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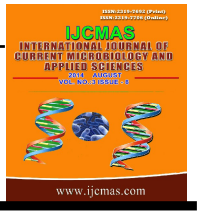

Original Research Article

Isolation, Characterization and Growth Optimization of Toxicogenic Molds from Different Animal Feeds in Tamilnadu

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A B S T R A C T

Samples of animal feeds, cereals and grains were collected from farms and retail shops in the intense farming areas of Tamil Nadu to assess their quality from the mycological perspective. Fifty one samples were analyzed for the presence of toxicogenic fungi by conventional mycological identification methods. The investigation revealed the presence of *Aspergillus* species as a predominant fungal contaminant followed by *Penicillium* and *Fusarium* species. Nearly 83% of feed samples tested were infected with *Aspergillus* species. Comparatively, the poultry feed seems to be more predominant animal feed that are infected with such mycotoxicogenic fungi. Among the *Aspergillus* species, *A. flavus* was the most predominant fungal species contaminating animal feed samples. Some of the feed samples tested were contaminated with more than one fungal species. Since favorable temperature and moisture are crucial for the growth of mycotoxicogenic fungi and subsequent production of mycotoxins, growth optimization studies were carried out for the fungal species isolated under various physical parameters like temperature, moisture and pH. The results revealed 28-30°C as an ideal temperature range for the optimal growth of *Aspergillus* species of fungi. Out of 51 feed samples tested for the moisture content poultry feed samples showed highest moisture level (114.3g/kg), similarly their mycological load was also estimated to be high. With respect to pH, the optimal range for fungal growth was observed at 4-4.5 for *A.flavus* and *A.fumigatus* and 7-7.5 for *A.niger*. Presence of mycotoxicogenic fungi in the animal feeds increases the risk of mycotoxin food poison for animals and in turn for human beings. Quality animal feed production will largely rely on the using of fungal free agricultural commodities and maintaining proper storage conditions of the agricultural produce and formulated animal feed.

Keywords

Fungi, *Aspergillus*, mycotoxins, animal feed, temperature, moisture.

Introduction

Animal feed plays an important role in the production of quality milk, egg and meat for human consumption. If the quality of feed is reduced both animal and human health will be affected. Various agricultural products are used as a primary raw material for the production of animal feed that are fed to cattle, poultry animals and pig. The most

commonly used agricultural product for the production of animal feed includes maize, ground nut, gingelly, soya, sorghum, wheat, barley etc., These agricultural products are exposed to various toxicogenic molds both at harvest and storage. While preparing animal feed from fungal infected agricultural produce, there is a high risk of feed contamination. While feeding the livestock with such contaminated feed the health of animals will be affected and in turn will also affect the human health. The fungal growth reduced nutritional value and could result in the production of mycotoxins (Frisvad et al., 2006) and allergenic spores (Adhikari et al., 2004) that constitute a risk factor for human and animal health. Fungi are the major contaminants of food and can cause rapid quality deterioration Sibonda et al., (1997) and Boysen et al., (2000). Filamentous fungi are able to grow on all types of food especially on a wide variety of agricultural products worldwide. Contamination of feed stuff can occur during handling, storage, processing and transport. Approximately19 genera of fungal species, among them *Fusarium* spp., Aspergillus spp., *Penicillum* spp. and *Stenocarpella* spp., affect maize (William and McDonald, 1983; Payne, 1999). Development of toxicogenic molds in the animal feed is favoured by factors such as condensation, heating, leakage of rain water, insect infection. Such toxicogenic species include *Aspergillus fumigates, Aspergillus parasiticus, Aspergillus flavus, Fusarium verticillioides, Monascus rubes, Penicillium roqueforti* and *Trichoderma viride* (Cleveland et al., 2003; Garon et al., 2006). Under certain environmental conditions, such as high temperature and high rainfall, maize grain is infected by ear rot fungi which produce mycotoxins (Bilgrami andChoudhary, 1998; Miller, 2001; Logrieco et al., 2002; Fandohan et al.,2005), Due to moldy growth on agricultural products results in the change in the texture, smell and taste of the infected foodstuff. This is because of the excretion of enzymes and volatile compounds by the fungus. In addition to these, filamentous fungi also produce certain toxic secondary metabolites called mycotoxins. A number of these fungal compounds are endowed with toxic effects towards animals and human beings and are accorded the collective term mycotoxins. Many species of *Fusarium, Aspergillus, Penicillium* and *Alternaria* are not only recognized plant pathogens but are also sources of the important mycotoxins of concern in animal and human health (Abramson, 1997; D'Mello et al., 1997; Panigrahi, 1997; Smith, 1997). These mycotoxins contaminate agricultural staples along the food production chain from field, harvest and transport to storage (Cast 2003). Mycotoxins can cause serious health implications for animals and can contaminate food of animal origin and endanger human health. The development of modern agricultural methods, together with large scale processing, has exacerbated the problem, resulting in outbreak of acute diseases termed mycotoxicosis (Newberne, 1974). These compounds can enter the food chain mainly by ingestion through the dietary channel of humans and animals (Aycicek, Aksoy & Saygi, 2005). The significant health risk posed in the consumption of mycotoxins contaminated food products by both animal and man is of utmost concern (Danike, 2002; D mello, 2003) in many developing countries. These secondary metabolites from fungi were also known to be carcinogenic (aflatoxin B1), nephrotoxic (citrinin) and estrogenic (zearalenone) (Bennett and Klich, 2003). Experimental and epidemiological evidence suggest that certain mycotoxins are associated with different forms of human cancer (WHO, 1993; Fink-Gremmels, 1996).

