



Trials of Control of Some External Parasitic Nile tilapia Diseases with Emphasis on Preparation of vaccine against *Ichthyophthirius multifiliis*

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Abstract : The objective was experimental application of new techniques in treatment trails (Medicinal plants) and trial of preparation of vaccine against *Ichthyophthirius multifiliis*. The study was carried out on 300 specimens of cultured Nile tilapia fishes (200 fingerlings and 100 adult fish) of different sizes and body weight. They were randomly collected seasonally from areas of study. In addition to 280 infested fingerlings Nile tilapia collected live from the same ponds of study and 120 healthy fish and transported to Lab. of hydrobiology department, NRC. The clinical signs of most examined fishes revealed some abnormalities on the external body surface of heavily naturally infested Tilapia fishes, represented as asphyxia, some of them aggregated on the surface and accumulated at the water inlet of the pond.

The postmortem findings of investigated fish revealed the presence of excessive mucus, white spots were observed in different parts of the body in some infested fishes. Numerous white dots in some gill filament as well as skin of infested fishes were observed. In some cultured *Oreochromis niloticus* tilapia fishes from Alsharkia district, observed small and large yellowish encysted and excysted metacercariae in branchial cavity and on skin as yellow to green pea or pomegranate seed like and arranged in group like structure (*Clinostomum cutaneum*). The different doses of medicinal plants (*Onion* and *Neem* extract) for treatment of *Ichthyophthirius multifiliis* & *Clinostomum cutaneum* parasites were applied. It was found that, the previous extract with different doses were not effective against *Ichthyophthirius multifiliis* parasite. Also, *Onion* and *Neem* extract not effect on stages of fish infested with *Clinostomum cutaneum*. The result of this study proved the importance of development of protecting vaccine against *Ichthyophthiriu smultifiliis* due to lack of effective nontraditional treatments.

Introduction

Aquaculture is one of the important sectors contributing significantly in the national economy of Egypt. *Ichthyophthirius multifiliis* (*Ich*) is one of the most important protozoan pathogens of cultured freshwater fish worldwide. In addition to, *Ich* is one of the most severe fish parasites which infest every growth stages, from fry to brood fish¹. The highest rate infection in *Oreochromis niloticus*, was followed by *Oreochromis aureus*, *Sarotherodon galilaeus* and *Tilapia zilli*. Also, the malachite green was drug of choice for controlling *Ich* infections; there have been relatively few

effective alternative strategies, besides it has carcinogenic effect². The potential antiparasitic medicinal plants was controlled of *Ich* based on our previous studies, and comprehensively evaluates in vitro and in vivo anti-*ich* activity of selected plant extracts. Also, **Xu et al.**³ recorded that high mortality of fish infected by *Ichthyophthiriasis* and causes heavy economic loss in cultured fish. Tilapia immunized with live theronts or sonicated trophonts acquired protection against *Ich* had a low infection and mortality compared to non-immunized fish. In addition, **De-HaiXu**⁴ reported that the vaccine against *Ich* induced protective immunity and have great impact by preventing the disease.

Clinostomum cutaneum is one of the most important digenetic pathogens of cultured freshwater fish worldwide. The highest rate infection in *Oreochrom isniloticus* was observed. It is an a parasitic disease affecting both wild and cultured tilapia as well as some freshwater fishes and characterized by the presence of small and large yellowish encysted metacercaria in the branchial cavity and excysted on gill filaments and skin^{5,6}. From the point of public health importance, the disease needs much care for persons who suffer from throat pain to exclude such parasite in human it causes “**Halzuun-like disease**”

Today, the world is interest to produce almost of antiparasitic and antibacterial substances from medicinal plants to replace antiparasitic and disinfectant alternatives. The more prevailing plants used in Egypt are garlic and *Artemisia vulgarism*⁷. Also, Neem and onion are considered one of the edible plants that not only antiparasitic, but also has beneficial effects on the immune system's⁸. Also, ethanolic extract of Onion and Neem are a good antiparasitic⁹. The ethanolic extracts from two tropical medicinal plants, were used in control of *Ichthyophthirius multifiliis* (*Ich*) which is the most pathogenic parasite of ornamental and cultured fish maintained in captivity. That recorded 90% reduction in numbers of *Ich* on fish after treatment in baths of **Sodium percarbonate** and ethanolic extract of Neem at 200 mg l⁻¹ compared to untreated controls¹⁰.

