

TOXIGENIC MOLDS ON FISH FEEDS-I : IMPACT OF CLIMATIC FACTORS

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ABSTRACT

The present communication is a survey report carried out to assess the incidence of toxic mycoflora on seven types of agriculture products/byproducts incorporated during fish culture as supplementary dietary items. Samples were obtained from various sources at Darbhanga, Madhubani and Samashtipur districts during summer, winter and monsoon months. Out of the total 1774 samples, only 894 appeared to be fresh visually reflecting average incidence of contamination around 46.6%. However, the apparently fresh samples, when subjected to culture, 26.9% of them were found to be contaminated. Thus, degree of fungal spoilage in feed ingredients in parts of north Bihar appears to be significantly high (73.5%). The present study illustrates the facts with special reference to *Aspergillus flavus*, *A. parasiticus* (elaborating aflatoxins) *A. ochraceous*, *Penicilium viradicatum* (elaborating ochratoxins) and *A. versicolor* (elaborating sterigmatocystin). The other strains already known for their toxigenic potentials that appeared on the present substrates included *A. niger*, *A. fumigatus*, *A. candidus*, *P. islandicum*, *Rhizopus* spp. and *Mucor* spp. Studies indicate that the prevalent climatic factors like temperature and relative humidity facilitate a congenial condition almost all through the year and in particular during summer and monsoon months. But water content of the substrates is a vital factor that further accelerates the pace of mycobial spoilage. A thorough sun-drying of the agricultural commodities before prolonged storage to bring water content below the "low risk limit" may significantly reduce the incidence of molds.

Keywords : Toxigenic molds, fish feeds, fungal spoilage

INTRODUCTION

Technological advancements in fish culture practices have made supplementary feeding indispensable. Besides hygiene, a balanced nutritional supplementation is now being observed as key to profitable aquaculture. Consequently, new feed formulations are being worked out incorporating food commodities of

animal and plant origins. However, the present scenario of aquaculture *vis-a-vis* fish feed availability in India is far from adequate and the small pisciculturists are dependent on local feed ingredients and formulations. Composition of such feeds essentially depend on easy availability of agricultural products or byproducts. In Bihar, the fish farmers usually utilise

mustard oil cake (MOC), ground pluses mixture (GPM), wheat bran (WB), rice bran (RB), wheat flour (WF), rice flour (RF), etc. alone or in combination to supplement the nutritional requirements of their standing fish crop.

On the other hand, the concern for mycobial spoilage of foodstuffs of both plant and animal origins, an age-old phenomenon, is now attaining new dimensions. Since the discovery of aflatoxins, secondary toxic mold metabolites, as an etiological agent in many of the epidemic outbreaks of farm animals and pets all over the globe (Verma, 1989), many others have been added to the list (Verma, 2001). In recent years, mycotoxicosis is becoming increasingly implicated in human (Wafa *et al.*, 1998; Stoev, 1999; Stoev *et al.*, 2001) and animal health problems (Stoev, 1998, Stoev *et al.*, 1998).

The present study illustrates the incidence of toxic fungi on agriculture products incorporated in fish feeds with special reference to *Aspergillus flavus* Link, *A. parasiticus* Spear (elaborating aflatoxins); *A. ochraceus* Wilh and *Penicillium viradicatum* Westl. (elaborating ochratoxins) and *A. versicolor* (Vuil.) Tiy. (elaborating sterigmatocystin). Impact of climatic factors on the incidence of these toxic fungi and the role of water content of the substrates have also been discussed.

MATERIAL AND METHODS

Feeds: Composition of fish feeds varies from area to area largely depending upon the availability of parent agricultural products. MOC is either pure yellow mustard (*Brassica campestris* L.) or

disproportionately mixed yellow and black (*B. napus* var. *dichotoma* Prain) mustards byproduct following extraction of oil. It often contains rapeseed (*B. campestris* var. *olifera* Dc.) in small quantities. The GPM is a coarse composition of pigeon pea (*Cajanus indicus* Spreng.) massor (*Lense esculentus* Monech.), gram (*Cicer arietinum* L.), moong (*Phaseolus mungo* L.) and urd (*Phaseolus radiatus* L.). Besides, wheat and rice flour, and their processing byproducts (wheat bran and rice bran) are widely used as animal feeds. Some of commercial rations (CR) available in the local market are also incorporated.

Survey and sampling: Geographical survey for the incidence of toxic fungi was made during winter (November-January), summer (April-June) and Monsoon (July-September) in three flood-prone districts of Bihar. Altogether, 26 sampling stations (Fig. 1) consisting of 12 block headquarters from Madhubani and seven each from Darbhanga and Samastipur districts were selected.

The samples were collected following the guidelines suggested by US Food and Drug Administration. Before sampling, history of each lot was depicted with special reference to their time of production/processing, processing practices, climatic condition during processing, storage period, storage condition and packaging details.