In general, only little is known about the incidence of Fungal contamination and presence of mycotoxins including Aflatoxin and *Fusarium* toxins in commodities such as poultry feed mixtures, cattle feed and cereal grains used to feed the livestock in Tamilnadu, animal feed samples and cereal grains were collected from intense farming areas of Namakkal and Coimbatore in the present investigation. The present study also constitutes a survey to obtain data for moisture, total mold count as well as mycotoxins of feed collected from farms, cereals from retail shops and feed manufacturing industry throughout Tamilnadu. As the various growth factors like moisture, temperature and PH are critical for the growth of mold and subsequent production of toxins, growth optimization studies were also carried out.

Materials and Methods

Sample collection

A total of 51 samples of feed mixtures, designed for poultry feeding, cattle feeding and pet food were obtained from different parts of farming areas in Tamilnadu during the period of September 2010 to April 2011. The poultry feed samples collected includes chicken broilers-starting, chicken broilers-growing and chicken broilers-finishing.

Cattle feed such as rice straw, oil seed cakes and commercial formulations of cattle feed were collected. Feed ingredients used to feed poultry and cattle such as maize, ragi, sorghum and wheat were also collected from local providers and retail shops for microbial analysis. These samples were collected in plastic bottles and were immediately stored at -20^0C until analysis.

Moisture content of feed samples collected

The moisture content of feed samples collected was determined according to the method by Silva (1991). Samples were previously weighed and dried at 105°C until they reached a constant weight. Moisture content of each sample was determined by the difference between dry weight and initial weight.

Mycological analysis

The cereals taken for the mycological analysis in the present investigation had no preservatives or additives that might interfere with fungal growth. Forages were cut in pieces of about 2 cm long and cereals were coarsely ground. Feeds prepared in this way were weighed into 100mL Erlenmeyer flasks, 5 g for forages and 10 g for cereals, followed by addition of equivalent amount of distilled water. Flasks were incubated in static culture at 37°C for up to 2 weeks and examined daily for fungal growth under binocular loupe. At day 6 and 14 of the incubation period distilled water was added to triplicate flasks to maintain the moisture content.

Cultivation of mold and identification

After observing the fungal growth in the flasks, the mycelia, conidia and spores were gently removed and fresh inoculations were done on the Potato Dextrose medium plates. Then the plates were incubated at 37°C for 3 days. By observing the inoculated plates under a Quebec chamber the colony morphology was noted with respect to colour, mycelium size and nature of colony on reverse side and lateral sides of the plates. Species identifications were done by following the standard taxonomic procedures. Tease mount preparation was also done for further identification of fungal species. For this study, materials like Lacto phenol cotton blue stain. A drop of Lactophenol cotton blue was placed on a

clean glass microscopic slide. With a straight wire slightly bent at the tip, a portion of the colony was removed and placed in a drop of Lactophenol cotton blue, covered with a coverslip and gentle pressure was applied to spread evenly. Then, it was examined microscopically giving sufficient time for the structure to take up the stain. The fungi isolated from animal feed were maintained on Potato Dextrose Agar. The fungus was routinely sub-cultured on Potato Dextrose agar slants for every month.

