Saprolegniasis on the eggs of the common carp (*Cyprinus carpio* L.) with the occurrence of micropredators at Al-Wahda fish hatchery, south of Baghdad.*

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Abstract

The aim of the present study is to find out if the occurrence of the zooplankton with the incubated eggs of the common carp (*Cyprinus carpio* L.) could increase the fungal infection causing saprolegniasis in the main fish hatchery in Iraq during the artificial propagation from April to June 2009. Four eggs treatments were used, the highest hatching rate was 85.8 % in treatment no. 2 in which filtered water was used to incubate the eggs and lowest hatching rate was 21 % in the negative control according to the availability of zooplankton and the absence of fungicidal treatment. Crustacea identified were: *Bosmonia*, *Cyclops*, *Daphnia*, *Moina*, Nauplii and aquatic insects were: *Chironomas*, *Notonicta*, *Culex*.

INTRODUCTION

Al-Wahda fish hatchery at Suwaira, south of Baghdad is the main central station of fish production in Iraq. It is controlled and operated by the State Board of Fisheries Department. It produces common carp *Cyprinus carpio*, silver carp *Hypophthalmichthys molitrix*, grass carp *Ctenopharyngodon idella* and other fish species belonging to Cyprinidae (White, 1988).

Saprolegnia is the main genus of water molds responsible for significant fungal infection of freshwater fish and their

eggs (Bruno and Wood, 1999). In Iraqi hatcheries *Saprolegnia* were isolated from fish and their eggs by Al-Shaikh and Rabee (1993), Al-Kazzaz (2002). The infection with *Saprolegnia* is termed saprolegiasis it causes a considerable economic problems in the fish farming industry. The following species were isolated from eggs and brood fish of brown trout hatchery: *Saprolegnia diclina*, *S. australis*, *S. ferax*, *S. furcata* and *S. parasitica* (Fregeneda-Grandes *et al.*, 2007). Mousavi *et al.* (2009) isolated different genera of Saprolegniaceae for the first time in Iran. Kanit and Kishio (2004) examined fungal infection in common carp eggs cultured at fish farms in Khon Kaen. Under laboratory conditions water molds species can infect the eggs of nine taxa of the genus *Oncorhynchus* and other genera of freshwater fish species (Czeczuga and Muszynska, 1996).

Dead fish eggs are a fertile medium for growth of zoosporic fungi (Post, 1983). The mycelium extends from the eggs into the water surrounding the eggs, when the fungi occurred over the dead eggs; they spread around healthy eggs, causing the infected eggs to lose oxygen for breathing and thus die. Fungal infection depends on the quality of eggs, eggs of poor quality have mortality rate twice as high as that of good quality (Espeland and Hansen, 2004). Injuries from handling and attaching by carnivorous crustacean like *Cyclops* which hurt the egg shell by scraping it with their thorny feet. On the other hand the insect larvae such as Chironomids bite the shell with their mandibles and destroy the eggs (Woynarovich and Horvath, 1980). Poor water quality such as water with low circulation , low dissolved oxygen, crowding, stress, decreasing temperatures and organic matter that surrounding the eggs make it easy to be attack by fungi and bacteria (Klinger and Francis-Floyd, 1996).

Saprolegnia species can infect dead fish eggs (Pottinger and Day, 1999) from these eggs the fungus can spread to live eggs via positive chemotaxis (Bruno and Wood, 1999) meaning that some chemical signals from the live eggs

cause the fungus to move towards them (Lawrence, 2000). Since 1936, infection caused by *Saprolegnia* treated with malachite green, but in 2000 the use of malachite green for food fish was banned in the EU because it has an affinity for DNA, altering their functions and structure owing to its mutagenic and carcinogenic properties (Meyer and Jorgenson, 1983), thus general public may become exposed to malachite green through the consumption of treated fish.

The objectives of the present study were to investigate the possibilities of the infection by *Saprolegnia* species of healthy and injured eggs with the occurrence of zooplankton and aquatic insects in the medium and to find out if the presence of zooplankton have a consequence on increasing the fungal infection and affecting the hatching rate of the incubated eggs (in the absence of mechanical filters).

MATERIAL AND METHODS

The present study was done at Al-Wahda hatchery of Suwaira, south of Baghdad during the artificial propagation of common carp from April to June 2009.
Glass zougjars of a capacity from 9-10 liters are the type of eggs incubation in Suwaira hatchery.

Eggs treatments

Positive control: incubation of eggs with the hatchery water treated with malachite green (8 ppm) as normal rotten treatment in the hatchery.
Negative control: incubation of eggs with hatchery water without malachite green.
Treatment 1: incubation with filtered water and zooplankton with density of 25 ind./L and zoospores density of 85 zoospores / L.
Treatment 2: incubation with filtered water only.
For eggs treatments a tank with capacity 250 liters was used for each treatment 150000 eggs for each zougjar and

the water flow was control by a pump. Five replicas were used for each treatment.