Each lot prior to sampling was visually inspected for fungal spoilage, if any. Thus, the samples were designated as fresh (F), partially infected (PI) and infected (I) depending upon the physical condition of the substrates. The random samples (minimum 500 g) were serially numbered, registered and brought to the laboratory,



1. Harlakhi	10. Lohna Road	19. Hayaghat
2. Basopatti	11. Sakri	20. Pusa
3. Jaynagar	12. Jogiara	21. Kisanpur
4. Laukaha	13. Jale	22. Samastipur (Hq)
5. Benipatti	14. Darbhanga (Hq)	23. Rosera
6. Madhubani (Hq)	15. Simri	24. Dalsingsarai
7. Rajnagar	16. Laheriasarai	25. Sahnur Patori
8. Phulparas	17. Bahera	26. Hassanpur Road
9. Jhanjharpur	18. Supaul	

Fig. 1 : Sampling sites in Madhubani, Darbhanga and Samastipur districts

Table 1 : Mold contamination of agricultural products/byproducts incorporated in animal fish feeds obtained from different sources in three districts of North Bihar**WINTER**

Type of substrate	Total number physical condition F/PI/I	Natural infestation (%) (A)	Contamination		Source		
			appearing after incubation (%) (B)	Total contamination (%) (A+B)	Farmer's godown	Retail shop	Wholesale depot
Mustard oil cake	116 47/27/42	59.4	22.4	818	60	30	26
Wheat flour	59 47/09/03	20.3	35.5	55.8	30	20	09
Rice bran	125 81/24/20	20.3	25.4	60.6	50	50	25
Soya flour	05 05/0/0		60.0	60.0	00	05	00
Wheat bran	59 39/07/10	33.8	32.2	66.0	30	15	14
Ground pulse mixture	127 62/23/42	51.1	25.1	76.2	60	40	27
Rice flour	58 55/03/00	5.1	36.2	41.3	25	18	15
Commercial rations	20 14/6/-	-	30.0	30.0	8	8	4

SUMMER

Mustard oil cake	126 70/28/29	44.4	31.7	76.1	63	57	06
Wheat flour	72 44/28/0	38.8	41.6	80.4	18	47	07
Rice bran	130 96/29/05	26.1	15.3	41.4	47	77	06
Soya flour	10 10/00/00	-	60.0	60.0	00	10	00
Wheat bran	60 20/31/09	66.6	25.0	91.6	20	37	03
Ground pulse mixture	128 46/47/36	64.0	19.5	83.5	64	55	09
Rice flour	60 41/10/09	31.6	50.0	81.6	19	37	04
Commercial rations	10 8/2/00	-	20.0	20.0	4	2	4

MONSOON

Mustard oil cake	133 36/61/33	55.6	22.5	78.1	63	68	02
Wheat flour	70 20/34/16	57.1	24.2	81.3	27	26	18
Rice bran	135 57/50/28	42.9	34.0	76.9	49	68	18
Soya flour	10 10/00/00	-	70.0	70.0	00	10	00
Wheat bran	59 13/21/25	61.0	16.9	77.9	32	27	00
Ground pulse mixture	125 50/40/35	44.0	32.8	76.8	65	56	04
Rice flour	57 10/23/24	47.3	12.2	59.5	21	36	00
Commercial rations	20 10/8/2	10.0	40	50.0	7	7	6

F=Fresh, PI=Partially Infected, I=Infected, T= Total

thoroughly mixed using pestle and mortar or electrical blender and reduced to working size of approximately 50 g by quartering (Dickens and Whitaker, 1982).

Culture: The sub-samples were subjected to culture using two routine techniques, *i.e.*, blotter technique and solid medium or agar plate technique following Verma (1989). In the first method, Whatman filter paper number 1 in sterilized petri plates and in the second, Asthana Howket 'A' (AH) and Capak's (CZ) agar media were simultaneously used. The cultures were incubated for 7-10 days at

30±2°C at the end of incubations, fungal colonies were initially identified on characteristic colour (Table 2) and finally on micromorphological features (Table 3) following Wyllie and Morehouse (1977).

RESULTS AND DISCUSSION

During 1999-2000, 1974 samples were collected. A seasonal record of mold contamination of these substrates and their physical condition under prevalent marketing practices are presented (Table 1, Fig. 2). Fungal colonies in culture start

Table 2 : Characteristic colony colour patterns of some toxic fungal strains

Strain	Characteristic colour of the colony	Medium
<i>A. flavus</i>	- Heads yellow-green*	AH and CZ
<i>A. ochraceous</i>	- Heads yellow to ochraceous	AH
<i>A. parasiticus</i>	- Heads dark yellow green*	AH and CZ
<i>A. versicolor</i>	- Heads light-yellow green	AH and CZ
<i>A. rubur</i>	- Heads blue-green to olive green	AH and CZ
<i>P. viradicatum</i>	- green to yellow -green	CZ

AH - Asthana Howker's A, CZ = Czapek's Agar medium, (5) *A. niger*

*Not easily distinguishable unless verified through microscopic features.