Growth optimization of fungal isolates

Growth regulating parameters like temperature, pH, and moisture were considered to study the optimal conditions for the growth fungi isolated from feed samples. For temperature optimization study, potato dextrose broth was prepared and sterilized by autoclave method along with chloramphenicol. A loop full of fungal cultures like *A. niger, A. flavus* and *A. fumigatus* were added into separate broth and maintained at five different temperatures (28 , 30, 32, 34 and 37). All the tubes were incubated for 24-48 hr to observe the increased growth of fungal isolates at a particular temperature. To study the effect of different pH on the growth pattern of fungal isolates, potato dextrose broth was prepared and sterilized as in the previous study. pH of the growth medium was adjusted by adding HCl for low pH and NaOH for high pH. Five different pH (4-5, 5.5-6.0 6.0-6.5, 6.5-7.0, 7-7.5) ranges were taken for studying the growth of fungi. After inoculating the fungal cultures the test tubes were incubated for 24-48 hr and observed for the increased growth of fungal isolates at a particular pH. For determining the optimal moisture content for the growth of fungi isolated from feed samples, test tubes containing the culture broth were maintained at particular moisture content throughout the incubation period. The moisture content was regulated by adding de ionized distilled water to the growth medium. Five different moisture percentage (5,7,9,11,13,) ranges were taken for the present investigation. After inoculating the fungal cultures, the test tubes were incubated for 24-48 h and observed for the increased growth of fungal isolates at particular moisture content. For biomass calculations in all the growth optimization studies, initial weight of the culture medium was noted. After the period of incubation, the culture test tubes were centrifuged at 1000 rpm and the supernatant was discarded. Then the pellet was transferred in to a pre-weighed Whatmann filter paper and dried overnight at 60^0C and weighed for determination of fungal biomass.

Results and Discussion

In the present study, we have collected 51 feed samples including poultry feed, cattle feed and common feed ingredients used in the production of animal feed. List of feed samples used in the study are presented in table 1. Initially all the feed samples were tested for moisture content. The average values for moisture were higher for poultry feed formulations and lower for the cereal grains (Table 2).

Initially all the feed samples were tested for moisture content. The moisture content of each feed sample was calculated by heating the feed sample up to 120^0C and until constant weight is reached. The difference between the initial weight and the final weight of the feed sample is calculated as the moisture content and expressed in g/kg of the feed sample. The average values for moisture were higher for poultry feed formulations followed by peanut cake, cattle feed and maize grains. Other feed ingredients showed lower moisture contents. The results are presented in Table 2.

To find out the mycological contamination of the feed samples, each sample was prepared as described in the materials and methods and observed for the fungal growth. Each sample was maintained in triplicate plates. The results of this study showed that, at the end of 14th day of incubation period, most of the feed samples were infected with moldy growth. The infection percentage of feed samples is presented in table 3. Fungal infection percentage was found to be higher in poultry feed compounds followed by cattle feed, peanut cake, cotton seed, maize, sorghum and wheat. The variation in the percentage of fungal infection corresponds with the composition of the feed Substrate that provides the ideal conditions for fungal development.

The results of the fungal identification studies are presented in table 4. The results show that, all of the feed samples are infected with *Aspergillus, Fusarium* and *Penicilium* species of fungi. Growth, morphological characteristics and colony colorations were considered for the fungal identification. *Aspergillus flavus,* was identified by its mat like surface of pale yellow to intense yellow green colour of the colony. It has a conidiophore of variable length, coarsely roughened, pitted and spiny structure. Sterigmata are single and double covering the entire vesicle and they point out in all directions. *Aspergillus niger* was identified by its colony characteristics which are woolly at first, white to yellow, then turning brown to black and the conidiophores are of variable length, pitted and spiny and the sterigmata are double covered vesicle which radiate from head. A*spergillus fumigates* was identified by its flat white colony which developed in 3 -6 days, later turned to bluish green to gray and the conidiophore were long with club shaped vesicle and a single row of phialids giving rise to long chain of ovoid conidia were observed. *Fusarium sp.*, was identified by its white and velvety colonies, which on maturation turned woolly to cottony with variation of colours mostly from pink to violet and the conidia was found to be pointed, sickled and septate macroconidia. *Penicillium puberulum* was identified by its initial fluffy and white colour which later turned to green or bluish green in colour and the conidiophore is long with branching phialids.

Fungal species identification studies show that, most of the feed sample were infected with Aspergillus sp. of fungi when compared to the other two species of fungi(*Fusarium* and *Penicilium*). Of the *Aspergillus* species, *A.flavus* is found to be the predominant fungal species that inhabits most of the feed samples (10/12), followed by *A.niger* (5/12) and *A.fumigatus* (3/12). *Penicilium* species is found to be the next to *Aspergillus* in colonizing the feed samples (6/12) followed by the *Fusarium* sp. (1/12). Further investigations in the present study are aimed at the *Aspergillus* species of fungi as they seem to be the predominant fungal colonies that inhabit nearly 83% of animal feed samples.

Growth optimization studies were carried out for all the three species of the *Aspergillus* to optimize the ideal conditions (temperature, pH and moisture) for the growth of fungal species. Five different temperatures were taken to observe the ideal temperature for the growth of fungi. The results of the effect of temperature on the fungal biomass are presented in table 5. The results of the present study show that, at the temperature range of 28-30^{0}C all the three Aspergillus species were found to show increased growth rate when compared to other temperatures.

