

ASSESSMENT OF MOULD CONTAMINATION OF TILAPIA NILOTICA AND *Mugil cephalus* FISH AND TRIALS TO REDUCE USING NATAMYCIN

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Abstract: Contamination of fish by fungi is considered one of the most serious causes of losses in aquaculture. Therefore, our study was firstly aimed to screen the fungal status of two commonly consumed fish species in Egypt, *Tilapia nilotica* and *Mugil cephalus*. Secondly, an experimental trial to investigate the antifungal effect of natamycin on *Tilapia nilotica*. A total of 60 fish samples including *Tilapia nilotica* and *Mugil cephalus* (30 of each) were randomly collected from various retail markets and shops at dissimilar sanitation levels at Kafrelsheikh Governorate, Egypt during the winter season, 2018. All samples were examined for fungal contamination. *Tilapia nilotica* and *Mugil cephalus* samples showed average mould counts of 3.63×10^2 and 1.65×10^2 CFU/g, respectively. Nine fungal species were isolated from two fish species. Seven and five species were isolated from *Tilapia nilotica* *Mugil cephalus*, respectively. The highest prevalent fungal species isolated from the two fish was *Aspergillus flavus*. Natamycin showed significant antifungal properties in a concentration- dependent manner. Thus, efficient hygienic handling, rapid cooling of fish can reduce the fungal contamination of fish. In addition, we highly recommend soaking or spraying fish with natamycin solution as an efficient strategy in reducing the fungal load of raw fish.

Key words: *Tilapia nilotica*; *Mugil cephalus*; fungi, natamycin

Introduction

Fish is a significant source of animal protein, vitamins, minerals and omega-3-fatty acids, however, on the same time, fish is a rapidly decomposed food if not properly handled and stored. With the growing demand for fish as human food, fish farming is rapidly extending all over the world to recover the need for animal protein. *Tilapia nilotica* and

Mugil cephalus are considered as two of the most popular fishes in Egypt.

Fish diseases and hypoxic stress play an important role in lowering fish production in aquacultures (1). Source of fungal infection in aquaculture may be contributed to the utilization of contaminated feed as revealed by positive correlation between decomposition of such feed and fungal infections (2). Fungal diseases consider a serious cause of lowering

fish production. Most fungi affecting fish considered opportunistic which infect fish when they are under stress or reduced immunity due to unfavorable ecological conditions. Fungal infections occur secondarily to viral or bacterial infections, or at mucosal loss resulting from trauma or severe handling (3). Intensive aquaculture conditions may cause transmission of fish diseases, especially fungal diseases, resulting in drastic economic losses.

Assessment of fungal contamination of fish by using mould count could evaluate the quality and shelf life of fish (4). Contamination of fish by different fungal species as *Aspergillus glaucus*, *A. sulphus*, *A. ochraceus*, *A. candidus*, *A. versicolor*, *A. wentii*, *A. oryzae*, *A. melleus*, *Mucor racemosus*, *Torula fuliginea* and *Cladosporium herbarum* was reported (5). Additionally Aspergilli observed to be the most widely recognized soil growth found in high numbers in water connected with biofilms and residue (6).

Thus, there is a great need for proper handling, storage and preservation of this precious food source. Using food preservatives to prolong the shelf life of fish and to control fungal contamination is another alternative way for fish preservation. Natamycin is a natural polyene macrolide antifungal compound generated from fermentation of bacteria such as *Streptomyces natalensis*. It had an antimicrobial effect, considerably fungicidal and active against all food spoilage moulds and yeasts with most strains. Despite its long-term use, resistance does not naturally occur unlike the chemical organic propionate and acid sorbate as preservative for which a number of resistant moulds and yeasts have been detected (7). Furthermore, natamycin is applied, in over 150 countries, in the food industry as a preservative (8). It can be added in a different ways: as an aqueous suspension (such as mixed into brine) sprayed on the product or into which the product is dipped, or in powdered form (along with an anticaking agent such as cellulose) sprinkled on or mixed into the product (9).

Therefore, this study was conducted to check the mycological contamination of fresh *Tilapia nilotica* and *Mugil cephalus* and to find

out the most suitable methods for decreasing of the fungal contamination of *Tilapia nilotica* using natamycin with different concentrations.

