A trial for control of some external parasitic diseases cultured *Oreochromisniloticus* in Egypt

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Abstract: The present study was carried out on 360 of fingerlings Nile tilapia of different body weights. They were collected live from the same ponds of study 120 fish from infested farms in the three previous governorate 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 aggregated on the surface, 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. Marbling appearance with numerous white dots in some gill filament of infested fishes. Some external protozoa, monogentictrematod and crustacean parasite were isolated, identified and recorded. Also, some physico-chemical parameters of pond waters represented in pH, salinity, ammonia and sulphates were examined in relation to the infestation rate with external parasites. The application of different doses of medicinal plants *(Allium Sativum* and *Artemisiasm vulgarism*) for treatment of external parasites. Also, humic acid and mannan-oligosaccharides (Bio-Mos®) were used in treatment. It was found that, the medicinal plants very effective causing a great damage to the most external parasites within few days. Also, humic acid and mannan-oligosaccharides (Bio-Mos®)were effective on some protozoal external parasites without side effect on infested fish.
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1. Introduction

The external parasites in cultured fish ponds have been increasing problem and leading to diseases that cause severe economical impact.

The protection is necessary to increase fish production by aquaculture. The convential methods are traditional for control of freshwater fish parasites (**Noga, 2010**). The most practical and effective preventive and curative non convential methods for treatment and control fish parasites**.**

Today, there is a great attention to the use of medicinal plants which have provided the models for 50% of western drugs **(Robbers *et al*., 1996).** Garlic is one of the edible plants that had a strong interest to scientists and recognized as an important medicinal plant which has a wide spectrum of actions; as antiprotozoal **(Harris *et al*., 2001).** *Artemisia vulgaris* is another plant that is promising to prevent fish diseases **(Ekanem and Brisibe, 2010).** The raw and squeezed garlic (*Allium sativum*) at 200 mg/l had potential to treattrichodinidsin eel **(Madsen *et al.*, 2000).** The crude extracts from two tropical medicinal plants, Mucunapruriens (Fabaceae) and Carica papaya (Caricaceae), used in treatment of the ciliate *Ichthyophthiriusmultifiliis* which is the most pathogenic parasites of fish maintained in captivity. The result was a 90% reduction in numbers of *I. multifiliis* on fish after treatment in baths of each plant extract at 200 mg l^{-1} compared to untreated controls (Buchmann *et al.,* 2003)**.** It is the more safety method for control of fish parasites used mainly in control of crustacean parasites of fish **Noor El Deen *et al* (2010).**. Bio-Mos® considered being as growth promoter at level of 4 g/Kg of fsh diet, with consequent improving the general fsh health and increasing the total fsh gain **(Mohamed *et al*., 2013**).

The present investigation was focused on the clinical picture of the examined infested *Oreochromisniloticus* with ectoprorozoal diseases. Besides, a trial for treatment was applied using some medicinal plants such as *Allium Sativum*and *Artemisia vulgarism*, humic acid and Bio-Mos®.

2. Materials and Methods

Fish for expermintal examinations:

A total of 360 infested *O niloticus* were divided into five equal groups. The first group G1 was treated with *Allium sativum*400 mg /kg diet, G2 was treated with *Artemisia vulgar is* 800mg */kg* diet, G3 was treated with a mixture of *Allium sativum*400 mg and *Artemisia vulgar is* 800 mg lKg diet as medicated feed. While, G4 was treated with **Bio-Mos®** 4 g/kg feed and G5 as control**.** Feeds were formulated from ingredients commercially available in Egypt according to **Noor El Deen & Razin (2009)**.

Medicinal plants:

Garlic (*Allium sativum*): a box of 1 kg imported from China in a conc. Garlic extract allcin 25% and

volatile oils 10% under a commercial name (Garlen Extra 4).

***Artemisia vulgaris*m:** Leaves were washed thoroughly in a running tap water to remove sand and debris**.** They were dried in a hot air oven at 60ºC for 8 hrs. The dried leaves were crushed to powder and extracted according to **Lee *et al* (2004)**.

Bio-Mos®. A mannan-oligosaccharides derived from the cell wall of the Saccharomyces cerevisiae yeast. It is a product manufactured by Alltech, Inc. UK. Biomoss was added within the ratio of 2 and 4 g/kg.

Clinical Examination:

Alive fish were clinically examined for the general behaviors, changes in colour, respiratory manifestation, feeding and any clinical abnormalities on the external body surface (skin, gills, eyes and mouth), also any external growth lesions like wounds, petechial hemorrhage, ulcers, slimness, sloughing of scales from the skin or eroded fins and cysts for detection of any parasites visible by necked eye according to the methods described by **Noga (2010)**.

Parasitological examination:

Smear scrapings from the different external body surface of the collected fishes were obtained. 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 examined microscopically **(Lucky, 1977).**

Water analysis:

A total of 36 water samples equally distributed through the different seasons, were collected from the different fish ponds; simultaneously with fish specimens for determination of pH, salinity, ammonia, alkalinity, oxygen and sulphate according to **Adams 1990**.

