# CONTROL OF AFLATOXIN IN MAIZE

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## ABSTRACT

In addition to *Aspergillus flavus* and *A. parasiticus* which produce aflatoxin many other toxin-producing fungi have been detected in stored maize in Thailand. It was shown that the aflatoxin content is low at harvest and increases during storage. Field drying and mechanical drying are the most effective measures for controlling aflatoxin contamination in maize. A rapid BGYF test was developed to estimate the aflatoxin level and is being used by many maize dealers. Among the chemicals tested for controlling the causal fungi only a mixed solution of ammonium bis propionate and propionic acid will give temporary control and prevent the growth of fungi in maize under high moisture conditions but will not destroy aflatoxin present before the treatment of the grain. The grain still must be dried. Maize, highly contaminated with aflatoxin, can be detoxified efficiently with ammonia and the resulting grain is safe and can be used for feeding cattle and swine but is not suitable for human consumption and international trade.

## Maize production in Thailand

Maize is presently the fourth most important crop produced in Thailand. Maize production is particularly important in the northern, northeastern and central regions. (Table 1, Fig. 1) Since maize cultivation is a low input system, farmers tend to increase the planting area rather than intensify production. Despite the increase of the area, production is still low when compared to that in other maize-growing countries. Only a few farmers accept the new production technology including the application of fertilizers, pest control and use of seed of new high-yielding varieties. The Fifth National Development Plan (1982-1986) set a target to increase the production by 4.5% per year. The increased production was intended to be achieved through the extension of the cultivated area. Several trials show that the yield increased by more than 2% when the recommended fertilization and cultural practices were applied. The Sixth National Development Plan (1987-1991) has set a goal of 5% increase of production. The promotion of maize quality improvement will be a priority. Maize is cultivated both in single and double cropping systems. Generally, varieties resistant to downy mildew are grown throughout the country. There are other hybrids produced by private seed companies available in the market, but the cost is the major limiting factor for the use of hybrid seeds. The total amount of maize produced in Thailand is equivalent to only 1% of the world production and Thailand accounts for only a small part of the total international trade. The maize importing countries of Asia and other countries prefer yellow color maize.

## Postharvest handling of maize

It is estimated that Thai maize production increased by about 11% during the period of 1960-1975. After this period, the extension of the cultivated area was limited and affected the total production increase. An increase of 2-5% on the average was obtained each year depending on the farm price and drought conditions. Normal planting period extends from mid-May to early June, depending on the onset of rainfall. Planting may start as early as from mid-March to mid-April. For single cropping the growing season starts from mid-May, but for double cropping planting must be performed earlier. The second crop can be planted in August/September and harvested in Octo-

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**Table 1 Estimated planted area, production and yield in major maize-growing regions**

| Regin | 1985/86 Planted area (rai) | Production (rai) | Yield (ton) | Average yield (kg/rai) |
|---|---|---|---|---|
| Northern | 7,566,655 | 7,268,451 | 3,042,553 | 5,925 |
| North Eastern | 3,119,853 | 3,078,534 | 1,202,532 | 2,447 |
| Central | 1,210,814 | 1,172,679 | 537,234 | 2,513 |

| | 1986/87 Planted area (rai) | Production (rai) | Yield (ton) | Average yield (kg/rai) |
|---|---|---|---|---|
| Northern | 7,431,431 | 6,949,942 | 2,687,464 | 5,937 |
| North Eastern | 3,176,232 | 2,866,322 | 1,012,226 | 2,253 |
| Central | 1,197,851 | 1,161,164 | 493,457 | 2,207 |

| | 1987/87 Planted area (rai) | Production (rai) | Yield (ton) | Average yield (kg/rai) |
|---|---|---|---|---|
| Northern | 7,039,457 | 5,385,255 | 1,697,075 | 4,871 |
| North Eastern | 2,708,235 | 2,352,159 | 763,477 | 2,205 |
| Central | 1,128,555 | 969,237 | 383,098 | 1,935 |

