Studies on some prevailing parasites affecting *Oreochromis niloticus* fingerlings with a trial of treatment

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ABSTRACT

Three hundred and fifty naturally infested *Oreochromis niloticus* fingerlings were collected from a cultured fish farm. They were examined for external Protozoa, monogenetic and digenetic parasites. The prevalence of isolated Protozoa showed high infestation rates with *Trichodina mutabilis* (71.3%), *Chilodonella hexasticha* (60%). Monogenetic flukes (*Gyrodactylus* rysavyi) had infestation rate of 40%, while digenetic larvae (Hetrophyid metacercariae) showed an infestation rate of 66.6%. The morphometric characters of isolated parasites were determined. Anorexia was the main clinical sign recorded with slimness of the skin, presence of scattered haemorrhagic spots, detached scales and restlessness. Mortality rate was 15 % among examined fish.

Comparative treatment of the infested fish with formalin (20 ppm) and potassium permanganate 2 ppm for 48 hr bath was recorded. Besides, the adverse effects of such drugs on water quality were studied. Such drugs showed effectiveness against *Trichodina*, *Chilodonella* and *Gyrodactylus,* although marked decrease in dissolved oxygen and increase in nitrite was recorded. The value pH decreased in case of formalin and increased in case of potassium permanganate.

INTRODUCTION

Oreochromis niloticus represents the main popular cultured fish produced in Egypt. Parasitic infestations, especially ectoparasites from Protozoa, monogenetic and digentic flukes are the most dangerous groups affecting skin and gills that induces slimness of the skin, irritation, destruction of gills, anorexia and impaired breathing (Lom, 1995). The synergistic action of the parasites may cause mortalities (Osman, 2001).

Ectoparasitic ciliates include species that are the most common parasites of fishes (Lom, and Dyková, 1992). *Trichodina* spp. as well as *Chilodonella* spp. causes 70% mortality among 2-month old fry of cultured grass carp (Uzbilek and Yilidiz, 2002). *Trichodina* glides rapidly over the gills and skin, affects all fish species causing direct or indirect death (Durborow, 2003). Trichodinids commonly occur in association with other ectoparasites, especially, monogenic and digenic flukes (Pearse, 1972).

Monogenean flukes are a group of parasites best described as flatworms commonly found on the gills, skin or fins of fishes have a series of hooks that attach to the fish causing irritation, excessive mucus production (Reed *et al.*

2003). Fish may undergo flashing and have fraying of fins. Severe infection (gills) may cause the fish to become dyspneic and die (Moeller, 2001). Numerous *Gyrodactylidae* spp. affect the skin, fins and also gills of a wide variety of marine and freshwater teleosts (Soulsby, 1982).

Digenetic trematodes are helminthes that require several hosts on which to complete their life cycle. The majority of digenetic trematodes that infect fish do so as a metacercaria or "grub" stage (Bowser, 1999). Metacercarial infestation in fish has economic losses (Paperna, 1991). The incidence of Heterophyid encysted metacercariae in gills of *Oreochromis niloticus* fingerlings in Abbassa aquaculture was 98.46% (Ramadan *et al.,* 2002) while it was 46% in Common Carp gill (Abd EL Hady, 2007).

Formalin bath is effective for controlling Protozoa and monogenean infestations in freshwater fish (Reed *et al.*, 2003). Potassium permanganate (KMnO4) is one of the widely used inorganic chemicals worldwide; it has been used as a water/bath treatment for protozoan and monogenetic flukes in commercial and ornamental fish at 2 ppm (Marecaux, 2006). Formalin causes decline of dissolved oxygen from 10.1 to 3.0 with 36 hr of treatment in addition to pH decline from 7.2 to 6.3 (Rowland *et al,* 2006), while potassium permanganate tended to increase pH and total alkalinity (Kori-Siakpere, 2008).

Therefore, the present work was carried out to detect the prevalence and intensity of different parasites in naturally infested *O. niloticus* fingerlings with a trial for treatment of such parasites either by using formalin or potassium permanganate with reference to water quality.

