**FUNGI ASSOCIATED WITH SPOILAGE OF SMOKED DRIED FISH**

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**ABSTRACT**

This study was carried out on fungi associated with spoilage of smoked dried fish. To achieve this 2 research objectives were formulated. The study made use of four fish samples. The Four fish samples used for the study were purchased from four different location A,B,C and D in Eke-Awka market. Test tube, Text tube rack, Conical Flask, Electric blender, Autoclave, Cork borer, Microscope and Inoculating loopThe study ultilized were used to carry out the study. The reagents used in the study include the Distill water and Ethanol. The preparation culture media(SDA Agar) for Fungi and mode for identification of microorganism were respective described in chapter three of the study. The evidence from the study have showed that there are fungi associated with smoked dried fish and those fungi possess mycotoxins that pose danger to human and animal health as they are toxic to vertebrates and other animals in low concentrations. This indicates that it is necessary to show a dose-response relationship between the mycotoxin and the disease which this study to has evidently shown. Hence, it can be said that most of the smoked dried fish vended in Eke Awka contain some level of fungi contamination. Based on the findings, the researcher recomended that proper care should be taken by fish vendors during processing and sell. And fishes meant for consumption should be properly washed and boiled in other to kill any presence of fungi and it micro toxin.

**CHAPTER ONE**

**INTRODUCTION**

**1.1 Background Of The Study**

Fish is highly nutritious with high protein content. However, it is a suitable medium for growth of microorganisms, if poorly processed (Oparaku and Mgbenka, 2012). The growth of microorganisms and other non-microbial activities such as lipid oxidation contribute to the deterioration of fish products (Martin, 2010). An increase in the ambient temperature triggers favourable conditions for microorganisms to thrive, which reduces the quality of fish and its potential keeping time leading to food loss (Abolagba *et al.*, 2011).

Preserving food and other perishable products like fish and meat generally involves processes that impede growth of microorganisms either by the addition of growth inhibiting ingredients or adjusting storage conditions by freezing or drying (Akise *et al*., 2013). Processing methods affect the microorganisms in fish in different ways, resulting in different types of micro-flora and different risks from spoilage organisms and pathogens.

In dried fish, the micro-flora are prevented from growing by the storage method used and the product may have a long shelf life in the preserved state. However, the microbial load of fish rarely indicates the quality of the fish, but gives an indication of the risk of spoilage induced since each of the organisms has different ways of affecting the health conditions of consumers of such contaminated fish (Gram *et al*.,2015). As result there is a need to investigate microorganisms such fungi that are associated to fish spoilage. This necessitated this study into investigating fungi associated with smoked dried fish vended in Eke –Awka Market in Anambra state.

**1.2 Aims/Objectives of the study.**

The aim of the study is to investigate the fungi associated with spoilage of smoked dried fish. Specifically, the objective of the study is to;

1. Isolated the fungi’s associated to smoked dried fish vended in Eke-Awka market.
2. To identify the fungi associated to smoked dried fish.

**1.3 Scope of the study**

The scope of the study covers the fungi associated with smoked dried fish that are vended in Eke- Awka market. The scope will also cover the isolation and identification of fungi associated to smoked dried fish.

**1.4 Justification**

* Smoked dried fish constitute a major source of animal protein for a vast majority of the population in Nigeria, particularly the rural areas.
* It is therefore important from toxicological points of view to investigate the growth and effects of fungi on smoked —dried fish.

**CHAPTER TWO**

**LITERATURE REVIEW**

Fungi are parasitic, that is they rely on a nutritional supply from a host (you) in order to survive. Without carbohydrate, they will soon perish. Carbohydrates are foods that produce sugar upon digestion. It is also important to re-establish good bacteria in the bowel(Ogbonna,2013). The bowel is an entire ecosystem containing good bacteria and harmless yeasts. A disruption within this ecosystem is called dysbiosis. Normally your intestinal ecosystem serves two functions. It protects and defends. When you take antibiotics, birth control pills and cortisone, they negatively impact this ecosystem. If you have taken any of these you need to supplement with "good bacteria". Health food stores carry probiotics in pill or powder form. It is impossible to restore your health without restoring the integrity of the intestines.

Microbial tests of fish and fish products are used by the industry for contractual and internal purposes and by the authorities to check that the microbiological status is satisfactory (Jay, 2015). The micro-flora in which fungi are a part consists of the microorganisms that normally live with the animals. Studies on microflora count and characterization are many in order to establish a baseline that is satisfactory for the consumption populace. Such microflora isolation studies have been reported by Martin (2011), Olawale *et al.* (2015), Adesokan *et al*. (2015), and Abolagba and Igbinevbo (2010) for smoked fish (*Clarias spp*) sold in markets in Benin Nigeria. It is generally accepted that fish with microbial load greater than106cfu/g is likely to be at the stage of being unacceptable from the microbiological point of view and unfit for consumption (Cheesbrough, 2010). Akise *et al.* (2013) reported that high microbial load and unfavourable composition from smoke-dried *Lutjanus agennes* (Red Snapper), *Mugil cephalus* (Mullet) and *Chrysichthys walkeri* (Catfish) during shelf storage poses a serious health concern for consumers and public health workers.

