Isolation and identification of fungi from African Catfish (*Clarias gariepinus* Burchell, 1822) skin ulcers cultured in Abia State, Nigeria

Ozioko, C. A.[1]* and Oginyi, J. N.[1]

[1]Department of Veterinary Microbiology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

***Corresponding Author**: christiananene333@gmail.com; +2348065997219

Abstract

Fifty African Catfish (*Clarias gariepinus*) with skin ulcers were purposively sampled from farms in five local government areas (Umuahia South, Ikwuano, Umuahia North, Osisioma and Aba North) in Abia state, Nigeria. The fungi associated with the skin ulcers were isolated and identified using macroscopic and microscopic morphologies. The frequency of isolation and prevalence of the fungal genera were calculated. The infection rate of fungi on fish samples from Umuahia South, Ikwuano, Umuahia North, Osisioma and Aba North was 100%, 80%, 60%, 100% and 100%, respectively. Five fungal genera (*Aspergillus*, *Saprolegnia*, *Penicillium*, *Aphanomyces* and *Fusarium*) were isolated from the samples. A total of 98 fungal isolates were identified with *Aspergillus* spps being the highest (55), while *Fusarium* spps (2) and *Aphanomyces* spps (1) were least. The prevalence of the fungal genera isolated was *Aspergillus*: 70%, 60%, 40%, 30% and 40%; *Saprolegnia*: 50%, 60%, 60%, 50% and 50%; *Penicillium*: 40%, 20%, 20%, 10% and 20% in Umuahia South, Ikwuano, Umuahia North, Osisioma and Aba North, respectively, while *Aphanomyces* and *Fusarium* showed 10% and 20% prevalence, only in Umuahia South. The frequency of isolation of *Aspergillus (*41.7%, 75%, 40%, 52.2% and 60%), *Saprolegnia (*25%, 14.3%, 50%, 52.2% and 26.7%), and *Penicillium (*8.3%, 10.7%, 20%, 17.4% and 13.3%) varied in Umuahia South, Ikwuano, Umuahia North, Osisioma and Aba north, respectively; whereas *Fusarium* (16.7%) and *Aphanomyces* (8.3%) were found only in Umuahia South. The fungal organisms have the potential to be pathogenic. Hence, there is need for screening of African Catfish with skin ulcers, to ascertain the presence of disease causing organisms and possibly avoid human consumption.

Keywords: Abia State, African catfish, Fungi, *Saprolegnia,* Skin ulcer

Introduction

In the tropics, fish is a major source of dietary protein and vitamins to the general populace (Kumolu-Johnson and Ndimela, 2011). Consequently, fish farming is an important source of food, income, jobs and entrepreneurship opportunities (CTA, 2007). Fish protein is highly digestible, and rich in lysine and sulphur-containing amino acids, which are essential for proper nutrition and healthy living. Fish consumption helps to complement high carbohydrate diets, contributing 22% of required protein in Sub-Saharan Africa (Bhaskar, 1994; FAO, 2003). However, in Nigeria, there is a deficit in meeting the FAO recommended standard of 12.5 kg per head per year of minimum fish consumption (FAO, 2009).

African Catfish (*Clarias gariepinus* Burchell, 1822) is a member of family Clariidae,

normally coloured black or dark grey on its back and fading to white on its belly. It is a nocturnal fish that feeds on living as well as dead organic matter (Osungbemiro *et al.,* 2014). The rearing of African Catfish, in Central and West Africa, dates back to the 1970s. Catfish farming is considered to be a lucrative venture because of the numerous agricultural by-products that could be used in fish production and the nutritional benefits (such as vitamins, minerals, proteins and saturated fats) accruable from the fish (Kato *et al.,* 2016). Commercial farming of African Catfish has significantly increased in Abia state and Nigeria, becoming a major source of relatively cheap fish protein.

