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WAYS OF REDUCING FOOD AND FEED CONTAMINATION WITH MYCOTOXINS

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Vegetable products are usually contaminated with mycoto-xins occurs, during the vegetation period when the toxins be-gin to grow on plants, during products storage and, occasional-ly during the processing of grain and oil-bearing crops.

A set of techniques is used in each of these stages to preclude or limit the formation of microorganisms and, hence, to reduce the danger of mycotoxins accumulation. Special pre-ventive measures have been developed and adopted in the past few years. These include the treatment of humid grain with ammonia or low molecular fatty acids. Besides, there is a possibility of natural decomposition of some mycotoxins during storage.

1. Prevention of mycotoxins formation on plants in
   the field

In certain areas of the world maize, peanuts, and nuts may be contaminated with aflatoxins during ripening. Zearale-none and trichothecene mycotoxins have been found in maize, wheat, barley and sorghum crops. In certain years, owing to weather conditions of mycotoxins formation on vegetating plants may be the main source of contamination of grain and seeds.

Prevention of mycotoxins formation in the field calls for an integrated approach and incorporates the cultivation of resistant and acclimatized varieties, the treatment of seeds and crops with fungicides, crop rotation and appropriate agri-cultural practices.

Let us consider some of such measures.

In most cases aflatoxins contaminate maize in those parts of the USA where grain ripens under conditions of air drought. A change in the time of sowing, cultivation of drought resistant hybrids or hybrids with a different vegetation period makes it possible to avoid unfavourable effects of stress droughty conditions facilitating the accumulation of aflatoxins in the field (Midstrom N. et al., 1981; Zuber M., Lillehoj E., 1979).

The improvement of cultivation practices, like the optimum density of sowing and proper nitrogen fertilization increase plant resistance and reduce the danger of aflatoxin contamination.

The choice of harvesting time is quite important. It is undesirable to carry out early harvesting of well-moisted grain since the danger of the emergence of toxin during storage increases and will involve additional outlays for drying. However, overripening of such crops as maize and sorghum worsens grain quality and facilitates accumulation of aflatoxins and zearalenone (Jones R., Duncan H., 1981).

Proper irrigation is particularly important for peanuts. Peanuts grown on humid soil may be affected by Aspergillus flavus, but droughty conditions weaken the plants and are particularly favourable for fungi infection and aflatoxin formation. Irrigation of peanuts a month prior to harvesting reduced aflatoxin contamination (Pettit R. et al., 1971).

Treatment of soil and sowings with fungicides may be recommended as a means of protecting peanuts against A. flavus. Destruction of weeds and vegetable remains which are a source

of A. flavus inocculum also facilitates aflatoxin control.
Crop rotation considerably reduces the fungus abundance in soil
(Pettit R. et al., 1971). Apparently peanuts should be cultiva-
ted on the same field once in three years at most.

Graincrops contamination with fusariotoxins may be
markedly reduced by limiting fusariosis of grain and grain
ears. However, the great variety and wide spread of fusariums
make this task is hard to solve by one technique only. The main
stages of preventing crops contamination with fusariotoxins
are the same as for aflatoxins. They include crop rotation,
farming practices, cultivation of resistant varieties and the
application of fungicides.

At present no fungicides capable of restricting fusario-
toxins synthesis on plants in the field are available. Treat-
ment of seeds and plants with Benomyl and Thiram increases the
germination capacity of seed and reduces grain contamination
with fusariosis by 41% but did not practically affects the
formation of vomitoxin (Martin R., Johnston H., 1982). To
cope with the problem, appropriate fungicides should be looked
for.

As for proper farming practices, special attention should
be paid to correct timing of sowing, control of soil over-
moistening and excessive crop density.

2. Prevention of mycotoxin formation during storage

The main task of storage is to preserve the quality
of the dry matter and to reduce its loss to minimum. This
is achieved by minimizing vital activity of biological com-
ponents of the grain mass: grain proper, organic admixtures,

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microorganisms, insects and mites.

Safe storage of vegetable products is ensured by regulating the main environmental factors: humidity, temperature, and oxygen content. Proceeding from this Trisvyatsky (1975) singles out three main regimes of grain mass storage:

- storage in a dry state, i.e. with a humidity within the critical level;

- storage in a cooled state, i.e. at temperatures which inhibit all vital functions in grain mass components;

- storage without air access or in modified gas media. Several multi-purpose technological methods are used to increase the grain mass stability under any storage conditions. The following methods are particularly important:

- grain cleaning;

- grain drying (thermal, air and solar);

- active ventilation by controlling air parameters or else by drying or colling the grain;

- preventive and exterminating methods of insects control.

These methods restrict the development of microscopic fungi and the danger of contamination of stored products with mycotoxins. Besides several often techniques are used, including treatment of grain and seed with chemical conservants and radiation sterilization.

2.1. Storage in a dry state

Storage of products in a dry state is especially wide spread technique. Grain and seed are stored with a humidity which is within or below critical. For different cultures the critical value differs depending on the chemical composi-

tion and anatomical structure.

Thus, the Regulations for the Storage of Food and Feed
Grain, Oil-bearing Seed, Flour and Hulled Grain No. 9-2 adopt-
ed in the USSR states that during short-term storage the level
of humidity for wheat, rye, barley and wheat should not exceed
15%; for grain maize, millet, sorghum, oats and grain rice —
14%; as for sunflower seed — 7%, castor-oil plants — 6%, beans,
peas, lentil, fodder beans, lupine —16%.

