

Comparative studies on the impact of Humic acid and formalin on ectoparasitic infestation in Nile tilapia *Oreochromis niloticus*

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Abstract: Naturally infested *Oreochromis niloticus* (*O. niloticus*) were collected and examined for external parasites (*Trichodina* and *Cichlidogyrus*). The aim of present study was to investigate the efficacy and difference in treatment of formalin as a chemical and Humic acid as a natural product in treatment of ectoparasitic infestation of *O. niloticus*. The prevalence of isolated parasites showed high infestation rate of *Trichodina* (100%) and *Cichlidogyrus* (65%). The main clinical signs of infested fish were slimy dark skin with signs of asphyxia, rapid opercular movement, aggregation of fish on the water surface with gulping the atmospheric air. Flashing of fish, detached scales with frayed fins with presence of hemorrhagic lesions on the skin, fins, gills with congested gills, sluggish movement, finally off food and loss of escape reflex was observed. Hematological parameters of infested fish showed significant decrease in red blood cell counts, hemoglobin percentage and packed cell volume. Total WBCs showed non significant difference. Serum total protein and cholesterol were significantly decreased, while, AST, ALT, alkaline phosphatase, urea sodium and potassium were significantly increased. Chemical treatment was used formalin 20 ppm for 24h. As a long bath and natural treatment using Humic acid 3 ppm for 24h. As along bath showed complete eradication of *Trichodina* and *Cichlidogyrus*. [Nature and Science 2010;8(2):121-125]. (ISSN: 1545-0740).

Key words: *Oreochromis niloticus*- *Trichodina* – *Cichlidogyrus*- Humic acid and formalin

1. Introduction

Ectoparasitic infestation causes mass mortality and great economic losses such mortalities which are due to the break down of epithelial integrity, resultant loss ionic and osmotic balance. Furthermore, open lesions in the dermis allow fungal and bacterial infections to establish. These together with anorexia, leading to high mortality (Noga 1996 and Eissa 2002).

All fish drugs are toxic to fish fortunately it usually takes higher concentrations of the drug to harm the fish than it does to harm the pathogen. In recent years considerable changes have been happened in attitude towards the use of chemical parasitocides in aquaculture and fish diseases with increasing public awareness concerning the quantities and types of chemicals used and their potential impact on the environment. There is a need for alternative measures to control diseases among farmed fish. Treatment of fish diseases is limited because of only few chemicals approved for use in aquaculture and there are not effective for all pathogens. Chemical treatment may be linked to side effects such as toxic stress (Meinelt *et al* 2000 and 2001). Application of chemical treatment by using formalin is widely used against many protozoan and monogenetic trematodes, showed a great success for eradication of such ectoparasites (Ebtsam and Yonuis (2003), However, as formalin is reducing agent on fish culture.

A trial for treatment for such parasites using natural substances as humic acid and Humates is considered of great interest and preferable. Therefore present study was carried out to investigate the prevalence and intensity of ectoparasites, *Trichodina*, *Cichlidogyrus* in naturally infesting *Oreochromis niloticus* monitoring their effect on hematological parameters and certain serum constituents with comparative treatment trials of them with either chemical treatment as formalin and natural treatment as Humic acid.

Materials and methods

Fish:

A total number of 100 naturally infested freshwater cultured *O. niloticus* of average weight 80-100 g were obtained alive from private fish farm in Kafr El-Sheikh Governorate in May 2009. Another group 20 apparently healthy and free from parasites was collected from El-Wafaa fish farm. all fish were held in glass aquaria with aerated chlorine free tap water thermostatically adjusted at 24±1°C. fish were fed commercial ration once a day, subjected for parasitic examination and blood examination and treatment with formalin and Humic acid.