Fish supply from natural water bodies is becoming limited due to high extraction to meet the demands of the rising human population. Hence, aquaculture has become an essential source of about 30% of fish production (Fletcher *et al.,* 1999). Unfortunately, increased production of fish has the potential to increase transmission of aquatic diseases (Shagar and El-Rafaee, 2012). Hence, infectious fish diseases have been recognized as major constraints to aquaculture development in Nigeria (Nkemakolam *et al.,* 2011). Infectious fish diseases are caused by fungi, bacteria, viruses, or parasites and could be mold-induced (Bassey, 2011). Some fungi are primarily pathogenic, while others are opportunistic, requiring predisposing factors such as malnutrition, poor handling, poor water quality, fluctuating water temperature or overcrowding; to establish infection (Shagar and El-Rafaee, 2012).

The entire body of fish is covered by epidermis, which plays an important role in fish homeostasis. Hence, the integrity of the epidermis is essential for defense against opportunistic pathogens in the aquatic environment, which can easily gain access to the fish through open wounds. Damage to the epidermis does not only provide access to infectious agents but also predisposes the fish to life-threatening osmotic stress (Edward, 2000). Pathogenic organisms in the epidermis can kill fish as a result of osmotic shock related to epidermal damage. Also, the ulceration of about 10% of body surface area can cause acute mortality due to osmotic stress (Bouck and Smith, 1979).

Fungal pathogens are among the most important disease causing organisms in fresh and cultured fish, resulting in high economic losses. These diseases present clinical abnormalities in the form of skin darkening, necrotic foci with sloughing of tail, body fins with petechial hemorrhages, cotton wool like growth on the various parts of the skin and sloughing of the uppermost layers of the skin. These fungal diseases are caused by *Aspergillus niger, Aspergillus flavus, Penicillium* spps., *Alternaria* spps., *Cladosporum* spps., *Fusarium* spps., *Mucor* spps. and *Saprolegnia* spps. (Marzouk *et al*., 2003).

Many fish farmers rely on antibacterial agents as the sole remedy for skin diseases, neglecting the possibility of treating these anomalies with antifungal agents or a combination of both. Nevertheless, it is pertinent to identify and treat fish skin diseases caused by fungi because of their public health importance (Olojo *et al.,* 2010;

Efuntoye *et al.,* 2012). Furthermore, there is limited information on the isolation and identification of fungi from the skin ulcers of commercially reared African Catfish in Abia state, Nigeria. Therefore, this study isolated and identified fungi species from skin ulcers of African Catfish from different localities in Abia State, Nigeria.

Materials and Methods

Sample collection

African Catfish showing apparent skin ulcers (Figure 1) were collected from fish farms located in five local government areas in Abia State, Nigeria. Ten samples were purposively collected from each farm in each local government area. The samples collected were transported in plastic containers with pond water to the Department of Veterinary Microbiology Diagnostic Laboratory, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria; for isolation and identification of fungi on the skin ulcers of the fish samples.

The fish samples collected were sterilized with sterile cotton wool soaked in 70% ethanol and thereafter rinsed with sterile distilled water. Thereafter, the lesions were scraped with sterile scalpel blade into sterile plastic bottles and mixed in 2 ml of sterile water. The mixture was homogenized in a manual homogenizer. Then, 0.1 ml of the homogenized mixture was spread over Potato dextrose agar and Sabouraud dextrose agar supplemented with Chloramphenicol (250 µg/ml) (Pitt and Hocking, 1997). The inoculated plates were taped, incubated at 25°C and examined for fungal growth every 24 hours. The observed colonies were repeatedly sub-cultured on fresh plates of Potato dextrose agar medium until pure cultures were obtained at 25°C for 7 to 14 days.

The fungal isolates were identified by examining their morphology and microscopic features (Dugan, 2006). The morphological characteristics (macroscopic) observed included growth rate, general topography, surface and reverse pigmentation. Microscopic examination was carried out using slide culture techniques (Domsch *et al.,* 2007). Slides containing the fungal growths were stained with 0.05% of Trypan blue in Lactophenol. The slides were observed under a light microscope and photographed. The fungi were identified using fungal identification keys and literature (Willoughby, 1994; Abolude *et al.,* 2013). Microscopic characteristics of fungi such as hyphae, conidial heads and arrangement of conidia were observed.