ber/November/December. Regional variations in rainfall affect the planting dates and time of harvest. Farmers try to plant early because an early crop gives a higher price. To gain this advantage, the farmers may have to replant several times as rain is unreliable in the early rainy season. For example, the crop in 1987/1988, in some planting areas, had to be replanted four times. In the years with maize shortage the farmers sometimes harvest an immature crop due to the high market demand. Normal maturity for the recommended vaieties is 110 days after planting, though some early maturing varieties of 90 days are available from the government seed centers or local experiment stations but they give lower yields. The single crop or the first crop in a double cropping system is harvested in the rainy season. Only hand picking and stripping of cobs are practiced for harvesting; mechanical harvesting is not implemented in Thailand. Stripped cobs are removed from the plants and packed in bags in the field. Harvested maize is transported by hand, by animals and engine-powered carts to storage cribs or local dealers. Some farmers move the cobs immediately after harvest to an area for shelling and sale. Most local dealers provide shellers for farmers' maize by contact. Shelling cost is borne by the farmers. Those who are not satisfied with the price offered will dry the cobs and keep them in a crib until the price becomes more acceptable. Since harvesting takes place mainly in the rainy season, it is difficult to dry maize. Farmers will keep maize on the cob either wet or dry while local dealers, who always face a shortage of storage facilities will keep maize only as shelled grain. A survey showed that 79% of the farmers sell their maize as soon as it is harvested mostly by contact with local dealers. Only 21% of the farmers store the crop and wait for a better price or until they are forced to sell. On-farm post-harvest drying and storage are seldom practiced by the farmers. These practices, are not considered worthwhile because the farmers keep the maize only for a short period. For longer storage, typically, farmers dry the harvested maize on the cob using straw

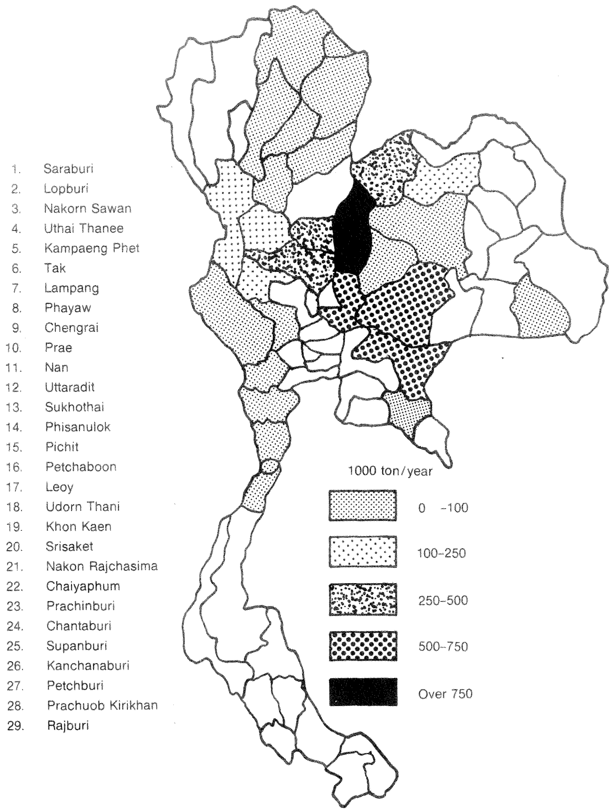


**Fig. 1 Maize-producing provinces in Thailand.**

mat on the ground. Then the dried ears will be kept in a storage crib. In some areas the ears are left on the plant before harvesting until the moisture content of the grain reaches 18%. Topping, which removes the upper part of the mature plant has been recommended for the double-cropping system. This method makes the land available to grow a second crop without the necessity to harvest the first crop. Ears can be left on the plant in the field for one month without aflatoxin formation.

During the harvesting season, local dealers are busy travelling to buy maize to be stored in godowns, bins and silos. All the purchased maize is wet. The dealers must dry the grain before storing it to increase its quality and value. Sun-drying on cement floors is the most common practice but the amount of rain limits the utility of the floors. Aflatoxin contamination can become a serious problem, when the wet grain can not be dried quickly. Research carried out by the Department of Agriculture indicates that if the wet grain cannot be dried within 72 hours, aflatoxin contamination is likely to occur. As shown in Fig. 2, local dealers will move the maize to Bangkok wholesalers for export or domestic use. Some local dealers also are involved in moving the grain directly from the producing area to overseas importers. Cooperatives also play a small part in the marketing channel. It was found that aflatoxin contamination in pre-harvested maize is relatively low but increases rapidly during the period when the grain is handled by local dealers. As the problem is becoming increasingly serious, the local dealers now pay more attention to solutions. Several types of mechanical dryers, storage facilities and silos with dryers have been set up in many parts of the country for drying and handling the grain. Some mold inhibitors also are used in domestic feeds.

