# A contribution in ectoparasitic infection and its control in cultured *Oreochromis niloticus* in Egypt

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**Abstract**

A total of 420 *Oreochromis niloticus* live fish specimens were collected from some farms fishes, (Central Lab for Aquaculture Research (CLAR) Abbassa, Sharkia and private fishes from Kalubia & Giza governorates), at different seasons. Inspected fishes were recovered infection with 4 genera of protozoan including; *(Trichodina heterodentata, Trichodina mutabilis), Chilodonella hexasticha, Myxobolus ellipsoids* and *Epistylis* spp.) with infection rates of 42.7%, 17.9%, 8.8% and 31.2%, respectively. Infection with *Cichlidogyrus halli* (monogenea), encysted metacercariae (digenetic larvae) and *Lamproglena monodi* (crustacea) were recorded in the examined with rates of 22.7%, 12.4% and 17.6%, respectively. Moreover, mixed infection with *Trichodina*, *Epistylis* and encysted metacercariae (EMC) were detected with rate of 3.6% in the examined fishes.

The main clinical sings of infected fish were slimy dark skin with signs of asphyxia, detached scales with frayed fins with presence of hemorrhagic lesions on the skin, fins, and gills with congested gills.Treatment trials on *O. niloticus* fishes with mixed natural infected *Tirchodina*, *Epistylis* and EMC by using three products were used ; 1st Chemical treatment with Per oxygen compound®, surfactant organic acids and buffer systems; the 2nd natural product Humate® (humic, ulmic and folvic acids) and the 3rd Probiotic product as (*Bacillus thuringenisis kurstak*). The experimental treatment result revealed that Peroxygen® compounds at concentration (2ppm./40 min. & 5ppm/20min.) and at concentration (1000ppm/30 min. & 1500 ppm /15min.) were sufficient completely eradicates *Trichodina* spp. *& Epistylis* but did not affect on EMC in different doses. However, the natural product as Humate® was proved to be the most effective product at concentration 3ppm/ 30 min. to eradicated *Tirchodina*, *Epistylis* and at the same concentration for 60 min. was to be killing EMC infection.

Hematological parameters of infected fish showed increase significant in RBCs & hemoglobin ($P \leq 0.005$) $2.2 \pm 0.03$ $x10^6/\mu l^6$ and ($P \leq 0.001$) $6.7 \pm 0.01g/dl$.

**Keywords:** *O. niloticus*, external parasites, clinical sings, Treatment, Peroxygen® compounds, Humate®, Probiotic product, Hematological parameters.

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## Introduction

In coastal countries as Egypt, the fish production was supplied widely from natural water resources including the inland natural and artificial lakes of different water salinities.

Parasitic diseases of fish have a superior position and have received a significant attention in Egypt of one of subtropical country Eissa et al., (2000). Away from their direct damage effect on fish tissues, parasitic agents may act as stress factors rendering the fish more susceptible to other diseases Hoffman et al., (1990). Also the drastic indirect effect played by fish parasites; their retardation of fish growth with combination of fish mortality-constitute the most economical impact concerning fish production.

External protozoa, monogenetic and digenetic trematodes (EMC) of freshwater fish could be considered as the most prevalent causes of diseases affecting skin and gills causing gill inflammation and distortion of normal anatomy which impairing their respiratory foundation. It is the primary site of nitrogenous waste excretion and plays an important role in ionic balance Woo (2006), Noga (2010) and Reda (2011) in skin causing irritation, inflammation and loss of the surface epithelium which this in turn open the way for secondary invaders Tantawy (2001).

The important to keep in mind that all fish drugs are toxic to fish. Fortunately it usually takes higher concentration of the drug to harm the fish than it does to harm the pathogen. Thus non chemo therapeutant should be used unless there are many products available that have never been tested in controlled laboratory studies. Therefore, in

recent year's considerable change have been happened toward using biological drugs alone or in combination some chemical drugs with safe use as possible as could. Chemical treatment may be linked to side effects such as toxic stress Meinelt et al., (2007).