Materials and methods

Fish:

A total of 300 specimens of *Oreochromis niloticus* (*O.niloticus*) (100 adult *O.niloticus* and 200 fingerlings *O.niloticus*) of different size and body weight were randomly collected from cultured fish farms of different areas of study. The collected fish were 60±15 g body weight. They were subjected to the clinical examination for detection of the prevalent infestations.

Clinical Examination:

Alive fish were clinically examined for the general behaviors, changes in colour, respiratory manifestation, off food and any white spots all over the body and cysts on branchal cavity visible by necked eye according to the methods described by **Austin and Austin**¹¹ and **Noga**¹².

Postmortum examination

All examined fish were sacrificed by cutting the junction between the vertebral column and the head and placed on their right side. The first cut was made in front of the anus through the abdominal wall with blunt sterile scissors. The second cut was made perpendicular to the first directly behind the branchial cavity. The collected fishes were examined macroscopically using the methods described by **Amlacher**¹³.

Parasitological examination:

Smear scrapings from the external body surface of the collected fishes, the trunk, head, fins and gills were obtained especially the parts showing white spots. Microscopic parasites were collected by a brush, special needle or dropper, Then washed for several times in warm saline solution and left in the refrigerator until the specimens has been died and completely relaxed.

The smears were obtained by scraping the outer layer of gill filaments and spread with a drop of normal saline, covered with a clean cover slip and examined microscopically¹⁴.

Preparation of permanent samples for Identification of parasites.

The obtained protozoa smears by scrapings were freshly examined, then fixed in methyl alcohol and stained by carmine stain. The fixed films were immersed into the diluted stain for 20 minutes. After staining, the film was rinsed with water and left one dry. The stained preparation was immersed for a few seconds into acetone. Cedar oil was then dropped on the dried preparation and examined with an immersion objective¹⁵.

Identification of parasites

The identification of the parasites was undertaken according to **Paperna**¹⁶.

1- Fish for experimental examinations:

A total of 280 infested *O niloticus* were divided into seven equal groups each 40 fish. The first group G1 was treated with Onion powder 400 mg /kg diet; G2 was treated with *Neem Extract* 200 mg /kg diet, G3 as control. Feeds were formulated from ingredients commercially available in Egypt according to **Noor El Deen & Razin**¹⁷. In addition to 120 fish were prepared for challenged test. Also, 120 healthy *O niloticus* were divided into two equal groups each 60 fish. For comparative between accidental infested group and after 6 months should be vaccinated and control one.

2- Medicinal plants:

Onion powder : A commercially available onion powder a box of 1 kg imported from China in a conc.

Neem Extract:

Preparation of aqueous *Neem* leaf extract *Azadirachta indica* (*A. indica*) leaves were obtained from the surrounding area of Kafr El Sheikh Fish farms, dried in a hot air oven at 60°C for 8 hrs. The dried leaves were crushed to powder and extracted, then dissolved in hot tap water, at a concentration of 500 g of dried leaves per liter of water, for 24 h at room temperature. The mixture was filtered and the extract (500 g/l) was used immediately in the experiments, in different dilutions. Leaves were washed thoroughly in a running tap water to remove sand and debris.

3-Parasite : *Ichthyophthirius multifiliis*.