Laboratory tests included:

Zoospores of water molds were isolated by centrifuging, the water at 3000 rpm for 10 minutes to settle down the zoospores and discards the supernatant. Zoospores were counted by a haemocytometer slide (Osman *et al.*, 2008), 85 zoospores /L. were placed in each zougjar. The zooplanktons were identified according to the (Edmondson, 1959). The density of zooplankton was determined by direct count. Temperature, pH and dissolved oxygen in the intake water of the zougjars were monitored every day through the treatment period.

The fertilization rates and hatching rates of the eggs were calculated according to Kitimasak (2009):

$$\text{Fertilization rate \%} = \frac{\text{amuont of fertilized eggs}}{\text{total amount of eggs}} \times 100$$

$$\text{Hatching rate \%} = \frac{\text{amount of hatching fry}}{\text{amount of fertilized eggs}} \times 100$$

Statistical analyses

The differences between two variables and between treatments within the incubation time were determined by a two-way ANOVA using the statistical program SPSS (11.0).

RESULTS

Saprolegniasis on the eggs were identified according to the morphological features of the infection, fungal filaments which form a white cotton wool or felt-like mat on the surfaces of the eggs, (Figure 1 A and B) in addition to the differences between embryos, dead eggs, infected eggs (Figure, 1 C) .

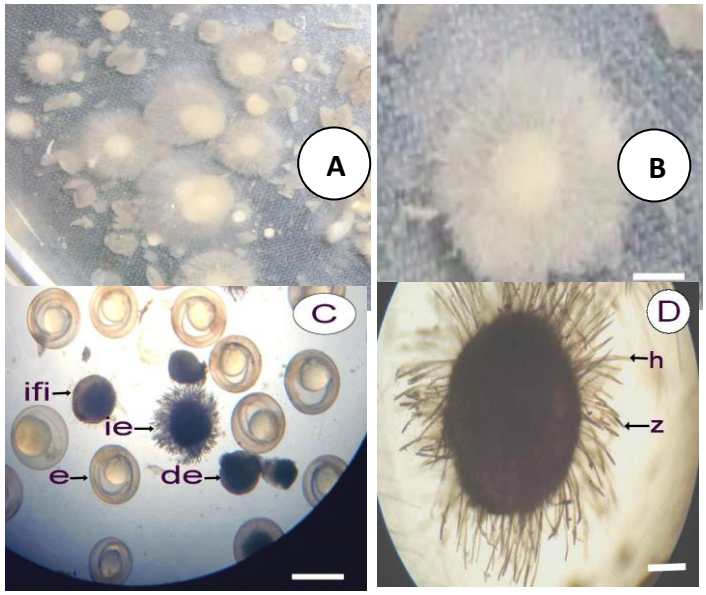
There was no infection observed by the naked eye in the first day of incubation (table 1). At the last day of incubation the highest infection rate (82.8 %) was observed in the negative control and the lowest infection rate (7.8 %) was noticed in the treatment no. 2 (Table 1).

Table (1): Percentage of infected eggs of *Cyprinus carpio* with Saprolegnia (upper row) and density of zoospores / l (lower row), within the incubation time.

Treatments / Incubation (hours)	Control +ve) Mean ± S.D	Control (-ve) Mean ± S.D	Treatment no.1 Mean ± S.D	Treatment no.2 Mean ± S.D
0	0±0 19.6± 5.6	0±0 98±1.2	0±0 85±0	0±0 0±0
24	3.2±0.2 149.8±3.2	3.2±1.3 42±4.7	0±0 40.2±3.4	0±0 22.2±1.9
48	36.4±3.3 269.8±8.6	57.6±2 1.2×10^{6}±0.6	13±0.7 81.4±4.6	2.6±0.8 55.6±3.6
72	63±1.2 1.8×10^{4}±0.8	82.8±3.8 1.6×10^{7}±0.6	25.6±3.9 1.7×10^{3}±1.1	7.8±2.1 98.4±0.5

At the last day of incubation the highest density of zoospores in the zougjars was 1.6×10^{7} zoospores /L in the negative control and the lowest density was 98.4 zoospores / L in the treatment no. 2 (Table 1).

The hatching rates in the last day of incubation in: positive control, negative control, treatment no.1 and treatment no.2 were 55 %, 21 %, 44.2 %, 85.8 % respectively. However, temperature, pH and dissolved oxygen during the period of incubation were: temp. 20-25 C, pH 6.0-7.9 and DO 3.6-6.4 mg/l. Four genera of crustacean were recognized these are: *Cyclops, Daphnia, Moina, Bosmoina* and nauplii of copepoda and two genera



Figuer (1): A and B Morphological features of the fungal nfection, C : de: dead egg, e: emberyo, ie: infected egg and ifi: initiatation of fungal infection), D: infected egg, h: hyphea and z: zoosporangium.Scale bar for A= 20 mm, B bar =10mm, C bar = 15mm, D bar = 5mm

of aquatic insects were also found: *Chironomus* and *Notonicta* in addition to the larval stages of *Culex*.
Table (2) shows an increased numbers of Crustacea and aquatic insects of the controls as compared to treatment no.1 and treatment no.2. The statistical analysis shows significant differences between controls and treatments.