**2.2 Microbial Activities of Fungi**

According to Josphus,(2011), fungi play a huge role in the breakdown of plant and animal matter and return it to the soil to be used again. Researcher has shown that the number of identified fungal species approaches 100,000, and it is estimated that there may be more than 1.5 million that have yet to be recognized( Musa,2015). Yeasts are single-celled organisms, whereas moulds and mushrooms are groups of cell clustered together. They can reproduce either by sexually- by sharing DNA, dividing and growing- or asexually, either by releasing spores into the environment that form new fungi, or by budding new yeast cells from a parent cell. Spores can lay dormant for many years until the condition are favorable for them to multiply.

**2.3 Microbial Spoilage of Food**

Composition of the microflora on newly caught fish depends on the microbial contents of the water in which the fish live. Fish microflora includes bacterial species such as Pseudomonas, Alcaligenes, Vibrio, Serratia and Micrococcus (Gram and Huss, 2010). Microbial growth and metabolism is a major cause of fish spoilage which produce amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable off-flavors (Dalgaard *et al.,* 2016; Emborg *et al.,* 2015; Gram and Dalgaard, 2012). For unpreserved fish, spoilage is a result of Gram-negative, fermentative bacteria (such as *Vibrionaceae*), whereas psychrotolerant Gram-negative bacteria (such as Pseudomonas spp. and *Shewanella spp*.).

Microbial spoilage impedes at a recommended storage temperature of -9 to -12°C. However, enzyme present in the fish will still play an important part in spoilage. About 10-60% of the viable microbial population die during freezing yet the remaining population gradually increase during frozen storage (Rahman, 2014). Morlier *et al*. (2010) reported that Anasakis simplex, a nematode found in certain fish, can be killed by freezing to -20°C and holding at least 24 h. Raj and Liston (2009) reported that only a 1 log10 reduction in numbers of Salmonella typhimurium when fish was frozen at -22°C and stored at -17.9°C for over 1 year. Miladi *et al.* (2011) found that freeze storage of L. monocytogenes in salmon does not decrease the potential survival of food-borne pathogens over a period of ten months at freezing temperature of -20°C.. Arannilewa *et al*. (2005) investigated the effect of duration of freeze storage on the chemical, microbiological and sensory profile of tilapia fish (Sarotherodun galiaenus). They reported decreases in the values of protein and fat by 27.9 and 25.92%, respectively. The total coliform count was increased from 3.0×103 -7.5×106 during storage

Yeasts fungi are opportunistic organisms. This means that, as intestinal bacteria die, yeasts thrive, especially when their dietary needs are met. They can use their tendrils, or hyphae, to literally poke holes through the lining of your intestinal wall. This results in a syndrome called leaky gut. Yeasts are not the only cause of this syndrome. Some scientists have linked non-steroidal and anti-inflammatory drugs as well as antibiotics( webmer,2014).

**2.4 Anatomy of Fungi Organism**

Fungal cells are overall very similar to human cells in that not only do they contain a nucleus with DNA, but they also share many of the same physiologic and biologic pathways required for growth and reproduction. They are more closely related to human cells, based on function and gene content than either viruses or bacteria(Hassan,2016).This similarity to human cell poses a challenge to researchers in that it is difficult to find a drug that will selectively kill the fungus and not the human. Another challenge is that fungal cells can be confused for either normal human blood cells or even malignant cancer cells. Without the proper staining techniques by microbiologists and pathologists, the identification of fungal organisms can be entirely missed. Treatment errors may then follow. It is possible to receive an antiobiotic for an infection when what you require is an antifungal. In fact the antiobiotic only worsens the fungal infection by destroying our intestinal bacteria that normally serves to protect against yeast overgrowth.

In a study of mycoflora of smoked–dried fishes sold in Uyo, Eastern Nigeria by Adebayo–Tayo *et al;* (2015),listed 12 different fungi associated with the smoked–dried fish samples. The associated fungi were:

* *Aspergillus flavus*
* *Aspergillus tereus*
* *Aspergillus fumigatus*
* *Absidia sp*
* *Rhizopus sp*
* *Aspergillus niger*
* *Mucor sp*
* *Clasdosporium sp*
* *Penicillium italiculum*
* *P. Viridatus*
* *Candida tropicalis*
* *Fusarium moniliformis*

**2.4 Scientific Classification of Fungi**

Kingdom: Fungi

Division: Ascomycota

Class: Eurotiomycetes

Order: Eurotiales

Family: Trichocomaceae

Genus: *Aspergillus*

Species: *A. spp*

Fungi *are* saprotrophic and pathogenic fungus with a cosmopolitan distribution ( Masayuki Machida *et al;* 2016). It is best known for its colonization of cereal grains , legumes , and tree nuts . Postharvest rot typically develops during harvest, storage, and/or transit. A. flavus infections can occur while hosts are still in the field (preharvest), but often show no symptoms ( dormancy ) until postharvest storage and/or transport. In addition to causing preharvest and postharvest infections, many strains produce significant quantities of toxic compounds known as mycotoxins , which, when consumed, are toxic to mammals ( Agrios &George, 2015). A. flavus is also an opportunistic human and animal pathogen , causing *aspergillosis* in immunocompromised individuals.