Similarly five different pH ranges were taken to optimize the ideal pH range for the growth of fungi. The results of the effect of

pH on the fungal biomass are presented in table 6. The results of the present study show that, at the pH range of 4.0-5.0 the two species of *A.flavus* and *A. fumigatus* were found to show increased growth rate when compared to other pH ranges, whereas *A.niger* showed highest growth activity at the pH range of 7.0-7.5.

Filamentous fungi are abundant in nature that contaminate almost all kinds of food and feed commodities meant for human and animal consumption. Contamination of agricultural commodities with toxicogenic molds is known to occur both at pre harvest and post-harvest conditions. Once the crop becomes infected with fungal pathogens at field conditions, the fungal growth continues with increasing vigor at post-harvest and storage. The results of the present study also show that the animal feed and feed ingredients collected from different parts of farming areas of Tamilnadu are infected with different species of toxicogenic fungi especially, the Aspergillus species. Nearly 83% of feed samples tested were infected with Aspergillus species. Comparatively, the poultry feed seems to be more predominant animal feed that are infected with such mycotoxicogenic fungi causing the possibility of mycotoxin contamination in the infected feed formulations. In addition to the threat of mycotoxin in the fungi infected feed, there will be a remarkable rapid quality deterioration.

Next to the *Aspergillus*, *Penicilium* sp. are found to infect the stored grains and compounded animal feeds of cattle and poultry (chiken), nearly 50% of the total feed samples tested. *Fusarium* sp. is known to infect a very few feed samples (16%) especially the rice straw. This result of the present investigation corresponds to the findings of Pitt and Hocking(1997) who reported *Fusarium* sp. mainly as field fungi. A large number of fungi are associated with grain mouldiness, but the most common are *A. flavus, A. parasiticus, F. graminearum, F. verticillioides, Penicillium spp*. and *Diplodia maydis* (Kpodo et al., 2000; Gonza´ slez, et al.,2003). *A.flavus* is considered to be mainly a storage fungi and its presence in the animal feed could lead to faster deterioration. Further, the capability of *A. flavus* strain in producing B-type AFs (AFB1 and AFB2) was reported by CAST (2003) and Somashekar et al. (2004). *A. fumigatus* is reported to be a saprophytic fungi (Cole et al., 1977; Pelhate, 1987; Schneweis et al., 2001; Smith and Lynch, 1973) contaminating silage and hay. This fungus is the main causal agent of invasive aspergillosis in humans and animals (Latge, 2001). The spores of A. fumigates are easily spread in the air making its exposure risk high for human and animals. Maize is one of the richest substrates for fungal contamination and aflatoxin production when compared to other cereal crops like wheat, barley, oat and sorghum. *A. flavus* is the major source of primary inoculums in maize fields (Scheidegger and Payne, 2003). Since maize is being used as one of the major feed ingredient in poultry feed formulations it is being infested with *Aspergillus* sp. of fungi.

In some of the feed samples tested for the toxicogenic fungi showed the presence of more than one species fungi especially poultry feed and cattle feed. Such incidence of co-contamination, particularly among toxicogenic members could aggravate the problem of feed spoilage. Rizzo et al., 2004 have reported such co-contamination in maize from Cameroon. This co-contamination of toxicogenic fungi in the animal feed increases the risk of production of multiple mycotoxins. Adverse climate conditions during harvesting and storage of the forage and cereals may be the cause for

fungal contamination. Tropical conditions such as high temperatures and moisture, monsoons, unseasonal rains during harvest and flash floods lead to fungal proliferation and production of mycotoxins (Bhat and Vasanthi, 2003).