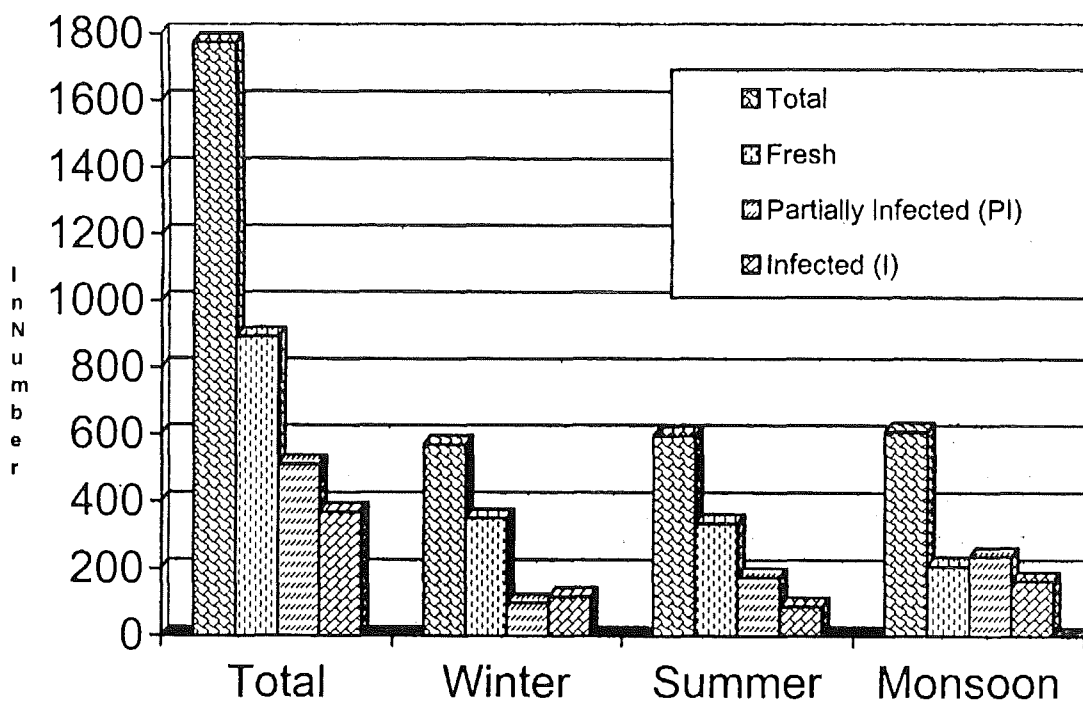


Fig. 2 : Seasonal record of mold contamination of the substrates

appearing on day 3 onwards, but assumes characteristic colour only after seven days. Colonies identified in the culture plates at the end of incubation have been summarily tabulated (Table 2). Characteristic micromorphological features of the strains under investigation are also presented (Table 3).

Sampling records indicate that a significant number of agricultural items open for sale in general market or stored for daily feeding by leading farmers were contaminated by a variety of fungal spores well known for producing potentially lethal toxins.

Out of the total collection (1774), only 894 samples appeared normal visually

reflecting natural spoilage/contamination of 49.6% samples. However, when these fresh-appearing samples were subjected to culture, 45.8% of the winter, 80.7% of the summer and 72.1% of the monsoon samples were found to be contaminated with the spores (Table 4). Apparently, they could not germinate due to the lack of favourable climatic factors and supportive water content of the substrate (WCS). Thus, altogether the degree of mycobial contamination becomes, as high as 73.5% (Table 5).

Analysis of the survey records revealed that the samples collected from retail shops presented the most critical scenario from

Table 3: Characteristic micromorphological features of the toxic fungal strains

Strain	Micromorphological features
<i>A. flavus</i>	Sterigmata almost entirely biseriate
<i>A. ochraceous</i>	Conidial heads globose or radiate, heads large, vesicles large and globose or nearly so, sclerotia sometimes occurring
<i>A. parasiticus</i>	Sterigmata uniseriate
<i>A. versicolor</i>	Sterigmata uniseriate
<i>A. rubur</i>	Phialides strictly uniseriate, conidiophore smooth
<i>P. viradicatum</i>	Conidophore heads are assymetrical, consisting of sterigmata, metulae and branches (often rough walled), sterigmata flask shaped, conidia sub-globose and rough walled

Table 4: Mycobial infestation appearing on fresh samples following incubation

Season	Total no.	No. of fresh Samples	Samples developing fungi following incubation	
			No.	%
Winter	569	350	154	45.8
Summer	596	335	166	50.7
Monsoon	609	209	158	72.1
Total	1774	894	478	53.5

the mycobial contamination point of view. A critical analysis of the degree of spoilage at different source levels (Fig. 3) indicated

maximum infestation during monsoon in the samples collected from retail shops followed by farmer's godown and wholesale depots.

