

FOOD AND AGRICULTURE ORGANIZATION **OF THE UNITED NATIONS**

Her Brough



UNITED NATIONS **ENVIRONMENT PROGRAMME**

Recommended Practices

for the **PREVENTION OF MYCOTOXINS**

in Food, Feed and their Products

prepared by The Food and Agriculture Organization of the United Nations in collaboration with The United Nations Environment Programme

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS



FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS



UNITED NATIONS ENVIRONMENT PROGRAMME

Recommended Practices

for the

PREVENTION OF MYCOTOXINS

in Food, Feed and their Products

prepared by

The Food and Agriculture Organization of the United Nations

in collaboration with

The United Nations Environment Programme



FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS ROME 1979 The designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations and of the United Nations Environment Programme concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

.

M-84

ISBN 92-5-100703-9

The copyright in this book is vested in the Food and Agriculture Organization of the United Nations and in the United Nations Environment Programme. Applications for permission to reproduce this book, in whole or in part, by any method or process, should be addressed, with a statement of the purpose and extent of the reproduction desired, to the Director, Publications Division, Food and Agriculture Organization of the United Nations, Via delle Terme di Caracalla, 00100 Rome, Italy.

© FAO and UNEP 1979

TABLE OF CONTENTS

Foreword11Introduction11PART I2Schedule of Recommended Practices2Schedule for the Control of Field Infection by Fungi of2.1Standing Crops2.1Schedule for Harvesting and Drying2.2Schedule for Storage of Crops2.3Schedule for Post-Harvest Processing including2.4Decontamination2.4PART II2.5Standing Crops and Control of Field Infection by Fungi.3.1References3Standing Crops and Control of Field Infection by Fungi.3.2Inferences3.3Id4Harvesting and Drying3.4Control of Field Infection by Fungi.3.4References3.5Storage3.6References3.8Marketing3.9Storage3.9Storage3.10References3.12Aransport3.10References3.12References3.12Starting Materials3.11Afferences3.12Aransport3.12Aransport3.13References3.13References3.13References3.13References3.13Starting Materials3.11Afferences3.12Aransport3.13Starting Materials3.13Areferences3.13Starting Materials3.13Areferences3.13 <t< th=""><th></th><th>Section</th><th>Page</th></t<>		Section	Page
Standing Crops2.14Schedule for Harvesting and Drying2.25Schedule for Storage of Crops2.35Schedule for Transportation2.47Schedule for Post-Harvest Processing including2.57Decontamination2.57PART II	Introduction PART I Schedule of Recommended Practices		1 4
Decontamination2.57PART II	Standing Crops Schedule for Harvesting and Drying Schedule for Storage of Crops Schedule for Transportation	2.2 2.3	
Recommended Practices38Standing Crops and Control of Field Infection by Fungi.3.18Control of Field Infection by Fungi3.213References3.314Harvesting and Drying3.416References3.527Storage3.628Transport3.735References3.8Processing3.9Decontamination3.10References3.11After Products4.1For Muldy or Myootoxin-ContaminatedPART III50Needs and Aids for Implementation of the RecommendedPractices4.1Star Base and Monitoring4.1Education and Extension4.2Star Base4.2Star Base4.2	Decontamination PART II	2.5	7 8
Products3.1348PART III50Needs and Aids for Implementation of the Recommended4Practices4Data Base and Monitoring4.1Education and Extension4.2	Recommended Practices Standing Crops and Control of Field Infection by Fungi. Control of Field Infection by Fungi References Harvesting and Drying References Storage Transport References Processing Decontamination Recovering Aflatoxin-free Products from Contaminated Starting Materials References	3.1 3.2 3.3 3.4 3.5 3.6 3.7 3.8 3.9 3.10 3.11	8 13 14 16 27 28 35 36 36 36 38 46
Practices450Data Base and Monitoring4.150Education and Extension4.251	Products	3.13	
	Practices Data Base and Monitoring Education and Extension	4.1 4.2	50 51

APPENDICES

Appendix 1 - Mycotoxins formed by different fungi and their	
association with foodstuffs	53
Appendix 2 - Probable mycotoxicoses caused by rusts and smuts	56
Appendix 3 - Schedule for treatment of bag stacks under warehouse	
storage for protection from damage by insects, moulds and rodents	57
Appendix 4 - Influence of moisture content on mould growth during	
storage of grains at different temperatures and relative humidities	62
Appendix 5 - Moisture content equilibrium values, maximum acceptable	
level for storage of produce	63
Appendix 6 - Assessment of the Aflatoxin Content in Feeds	64
Appendix 7 - List of some institutions working on mycotoxins	66

FOREWORD

This publication is issued by FAO under FAO/UNEP Project 0107-75-01, "Control of Environmental Contaminants in Food: MYCOTOXINS". It has been prepared based on:

- (a) A document ("Recommended Practices for the Prevention of Mycotoxins in Food, Feed and their Products" 1976) elaborated by the Central Food Technological Research Institute (CFTRI), Mysore, India, under the guidance of Dr. B. L. Amla, Director, and Dr. V. Sreenivasa Murthy, Dr. S. C. Basappa and Mr. S. K. Majumder, Senior Scientists of the Institute;
- (b) A draft guideline (AGS MISC/77/7, June 1977) using the above-mentioned document of CFTRI arrived at during a meeting (21-25 February 1977) attended by Dr. V. Sreenivasa Murthy and the collaborators Dr. M. Jemmali (Institut National de la Recherche Agronomique, Service des Mycotoxines, Paris, France), Dr. Homero Fonseca (Depto. de Technologia Rural ESA, Luiz de Queiroz, Universidade de Sao Paulo, Piracicaba, S.P., Brazil) and Prof. J. W. Dickens (Agricultural Research Service, U.S. Department of Agriculture, North Carolina State University, U.S.A.) The collaborators also furnished recent information on their respective regions which was very valuable in the preparation and finalization of this publication;
- (c) The critical discussion of the above draft guideline and the recommendations of the Joint FAO/WHO/UNEP Conference on Mycotoxins (Nairobi, Sept. 1977); and
- (d) The final revisions undertaken by the consultant, Dr. T. W. Coombs (Ministry of Agriculture, Fisheries and Food, London, U.K.)

Within FAO, responsibility for finalizing this document rested with the Agricultural Services Division, assisted by the Plant Production and Protection Division and the Food Policy and Nutrition Division. Comments and suggestions for possible future editions of this publication should be sent to:

> The Chief, Food Standards and Food Science Service Food Policy and Nutrition Division Food and Agriculture Organization of the United Nations 00100 Rome, Italy

1. INTRODUCTION

Mycotoxins are a group of toxic chemical compounds produced by certain strains of a number of species of fungi when they grow under favourable conditions on a wide variety of different substrates. As their generic name implies, these compounds are toxic to man and animals causing diseases collectively known as mycotoxicoses. Fungi capable of producing such compounds are usually described as toxigenic.

Although an examination of the early literature reveals that the existence of mycotoxins has been known for over two centuries, it is only since 1961, after the discovery of aflatoxins, that these compounds have attracted considerable attention. On the basis of their target organ or their pathological manifestations they can be recognized as carcinogens, hepatotoxins, nephrotoxins, tremorgens, neurotoxins, etc. With the development of sensitive methods for their detection and analysis, widespread occurrence of such compounds in almost all foods or feeds is becoming evident. This has led to apprehensions about the safety of the food materials we consume daily. A list of mycotoxins found naturally in agricultural commodities, and of mycotoxicoses caused by rusts and smuts, is given in Appendices 1 and 2.

In developing countries, failure to conserve all food resources may seriously aggravate the problems of hunger. In regions where demand exceeds supply, the situation is made worse by high food losses due to insects, micro-organisms, rodents and birds. Climatic conditions in many regions are suitable for agricultural production throughout the year, but they are also ideal for mould growth which can cause an enormous amount of food spoilage. In these same regions the per capita food consumption is low and the ravages of infections and nutritional deficiencies are high. The limited supply of food has forced people to avoid rejecting any material that can be used as food even if moulds have changed its organoleptic quality. It is for this reason that safety of foods that are liable to attack by toxigenic fungi has to be ensured.

In industrialized countries, the problem of contamination of foodstuffs by aflatoxins and a few other mycotoxins is assuming increased importance. Further potential toxins are coming to light as a result of the detailed examination of foods likely to be at risk of fungal contamination. During the last twenty years, efforts have been devoted towards the study of the origins, nature, distribution and possible hazards to human and animal health of mycotoxins in food and feedingstuffs.

Since contamination by mycotoxins may not be completely avoided at the present time, a number of countries throughout the world have established limits in food and feedingstuffs for a few of the mycotoxins. The aflatoxins, derived from toxigenic strains of <u>Aspergillus flavus</u> and <u>A. parasiticus</u> are by far the most commonly controlled mycotoxins in this

respect. Analytical methodology is capable of detecting aflatoxins down to levels of about 1 part per billion (1 ppb or 1 microgramme per kilogramme). Problems of sampling are recognized to be of crucial importance in attempts to control contamination of non-homogeneous lots of foods and feeds. Current information at national level concerning limits set for aflatoxins in foods and feedingstuffs is available from the Health or Agricultural departments of many countries. As such limits are subject to continual review in the light of current information no attempt is made to list them in this guideline. The purpose of this publication is to recommend practices for the prevention of mycotoxins in food, animal feed and their products. It has been prepared following the discussions by the Joint FAO/WHO/UNEP Conference on Mycotoxins (Nairobi, Kenya, 19-27 September 1977) of the draft of a guideline prepared for FAO/UNEP by the Central Food Technological Research Institute, Mysore, India with the assistance of specialists from Brazil, France and the United States of America. It recognizes that effectiveness of preventive and protective practices to be adopted throughout the food chain from the time of sowing, through cultivating, harvesting and processing to storage and marketing depends on a thorough understanding and rigid control of the various factors involved, if the incidence of known important mycotoxins is to be minimized.

Other papers which provide information relevant to problems of mycotoxins and their control are:

a) "Global Perspective on Mycotoxins" (tentative title) an edited collection of major working papers for the Joint FAO/WHO/UNEP Conference on Mycotoxins (see "b" below), dealing with occurence, health and toxicological aspects and affects on the environment and on trade and commerce;

b) "Report of the Joint FAO/WHO/UNEP Conference on Mycotoxins", FAO Food and Nutrition Paper No. 2, FAO, Rome 1977 (includes recommendations);

c) "Mycotoxin Surveillance -- A Guideline", FAO Food Control Series No. 4, FAO, Rome 1977.

The publication is intended to provide information that will be useful to workers in extension services, agriculture, industry and trade. It is recognized that extension needs at the grassroots level will include publications and audio-visual educational materials which must be tailored to the particular audience. It is hoped that this publication will serve as stimulus for the development and use of such extension materials.

This publication is in three parts. Part I gives a schedule of practices recommended respectively for standing crops, for harvesting and drying, for storage of crops, for transportation and for processing (post-harvest, including decontamination). Part II is a discussion or explanation of factors relating to the recommended practices. Part III focuses on needs and aids for implementation of the recommendations.

The commodities mentioned in the discussion (Part II) are those that form the bulk of the food and feedingstuffs in some countries. The mycotoxins considered are those known to be important contaminants in these commodities. Although adherence to the recommendations should minimize the risk of mycotoxin contamination, methodologies to remove, inactivate or destroy contaminant mycotoxins are also suggested in order to attempt some utilization of material which would otherwise require total rejection. References are also given to existing practices, current trends in research, and their likely outcome in relation to the problems posed by mycotoxins.

It is recognized that all of the recommended practices cannot be of universal applicability. Nevertheless, they should provide (see Part III) sufficient information for national authorities to take appropriate action, using or improving the infrastructure and practices existing in their country. PART I

2. SCHEDULE OF RECOMMENDED PRACTICES

- 2.1 Schedule for the Control of Field Infection by Fungi of Standing Crops
 - 2.1.1 Reduce insect and fungal damage by the proper use of approved insecticides and fungicides, and other appropriate practices integrated into a pest control programme.
 - 2.1.2 Use approved fungicides, as appropriate, to reduce or prevent moulding of crops, selecting the fungicidal treatment appropriate to the crop in hand.
 - 2.1.3 Sow crops at the recommended spacings for the species and/or varieties grown in order to avoid overcrowding of plants.
 - 2.1.4 Remove or destroy wild grasses in the vicinity of crops to eliminate reservoirs of fungal inoculum.
 - 2.1.5 Remove or destroy weeds during the lifespan of the growing crop to prevent them from competing with the crop.
 - 2.1.6 Practise crop rotation as a routine.
 - 2.1.7 Irrigate the entire crop evenly, ensuring that the individual plants have an adequate supply of water.
 - 2.1.8 Destroy or bury all dead organic matter, crop residues and alternative host plants together with fungal infected plant material before preparing the ground for a new crop.
 - 2.1.9 Sow and harvest at times which avoid, as far as is practicable, times when mould infections are more likely to occur.
 - 2.1.10 Avoid mechanical damage to crops during cultivation.

2.1.11 Harvest crops at full maturity.

The factors relating to the above recommendations are discussed in some detail in relation to both the control of field infection by fungi and to individual crops in Part II, see pages 8-16.

2.2 Schedule for Harvesting and Drying

2.2.1 Harvest crops at full maturity.

- 2.2.2 Avoid mechanical damage during harvesting.
- 2.2.3 Dry crops immediately after harvesting, particularly if crops are harvested at high moisture levels.
- 2.2.4 Dry crops as rapidly as possible, bearing in mind that sun drying in conditions of high humidity is likely to result in mould infestation of the crop.
- 2.2.5 Avoid re-wetting the crop during and after the drying process by ensuring adequate protection from rain during sun drying, and by avoiding conditions of high humidity induced by the use of covers upon which water vapour condenses when the air temperature falls at night.
- 2.2.6 Dry crops to safe moisture levels before storage.

The factors relating to the above recommendations are discussed in some detail in relation to the harvesting and drying practices used in various countries for various crops in Part II, see pages 16-27.

