Aflatoxins Investigation on Dried Fishes of Tuticorin, South East Coast of India

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Abstract

The aim of the current study was to determine the toxigenic fungus on dried fishes of Tuticorin such as Chirocentrus dorab, Saurida tumbil, Epinephelus merra, Sillago sihama, Atule mate, Aprion virescens and production of fungal toxins. The quantitative enumeration of fungi as colony-forming units per gram (CFU/g) was between 3.5×10⁵ - 2.0×10⁶ CFU/g during 6th month of storage period but the initial fungal counts in the sun-dried fishes from market were between 2.8×10² to 1.2×10³ CFU/g. Moisture and pH content of the dried fish samples varied with storage time and it was between 8.5 - 33 % and 5.10 - 7.95 respectively. Twenty three different fungal species were found and it was associated with the dried fishes samples sold in the dried fish markets. The associated fungi were Aspergillus flavus, A. terreus, Aspergillus fumigatus, Absidia sp., Rhizopus sp, A. niger, Mucor sp, Penicillium sp, Fusarium moniliformis, A. oryzae, Trichoderma sp, Geotricus candidum, A. sulphureus, A. terricola, A. awamori, A. flavipes, A. versicolor, A. tamari, Eurotium sp, Alternaria sp, A. parasiticus, A. sydowii and A. ochraceous. The Aspergillus flavus and A. niger had the highest rate of occurrence among the isolated fungi. The concentration of aflatoxin B1 and G1 were also assessed in the stored dried fish sample and these were found in the dried fish sample ranged between 0.001 - 5.492 μg/kg and 0.01 - 2.296 μg/kg respectively. Quantification of Aflatoxins from the dried fish revealed samples were contaminated with Aflatoxins but they did not exceed the legal limits and confirms potential exposure of this toxin from dried fish infected with fungi. Prolonged intake of dried fish with these metabolites may constitute potential public health hazard. Adequate cooking could help in reducing mycoflora of dried fish. This study demonstrates the importance of proper processing and handling of fish in order to safeguard public health. Findings of this study will increase the knowledge base towards adoption of improved handling and drying methods hence minimize fungal growth and possible aflatoxin contamination in the dried fish sector. This study also showed that long time stored dried fish displayed for sale at market sites of Tuticorin were contaminated with more aflatoxin which poses a great threat on the health of the consumers. However, fish samples should be well salted and dried to reduce the moisture content, the samples for sale should be kept in a covered container or show glass to reduce settling of droplets and spores and retailers to reduce long time storage of dried fishes to reduce contaminations.

Key words: Dried fish, Retail shops, Fungal species, Aflatoxin content.

1. Introduction

Fish is a highly nutritious food and an excellent source of proteins, vitamins, minerals and essential fatty acids. Interest in fish consumption has increased over the years, due to its health benefits it impart being a rich source of omega -3 fatty acids that reduces cholesterol levels and the incidence of heart disease and pre-term birth (Siscovick et al., 2000). Dried fish are very important parts of the traditionally accepted diet for many in developing countries as well as a
mold. Fish is a rich substrate for the growth of microbes especially fungi. The fungi introduced to the fish either through the environment or poor personal hygiene or improper processing of fish act as a substrate to grow and produce their metabolites such as aflatoxins which are toxic and detrimental to the consumers well being (Adebayo-Tayo et al., 2006). Fungal growth on dried fish indicates the onset of spoilage and deterioration of the product (Pitt and Hocking, 2009). Now a days this is the main problem while storing dried sea foods because products of animal origin that are likely to spoil from fungal growth include cured fermented meat products such as salted, dried and smoked seafood. This could result in negative effects such as discolouration, rotting and the production of off odour making food unmarketable incurring huge losses to manufacturers and/or distributors of the food. During storage process, sea foods infected with fungi which are known to cause deterioration to stored products. Spores of spoilage fungi may be present on the sea food going to storage and improper storage conditions results in germination of spores and subsequent deterioration. In addition, during storage of dried fish products, wholesalers and retailers store the products in poorly ventilated stores where pest can gain access and may directly inoculate them with fungi. The market place where the fish products are displayed for sale most times are not clean or hygienic, such as in open trays without coverage. This in turn allows the dusts and fungal spores to settle on the product and lead to fungal invasion, production of toxins and spoilage.

Another risk of fungal contamination in sea food is mycotoxin production with its resulting carcinogenic and mortality effects on consumption. Since long time, aflatoxin contamination represents a potential threat to human health and animals due to its carcinogenic, teratogenic, immunosuppressive and other adverse effects and because of its retention capability by animal tissues. Many species especially those belonged to Aspergillus are known to produce many types of toxins such as aflatoxins, ochratoxins and sterigmatocystine. These mycotoxins exhibit toxic, mutagenic effects in human beings (Motalab et al., 2008). So far extensive research in this area has led to the discovery of more than 100 toxigenic fungi and 300 mycotoxins from different food products across the world (Keller et al., 2005). The toxicity of these compounds can vary quite widely with a range of undesirable health effects that could either be acute or chronic.

