Mycotoxin contamination of foods and feeds in Nepal

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SUMMARY

Monitoring of mycotoxins in food commodities was first initiated in Nepal in the year 1978 as a consequence of FAO/UNEP Regional Monitoring of Food Contaminants Project involving four countries in which Nepal and India were

placed in one group and Pakistan and Sri Lanka on the other. Since then 850 food samples mainly comprising of Cereals, Pulses, Oilseeds and Spices and about 150 samples of feed and feed ingredients were monitored for aflatoxin content covering the period of 1980 -1987. Result has indicated that corn and peanut are the two commodities most prone to aflatoxin contamination. Ecologically, Terai Southern Plain area is more susceptible to aflatoxin hazard compared to hills and mountainous area. Coincidentally, toxigenic fungi (Aspergillus flavus) predominated in Terai since from the onset of storage as contrary to hills and mountainous areas. Further, A. flavus spp. isolated from Terai area produced higher amount of aflatoxin B. (6334 ppb) than from the hills and mountains. Thus, the potency of the strains has shown altitude bias. Maximum level of aflatoxin B. is found in corn (321 ppb) and peanut as raw shelled (634 ppb). Aflatoxin content in feed and feed ingredients are relatively higher because low quality cereals or damaged grains are usually used for feed and feedstocks. Maximum level of aflatoxin B. in Poultry feed is found to be 1100 ppb.

INTRODUCTION

Mycotoxin are considered to be potent liver carcinogens. A host of toxigenic fungi produce varieties of toxic metabolities contaminating foods and compel them unsuitable for consumption. Meagre resources of developing countries need to be channelled to increased production by avoiding unwanted natural contaminants like mycotoxins. Increasing production is the national goal of every developing nation and therefore the measures to avert losses during preservation, processing, storage and marketing, have to be afforded due priority in the developmental plans particularly with regard to the invasion of toxic fungi. Mycotoxin monitoring programme has been integrated with the study of causative fungi for developing a viable strategy to control the mycotoxin contamination in food and feed.

Central Food Research Laboratory (CFRL) under the Ministry of Agriculture has taken the lead in the study of mycotoxin and toxigenic fungi in coordination with other related agencies like Rural Save Grain Project and Teaching Hospital for finding clues to disease syndrome by consuming suspected food commodities.

OBJECTIVES

1. To monitor aflatoxin content in different food and feed products.
2. To investigate causative micro-organism and study their growth pattern and potency to form aflatoxin.
3. To determine ecological distribution of toxic fungi in relation to aflatoxin production.
4. To investigate aflatoxin production in various storage system of corn in Kathmandu Valley for recommending suitable one for the intended purposes.

INCIDENCE OF AFLATOXINS IN FOOD AND FEEDS

Karmacharya et al (1988) reports the analytical data of aflatoxin in food and feeds.

Table 1. shows the occurrence of aflatoxin contamination in different food commodities. Out of total 582 samples of corn and peanuts products, and wheat flour, and parboiled rice, 109 (18.7%) samples were contaminated with aflatoxin. Further, the number of samples which exceeded permissible limit of 30 ppb of aflatoxin B1 as suggested by FAO, were 35 (6%) samples of tested food

commodities. Aflatoxin problem as revealed by this study is of no serious magnitude. However, it warrants careful monitoring of mycotoxin for controlling mould infection in stored products.

Table 1 Occurrence of aflatoxin B1 in food commodities (1980 -1987)

Corn and peanut are the two risk commodities from the point of aflatoxin contamination. Out of 35 samples exceeding 30 ppb of aflatoxin, 90 of contaminated samples comprised of corn and peanut products. Thus special impetus should be given to prevent aflatoxin hazard in these commodities.

Table 2. shows list of 255 samples of different food commodities mainly comprising of raw rice, beaten rice, wheat, flour, pulses, spices etc; which did not show presence of aflatoxin. The sampling period covered almost seven years. However, it suffers from some deficiencies of basic informations with regard to the exact origin of samples. Therefore, it has been planned to collect appreciable number of samples from different ecological areas to determine the extent of problem and thereby to employ counter measures to avert it.

Table - 2 Aflatoxin negative samples of different food commodities (1980 -

1987)

	Total	
Commodities	**Samples**	**Remark**
Rice (Rangy	41	ND
Beaten rice	13	ND
Wheat	32	ND
Maida (Wheat flour)	32	ND
Barley	8	ND
Pulses	43	ND
Peanut (Fried and salted)	3	ND
Oil seed (Sesame mustard)	9	ND
Vegetable Oil	12	ND
Betal nut	15	ND
Spices (chill), cumin, black pepper)	43	ND

Gundruk (fermented & dried vegetable leaves)	4	ND

ND = Not Detected.

Table 3. shows the occurrence of aflatoxin in feed products. 116 samples of different feeds comprising mainly of poultry, cattle and pig were tested for aflatoxin content. 55 (47.4%) samples showed positive aflatoxin content ranging from trace to 1100 ppb in case of poultry feed. Further, it indicates that 20 (12.9%) samples exceeded the permissible limit suggested by EEC countries for animal feed. Data presented here mainly refer to poultry feed. Therefore, it is necessary to collect more samples of feed and feed ingredients to ascertain the safe product of feed and hence minimise aflatoxin problems in these products.

Table 3 Occurrence of aflatoxin In different feeds (1983-1987)

TOXIGENIC FUNGI ISOLATED FROM CORN

Nepal has a varied topography stretching from the plain area of Terai to the rugged terrace of hills and mountains. Most of the surplus cereals are produced in the plain of terse where ambient temperature and humidity are relatively high. These grains are supplied to deficit hilly areas, after nearly six month's storage. Corn is also grown in plain, hills and mountains. Corn cob is harvested at high moisture content (18-21), dried and then stored cob with husk for about six month before being shelled. milled and consumed. Thus, it is not surprising that most of the corn samples are occasionally infected by mould producing toxin. Earlier studies by Karki et al (1979) also conforms that corn might be the prominent problem area of aflatoxin contamination especially during storage and movement of grains from the plain Terai to the deficit hilly areas. Thus, corn can be a problem from food safely standpoint and hence this aspect has to be dealt with utmost care and attention.

In Hills/Mountains and Kathmandu areas, the predominant mould flora were in the order of Fusarium spp., followed by Aspergillus spp (A. flavus A. niger, A. fumigates), Penicillium spp. and Rhizopus, Where as this trend was different in

Terai and inner Terai regions and therefore the prominent flora were in the order of A. flavus, Fusarium spp. Penicillium spp, A. niger A. fumigatus, and Rhizopus spp. (Table 4). While Fusarium spp. was found dominant in hills/mountain, and Kathmar du Terai samples showed preponderance of A. flavus However, these data reveal that Fusarium toxin may prevail in the hills/mountain area as contrary to the aflatoxin problem in Terai. Therefore, occurrence of various toxin as a metabolic product of causative fungi has to be investigated in order to arrive at a practical solution of this natural contaminant.

Table 4 Occurrence and identification of internal mold flora in corn samples.

POTENCY OF ASPERGILLUS FLAVUS SPP. FOR AFLATOXIN PRODUCTION

Nineteen representative strains of A. flavus isolated from corn grown at different altitudes, were selected for their toxin production. Corn grown from the following localities were collected for investigation.

a. **Hills/Mountain: Elevation 1517-2300 meter (Khopasi, Dhading, Kavre, Jumla)**
b. **Kathmandu Valley: Elevation 1330-1350 meter (Kathmandu, Lalitpur, Bhaktapur)**
c. **Terai /Inner Terai: Elevation 72-474 meter (Biratnagar, Mahendranagar, Nijgadh & Hetauda)**

A. flavus isolated from Terai and inner Terai produced aflatoxin B1 & B2 at the concentration of 6 gm/kg and 0.9 mg/kg respectively (Table 5). This finding has indicated that A. flavus isolated from hills/mountain and Kathmandu region seem to have lower potency of aflatoxin production. Two strains from Kathmandu Valley produced all four aflatoxins B1, B2, G1 and G2. However, two strains from Terai, one from Kathmandu Valley and one from mountain areas did not produce aflatoxin. A. flavus isolated from terse produced the highest amount of aflatoxin compared to valley and other hills/mountainous region.

This result shows that there is a need of precautionary post-harvest measures to minimise the possible aflatoxin hazards. In short, aflatoxin can become a big

menace in Terai area where temperature is relatively high and the humidity is also favourable for the growth of A. flavus. However, a careful strategy needs to be initiated to determine the extent of damage caused by A. flavus along the coastal area of river belt. These areas seem to be prone to A. flavus invasion. For instance, the Terai and inner Terai area extending to river beds of Gandaki, Koshi and Karnali need to be investigated for probable hazards of A. flavus and subsequent toxin production. Also the use of other additives like spices and mould inhibitors and even some acid producing bacteria should be investigated from the point of controlling aflatoxin contamination in food products.

AFLATOXIN CONTENT IN CORN AT DIFFERENT STORAGE SYSTEM AFTER 10 MONTH'S STORAGE AT KATHMANDU

Karki et al (1988) reports the occurrence of A. flavus and subsequent aflatoxin production in corn at different storage structures of Kathmandu Valley.

Table 5 Variation in potentiality of A. flavus strains isolated from corn grown at different altitude In Nepal.

Fig. 1 shows the distribution pattern of A. flavus propaguels and aflatoxin production at different storage systems employed in Kathmandu Valley. No aflatoxin was detected on the sample stored at Thangro (outdoor open storage system around a pole and four support base). The sample stored at Bhakari (bamboo bins) contained 1.5 ppb aflatoxin B.. However, the aflatoxin content in Ghyampo (clay jar) and metal bin was found to be 40 and 125 ppb aflatoxin B1 respectively. This clearly indicates that Thangro seems to be an appropriate storage system for corn as evidenced by absence of A. flavus and aflatoxin production. Furthermore, Bhakari type of storage can be improved for preventing mold infection and relative toxin production. It needs further elaboration in different agro-climatic regions.

AFLATOXIN IN RELATION TO LIVER CANCER

In order to establish a close relationship of human mycotoxicoses with that of the aflatoxin content in the diet intake, it is very important to employ a comprehensive screening programme. For this purpose, daily consumption of aflatoxin is needed to analyse a large number of foodstuffs collected from market and domestic households. Rensburg (1977) reports a significant statistical relationship between the incidence of liver cancer and the intake of aflatoxin as shown in Table 6.

Table 6 Incidence of liver cancer (cases per 100,000 people per annum) and aflatoxin Intake (mg/kg body weight per day) In Africa and Asia.

Region	Incidence of liver cancer	Aflatoxin content
Kenya- highlands	0.7	3.5
Thailand - Songkhal	2.0	5.0
Swaziland - highlands	2.2	5.1
Kenya - medium altitude	2.9	5.8

Swaziland - medium altitude	4.0	8.9
Kenya - lowlands	4.2	10.0
Thailand - ratburi	6.0	45.0
Swaziland - lowlands	9.7	43.1
Mozambique - inhambane	13.0	222.4

Source: S.J. Van Rensburg p699, in R.J. Rodricks, C.W. Hesseltine M.A. Mehlamann: Mycotoxins in Human and Animal Health. Path. tox. Fubl. Inc. Fark Forrest, IL, 1977.

The following inferences can be drawn :

a. **Highland areas are relatively dry and thus mold infection are reduced producing low level of aflatoxin.**
b. **The daily aflatoxin intake in these regions is less than 5 mg/kg body weight. If the daily intake is doubled to 10 mg/kg as observed in several humid lowland areas (Kenya), the incidence of liver cancer increased by 100.**

This information is highly pertinent to our conditions and the findings on toxigenic mould and aflatoxin content in corn. In our studies, aflatoxin content and A. flavus propaguels are found to be increased in lowlands than in the highlands. However, there is dearth of information with regard to liver cancer cases according to altitude ranges. However, it reflects two main issues to be considered.

a. **Consumption pattern of two risk commodities (corn and peanut) in different area.**
b. **Liver cancer cases amongst the consumers of this area.**

In Nepal, most people living in hills consume corn as the staple food. But Terai is the risk area for aflatoxin production, Thus the magnitude of problems concerning production, storage, and consumption of risk commodities in different areas have to be carefully determined to monitor the level of toxin present in food and the probable mycotoxin cases in different areas.

Future Course of Action:

1. **To prepare aflatoxin mapping of different food commodities at different localities.**
2. **To initiate monitoring of other mycotoxins (Ochratoxin, Zeralenone etc) in view of their causative fungi for possible intervention.**
3. **To introduce efficient post-harvest technology systems through existing networks.**
4. **To employ mould inhibitors for effective conservation and control of aflatoxin production.**
5. **To communicate crucial matters of health hazards to farmers, grain handlers, and marketing people for exhancing safety & minimising losses.**
6. **To enforce the regulation of aflatoxin level in foods and feeds.**
7. **To use heat treatment (roasting) for decontaminating aflatoxin level in foods through extension system.**

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Contents - Previous - Next

Home"" """"> ar.cn.de.en.es.fr.id.it.ph.po.ru.sw

The prevention and control of mycotoxins in Thailand

Contents - Previous - Next

Mycotoxins research in Thailand during the 10 years has concentrated on aflatoxin with particular reference to maize and peanuts. Maize is the second crop next to rice which is mainly produced for export purposes. The aflatoxin content has become a mayor factor affecting the export of maize in a recent years. At present, such problem seems to be under control after the Government of Thailand has brought the problem into focus in local traders and provided more support in both management and technical aspects.

INCIDENCE OF AFLATOXIN CONTAMINATION IN THAILAND

Based on the testing of 90 samples pre-harvest aflatoxin contamination of

maize was found to be negligible in five Thai provinces from 1980 to 1983. A recent joint Department of Agriculture-UK based on 46 maize samples collected from two provinces during the major harvest period in the 1985 rainy season, confirms this low incidence of pre-harvest contamination, where a mean total aflatoxin content of less than 4 ppb and a range of 0 to 27 ppb. were found. Interestingly, most of the 46 batches monitored at harvest had been dried in the field from one to four weeks, but this did not result in any significant aflatoxin contamination. In the rainy season of 1986, a zero level of aflatoxin contamination has been detected from 4 experiment plots in 3 provinces, according to the "On-Farm Testing Program" run by Kasetsart University researchers.

Storage of undried ears, especially temporary farm storage for one to six weeks before the crop is sold, has been identified as the significant source of initial contamination. A mean aflatoxin content of 45 ppb with a range of 10 to 95 ppb was found in ten samples of freshly shelled maize in 1984, and an aflatoxin level of 74 ppb, with a range of 3 to 299 ppb, was found in 19 samples from two provinces in 1985. Effective aflatoxin control measures are needed soon after harvest, preferably within 48 hours. Although the critical initial

contamination occurs during ear storage, high levels of aflatoxin contamination are usually associated with delays due to incidence of rain during sun drying, and in transportation or storage systems of either undried or partially dried grain.

AFLATOXIN RESEARCH

All new aflatoxin research is coordinated by the National Committee on Mycotoxin Control in Agricultural Commodities and its three subcommittees, which cover the areas of research and development, extension and marketing. The committee is composed of representatives drawn from both the private sector and the government, including the Cooperatives Promotion Department, Office of Agricultural Economics, Department of Agriculture, Department of Agricultural Extension, Department of Foreign Trade, Board of Trade of Thailand, Bank of Agriculture and Agricultural Cooperatives and the National Economic and Social Development Board.

The duties and responsibilities of the committee are to:

- **Publicize the nature and importance of aflatoxin, especially with regard to export commodities;**
- **Devise and evaluate effective aflatoxin control measures applicable to each stage in the marketing chain, and then vigorously promote these techniques through radio, television, newspaper advertisements, leaflets and posters;**
- **Coordinate aflatoxin research and maximise collaborative studies to increase efficiency and reduce excessive duplication of projects;**
- **Encourage mechanical drying of maize upcountry to minimize delays in drying wet grain to a safe moisture content, and hence reduce the risk of aflatoxin contamination; and**
- **Regularly monitor the aflatoxin content of maize and peanuts intended for export, so that any problems can be recognized early and remedial action can be taken promptly.**

STUDY ON THE ENVIRONMENTAL CAPABILITY OF THE PATHOGEN FOR AFLATOXIN PRODUCTION IN MAIZE

The experiment was conducted to determine the effects of various times and moisture contents on aflatoxin levels in storage conditions. The results indicated that after one week and two weeks 35 ppb and 25 ppb of aflatoxin were detected at the levels of 26, 30% and 14% of moisture content respectively. However, after one month of storage no aflatoxin was detected at 11 % moisture content.

METHODS OF PREVENTING AFLATOXIN

Method of preventing aflatoxin were studied including mechanical drying, chemical treatment and planting time.

Mechanical Drying

A Batch Type system has been tested and found that this method is practical. However, the drying process is slow while batches of maize grain should be placed in the dryer within 48 hours.

Chemical Treatment

Only ammonia and a mixture of ammonium polypropionate and propionic acid at a ratio of 8: 2 were economically effective in preventing aflatoxin contamination.

Planting Time

Maize can be planted all year round but harvesting time should coincide with dry season to avoid aflatoxin contamination.

In-Field Drying

Leaving mature maize in the field for 2-4 weeks before harvesting has proved that aflatoxin formation was minimised. This method enhanced the reduction

on costs for drying. The second crop could be grown immediately after cutting off the top.

PRACTICAL APPLICATIONS OF RESEARCH RESULTS

Results from a joint project between the government and private sectors on prevention of aflatoxin maize at Amphur Phayuhakiri, Nakhon Sawan Province are as follows:

Delaying the Time of Maize Cultivation to Avoid Aflatoxin Contamination

Soybean was planted in May, 1986. After harvesting soybean, maize was planted in August. Aflatoxin prevention in maize was successful because maize was harvested in December when the moisture content was relatively low (i.e. < 14%) due to recent drought.