Materials and methods

Samples Collection

Sixty random fish samples of *Tilapia nilotica* and *Mugil cephalus* (30 each) were collected from different retail markets and shops at various sanitation levels at Kafrelsheikh Governorate, Egypt. The collected samples were packed in a sterile polyethylene bags, closed and cooled in an insulated box contained crushed ice, then immediately transported to the laboratory for further examination. The fish samples were subjected to mycological examinations.

Mycological examinations

Preparation of samples

All fish samples were prepared under complete aseptic conditions, 25 g of each sample cut-off, weighed and homogenized with 225 ml of 0.1% of sterile buffered peptone water (LAB104, LAB M, UK) for 1-2 minutes at 2000 rpm using sterile homogenizer (type M-p3-302, mechanic, precyzina, Poland), such homogenate represents the dilution of 10^{-1} . The homogenate was kept for 5 minutes at room temperature. One ml from the original dilution (10^{-1}) was aseptically transferred to another sterile tube containing 9 ml of sterile buffered peptone water 0.1% and further ten-fold decimal serial dilutions were prepared (10).

Determination of the total mould counts

The total mould counts were determined by culturing duplicate plates of Sabouraud's dextrose agar media (Oxoid, Basingstoke, UK) supplemented with chloramphenicol 100 mg/L followed by dark incubation at 25°C for 5-7 days. During incubation time, the plates were examined daily for the fungal growth. Estimation of total mould was obtained by direct counting of the cultured agar plates (10).

Identification of some isolated moulds

Colonies of different fungal species inspected under microscope. Macroscopic and microscopic characteristics of the developing cultures were utilized (11, 12, 13 and 14).

Experimental work

In a trial for reduction of mould contamination of *Tilapia nilotica* fish using diluted solutions of natamycin (food grade- China), different concentrations were used under 0.5, 1 and 2.5g/l. A total number of 40 *Tilapia nilotica* fish (weigh about 200 g/each) were divided to 4 groups (n=10 fish). The first group was soaked in sterile distilled water for 30 min and kept as a control (non- treated) group. The second, third and fourth groups were soaked with repeated shaking for 30 min at room temperature in 0.5g/l, 1g/l and 2.5 g/l natamycin solutions, respectively. Total mould count was conducted as mentioned before.

Statistical analysis

All results were expressed as (means $\pm$ SE). The values were analyzed statistically by Graph Pad Prism5 software.

Results and discussion

*Evaluation of fungal status of *Tilapia nilotica* and *Mugil cephalus**

Contamination of fish by fungi may be due to presence of microorganisms on their skin surfaces, intestine and in the gills. In many parts of Egypt and other developing countries, fish is sold in the fish markets in open air, kept in wooden boxes and covered with ice. Poor handling of the fish might lead to rupture of the intestinal tract and spoilage of the fish body with the fish intestinal contents. Thus, in the first part of this study, the fungal status of the examined fish species was investigated via estimation of total mould counts. During catching, handling, transportation and processing, contamination may occur which leads to introduction of pathogens into the fish meat. The sources of these pathogens may be from the surrounding environment (15). Many factors affect the microbiological characters of

different sea food types like species differences, environment, methods of catching, on board, handling, fishing vessels, sanitation, processing, preservatives and packaging (16). *Tilapia nilotica* is commonly contaminated with mould and yeast than other types of fish which may fed on feed contaminated with moulds and yeasts (17). Also, the presence of high number of fish in limited areas encourages the growth, multiplication of mould and enhances spread of food borne outbreak infections.

In the present study, *Tilapia nilotica* had higher total mould count than *Mugil cephalus*. The mean values of the total mould counts were $3.63 \times 10^2 \pm 8.75 \times 10$ and $1.65 \times 10^2 \pm 4.78 \times 10$ CFU/g in the examined *Tilapia nilotica* and *Mugil cephalus*, respectively (Table 1 and Fig. 1). This may be attributed to the higher moisture content of *Tilapia nilotica* than *Mugil cephalus* which may cause a higher contamination with mould (18). These results were in agreement with (19) who recorded higher mould counts in *Tilapia nilotica* sold in Ed Dueim, Sudan. Furthermore, some studies (20, 21) revealed higher mould counts in *Tilapia nilotica* and *Mugil cephalus* collected from different fish farms and natural water channels in Kafrelsheikh and Dakahlia Governorate, Egypt. This fungal contamination may be indicate inadequate sanitary measures performed starting from the point of catching. The conditions of the environment in the refrigerators, cooling boxes, anglers' hands and clothes are very suitable for the development of mould spores (22 and 23). Fungal contamination of fish may lead to their spoilage and production of mycotoxins with potential health hazards to human due to their carcinogenic effects, liver diseases and organ damage (24).