Aflatoxins have a high melting point of 250°C. It has been proved that food items do carry residue of the toxin. Thus, it is certain that human beings are exposed to aflatoxins through contaminated food items among which fish is an important component (Murgani, 2000). Several studies on the assessment of the risk potential of dried fish through improper cooking (Jonsyn and Lahai, 1992; Mugula and Lyim, 1992) and some species are resistant to cooking also (Bukola et al., 2008; Ali et al., 2011). Adebayo-Tayo et al. (2006) reported several toxins produced from Aspergillus species among that Aflatoxin B1 and G1 were the most important causing agents. However, little information is available on the mycotoxin production in dried fishes of Tuticorin region; hence this study is aimed at evaluating the aflatoxin evaluation of dried fish over a six month storage period.

2. Materials and Methods

2.1 Sample Collection

Samples of dried fishes such as Chirocentrus dorab, Saurida tumbil, Epinephelus merra, Sillago sihama, Atule mate and Aprion virescens were purchased from local markets. Approximately 100 g of dried fishes harbor in visible fungi were collected randomly, that is one to two samples per supplier and directly placed in individual sterile polythene bags using aseptic techniques and transferred to the laboratory. Here all the samples were stored in room temperature for 6 months to examine the fungal population and also aflatoxins production. Sampling was done for every month and all the analysis was done in duplicate. During analysis all the samples were surface sanitized using 70% ethanol and rinsed with sterile distilled water before grinding. Surface sanitation aimed to remove fungal spores from the surface and ensure enumeration of those fungi actually invading and contaminating food (Samson et al., 2010). For the enumeration of fungi the Potato dextrose agar was used. Fungi were isolated from surface sanitized samples by application of direct and dilution plating. For direct plating, the ground samples were sprinkled

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directly onto the isolation agar in three replicates. For dilution plating, 10 g of the ground sample was weighed and macerated for 2 minutes with 90 ml of 0.2% peptone water. Serial tenfold dilution were made (up to $10^6$) and spread plated onto isolation agar in three replicates and incubated at 25°C for 7 days. Colonies with visually distinct cultural characteristics were inoculated onto appropriate media for the identification.

2.2 Identification of Fungi

The fungi were identified to species level using the media, methods and plating regimen of Pitt and Hocking (2009). The morphological and microscopical examinations, and necessary slide culture technique was carried out according to (Arx, 1976). Penicillium species were identified according to (Raper and Thom, 1949; Samson, 1979; Pitt, 1988). Aspergillus species were identified according to Raper and Fennell (1967). Petri-plates were examined by eye under natural day light and colony colours including reverse colours were checked by day light. Microscopic features of the isolates were determined using a wet mount (Pitt and Hocking, 2009) or by the transparent tape technique (Samson et al., 1995).

A wet mount was prepared by cutting off a small portion of the colony using an inoculation needle and placing it on a slide with the aid of a drop of 70% ethanol. A drop of lactophenol cotton blue was added when required. A cover slip was placed on top and excess liquid removed by gently blotting with tissue paper before examination. For the transparent tape technique, a length of transparent adhesive tape was gently pressed down onto the surface of the colony and transferred to a glass slide containing a drop of lactophenol cotton blue. The tape was pressed at the extreme ends of the slide. The slides were viewed using a high resolution light microscope initially under 40 X objective for examining major structures and under 100X using oil immersion for studying details of the spores and surface textures. Microscopic features of the isolates such as conidial heads, stipes, shapes of conidia, and roughness of conidial walls and existence of ascospores were determined.

2.3 Confirmation of Species

The major aflatoxins producing species A. flavus and A. parasiticus have some resemblance to A. tamarii on culture media. Aspergillus flavus and A. parasiticus agar (AFPA) is recommended as a useful diagnostic to distinguish. Isolated colonies were inoculated on to AFPA and incubated at 30°C for 42 - 48 hours. At the end of the incubation period, plates were checked for reverse colouration under daylight. Colonies of A. flavus and A. parasiticus are distinguished by bright orange yellow reverse clouration.

