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PARASITOLOGICAL AND HISTOPATHOLOGICAL STUDIES ON THE GRASS CARP, *CTENOPHARYNGODON iDELLA* INFECTED WITH *TRICHODINELLA ePIZOOTICA*

BY

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SUMMARY

The histopatholgocal effect of trichodinid, *T. epizootica* was experimentally determined on the gill filaments of grass carp *[Ctenopharyngodon idella]*. After one month intection, the parasite caused high mortalities among fish weighting 10-15 grams. On the gill filaments of heavily infected fish, the parasite induced hyperplastic proliferation, desquamation of the epithelial lining, vacuolar degeneration, edema, necrosis, melano-macrophage cell aggregations and dilatation of the branchial blood vessels.

For control purposes, formalin was used at concentrations of 25, 50, 100, 200, 300 and 400 ppm to kill the parasite after 24 hours, 24 hours, 65, 35, 15 and 7 minutes respectively. Copper sulphate at concentrations of 0.5, 1, 2, 5, 10 and 20 ppm was used to eradicate the parasite after 24, 24, 24 hours, 40, 10 and 5 minules respectively. Malachite green could be also used to exterminale the parasite after 24 hours, 35, 35, 25 and 20 minutes at concentrations of 0.3, 2, 4, 6, 8 and 10 ppm respectively.

INTRODUCTION

The herbivorous fish, grass carp, *Ctenopharyngodon idella* has been introduced in Egypt for biological control of aquatic weeds harboring the water flow in canals and drains. This fish can not reproduce naturally under common Egyptian climatological circumstances. As a result, a fish hatchery, Delta Breeding Station "D. B. S." was constructed by Research Institute of Channel Maintenance. This phytophagous fish was noticed to be a target of many parasitic forms (Szakolezai and Molnar, 1966; Musselius and Strelkov, 1968; Molnar, 1971; Stepanova, 1971; Riley. 1978; Shirman and Smith, 1983; Abdel Meguid, 1989, 1995). One from those parasites are ectoparasitic trichodinids. The trichodinid parasites are regarded as a main cause of death among many fishes (Amlacher, 1970, Ahmed, 1976; Paperna, 1980; McArdle, 1984; Eisa et al., 1985; Abdel Meguid, 1989, 1995). The gill lesions induced by these parasites included mainly excess of mucus production, hyperplasia, necrosis and edema (Lom, 1970; Ahmed, 1976; Paperna, 1980; McArdle, 1984; Eisa et al., 1985; Abdel Meguid, 1989).

Several concentrations of formalin have been tried for eradicating trichodinids (Snow, 1962; Allison, 1963; Klinke and Elkan, 1965; Meyer, 1962; Amlacher, 1970; Paperna, 1980; Ramadan and Abdel Meguid, 1986; Abdel Meguid, 1989); Also, few attempts have been done to control the parasites using either copper sulphate (Meyer, 1967; Ramadan and Abdel Meguid, 1986; Abdel

Meguid, 1989) or malachite green (Molnar, 1971; Herman, 1972).

It is generally believed that the smaller varieties of trichodinids such as *Trichodinella sp.* are more pathogenic (Brown and Gratzek, 1980). However, this assumption has never been verified because *Trichodinella sp.* occurred with other ectoparasites on the gills of fishes in mixed infection (Molnar, 1970). The objective of the present study was to describe the morphological structure of *T. epizootica* and to evaluate the magnitude of gills injury experimentally induced by this parasite. Additionally, efforts were made to control the parasite using different concentrations of formalin, copper sulphate and malachite green.

MATERIALS AND METHODS

To determine the morphotogical structure of *T. epizootica,* 50 parasites from the gill filaments of infected grass carp were carefully collected, fixed in methyl alcohol and stained with haematoxylin or subjected to silver impregnation technique (Abdel Meguid, 1989).

To examine the effect of the parasite on the gill filaments of grass carp, 5 heavily infected fish weighting about 10-15 grams were collected from a pond at Deita Breeding Station (D. B. S.) and stoked in a small cage previously installed in a basin its capacity was 2000 liters containing 50 unparasitized grass carp weighting 10-15 g. The basin was provided with a source of aeration. The experment was done at water temperature ranged between 20 to 23 C. With a straight forward life cycle, the protozoan parasites could spread and infect the unparas. itized population . After one month infection, 10 infectd fish were collected. Host gills with attached parasites were fixed in Bouin's solution, embedded in paraffin wax, sectioned at 4-5 mircons and stained with haematoxylin and eoxin.

