Vet.Med.J.,Giza. Vol.49, No.4. (2001): 497-506.

EFFICACY OF BIO-CLEAN FOR CONTROL OF SOME ECTO-PARASITES INFESTING *OREOCHROMIS NILOTICUS* IN AQUACULTURE

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Received: 7.5.2001
Accepted :30.6.2001

SUMMARY

In the present study , attempts were made to observe the clinical signs of naturally infested Nile tilapia (*Oreochromis niloticus*) with ectoparasites of the ciliated Trichodina and aquatic leeches (*Glossiphonia spp.*). The prevalence of both parasites were 100% and 30% respectively. The histopathological investigations revealed sever inflammation with inflammatory cells infiltration in skin as well as coagulative necrosis (Zenker's necrosis) and oedema of the muscle bundles. Significance of biological control measures using the Bio-clean product (FER Mone-2/B) [A biological mixture of *Bacillus thuringiensis var Kurstaki* (BTK) and Beauveria bassiana (B.b)] as a new method to eradicate such parasites was evaluated. The half lethal concentration (LC-50) was 85 mg/L / 48 hours. The bio-clean product was safe to treat *Trichodina spp.* infestation in fish farms at a dose of 25 mg/L. On the other hand, this product was not suitable for treatment of Leeches (*Glossiphonia spp.*) due to its effective dose (40 mg/L) was narrow safety margin.

INTRODUCTION

Ectoparasites constitute the most important parasites infesting cultured fishes and ornamental fishes under unhygienic environmental conditions.

The trichodinids are considered as ecto-commansal present on the surface of the fish body, gills in small numbers which in turn become highly pathogenic with tremendous increase in numbers under unfavourable environmental stress conditions with the result of growth reduction and high mortality (Janovy and Hardin, 1987). The clinical signs in infested fishes are manifested with increased sliminess and irrita-

tion of the skin (Schaperclaus, 1992). The histological investigation has shown skin hyperplasia as well as degeneration, necrosis of the lining epithelium (Paperna, 1996).

Leeches are semi-permanent parasites infesting the fish body leading to direct harmful effect manifested by sever emaciation, haemorrhage, skin injuries and ulcerations as well as indirect harmful effect through transmission of other bacterial, fungal or even protozoa agents (Woo, 1995). The histopathological impact of these leeches is usually manifested by inflammatory and hyperplastic changes of epidermis with displacement and erosion of the dermis.

In the last few years, biological eradication programs using the biological materials were developed aiming for replacing the chemicals used for destruction of the most harmful parasites infesting terrestrial animals. In this field, several organisms are currently used in pest and parasitic control as spore-forming bacterium (*Bacillus thuringiensis*) and fungus (*Beauveria bassiana*) which accounting for 92% of the biological control market (Powell, 1993). Bacillus thuringiensis is toxic to black fly and the indirect (food chain) toxicity to fish are particularly lacking even though black flies are important food items for fish (Davies, 1991) Application of Beauveria bassiana alone or in combination with conventional insecticides was established with no adverse effects on fish, birds and human (Hinz and Wright, 1997). The biological control with Beauveria bassiana could be the most effective way to reduce the land-dwelling leech (Sasaki and Tani, 1997). The present study was planned to investigate the clinical signs, histopathological changes of the naturally infested fish with the cillited Trichodina spp. and the aquatic leeches (*Glossiphonia spp.*) as well as evaluation of the biological measures using the Bacillus thuringiensis bacteria and the Beauveria bassiana fungus for controlling such problems.

MATERIAL AND METHODS

Fish :

A total number of 160 freshwater cultured *Oreochromis niloticus* (*O. niloticus*) of different sizes (16-23 cm) and weights (80-200 g.) were collected alive from Abbassa Fish Hatchery in March, 2000. The fish were transported to the lab of Fish Disease Department, Animal Health Research Institute, Dokki, Giza, in large plastic bags under good transport conditions. The fish were examined clinically for any abnormal lesions according to Noga (1996). Parasitological examination of skin, fins and gills was carried out according to Paperna (1996). Tissue specimens from skin lesions were fixed in neutral 10% formalin, dehydrated in alcohol, cleared and embedded in paraffin blocks after which they were sectioned and stained by hematoxyline and eosin (Paperna, 1996).