The prevalence and frequency of isolation of the various fungal genera were calculated using equation 1 and 2, respectively.

$$\text{Prevalence (\%)} = \frac{\text{Number of positive samples}}{\text{Total number of samples collected}} \times 100 \text{ ----- (1)}$$

$$\text{Frequency (\%)} = \frac{\text{Number of fungal isolates}}{\text{Total number of fungal isolates}} \times 100 \text{ ------ (2)}$$

Data analysis

The data obtained were processed using descriptive and inferential statistics in Microsoft Excel Software (2007).

Results

Most of the fish samples were positive for multiple fungal growths on the media. The infection rate of samples from Umuahia South, Ikwuano, Umuahia North, Osisioma and Aba North was 100%, 80%, 60%, 100% and 100%, respectively (Table 1). A total of five fungal genera: *Aphanomyces* spps., *Saprolegnia* spps., *Aspergillus* spps., *Fusarium* spps. and *Penicillium* spps. were isolated from the *Clarias gariepinus* samples (Table 1). Figure 2 showed pure cultures of *Fusarium* and *Saprolegnia* spps. The microscopic structures of isolates from the five genera are shown in Figures 3 – 5.

A total of 98 fungal isolates were identified during the study (Table 2). The number of *Aspergillus spps.* isolated was highest (55), while *Fusarium* spps. (2) and *Aphanomyces* spps. (1) were least. *Aspergillus* spps. showed the highest frequency of isolation in samples from Ikwuano (75%), while *Saprolegnia* spps was highest in samples from Umuahia North (50%). *Aspergillus* spps., *Saprolegnia* spps. and *Penicillium* spps. showed the least frequency of isolation in samples from Umuahia North, Osisioma and Umuahia South, respectively (Figure 6).

Table 1: Fungi genera isolated from African Catfish obtained from fish farms and the fungal infection rate (%) in different local government areas in Abia State, Nigeria

Location	Fungi genera	Fungal infection rate (%)
Umuahia North	*Aspergillus* spps.	
	Saprolegnia spps.	60
	Penicillium spps.	
Umuahia South	*Aspergillus* spps.	
	Saprolegnia spps.	
	Penicillium spps.	100
	Fusarium spps.	
	Aphanomyces spps.	
Ikwuano	*Aspergillus* spps.	
	Saprolegnia spps.	80
	Penicillium spps.	
Aba north	*Aspergillus* spps.	
	Saprolegnia spps.	
	Penicillium spps.	100
Osisioma	*Aspergillus spps.*	
	Saprolegnia spps.	100
	Penicillium spps.	

Figure 1a and b: Ulcerative skin lesions on African Catfish samples collected from selected fish farms in Abia State, Nigeria

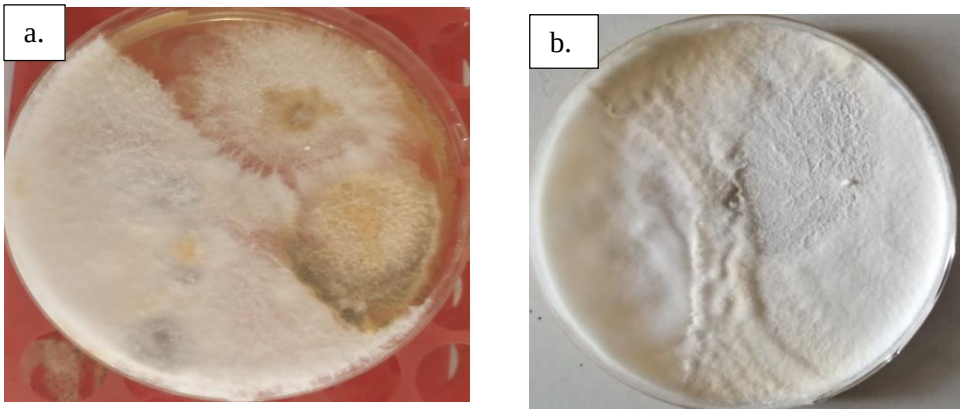


Figure 2. Pure cultures of fungal isolates on Potato Dextrose Agar (PDA) [(i) *Fusarium* spps.] and Sabouraud Dextrose Agar [(ii) *Saprolegnia* spps.]