To control the parasite, heavily infected fish were subjected to different concentrations of fomalin (25, 50, 100, 200, 300 and 400 ppm); copper sulphate (0.5, 1, 2, 5, 10 and 20 ppm) and malachite green (0.3, 2, 4, 6, 8 and 10 ppm). Ten heavily infected fish were used for each treatment after placing them in aquaria of 20 liters capacity provided with aeration. For high concentration treatments (short bath treatments), samples of the gills were examined under the microscope every 5 minutes to determine the time of parasite death. For low concentration treatments (long bath treatments), samples of the gills were examined after 24 hours. Each experiment was repeated 3 times and the average of times was considered to determine the lethal concentrations to the parasite. All experiments were carried out at a temperature of 22 C using filtered Nile water having the follwing chemical characteristics: PH = 7.5 NH3= 0.05 mg/L, NO2= 0.0 mg/L, Cl=20mg/L, total hardness = 7D and alkalinity=3 mg/L.

RESULTS

Trichodinella epizootica showed a disc-shaped body with a well developed adhesive disc (Fig. 1A). This disc was surrounded by a bordering membrane and a clear denticular ring. The denticle exhibited an outer flat blade, a central cone and a poorly developed inner ray. The macronucleus appeared as a horse-shoe shaped appearance (Fig. 1B). The micronucleus was very small laying near the terminal end of the macronucleus in + y position. Measurements of *T. epizootica* were listed in Table (1).

Trichodinella epizootica occurred very abundantly on the gill filaments of grass carp. In heavily infected gills, most of the tricodinid parasites cupped over the top edge of the secondary lamellae (Fig. 3). Also, they showed scattered distribution along the gill filaments and between the secondary lamellae (Figs. 4, 5). The parasite

caused severe mortality among host fish within one month infection. On the gill filaments of the experimentally infected grass carp, it appeared that the extent of gill damages were highly related to the number of parasite harbored.

degeneration and necrosis (Fig. 3). Also, the secondary lamellae revealed an accumulation of edematous fluid (Fig. 5) which might be replaced by fiberllar materials. Furthermore, the parasite induced drastic mucus cell hyperplasia,

Table (I) Biometric dimensions of *T. epizootica* from different hosts (measurements in microns)

	Stein (1968)	Stein (1968)	Present study
Host	(1)	(2)	(3)
Diameter of body	18.0 - 30.0	27.2 - 45.8	17.0 - 29.0
Diameter of adhesive disc	15.0 - 21.0	18.6 - 30.0	15.0 - 20.0
Diameter of skeletal ring	10.5 - 18.0	15.7 - 20.0	09.0 - 13.0
Length of outer blade	01.5 - 03.0	01.4 - 02.9	02.0 - 03.0
Number of denticles	19.0 - 23.0	19.0 - 27.0	20.0 - 24.0
Diameter of macronucleus	12.0 - 21.0	10.0 - 27.2	11.0 - 20.0
Body/adhesive disc diameters ratio	01.1 - 01.5	01.2 - 01.8	01.1 - 01.5
Body/skeletal ring diameters ratio	01.4 - 02.0	01.4 - 02.4	01.9 - 02.2
Adhesive disc/skeletal ring diameters ratio	01.1 - 01.6	01.1 - 01.7	01.5 - 01.7
Body/macronucleus diameters ratio	01.0 - 01.9	01.6 - 02.7	01.3 - 01.5
Skeletal ring/ macronucleus diameters ratio	00.7 - 01.1	00.7 - 01.4	00.7 - 00.8

(1) *Carassius auratus gibelio*
(2) *Lota lota*
(3) *Ctenopharyngodon idella*

Microscopically, the gill filaments of heavily infected fish initially responded to the presence of the parasite by showing extensive epithelial proliferation (Figs. 3, 4, 5, 6) comparing to unparasitized gill filaments (Fig. 2). This hyperplasia extended along the whole gill filaments resulting a fusion of the secondary lamellae (Fig. 4). Moreover, the comprehensive epithelial tissue proliferation exhibited desquamation of the epithelial cells, vacuolar prevascuolar aggregation of melanin-carying cells (Fig. 6) and congestion of lamellar blood vessels. In many cases, the parasite appeared surrounded with necrotic epithelial tissues, tissue fragnents and nuclear elements (Fig. 7) which might form a cyst like structure. In some cases, the host responded to the parasite invasion by an accumulation of inflammatory cells mainly lymphocytes in areas of parasitic infestation (Fig. 8).

