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PRELIMINARY STUDIES ON THE OCCURRENCE OF MYCOTIC AND MYCOTOXIN CONTAMINATION IN AQUACULTURE FEEDS USED IN SAUDI ARABIA

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ABSTRACT

The present study was carried out on three kinds of aqua feed used for feeding of marine fishes and shrimp in Fish Farming Center (FFC), Jeddah, Saudi Arabia namely; Grower seabass, Grower tilapia and Grower shrimp. Random feed samples were collected at regular intervals every two weeks for 4 months from the feed storage room. Total mycotic counts, identification of moulds, concentration of aflatoxin and ochratoxin as well as proximate analyses of feeds were determined and the correlation between them was estimated. Moreover, the storage conditions were monitored all over the period of experiment. The mycological investigation revealed that the grower shrimp diet was more contaminated than the other two types of feeds, where the total mycotic counts reached to 3.666×10^7 cfu/gm after 4 months, while the total mycotic counts reached to 2.867×10^4 cfu/gm and 8.3×10^5 cfu/gm in grower seabass and grower tilapia diets respectively. *Aspergillus* species were dominant in all kinds of aquafeeds tested in addition to *Mucoraceace* fungi in grower seabass feed. The identified *Aspergillus* sp. were classified into *A. flavus*, *A. fumigatus*, *A. ochraceaus*, *A. niger* and *A. terreus*. *A. flavus* was predominant in all types of feed in addition to *A. fumigatus* in grower shrimp, while *A. ochraceaus* was recorded by 1, 6 and 7 isolates in grower seabass, grower tilapia and grower shrimp feeds respectively. Aflatoxin and Ochratoxin were increased in concentration by increasing the period of storage which reached 1.91 ±0.11 and 2.89 ±0.16, 79.0 ±4.7 and 24.60 ±3.21, and 98.55 ±5.20, 66.50 ±5.65 ppb in grower seabass, grower tilapia and grower shrimp respectively. The proximate analysis of the ratios revealed that the nutritive value decreased after 30 days in grower shrimp diet and after 48 days in grower tilapia

diet, while the nutritive value of grower seabass diet was not affected during this investigation. It is worthy; to mention that the storage conditions were more than the suitable levels, even the temperature or humidity as well as moisture contents of the diets. So, we concluded that the aquafeeds should be stored in good and define conditions as well as for a specific period to prevent mycotic contamination and if we need to store them for long period, fungal and its metabolites inhibitors "antifungal and antimycotoxin" should be added.

INTRODUCTION

The aquaculture industry in the kingdom of Saudi Arabia has its success story since 1978. The improvements in aquaculture have played a major role in replacing seafood imports and enhancing export opportunities. Unfortunately, aquaculture industry all around the world is facing serious problems such as environmental and nutritional ones, which ends up huge economic losses. Aquaculture feeds, feed quality and source, besides growth and feed conversion rate (FCR) are considered the main points of view when justice on any project of fish culture. So, the appearance of the problem of increased FCR in the Fish Farming Center go ahead for evaluation of the feed used for feeding of cultured fish and shrimp during growth stage. The evaluation includes different lines such as total mycotic count, identification of moulds, detection of prevalence mycotoxins, proximate analysis feeds and monitoring of storage conditions.

Mold grow over a temperature range of 10-40 °C, pH range of 4-8 and humidity levels greater than 62% as well as more than 12-13% moisture, while yeasts require free water (Lacey, 1991). Mold growth and mycotoxin production are related to weather extremes, to inadequate storage practices causing low feedstuff quality and faulty feeding conditions (Doerr *et al.*, 1982).

Generalized *Aspergillus* and *Penicillium* genera are considered as the most important pathogenic molds even on livestock including fish and feed ingredients (Refai, 1987). However, both genera will develop and produce toxins at much lower temperatures as well as slightly higher ones, fusarium, on the other hand, is considered a "cool" mold growing optimally at temperature more moderate than *Aspergillus* (Frey *et al.*, 1979).

An important first consideration of mycotoxins is the concept of number and diversity of its secondary metabolites. Over 350 mycotoxins are known to be affected on feedstuff (Doerr, 1994). Aflatoxins are a family of extremely toxic, mutagenic and carcinogenic compounds produced by *A flavus*. Toxigenic *A flavus* isolates produced four major aflatoxins B_1, B_2, G_1 and G_2 (Cotty *et al.*, 1994). Ochratoxin A (OTA), is produced by fungi such as *A.ochraceous* and some *penicillium* fish feeds (Palli *et al.*, 1999).