Fungal species isolation from fish

Moreover, to verify the most common fungi in both *Tilapia nilotica* and *Mugil cephalus* phenotypic identification of fungal isolates was carried out. Results in table (2), showed 9 fungal species were isolated from two fish species. Seven species was isolated from *Tilapia nilotica* while five species was isolated from *Mugil cephalus*.

Figure (2) showed that *Tilapia nilotica* had the highest value of isolation percentage of *Aspergillus flavus* (86.95%) followed by *A. niger* (73.91%), however, the lowest values were obtained for *A. ochraceus*, *A. parasiticus* and *Alternaria* species as (8.69%). *Mugil cephalus* had *A. flavus* as the highest value of isolation percentage (78.94%) followed by *Cladosporium* species (47.36%), while the lowest values were obtained for *A. niger* (10.52%) (Fig. 3). These findings were in concurrence with detailed information by (2). It was found that aspergillomycosis in African *Tilapia* (*Oreochromis species*) is caused by *A. flavus*, *A. japonicas* and *A. terreus* (25). Also, we found *Cladosporium* species at a lower rate as described beforehand (26). The incidence of contamination of fish feed by *Aspergillus* species, especially *A. flavus* at high moisture levels lead to increase fungal growth during the storage period of feed at increased dampness levels (27-29). The polluted water supply, worker's hands and feeds, represent very important role on the health status of fish (30, 31).

In agreement with our results, (32) isolated *A. niger*, *A. flavus*, *A. versicolor*, *A. parasiticus*, *Rhizopus spp.*, *Mucor spp.*, *Phoma herbarum* and *Trichoderma hamatum* from *Tilapia nilotica*. Moreover, (9) isolated 14 and 10 fungal species from *Tilapia* and Mullet, respectively. The highest was *A. niger* (100%) in *Tilapia*, while *A. flavus* was the highest (83.3%) in mullets. (33) also isolated 7 genera of mould from different types of fish. Variety in the results are most added to contrasts in land territory, temperature, dampness, and hygienic conditions (34). Therefore, the fungal contamination of fish could be due to incorrect sanitation during transportation, handling, catching, storage, marketing and manufacturing of fish (35 and 36).

Reduction of mould contamination in Tilapia nilotica fish using natamycin

Fungal contamination of fish had several implications starting from organoleptic changes may lead to fish spoilage, which is an unacceptable metabolic process which causes foods to be rejectable and undesirable for human consumption due to changes in sensory characteristics. Fish is also very liable to spoilage due to enzymatic and chemical activities, the breakdown of protein, carbohydrates and fat of fish result in the progress of off-odor, off flavor and formation of slime which make the fish rejectable for human consumption. Thus, one major task of the food hygienists and microbiologists is to find ways to prolong the shelf life of fish and to decontaminate or decrease fungal contamination levels of fish. Thus, in the second part of this study, trials to control the outgrowth of moulds contamination of *Tilapia nilotica* fish using natamycin was evaluated.

Natamycin could significantly reduce fungal contamination of *Tilapia nilotica* fish and extend the shelf life of fish in a concentration-dependent manner (Table 3 and Fig. 4). For instances, natamycin 2.5g/l significantly reduced total mould count (77.09%). Similarly, (37) also declared the anti-fungal activity of natamycin in Saloio cheese. Fungal infections in fish have expanded probably due to an absence of a strong anti-fungal therapy, and for the development of fungicidal resistant strains (38, 39) and the poisoning of the most relevant anti-fungal components (40). So, there is a mandatory need to look for new strategies against fungal contamination in fish (40 and 41). As natamycin is approved for surface treatment of cheese, sausages, yoghurt and many foods all over the world as a food additive and its use is considered to be safe, it could be used to control mould growth on fish.