2.3 Schedule for Storage of Crops

- 2.3.1 Ensure that storage structures are dry and do not permit the entry of water either by seepage of ground water or other leakage.
- 2.3.2 Stack bagged grain on dunnage or pallets to avoid upward movement of ground water, unless the floor incorporates a membrane impermeable to water vapour.
- 2.3.3 Ensure that only high quality, mould and insect free crops, dried to safe moisture levels for the particular crop, are stored.
- 2.3.4 Control insect infestation in both storage structures and stored bulk grain by preventive/corrective treatment with approved insecticides. Elimination of crawiing insects from grain avoids the deposition of fungal spores and fungi, and minimizes the development of "pockets" of grain with elevated moisture levels where fungal growth invariably occurs.
- 2.3.5 Store at low temperature whenever possible, as fungal growth resulting in mycotoxin contamination is correlated directly with temperature increase.

- 5 -

Certain Fusarium species are an exception to this general rule in that they can produce mycotoxins at low temperatures. In these cases, storage under nitrogen may be effective.

- 2.3.6 Farm Storage
 - (a) Fumigate and dry produce already infected in the field before storage:
 - (b) Winnow and sieve out immature, discoloured and broken kernels;
 - (c) Store the produce in moisture-proof structures or containers which are amenable to fumigation treatment:
 - (d) Inspect the stored produce regularly, using suitable fumigants to control any insect infestation where necessary.

2.3.7 Large-Scale Storage

·高山縣公司941-1月1日上部日

ALL A MALEST MILLING

t there is here a

- (a) Avoid storing produce of a moisture content that exceeds that recommended in Appendix 5.
 - Ensure that the warehouse floor is moisture-proof (b) and rodent-proof. Use polyethylene sheet or wooden pallets under the stacks. Ensure that the warehouse can be both properly ventilated and rendered airtight to allow fumigation as and when necessary.
- (c) Use suitable, approved fumigants.
 - (d) Reduce moisture levels in the stored produce by aeration if the relative humidity permits.
- (e) Prevent cross infestation of different lots of produce in the warehouse by prophylactic treatment with suitable approved pesticides. State Made - sulvisions
 - (f) Prevent access by rodents and birds.

The factors relating to the above recommendations are discussed in some detail in Part II, see pages 28 - 34 . 12825 10 1.

> . The second is the second and second the state but it in the second intertains of a 175 of the mention is with a

- 2.4 Schedule for Transportation
 - 2.4.1 Check on and correct undesirable conditions in commodities during transport.
 - 2.4.2 Disinfest empty transport containers and vehicles periodically with a suitable approved fumigant or other pesticide.
 - 2.4.3 Avoid warehousing food shipments whose moisture content exceeds that recommended in Appendices 4 and 5 for the appropriate commodity.
 - 2.4.4 Avoid reabsorption of moisture during shipment or other transportation by the use of tarpaulins, by ballooning, or by using airtight containers as appropriate.
 - 2.4.5 Utilize insect-proof or insect-resistant packaging materials, or containers rendered insect-repellent and rodent-repellent by chemical treatment.

Some factors relating to the above recommendations are discussed in Part II, see pages 35-36 .

- 2.5 Schedule for Post-Harvest Processing including Decontamination
 - 2.5.1 Avoid mechanical damage to crops during threshing or decortication.
 - 2.5.2 Protect crops during processing from any conditions which favour fungal growth.
 - 2.5.3 Expedite those stages of processes that necessarily involve some rehydration of the raw material.
 - 2.5.4 Segregate by physical means the damaged portion of any crop before processing commences.
 - 2.5.5 Ensure inactivation of mycotoxins by methods that will not result in the introduction of new toxic substances into the food or feed chain.

The factors relating to the above recommendations are discussed in some detail in Part II, see pages 36-48.

PART II

3. DISCUSSION OF FACTORS RELATING TO THE

SCHEDULE OF RECOMMENDED PRACTICES

3.1 Standing Crops and Control of Field Infection by Fungi

3.1.1 MAIZE

Studies have shown that the aflatoxin producing fungus, <u>Aspergillus flavus</u>, can infect corn before harvest (1). The presence of the fungus and of aflatoxin in maize ears has been associated with kernel injury caused by the European corn borer on the standing crop (2). Other studies have provided presumptive evidence for regional differences in <u>A</u>. <u>flavus</u> infection of field maize with indications that maize grown in warm, humid areas is highly susceptible (3). Examination of the factors contributing to the problem showed that growing maize under stress conditions, such as dense population of plants or reduced fertilization, appeared to increase the incidence of aflatoxin contamination (4).

Examination of maize strains for differences in susceptibility to <u>A</u>. <u>flavus</u> infection could reflect simply a difference in protection offered by the husk against insect attack which is responsible for introducing the micro-organism into the kernel region of the ear (5). Insecticide treatment of developing ears reduced insect damage, but failed to preclude <u>A</u>. <u>flavus</u> infection and subsequent aflatoxin production (6).

Laboratory studies have presented some evidence for the existence of groundnut varieties that resist invasion by aflatoxin producing fungi (7). In contrast, hybrids of maize experimentally infected with <u>A. flavus at the late milk to early dough stage of</u> kernel development did not vary in extent of infection of <u>A. flavus</u>; toxin production, however, varied significantly among the hybrids (8). Available research information does not provide an unequivocal basis for establishment of prevention techniques in the field. There are no known techniques to prevent contamination by zearalenone or some other mycotoxins in standing crops of maize. Frequent mould and mycotoxin contamination of this crop in certain regions should stimulate increased concern for appropriate harvesting and storage of the commodity.

3.1.2 WHEAT, RYE, OATS AND BARLEY

Rusts and smuts are the two important fungal diseases that affect these crops. In recent years they have been suspected as causes of mycotoxicoses (9). The methods developed to prevent these diseases on standing crops are not adequate and hence general good agricultural practices, such as crop rotation and field sanitation, and the growing of resistant varieties are recommended. So far there is no evidence of <u>A</u>. flavus infection on standing crops of the above cereals.

The outstanding example of a fungal disease of cereals causing mycotoxicosis in animals and man is ergot. As the principal cereals vulnerable to attack by the causative fungus <u>Claviceps purpurea</u> are rye and durum wheats, the disease can be kept under control (10) by a system of rotation with the commoner wheats, or with oats or barley which are rarely attacked. Rotation avoiding cereals will help in the reduction of inoculum in the field. <u>C. purpurea</u> also infects other hosts which serve as sources of inoculum to the rye crop. The prevalence of sclerotia on such a wide range of pasture and wild grasses makes it imperative to eradicate as far as possible all such hosts, especially if they occur in wet, sheltered borders of rye fields.

As the disease affects infloresence only, hay should be cut while still green before ergots develop (11). Since ergots are not long lived, deep-ploughing, together with a rotation of 2 or 3 years before re-planting rye grains, is usually enough to starve these bodies in the soil. Only seeds which do not contain the fungus should be planted. Mould often becomes established on sorghum grain before harvest. Invasion generally begins while seeds are immature and may continue until the seed is adequately dried after harvest. Humid conditions during seed development greatly enhance infection. Fungi generally associated with mouldy grain are species of <u>Alternaria</u>, <u>Curvularia</u>, <u>Fusarium</u>, <u>Cladosporium</u>, <u>Penicillium</u>, <u>Oidium</u>, <u>Nigrospora</u> and <u>Phoma</u> (12). The head moulds of sorghum also include <u>Aspergillus flavus</u> which has been found to produce aflatoxin on the earhead of sorghum (13). In addition to mycotoxin development, head moulds are of particular concern in seed production, and they may also reduce the nutritive value of the grain.

Growing resistant varieties, seed treatment, crop rotation, destruction of infected parts and field sanitation are some of the general practices that can control the head moulds as well as smuts and ergot.

3.1.4 PEARL MILLET (PENNISETUM THYPHOIDEUM)

The fungus <u>Claviceps macrocephala</u> which causes ergot diseases of millet infects the standing crop at the inflorescence stage. It attacks a number of <u>Pennisetum</u> species (14). The conidia of the fungus retain their viability up to 13 months. Thus the fungus can survive from year to year through the agency of conidia. Cool, humid weather favours infection. Control methods suggested above for rye should be followed.

3.1.5 GROUNDNUTS

Since fungi are distributed both in soil and air, the infection of groundnuts with toxigenic fungi may occur before groundnuts are removed from the soil or during subsequent harvesting, drying, handling and storage. In many cases the entry of <u>Aspergillus flavus</u> into the tissue of standing crops is facilitated by damage from nematodes, mites, termites and other small animals.

It has been observed that drought conditions favour infestation by lesser cornstalk borer (Elasmopalpus lignosellus).

which damages pods and feeds on kernels (15). <u>A</u>. <u>flavus</u> propagules may be carried by the insect to ideal sites for infection where the kernel is damaged. Other small animals like nematodes and mites which inhabit the soil may infect the groundnuts with <u>A</u>. <u>flavus</u> when they attack them for food and moisture. Injury of the pods by agricultural implements also favours infection.

Although groundnuts may be invaded by A. flavus when growing in moist soil, drought conditions appear to be more favourable both for infection by the fungus and for aflatoxin production (16). During drought, temperature and moisture contents of the groundnuts and soil appear to become favourable for A. flavus growth. Irrigation of fields at least one month prior to harvest has been found to reduce aflatoxin contamination in groundnuts (16). This is probably because moist soil reduces attack by soil insects and mould and because groundnuts previously damaged by insects or mould may shed from the plants before they are harvested. Both foliar and soil fungicides have failed to reduce A. flavus and aflatoxin contamination before harvest (17). Groundnut varieties resistant to insect attack or insecticides may be used to control the lesser cornstalk borer in non-irrigated fields and soil fumigation may be used to control nematodes (15).

Since weeds compete with the growing plants of groundnuts and interfere with good harvesting and drying, weed control is an important practice to help reduce the risk of aflatoxin contamination (18).

Care should be taken not to damage pods during cultivation since such damage may allow mould growth. Crop rotation and winter cover crops have been reported to reduce <u>A</u>. <u>flavus</u> propagules in the soil (17). Groundnuts may be grown satisfactorily in rotation with a number of crops like cotton, tobacco and cereals. Many experimental stations recommend that groundnuts should not be grown in the same soil more often than once every 3 years.

Varieties like Junagadh 11 are being tested under some Indian conditions and resistance to toxin elaboration appears to be a fairly stable character (19). However, there is need for constant surveillance of such resistant varieties under different agro-ecological conditions. Attempts are being made by several countries in the world in order to explore the possibilities of evolving varieties of groundnuts resistant to <u>A. flavus</u> growth and to aflatoxin formation.

3.1.6 COTTON

<u>A. flavus</u> has been known for many years as the cause of boll rot in the cotton plant (20). A bright greenish-yellow fluorescence (BGY) on the lint when it is exposed to long-wave ultraviolet light is caused by <u>A. flavus</u> and is symptomatic of this boll rot. BGY is a useful presumptive indicator of the presence of <u>A. flavus</u> and aflatoxin (21). Invasion of the cottonseed boll by insects and ultimately by <u>A. flavus</u> generally occurs before full boll opening and is maximal at an optimum temperature range of $30-35^{\circ}$ C (22). Aflatoxin production apparently occurs in undamaged bolls only from the time they start to open until the seeds are dry as a consequence of greater aeration (22). Thus the more rapidly cotton bolls open and dry, the lower the potential for aflatoxin contamination of the seeds.

Ashworth et al. (23) have implicated the pink bollworm (Pectinophoro gossypiella Saunder) exit hole as a major portal by which the fungus enters the boll. Lygus (Lygus hesperus Knight) and stink bug (Chlorachroa sayi Stal.) have been implicated as means of A. flavus propagule dissemination into these exit holes (24).Internal injury to locules by insects such as the pink bollworm slow the rate of boll opening and often prevent the boll from opening completely and the locules from fluffing fully. High moisture conditions surrounding bolls also contribute to a slower boll opening rate (25). The combined effect of both conditions is to prevent locule fluffing and drying and to maintain higher moisture levels in seeds for prolonged periods of time. This drying delay probably increases the opportunity for seed invasion and subsequent aflatoxin elaboration by A. flavus. Management practices like defoliation of the lower third of cotton crop with DEF (S.S.S-tri-butylphosphorotrithionate) accompanying the 4 by 4 skip row pattern in order to improve aeration of the plants and lower humidity has been found to reduce aflatoxin (26). Aflatoxin accumulation in Deltapine 16 cottonseed, grown in Arizona in a 3-year study (27) was significantly influenced by the timing of irrigation termination and the level of pink bollworm infestation. In 1971 and 1972, termination of irrigation by early August resulted in significantly less aflatoxin than in plots where two additional irrigations were applied. Significantly less aflatoxin also was found in the 1971 and 1973 plots where low levels of pink bollworm infestations were maintained by treating the plots with insecticides like methylazinphos and methomyl.

Thus, the available information suggests that <u>A</u>. <u>flavus</u> infection and aflatoxin formation in the seed of standing crops of cotton may be prevented to a significant extent by following controlled irrigation at boll stage to reduce humidity and by controlling the insect infestation by the use of suitable insecticides.

3.2 Control of Field Infection by Fungi

Crops such as sorghum, maize, paddy (rough-rice) and beans are liable to internal and external pre-harvest attack by insects and moulds in the field (29). The oviposition by storage pests or mechanical injury by insects and other arthropods are largely responsible for fungal infection. The preventive measures for controlling oviposition by the insects on the kernels and pods may be achieved by application of repellents or insecticidal compositions (30). Pre-harvest disinfection and prophylactic application of chemicals such as captan, thiram, zineb, propionic acid and acetic acid have been found to reduce incidence of fungal contamination of grains. This spraying operation, if carried out during the milky and post-milky stages, may prevent internal infection. Combinations of pesticides of low mammalian toxicity such as malathion and captan at 0.3% concentration applied with a low-volume nozzle have given prophylactic effects on treated panicles of paddy, sorghum, maize and legumes. A low-volume nozzle attached to a hand sprayer (knapsack sprayer) provides a way to apply the low-volume spray. It is necessary to watch out for development of resistance to pesticides.

It is quite clear that kernels which are damaged by mechanical injury during agronomic operations are more subject to mould infection. During post-harvest handling, particularly of the wet crop, the prevention of mechanical injury and of attack by fungi and storage insects are of prime importance. Pre-harvest sanitation and post-harvest safe handling are essential to reduce moulding of the kernels.

