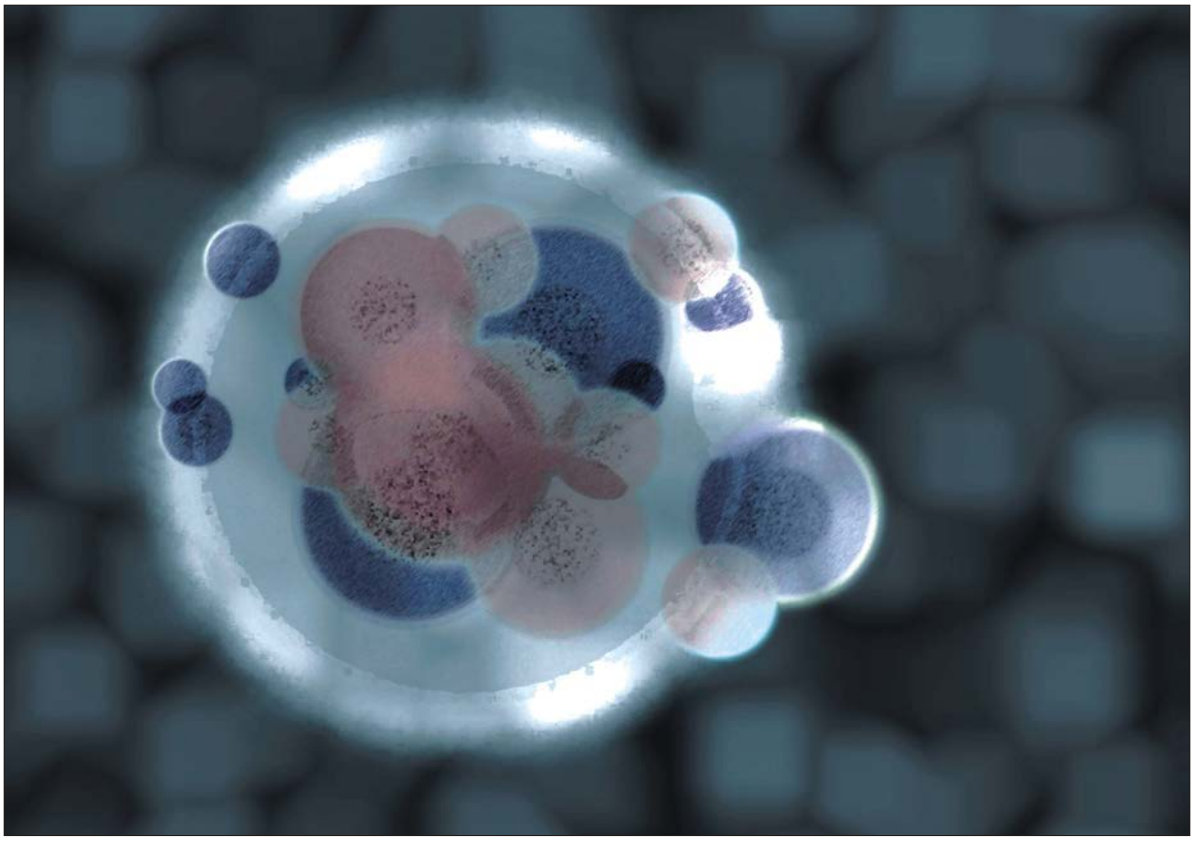

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Research Article
Mass Mortality in Cultured Nile Tilapia *Oreochromis niloticus* in Kafr El-Sheikh Province, Egypt Due to Saprolegniosis with Emphasis on Treatment Trials

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Abstract

Background and Objective: Saprolegniosis is considered the major aquatic mycotic harmful disease due to its impact on commercial fish culture. The present investigation was carried out to determine the drug of choice for treatment saprolegniosis in *Oreochromis niloticus* (*O. niloticus*). **Materials and Methods:** The present study was carried out on 500 cultured Nile tilapia *Oreochromis niloticus* in earthen fish ponds that suffered from saprolegniosis, which is considered as the most important cause of mass mortalities in cultured freshwater fish in winter in Egypt. **Results:** The fishes were subjected to full clinical, postmortem, identification and hematological and biochemical examination, trials also done for treatment on 240 apparently diseased *O. niloticus* using humic acid for control of saprolegniosis in a dose of 125 ppm for 10-12 min for 3 successive days as a bath and hydrogen peroxide was given as bath 75 μL L^{-1} for 10-12 min and sodium chloride was given as bath 375 ppm for 10-12 min for three successive treatments. **Conclusion:** The treatment of choice for saprolegniosis in affected pond was humic acid which were effective, economic and safe for fish.