Table 1: Total mould count (cfu/g) of the examined fish samples (n=60)

Fish type	No.of positive samples		Min.	Max.	Mean±SE.M
	No.	%			
<i>Tilapia nilotica</i>	23	76.66	3.4×10	9.8×10^2	$3.63 \times 10^2 \pm 8.75 \times 10$
<i>Mugil cephalus</i>	19	63.34	1.8×10	2.6×10^2	$1.65 \times 10^2 \pm 4.78 \times 10$

Table 2: Incidence of fungal species in examined fish samples

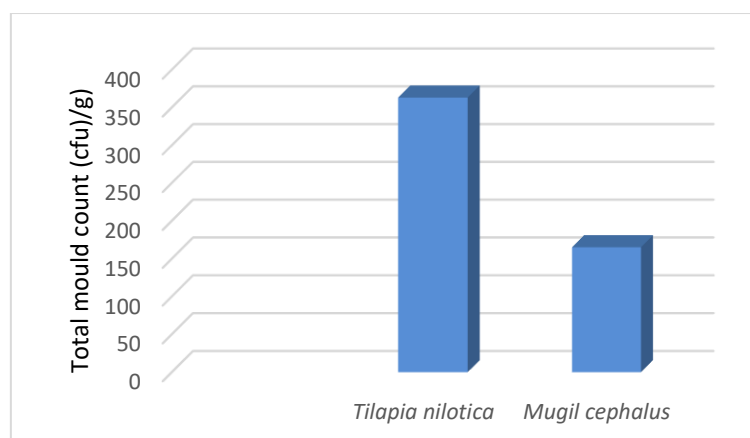
Identified mould spp	<i>Tilapia nilotica</i>		<i>Mugil cephalus</i>	
	No. of positive samples(23)	%	No. of positive samples(19)	%
<i>A. flavus</i>	20	86.95	15	78.94
<i>A. niger</i>	17	73.91	2	10.52
<i>A.ochraceus</i>	2	8.69	5	25.31
<i>A.parasiticus</i>	2	8.69	0	0
<i>A.versicolor</i>	0	0	6	31.57
<i>Alternaria spp.</i>	2	8.69	0	0
<i>Cladosporium spp.</i>	0	0	9	47.36
<i>Rhizopus spp.</i>	8	34.78	0	0
<i>Mucor spp.</i>	4	17.39	0	0

The table expressed as the percentage was calculated in relation to the number of positive examined fish samples

Table 3: Effect of natamycin with different concentrations in *Tilapia nilotica* fish

Concentration	Total mould count	Reduction%
Control(nontreated)	$3.63 \times 10^2 \pm 8.75 \times 10$	0
Natamycin 0.5g/l	$2.73 \times 10^2 \pm 3.7 \times 10$	24.83
Natamycin 1g/l	$1.76 \times 10^2 \pm 1.45 \times 10$	51.4
Natamycin 2.5g/l	$0.83 \times 10^2 \pm 3.71 \times 10$	77.09

$$\text{Reduction \%} = \frac{\text{Control} - \text{After treatment}}{\text{Control}} \times 100$$