It may be possible to segregate <u>A</u>. <u>flavus</u> contaminated groundnuts at the field level. Close observation of the groundnut fields prior to digging may reveal areas of plant stress such as isolated pockets of severe drought stress that are often the areas of <u>A</u>. <u>flavus</u> infection and insect infestation. In irrigated fields the areas missed by the irrigation system may contain high concentrations of insect damaged and <u>A</u>. <u>flavus</u> infected groundnuts. Harvests from such areas could be handled and processed separately to remove the aflatoxin contaminated kernels. If <u>A</u>. <u>flavus</u> growth is confirmed by careful examination, the groundnuts should be diverted to non-food use or decontaminated by removal of all mouldy kernels.

Careful examination of standing crops of grain and separate harvesting of mouldy heads would prevent the mouldy grain from being mixed with other grain during harvesting. If the heads are harvested intact then the mouldy heads should be removed before the grain is threshed. In the case of maize, the ears with mouldy kernels should be separated before shelling to avoid mixing the mouldy kernels with non-mouldy ones.

- 3.3 References to Sections 3.1 and 3.2 Standing Crops and Control of Field Infestation by Fungi
 - Rambo, G.W., Tuite, J., and Crane, P. (1975) Phytopathology, <u>64</u>, 797.
 - Lillehoj, E.B., Fennel, D.I., and Kwolek, W.F. (1976) Science, 193, 495.
 - Burnside, J.E., Sipple, W.L., Forgaes, J., Carll, W.T., Atwood, M.B., and Dott. E.R. (1957). Am.J. Vet. Res. 18,817.
 - Anderson, H.W., Nehring, E.W. and Wichsor, W.R. (1975)
 J. Agr. Food Chem. 23, 775.
 - Lillehoj, E.B., and Zuber, H.S. (1975). In proceedings of the 13th Annual Corn and Sorghum Research Conference. U.S.D.A.
 - Widstrom, N.W., Sparks, A.N., Lillehoj, E.B. and Kwolek, W.K. (1975) J. Eco. Entomol, <u>68</u>, 855.
 - Nagarajan, V., and Bhat, R.V. (1972). J. Agr. Food Chem. 20,911.
 - Laparde, J.C., and Manwiller, A. (1976). Phytopathology, <u>66</u>,675.
 - 9. Martin, P.M.D. and Gilman, G.A. (1976) "A consideration of the Mycotoxin hypothesis with special reference to Mycoflora of maize, sorghum, wheat and groundnuts", published by T.P.I., London.
 - Butler, E.J., and Jones, S.G. (1949) in "Plant Pathology" McMillan and Co. Ltd. p. 445.
 - Sampson, K., and Western, J.H. (1971) Cited in "Plantpathology" McMillan and Co., by Butler and Jones.

- 12. Tarr, S.A.J. (1962). "Diseases of Sorghum, Sudan grass and Broom corn" published by Commonwealth Mycological Institute, Kew, Surrey.
- 13. Thripathi, R.K. (1973) Ind. Expl. Biol. II. 361.
- 14. Singh, R.S. (1968) cited in "Plant diseases" Oxford and IBH Publishing Co. New Delhi.
- 15. French, J.C., and Morgan, L.W. (1972). J. Amer, Peanut Res. and Educ. Assoc. <u>4.41</u>.
- Pettit, R.E., Taber, R.A., Sehraeder, H.W., and Harrison, A.L. (1971), Appl. Microbiol. 22,629.
- 17. Pettit, R.E., and Taber, R.A. (1973). J. Amer, Peanut, Res. and Educ. Assoc. 5, 195.
- 18. Dickens, J.W. (1976) Journal Series of the North Carolina Agr. Ept. Sta.
- Nagarajan, V., Bhat. R.V., and Tulpule, P.G. (1974) Phoc. Nut. Soc. India <u>16</u>,52.
- Marsh, P.B., and Kerr, T. (1961) Plant disease reporter 45, 550.
- Ashworth, L.J., McMeans, J.L., Pyle, J.L., Brown, C.M., Osgood, J.W. and Ponton, R.E. (1968). Phytopathology. 58,102.
- 22. Ashworth, L.J. McMeans, J.L., and Brown, C.M. (1969) Phytopathology. <u>59</u>,669.
- Ashworth, L.J., Rice, R. E., McMeans, J.L., and Brown, C.M. (1971). Phytopathology <u>61</u>,488.
- 24. Stephensen, L.W., and Russel, T.E. (1974) Phytopathology, 64,1502.
- 25. Kerr, T. and Tharp, W.H. (1956) Cottongin Oil Mill Press, 57,8.
- Ashworth, L.J. (unpublished data) cited by R.W. Howell in "Toxic Micro-organisms". Edited by M. Herzberg (1970) proceedings by U.J.N.R. Meetings.

- 27. Russel, T.E., Watson, T.F. and Ryan, G.F. (1976) Appl. Environ. Microbiol, 11, 711.
- Christensen, C.M. and Kaufmann, H.H. (1965) <u>31</u>,711 Ann. Rev. Phytopath. <u>3</u>,69.
- Majumder, S.K., Raghunathan, A.N. and Rangaswamy, J.R. (1973) Ann. Technol. Agri. <u>22</u>,483.
- Majumder, S.K., Krishmamurthy, K., and Godavari Bai. S.C. (1961) Nature (London) 192,373.

3.4 Harvestin; and Drying

During the harvest and in post-harvest handling, particularly of the wet crops, the prevention of mechanical injury is of prime importance. The following describes harvesting and drying practices for corn, rice and groundnuts in various countries. The descriptions are not given as recommended practices.

3.4.1 HARVESTING MAIZE

The time of harvest is fixed at the maturity of the grain. In France 75 percent of the maize is combine harvested, the drying being done by hot air (110-120°C). The 25 percent remainder is stored on the cob (ears) in "cribs". Shelling or threshing is done after the grain dries to 15 percent moisture content. The practice of crib storage may result in contamination by zearalenone and trichothecenes (1).

In Brazil, maize is usually dried on the plant before harvest. If it is intended to be used as feed, the ears are stored. If it is to be sold, the grain is threshed and stored in sacks. The major source of possible contamination is improper storage with the possibility of getting wet by rain (2).

3.4.2 HARVESTING RICE

Rice is ordinarily harvested at moisture contents above safe storage levels. The criteria for determining the proper harvest. time include maturity in relation to panicle colour, age of the paddy crop and moisture content of the paddy. Moisture is the most important factor which decides the quality of the grain during storage.

Harvesting at a late stage of maturity when the moisture content is around 15-17 percent seems to be the traditional practice, but shedding losses and heavy milling breakage have induced farmers to harvest at 20-24 percent moisture level (3). This moist paddy must be dried to a safe moisture content of 14-15 percent before storage. Machine harvested paddy usually has much higher moisture levels which makes pre-storage drying obligatory. Likewise, rice produced by dehusking high moisture paddy needs pre-storage drying.

Rice will readily gain or lose moisture until it is in equilibrium with the moisture of surrounding air. The equilibrium moisture content is dependent primarily on the relative humidity of the air and depends to a lesser extent on air temperature (4). The equilibrium moisture content is different for rough rice, brown rice, milled rice and bran (4). High relative humidity of the atmosphere interferes with drying of rice to safe levels and also favours moisture absorption by rice during storage.

3.4.3 HARVESTING GROUNDNUTS

To facilitate removal of groundnuts from hard soil in many growing areas, harvesting is done during rainy months. Under dry weather conditions it is done after soaking the soil with flood irrigation. Mechanical injury to the pods by either arthropod activity or tillage may induce fungal infection. Injury inflicted during harvesting is also responsible for kernel penetration by saprophytic fungi.

In more developed countries, windrow harvesting is the generally accepted method for groundnuts. A digger-shaker windrowing machine is normally used to dig the plants, shake off soil from the roots and pods and invert the plants in a windrow. Most of the pods are on top of the inverted windrow and exposed to sunlight and aeration which aid drying. After several days, partially dried groundnuts are picked, piled 1.5 m deep on perforated floors and dried to an average moisture content of less than 10 percent by forcing heated air up through the pile. In the absence of mechanical drying facilities, particularly where farm holdings are small, suitable treatment for the control of fungal infection in moist pods is very necessary. In certain regions of India, the pods are left intact with haulms for a few days on the ground. This brings the moisture level down to about 15-20 percent. After separation, the pods are further dried by spreading on a paved floor. In other regions, stacking the plants with pods facing outside and haulms towards the centre is practised. In all such cases, low humidity weather conditions help in quick drying. In humid or rainy weather, the drying becomes difficult and there is an opportunity for growth of <u>Aspergillus flavus</u>. In such situations, the pods can be protected by using fungicidal fumigants.

In Brazil the groundnut plants are left inverted so that pods are exposed to the air and sun for drying. Since the grower is anxious to market the crop and receive payment, the pods are often harvested before they have dried to a safe moisture level for storage. The pods are placed in sacks and taken to the oil mill where they are easily marketed regardless of moisture content since the buyer adjusts the price according to the weight of moisture above 8 percent.

In some regions of Africa threshing is done manually and care is taken to avoid breaking the pods. After threshing the pods are spread on the soil in thin layers to dry. In the Sahel Zone the groundnut varieties planted are ready for harvest after the end of the rainy season; the groundnuts dry without moulding and there is a low incidence of aflatoxin contamination. In the equatorial zone, aflatoxin contamination is more prevalent because the crop must be harvested and dried during rainy, humid weather and moulding often occurs.

3.4.4 DRYING PRACTICES

Drying is an essential step in the preservation of grains against fungal attack. At harvest, cereal grains or oilseeds often contain too much moisture for safe storage. In places where harvesting is done in dry weather, the problem of mycotoxin contamination does not reach alarming proportions. But it becomes a serious problem in areas where harvesting is done in very humid weather. In such cases, drying of the produce is an extremely important factor in solving mycotoxin problems. During drying operations, mechanical damage must be prevented and the temperature and duration of exposure of the produce to heated air must not be excessive, or quality losses will occur.

The normal practice is to spread the commodity on a paved floor and expose it to the heat of the sun and to natural air currents. Frequent stirring is required to achieve uniform In most places this is done manually. Spreading the drying. commodity on a loosely woven material or screen suspended above the ground will improve aeration and reduce but not eliminate the need for stirring. In cloudy and rainy weather, insolution is not sufficient to dry the commodity to safe moisture levels in a reasonable time; drying by artifical means must then be resorted to. The FAO Agricultural Development Paper No 90, "Handling and Storage of Food Grains in Tropical and Subtropical Areas" (1970) describes natural and artificial methods of drying groundnuts to reduce the risk of aflatoxin contamination. Normal practice is to heat air by burning fuels, by utilizing heat supplied by internal combustion engines, or by use of electrical-resistance heaters and then to pass the heated air through the commodity.

3.4.5 DRIERS FOR RICE (AND OTHER CROPS)

Various types of driers using forced natural air or forced heated air are used in different countries (5). Some of the driers used for rice are described below:

(i) Sack drier

This is a simple unit (Figure 1) wherein bags of grain or nuts are laid flat over holes cut in the floor of a tunnel system so that air can be forced up through the bags from an air chamber underneath. A single layer of sacks and air at 115-120°F at a rate of 110 cfm/sack can reduce the moisture content from 48 to 8 percent in 16 hours. It is being used for rice drying in many countries in the tropics, e.g. in the Philippines and in Madagascar. A drying system of this type used in Madagascar takes 228 bags of rice at a time. The chief disadvantages of these types of drier are high labour

cost, low drying capacity and need for close supervision.

(ii) Horizontal warm-air drier

This type of drier (Figure 2) is not often used for drying of rice. It is manufactured mostly by Italian and French

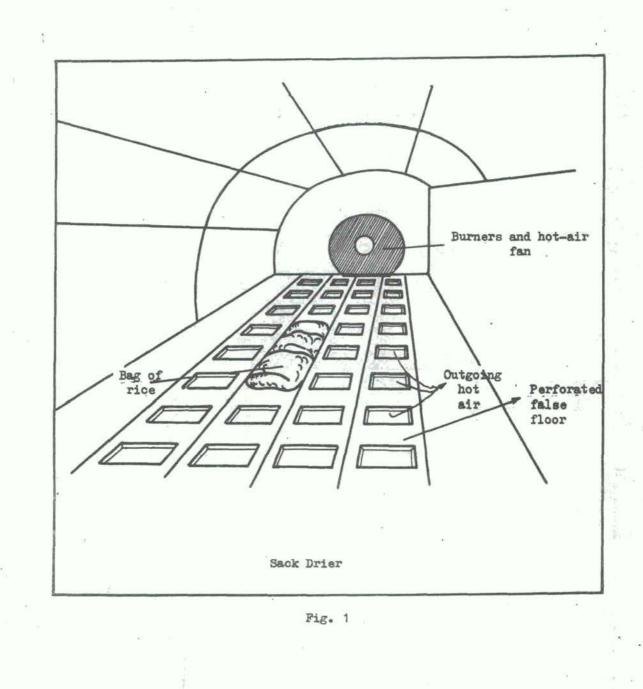
firms. These driers consist of one or more drying chambers. Each chamber is divided by horizontal, equidistant screenbottom trays fitted to horizontal pivots. A control lever on the outside swivels the trays into a horizontal position for drying, and a vertical position to discharge the kernels onto the tray underneath. The moist grain or nuts are fed to the top tray. Retention time on each tray is set equal to the total drying time divided by the number of trays. The hot air is fed through each tray at 95-100°F. Units with a capacity of 25 tons in 24 hours are available.