Food, fodder and seed grain meant for long-term storage
should be dried down to the following humidity: wheat, rye,
barley, oats, buckwheat, rice — 13-14%, maize and millet —
12-13%, peas — 15%.

Lower humidity levels are recommended for countries with
tropical and subtropical climate where high temperatures speed
up microbiological processes and low down the equilibrium
humidity of grain (Table 1).

The lower humidity limit for <u>A. flavus</u> growth and afla-
toxin synthesis on natural substrates is as follows: wheat,
maize and sorghum — 18.5%; rice-groats — 16.5&, rice grain —
17.5%; soya — 17-18%, peanuts and other oil-bearing crops —
9-10%.

However, to exclude completely the possibility of micro-
biological processes contributing to a gradual increase of both
humidity and temperature and, hence, to prevent the formation of
mycotoxins many specialists recommend that humidity
be reduced to RH = 70% and even to RH = 60% (Kurata et al.,
1973).

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Table 1

Maximum allowable humidity limits for storage of grain
and seed in countries with tropical and subtropical
climates (at approximately 27°C)

Crops	Humidity at RH = 70%, %
Maize	13.5
Wheat	13.5
Sorghum	13.5
Rice-grain	15.0
Rice-groats	13.0
Vigna	15.0
Beans	15.0
Shelled peanuts	7.0
Cotton seed	10.0
Cocoa beans	7.0
Copra	7.0
Coconuts	5.0

Hanssen E. and Yung M. (1973) suggest the following con-
ditions of safe storage should be met based on controlled hu-
midity and temperature of grain (Table 2).

Table 2

Product	Humidity	Temperature, °C	Relative air humidity
Wheat	12	6	60
Rye			
Maize			
Peanuts	6	4	65
Cashew	6	8	65
Walnuts	6	4	65
Almonds	6	6-10	65

The rigid requirements for humidity of stored products
can be explained by the fact that humidity and temperature of
the grain mass, which are generally safe for storage, cannot
guarantee the absence of microorganisms growth and formation of
mycotoxins if the product humidity is not equalized or is
under impact of temperature gradient. Under fluctuation of
ambient temperature or when different portions of grain have
different temperature the grain moisture starts shifting to-
wards cooler layers where it is condensed. Free moisture marked-
ly intensifies microbiological activity (Table 3).

Table 3

Effect of temperature fluctuations on moisture distribution
and number of microorganisms in sorghum grain
(Majumder et al., 1965)

Material	t°C fluc-tua-tions	Ini-tial humi-dity, %%	Humidity, %			Numbers of micro-organisms		
			top	middle	bot-tom	top	middle	bottom
Aluminium	11	18	18	18	19	16	9	80
Copper	10	17	17	13	18	16	10	24
Iron	15	22	22	16	24	182	152	222
Ferro-concrete	15	17	17	14	18	12	13	16
Ferro-concrete	18	20	20	17	20	42	13	93

The growth of A. flavus and, hence, the formation of aflato-
xins has been revealed in maize grain at a 13.5% humidity
owing to shifts in grain temperature and moisture in case of
non-uniform heating (Sellam M., Christensen C., 1976). Afla-
toxins were accumulated in a mixture of wet (26.6-27.9%) and
dry (9.8%) maize at a 14% humidity of the mixture on average.

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Though after eight weeks of storage, moisture in dry maize remained below 13%, the level of aflatoxin B_1 in it was as high as 500 μg/kg. Forced ventilation of the grain lots may prevent local formation of aflatoxins through levelling both the product humidity and temperature.

2.2. Storage of products under low temperatures

Most microscopic fungi affecting vegetable products in storage belong to mesophils (T_{min} - 5-10°C; T_{opt} - 20-35°C; T_{max} - 45°C). Several penicillae are similar by their temperature requirements, to psychrophillic organisms (T_{min} - 0°C; T_{opt} - 20 to 25°C; T_{max} - 30 to 35°C).

Low storage temperatures (0-10°C), slow down markedly the formation of mesophilic fungi and psychrophillic organisms. In experiments conducted by Mikhalovsky (Mishustin E., Trisvyatsky L., 1963) the numbers of fungi in a grain mass with a 18.2% humidity went up 2730-fold at 20°C and the grain could not be longer used as food or seed material; whereas the number of fungi in the same grain at 8°C increased 5.5-fold only.

Formation of aflatoxins appears to be inhibited by low temperatures since the bottom temperature limit for aflatoxin synthesis lies within 10°-12°C. This limit, however, depends on both the substrate and the strain peculiarities. In the areas where temperature during harvesting does not exceed 15°C, the danger of fungi formation and the accumulation of aflatoxins is much lesser than that in tropic and subtropic regions.

At the same time, low temperatures only slow down or temporarily discontinue the formation of microflora. Mycotoxicologically a coolled state storage is not a means of preventing wet grain and seed from being affected by psychrophilic

penicillae and fusariums after a long-term storage. As a result there is always a possibility of vegetable product contamination with ochratoxin A, citrinin, penicillic acid or patulin. Low temperatures occasionally stimulate the synthesis of fusariotoxins (zearalenone and toxin T-2). According to Harwig J. and Chen V. (1974) long-term storage at +5°C does not prevent a high-level accumulation of wheat and barley with ochratoxin and citrinin. In case of high humidity (29-31%) ochratoxin is formed in grain crops even at 4°C (Krogh P., 1979). P. cyclopium, P. martensii, P. palitans, P. puberulum and penicillic acid in substrates were found to develop on maize, rice, barley, millet, oats and wheat following a long storage at 18°C (Ciegler A., Kurtzman C., 1970).