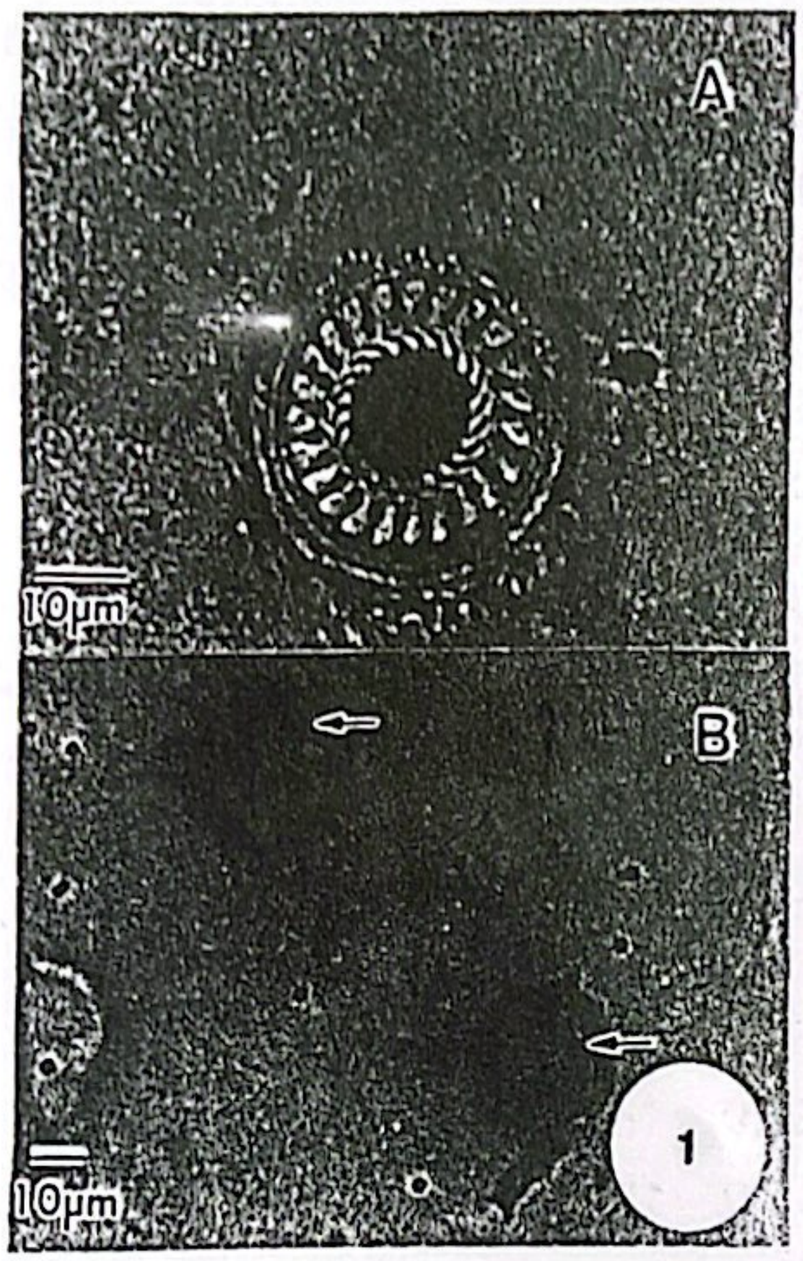

Fig. 1. (A): Photomicrograph of silver impregnated *Trichodinella epizootica* showing the adhesive disc. (B). *T. epizootica* stained with haematoxylin and eosin showing the macronucleus (arrows).