2.4 Analyses of Aflatoxins Contamination

Aflatoxins were analysed using the solvent efficient thin layer chromatography (TLC) method according to AOAC method 993.17 (AOAC, 1995). About 50 g of ground sample was extracted with 200 ml of methanol-water mixture (85:15) and filtered. Then 40 ml of the extract was partitioned in a separating funnel using 40 ml of 10% NaCl and 25 ml of hexane. Aflatoxins were then extracted with duplicate 25 ml of chloroform from the aqueous phase and evaporated to dryness in a steam bath. Aflatoxin extracts were dissolved in 3 ml of dichloromethane and purified in a glass column packing (22 x 300 mm) of 10 g silica gel 60 M and 0.5 g Na2SO4. The packing of the column was done as described in the AOAC method 968.22 (AOAC, 1995). The column was initially conditioned with 30 ml of hexane and 30 ml of dichloromethane. Aflatoxins were then eluted with 3 portions of 30 ml chloroform-acetone mixture (9:1) and the collected elute evaporated to dryness on a steam bath under nitrogen stream. Estimation of aflatoxins was carried out according to the methods recommended by AOAC (1984) and described by Alhussaini (2012). For this pre-coated silica gel plates were used. Rectangular glass jar was used for developing chromatoplates. A suitable volume of solvent mixture (chloroform: methanol, 97:3 v/v) was placed in the bottom of the jar so that the starting spots on the plates would be 1 cm above the upper surface of the solvent mixture. The chromatographic plates were activated by heating 1 hour at 120°C in a hot air oven, and removed immediately to desiccators to cool. Of the extracted samples 5, 10 and 15 μl were spotted on three different points on a ruled base line of the TLC plates. Also 5, 10 and 15μl of the aflatoxin standard were spotted on another three points near the previous sample extract spotted points. Parallel starting spots, 2 cm from each side of the plate and 1.5 cm apart, were made with micropipettes from chloroform extracts with reference aflatoxins. Spots were left to air dry. Prepared plates were then transferred to the chromatographic jar, developed to a suitable distance (10 cm), and removed. The solvent front was marked and the plates were air dry. Spots were viewed under UV light (366 nm) and the outline of each fluorescent spots was marked by sharp pin. Retention factor (Rf) values, colors, and intensities of the spots were compared with reference mycotoxins (El-Bazza et al., 1982). The dilution-to-extinction (Coomes et al., 1965) and comparison of standards (Aflatoxin B1 and G1) (AOAC, 1984) techniques were used for estimation of aflatoxins concentrations (Alhussaini, 2012).
2.5 Determination of pH

2 grams each of the fish samples were weighed in triplicates. Water was added and mixed thoroughly to make a fish slurry. The pH readings were taken using digital pH meter equipped with a glass electrode. The electrode was rinsed and immersed into the fish slurry. The pH readings were then recorded.

2.6 Moisture Content

The moisture content of dried fish samples were carried out at the initial stage of collecting the dried fish and for six month storage using hot air oven (105°C) as AOAC (1990).

3. Results

The results of initial fungal count in the dried fishes collected from the market and the fungal count in same samples stored at room temperature for 6 months were represented in Table 1. Initial High (1.2×10³) and low (2.8×10²) fungal count was observed in Saurida tumbil and Atule mate. The fungal count was fluctuated monthly during the storage. High total fungal count of 2.0×10⁶ (cfu/g) was observed in 6th months stored Saurida tumbil, Chirocentrus dorab and Aprion virescens (1.1×10⁵), Atule mate (0.9×10⁵), Epinephelus merra (6.9×10⁴) and Sillago sihama (3.5×10⁴).

The moisture content of the dry fish was presented in Table 2. Moisture content of the selected fishes ranged between 12.3 to 28.88% in Chirocentrus dorab, 15 - 30.0% in Saurida tumbil, 13.4 - 32.0% in Epinephelus merra, 9.0 - 19.76% in Sillago sihama, 15.66 - 29.50% in Atule mate and 17.0 - 25.40% in Aprion virescens. Higher and lower moisture content was observed in Epinephelus merra (32.0%) and Sillago sihama (19.76%) after 6 months of storage.

The pH of the dry fish samples are shown in Table 3. The dry fish samples had acidic nature and the pH ranged from 5.10 - 6.65. The pH decreased after one month of storage and it was slightly increased or decreased resulting from the production of toxin. The pH of the dry fish during the storage period support the fungi and production of mycotoxin.

The fungal species of the dry fish samples are presented in Table 4. Mycobtiota of dry fish samples revealed a total of twenty four species belonging to nine genera such as Aspergillus, Alternaria, Mucor, Eurotium, Fusarium, Penicillium, Trichoderma, Rhizopus and Absidia. Aspergillus species (Fig 1) was the most dominant fungal group in this study. A. niger. A. fumigates, A. ochraceus and A. flavus are the most prevalent species isolated from all the dry fish samples. The fungal species varied between the months, the most pre-dominant; however aflatoxins producing fungi A. flavus was dominant in all the selected fish species.

The incidence of aflatoxigenic and non-toxigenic strains of A. flavus was represented in Table 5. Aflatoxins production was noticed as blue fluorescence on silica gel TLC plates under UV (365 nm) light. The frequencies of the A. flavus isolate regard to aflatoxins and blue florescence producing ability. These results indicated that among the total of 37 A. flavus isolates, 17 isolates were able to produce AFB1 and 10 isolates were able to produce AFG1.

The result of aflatoxins concentration in dry fish samples is shown in Table 6 and 7. Aflatoxins were detected in all the six dry fish samples. The concentration of aflatoxins B1 initially ranged from 0.001 - 1.2 µg/kg-1 and during the storage period it increased to 2.132 - 5.492 µg/kg-1. The aflatoxins B1 production was very high in the Chirocentrus dorab (2.104 µg/kg-1) and it was low in Sillago sihama (1.642 µg/kg-1). In the case of aflatoxins G1 was initially absent in Epinephelus merra, but following storage aflatoxin was found. Compared to AFB1 the toxin AFG1 concentration was low in all the fishes. Both B1 and G1 aflatoxins were serially increased during the storage period.