According to Hacking A. et al. (1977) a 14% humidity can be considered as the most suitable for safe storage of barley since at a 18% humidity level zearalenone is being accumulated even at a near 0°C temperature. The lowest temperature for some species of fusariums is -7°C. Therefore the optimum temperature for the formation of toxin T-2 and diacetoxiskirpenol is in the range of 8-15°C (Smalley E. et al., 1974).

Hence storage in a coolled state of wet and humid grain can be considered as a temporary measure prior to grain drying. It should also be taken into account that grain heating by ambient air, may result in a rapid moulds formation and the risk of the mycotoxins development becomes much greater.

The storage of dry grain at low temperatures is the best way of storing which precludes the development of fungi and the synthesis of mycotoxins.

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2.3. Storage in air-tight conditions

The creation of oxygenless conditions during grain storing greatly diminishes the activity of microbiological processes. One of the way of achieving this is to use natural accumulation of CO_2 in autoconservation, storage in a modified gas medium and in a vacuum.

The notion of fungi as of strict anaerobs is generally accepted. The lower limits of oxygen content in the atmosphere which would restrict the development of fungi, however, varies from one species to another. When high concentrations of CO_2 accumulate as a result of life activity, we may observe in many species a shift from aerobic respiration to anaerobic. Moreover, the majority of toxigenous fungi belong to soil organisms and, therefore, they are physiologically resistant to high CO_2 concentrations owing to poor conditions of gas metabolism in soil.

According to Diener and Davis (1969) the inhibition of aflytoxin synthesis has been observed in the atmosphere which contain more than 20% of CO_2. Aflatoxins did not grow in general in the presence of 80-100% of CO_2. A reduction in oxygen content to 5% sharply inhibited toxigenesis. Similar findings have been obtained for A. parasiticus.

Atmospheric composition acts differently depending on temperature. At 25°C atmosphere consisting of 60% of CO_2 and 20% of O_2 completely suppresses aflatoxin formation, similar effect was observed in a gas medium at 15°C, containing 20% of CO_2 and 5% of O_2.

Aflatoxin accumulation was less intensive in artificial gas mixtures with up to 13.6% of CO_2 than in the air but

in either case <u>A. flavus</u> growth is inhibited (Wilson D. et al., 1976, 1977), though an increase in CO_2 concentration led to a greater lag period of spore formation and toxin synthesis (Epstein E. et al., 1970).

A substitution of ordinary air for the modified gas medium produced rapid spoiling and accumulation of aflatoxins (Wilson D., Jay E., 1975).

Similar regularities were established by Orth R. (1976) for patulin and sterigmatocistin. The toxin forming ability becomes less pronounced when producent strains are cultivated in the presence of 40% of CO_2 and 6% of O_2 their growth was suppressed completely in an atmosphere containing 90% of CO_2 and 10% of air or 90% of nitrogen and 10% of CO_2.

Atmospheric pressure fluctuation in a rather wide range did not affect the synthesis of patulins <u>P. expansum</u> and <u>P. patulum</u>. The growth of toxin was suppressed only at a pressure of 60 m Hg (Adams K. et al., 1976).

Thus, artificial gas mixtures enriched with CO_2 or the atmosphere of inert gases may serve as a temporary environment to store vegetable material of increased humidity as a means of preventing mycotoxin synthesis.

Thus, a modified atmosphere may be used for storing and transportation of fruit (Sommer et al., 1974) and during temporary storage of humid maize and peanuts (Wilson D. et al., 1977).

2.4. Technological methods used in storage

A number of techniques are used to bring the grain into a state stable for storage: drying and cleaning of the grain mass from admixtures, their active ventilation, disinfection, chemical conservation, etc.

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Bringing a lot of grain or seed to a homogeneous state in terms of humidity, admixtures and other indicators, creates conditions for greater stability of the lot in storage.

Grain masses with no signs of pest infestation, cleaned from dust and other admixtures contain, naturally, smaller numbers of microorganisms, are more resistant in storage in a dry or cool state.

2.4.1. Grain cleaning

After harvesting and during storage, grain and seed of many crops are cleaned mainly to remove a larger part of inadequate (damaged, crushed, substandard) seed, organic and mineral admixtures.

This increases the grain mass stability in storage since a majority of microorganisms is concentrated in the mineral and organic admixtures and in damaged kernels (Table 4).

Table 4

Numbers of microorganisms in wheat grain before and after cleaning (Trisvyatsky L., 1975)

Product	Number of microorganisms, thous/g	In % to uncleaned mass
Grain mass before cleaning	3,800	100
Grain mass after cleaning	2,460	65
Dust after primary cleaning	42,600	11,121
Grain waste	52.000	1,369

Mechanically damaged grain, and also organic admixtures are more susceptible to attack by microorganisms and, specifically by microscopic fungi and, therefore, may contain aflatoxins more often than other grain; hence, timely cleaning of grain and a lessening of mechanical damage reduces the potential of formation of mycotoxins and facilitates the withdrawal of the more contaminated seed from the overall mass.