Under certain condition, the moldy contamination of the feeds deals with changes in nutrient profile of the moldy grains. When the mold numbers reach and exceed the level of 10^6 colony forming units/gram, as much as 5% reduction in the total energy, and 7% reduction in protein may occur. Of interest was the loss of crude fat. At mold levels of 1-5 x 10^6 cfu/g, suggestion of discounting that grain value by as much as 5-10 % (Doerr, 1994).

Mold growth and mycotoxin production are related to weather extremes, to inadequate storage practices causing low feedstuff quality and to faulty feeding conditions (Doerr *et al.*, 1982).

Because feedstuffs can be contaminated pre-harvest, control of additional mold growth and mycotoxin formation is dependent on storage management (Coulumbe, 1993). In wet feed such as silage, higher moisture levels allow mold growth, if oxygen is available.

Economic losses from mycotoxicosis are reported, such as in fish from chronic infection as well as increasing feed conversion ratios and mortality among fish (Fuchs *et al.*, 1986; El-Shaboury, 1998). Sometimes mycotoxins occur at concentrations high enough to cause major losses in health and performance of fish.

Therefore, the present work was carried out to study the effect of mold growth and mycotoxins produced on feed quality of different kinds of aquafeeds of cultured marine fish and shrimp stored in uncontrolled temperature and humidity for long period (4 months) in the Fish Farm Center.

MATERIAL AND METHODS

Investigation included eighty one feed samples of three kinds of diets from feed store room in the Fish Farming Center used for feeding of cultured marine fish (27 sample /each). The aquafeeds were manufactured locally by the two large companies called National Prawn Company NPC

(for grower shrimp feed) and ARASCO (for grower seabass and grower tilapia). The study was performed for 4 months on the feeds namly:

Feeds	Calculated analysis	%
a- Grower seabass Feed mill (Marine fish 48% protein) sinking 6 mm	Crude protein Crude fat Crude fiber Ingredients: Cereal, Fishmeal, Fish oil, Antioxidant, Fungicide, Vitamins, and Minerals	48 10 1.5
b- Grower tilapia (Tilapia32% protein) sinking 4 mm	Crude protein Crude fat Crude fiber Ingredients: Cereal, Soya bean meal, Fishmeal, Fish oil, Lysine, Methionine, Choline chloride, Vitamins and Minerals	32 6 4
c- Grower shrimp feed	Moisture Crude protein Crude fat Crude fiber Crude ash Calcium Phosphorus Ingredients: Fishmeal, Soya bean meal, Wheat, Vitamins and Minerals premixes, Fish oil, Lecithin and other	11 max 30 min 15 max 4 max 15 max 2.5 max 1 min

Feed samples were taken before storage then the following samples were obtained at regular intervals every 15 days and exposed to mycological and proximate analysis.

Mycological evaluation

a- Preparation of feed samples for total Mycotic count:

Feed samples were taken randomly. 3 samples from each kind were obtained at each period (9 samples from all kinds of ratios), and exposed to examination. 10 grams from each sample was dissolved in 90 ml of sterile physiological saline and ten-fold serial dilution procedures were carried out. Next, one ml from each dilution were cultured on Sabowraud's dextrose agar, Czapek's Dox agar media and corn meal agar, and then incubated at 28-30 °C for 7-10 days. Later on, the growth colony was examined mycologically (APHA, 1972).

b- Mycological examination:

Mycological examination was done according to Dade and Gunnell (1969), where after streaked feed samples on different media; the resulting fungal colonies were subcultured on plates of SDA, and grown at room temperature (25 °C) (Collins and Lyne, 1984).

To examine fungal structure in pure culture, preparation was made either direct from the culture, or using the technique of slide culturing (Larone, 1976). These preparations were mounted in Lactophenol Cotton Blue (LPCB). For describing the fungus, the new terminologies proposed by (Frey *et al.*, 1979; Refai, 1987; Lightner *et al.*, 1988) were used.

C- Detection of mycotoxins Aflatoxin B_1 and Ochratoxin A residue in feed samples:

Random feed samples were taken from feed samples after each period and examined for residues of AFB_1 and OTA by using the Aflatest ™ and Ochratest ™ method for samples 0-100 ppb according to the manufactures procedure by VICAM Aflatest ™ and Ochratest ™ (USA).

Proximate analysis of the diets

Three feed samples from each kind of the tested ratios were withdrawn at the same period of mycological examination for proximate analysis. Crude protein content was determined using Kjeldahl methods (Auto Kjeldahl System, Buchi B-324/435/412, Switzerland), Lipid contents determined using ether-extraction method, moisture content was determined by drying sample in a dry oven at 105 °C for 24 hours, and fiber content was determined using automatic analyzer (Fibertec, Tecator, Sweden), all methods were performed according to standard of AOAC (1990).