Table 5 : Fungal infestation appearing on various substrates following incubation

Substrate	Winter		Summer		Monsoon	
	Fresh samples (no.)	Infestation appearing after incubation (%)	Fresh samples (no.)	Infestation appearing after incubation (%)	Fresh samples (no.)	Infestation appearing after incubation (%)
MOC	47	22.4	70	76.1	39	78.1
WF	47	35.5	44	80.4	20	81.3
RB	81	24.4	96	41.4	57	76.9
SF	05	60.0	10	60.0	10	70.0
WB	39	32.2	20	91.6	13	77.9
GPM	62	25.1	46	83.5	50	76.8
RF	55	36.1	41	81.6	10	59.5
CR	14	30.0	8	20.0	10	50.0

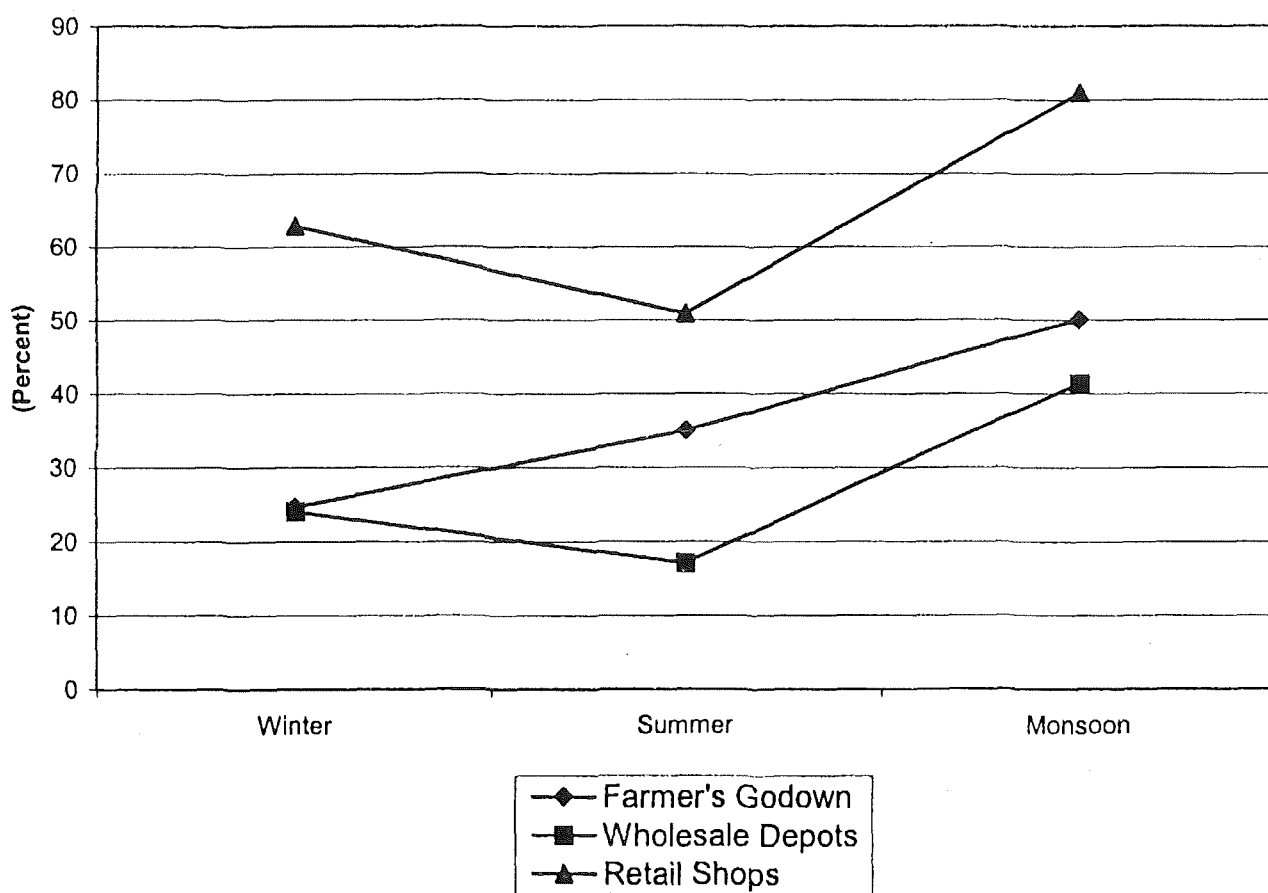


Fig. 3 : Degree of mycobial spoilage of agriculture commodities sourcewise

Variable records of mycobial contamination of different substrates, (Fig. 4) besides those appearing naturally may be attributed to the variation in the climatic factors like temperature, relative humidity (RH) and WCS.