MATERIAL AND METHODS

1-Fish: A total number of 350 naturally infested freshwater cultured *O. niloticus* fingerlings with a total length of 7-10 cm and body weight of 12-15 g were collected from a cultured fish farm at Giza. Fish were transported alive in large plastic bags to the Fish Diseases Department, Animal Health Research Institute, Dokki, Giza. They were acclimatized to Lab conditions in glass aquaria with aerated chlorine free tap water, while temperature was thermostatically adjusted at 23.2 ±1 ^{o}C. Fish were fed commercial ration twice a day.

2-Chemicals:

A- Potassium permanganate was available in the form of crystals from El-Nasr Co - Egypt. It was used at a dose of 3 mg for 24 h according to Marecaux, (2006).

B- Formalin obtained from Sturat Adwia Co., Egypt and used by a dose of 20 ppm for 24 h according to Lom (1995).

3-Experimental design:

A- Clinical picture:

Two hundred naturally infested fish were continuously examined for gross clinical picture according to Noga (1996).

B- Parasitological examination:

Naturally infested fish were examined according to the method described by Paperna (1996) for the presence of external Protozoa, monogenetic and digenetic parasites, where the prevalence, intensity and identification of each parasite were recorded.

C-Treatment trial: 150 naturally infested *O. niloticus* fingerlings were divided into 3 equal groups; 50 each. First group was treated with 20 ppm formalin, while, the 2nd group was treated with 2 ppm potassium permanganate as permanent bath according to Lom (1995) and Marecaux (2006). The 3rd infested group was kept as a control group for parasitic investigation and for studying the adverse effects of the used drugs. Temperature was adjusted at 23.2 ±1 ^{o}C at the beginning of experiment. The severity of infestation with the parasites was assened by counting the parasites per microscopic field pre and post treatment, according to El-Khatib (1993). For studying the effects of the tested drugs on ectoparasitic infestation, skin and gill smears were taken just before treatment and after the exposure to the used drug. Also water sample was taken just before and after 48 hr of exposure to the used drug for studying the effects of the tested drugs on the water quality, while water temperature was measured daily.

D-Water quality:

1-Temperature: The temperature (^{o}C) was measured by dipping a dry bulb thermometer to about 10 cm below the water surface in each stabilize and allowed to equilibrate for 5 min before the reading was taken.

2-Hydrogen ion concentration (pH): Ten (10) ml of water sample was drawn from the aquaria and transferred into a beaker; and the pH reading was taken with a pH meter.

3-Dissolved oxygen: was determined by using oxygen meter

4-Nitrite (NO_2): Determination of nitrite by kits of Hanna instruments, Standered methods for the examination of water and Wastewater, 18th edition (1992).

RESULTS AND DISCUSSION

Clinical examination:

The present study revealed that naturally infested *O. niloticus* fingerlings with parasites revealed in a sequence of progressive signs as slimy pale skin, detached scales and presence of haemorrhagic lesions on the skin, fins and gills, anorexia and signs of asphyxia, manifested by rapid breathing and aggregation of fish near the water surface. The skin where the flukes were attached, showed areas of scale loss and may ooze a pinkish serous fluid in some fish. Heavy gill infestations revealed increased respiratory rate and fish were less tolerant to low oxygen conditions. Gulping air at the water surface and "Piping" may be observed in fish with severe respiratory distress. Large numbers of parasites on either the skin or gills may result in mortality. Mortality rate among examined fish was 15%. Most of these clinical signs coincided with those observed by Post (1987), Schaperclaus (1992), Noga (1996), Dey and Chandra (1998) and Yousef

(2008). Damaged gills, filaments caused by trematodes infestations were reported as the main cause of asphyxia and respiratory dysfunction (Cone, 1995; Reed *et al.,* 2003). Scales sloughing as well as skin, fins and gills haemorrhages, could be attributed to continuous irritation of *Trichodina* adhesive discs and the feeding activities of monogenetic flukes and the action of their hooks (Schaperclaus, 1992; Reed *et al.,* 2003) In our study, respiratory dysfunction may also be due to penetration and encystation of metacercarial in gill tissues. This agrees with Lemy and Esch (1984) and Farstey (1986).