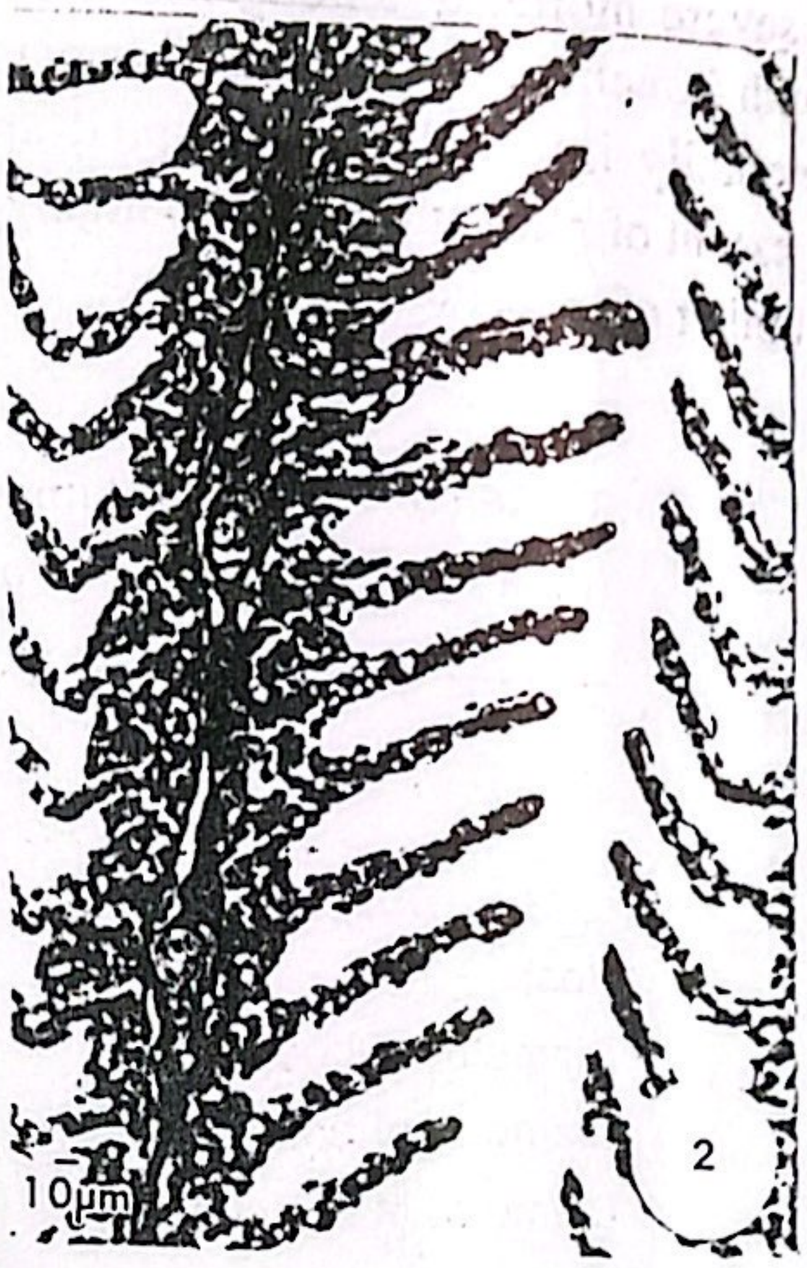

Fig. 2. : Section through the gill filaments of unparasitized grass carp showing normal gill architecture.