- Collect Ichthyomonts from naturally heavy infected cichlide fish (5 days post infection) through gently scraping of skin to dislodge the tomonts according to **McCallum**¹⁸.
- The isolated tomonts were put on a 250 µm mesh sieve to retain fish skin and mucus, then put over a second 75 µm mesh sieve and flushed with running water.
- The trapped tomonts over the 75 µm mesh sieve were collected and washed with water using wash bottle, then transferred into one – liter glass beaker containing 500 ml water as a modification of the method of **Noe and Dickerson**¹⁹.
- The trapped tomonts over the 75 µm mesh sieve were collected and incubated at 24±1°C for two hours, then the PBS was decanted to get rid of other contaminants and 100 ml tap water was added to the beaker and incubated at 24±1°C for 20-24 hours for development of theronts.
- Developed theronts were filtrated through 38 µm mesh sieve and examined under binuclear microscope for viability.
- Theronts were counted with a sedgwickr after cell (VWR Scientific products, Atlanta, GA, USA). Live theronts were prepared after concentration by centrifugation at 500 xg for 5 min., collected and sonicated for 3-5 minute then centrifugated in cooling centrifuge then collect supernatant to detect amount of protein according to **Burkart et al.**²⁰.

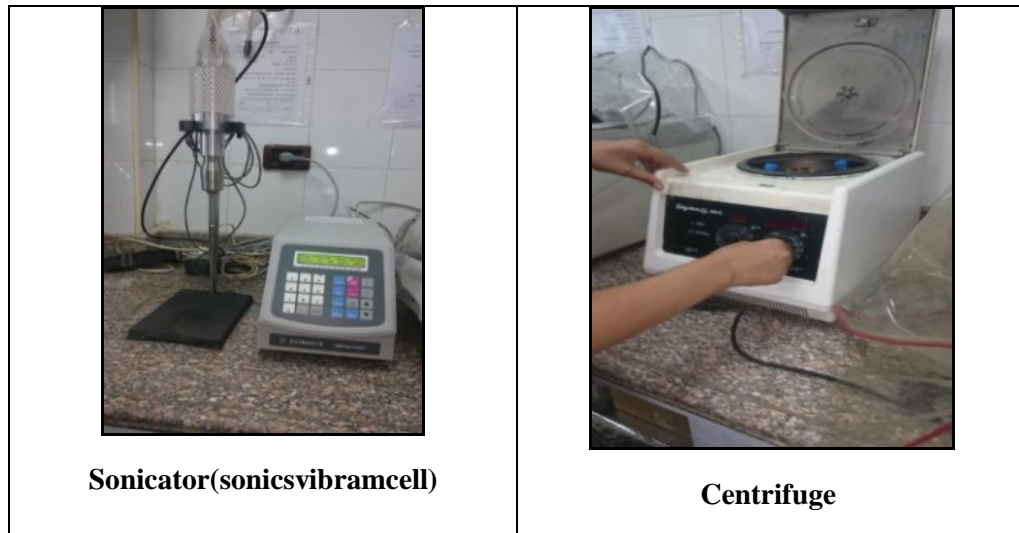
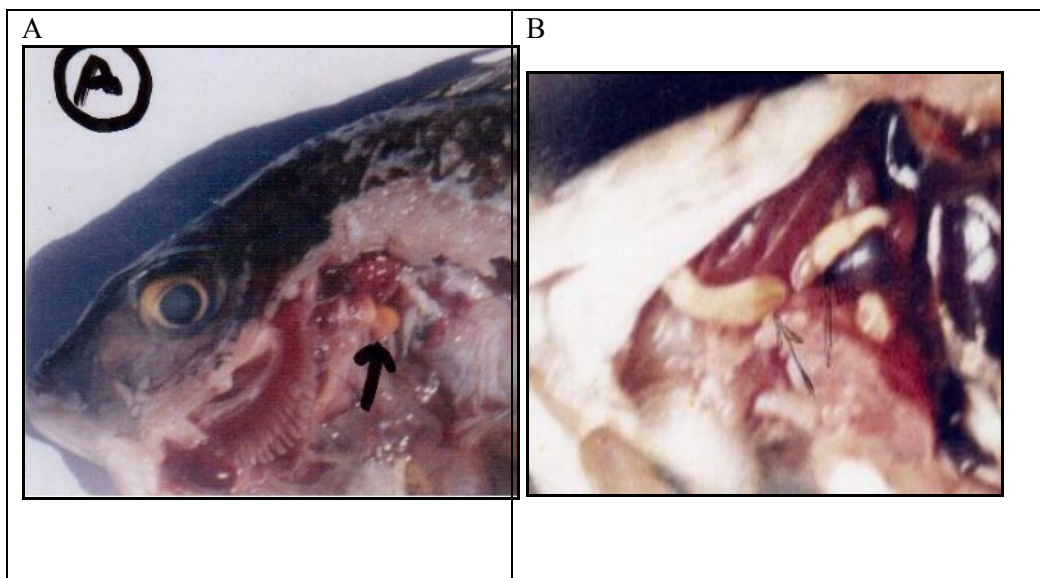


