RESEARCH PAPER | Zoology | Volume : 4 | Issue : 6 | June 2014 | ISSN - 2249-555X

Studies on Fungal Contamination of Three Fish Species, Sold in Local Market of Nanded, Maharashtra.

KEYWORDS: Fungi, *Oreochromis mossambicus, Labeo rohita, Puntius* sp.

Dr. Dinesh D. Wanule
Asst. Prof., Department of Zoology, Birla College of Arts, Science and Commerce, Kalyan (W)-421304 (MS) India

Dr. V. Jalander
Academic Consultant, Department of Botany, Telangana University, Nizamabad -503322 (Telangana) India.

Mr. Jayvardhan Vithalrao Balkhande
Research Scholar, Department of Zoology & Fishery Science, N. E. S. Science College, Nanded-431605 (MS) India

ABSTRACT *Fishes are an important Source of nutrients and consumed as food since ancient time. In the present investigation all three fish species were contaminated by fourteen different fungal species. Oreochromis mossambicus fishes were found to be heavily contaminated fish with total 96 fungal colonies, belong to 12 different fungal species. The highest contamination was due to Cladosporium sp. with 33.33 % followed by Rhizoctina sp with 15.62%. Puntius sp of fishes were found to be contaminated fish with total 71 fungal colonies belong to 10 different fungal species. The highest contaminant was due to Aspergillus niger i.e. 29.57% followed by Rhizoctina sp with 28.16% fungal colonies, while Labeo rohita fishes were found to be least contaminated fish with 26 fungal colonies belong to 9 different fungal species in which highest contaminant was , A. niger with 19.23% followed by White sterile mycelium with 15.38%. .*

Introduction:
Post-harvest losses cause great economic damage to fishermen. Bad handling of fishes, microbial contamination, absence of processing and marketing infrastructures cause the post harvest losses. According to G.N. Shimang post-harvest losses in fisheries were estimated about 10% of the total income of fisherman. B. Olayemi et.al. from Nigeria reported presence of *Aspergillus* species, *Rhizopus* sp. *Mucor* sp. , *Achyla* Sp. And *Saprolegnia* sp. on sixty five fishes belongs to six genera [1]. Bukola.c. Adebaya-Tayo et.al, isolated *Aspergillus flavus, A. tereus, A.fumigatus, Penicillium viridatus, Candida tropicalis* and *Fusarium monaliformis* from smoke dried fishes sold in Uyo, Eastern Nigeria [2]. A report published in monthly newsletter of National Center for Disease Control dated December, 2009 from India reported worldwide, food-borne diseases are a major health burden leading to high morbidity and mortality. It also reported that 44 people were suffered due to food poisoning outbreak of Salmonella weltevreden and Salmonella wein found in non-vegetarian food (chicken and fish) from Mangalore in 2008-09[3].

In present study fishes sold in local market of Nanded city were investigated for fungal contamination, level of contamination and diversity of fungi.

Materials and Methods:
Three food fish species, *Oreochromis mossambicus*, *Labeo rohita and Puntius* Sp. were procured for the purpose of study from local market in Nanded city (Maharashtra, India). Six fishes of each species were purchased from local market, Nanded and kept separately in sterile polythene bags and brought to the laboratory. Acidified potato dextrose agar was used as media for isolation of fungal species. Modified leaf print method (Aneja, 2003) [4] was used for investigation of different fungal species. Fishes were aseptically impressed on agar media and incubated at room temperature (28 ± 2 C) for seven days. Six replicates were run for each sample. These plates were examined for fungal colonies. Appeared fungal colonies were identified on the basis of morphological, vegetative and reproductive characters using microscopic and macroscopic examination of cultures as described by Barnett H.L. et.al, (1972)[5] and Subramanian C.V. (1961)[6]. Similarly percentage of different fungal species was also calculated.