Storage Conditions

Feeds storage conditions were measured continuously during the duration of the storage. These include room temperature and humidity.

Statistical analysis

One-way ANOVA, Two-way ANOVA and Duncan's multiple range test (Duncan, 1955) were used to analyze the significance of difference among the means of treatment using SAS program Robbins (1986).

RESULTS

The occurrence of molds and mycotoxins contamination in three kinds of diets used in aquaculture feed was evaluated by measuring the total Mycotic count, identification of the isolated fungus, detection of the

residues of mycotoxins in the feed and proximate analysis of the diets for the regular intervals at the same storage conditions.

Total Mycotic Counts (TMC):

The obtained results showed that the total mycotic count was very high in the diets of grower shrimp and grower tilapia compared to the diet of grower seabass all over the periods of investigation (Table 1). From Table 1, results reached that there were no significant differences ($P>0.05$) in total mycotic count in diet of grower seabass during first two months of storage, while there is a significant difference in TMC during first month of storage in case of diet of grower shrimp and tilapia. The significant increase of the total mycotic count in the diet of grower shrimp and tilapia appeared from the first sample until last sample (after 4 months) but this elevation is more clear in the diet of grower shrimp than grower tilapia. On the other hand, the TMC in the diet of seabass, tilapia and shrimp during initial sample was 1.366×10^3 cfu/g, 2.1×10^4 cfu/g and 1.533×10^4 cfu/g respectively, whereas these counts at the end of study were 2.867×10^4 cfu/g, 8.3×10^5 cfu/g and 3.666×10^7 cfu/g respectively.

Mycological examinations:

Results of mycological examinations revealed that the diet of grower shrimp was highly contaminated by different fungi and yeast species than diets of grower tilapia and grower seabass (Table 2).

Concerning *Aspergillus* sp. and *Fusarium* species, as the most predominant isolated fungi it was found that the grower shrimp diet was highly contaminated by *Aspergillus* species (33 isolates) compared to grower tilapia diet (18 isolates) and grower seabass diet (5 isolates), while the grower tilapia diet was more contaminated by *Fusarium* species (4 isolates) (Table 2 & Fig. 1).

Identification of *Aspergillus* species, which were isolated from feeds showed that the *Aspergillus flavus* came in the first rank in diet (12 isolates) and only one isolate was reported in diet of grower seabass (Table 3 & Fig. 2). On the other hand, from Table (3) it was found that the *Aspergillus ochraceous* was isolated from all kinds of diets but with high incidence in diet of grower shrimp (7 isolates) and diet of grower tilapia (6 isolates), while only one isolate was detected in diet of grower seabass (Fig. 3). Moreover, it was found that *Aspergillus fumigatus* (Fig. 4) and *Aspergillus terreus* (Fig. 5) were isolated only from grower shrimp diet, 10 and 2 isolates respectively, while the *Aspergillus niger* was isolated from grower seabass diet (2 isolates).

Aflatoxins and Ochratoxins residues in feeds:

Because *Aspergillus flavus* and *Aspergillus ochraceous* were the most common isolates of mold in this study, residues of total Aflatoxin and Ochratoxin in the feeds were carried out. Table (4) revealed that the Aflatoxin was accumulated in high concentration in diet of grower shrimp and diet of grower tilapia, which reached to 98.55 ±5.20 ppb and 79.00 ±4.7 ppb after 4 months of storage respectively. the results also revealed that the diet of grower tilapia and grower shrimp were contaminated by high concentration from all types of mycotoxins of Aflatoxin and Ochratoxin at levels of 79.00 ±4.7, 34.30 ±2.10 ppb and 19.50 ±3.10 and 66.50 ±5.65 ppb respectively at the end of the study.

It is worthy to mention that the diet of grower seabass was the lowest type of feeds contaminated by the mycotoxins, where the levels of Aflatoxin and Ochratoxin after 4 months from storage in this diet were 1.91 ±0.11 and 2.89 ±0.16 ppb respectively.

Proximate analysis:

From Table (5), it is clear that the proximate analysis of the diet of grower tilapia and grower shrimp were highly affected by mycotic and mycotoxin contamination as compared to the diet of grower seabass. The percentages of crude protein, lipids and fiber in both grower tilapia and grower shrimp diets after 4 months from storage reached to 20.8 ±1.13, 22.43 ±1.25, 3.00 ±0.37 and 8.07 ±0.37, 5.97 ±0.25, 5.83 ±0.68 respectively, and these levels at the end of study for sea bass diet were 44.90 ±1.12, 8.51 ±0.29 and 2.10 ±0.06 respectively.