4. Discussion

Fungal contamination of fish is considered the main cause of spoilage as off flavor and unpalatable taste and it may constitute a public health hazard as well as many of economic losses (El-Ahl, 2010). Fish are more liable to contamination with fungi from animal and human reservoirs which may contaminate the water in the fishing area. Furthermore, contamination during handling and processing may also occurred. The contamination was increased in cases of fish caught from polluted areas. The fungal contamination of dried fish could be attributed to improper sanitation during catching, handling, manufacturing, storage, transportation and marketing of fish (Ward and Baji, 1988). Both direct and dilution plating was used in this study for the isolation of fungi from the product. Direct plating was more effective and resulted in enumeration of a wider range of species compared with dilution plating. So far several species of Aspergillus have been isolated from dried meat and these species are known to produce toxic substances and this was agreed with this present study results. Most food products contain a carbon source that most common fungi are able to use. In protein rich substrates of dried fish, amino acids may be used as a carbon source by the fungi (Pitt and Hocking, 2009).

Fish are exposed to direct sunlight for drying and the dry fish are maintained at the ambient temperature for storage and sale. Normally the dry fish...
were stored in retail shops for a maximum of 6 months in ambient temperature without packing. The low water activity of the product together with the high ambient temperatures in the tropics creates an environment for potential proliferation of many toxigenic fungi in dried food commodities. Dried fish from warmer climates have been reported to harbor potentially toxigenic fungi such as *Aspergillus flavus* and potent mycotoxins such as aflatoxins have been detected from some of these products (Wheeler et al., 1986; Atapattu and Samarajeewa, 1990; Fafioye et al., 2002; Adebayo-Tayo et al., 2008). Prolonged exposure of mycotoxins through continuous consumption of mouldy fish could lead to chronic sickness and potentially death. During the storage of dry fish good storage practices are not adhering by wholesalers hence stores are not well ventilated and pest can easily gain access into the stores. Dry fish displayed in the market is not always hygienic and this is another avenue for microbial contamination. Very often, retailers display the dry fish samples in open trays beside the gutter on refuse heaps; this also encourages fungal attack and subsequent production of toxins. This is in agreement with the report of Akande and Tobor (1992).

During storage, temperature of the surroundings is an important factor for fungal growth. Higher temperature (>20°C) support the growth of *Aspergillus* species, while lower levels (< 20°C) tend to favor cold tolerant fungi such as *Penicillium* (Pitt and Hocking, 2009). The low water activity of dried foods and the elevated ambient temperatures of tropical countries provide favorable conditions for the growth of *Aspergillus* species. In the present study also the storage environment is very suitable for the growth of the fungus. The optimum temperature for *A. flavus* is near 33°C, a minimum near 10 - 12°C and maximum near 43 - 48°C (Holmquist et al., 1983; Pitt and Hocking, 2009). Optimum temperature for *A. tamarii* is reported as 33°C (Ayerst, 1969; Pitt and Hocking, 2009). In this study area of Tuticorin, normally the temperature of storage was 39°C during the study.
Table 4: Fungal species isolated from the dried fish during the storage period

<table>
<thead>
<tr>
<th>Fish samples</th>
<th>Months of storage</th>
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<td>1</td>
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</table>

The use of unwholesome raw materials, unhygienic handling prior to production and failure to meet the production parameters can all render the dried fish product unsafe. Furthermore, inadequate storage and frequent handling involved in post production and sale pre-disposes it to contamination. Most of these products are held at ambient temperature and sold in open markets in poor packaging and sometimes without packaging dry fish are stored for long period. As such post process contamination can increase the microbial count above $10^9$ CFU/g of fish (Sikorski et al., 1998) and it was agreed with the present results.

Tamil Nadu is one of a tropical state with an average day time temperature between 28°C to 30°C. The high ambient temperature, together with lower water activity of the fish, pre-disposes it to conditions suitable for the most prevalent species isolated are close to the environmental temperatures. Packaging is important to prevent exposure of dried product to fungal contamination and re-absorption of moisture during storage and handling. Requirements for suitable packaging for dried fish include inertness, leak, proof, impermeability to oxygen and moisture, low transparency and resistance to abrasion and puncture (Gopal and Shankar, 2011). Bulk packaging material commonly used in the tropics include waxed corrugated cartons, dead wood and plywood boxes, bamboo baskets, gunny bags, dried palmirah or coconut palm leaves and multiwall paper sacks. Low density polyethylene (LDPE) packages and recently pouches made of polyester laminated with polythene are used for individual consumer packages. In the present study during the collection of fishes all fishes were displayed open in tray, after that in laboratory it was stored in low density polyethylene bags up to six months.
The fact that the product is often consumed without further processing or only minimal processing imposes as additional risk to the consumer. Tatiana et al. (2013) reported that total fungal counts ranged from <1 x 10^2 to 4.7 x 10^4 CFU/g sampled from tilapia farms in the Rio de Janeiro State, Brazil. However, in our study, samples analyzed initially shows the fungal count of 10^2 to 10^3 CFU/g, but it was exceeded the levels proposed as hygienic feed quality limits in DRBC (1 x 10^4 CFU/g; Good Manufacture Practice, 2008) during the 4th month of storage. Wogu and Iyayi (2011) reported mycoflora of some dried fish varieties in Benin city, Nigeria resulted highest mycoflora count (17.833 x10^3 CFU) was recorded in Tilapia sp. lowest count was of (11.16 x 10^3 CFU) was recorded in Drepane africana. The microbial load on fish rarely indicate the quality of the fish but gives an indication of the risk of spoilage induced since each of the organisms had different ways of affecting the health conditions of consumers of such contaminated fish (Gram et al., 2000). It is generally accepted that fish with microbial load >10^6 CFU/g is likely to be at the stage of being unacceptable from the microbiological point of view and unfit for consumption (Cheesbrough, 2000). In the present study all the dry fishes was acceptable for 2 months. Akande and Tobor (1992) reported the microbial levels obtained in this report which is 10^4 could be considered hazardous to consumers because of the possibility of the presence of enterotoxigenic strains.