Drying

The experiments to control aflatoxin were conducted by drying 18 tons of maize grains for 3 hours in 3 times. The results showed that the moisture content of seeds was 30-32% before drying and 13-16% after drying. No increase in aflatoxin content was detected before and after the drying phase.

Chemical Treatment

Sixty metric tons of maize seed treated with ammonium polypropionate + propionic acid in a ratio of 8:2 under 22:25% relative humidity showed no increase in aflatoxin content during the 6 weeks post treatment.

A training program for aflatoxin control was organized for officers from the Agricultural Extension Department, the Bank of Agriculture and Agricultural Cooperatives, Department of the Cooperative promotion, as well as representatives from the Thai Maize and Produce Traders Association.

The recommendations for aflatoxin monitoring and control were published and distributed to farmers, local dealers and exporters, etc.

The addition to the control of aflatoxin in Agricultural Food Commodities level of toxin contamination was established by the Food and Drug Adminstration (FDA) of Thailand. According to the relevant notification of the Ministry of Public Health prescribed by virtue of Food Act B.E. 2522 (1979), foods shall not contain any microbiological toxin or metabolic products in a manner that may be harmful to health. The maximum allowed level of aflatoxin in oil, fat, peanut oil and coconut oil is 20 ug/kg.

In respect to commodities for export, the level of aflatoxin may follow any requirement (imposed by the importing country), which might also be aligned with the standard level recommended by the Codex Committee on food additives.

ANALYTICAL SERVICES

Aflatoxin analysis is routinely done at a number of laboratories in Thailand. Unfortunately, sampling methods, sample preparation and analytical methods

vary widely, although efforts are being made to standardise 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 thinlayer chromatography (TLC). Sophisticated techniques, such as high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC), are being introduced.

FUTURE RESEARCH

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

- **Continued work on inhibition of aflatoxinproducing fungi by chemical treatment;**
- **Aflatoxin detoxification;**
- **Evaluation of the UTP system for producing lowaflatoxin 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 semiquantitative, 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 to provide funding, training and staff is still needed; 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 provide 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 Agricultural Research Centre (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 Agricultural Research Centre supplies senior researchers, training, analytical

equipment and software.

In 1986-1991, Japanese International Cooperative Agency (JICA) agrees to finance Maize Quality Improvement Center at the Department of Agriculture a budget which comprise of 66% for aflatoxin prevention. The total budget committed was for building and sophisticated equipment at a cost of approximately 100 million baht.

Contents - Previous - Next

Home"" """"> ar.cn.de.en.es.fr.id.it.ph.po.ru.sw

Mould infection and aflatoxin contamination in foodgrains in Vietnam

Contents - Previous - Next

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Vietnam is a tropical agriculture country. The climate conditions are favourable for the year-round agriculture practice but at the same time they make a good environment for moulds and insects to develop. Moulds proliferation leads to the production of mycotoxins and to the spoilage of food, both in quantity and quality.

The study on mycotoxins and methods for their prevention and control has become a prominent problem in our country and in recent years it retains the interest of our scientists. Lucas et al. determined the content of aflatoxin in rice in different stores in Saigon (1971), Bui xuan Dong did some preliminary researches about moulds infection in groundnut in Hanoi stores and poisoning effect of moulds that produce mycotoxins in ducklings (1976). Pham van Phung, Nguyen Lan Dung and Nguyen van Ngoan reported that from 15

strains of isolated Aspergillus flavus, 6 have the potential of aflatoxin production. Le van To reported about mould infection in animals feed: 7 from 9 strains isolated produce mycotoxins and the perspective to use Athrobactor sp. to test the activity of Dicoumarolmycotoxin. Veterinary Doctor Nguyen van Phong (1984) found degenerative lesions and tumors in liver of ducklings and ducks suspiciously died from mycotoxin poisoning (1984).

These facts clearly showed that in Vietnam, there are problems of mould infection and consequently aflatoxin contamination on foodgrains.

Thanks to the encouragement of FAO and UNDP and their supply of analytical standard reference samples of aflatoxin, our Post-harvest Technology Institute could carry out well a national research subject about the level of mould infection, methods of elimination of moulds and about aflatoxin contamination on foodgrains.

The title of the research is "Study on the level of moulds contamination on foodgrains and methods for prevention of mycotoxins caused by moulds".

Research was carried out on three main kinds of foodgrains: paddy, rice and maize, aiming at the following objectives:

1. **Determination of moulds infection in foodgrains in Vietnam especially of the moulds that produce mycotoxins.**
2. **Preliminary application of some processes for moulds prevention and control in the warehouses.**
3. **Determination of Aflatoxin contamination on foodgrains in Vietnam.**
4. **Selection of appropriate methods for determination of Aflatoxin contamination level on foodgrains in order to establish the standard level of permitted aflatoxin contamination on human food and animal feed in Vietnam.**

Several obtained results are as follows:

MOULDS INFECTION IN RICE GRAINS

- **The paddy grains of rainy crop (Summer-Autunm crop) stored for 3 months were used as sample for the study, the moisture content from 15, 7 to 17%. We have isolated moulds from Aspergillus and penicillis. A. flavus infected is 20% and A. fumigatus 15% .**
- **With the rice samples that are infected by moulds, we have isolated 21 species from Aspergillus, penicilia, curcularis, aureobasidius and cladosporium. A. flavus infection is about 28%.**

MOULDS INFECTION IN MAIZE GRAINS

Maize harvested in rainy season is very difficult to be dried and stored properly. Therefore, it could be infected by moulds with a very high percentage. In many tested samples, nearly 100% were infected by moulds.

STUDIES IN SOME METHODS FOR PREVENTION AND CONTROL OF MOULDS IN

WAREHOUSES

Methyl bromide and phosphine are two common chemicals usually used for grain disinfection from insects. We tried to use these substances to kill moulds in an attemp to combine with the disinfection effect on moulds as well as on insects. The results showed that:

- **Methyl bromide: In laboratory conditions, the dose of 32 b/m can kill 100% of moulds. Unfortunatelly, in field conditions, only 78-82% of moulds were killed. This lower result is due to the chemical adorption by the grain mass. In field application, we used doses of 40 mg/m and 48 mg/m. Both doses have similar results to the 32 mg/m dose but 48 mg/m can kill 94-98% of mould in the foodgrains warehouses with the capacity of hundred tons.**
- **Phosphine: dose of 50 g/m can kill only the growth period of moulds and cannot kill Spores of moulds.**

By using the mixture of phosphine and amoniac, at the dose of 50 mg/m +50 mg/m, we found that all the moulds were killed. Thus, we can use methyl

bromide and the mixture phosphine and amoniac to disinfect paddy grains in big capacity warehouses.

AFLATOXIN CONTAMINATION IN FOODGRAINS IN VIETNAM

- **On the 35 rice samples, tested by using DD. Doromina and L Makaimenko method, we were not able to detect Aflatoxin presence.**
- **On mould infected maize samples, we discovered aflatoxin with high level > 19 ppb. Yellow maize is infected more heavily than white maize. Using the analytical reference standards of aflatoxin given by FAO, we can test aflatoxin in exported foodgrains.**

We believe that with the results obtained from this training course on new methods for testing aflatoxin and with further assistance of FAO/UNDP and RAS/86/189 project we could intensify our control activities of foodgrains including the control of aflatoxin.

On behalf of all staff members of Post-harvest Technology Institute and its branch in Ho Chi Minh City, I would like to express once again our grateful acknowledgements to FAO/UNDP and Project RAS/86/ 189 and to all our colleagues here. We hope that our cooperation will be well developed.

Thank you very much.

Contents - Previous - Next

Home"" """"> ar.cn.de.en.es.fr.id.it.ph.po.ru.sw

Mycotoxins in foodgrains in some Asian countries

Contents - Previous - Next

JOINT FAO/WHO/UNEP SECOND INTERNATIONAL CONFERENCE ON MYCOTOXINS

Bangkok, Thailand, 28 September - 3 October 1987

Marianne Flach
FAO Regional Network on Grains Post-Harvest Technology and Quality Control, Bangkok

INTRODUCTION

The FAO Project "Inter-Country Cooperation in Post-Harvest Technology and Quality Control of Foodgrains", located in Bangkok, is operating since 1983. Thirteen countries in Asia are members of this Network: Bangladesh, Burma, China, India, Indonesia, Republic of Korea, Malaysia, Nepal, Pakistan, Philippines, Sri Lanka, Thailand and Vietnam.

In 1986 a questionnaire on Grain Quality Control was sent out to the member countries, as a part of the activities of the Network. Part of the aims of this questionnaire was to find out concerning mycotoxins:

* The current situation in the region with respect to the major grains in each country;
* The existing quality criteria for the major foodgrains in the region at post-harvest level.

The National Coordinators in each member country were asked to supply information countrywide or on provincial basis which would indicate the relative distribution and degree of contamination in each country. With the collected information recommendations were made in order to avoid duplication of work and make full use of already to existing information.

This paper will give an overview on the work that was done in Asian countries concerning mycotoxins. It appears that since the First International

Mycotoxin Conference, 10 years ago, a lot of work has been undertaken. However, it focusses in all countries on aflatoxin. Furthermore, it should be stressed here that the gathered information is not complete:

* Information is only given for the Network member countries and

not for the whole of Asia;
* Information is only given for foodgrains;
* There may be more information on research going on in the member countries, which we are not aware of, especially when reports are written in the local language.

Nevertheless, the available information is quite substantial, and it is possible to draw general conclusions and make recommendations.

AFLATOXIN IN FOODGRAINS IN SOME ASIAN COUNTRIES

Introduction

In Table 1. an overview is given of results of research and monitoring programmes in the different countries. Laboratory trials are not included, since the questionnaire focussed on the current situation concerning mycotoxins. Data are available for 11 of the 13 member countries, Burma and

Vietnam do not have reports available.

TABLE 1. AFLATOXIN CONTENT OF VARIOUS GRAINS IN SOME ASIAN COUNTRIES, BASED ON FIELD STUDIES

TABLE 1. AFLATOXIN CONTENT OF VARIOUS GRAINS IN SOME ASIAN COUNTRIES, BASED ON FIELD STUDIES (continued)

TABLE 1. AFLATOXIN CONTENT OF VARIOUS GRAINS IN SOME ASIAN COUNTRIES, BASED ON FIELD STUDIES (continued)

The foodgrains most at risk are maize, peanuts and parboiled rice. Polished rice, not parboiled, seems to be relatively free from aflatoxin, but it appears that aflatoxin is concentrated in the bran layer (1). However, not much information is available on this, and none of the countries reported on it. Unrefined peanut oil as a processed product was mentioned by some countries to be contaminated with aflatoxin.

Most of the work in the region consisted of surveys to indentify the problems. Considerable work also has been put into methodologies for the detection and quantification of aflatoxin. However, sampling, sample handling and analysis is not standardised, and this makes interpretation and comparison of results difficult. Conditions in laboratories may not always be safe enough. Some countries have done studies on finding out at which stage in the post-harvest handling of foodgrains contamination is likely to occur and on how to prevent it rather than destroying the toxin. The results of those studies will be very valuable for other countries as well. A few countries have set up maximum levels for mycotoxins in grains.

In the following paragraphs country situations will be described for each commodity at risk. Based on this information recommendations will be made for future activities.

AFLATOXIN IN MAIZE IN SOME ASIAN COUNTRIES

Introduction

The top producers among the member countries are China, India, Thailand, Indonesia and the Philippines. Thailand and Burma export maize. The other countries except Pakistan are importing maize. The percentage of production retained at the farm varies. About half is retained by the farmers in Pakistan and Sri Lanka but 90% in Nepal. In Thailand however, two thirds is for export, the remainder is marketed locally, mainly for feed purpose.

China

Maize is considered to be a high risk commodity in China. The main cause is the absence of drying facilities and harvest during the rainy season. Research work has been done on aflatoxin, and also on other mycotoxins, however, at this moment there are no reports available in English.

India

In India, a study done in 1982 (3), shows that 33 out of 100 maize samples

contained aflatoxin up to 321 ppb. Six out of 10 maize flour samples had aflatoxin levels upto 179 ppb.

Indonesia

The mean aflatoxin content of maize in North Sulawesi is significantly higher than in other provinces, however, the mean aflatoxin levels in other provinces is already above 100 ppb. As maize is the second staple food in certain regions of Indonesia (e.g. South Sulawesi, where per capita consumption per day can be 300-500 g), these levels give rise to considerable concern.

Another study (7) compares aflatoxin content in maize samples in East Java, North and South Sulawesi and relates it to drying and storage practices. It was found that in both North and South Sulawesi levels were higher than in East Java. In North Sulawesi; where the levels were highest, farmers usually leave the maize intact with the plant in the field for drying, for one month, after maturity. No drying is required before shelling, but fungal and insect damage occurs. In South Sulawesi farmers may store the maize for 1-2 years before selling. Although the maize is stored on the cob, the long period may enhance

aflatoxin formation.

Nepal

During 1980-85 101 samples of maize and maize products were analysed for aflatoxin content. Thirty samples had aflatoxin B1 in the range of trace to 321 ppb, with 10 samples above 30 ppb (12).

In 1983-84 a study was done on aflatoxin content of maize samples from different periods after harvest (13). The data show that:

- **Aflatoxin content at harvest is low;**
- **Aflatoxin content after some period of storage is lower in maize cobs than in shelled maize, and highest in maize flour;**
- **The contamination problem is more serious in the Terai area, which has a warm, humid climate.**

One of the main problems causing mould growth is the fact that most of the maize, especially in the Terai area, is harvested during the rainy season and

farmers do not have drying facilities. Table 3. shows some data. Farmers usually store for up to 1 year, as maize is an important staple food in Nepal. About 90% may be retained at the farm, there is little marketable surplus. The three different storage types, namely outside on an elevated structure covered by straw, hanging under the eaves of the house, and inside the house in baskets, have not been studied in relation to aflatoxin, but it may be worthwhile to find out which type is more prone to aflatoxin formation and how it can be improved.

Pakistan

During 1980-85 a study was done, during which samples were collected, from local markets, government stores etc. Maize and maize products mainly came from Lahore. Fifty-three samples were analysed, 14 (26%) contained aflatoxin in the range of 133-800 ppb (14)

Philippines

A study was done in 1982 to determine the effects of different drying methods

on aflatoxin contamination of maize (22). The drying methods compared were mechanical drying, sun drying and crib drying. Both white and yellow maize were analysed. The 20 ppb level was reached in the white variety after 40, 35 and 15 days from harvest for the above mentioned methods respectively. For the yellow variety this level was attained after 35, 15 and 10 days.

The level of aflatoxin in maize differs for the various regions is the Philippines. This is apparently due to marketing, climatological and agricultural factors. For instance, one of the major maize producing areas, Cagayan Valley, consumes only maize that it produces and mills, and sends the surplus directly overland to other areas. The market flow is simple and involves few middemen. Moreover, harvesting is during the dry season. Average aflatoxin levels are in the range of 1221 ppb A second major maize producing area is South Western Mindanao. Here the maize is harvested during the wet season. Millers throughout Mindanao cannot cope with the maize supply and, therefore, part of the maize is shipped to Cebu for milling. Thereafter, the milled maize will be reshipped to Mindanao or onwards to other islands. This market flow involves several middlemen and delays. However, average aflatoxin levels are not yet alarmingly high, although above

acceptable level, namely 60 ppb (16).

A study at on-farm level of maize handling states that shelled maize should be dried immediately to a safe level in order to keep aflatoxin content within safe levels. If immediate drying is not possible, than grains should be stored in unshelled form with husk, with adequate aeration for not longer than a week. At offfarm level, e.g. at trader level, it appeared that maize may be heavily contaminated already at the moment of arrival (23).

Thailand

Several studies have been done concerning aflatoxin in maize. In general it can be said that in Thailand, as in other countries in Asia, aflatoxin at harvest level is low. Two months storage at farm level does not necessarily result in high aflatoxin level, one study shows that after two months the aflatoxin content was 21 ppb. However, much depends on climatic conditions during harvesting, most of the maize is harvested during the rainy season. Also the conditions of the store are important. At silo and middlemen level aflatoxin content is often around 100 ppb (19, 20).

An interesting project is being undertaken in Thailand to combat the problem of aflatoxin in maize, a bilateral project with the UK. Results revealed the following during Phase l:

> *** Aflatoxin contamination at harvest is low, but increases significantly during storage;**
> *** Mechanical drying is capable of "freezing" aflatoxin levels provided that the drying period does not exceed 48 hours;**
> *** Both mechanically and sundried maize can be stored for at least 2 months without any increase in aflatoxin.**

During Phase II low aflatoxin maize was successful produced in the rainy season by mechanically drying freshly harvested maize, which was field-dried 1-4 weeks prior to harvest. Another test during Phase II showed that one-stage drying is able to produce low aflatoxin maize, whereas two-stage drying is too slow. Farmers prefer to sell their maize as quick as possible after harvest, however, marketing problems may force them to store the maize although they might not have good storage facilities. Therefore, like in the Philippines, maize arriving at the primary merchant may have a high aflatoxin

level already. Another problem is that with the existing pricing mechanism in Thailand there is no incentive for farmers and primary merchants to dry their maize below 18% m.c.

At present the project is in the third phase. Emphasis is now on producing low aflatoxin maize by giving premiums. Steps are undertaken together with the private sector (feedmillers) to include aflatoxin content in the price determination (24, 25).

AFLATOXIN IN PEANUTS IN SOME ASIAN COUNTRIES

Introduction

The main producers of peanuts or groundnuts are: India, China, Indonesia, Burma and Thailand. Peanuts are mainly used locally as food, either fresh or processed (edible oil, flour, peanut butter, crackers, candies etc.). Little is used in the member countries for feed. The quantity retained at the farm is

more than 90% in Indonesia and Korea, in other countries not more than 35%.