RH fluctuates between 68-85 and 59-74%, respectively. During monsoon, the temperature maintains almost the same trend as observed in summer, but the RH increases up to 96%. Thus, during summer while the temperature range favours

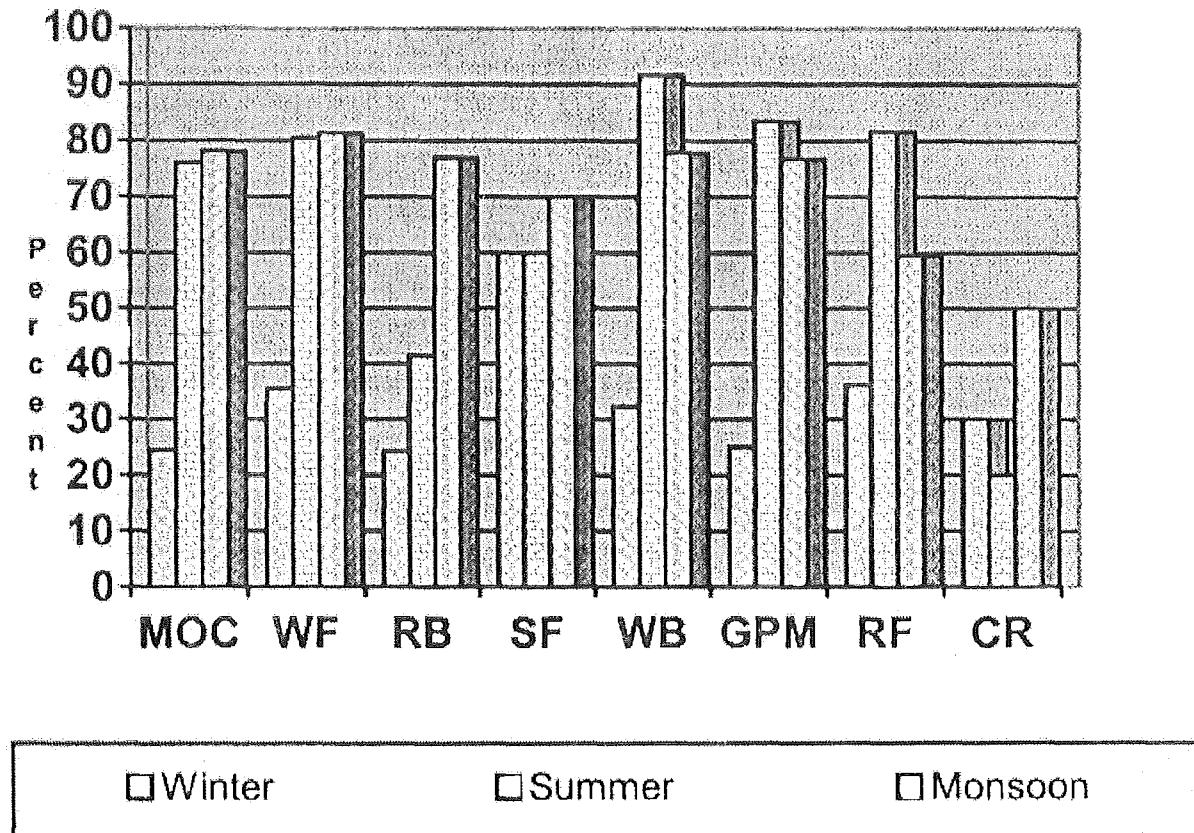


Fig. 4 : Degree of mycobial spoilage of agricultural commodities at source - seasonal

The present area of investigation constitutes the core of the Indo-Gangetic planes. The climatic variation in different seasons is characterised by extreme heat and cold, and high RH added with recurrent floods. The area, faces a wide range of temperature (4-38°C) and RH (59-96%) variations. During winter and summer, the temperature usually ranges between 6-15°C and 28-38°C, and the corresponding

mycobial growth, the RH or WCS is hardly supportive. Only the substrates with high WCS that gain moisture from other sources reflect mycobial spoilage during summer. Similarly, during winter, the moisture gained over by the substrate in preceding monsoon rains are sustained within the substrates since the low range of atmospheric temperature prevents evaporation.

Fungal spores infesting any substrate virtually require a favourable temperature range with high RH and a substantial WCS. The spores of the fungi retain their viability for longer periods, even several years under favourable conditions and undergo germination on finding congenial climatic factors. Table 6 indicates that the present substrates in prevalent storage conditions contains WCS variable between 5 and 12%, the highest in the cases of MOC and GPM followed by wheat bran (11%). An earlier report (Austwick and Ayerst, 1963) suggests that 5.6% WCS in fresh oilcakes usually do not support mold invasion and their subsequent growth. Comparison of the two records clearly suggests the role of increased RH in elevating WCS. It may also be inferred (Table 6) that in prevalent climatic conditions, average WCS of 8.17% corresponds to 59-74% RH. With increase in RH during winter and monsoon, there is an apparent average gain of 3.6 and 5.3%,

respectively, over the WCS values recorded during summer. Thus, WCS becomes a significant factor that cannot prevent contamination (mycobial infestations are usually air/vector borne), but can impose check on further propagation of contaminating spores. As a matter of fact, it is the climatic conditions that makes the tropical and subtropical countries a congenial ground for mycobial spoilage (WHO, 1979).