It is of interest to report that there were no significant differences in the percentages of crude protein, lipids and fiber all over the period of the study in the diet of grower seabass, whereas these percentages at first sample (before storage) were 47.77 ±1.23, 9.70 ±0.33 and 1.68 ±0.04 respectively.

Feed storage conditions:

The storage condition during this study were temperature (28 - 32 °C), relative humidity levels (73 - 82 %) and moisture levels in the diets of grower seabass (3 -3.2%), grower tilapia (16 - 17%) and grower shrimp (18 - 18.3%).

DISCUSSION

Molds of fungi grow in multicellular colonies, compared with yeasts which are single cellular fungi. Molds can grow and mycotoxins can be produced pre-harvest or during storage, transport, processing or feeding.

Molds growth and mycotoxin production are related to weather extremes (causing plant stress), to inadequate storage practices, to low feedstuff quality and to faulty feeding conditions (Doerr *et al.*, 1982). In general, environmental conditions, e.g. heat, water and insect damage, cause plant stress and predispose plants in the field to mycotoxin contamination (Coulumbe, 1993).

In the present study, the results revealed that the feeds of grower tilapia and grower shrimp were more contaminated with molds than feed of grower seabass (Table 1). The possible explanation of these results is the presence of mold inhibitors in the feed of grower seabass which reduce the total mold count. Similar results obtained by (Joff, 1986; Desjardins *et al.*, 1993; Trial *et al.*, 1995) who used mold inhibitors such as acetic and propionic acids to lower the pH of the feed to prevent mold growth.

Mold spore counts may not be very useful and are only a gross indication of the potential for toxicity, but mold identification can be useful to suggest which mycotoxins maybe present (Scott, 1990). Tables (2 and 3) cleared that the incidence of different types of molds in the studied feeds. *Aspergillus* sp. especially *Aspergillus flavus* and *Aspergillus ochraceous* were most common in grower tilapia and grower shrimp feeds, in addition to *Aspergillus fumigatus* in grower shrimp feed only. Moreover, *Fusarium* sp. was recorded but at lower incidence than *Aspergillus* sp. especially in the grower tilapia feed. The encountered results were nearly similar to those mentioned by (Christensen *et al.*, 1977; Doerr, 1994; Trenholm *et al.*, 1988; Whitlow and Hagler, 2002) who noted that *Aspergillus* sp. and *Penicillium* sp. are storage fungi, while *Fusarium* sp. are generally field fungi. These results could be attributed to the higher moisture levels in the grower tilapia and grower shrimp feeds than grower seabass feed, in addition to the presence of mold inhibitors in grower seabass feed (Boyacioglu *et al.*, 1992; Gareis and Ceynowa, 1994).

Naturally contaminated feeds are more toxic than feeds with the same level of a pure mycotoxin supplemented into the diet (Applebaum *et al.*, 1982; Forster *et al.*, 1986; Smith and MacDonald, 1991). Concerning the mycotoxins residues (Table 4), the results showed that Aflatoxin was accumulated in high levels in grower shrimp and grower tilapia feeds after 4 months from storage reaching 98.55 ±5.20 and 79.00 ±4.7 ppb respectively, while it was not detected in grower seabass diet. On the other hand, Ochratoxin was accumulated in higher level in diet of grower

shrimp than in grower tilapia after the study period with a level of 66.50 ±5.65 and 24.60 ±3.21 ppb respectively, while not detected in grower seabass.

The above mentioned results may be attributed to the high level of moisture contents of feed which increase incidence of moldy feedstuff which increase the secretions of mycotoxins (Juli *et al.*, 2005). One possible explanation for the Aflatoxin and Ochratoxin increment in grower tilapia and grower shrimp diet more than grower seabass is the presence of toxin inactivator as feed additives or mold inhibitors which lead to destroy and dilute these mycotoxin (Whitlow and Hagler, 2002).