The moisture content of the dried fishes however correlated with the fungal count of this study. Moisture is an important factor for the growth of fungi (Onyuka et al., 2014). Moisture relates to the availability of water in the food for the use by microorganisms and hence is the primary defining factor for the stability of dried stored products in any practical situations (Pitt and Hocking, 1991). Probably the two most important environmental components favoring fungal growth and AF production are hot and

<table>
<thead>
<tr>
<th>Fish samples</th>
<th>A. flavus isolates (no)</th>
<th>B1 Aflotoxin producing isolates (no)</th>
<th>G1 Aflotoxin producing isolates (no)</th>
<th>Nontoxigenic isolates (no)</th>
<th>Blue fluorescent Under 365 nm UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chirocentrus dorab</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Saurida tumbil</td>
<td>12</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Epinephelus merra</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Sillago sihama</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Atule mate</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Aprion virescens</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
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</table>

Table 6: Quantitative estimation of Aflotoxin B1 (µg/kg-1) on dried fishes during the storage period

<table>
<thead>
<tr>
<th>Fish samples</th>
<th>Initial</th>
<th>Months of storage</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saurida tumbil</td>
<td>0.09</td>
<td>0.19</td>
<td>0.3</td>
<td>1.669</td>
<td>2.31</td>
<td>2.90</td>
<td>4.204</td>
<td></td>
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<tr>
<td>Epinephelus merra</td>
<td>0.45</td>
<td>1.18</td>
<td>1.5</td>
<td>2.0</td>
<td>3.111</td>
<td>3.661</td>
<td>4.111</td>
<td></td>
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<tr>
<td>Sillago sihama</td>
<td>0.28</td>
<td>0.31</td>
<td>0.4</td>
<td>0.80</td>
<td>1.261</td>
<td>1.980</td>
<td>2.132</td>
<td></td>
</tr>
<tr>
<td>Atule mate</td>
<td>0.02</td>
<td>0.21</td>
<td>0.5</td>
<td>1.02</td>
<td>1.141</td>
<td>2.322</td>
<td>2.570</td>
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<tr>
<td>Aprion virescens</td>
<td>0.001</td>
<td>0.13</td>
<td>0.2</td>
<td>1.146</td>
<td>2.191</td>
<td>3.311</td>
<td>3.444</td>
<td></td>
</tr>
</tbody>
</table>

Table 7: Quantitative estimation of Aflotoxin G1 (µg/kg-1) on dried fishes during the storage period

<table>
<thead>
<tr>
<th>Fish samples</th>
<th>Initial</th>
<th>Months of storage</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saurida tumbil</td>
<td>0.243</td>
<td>0.422</td>
<td>0.92</td>
<td>1.28</td>
<td>1.72</td>
<td>1.9</td>
<td>1.990</td>
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<tr>
<td>Epinephelus merra</td>
<td>-</td>
<td>-</td>
<td>0.222</td>
<td>0.84</td>
<td>1.02</td>
<td>1.176</td>
<td>1.642</td>
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<tr>
<td>Sillago sihama</td>
<td>1.17</td>
<td>1.33</td>
<td>1.47</td>
<td>1.86</td>
<td>204</td>
<td>2.12</td>
<td>2.296</td>
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</tr>
<tr>
<td>Atule mate</td>
<td>0.01</td>
<td>0.810</td>
<td>1.10</td>
<td>1.27</td>
<td>1.38</td>
<td>1.90</td>
<td>1.901</td>
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<tr>
<td>Aprion virescens</td>
<td>0.019</td>
<td>0.561</td>
<td>0.82</td>
<td>1.198</td>
<td>1.399</td>
<td>1.401</td>
<td>1.813</td>
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humid conditions. Although the optimum temperature and moisture content for growth and toxin production for the various aflatoxigenic fungi varies, many of them achieve best growth and toxin synthesis between 24 and 28°C (Schindler, 1977) and moisture content of at least 17.5% (Trenk and Hartman, 1970; Ominski et al., 1994). In the present study most of the samples had moisture content above 12%. Fungal growth often tolerates lower water activity than bacteria and the reduced water activity of dried fish predisposes these products to fungal attack and potential mycotoxin -