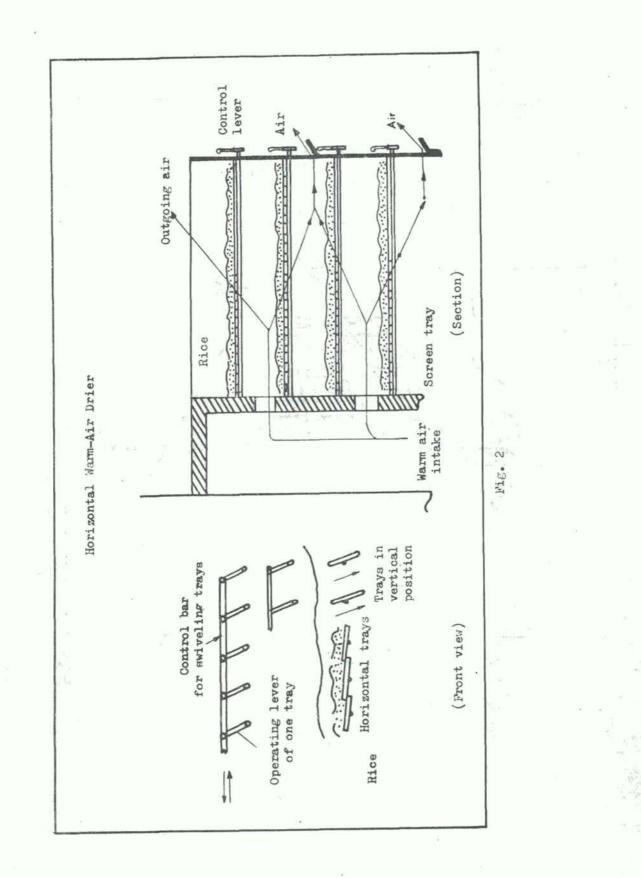
(iii) Mixing-type columnar drier with baffles

This drier is fitted with baffles (Figure 3) which conduct rice downward in a zig-zag path as heated air is forced through the descending grain. The warm air blown into the centre of the drier penetrates into the rice through the vents on the inner side and escapes through the outer side. In order to dry 60 tons of damp rice in 24 hours, a drying system should comprise four successive drying units and a 22-h.p. motor. The housing structure would cover 80-90 m².

(iv) LSU mixing-type drier

The Lousiana State University Drier (Figure 4) is more complicated. It consists of a bin in which layers of air channels are installed. Heated air is introduced at many points in the descending material through these channels which are inverted-V-shaped. One end of each channel is open and the other end is closed. Alternate layers are air-inlet and air-outlet channels. In the inlet layers the openings face the air-inlet plenum chamber; in the outlet layers they face the exhaust. Each layer is offset so that the top of the channels split the streams causing mixing. The discharge at the base of the column regulates the flow of material. In most models heated air is supplied by a blower. The capacity varies from 2 to 11 tons, and the drier height from 4.6 to 13 m. The number of air channels range from 60-160 according to the size of the drier, and power requirements from 10 to 40 h.p. Batteries of driers can be installed. Though large quantities can be handled under controlled conditions, these units are complicated and expensive.





27

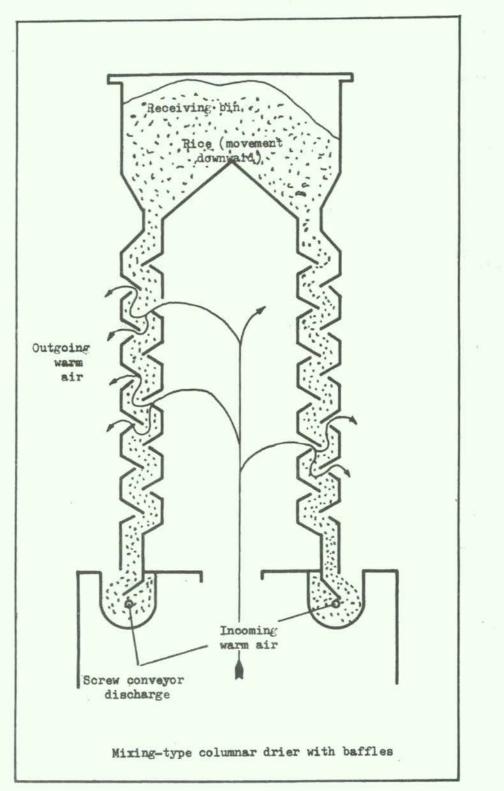


Fig. 3

- 23 -

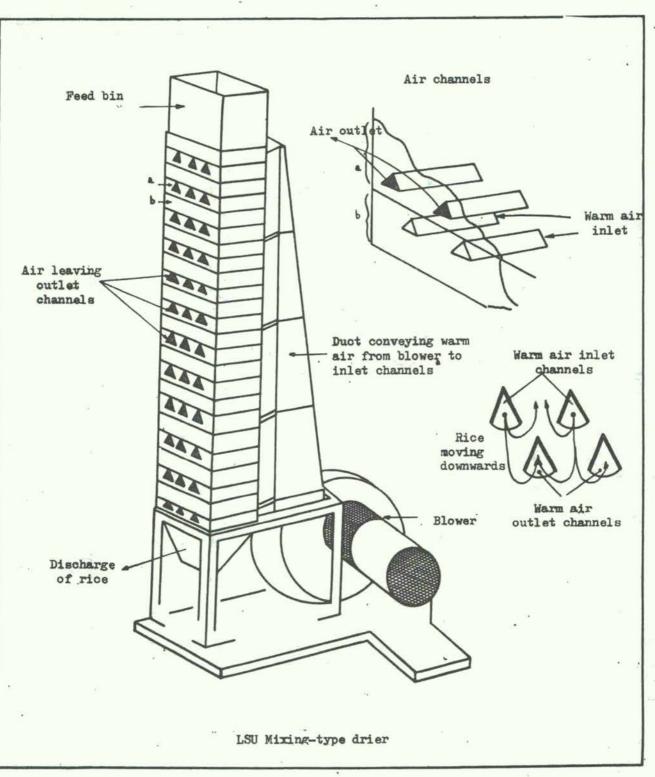


Fig. 4

- -- power for handling, conveying and aeration equipment
- -- fuel (fuel oil, natural gas, butane, coal, wood) to heat air
- -- labour
- -- repairs and maintenance

-- humidity and temperature control

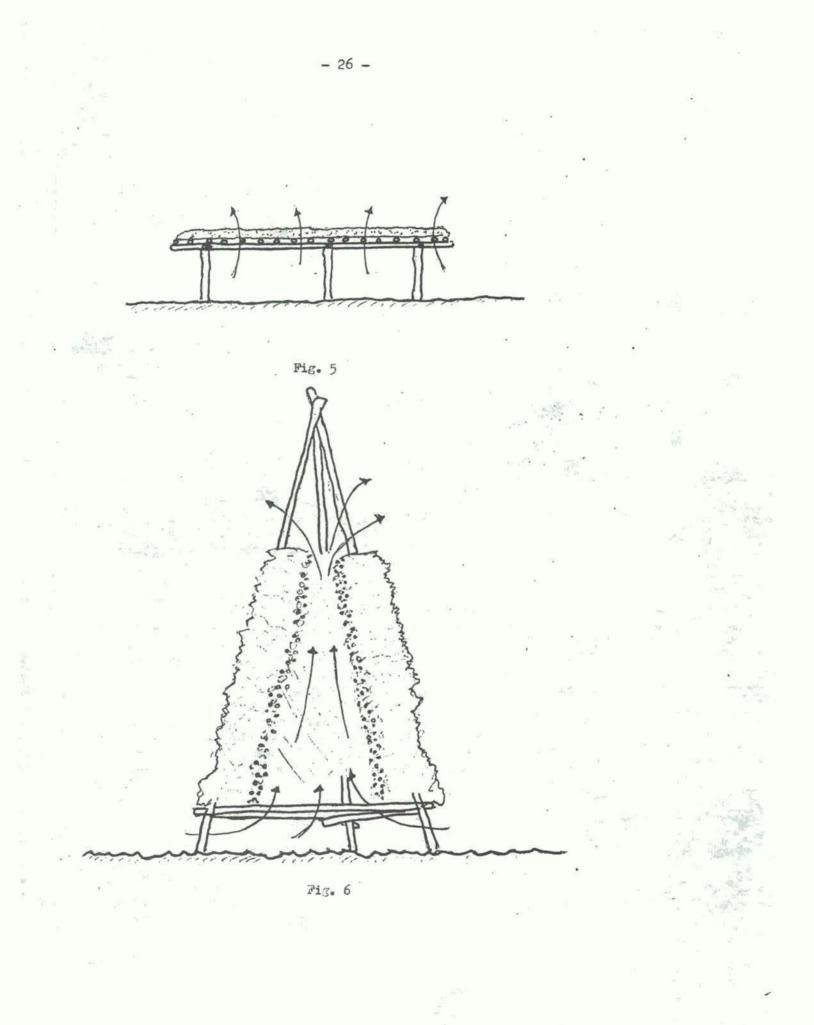
-- amortization

-- interest on capital.

3.4.6 GROUNDNUT DRYING

A procedure for groundnut drying in some African countries is usually performed in two steps. After digging and shaking, the groundnut plant is left in windrows in the field. Under favourable conditions, the groundnuts will dry down to a moisture content of about 20 percent. Sometimes the plants are stacked in the field, usually with the pods on the outside to expose them directly to the sun. The haulms may be stacked around poles or on platforms elevated about 0.5 m above the ground (Figure 5). Another suitable method is that of stacking the plants on some type of rack, with the pods facing an internal cavity (Figure 6). Correct windrowing and improved methods of stacking are inexpensive improvements which would give immediate results (from Carl Bro International A/S, Copenhagen, 1976), (6).

In the second step, the pods are picked from the haulms and brought to the farmhouse. Additional drying is done at this stage. The usual method is to spread the pods on a dry area of the ground and allow to dry in the sun, with periodical turning. In recent years, solar energy collectors for heating air to dry the commodity are being introduced for efficient use of the sun's energy.



The cost of drying can be estimated in terms of capital expenditure, labour and running costs. With natural drying usually only labour costs have to be considered. Quite often this is negligible in real terms as the farmer and his family perform the operations of spreading, collecting and re-spreading. With artificial drying, however, capital expenditure may vary considerably, the cheapest being the in-sack drier and the more expensive being the continuous flow driers which require trained people to maintain them. The cost of drying in most of these latter cases exceeded US\$3 per ton in 1976.

3.5 References to Section 3.4 Harvesting

and Drying

- Report from Dr. Jemmali, one of the collaborators in the preparation of this manual.
- Report from Dr. Homero Fonseca, one of the collaborators in the preparation of this manual.
- Shiva Subrahmanian, S., Sheik Dawood, A., and Krishnan, R.H. (1972), Madras Agr. J. 59, 574.
- Roy Adair, C. (1972) cited in "Rice Chemistry and Technology" by American Association of Cereal Chemists, Ed. E.E. Houstan.
- Angladette, A. "Rice drying principles and techniques", Informal Working Bulletin, 23, FAO of the UN.
- Carl Bro. International A/S, Copenhagen, Denmark, "Aflatoxin-free Groundnut Production in Developing Countries".

3.6 Storage

3.6.1 BASIC PRINCIPLES

Prevention of attack by fungi and stored product insects and other pests is of prime importance in post-harvest storage. The basic principles of grain storage are relatively simple. Firstly, the product to be stored should be conditioned to a stable state in which respiration of the seeds and of associated micro-organisms are reduced to a minimum. This is achieved by keeping the moisture content of the grain and the ambient humidity very low.

Secondly, the product should be placed in a container or structure that will maintain a suitable environment and prevent or restrict the entrance of insect and animal pests.

Lastly, the grain should be accessible throughout the storage period for additional treatment if necessary to maintain good condition particularly with respect to heating and moisture absorption.

Since in many cases agricultural crops are seasonal, they must be stored from one season to the other. In rural areas, individual farmers have their own storage structures in which the grains are stored. The storage structures vary in type of construction, size and materials for construction (1,2). Above-ground structures range from cubicles and small rooms to large warehouses. Masonry bins, wooden cubicles, straw and bamboo structures, and clay containers of baked and unbaked materials are used in the developing countries. For commercial purposes, storage structures may be silo-like elevators, large-scale bulk storage warehouses or bag-storage godowns.

In order to prevent losses in quality or quantity, the product (crop) must be protected from insects, rodents, moulds and biochemical deterioration. Protection from moulds has become very important due to increased awareness of the hazards of mycotoxins to human and animal health. Temperature and moisture control are important considerations for prevention of moulding. Insect control is also important since insects may cause high-moisture pockets in the stored product, create sites for mould infection by penetrating the product and carry mould spores to infection sites. Processed products such as flour,

- 28 -

meal and groundnut cake must be protected during storage since viable mould spores are present and if conditions are favourable for mould growth, mycotoxins may be produced.

3.6.2 CONTROL OF MOISTURE AND TEMPERATURE

Proper drying of the product before placing it in storage is necessary. The influence of moisture content on mould growth during storage of grain at different temperatures and humidities is indicated in Appendix 4. It is important that the commodities be dried below a moisture content that will support mould growth. Recommended moisture contents for commodities going into storage are listed in Appendix 5.

Recent work has shown that the measurement of water activity (free water not bound to constituents in the produce) rather than the equilibrium moisture content of produce is more directly related to fungal growth. Suitable instruments for use in the field are now available for this measurement. At present most moisture testers being used in developing countries do not measure water activity.

As previously mentioned there is a large variety of storage structures used throughout the world. Requirements for ventilation and aeration for small bulk storages or when the product is stored in bags or other small containers are different than for the large bulk storages. Whenever moulding and caking occur in these different types of structures, care should be taken to determine the cause of the problem and corrective action should be taken. In general, the storage structure must be constructed to prevent leaking or diffusion of moisture through the roofs, walls or floor of the structure.

Convection currents can produce pockets or layers of high moisture grain even in properly dried grain when it is stored in large bulk. Therefore, it is necessary to have well ventilated storage warehouses to allow the warm, moist air to escape from the warehouse rather than have the moisture condense on interior surfaces of the building and rewet the grain. Escape of the warm, moist air from the warehouse will also facilitate cooling of the grain and thus reduce mould growth and insect activity. In some cases, natural ventilation of the headspace in bulk storage warehouses is not sufficient to prevent moisture condensation, moulding and caking of the surface layer of grain. Aeration of the grain by fans to exhaust the warm moist air from perforated ducts placed underneath the bulk-stored product is then necessary. Aeration exhausts the warm, moist air before it comes in contact with cool grain or cool interior surfaces of the warehouse where moisture condensation may occur. It also draws cool, dry air into the grain which cools it and reduces mould growth and insect activity.

When weather conditions are warm and humid, ventilation or aeration may be detrimental rather than helpful. If the relative humidity and temperature of the air are higher than conditions within the warehouse, ventilation or aeration may increase the moisture content and temperatures of the grain. In warm, tropical areas where such conditions are prevalent it may be necessary to provide ways to avoid moisture absorption by the dry product during storage. Use of a ballooning technique when the crop is dry will prevent moisture penetration and absorption by the crop during the humid season.