Parasitological examination: Based on the morphological and parasitological examination of the isolated parasites from skin, fins and gills of the infested *O. niloticus* fingerlings, the recovered parasits belonged to the genera of *Trichodina, Chilodonella* (ciliated Protozoa), *Gyrodactylus* (Monogenea) and hetrophyid metacercariae (Digenea) by ratios of 71.3, 60, 40 and 66.6% respectively, while the degree of infestation was moderate as shown in table (1). The high prevalence of *Trichodina* may be due to the low specificity to its host species which agrees with Lom (1995). The obtained results disagree with that of Vargas *et al.* (2000) who reported *Trichodina* spp. and monogenea in fingerlings of Nile tilapia by raties of 36 % and 15 % respectively.

Table (1): Prevalence and intensity of infestation with external parasites recovered from *Oreochromis niloticus* fingerlings.

Parasites	No. examined fish	Total infestation		Intensity of infestation* (No. / M.F)
		No.	%	
1- *Trichodina*	150	107	71.3	15-20
2- *Chilodonella*		90	60	10-13
3- *Gyrodactylus.*		60	40	3 -10
4- Hetrophyid metacercariae		100	66.6	3 -5

* Number of parasites per microscopic field

The morphological characteristics of *Trichodina mutabilis* (Kazubski et Migala, 1968) (Fig.1).

Kingdom: Protozoa, Phylum**:** Ciliophora; Class: Ciliatea; Order: Mobilina Family: Trichodinidae, Genus: Trichodina, *Trichodina mutabilis* (Kazubski et Migala,1968) is medium to large disc shape provided with several rows of cilia at the circular periphery and a circle of more centrally laying hooklets. Denticle blade oblong, largely barrel to radial disc, with squared distal end, central part narrow with oblong to round overlapping end.

The morphological characteristics of *Chilodonella hexasticha* (Kiernik, 1909) (Fig. 2).

Kingdom: Protozoa, Phylum: Ciliophora, Class: *Ciliata,* Order: *Cyrtophorida* Family: Chilododontidae, Genus: *Chilodonella, Chilodonella hexasticha* (Kiernik, 1909) is a ciliated large protozoan, heart-shaped ciliate (60 to 80 m) with bands of cilia along the long axis of the organism. The ventral side was flat

with barrel ciliary rows, the dorsal surface was slightly convex and lacked cilia except in the oral groove at the extreme anterior end (35-39 um) in length and (23-25 um) in width. The macronucleus was rounded .The morphological characteristics of *Trichodina mutabilis* as well as *Chilodonella hexasticha* were nearly similar to that described by Lom and Dyková (1992), Lom (1995), Noga (1996) and Paperna (1996). The morphological characteristics of *Gyrodactylus rysavyi* (Ergens, 1973) (Fig. 3). Phylum: Platyhelminthes, Class: Trematoda Subclass: Monogenea Order Gyrodactylidea, Family Gyrodactylidae Genus: *Gyrodactylus. Gyrodactylus rysavyi* (Ergens, 1973). It is described as flatworms, with two lobed projections on the anterior end and the chief attachment organ (opisthaptor) is posterior, more or less discoid, muscular, provided with paired anchors and a twenty smaller hooks. Each individual parasite has both male and female reproductive organs. The morphological characteristics agree with that reported by Moravec *et al.* (1991), Cone (1995) Noga (1996) and Reed *et al.* (2003).

The detected hetrophyid metacercariae from gills (Figs. 4 & 5) are spherical encircled by two thin layers and measure 0.13-0.20 mm in diameter The oral and ventral sucker are well developed. The oral sucker terminated with circumoral spines as reported by Tantawy (1997) and Abu El- Ezz *et al.* (2000).