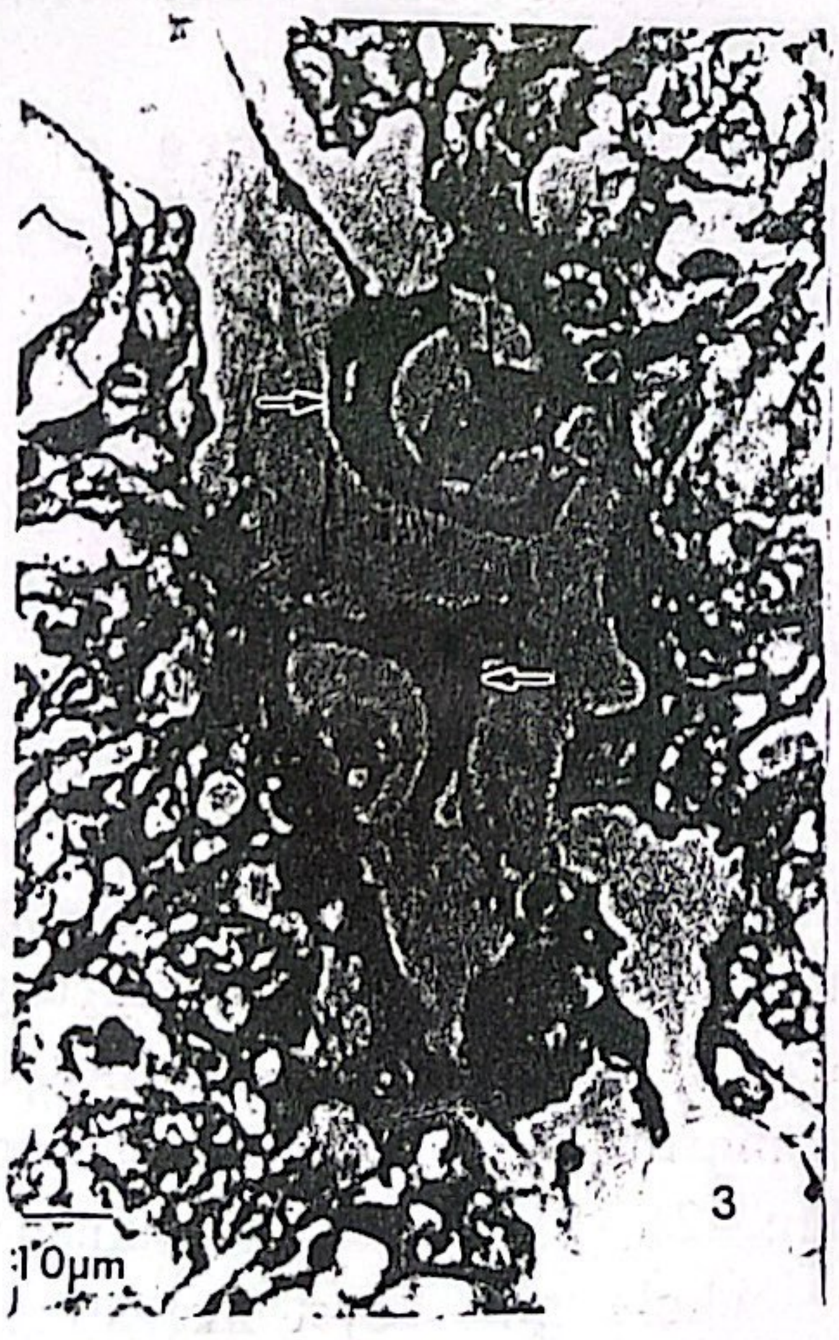

Fig. 3. :Section through the infected gill filaments of grass carp showing the parasites (arrows) holding the top edge of the secondary lamellae.