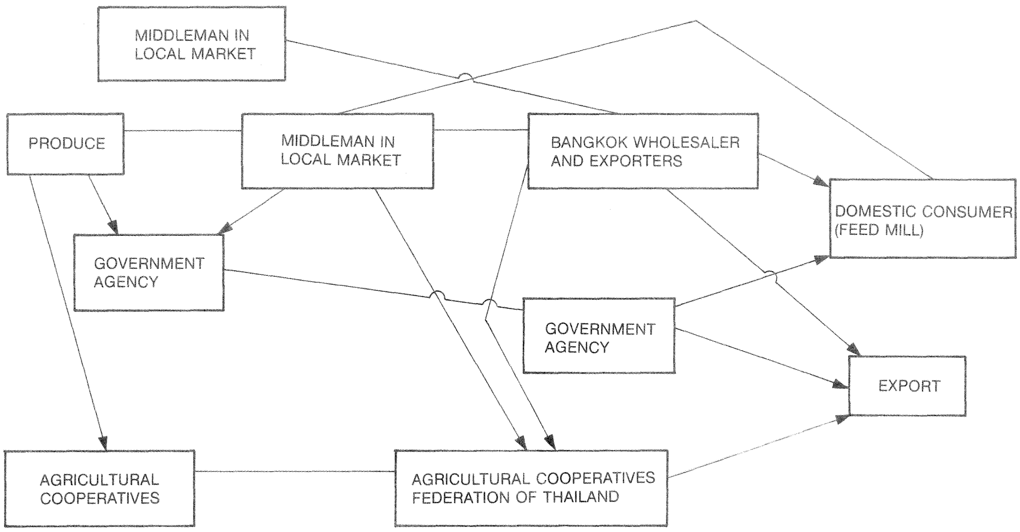


**Fig. 2 Marketing Channel for Maize in Thailand.**

## Control of aflatoxin in maize

Aflatoxin content has become a major factor affecting the export of maize and most importers have set aflatoxin limits, usually in the range of 20 to 100 ppb. Aflatoxin restrictions and the world surplus of maize have made markets increasingly difficult to find and prices have tended to fall until the recent US drought. Since Thailand exports more than 70% of the production, a discount of 5% of FOB price due to a contamination would cost more than US$25 million per year. Thailand, therefore, assigned top priority to research on aflatoxin control in maize. This work, coordinated by a national committee, has made rapid progress and many of the aflatoxin control measures that have been devised are being implemented and/or evaluated on a commercial scale. Research in aflatoxin involves several different fields.

## Chemical treatment

The Division of Plant Pathology and Microbiology of the Department of Agriculture has recently completed the screening of seven reagents in the laboratory for their effectiveness in preventing or reducing aflatoxin contamination of maize. Only three of the reagents were found to be effective, i.e. sodium bisulphite, ammonia, and propionic acid: ammonium bis propionate at a ratio of 9:1. Sodium bisulphite and ammonia treatments both result in grain with a strong residual odor; the ammonia treatment also produces darker grain. The most promising reagent is the propionic acid-based fungicide formulation, which has been shown to effectively control both mold growth (*A. flavus*) and aflatoxin formation, while not adversely affecting the physical quality of the grain. The cost of the fungicide treatment may be offset by higher prices for better quality grain. Future work aims to reduce costs by minimizing the inclusion rate and improving the application method.