The effect of potassium permanganate and formalin on the degree of parasitic infestation and mortality rate of *O. niloticus* fingerlings were shown in Table (2).

Table (2): Effect of formalin and potassium permanganate on the intensity of parasitic infestation in *Oreochromis niloticus* fingerlings.

Ectoparasites / Group	*Trichodina*	*Chilodonella*	*Gyrodactylus*	Heterophyid metacercariae
1st infested fish group pre treatment	++	+	+	++
1st infested group post treatment with potassium permanganate	-	-	-	++
2nd infested fish group pretreatment	++	+	+	++
2nd infested group post treatment with formalin	-	-	-	++
3rd infested group pretreatment	++	+	+	++
3rd infested group without treatment	+++	++	++	++

\+ Light infestation protozoa 1- 10, monogenia 2- 5 ,
++ Moderate infestation: protozoa 12- 17, monogenia 10- 20. digenia 3-5
+++ Heavy infestation protozoa 20- 30, monogenia 21- 35.

The two used drugs were highly effective against ectoparasitic infestations *(Trichodina*, *Chilodonella* and *Gyrodactylus).* These results nearly agree with findings given by Stuart (1983); Younis (1993, 1999), Brown (2000),

Tantawy and Younis (2003) and Rowland *et al.* (2006). The obtained results agree also with Reed *et al.* (2003) who mentioned that formalin bath is effective for controlling protozoan and monogenean infestations in freshwater fish. From the present results, the two drugs did ret affect heterophyid metacercariae, which remain viable, surrounded by thick fibrous capsule and need special treatment as recorded by Tantawy (2004). Also, from Table (2) it is clear that, parasitic infestations were increased in parasitic non treated group with 20% mortality rate. This agrees with Uzbilek and Yilidiz (2002) who recorded 70% mortality among 2-months old fry of cultured grass carp infested with *Trichodina* and *Chilodonella* and Kim *et al.* (2002) who recorded that monogeneaus caused losses among 15 species of ornamental fish in korea.

Comparative study on the effect of formalin and potassium permanganate on water quality was recorded in Table (3) Both of them cause decrease of dissolved oxygen .This result agrees with Hazen and Sawyer (1992 who reported that 5mg /L of formalin remove 1g of dissolved oxygen. This is one reason why using of formalin in ponds is discouraged. It also agrees with Kori-Siakpere (2008) who recorded that potassium permanganate decrease oxygen level. Potassium permanganate loses two oxygen atoms and is reduced to insoluble manganese dioxide MnO_2. The lost oxygen atoms react aggressively with parasites altering their structure and properties which lead to their mortality. Insoluble manganese dioxide may precipitate on the gills causing respiratory problems. This agrees with results of Hazen and Sawyer (1992), Rowland *et al.* (2006) and Kori-Siakpere (2008).

Table (3): Effect of formalin and potassium permanganate as water bath for 48 hr on water quality at 23.2±1 ^{o}C

drug / parameter	Formalin 20 ppm	Potassium permanganate 2ppm	Control (Non treated)
Dissolved oxygen (DO)	6.0^{o}C	6.4	7.5
Nitrite (NO_2)	0.45 mg/L	0.40 mg/L	0.35 mg/L
pH	5.5	8.8	7.2

So during treatment with potassium permanganate and formalin, the oxygen supply to the treated fish should be increased to avoid the bad effect of oxygen decrease.

It is also clear from Table (3) that nitrite and pH were increased than in the control except in case of formalin, where pH was decreased. This agrees with Rowland *et al*. (2006) and Kori-Siakpere (2008). These changes represent a stress factor to fish as reported by Noga (1996) that at low pH, the toxic metal becomes rapidly dissolved in water and can flux through the permeability of gills causing toxicity, while in alkaline water ammonia changes into unionized form that can enter the body of fish through the permeability of gills and cause

mortality. These results agree also with that of Hazen and Sawyer (1992) who recorded that nitrite and pH should be closely monitored following treatment with potassium permanganate. Also, Noble and Summerfelt (1996) reported that high nitrite acts as a physical streamer affecting on defense mechanism and immune response of fish especially younger fish.