The technique consists of placing 0.06 mm polyethylene sheet on the floor over treated gunny-cloth dunnage, enveloping the whole stack with another 0.06 mm polyethylene sheet from the top and sealing the seams with clamps. Finally, the plastic envelop thus made must be protected from rodents and is protected against insects by the application of an insecticidal spray. Protection from mould damage is also achieved by the ballooning technique (see Appendix 3).

3.6.3 CONTROL OF STORAGE INSECTS

The relationship between insect damage and mould activity is two fold:

- (a) Even in dry grain, the metabolic activities of insects raise moisture levels and provide a favourable environment for fungal growth (1);
- (b) The insects invariably carry with them a variety of microflora including fungi.

These are the reasons for the common observation that invasion by insects is almost always accompanied by some fungal infection.

Even in bulk storage, disinfestation measures for insect control reduces fungal growth. Majumder et al. (3) reported an increase of 10 to 30 percent in mould growth when insects were left uncontrolled in commodities like wheat, green gram, Bengal gram, red gram, coffee bean, chicory and coriander, and an increase of up to 475 percent in sorghum. In addition to insects, mites also are responsible for establishing populations of fungi. More than 36 genera of mites were found to be associated with 27 genera of fungi obtained from wheat, barley and oats (4).

Some control methods for storage insects are included in Appendix 3. Refer to FAO Agricultural Studies No. 79 for more informatinn.

3.6.4 CHEMICAL CONTROL OF MOULDS

Many different chemicals have been tested for control of mould on moist grain. Compounds like aureofungin, thiram, captan, orthophenyl phenate, benlate, Bordeaux mixture, and organic acids like propionic, sorbic, lactic, acetic and benzoic acid have given promising results (1,2). Salts of organic acids are also suitable for seed treatments.

However, the results have shown that, for long-term storage, these organic acids and protectants cannot inhibit the inherent enzymatic activity of the seeds above a moisture content of 16 percent at tropical temperatures. At temperatures below 15° C on the other hand, all of these protectants inhibited fungi and prevented enzymatic deterioration of the grains in long term storage (2,6).

As an adjunct to natural drying, fumigation with gaseous sterilants has given promising results. Large-scale trials on groundnuts have shown that the saprophytic fungi could be controlled in the post-harvest period by using fumigants (3).

Out of the solid fumigant formulations screened, ammonia and phosphine combinations were found to be the best (5). Concentrations of 50 mg of phosphine together with 50 mg of ammonia per litre provide the fungicidal action under the field conditions. The species of sporulating bacilli present on groundnuts could not, however, be controlled with ammoniaphosphine combinations even at dosages of about 200 mg per litre. However, fungal growth could be checked (1).

See Appendix 3 for more detailed instructions and information about fumigation; also see FAO Agricultural Studies No. 79 (9).

Growth of <u>A</u>. <u>flavus</u> on maize during storage could be effectively controlled by the use of grain preservatives like 2 percent ammonia and 1 percent propionic acid, but this may not be economical. In a recent study (6) preservation of high-moisture maize cobs in cribs by dusting each layer with lindane (0.5 percent active component) where approved has proved very helpful in reducing storage losses by insect damage and arresting mould growth caused by insects.

3.6.5 RECOMMENDED PRACTICES DURING STORAGE

The two basic and guiding principles of storage are to keep the commodity dry and to keep it free from insect, fungus and rodent damage. Dry materials can absorb moisture from the environment. This has to be prevented by suitable means. Insect damage is avoided by preventing infestation of or disinfesting the material prior to storage followed by a continuing control programme which may need to include periodic fumigation of the materials. Rodent damage must be prevented by using rodent stoppage, rodenticides, removal of harbourage and fumigating nearby rat burrows.

A number of specific recommendations relating to storage are the following.

- (a) For safe storage the maximum moisture content (wet basis) should be as follows: 9 percent for groundnut kernels, 13.5 percent for maize, 13.5 percent for sorghum, 15 percent for paddy and 15 percent for grain legumes (beans). If the moisture contents are above these safe limits, drying is necessary prior to storage (see Appendices 4 and 5).
- (b) Remove chaff, immature grain, discoloured seeds, insect frass, weed seeds, stones, grit other refractory material by sieving and winnowing. The maximum foreign matter and total refractories should not exceed 4 percent at the farm level.

- (d) Treat the gunny bags (burlap), cloth or other textile bags for infection and infestation by either fumigation or insectproofing with approved pesticides (malathion or lindane has been used in the past). The level of the active ingredients on treated containers should not exceed 50 mg/ft².
- (e) Maintain temperature and relative humidity records in the warehouse with thermohygrographs, wet- and dry-bulb thermometers or other suitable instrument.
- (f) Relative humidities above 70 percent cause the moisture content of groundnuts, maize, sorghum, paddy and grain legumes to exceed safe moisture contents in storage at temperatures above 20°C. The relative humidity should be kept below 70 percent by proper ventilation or aeration during favourable weather conditions. Prior to and during the rainy months and during continuous high humidity in the atmosphere the stacks should be kept in or under plastic sheeting. (The plastic sheet "ballooning" technique is further described in Appendix 3).
- (g) In order to control any infestation present in either the warehouse or on/in the bag stacks, prophylactic spraying of the structure and the exposed bag surfaces or prophylactic cloth stack covers impregnated with suitable and approved pesticides may be employed. If there are signs of infestation within the commodities, funigation should be carried out with funigants effective against all stages of insects (including eggs) and harmful fungi.
- (h) For large-scale fumigation, methyl bromide or mixtures of ethylene dibromide and methyl

bromide have proved to be effective when applied under tropical temperature conditions. In non-airtight warehouses, fumigation sheets made of balloon film, PVC coated aluminized fabric, or high density polyethylene laminated with woven nylon, are sufficient to retain these fumigants during the exposure period. A combination of ethylene dibromide and methyl bromide 1:3 at 2 lb/1000 ft³ (32 g/m³) has proven adequate in India for complete disinfestation with respect to the storage insects and harmful fungi. Phosphine generating fumigant products are also widely used with safety.

- (i) Aerate the stack after the exposure period to free the stack from absorbed fumigants. Detectors should be used for verifying the complete aeration of the stack.
- (j) In non-rodent-proof warehouses, stacks should be protected from rodents by rodent harbourage removal, rodent stoppage techniques, rodenticidal baits and tracking powders as appropriate. FAO Agricultural Development Paper No. 90 has more information (5).
- (k) Funigate rat burrows in a safe manner with a suitable and approved funigant; phosphine, hydrogen cyanide and a combination of 60:40 (w/w) ethylene dibromide-methyl bromide have been reported effective.

If appropriate techniques such as these are followed according to approved procedures, rodents will be controlled and the terminal residues of the fumigants and pesticides will not exceed the permissible limits prescribed by many countries (7). 3.7 Transport

Transport from rural areas to urban centres or to processing plants may be carried out in carts, trucks, harges, ships and railways.

Infestation present in the transport containers may provide sources of contamination to the stocks which are carried by the transport system. It is therefore necessary to disinfest empty transport containers periodically by spraying with approved insecticides or by appropriate fumigation. Where approved for use, pesticides such as lindane, malathion methylbromide and ethylene dibromide are effective in disinfesting transport containers. Material residues from previous shipments should first be removed from the containers.

If the transit is prolonged, fumigation of the full transport containers on reaching the destination can be helpful in reducing infestation.

During transit in rainy and humid weather and also during river and ocean transportation, moisture may be absorbed by the consignment. Therefore care should be taken to ensure that the shipment has a safe moisture content before warehousing. Use of tarpaulins, ballooning or air-tight containers will prevent moisture uptake during transportation on water and also during rainy and humid weather.

Use of packaging materials resistant to insect penetration or which have been made insect-repellent by application of an approved chemical treatment, together with ballooning of the bag stacks, help overcome the hasards of moisture absorption and prevent cross-infestations during handling and transportation. These methods can be used for large-scale transport, storage and distribution systems of milled and processed sorghum, maize, rice, groundnuts and other products (7).

- 3.8 References to Sections 3.6: Storage and
 - 3.7: Transport
 - Majumder, S.K. and Natarajan, C.P. (1963) World Review of Pest Control 2, 25.
 - Sinha, R.M., and Wallace, H.A.H. (1965) Canad. J. Plant Sci <u>45</u>, 48.
 - Majumder, S.K., Narasimhan, K.S., and Parpia, H.A.B. (1965) in "Mycotoxins in Foodstuffs" Ed. G.H. Wogan. MIT Press.
 - 4. Sinha, R.N. (1961) Canad. Ent. 93, 609.
 - FAO Agricultural Development Paper No. 90, (1970) "Handling and storage of food grains in tropical and subtropical areas" 350 pp.
 - Patel, A.U., and Adesuyl (1975) Trop. Stored Inst. Inf. <u>29</u>, 33.
 - Majumder, S.K. (1970) Proc. III International Congress of Food Sci. and Technol., Washington, D.C. p. 513.
 - Heiss, R. (1970) An International Guide-Principles of Food Packaging FAO 225-269.
 - 9. FAO Agricultural Studies No. 79, "Manual of Fumigation for insect control". 381 pp

3.9 Processing

During processing many commodities are exposed to conditions which may favour mould development and aflatoxin contamination. When this happens extreme care should be taken to eliminate or reduce the risk associated with the practice, or other processing methods should be developed. The following are examples of processes which increase the risk of mycotoxin contamination.

3.9.1 RICE PADDY

Milling of rice to remove the husk and some bran is a universal practice to enhance the eye appeal as well as the digestibility. Since certain vitamins are associated with bran, nutritionists advocate only partial removal of the bran. Bran retention on rice is known to reduce the keeping quality to a marked degree. The presence of sugar in the bran makes the unpolished or partially polished rice more hygroscopic than the polished rice. Therefore, rice with bran retains more moisture than does polished rice and becomes more vulnerable to insect and fungal attack.

Parboiling of rice is extensively practised to increase yields of head rice and to improve cooking and nutritional quality. In the traditional method of parboiling, paddy is steeped in water for three days followed by steaming, drying and milling. The steeping period is long enough to permit bacterial growth which may cause discolouration and development of off-flavours. Although there is no information on this subject, the possibility of the rice grains carrying some bacterial toxins exists.

A modern method of parboiling involves the use of hot water for steeping, thereby reducing considerably the period of steeping and limiting opportunity for microbial growth.

Inadequate drying of the parboiled rice to safe moisture levels or delay in drying due to high humidity or rainy weather allows moulds to multiply and possibly produce toxins. In southern parts of Karnataka (India), farmers preserve parboiled rice in straw baskets and store them in the kitchen over the hearth. A recent survey carried out by the Central Food Technological Research Institute of Mysore revaled that such rice was practically free from microbial growth, whereas parboiled rice stored in the usual way in boxes, bins or bags absorbed moisture from the atmosphere and became mouldy. (Whether this storage practice involves factors other than microbial growth which may influence human health was not determined.) This survey also revealed that the most common fungi contaminating parboiled rice were species of Aspergillus and Penicillium, and many of the moulded samples contained aflatoxin.

After parboiling, paddy can be spread on a paved floor for sun-drying. Turning of the paddy periodically by manual operations helps uniform drying. Parboiled rice should be stored at moisture contents lower than 14 percent.

In summary, retention of bran by under-polishing rice favours insect and fungal damage. Inadequate drying before storage of parboiled rice aids fungal growth and mycotoxin formation.

3.9.2 PULSES

Milling of pulses may be done by loosening the husk by mixing with oil and water, sun-drying and dehusking in a mill.

In the processing of red gram dhal (split gram) the kernels are first steeped in water for 4-12 hours, then the soak water is drained off and the kernels are mixed with a paste of red earth and water. The mixture is kept heaped for 16 hours. This is followed by spreading of the mixture in a drying yard. A procedure of spreading during the day and heaping during the night is repeated for 2-4 days until the grain is completely dehusked. Careful handling and drying is necessary to avoid moulding during this process.

More than 70 percent of the grain legumes are stored and consumed in rural areas where facilities for quick drying are inadequate. As a result they are often stored at high moisture contents. Since fungal growth invariably occurs on such products, millers should exercise due care in procurement of sound lots. Millers should also adopt improved methods that ensure minimum delay in drying if legumes are milled following conditioning (moistening) to technologically desirable levels.

Delayed drying of legumes conditioned by moistening prior to milling may lead to spoilage by fungal growth.

3.10 Decontamination

In the preceding sections, factors favouring fungal growth on foodgrains and oilseeds during cultivation, storage, handling and processing have been discussed and methods for preventing microbial growth have been described.

It may not always be possible to protect all the grains produced in a country. During storage, the grain that escapes the usual treatment to limit fungal growth may become contaminated A decontamination process must be technically and economically viable. In addition, an acceptable process must meet the following criteria. The process must:

- (i) destroy, inactivate or remove the mycotoxin;
- (ii) not produce or leave toxic or carcinogenic/ mutagenic residues in the final products or in food products obtained from animals fed decontaminated feed;
- (iii) retain the nutritive value and the acceptability of the product;
- (iv) not significantly alter important technological properties;
- and ideally must

contaminating toxins.

(v) destroy fungal spores and mycelia which could, under favourable conditions, proliferate and form new toxin.

Methods of decontamination currently extant can be classified as involving:

- (a) physical separation of mycotoxin contaminated materials;
- (b) removal of mycotoxins; and
- (c) inactivation or total degradation of mycotoxins.

Extensive studies have been carried out on aflatoxins, but only limited information is available on other mycotoxins. Since aflatoxin is the most potent and widely distributed of all these toxins so far discovered, a closer examination of the information available on aflatoxin decontamination is attempted in the following pages. Although none of the tested chemical and solvent treatment processes have yet proved commercially feasible, several are under active investigation for different commodities. Therefore, this publication will describe only variously recommended methods of physical separation of contaminated material and inactivation of aflatoxin by sunlight in groundnut oil and by heat treatments.

It should, however, be noted that work is currently proceeding at a number of centres on processes utilizing solvent extraction (e.g. methoxymethane) or chemical detoxification with gaseous ammonia or hydrogen peroxide. Although solvent extraction has not so far proved successful, particularly on economic grounds, treatment by gaseous ammonia has been demonstrated to be efficacious for cottonseed meal, groundnut meal and maize. Studies are still under way to determine the safety-in-use of the detoxified products for animal feedingstuffs.

