TREATMENT TRIALS OF INTUGMENTRY MYCOSIS (ACHLYA KELBSIANA) CAUSING MASS MORTALITIES AMONG MUGIL CEPHALUS FRIES IN DAKAHLIA PROVINCE

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ABSTRACT

An epizootic outbreak occurred among Mugil cephalus fries during February 1999, where the most clinical signs observed were cottony-wool like masses on the head, skin and fins. No external parasites were observed in skin smears of infected fish. The causative agent proved to be Achlya kelebsiana .

Treatment trials with levamisal HCl, formalin, acetic acid, hydrogen peroxide and iodophor were able to increase the survival rate of infected fish 83.4% , 76.7% , 73.4% , 70% and 70% respectively , whereas the infected non treated control group had 40% survival rate , Good fish viability after therupeutic treatments were noticed among levamisol and formalin treated fish than others . On the other hand, acetic acid and formalin showed a good results in eleminating the funyi from aquarium woter .

INTRODUCTION

Interest in diseases of grey mullets (Mugilidae) continues to grow with the increasing development of mullet cultivation in marine, brakish and freshwater .

Fungal infections of fish by Oomycetes, commonly known as water molds, are wide spread in freshwater as saprophytic opportunistics, multiplying on fish that are physically injured, stressed or infected **(Pickering and Willoughby , 1982)**.

Only Saprolegnia, Achlya and Aphanomyces represent the most economically fuugal species group affecting wild and cutlured fish as they responsible for significant infections which appear as a conspicuous, circular or crescent-shaped white, cotton-like mycellum particularly arround the head and caudal fins. **(Aller et. al., 1987; Willoughby, 1989; Hatai and Hoshiai, 1993; Noga, 1993 and Bly et. al., 1996)** .

For many years, infections have been largely controlled with malachite green **(Bailey, 1983; Willoughby and Roberts, 1992)**. Unfortunately, the potential teratogenic or mutagenic properties of malachite green was recorded by **(Meyer and Jorgenson, 1983; Fernandes et al., 1991)**.

The search for alternative chemical treatments or other means of controlling fungal infections has studied by many investigations **(Bailey, 1983 and Bangyeekhun et al., 2001)** .

Basically, the most successful strategy is a compination of farm management, husbandry praetices and chemical bath treatments .

The aim of this study is the approach for identifying one of the causes of disease problemes causing a mass mortalities of fries of Mugil cephalus .

Evaluation of some chemical compounds as fungicides to be used in fish aquaculture for controlling ulcerative dermal neerosis syndrome (UDN) caused by Aehlya klbsiana have been tried .

MATERIAL AND METHODS

Outbreaks of high mortality occurred in Mugil cephalus in Dakahlia were reeorded province in the spring of 1999 (Mareh) . Cultured fish were hold in earthen pond at a stocking density of 50,000 fries / fedan with water temperature ranged from 19 to 22°C throughout the spring season and with an average body weight of 10gm.

Naturally infection fish :

Tow hundred of diseased Mugil eephalus fries were transported alive to Fish Disease and Management Laboratories at both Faculty of Veterinary Medicine, Mansoura univ. and Faculty of Veterinary Medicine at Moshtohor , zagazig univ.

The fish were reared in all glass aquaria supplied with dechlorinated tap water and good airation .

External examination for any lesion was performed for the skin, fins, eyes and gills according to **(Noga 1995)** .

Direct microscopical examination :

Skin scrapings were taken from areas showing cotton wool like appearance and skin ulcers . The scrapings were teased using two needles on a slide with drop water, covered with cover slide and subjected to microscopical examination .

Mycological isolation and identification :

Specimens from diseased fish were inoculated onto glucose yeast extract agar (G Y agar) con-

taining 10 g glucose, 2.5 g yeast extract and 12 g agar in 1 L distilled water (Willoughby , 1970) also G Y broth was used .

Chloramphinicol was added to the medium for bacterial growth inhibition . Initial isolation on G Y agar, then subcultured at least twice on the same medium, inoculated into agar slop and incubated at room temperature .

For zoospore formation the mycelia were transformed to sterilized tap water at 25°C after being washed twice with sterilized tap water . Sexual organs production was enhaneed by inoculating the mycelia into sterilized tap water containing needle punctured hemp seeds and incubated at 15°C for 10 days .