Thus, studies by Shotwell O. et al. (1972) indicated that aflatoxins are concentrated in individual damaged kernels of maize and their content may reach 88-101 mg/kg. In two out of 13 lots of maize toxins were found in a high concentration in fractions of organic and waste admixtures. Ashworth L. et al. (1968) found that 0.3% of cotton seed contain on average practically all aflatoxins of the damaged lot. The situation is the same for other kinds of seeds.

For instance, 70% of all aflatoxins formed in a lot of rice grain were concentrated in shelled kernels. When the content of shelled kernels grows from 0% to 8%, the level of rice contamination with aflatoxins increases from 667 to 10,000 µg/kg, the contamination level of damaged kernels raises too.

After trashing and separation of maize which was stored in cobs subject to spoilage we observed a reduction in the level of maize kernel contamination with zearalenone and aflatoxins. A greater part of damaged fragile kernels was crushed during trashing and removed with wastes, as a result the concentration of mycotoxins and fluorescing steroid substances of mycogenic origin in the damaged kernels increased.

The removal from the grain mass of crushed, damaged and mechanically injured kernels greatly increases grain resistance in storage and brings down the intensity of aflatoxin formation.

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Rice grain harvested manually, with no mechanical damage or
crushed kernels, even at a 18-26% humidity was but slightly
damaged by <u>A. flavus</u>, whereas, aflatoxins were not found in it
even after 40 days of storage at 27°C (Lvova L., Orlova Z.,
1976).

Aflatoxins were accumulated more intensively in small frac-
tions of wheat grain than in larger kernels (Merkulova T. et
al., 1982). Size fractioning is an additional technique of
grain decontamination.

Thus, separation may be used as a means of preventing
the development of fungi and accumulation of aflatoxins as
well as for partial decontamination of seed and grain mass. In
the latter case, individual ways of isolating damaged kernels
should be worked out for each crop. The case in question is
the repeated cleaning of peanuts with the application of pneu-
matic separation which has been introduced in the United States
for imported lots and which has made it possible to reduce the
amount of sorted out lots from 32% in 1968 to 1% in 1971-1974
(Stoloff L., 1976).

2.4.2. <u>Drying</u>

Drying is without doubt a radical means of preventing myco-
toxin contamination of grain.

At the time of harvesting many crops quite often have
a higher humidity than is allowed for safe storage. The myco-
toxin problem becomes particularly grave for those areas where
harvesting coincides with the onset of humid period. In that
case drying is the main means of settling the mycotoxin prob-
lem.

A shortening of grain and seed storage time prior to drying considerably lessens the danger of contamination of grain with toxins. The recommendations of the department of agriculture of the United States suggests, to avoid the myco-toxin contamination, to dry wet maize not later than 24 hours following harvesting. The safe storage time is 2-3 days for maize and peanuts and 5-7 days for the other grain crops.

One can distinguish a thermal drying using dryers of various design, a solar and air drying. The last two methods of drying are used for peanuts in the areas of warm climate, specifically in small farms which do not have mechanical dryers. Traditional air and solar drying takes up a lot of time and facilitates peanut contamination. Peanut kernels or complete plants with the beans in them are dried on grid platforms and frames placed in open air. There may be rewetting during drying owing to high relative air humidity as well as due to rainfall and moisture condensation under the plastic covering. All this makes drying longer and may result in aflatoxins formation.

Thermal drying is brief and most effective. When it is used one should carefully follow the uniformity of drying, specifically of wet grain and its concurrent cooling. Inadequately dried and uncoooled grain may be a cause of focal self-heating.

Owing to thermostability, aflatoxins are not destroyed in conventional drying patterns. Ochratoxins also withstand temperature up to 270-300°C. Approximately 95% of aflatoxins in wheat of a humidity of 22-30% were destroyed only after four hours of heating at 120°C (Tomova S., Bonchef N., 1977). Some other mycotoxins are less thermoresistant. Heating at a

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temperature exceeding 65°C facilitates rapid destruction of citrinin and rubatoxin (Jackson L., Ciegler A., 1978; Engstrom G., Richard K., 1981).

2.4.3. Active ventilation

Active ventilation of grain may be used to prevent the appearance of mycotoxins provided it ensures timely and sufficiently rapid decrease in temperature (or humidity) throughout the grain mass to a level which precludes the formation of fungi. Besides this, active ventilation facilitates the temperature equalization in the grain mass so as to avoid thermomoisture diffusion and moisture condensation. Effectiveness of active ventilation greatly depends on air discharge per ton of grain, taking into account its state, temperature and air humidity.

Time of grain cooling should not exceed duration of its safe storage at a given humidity and temperature. For instance, grain rice at a 17-19% humidity and temperature of 15-20°C should be cooled for 24 hours and for 5 days at a temperature below 15°C. The absence of an obvious increase in the numbers of microorganisms, including microscopic fungi should be considered as a criterion of the proper timing of cooling and correct choice of air discharge rate.

When active ventilation fails to ensure removal of surplus biological heat from grain and its timely cooling or drying it can only activate mycotoxin synthesis which is further promoted by additional access of oxygen capable of increasing aflatoxin formation rate from 3 to 100 fold (Hesseltine C., 1966).