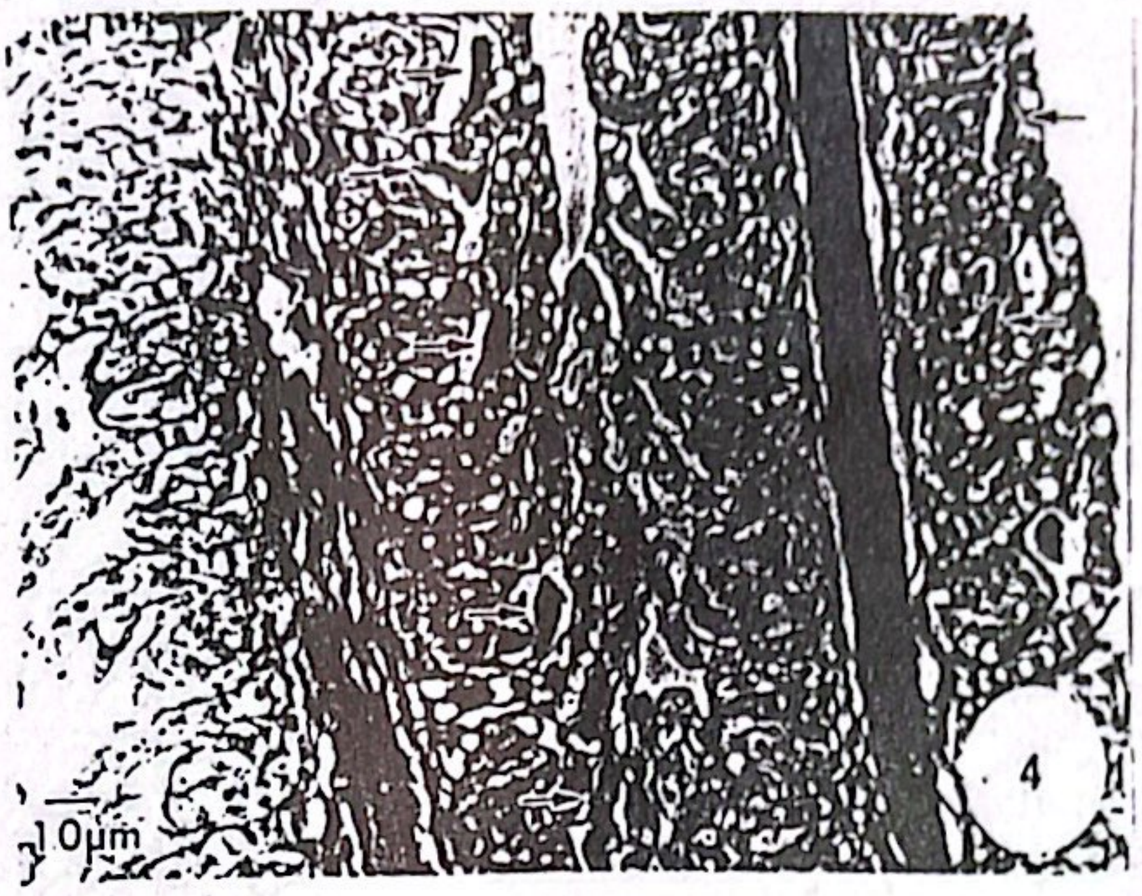

Fig. 4.: Section through the infected gill filaments of grass carp showing excessive epithelial proliferation among the secondary gill lamellae. Note the presence of the parasites (arrows) between the secondary gill lamellae.

Fig. 5.: Seciton through the infected gill filaments of grass carp showing an accumulation edematous fluid (arrows).