China

Both peanuts and peanut oil (unrefined) may contain high levels of aflatoxin. The major cause seems to be rain during harvest and no drying facilities. This is especially so in the southern part of China. Contamination also occurs in a later stage, during storage. No exact data are available since there are no reports in English.

India

One study reveals that out of 124 shelled peanut samples 19 (15%) were found to have aflatoxin upto 120 ppb (3). More studies have been done but data are not available at present.

II.3.4 Indonesia

Peanuts are considered to be a high risk commodity in Indonesia. It was found

(5) that contamination of peanuts usually happens at the retailer stage, and it is explained by the fact that retailers keep the nuts in uncovered containers, and they may keep the nuts for quite some time. The survey was conducted during the dry season, and levels of aflatoxin B1 at retailer stage varied from 7-2000 ppb, while at distributor stage the level was 7 ppb.

The effect of home storage on aflatoxin in peanuts has also been studied (5) and it appeared that the first traces of aflatoxin were detected only after 10 weeks of storage, but thereafter the level increased rapidly to 912 ppb aflatoxin B1 and 740 ppb aflatoxin G1 in the 28th week. However, nothing was mentioned about the way the peanuts were stored.

Another study (26) reports varying levels of aflatoxin content in peanut samples, see Table 4. Fresh and dried, unshelled peanuts contain no aflatoxin, salted peanuts had low levels and press-cake and shelled peanuts may have alarmingly high levels.

Malaysia

Several monitoring programmes on aflatoxin have been carried out between 1976 and 1984. Peanuts with shell show no or low levels of aflatoxin, shelled peanuts may sometimes have high aflatoxin levels, and among peanut products also high levels may be found, e.g. peanut butter (locally made contains more than imported), peanut candy, peanut cake and peanut gravy.

A wide variety of Malaysian dishes uses peanut as one of the ingredients and further research should be emphasized in order to trace at what stage contamination occurs and how to prevent it (11).

Pakistan

A survey was done on aflatoxin in food and feed stuffs in Pakistan during 1981-85 (14). Samples of different commodities were collected from local markets, government stores etc. Samples came from different areas of Pakistan. Forty-two samples of peanuts were analysed, and 10 contained aflatoxin B1 and B2, in the range of 24-80 ppb.

Thailand

Peanuts are widely used in Thailand, as food snacks, feed and in the production of vegetable oil. A survey was done in 1982 to study the incidence of aflatoxin in peanuts. At harvest time the aflatoxin content is still low, but shelled peanuts have a rapidly increasing aflatoxin level. Some unshelled samples however could also have an aflatoxin level of 75 ppb, even one week old samples. All peanuts were collected from middlemen stores. Peanuts are at present harvested during the rainy season and no artificial dryers are available with farmers or middlemen (21).

AFLATOXIN IN PARBOILED RICE IN SOME ASIAN COUNTRIES

Introduction

Milled rice usually contains no or very low levels of aflatoxin. However, several countries (Bangladesh, India, Sri Lanka) make note of sometimes high aflatoxin levels in parboiled rice. It is mainly the Indian subcontinent where parboiled rice is consumed, and Thailand exports parboiled rice. Only one

survey in Sri Lanka, tries to relate the process of parboiling to aflatoxin formation and is therefore mentioned here.

Sri Lanka

An extensive survey (17) was done on the occurrence of aflatoxin in parboiled rice and raw processed rice in the major rice producing areas of Sri Lanka. A total of 485 samples were analysed for aflatoxin B1 and G1. Results are shown in Tables.

Table 5. Mean aflatoxin content of raw and parboiled rice in Sri Lanka, 1984

District	Mean aflatoxin content (ppb)			
	Raw rice		Parboiled rice	
	B1	G1	B1	G1
Anaradhapura	49.0	493.2	185.5	963.2
Colombo	0	12.0	92.5	360.5

Galle	4.7	19.2	70.3	192.8
Jaffna	0	24.0	92.5	240.4
Kandy	17.0	10.5	85.0	320.2
Nuwara Elija	0	9.5	60.3	175.6

The high level of aflatoxin G1 in raw rice in Anaradhapura cannot be explained here. All parboiled rice samples show high levels of aflatoxin. It was stated in the report that commercial parboiling resulted in higher levels of aflatoxin than household parboiling. Household parboiled rice is cleaner and odourless since the process is under careful control. Commercial parboiling, however, means sometimes soaking in cold water for 2 to 4 days before steaming, and it seems that the water is very often being reused, resulting in mouldy paddy with a strong fermented odour.

II.5. QUALITY CRITERIA FOR MYCOTOXINS IN GRAINS

China, India, Malaysia, Philippines and Thailand have established national maximum levels for aflatoxin:

China:	**Maize & products**	**20 ppb**
	Rice	10 ppb
	Legumes	5 ppb
	Infant food	0 ppb
India:	Food	30 ppb
	Peanut meal (export)	120 ppb
Malaysia:	Food	35 ppb
Philippines:	Food	20 ppb
	Feed	200 ppb
Thailand:	Food	20 ppb

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. **Research and monitoring programmes focus in all countries on aflatoxin.**
2. **Commodities most at risk concerning aflatoxin contamination are maize, peanuts, and parboiled rice, and levels found are often high.**
3. **Considerable work has been put into methodologies for the detection and quantification of aflatoxin, however, sampling, sample handling and analysis is not standardised. Care should be taken in interpretation and comparison of results.**
4. **Some countries have done studies on finding out at which stage in the post-harvest handling of foodgrains contamination is likely to occur and how to prevent it rather than destroying the toxin. For instance, it can be concluded that:**

*** In many countries maize and peanuts are harvested in the rainy**

season, and no drying facilities are available.
*** In Nepal most contamination occurs during on-farm storage, and susceptibility to aflatoxin formation is highest in maize flour, followed by shelled grains and lowest in cobs with husk.**
*** In Thailand maize is usually for export, and farmers only keep maize at the farm while awaiting better prices. Most farmers, however, sell quickly, and prevention of aflatoxin formation could be achieved by: quick mechanised drying at primary merchant level, fast marketing chain and premiums for aflatoxin free maize.**
*** Contamination of peanuts in Indonesia often finds place at retailer stage, since they keep the peanuts for long time in uncovered containers. However, more information is needed.**
*** Contamination of parboiled rice probably takes place during the soaking and drying process. However, more information is needed.**

Recommendations

1. **Setting up and coordinating of research and monitoring programmes on other mycotoxins.**
2. **Standardization of sampling, sample handling and analysis.**
3. **Testing of economic viability and acceptability of existing drying technologies.**
4. **Developing a field kit for analysis.**
5. **Improving of on-farm storage facilities.**
6. **There is a need for more coordination and streamlining of programmes in Asian countries. More use should be made of each others experiences and capabilities.**

The "ASEAN Mycotoxin Expert Group on the Formulation of an ASEAN Aflatoxin Control Program" recommended the establishment of an ASEAN Mycotoxin Regional Training Centre and Reference Laboratory. Funding was requested Through the ASEAN Food Handling Bureau from the EG However, up till now the proposal was not yet approved. It may be worthwhile to consider the establishment of a Regional Training Centre not only for the ASEAN Region, but for Asia and Pacific countries as a whole.

In this respect it may be worthwhile to mention that a similar centre for pesticides: "Regional Pesticide Training Centre and Service Laboratory" for Asia and the Pacific Region was proposed for setting up in Chiang Mai in Thailand.

It may be considered to, instead of setting up separate laboratories and centres for pesticides and mycotoxins, to set up a regional centre for quality control as a whole.

Table 2. Mean and highest incidence of aflatoxin contamination of maize by province, Indonesia, 1983 (6)

Province	No. of samples	Incidence positive (%)	Aflatoxin B1 level (ppb)	
			mean	highest
East Java	15	100	149	390
Lampung	12	92	144	350
North Sulawesi	12	100	464	790

South Sulawesi	12	100	108	250
West Nusa	12	83	186	1140
Tenggara				

Table 3. Aflatoxin content of maize samples related to product In Nepal, 1983-84

Area	**Product**	**No. of samples**	**No. with aflatoxin**	**Range (ppb)**
Kathmandu Valley				
+ Hills	harvest level	11	0	—
"	cobs, stored	26	2	tr - 30
"	shelled grains, stored	44	6	tr - 10
"	flour, stored	30	13	tr - 546

Terai Area	harvest level	8	0	
"	cobs, stored	32	6	10 - 215
"	Shelled grains, stored	64	27	tr - 6382
"	flour, stored	46	33	tr - 1418

Table 4. Aflatoxin In fresh peanuts and peanut products in Indonesia, 1984

Food Item	**No. of samples**	**No. with aflatoxin**	**Aflatoxin content (ppb)**	
			B1	**G1**
Fresh peanuts	11	0	0	0
Dried, unshelled peanuts	6	0	0	0
Salted peanuts	6	4	0-28	0

Press cake	6	6	46-3080	0
Shelled peanuts I	14	4	20-1262	0
Shelled peanuts II	6	3	2 7	3-7
Shelled peanuts III	8	2	0-30	6-8
Shelled peanuts IV	4	4	7 882	0 110

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Contents - Previous - Next

Home"" """"> ar.cn.de.en.es.fr.id.it.ph.po.ru.sw

Reduction of post-harvest losses in stored grains

Contents - Previous - Next

SUMMARY OF MISSION REPORT UNDER

UNDP/FAO/THA/82/004

These were the most often observed practices at the farm level that contribute to the high level of aflatoxin. Harvesting and storing moist grain for a considerable time in mould infested storage bins summarise most often followed by Thai farmers. Changes in these areas should lower the aflatoxin contamination considerably. If the maize moisture content is high, every effort should be made to keep the grain as cool as possible.

Silo and exporters

By the time maize reaches terminal silo, the damage to the maize with respect to aflatoxin is history. The silos visited are well managed storage facilities and do not offer a conducive environment for fungal growth. The terminal silos practice sound and effective measures to prevent grain deterioration. It is this consultant's opinion and many commentors' that silo grading of income grain is not according to the official method established by the Thai government. The official grading standard seems to be subject to change without notice throughout the marketing system. The official grading standard is influenced

by present supplies, demands and prices. Maize grades used, such as super A, by the terminal silos do not exist in the official grading standard published by the Thailand government.

Within the past five years, the terminal silos have greatly increased the storage and drying capacity, mainly to meet international maize standards required for moisture content. However, most of the

increased capacity is concentrated at or near point of export. If some of the drying capacity was centered inland near the major maize production area, a high percentage of the maize could be dried before mould growth occurs. The use of large highly efficient dryers, presently located at the end of the marketing system, could be very cost-effective method in reducing mould growth in Thai maize. Under the present system, moist maize is transported and then dried. The better system would be to dry the maize and then transport to the terminal silo. Under this system, the maize will be preserved before transporting. The installation of regional high efficient dryers should produce a high quality maize that is free of aflatoxin contamination and would demand a premium price on the international export market.

Exporter Importer Interface

Many exporters have commented that maize determined by analysis to be low in aflatoxin contamination at time of export, but when received by importer, had significantly higher level of aflatoxin (i.e. increased from 20 PPB to 100 PPB). This could be caused by a number of factors on the exporter side, improper sampling, improper analysis, or an increase in aflatoxin contamination anywhere along the exportimport interface. Due to tremendous economic gain or loss that can occur when the price of maize is related to level contamination, it is highly recommended that proper sampling and analysis be used before the Thai maize is exported.

Contents - Previous - Next

Home"" """"> ar.cn.de.en.es.fr.id.it.ph.po.ru.sw

Inspection, sampling and analysis of maize and

groundnuts for aflatoxin

Contents - Previous - Next

by George M. Ware

Farm Level

The effect of environmental and cultural factors on aflatoxin contamination in maize have been studied and have identified the 5 major factors that affect the development of toxin-producing fungi; inoculum level, moisture, temperature, time, and damaged kernels, Each of these variables play a complex role in toxinproducing capability of fungi. From the farmer prospective the following practices are major contributors to the overall high aflatoxin level in maize.

Moisture

1) Harvesting before maturity where the moisture of the grain is high (greater than 25%).

Damage

2) Shelling grain with high moisture content causes severe hernel damage.

Time

3) Temporary storage of moist grain (very common) (Known as bulking up in the maize trade)

Inoculum Source

4) Storage of grain in mould infested bin (last year mould spores will infect this year crop). A bottle of bleach and water will reduce the inoculum level in many storage bins and it is inexpensive.

In most cases this will require more than one sample and one analysis. Because of the variability associated with test methods for aflatoxin in maize, analyses

of samples from good lots may indicate that the lots are bad (low level of aflatoxin); (exporters' risk) and analyses of samples from bad lots (high level of aflatoxin) may indicate that the lots are good (importer's risk). Testing program for aflatoxin has been developed for maize in the United States. This testing program involves taking a 4.5 kg. sample from a lot, grinding the entire sample and subdividing into five 50 9. samples. The aflatoxin is solvent-extracted from the maize meal by the AOAC CB method. If the analysis of one sample is less than 20 PPM, the lot is accepted. Otherwise, two more samples are analyzed. If the difference between each of the 3 analyses and their average (A) is less than 0.3 A. The lot is accepted when a is less than 25 PPB and the lot is rejected when the average is greater than 25 PPB. When any one of the 3 analyses is not within the range of (A 0.3 A) PPB. the fourth and fifth samples are analyzed. The lot is accepted when the average of the 5 anlyses is greater than 25 PPB.

Even with this elaborate test program, the exporter still has a 1% probability of making a wrong decision based on the results of 5 analyses, and the importer has a 7.3% probability of making a wrong decision.

Decisions based on mini-column method, that is not qualitative, will expose the exporter to a great risk and likelihood of being wrong with respect to the amount of aflatoxin in particular lot of maize being exported. Also, the importer has the same increase risk of being wrong using a non-quantitative method.

Special Precaution and Decontamination Procedures

Handle aflatoxins as very toxic substance, use gloves when handling standards. Perform manipulations under hood whenever possible. Take special precautions with toxins in dry form because of electrostatic nature and tendency to disperse in work area. Decontaminate all volumetric flasks used in making standard solutions and all vials containing concentrated sample extract or reference standard solutions by the following procedure. Prepare decontamination solution by adding 3 ml of sodium hypochlorite solution (household bleach) per microgram of each aflatoxin. After mixture is left for several hours or overnight, an equal volume of 5% aqueous acetone is added.

After 30 minutes, the mixture may be safely discarded. Oils and solids extracts should be shaken well with the sodium hypochlorite solution. Large volume of organic solvent should be evaporated and then add the sodium hypochlorite to residue. It is very important to add the acetone to sodium hypochlorite solution. One of the products of treatment of waste containing aflatoxin b1, -2, 3 dichloride, which is also carcinogenic. Rinsings of glassware and TLC plates should be done in sink in washer room to minimize bleach use in area where aflatoxin analysis is ongoing.

Use dust mask when grinding grains, feeds or any other products generating dust into atmosphere during preparation. Sample preparation area should be specially cleaned after preparation of sample containing high levels of mycotoxin.

Extraction and clean-up steps should be performed under hood whenever possible. Since aflatoxin is destroyed by alkali and strong acids, do not handle samples in same area where vapors of strong acid/base may be present. Spot TLC plates under hood and avoid exposure of spotted plates to reactive vapors such as O3, SO2 and HC1, which effect stability of aflatoxins on plates

and which can also effect the absorbents. Dry plates thoroughly before exposure to UV light since presence of solvent enhances degradation of aflatoxins expased to UV light including fivorescent Light

Sampling Procedures

Samples may be taken from crops growing in the field, during handling, during storage, and at other points in the production, marketing, and processing system.

Stream Sampling

The most effective sampling method is to take small portions from a moving stream at periodic intervals and combine the portions into a sample. The stream should be sampled frequently but the amount taken at each interval

should be small to avoid accumulating too large a sample. The samples must be taken from the stream throughout the time the lot is moved.

Probe Sampling

Probe sampling is probably adequate for lots which have relently been blended by harvesting lots which have relently been blended by harvesting or handling operations. Recommended methods for taking probe samples are published by the American Oil Chemists Society Official Methods of Analysis.

Field Sampling

Diagram each field and divide the field into approximately N equal sections. If the field is rectangular, a count of rows for width and a count of paces for length can be helpful in dividing and identifying N equal field sections. Devise

a method to randomly (table of random number) select one ear from each section. Shell and composite the N ears in a mesh bag as a single sample.

Sample Size

Sampling must yield a minimum of one 4.54 kg. sample per unit sample. If more than 4.54 kg. is taken, reduce by dividing or by blending and quartering.

Note on Sampling Methods

Stream sampling is preferable to probe sampling which in turn is preferable to field sampling of ears of corn. For example, the estimated coefficients of variation (%) among aflatoxin test when the indicated number of ears of maize is randomly selected from a field with an average of 100 ppm aflatoxin a 133% for 36 ears harvested and shelled, 83% for 100 ears, 62% for 200

ears, 48% for 400 ears, and 39% for 800 ears. However, if the same entire field is harvested, shell and a stream sample is taken the estimated coefficient of variation is only 29%

Sample Collection Report

A sample collection report should have a sample number and give the date sample was collected, where collected, observation at collection site (insects, rodents), description of sampling method used to collect sample, size of lot sample was drawn from, where sample is stored in laboratory and a description of method used to prevent aflatoxin accumulation during period of sample collection and sample analysis.

Handling and Safe Storage

Each sample should be handled in a manner that minimizes the post-collection production of aflatoxin. Moist samples should be held in cloth or paper bags, cooled if feasible and transported to drying facilities as soon as possible. Samples should not be placed in plastic bags unless seeds are dead and dry, or any other confined area where humidity and temperature can increase around them.