Calderwood D. and Schroeder H.(1968) observed aflatoxins formation in freshly harvested rice grain by the second or third day of its storage in aerated hoppers. The specific air supply at a rate of 0.5 c.f.m. per barrel of grain envigorated aflatoxins formation amounting to 857 μg/kg whereas air supply at a rate of 1 c.f.m. per barrel reduced the toxin growth rate to 58 μg/kg.

At a 22.5% humidity aflatoxins contamination level for maize was 1.78 mg/kg by the 7th day of its storage at the airflow rate of 0.1 m/sec (Thaler M. et al., 1979).

Lvova L. et al., in their experiments (1979) with a bulk lot of wet maize and with maize, in cobs, maintained for a long time favourable conditions for the formation of A. flavus, i.e. at the grain temperature at 30-45°C, owing to inadequate air supply (40 m^3 per hour). Examination of the grain samples revealed the presence of aflatoxins at considerable concentrations (up to 2,000 mg/kg), the level became somewhat lower towards the end of the experiment, by the 16th-18th day (Table 5).

Slow cooling of wet maize by active ventilation up to 1-5°C created conditions necessary for the development of penicilli and A. flavus. Aflatoxins were found in grain cooled within 4-8 days (16-95 μg/kg). A 12-hour interval in maize cooling resulted later in the formation of A. flavus and its accumulation at high concentrations by the fifth day (1,000,000 μg/kg).

Table 5

Dynamics of aflatoxin accumulation in a poorly ventilated
bulk lot of maize in cobs (W = 31-32%)

Storage, days	Temperature, °C	Germination, %%	Storage fungi, colonies/ 100 kernels	Aflatoxin B_1, µg/kg	Green fluorescing substances, mg/kg
0	20	90	14	0	0
9	30	94	80	133	9.5
11	35	92	116	999	6.5
12	40	78	118	1330	19.0
13	50	72	152	2000	27.5
16	60	28	106	1330	1.5
18	60	0	40	167	5.0

Active ventilation in warm climate can lesson but not
preclude the danger of mycotoxins formation. Schroeder H. and
Calderwood D. (1968) in their experiments stored freshly harvested rice-grain in aerated hoppers at different air flow
rates, humidity and temperature of ambient air. During   the
warm season(August-September) at 26-35°C up to 743 µg/kg aflatoxins were detected in the rice, as compared with its storage
in October when no aflatoxins were detected. Under tropical
and subtropical climate it is expedient to use active ventilation by refrigerated air.

During the drying of maize grain from 35-39% to 12% humidity by means of active ventil tion, the formation of aflatoxins, patulin and zearalenone had been established. Grain temperature was 8-15°C, the air flow velocity - 0.1-0.25 m/sec,

duration of drying of the upper layer - 20 days, of the lower
- 5 days. Patulin was found in 7 days of ventilation in the
upper layer while zearalenone was detected after 14 days
of drying practically in all layers (Thaler M. et al., 1979).
Most likely active ventilation of freshly harvested maize with
a high degree of humidity in the mid-European climate cannot
guarantee against the development of mycotoxins. It seems to
be more expedient to dry maize at air velocity equal to $W = 20\%$
with subsequent active ventilation with outside or refrigerated
air (dry aeration).

Addition of dehydrated ammonia to air flow seems to be
a promising method of suppressing microbiological develop-
ments in case of prolonged ventilation of wet maize. During
a 10-week drying which comprised ten weeks, the discharge
of ammonia did not exceed 0.05% of the grain weight. Expendi-
tures with this method of drying amounted to 44% of the out-
lays under conventional drying in thermal driers (Merit H.,
1980).

Under conditions of cool and temperate climate, a well-
designed and arranged system of ventilation is capable of
reducing   grain damage due to the development of microorga-
nisms and the risk of mycotoxins formation. Even in a warm
climate, selective ventilation taking into account parameters
of the supplied air may limit harmful consequences of micro-
biological processes.

3. Role of insects in contamination of grain
with mycotoxins

Grain and seed damaged by insects in the field facili-
tate penetration of fungi in such seeds and kernels. Insects

are also vectors of fungi spores. During storage the develop-
ment of insects and mites leads to greater humidity and tempe-
rature of stored material which without fail entails more ac-
tive microbiological processes.

Consequently, the lowering in the numbers of insects and
prevention of damage caused by them to grain both in the field
and in storage is an indispensable component of the mycotoxin
control system.

As a whole, the number of pests on plants should be con-
trolled by several measures: cultivation of pest resistant
varieties, crop rotation, proper farming practices, and reason-
able application of insecticides.

Studies conducted in the USA have shown the infestation
of maize in the field with earworm is much lower in the pest
resistant varieties having longer and denser husks. Triple
treatment with Seven during the heading of stigmas greatly
reduced the number of earworm and the accumulation of afla-
toxins (Lillehoj E. et al., 1975 and 1976). In Gardona treat-
ed corn the number of earworm was lower by 60%, aflatoxins
contamination - by 50%.

In a number of cases, however, the decrease in the insect
population was not accomponied by a corresponding drop in the
level of aflatoxins.

The development of insects in stored products (wheat,
coffee, coriander) led to a 10-30% increase in the number of
fungi and by 475% in sorghum (Majumder S. et al., 1963). Mites
also contribute to increase in the fungi population. More than
36 species of mites are associated with 27 species of storage
fungi (Sinha R., 1961).

An increase in the insect abundance even in a comparatively dry grain (14.7%) facilitated the development of the more xerophytic species of fungi which subsequently may lead to self-heating of grain (Table 6).