## Mechanical drying

The UK-Thai Aflatoxin in Maize Project(1) has identified a set of criteria, called the UK-Thai Project(UTP) System, which have been shown to reliably produce low aflatoxin content maize during the rainy season. With the UTP system, maize is first field-dried on the stalk for one to two weeks before harvesting to reduce the moisture content to 18 to 22%. It is next shelled within 24 to 48 hours of harvest, and loaded into a dryer within 12 hours of shelling. Thus, within 48 hours, it is dried to 14% moisture content, 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% moisture content by the UTP system can be safely stored for a minimum of two months with no increase in aflatoxin content. Using this system, 25 three-ton batches of maize were successfully processed with a mean total aflatoxin content of 2.5 ppb and a range of 0 to 16 ppb at drying sites in two provinces. The system is now being used commercially for about 50,000 tons of maize.

## Improved farm storage

A USAID-funded project conducted by the Department of Agriculture was begun in 1985. The project aims to develop and evaluate improved farm storage and drying methods. In 1985, cribs of three sizes (0.5, 1.0 and 2.5 meters) were tested, as was a solar dryer developed by the Asian Institute of Technology (AIT), Bangkok.

## Mycological studies

A collaborative study, involving the Division of Plant Pathology and Microbiology, the Department of Agriculture and the Tropical Agriculture Research Center (TARC) of Japan, is being conducted on the incidence and occurrence of *Aspergillus flavus*. A very high incidence of *A. flavus* has been found in soil samples, especially in the soil around drying facilities and warehouses. No *A. flavus* spores were detected in the atmosphere in maize fields, but high levels of spores were found in the air in warehouses used for maize storage.

A mycological study of maize was also carried out by the United Nations Development Programme/FAO in collaboration with the Thai Department of Agriculture in January and February, 1985. The work confirms the low concentrations of *A. flavus* spores in the air in maize fields during the dry season, as well as the high concentration of spores in warehouses. *Aspergillus flavus* contamination in stored maize was found to be closely associated with weevil infestation (*Sitophilus zeamais*); the insects carried extremely high concentrations of *A. flavus* spores. Virtually no *A. flavus* was found before harvest in the second, dry-season crop of maize, but concentrations increased slowly during temporary storage of ears and grain. It was also found that only *A. flavus* and *A. parasitica* produce aflatoxin in maize.

## Quality control methods for aflatoxin

The UK-Thai project has collected data which strongly indicate that an adaptation of the bright greenish-yellow fluorescence (BGYF) test can be used in Thailand to identify the level of aflatoxin present in maize. Batches were classified according to the number of observed BGYF particles (e.g. 0, 3, 5, 10 counts). Correlation between these BGYF counts and the mean total aflatoxin content of all batches within each classification was found to be highly significant (correlation coefficient, r=0.92). Sampling was found to be a critical factor when working toward a 20 or 30 ppb aflatoxin limit. The good correlations were only found when a 10-kg representative sample was coarsely ground with a hammer mill fitted with a 6-mm screen and the sample subdivided into four 125-g subsamples. Aflatoxin quality-control procedures based on the BGYF test have been devised for monitoring the production of low-aflatoxin content maize, and for assisting grain management at regional and export storage facilities. Monitoring is best done in conjunction with minicolumn testing to minimize consumer risk.

## Analytical services

Aflatoxin analysis is routinely performed at a number of laboratories in Thailand. Unfortunately, the sampling methods, sample preparation and analytical methods vary widely, although efforts are being made to standardize them. Inspection companies offer an aflatoxin analysis service that is predominantly semiquantitative, based on minicolumn determination which is sometimes linked to a fluorotoxin meter. Fully quantitative aflatoxin determination is mainly performed in government laboratories, using quantitation by thin-layer chromatography (TLC). Sophisticated techniques, such as high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC), are gradually being introduced, and should soon enable a faster and more accurate analysis of sample.

## Future research

Future research has been approved by the national committee in the areas of:

* Continued work on inhibition of aflatoxin-producing fungi by chemical treatment;
* Alfatoxin detoxification;
* Evaluation of the UTP system for producing low-aflatoxin maize on a commercial scale;
* Determination of the feasibility of increasing the proportion of second-crop, dry-season maize, which is known to have a low aflatoxin content, and to determine where such changes might be most appropriate;
* Study of aflatoxin distribution in low-aflatoxin content batches in order to devise appropriate sampling plans for use throughout the maize marketing chain;
* Development and evaluation of analytical techniques, both fully quantitative and semi-quantitative, for use in quality control;
* Reduction of the risk of aflatoxin contamination in unshelled maize, e.g., in crib storage and extended field drying; and
* Study of the risk of aflatoxin contamination associated with maize shipping, and the development of suitable control measures.