3.10.1 PHYSICAL SEPARATION OF MYCOTOXIN

CONTAMINATED MATERIALS

Fungal infection of any seed or grain usually imparts characteristic colour or other physical properties (see Figures 7 and 8). Hence, separation of such seeds or grains by some means is an effective approach to minimize mycotoxin contamination.

Colour sorting is widely practised for groundnuts, coffee berries and other similar-sized materials, either by using an electronic sorter, or by manual hand-picking (see Figure 9) or by using both for efficient removal.

With wheat or millet, ergot contamination is frequently encountered and the ergot seeds are removed either by a flotation technique (1), by suspending the grains in sodium chloride solution, or by air classification. Since the ergot sclerotia are lighter than sound seeds they can be removed by any one of these methods.

In all stages of handling and processing extreme care should be exercised to remove any unit of material that has become mouldy or is suspected to contain mycotoxin. A bag, clump, batch or other unit of mouldy material may be easily set aside during storage, handling or processing, but it is

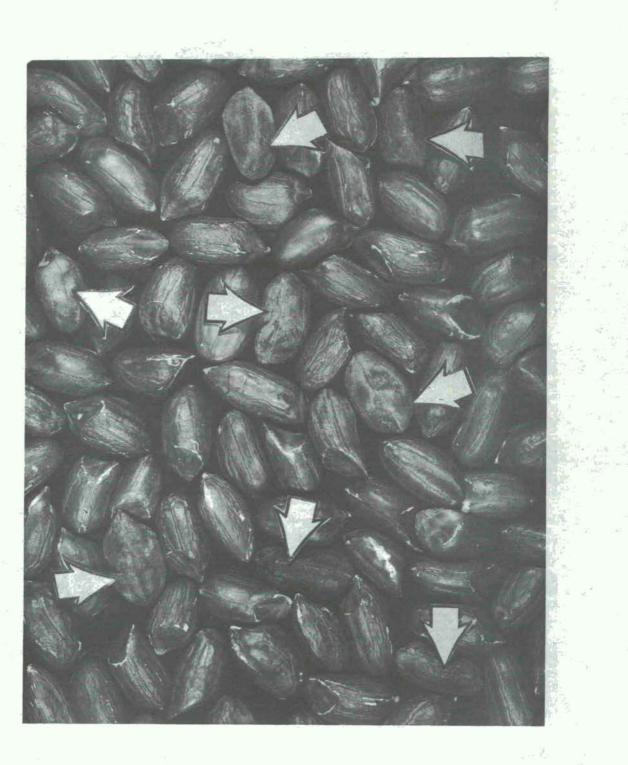


Figure 7. Groundnuts. Arrows point to off-colour kernels.

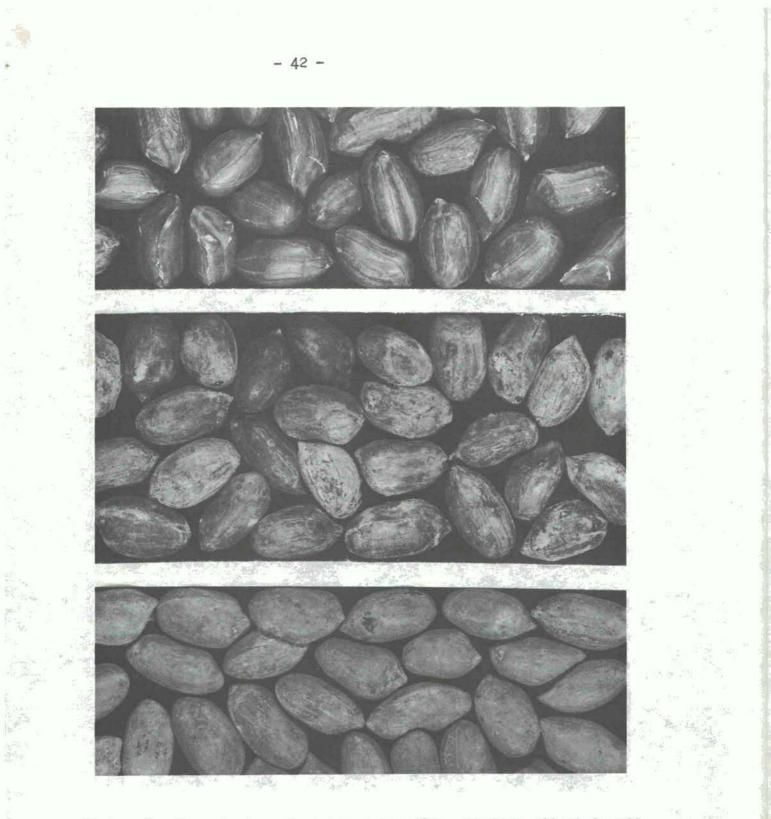


Figure 8. Groundnuts. Top: sound kernels; Centre: affected, offcolour kernels; Bottom: affected, discoloured kernels.

very difficult to remove individual grains of this mouldy material after it has been mixed with non-mouldy grains. The segregated mouldy portion of the product can then be treated separately to remove the mouldy grains, or it can be diverted to suitable non-food use. This is much less costly than having to treat the entire lot of material.

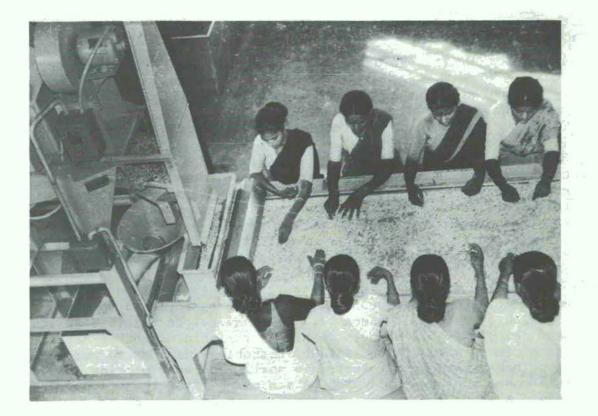


Figure 9: Physical separation of mycotoxin contaminated (discoloured) groundnut kernels by hand-picking in an oil mill. Examination of stored products will indicate whether moulding or caking has occurred. Such material should be removed from the warehouse before it becomes mixed with other non-mouldy material. High-moisture material should

also be set aside for drying and other special treatment.

Small, shrivelled kernels, insect-damaged kernels and broken kernels which often contain high concentrations of aflatoxin may be removed by sizing over screens and/or by aspiration.

3.10.2 PHYSICAL SEPARATION OF AFLATOXIN

CONTAMINATED GROUNDNUTS

There are many steps which may be employed to help remove aflatoxin contaminated kernels from lots of groundnuts. The economics of each step must be considered to see whether the increased value of the groundnuts will justify the costs. Since the incidence of <u>A</u>. <u>flavus</u> infection and aflatoxin contamination is usually highest in those kernels inadvertently shelled by harvesting and handling operations, it is helpful to remove those kernels by a screening operation before the lot goes to the sheller. Furthermore, since kernels in damaged pods usually contain more aflatoxin, they should be removed by sorting if it is economically feasible to do so.

After shelling and sizing operations, the shelled kernels may be scanned with electronic sorters or hand picked to remove discoloured and mouldy kernels. This may not be feasible if the skin colour of good kernels is highly variable. Another method is to remove the skin or testa by blanching and then to remove discoloured kernels by electronic sorting or hand picking. In this case those kernels which retain skins after the process should also be removed by hand picking or colour sorting since mould often makes the skin adhere to the kernel.

3.10.3 REMOVAL OF AFLATOXIN IN OIL

A major portion of the aflatoxin in crude groundnut oil is in the suspended state and this can be separated by a suitable filter. The remaining toxin can be removed by adsorption on a suitable adsorbent. A filter has been developed (2) which can easily be adopted in modern oil mills in place of the cloth filter now being used. This new filter, which may remove the toxin to an extent of 95-100 percent, appears to be a simple approach to solve the problem of aflatoxin in unrefined groundnut oil.

3.10.4 INACTIVATION OF AFLATOXIN BY HEAT

If aflatoxin cannot be completely removed, the next best approach is to inactivate it, either by irreversible modification of the compound chemically, or by alteration of the active groups in the molecule.

In recent studies at the Central Food Technological Research Institute, Mysore, India, destruction of nearly 70 percent of aflatoxin was recorded upon cooking of rice under steam pressure (3). Cooking at atmospheric pressure can destroy about 50 percent of the toxin. Pressure cookers are now being extensively used for cooking food, particularly in urban households. This can help to minimize the hazards of this toxin to an appreciable degree.

Dry roasting and oil roasting of groundnuts reduces aflatoxin levels to a significant degree. About 65 percent reduction in aflatoxin B1 level by oil roasting and about 69 percent reduction by dry roasting has been reported (4). In a subsequent study, dry roasting was found to reduce by 40-50 percent the aflatoxin B1 content originally present (5). A serious threat remains, however, from foods which even after cooking contain high concentrations of aflatoxin and from those foods which are frequently contaminated with aflatoxin and are not subjected to proper heat treatment.

3.10.5 INACTIVATION OF AFLATOXIN BY LIGHT

Light has been successfully employed to destroy aflatoxin in unrefined groundnut oil. Recent studies (5) have shown that visible light is more effective than either ultraviolet or infrared light and that sunlight is by far the best agent for total destruction of aflatoxin. Trials carried out at the Central Food Technological Research Institute, Mysore, India show that oil in small clear bottles exposed to direct sunlight for an hour will be decontaminated. The photodegraded aflatoxin was not regenerated even after a long storage period judging from absence of toxicity of treated groundnut oil in animal tests. Large-scale trials have not been done. However, commercial feasibility of detoxification depends on necessary arrangements for maximum exposure of the oil to sunlight for at least one hour. It also depends on the intensity of the sunlight. Exposure for one hour during the brightest part of the day when the lux units will be in the order of 50,000 should be adequate for total destruction. Finally, commercial feasibility may also depend on avoidance of adverse changes in flavour.

In summing up the practical methods to decontaminate infected foods, it can be stated that physical separation of grains or oilseeds is an efficient and feasible method of minimizing mycotoxin contamination. This is effected either by manual operation or with the help of an electronic sorter. Cooking rice under steam pressure, roasting groundnuts, photodegradation of the aflatoxin in groundnut oil and filtration of the oil through a special adsorbent filter are some of the practicable approaches towards minimizing aflatoxin contamination in these products.

3.11 Recovering Aflatoxin-free Products from

Contaminated Starting Materials

3.11.1 MAIZE

A study has been reported of the distribution of aflatoxin among various product fractions when the toxin contaminated maize is milled (7). Grits contained only one-tenth of the aflatoxin concentration in the lot of whole kernels from which they were produced. Aflatoxin B_1 level of meal was 13-16 percent of that in whole maize, and for flour the level was about 30-70 percent depending on initial aflatoxin concentration. Wet-milling of maize is used to produce starch, oil and other products. Studies on aflatoxin contamination during processing show that the starch, oil and most other products are aflatoxin-free and that 80-90 percent of the aflatoxin is concentrated in the gluten feed fraction (steepwater, fibre and spent grain) which must be discarded or diverted to suitable uses (8).

3.11.2 GROUNDNUTS

When oil is expressed from groundnuts, about 85 percent of the aflatoxin will remain in the presscake (9). Proper refining leads to groundnut oil in which aflatoxin is not detectable. In section 3.10.3 on decontamination, a method is described for removing aflatoxin contamination from unrefined groundnut oil by a filtering process, and another method for destroying it by exposure to sunlight. The aflatoxin-contaminated presscake may be used for animal feed if the final feed product has acceptably low levels. Presscake with very high levels of aflatoxin contamination should be used for fertilizer. Detoxification methods (10,11) may also be employed for meal, but they are not widely practised at present because of cost.

3.12 References to Sections 3.10 and 3.11:

Decontamination and Recovering aflatoxin-free

products from Contaminated Starting Materials.

BULL BUL

- Singh, R.S. (1968) in "Plant diseases" p. 168 Oxford and IBM Publishing Co., New Delhi.
- Basappa, S.C. and Sreenivasa Murthy, V. (unpublished data - Central Food Technological Research Institute, Mysore, India).
- 3. Fasiha Rehania and Sreenivasa Murthy, V. (unpublished data - Central Food Technological Research Institute, Mysore, India)

- Eschor, F.E., Koehler, P.E. and Ayers, J.C. (1973) J. Food Sci. 38, 889.
- Waltking, A.E. (1971) J. AOAC 54, 533. 5.
- 6. Shantha, T., and Sreenivasa Murthy, V. (1977) Ind. J. Techn. (In press.)
- 7. Brekke, O.L., Paplinski, A.J., Nelson, G.E.N., and Griffin, E.L. (1975) Cereal Chem. 52, 205.
- Yahl, K.R., Watson, S. A., Smith, R.J. and Barabolok, R (1971), Cereal Chem. 48, 385.
- Basappa, S.C., and Sreenivasa Murthy, V 9. (1974), J. Food Sci. and Technol. 11, 196.
- 10. Sreenivasa Murthy, V., Parpia, H.A.B. Srikanta, S. and Shankar Murbi (1967) J.A.O.A.C. 50, 350.
- Hann, G.E., Codifer, L.P., Gardner, 11. H.K., Koltum, S.P. and Dollear, F.G. (1970) J. Amer, Oil Chem. Soc. 47, 173.

3.13 Utilization of Mouldy or Mycotoxin-Contaminated

Products

Since it is not always possible to prevent moulding or mycotoxin contamination in agricultural products, it is extremely important to provide alternative uses for contaminated products in order to reduce economic loss to the producer and to encourage diversion of these products to acceptable uses. Traditional processing produces mycotoxin-free components from some commodities. Traditional milling and blending operation used for animal feed will also reduce mycotoxin concentration to acceptable levels for selected animals. However, the production efficiency for some animals may be reduced by even a low concentration of mycotoxins and there is a risk that mycotoxin contamination may be transferred to some animal products used as food. These problems and approaches to solutions are also discussed in some detail in the publication "Mycotoxin Surveillance -- A Guideline", FAO Food Control Series, No.4.