Climate represents the key agro-ecosystem driving force of fungal colonization and mycotoxin production (Magan, Hope, Cairns, & Aldred, 2003). The most important parameters in the fungal growth and mycotoxin production are moisture and temperature. Storage temperatures between 25°C and 30 °C and a relative humidity of 97% favour the production of toxins during storage (Otniel Freitas-Silva and Armando Venâncio, 2011). As most of the feed samples tested in the present study were infected with *Aspergillus* sp. of fungi (10/12), their growth kinetics was studied with respect to temperature, moisture and pH. Animal feeds tested for moisture in the present investigation showed highest level for poultry feed samples (114.3g/kg) and lowest for ragi (73.5g/kg). These moisture conditions provided an ideal condition for the growth of fungi, especially in poultry feed samples. Aspergillus species develop when conditions such as temperature and humidity/water activity favour their proliferation. In the case of A.*parasiticus***,** temperatures of 25-30°C are optimal for maximising aflatoxin production (J.P.F. D'Mello et al., 1997). In the present study also *A. flavus, A. fumigatus* and *A. niger* were observed to show their highest growth activity between the temperature range of 28-30°C. Crops grown in warm climates have greater likelihood of infection by aflatoxin producers and in some regions, infection only occurs when temperatures rise in association with drought (Sanders, Blankenship, Cole, & Hill, 1984; Schmitt & Hurburgh, 1989). The agro- ecosystem in which the agricultural crops are raised in India and in particular at Tamilnadu provides an ideal condition for the infection by aflatoxin producers. Cereals and grains produced in such climatic conditions are being used as primary raw materials for the manufacture of animal feed make it susceptible for toxicogenic fungal growth. The same has reflected in the results of the present investigations where most of the feed samples are infected with aflotoxin producers. Like temperature and moisture, pH is also a limiting factor for the growth and toxin production for fungi. Results of the present study indicate that *A.flavus* and *A. fumigatus* showed highest growth activity at low pH (4.0-5.0) in contrary to the *A. niger*, which had highest growth activity at pH range of 7.0-7.5. A report of similar kind has been shown by María L et al., 2010 in which the pH of fruits (<2.5 to 5.0) is tolerable for many fungal species. The mycotoxins most commonly present in fruits and fruit products are aflotoxins, ochratoxin A, patulin and *Alternaria* toxins (Drusch S et al., 2003. MossMO et al., 2008 and Barkai-Golan R et al 2008).

The results obtained in the present study showed high degree of fungal contamination of animal feed and feed ingredients. This renders them unfit for animal consumption and significantly lowers the value of grains as an animal feed and as an export commodity. Presence of more than one genera and species of fungi in the animal feeds tested increases the risk of multiple mycotoxins. In order to assure the quality of food and feeds used for human and animal consumption, feed ingredients like cereals and grains have to be checked for the presence of toxicogenic molds and mycotoxins. Since fungal contamination of animal feed and feed ingredients is known to occur both at pre and post-harvest stages, care has to be taken to avoid fungal contamination by adopting good agricultural

practices and sanitary conditions at storage. For minimizing the fungal infestation in the agricultural commodities at pre-harvest stage, proper planning of planting date of the crop, good irrigation practices, and pest management have to be followed and in fact these practices have considerably reduced the problem of fungal infestation and subsequent production of mycotoxins. Munkvold, 2003 and Champeil et al., 2004 have also reported that good cultural practices at agriculture have limiting effects on infection and subsequent mycotoxins accumulation. Fungal infestation at post-harvest stage largely relies on proper storing of them for future usage. Storage of agricultural commodities and animal feed formulations for longer period of time poses a great threat of fungal infestation and remains as a big challenge in the developed and developing countries. Improper grain storage and processing conditions have favored the development of storage and contaminant fungi. Imposing proper storage conditions of cereals, grains and formulated animal feed will largely help to overcome the problem of fungal infestation at storage. Multivariate statistics can be used to examine the interactions between factors to assist safe storage (Wallace & Sinha, 1981). Since moisture content of cereals and grains play an important role in fungal infestation, proper drying of agricultural commodities before storage is important. Rapid drying of agricultural products to low moisture level is critical as it creates less favorable conditions for fungal growth and proliferation.

Hamiton (2000) reported that drying harvested maize to 15.5% moisture content or lower within 24–48 h would reduce the risk of fungal growth and consequent aflatoxin production. Awuah and Ellis (2002) demonstrated that when groundnuts were dried to 6.6% moisture level, they were free of fungi regardless of the local storage protectant used for 6 months. Storage go-downs have to be properly checked for water leakage, intruding of rats and insect pest infestation that could create an ideal condition for fungal growth by increasing the moisture content in the agricultural commodities and feed materials. Avantaggio et al. (2002) found that insect damage of maize is good predictor of *Fusarium* mycotoxins contamination. Biological activity of these insects and mammalian pests can increase moisture content through condensation of moisture resulting from respiration, low temperatures and inert atmospheres (Lanyasunya et al., 2005; Turner et al., 2005), thereby creating an ideal condition for the development of toxicogenic moulds.

Climate and other growth-related factors that influence the growth of toxicogenic molds in the animal feed provide an ideal condition for the release of secondary metabolites by these fungi. Once these secondary metabolites called mycotoxins are formed in the animal feed they are inevitably consumed by the farm animals leading to serious risks for animal health. The amount of toxin produced by some strains is enough to pose a potential animal health problem if found in feeds (Morgavi et al., 2004; Upperman et al., 2003;Watanabe et al., 2003). Human exposure to mycotoxins may result from consumption of mycotoxin contaminated animal products such as meat, milk and eggs. Bennett and Klich, 2003 have also reported Human food contamination with mycotoxins at various stages in the food chain. It is important to mention that experimental exposure of rat to mycotoxins like AFB 1 and AFB 2 can induce profound and persistent histopathological damages, including apoptotic and karyomegalic or mitotic changes in the nuclei of tubular epithelium in kidneys of rats as well as DNA-adducts formation (Miljkovic et al., 2003).