## Cooperative research

Much of the aflatoxin research in Thailand can now be considered to be coordinated and cooperative, due to the influence of the national committee. Assistance from other countries which provide funding, training and staff is still needed as such support has played a significant role in aflatoxin research in the past. Various foreign agencies have given support to the Department of Agriculture through bilateral or multilateral assistance.

The United Kingdom has provided training, equipment, staff and volunteers to join in collaborative projects with Thai researchers, at a value of approximately 15 million baht (US$600,000). The United States Agency for International Development in phase 1 of its contract, has approved a soft loan of approximately US$200,000 and a grant for research staff and overseas training and study tours for Thai scientists for 1985 and 1986. The United Nations Development Programme (UNDP) has approved funds of US$38,500 for 1985 and 1986. In addition, the Tropical Agriculture Research Center (Japan) has approved a cooperative project with the Division of Plant Pathology and Microbiology of the Department of Agriculture on quality and preservation of maize by preventing aflatoxin contamination. The Tropical Agriculture Research Center supplies senior researchers, training, analytical equipment and software.

## Acknowledgements

Credit for the rapid progress in the battle against aflatoxin must go to the National Committee

on Mycotoxin Control in Agricultural Commodities and its constituent organizations. Particular mention should be made of the research carried out by the Department of Agriculture, through its own research and in collaboration with the United Nations, Japanese teams (TARC and JICA), USAID and British teams.

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## Discussion

**Uritani, I.** (Japan): I have learned that you are making great efforts to prevent the contamination of maize with aflatoxin. Have you used desiccants such as silica gel or calcium hydroxide to dry the grains (moisture content of less than 15%) in addition to natural drying by sunshine?

**Answer:** We have thought about it and considered using desiccants to absorb moisture from the hot air blown through the moist grains and recycle the hot air which is almost dry through the grains again. This way we hope that the efficiency of the drier would increase. We hesitate to use calcium hydroxide due to the residues and the use of silica gel may be too expensive.

**Fajemisin, J.M.** (IITA): 1. It is not advisable to make a general statement about the effectiveness of the use of cribs for the whole of Africa as the effectiveness of the cribs depends on the climate conditions. When it is too humid the cribs are not effective, when they are too wide the rate of circulation of the air will not be satisfactory. You also referred to leaving the ears for a period of 1 and a half months after the removal of the tops. Under these circumstances you may encounter storage problems associated with the infestation with *Sitophilus* which may promote the contamination with the *Aspergillus flavus* fungus. The prevention of aflatoxin contamination depends on sanitation and the reduction of the moisture content of the grains. 2. You mentioned that you do not use a very sophisticated equipment for testing the aflatoxin level. Do you correlate the amount of aflatoxin based on the number of infected kernels by *Aspergillus flavus*, i.e. counting the ears or grains and determine the percentage of kernels infected? In other words, do you determine the level on the basis of physical counts of infected kernels or carry out chemical analyses?

**Answer:** 1. Thank you for your comment. Indeed the cribs were not effective in Thailand. 2. We use both methods.

**Uddin, K.S.** (Bangladesh): 1. Have you considered a radio-active approach to remove *Aspergillus flavus* and *A. parasiticus* from the seeds of maize? 2. Have you any program for the development of resistant varieties?

**Answer:** 1. No. As maize is not a cash crop, this method would not be economical. 2. We are in the process of breeding resistant varieties but so far no results have been obtained.

**Garcia, P.R.** (The Philippines): I read from your abstract that the BGYF test was developed to estimate the aflatoxin level and is being used by many dealers. However the BGYF test is a presumptive test (qualitative test) which can not be used for quantitative purposes. What is the chemical test you use for quantifying the level of aflatoxin in corn?

**Answer:** The BGYF test is used only by local dealers to separate contaminated from healthy grains (50 ppb level) in silos. For export certificates, we do not use this test but AOAC or minicolumn methods or TLC.