A trial for treatment such parasites using natural products such as Humate is considered of great interest and preferable Noor El- Deen et.al. (2011). Therefore, present study was carried out to investigate the prevalence of ectoparasites infecting *O. niloticus*. Experimental treatment of natural infecting fishes by using three products either Chemical as Peroxygen® compounds, natural as Humate® and Probiotic as *Bacillus thuringenisis kurstak* and evaluated as eradicate of ectoparasitic infection. Efficient of treatment on some hematological parameter was also evaluated post treatment.

**Materials & Methods**

Fishes A total of 420 live *O. niloticus* fishes were collected alive from: (Central Lab for Aquaculture Research (CLAR) - Abbassa, Sharkia and private fishes from Kalubia & Giza governorates), at different seasons of the year. Fishes were inspected for ectoparasitic affection in this fishes. The fishes were transported alive to the laboratory of fish diseases at Animal Health Research Institute, Dokki, Giza, Egypt. In large tanks filled with water of the same sources supplied with battery air pumps and examined as soon as possible. Fish skin, fins and gills were firstly examined by the naked eye for detection of any macroscopically visible lesions. Samples of mucus were scraped gently from the skin, fins and gills, then spread on a clean slide and freshly examined under phase contrast microscope for the presence of external protozoan. Some of the positive slides were air-dried and stained according to Klein's dry silver impregnation method. Other positive slides were also air-dried, fixed with absolute methanol and stained with 10% Giemsa stain Lucky (1977). All detected parasites; were examined freshly, fixed and stained according to Pritchard & Kruse (1982) then identified according to; Dykova & Lom (1992), Paperna (1996) and Woo (2006).

Products: as described in details by Yousef (2008) were used three products for treatment of naturally infected fishes including; Chemical product as Virkon-s® (Peroxygen compounds®, surfactant organic acids and an organic buffer system, it is a product of Dupont Animal Health Solutions United Kingdom); natural product as Biofarm® (Humate= humic acid, ulmic acid and folvic acid with the trace minerals it is a product of Farmavet International Istanbul-Turkey) and probiotic product as Protecto *(Bacillus thuringenisis kurstak* ) from Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

**Experimental design**

A. Determination of half-lethal concentration ($LC_{50}$) of drugs used for estimation the toxicity of the used drugs for treated fishes, The $LC_{50}$ test was performed for each used product in treatment experiments according to fish test Yousef (2008). A total number of 190 *O. niloticus* apparently healthy and parasitologically negative were divided into 19 groups of 10 fish each used for determination of $LC_{50,}$ after exposed fish groups to different concentrations for each product.

Experiment (Table 1): A total of 140 a live *O. niloticus* naturally mixed infected with (*Trichodina* spp., *Epistylis* and EMC.), were divided into 3 main groups (gp) as; $1^{st}$ gp. 40 fishes, $2^{nd}$ & $3^{th}$ gp. each 50 fishes maintained in a separate glass aquaria. Fishes were subjected to different treatments using Virkon-s®, Biofarm® and Protecto.

Measuring of some hematological parameters; blood samples were collected from the living fish, caudal vessel according to Lucky (1977). The following blood parameters were evaluated in fishes include; Haematocrite value (PCV) Blaxhall & Diasely (1973) Hemoglobin (HP) estimation Foda (1973), total RBCs and differential Leucocytic count, according to Kanaev (1985). Blood samples were collected before and 15 days after treatment to investigate the hematological changes in the treated fish.

**Results**

Inspection a total of 420 *O. niloticus* fishes collected alive from fish Abbasa aquaculture, private fish farms at Kalubia & Giza governorates, at different seasons of the year. Prevalence of recovered protozoan in Table (2) revealed infections with 4

genera which including;*(Trichodina heterodentata ,Trichodina mutabilis),Chilodonella hexasticha, Myxobolus ellipsoids* and *Epistylis* spp. with infection rates 42.7%, 17.9%, 8.8% and 31.2%, respectively. Infection by trematodes and crustacean parasites including; *Cichlidogyrus halli*, encysted metacercariae (EMC) and *Lamproglena monodi* with prevalence 22.7%, 12.4% and 17.6%, respectively. Mixed infection with *Trichodina, Epistylis* and EMC and mixed infection with *Cichlidogyrus halli* and *Lamproglena monodi* were detected in *O. niloticus* at rates of 3.6%.