Table.1 List of feed and feed ingredients used in the study

S.No	Name of the sample	No. of samples
1.	Poultry feed- Starter	5
2.	Poultry feed- middle	5
3.	Poultry feed- finisher	5
4.	Cattle feed	4
5.	Gingelly Oil seed cakes	4
6.	Pea nut cake	4
7.	Maize	4
8.	wheat	4
9.	sorghum	4
10.	Rice straw	4
11.	Ragi	4
12.	Cotton seed	4

Table.2 Mean values of moisture of feed samples

S. No	Name of the sample	Number of samples	Moisture g/kg mean ±SD
1.	Poultry feed starter	5	114.3±16.5
2.	Poultry feed middle	5	110.6±9.6
3.	Poultry feed finisher	5	108.4±5.9
4.	Cattle feed	4	101.8±3.8
5.	Gingelly oil seed cake	4	99.4±6.9
6.	Pea nut cake	4	103.4±6.3
7.	Cotton seed	4	99.3±4.8
8.	maize	4	102.3±2.4
9.	wheat	4	85.5±9.7
10.	sorghum	4	87.3±6.3
11.	Rice straw	4	45.3±2.3
12.	Ragi	4	73.5±4.9

Fig.1 Mean values of moisture of feed samples

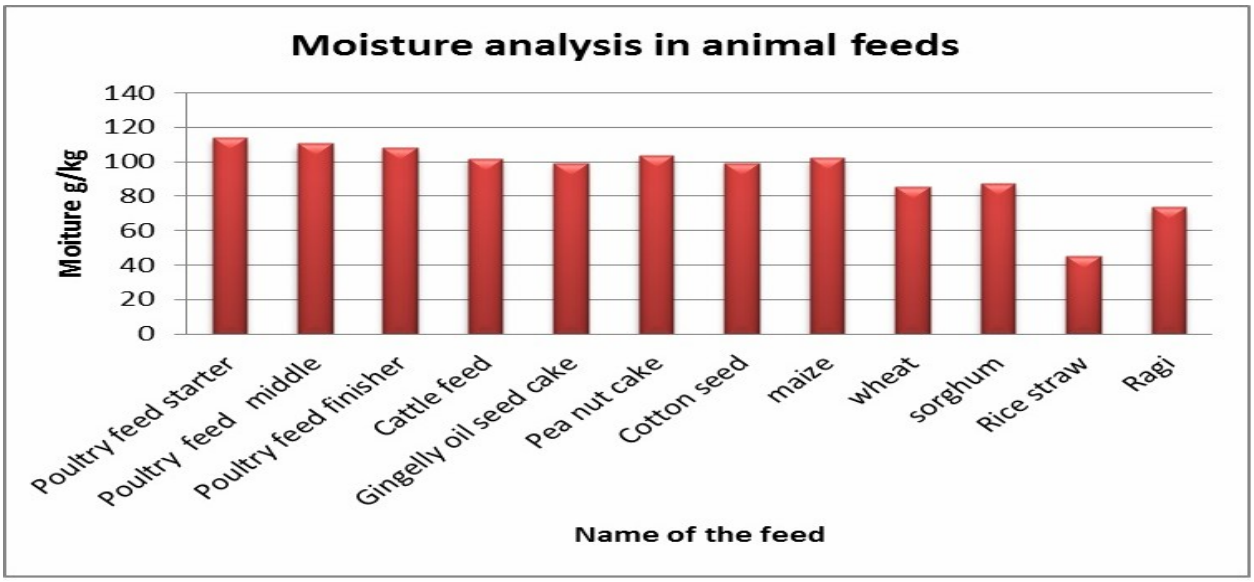


Table.3 Percentage of fungal infection in the feed samples

S. No	Name of the sample	Number of samples	Number of samples plated	Number of plates infected	% of infection
1.	Poultry feed starter	5	15	15	100
2.	Poultry feed middle	5	15	14	93
3.	Poultry feed finisher	5	15	14	93
4.	Cattle feed	4	12	10	83
5.	Gingelly oil seed cake	4	12	8	66
6.	Pea nut cake	4	12	8	66
7.	Cotton seed	4	12	8	66
8.	Maize	4	12	7	58
9.	Wheat	4	12	7	58
10.	Sorghum	4	12	7	58
11.	Rice straw	4	12	6	50
12.	Ragi	4	12	6	50