Significant mould growth and aflatoxin production can occur in just a few hours and this must be prevented. As soon as possible after collection, the samples should be dried at approximately 80-90 C for 3 hours or more to reduce the grain moisture to about 12-13% (where moulds are to be studied, 60C for a longer time is recommended). If samples were refrigerated before drying, they should be dried immediately and kept dry until analysed.

Sub-Sampling

The entire 4.54 kg. sample should be ground to pass a No. 14 sieve, thoroughly

blended, and properly sub-divided to a 1 kg. sample. The entire 1 kg. sample should be ground to pass a No. 20 sieve, thoroughly blended, and properly subdivided to 50 9 analytical samples. When feasible, the samples may be ground and sub-sampled immediately after collection. The subsamples can be analyzed immediately, stored under refrigeration, or dried for storage.

Methods of Analysis

The method for analysis should be selected in advance and used uniformly throughout the survey. Official methods that have been collaboratively studied should be used as the method of analysis for aflatoxin. Unofficial methods or methods reported in literature should be validated with respect to the method accuracy and variability before survey is started. The selected method should be retested during the survey by periodic analyses of blank, standards and "spiked" samples. Analytical procedures once set must be followed to the letter. No alternative procedures are permitted unless expressly authorised in operations manual or by project leader.

Laboratory Report

A standard format for reporting data should be adopted and used throughout the survery. Laboratory report form should include sample number, date analysed, method used, commodity, analyst's name, all calculations of results, source of standards and concentration of standard.

Preparation and Storage of Aflatoxin Standards

Aflatoxin standards should be prepared and stored as per AOAC Official Book of Method 14 edition.

Survey Sample

A survey summary should be used to capture data generated by the survey. This form should be kept by the project leader of the survey and can be used to summarize the progress of the survey. The form should include sample number, date collected, date analysed, analyst's name, analytical result, and space for reporting miscellaneous observations, such as recovery from spiked sample and/or duplicate samples analyzed at different times by different analysts.

RECOMMENDATIONS

Inspection/Sampling/Analysis

1. **Develop protocols for surverys, sampling, postcollection handling, and analysis of grain sample involved in the aflatoxins.**
2. **Maize grading standard should be standardized throughout maize marketing system.**
3. **Develop a standard operating procedure for aflatoxin analysis.**

Standardization of operation procedure will aid in the assessment of the true aflatoxin concentration.

4. **Develop the necessary mechanism or an agreement with the largest importer of Thai maize to obtain information about the aflatoxin contamination levels found at time of import. This project could be a very cost-effective way in obtaining contamination levels in Thai maize and will aid in resolving the problems associated with level of aflatoxin found at time of export and the level of aflatoxin found at time of import.**
5. **Develop and evaluate a testing program for aflatoxin in Thai maize.**
6. **Establish aflatoxin certification laboratory. This laboratory should be subjected to periodic proficiency testing.**
7. **Establish a centralized aflatoxin standard distribution system for Thailand.**
8. **Develop a national aflatoxin check sample program.**
9. **Centralize data collection from both government and private sector.**
10. **Maintain a high level of qualified analysts in aflatoxin area in government and private sector. Training should be given periodically in aflatoxin survey techniques, quality control, sampling and analytical techniques.**

Prevention and Control

The development of reliable aflatoxin prevention programs requires considerable scientific knowledge about when and where in the marketing system that aflatoxin contamination occurs. Guidelines based on lesser scientific knowledge most often are costly and ineffective. The environmental conditions that Thai maize is subjected to during post-harvest handling will determine the contamination level at the exporter's silo. Reducing moisture content of maize to below the safe level (13-14%) before any mould growth occurs is at present the safest and most reliable method of preventing aflatoxin contamination. However, reducing moisture content of very large volume of maize in a short period of time in high humidity environment is costly. Other methods should be investigated (particularly aeration, on cob drying on plant, effects of insects, and inoculum levels in bin) to determine if control of these environmental conditions will retard the accumulation of aflatoxin during post-harvest processing. Optimization of these parameters may increase the time moist maize may be safely stored before final drying

and final storage.

Guildelines for Mould Control and Aflatoxin Prevention in High-Moisture Maize

- **Reduce the moisture and temperature of shelled maize. Recognize that high levels of moisture and temperature, together with damage to kernels, are the chief reasons for the growth of molds in shelled maize. Mould growth is negligible when maize moisture content is below 13%.**
- **Never exceed the maximum storage time for a given combination of moisture and maize temperature (see table 1). Remember that the maximum time begins with harvest.**
- **Dry wet maize to 12 to 14 percent moisture immediately after harvest. This is the surest method of limiting mould growth.**
- **If you must hold wet maize at moisture levels above 20 percent -without even a small amount of drying, maintain maize temperatures as low as posible under some form of aeration or maintain maximum ventilation as possible.**

- **Follow those cultural practices most likely to produce a vigorous maize crop and make it fully mature before harvesting. If maize is fully mature, there is less infection and mould growth while ears are still on the plant.**
- **Keep damage to kernels to a minimum. Causes of damage include: high-moisture shelling, high speed operation of sheller, drying process, loading and unloading, equipment and Insect damage in field and in storage.**
- **Use caution in blending lots of maize that differ substantially in either quality or moisture content. Do not blend maize of 20 percent moisture with 10 percent maize in the belief that the mixed batch will equalize overall at 15 percent. It will not unless the mixing is unusually thorough.**
- **After the maize has been dried to a moisture level adequate for storage, provide sufficient air flow to bring the maize to a uniform temperature.**
- **Check maize regularly for moisture, heat, mold, insects or off odors.**
- **Follow good sanitation practices, good insect control, and disease control practices.**

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Contents - Previous - Next

Home"" """"> ar.cn.de.en.es.fr.id.it.ph.po.ru.sw

Aflatoxin research on grain in Asia - its problems and possible solutions

Contents - Previous - Next

T.D. Cardona, S.G. Ilangantileke and A. Noomhorm

0strong>ABSTRACT

Cereals, especially maize, are the major source of carbohydrates in Asia and are important as export products in some Asian countries. As export crops, grain quality has been critically monitored to meet international standards. Mycotoxin development in many stored cereal grains has constantly hampered the availability of good quality grains in Asian countries. Of the major problems, the fungi Aspergillus flavus and Aspergillus parasiticus have been identified as the quality deterrent producing aflatoxin contaminated grain when stored. In the last decade, aflatoxin levels on produce was found to exceed an acceptable level limit of 50 ppb stipulated in most export specifications.

Aflatoxin contamination has affected maize and peanuts in Thailand arid India and also in coconut in the Philippines, Sri Lanka and other Pacific countries. Aflatoxicoses both in humans and animals and liver cancer among humans have been more prevalent in areas where maize constitutes a major part of the diet. Because of these findings, research has been focused on identifying causal factors and formulating effective preventive and control measures against aflatoxin. Many private and government organisations have embarked on aflatoxin research. However, many problems are associated with the actual research activities concerning aflatoxin.

This study reviews aflatoxin research on grain in the Asian region. In the review, constraints for aflatoxin research are identified and are research methodologies such as sampling techniques; financial restrictions to procure sophisticated equipment and chemicals for precise aflatoxin analysis; scarcity of highly trained and experienced personnel for aflatoxin research; absence of facilities for safe aflatoxin research; unawareness among farmers about aflatoxin hazards; stress factors when the crops are still in the field due to inadequate infrastructure for production; policies on aflatoxin contaminated produce and other possible problems towards the reduction of aflatoxin

contaminated produce.

This paper, in addition, attempts to suggest solutions which hopefully, will minimize the constraints in aflatoxin research, based on those identified.

INTRODUCTION

Mycotoxins are toxic secondary metabolises produced by fungi. For centuries, these mycotoxins have been associated with quality degradation of many agricultural products which cause considerable changes in texture, flavor and colon Although many toxic mould metabolises have been isolated through laboratory cultures from agricultural products, only seven have been found to have possible significant occurrence in naturally contaminated foods and feeds. These are aflatoxin, zearalenone, ochratoxin A, citrinin, trichothecenes patulin, penicillic acid and the ergot alkaloids. Among these mycotoxins, aflatoxins have gained considerable attention because they are most toxic and potent carcinogen even in small quantities (FAO, 1979, Mirocha and

Christensen, 1982, and Campos, 1987). In 1981, the maximum limit for aflatoxin in food (aflatoxin B. or the sum of aflatoxins B1, B2, G1 and G2 vary from zero to 50 parts per billion (ppb) (Van Egmond, 1987 and Jewers 1987).

Aflatoxins are a family of related bisfuranocoumarin compounds produced by fungi Aspergillus flavus and Aspergillus parasiticus. The term aflatoxin was derived from Aspergillus (A-) falvus (-fla-) and toxin. It has been reported that out of the known strains of Aspergillus flavus and Aspergillus parasiticus, only about one-half produce toxins. There are 14 known aflatoxins but most of these are metabolises formed endogenously in animals administered by one major toxins, i.e., aflatoxin B. (AFB1) aflatoxin B2 (AFB2), aflatoxin G. (AFG1) and aflatoxin G2 (AFG2) (B= blue fluorescence and G = green fluorescence). Aspergillus flavus was produces AFB1 and AFB2 whilst Aspergillus parasiticus produces the four mayor toxins (Lillehoj, 1986).

Aflatoxins are often isolated in cereals, cereal products, groundnuts and beans. They have also been detected in cotton seeds, copra, nuts, cassava, eggs and cheese. This is alarming in Asia since cereals and groundnuts are the major items in the diet. The situation may be aggravated by the fact that

there is a relatively high incidence of aflatoxin in warmer areas which is true in most Asian countries. In addition, harvesting seasons in Asian countries usually fall on the onset, of the wet season. During this period, sun drying may not be possible which is the usual method employed by most of the farmers. Thus, grain enters the storage system at high moisture content. Aflatoxin contamination occurs when the crops could not be dried within 48 hours (FAO, 1979, llangantileke, 1987, Campos, 1987, Sanchis et al., 1988 and Luxsanakoses, 1989). Furthermore, with the green house effect or global warming, alteration of the climatic conditions in most Asian countries have been observed.

Over the past decade aflatoxicoses both in humans and animals and liver cancer among humans have been reported in India (FAO, 1979 and Bilgrami and Sinha, 1986), Thailand (RIO, 1988, Angsubhakorn, 1989 and Boon-long et al.), Philippines (Ilag, 1984 and RIO, 1988), Taiwan (Angsubhakorn, 1989 and Pitt, 1989a) and Indonesia (Machmud, 1987). Aflatoxicosis caused acute liver damage, liver cirrhosis, induction of tumours, attack on the central nervous system, skin disorders and hormonal effects (Pitt, 1989a). Epidemiological studies have indicated a relationship between aflatoxin intake and incidence of

liver cancer in several developing countries. Pitt (1989b) reported that there is high incidence of human liver cancer in Central Africa and parts of Southeast Asia. Studies have shown a correlation between the logarithm of aflatoxin intake and the occurrence of primary liver cancer. Aside from health hazards, aflatoxins cause economic and nutritional losses. Because of these findings, several government as well as private institutions have been engaged in aflatoxin research with the aim of identifying the factors enhancing its contamination and formulating effective strategies for its prevention and control. However, several problems have been associated with aflatoxin research.

This paper envisions to review the aflatoxin research on grain in Asia and subsequently identify problems that have been encountered. Consequently, recommendations leading to the solutions on the identified problems shall be made.

REVIEW OF LITERATURE

The toxic effects of aflatoxins have been described as early as 1913 but the toxin was not isolated and the subject was forgotten. Aflatoxicosis in swine had been reported in 1940 in Georgia, USA. The death of the swine was traced as a result of feeding mouldy maize. Similar incidence happened in 1950 at Alabama (FAO, 1979 and Schaible, 1979). In 1959, a singular event occurred in Britain specifically in East Anglia where thousands of turkey poults died over a very short period of time. it was quickly demonstrated that these birds died from a poison present in the pelleted feed which formed a major part of the diet. The major raw materials used were protein rich plant products such as groundnut meal imported from tropical and subtropical countries. Examination of the incriminated groundnut meal revealed the presence of mould mycelium and thin layer chromatography showed the presence of several new compounds which fluresce intensively under ultraviolet light. The mould was shown to be Aspergillus falvus and its highly toxic metabolises were called aflatoxins. The acute toxicity of aflatoxin is shown in Table 1. Aflatoxins have been reported as a potent carcinogens and there have been many cases of aflatoxicosis in humans. These findings triggered off an intensive international research effort resulting in a massive

literature on this single group of mycotoxins. In early 1960's the term mycotoxin and aflatoxin were synonymous (Smith and Moss, 1985). Araullo et al. (1976) and Lillehoj (1986) considered the outbreak of unknown disease in England as the first evidence of aflatoxin poisoning.

Table 1. Acute toxicity of aflatoxin B1 expressed as a single oral dose LD50

Species	**LD50 mg kg (-1) bodyweight**
Rabbit	0.30
Duckling (11 day old)	0.43
Cat	0 55
Pig	0.60
Rainbow trout	0.80
Dog	0.50 - 1.00
Sheep	1.00 - 2.00
Guinea pig	140 - 2.00

Baboon	2.00
Chicken	6.30
Rat (male)	5.50 - 7.20
Rat (female)	17.90
Macaque (female)	7.80
Mouse	9.00
Hamster	10.20

a/ The LD50 is the dose that will cause the death of 50 percent of a statistically significant population. It is usually obtained by extrapolation from dose response experiments.

Aflatoxins are important because some are extremely toxic to many kinds of animals; 10 ppb AFB1 consumed regularly by rats may eventually result in fatal liver cancer and in somewhat larger amounts-a few hundred ppb-aflatoxin cause a great variety of ill effects in wild and domestic animals. An aflatoxin contamination level of 20 ppb is permitted in feed grains and in feeds

in the U.S.A. but in foods intended for human consumption the tolerance is zero. However, these guidelines were then revised. North Carolina agricultural officials reported that maize containing up to 200 ppb could be fed to heifers (older than 6 months), calves and bulls and to non-lactating brood cows. Maize containing aflatoxin higher than 20 ppb should not be fed to lactating animals, used as any starter ration or feed to poultry. All other animals could be fed with maize containing aflatoxin levels up to 100 ppb. For human consumption, a level, below 20 ppb is enforced (Mirocha and Christensen 1982 and Pitt, 1989b).

The natural incidence of aflatoxins in food and feed is influenced by climate. Practically, all tropical countries encounter the problem of aflatoxin contamination. Unfortunately, the climatic conditions in Asian countries are very favorable for aflatoxin producing fungi (Bilgrami and Sinha, 1986). This situation is aggravated in places where harvest season coincides with the wet months since most farmers rely on sundrying to reduce the moisture content of freshly harveshly harvested crops (llangantileke, 1987). Aflatoxin contamination has been frequently encountered in cereals especially maize, rice, groundnuts and other oily products. Aflatoxicoses both in humans and

animals as well as primary liver cancer have been reported in many Asian countries where the above mentioned products have been a constituent of the diet (Campos, 1987 and Pitt, 1989a).

In India, maize is grown in 16 states in India which has a total production of 6.1 million tons as cited by FAO (1979) a large part of which is consumed as human food. Aflatoxin has become a problem since droughts and floods occur alternately in various states during the growing and harvesting seasons. Although, there was no systematic survey of maize in India, it was reported that aflatoxin contaminated maize caused toxicosis in two western states in 1974.

India is the second largest producer of rice in the world. It was reported that aflatoxin has been a problem in rice. However, only limited information is available. In one study conducted in Magalore, Karnatake, 6% of all sample gathered revealed the presence of aflatoxin. Reddy et al. (1986) reported that the incidence of Aspergillus flavus colonies in broken rice ranges from 11 to 12%.

Aftatoxin was also detected in groundnuts. In a study conducted in Andhra Pradesh in 1965, aflatoxin levels were in the range of 1,000 and 5,000 ppb in 12% of 743 in-shell and 141 groundnut cake samples. Aflatoxin levels were highest in samples of the rainy season crop. The 1965-1967 survey in Gujarat, Andhar Pradesh and Madras revealed that 10-40% of grounPradesh and Madras revealed that 10-40% of groundnuts and 82% of groundnut cakes conducted in the western coast in 1967 68 revealed that nearly 50% of 500 groundnut samples collected had aflatoxin B1 at levels between 100 to 250 ppb. In 1976, 50% of the groundnut cake samples collected mostly in Madhya Pradesh region were positive for aflatoxin (FAO, 1979 and Reddy et al., 1986).

One of the earliest report on aflatoxicosis in animals was in Andhra Pradesh on 24 Murrah buffaloes. The symptoms were a loss of appetite, diarrhea and dullness. Histopathological studies revealed centrilobular hepatic cell necrosis, bile duct proliferation and central vein occlusion. Aflatoxicoses were also reported in Karnataka which resulted in the mortality of 58 crossbred castles, abortion in pregnant buffaloes and death of 2219 chicks. Large-scale mortality of ducklings in government farms and swine at breeding farm were reported in

Kerala. In Kulu Valley, more than 4000 rabbits died due to the consumption of aflatoxincontaminated pellets. Large-scale mortality of dogs were also reported in Western India 1974 due to feeding on food remnants containing high levels of aflatoxin (Bilgrami and Sinha, 1986).