From the present study, it was concluded that ectoparasitic infestation in *O. niloticus* should be diagnosed and treated quickly to avoid their rapid spread among fish farms, minimizing the morbidity and mortality rates and to avoid secondary bacterial or fungal infections. Also, treatment either by potassium permanganate or formalin seems to be of a great success for the control of parasitic infestation under enough oxygen supply to avoid the bad effect of decreased oxygen.

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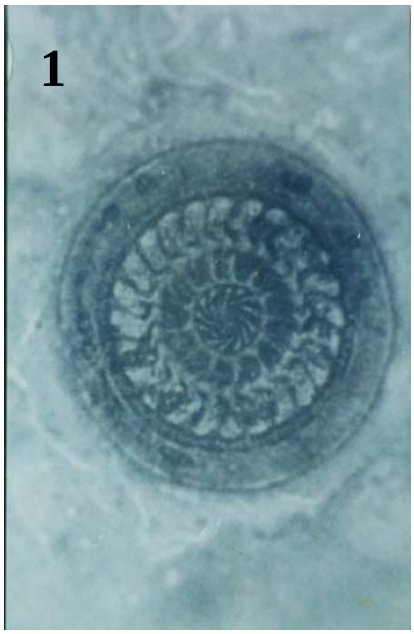
Fig. (1): *Trichodina mutabilis,*
Silver imprgnation X40

Fig. (2): *Chilodonella hexasticha.*
Silver imprgnation X40

Fig. (3): *Gyrodactylus* rysavyi
1-Prohaptor (2 lobes).
2-Uterus.
3-Embryos.
4-Central hooks.
5-Marginal hooklets.

Fig. (4):Hetrophyhid metacercaria in gill filament (X 100)
1-Oral sucker with circumoral spines.
2-Ventral sucker.
3-Thick fibrious cyst wall.

Fig. (5): Hetrophyhid metacercariae in gill f ilaments (X 40)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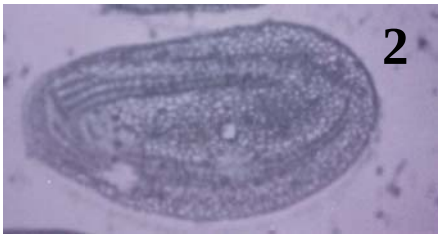
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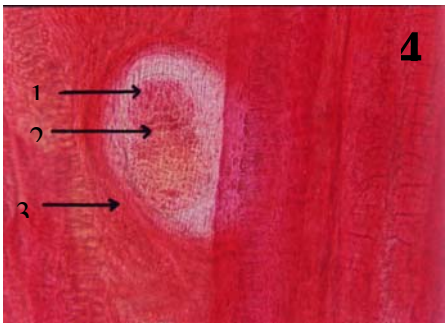
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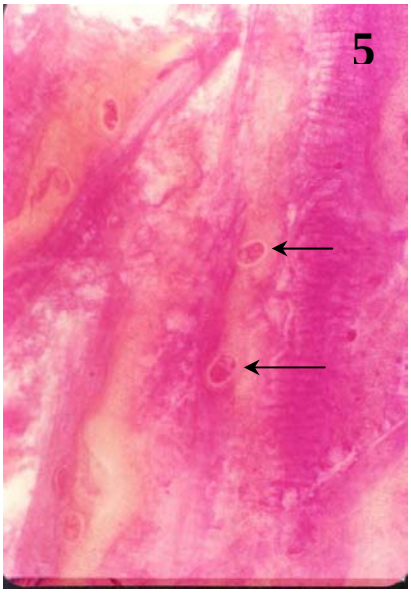
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