The known dietary factors that interact with mycotoxins include nutrients such as fat, protein, fiber, vitamins and minerals (Smith *et al.*, 1971; Brucato *et al.*, 1986; Coffey *et al.*, 1989). The present findings show a decrease in the nutritive values of crude protein and total lipids of both grower tilapia and grower shrimp diets after 4 months from storage which reached 20.8 ±1.13, 22.43 ±1.25 and 3.00 ±0.37, 8.07 ±0.37 respectively, while the same nutrients values were not affected in grower seabass diet (Table 5). These results are in partial agreement with those of Doerr (1994) who reported that as mold numbers reach and exceed the level of 10^6 colony forming unit/gram, as much as 5% reduction in the total energy, and 7% reduction may occur in protein as well as loss of crude fat, and at mold levels of 1-5 × 10^6 CFU/g. He also suggested discounting such grain value by as much as five to ten percent.

This study revealed that Aflatoxins present in higher concentration than the recommended ingredients for domestic animals by FDA.

Aflatoxins can cause disease indirectly through their effects on essential nutrients in the diet. For example, AF affects fat soluble antioxidants in feeds, such as vitamin A, and water soluble antioxidants and vitamins, such as vitamin C (necessary for immune function) and thiamin (necessary for metabolic and nervous function). Hence it is not surprising that aflatoxins have been shown to depress the immune system, making fish more susceptible to bacterial, viral or parasitic diseases (Doerr, 1994). In tropical and subtropical conditions, this potential is further increased due to storage under humid and hot conditions.

The results suggested that when feeds are stored for long periods (more than two months) or under poor storage conditions, fish health problems might arise, not only from molds and mycotoxins, but also from loss of nutrient quality. Further studies would be practical only if indeed

seriously high levels of mycotoxins occur or are found in our feed before we are able to use them completely.

A possible remedy for feeds that do contain small amounts of mold is to add mold inhibitors and toxin inactivator to the stored feeds to manage and control the molds and mycotoxicosis in fish feeds.

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Table 1: Total mycotic counts CFU/g. in three kinds of culture's feeds used in Saudi Arabia.

Periods of sampling	Kinds of Feeds		
	Grower Seabass	Grower Tilapia	Grower Shrimp
	Means ± SE	Means ± SE	Means ± SE
Before storage	1.366 ± 03 ^{Bcb}	2.100 ± 04 ^{Ba}	1.533 ± 04 ^{Ba}
After 15 days	3.400 ± 03 ^{Bb}	3.633 ± 04 ^{Ba}	2.267 ± 04 ^{Ba}
After 30 days	2.000 ± 03 ^{Bb}	4.600 ± 04 ^{Ba}	3.367 ± 04 ^{Ba}
After 45 days	2.767 ± 03 ^{Bb}	2.633 ± 04 ^{Aa}	4.300 ± 04 ^{ABa}
After 60 days	2.833 ± 03 ^{Bb}	2.266 ± 05 ^{Aa}	1.933 ± 05 ^{Aa}
After 75 days	1.967 ± 04 ^{ABc}	3.300 ± 05 ^{Ab}	2.167 ± 06 ^{Aa}
After 90 days	2.000 ± 04 ^{Ac}	7.700 ± 05 ^{Ab}	2.800 ± 06 ^{Aa}
After 105 days	2.667 ± 04 ^{Ac}	7.533 ± 05 ^{Ab}	4.333 ± 06 ^{Aa}
After 120 days	2.867 ± 04 ^{Ac}	8.300 ± 05 ^{Ab}	3.666 ± 07 ^{Aa}

CFU/g.: Colony forming unit per gram feed or diet.
Means with different letters in the same column differ significant ($P<0.05$).
Values receiving same superscript are statistically insignificant ($P>0.05$).

Table 2: Occurrence of mycotic contamination in three kinds of culture's feedsused in Saudi Arabia. (Number of mold species per 3 samples of diet every timeas affected by storage period.

Periods of sampling	Number of Mold species in Kinds of Feeds																	
	Grower Seabass						Grower Tilapia						G rower Shrimp					
	Aspergillus sp.	Penicillium sp.	Fusarium sp	Dematiceaus Fungi *	Mucoraceace Fungi **	Yeast sp.	Aspergillus sp.	Penicillium sp.	Fusarium sp	Dematiceaus Fungi	Mucoraceace Fungi	Yeast sp.	Aspergillus sp.	Penicillium sp.	Fusarium sp	Dematiceaus Fungi	Mucoraceace Fungi	Yeast sp.
New (Before storage)	-	1	-	-	2	-	1	-	-	1	1	-	3	-	-	-	-	1
After 15 days	1	1	-	-	-	1	1	-	1	-	-	1	2	-	-	1	-	-
After 30 days	-	-	-	1	2	-	2	-	-	-	1	-	3	-	-	-	-	-
After 45 days	1	1	-	-	-	1	3	-	-	-	-	-	4	-	-	-	-	-
After 60 days	1	-	-	-	2	-	2	-	1	-	-	-	4	-	-	-	-	1
After 75 days	1	-	-	-	2	-	2	-	1	-	-	-	6	-	-	-	-	-
After 90 days	-	-	-	1	2	-	3	-	-	-	-	-	4	-	1	-	-	-
After 105 days	-	1	-	-	2	-	2	1	-	-	-	-	5	1	1	-	-	-
After 120 days	1	1	-	1	-	-	2	-	1	-	-	-	4	-	-	-	-	-
Total Number of Isolates	5	5	-	3	12	2	18	1	4	1	2	1	35	1	2	1	-	2