Parasitological examination :

Random moribund and apparently healthy fish samples were collected . Skin and gill scrapings were examined done according to **Noga, (1995)**. Slides were directly examined microscopically

Treatment trials :

One hundred and eight naturally infected and apparently healthy Mugil cephalus frics were caught and transported alive to the fish laboratory in Faculty of Veterinary Medicine Mansoura University. Fish were devided into 6 groups (30 fish/group) and distributed to the experimental glass aquaria (70 x 50 x 30cm). Each aquarium was aerated and contained dechlorinated tap water (80 litre) . The water temperature , dissolved O_2 and PH were standardized as 10°C ± 1 , 4.2 mg/L and 7.2 respectively .

Each aquarium was used as a separate treatment and treated as follow :

Treatment I : using formalin at a dose of 50 mg/L

" **II** : using potentiated iodine = iodophore (petadine ¤ 1% solution)at a dose of 30 mg free iodine/litre

" **III** : using hydrogen peroxide 3% solution) at a dose of 10 ml/L equall to 300 ppm. (15 mints)

" **IV** : using Acetic acid (glacial Aectic acid 96%) at a dose of / 1ml / litre = / 1ppm (10 mints)

" **V** : using levamisole hydrochlorid at a dose of 10 mg/L

" **VI** : fish kept without treatment and surved as control non-treated.

Chemical fungicides were added to water at the first and 3rd days of the treatment trial experiment .

All treated fish were observed for 7 days after the onest of the treatment. Daily observation of fish viability and mortality in each treatment aquarium were caleulated.

After the 7th day of observation a water sample from each aquarium was taken under complete aseptic condition in a sterile test tube and cultured on sabaroud's dextrose agar plate.

RESULTS

Clinical signs of naturally infected fish :

Affected fish in early infection showed white or grey skin lessions whieh developed rapidly causing destruction of the epidermis. Scverly affected fish showed a cotton-wool like tufts on the skin and head as showed in (Fig1) , as well as lethargy and imbalance .

Mycological examination :

Isolation :

Gross examination of specimens on glucose peptone yeast extract agar (GPA) showed white cottony hyphal growths 2-4 days post-inoculation, at 20°C, while old cultures appeared brownish to black coloration .

Identification

Microscopical examination revealed short to long threads without numerous branches. Primary sporangia were abundant and secondary sporangia were also seen. Sporangia released their contents either in achyloid manner as normally occur in achlya (Fig 2) or through numerous mouths (Fig 1). Some spores did not escape and encysted within the sporangia which may be sprout inside and swim in rapidly swarm. In old culture abundant gemmae were formed by segmentation of the hyphae giving more or less dense rods in catenulated manner (Fig 6). The gemmae sprout after a rest period by threads forming a small sporangia (Fig 5). Oogonia were numerous of spherical or pyriform shape and filled with eggs 1-7, mainly 4 (Fig 3). Slender diclinous antheridial branches were seen enclosing the oogonia and usually more than one. The mature egg appears as sphere with eccentric nuclus and large oil droplet (Fig 4).

Parasitological examination from the skin scrape preparation the results of parasitological examination of skin of infected fish revealed absence of any ectoparasites.

Treatment trials

As shown in table (1), when the effect of some chemical compounds (formalin, iodophor, hydrogen peroxide, acetic acid and levamisol HCL) were studied as chemotherapeutic drugs (bath treatment) for treatment of mugil cephalus fries from the natural infection from Achlya kelebsiana .

It is clear that fish treated with levamisol had a good survival rat (83.4%) with a good fish viability followed by formalin (76.7%) and good fish viability, then acetic acid (73.4%), followed by iodophor and hydrogen peroxide (70%), whereas the non-treated control group had 40% survival with poor viability .

The results of water culture on Sabaroud's dextrose agar from each treated glass aquaria after 7 days from the onset of the treatments showed that both acetic acid and formalin treated waters had no fungal growth after 7 days, whereas levamisol treated water had a few fungal growth .

Finally, iodophor and hydrogen peroxide treated waters had a moderate fungal growths compared with the control non treated water that had an over growth of fungi .

