International Journal of Research and Development in Applied Science and Engineering (IJRDASE)
ISSN: 2454-6844

Mycotoxins In Fish Feed of Bihar

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Abstract-- **The present communications is a survey report to obtain the incidence of toxic mycoflora on seven types of agricultural products and their byproducts incorporated in fish culture as supplementary dietary items. Samples were obtained from various sources of Darbhanga, Madhubani and Samastipur districts during summer, monsoon and winter months. Altogether 1774 post-harvest samples were screened. Studies revealed an alarming level of mycobial contamination by Aspergillus group of fungi dominated by A. flavus (51.5). The other species of the group recorded were A.niger (50.8), A.ochraceous (28.1), A.parasiticus (21.1). A.versicolor (12.2), A.rubur (6.9) and A.fumigatus (2.2). Among the penicillium group P.viradicatum (17.7) and P.islandicum (14.2) infestation was prominently recorded. Analysis of the data indicated that the degree of infestation was maximum on the samples obtained from retail shops. The incidence of toxic mycoflora at source level may be expressed as retail shop> farmer's godown> wholesale depots> oil mills. Studies made so far emphasizes the need of elaborate investigation on this score since most of the fungal species recorded, hold recognition for their toxic and carcinogenic potentials. The frequency of occurrence on the present substrates also indicate some disastrous consequences at any opportune moment affecting the fish culture in this geographical region of Bihar. As a consequence of aquaculture vis-à-vis fish culture becoming more and more spectacular, the outlook has undergone tremendous changes attaining unimaginably large dimensions. In particular, freshwater aquaculture holds the future of the fisheries of our country. At this juncture when further intensification of freshwater aquaculture is being emphasized, it becomes highly relevant that all possible constraints are considered in their proper perspective.**

1. Introduction:

Adequate health management is the key of intensive fish culture programme. With recent researches and technological applications in the field, we are heading towards blue revolution, but the same has also brought many drawbacks often causing serious concern through epidemic outbreaks. Intensive researches are being carried out to delineate the stress factors, primarily identified as parasites, fungi, bacteria, virus and organism-environment interaction. But one vital aspect, "aquaculture feed quarantine", that practically forms the basis of the aquaculture output and is poised to play a significant role during the present era has largely been ignored. The reason may arguably be attributed to the fact that any epidemic outbreak as contaminated feed supply has not so far been registered here. Ina country where first incidence of hepatoma epidemics in human beings due to aflatoxin contamination was recorded, it cannot be argued that our animal feeds are free from mycotoxins. The reasons behind non-documentation of such instances possibly are:

i. Bulk of the inland fish culture operations, except in organized farms, are still dependent on natural feed. Application of supplementary feed is rather a "ceremonial phenomenon".
ii. Lack of interrogative scientific approach and general awareness (We do not inspect the death of our farm animals, especially fish from mycotoxicosis point of view and if at all we do, we try to find out characteristic toxic described in foreign texts).
iii. Lack of early clinical symptoms (pathogenomic trait).

Toxic fungi though are ubiquitous in occurrence, their magnitude of incidence varies in different geographical regions depending upon the prevailing climatic factors that decide their invasive potentials. growth and toxigenic potentials. It is the climatic factors that make the tropical and subtropical regions a congenial ground for mold invasion. In this perspective, mycotoxin contamination of aquaculture feeds in India demands exclusive and thorough investigation not only to protect aquaculture crop but also to prevent possible poisoning of the secondary consumers. Carry-over of the mycotoxins to man through their flesh, as absorbed in other laboratory and farm animals, cannot be ruled out.