Frid Mr. and

There is very little knowledge about the effects of low concentrations of mycotoxins on the susceptibility of animals to diseases and the efficiency of their production of meat, milk or work. It has been demonstrated that some of the ingested mycotoxins may be transmitted to animal tissue and to milk. Cows may transmit up to 3 percent of ingested aflatoxin to their milk as aflatoxin M_1 . Although this is a low percentage, the risk is increased because milk is often consumed by children or young animals who are known or suspected to be more susceptible to the effects of the toxin than are adults or older animals. It is therefore essential that the aflatoxin levels in animal feeds should be kept low.

Proper proportions of the contaminated material in the ration will lower the concentration of mycotoxin by dilution. Different tolerances for mycotoxins in the ration should be set according to the animal species, animal age and end use of the animal products. An example of such blending practice based on effects on animal health or productivity is given in Appendix 6, but it should be emphasized that this is only an example. Regulations in relation to such practices exist in many countries. Such regulations as allow admixture in this way have been developed in the light of up-to-date research and toxicological interpretation in relation to the total food chain. If blending practices are contemplated in any situation, information on current regulatory requirements is essential.

In summary, blending of toxin-contaminated material with toxin-free material reduces the effective level of mycotoxin. Whereas in maize processing most of the aflatoxin is concentrated in the gluten feed fraction, in groundnut expeller processing 15 percent of the total aflatoxin resides in the oil and the remaining 85 percent in the meal. Aflatoxin-contaminated groundnut meal can be used in animal feeds, provided that in the final mix aflatoxin levels are within permissible limits, e.g. for cattle and poultry. Groundnut oil on the other hand can be either refined or passed through an adsorbent-coated filter or the aflatoxin photodegraded by exposure of the oil to sunlight for one hour. PART III

0 5

4. NEEDS AND AIDS FOR IMPLEMENTATION

OF THE RECOMMENDED PRACTICES

4.1 Data Base and Monitoring

The most important prerequisite for any action programme is to know which of the commodities grown in any region are susceptible to fungal damage, and at what stage or stages they are vulnerable to fungal infection leading to mycotoxin formation. Such information is only obtained by comprehensive survey programmes designed to identify

(a) high risk crops and commodities, and

(b) high risk regions.

To these parameters must be added, with particular reference to those areas of the world in which significant proportions of susceptible crops are consumed by the indigenous population, the identification of

(c) high risk populations and age groups.

Comprehensive survey programmes to establish data deemed reliable to effect judgements in these three areas would include analysis of the food and feedingstuffs for mycotoxins at all stages in the food chain from growth, through harvesting, drying, storage, transportation and post-harvest processing, including decontamination if necessary, together with a knowledge of household uses of both raw materials and products.

The reliable identification of the "high risk" parameters would enable appropriate channeling of both effort and resources to those links in the food chain where maximum effect could be achieved. In the case of high risk populations and age groups, education and the encouragement of improved food and (child) feeding habits would require the provision, in some cases, of nutritionally adequate and acceptable alternative diets.

4.2 Education and Extension

The identification of high risk commodities, high risk regions and high risk populations carries with it an obligation to inform populations of the risks involved in the consumption of mould-infected foods.

Farming communities should be encouraged to adopt changes in agricultural practices designed to eliminate or drastically reduce the occurrence of situations in which fungal growth is favoured. The role of the extension worker is vital to this part of any educational programme, since he or she is the means by which information is relayed to farmers, traders, public health workers and the consumers. The full panoply of the modern 'communications industry' should be utilized by the extension worker as appropriate or available, including talks at all levels, local-language pamphlets, audio-visual aids and demonstrations.

4.3 Marketing, Distribution and Incentives

It must be recognized that different types of marketing systems exist throughout the world for crops and commodities liable to mycotoxin contamination. Thus, in some countries, small farmers produce a limited number of commodities, mainly for their own consumption, or for local sale. It is also not uncommon for just that part of a crop which is surplus to family or even village requirements to be offered for sale in the larger market. This part would not necessarily be subject to quality control procedures or appraisal suited to a national or international market. On a larger scale marketing boards may operate particularly for some commodities such as groundnuts for which a wider national and international demand exists. Such marketing boards have over a period of time developed systems of grading and quality control. These practices, developed largely in response to the stipulations of importing countries based on public health and quality criteria, have resulted in improved and more stable prices for producers and exporters.

It is therefore clear that one incentive for the development of improved marketing and distribution systems, -- in addition to appropriate adoption of the various recommendations in Part I of this publication -- exists when a higher price can be obtained for better quality and uncontaminated produce.

In those situations where the government of a country initiates or controls the bulk of the trade in crops and commodities liable to mycotoxin contamination, there is merit in the provision of adequate price incentives for product conforming to criteria of cultivation, harvest, handling, storage and transportation, designed to ensure that only high quality, uncontaminated produce appears in world trade.

APPENDIXES 1 to 7

APPEAR ON THE FOLLOWING PAGES

	Some toxic effects
Groundnuts and products, rice, maize, other nuts and seeds, cottonseeds, coconut, wheat, tree nuts, milk, cheese	Liver and kidney carcinoma, bileduct proliferation, fatty infiltration of liver of animals
Cereals	Hepatoma in rats
Cereals	Liver and kidney pathology of rats
Cereals	Antimicrobial and toxic to mice
Cereals	Antimicrobial and mammalian toxicity
Cereals	Toxic to man and animals
Maize and other foodstuffs	Sustained trembling in mice
	Liver toxicity Hepatoma
	Nephrosis and liver damage
	Hepatotoxin hepatoma of animals

- 53 -

Toxin	Organiam	Foodstuffs affected	Some toxic effects
12. Citrinin*	P. citrinum	Rice	Automicrobial nephropathy of animals
13. Citreoviridin	P. citreo-viride and other Penicillium spp.	Rice	Mammalian paralysis
14. Rubratozins	P. rubrum	Maize	Fatty infiltration of liver of rats
15. Patulin*	P. expansum A. clavatus	Apyle Rice Feeds	Antimicrobial phytotoxic, carcinogenic in rats
16. Penicillic acid*	P. puberulum P. cyclopium	Maize	Antimicrobial carcinogenic in rats
17. Cyclopiazonic acid	P. oyclopium	Food and foodstuff	Convulsions in rats. Severe X lesions in spleen and kidney 1
18. Psoralens	<u>Sclerotinis</u> Sclerotiorum	Celery plant	Toxic to man and animals dermatitis
19. Stachybotrys toxin	<u>Stachybotrys atra</u> <u>S. alternan</u> s	Straw	Farm and other animals and man. Stachybotryotoxicosis (Dermal Toxicity)
20. ATA toxin	Furarium sporotrichioides	Oats, Wheats, Barley	Alimentary toxic Aleukia of animals and man (blood dyserasia)
21. Diacetoxy- scirpenol*	<u>F. scirpi</u> F. tricinctum	Wheat, Oats, Rye, Maize	Skin necrosis and eye damage in rats
22. T-2 toxin*	$\frac{\mathbf{F}}{\mathbf{F}}, \frac{\text{tricinctum}}{\text{nivale}}$	Cereals, Maize, Fescue grașs	Necrosis of the epidermal tissue of rats "Fescue Food of Cattle"
23. Nivalenol* Deoxynivalenol*	F. nivale	Rice	Inhibition of DNA synthesis
		State of the second	

Appendix 1 (continued)

* Mycotoxins detected as natural contaminants

- 54 -

							- 55 -		
	effects	of protein syn- nice	tile and	in animals	inimals	cattle and			
	Some toxic effects	Inhibition of prot thesis in mice	Fescue-foot in cattle and tail necrosis	Hyper-estrogenic in animals	Facial eczema of animals	Slobbering in catt horses	Gangrene	18 19	
Appendix 1 (continued)	Foodstuffs affected	Rice and cereals	Maize, Fescue, Cereals, Hay	Maize, Hay, Barley, Feed	Pasture	Hay, Red Clover			
	Organism	F. nivale	F. nivale	F. graminearum	Pithonyces chartarum	Rhizoctonia leguminicola	Claviceps spp.		* Mycotoxins detected as natural contaminants
	Toxin	24. Fusarenone	25. Butenolide	26. Zearalenone*	27. Sporidesmins*	28. Rhizoctonia toxin	29. Ergot	ж (н Т	* Mycotoxins detec

Appe	ndix	2

Probable mycotoxicoses caused by rusts and smuts1/

Country	Organism	Animal	Symptoms
England	<u>Tilletia</u> tritici	Dog	Epileptiform convulsions and acute cerebral meningitis
England	T. tritici	Chickens	Lesions on comb, wattles and mucous membrane
JSA	<u>Ustilago</u> zeae	Humans	Toxicosis
Rumania	Puccinia graminis	Horses	Fatal intoxication, salivation and stomatitis
Russia	Smat	Pigs	Conjunctivitis, irritation of upper respiratory tract, edema of lungs
Cgypt	Puccinia sp.	Buffalo, Sheep, Rats	Stimulation of uterine muscles
dussia	<u>Ustilago</u> <u>hordei</u>	Pigs	Conjunctivitis icterus of mucous membranes and skins, pulmonary edema (acute form) encephalitis (chronic form), blood alteration
			Ph. Progetime
lussia	<u>Ustilago</u> <u>hordei</u>	Cattle	Toxicosis as above
dussia dussia	<u>Ustilago</u> <u>hordei</u> <u>Tilletia</u> <u>laevia</u>	Cattle Mice Rats	Toxicosis as above Toxicity and death No effect

1/ Martin, P.M.D., and Gilman, G.A. (1976). A consideration of the mycotoxin hypothesis with special reference to the mycoflora of maize, sorghum, wheat and groundnuts. Tropical Products Institute, London.

Appendix 3

Schedule for treatment of bag stacks under warehouse storage for protection from damage by insects, moulds and rodents

The satisfactory storage of commodities under warehouse conditions requires a two-pronged approach:

(a) to eradicate a resident or initial infestation, and

(b) to prevent cross-infestation from outside sources.

This means that both curative and prophylactic measures are required for sound storage of the commodities. The curative measures such as fumigation or heat disinfestation as well as the protective treatments are to be employed <u>in situ</u> in the warehouse. The code of practice for safe handling is as follows:

The bag stacks should be built on moisture-proof and insect-proof dunnage leaving enough alleyways and space between the stacks and walls and among stacks.

Stacks should meet criteria such as stability, optimum utilization of space and consideration for the bearing capacity of the lowest layer of the bag stack.

Prior to stacking, dunnage should be treated with an appropriate pesticidal composition (e.g. based on lindane or malathion) in high viscosity oil. Approved usage rates of most such insecticides would result in application of the active ingredients at 50 mg/ft² of the surface area. This treatment can exert lethal effects on crawling insects and mites and prevent termite attack from the soil.

Rodents are responsible for polluting stored materials with their excrets and hair. Many harmful species of micro-organisms are carried and dispersed by insects and rodents.

(2)

(1)

Rodent control measures include removal of rodent harbourage, rodent stoppage, premise sanitation, prudent use of rodenticide tracking, powders, traps, application of poison bait in bait containers and fumigation of rat burrows in a safe manner.

The burrowing habits of many rodents, such as <u>Bandicota</u>, <u>Tatera and Milardia</u>, are well know. <u>Rattus rattus</u> and <u>Mus</u> <u>musculus reside within the precints of the storage structures</u>, and control measures for these rodents depend on the application of attractive poison bait or efficient traps. Mice may complete a life cycle without leaving a stack, making them a difficult pest to control.

The storage structure itself could be made more rodent-proof by proper design of the plinth. 'Rodentproofing of the warehouse may be improved by providing a functional ramp slightly away from the main building. The gap may easily be bridged whenever necessary by providing a suitable plank. The building wall should be smooth, the floor should be damp-proof, doors and windows capable of being made gas-tight and the roof suitable for fumigation.

Risk of product deterioration occurs during transport and distribution and when products stored in warehouses are subjected to cross-infestation by stored product insects and by rodents.

None of the packaging materials such as jute bags, cotton cloth bags, plastic-laminated thin-walled containers, paper bags and their laminates seem to answer all the functional requirement for safe transport and distribution. All of these are liable to succumb to attack by moulds, rodents or insects. Suitable chemical treatment to increase resistance to attack by insects is often required. Since e.g. malathion and other pesticides can be stabilized in appropriate oils and these compositions do not pose a problem of transfer from the treated surface to the commodities stored in the bags, the outer surfaces of the stack can readily be made more nearly insect-proof by prophylactic spraying.

For large-scale treatments of empty bags, a machine has been designed. A spray emulsion, containing e.g. lindane is diluted with water and sprayed on the bags. With a skilled operator about 2500-3000 bags can be treated in 8 hours using the pest-proofing machine. Drying is quite rapid as the moisture increase of the bag immediately after spraying does not exceed 2 to 3 percent. The cost of insect-proofing empty gunny bags worked out to about 25 (Indian) paise or 3(US) cents, some years ago. The transfer or migration of residues

(3)

to grains stored in these bags does not exceed customary tolerance limits.

In most cases insecticide treatment of bags of foodstuffs is required during storage in the warehouse. This is usually done with insecticide formulations that are water emulsifiable or wettable powders. To spray the interior of the loaded warehouse with a waterdispersible composition at times of extremely high relative humidity may invite the danger of moisture increase and fungal growth. Dusting with residual pesticidal formulations also may be difficult to carry out without increasing food contamination hazards.

The following technique of an <u>in situ</u> prophylactic spray treatment, using an oil-based formulation, has been developed for stacks of packed commodities in conjunction with a fumigation treatment.

It has been reported that a combination of groundnut oil, a high-viscosity mineral oil and batching oil considerably increased the toxicity of some insecticides and their stability on the treated surface. The prevention of cross-infestation has been stated to be achieved by careful selection of solvents, synergists and pesticides, and by proper application of formulations on the outer surfaces of the stacks.

The web clearances of the sack materials should be considered before direct spraying on the bag stack; impinging droplets of spray should not readily infiltrate through the bags. Direct spraying can be carried out safely on A-twill, B-twill and DW types of bag filled with commodities. The bags with higher web clearance should be protected by spreading over the stacks a prophylactic cloth impregnated by chemical treatment using, e.g. lindane and malathion in high viscosity oil. Lightweight unbleached muslin has been reported to serve well as a protective covering, treated or untreated.