DISCUSSION

Infections of fish involving members of Oomycetes are reported extensively on both wild and farmed fish **(Willoughby, 1970; Neish and Hughes, 1980)** and are considered ubiquitous in freshwater ecosystem **(Waterstrat, 1997)**.

The first report of water mould disease was noted in Atlantic salmon in the Republic of Irland, with epizootics subsequently occurring in Great Britain **(Munro, 1970)** and Europe **(De Kinkelin and Le Turdu, 1971)**. Many researches reported infections on salmonids **(Willoughby, 1986)**, in Atlantic salmon batcheries in Irland **(Smith, 1994)** and in Norway **(Langaved, 1994)**. In Japan, epizootics have occurred in farmed coho salmon **(Hatai and Hoshiai, 1992, 1993)**, whereas in France mortality was recorded in cultured roach **(Papatheodorou, 1981)**. In Nigeria, **Ogbonna (1989)** showed that a wide range of Oomycetes have been found in various freshwater fish. Oreochromis niloticus cultured in South Africa, were reported as common host for saprolegnia species **(Oldewage and Van As, 1987)**. In Brazil, silver mullet, Mugil curema, reacclimatized in fresh water were susceptible to saprolegnia sp. **(Conroy et al., 1986)**. Another species of mullet, Liza abu and carp cultured in Iraq were infected with Achlya polyandra **(Butty et al., 1989)**. Freshwater tropical fish commonly living in waters of 32°C have also shown clinical signs of Achlya kelebsiana infection **(Ogbonna, 1989 and Sati, 1991)**.

The incidence of infection with Achlya sp. was found to be influenced by the physicochemical characteristics of water. The maximum disease severity (50-60%) occurred in March with water temp (22-24°C), moderet dissolved oxygen content 6.5 mg/L and PH 8.5, while lowest level of infection (1.5%) was recorded at high temp (29-32°C) during June **(Khulbe et al., 1994)** Such. results hearly agree with the findings of the present study .

Mechanical damage from high stocking densities of cultured fish seems to be responsible for an increased incidence of Achlya infection, such observation was recorded by **Richards and Pickering, (1979)**.

Concerning to the clinical signs associated with Achlya kelebsiana infection were respresented as cottony patches which scattered on the body surface, head and fins. such reeorded signs were reported by many authors **(Sekino et al., 1987 and Singhal et al., 1987, El-Hissy et al., 1989 and Noga, 1995)**.

The actual eause of death is likely to be associated with impaired osmoregulation **(Gardner, 1974; Hargens and Perez, 1975)**, or may be due to respiratory failure when infection is associated with gills **(Bruno and Stamps, 1987)**.

Malachite green has been used in fish aquaculture facilities worldwide to eontrol or prevent freshwater fungal outbreaks for more than 40 years **(Foster and Woodbury, 1936)** and is typically administrated as a bath or flush treatment for infeeted fish and eggs .

Several studies assoeiated with malachite green have potentially mutagenie properties **(Clemmensen et al., 1984)**. In addition, there are implication for this compound as a teratogen **(Meyer and Jorgenson, 1983)** .

In recent years, the search for alternative methods and eompounds for the control of Oomycetes outbrcaks has increased and the efficacy of many potential fungicides have been tested **(Bialey, 1983, 1984, Bialey and Jeffery, 1989)** .

The present study used some chemical compounds as fungicides, showed that levamisol hydrochloride treated fish had a higher survival rate followed by formalin, acetic acid, iodophor and hydrogen peroxid. The results of **Lilley and Inglis, (1997)** were in accordanee with the present results wbere they concluded that the use of hydrogen peroxide have some potential for fungicidal treatment of Aehlya. and saprolegnia sp. infection in crayfish and they also found tbat peracetic acid and iodophore may used as water disinfectants. Other investigators recòrded that formalin ean be the effective fungicide against epizootic outbreaks **(Bialey and Jeffery, 1989, Marking et al., 1994 a,b , Bly et al., 1996; Schreir et al., 1996)** .

Several investigators **(Dawson et al., 1994, Marking et al., 1994a , Waterstart and Mark-**

ing, 1995 and Schreier et al., 1996) have reported on the effectiveness of hydrogen peroxide for controlling saprolegnia on developing eggs of rainbow trout and Chinook salmon. Also, effective mixture of 20% hydrogen peroxide and 5% peracetic acid at a dose of 100 mg/L water founded to decrease fungal infection on rainbow trout eggs and subsequent improve the hatch rate **(Marking et al., 1994 a)** .