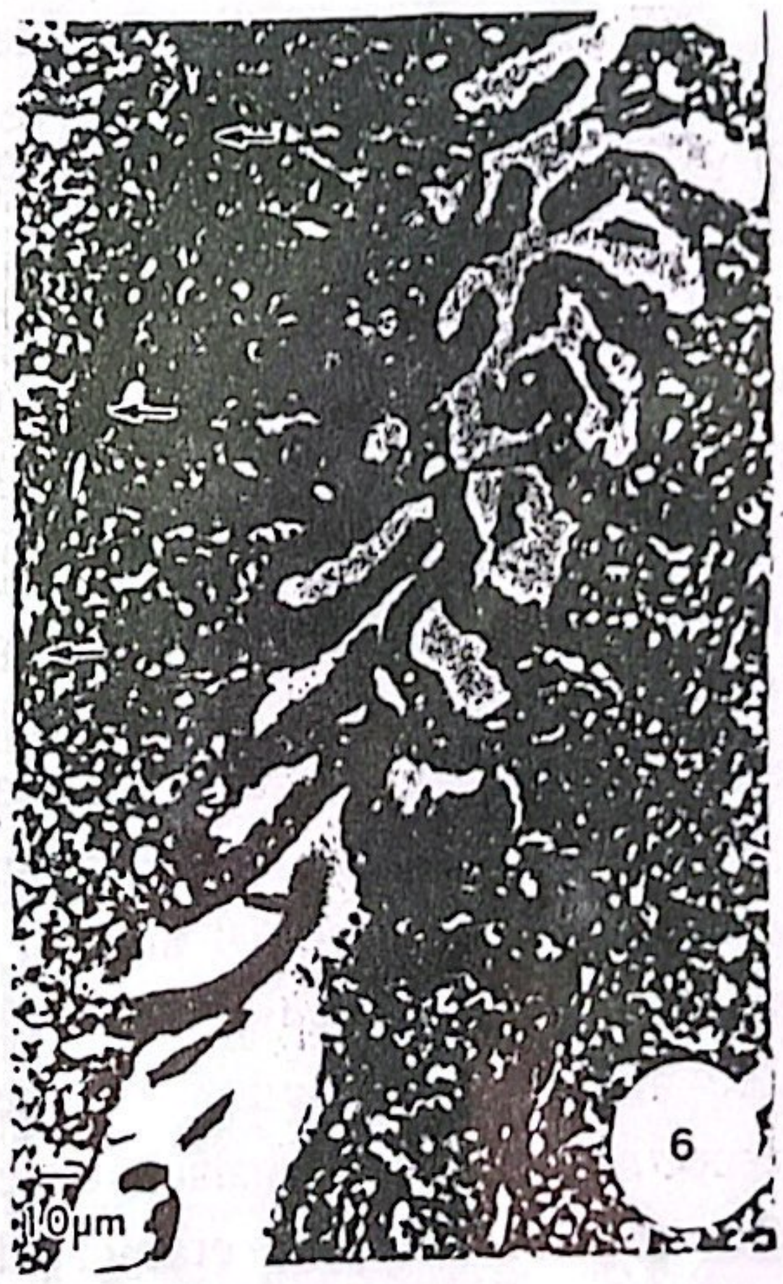

Fig. 6. :Secton through the infected gill filaments of grass carp showing an aggregation of melanin-carying cells (arrows).

Fig. 7.:Section through the infected gill filaments of grass carp showing tissue fragments and nuclear elements around the parasite (arrow).

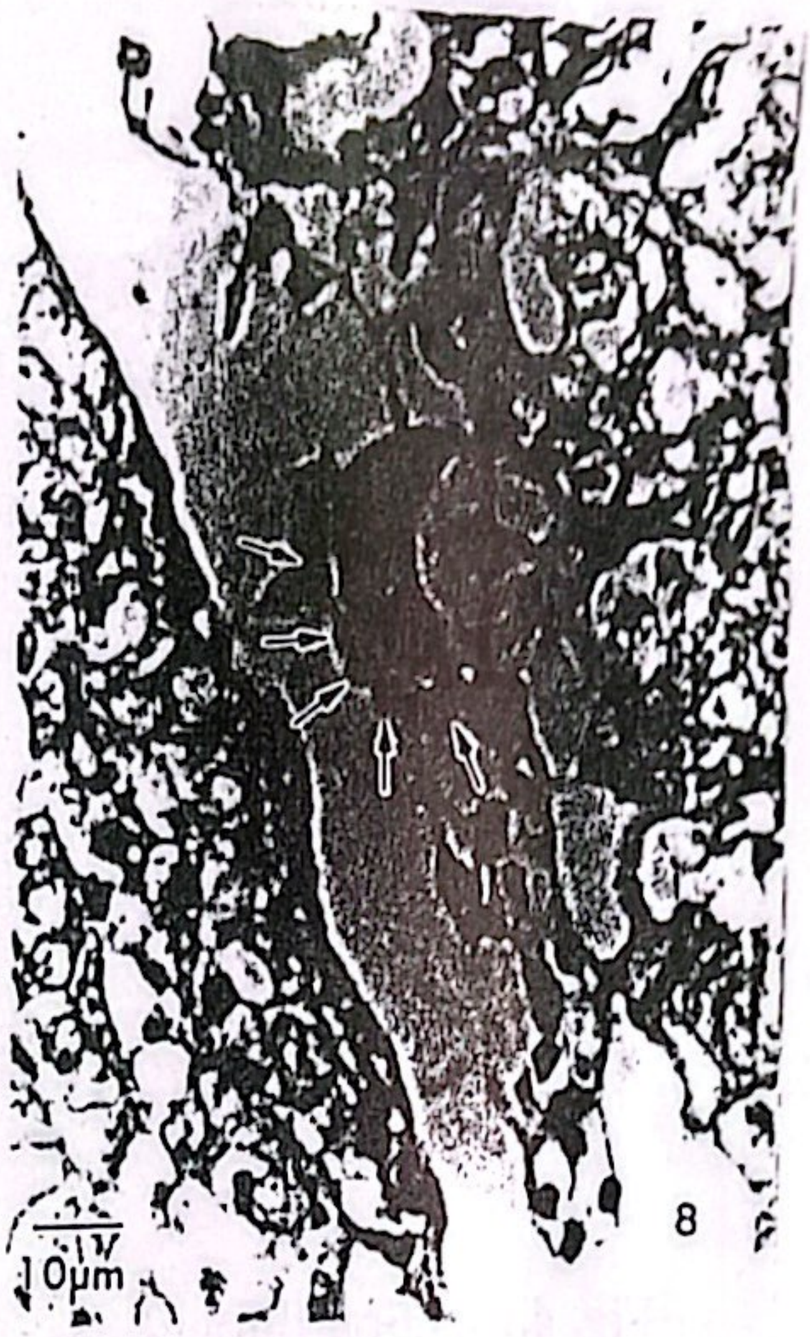

Fig. 8.: Section through the infected gill filaments of grass carp showing an accumulation of lymphocytes (arrows) in areas of the parasite.