The absorption of moisture during storage in atmospheric conditions may create certain problems. Groundnut, coffee and rice stored in coastal areas and also in humid weather in other parts of a country during the rainy season can be fumigated, e.g. with methyl bromide or ammonia-

(4)

- 59 -

phosphine (1:1.6) mixtures, and protected during subsequent storage by the ballooning technique. The ballooning technique prevents absorption of moisture from the atmosphere. It also renders the stack resistant to cross-infestation by insects. Ballooning has proved extremely useful under the existing facilities of storage in coastal and other humid areas. Huge capital investment for the construction of costly warehouses, with humidity control of silos and elevators, can be avoided with the application of this appropriate technology. Caution must be observed, however, that condensation and resulting localized moisture problems do not occur.

When weather conditions prevented proper drying, this fumigation technique has been successfully applied to freshlyharvested groundnuts in India. Freshly harvested groundnut pods containing moisture below 35 percent were enclosed in two polyethylene sheets (250 gauge, 0.06 mm) and after measuring the volume of the heap, required amounts of reaction mixtures or fumigants were placed beneath the sheet at different points (see Figure 10). The exposure period varied between 36 and 72 hours depending on the climatic conditions. In situ generation of the fumigant within the polyethylene enclosure led to partial sterilization of the pods, which were subsequently sun-dried. The gas mask(s) fitted with the appropriate canister(s) must always be used as directed during such fumigations.

It must be cautioned that phosphine is highly toxic to human beings and the permissible limit of the gas in the atmosphere is of the order of 0.05 ppm. This technique should therefore not be conducted in a closed building without appropriate precautions; a shed with proper ventilation may be suitable. During aeration the desorption of phosphine is relatively rapid, while ammonia shows some lingering action. The traces of ammonia usually disappear during drying. Ammonia, however, does not present known toxicological hazards to consumers when present on groundnut pods. For more information on fumigation refer to FAO Agricultural Studies No. 79, "Manual of Fumigation for Insect Control" — 1969, 381 pp.

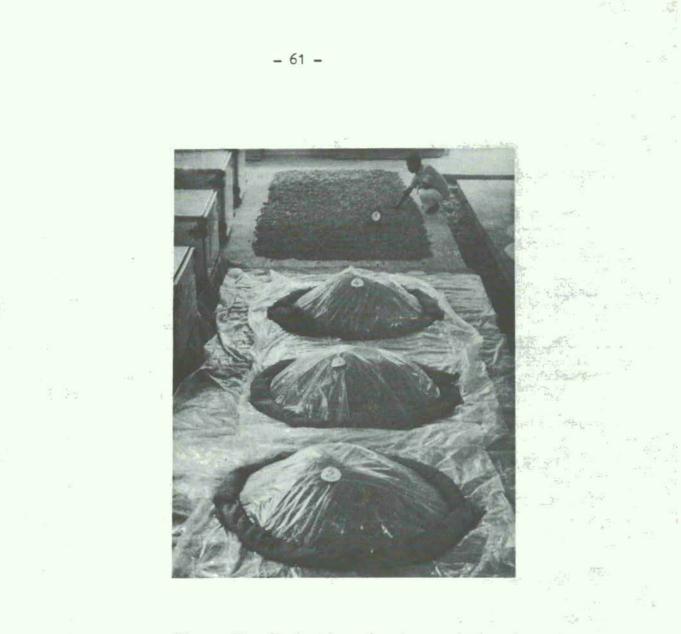


Figure 10: Fumigation of wet groundnut pods under polyethylene sheets

Appendix 4

- 20-

Influence of moisture content on mould growth during storage of grains at different temperatures and relative humidities

4 D - 1	A	and and	Rel	lative Humi	dity	
Commodity	Temp. range	30%	50%	70%	80%	90%
1 - a - 1 - 1	°C	Moie	sture conte	ent (wet be	usis)	
Rice-polished	28-31 10-11	9.0 9.9	10.6 11.0	12.6	16.0*	23.0* 22.5
Rice-parboiled	28-31	9.2	10.9	13.6	15.1	22.8*
	10-11	8.3	11.3	13.9	14.5	20.5*
Wheat	28-31	8.6	10.5	13.2	18.0*	20.4*
	10-11	8.4	11.0	13.2	14.1	23.0*
Sorghum	28-31 10-11	8.9 8.3	11.0	13.6 13.5	17.5* 14.2	21.6* 22.1*
Bengal gram	28-31	5.1	9.6	14.0	22.7*	26.8*
	10-11	6.6	10.1	11.2	16.4*	25.0*
Green gram	28-31	6.2	9•3	11.8	14.7*	26.3*
	10-11	7.5	9•2	11.0	13.9	31.6*
Horse gram	28-31	6.6	9.1	11.6	16.6*	25.3*
	10-11	7.0	9.3	10.7	14.0	26.0
Groundnut	28-31	3.0	4.8	6.6	10.3*	18.0*
kernel	10-11	3.7		5.9	8.4	16.8*
Cumin	28-31	9.9	11.6	14.0	22.0*	30.0*
	10-11	6.8	7.0	9.2	21.8*	28.0*
Coriander	28-31	9•7	11.4	16.9	24.5*	30.0*
	10-11	7•2	8.3	10.2	22.0*	29.6*
Coffee bean	28-31 10-11	5.5.	7.6 7.2	9.0 8.8	14.5 14.0	20.0* 18.2*

Visible mould growth
 From Majumder et al., 1965 in "Mycotoxins in Foodstuffs"
 Ed. C.H. Hogan, MIT Press, Cambridge Mass. USA.

and the second second

Appendix 5

Moisture content equilibrium values (at a temperature of about 27°C) for a range of produce at 70 percent relative humidity, the maximum acceptable level for storage for any sample (1)

								moist	ilibrium ure conten percent R
Maize		•••	••				••	••	13.5
Wheat	••	•••	••	•••	••	•••	••	••	13.5
Sorghu	m	••	••	••	••	••		••	13.5
Millet	••	••*	••	••	10 10 •	••	••	••	16.0
Paddy	••	••	••	••	•••	••	••	••	15.0
Rice	• •	••	•••	•••	••	•••	••	••	13.0
Cowpeak	8	••		••	••	••	• •	••	15.0
Beans	••	••		••		••	••	••	15.0
Ground	nuts	(sh	elle	d)	••	••	••	••	7.0
Cotton	seed	•••	•••	•	֥	à.•	••	••	10.0
Cocoa 1	bean	3		••	••		••	••	7.0
Copra		••	••	••	••	••	••	••	7.0
Palm ke	ernel	ls	••		••	••	••		5.0

 From FAO Agricultural Development Paper No. 90, 1970, on "Handling and Storage of Food Grains in Tropical and Subtropical areas", p. 53. (学)的学校

1	4	0	1
100	•	1	I
1	2	1	
	201	101	l
1	\$	2	1
1	7	٩	ł

Assessment of the Aflatoxin Content in Feeds and Maximum Permissible Admixture of Aflatoxin-containing Components to Mixed Feeds - 1969 Recommendations* made for Federal Republic of Germany (Agricultural Experimental and Research Institute, Kiel, Germany: Quoted from

Aflatoxin content*	Type of feed	Admixture of arlatoxin- containing components to the feed	Maximum total af- latoxin content per kg mixed feed
Slightly positive	Duck feed	no admixture	0
(up to 0.1 mg/kg)	Turkey starter feed	no admixture	0
	Chick feed	no admixture	0
	Pig rearing feed	no admixture	0
	Lamb rearing feed	no admixture	0
	Calf feed	no admixture	0
	Turkey finishing feed	max 5 %	(0.005 mg)
	Broiler finishing feed	max 5 %	
*	Pig feed	max 7.5 %	(0.0075 mg)
1 1 1	Layers feed	max 7.5%	(0.0075 mg)
	Cattle finishing feed	max 7.5%	(0.0075 mg)
- 41 -	Dairy cattle feed	max 15 %	(0.015 mg)
15 1 - 21 17	Sheep feed	max 15 %	
Moderately to	Turkey finishing feed	max 2.5 %	0.025 mg
strongly positive	Brotler finishing feed	max 2.5 %	
(0.1 to 1.0 mg/kg)	Pig feed	max 3.75%	_
	Layers feed	max 3.77%	
	Cattle finishing feed	max 3.77%	0.038 mg
	Dairy Cattle feed	max 7.5%	
	Shean feed	mar 7.5 %	0.075 me

一門の時間の

「山田山町

Appendix 6 (continued)

Very strongly Dairy cattle feed positive Sheep feed (1.0 to 2.0 mg/kg) Over 2.0 mg/kg Unusable as a feed ingredient	ed max 2.5 % max 2.5 % max 2.5 % eed ingredient	(0.05 mg) (0.05 mg)
30	eed ingredient	er Refe
50 3 3 3 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5		7
515. "Y		
(4) 7 = 12 - 7		

are no longer considered "slightly positive". These recommendations are cited as an example only, based on scientific studies with, and considering only the health and productivity of, animals. They should not be taken as applying currently in the Federal Republic of Germany or elsewhere.

10

- 66 -

Appendix 7

List of some institutions working on mycotoxins

The following is a short list of some of the institutes known to be actively working on various aspects of mycotoxins. Others working in this field are invited to provide brief information on their mycotoxin related activities for possible inclusion in updated versions of this publication or others as appropriate. Send advice to: Food Science, Control and Consumer Protection Group, Food Standards and Food Science Service, Food Policy and Nutrition Division, FAO, 00100, Rome, Italy.

<u>Brazil</u> 1. Universidade de Sao Paulo Depto. De Tecnologia Rural E.S.A. Luiz de Queiroz 13.400-Piracicaba, S.P.

(a) Aflatoxin(b) Ochratoxin

Instituto Adolfo Lutz
 Av. Dr. Arnaldo
 Sao Paulo, S.P.

(a) Aflatoxin(b) Ochratoxin

Canada Health and Welfare Canada

- 1. Health Protection Branch Ottawa, Ont. KIA
 - (a) Analysis of mycotoxins(b) Toxicology of penicillium toxins

 Health of Animals Branch Animal Pathology Division 115 Veterinary Road Saskatoon, Sask. S7N 2R3

(a) Analysis of mycotoxins(b) Toxicology of mycotoxins

Denmark

Federal

Germany

Republic of

Institute of Hygiene and Microbiology, Royal Veterinary and Agricultural University Copenhagen

(a) Pathology of mycotoxins

Federal Centre for Meat Research 1. Oscar-von-Miller Strasse 20 8650 Kulmbach

> (a) Penicillium toxins (b) Fusarium toxins

2. Federal Health Office Max Von Pettenkofer-Institute Thielallee 88/92 D1000 Berlin 33

> (a) Analysis of mycotoxins (b) Toxicology of mycotoxins

Institut National de la Recherche Agronomique Service des Mycotoxines 16 rue Nicolas Fortin 75014 Paris

(a) Analysis of mycotoxins

India

France

National Institute of Nutrition 1. Hyderabad, A.P.

- (a) Toxicology of mycotoxins
- (b) Epidemiological surveys
- (c) Resistant varieties of seeds
- Vallabh Bai Patel Chest Institute 2. New Delhi
 - (a) Biosynthesis of aflatoxin

India (continued)

3. Central Food Technological Research Institute Mysore

- 68 -

- (a) Analysis, prevention, control and detoxification
- (b) Isolation, characterization and metabolism of fungi

day a statte

- (c) Toxicology
- (d) Epidemiological studies
- (e) Insect infestation control
- 4. The Overseas Merchandise Inspection Co. Dr. A. B. Road Worley Bombay-25

(a) Analysis of aflatoxin

Israel

The Hebrew University Department of Botany Laboratory of Mycology and Mycotoxicology Jerusalem

(a) Toxicology of mycotoxins

Japan

 The Institute of Medical Science University of Tokyo Takanawa Tokyo

(a) Penicillium Toxins

2. Food Research Institute Ministry of Agriculture and Forestry Tokyo

(a) Toxigenic fungi on stored foods

The Netherlands

National Institute of Public Health P.O. Box 1, Bilthoven

- (a) Microbiological aspects of mycotoxins
- (b) Analysis
- (c) Toxicology
 - Toxins: aflatoxins, ochratoxin A, patulin, penicillic acid, sterigmatocystin, citrinin

Sri Lanka

University of Sri Lanka Department of Bacteriology Peradeniya

(a) Analysis(b) Detoxification of aflatoxin

United Kingdom

 Imperial Chemical Industries Ltd. Central Toxicology Laboratory Alderley Park, Cheshire

(a) Toxicology of mycotoxins

 Ministry of Agriculture, Fisheries and Food Food Science Division Great Westminster House Horseferry Road, London, SWIP 2AE

(a) Surveillance work

- Tropical Products Institute 56/62 Gray's Inn Road London, WCIX 8LU
 - (a) Analysis of mycotoxins(b) Stored product protection

United States

United States Department of Agriculture, Science and Education Administration

 Agricultural Experiment Station North Carolina State University P.O. Box 5906 Raleigh, NC 27607 United States (continued)

- (a) Control of mycotoxins(b) Analysis of aflatoxin
- (c) Extension to farmers
- Southern Regional Research Centre P.O. Box 19687 New Orleans, LA 70179
 - (a) Analysis
 - (b) Detoxification and control
- Northern Regional Research Center Peoria, IL 61604
 - (a) Microbiological and entomological aspects of mycotoxins
 - (b) Control of aflatoxin
 - (c) Detoxification of aflatoxin
 - (d) Analysis
- Agricultural Experiment Station P.O. Drawer ED College Station, TX 77840
 - (a) Microbiological aspects of mycotoxins
- 5. University of Minnesota Department of Plant Pathology Minneapolis, Minn.
 - (a) <u>Fusarium</u> toxins
 - (b) Grain microbiology
- Massachusetts Institute of Technology Department of Nutrition and Food Science Cambridge, Mass.
 - (a) Toxicology of mycotoxins
- 7. Auburn University and Agricultural Experiment Station Botany and Plant Pathology Department Auburn, AL 36830
 - (a) Microbiological aspects of mycotoxins

United States (continued)

- 8. United States Department of Health, Education and Welfare Food and Drug Administration (various Divisions, viz. Chemistry and Physics, Toxicology) 200 C Street, SW Washington, DC 20204
 - (a) Analysis
 - (b) Toxicology(c) Mycology

M-84

ISBN 92-5-100703-9