* = Dematiceaus fungi: Alternaria species, cladosporium sp.

** = Mucoraceace fungi: Mucor sp., Rhizopus sp., Absidia sp.

Table 3: Occurrence of *Aspergillus* species in three kinds of culture's feeds used in Saudi Arabia. (Number of *Aspergillus* species per 3 samples of diet every time) as affected by storage period.

Periods of sampling	Number of *Aspergillus* species in Kinds of Feeds														
	Grower Seabass					Grower Tilapia					G rower Shrimp				
	A. flaves	A. fumigatus	A. ochraceous	A. niger	A. terreus	A. flaves	A. fumigatus	A. ochraceous	A. niger	A. terreus	A. flaves	A. fumigatus	A. ochraceous	A. niger	A. terreus
New (Before storage)	-	-	-	-	-	1	-	-	-	-	2	1	-	-	-
After 15 days	-	-	-	1	-	1	-	-	-	-	1	-	1	-	-
After 30 days	-	-	-	-	-	1	-	1	-	-	2	-	1	-	-
After 45 days	-	-	-	1	-	2	-	1	-	-	2	1	1	-	-
After 60 days	1	-	-	-	-	1	-	1	-	-	1	2	1	-	-
After 75 days	-	-	1	-	-	2	-	-	-	-	2	2	1	-	1
After 90 days	-	-	-	-	-	2	-	1	-	-	2	1	1	-	-
After 105 days	-	-	-	-	-	1	-	1	-	-	2	2	-	-	1
After 120 days	-	-	-	1	-	1	-	1	-	-	2	1	1	-	-
Total Number of Isolate	1	-	1	3	-	12	-	6	-	-	16	10	7	-	2

Table 4: Mycotoxins contamination in three kinds of culture's feeds used inSaudi Arabia as affected by Sp.

Periods of sampling	*Kinds of Feeds*					
	Grower Seabass		**Grower Tilapia**		**G rower Shrimp**	
	Aflatoxin (ppb) Mean ± S.E	**Ochratoxin (ppb)** Mean ± S.E	**Aflatoxin (ppb)** Mean ± S.E	**Ochratoxin (ppb)** Mean ± S.E	**Aflatoxin (ppb)** Mean ± S.E	**Ochratoxin (ppb)** Mean ± S.E
Before storage	- ^{De}	- ^{Ce}	0.40 ^{Ed} ±0.02	0.30 ^{Ed} ±0.01	6.50 ^{Ea} ±1.08	2.50 ^{Gb} ±0.70
After 15 days	0.30 ^{Cd} ±0.04	- ^{Ce}	4.00 ^{Db} ±0.7	0.65 ^{Ed} ±0.01	21.00 ^{Ea} ±2.10	4.20 ^{Fb} ±0.85
After 30 days	1.10 ^{Be} ±0.08	- ^{Cf}	7.00 ^{Dc} ±0.9	1.80 ^{De} ±0.01	45.00 ^{Dc} ±2.10	10.30 ^{Eb} ±1.20
After 45 days	1.40 ^{Bf} ±0.11	- ^{Cg}	19.0 ^{Cc} ±1.80	5.20 ^{Ce} ±0.81	62.50 ^{Ca} ±2.30	25.60 ^{Db} ±2.50
After 60 days	1.70 ^{Af} ±0.17	- ^{Cg}	21.0 ^{Cc} ±1.20	13.50 ^{Bd} ±1.21	76.50 ^{Ba} ±2.80	33.20 ^{Cb} ±2.90
After 75 days	1.50 ^{Ag} ±0.09	0.10 ^{Bf} ±0.03	55.0 ^{Bb} ±3.70	14.30 ^{Be} ±1.31	83.00 ^{Ba} ±3.52	38.70 ^{Cc} ±2.90
After 90 days	1.68 ^{Ag} ±0.07	0.18 ^{Bg} ±0.03	58.0 ^{Bb} ±2.7	18.25 ^{Ae} ±2.13	87.00 ^{Aa} ±3.85	47.20 ^{Bc} ±3.40
After 105 days	1.83 ^{Ag} ±0.03	2.60 ^{Ag} ±0.04	68.0 ^{Ab} ±3.10	19.50 ^{Ae} ±2.91	91.00 ^{Aa} ±4.20	56.60 ^{Ac} ±4.25
After 120 days	1.91 ^{Ag} ±0.11	2.89 ^{Ag} ±0.16	79.0 ^{Ab} ±4.70	24.60 ^{Ae} ±3.21	98.55 ^{Aa} ±5.20	66.50 ^{Ac} ±5.65