Results and Discussion:
The present study showed all three fish species were found 100% contaminated by different fungal species. *Oreochromis mossambicus* fishes were found to be heavily contaminated fish with total 96 fungal colonies, belong to 12 different fungal species i.e. Aspergillus flavus, A.niger , A.fumigatus, Aspergillus sp.1, *Aspergillus sp.2, Cladosporium sp ., Alternaria sp. , Cladosporium herbrum Rhizopus stolonifer Rhizoctina sp.*, White sterile mycelium and Brown sterile mycelium . The highest contamination was due to *Cladosporium* sp. with 33.33 % followed by *Rhizoctina* sp with 15.62%. *Puntius* sp of fishes were found to be contaminated fish with total 71 fungal colonies belong to 10 different fungal species i.e. *Aspergillus flavus, A.niger, Aspergillus sp.1, Rhizopus stolonifer, Rhizoctina sp., A. nidulance Fusarium* sp., White sterile mycelium Brown sterile mycelium. The highest contaminant was due to *Aspergillus niger* i.e. 29.57% followed by *Rhizoctina* sp with 28.16% fungal colonies, while *Labeo rohita* fishes were found to be least contaminated fish with 26 fungal colonies belong to 9 different fungal species i.e. *Aspergillus flavus , A.niger , Aspergillus sp.1, Cladosporium herbrum, Rhizopus stolonifer, Rhizoctina sp., A. nidulance Fusarium sp. ,* White sterile mycelium Brown sterile mycelium in which highest contaminant was , *A. niger* with 19.23% followed by White sterile mycelium with 15.38%.

Fish is a vital source of food for people. Fishes are rich in protein content and thus provide a cheap source of protein for poor people. According to FAO fish is a food of excellent nutritional value, providing high quality protein and a wide variety of vitamins and minerals, including vitamins A and D, phosphorus, magnesium and selenium, . Fish protein is easily digestible and favorably complements dietary protein provided by cereals and legumes that are typically consumed in many developing countries [7].

James H. et.al. (2001) reported that, fish is a particularly important protein source in regions where livestock is relatively scarce, fish supplies <10% of animal protein consumed in North America and Europe, but 17% in Africa, 26% in Asia and 22% in China (FAO, 2000). He also reported that Fish has substantial social and economic importance. The FAO estimates the value of fish traded internationally to be US$ 51 billion per annum (FAO, 2000). Over 36 million people

Volume : 4 | Issue : 6 | June 2014 | ISSN - 2249-555X

are employed directly through fishing and aquaculture (FAO, 2000), and as many as 200 million people derive direct and indirect income from fish [8].

Lack of processing, no availability of marketing infrastructure, poor storage conditions, unhygienic handling are some major causes of postharvest losses in fishery. According to FAO the majority of mycotoxins of pathological importance were produced by filamentous fungi, belong to *Aspergillus, Penecillium* and *Fusarium* genera and include alpha toxins B1-B2 G1-G2, Fusarium toxins Zearalenones, tricotheecenes and vomi toxins. Mayer J Newman reported that presence of alpha toxins and antimicrobial in food affects food safety. He also mentioned that cooking of meat kills microorganism, they may survive gentle frying and roasting if meat was not defrosted. Special care should be taken to avoid food contamination of fishes. Rudolf Kreuzer (1974) suggests "It is wise to prevent contamination of fishes at all stages"[9].

The study also reveals that the fishes carry different pathogenic fungi which may have potential to cause serious infections to fish handlers like fishermen, fish sellers and customers. The airborne fungi like *Aspergillus* species, *Rhizopus* species, and *Penicillium* species may settle on fishes. Bad handling, keeping it open for selling without any processing and packaging may bring fishes directly in contact with microbes. *Aspergillus* species like *A. niger* and *A. flavus* cause postharvest damage in fruits and vegetables. Some Aspergillus species cause eye infections, ear infections, bronchopulmonary allergic diseases and Mycotoxicosis in man [10]. Aspergillus flavus, A.niger and A.fumigatus are involved in causing Aspergillosis[11]. People with leukemia or other cancers are unable to contain the organism in the lungs and may develop widespread disease involving liver, kidneys, skin and brain [12]. Diseases of nails and mycetoma of scalp, trunk or limbs which are rarely lives threatening but a chronic disease which cripples the patient are caused by *Aspergillus nidulans* [13]. Some *Rhizopus* species which attack on fruits, medicinal plant parts also cause mucormycosis in humans[14] .