Aspergillus terreus , also known as Aspergillus terrestris , is a fungus (mold) found worldwide in soil. Although thought to be strictly asexual until recently, A. terreus is now known to be capable of sexual reproduction ( Arabatzis. , *et al;* 2013). This saprotrophic fungus is prevalent in warmer climates such as tropical and subtropical regions. Aside from being located in soil, *A. terreus* has also been found in habitats such as decomposing vegetation and dust. *A. terreus* is commonly used in industry to produce important organic acids, such as itaconic acid and cis -aconitic acid, as well as enzymes, like xylanase (Shimada *et al.* 2012).

*Aspergillus fumigatus is* a fungus of the genus Aspergillus , and is one of the most commonAspergillus species to cause disease in individuals with an immunodeficiency( Michelle,2014) . *A. fumigatus*, a saprotroph widespread in nature, is typically found in soil and decaying organic matter, such as compost heaps, where it plays an essential role in carbon and nitrogen recycling. Colonies of the fungus produce from conidiophores thousands of minute grey-green conidia (2–3 μm) that readily become airborne. For many years, *A. fumigatus* was thought to only reproduce asexually, as neither mating nor meiosis had ever been observed. In 2008, however, *A. fumigatus* was shown to possess a fully functional sexual reproductive cycle, 145 years after its original description by Fresenius ( O'Gorman; *et al.* 2016). Although *A. fumigatus* occurs in areas with widely different climates and environments, it displays low genetic variation and lack of population genetic differentiation on a global scale ( Rydholm C; et al. 2006). Thus the capability for sex is maintained even though little genetic variation is produced.

The fungus is capable of growth at 37 °C or 99 °F ( normal human body temperature ), and can grow at temperatures up to 50 °C or 122 °F, with conidia surviving at 70 °C or 158 °F—conditions it regularly encounters in self-heating compost heaps. Its spores are ubiquitous in the atmosphere, and it is estimated that everybody inhales several hundred spores each day; typically these are quickly eliminated by the immune system in healthy individuals. In immunocompromised individuals, such as organ transplant recipients and people with AIDS or leukemia , the fungus is more likely to become pathogenic , over-running the host's weakened defenses and causing a range of diseases generally termed aspergillosis . Several virulence factors have been postulated to explain this opportunistic behaviour (Abad *et al.* 2010).

**Absidia fungi:** is a genus of fungi in the family Cunninghamellaceae . The best-known species is the pathogenic Absidia corymbifera, which causes zygomycosis , especially in the form of mycotic spontaneous abortion in cows. It can also cause mucormycosis in humans. It is an allergenic that could cause mucorosis in individuals with low immunity. It usually infects the lungs, nose, brain, eyesight and skin. Absidia spp. are ubiquitous in most environments. They are often associated with warm decaying plant matter, such as in compost heaps ( Rydholm, *et al*.2016).

**Rhizopus**: This is a genus of common saprophytic fungi on plants and specialized parasites on animals. They are found on a wide variety of organic substrates, including "mature fruits and vegetables", jellies, syrups, leather, bread, peanuts, and tobacco ( Kirk *et al.* 2008). Some Rhizopus stolonifer species are opportunistic agents of human zygomycosis (fungal infection) and can be fatal. Rhizopus infections may also be a complication of diabetic ketoacidosis ( Chinn *et al.* 2015). This widespread genus includes at least eight species (Abe *et al.* 2010). Rhizopus species grow as filamentous, branching hyphae that generally lack cross-walls (i.e., they are coenocytic ). They reproduce by forming asexual and sexual spores. In asexual reproduction, sporangiospores are produced inside a spherical structure, the sporangium . Sporangia are supported by a large apophysate columella atop a long stalk, the sporangiophore(Ogbonnaya,2014). Sporangiophores arise among distinctive, root-like rhizoids. In sexual reproduction, a dark zygospore is produced at the point where two compatible mycelia fuse. Upon germination, a zygospore produces colonies that are genetically different from either parent.

***Aspergillus niger*** : this is a fungus and one of the most common species of the genus Aspergillus. It causes a disease called black mould on certain fruits and vegetables such as grapes, apricots, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of Stachybotrys (species of which have also been called "black mould") (Samson RA; *et al*. 2001). Some strains of A. niger have been reported to produce potent mycotoxins called

*ochratoxins ;* ( Abarca *et al.* 2011) other sources disagree, claiming this report is based upon misidentification of the fungal species. Recent evidence suggests some true A. niger strains do produce ochratoxin A ( Schuster ;*et al.* 2012). It also produces the isoflavone orobol .