Means with different letters in the same column differ significant (P<0.05).
Values receiving same superscript are statistically insignificant (P>0.05).

Table 5: Proximate analysis of three kinds of culture's feeds used in Saudi Arabia as affected by Sp.

Periods of sampling	Mean personages % of nutrients in the three kinds of culture's Feeds								
	Grower Seabass			Grower Tilapia			G rower Shrimp		
	Crude protein Mean ±S.E	Crude Lipids Mean ±S.E	Crude fiber Mean ±S.E	Crude protein Mean ±S.E	Crude Lipids Mean ±S.E	Crude fiber Mean ±S.E	Crude protein Mean ±S.E	Crude Lipids Mean ±S.E	Crude fiber Mean ±S.E
Before storage	47.7^{A} ±1.23	9.70^{A} ±0.33	1.68^{A} ±0.04	31.7^{A} ±1.23	5.83^{A} ±0.24	4.43^{A} ±0.19	33.63^{A} ±1.68	14.67^{A} ±0.96	4.67^{A} ±0.39
After 15 days	46.27^{A} ±1.61	9.50^{A} ±0.34	2.03^{A} ±0.07	30.13^{A} ± 1.12	4.83^{A} ± 0.27	4.77^{A} ± 0.25	30.13^{A} ± 1.15	13.65^{A} ±0.93	4.60^{A} ±0.32
After 30 days	46.13^{A} ±1.08	9.20 ±0.22^{A}	2.10^{A} ±0.06	29.00^{A} ± 1.09	4.27^{A} ± 0.19	4.77^{A} ± 0.25	29.13^{B} ± 1.12	12.83^{A} ±0.85	4.67^{A} ±0.42
After 45 days	46.00^{A} ±1.12	9.13^{A} ±0.16	2.17^{A} ±0.08	27.40^{B} ± 1.11	4.13^{A} ± 0.16	4.83^{A} ± 0.13	27.87^{B} ± 1.33	11.93^{B} ±0.79	5.17^{A} ±0.56
After 60 days	45.70^{A} ±1.04	9.00^{A} ±0.17	2.23^{A} ±0.09	26.57^{B} ± 1.20	3.83^{B} ± 0.22	5.20^{A} ± 0.27	27.33^{B} ± 1.14	8.63^{C} ±0.72	5.20^{A} ±0.47
After 75 days	45.17^{A} ±1.16	9.00^{A} ±0.26	2.13^{A} ±0.12	26.23^{B} ± 1.23	3.90^{B} ± 0.42	5.27^{A} ± 0.29	26.87^{B} ± 1.21	8.70^{C} ±0.68	5.23^{A} ±0.38
After 90 days	45.03^{A} ±1.12	8.33^{A} ±0.12	2.13^{A} ±0.42	24.83^{C} ± 1.09	3.53^{B} ± 0.33	5.63^{A} ± 0.28	26.17^{B} ± 1.20	8.67^{C} ±0.56	5.40^{A} ±0.38
After 105 days	45.0^{A} ±1.16	8.77^{A} ±0.14	2.20^{A} ±0.14	22.07^{C} ± 1.14	3.17^{B} ± 0.18	5.80^{A} ± 0.18	25.1^{C} ± 1.31	8.63^{C} ±0.49	5.77^{A} ±0.67
After 120 days	44.9^{A} ±1.12	8.51^{A} ±0.29	2.10^{A} ±0.06	20.80^{C} ± 1.13	3.00^{B} ±0.37	5.97^{A} ±0.25	22.43^{C} ±1.25	8.07^{C} ±0.37	5.83^{A} ±0.68

Means with different letters in the same column differ significant ($P<0.05$).
Values receiving same superscript are statistically insignificant ($P>0.05$).