Iodophores are used to disinfect eyed ova to destroy fungal hyphae on the egg surface **(Alderman and Polgase, 1984)**, whereas **Marking et al., (1994 a)** succeded in treating rainbow trout eggs with fungi growth with iodophor at a concentration of 100 mg/L, on the other hand, **Waiser and Phelps, (1993)** succeded in treating channel catfish with iodine wich administrated twice daily as afish treatmentt at 50,100 and 200mg il.

Table (1) : Effect of some chemical compounds on *mugil cephalus* fries as chemotherapeutic drugs against *Achlya kelebsiana*

Drug	Cumulative mortality	Mortality %	Survival %	viability of survival fish
Formalin	7/30	23.3	76.7	+ + +
Iodophor	9/30	30	70	+ +
H_2O_2	9/30	30	70	+ +
Acetic acid	8/30	26.6	73.4	+ +
Levamisol HCl	5/30	16.6	73.4	+ + +
Control (non-treated)	18/30	60	40	+

Fig. 1 : Cotton wool like grow over the head of mugile cephalus fry (arrow).

Fig. 2 : Sporangia of Achlya klebsiana which partially emotied by numerous mouths (arrows).

Fig. 3 : Oogonium of Achlya klebsiana with eggs.

Fig. 4 : Oogonium of Achlya klebsiana with eggs.

Fig. 5 : Oogonium of Achlya klebsiana with many dielinous antheridial attachment

Fig. 6 : Mature egg of Achlya klebsiana.

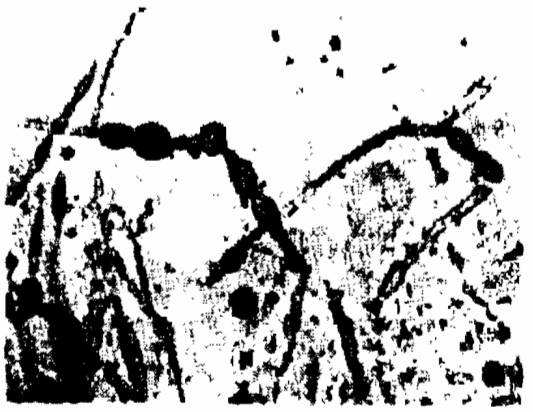

Fig. 7 : Gemmae of Achlya klebsiana with Sprouted small sporangia.

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الملخص العربى

محاولات علاجية لفطر الجلد (أكلياكليبسيانا) المسببة لنفوق جماعى بين ذريعة البورى الحر بمحافظة الدقهلية

المشتركون فى البحث

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قسم أمراض الأسماك ورعايتها - كلية الطب البيطرى - جامعة الزقازيق بمشتهر

فى فبراير عام ١٩٩٩ وقعت نسبة نفوق عالية بين زريعة البورى الحر بمحافظة الدقهلية وكانت أهم العلامات المرضية المصاحبة للنفوق ظهور كتل من الزغب القطنى على منطقة الرأس والجلد وزعانف الزريعة المصابة. لم تلاحظ أى إصابات طفيلية خارجية على الجلد عند فحص مسحات منه. ويعزل المسبب وتصنيفه فوجد أنه فطر الأكليا كليبسيانا. تم إجراء محاولات علاجية للزريعة المصابة بكل من الليفاميزول والفورمالين وحمض الخليك وماء الأكسجين ومحلول اليود كحمامات علاجية ووجدنا نسبة الإعاشة كانت ٤ر٨٣٪ - ٧ر٧٦٪ - ٤ر٧٣٪ - ٧٠٪ - ٧٠٪ على التوالى. بينما كانت نسبة الإعاشة فى المجموعة الضابطة الغير معالجة ٤٠٪. لوحظ تحسن بدرجة جيدة لحيوية الأسماك بعد العلاج بالليفاميزول والفورمالين عن العلاج بالمركبات الأخرى. كما أظهرت النتائج أن العلاج بالفورمالين وحمض أخليك لهما قدرة على إزالة الفطر من مياه الأحواض الزجاجية المستخدمة.