Enough food is produced globally to feed the planet but even though more than one billion people go to bed hungry every night. Hunger is a leading cause of death, killing more than AIDS, tuberculosis and malaria combined. A child dies of hunger every 6 seconds (U.N. World Food Programme - WFP), A quarter of children in developing countries is underweight (WFP) [15]. Under such circumstances we should take care of our harvested food and need to protect it from spoilage due to microbial contamination. Proper guidelines and training programmes to be provided to fishermen and people to make them aware about food contamination, health risk through contaminated food, food handling, processing and packaging.

Table 1: Total number of fungal colonies isolated from *Oreochromis mossambicus*

Sr.	Name of fish	*Oreochromis mossambicus*									
	Name of fungi	Rpl1	Rpl2	Rpl3	Rpl4	Rpl5	Rpl6	Total	Mean	SD	Contamination percentile
1	*Aspergillus flavus*	2	0	1	2	1	2	8	1.33	0.82	8.333333333
2	*A.niger*	1	1	0	1	0	1	4	0.8	0.45	4.166666667
3	*A.fumigatus*	1	0	0	0	0	1	2	0.33	0.52	2.083333333
4	*Aspergillus* sp.1	2	1	0	1	0	0	4	0.67	0.82	4.166666667
5	*Aspergillus* sp.2	0	0	0	1	0	1	2	0.33	0.52	2.083333333
6	*Cladosporium* sp.	3	12	5	6	2	4	32	5.33	3.56	33.33333333
7	*Alternaria* sp.	1	0	0	0	0	1	2	0.33	0.52	2.083333333
8	*Cladosporium herbrum*	2	2	2	1	0	3	10	1.67	1.03	10.41666667
9	*Rhizopus stolonifer*	0	0	1	0	1	1	3	0.5	0.55	3.125
10	*Rhizoctina* sp.	0	8	1	2	0	4	15	2.5	3.08	15.625
11	*A. nidulance*	0	0	0	0	0	0	0	0	0	0
12	*Fusarium* sp.	0	0	0	0	0	0	0	0	0	0
13	White sterile mycelium	2	2	0	2	0	3	9	1.5	1.22	9.375
14	Brown sterile mycelium	1	0	0	2	0	2	5	0.83	0.98	5.208333333
	Total	15	26	10	18	4	23	96	16	8.17	100

Table 2: Total number of fungal colonies isolated from *Puntius* Sp.

Sr.	Name of fish	*Puntius* Sp.									
	Name of fungi	Rpl1	Rpl2	Rpl3	Rpl4	Rpl5	Rpl6	Total	Mean	SD	Contamination percentile
1	*Aspergillus flavus*	0	3	0	0	1	1	5	0.83	1.17	7.042253521
2	*A.niger*	3	5	2	6	2	3	21	3.5	1.64	29.57746479
3	*A.fumigatus*	0	0	0	0	0	0	0	0	0	0
4	*Aspergillus* sp.1	0	1	1	0	1	0	3	0.5	0.55	4.225352113
5	*Aspergillus* sp.2	0	0	0	0	0	0	0	0	0	0
6	*Cladosporium sp.*	0	0	0	0	0	0	0	0	0	0
7	*Alternaria* sp.	0	0	0	0	0	0	0	0	0	0
8	*Cladosporium herbrum*	0	0	0	0	0	0	0	0	0	0
9	*Rhizopus stolonifer*	0	0	1	0	1	0	2	0.33	0.52	2.816901408
10	*Rhizoctina sp.*	2	1	6	4	4	3	20	3.33	1.75	28.16901408

Volume : 4 | Issue : 6 | June 2014 | ISSN - 2249-555X

11	*A. nidulance*	0	0	2	0	2	0	4	0.67	1.03	5.633802817
12	*Fusarium* sp.	0	0	1	0	0	0	1	0.17	0.41	1.408450704
13	White sterile mycelium	0	1	1	1	1	1	5	0.83	0.41	7.042253521
14	Brown sterile mycelium	2	2	1	2	2	1	10	1.67	0.52	14.08450704
	Total	7	13	15	13	14	9	71	11.8	3.13	100