Fig.2 Percentage of fungal infection in the feed samples

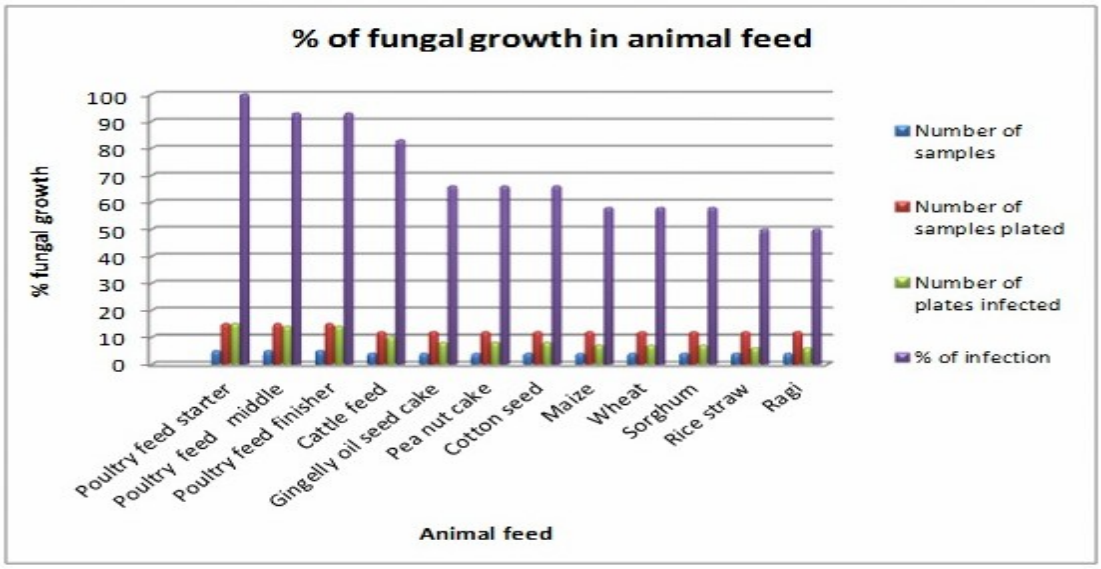


Table.4 Different fungal species identified in feed samples.

	Name of the sample	sp. identified
	Poultry Feed Starter	*r, A. flavus*
	Poultry Feed Middle	*atus, A.flavus*
	Poultry Feed Finisher	*s, P.puberulum*
	Cattle Feed	*m sp., A.flavus*
	Gingelly Oil Seed Cake	*r, A. flavus*
	Peanut Cake	*m sp.,A.flavus*
	Cotton Seed	*m sp.,A.flavus*
	Maize	*r, A. flavus*
	Wheat	*r, A. flavus*
	Sorghum	*m sp., A.fumigatus, A.niger*
	Rice Straw	*m sp A. fumigatus*
	Ragi	*m sp.,A.flavus*

Table.5 Effect of pH on fungal biomass.

S.No	Organisms	pH ranges				
		4.0-5.0	5.5-6.0	6.0-6.5	6.5-7.0	7.0-7.5
1	***A.niger***	0.26 ± 0.00^{a}	0.32 ± 0.01^{a}	0.32 ± 0.00^{a}	0.38 ± 0.00^{a}	0.44 ± 0.00^{a}
2	***A.flavus***	0.48 ± 0.00^{b}	0.47 ± 0.00^{b}	0.36 ± 0.00^{b}	0.36 ± 0.00^{b}	0.46 ± 0.00^{b}
3	***A.fumigatus***	0.41 ± 0.00^{c}	0.33 ± 0.00^{a}	0.39 ± 0.00^{c}	0.34 ± 0.00^{c}	0.37 ± 0.00^{c}