The Aquaculture in Bihar is an essential subsector of agriculture that provides food, employment, and other economic resources for the state (Prasad 2002). Fish production can be threatened when feeds are contaminated by fungi and their toxic metabolites. Several mycotoxins, including aflatoxins (AFs), cyclopiazonic acid (CPA), fumonisins (FUMs), nivalenol (N IV) and zearalenone (ZEN) have been reported to contaminate fish feed and their ingredients (Prasad 2002,Das 2002,Verma 1990, Labuda et al. 2005; De Boevre et al. 2012; Ezekiel et al. 2012a; Njobeh et al. 2012; Rodrigues and Naehrer 2012;). The occurrence of mycotoxins in feed ingredients depends on several factors that include climatic conditions, diversity of fungi contaminating the crops, harvesting methods of the individual crops, storage practices, and seasonal variations, while the types and levels of mycotoxins in the feed largely depend on the mycotoxins in the individual feed ingredients, the mix/ proportion of feed ingredients, feed processing techniques, and storage practices (Warth et al. 2012;). Mycotoxins pose a huge threat to the safety and security of livestock first and then to human beings that consume them due to their different toxic effects and their probable synergistic properties (Shephard 2008; Hossain et al. 2011;

ISSN: 2454-6844

Njobeh et al. 2012). When animals ingest feed contaminated with high mycotoxin concentrations, mycotoxicoses, often marked by reduced animal productivity (reduced body weight gain, reduced litter sizes, deformed offspring, reduced egg production) and immune suppression (Shareef 2010; Hossain et al. 2011), could result to severe economic losses. Fish feed ingredients are derived from a variety of raw materials that originate from plants and animals. It is usually a mixture of cereals that serves as energy source, animal protein sources (fish meal, meat, and bone meal), and plant protein sources (soybean meal). Maize, the predominant grain used in Fish feeds, can be contaminated by mycotoxins from Aspergillus, Fusarium and Penicillium during processing and storage (Zinedine and Manes 2009;).

Globally, mycotoxins in finished Fish feed have been reported (Labuda et al. 2005; De Boevre et al. 2012; Ezekiel et al. 2012a; Njobeh et al. 2012; Rodrigues and Naehrer 2012), but there is sparse information on the source tracking of mycotoxin contamination of the feed by individual ingredients, especially in Bihar. Hence, this study aimed at investigating mycotoxins in Fish feed and the ingredients used in locally formulating the feed in Bihar with a view to associate contamination of major ingredients to overall contamination in finished feed. This survey provides snapshot data for the consideration of other cereal-based ingredients and protein sources that are less prone to AFs and FUMs contamination in feed formulation.

2. Mycotoxins and mycotoxicosis

During the last century, a new chapter started with suspected role of fungi in producing potentilly lethal toxins. Many of mass mortality of farm animals created serious concern and a defined term, 'mycotoxin (Gr. Mykes = fungus, toxicum toxin), came into existence. Mycotoxin is a generic term used to describe metabolites produced by mold(s) in food stuffs or feed, which can cause illness or death on ingestion, skir. contact or inhalation. The disease caused due to intake of mycotoxins is termed mycotoxicosis'. The year 1960 witnessed a real breakthrough in mycotoxicology when 'turkey-x-diseases' and 'rainbaow trout hepatoma epidemic were reported incriminating aflatoxins, secondary toxic metabolites produced by Aspergillus flavous Link Group of fungi, in its etiology. A group of scientists, in spite of numerous constraints of epidemiological evidences, were able to discover 'aflatoxicosis' as a distinct disease syndrome and implication of 'aflatoxin family of compounds as potential hepatocarcinogens in human and animal food chain. Soon, during the 1970's following recognition of A flavus as storage fungi, a thrilling awareness was felt among scientists and others concerned with production, handling and manufacturing of feed/food products, and livestock and poultry producers.

It is true that the fungal toxins, which have attracted the most attention since their discovery, are aflatoxins associated with A flavus and A parasiticus. It was primarily because the toxin, particularly aflatoxin B, is not only the most motent naturally occurring hepatocarinogen, but a strong mutagen, tetragen and immunosupressant too. But, from the aquaculture point of view, may other also deserve due attention (Table 1). Almost all these mycotoxins enter the system through contaminated food and feeds of either plant or animal origin. Even their presence in edible tissue (flesh), and eggs of farm animals fed with contaminated rations has been reported.