The effect of aflatoxins on primates was studied in the National Institute of Nutrition in Hyderabad on monkeys. The quantity of aflatoxin M, excreted in the urine of albino rats, guinea pigs and monkeys was studied at the Central Food And Technological Research Institute in Mysore. Also, the effects of aflatoxins on humans were studied. Circumstantial evidence suggests the involvement of the toxin in human disease and deaths. Indian childhood cirrhosis (ICC) a serious disorder of the liver in children was reported to be caused by aflatoxins through mothers' milk and foods such as parboiled rice and unrefined peanut oil. Malnourished children who had consumed aflatoxincontaminated protein flour developed hepatic lesions similar to that of ICC. Urine and liver extracts from ICC children were examined and subsequently, the presence of aflatoxins was confirmed in about 7% of the urine samples of 332 children. The scientists of the National Institute of Nutrition in Hyderabad, on the other hand, undertook detailed investigations

on the outbreak of acute hepatitis in the tribal belts in Western Indian where 100 people died. Death caused by consumption of maize grains heavily contaminated with aflatoxins up to 1560 ppb (Bilgrami and Sinha, 1986, Pitt, 1989a and Pitt, 1989b).

In Thailand, aflatoxin has been a great problem in maize. Recently, Thai maize has lowered its foreign market ability due to a high incidence of aflatoxin (Tangthirasunan, 1989 and Yoshlyama, 1989). This problem is very significant since Thailand ranked fourth as a maize exporter in the world. The major buyers of Thai maize were Hongkong, Japan, Taiwan and Singapore. The aflatoxin levels for these countries are: Japan 10 ppb (AFB1) Hongkong 20 ppb (AFB1 + B2 + G1 + G2 + M1 + M2 + P1 + Aflatoxicol); Taiwan (ROC) 50 ppb (AFB1) and Singapore 0. Thai maize frequently exceeds these levels in the rainy season. Because of this, maize is traded at $10$20 per ton below than that of maize from other sources. The annual cost to Thailand of the aflatoxin and related quality problems, is not less than US$50 million. (Buangsuwon, 1986, Van Egmond, 1987a and RIO, 1988).

The main cause of initial aflatoxin contamination in maize is during temporary

farm storage of undried ears for one to six weeks before the crop is sold. In a survey made in 1967-1969, the average level in the contaminated samples was found to be 2,730 ppb. In 1985, a mean aflatoxin level of 74 ppb with a range of 3 to 299 ppb was reported (FAO, 1979 and Buangsuwon, 1986).

The incidence of aflatoxin contamination in rice in the market was determined by a survey conducted in 1967-1969. Aflatoxin was detected in raw rice in the range of 20 to 98 ppb in 2% samples out of a total of 364 samples. In another survey conducted in 3 locations, Singburi was reported to have, 3% of the samples of prepared rice contaminated with aflatoxin at levels up to 600 ppb; in Ratburi, 10% were contaminated with levels up to 180 ppb and in Songkhla, 1% contained aflatoxin at concentrations up to 71 ppb. Aflatoxin was found to be highest in the rainy season and lowest in the hot season (FAO 1979).

Aflatoxin is also a problem in peanut. In a survey conducted in 1967-1969,49% had an average aflatoxin level of 1,530 ppb. The highest level observed was 12,300 ppb out of 219 samples collected from 100 towns and villages (FAO, 1979 and Campos, 1987). Buangsuwon (1986) reported that a higher incidence of aflatoxin contamination was found in shelled peanuts, especially in samples

of damaged kernels where 9 out 15 samples had aflatoxin contamination ranging from 0 to 350 ppb and in samples of damaged kernels in which all 20 samples had readings of 15 to 1350 ppb.

Aflatoxin has been linked to outbreaks of Raye's syndrome which was an epidemic in Northeast Thailand affecting children up to the age of adolescence. It was reported that aflatoxin was found in 22 out of 23 children affected by the disease. Similar symptoms to those of Reye's syndrome were obtained by feeding large quantities of aflatoxin to Macaque monkeys (RIO, 1988) and Angsubhakorn, 1989).

The incidence of primary liver cancer was found to be correlated with the consumption of aflatoxin contaminated foods. One case of acute aflatoxicosts was reported causing the death of a child consuming contaminated rice. The child was said to have consumed steamed rice contaminated with aflatoxin for two days approximately in the range of 6 mg daily prior to the total illness (FAO, 1979).

The mycotoxin research in the Philippines has been concentrated solely on

aflatoxin since it is a highly potent poison affecting several animal species and is a common contaminant of many important agricultural produce including maize, peanut and copra. Other mycotoxins have not so far been reported to occur in the country although the Food and Nutrition Research Institute has reported the presence of a small amount of ochratoxin and zearalenone in maize. A survey conducted in 1972, revealed that the aflatoxin content of various agricultural crops and their byproducts were in large quantity. Malze samples were usually laden with the toxin. Some samples showed no visible sign of Aspergillus falvus but contained aflatoxin while others had Aspergillus falvus but no toxin. It was reported that maize grown in Visayas and Mindanao had more toxin than those grown in Northern Luzon. Palay, soybean and millet however, were noted to be poor substrates for aflatoxin production (Ilag, 1984).

The risk potential from aflatoxin in the Philippines is alarming because of the prevailing high temperature and relative humidity which favors fungal growth. In addition, heavy rain coincides with the peak harvest months of staple cereals, particularly maize. As a consequence, handling of high moisture grain is commonly encountered and subsequently leads to aflatoxin formation

(Garcia and flag, 1986).

Studies on rice indicated that under current handling practices, palay or rough rice contain low levels of aflatoxin or none at all. However, milled or brown rice when inoculated with toxicogenic strains of Aspergillus falvus and Aspergillus parasiticus, yieladed very high levels of the toxin. Aflatoxin B1 in brown rice was found to be concentrated on the bran layers whereas the polished rice contained no toxin or only traces of aflatoxin (Ilag, 1984).

A study by the Food and Nutrition Research Institute on the aflatoxin content of various food items in the country revealed that groundnuts under natural condition contain high level of aflatoxin (Ilag, 1984). Lagunda (1989) reported that aflatoxin build-up in the 4 day windrowed and mechanically stripped pods was observed at 15 days of storage. The aflatoxin level increased from an initial value of 0.432 ppb up to 108.559 ppb after 45 days of storage. He concluded that 4 day curing and mechanical stripping is not recommended due to the build-up of aflatoxin.

A study by Jayme et al. (1982) showed that primary liver cancer (PLC) ranks

first among malignancies that reach autopsy. It was concluded that there is a strong positive association between the ingestion of increasing levels of aflatoxin and the rising risk of developing primary liver cancer. This effect is synergistically aggravated by alcohol consumption.

There has been no report on aflatoxin incidence on maize in Taiwan. As cited earlier, Taiwan is one of the major importers of maize in Asian countries. Since the aflatoxin level is strictly stipulated in trade, the level of toxin is within an acceptable limit of 50 ppb.

A study published in 1968 reported that no aflatoxin was detected in rice collected from markets in Taiwan. However, aflatoxin was found in one of the samples of mouldy rice that was collected from families in which 25 people became ill and 3 children died of unknown cause. Of the five samples analyzed one contained 200 ppb of aflatoxin B1 (FAO, 1979).

Aflatoxin in groundnut was also studied in 1966. Samples taken from 3 out of the 8 oil mills revealed the presence of aflatoxin B. in the range of 40 to 430 ppb. Groundnut cake samples collected from 4 to 12 oil mills were positive for

aflatoxin and contained 80 to 290 ppb of AFB1 (FAO, 1979).

In Indonesia, there is no report on the incidence of aflatoxin in maize as of 1977. However, Widiastuti et al. (1988) cited that cyclopiazonic acid has been found to be accompanied by other mycotoxins especially aflatoxins from samples collected from Bogor poultry feedmill. FAO (1979) cited that aflatoxin in trace amounts was detected in rice handled by the distributors but was negative at the storage facilities where rice bought from the farmers were stored. Analysis of four samples suspected to be contaminated showed the presence of aflatoxin G1 in one at a concentration of 15 ppb.

Groundnut is an important crop in Indonesia. About 10% of the crop was exported as reported by FAO in 1979. A comparison of aflatoxin contamination of groundnut samples collected from 3 points in 1970 showed that higher level of aflatoxin in retail groundnuts was attributed to inadequate protection from wetting during rainy weather. Aflatoxin levels were found in groundnut press cake taken in West Java. It was reported that higher levels of aflatoxin was found in groundnuts used for making groundnut oil than those destined for the retail market. This happened because lower grades of

groundnuts were used for oil extraction. Machmud (1988) reported that about 60-80% of marketable groundnuts were contaminated with aflatoxin at levels from 40-4100 ppb and retail groundnuts were highly contaminated.

PROBLEMS ON AFLATOXIN AND AFLATOXIN RESEARCH

Over a decade of aflatoxin research on grain in Asian countries and other tropical and subtropical countries, have caused researchers to encounter several problems. These problems encompass the physical, analytical, socio-political, pathological and mycological aspects. Some of these problems or constraints are enumerated as follows:

1. Sampling, Subsampling and Sample Handling

Blanc (1987) commented that no sampling method for aflatoxin tests in groundnut kernels and cakes is considered totally reliable. This is not only true in groundnut but also in other agricultural commodities particularly cereals.

Hongsuwong (1989) stated that sampling error is usually the largest contributor to the total error. This would have a great impact considering that one positive aflatoxin analysis on a single shipment on any commodity could lead to the rejection of the entire lot.

Sampling for aflatoxin determination has been a critical factor in aflatoxin research since aflatoxin is extremely variable in nature. Huff (1980) reported that concentration of aflatoxin in artificially inoculated maize was not even homogeneous. Lee et al. (1980) said that aflatoxin is very heterogeneous and that kernels containing a high level of aflatoxin were often adjacent to aflatoxin negative kernels. To date, no absolute quantity of sample has been recommended for a single aflatoxin prone commodity. Most researchers suggested that 5-10 kg representative sample should be taken from a sample lot for analysis (Buangsuwon, 1986).

Jones (1972) commented that it is difficult to lay down strict rules on sampling. Various trade bodies also have their own sampling procedure. Van Egmond (1987) reported that only limited information is available about the use of sampling plans.

RIO (1988), pointed out that sampling and more specifically sample division using whole maize kernels rather then ground kernels, is seen as the major source of error in official inspection and for quality control testing.

FAO (1982) reported that sampling of lots of food or feed selected for examination is one of the most difficult and often most costly aspects of conducting a survey. The size of the sample to be taken as representative of a particular lot of food depends on the type of commodity to be examined.

2. Lack of Funds and Other Resources

In all research activities, adequate funding is a dire need to accomplish the objectives satisfactorily. The government of any country where mycotoxin problems have been identified should provide sufficient funds for research as well as extension activities. Bhat (1987) reported that the funding pattern for mycotoxin prevention and control strategies is minimal. The political commitment of most governments is generally directed for increased production of agricultural commodities and less priority is provided for postharvest techonolgy aspects especially on mycotoxin control programs.

Moreover, Blanc (1987) and Campos (1987) pointed out that research and extension programmes on aflatoxin could not be implemented due to the lack of funds.

Considerable resources are necessary on aflatoxin studies specially on a large scale program. Aflatoxin is not only a problem among agriculturists and food scientists but also to veterinarians, biologists, microbiologists, and other related fields. Hence, a multidiscriplinary approach to overcome the constraints might be feasible since aflatoxin itself is not only a postharvest problem. These programmes need adequate resources in terms of personnel, energy source, capital investment and analytical and technical facilities (FAO, 1977, Garcia and flag, 1986 and Campos, 1987).

Laboratories in most Asian countries are not as sophisticated as those in developed countries. Technical apparatus such as Thin Layer Chromatography (TLC), High Performance Liquid Chromatograph (HPLC) are necessary for the accurate detection of mycotoxin contamination. In Asia, TLC is the most common apparatus for aflatoxin detection but this equipment is not as accurate as the HPLC.

Recently, a semi-quantitative approach to detect aflatoxin have been developed for faster and is less expensive than the quantitative method. May and Baker of U.K. has developed an aflatest kit using a minicolumn. This method is faster and useful for silo operators as tested in Thailand. Another is the method developed by the Plant Pathology section of the Department of Agriculture in Thailand. It employs a minicolumn principle to extract toxin that could be prepared easily in the laboratory (Simmonds, 1988).

3. Availability of Chemicals for Analysis and Aflatoxin Control

For quick and efficient analysis, chemicals should be readily available. In most cases, reagents for aflatoxin analysis are usually imported (Garcia and llag, 1986). These chemicals may only be available in big cities of the Asian countries. Perhaps, this could be attributed to the fact that aflatoxin is not yet known especially in the hinterland villages.

Chemical control has been reported to be one of the alternatives for aflatoxin control but chemicals intended for this purpose may not be readily available and might be very expensive. In addition, farmers may not be familiar with the

correct application procedure (llangantileke, 1987).

4. Scarcity of Highly Trained and Experienced Personnel

Effective research needs experienced and highly trained personnel. In countries like U.S.A., U.K. and the rest of those pioneers in aflatoxin research, highly qualified personnel may not be a problem. In developing countries however, there is a dearth of trained personnel to embark on large scale multidisciplinary programs for the prevention and control of aflatoxins in various food and feed products (Ilag, 1984; Garcia and llag, 1986 and FAO, 1977).

5. Inadequate Facilities for Safe Aflatoxin Research

One of the major hindrances for a successful research program especially on dangerous substances like aflatoxin is the safely of the personnel. Those who are aware of aflatoxin health hazards, are unwilling to venture on this research activities if their health is at risk. Angsubhakorn (1989) cited cases where human risk was involved. He cited two chemical engineers in

Czechoslovakia who worked on a method for sterilising peanut meal infected with Aspergillus falvus and subsequently died of lung cancer. One had worked for only three months and developed symptoms of lung cancer three months later. In another instance, two British biochemists developed cancer of the colon after exposure to purified aflatoxins. One of them had worked with aflatoxin for three years and developed symptoms seven years later. The second had done this work for 12 months end developed symptoms 2 years later.

6. Lack of Maintenance of Laboratory Facilities

Analytical laboratories must be properly maintained to obtain reliable and reproducible results. Sophisticated equipment for measuring minute quantity (in ppb) should be routinely checked for accuracy. However, highly trained personnel may not be always available in Asia or developing countries since most if not all of these sophisticated equipment are imported (Blanc, 1987).

7. Lack of Awareness on Aflatoxin Hazards

Prevention of aflatoxin contamination at the grassroots level is perhaps, the most effective method for aflatoxin control programs. It has been reported that aflatoxin is solely a postharvest problem but it was demonstrated by several researchers that infection starts when the crop is still on the field (Payne, 1986; Jones, 1986; Fortnum, 1986) because Aspergillus flavus is present in soil and air. Majority of the farmers are not aware of these findings. Some farmers would ignore the advise from their cofarmers especially when money is involved. These could be proven by non-adoption of improved postharvest practices to prevent aflatoxin contamination on freshly harvested produce. Similarly, middlemen and other grain handlers are not aware of all the danger of aflatoxin to both human and animals (FAO, 1977).

8. Stress Factors on Field Crops

Stress factors such as drought, floods, typhoons, etc. are beyond the control of the farmers. These factors would be aggravated in tropical and subtropical countries since warm and humid climate prevail. A relatively high incidence of aflatoxin in warmer areas of temperate regions and in subtropical and tropical climates have been reported. A typical example was the severe drought in

Southwestern United States which caused economic damage on the maize farmers due to contamination of aflatoxin. Jones et al as cited by Payne (1986) reported that plants exposed to drought stress in the field had more infected kernels than samples from irrigated plots.

9. Inadequate Infrastructure

Infrastructure is divided into two parts, one would be the rural infrastructures which includes roads, bridges, etc. while the other would be the grain storage structures such as silos, warehouses and godowns. The former plays an important role in moving the freshly harvested produce to central drying facilities, storage structure and access to probable buyers. If these would be provided by the government in one country, there would be a free movement of crops intended for market. However, this would depend on the market price. Usually at harvesting time, the price decreases and farmers are reluctant sell their produce. They prefer to crib store their produce for 2 to 3 months before selling (RIO, 1988).

Grain storage structures are very important for preserving the quality of the

produce. A safe storage place must be provided for the produce until it is needed for consumption since grain production is seasonal and consumption is continuous (Bailey, 1982). In developing countries such as in Asia, grain storage structures are still inadequate perhaps, due to the financial capability of most farmers to construct good grain storage structures.

10. Government Policies

Mycotoxin regulatory programs are being introduced in some Asian countries. These regulatory programs are introduced primarily to protect the export market of agricultural commodities. These regulations are being strictly enforced or else the importing countries would reject the commodities, resulting in a loss of valuable foreign exchange earnings. On the other hand, domestic regulatory measures on aflatoxins received very little attention. In India, mycotoxin legislations have been introduced but the implementation was found to be inadequate. This might be due to the interference connected to businessmen dealing with aflatoxin products. Most farmers feel that it is another government ploy which calls for additional investment with no incentives given for the aflatoxin free produce (Bhat, 1987). There has been

no heavy penalty on the violators of aflatoxin regulations.

Aflatoxin contaminated products are allowed to enter in the market. Governments should have a regulation to reject those food and feedstuffs which have an aflatoxin level above the acceptable limit. However, an equipped laboratory would be needed with adequate and well-trained staff to perform such analysis.

11. Trade Practice

Trade of agricultural produce in most countries is carried out by weight. As much as possible the farmers prevent loss of excessive moisture to have a high income or return of their produce. This situation is a good entry of toxigenic fungi (FAO, 1997).

In groundnut trade, hand-picked selected (HPS) produce commands higher demand due to minimal or no damage. The damaged groundnuts would be for local or domestic trade, thus increasing the chance of ingestion of contaminated groundnuts by the local community. This has been manifested by

a high incidence of primary liver cancer in Asian countries since no special attention is given on the produce for home consumption. A common practice is to set aside produce with good quality while the rejects would be for home use (FAO, 1977).