Table 6 further indicates that the present substrates exhibit variable capacity to gain water in different seasons. This practically depends on the physical state, *viz.*, affinity towards water molecule, degree of saturation, drying character, etc. of the substrate. It has been observed that the dried substrates gain more humidity than others. Guilbot (1960) pointed out that at a given temperature, for each concentration of water in the substrate, there is a corresponding RH representing the ease

Table 6 : Range of relative humidity, water content of the substrate and temperature favouring sporulation/growth of some toxic fungi

Species	RH (%)	Water content (%)	Maximum and minimum temperature (0°C)
<i>A. flavus</i>	80	15 - 16	10 - 45
<i>A. parasiticus</i>	82	14 - 15	10 - 45
<i>A. niger</i>	85	16 - 17	10 - 40
<i>A. versicolor</i>	81	---	10 - 42
<i>A. rubur</i>	70	12 - 13	10 - 45
<i>A. ochraceous</i>	80 - 82	16	14 - 40
<i>A. nidulanse</i>	84	15 - 16	10 - 42
<i>A. candidus</i>	75	15 - 15.2	10 - 55
<i>Penicillium</i> spp.	82 - 86	15 - 16	0 - 35
<i>Rhizopus</i> spp.	90 - 92	17 - 18	0 - 35
<i>Mucor</i> spp.	88 - 89	17 - 18	30 - 60

with which the substrate loses water to the atmosphere. At low RH, water is bound to the substrates by a significant bonding energy, but as the RH increases, the availability of water also increases, leading to feeble bonding. It is this degree of mobility of water, which makes it possible for the molds to grow on solid substrates.

An earlier report (Moreu, 1974) suggests that many of the toxic molds including the ones included in the present study grow conveniently between 0 and 45°C on the substrates having 15-18% WCS or corresponding range of RH (75-96%). During summer, WCS of the substrates varied between 5 and 12% depending upon their physical state, and these substrates in normal storage condition exhibit a gain of 3.6% during summer and 5.3% during monsoon. The phenomenon obviously provides ample opportunity for the infesting molds to grow over the substrate.

The pace of mycobial spoilage is further compounded by the prevalent practices like storage in direct contact of soil, use of packaging materials prone to wetting and delayed drying, localisation of storehouses in damp areas, poor ventilation, careless marketing, etc. These practices are detrimental and further accelerate the pace of mold spoilage. The climatic factors like temperature and RH are beyond manipulation, and fungal infestation of the substrates due to air-bound movement of spores cannot be completely checked. The agricultural commodities from sowing to final storage are always at risk of getting contaminated. But, the WCS before short or prolonged storage can easily be brought down to a "low-risk-range" by adopting simple and thorough sun-drying. The

substrates handled with care during pre and post harvest conditions and stored at 5-7% WCS face minimum risk of mycobial infestation.

Record of the fungal strains isolated from the present substrates during different seasons and their order of preference for the substrates under investigation are shown in Table 7. *A. flavus* (Fig. 5) appeared as the most dominant mycoflora in general followed by *A. niger* (Fig. 6) almost throughout the year. Both *Aspergillus* spp. and *Penicillium* spp. have been recognised as storage fungi (Christensen and Kaufman, 1965). However, there is now overwhelming evidence for pre-harvest invasion of the crops by these strains mainly carried out by the insects (Lillehoj *et al.*, 1976). The growth of *A. flavus*, *A. parasiticus* (Fig. 7) and *A. niger* is favoured by humidity in excess of 85% RH (Davis and Diener, 1970) corresponding to WCS of 12-15%. It has been suggested that 32°C temperature is optimum for the growth of *A. flavus* and 35°C for *A. niger*. The latter grows conveniently even at a comparatively low RH of 77% (Ayrest, 1969). Jackson (1965) suggested that *A. flavus* is capable of growing over the temperature range of 6-54°C and optimum growth occurs between 30 and 35°C. Contaminations due to *A. flavus* and *A. parasiticus* are usually indistinguishable. These species are frequently discussed together on account of their taxonomic and physiological similarities, *viz.*, common colour of the colony, physiological requirements, climatic conditions and most significantly, production of similar secondary toxic metabolites, the aflatoxins. The only micromorphological feature that allows one

to separate the two species is the uniseriate phialides (Fig. 7) in case of *A. parasiticus* which is biseriate in *A. flavus* (Fig. 5).

According to Raper and Fennel (1965), the group *A. ochraceous* (Fig. 8) comprises about nine species, but only a few of them are common invaders. The species is somewhat xerophytic (Christensen, 1962) growing at about 80-82% RH and has commonly been reported from heating grains. Natural occurrence of *A. ochraceous* in food and feeds of plant origin has been widely reported (Stoev, 1999) and ochratoxin A, the secondary toxic metabolite of *A. ochraceous*, has been identified to cause mycotoxic porcine nephropathy in pets (Stoev *et al.*, 2001) and man (Wafa, *et al.*, 1998; Stoev *et al.*, 1998).