Fig 1. Fungal species isolated from the dried fish during the storage period
contamination. Some fungi associated with dried sea foods have the ability to grow at very low water activity levels that do not support the growth of many other organisms. Such fungi are termed xerophils, literally means “dryness loving”. According to Pitt (1975), a xerophilic fungus is capable of growth, under at least one set of environmental conditions, at a moisture content of above 15%. Fungal growth can be totally inhibited by rapid drying of the product to moisture content below 7% and maintaining this level during storage (Samson et al., 2010). In the present study, during the storage moisture content was increased and it was reached above 25%. But in the case of Sillago sihama shows initial moisture content was 9.0% and it was drastically increased every month and it was reach 19.76%. Higher moisture content was observed on Epinephelus merra, pink discolouration was also observed on 5th month onwards but the other fishes had no pink colouration. Higher moisture content was able to produce the toxin at higher amount than compared to initial storage period.

The fluctuation in fungal load in fish samples suggests that intrinsic factors which include physical, chemical and structural properties of the fish such as water activity, pH, redox potential (Eh), available nutrients and natural antimicrobial substances and extrinsic factors such as storage time, temperature, humidity, and the composition of storage atmosphere may have played a role (Adams and Moss, 2008). The initial pH of the samples ranged from 5.10 - 6.65 and during storage fluctuation in pH was observed and showed little variation from 4 -7 and these values vary with the fishes. Most fungi can tolerate a broader pH range, generally pH 3 - 8, compared to bacteria and other microbes (Wheeler et al., 1991). The optimal pH range for the growth of A. flavus is a broad range between 3.4 to 10 (Wheeler et al., 1991; Gock et al., 2003) and Eurotium is from 4.5 to 5.5 (Gock et al., 2003). Hence the pH of the experimental samples of this study is optimal for the growth of these fungi. The slightly acidic pH together with the high ambient temperatures of the study area was eminently suitable for fungal growth and mycotoxin production.

Samples were also cultured after surface sanitation to remove any environmental contamination and allow enumeration of fungi that was actually invading the fish. Furthermore, the surface of the dried fish is often removed by trimming before consumption or use especially if visible fungal growth is present. Hence this could be a more accurate estimation of the risk resulting from the consumption of this product. Spores of Aspergillus and related species are highly resistant to sunlight and can survive even after the sun drying. Hence it is not surprising that they have been isolated from dried fish which has often been exposure to extensive drying under the sun (Essein et al., 2005). In this study fungal species were isolated from the dry fish for 6 months are A. niger, A. flavus, A. parasiticus, A. ochraceous, A. fumigatus, A. sydowi, A. tereus, Eurotium sp, A. versicolor, A. tamari, A. sulphurious, Fusarium, A. oryzae, A. flavipes, Alternaria and Penicillium species were isolated from most of the selected species. Most of these moulds can grow well on substrates of relatively low moisture content (Munimbazi and Bullerman, 1996). A. flavus, A. fumigatus, A. niger, A. ochraceous and A. sydowi are common isolates from smoked and dried fish originating from the tropics (Wheeler et al., 1986; Atapattu and Samarajeewa, 1990; Essein et al., 2005; Adebayo Tayo et al., 2008; Fatorio et al., 2008). Penicillium species were reported as the second most frequently occurring genus on such products (Ahmed et al., 2004; Essein et al., 2005) although they are more common contaminants of food from the temperate areas. Jonsyn and Lahai (1992) reported dried salted fish from markets in Burundi show infection with species of A. niger, A. flavus, A. fumigatus, A. glaucus, A. restrictus, Basipetospora halophila, Cladosporium herbarum and Penicillium chalybeum. Ahmed et al. (2004) reported traditional Egyptian salted fish were contaminated with A. niger, A. nidulans, A. terreus, Eurotium and Penicillium species. Fungi associated with dry fish in Sri Lanka, 33 out of 61 cultures isolated were Aspergillus with A. niger as the most prevalent fungus (Atapattu and Samarajeewa, 1990). Other Aspergillus species isolated include A. flavus, A. fumigatus, A. restrictus in dried salted fish was also reported by Andrew and Pitt (1987) and Pitt and Hocking (2009). A. niger was also the most common species isolated from Malaysian salted dried fish (Ito and Abu, 1985). Salted and sundried fish from India were frequently contaminated with A. flavus, A. niger, A. fumigatus, A. oryzae, A. sydowi and Penicillium species (Prakash et al., 2011). Wheeler et al. (1986) reported in their study frequently isolated fungal species are E. rubram, E. repens, E. chevalieri, A. niger, A. flavus, A. sydowi, A. wenti. All these statements were correlated with this study.

In this study, Aspergillus species were dominant than all other species. Aspergillus sp. is closely associated with the soil. Therefore, it is possible that the sun drying of fishes on the bare ground surfaces could have exposed dried fish to high mould infestation particularly from Aspergillus sp. Aspergillus contributes to spoilage through production of lipolytic and proteolytic enzymes, giving rise to off flavours, odours and production of toxins (Marth, 1998).