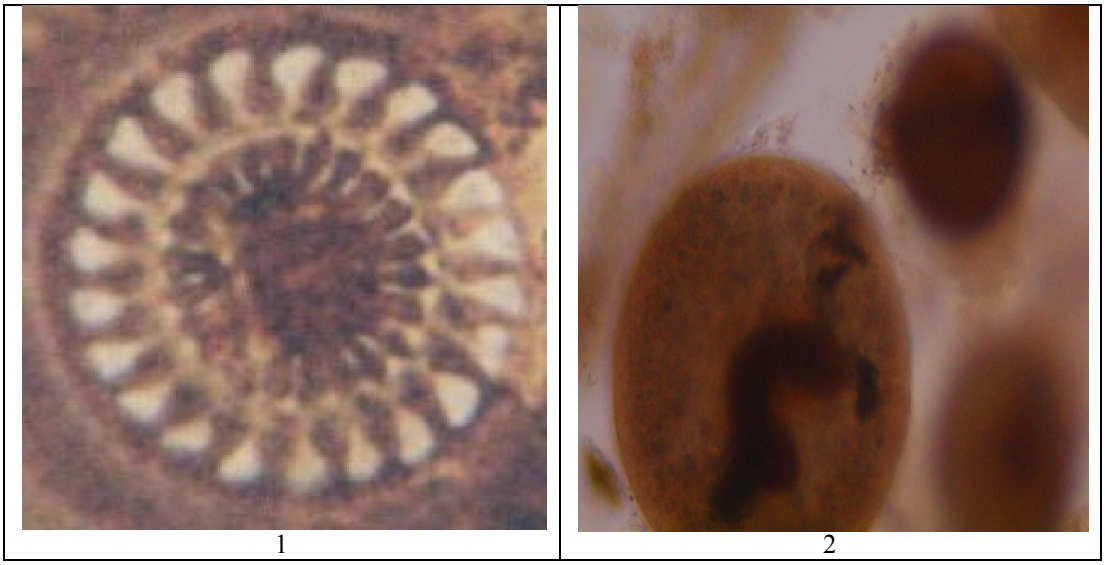
3. Results

Clinical picture:

The clinical examination of most examined fishes was observed some fish aggregated on the surface of aquarium. Also, fishes showed gills were appeared swollen and be covered with thick mucus. The scale external body surface as well as sloughing.

Parasitological findings:

Microscopic smears were taken from skin and gills of examined fish, showed a peritrichus ciliated protozoan. Such ciliated protozoan was identified as *Trichodinaheterodentata Duncan, 1977* **(Fig,1).** A holotrichous protozoan was identified as *Ichthyophthiriusmultifiliis* **(Fig,2).**



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Plate,1. Showing magnified *Trichodinacalifornica* (1) Stain: Gimsastain X 400and*Ichthyophthiriusmultifiliis*(wet mount) (2).

Water parameter:

There was a reverse proportion between the infestation rate and water quality (ammonia from 0.5 mg/l - 0.6 mg/l, sulphate from 70ppt - 200ppt and pH from 6.1- 7.3). Where, the increases of water salinity (from 8ppt to 15 ppt) were accompanied with the decrease of parasitic infestation (Table,1).

Table 1: -showing parameters of water quality at different localities.

Locality	Parameters of Water Quality				
	Ammonia mg/l	Dissolved oxygen mg/l	Water salinity ppt	Alkalinity ppt	PH
Alsharkaya fish farms	0.3-0.6 (0.5)	5-6	7- 8 (8)	151-210 (196)	7.1- 75(7.3)
Kafr El Sheikh fish farms.	0..4 - 0.7(06)	5-7	8-10 (9)	92-154 (123)	5.7- 7 (6.2)
Alfayoum fish farms	0.3 - 0.6(0.5)	5-7	12- 18(15)	46-68 (57)	5.2- 6.7 (6.1)

Treatment trails:

Medicinal plants.

Treatment trails on 120 infested fish from the same ponds of examined (30 fingerlings *O. niloticus* in each group) infested with external parasites were applied using mixtures of medicinal plants (garlic and *Artemesa vulgarism*). The results of treatment of such naturally infested tilapia after using medicinal plants were described in Table (5).

It was noted that, the best suitable and effective concentration of mixtures of medicinal plants (garlic and *Artemesa vulgarism*) used in ponds without harmful effect on fish causing a greet damage to the parasites was (400 mg garlic + 800 mg *Artemisavulgarisum* / kgm ration) for 15 days.

Table 5: Showing the different treatment trials on external protozoans and momogena.

Group	Medicinal plant	Dose mg/kg	Trichodina	Chilodonella	Icthyoboda	Ich	Cichlodgyruis
1	Garlic	400	++	++	+	_	+
2	Artemisa	800	++	++	+	_	+
3	Garlic +artemisa	400+800	+++	+++	++	_	++
4	Control	-	-	-	_	_	_

+= Lowpotency, ++ =moderate potency, +++=high potency, - = no potency.

Humic acid

Treatment trails of 120 infested fish from the same ponds of examined (30 fingerlings *O. niloticus* in each group) infested with external parasites were applied using mixtures of humic acid. The results of treatment of such naturally infested tilapia after using humic acid were described in Table (6).