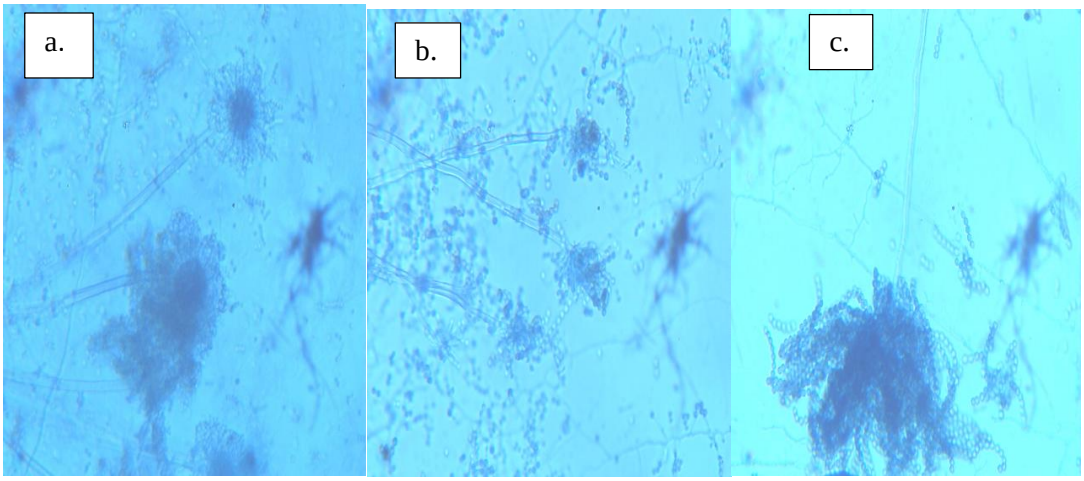
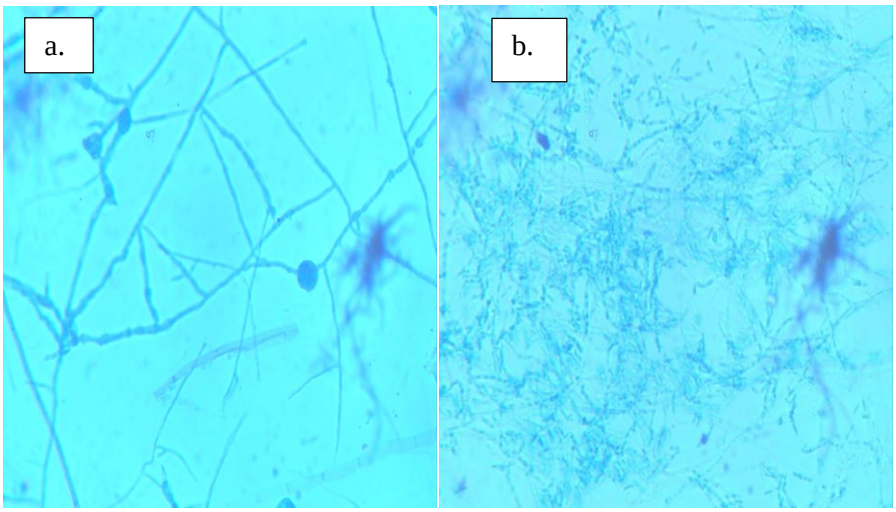