The damaged groundnut would usually be destined for oil mills. It has been reported that oil has high aflatoxin levels and unrefined oil is still consumed extensively in preference to refined oil due to the high cost of the latter.

Also, most traders have poor storage facilities. During storage, the produce would be most likely attacked by insect pests which would provide a ready access of toxigenic fungi into the grain and subsequent mycotoxin contamination. Moreover, businessmen hoard the produce during the lower price stage and sell them when the price is favorable.

12. Inadequate Extension Programs

Considerable information has been gathered concerning the conditions that lead to mould growth and mycotoxin contamination during growing, harvesting

and storage of crops on the farm and on the practices that can be followed to avoid or minimize contamination. Information is also available on the levels of aflatoxin in feeds, safe storage and handling practices, transport of agricultural commodities, selection of foodstuffs by housewife and other related informations. However, all this information may not reach the farmers, traders and all those who need to be informed, due to inadequate extension services, lack of community and demonstration agents, non-existence of farm organisations, non-cooperation of traders organisation and no regular radio and television programs (FAO, 1977).

Go To Next Page

Contents - Previous - Next

Home"" """"> ar.cn.de.en.es.fr.id.it.ph.po.ru.sw

SOLUTIONS TO THE IDENTIFIED PROBLEMS

Aflatoxin control programs offer opportunities for economic gains as well as health improvement. It is now recognised in many developing countries that reducing aflatoxin residue levels in food or feed can confer international trade advantages. In addition, there may also be long-term health benefits for the local population (Dichter, 1987). Several researchers have suggested possible solutions. Some solutions are given as follows:

1. Specify an Appropriate Sampling Scheme for Different Types of Crops

The International Association of Oilseed Crushers Standard Procedure (IAOCSP) has come up with a sampling procedure for oil seeds but this procedure is not rigorous enough to be used in aflatoxin research and trade (Jones, 1972). Therefore this method should be re-evaluated. Intensive testing would be done on the improved procedure before adopting it as sampling standard.

A design of sampling plans that would provide representative samples of lots

of contaminated commodities could be another way to overcome the variability of aflatoxin. Moreover, research would be necessary on the distribution characteristics of aflatoxin as a prerequisite on the design of statistically valid sampling plans. Such plans are essential in reducing sampling error as a possible major source of variance in analytical results obtained by the buyer and seller of a given lot of produce.

Sampling should be done by trained personnel. This is one of the causes of variations. As much as possible, a lot for sampling, i.e., train, ship, etc. should be made by the same person or group of persons to eliminate personal bias.

The methods to increase the precision of aflatoxin test are to increase the sample and subsample sizes and increase the number of analysis. The US Food and Drug Administration (FDA) has advocated a minimum sample size of 6.8 kg. A properly drawn 21.8 kg. (48 lb.) sample is a representative of 45.5 tons. Currently, the FDA recommends 66 kg. (144 lb.) for the control of aflatoxin in peanuts. Increasing the sample size has the advantage of simultaneously reducing the number of good slot rejected and the number of bad slot accepted (Hungsuwong, 1989). Pithaya-Acharlyakul (1989) cited that four 2-

kg. subsamples should be set aside for aflatoxin analysis. Each subsample should be crushed, sieved, mixed and reduced by increment reduction method to obtain 50 gm. for analysis. The procedure for the specification of 50 ppb aflatoxin level is given as follows:

1st analysis = over 75 ppb

- rejected and not qualified for 2nd analysis

= up to 75 ppb - allowed for 2nd analysis 2nd analysis = less than 25 ppb - the lot is accepted

= over 25 but less than 75 ppb - allowed for 3rd analysis 3rd analysis = average with former 2 analysis is 50 ppb or below - can be accepted

= average with former 2 analysis is over 50 ppb - rejected - rejected

2 Improvement of the Traditional and VillageLevel Storage Structures

Storage facilities are of particular importance since bulk handling and storage

of food grains have become a necessity in many parts of the world due to the seasonality of the crop and because consumption is continuous. Several types of storage structures which have been used by the farmers for a long time. Some of them are good in terms of their structure and appearance but still inadequate for fungal control. Research should be undertaken to find methods to improve the systems . Bhat (1987) stated that the national agencies in different developing countries should emphasise on project which are not capital intensive like improving the traditional existing storage structures. Also, a design of appropriate storage structures suitable for different commodities in different regions should be encouraged. A good storage structure prevents insect and rodent infestations, protects from moisture and humidity changes and mould infection. An improved storage is perhaps, better than introducing a new storage structure due to the financial capability of the farmers. A good example of this is the improvement of the traditional crib store of maize in African regions. FAO (1980) concluded that the use of improved cribs which fully exploit the drying capabilities of natural air appear to offer the most practical and economical method drying and storing maize cobs.

Small-scale middlemen in the village should also be encouraged to make a good warehouse which would give ample protection to the produce while in temporary storage.

3. Adoption of New Cultural Practices

Cultural practices have been proven to influence aflatoxin contamination of the crops. Appropriate agronomic practices such as correct water application, application of fertilisers, control of insect pest and field hygiene must be practiced by the farmers (Jones, 1986, Blanc, 1987, Machmud and Burford, 1989 and Nibe, 1989).

4. Breeding of Aflatoxin Resistant Varieties

Unfavorable climatic conditions could not be controlled by farmers thus, planting of aflatoxin resistant varieties may alleviate the situation. Darrah (1986) reported that no germplasm which would reduce aflatoxin B1 infection in maize kernels have been identified. Therefore, intensive breeding programs for cereal and groundnut should be mace and implemented. These would take

time since these will require thorough screening and location testing but once successful, these would serve as breakthrough in aflatoxin reduction. Suttajit (1989), pointed out that the development of 'superplant' against pathogenity utilizing genetic resistance to mycotoxin contamination should be encouraged.

5. Inhibition of fungal Growth

FAO (1979) and Suttajit (1989) pointed out that the inhibition of fungal growth can be achieved by physical, chemical and biological means. These include immediate reduction of moisture content on freshly harvested produce, minimize harvesting damage and provide a good storage, use of synthetic fungicides and the use natural products from plants.

Since sundrying may not be always feasible during the harvesting period, mechanical dryers are recommended. However, large capital investment is necessary. Mechanical dryers could be set-up in a strategic and accessible place in certain villages so that farmers in that village could dry their produce easily if sundrying is not feasible. This necessitates firstly, careful planning such as staggered planting so that crops from all farmers could not be

harvested and stored at the same time and secondly, observance of recommended cultural practices. Another way is to provide a mobile dryer (Mekvanich, (1989). This would be appropiate in areas where rural transportation infrastructure crisscross the hinterland villages. The Department of Agriculture in Thailand has designed, developed and tested a mobile dryer.

Ilangantileke (1987) and Cardona (1988) reported that chemical treatment for prevention of fungal growth on maize has a promise. The results of their studies revealed that Nilspor Plus, a propionic acid based fungicide could suppressed aflatoxin development on freshly harvested maize. Tanboon-ek (1989) reported that ammonium bis propionate and propionic acid would give temporary control and prevention on fungal growth in high moisture maize but would not destroy the aflatoxin present before the treatment.

Suttajit (1989) cited that the natural products from plants such as allicin from garlic and onion extracts, clove oil, cinnamon extracts, black and white pepper could be used for fungal control. Bhat (1987) stated that dried leaves of certain plants are mixed with foodgrains to be stored to prevent insect and

mould infection.

6. Promote Aflatoxin Hazard Campaign

The aflatoxin problem is not very well-known to all farmers and perhaps, to legislators, businessmen and other grain handlers in the Asian countries. It is only popular to the researchers and medical personnel. Once people are made to understand the danger of aflatoxin, they would be careful in selecting food and feedstuffs. The incidence of aflatoxincosis in humans has commonly observed among the rural people. Therefore, the national agencies of every country should initiate a program to educate the peasant or small farmers. Government and private agencies should join hand to disseminate the information particularly to the most remote villages. Radios and televisions must have a regular program about aflatoxin hazard. Pamphlets and posters would also be used. Moreover, there must be regular features on the subject in widely circulated daily newspapers of Asian countries.

7. Sponsor Seminars and Symposia

Seminars and symposia are good avenues to exchange findings and to get acquainted with the research work from other research agencies. This activity should be promoted locally and internationally. In Asian countries, the ASEAN Grain Postharvest Programmes (AGPP) organises an annual seminar on grain postharvest technology to update scientists in the ASEAN region on advances in research and development in grain postharvest technology. Such a gathering gives researchers a chance to evaluate the past and present studies and consequently plan for future research priorities.

8. Establishment of Mycotoxin Surveillance Programs

The government planners would be encouraged to establish aflatoxin surveillance programs. This would need resources but FAO (1977) stated that this program would be feasible and desirable with the start of modest resources. Experience could be earned as time goes by and all other resources could be secured especially if the performance rating is high. Furthermore, FAO (1977) cited that entry of the programme would start with the establishment of food control laboratories, training of inspectors and analysts in the principles and practices of sampling, sample preparation and mycotoxin

analysis.

9. Consultation with Local Veterinary and Medical Authorities

Aflatoxicosis both in humans and animals has been reported. Consultation with persons dealing with aflatoxicosis problems would provide insights for the researchers to initiate survey and regulatory control activities. International bodies such as WHO and FAO might gather information on diseases on humans and animals that are of unkonwn origin and identify those for which mycotoxin hypothesis may be wanted Such work including investigations are needed before one can begin to estimate the extent to which the mycotoxins contribute to disease (FAO, 1977).

10. Incentives for Removal of Mould Contamination

One of the most effective methods for reducing mycotoxins in the food supply is to encourage diversion of mouldy grain and oilseeds to non-food uses or to processing industries that recover one or more mycotoxin free products. When alternative markets do not exist for the mouldy product or if the price is much

less than for non-mouldy products, there is a large economic incentive to blend the mouldy with non-mouldy products, there is a large economic incentive to blend the mouldy with non-mouldy products so it can be marketed for food uses (FAO, 1977).

11. Work on Feasible Detoxification Techniques

Since aflatoxin is a naturally occurring contaminant of several staple food products, it cannot be completely eliminated without compromising the food supply and creating significant economic losses (Dichter, 1987). The removal of aflatoxin from contaminated produce entails additional cost therefore, a cheap method of detoxification must be work out. Various studies have been done on aflatoxin removal of inactivation based on physical, chemical and biological processes (Brekke et al. 1977, Huff and Hagler, 1982 and Lillehoj, 1987). Ammonia has been found to be the most efficient detoxification method (Huff, 1980) but further work must be undertaken on this aspect. Tanboon-ek (1989) commented that maize with high aflatoxin content can be detoxified efficiently with ammonia and the resulting grain is safe and can be fed for cattle and swine but not suitable for human consumption. Therefore, further

studies should be carried out so that an appropriate detoxification technique could be attained.

12. Collaborative Research

Since aflatoxin research needs considerable resources, a cooperative research activity may be a better approach. These have been done in the Philippines, Thailand and India and the outcomes seem to be very encouraging (Bilgrami and Sinha, 1986, Buangsuwon, 1986 and Garcia and Ilag, 1986). It would be appropriate then to strengthen cooperative research within one country and between other countries.

13. Training Programmes

The technical expertise of those who work on aflatoxin, especially those who would perform sampling and laboratory analysis is a crucial factor for effective aflatoxin control programmes. Training is a very important component and it would be logical to sponsor training programmes periodically. A recent training activity was done by Thailand and was

supported by FAO, AGPP and the Royal Thai Government. This training program had participants from different countries in Asia.

14. Inclusion of Mycotoxins in the University Curriculum

Campos (1987) recommended for a possible inclusion of mycotoxin in the curriculum is the department of agronomy, veterinary sciences, food technology, medicine. chemistry and pharmacy. Bhat (1987) stated that the information on mycotoxins has to be incorporated in the syllabus at various level for certificate, diploma and the courses offered for agricultural extension workers. This would have a great future benefits for all countries.

15. Promulgation of Policies on Aflatoxin.

Policymakers in the national government should be aware of the health benefits as a result of the preventive health strategy that successfully reduced human exposure to mycotoxins. Dichter (1987) commented that decreased exposure to aflatoxin would result in fewer deaths from liver cancer, a chronic disease, which takes perhaps 20 or more, years to develop.

Aflatoxin legislation has been enforced in other countries like U.K., U.S.A., Canada and the E.E.C. member countries. An aflatoxin legislation should also be instituted in the developing, countries where incidence of Aflatoxicosis is high. However, Campos (1987) cautioned that in introducing an aflatoxin legislation, a certain country should not copy the legislation of other countries and adopt it in toto as its own national standard.

The aflatoxin tolerance or limit varies widely from one country to another. This should be standardized and the tolerance level should be reduced. Van Egmond (1987) cited that harmonization of mycotoxin regulation is highly desirable. Effort should be made to standardize and harmonize the methods of determining the aflatoxin level in bulk commodities to eliminate or minimize injustices.

CONCLUSIONS

Some problems associated with aflatoxin research have identified. Effects of

aflatoxin on humans and animals were also reviewed. Consequently, recommendations from different agencies working on aflatoxins are thoroughly reviewed.

If the recommendations from all these agencies would be implemented, perhaps, aflatoxin problems in Asian countries would be minimized if not solved.

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Contents - Previous - Next

Home"" """"> ar.cn.de.en.es.fr.id.it.ph.po.ru.sw

UK-THAI maize project

Contents - Previous - Next

BACKGROUND

In Phase I of the project (1984) it was established that mechanical drying to 14% moisture content could effectively prevent further aflatoxin

contamination. However, using driers at the local merchant and regional silo level did not produce maize with low levels of aflatoxin as incoming maize was already well in excess of 30 ppb.

During Phase II (1985), maize was collected and loaded into a drying system immediately after harvest (within 48 hours) in order to freeze aflatoxin at the lowest possible levels. The results of phase two were as follows:

1. Low aflatoxin maize was successfully produced during the rainy season in 35 trials in Loei and Lopburi provinces by mechanically drying freshly harvested maize with a history of 1 to 4 weeks pre-harvest drying in the field. The mean total aflatoxin content of maize produced during these trials was 2.5 ppb (range 0 to 16 ppb).

2. The control survey confirmed that maize at local merchants (with a history of 1 to 6 weeks temporary farm storage) and at regional merchants/ silos was already too high in aflatoxin content (in excess of 50 and 100 ppb respectively) to allow the production of low aflatoxin maize. This emphasises the need to use freshly harvested maize and mechanical driers to effectively control

aflatoxin.

3. Slow drying, taking longer than two days, was shown to be inadequate in controlling aflatoxin contamination, confirming a finding in Phase I.

OBJECTIVES OF PHASE III

- **To produce dry (14.5% moisture) maize that is low in aflatoxin content.**
- **To demonstrate that investment in drying equipment up-country can be profitable.**
- **To develop marketing systems that will promote quick release of maize after harvest.**
- **To demonstrate that farmers and merchants who cooperate to produce high quality (low aflatoxin) maize will obtain higher rewards than obtainable from the traditional system.**

APPROACH TO PHASE III AND BRIEF DESCRIPTION

Phases I and II had demonstrated that dry, low aflatoxin maize COULD be produced in the rainy season. The aim of Phase III was to apply the Phase I/II findings within the existing maize marketing chair) during the rainy season harvests of 1986/87 and 1987/88 in order to establish commercially realistic improvements to the marketing chain; produce commercial quantities of low aflatoxin maize; and monitor "project" maize from farmer to final buyer.

a) Drying and Pricing

Selected merchants and buyers with suitable grain driers were invited to participate in the project by offering to buy BAAC farmer clients' maize at a premium price. Project staff, in conjunction with BAAC, identified and contacted up-country merchants with mechanical driers. Project staff monitored the quality and costs of maize harvested, shelled and dried in various locations throughout the country.

The main points agreed with participating merchants were as follows:

1. **An agreed tariff for weight deduction on wet maize.**
2. **Use of commercial moisture meter for project maize.**
3. **Agreed base price for project maize at 14.5% MC.**
4. **Agreed premium for quality based on grade and/or aflatoxin content.**
5. **Agreement for monitoring of drier and maize by project staff.**
6. **Agreement on other facilities available to the project.**

In year one agreements were reached between local merchants and export silos. Direct selling links from farmer to end silo were also established.

In year two agreements were negotiated with quality conscious feedmillers on premiums and aflatoxin testing.

b) Interviews/Seminars

Farmers and merchants were interviewed to establish the difficulties in changing their current practices when trying to switch to the UTP system. This followed the work of phases I & II which showed that maize dried within a specified short time after harvest was low in aflatoxin content.

Two seminars were held with the main objectives of informing project participants and concerned parties of the project aims and implementation schedules. There was also a strong educational element aimed particularly at the BAAC credit officers.

c) The problems of drought and pre-harvest contamination

In 1987 drought conditions led to crop failures and pre-harvest incidence of aflatoxin in serveral sites. Altough maize was dried within the time limits of the semi-UTP system, aflatoxin levels at these sites were very high.

d) Evaluating the role of mould inhibitors

An alternative to fast drying of freshly harvested maize is to apply a chemical that will prevent fungal growth and hence aflatoxin. Ammonium bis-propionate (NILSPOR PLUS) solution has been shown to prevent deterioration in grain and fodder crops resulting from fungal and bacterial action.

A trial was undertaken to demonstrate that NILSPOR PLUS, when applied to

fresh maize (that had been subjected to Aspergillus flavus inoculant), would prevent aflatoxin development under conditions found on Thai farms. A suitable spray applicator was also developed for use by farmers.