According to Akande and Tobor (1992), in artisanal fishery, freshly caught fish are covered with damp sacks and at times, they are mixed with wet grass
or water weeds to reduce the temperature. Fish treated this way is prone to contamination with microorganisms such as bacteria and fungi. This indicates that spoilage of fish starts right from the aquatic ecosystem. Handling fishes are also prone to microbial attack especially in artisanal fishery due to unhygienic methods of reducing temperature. During the salting and drying, salts used in artisanal fishery and the overloading of the fishes on the trays leads to improper processing which in turn encourages fungal attack. A. flavus is ubiquitous in the air of many tropical countries (Gupta et al., 1993; Adhikari et al., 2004; Hedayati et al., 2007) and hence it is no surprise that a very high percentage of fish was contaminated with this species in this study. Experimental fish are mainly sundried in open spaces accessible to any contamination from the environment. The product is also commonly stored in bulk and sold in open markets without any packaging, increasing the chances of air borne and contact contamination. These results are in agreement with many other studies where A. flavus was the dominant fungus on dried fish or similar products (Wheeler et al., 1986; Atapattu and Samarajeewa, 1990; Essein et al., 2005; Adebayo-Tao et al., 2008; Fatioye et al., 2008; Edema and Agbon, 2010 ). The presence of A. flavus in the experimental samples might probably make its consumption hazardous to health.

A. ochraceous was another common Aspergillus species isolated in this study. This is a widely distributed across the world especially as a marine organism (Samson et al., 2010). It is also halotolerant and therefore not surprisingly could be associated with marine products such as dried fish. A. ochraceous was a common isolate of dried fish of Jakarta markets (Wheeler et al., 1986). Ochratoxin (OTA) was produced from A. ochraceous major contributors to mycotoxin in tropical foods. A. niger was another important Aspergillus species which was frequently encountered in this study. A. niger is dominant in sundried food products due to the characteristics black spores to the sunlight (Pitt and Hocking, 2009). A. niger was the dominant species on a variety of dried fish originating from local markets of Malaysia (Ito and Abu, 1985). It was also a frequent contaminant of smoked dried fish or similar products from other tropical countries (Jonsyn and Lahai, 1992; Munimbazi and Bullerman, 1996; Essein et al., 2005; Edema and Agbon, 2010; Prakash et al., 2011). Another Aspergillus isolates such as A. sydowii, A. terreus and A. candidus do not produce any mycotoxins of significance and hence are less concern.

Eurotium species have been frequently isolated from dried fish. Dried marine products from Japan and Southeast Asia were found to be dominated by this genus. In a study salted dried fish originating from Pakistan, Malaysia, Thailand and Hong Kong, Eurotium sp. were the most frequently isolated fungi (Phillips and Wallbridge, 1977). The presence of Trichoderma sp can also be as a result of the fish which has been kept on contaminated soil. Trichoderma sp is mainly isolated from forest and soils. The occurrence of A. fumigatus in dried fish samples is an indication that fish samples may have been contaminated by atmosphere during storage. Fusarium species are predominantly plant pathogens that grow and produce mycotoxins in stored maize, wheat crops and sometimes in protein rich fish products. All these microorganisms isolated in this study are of food processing and public health concerns and hence hazardous and injurious to human health if consumed. Fungi belonging to these species can produce a wide range of secondary metabolites under favourable conditions some of which are toxic.

Approximately 50% of Aspergillus flavus strains isolated from dried fish was able to produce AFs. The percentage of aflatoxigenic fungi among A. flavus isolated from dried fish is depend on several factors including the type of fish, environmental conditions, culture conditions, detection method, etc. Quantification of aflatoxins from the product revealed that the observed fungal counts are above the legal limits and confirms potential exposure to significant level of toxin from fungal infected fish. Various fungi are capable of producing toxic secondary metabolites named “mycotoxins” such as aflatoxin, ochratoxin, patulin and zearalenone (Bennett and Klich, 2003). Among the toxigenic aflatoxins (AFs) are important fungal toxic compounds, which are produced by an expanding list of closely related fungi mainly belong to Aspergillus section Flavi specially Aspergillus flavus and A. parasiticus (Eaton and Groopman, 1994). These fungi are widely distributed in soil, air, organic materials and all over the world (Bennett and Klich, 2003). Toxigenic A. flavus strains that possess all necessary genes for AF biosynthesis produce either AFB1 and/or AFG1 (Eaton and Groopman, 1994). AFB1 and G1 currently one of the most important mycotoxins that is under regulation by the Food and Drugs Administration (FDA) (Coulombe, 1993). Aflatoxin production has also been reported to occur at favourable temperature conditions of 28-30°C and relative humidity of 90% (Arun et al., 1987). Aflatoxins have been extensively studied due to their ubiquitous nature and toxicity in the worlds food supply (Theuver and Rubenstein, 2011). Aflatoxins occur mainly in the forms B1, B2, G1 and G2, so called because of the fluorescent colors blue or green displayed on exposure to long wave UV light. Aflatoxins are mutagenic, teratogenic and carcinogenic
and can cause acute liver damage including cirrhosis (Wang and Tang, 2005 and Groopman et al., 2008). Long term exposure could lead to general adverse health effects such as immune suppression, childhood stunting and interference with protein uptake (Williams et al., 2004). Although acute toxicity of aflatoxins is rare, several incidences including fatalities have been reported due to the consumption of contaminated foods (Lewis et al., 2005).