**2.8 *Mucor spp***

Scientific classification

Kingdom: Fungi

Division: Zygomycota

Class: Mucormycotina

Order: Mucorales

Family: Mucoraceae

Genus: Mucor

Mucor is a microbial genus of approximately 6 species of moulds commonly found in soil, digestive systems , plant surfaces, rotten vegetable matter and iron oxide residue in the biosorption process.

According to Akande and Tobor,(2016) 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 smoke drying period, smoking kilns used in artisanal fishery and the overloading of the fishes on the trays leads to improper processing which in turn encourages fungal attack (Eyo, 2011). During storage of smoked dried fish products, good storage practices are not adhering by wholesaler hence stores are not well ventilated and pest can easily gain access into the stores. The environment in which fishes are displayed in the market is not always hygienic and this is another avenue for microbial contamination. Very often, retailers display the smoked-dried fish samples in open trays beside the gutter on refuse heaps, this also encourages fungi attack and subsequent production of toxins.

**2.3 Effect of Fungi Mycotoxins Animals**

Mycotoxins are secondary metabolites of molds that have adverse effects on humans, animals, and crops that result in illnesses and economic losses. The worldwide contamination of foods and feeds with mycotoxins is a significant problem(Canel,2014). Aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins, and ergot alkaloids are the mycotoxins of greatest agro-economic importance. Some molds are capable of producing more than one mycotoxin and some mycotoxins are produced by more than one fungal species. Often more than one mycotoxin is found on a contaminated substrate. Mycotoxins occur more frequently in areas with a hot and humid climate, favourable for the growth of molds, they can also be found in temperate zones(Taro,203). Exposure to mycotoxins is mostly by ingestion, but also occurs by the dermal and inhalation routes. The diseases caused by exposure to mycotoxins are known as mycotoxicoses. However, mycotoxicoses often remain unrecognized by medical professionals, except when large numbers of people are involved. Factors influencing the presence of mycotoxins in foods or feeds include environmental conditions related to storage that can be controlled. Other extrinsic factors such as climate or intrinsic factors such as fungal strain specificity, strain variation, and instability of toxigenic properties are more difficult to control(Charls,2014). Mycotoxins have various acute and chronic effects on humans and animals (especially monogastrics) depending on species and susceptibility of an animal within a species(Onyeka,2010). Ruminants have, however, generally been more resistant to the adverse effects of mycotoxins. This is because the rumen microbiota is capable of degrading mycotoxins. The economic impact of mycotoxins include loss of human and animal life, increased health care and veterinary care costs, reduced livestock production, disposal of contaminated foods and feeds, and investment in research and applications to reduce severity of the mycotoxin problem. Although efforts have continued internationally to set guidelines to control mycotoxins, practical measures have not been adequately implemented(Stephen,2015)

Toxigenic molds are known to produce one or more of these toxic secondary metabolites. It is well established that not all molds are toxigenic and not all secondary metabolites from molds are toxic. Examples of mycotoxins of greatest public health and agro-economic significance include aflatoxins (AF), ochratoxins (OT), trichothecenes, zearalenone (ZEN), fumonisins (F), tremorgenic toxins, and ergot alkaloids. These toxins account for millions of dollars annually in losses worldwide in human health, animal health, and condemned agricultural products. Factors contributing to the presence or production of mycotoxins in foods or feeds include storage, environmental, and ecological conditions. Often times most factors are beyond human control ( Hussein & Brasel, 2011 ). Ochratoxin A (OTA) is a secondary metabolite produced by several species of Aspergillus and Penicillium . The toxin, which is a nephrotoxic and nephrocarcinogenic compound, has mainly been found in cereals as well as in other products like coffee, wine, dried fruits, beer and grape juice. It occurs in the kidney, liver and blood of farm animals by transfer from animal feed. Although its genotoxic power has so far not been definitively established, zearalenone (ZEA), produced by various species of Fusarium , in particular Fusarium graminearum and Fusarium culmorum , has an osteogenous action and is significantly toxic to the reproductive system of animals ( Milicevic *et al.,* 2010 ).

Human food can be contaminated with mycotoxins at various stages in the food chain (Bennett & Klich, 2013) and the most important genera of mycotoxigenic fungi are Aspergillus , Alternaria , Claviceps , Fusarium , Penicillium and Stachybotrys. The principal classes of mycotoxins include a metabolite of A. flavus and Aspergillus parasiticus , aflatoxin B (AFB ), the most potent hepatocarcinogenic substance known, which has been recently proven to also be genotoxic. In dairy cattle, another problem arises from the transformation of AFB and AFB into hydroxylated metabolites, aflatoxin M and M (AFM and AFM ), which are found in milk and milk products obtained from livestock that have ingested contaminated feed (Boudra et al., 2007 ). In 1993, the WHO-International Agency for Research on Cancer (WHO-IARC, 1993a,b ) evaluated the carcinogenic potential of AF, OT, trichothecenes, ZEN, and F. Naturally occurring AF were classified as carcinogenic to humans (Group 1) while OT and F were classified as possible carcinogens (Group 2B). Trichothecenes and ZEN, however, were not classified as human carcinogens (Group 3). The health hazards of mycotoxins to humans or animals have been reviewed extensively in recent years ( Yaling *et al*., 2011; Averkieva, 2009 ).