This work was carried out at AIT, Rangsit. Farm conditions were simulated during Aprill/May 1988 using maize that was inoculated with Aspergillus flavus spores. During 1986/87 a preliminary trial was carried out to compare two methods of chemical application. It was found that spraying was more suitable for farm application than soaking or dunking for a short time.

e) Surveys

The economics of the early selling of maize in a falling market and the attitudes of farmers to early selling were considered. Two farmer surveys were conducted to gather information on farmer attitudes and selling practices.

f) Sampling and testing for aflatoxin

In order to monitor aflatoxin levels of both project and control maize, small laboratories were set up at one export silo and at one up-country merchant's godown. All samples were analysed by the TCO/ODNRI mycotoxin specialist at the Department of Agriculture. DOA and ODNRI were responsible for analysis of the coded samples.

SUMMARY OF ACTIVITIES AND RESULTS

Activities Year 1

Agreements were reached with two up-country maize merchants for the purchase, drying and monitoring of project maize.

Various services were agreed with the first merchant, Mr. Prapak Tarwat in Tak Fa District, Nakon Sawan Province. The supply of maize was organised by the BAAC branch office under supervision of the BAAC credit officers.

The second drier was at Wangshampoo District, Petchaboon Province. This was the sister ST3 batch type drier, on loan from the Department of Agriculture. Maize was supplied through the merchant, Mrs. Rampai Wongprayoon. Costings and performance of the machine were monitored by the project staff. A series of forms were designed in order to monitor the maize from harvesting through to final sale/aflatoxin analysis.

In Lopburi and Saraburi Provinces arrangements were made for the sale of project maize straight from shelling to the silos in the Tha Rua District, Ayuthaya Province.

All sampling and analytical work was performed at the two Tha Rua silos by the Seed and Post Pathology Branch of the Department of Agriculture, under the supervision of the Technical Cooperation Officer.

A farm survery was carried out in Petchaboon Province.

A project was carried out at the Asian Institute of Technology on the application of propionic acid at the farm level in the control of aflatoxin in

stored maize cobs.

Results Year 1

Harvesting

The labour input required to harvest, within two days, sufficient maize to warrant shelling and transport is beyond the means of most farmers.

Often labour is not available and farmers do not have the cash to pay them. Thus the UTP criteria were not rigidly adhered to and farmers were advised, through the credit officers, to harvest as quickly as circumstances permitted.

In the 1986/87 harvest, premiums were only payable for maize less than 20 ppb. Farmers were reluctant to speed up their harvest, thus incurring additional costs, for a premium that was not flexible enough to accommodate maize above 20 ppb.

Project maize in 1986/87 was from Lopburi, Saraburi, Nakon Sawan and

Petchaboon provinces. From beginning of harvest to start of shelling there was an average time lapse of 9 days.

Shelling and Drying

Shelling to delivery to drying silo was completed within 24 furs, with the exception of Petchaboon, which varied between 1.5-3 days. This reflects the difficult terrain in Petchaboon where the maize had to be brought down from the mountain by tractor and then loaded onto a pick up truck for delivery to the ST3 batch drier. Project maize going into the Tha Rua silo had a special dispensation and was allowed to go to the head of the queue and thus there was no delay before drying.

Tonnage Collected/Aflatoxin Levels

293.7% tonnes of project maize was collected from the 4 sites. 151.6 tonnes, or 51.6% of the total tonnage collected, was less than 20 ppb. The low tonnage collected reflects the unwillingness of farmers to implement a UTP or Semi-UTP system.

Chemical Control

Chemical usage in the dipping tests was in excess of 100 litres/tonne of maize. Spraying systems were more practical and more financially viable. Laboratory determination indicated a control on fungal activity by different treatments, although in the study very low aflatoxin levels were detected, possibly due to low levels of atmosphere contamination in the research area.

The main recommendation was for further work into the design of a spraying system.

Activities Year 2

Two pre-implemention seminars were held (See Appendix 4); The first was held with the aim of informing the maize industry of the proposed project activities. The second seminar, in which all project participants attended, focussed on project implementation.

The project team worked with a total of six upcountry maize merchants. Due

to a prolonged drought in the planting/growing season only four merchants operated their businesses in the harvesting/buying season. These were in Petchaboon, Chantaburi, Nan and Phrae Provinces.

Maize was supplied through BAAC branch offices. A merchant drier survey was carried out by project staff at three drier sites. Because maize loses its identity in a continuous flow drier, samples were taken and sun dried during unloading at the drier intake.

A second farmer survey was undertaken and preharvest cob samples were taken to determine the incidence of pre-harvest aflatoxin contamination. Work was done at the drier in Petchaboon on using the BGYF as a presumptive aflatoxin check.

A sub-project was undertaken involving the collection and delivery of maize to an export silo in Tha Rua. The objective was to spray the shelled maize with propionic acid to control fungel development. Samples were taken from incoming project maize and were analysed on site using a commercial aflatoxin analysis kit and by using the BGYF. Pricing difficulties between the

Tha Rua silo and the final buyer meant that the maize collected was not sprayed.

The Asian Institute of Technology undertook a second year of work on the application of chemicals on cob maize. Work involved the design and construction of an on-farm, man powered, continuous flow cob sprayer. The sprayed cobs were then stored and sample were taken to check aflatoxin levels and propionic acid residues. The samples were analysed at AIT using the commercial aflatoxin analysis kit, Aflatest.

All sample generated in the projects were analysed by TLC at the Seed and Post Harvest Pathology Branch, Department of Agriculture, under the supervision of the Technical Cooperation Officer.

Results Year 2

Harvesting

The average harvest time for project maize was 6 days. The short time taken

to harvest reflects the fact that project maize was often delivered in small quantities. The average time from the end of harvest to the beginning of shelling was 16 days. In both 1986/87 and 1987/88 it was difficult to persuade farmers either to speed up their harvest or to sell soon after completion of harvest. Pre-harvest aflatoxin contamination, positively identified at two drying sites (Petchaboon and Chantaburi), lead the project team to conduct pre-harvest sampling to identify the level of aflatoxin contamination.

Shelling and Drying

The end of shelling to delivery to drier was within 24 furs. In most cases project maize delivered to the silo was dried within 3 furs. In a few cases however, there was a time delay of up to 12 hrs before the maize was loaded into the drier. Monitoring of project maize stopped at the up-country driers because of mixing within the drier and the difficulty of judging when project maize entered and left the drier.

Tonnage Collected/Aflatoxin Levels

2098 tonnes of project maize was collected. There was no negative aflatoxin (less than 20ppb). maize. Of the total tonnage tested for aflatoxin 9.25% was between 20-50ppb. There was no negative aflatoxin (less than 20ppb). The remainder was above 50ppb. (This is explained largely by the incidence of preharvest contamination resulting from the drought).

Siam Grain

A total of 1422.7 tonnes was collected. From the tonnage tested, 6.21% was less than 20ppb, 9.2% was between 20-50ppb and the remainder was above 50ppb.

Chemical Control

A continuous flow rotary drum sprayer was designed and built at AIT. Control and aflatoxin innoculated maize was treated. A knapsack sprayer was used linked to the rotary drum. Work was carried out using an Ultra Low Volume spray system.

Results indicated complete cob coverage by the chemical and low chemical application rates, especially with the ULV spray system. The main recommendations were further development of the machine and implementation of farm based trials.

MAIZE PRODUCTION AND MARKETING IN THAILAND

Maize production in Thailand peaked in 1985/86. The planted and harvested area was 12.377 million rai and 11.990 million rai respectively. This is a five fold increase since 1965. Yields have fluctuated yearly due to climatic conditions and generally there has been little significant increase in yields. The increase in production reflects an increase in the cultivated area.

The 1986/87 harvest was an estimated 4.2 million tonnes with 11.982 million rai planted and 11.181 million rai harvested. World maize production was also high and this was responsible for severely depressing the Bangkok silo price. Farmers were reluctant to sell and many stored their maize through until

Nov./Dec./Jan.1986/87 when they were forced to sell to repay their loans.

Low prices in 19 6/87 meant that there was less credit available for the 1987/88 season. This factor, coupled with a severe drought that killed off much of the first planting, lead to a 37% drop in harvested area and a harvest estimated at 2.31 million tonnes. Domestic demand caused a rapid price escalation that made Thai maize uncompetitive on the world market. The Grade A Bangkok daily price rose from B2166/tonne on the 1st July 1987 to B3632/tonne on 31st Jan.1988. Farmers stored their maize in order to take advantage of a rising market.

Maize is typically a low input-low output system. It would appear that most farmers would prefer to sell their maize as soon as possible after harvest. Few farmers however, are able to sell soon after harvest. There are the physical problems of access, transport, availability of sheller etc. There are also problems of rising and falling markets, weight penalties for wet maize and credit terms that might delay the farmer's sale.

Since the loss of the Japanese and Taiwanese markets there has been

considerable expansion in the Southeast Asian (Korea, Singapore, Malaysia) and Middle Eastern markets (Saudi Arabia, Kuwait, Iraq, Iran).

There is also a regional variation in the timing of the maize harvest, the likely moisture content at harvest, deliveries onto the market and need to store, particularly because of inaccessibility during the wet season.

There are maize growing areas which concentrate on early planting, harvest and early sale. This maize is harvested and sold around June/July because prices usually start falling as the bulk of the maize crop is harvested. This maize is usually of high quality/low aflatoxin and feed millers are willing to pay above the market price to secure this maize.

There are two official grades of Thai maize-1 and 2. Although aflatoxin levels are not contained in either US or Thai maize standards, importers are increasingly specifying aflatoxin levels. In certain cases aflatoxing levets at loading are above those specified in the contract although pre loading levels might be within the aflatoxin specification.

The overseas markets for Thai maize, in Southeast Asia and the Middle East, are strategically placed for small shipments in bags. Traditionally these markets have been less quality conscious but there is a danger of Thailand losing a share of these markets if higher quality maize, particularly low aflatoxin maize, could be economically sourced from other maize producing countries.

The markets for quality maize in Thailand are with the feed millers, many of which are vertically integrated. They consistently pay higher prices than export silos for maize of the same quality.

Approximately 31% of Thai maize was used domestically for feed up to 1985. In 1986187 and 1987/88 the figures were 37% and 86% respectively. The tonnage used domestically rose between 198611988. The jump in 1987/88 can be attributed to three main factors. 1) The small 1987/88 harvest. 2) The increase in livestock numbers. 3) The high price of paddy rice.

Observations on the 1985 harvest suggest that, theoretically, it is worth spending up to $10 per tonne to ensure maize is below 20ppb and $5 per tonne

to meet grade A specification - $15 per tonne in all. This statement is still true if the price differential between Thai and US maize is $15 or more per tonne. However the extent to which sellers may be able to achieve a high price for quality depends upon market conditions. Price differentials between Thai and US maize in 1987/88 season showed Thai maize at an average of $26.48/tonne above US maize.

Given the current Thai maize situation, a system of pledging maize at an upcountry drier with a premium for quality might offer a realistic way out. Here an early release from the farm and timely drying could result in quality maize and a realistic price to the farmer. The limitations of the weight correction for moisture content in Thailand, with its lack of incentive for merchants to dry to 14% moisture content, was noted in Phase I and 11 reports. An alteration to the weight correction scale is recommended to increase the attractiveness of drying as near to the areas of production as possible.

MECHANICAL ASPECTS OF DRYING AND SUNDRYING

It was noted in both Phases I and II that the delays in moving the maize from the farm to the existing buying centres is largely responsible for the development of high levels of aflatoxin in the grain.

At the farm level little attempt is made to dry the harvested cobs. Although field drying is a widely accepted practice, there is a serious constraint on field drying in areas where double cropping is practised. Mung bean and soya bean are frequently planted after maize and farmers will not field-dry their maize because of the need to plant the next crop while there is sufficient moisture in the soil, and a possibility of late rain.

A few farmers will attempt to sundry a portion of the harvested cobs prior to crib storage. The cobs are often spread on a sheet of plastic, galvanised sheet or tarpaulin. The farmer must constantly be in the vicinity, however, to collect up the cobs before impending rain. For this reason many farmers are reluctant to attempt on-farm sun-drying.

Sun-drying is still the most widely accepted upcountry method for reducing moisture content. Merchants make every effort to obtain the maximum moisture reduction, usually to the detriment of maize quality. When the merchant makes a decision, based on weather conditions, to sun-dry his maize he is then committed to pay the sun-drying costs. If it starts raining and the merchant cannot achieve a moisture reduction to cover his sun-drying costs, then he has the choice of selling the maize, perhaps incurring a loss on the sun drying costs, or he can hold the wet maize and wait for weather conditions to improve to continue sundrying. If the merchant chooses the latter course then the maize quality will suffer adversely.

Whilst some merchants appreciate the improvement in maize quality through mechanical-rather than sun-drying means, most merchants are interested in the margin over drying costs. Quality premiums are generally not big enough at present to warrant investment in mechanical driers to produce quality.

Maize that can be sun-dried, under sunny conditions in one day, is often of high quality but the sun-drying floor must be clean and tractor use must be limited to avoid cracking the kernels.

Mechanically dried maize can also be of high quality provided that the maize is dried at the correct temperature and is free from smell and uncombusted burner fuel. A pre-cleaner should also be incroporated to reduce dust and chaff.

The continuous flow driers monitored in the project make it difficult to keep maize of differing quality apart, due to the mixing of the maize within the drier This can make it difficult for merchants to offer a premium for specific loads.

Merchants with driers, in general, appear not to link their purchasing to the drier's capacity. Some merchants use their drier in combination with their sundrying floor, while others do not use their sun-drying capability at all.

There is little opportunity to mechanically dry other crops and spread overheads. Mung bean and soya bean, crops which are commonly grown after maize, are harvested in the dry season. Rain-fed rice is harvested in the dry season. In areas where water is available, and rice is double cropped through irrigation, there is little maize grown. There are also very few upcountry

merchants who trade in both rice and maize. Most rice traders, often with a rice mill, trade only in the one commodity.

SUMMARY OF ANALYTICAL STUDIES AND SAMPLING PROCEDURES

In the first year all quality assessment was carried out in exporters' silos in Tha Rua, Ayuthaya Province. Sampling was carried out on incoming trucks as detailed in section 7.2.1 In the second year similar sampling procedures were taken at the up-country silos which participated in the project.

In year 1 BGYF tests were compared with minicolumn analysis at the exporters' silos. In year 2 BGYF tests were carried out on all incoming maize at the Petchaboon silo. At the export silo in Tha Rua, Aflatest and BGYF analyses were undertaken. Aflatest was also performed on the experimental batches at AIT. All these results were confirmed by HPTLC analysis at the Department of Agriculture.

CHEMICAL TREATMENT

It is estimated that some 40% of maize-producing areas are inaccessible during the rainy season. This is particularly the case in the newly developed areas that have been claimed from the mountainous forest reserves. In these areas it is not practical to bring freshly harvested maize down to the villages and main roads until the dry season enables traffic access.

To avoid fungal attack of cobs that are harvested during this period would require either that a drier is located very close to the fields or that some alternative form of treatment is considered. Whilst mechanical drying is possible in certain areas, another possibility is the use of chemicals such as ammonium his propionate solution to repress the growth of fungi.

Work has already been carried out to assess the effect of chemical control on shelled maize prior to loading on to export vessels during the rainy season. Little has been done, however, to research the effectiveness of chemical

control on maize cobs immediately after harvesting. Development of application techniques and application rates would be necessary to develop a suitable system that would would be effective at farm level. Any applicator so developed would need to be cheap and simple to use.

To develop this work, AIT were approached to set up a programme to compare dunking of cobs in the chemical solution with spraying, using a purpose built sprayer suitable for farmers' use. The rationale behind this approach follows from the work done in Phases I & II of this project. This showed that normally maize is low in aflatoxin level immediately after harvest but that the level builds up rapidly in farm storage during the wet harvesting months of August and September. If treatment with chemical at this time, immediately after the harvest, it is successful in controlling fungal growth the resulting maize should be low in aflatoxin content. The detailed objectives, methodology, findings, conclusions and recommendations are to be found in Appendix 1. After the first year's study it was decided that further work was necessary to refine the application of spray chemical. The second year's work is presented in Appendix 2.

CONCLUSIONS

Summary of Conclusions from the Project

Over the four year period of the project a great deal has been learnt and is recorded in the reports on the three phases. Before making the final recommendations from the project it well be helpful to summarise the conclusions of the project in relation to its overall aim of finding an economic solution to the problem of aflatoxin in maize in Thailand.

From Phase I it was concluded that:

a. **mechanical drying to 14% mc (no part exceeding 15% mc) can effectively prevent further aflatoxin contamination. In other words, drying to 14% will freeze the aflatoxin level but it will not reduce it. Further, there was strong evidence that drying to 14% should be completed in less than 48 hours so as to avoid the transport and temporary storage of wet maize** which were found to lead to a substantial increase in aflatoxin content. Low aflatoxin maize, therefore, cannot be produced by installing mechanical driers alone because maize at this stage in the marketing chain usually already contains aflatoxin in excess of 50ppb.

b. dry maize (14%) can be stored for a least 2 months without any increase in aflatoxin over its initial level.
c. drying maize is economic down to 18% mc only. Drying below 18% is not economic for farmers or primary merchants under the existing pricing mechanisms widely practised in Thailand.