Plate 1, showing Sonicator (sonicsvibramcell) & Centrifuge

Results

Clinical examination:

The most examined fishes showed on slight and heavily naturally infested fishes (fingerlings and adult of *O.niloticus*), represented as respiratory manifestations, some aggregated on the surface, and accumulated at the water inlet of the pond. Others appeared dull with loss of escape reflex. Also, fishes showed **gills will appear swollen and be covered with thick mucus**. The scale external body surface as well as sloughing. On some examined tilapias showed small and large yellowish encysted metacercariae in the branchal cavity or on gill filament and skin (**Plate 2A&B**).



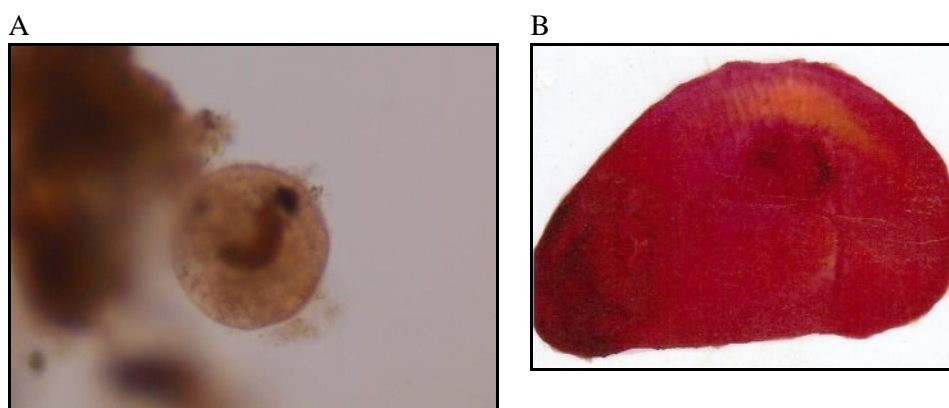
Plate,2: Showing a large number of orange encysted metacercariae scattered in the branchial cavity and on the gill arch *Oreochromis niloticus* (arrow) (A), excystedmetacercariae on branchial cavity (arrow) (B).

Parasitological examination :

External body surface.

Microscopic smears were taken from skin and gills of examined fish, showed a holotrichous ciliated protozoan as the entire surface area is ciliated with a meridional row. The mature parasite (trophont) is round to oval in shape, ranging from 0.5 up to 1.5 millimeters in diameter and considered as the largest external protozoan affecting fish. The macronucleus is embedded in the protoplasm and well characterized by a horseshoe C shape. Such flagellated protozoa were identified as *Ichthyophthirius multifiliis* (**Plate 1A**).

Macroscopic white clusters were taken from branchal cavity of examined fish, showed cysts were observed as a large orange to yellowish white peas shaped cysts and embedded in the branchal cavity. The excysted metacercariae were a leaf-shaped, and fairly large if compared with other metacercariae. The oral sucker subterminal and the ventral one was situated near oral sucker. The pharynx lead to bulb shape oesophages. Such cysts were belonged to genus *Euclinostomum* and identified as *Clinostomum cutaneum* (**plate 1B**).



Plate, 3: Magnified *Ichthyophthirius multifiliis* (wet mount) (A) , *Clinostomum cutaneum* (B), Stain: carmine stain X 400.

Treatment trails:

Medicinal plants.

Treatment trails on 280 infested fish from the same ponds of examined (120 fingerlings *O. niloticus* in each group) infested with external parasites were applied using medicinal plants (*Onion* extract and *Neem* extract). The results of treatment of such naturally infested tilapia after using medicinal plants were described in **Table (1)**. It was noted that, the different concentration of (*Onion* and *Neem*) extracts of were used in ponds without harmful effect on fish causing no effective on Ich for 15 days.