Table 6

Relationship between the number of granary weevils and microbiological processes in wheat grain

What grain infested with granary weevil, %	Bacteria, thous/g	Fungi, thous/g				
		Total	Penicil- lium spp.	A. glau- cus	A. can- didus	Yeast
0	44.2	0.8	0.4	0.2	0.1	0
1	34.5	5.3	1.7	2.2	0.1	1.2
5	602.0	27.3	10.0	15.0	1.0	1.3
10	630.0	17.3	0.3	14.6	1.2	1.1
25	1,360.0	137.0	0.2	111.0	24.0	1.6

Preventive and exterminating measures are used to protect products against pests during storage. Preventive measures of control aimed at obstructing infestation of products with insects and, hence, precluding the development of pests.

Measures of extermination are based on chemical treatment of grain in storages, at flour mills using organochlorine and organophosphorus pesticides and also halogen containing formulations (malathion, lindan, methylbromide, phosphine, carbophos, dichlorphos, naled, dichlorethane, etc.).

Alongside with insecticides there are formulations with a fungicidal action. Application of chlorpicrine vapour to grain can stop its self-heating (Mishustin E., 1963). Dichlorethane is a potent inhibiter of mould fungi growth. A combination of methylbromide and dibromethane can completely destroy

not only insects but storage fungi too.

A possibility of suppressing the synthesis of aflatoxins and other mycotoxins by insecticides has been studied extensively. The results are contradictive. Treatment of grain contaminated with such toxigenic strains as A. flavus, A. parasiticus, A. ochraceus, P. viridicatum, phosphine, carbotetrachloride and carbon disulfide stimulated in most cases the formation of aflatoxins and ochratoxin (Hesseltine C., 1974).

It was found in laboratory experiments that dichlorphos and naled at a concentration of 10-20 mg/kg suppressed the synthesis of aflatoxins (Cundu R., Harein P., 1973; Draughon F., Ayers F., 1981). Considerable inhibition was also observed when toxigenic strains were treated with sevin, landrin and peretrines. Dichlorphos suppresses the synthesis of aflatoxins in early stages, most likely in the averufine stage (Yao R., Hsieh D., 1974). Ayers F. and collaborators have shown in their studies that dichlorphos also strongly inhibits the synthesis of zearalenone in F. graminearum, citrinin in Penicillium spp. and ochratoxin in A. ochraceus.

Insecticides, however, are rarely used to control mycotoxins since, this requires higher concentrations of the insecticides than those used for desinsection. Insecticides while inhibiting mycotoxin synthesis may slightly suppress the vegetative growth of fungi. Therefore, in subsequent storage of a treated product one can observe even intensive formation of mycotoxins owing to the weakening of the grain proper. Wheat, inocculated with A. niger and fumigated by methylbromide (100 mg/l), was again infested by the fungi at a rate of 20% after 16 days of storage, and the infestation was 100% after

the passage of 29 days. The results of experiments conducted
by Paster N. et al. (1979) have shown that the application of
methylbromide in commercial doses which are used to control
insects far from destroying the vegetative forms of micelial
fungi, fails to prevent further development of fungi due to
the weakening of the grain stability to infestation with
microorganisms.

The treatment of grain by a range of fumigants (phostoxin,
chlorpicrin, carbon tetrachloride, etc.) at concentrations
from 4 to 25 fold higher than those conventionally used did
not reduce the content of aflatoxins accumulated in the grain
(Brekke O., et al., 1978).

4. Prevention of mycotoxin formation by chemical and physical means

Considering that plant products with increased humidity
are easily infested with fungi and bacteria, scientists and
experts all over the world started using chemical and physi-
cal ways to control microorganisms formation. Promising results
have been received in controlling mycotoxin formation by such
fungicides as tiram, captam, botran, benlat, relfungin; organic
acids (propyonic, sorbic, lactic, acetic, benzoic) and their
salts. (Bell D. et al., 1972; Herting D. et al., 1974; Bothast
R. et al.m 1976; Buchanan R., et al., 1976; Chipley J. et al.,
1980).

Treatment of grain with ammonia (2% of grain weight) is
also a promising technique. Application of ammonia has made it
possible to preclude aflatoxin formation and to keep humid grain
for 29 weeks (Vandegraft E. et al., 1975). Notwithstanding

a foreign smell, the grain after being treated with ammonia at
a rate of 2% of grain mass was willingly eaten by animals
(Bothast R., et al., 1973). In a number of cases ammonia
has been used for fumigation in a mixture with phosphine
(100 mg/l); this mixture completely inhibited the growth of
fungi (Majumder S., Natarajan C., 1963).

Treatment with volatile fatty acids has been checked on
maize, sorghum, oats, wheat, barley, peanuts and peanut cake,
on some combined feeds too. Diluted solutions of acids have
a stronger fungicidal action. Synergic action has been demon-
strated for some acids. The dosage should grow with the increase
in grain and seed humidity. Treated grain, according to Herting
D et al. (1974) may be stored for more than 12 months.

However, a long-term storage of treated products at a hu-
midity exceeding 16% and high temperature may lead to enzymatic
changes in the grain which worsen its quality. Under 15°C
many protectants inhibit the growth of fungi and the enzymatic
damage to wet grain.

Fungicidal activity of acids becomes less pronounced when
large masses of grain are treated where non-uniform application
of the protectant creates foci of intensive microbiological
processes. The problem of harmful chemical residues obstructs
large-scale application of fungicides.