Drugs:

Formalin: Obtained from El-Gomheria Company Egypt
Humic acid: (Biofarm) from Grand Vet Company

.Egypt

Experimental design:

A group of 100 naturally infested *O. niloticus* fish were examined according to the method described by Paperna (1996) for the presence of external protozoa and monogenetic flukes with recording the prevalence and intensity of each parasite. The infested fish were continuously examined for gross clinical signs and lesions according to Noga (1996). After recording the prevalence and intensity of each parasite, the groups of infested fish were divided into 5 groups of 20 fish each. First 4 groups were used for treatment with formalin and Humic acid for investigating the hematological and biochemical studies, whereas the 5th group was left without treatment for studying the effect of parasites and mortality rate. Another group of 20 apparently healthy (parasite free) 6th group was kept as control for hematological and biochemical examination. Blood samples were collected from caudal vein (Lucky, 1977) for estimating total erythrocytic count, total leucocytic count, packed cell volume (PCV) and hemoglobin content according to method described by Lucky, 1977. Prepared serum samples were analyzed for estimation total protein (Wotten and Freeman, 1982), total cholesterol (Richmond, 1973), serum aspartate and aniline transaminases (AST and ALT) (Reitman and Frankel, 1957), Alkaline phosphatase (Kilchling and Feribury, 1951), serum urea (Fawcett and Scott, 1960), serum creatinine (Hudson and Rapoport, 1968) and finally sodium and potassium (Colline and Palkinhome, 1952). The degree of infestation of the parasite was determined by counting the parasites per microscopic field pre and post treatment according to Nahla (1993). Mortality rate pre and post treatment was also recorded. Soon after blood sampling, the first 5 infested groups were subjected to treatment, first group treated by using commercial grade of formalin 20 ppm as long bath for 24 h. 2nd were subjected to treatment with formalin at 5 ppm for 48 h and 3rd groups were subjected to treatment with Humic acid at 1 ppm for 48 h and 4th group subjected to treatment with Humic acid with 3 ppm for 24 h. The 5th group was kept as control without treatment for parasitic infestation. Another blood sample was taken from the completely cured infested group after 2 weeks and the 6th parasite free non treated control group for comparative study with other infested groups treated with formalin and Humic acid and control one for measure the hematological and biochemical parameters. Blood was collected from the caudal vein of five fish in each group at the

beginning and at the end of the experiment. The blood sample was divided into two portions. The first portion was kept as a whole blood in heparinized tubes for hematological examination. Serum was separated from the second portion for biochemical analysis.

Clinical examination:

Clinical examination for *O. niloticus* performed according to Austin and Austin (1986)

Results

Clinical signs of *O. niloticus* infested with external parasites:

The study revealed that naturally infested *O. niloticus* with *Trichodina sp.* and *Cichlidogyrus* showed slimy dark skin with signs of asphyxia, rapid opercular movement, aggregation of fish on the water surface with gulping the atmospheric air. Flashing of fish, detached scales with frayed fins and presence of hemorrhagic lesions on the skin, fins **Fig (A)** and gills and congested gills, sluggish movement and finally off food and loss of escape reflex.

Microscopical examination :

Microscopical examination of skin, fins and gill smears from infested fish showed *Trichodina sp.* **Fig (B)** and *Cichlidogyrus sp.* **Fig (C)**. High infestation rate with *Trichodina* 100% and *Cichlidogyrus* 65%. The intensity of infestation of *Trichodina* was higher than that of *Cichlidogyrus*. The effect of formalin and Humic acid treatment, the degree of parasite infestation and mortality rate were compared and recorded in **table (1)** from the obtained results that formalin 20 ppm for 24 h. as long bath was highly effective against ectoparasitic infestation, on the other hand the use of Humic acid at the dose of 3 ppm for 24 h. showed complete eradication of *Trichodina* and *Cichlidogyrus*. Concerning the effect of ectoparasitic infestation and monogenetic trematodes on the hematological picture **table (3)** showed significant decrease in the red cell counts (RBCs), hemoglobin (Hb) and hematocrite value (PCV) in infested fish in comparison to parasite free control group. There were no significant alteration in total leucocytic counts, there were disturbance in serum constituents of infested fish expressed by decrease of total protein and cholesterol and significant increase in the activity of AST, ALT, Alkaline phosphatase, urea, sodium and potassium levels as shown in **table (3)** serum creatinine level showed non – significant alteration in both infested and non infested.