Table 1: Some important mycotoxins, their source and characteristic toxic effect

Mycotoxin	Chief source	Toxic action
Aflatoxins	Aspergillus flavus **A. parasiticus**	Hepatocarcinogenic mutagenic teratogenic and immunosupressant
Ochatoxin A	A.ochraceous **Penicillium veridicatum** P cyclopium	Hepatotoxic and nephrotoxic
Citrinin	P citrinum **P. verdicatum**	Nephrotoxic
Sterigmatocystin	A versicolor A flavus A rubur	Degenerative changes in liver and kidney
Zearalenon	Fusarium roseu **F . moniliforme**	Genital hypertrophy, growth promoting activity
Trichothecenes	Fusarium spp	
Patulin	A clavatus	Neurotoxic, eye irritant and toxic changes in alimentary canal
Penicillic acid	A clavatus **P. puberulum**	Convulsions. Coma and death
Ergot alkaloids	Claviceps spp. Aspergillus spp Penicillium spp.	Gangrenous and convulsive
PR toxin	Pencillium spp.	Ataxia, anoxia
Kojic acid	A flavus A. oryzae	Convulsive at large dose
Viriditoxin	A viridi natans	Gastric irritant, tetany
Rubratoxin	P. rubrum	Hemorrhages in body, kidney and liver necrosis

3. Mycotoxin problem in India:

In most of the tropical and subtropical countries, climatic conditions are such that there exists a two crop-system' and also a wide range of crop damaging agents. Besides, the tendency of 'grow-more-store- more without adequate storage facility provide ample opportunity for microbial spoilage of substrates. It is also in these regions, that the commodities, even if they are aesthetically and organoleptically unacceptable, are frequently marketed. Thus, bulk of the life depending upon agricultural products is prone to toxic effects of mycotoxins. The fungal spores are air-born and are inevitably deposited on the surface of the material, raw or processed, that remains exposed in the substrate. However, presence mold(s) does not necessarily indicate toxin in the substrate, because not all the strains are toxigenic. Scientific reports suggest that in India, 26% of the total fungi possess toxigenic potentials, but their incidence is even higher in parts of the country facing prolonged and high climatic temperature with high relative humidity/rainfall and recurrent flood (discussed ahead).

4. Inland aquaculture mycotoxins:

It is often asked what a fish has to with molds contaminating the agriculture products? Possibilities of mycotoxin exposure to fish with special reference to aflatoxins have been chematically presented.

Recent technological applications making artificial feeding inevitable during aquaculture has increased the risk of mycotoxin exposure too fish manifold Almost all feed ingredients are potent substrates facilitating mold invasion and subsequent toxic metabolite production Numerous reports are available substantiating the fact Improper handling of these commodities, unsanitary storage and reckless application are sure to bring epidemic outbreaks in the ensuing days of aquaculture. However, the extend of damage will depend chiefly on the toxigenic potentials of the infesting strain of fungi. A strain having low toxigenic potentials may render a lot of crops having no adequately operating defense system of their own prone to secondary infections reflecting low productive potentials. Unfortunately, the immunosuppressive potentials of mycotoxins, particularly aflatoxin B have not yet attracted due scientific attention. But, if the status of present aquaculture practices in India is investigated thoroughly, the damage being caused by mycotoxins will come up on the surface. In fact, usually we practice 'annual-crop-harvesting'. This provides no time for characteristic chronic response of mycotoxins to be expressive and acute exposure in the field conditions is a rate phenomenon. But, the immunosuppressive potentials start affecting the crop administered with contaminated ration much earlier than do the carcinogenic influences. Investigation on this score demands due attention of the sponsoring agencies as well as an enthusiastic research approach of the workers in the field.