Sharma (1992) reported that the two major metabolites of Aspergillus sp. called aflatoxins were designated B1 and G1 because they fluoresce blue (B1) and green (G1) when exposed to long-wave ultraviolet light. In this study aflatoxin was detected in all of the samples. The concentration of aflatoxin B1 and G1 ranged between 0.001 - 5.492μg/kg and 0.01 - 2.296μg/kg respectively. This indicate that the dried fish samples have been contaminated by fungi especially Aspergillus flavus, which produced the toxins. Aflatoxins are highly carcinogenic, causing hepatoma (cancer of liver) and have also been associated with acute hepatitis in man, mostly in the developing world (Krog, 1992; Prasad, 1992; Eaton and Groopman, 1994). Most of detected levels of aflatoxins were over the permissible limits in food reported by (WHO, 1979) (who stated that the aflatoxins must be not more than 15 ppb and 5μg/kg) and (FAO,1995; FDA, 2000) (who stated that the levels of aflatoxins must be not more than 20 ppb/6μg/kg in food). Hence, most of detected levels were health hazard for consumers where, cases of carcinogenic effects for internal vital organs are resulted particularly for liver and kidney. According to the regulatory levels for aflatoxins issued by the Food and Drug Administration (FDA) of the United States (The FDA regulatory levels for aflatoxin intake for humans and all animal species is maximum of 20ppb). Adebayo-Tayo et al. (2006) reported different results in marketed dried fish stored for sale in Uyo, Akwa-Ibom State, whereby the presence of aflatoxin were in higher concentrations (The aflatoxin concentrations were between 1.5 – 8.1 μg/kg) in the samples which might make their consumption hazardous to health.

Jonsyn and Lahai (1992) mentioned that 20 samples of dried fish obtained from homes and markets in Njala, Sierra Leone were contaminated with four Aspergillus species, A. flavus, A. ochraceus, A. tamarii and A. niger. Varying amounts of aflatoxins B1 and G1 were detected in the moldy fish. All these fungal species have been previously reported to have the ability to produce mycotoxins (Bugno et al., 2006; Reddy et al., 2010; Ihesiulor et al., 2011). According to Pohland and Wood (1997) 70-80% of the Penicillia are potential producer of mycotoxins. Contamination of fish with mycotoxin producing fungi could lead to accumulation of these toxins in fish tissues. The risk for mycotoxins contamination may be occurred as a result of using the contaminated fish tissues, especially in great quantities. The result supports our hypothesis that the contaminated fishes with fungi contain mycotoxin residues that affect the public health. Bukola et al. (2008) detected aflatoxins B1 and G1 concentrations ranging from 1.50 - 8.10μg/kg and 1.81 - 4.5μg/kg respectively. This finding is instructive as consumption of contaminated smoked fish could pose serious health problems. Wheeler et al. (1986) in their study of Indonesian dried fish conducted aflatoxins analysis on several fish from which A. flavus was isolated and found none of the samples to be positive for the toxin. The high moisture content of the samples (22.7 to 27.6%) could have supported toxin production agreed with this study. In another study, TLC analysis of six moldy fish extracts of dried fish was positive for aflatoxins B1 and G1 (Jonsyn and Lahai, 1992). In Tanzania, 16% of A. flavus isolated from traditional cured fish from markets was aflotoxigenic and contamination levels of up to 18.5μg/kg (Mugula and Lyim, 1992). Nwokolo and Okonkwo (1978) reported dried fish from semi-Savannah and forest areas in Nigeria, contaminated with high toxin levels of 400 - 800ng/g. Most countries have applied a regulatory limit of 2 or 4ng/g for aflatoxins B1 and 4 to 20ng/g for total aflatoxins in a wide range of foods (FAO, 2004). The presence of toxin above the threshold limits in dried fish indicates widespread exposure of aflatoxins through regular consumption of these foods.

5. Conclusion

Many methods can be applied to control the growth of fungi and mycotoxin production in dried fish. The most practical of these would be to reduce the water activity to a sufficiently low level to inhibit toxigenic fungi. Other strategies such as modified atmospheric packaging or biological control could also be adopted. A high probability of co-occurrence of these mycotoxins is expected to increase the food safety risk due to possible synergistic effects. Hence consumption of products infected with fungi is likely to pose a health hazard. Improper salting and drying of fishes may lead to insect infestation, fungal attack, fragmentation and degradation of the product. Since most of the moulds isolated are probably contaminants rather than originating in the fish samples, better methods of preservation (drying and storage) will reduce their incidence or eliminate them. The main conclusion of this study is the confirmation of the infection of dried fishes with mycotoxin producing fungi. Since most of the fungus isolated was possible contaminants rather than originating from the fish.  

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samples, better preservation and handling would reduce mycoflora proliferations.

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