Table 3: Total number of fungal colonies isolated from *Labeo rohita*

Sr.	Name of fish	*Labeo rohita*									
	Name of fungi	Rpl1	Rpl2	Rpl3	Rpl4	Rpl5	Rpl6	Total	Mean	SD	Contamination percentile
1	*Aspergillus flavus*	0	1	0	0	1	0	2	0.33	0.52	7.692307692
2	*A.niger*	1	1	0	2	0	1	5	0.83	0.75	19.23076923
3	*A.fumigatus*	0	0	0	0	0	0	0	0	0	0
4	*Aspergillus* sp.1	0	0	2	1	0	0	3	0.5	0.84	11.53846154
5	*Aspergillus* sp.2	0	0	0	0	0	0	0	0	0	0
6	*Cladosporium* sp.	0	0	0	0	0	0	0	0	0	0
7	*Alternaria* sp.	0	0	0	0	0	0	0	0	0	0
8	*Cladosporium herbrum*	1	0	1	0	0	0	2	0.33	0.52	7.692307692
9	*Rhizopus stolonifer*	0	0	0	0	0	1	1	0.17	0.41	3.846153846
10	*Rhizoctina* sp.	0	2	1	0	0	0	3	0.5	0.84	11.53846154
11	*A. nidulance*	1	0	0	0	0	0	1	0.17	0.41	3.846153846
12	*Fusarium* sp.	0	0	1	1	0	0	2	0.4	0.55	7.692307692
13	White sterile mycelium	1	0	1	1	0	0	3	0.5	0.55	11.53846154
14	Brown sterile mycelium	0	1	2	0	1	0	4	0.67	0.82	15.38461538
	Total	4	5	8	5	2	2	26	4.33	2.25	100

REFERENCE

1. A.B. Olayemi et.al., Microbiological Studies on Freshwater Fishes from the Asa ,Ilorin ,Nigeria ,Jr.Aqua.trop.,5(1990) 135-139 pp published by Oxford & IBH Publishing Co.Pvt.Ltd. | 2. Bukola C et.al, Mycoflora of smoke dried fishes sold in Uyo ,Eastern Nigeria , World Jr. of Agricultural Science 4(3) :346-350 pp ,2008 | 3. http://www.nicd.nic.in/writereaddata/linkimages/dec_091047732317.pdf dated 15-04-2014. | 4. Aneja, K.R. (2007). Experiments in Microbiology, Plant Pathology and Biotechnology. New Age International (P) Limt. Publishers. New Delhi | 5. Barnett, H.L. and Hunter, B.B. (1972). Illustrated genera of imperfect fungi. Burgess public company, Minneapolis, Minnesota. | 6. Subramanian, C.V. (1971). Hypomycetes. "An account of Indian species, except Cercosporae." ICAR publ. New Delhi. Pp.930. | 7. http://www.fao.org/FOCUS/E/fisheries/nutr.htm accessed on 8-8-2010 | 7. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1084135/ accessed on 8-8-2010 | 8. Rudolf Kreuzer (1974) Fishery Product Published by arrangement with the food and agriculture organization of United Nation by Fishing News, England. | 9. Christensen, C.M. (1975). Molds, Mushrooms and mycotoxins. Minneapolis | University of Minnesota press, USA. | | 10. Segretain, G. (1959). Mycopath. Mycol. App., 11: 327-353. | 11. www.Britannica.com. Accessed on 15-07-2009 | 12. Baylet, R., camain, R.Chabal, J. and Izara, R. (1968). Bull. Soc. Med. Afr. noire Lang. F.13: 311-313 | 13. Anaissie, E.J., Bodey, G.P.Kantarjian, J.Ro, Vartivarian, S.E., Hopter, R., Hoy, J. and Rolston, K. (1989). New spectrum of fungal infections in patients with cancer. Rev. Infect Dis. 11: 369-378. | 14. Anthony Faucci et.al. (2008) Harrison's Principles of Internal Medicine 17th edition Volume I Publisher McGraw Hill Companies USA pp .1364 | 15. http://www.alertnet.org/db/topics/HUNGER.htm/ accessed on 8-8-2010 |