**2.4 Occurrence and Significance of Mycotoxins in Foods**

Mycotoxicoses in humans or animals are characterized as food or feed related, non-contagious, non-transferable, non-infectious, and non-traceable to microorganisms other than fungi. Clinical symptoms usually subside upon removal of contaminated food or feed. A wide range of commodities can be contaminated with mycotoxins both pre- and post-harvest (CAST, 2013 ). Aflatoxins (AFTs) are found in maize and peanuts, as well as in tree nuts and dried fruits. OTA is found mainly in cereals, but significant levels of contamination may also occur in wine, coffee, spices and dried fruits. Other products of concern are beans, roasted coffee and cocoa, malt and beer, bread and bakery products, wines and grape juices, spices, poultry meat and kidneys, pig kidneys and pork sausages ( Milicevic *et al.,* 2011 ).

**Aflatoxins**

Aflatoxin contamination has been linked to increased mortality in farm animals and thus significantly lowers the value of grains as an animal feed and as an export commodity. Milk products can also serve as an indirect source of aflatoxin. When cows consume aflatoxin-contaminated feeds, they metabolically biotransform aflatoxin B into a hydroxylated form called aflatoxin M ( Van Egmond,2009). Aflatoxin is associated with both toxicity and carcinogenicity in human and animal populations. The diseases caused by aflatoxin consumption are loosely called aflatoxicoses. Acute aflatoxicosis results in death; chronic aflatoxicosis results in cancer, immune suppression, and other “slow” pathological conditions. The liver is the primary target organ, with liver damage occurring when poultry, fish, rodents, and nonhuman primates are fed aflatoxin B . There are substantial differences in species susceptibility. Moreover, within a given species, the magnitude of the response is influenced by age, sex, weight, diet, exposure to infectious agents, and the presence of other mycotoxins and pharmacologically active substances. Thousands of studies on aflatoxin toxicity have been conducted, mostly concerning laboratory models or agriculturally important species ( Cullen and Newberne, 2014 ).

**Ochratoxins**

Ochratoxin A (OTA) is produced by fungi of the genera Aspergillus and Penicillium . The major species implicated in OTA production includes *Aspergillus ochraceus, Aspergillus carbonarius , Aspergillus melleus , Aspergillus sclerotiorum , Aspergillus sulphureus , Pichia verrucossum* . However, *Aspergillus* *niger* and *Pichia* *purpurescens* are less important OTA producers ( Benford *et al.,* 2011 ). OTA is a frequent natural contaminant of many foodstuffs such as cocoa beans, coffee beans, cassava flour, cereals, fish, peanuts, dried fruits, wine, poultry eggs and milk ( Weidenborner, 2011 ). The mycotoxin was reported in 35% in “under-five clinics” of breast milks in Southern province of Sierra Leone with up to 22% cooccurrence with aflatoxins. However, the scientists observed that whenever OTA was detected in high levels, AFB was absent or present at very low levels and vice versa which suggests some sort of competition between these toxins either at the production level in foodstuffs or in their rate of absorption in the gastrointestinal tract. OTA has also been reported as a contaminant of tiger nuts and fermented maize dough in West Africa ( Kpodo, 2010 ).

**. Fumonisins**

Fumonisins (B and B ) are cancer-promoting metabolites of Fusarium proliferatum and Fusarium verticillioides that have a long-chain hydrocarbon unit (similar to that of sphingosine and sphinganine) which plays a role in their toxicity. Fumonisin B (FB ) is the most toxic and has been shown to promote tumor in rats and cause equine leukoencephalomalacia and porcine pulmonary edema. The naturally co-occurring aminopentol isomers (formed by base hydrolysis of the ester-linked tricarballylic acid of FB ) have been suggested to exert toxic effects due to their structural analogy to sphingoid bases (Humpf *et al.*, 2013 ). Consumption of fumonisin has been associated with elevated human oesophageal cancer incidence in various parts of Africa, Central America, and Asia and among the black population in Charleston, South Carolina, USA. Because fumonisin B reduces uptake of folate in different cell lines, fumonisin consumption has been implicated in neural tube defects in human babies. Some correlation studies have suggested a link between the consumption of maize with high incidence of F. verticillioides and fumonisins and the high incidence of human oesophageal carcinoma in certain parts of South Africa (Marasas *et al.,* 2014 ).