Key words: Tilapia, saprolegniosis, hydrogen peroxide, humic acid, sodium chloride

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish aquaculture has been established that the greatest losses of fish are caused by infectious diseases[1]. Fungal infections are one of the main causes for mortalities and great economic losses in cultured fishes[2,3]. The most common fungal infection was saprolegniosis which is the major aquatic mycotic winter freshwater fish disease, frequently influences wild and cultured fishes[4].

Nearness of saprolegniosis in fishes is accompanied with stress factors, such as, scraped skin, wounds or ulcers on the intact skin, sexual development, poor water quality and sudden fluctuation of water temperature[5-7]. In the present time, mycotic infections with members of the family Saprolegniacae are widely reported in freshwater fishes[8]. In Egypt, saprolegniosis is considered the most important mycological disease that infect fishes due to sudden drop of water temperature and was frequently affect fishes exhibiting fungal skin lesions which, recognizable as cottony-white growth on the epidermis of the affected fishes[9]. Saprolegniosis is very difficult to treat once it have taken hold[7,10].

Several medications have been used to diminish the economic losses and reduce the number of fish suffering from such disease. Malachite green and potassium permanganate are considered the most effective fish fungicides also formalin, the cheapest and effective alternative chemical has an acute impact on the aquatic environment[11,12]. However, there is a critical requirement for new treatment against saprolegniosis in fish.

The use of humic acid is a potential chemotherapeutic compound looked upon aquaculture community with increasing interest. The compound is considered environmentally compatible as well as being an effective treatment for a variety of external fish disease, fungicide, bactericide and parasiticide on fish[13]. Hydrogen peroxide, sodium hydroxide and humic acid have a law regulatory priority classification by United States Food and Drug Administration for use as fungicide on fish[10].

Therefore, the present study was aimed to focus on saprolegniosis as a major fungal disease effect on cultured freshwater fish especially tilapia *O. niloticus* concerned on the clinical picture, isolation and identification of the causative agent as well as the optimal preventive trials to control saprolegniosis in naturally infected *O. niloticus* using chemicals safe for both environment and human being.

MATERIALS AND METHODS

Naturally infected fish: A total number of 500 *O. niloticus* fish showing skin lesions were collected from private fish farms in Kafr El sheikh province during the period of December, 2016 and March, 2017, with an average body weight 80±10 g and total length 10±3 cm. The collected fishes were transported in an ice box to the Lab of Hydrobiology, National Research Centre, Egypt for full mycological examinations.

Fishes for experiment: A total number of 240 naturally infected *O. niloticus* with saprolegniosis of an average body weight 80±10 g were collected from earthen pond of the same farms and transported to the wet laboratory and acclimated to the conditions for 2 weeks, maintained at 25±2°C in glass aquaria of 40×50×80 cm^3, they were supplied with chlorine free water, the temperature was thermostatically adjusted at 25±2°C and fishes were fed diet with 32% crude protein according to their body weight.

Experimental design: The experiment for treatment was carried out on 240 *O. niloticus* in wet laboratory using 12 aquaria for 4 groups, 3 replicate for each group of treatment as shown in Table 1 to study the effect of best concentration of both drugs on condition and behavior of healthy fishes. All groups were observed during the experiment regarding clinical signs and mortality rate according to the method of Easa and Amin[14].

Clinical examination and postmortem examination: The clinical examination was done in naturally infected fishes for the presence of the characteristic clinical picture of saprolegniosis and the mortality rate were recorded according to Lucky[15].

Mycological examination

Medium: Sabouraud dextrose agar (SDA) (Adwic SCG) was used with addition of chloramphenicol 50 mg mL^{-1} for isolation of fungus and was prepared by dissolving 65 g L^{-1} of distilled water by gentle heating and sterilized in autoclave at 121°C for 15 min[16].

Isolation and identification: It was carried out on naturally infected fishes, samples taken from fish showing skin lesions were collected and inoculated into SDA medium plates and incubated at 20±2°C for 3-4 days, subculturing on the same medium was done for purification.

All positive cultures were examined for colonial growth, morphological features and microscopical characteristics. The morphological features included appearance of the cultures, rate of growth, texture of the surface of colonies and color according to Willoughby[17]. Microscopical examination was done for wet mount of the skin lesions and mycelia cultured on (SDA) to detect septation of hyphae.