Table (1) effect of formalin and humic acid on infestation in *O.niloticus*

	Non infest. non treat. fish	Infest. fish treat with formalin		Infest. fish treat with humic acid		Infest. non treated fish
		5 Ppm /48h.	20 ppm/24h.	1 ppm/48h.	3 ppm/24h.	
<i>Trichodina</i>	-----	+	-	-	-	++++
<i>Cichlidogyrus</i>	-----	++	-	++	-	+++
Mortality	12%	14%	19%	1%	2%	25%

+ light infestation=protozoa, 1-10 monogenea 2-5
 ++ moderate infestation =protozoa, 12-15 monogenea 8-10
 +++ heavy infestation =protozoa, 15-20 monogenea 10-20

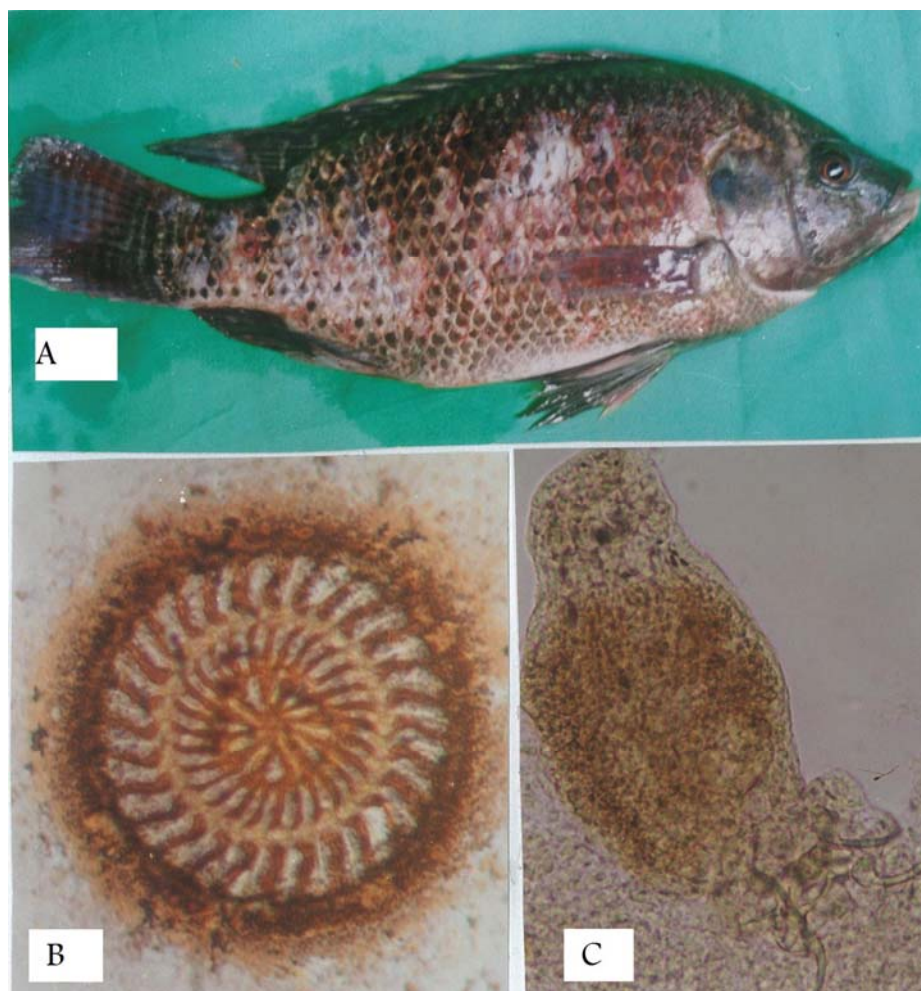
Table (2) effect of formalin and humic acid on hematological picture of *O.niloticus* infested with *Trichodina* and *Cichlidogyrus*.

Group Parameter	Control non infested fish	Infested Fish before treated	Infested two weeks post treat. with formalin	Infested two weeks post treat. with humic acid
			20 ppm/24h.	3 ppm/24h.
RBCs count	1.63±0.081	1.22±0.061	1.39±0.069	1.62±0.081
Hb	6.3±0.315	5.7±0.285	5.9±0.295	6.3±0.315
PCV%	22.4±1.120	17.0±0.850	19.3±0.965	21.3±1.065
WBCs	4.5±0.225	4.63±0.231	4.35±0.217	4.12±0.206

Table (3) effect of formalin and humic acid on some serum constituents of *O.niloticus* infested with *Trichodina* and *Cichlidogyrus*.