Fish infected with protozoa *Trichodina* spp., *Chilodonella, and Epistylis,* showed slimy pale skin with sever blood spots scattered on the body especially at the base of fins with detached scales in *O.niloticus.* while, the examined fishes infected with EMC which appear as black spots on pectora fin (Photo 5) & on the gills (Photo6). Moreover, the infested *O.niloticus* by *Cichlidogyrus halli* showed loss of appetite and sluggish movements, swimmed near the surface of the water with increased breathing frequency, stretched gills covers and expanded pale and sticky gills. Infected *O. niloticus* were anaemic and exhibited symptoms of anoxia and detached scales ( Photos 1 - 4 ).

Concerning seasonal prevalence in investigated *O. niloticus* are illesterated in table (2). Demonstrated that highest infection rate with *Trichodina* spp was recovered in winter (63.7%) followed by autumn (44.7%). The EMC infection was only found in spring with rate of 82.5%. While the highest infection rate with *L. monodi* was recorded in spring (49.2%). The mixed infection of *EMC, Epistylis and Trichodina* were recorded in spring with prevalence of 23.7%. While, the mixed infection with *L. monodi and C. halli* were recorded in summer only with prevalence rate of infection 15.6%.

The results of experimental treatment were showed in the Table (3) of naturally mixed infected *O. niloticus* revealed that the most efficiency of Biofarm® at concentration 3ppm/30min. to eradicate *Trichodina, Epistylis* and for 60 min. exposure period was killed EMC. In the same time, Virkon–s® and Protecto at concentration 2ppm/40min. & 5ppm/20 min. and1000ppm/ 30 min. & 1500 ppm /15min.were succeed to eradicate *Trichodina* spp. *& Epistylis* without effect on the EMC.

Concerning the effects of treatment program on the hematological pictures of the treated fishes. The result in table (4), revealed significant important in all of the evaluated

parameters post treatment. Significant increase in RBCs ($p \leq 0.001$) $2.6 \pm 0.02 \times 10^6/\mu l^6$ and hemoglobin ($P \leq 0.001$) $6.7 \pm 0.01 g/dl$ at were recorded after treatment with Biofarm® at concentration 2ppm & 3ppm. Moreover, Virkon–s® at 2ppm induce significant increase in PCV ($P \leq 0.001$) $23.7 \pm 0.04\%$.While, the Protecto® 1000ppm cause high significant increase in hemoglobin and PCV ($P \leq 0.001$) $8.2 \pm 0.03$ g/dl & ($P \leq 0.001$) $23.0 \pm 0.05\%$, respectively.

**Table (1): Design of experimental treatment of naturally infected *O. niloticus***

| Different products used for treatments | Five Subgroup of infected fishes (Each consists of 10) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **1st** | **2nd** | | **3th** | | **4th** | | **5th** | |
| | | Dose | Duration time | Dose | Duration time | Dose | Duration time | Dose | Duration time |
| Virkon- S® | Non treated = control | 1ppm | 3days | 2ppm | 40 min. | 5ppm | 20 min. | - | - |
| Biofarm ® | | 0.5 ppm | 2days | 1ppm | 2 days | 2ppm | 90 min. | 3ppm | 30 min. |
| Protecto | | 100 ppm | 24 hours | 500 ppm | 24 hours | 1000 ppm | 30 min. | 1500 ppm | 15 min. |

**Table (2): Prevalence of infected by ectoparasites from the examined 420 *O. niloticus***

| Season<br>Parasites | Autumn | Winter | Spring | Summer | prevalence |
|---|---|---|---|---|---|
| *Trichodina* | 44.7 | 63.7 | 31.7 | 15.6 | 42.7 |
| *Chilodonella* | 30 | 27.4 | 0 | 0 | 17.9 |
| *Myxobolus* | 0 | 0 | 0 | 38.5 | 8.8 |
| *Epistylis* | 38 | 47.9 | 26.7 | 0 | 31.2 |
| *Cichlidogyrus halli* | 20.7 | 20.5 | 39.7 | 15.6 | 22.7 |
| *EMC* | 0 | 0 | 82.5 | 0 | 12.4 |
| *Lamproglenea* | 0 | 0 | 49.2 | 44.8 | 17.6 |
| Mixed infection | | | | | |
| *Trichodina, Epistylis,EMC* | 0 | 0 | 23.7 | 0 | 3.6 |
| *Lamproglenea & Cichlidogyrus halli* | 0 | 0 | 0 | 15.6 | 3.6 |