Table(1) : Showing the different treatment trials on *Ichthyophthirius multifiliis* & *Clinostomum cutaneum*.

Group	Medicinal plant extract	No of fish	Dose mg/kg	Ich	<i>Clinostomum cutaneum</i>
1	<i>Onion</i>	40	200	—	-
		40	400	-	-
		40	600	-	-
2	<i>Neem</i>	40	100	—	-
		40	200	-	-
		40	300	-	-
3	Control	40	-	—	-

Preparation of vaccine:

Some experimental infested tilapia after vaccinated with the sonicated preparation showed less mortality rate than control one **Table (2)**.

Table (2): Survival of Nile tilapia exposure to Sonicated trophont IP injection and control.

Immunization groups	Number of examined fish	Number of dead fish	Survival (%)
Sonicated trophont IP injection	60	12	80
Control	60	45	25

Discussion

In Egypt, more attention is being focused nowadays to the improvement of fish aquaculture to solve the shortage of animal protein.

The present study deals with different external parasitic diseases among naturally infested cultured *Tilapia sp* in relation to the different districts (Kafr El- Sheikh, Alsharkia and Alfayoum) fish farms with treatment and trials of preparation of vaccine against (Ich)*Ichthyophthiriu smultifiliis*.

The parasitic external ciliated protozoan parasite that may infest almost all freshwater fish species caused significant economic damage to the aquaculture industry specially *Ichthyophthirius multifiliis*(Ich)²¹.

Concerning the clinical signs in tilapia sp infested with ciliated protozoa Ich was causes white nodules covering the skin. Also showed signs of skin irritation were manifested by rapid swimming behavior and aggregation of fishes around the water inlet. Some examined tilapias showed small and large yellowish encysted metacercariae in the branchial cavity or on gill filament and skin. The same clinical signs were previously obtained by Noor El Deen *et al*²² and Hamoda⁶.

The ciliated protozoan, *Ichthyophthirius multifiliis*, was morphologically and parasitologically identified. Such results were nearly similar to the descriptions given by Kabata²³.

Concerning the results of parasitological examinations of Tilapia fishes, isolation of Ichsp was provided with²². The morphological characters of these parasites were nearly similar to descriptions given by El-Seify *et al*²⁴.

The digenetic trematod, *Clinostomum cutaneum*, was morphologically and parasitologically identified. Such results were nearly similar to the descriptions given by Eissa²⁵. The *Clinostomum* can pose a sever public health problems most commonly as laryngopharyngitis that is commonly called in Arabic countries as "Halzuun-like disease".

Regarding to the treatment trails it was revealed that the usage of medicinal plants such as (Onion and Neem) extracts were effective in treatment of some external parasitic diseases in *O. niloticus* where the best suitable and effective dose causing a great damage of parasites without harmful effect on fish was the combination between (400 mg Onion extract and 200 mg Neem extract / kg diet) for 15 days. This result agrees with Noor El-Deen and Razin¹⁷ and Sahandi *et al*²⁶. From the present study it is indicated that Onion, Neem extracts not effective on Ich.

Concerning the survivability of vaccinated with the sonicated prepared of theronts higher than control one. This result may be attributed to acquired immunity. These results were agree with that recorded by Alvarez-Pellitero²⁷ and De-Hai Xu⁴.

Finally, the trials of preparation of vaccine against Ich through injection of the sonicated serum to group of healthy fish and after 180 days of exposure to experimental infection, the observation was the survivability of injected fish showed 80% than control 25%. This result may be attributed to acquired immunity and can be considered as a preliminary step for preparation of commercial vaccine.

Conclusions:

Medicinal plants not effective on **Ich** external parasites and more save on fish than most of chemical anti-parasitic drugs. Preparation of vaccine against **Ich** should be vitro application. Yellow grub disease considered as the more zoonotic and prevailing external zoonotic disease in adult tilapia at Alsharkia district.

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