It was noted that, the best suitable and effective concentration of humic acid used in ponds without harmful effect on fish causing a greet damage to the parasites was 5 ppm in water of pond for three successive days.

Table 6: Showing the different treatment trials on external protozoans and momogena.

Group	Medicinal plant	Dose ppm	Trichodina	Chilodonella	Icthyoboda	Ich	Cichlodgyruis
1	Humic acid	3	++	++	+	_	+
2	Humic acid	5	++	++	++	_	++
3	Humic acid	10	++	++	++	_	++
4	Control	-	-	-	_	_	_

+= Low potency, ++ = moderate potency, +++= high potency, - = no potency.

Bio-Mos®

Treatment trails of 120 infested fish from the same ponds of examined (30 fingerlings *O. niloticus* in each group) infested with external parasites were applied using Bio-Mos®. The results of treatment of such naturally infested tilapia after using humic acid were described in Table (7).

It was noted that, the best suitable and effective concentration of Bio-Mos®. used in ponds without harmful effect on fish causing a greet damage to the parasites was 5 ppm in water of pond for three successive days.

Table 7: Showing the different treatment trials on external protozoans and momogena.

Group	Medicinal plant	Dose ppm	Trichodina	Chilodonella	Icthyoboda	Ich	Cichlodgyruis
1	**Bio-Mos®**	2	++	++	+	_	+
2	**Bio-Mos®**	3	++	++	++	_	++
3	**Bio-Mos®**	4	++	++	++	_	++
4	Control	-	-	-	_	_	_

+= Low potency, ++ = moderate potency, +++= high potency, - = no potency.

4. Discussion

In the present study, the main clinical pictures of infested freshwater fishes were aggregation on the water surface and rubbing the body against the sides of aquaria to get rid of the irritation induced by the parasites on the skin, fins and gills. They appeared asphyxiated and exhausted this may be due to low respirated oxygen of destructed gill epith which caused by feeding activity, attachment, fixation and locomotion of trichodina causing massive destruction of the respiratory epithelial cells. This result may be similar to that recorded by **Eissa *et al* (2010).** Sloughing of scales was noticed. In addition erosions and ulcerations on trunk area of the skin and sloughing of scales. The same clinical signs were previously obtained by **Noor El Deen *et al* (2010).**

Concerning to the water analysis, it was revealed that ammonia 0.5mg/l in Alsharkia and Alfayoum while in Kaf El Sheikh 0.6mg/l. These results may be attributed to the farms area in Kaf El Sheikh its water supply coming directly from agriculture discharges. Dissolved oxygen 5-6 mg/1 in Alsharkia while in Alfayoum and Kaf El Sheikh 6-7 mg/l. These results may be attributed to the intensity of fish in Alsharkia farms higher that that cultured in Alfayoum and Kaf El Sheikh. Water salinity 8, 9 and 15 mg/1 in Alsharkia, Alfayoum and Kaf El Sheikh respectively. These results may be attributed to type of water drench in each area. alkalinity 196, 123 and 57 mg/l inAlsharkia, Alfayoum and Kaf El Sheikh respectively. While, pH 7.4,6.2 and 6.1 in Alsharkia, Alfayoum and Kaf El Sheikh respectively.These results may be attributed to its water supply coming directly from industrial discharges.These results were nearly similar to thefindings met with (**Elewa and Mahdy, 1988).**

Regarding to the treatment trails it was revealed that the usage of medicinal plants such as (garlic and Artemisia vulgarism) 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 garlic and 800 mg Artemmisia/ kg diet) for 15 days as no viable external parasites were observed on skin and gills. This result agrese with **Ankri and Mirelman (1999)** who recorded that isolated, identified and synthesized an oxygenated sulfur amino acid that is present in large quantities in garlic cloves andwhich they named allicin and that recorded by **Noor El-Deen and Razin (2009) and Mesalhy *et al* 2008** who found that medicinal plants act as saponin effect (allicin and artemisenic acid) on the parasite. They also reported that crude extracts of either garlic at 400 mg or *Artemisia vulgarism* at 800 mg/kg diet significantly eliminated Trichodinasp infestation in tilapia. *A. vulgarism* will be used as alternative to chemicals to treat of infected tilapia with Trichodina sp. This variance may be attributed to the diffrenece in drug consistansy and mode of application.

Finally, from the present study it is indicated that garlic, *Artemisia vulgarism* (Sheih) and humic acid and mannan-oligosaccharides (Bio-Mos®) are effective, safe and cheap for treatment of external parasites in *O. niloticus*. The medicated feeds not only affect internally but also externally as feeds present in water and affect directlyon external parasites. Also, It was noted that, the best suitable and effective concentration of humicacid andmannan-oligosaccharides (Bio-Mos®) used in ponds without harmful effect on fish causing a greet damage to the parasites was 5 ppm in water of pond for three successive days.

The present findings indicated that the used medicinal plants andhumic acid and mannan-oligosaccharides (Bio-Mos®) improve fish resistance, effective, safe as well as they are cheap comparatively with chemotherapy.

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