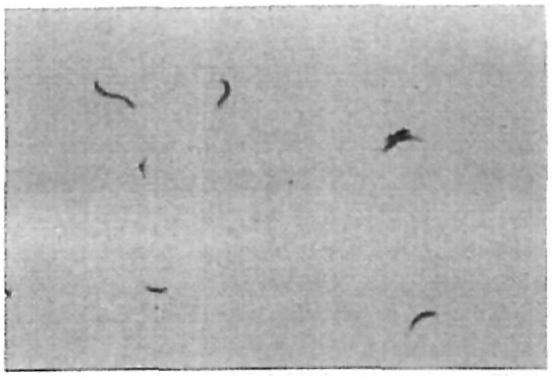

Fig. 1: Fusarium sp. showing macro conidia and micro conidia which is fusiform.

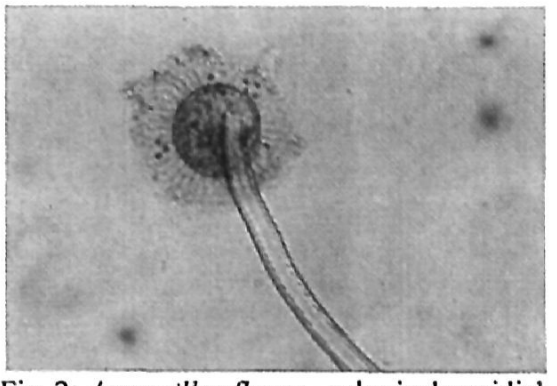

Fig. 2: *Aspergillus flavus* - spherical conidial head

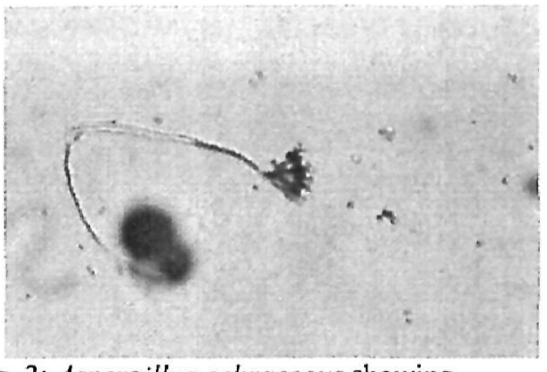

Fig. 3: *Aspergillus ochraceous* showing globular form of conidial.

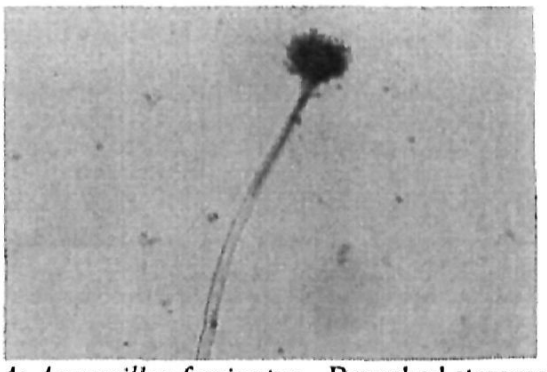

Fig. 4: *Aspergillus fumigatus* – Branched stregmata and philidea of conidial head.

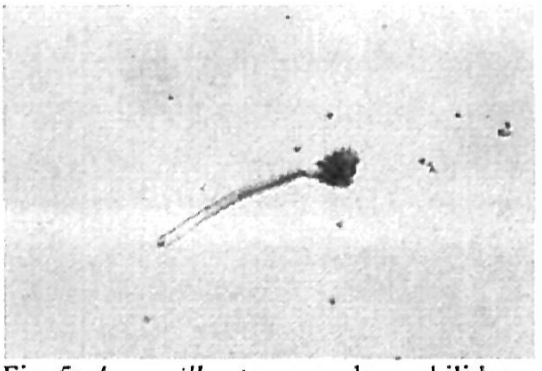

Fig. 5: *Aspergillus terreus* – long philidea on the conidial head.

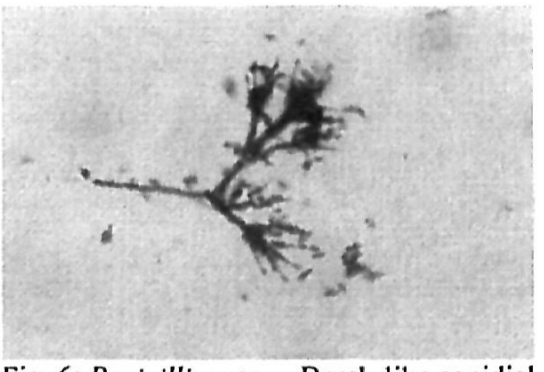

Fig. 6: *Penicillium* sp. – Brush-like conidial head.