5. Fish feeds: Incident toxic fungi and their toxins:

Dearth of literature on this score makes the presentation rather incomplete in national context. However, some isolated reports on mycotoxin contamination of agricultural commodities and the investigation carried out by us indicate that the fungi belonging to Aspergillus, Penicillium, Rhizopus, Curvularia, Mucor and Alternaria are the most common invaders (Table 1). In particular, the genus Aspergillus followed by Penicillium appear as dominant mycoflora. During monsoon, however, Rhizopus and Mucor often overcast all other strains in routine cultures. The genera Aspergillus and Penicillium are well recognized as storage fungi, but there is now overwhelming evidence for pre-harvest invasion of the crops carried out by insects.

Records of fungal strains isolated from fish feed ingredients during different seasons (winter, summer and monsoon), their preference for the type of substrate as well as climatic condition, and per cent contamination of the samples screened (1774 in total).

Available data suggest that the germ hulls contained 90% of the toxin traced in the substrate. Similarly, when brown rice was milled, about 60-80% of the aflatoxins are separated with bran and polish About 85-90% of the aflatoxins present in the kernels is left In the cake processed in the expeller mill. The protein isolated from peanut contained 60-70% of the toxin originally present in the cake.

Our observations suggest that 51.1% of A. flavus, 30.8% of A. parasiticus and 50% of A. rubur isolated from fish feeds in parts of North Bihar were aflatoxigenic elaborating 2-100 mg/kg of toxin (aflatoxin B). Similarly, 42.8% of A. ochraceous and 37.5% of P. viridcatum elaborated ochratoxin-A in amounts ranging between trace and 50 mg/kg. Only 35% of A. versicolor bore capabilities of producing 8-10 mg/ kg sterigmatocystin.

6. Influence of nutritional and climatic factors:

Most of the aquaculture feeds and their ingredients have good nutritional profile to support growth of a wide range of toxic fungi. Besides, in case of oil cakes, the left-out amount of oil becomes an important factor affecting the rate of toxic metabolite production. Different forms of carbohydrates (glucose, mannose sucrose, fructose) favour aflatoxin production, as does glyceraldehydes. Peptone or certain amino acids, especially glycine and glutamate as nitrogen source, gives high yield of the toxin.

The climatic factors like temperature, relative humidity (RH) and water content (WC) of the substrate play vital role in accelerating the pace of mycobial spoilage. Feed ingredients under sale at retail shops are most vulnerable and reflect maximum per cent contamination.

High incidence of a particular mold during a period of the year indicates its fascination for the prevailing climatic factors. Screening of altogether 1774 samples (winter - 569; summer -596 and monsoon 609) indicated that monsoon months are most congenial for many of the toxigenic strains.

The climatic conditions in most parts of the country are characterized by extreme hot and cold, high RH added with widerspread flood RH during summer and winter is relatively low in comparison to monsoon. During summer, while temperature shows a favourable range for many of the toxic molds (Table 2) RH and WC are hardly supportive (Table 3). In contrast, during winter, the moister gained over by the substrates during preceding monsoon is sustained since low atmospheric temperature prevents evaporation. The monsoon with high temperature range and RH, thus provides a congenial period for the molds to invade and dominate over the substrates. During summer, only those substrates having elevated content or gaining moisture from any other source, and during winter heating of substrates in storage make them prone to mycobial spoilage.

Table 2: Range of relative humidity, water content of the substrate temperature favouring sporulation/growth of some toxic fungi.

Species	RH (%)	Water content (%)	Maximum and minimum temperature (°C)
A flavus	80	15-16	10-45
A parasiticus	82	14-15	1045
A niger	85	16-17	10-40
A versicolor	81	_	10-42
A rubur	70	12-13	10-45
A ochraceous	80-82	16	14-40
A nidulanse	84	15-16	10-42
A candidus	75	15-15.2	10-5555
Penicillium spp	82-86	15=16	0-35
Rhizopus spp.	90-92	17-18	30-36
Mucor spp.	88-99	17-18	30-60

We cannot purposely manipulate the climatic factors and so WC of the substrates becomes a critical factor that if monitored properly cannot prevent contamination, but can check the germination and growth of the fungal spores contaminating the substrates. Approximately 40-60-day old agricultural products kept under normal storage conditions contain WC variable between 5 and 12% (Table 3). Normally, 8.17% WC of the substrate corresponds to 59-74% RH. With further rise during winter (68-80%) and monsoon (up to 96%), there is an apparent average gain of 3.6 to 5.3% moisture content over summer (Table 3).