**Trichothecenes**

The trichothecene mycotoxins (TCT) comprise a vast group of over 100 fungal metabolites with the same basic structure. Several fungal genera are capable of producing TCT; however, most of them have been isolated from Fusarium spp. All trichothecene contain an epoxide at the C , positions, which is responsible for their toxicological activity. At the cellular level, the main toxic effect of TCT mycotoxins appears to be a primary inhibition of protein synthesis. TCT affect actively dividing cells such as those lining the gastrointestinal tract, the skin, lymphoid and erythroid cells. The toxic action of TCT results in extensive necrosis of the oral mucosa and skin in contact with the toxin, acute effect on the digestive tract and decreased bone marrow and immune function (Schwarzer, 2009 ). The trichothecene mycotoxins occur worldwide in grains and other commodities. Toxin production is greatest with high humidity and temperatures of 6–24 °C. Natural occurrence of TCT has been reported in Asia, Africa, South America, Europe, and North America (Scott, 2011). Trichothecenes have been detected in corn, wheat, barley, oats, rice, rye, vegetables, and other crops. They are common contaminants of poultry feeds and feedstuffs and their adverse effects on poultry health and productivity have been studied extensively (Leeson et al., 1995 ). Examples of type A TCT include T-2 toxin (T-2) and HT-2 toxin (HT-2), and diacetoxyscirpenol (DAS). Fusarenone-X (FUX), deoxynivalenol (DON), and nivalenol (NIV) are some of the common naturally occurring type B TCT. Types A and B trichothecene are distinguished by the presence or absence.

**Zearalenone**

Zearalenone is a mycotoxin produced by *F. graminearum* and other *Fusarium* molds using corn, wheat, barley, oats and sorghum as substrates. It is a non-steroidal compound that exhibits oestrogen-like activity in certain farm animals such as cattle, sheep and pigs. Zearalenone is a phenolic resorcyclic acid lactone with potent oestrogenic properties, produced primarily by *Fusarium* (Schwarzer, 2009 ). Zearalenone is a phytoestrogenic compound known as 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcylic acid μ-lactone. It is a metabolite primarily associated with several Fusarium species (i.e. *F. culmorum , F .graminearum* , and *F . sporotrichioides*) with *F . graminearum* being the species most responsible for the oestrogenic effects commonly found in farm animals. Alcohol metabolites of ZEN (i.e. α-zearalenol and β-zearalenol) are also oestrogenic ( Cheeke, 1998a ).

**Moniliformin**

Moniliformin (i.e. a potassium or sodium salt of 1-hydroxycyclobut-1-ene-3,4-dione,) is produced by several Fusarium species (mainly F . proliferatum) and is usually found on the corn kernel. It can be transferred to next generation crops and survive for years in the soil. Although both FB and moniliformin are produced by the same fungal species (F . proliferatum ) no structural resemblance is found between the two toxins ( Price et al., 1993 ).

**2.5 Negative Effects of Mycotoxins on Humans**

Mycotoxicoses, like all toxicological syndromes, can be categorized as acute or chronic. Acute toxicity generally has a rapid onset and an obvious toxic response, while chronic toxicity is characterized by low-dose exposure over a long time period, resulting in cancers and other generally irreversible effects (James, 2015 ). Prior to the discovery and implementation of modern milling practices, Fusarium species have been implicated in several human outbreaks of mycotoxicoses. Cereal grains contaminated with *F. sporitrichoides* and F. poae were implicated in alimentary toxic aleukia in Russia from 1932 to 1947. Symptoms included mucous membrane hyperaemia, oesophageal pain, laryngitis, asphyxiation, gastroenteritis, and vertigo (Lewis *et al.,* 2015 ).

Aflatoxicosis is a toxic hepatitis leading to jaundice and, in severe cases, death. Repetitive incidents of this nature have occurred in Kenya , India, and Malaysia ( Shephard, 2014; Lewis *et al.,* 2015 ). AFB has been extensively linked to human primary liver cancer in which it acts synergistically with HBV infection and was classified by the International Agency for Research on Cancer (IARC) as a human carcinogen (Group 1 carcinogen) (IARC, 2015 ). This combination represents a heavy cancer burden in developing countries. A recent comparison of the estimated population risk between Kenya and France highlighted the greater burden that can be placed on developing countries (Shephard, 2016 ).