From Phase II it was conclude that:

a. maize with low levels of aflatoxin could be produced during the rainy season using FRESHLY harvested maize and single stage mechanical drying- a system defined as the UK-Thai project system (UTP). The system called for:

    i. field drying maize for 1-4 weeks before harvesting to reduce

moisture content to less than 22% mc. (Field drying is an important component of the UTP system because it reduces the cost of mechanical drying, helps to stagger the harvest and can probably improve shelling efficiency). Field trials conducted by the UK TCO project confirmed that drying to a moisture content of 22% could be achieved within 2 weeks during the rainy season. There was no increase in aflatoxin or decrease in physical quality over a 6 week period of field drying. However field drying should not be practised when drought has predisposed the crop- to pre-harvest contamination.

ii. **shelling within 24 hours, certainly 48 hours, and loading the shelled maize into the drier within a further 12 hours.**
iii. **completing the drying process to a moisture content of 14%, with no part exceeding 15%, within 48 hours.**

b. **the BGYF test is a suitable quality control method for monitoring freshly harvested maize for use in a UTP, and probably a semi UTP, method of aflatoxin control. Quality control using visual quality checks can almost** certainly help to reduce the level of aflatoxin accepted, but it is not able to select and accumulate low aflatoxin maize. Additional quality control tests are required to achieve this including the BGYF test and the Holaday-Velasco mini-column (see Chapter 7.8 Conclusion No.8)

c. slow drying, taking longer than 2 days, was shown to be inadequate for controlling aflatoxin contamination.
d. maize temporarily stored on the farm-for 1-6 weeks-was found to have a mean total aflatoxin content which was already in excess of 50 ppb, hence the need for drying freshly harvested maize.
e. the % of mouldy grains was much lower in the fresh maize dried to 14% within 48 hours.

From Phase III it is further concluded that:

a. merchants are in general unwilling to offer a premium to farmers unless they can see that a premium is in turn available from their end-buyers. Feedmillers are generally willing to offer a higher price for quality maize than exporters, since the exports are at present unable to secure a substantial premium for quality on the world market. The premium paid for quality within Thailand is essentially a function of demand and reflects the anxiety of buyers to secure supplies.

b. farmers are reluctant to enter into forward agreements on date of sale and require any premium offered for quality to be guaranteed. The current premiums are inadequate to justify farmers speeding up the sale of the crop. It is generally more profitable for farmers to delay sales until the maize dries out and prices rise in December, unless there is some creditrelated pressure to sell. Smaller farmers tend to sell earlier than large farmers. Due to an increase in the amount of information available on radio and TV, farmers are now much more aware of price trends.

c. farmers and merchants have both found it difficult in practice to adhere to the requirements of the UTP system, although farmers in the early planting areas (eg Prachinburi) can implement a "semi-UTP" system and usually sell as quickly as possible after harvesting.

d. there are discrepancies between moisture meters used in the industry and farmers have little knowledge of the moisture content of stored maize.

e. sampling, and more especially sample division using whole maize kernels rather than ground kernels, is seen as the major source of error for official inspection and for quality control testing. A more rigorous sampling plan, such as the plans based on the attribute principle that are currently being devised by the TC project, would be required in order to improve the accuracy of inspection at preloading and loading. For quality control of truck-loads of maize a 5 kg sample, similar to the 4.5 kg sample taken in the USA, is considered to be a reasonable practical compromise as a first step, provided that the whole sample is coarse-ground prior to sample division. However, this could result in the rejection of substantial quantities of maize.

f. drought stress in Thailand CAN cause preharvest aflatoxin contamination.
g. approximately 113 of all maize in Thailand is grown in mountainous areas which cannot be evacuated during the rains.
h. maize is not a particularly profitable crop for farmers to grow at present, hence there is a reluctance to move into high input production.
i. sampling and inspection at the ports is inadequate to pressure exporters to meet international quality criteria. Many export cargoes are shipped

with aflatoxin content levels that are above contract specification. Physical problems in collecting preshipping samples contribute greatly to this.

j. **little is known about crib drying in Thailand, although it is a widespread practice and much of the aflatoxin contamination occurs in crib and other types of on-farm storage. Studies aimed at determining the maximum length of cob storage that can be recommended in a semi-UTP aflatoxin control system have so produce maize in the rainy season which has a high probability of containing less than or equal to 20 ppb total aflatoxin, it is necessary to limit the storage period of wet cobs to 2 days. If the aflatoxin limit is raised to 50 ppb, then it may be possible to extend cob storage and a practical limit may be 5 days.**

k. **mechanical driers should be located as near to the point of harvest as is economically and commercially feasible. There are an increasing number of suitable driers being manufactured in Thailand. Merchants who buy driers generally have little knowledge of the theory and practice of drying.**

l. **drying costs affect a merchant's competitive ability to buy maize and a**

cheap fuel source is the most obvious potential cost saving for a mechanical drier. There is therefore scope for developing and evaluating alternative power sources for drying, including biomass, particularly for the smaller driers that would be suitable for primary merchants.

m. **results of trials at AIT on the use of propionic acid for inhibiting the activity of A flavus, and therefore aflatoxin, in unshelled cob maize are encouraging. Further work should be undertaken on developing the onfarm cob sprayer utilising an Ultra Low Volume sprayer system. Field trials should be undertaken to assess the practical and financial viability of such a machine.**

n. **the BGYF test can be used for accumulating consignments with a relatively low level of aflatoxin and identifying batches of maize with a high mean total aflatoxin content. However, the efficiency of the test tends to be too dependent on the aflatoxin profile of the aflatoxin under to be recommended as an official quality control procedure for use by merchants. The HolidayVelasco mini-column, with some slight modifications, has been shown to be very effective as a quality control test and should prove suitable for use at regional merchants, maize** collection centres, export silos and feed mills. The method is fast, cheap and reliable but does require accurate standards and some laboratory equipment-including a dessicator and fume hood.

o. a recent report concluded that maize, above 20% mc, when mechanically shelled suffered from a very rapid increase in A flavus contamination. It is recommended that further research is done on this. In the event that this is shown to be the case then work should be undertaken to produce an "improved" sheller.

p. the TMPTA moisture scale remains a major disincentive to up-country drying. This report shows that the scale can be modified to give such incentive without markedly reducing the benefits to other parties in the marketing chain.

q. studies carried out in Phase I and 11 of this project showed that maize containing a mean level of aflatoxin less than or equal to 20ppb (or 50ppb) cannot be produced by simply installing mechanical driers at primary or secondary merchants, because freshly shelled maize arriving at these stages in secondary merchants, because freshly shelled maize arriving at these stages in the marketing chain was found to have a mean

aflatoxin content in excess of 50ppb. However, forty-one percent of the incoming maize did contain less than 50ppb total aflatoxin, so implementation of the UTP drying criteria, as explained in Chapter 7 rec. no.2, could help to produce significant quantities of maize within the 50ppb aflatoxin limit, during the rainy season.

r. **field-drying is an important component of the UTP system because it reduces the cost of mechanical drying, helps to stagger the harvest and can probably improve shelling efficiency. Field-trials conducted by the Thai-UK Technical Cooperation project confirmed that drying to a moisture content of < 22% could be achieved within 2 weeks during the rainy season. There was no significant increase in aflatoxin, or any decrease in physical quality, over a 6 week period of field-drying. Field-drying should not be practised when drought has predisposed the crop to pre-harvest aflatoxin contamination.**

s. **the May and Baker "Aflatest" kit was evaluated in the field and the overall performance suggested that, with some minor modifications to the florisil tip and the standards, the efficiency could probably be improved to match the best of the mini-column methods. The Aflatest was found to be** safer to use than minicolumn methods and did not require the use of a fume cupboard. Capital costs could be minimised by using an Aflatest kit, but running costs would be about B325 per sample.

RECOMMENDATIONS

A summary of recommendations is given below. These have been widely discussed and agreed with the various sectors of the Thai maize industry.

1. Harvest and Post Harvest Practice

Farmers should be encouraged to allow standing crops of maize after crop maturity to dry out for one or two weeks before harvesting.

Encouragement should in the first instance be undertaken by the relevant Government Agencies i.e. DOAE and BAAG Once the benefits of field drying are widely accepted, maize merchants with good silo facilities should be

advised by DOAE that the pursuit of such practices by farmers will lead to better quality (and hence higher prices)

2. Merchants should be encouraged by agencies such as DOAE and BAAC to dry maize rapidly after harvest; specifically to shell maize within 5 days of harvest; start grain drying immediately or within 12 hours of shelling; and use drying systems capable of drying maize to 14% mc (no part exceeding 15% mc) within a 48 hour period.

3. Drying to 14% MC

The specification for moisture content for Grade 1 export maize should be reduced to an average moisture not exceeding 14% by weight, but the MC of any portion must not exceed 15% by weight.

As the Office of Commodity Standards (part of the Commerce Ministry) are responsible for the official export standards, it is the Minister of Commerce who should authorise a review of possible changes for export maize.

4. Aflatoxin quality control should be used by all merchants with drying facilites to identify truck loads of maize which are whithin a 50 ppb limit and these loads should be kept separate and dried as in 2) above.

Encouragement should be undertaken by Government Agencies such as DOAE and BAAC as well the TMPTA and Feed Millers Association.

5. Incentives for Quick Release of Maize After Harvesting

Pledging and contract schemes should be developed to encourage farmers to release their harvested crops immediately after harvest.

BAAC has had experience of pledging schemes such as the large scale scheme for paddy rice in 1987/8. BAAC is recommended to carry out a pilot scheme with one or two approved merchants. Obviously such a scheme will only be brought into action if market conditions are such that farmers are anticipating rise in price.

In addition, Feed Millers who have identified supplying merchants following

acceptable quality control procedures should experiment with forward contracts for quality maize. A feature of such contracts should be that farmers benefit through early release of maize. This might be achieved by a premium price linked to an input supply scheme (often referred to as a total package scheme).

6. TMPTA Weight Scale

To provide incentive for farmers and traders to dry maize mechanically, the TMPTA weight scale should be altered to provide an incentive of at least 20 kgs per tonne per one per cent of moisture removed down to 14% moisture. Such a scale should replace the existing scale which does not give any incentive to dry below 18% mc.

As the name implies, the scale was initiated by the maize exporters at a time when Thailand's maize industry was developing. It is suggested that the Feed Millers Association should agree with TMPTA before approaching the Minister of Commerce.

7. Recognition of Silo Operators

Merchants operating in the maize producing areas, who are prepared to dry mechanically maize that has been freshly harvested and will offer a premium to the farmers, should be encouraged, recognised and approved.

Encouragement can be given by quality conscious buyers such as feed millers. Government organisations such as BAAC and DOAE should establish a scheme of approval and promote those individual merchants who follow there practices.

8. BOI Privileges

In order to encourage merchants to pursue the production of quality maize through mechanical drying and to encourage local farmers' participation, BOI should give consideration to awarding privileges to such merchants who make application.

Feed Millers will stand to gain most from the development of such improved

up-country merchants and will be the most likely group to succeed in persuading the 801.

9. Training of Merchants

Training should be offered to merchants in the following aspects of their business in order to improve quality:

Silo management

Choice of suitable plant and equipment

Cost control and financial planning

Quality Control

Communication skills with farmers

This training should be offered by the TMPTA and Feed Miller's Association, in cooperation with DOAE and DOA.

10. Laboratories in Maize Growing Areas

Encouragement should be given to the establishment of laboratories to pursue better methods of quality control in the maize producing areas. The function of such laboratories should include:

a.) Standardising and assessing moisture content of maize (and other crops)

b.) Monitoring and reporting the quality of local crops pre and post harvest. This will include assessment of aflatoxin content in the area.

c.) Supplying mini-column kits, including the regular supply of mini-columns, to merchants.

d.) Carrying out aflatoxin analysis of samples submitted by local traders and farmers.

e.) Recommending and teaching the adoption of good quality control techniques.

f.) Publicising and utilising agreed national methods of sampling.

g.) Testing and publicising other new quality control techniques as they become available.

Establishment of a laboratory in Tha Rua may be economically feasible for an existing inspection company. In the maize growing areas the Department of Agriculture should be encouraged to investigate the setting up of laboratories in their field crop-stations such as Petchaboon and Tak Fa, Nakon Sawan.

11. Inspection of Export Consignments

The Government should establish standard methods for sampling consignments of maize of different size in bags and in bulk. The standard method should apply equally to export shipments and domestic trade.

The Office of Commodity Standards should propose suitable sampling methods for consultation by the inspection companies and exporters. After consultation agreed methods should become standard practice and widely known. (see

7.9.4)

12. Sampling for Quality Control

Sampling and sample handling procedures should be modified to reduce the risk of batches passing PreLoading and Loading inspection, when they actually exceed the specified aflatoxin limit. As a first action Inspection Companies should install mills at each of the silos at which they are working. A minimum bulk sample size of 5 kg should be taken for aflatoxin quality control testing. The sample should be collected from throughout the batch, and should be coarse-ground before division to 2 kg and then fine-ground to pass a 1 mm screen before further division. For BGYF testing a 2.5 kg sample of kernels will usually be adequate. In The longer term, sampling plans designed to further reduce Consumer (Buyer) risk, such as that currently being devised by the Thai-UK Post Harvest Technology, (Aflatoxin in Maize) project, should be considered.

13. Cost of Mechanical Drying

Research should be carried out into the economics and efficiency of using biomass as an energy source for mechanical drying. Further development of biomass burners and gasifiers is needed, especially for small batch driers that would be suitable for primary merchants.

The responsibility for such research lies with the Division of Agricultural Engineering (DOA) in cooperation with the Trade Associations.

14. Farm Storage

The evidence gathered by the Thai-British project clearly indicates that temporary farm storage of undried maize cobs leads to a build up of aflatoxin contamination. Because farm storage is so widely practised and will be hard to change, it is recommended that further research work is undertaken on field drying and temporary farm storage.

15. Training & Education of Farmers

More extension work is needed to educate farmers in the principles of post

harvest management and marketing. Farmers should be taught about the effect of moisture content on the price. Market information should be explained so that they can learn how to compare broadcast prices in baht per picul with their own local prices.

In particular, this training should include:

The effect of moisture content on deterioration and toxin formation

The effect of moisture content on the sale price

The benefits of field drying

The relevant broadcasting authority should be made aware of these problems.

16. Asian Institute of Technology

Year two results from the Asian Institute of Technology indicate that the continuous flow cob sprayer, with minor modifications, could be used at the farm level. Further work should be undertaken with the aim of producing a

practical and economically viable prototype for commercial production.

Contents - Previous - Next

Home"" """"> ar.cn.de.en.es.fr.id.it.ph.po.ru.sw

History of the maize quality improvement research centre and project activities

Contents - Previous - Next

T. Yoshlyama

BACKGROUND

Maize is a very important crop for the Thai agriculture economy, ranking second only to rice in acreage under cultivation. The product, however, is

contaminated by an active carcinogenic substance called aflatoxin. The problem has hindered the development of maize production and has also become a barrier in maize trade. The Government of Thailand has tried to cope with this problem by enacting comprehensive measures in combined effort with various authorities concerned with this subject, universities, research institution and non-government sectors.

Under such circumstances, the Government of Thailand asked the Government of Japan for Technical Cooperation in solving the problem of aflatoxin contamination in maize.

Subsequently, the Government of Thailand and the Government of Japan had agreed to establish the Centre and dispatched the experts from Japan International Cooperation Agency (JICA) to implement the Maize Quality Improvement Research Centre Project from December 15,1986 to December 14, 1991 which is under responsibility of Department of Agriculture (DOA).

OBJECTIVES OF THE PROJECT

The project aims at extending the technology for contributing to the improvement of maize quality by controlling aflatoxin contamination through the technical cooperation between both countries. The research activites are carried out mainly at the Maize Quality Improvement Research Centre (Bankhen) and Praphuttabaht Field Crops Experiment Station.

FACILITY AND EQUIPMENT

The facilities of the Centre consist of Main building, Annex and other buildings. They were constructed at Bangkhen and handed over to DOA including with equipment provided as Japanese Grant Aid on March 21,1988. Besides, more necessary equipments are supplied under the technical cooperation throughout the project.

ORGANIZATION STRUCTURE AND ADMINISTRATION SYSTEM

1. **Working group for the Project was organized by the members of Thai personnel and Japanese experts.**
2. **Administration System**

The management for the Project is conducted by following Committee;

   a. **Joint Committee**
   b. **Coordinating Committee**
   c. **Coordinating Sub-Committee**

3. **Japanese Experts**
4. **Training of Thai Counterpart**

RESEARCH ACTIVITIES

In order to improve the quality of maize, while controlling aflatoxin contamination, research activities under project will comprise three fields; agronomy, postharvest and microbe. The main research areas aim to identify surveys and experiments, and then to improve individual technologies. The Project establish effective measures to prevent and/or reduce aflatoxin contamination in Thai maize.

a. **Research Implementation Plan I-Agronomy**
b. **Research Implementation Plan II-Post Harvest**
c. **Research Implementation Plan III-Microbe**

Contents - Previous - Next

Home"" """""> ar.cn.de.en.es.fr.id.it.ph.po.ru.sw

Appendix I. List of participants

Contents - Previous

INDONESIA:

1. DEWA NYOMAN NGURAH RAKA JAYA Agricultural Services of Bali

2. SINTA WIJAYA Badan Urausan Logistik Jakartra

MALAYSIA:

3. LPN REPRESENTATIVE (To Mr. Wang Yiung syan)

4. MARDI REPRESENTATIVE (To Mr. Kamarozaman Bijang)

PHILIPPINES:

5. MS. EVANGELINE TAMPOC
Chief, Chemical Laboratory Section
Technical Resource Development Directorate
National Food and Authority

Quezon City

6. DA REPRESENTATIVE (To Ms. Elenita Mateo)

7. MR. RAUL PAZ (To be invited thru Dr. Andales)
DR. ANDALES)
Researcher
National Postharvest Institute for Research and Extension
CLSU Campus
Munoz, Nueva Ecija
SINGAPORE:

8. MR. RONALD TAN YONG KWANG Singapore Storage and Warehouse Pte Ltd.

9. MS. LAU WOEI YUEN Department of Zoology National University of Singapore

THAILAND:

10. MRS. AREEPAN UPANISAKORN
Subject Matter Specialist
Central Regional Agricultural Extension Office
Department of Agricultural Extension
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Contents - Previous