Table 1: Showing application of chemotherapeutic treatment for control the infected *O. niloticus* with saprolegniosis

Groups in each 60 fish	Treatment	Concentration	Mode of treatment	Time of exposure (min)	Duration	Follow up through 10 days		Fungal growth and clinical signs	Survival rate (%)
						Mortality rate	Survival rate		
1	Control infected non treated	-	-	-	-	54	6	+++	10
2	Hydrogen peroxide (H_2O_2)	75 µL L^{-1}	Bath	10-12	3 successive days	24	36	+/--	60
3	Treated with sodium chloride	0.375 mL L^{-1} (375 ppm)	Bath	10-12	3 successive days	30	30	++/--	50
4	Treated with humic acid	0.125 mL L^{-1} (125 ppm)	Bath	10-12	3 successive days	12	48	+/---	80

+: Signs still occur, -: Signs disappear

Ration and chemicals used for treatment:

- Sodium chloride commercial salt
- Hydrogen peroxide 70% concentration from El-Nasr Company
- Humic acid: (Biofarm) from Grand Vet Company, Egypt

Biochemical analysis: Blood samples were taken from caudal blood vessels under anesthetic condition using neutralized MS222 (200 mg L^{-1}) to study the effect of hydrogen peroxide, humic acid and sodium chloride on the blood chemistry parameters of treated *O. niloticus* according to Wotton and Freeman[18].

Statistical analysis: All data were subjected to one-way analysis of variance (ANOVA) at a 95% confidence limit, using SPSS software, version 16[19].

RESULTS

Clinical and postmortem examination: The characteristic feature of saprolegniosis infection was the mass mortality of *O. niloticus* fish during winter season (Fig. 1). The characteristic cotton wool like masses found on the head and ulcerated areas on the external body surface of fish, as shown

Fig. 1: Showing mass mortality of *O. niloticus* infected with saprolegniosis during winter season in earthen pond in Kafr El-Sheikh governorate

Fig. 2(a-d): Showing (a) Cotton wool like tufts on the dorsal, above the head, (b) *Saprolegnia* cotton wool like on swimming *O. niloticus* with exophthalmia and cloudiness of eyes (arrows), (c) Pale to grayish gills, serous fluid in the abdominal cavity, distended gall bladder with inflamed congested liver and kidney and (d) Gray cotton wool like tufts on old ulcer on the lateral side of tilapia fish

Table 2: Showing the effect of treatment of different groups on hematological parameters and blood chemistry

Treatments	PCV	Hb	Total proteins	Albumin	Globulins	A/G	Ca^{++}	Glucose	ALT	AST
1	27.1±1.2^{a}	5.30±0.13^{a}	4.30±0.19^{a}	1.42±0.16^{a}	3.07±0.18^{a}	0.443±0.019^{a}	7.30±0.51^{b}	64±3.2^{b}	31±4.1^{a}	73±4.4^{b}
2	25.2±1.2^{a}	5.70±3.2^{a}	4.70±0.35^{a}	1.30±0.26^{a}	3.12±0.22^{a}	0.431±0.014^{a}	6.83±0.64^{b}	78±5.2^{b}	37±3.6^{a}	83±5.4^{b}
3	27.4±1.6^{a}	4.90±0.13^{a}	4.23±0.16^{a}	1.32±0.11^{a}	2.72±0.12^{a}	0.481±0.022^{a}	6.20±0.46^{b}	85±5.3^{a}	37±2.7^{a}	91±6.2^{a}
4	25.3±1.3^{a}	5.10±0.13^{a}	4.20±0.17^{a}	1.37±0.11^{a}	2.95±0.13^{a}	0.476±0.023^{a}	7.30±0.52^{b}	97±6.2^{a}	41±3.9^{a}	115±8.6^{a}

PCV: Packed cell volume, Hb: Hemoglobin, A/G: Albumin/globulin, Ca^{++}: Calcium, ALT: Alanine transaminase, AST: Aspartate transaminase, Each value represent Mean±SE, N = 5, Small letters a, b in the same column represent a significant change against letters a by LSD using ANOVA at $p \leq 0.05$

in Fig. 2a, b and d. *Saprolegnia* lesions are central dark white patches on the skin which have a cotton wool-like appearance submerged when the hyphal components stretch out. The early injuries were round about and reach out until they blend. The patches can then end up noticeably dull dim or dark colored as the mycelium traps mud or debris. The head was the most predilection site of infection followed by skin and gills. The main postmortem lesions were appearance of cotton wool tufts on gills and congestions in internal organs as shown in Fig. 2c.