Similar means of chemical treatment may be regarded as an
addition to drying and is used mainly for fodder grain.

Thus, propyonic acid (primarily Luprosil) is applied in
a number of countries to conserve fodder grain. The applica-
tion of 0.3% of Luprosil completely inhibited aflatoxin for-
mation in mixed feeds; 0.5% - in peanut cake (Cohler W.,

Fink F., 1977). A Japanese preparation gasole which is based
upon on a mixture of organic acids prevented the formation of
zearalenone. F. graminearum, in this case Luprosil was not ef-
fective (Kallela K. et al., 1981).

Radiation treatment of grain has been initially intended
as a method of checking the number of insects. The rates of
radiation treatment which are recommended by FAO (JAEA) WHO
to control insects, i.e. 15-100 krad, are much too low to des-
troy fungi. Higher radiation levels kill first grain and later
fungi. When doses which kill fungi are applied, the grain ac-
quires some foreign smell and aftertaste.

Irradiation at doses up to 250-300 krad usually stimulat-
ed the formation of aflatoxins B_1 and G_1, ochratoxin (Applega-
te K., Chipley J., 1974, 1976; Riyadarashini E. et al., 1979).
Most likely an alteration in the chemical composition of the
substrate during irradiation, and specifically the accumula-
tion of free fatty acids, stimulated aflatoxin synthesis. It
is also possible that this was facilitated by the weakening of
the resistance of irradiated grain to fungi infestation.

Though it has been found in a number of cases that irra-
diation has an inhibiting action upon the synthesis of afla-
toxin and patulin, we believe that the misgivings of Apple-
gate K., Chipley J. (1976) that radiation treatment for des-
insection may be dangerous since it envigorates the synthesis
of mycotoxins when the grain is infected with toxigenic strains.

5. Destruction of mycotoxins in storage

The concentration of mycotoxins in stored contaminated
products may occur naturally, without being associated with
man's intervention.

Though the process is not a panacea we believe it neces-
sary to consider the comparative stability of some mycotoxins
since in a number of instances real levels of contamination
of products depend both upon the rate of accumulation of myco-
toxins and the intensity of their binding or destruction.

Patulin, in natural conditions, occurs primarily in
fruit, vegetables and products of their processing. It is very
rare in cereal grains (Frank H., 1972) though patulin produ-
cents — A. clavatus, A. terreus, P. patulum, P. expansum
belong with the conventional fungal flora of cereal crops. One
of the reasons is the rapid binding of patulin by SH-groups of
vegetative proteins (Stott T., Bullerman L., 1975). The stabi-
lity of the toxin depends on humidity and the substrate. The
half-life of patulin in barley, maize and wheat (t = $25^{o}C$;
RH = 70%) was 12.7; 4.4 and 4.4 days, whereas at RH = 90% it
was 6.8; 2.4 and 1.9 days, respectively (Harwig J., 1977).

The derivatives of patulin and penicillic acid together
with sulphydril components are of much lesser toxicity than
patulin (Lieu F., Bullerman L., 1978; Hofmann K. et al., 1971;
Ciegler A. et al., 1972).

Similar mechanism of binding with sulphydril groups of
proteins has been found in citrinin (Harwig J. et al., 1977).
The half-time of half-life of citrinin at RH = 90% in barley,
maize and wheat was just as small as it is in patulin: 1.8;
10.4 and 3.0 days.

Spicher G. (1981) believes that even if patulin and citrinin are the causes of mycotoxicosis this cannot always be demonstrated owing to their rapid desintegration in grain and grain products.

Penicillic acid is also comparatively unstable (Scott P. et al., 1972). This property, to a certain extent, may explain why it is rarely found in vegetative materials unlike the broad spread of producent fungis. It has been impossible to find penicillic acid in the substrate following 48 hours of storage (Lieu F., Bullerman L., 1977).

Rubratoxin B, owing to its quick desintegration at high ($> 23^{\circ}$C) temperatures can neither be regarded as a serious contaminant of vegetative material. Therefore, it occurs very rarely under natural conditions (Engstrom G., Richard J., 1981).

Aflatoxins are characterized by a comparatively high stability but its degree varies subject to the substrate. Noticeable decrease in their concentration had been found in maize meal after 140 hours of storage. Content of aflatoxins in peanut meal or in peanut butter was neither changed after 24 months of storage at 23°C and RH = 50% (Baur F., 1975). However, Waltking A. (1971) stored fried and wet peanut butter for six months at room temperature and found that aflatoxins were strongly destroyed in wet butter. Less than 50% of aflatoxins remained in wheat flour after 350 days of storage (Atli A., Kosker O., 1980).

We have found that rice grain has a considerable rate of destruction of aflatoxin B_1 and it was practically one and the same at temperatures of $10-30^{\circ}$C and inversely dependent upon

the toxins' initial concentration. After the passage of nine
months in storage rice grain which contain 20, 350 and 640 μg/kg
of aflatoxin B_1, the average detected amount of the initial
aflatoxin was 93, 14 and 8%, respectively. After the passage of
12 months aflatoxins could not be detected in all variants by
chemical techniques (Fig. 1).