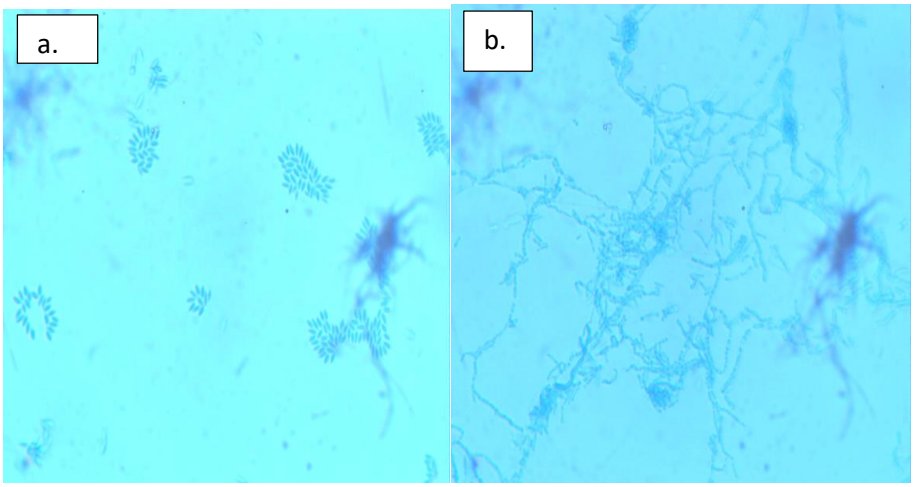
Figure 3. Microscopic morphologies of *(a) Aspergillus niger, (b) Aspergillus flavus and (c) Aspergillus parasiticus* isolated from African Catfish with skin ulcers in Abia state, Nigeria (X40)



Figures 4 Microscopic morphologies of (a) *Saprolegnia* and (b) *Penicillium species* isolated from African catfish with skin ulcers in Abia state, Nigeria (X40)

Table 2. Distribution of fungal isolates among the five local government areas in Abia State, Nigeria

Location	Number of fungal isolates				
	Aspergillus sp.	*Saprolegnia sp.*	*Penicillium sp.*	*Fusarium sp.*	*Aphanomyces sp.*
Umuahia North	8	10	2	-	-
Umuahia South	5	3	1	2	1
Ikwuano	21	4	3	-	-
Osisioma	12	7	4	-	-
Aba North	9	4	2	-	-
Total	55	28	12	2	1



Figures 5. Microscopic morphologies of (a) *Fusarium* and (b) *Aphanomyces* species isolated from African Catfish with skin ulcers in Abia state, Nigeria (X40)

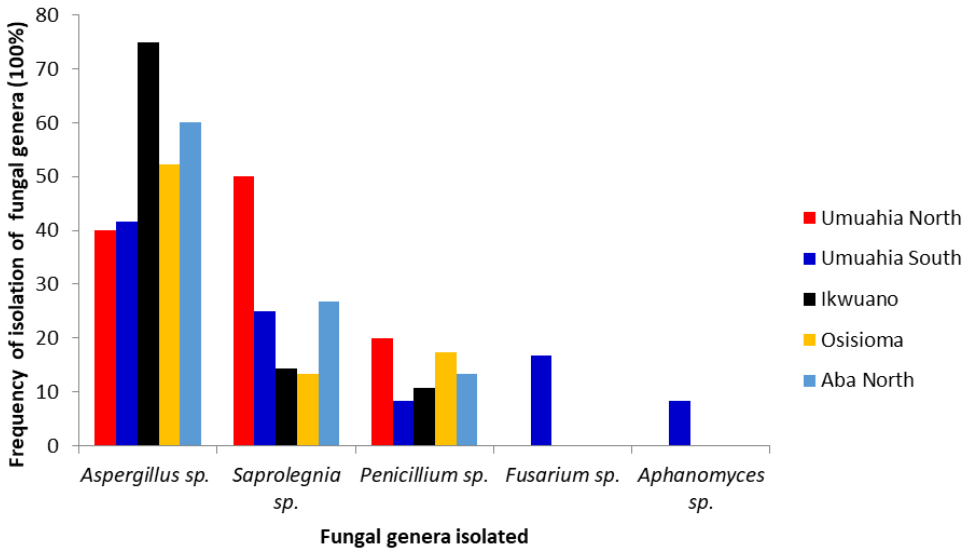


Figure 6. Frequency of isolation of fungal genera from the African catfish skin ulcers from five local government areas in Abia state, Nigeria