Mycological examination: The positive colonies on (SDA) at 20°C for 3-4 days started with cysts of long hairs with white cottony color after that became grey then black after 96 h. incubation, as shown in Fig. 3a.

The wet mount of skin, gills and mouth lesions showed masses of mature and immature sporangia mycelia filled with large number of sporangiospores, the hyphae appeared profusely branched and were non septated, these morphological findings were characteristic of the *Saprolegnia* species as shown in Fig. 3b.

***Saprolegnia parasitica* isolated from different organs and tissues:** *Saprolegnia parasitica* isolated from skin and fins with higher percentage (54 and 22%), respectively followed by gills and mouth 14 and 13%, respectively. The highest percentage was isolated from skin while the lowest percentage was isolated from mouth.

Experimental treatment trials: Humic acid showed a good result in the treatment shown in Table 1 and control of fungal growth and clinical signs in affected fish in aquaria especially in a dose of 0.25 mL L^{-1} (125 ppm) for 10-12 min, 3 successive days. The mortality rate reach 20% and the survival rate reach

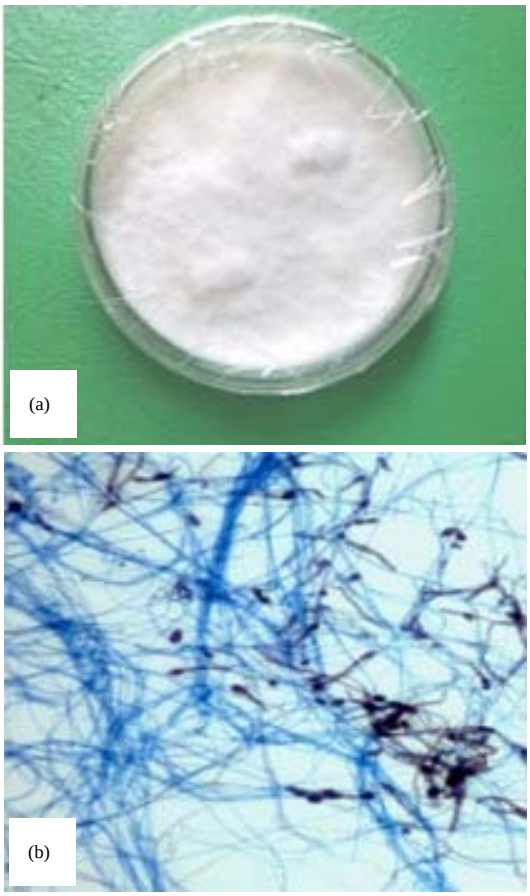

Fig. 3(a-b): Showing (a) Dextrose sabouraud agar medium cultured with *Saprolegnia* isolated from infected *O. niloticus* and (b) Wet mount of mycelia isolated from lesions showed masses of mature and immature sporangia filled with large number of sporangiospores, the hyphae appeared profusely branched and were non septated (arrow)

80% followed by hydrogen peroxide at the dose 75 µL L^{-1} as a bath for 60 min every other day for 7 treatments. Survival rate was 60% and mortality rate was 40% followed by sodium chloride survival rate was 50% and mortality was 50% with no effect on healthy state of treated fish.

Hematological parameters and blood chemistry: There are no significance changes in blood parameters and chemistry of fish treated with humic acid except increasing in glucose level and AST as shown in Table 2.

DISCUSSION

Saprolegnia is the major aquatic fungus affecting Nile tilapia *O. niloticus*[11]. In Egypt, the mycotic diseases constitute one of the most important diseases causing troubles in fresh culture with several losses especially saprolegniosis[20,21]. Saprolegniosis infection may contribute to heavy mortality among fishes and are wide spread in fresh waters ecosystem and affect wild and cultured fishes. Also, involving both living and dead eggs was considered as single largest cause of economic losses in aquaculture, second only to bacterial disease in economic importance[1,6,7,9,10].

The characteristic feature of saprolegniosis infection was the mass mortality of *O. niloticus* fish during winter season. The massive mortalities among affected farms may be attributed to the rapid decrease in water temperature induced immunosuppressant to such fishes. In addition, the low water temperature favored high levels of *Saprolegnia* species zoospores, the immunocompromised fish associated with rapid proliferation of *Saprolegnia* and production of high levels of zoospores, resulting the free swimming zoospores attached to skin and musculatures of fish, encysted and later germinated to penetrate the skin and muscles and after days the gross fungal lesions led to the observed fish mortalities. The results also revealed that *O. niloticus* highly susceptible to *Saprolegnia*, this may be due to that the extreme low temperature constitute a great stress on tilapia fish. In addition of presence of wounds and ulcers on infected fish leads to disturbance of osmoregulation of infected fish.