In laboratory cultures, Sansing *et al.*, (1973) obtained good ochratoxin at 25°C. In general, 80-82% RH with 6% WCS favours sporulation and growth of the fungi between 10 and 40°C. Schindler and Nesheim (1970) obtained good yield of the toxin with shredded wheat after 19 to 21 days at 21 to 23°C. Almost all species of this group produce two important mycotoxins, ochratoxin and/or penicillic acid (Wyllie and Morehouse, 1977).

The group *A. versicolor* includes 17 species (Raper and Fennel, 1965), but only *A. versicolor* and *A. sydowi* are known to be mycotoxigenic elaborating sterigmatocystin. Earlier reports on the occurrence of this species suggest the strain to be uncommon on cereals and grains, but invade wheat paste products and shredded grains (Wyllie and Morehouse, 1977). Its present incidence on wheat and rice flour deserves consideration. The species usually

invades the substrates rich in free nitrogen with the onset of deterioration (Lellehoj and Ciegler, 1968). During the present investigation, no fresh sample even under optimal climatic condition was found to be contaminated with *A. versicolor*, but the same substrates when left for 20-22 days with elevated WCS got contaminated with the strain.

A. ruber belong to *A. glaucus* group that comprises most commonly occurring fungi on stored products because of their ability to grow at low RH of 75-85%. Kulik and Holadey (1966) have suggested that some strains of *A. ruber* may produce aflatoxins.

Some of the other members of *Aspergillus* group invading the present substrates included *A. candidus*, *A. nidulanse* and *A. terreus*. *A. candidus* has been reported from a variety of substrates during storage and is a predominant fungus of refrigerated dough products (Graves and Hesseltine, 1966). *A. nidulans*, like *A. versicolor*, is known to produce sterigmatocystin and significant amount of penicillic acid (Ditchburn *et al.*, 1974). Lafont and Lafont (1969) have frequently isolated the strain from corn, wheat and barley kernels, and is found to be very common on animal feed in France. *A. nidulans* has usually been reported from heating corn often accompanied by *A. flavus* or *P. viradicatum*.

Among the *Penicillium* group, *P. viradicatum* (producing ochratoxins) and *p. islandicum* (producing luteoskyrin), like *A. flavus*, appear as common invaders on the present substrates. Both these strains require high RH justifying their abundance during monsoon (Table 7). Similarly,