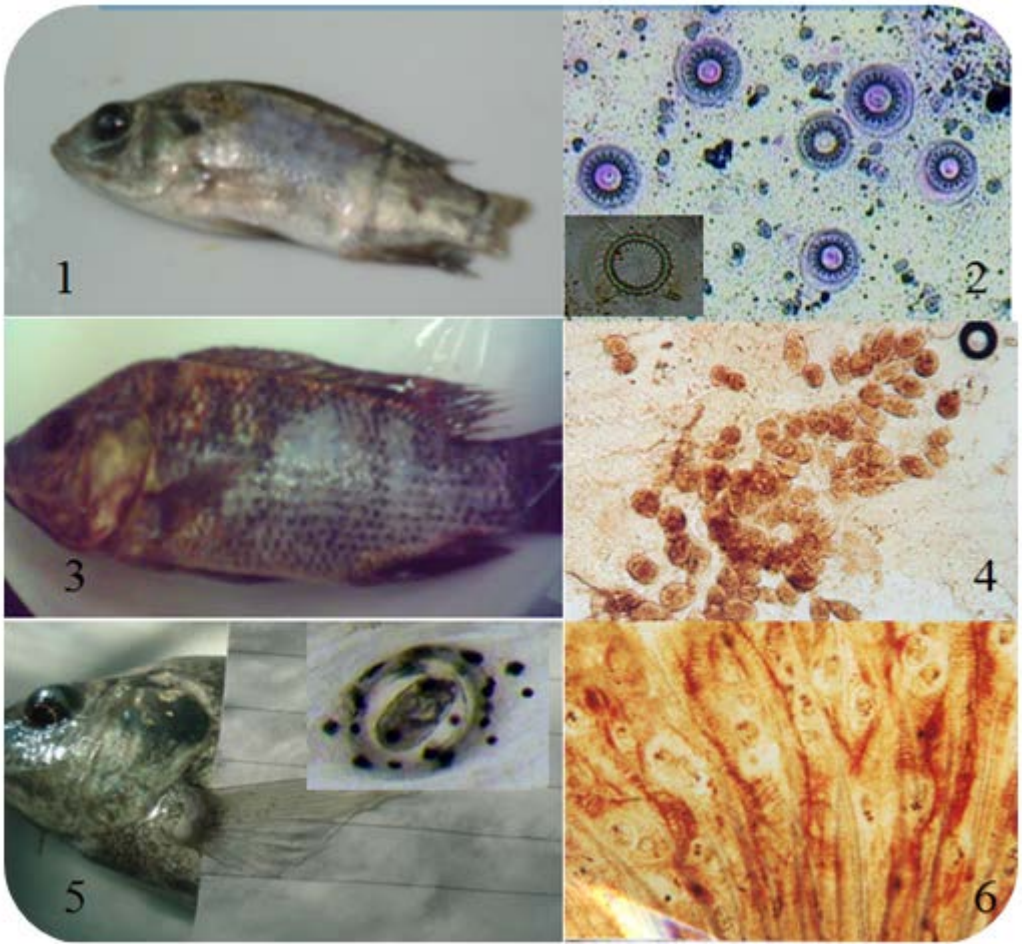
**Table (3): Efficacy of tested products on ectoparasitic infection on *O. niloticus***

| products | Dose/ hours | Parasites | | |
|---|---|---|---|---|
| | | *Trichodina* | *Epistylis* | *EMC* |
| Virkon- s® | 1 ppm/ 72 hrs | ++ | ++ | ++ |
| | 2 ppm/ 40mins | - | - | ++ |
| | 5 ppm/ 20mins | - | - | ++ |
| Biofarm® | 0.5 ppm/ 48 hrs | +++ | +++ | +++ |
| | 1 ppm/ 48 hrs | ++ | ++ | ++ |
| | 2 ppm/ 1.5hrs | - | - | + |
| | 3 ppm/ 30mins | - | - | Kill |
| Protecto | 100 ppm/ 24 hrs | ++ | ++ | ++ |
| | 500 ppm/ 24 hrs | + | + | + |
| | 1000 ppm/ 30mins | - | - | + |
| | 1500 ppm/ 15mins | - | - | + |
| Control | | +++ | +++ | +++ |