Saprolegniosis considered as localized infection not systemic infection, generally are external and appear any anywhere over the body surface especially fins, eyes, gills and ulcerated area on the body. The clinical signs appear on the fish suffered from saprolegniosis were represented as grayish white cotton like tufts on fins (dorsal, caudal and pectoral fins) and mouth, emaciation and death occurred due to blindness and the affected fish unable to feed. Also, distress occurs due to fungal growth on gills so, the gills become very pale due to the excessive mucus secretion and the fungal growth. In some cases, the affected fish showed muscle lesions as erythema and ulceration due to the lytic action of bacteria, so saprolegniosis is considered as secondary invader to the bacteria, these results recorded by Noga[22], Bruno and Wood[23], Hussein and Hatai[24], Aly and El-Ashram[25], Hussein *et al.*[1], El Ashram *et al.*[26], Chauhan[10] and Ganguly *et al.*[6].

The main postmortem lesions appeared as pale gills due to respiratory distress and excessive mucus secretion. The enlarged liver, kidney, spleen and gallbladder may be due to systemic bacterial infection and not attributed to saprolegniosis, these results agree with Paperna *et al.*[27], Bailey[28], Noga[22] and Roberts[29].

Regarding the result of mass mortalities may be attributed to environmental stress or secondary bacterial infections. These results observed by Aly and El Ashram[25], El Ashram *et al.*[26] and Mustafa[30].

The results of mycological examination showed that the identification of the genus of the fungus by wet mount procedures and isolation increase the incrimination and responsibilities of *Saprolegnia parasitica* to the winter kill syndrome. Isolation of *Saprolegnia parasitica* which appeared as branched non septated tubular hyphae. They were isolated from skin and fins with higher percentage followed by gills and mouth. The highest percentage was isolated from skin while the lowest percentage was isolated from mouth, these results agree with Marzouk *et al*.[31], Marzouk *et al*.[32], Refai *et al*.[33]and Eissa *et al*.[9].

The present study revealed that humic acid gave an encouraging result in the treatment and disappearance of fungal growth and clinical signs in affected fish experimentally especially in a dose of 0.25 mL L^{-1} (125 ppm) for 10-12 min for 3 successive days, the mortality rate reached 20% and the survival rate reach 80% followed by hydrogen peroxide at the dose 75 µL L^{-1} as a bath for 10-12 min for 3 successive days. Survival rate was 60% and mortality rate was 40% followed by sodium chloride in a dose of 0.375mL L^{-1} (375 ppm) for 10-12 min for 3 successive days, the survival and mortality was 50%. These results agree with that recorded by Derksen *et al*.[34] and Sherif and Abdel-Hakim[35].

The results of application of humic acid for control of *Saprolegnia parasitica* revealed good results for elimination of saprolegnia growth followed by hydrogen peroxide with no effect on behavior of treated fish and also on healthy fish. These results agree with that recorded by Howe *et al*.[36].

Dealing with the results of hydrogen peroxide, humic acid and sodium chloride on blood parameters showed that no significance difference between control healthy non treated and infected treated except glucose which was higher in humic acid treated fish than treatment. These results may be attributed to the catabolic effect on the diseased fish. Also, AST was higher in humic acid treated fish than others. These results agree with Tort *et al*.[37], El Genaidy *et al*.[38] and Zaki and Fawzi[39].

CONCLUSION

The most effective method for controlling and preventing saprolegniosis in fish ecosystem is a combination of good fish management as good water quality and avoid adverse water temperature and proper treatment of infected fish. The drug of choice was humic acid followed by hydrogen peroxide and sodium hydroxide. Humic acid have no effect on healthy condition and blood parameters of treated fish. The treatment of choice for saprolegniosis in affected pond were humic acid and sodium chloride which were effective and economic. While, hydrogen peroxide was effective but not economic. The result of humic acid treatment revealed that the drug was effective as the mortality rapidly decreased, the second day after treatment and stopped completely after 3 days of treatment *O. niloticus*.

SIGNIFICANCE STATEMENT

This study discovers the treatment of choice that can be beneficial for control and treatment of saprolegniosis in *Oreochromis niloticus* in Egypt. This study will help the researcher to uncover the critical areas of control and treatment of one of the most dangerous mycotic disease especially in cold weather or winter that many researchers were not able to explore. Thus a new theory on control mycotic diseases may be arrived at.

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