Different substrates exhibit variable capacity to gain water depending upon their physical condition viz, affinity towards water molecules, degree of saturation and drying character. The dried substrates gain humidity more rapidly than the others. At given temperature, for each concentration of water in the substrate, there is a corresponding RH representing the ease at which the substrate looses water to the atmosphere At low RH, water is bound to the substrate by a significant bonding energy, but as the relative humidity increases, the availability of water also increases the bonding becoming more and more feeble. It is this degree of mobility of water that makes it possible to grow on solid substrates.

Table 3: Water content of the substrate during winter, summer and monsoon in 50 g sample size.

Item	Winter			Summer		Monsoon		
	Dry weight (g)	Water content (%)	% gain	Dry weight (g)	Water content (%)	Water weight (g)	Dry weight (%)	% gain
MOC	43	14	+2	44	12.0	41	18	+6
WF	46	12	+16	46.8	10.4	44	16	+5.6
RB	44.5	15	+5	47	10.0	43.0	18	+8
SF	47	06	+3.5	47.5	5.0	44.5	11	+6
WB	42	16	+6	44.5	11.0	41.0	18	+7
GPM	41.5	17	+5	44	12.0	41.0	18	+6
RF	44.5	11	+4	46.5	7.0	44	12	+5
CR	46.0	8	+2	47	6.0	44.5	11.0	+5
Average gain over summer= 3.6%			Average = 8.17%			Average gain over summer = 5.3%		

7. Sensitivity of fish and pathological features :

The only mycotoxins tested thoroughly to register the sensitivity of inland fish species and pathological consequences are aflatoxins. Studies on ochratoxin-A and sterigmatocystin against air-breathing teleosts are underway.

The sensitivity of fish aflatoxins varies with age, sex, strain, route of exposure, physiological and genetical makeup of the host and nutrient factors. The present description includes the findings made on inland species. The air-breathing teleosts *(Channa punctatus, Heteropneusts fossilis and Clarias batrachus*) are fairly tolerant and capable of bearing high toxin load f 40 mg/kg b.w. of crude aflatoxin extracts administered intraperitonically. Major carp, *Catla catla*, is as sensitive as rainbow trout (*Salmo gairdner*). A few other aquaculture candidate species lime shrimp, finfish and *Oreochromis mossambicus* have also been tested, but the information available is scanty.

The characteristic clinical response in laboratory conditions includes weakness, loss in appetite, darkening of body colour and increased mucus over body surface in advanced condition. Gills indicating anaemia is thoroughly observed.

Anatomical inspection reveals multiple hemorrhages on liver, viscera and visceral fats. Gross swelling may involve spleen, head kidney and parts of gastrointestinal tract. The inflammatory reactions in liver are often associated with hyperemia in early stages. Such swelling may be located elsewhere depending upon the severity of the exposure. In C. catla, distended heart and thickening of pericardial sac besides congestion in liver, kidney and spleen may also be observed in advanced conditions, nodular outgrowths on the liver exhibit two general patterns.

- The characteristic light yellow, well vascularised. Protruding lesions that contrast sharply from surrounding normal liver tissue. It can even be detected when the outgrowths are no more than 1-2 mm in size, usual along the ventral edge of the anterior surface.

- A second form, more translucent, greyish-white and smooth in appearance that often develops into multicentric or large necrotic tumor.

Nodular outgrowths in primary stages present a variety of features. Multicentric, small, round or oval, gray tumors are frequently observed. Large single tumors are rather rare. Metastatic lesions on other internal organs (kidney and parts of intestine) and a condition simulating lymphocytic leukemia have also been reported.