The causes of the decrease in aflatoxin content could be
oxidation with air oxygen or with peroxide compounds which ac-
cumulated in grain and grain products in long storage owing to
the destruction of polyunsaturated fatty acids. From this point
of view it is possible to explain the comparative stability of
aflatoxins in peanut butter which contains less polyunsaturated
fatty acids than the fat component of wheat and rice grain.
Similar mechanism of aflatoxin oxidation has been for the first
time experimentally demonstrated by Ciegler A. et al. (1966).

The destruction of aflatoxins in prolonged storage of
other crops has not been studied. The chemical composition of
grain and specifically the distribution of aflatoxins (surface
or deep) may, most likely, materially influence this develop-
ment.

Fusariotóxins are distinguished by extreme stability. Ac-
cording to Joffe A. the active ingredient of alimentary-toxic
aleukia was preserved in grain for six years. The toxicity of
grain infested by Stachybotrys alternaus or did not go down
after 10-12 day storage (Mishustin E., Trisvyatsky L., 1963).

6. Prevention of m otoxin formation

We have thus considered the factors which determine the
formation of aflatoxins in real conditions and also effects
of individual methods of storage on this process. The findings

have made it possible to recommend a range of general preventive measures which forestall the development of microorganisms and accumulation of mycotoxins in different vegetable products during cultivation, harvesting and storage. The main provisions have been taken from the documents of a Joint FAO/UNEP Conference on Mycotoxins held in Nirobi in 1977.

1. Observing rules of farming practice, control of insects in the field to obtain a harvest of wholesome seed which are not infested with fungi and insects.

2. Cultivation of varieties which are resistant to infestation with A. flavus and Fusarium spp. and to the formation of mycotoxins.

3. Proper timing of harvesting of seeds and grain at the moment of their full ripening, prevention of delayed harvesting since this makes seed tissue more susceptible to infestation with fungi.

4. Minimizing mechanical damage and shoving of scaly crops during harvesting and post-harvesting treatment of grain.

5. Immediate drying of grain to a level which is safe for storage and subsequent maintenance of this humidity.

6. In case of impossibility of rapid drying of grain it is recommended to cool it by active ventilation within a short period of time. Avoiding weak ventilation patterns which facilitate the development of fungi and accumulation of toxins.

7. Removal of inadequate, unripe, decoloured and crushed grain and seeds as well as organic waste and weeds from the grain mass.
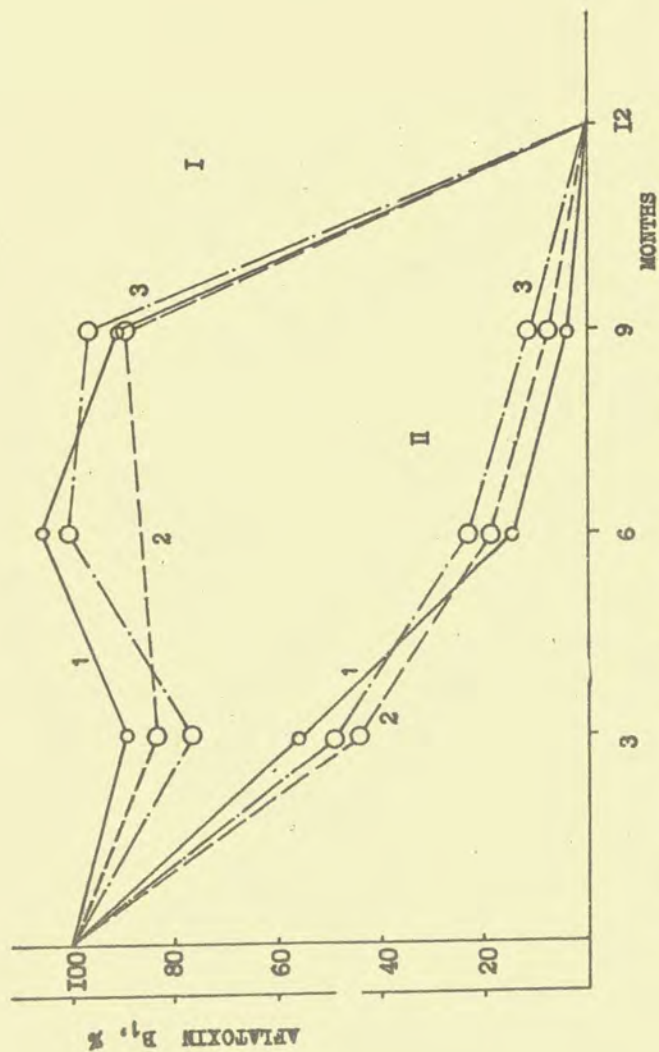
Fig.1. Alteration in aflatoxin B_1 content during long-term storage. Initial content of aflatoxin B_1: I – 20 $\mu g/kg$; II – 640 $\mu g/kg$. Storage temperature: 1 – 10°C; 2 – 20°C; 3 – 30°C.

8. Large-scale application of insecticides to prepare premises, prevention of infestation with insects and insect control during storage.

9. Regular checking of temperature and humidity in storage.

10. Storage under constant temperatures and relative humidity. Migration and condensation of moisture owing to the temperature gradient in stored mass of grain can lead to accumulation of moisture and further development of fungi.

11. Storage of products, as far as possible, in a cool state since most mycotoxins, with the exception of fusariotoxins are not formed at low temperatures.

12. Storage of products in modified gas media and in an atmosphere of inert gas.

13. Application of conservants for the treatment of wet fodder grain.