Discussion

The fungal infection rate was high, ranging from 60% to 100%. This finding was not in conformity with the observation of Ibrahim *et al.* (2016) who reported fungal infection rate of 32% on skin lesions of fish in Sudan. The high infection rates recorded in Umuahia South, Osisioma, Aba North and Ikwuano might be attributed to environmental conditions and other management practices that could predispose African Catfish to fungal infection. The African Catfish skin is susceptible to skin infection especially if

exposed to unfavorable conditions that could disrupt the skin barrier, because of its scale less nature (Hussein *et al*., 2001). Some of these factors include fluctuation in water temperature, increased salinity, high or low pH, poor management of pond water, overstocking, and fertilization of the pond with organic manure.

Five fungi genera: *Aspergillus*, *Saprolegnia*, *Penicillium*, *Fusarium* and *Aphanomyces*, were the major isolates identified in the study. The presence of these organisms on the skin ulcers may be due to their opportunistic nature. The species have also been isolated from skin lesions of diseased African Catfish (*Clarias gariepinus*) in Egypt (Refai *et al.,* 2010). Refai *et al.* (2004) reported that some of the opportunistic fungi are capable of eliciting virulence factors under suitable environmental conditions. For instance, *Saprolegnia* spps. and *Aphanomyces* spps. have been shown to be responsible for primary ulcerative mycosis in fish (Edward, 2000). Epizootic ulcerative syndrome (EUS) was also reported to be caused by a single Oomycete species (*Aphanomyces invadans*) in Asia (Lilley *et al.*, 1997).

Some of the fungal isolates obtained in this research are of veterinary and medical importance e.g. *Aspergillus niger*, *A. flavus* and *A. parasiticus* (Beck-Sague and Jarvis, 1993; Denning, 1996). Hence, the isolation of these microbial agents from African Catfish skin ulcers indicates a health threat to farmers and consumers. Furthermore, *Aspergillus* species have been associated with disease outbreaks in fish culture (Tsadu *et al.*, 2006); and *Aspergillus niger* is a human pathogen and environmental contaminant (Denning, 1996*).* Also, *Aspergillus flavus* and *Aspergillus parasiticus* may predispose fish to aflatoxin contamination and consumers to aflatoxicosis (Ghadeer and Al-Delamiy, 2012).

The high frequency of isolation of *Saprolegnia* spps. from farms in Umuahia North suggests a potential economic threat, because the fungus has been identified as one of the major causes of ulcerative mycosis which leads to high mortality in freshwater fish (Edward, 2000). The potential severity of *Saprolegnia* spps. was corroborated by Rahayu *et al.* (2017); who reported that the fungus could cause considerable losses for farmers because of its potential to quickly transmit and rapidly spread to other catfish in the pond. The differences in frequency of isolation of fungi from the different locations could be attributed to environmental factors, farm management practices and/or contamination of fish feed (Saleem *et al.*, 2012). Moreover, the fungi diversity and their potentials to produce toxins may have contributed to the skin ulcers. Most often, poor pond management increases the chances of occurrence of fish infection. Hence, good pond hygiene and fish health management, through the use of good quality inputs such as feed and water are essential. In addition, regular fish health monitoring should be practiced to ensure early diagnosis for better disease prevention and control on fish farms in the localities sampled.