8. Detoxification:

Following the prevalent handling and storage practices, mycotoxin contamination of food/feed appears to be inevitable. We will have to have preventive and safety measures. Out of the methods suggested for detoxification, only those having prospective values in relation to animal feeds have been emphasized.

I. Physical removal of infected part of the substrate by hand picking or colour sorting through electronic equipment is one of the most successful and suitable methods. Hand picking of peanut kernels on conveyer belts in some oil mills of southern India reduced aflatoxins to a great extent in oil mills.

II. Attempts to degrade aflatoxins by heat treatment have been only partially successful, since aflatoxins are resistant to thermal activation Methods employed include normal cooking to microwave treatment and autoclaving. This affected only moderate fluctuation, variable between 49 82% with a maximum of 95%. following microwave treatment.

III. Use of gamma radiation did not act effectively, but UV radiation gave promising results.

IV. Exposure to sunlight for more than 16 hours destroys 77-80% aflatoxins.

V. Use of moderately polar solvents in which aflatoxins are soluble, ammonia treatment process (at low concentration of 0.2-2.0, 50 psi pressure, and 80-120 C temperature for 30-60 minutes), hydrogen peroxide and ozone treatment are effective methods, but, they all alter the organoleptic and nutritional quality of the feeds.

VI. Use of certain microorganisms has also been attempted, but they may utilize the food for their own growth and elaborate some undesired metabolic wastes in the substrates.

VII. Aflatoxin-degrading enzymes responsible for conversion of aflatoxin-B, to aflatoxicol have been isolated. potentially purified and characterized These may find application as potential delexifying agent in future.

9. Control

The joint FAO/WHO/UNEP conference (1977) on mycotoxins has made wide recommendations with reference to aflatoxins. For aquaculture feeds, the following measures may effectively prevent mycobial spoilages.

I. Since most of the basic feed ingredients are agricultural products or their byproducts, use of resistant varieties of seeds for producing the crop should be encouraged.

II. Commodities before being used as feed ingredient must be thoroughly inspected for mycobial spoilages.

III. Harvesting and storage of the substrates under conditions of high humidity and moisture level should be avoided. Harvest drying should be as rapid as possible bringing water content level preferably less than 10%.

IV. Prolonged sun drying, in conditions of high humidity and rewetting of the substrates, in any way, may have deleterious effects.

V. Good warehousing facilities are essential for adequate storage. Storage structures should be maintained perfectly dry and entry of water either by leakage or seepage should be effectively prevented.

VI. Stacks of bagged materials should be arranged in such a way that it prevents upwards water mover available by any means, even in the form of vapour. Use of jute bags should be discouraged.

VII. Constant temperature and RH should be maintained through out the storage period. Measures to protect moisture migration and condensation resulting from thermal gradients must be taken into account.

VIII. Protective insecticidal measure should be used for prevention of insect pest invasion. Rodents, in particular, must effectively be tackled.

IX. General awareness among the farmers to produce and market fungi/toxin-free product and for purchaser, to buy a fresh product is essential. If the feed formulators and processors demand safe product, the farmer/the middleman will be forced to take necessary organized against mold spoilage.

10. Conclusion:

Mycotoxin contamination of food and feeds is a recurring phenomenon, but attitude towards it has been far from consistent. Dr. Leo A. Goldblatt pointed out: "Mycotoxin constitutes serious and ever present environmental health hazard. It cannot be eliminated from our food supply. We must, as such, learn to live with them".

In India. when feed-dependent aquaculture system is destine to overcast all traditional practices, efforts to make the feed supply free from factors bringing devastating endpoints are really imperative. There are five ways in which we may tackle the problem.

I. A thorough organized survey of different geographical regions of the country to identify the toxic fungal strains, assessment of their toxic potentials and subsequent toxic manifestations.
II. Measures to prevent fungal growth on the substances
III. Removal or detoxification of the toxic metabolites after elaboration on the substrates.
IV. Creating general awareness among the people concerned with aquaculture practice.
V. Establishment of aquaculture feed quarantine.

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