Plate 1

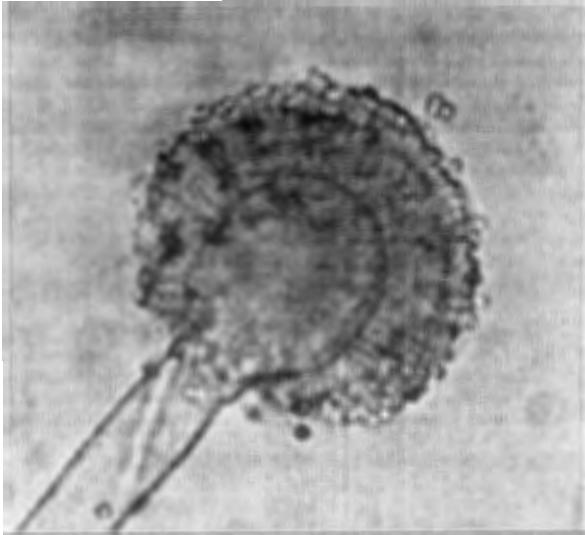


Fig. 5 : Conidial head of A. flavus showing biseriata phialides x 400



Fig. 6 : Conidial head of A. niger x 400

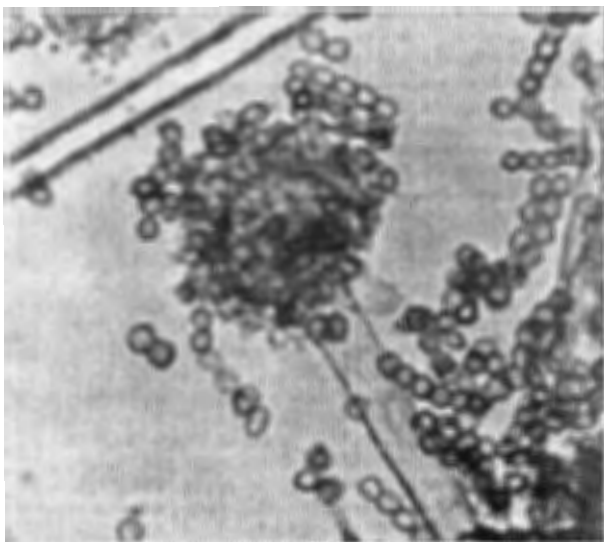


Fig. 7 : Conidial head of A. parasiticus showing uniseriate phialides x 400

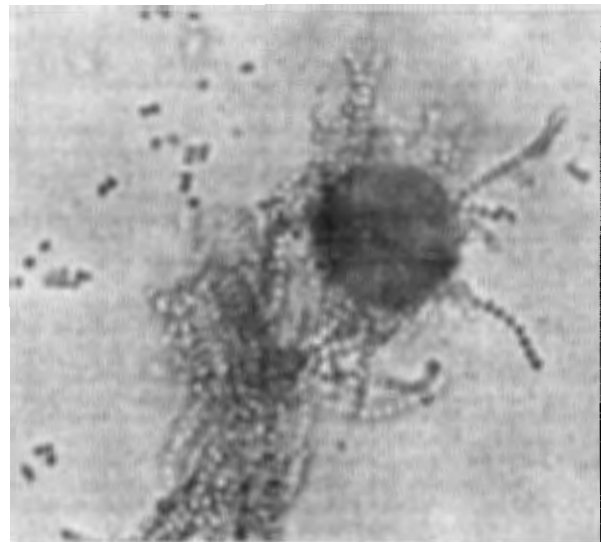


Fig. 8 : Conidial head of A. ochraceous x 400

Table 7: Seasonal incidence and substrate preference of the fungal strains isolated from agricultural products and their byproducts

Fungi	Seasonal incidence			Substrate preference shown in order of abundance	Positive samples (%)				Toxins produced
	W	S	M		W	S	M	Av	
<i>A. flavus</i>	+++	+	+++	MOC, GPM, WF, RF, WB, RB, SF	51.0	40.6	63.0	51.5	Aflatoxins
<i>A. ochraceous</i>	++	++	+	WF, WB, RF, GPM, MOC	38.0	27.5	19.0	28.1	Ochratoxins
<i>A. niger</i>	++	++	++	GPM, MOC, RF, WF, WB, RB	52.2	42.0	58.4	50.8	Oxalic acid
<i>A. paraciticus</i>	+	+	++	GPM, MOC, WF, SF,	42.0	12.4	23.5	25.9	Aflatoxins
<i>A. rubur ?</i>	+	+	+	MOC, RB, WB, GPM	8.5	7.0	12.6	6.9	Aflatoxins
<i>A. fumigatus</i>	+	-	-	MOC, RF, GPM	6.8	-	-	2.2	Gliotoxin
<i>A. versicolor</i>	+	++	-	WF, RF, GPM	16.0	20.5	-	12.1	Sterigmatocystin
<i>A. candidus</i>	+	+	-	RF, GPM	-	5.4	-	0.8	Candidulin
<i>A. nidulanse</i>	+	+	-	GPM, MOC, WF, SF	12.0	12.4	-	8.1	Nidulin
<i>Penicillium</i> spp.									
<i>P. cittinum</i>	+	-	++	MOC, GPM, RB, WB, RF	32.5	-	52.0	28.1	Citrinin
<i>P. viradicatum</i>	+	+	++	RB, GPM, WB, MOC, RF, WF	20.6	-	32.5	17.7	Ochratoxins
<i>P. islandicum</i>	+	+	++	MOC, RB, GPM	6.6	10.0	26.0	14.2	Luteoskynin
<i>P. cyclopium</i>	+	+	++	MOC, GPM, WF	7.0	11.0	16.0	11.3	Penicillic acid
<i>Rhizopus</i> sp.	-	-	+++	MOC, GPM, RB, WB, WF, RF	-	-	42.0	14.0	
<i>Curvularia</i> sp.	+	+	-	MOC, RB, WB, GPM	8.0	11.5	-	6.5	
<i>Mucor</i> sp.	-	-	++	MOC, RB, WB, GPM, RF, WF	24.0	11.5	-	6.5	
<i>Alternaria</i> sp.	-	+	-	RB, WB, GPM, MOC	-	9.0	-	3.0	
	-	-	+	GPM, WB	-	-	6.0	2.0	

? = not confirmed

Rhizopus spp. and *Curvularia* spp. also require high RH (90-92%) with WCS as high as 17-18%. Incidence of these fungi on substrates during summer or winter indicates either a high WCS or gain of moisture from any extra sources.

Penicillium spp. are seemingly always involved when food/feed, raw materials or finished products in field or storage are undergoing deterioration. The present strain of *P. viradicatum* is now known to be an ochratoxin A producer along with some other species of the group, viz., *P. palitans*, *P. commune*, *P. variable* and *P. cyclopium* (Stoev, 1999).

Present study, thus, indicates the gravity of the problem of mycobial spoilage of agricultural products and their byproducts incorporated as animal/fish feeds. Since most of the toxic strains invade the substrates during storage, in particular when the commodities have not been properly processed or stored, an assessment of the prevalent practices of storage and creating general awareness on the people concerned are essential.

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