**Table (4): Effect of drugs on haematological picture of *O.niloticus* infected with a mixed infection of *Trichodina, Epistylis* and EMC (mean ± SE),n = 5**

| Blood parameters / Group | | Hb g/dl | PCV % | RBCs x $10^6$/µl$^6$ | WBCs x $10^3$/ µl$^3$ |
|---|---|---|---|---|---|
| Naturally Infected group | | 5.47 ± 0.02 | 18.7 ± 0.05 | 1.4 ± 0.01 | 14.5 ± 0.02 |
| 2 weeks post treatment | Virkon- S® (2 ppm) | 6.5 ± 0.08** | 23.7 ± 0.04**** | 1.7 ± 0.15 | 11.7 ± 0.01**** |
| | Virkon- S ® (5 ppm) | 5.8 ± 0.1 | 21.0 ± 0.05**** | 1.8 ± 0.02 | 11.2 ± 0.15**** |
| | Biofarm ® (2 ppm) | 4.4 ± 0.01**** | 19.7 ± 0.06** | 2.6 ± 0.02**** | 11.4 ± 0.0**** |
| | Biofarm® (3 ppm) | 6.7 ± 0.01**** | 16.0 ± 0.05**** | 2.2 ± 0.03*** | 9.80 ±0.01**** |
| | Protecto (1000 ppm) | 8.2 ± 0.03**** | 23.0 ± 0.05**** | 2.1 ± 0.01**** | 13.6 ± 0.01**** |
| | Protecto (1500 ppm) | 4.7 ± 0.02**** | 17.0 ± 0.02**** | 2.2 ± 0.01**** | 10.8 ± 0.02**** |

* Slight significant at P < 0.05, ** Moderate significant at P < 0.01, *** High significant at P < 0.005, **** High significant at P < 0.001




**Photo(1&3): *Oreochromis* spp. infected with *Trichodina and Epistylis* showing sever skin ulceration and tail erosion .and detached scales.**
**Photo(2): stained *Trichodina spp.* species stained with Giemsa stain & silver impregnation (X40).**
**Photo(4): Unstained *Epistylis* species in skin wet mount (X10)**
**Photo(5-6):*Oreochromis* spp. infected with black spots on pectora fin & Encysted metacercaria in gills of *O. niloticus* (X100).**

## Discussion

The present investigation was carried out as a field survey for monitoring external parasites in cultured *O. niloticus* fishes under different cultural conditions. The Prevalence of infection with *Trichodina* spp. reached 42.7% this result was nearly similar with that recorded by Yousef (2008). On the other hand, consider higher rate of infection of *Tilapia* spp. with the same parasite was observed by Abu El-Wafa (1988),

who recorded an infection on 66% in Egypt. However, lower results were obtained by El-Khatib, (1989) and Hassan, (1992) 30% & 12.5%. The present difference in the prevalence of infection of the examined fishes with *Trichodina* spp., from those previous workers, could be attributed to the different environmental conditions during the period of the present investigation and different locality.

The seasonal variation of *Trichodina* spp. infection rate in *O. niloticus* was increased in winter and autumn (63.7% & 44.7%), and decreased in spring and summer (31.7% & 15.6%). These findings supported those recorded by Saha et al., (1995). This result was disagreement with El- El-Seify et al., (2011) who, stated that the seasonal incidence of protozoal infection was high in spring among *O. niloticus* in Egypt. In this study *Myxobolus* spp. were recorded with prevalence rate (8.8%) in the examined fishes, this result nearly agreement with Bichi and Dawaki (2010), who recorded the total prevalence rate of infection was (2.49%) among *O. niloticus* from Nigeria. While, the seasonal prevalence rate (38.5%) which infected the investigated *O. niloticus* only recorded in summer. In the present study, the infection *C. halli* rate reached higher level in spring 39.7%. These findings agree with mentioned by El-Khatib (1989), who worked in Egypt on tilapia sp. infected with *Cichlidogyrus* sp. and reported that the infection peak was found during spring. But, Mostafa et al., (1991) & Martins et al., (2010) who, stated that the infection rate of *Cichlidogyrus* sp. in tilapia spp. was recorded in summer 20.7% & 80%.