DISCUSSION

Saprolegniasis is considering amajor problem especially in fish hatcheries. In the present study there was no infection rate in the first day of incubation because the infection to be

established zoospores must be attracted chemotactically to the host or to wounds by scratches (Dieguez - uribeondo *et al.*, 1994). Chemotaxis facilitates the finding of a host but may not contribute significantly to host specificity, after the occurrence of chemotaxis adhesion take place and then germination. The lack of fungicidal application may increase the infection to the highest percentage (82.8 %; Table 1), there were significant differences ($p<0.05$) between treatments in the infection rate.

The absence of mechanical filters in the hatchery that permits the establishment of zooplankton and aquatic insects. Moreover, photoatracion to light of the hatchery during the night will increase the density of these organisms in the zougjars and and increases the infection rate and the hatching rate declined. It well known that the adults and advanced copepodid stages of cyclopoids are micropredators to the early life stages of Cyprinidae (Piaseuki *et al.*, 2004).

Cyclops was recorded in higher densities in contrast to other zooplankton taxa, a similar finding was attained by Piaseuki (2000) who noticed that *Aganthocyclops robustus* underwent a very fast development in summer, specially in carp and other warm water fish species cultures and attacks resulted in considerable mortality of fish larvae depending on the *Cyclops* density and the quality of food.

Table (2): Densities (ind/l) of the Crustacea and aquatic insects in the zougjars during the incubation time.

Treatments	Control (+ve) Mean ± S.D				Control (-ve) Mean ± S.D				Treatment no.1 Mean ± S.D				Treatment no.2 Mean ± S.D			
Incubation (hours)	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72
Crustacea (ind/l)	8.6 ±5.1	12.8 ±8.16	16 ±3.1	51.2 ±7.3	16.4 ±4.3	12.2 ±3.2	20.2 ±6.1	14.6 ±3	16.4 ±1.1	6 ±2.1	5 ±1.2	9 ±2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
aquatic insects (ind./l)	9.8 ±3.1	13.2 ±2.5	17.8 ±3.1	27.2 ±3.1	20.4 ± 3.5	6.8 ±4	15.8 ±1.7	34.8 ±1.9	8.4 ±3.1	2.8 ±1	4.2 ±1.9	10.8 ±1.9	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Total (ind/l)	18.4 ±6.2	26 ±6.2	33.8 ±2.2	78.4 ±9.2	36.8 ±6.8	19 ±5.3	37.8 ±3.8	49.4 ±4.9	24.2 ±2.9	8.8 ±3	9.2 ±2.7	19.8±3.9	0 ± 0	0 ± 0	0 ± 0	0 ± 0

It can be conclude of that the occurrence of zooplankton and aquatic insects affect the hatching rate by attacking and weakening the eggs. The use of other fish as biological filters like some species of silver carp (*Hypophthalmichthys molitrix*) or Mosquito fish *Gambusia affinis* may be recomended to reduce the density of micropredator in the settling ponds of the hatchery, or using environmentally friendly methods like LDE-based light traps as a potential control of micropredators (Flamarique *et al*., 2009).

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الاصابة الفطرية saprolegniasis في بيوض اسماك الكارب الاعتيادي (*Cyprinus carpio* L.) وعلاقتها بتواجد المفترسات الصغيرة في مفقس الوحدة المركزي للاسماك في قضاء الصويرة، جنوب بغداد

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الخلاصـــة

تمت هذه الدراسة خلال عملية التكثير الاصطناعي لاسماك الكارب الاعتيادي *Cyprinus carpio* في مفقس الوحدة المركزي الواقع في قضاء الصويرة وسط العراق للمدة من شهر نيسان لغاية حزيران 2009 لاثبات ان تواجد الهائمات الحيوانية مع البيوض يزيد من نسبة الاصابة الفطرية saprolegniasis للبيوض وحيث ظهرت فروق معنوية بين المعاملات الاربعة اذ ان اعلى نسبة فقس بلغت 85.8 % في المعاملة رقم اثنان واقل نسبة فقس كانت 21 % في السيطرة السالبة. اما الهائمات لحيوانية المشخصة شملت القشريات والحشرات المائية، وكانت القشريات تضم : *Bosmonia* و*Cyclops* و *Moina* و*Daphnia* ويرقات مجذافية الاقدام Nauplii والحشرات المائية:*Chironomas* و*Notonicta* والطور اليرقي *Culex*.