Fig.3 Effect of pH on fungal biomass

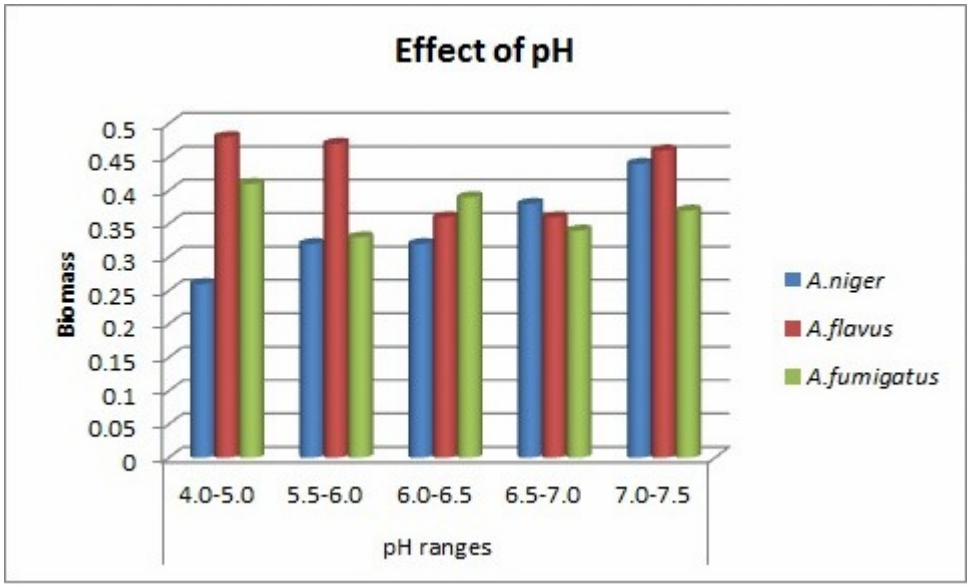
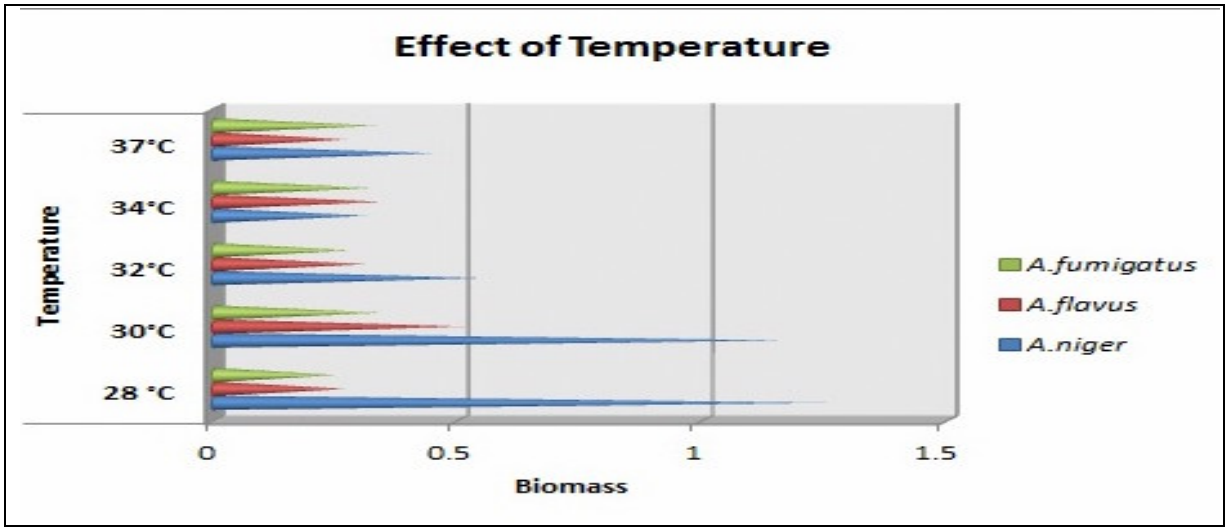


Table.6 Effect of temperature on fungal biomass

S.No	Organisms	Temperature				
		28 °C	**30°C**	**32°C**	**34°C**	**37°C**
1	*A.niger*	1.25±0.00[a]	1.17±0.01[a]	0.55±0.00[a]	0.31±0.00[a]	0.45±0.00[a]
2	*A.flavus*	0.27±0.0[b]	0.51±0.00[b]	0.31±0.01[b]	0.34±0.00[b]	0.27±0.00[b]
3	*A.fumigatus*	0.25±0.00[c]	0.34±0.00[c]	0.28±0.12[c]	0.32±0.01[c]	0.33±0.00[c]

Fig.4 Effect of temperature on fungal biomass



Fungus was reported to be a frequent contaminant in Bulgarian feeds, suspected of causing spontaneous porcine and chicken nephropathy (Mantle and McHugh, 1993; Stoev et al., 1998; Stoev et al., 2002).

The results obtained in the present investigation show the high levels of multiple fungal infestations in the maize containing animal feed samples. So, there could be a possibility for the presence of the secondary metabolites called mycotoxins of the fungi in these animal feeds. Care has to be taken to check these animal feeds for the presence of mycotoxins otherwise they could be consumed by the farm animals creating health implications in live stocks. Through the food chain these mycotoxins can enter human beings and can cause various toxic effects leading to immunosuppressive, genotoxic, cytotoxic, and apoptotic effects (Nieminen et al., 2002; Upperman et al., 2003;Waring et al., 1988).

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