The genus *Lamproglena* is the most primitive group of Lernaeidae Thomas and Hameed (1984) and the second major gill freshwater parasite cyclopoid Piasecki et al., (2004). In the present study, *L. monodi* was recovered only from *O. niloticus* at a rate of 17.6%. Regarding, high infection rate was recorded in spring and summer with 49.2% and 44.8%, in autumn and winter with 0% rate. These results agree with Eissa and Gharib (2005), who found that the more prevalent of *L. monodi* in *O. niloticus* in summer followed by spring and autumn. On the other hand, disagree with Martins et al., (2010), who found that the hot season being the less favorable for *Lamproglena* infection.

Concerning, the treatment trials for investigated ectoparasites the result proved that Virkon-s® appeared to be quite enough for eradication of *Epistylis and Trichodina* in two concentrations and no effect for EMC. The mode of action of Virkon-s® depends

on strong oxidizing system. It is a commercial disinfectant used to inactivate virus infection as human enterovirus 71 (HEV 71) Chan and Abu Bakar, ( 2005) and for reduction of *Staphylococcus aureus* and *Salmonalle enterica*, Dunowska et al., (2005) and for control of virus and bacteria in shrimp hatchery for water treatment and pond preparation. Presently, treatment reflect significant ($P \leq 0.001$) improvement in evaluated hematological parameters of Virkon-s® in PCV ($P \leq 0.001$) $23.7 \pm 0.04\%$ using 2 ppm.

On the other hand, the use of Biofarm® (Humate) at the dose of 3ppm /30 min. was more efficient to eradication *Trichodina*, *Epistylis*, and killed EMC, this result agreed with the result met by Noor El- Deen et al., (2012) who found Humic acid at the dose of 3ppm/ 24h.was highly effective to eradication *Trichodina* spp. and *Cichlidogyrus.*

Using of natural treatment for eradication parasitic diseases is safer than chemical treatment as formalin which has side effects on fish and water as it is reducing agent lowers oxygen level in water, toxic to fish and of public health importance in food fish when there is a residue in fish musculature Brown (2000). Concerning the effects of ectoprotozoal infection and digenetic trematodes on hematological picture, significant increase in hemoglobin and RBCs ($P \leq 0.001$) $6.7 \pm 0.01$g/dl, and ($P \leq 0.005$) $2.2 \pm 0.03$ $x10^6/\mu l^6$ in infected fish in comparison to parasite free control group.

Regarding treatment with Protecto as a probiotic product, it was proved that the concentrations of 1000ppm/30min. & 1500ppm/15min. were sufficient to eradicate *Trichodina* spp. and *Epistylis* while did not show effect on EMC. As the mode of action of Protecto is through the activity of this beneficial bacteria to lyse and engulph the protozoal cilia from the underlying fish tissues and destruction of the internal structure of parasites starve to death. These results supported those of Gill et al., (1992), who proved the success of biological control of external protozoal parasitosis using *Bacillus thuringiensis kurstaki*.

In conclusion, ectoparasitic infection of investigated fish should be diagnosed and treated as quick as possible to avoid fish motilities and economical losses and using natural treatment such as Biofarm® (Humate) is recommended and preferable than chemical and probiotic treatment in fish ectoparasitic infection as their application is cheap and safe on fish.

## Conclusion

Based upon the results of the surveys, it is concluded that treatment of infected *Oreochromis niloticus* by external parasites exhibit three products variations (chemical, natural and piropiotic), Humate® was proved to be the most effective product at concentration 3ppm/ 30 min. to eradicated *Tirchodina, Epistylis* and was to be killing EMC infection. Hematological parameters of infected fish showed increase significant in RBCs & hemoglobin. These information can be of important assets to any future research.

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