Group Parameter	Control non infested fish	Infested fish before treated	Infested two weeks post treated with formalin	Infested two weeks post treated with humic acid
			20 ppm/24h.	3ppm/24h.
Total protein	4.8±0.24	4.4±0.22	4.6±0.23	4.7±0.23
Cholesterol	142±6.20	119±5.95	132±6.60	138±6.90
AST	95±4.75	112±5.60	1.5±0.075	97±4.87
ALT	26.3±1.31	29.2±1.46	28.1±1.40	27.3±1.36
Alkaline phosphatase	19.3±0.96	25.3±1,26	22.3±1.11	20.2±1.01
Urea	2.61±0.13	3.7±0.18	2.70±0.13	2.67±0.13
Creatinin	0.57±0.028	0.60±0.03	0.59±0.02	0.58±0.02
Sodium	132±6.60	138±6.90	135±6.75	133±6.65
Potasium	6.2±0.31	6.8±0.34	6.5±0.32	6.3±0.31

Fig. (A) : Showing skin and fin hemorrhage in tilapia ,(B) *Trichodina* sp and (C) *Cichlidogyrus*



Discussion

Present study aimed to investigate the difference of by chemicals treatment represented by formalin and natural products treatment represented by Humic acid to ectoparasitic infestation, *Trichodina* and *Cichlidogyrus* in *O.niloticus*. The main clinical signs observed were slimy dark skin ,asphyxia with aggregation of fish near the water surface these clinical sings coincide with the finding of Osman (2001) and Eissa (2002).Scale sloughing, skin and fins hemorrhages could be attributed to continuous irritation of adhesive discs of *Trichodina* and movements with feeding activity of *Cichlidogyrus* Eissa (2002). Asphyxia and respiratory dysfunction may be attributed to damaged gill filaments which caused by *Cichlidogyrus* infestation (Osman 2005).Concerning the effect of formalin and Humic acid it appears from the results that formalin was highly effective against ectoparasitic infestation at the dose of

20 ppm for 24 h. these results nearly agreed with finding given by Woo (1995) and Ebtsam and Younis (2003), on the other hand the use of Humic acid at the dose of 3ppm for 24h. was highly effective in eradication *Trichodina* and *Cichlidogyrus* these results nearly agreed with the results met by Heidrick, and Schneider. (1999) and Nesreen (2008). The use of natural treatment for controlling parasitic diseases is safer than chemical treatment as formalin which have 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 residues in fish musculature Brown (2000). Concerning the effects of ectoprotzoal infestation and monogenetic trematodes on hematological picture, significant decrease in the mean red cells counts, hemoglobin percent and main hemochrite value in infested fish in comparison to parasite free control group these results

may be due to hemorrhage occur in damaged and destructed skin and gill filaments as the results of parasitic movements and feeding activity also, due to off food of infested fish (Schaperclaus,1992; Eissa, 2002;Osman, 2005).There were significant alteration in total WBCs count in infested fish. These results may be attributed to stimulation of hemobiotic organs and immune system (Roberts,1989;Ebtsam and Younis ,2003).Disturbance in serum constituents of infested fish was represented as decrease in serum total protein and cholesterol as well as progressive significant increase in activity of AST,ALT, Alkaline phosphatase, urea, sodium and potassium levels, these may be due to progressive irritation and destruction caused by *Trichodina* and *Cichlidogyrus* in case of heavy infestation. Serum liver enzymes (AST and ALT) increased as the results of injury and inflammation of tissues and gills due to parasitic infection. Serum urea significantly increased, these may be attributed to increased ammonia level as a result of disturbance in excretory and respiratory functions of destructed gill lamellae which leads to self poisoning with ammonia (Schaperclaus,1992 and Ebtsam and Younis ,2003).Serum creatinine level showed non significant change in both infested and non infected *O.niloticus*.

Significant osmo-regulatory disturbance was also recorded and expressed as marked increase in serum sodium and potassium levels, which was represented clearly in mortalities rate of infested fish. These may be attributed to destruction of skin epidermis and gill lamellae caused by parasitic infestation. After two weeks of treatment, progressive changes in blood picture and serum constituents were some what improved and returned towards control group. In conclusion, that ectoparasitic infestation in fish should be diagnosed and treated as quick as possible to avoid fish motilities and economical losses and using natural treatment such as Humic acid is recommended and preferable than chemical treatment in fish ectoparasitic infestation as their application is cheap and safe on fish.

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