For control purposes, the present study showed that the low concentrations of 25 and 50 ppm formalin could be used to exferminate the parasite within 24 hours for both concentrations. However, at the high concentrations of 100, 200, 300 and 400 ppm formalin, the parasite died after 65, 25, 15 and 7 minutes of exposure respectively. The concentrations of 0.5, 1 and 2 ppm copper sulphate could be successfully used to eradicate *T. epizootica* within 24 hours. On the other hand, at the high concentrations of 5, 10 and 20 ppm copper sulphate, the parasite could be exterminated after 40, 10 and 5 minutes of exposure respectively. Using of malachite green at the concentration of 0.3 ppm eradicated the parasite within 24 hours. However, at the concentrations of 2, 4, 6, 8 and 10 ppm malachite green, the parasite was completely exterminated after 55, 35, 35, 25 and 20 minutes of exposure respectively.

DISCUSSIONS

The characteristic measurements of the body and certain organs of *T. epizootica* as determined by Stein (1968) and the present study are shown in Table (1). They are showing great resemblance.

The experimental study showed that *T. epizootica* reduced, more severely, the survival rate of fingerlings grass carp weighting 10-15 grams. On the gill filaments of the parasitized grass carp, the extent of gills alterations were highly dependent on the degree of the parasite infection. The microscopical gill lesions were qualitatively similar to those previously reported on the gill filaments of fishes infected with *Trichodina sp.*

(Ahmed, 1976; McArdle, 1984; Eisa et al., 1985; Abdel Meguid, 1989). The lesions were characterized by vacuolar degeneration and desquamation of the epithelial cells. These lesions were usually associated with aggregation of melanin-carrying cells and congestion of lamelar blood vessels. The present study revealed also excessive epithelial proliferation resulting in an adhesion of the secondary lamellae. Similar host responses have been reported by Abdel Meguid (1989) on the gill filaments of grass carp infected with *Trichodina nobilis*. Furthemore, the present study showed an accumulation of inflammatory cells such as lymphocytes in areas around *T. epizooitca*.

For the control of trichodinids, formalin in short-term bath has been mostly recommended by many authors (Snow, 1982; Allison, 1963; Klinke and Elkan, 1965; Meyer, 1962; Amlacher, 1970; Papexra, 1980; Ramadan and Abdel Meguid, 1986; Abdel Meguid, 1989). However, there were variations of extermination time even at the same concentration. These variations were highly related to the type of host, species of parasite as well as the temperature and quality of water inhabited by the parasitized fishes (Abdel Meguid, 1989). Interestingly, the present study showed that *T. epizootica* was more resistable to formalin than *Trichodina nobilis* (Abdel Meguid, 1989). At the concentrations of 200, 300 and 400 ppm formalin, *T. epizootica* died after 25, 15 and 7 minutes respectively. On the other hand, *Trichodina nobilis* was killed after 15, 10 and 5 minutes at the same concentrations respectively (Abdel Meguid, 1989). Moreover, long bath treatment showed that *T. epizootica* could be eradicated from the grass carp within 24 hours at both concentrations of 25 and 50 ppm formalin. For the same purpose, Abdel Meguid (1989) used 50 ppm formalin to expel *Trichodina nobilis* from the grass carp within 24 hours. Similar to *Trichodina nobilis* (Abdel Meguid, 1989), the present study proved that copper sulphate was highly effective to control *T. epizootica*. The parasite was completely eradicated from the gill filaments of grass carp after 24 hours, 40, 10 and 5 minutes at the concentrations of 1, 5, 10 and 20 ppm copper sulphate respectively. Finally, the present study showed that malachite green could be also used to exterminate *T. epizootica* after 24 hours, 55, 35, 25 and 20 minutes at the concentrations of 0.3, 2, 4, 6, 8 and 10 ppm respectively.

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