**Figure 1:** Total mould count (cfu/g) of the examined samples

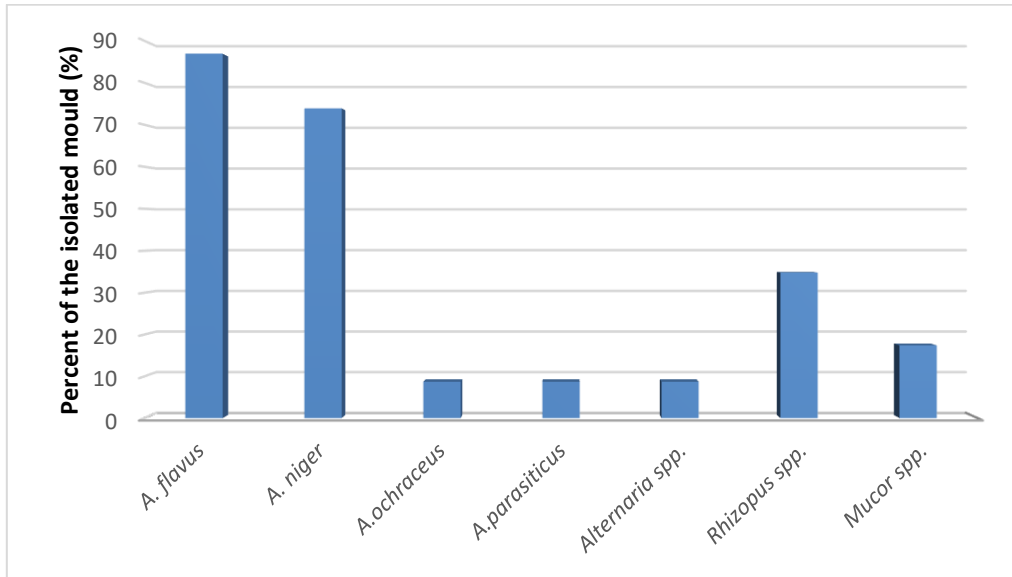


Figure 2: Percent of the isolated mould from *Tilapia nilotica* fish

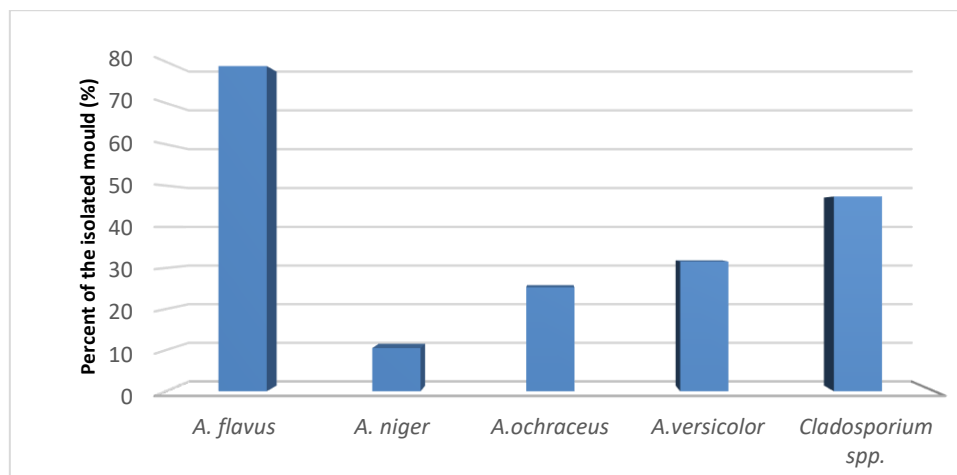


Figure 3: Percent of the isolated mould from *Mugil cephalus* fish

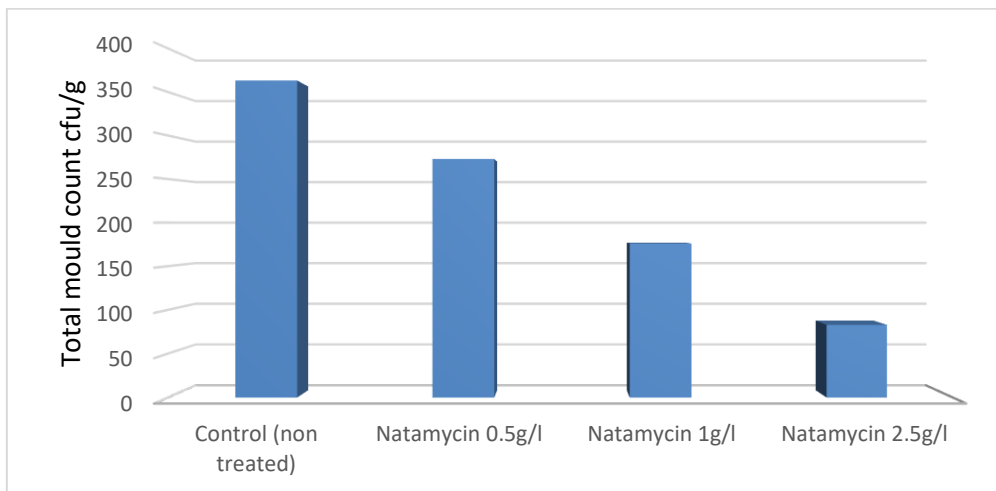


Figure 4: Effect of natamycin with different concentrations in *Tilapia nilotica* fish

Conclusion

Efficient hygienic handling, rapid cooling of fish, using of clean water during fish wash could reduce the initial fungal load of fish. It is critical that the proprietors of the fish's ranches and administrative specialists ought to adjust a superior technique to counteract fungal contamination of fishes amid developing periods and amid taking care of, preparing, transportation and processing. In addition, we highly recommend soaking or spraying of fish with natamycin solution as an efficient strategy for reduction of the fungal load of raw fish.

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Conflict of interest

The authors declare that they have no conflict of interest.

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