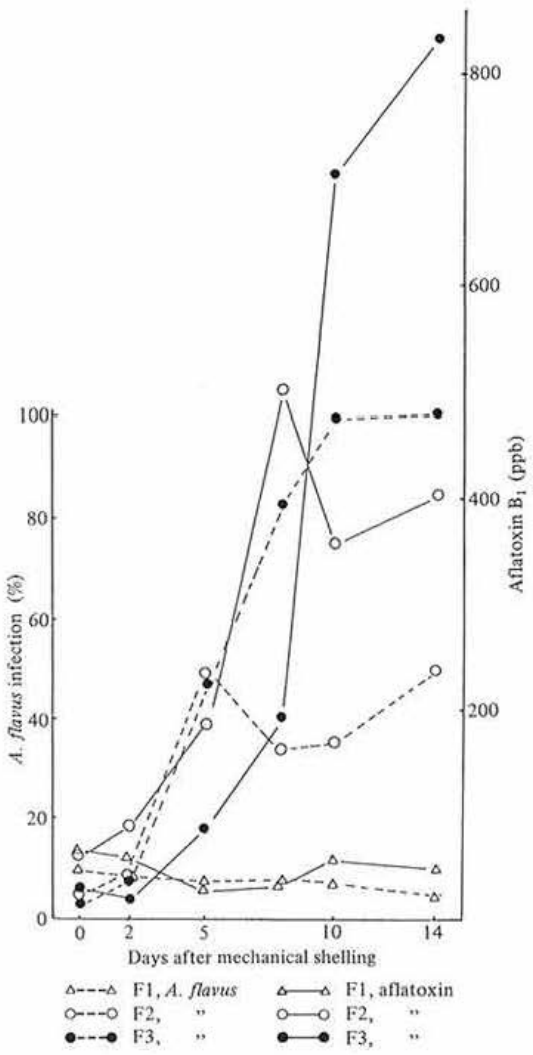

Fig. 8. Relationship between infection with *A. flavus* and aflatoxin B_1 contamination
Initial mc: F1; 16.9%, F2; 20.3%, F3; 23.0%.

with *A. flavus* in a short time (Fig. 7). Mold spread over the kernel surface in 2 to 5 days when the mc was ca. 20 to 30% (Fig. 8), and in a few more days when the mc was 20% or less and 30% or more.

(5) Undried shelled maize was not infected with *A. flavus* if the mc was less than 17%.

(6) Shelling process caused physical damage to the maize kernels, and it was the time when they were inoculated with *A. flavus* simultaneously.

(7) Thus the shelling process was found to initiate the *A. flavus* infection. When the maize kernels were in the ears, mold growth was slow or inhibited[8].

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