Conclusion

In this study, five fungi genera were isolated from the African catfish skin ulcers, with

Aspergillus spps., *Saprolegnia* spps., and *Penicillium* spps being the most common. Some of the isolated fungi could spread rapidly; cause high mortality and produce toxigenic strains that could contaminate the fish. Therefore, the fungi isolated pose a serious health challenge to the final consumers of the fish. Fish farmers should pay serious attention to the welfare of their fish stocks through health monitoring, appropriate diagnosis and institution of measures to prevent or control possible disease outbreaks.

Acknowledgements

The authors acknowledge the Head and Laboratory Technologists of the Department of Veterinary Microbiology, College of Veterinary Medicine, Michael Okpara University Agriculture, Umudike, Abia State, Nigeria; for their technical assistance and expert advice.

Conflict of Interest

We, the authors, have declared no conflicts of interest during or after the conduct of this research and the publication of the findings.

References

Bassey, S. E. (2011). A concise dictionary of Parasitology. Zetus Concepts, Port Harcourt: pp. 115.

Beck-Sague, C. and Jarvis, W. R. (1993). Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980-1990. National Nosocomial Infections Surveillance System. *Journal Infectious Diseases* 167: 1247-1251.

Bhaskar, M. (1994). Changes in the liver protein fractions of *Tilapia mossambica* (Peters) on acclimation to altered pH media. *Fish Resources* 19: 179-196.

Bouck, G. R. and Smith, S. D. (1979). Mortality of experimentally descaled Smolts of Coho salmon (*Oncorhynchus kisutch*) in fresh and salt water. *Transactions of the American Fisheries Society* 108:67-69.

Center for Agriculture and Rural Corporation (CTA) (2007). Fish farming angles of aquaculture. Spore No. 132.

Denning, D. W. (1996). Therapeutic outcome in invasive Aspergillosis. *Clinical Infectious Diseases* 26: 781-805.

Domsch, K. H., Gams, W. and Anderson, T. H. (2007). Compendium of soil fungi. 2nd taxonomically revised edition by W. Gams. IHW, Eching.

Dugan, F. M. (2006). The identification of fungi: An illustrated introduction with keys, glossary, and guide to literature. *The American Phytopathology Society*, St. Paul, Minnesota, USA, 176 p. ISBN 0-98054-336-4.

Edward, J. N. (2000). Skin ulcers in fish: Pfiesteria and other etiologies. *Toxicologic Pathology* 28 (6):807-823.

Efuntoye, M. O., Olurin, K. B and Jegede, G. C. (2012). Bacterial flora from healthy *Clarias gariepinus* and their antimicrobial resistance pattern. *Advance Journal Food Science Technology* 4 (3): 121-125.

FAO (2003). The State of Food Insecurity in the World (SOFI 2003). Rome: Food and Agriculture Organization, pp. 36

FAO (2009). The state of world fisheries and aquaculture 2008. Rome, Italy: Food and Agriculture Organization of the United Nations. See http://www.fao.org/fishery/sofia/en.

Fletcher, G. L., Shear, M. A. and Goddard, S. V. (1999). Transgenic fish for sustainable aquaculture In: sustainable aquaculture: food for the future, Svenning N., Reinertsein, H. and New, M. (Eds). Balkema, Rotterdam, pp: 193-201.

Ghadeer, A. O. and Al-Delamiy, K. S. (2012). Aflatoxin B1 production by *Aspergillus flavus* in different media and containers and the antifungal activity of garlic and black cumin. *Research Journal Engineer Applied Science* 1: 117-121.

Hussein, M. M., Hatai, K. and Nomura, T. (2001). Saprolegniasis in salmonids and their eggs in Japan. *Journal Wildlife Diseases* 37: 204-207.

Ibrahim, N., Abdel Hafeez, H. N., El-Sanousi, S. M. and Shuaib, Y. (2016). Aerobic bacteria and fungi from skin lesions of fish in Khartoum State. Environmental Science. *Journal of Advance Veterinary and Animal Research* 3 (44): 375-385. http://doi.org/10.5455/javar.2016.c176.