The largest risk of AF to humans is usually the result of chronic dietary exposure. Such dietary AF exposures have been associated with human hepatocellular carcinomas, which may be compounded by hepatitis B virus. Approximately 250,000 deaths are caused by hepatocellular carcinomas in China and Sub-Saharan Africa annually and are attributed to risk factors such as high daily intake (1.4 μg) of AF and high incidence of hepatitis B (Wild *et al*., 2014 ). Aflatoxins have been found in tissues of children suffering from Kwashiorkor and Reye’s syndrome and were thought to be a contributing factor to these diseases. Reye’s syndrome, which is characterized by encephalopathy and visceral deterioration, results in liver and kidney enlargement and cerebral edema (Blunden *et al.,* 2017 ). Aflatoxin has long been linked to Kwashiorkor, a disease usually considered a form of protein energy malnutrition, although some characteristics of the disease are known to be among the pathological effects caused by aflatoxins in animals. Aflatoxin exposure was associated with reduced levels of secretory immunoglobulin A (IgA) in Gambian children ( Turner *et al.,* 2013 ). Changes in differential subset distributions and functional alterations of specific lymphocyte subsets have been correlated with aflatoxin exposure in Ghanaian adults and indicate that aflatoxins could cause impairment of human cellular immunity that could decrease resistance to infections (Jiang *et al.*, 2015 ). Of the other health risk factors, the morbidity and mortality associated with unsafe sex, unsafe water and indoor smoke, arises from infectious diseases, such as HIV/AIDS, infectious diarrhoea and lower respiratory tract infection, respectively. The immunological suppression associated with aflatoxin and possibly DON could adversely affect all these outcomes. The modulating effect of aflatoxins in cases of zinc, iron and vitamin A deficiency in human health is less clear, but evidence from animal nutrition would suggest it could be significant ( Williams *et al*., 2014 ). Fumonisins have been implicated in one incident of acute food-borne disease in India in which the occurrence of borborygmy, abdominal pain, and diarrhoea was associated with the consumption of maize and sorghum contaminated with high levels of fumonisins. Fumonisin B , the most abundant of the numerous fumonisin analogues, was classified by the IARC as a Group 2B carcinogen (possibly carcinogenic in humans) ( IARC, 2012 ). Fumonisins, which inhibit the uptake of folic acid via the folate receptor, have also been implicated in the high incidence of neural tube defects in rural populations known to consume contaminated maize, such as the former Transkei region of South Africa and areas of Northern China ( Marasas *et al.,* 2014 ).

Both DON and ZEN from toxic Fusaria have been linked to scabby grain toxicoses in the USA, China, Japan, and Australia. Symptoms included nausea, vomiting, and diarrhea. Fumonisin B was associated with an illness outbreak in India with symptoms of acute onset of abdominal pain and diarrhea. Fumonisins also have been implicated in oesophageal cancer in China (Yoshizawa *et al*.,2014 ). However, with limited causal relationships and the presence of several confounding factors, data compiled by the International Agency for Research on Cancer were not conclusive for F carcinogenicity in humans ( Casegnaro and Wild, 2015 ). Trichothecenes have been suggested as potential biological warfare agents. For example, T-2 toxin was implicated as the chemical agent of ‘yellow rain’ used against the Lao Peoples Democratic Republic from 1975 through 1981 ( Peraica *et al.,* 2009 ). In an investigation of similar biological warfare agents in Cambodia from 1978 to 1981, T-2 toxin, DON, ZEN, nivalenol, and DAS were isolated from water and leaf samples collected from the affected areas (Peraica *et al*., 2009 ).

Clinical symptoms preceding death included vomiting, diarrhea, hemorrhage, breathing difficulty, chest pain, blisters, headache, fatigue, and dizziness. In addition to nephritic congestion, autopsy findings included necrosis of the lining of the stomach and upper small intestine, lungs, and liver. It should be noted, however, that the origin of the samples of yellow rain is still a subject of debate. For example, one theory attributed the source of illnesses to unidentified endemic factors because the yellow rain was found to be a native bee fecal material devoid of mycotoxins ( Seeley *et al.,* 2014).

**CHAPTER THREE**

**MATERIALS AND METHOD**

**3.1 Sample Collection**

The Four fish samples used for the study were purchased from four different location A,B,C and D in Eke-Awka market which is the largest market in Awka, named after one of the four market days. It is Located on a former community burial ground in the center of the city. Eke Awka has grown from a small market serving the needs of residents of the Agulu, Ezi-Oka and Amikwo sections of Awka to functioning as the main retail outlet for the city and neighbouring towns. It houses an estimated 5,000 lock-up shops and stalls all tightly packed into less than 35,000 square meters of space and has become infamous for causing tremendous traffic chaos with a medley of shoppers, buses, wheel barrows all jostling for the limited amount of space available.

**3.2 Equipment Used**

The equipment’s used in carrying out the study are as follows;

Test tube

Text tube rack

Conical Flask

Electric blender

Autoclave

Cork borer

Microscope

Inoculating loop

**3.3 Reagent Used**

The reagents used are as follow;Distill water and Ethanol

**3.4 Preparation Culture media(SDA Agar) for Fungi**

2g of the SDA was measured into 250ml beaker and 50ml distil water was added to the beaker containing the Sabourgaud agar(SDA). The mixture was shake to attain homogeneous mixture then made air tight with cotton wool and autoclaved at 1210c at the pressure of 15psi for 15 minutes. The media was poured into five petri dishes labelled respectively. The media was allowed to cooled then, 1ml of serial dilution of spoilt fish sample was introduced to the media in the petri dish and covers. The cultured petri dish of the fungi was kept for 72hrs to observe growth.

**3.5 Identification of Microorganism**

The growth of the colonies of the organisms were isolated and smell of each of the organism was made on a slide then stained in 1% methylene blue. The prepared slide was view under microscope. The morphological properties and anatomical structure of the organism viewed was match with the fungi identification kit and identified accordingly.

**CHAPTER FOUR**

**RESULT AND DISCUSSION**

The result of the finding is shown presented in the table shown below

**Table 1: Fungi isolated from the sample collected from four location in Eke- Awka Market**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Samples** | **Location A** | **Location B** | **Location C** | **Location D** |
| Claras garipenius | Aspergilius spp, Fusarium spp | Aspergilius spp, Fusarium and , mould fungi | Aspergilius spp, and Fusarium germanium | Aspergilius spp, fusarium hair |
| Orochromis niloticus | Aspergilius niger and Fusarium germanium | Aspergilius spp, Fusarium germanium,fusarium fumigatus | Aspergilius germanium , Fusarium hair and , mould fungi | Aspergilius spp, Fusarium and , mould fungi |
| Lampris | Aspergilius niger | Aspergilius germanium , Fusarium hair | Aspergilius fumigatus |  |
| Betta splendens | Aspergilius spp, Fusarium and , mould fungi | Aspergilius spp, Fusarium and , mould fungi | Aspergilius niger | Fusarium germanium |

Table 1 shows that from the there are various fungi organism that are found associated to smoked dried fish vended in Eke-Awka market. These fungi ranges from *Fusarium germanium, Aspergilius* *niger*, *Fusarium* hair, and *asprgilius* *fumigatus.*  These result may be due to the fact that the smoked dried fish samples are processed under unhygienic condition, or the sample were contaminated as result of their exposure in the market palce. The contamination of the sample may also be attributed to the nature of package and place of storage.

**Table 2: Physcio-Chemical Parameters**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Samples** | **weight before Experiment** | **weight after experiment** | **Moisture content before experiment** | **Temperature** |
| claras | 103g | 49g | 8% | 25±10C |
| Orochromis niloticus | 112g | 56g | 10% | 25±10C |
| Lampris | 98g | 45g | 6% | 25±10C |
| Betta splendens | 67g | 32g | 8% | 25±10C |

Table 2 shows that the weight of *claras garipinus* was 103g before the experiment, and 48g after the experiment. The moisture content and temperature was observed to to be 8% at 25±10C. this provided the conducive environmental condition viable for the fungi to grow on the fish samples. For *Orochromis* *niloticus,*  the weight decressed from 122g to 56g, 89g to 45g for *Lampris* and 67g to 32g for *Betta splendens* at environment condition of 10%,6% and 8% under room temperature of 25±10C respectively. The reduction in the weight of the sample was due to the fungi activities on the fish samples. This indicates that the infestation of fungi organism with time leads to corresponding degradation of the samples.

**Table 3: One way analysis of Variance (ANOVA)**

**Source of Variation DF SS MS F P**

Between Groups 3 908.333 302.778 0.153 0.025

Residual 8 15784.667 1973.083

Total 11 16693.000

P =0.05%

Table 3 shows that the infection of the sample by fungi and it association to is significant at P<0.05. At this level of significance, it can be said that the association of fungi on the sample is evident.

**CHAPTER FIVE**

**DISCUSSION, CONCLUSION AND RECOMMENDATION**

**5.1 Discussion**

Findings of the study indicated that various fungi organism are associated to smoked dried fish samples and this fungi are responsible for the degradation and micro toxin contamination of most food such as fish. Some of this fungi ranges from *Fusarium germanium, Aspergilius* *niger*, *Fusarium* hair, and *asprgilius* *fumigatus.*  The finding of the study also corroborate with the findings of Such Martin (2011), Olawale *et al.* (2015), Adesokan *et al*. (2015), and Abolagba and Igbinevbo (2010) for smoked fish (*Clarias spp*) Akise *et al.* (2013) reported that microflora isolation from fish sample high microbial load and unfavourable composition from smoke-dried *Lutjanus agennes* (Red Snapper), *Mugil cephalus* (Mullet) and *Chrysichthys walkeri* (Catfish).

The findings also showed the contamination of this fish samples are due to the method of processing, unhygienic condition and exposure in the market place to contaminated air and materials. The findings also indicate that that the contamination of this samples under favorable condition like temperature and moisture, the fungi causes great reduction in weight of the fish samples due to degradation. This findings agree with the findings of Josphus,(2011) who said fungi play a huge role in the breakdown of plant and animal matter and return it to the soil to be used again.

**5.3 Conclusion**

Evidence from the study have shown that there are fungi associated with smoked dried fish and those fungi possess mycotoxins that pose danger to human and animal health as they are toxic to vertebrates and other animals in low concentrations. The implication of this is that most consumers might have been consuming these metabolites and their prolonged intake may constitute a health hazard. In other to demonstrate that a disease is a mycotoxicoses, it is necessary to show a dose- response relationship between the mycotoxin and the disease which this study to has evidently shown. Hence, it can be said that most of the smoked dried fish vended in Eke Awka contain some level of fungi contamination

**5.4 Recommendation**

Based on the findings of the study, it is recommended that proper care should be taken by fish vendor during processing and sell. Fish meant for consumption should be properly washed and boiled in other to kill any presence of fungi and it micro toxin.

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