Kato, C. D., Mugaanyi, M. B., Majalija, S., Tamale, A., Musisi, N. L. and Sengooba, A. (2016). Isolation and identification of potential probiotics bacteria from the gut of *Oreochromis niloticus* and *Clarias gariepinus* in Uganda. *British Microbiology Research Journal* 17 (5): 1-8.

Kumolu-Johnson, C. A. and Ndimela, P. E. (2011). A review on post-harvest losses in artisanal fisheries of some African countries. *Journal of Fisheries and Aquatic Science* 6: 365-378. http://doi.org/10.3923/jfas.2011.365.378.

Lilley, J. H., Hart, D., Richards, R. H., Roberts, R. J., Cerenius, L. and Soderhall, K. (1997). Pan-Asian spread of a single fungal clone results in large scale fish kills. *Veterinary Record* 140: 653-654.

Marzouk, M. S., Simira, S. R. and El-Gamal, M. H. (2003). Mycological investigations on cultured Tilapia in Kafer El-Sheikh Governorate, Kafer Ei-Sheikh. *Veterinary Medicine Journal 1nternational* (2): 97-144.

Nkemakolam, A. N., Ebenezer, D. O. and Aimuanmwosa, F. E. (2011). Application of probiotics in Nigeria aquaculture: Current status, challenges and prospects. *International Research Journal Microbiology* 2 (7): 215-219.

Olojo, E. A. A., Amusa, N. A., Osho, A. and Badejo, V. O. (2010). Commensal bacterial flora of *Syndontis nigrita* and *Clarias gariepinus* from River Osun, Southwest Nigeria. *Research Journal Applied Science* 5(3): 231-235.

Osungbemiro, N. R., Sanni, R. O., Olaniyan, R. F. and Olajuyigbe, A. O. (2014). Bacteria flora in the gut and respiratory organs of *Clarias gariepinus* in fresh and brackish water habitat of Ondo State, South west, Nigeria. *WASET Journal of Biomolecular*

Agricultural, Food and Biotechnological Engineering 8: 558-561.

Pitt, J. I. and Hocking, A. D. (1997). Fungi and food spoilage. 2nd Edition, Blackie Academic and Professional Publishers, University Press, Cambridge, Great Britain. 59-171.

Rahayu, K., Sudarno, K., Hendi, K. and Yudha, P. (2017). Isolation and identification of *Aeromonas hydrophila* and *Saprolegnia* sp. on Catfish (*Clarias gariepinus*) in floating cages in Bozem Moro Krembangan Surabaya. IOP Conference Series: Earth and Environmental Sciences: 55012038.

Refai, M. K., Attia, S., Saleem, R. M. and ELDahshan, E. M. (2004). Studies on the pathogenicity of *Aspergillus fumigatus, A. flavus* and *A. niger* isolated from chickens and their environment. Egypt. *Journal Comparative Pathology and Clinical Pathology* 17(2): 193-205.

Refai, M. K., Laila, A. M., Amany, M. K. and Shimaa, El-S. M. A. (2010). The assessment of mycotic settlement of freshwater fishes in Egypt. *Journal of Animal Science* 6 (11): 823-831.

Saleem, M. J., Hanan, A., Nisa, A. U. and Qasir, T. A. (2012). Occurrence of aflatoxin in maize seed under different conditions. *International Journal of Agricultural Biology* 14: 473-476.

Shagar, G. E. and El-Refaee, A. M. (2012). Studies on cultured silver carp (*Hypophthalmichthys molitrix*) diseases induced by some bacterial, fungal and parasitic pathogens in Sharkia Governorate. *Journal of the Arabian Aquaculture Society* 7 (2): 221-238.

Tsadu, S. M., Ojutiku, R. O. and Ayanwale, A. V. (2006). Survey of fungal infestation of some fish species from Tagwai dam, Minna, Niger State. *Journal Tropical Biosciences* 6: 1-5.

Willoughby, L. G. (1994). Fungi and fish disease (Pisces Press, Stirling, United Kingdom). Pp 57.