Bio-clean (FER MONE-213) :

A biological mixture of Bacillus thuringiensis var Kurstaki (BTK) and Beauveria bassiana (B.b.) was performed according to Wipfli et al., (1994).

The LC_{50} testing of Bio-clean (FER MONE-213) :

A total number of 100 O. niloticus were divided into 10 groups of 10 fish each. The fish in each group were exposed to different concentration of Bio-clean namely 25, 40, 55, 70, 85, 100, 115, 130, 145 and 160 mg/L. respectively. The number of dead fish were recorded at 48 hours post exposure.

Bio-clean treatment of the naturally infested fish with Trichodia spp. and leeches :

A total number of 60 alive, naturally infested *O. niloticus* were divided into 3 groups of 20 fish each. The fish in the 1st group were exposed to 10 mg/L of the Bio-clean, while the 2nd and 3rd groups were exposed to 25 mg/L and 40 mg/L respectively. Ten fish from each group were examined parasitologically one hour post-exposure to the Bio-clean, while the other 10 fish in each group were examined in the same manner, but after 2 hours of exposure.

RESULTS

Gross pathological findings :

Gross examination of the infested fish revealed the presence of gray-bluish film of mucous on the skin with detachment of scales. Pale skin with several haemorrhagic spots on the fish skin (Fig.1) were detected. Skin erosions and ulcerations (Fig. 2) were also reported in some infested fish..

2. Parasitological examination :

The parasitological examination revealed the infestation of some *O. niloticus* fish with the ciliated, ecto-parasites *Trichodina spp.*, while others were infested with *Trichodina spp.* and the aquatic leeches (*Glossiphonia spp.*) (Table 1).

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

Parasite	No.of examined fish	No. of infested fish		Single infestation		Mixed infestation		Intensity of infestation (MF)
		No.	%	No.	%	No.	%	
Tricodina spp.	100	100	100	70	70	30	30	10-15
Glossiphonia spp	100	30	30	0	0	30	30	3-5

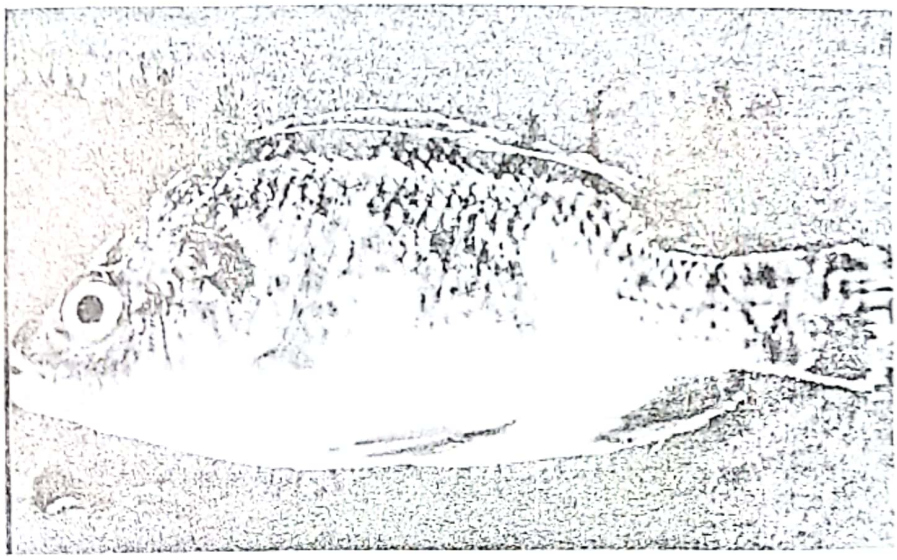

Fig. (1) :*O. niloticus* showing pale skin

Fig. (2) :*O. niloticus* showing skin injuries, ulcerations and eroded fins

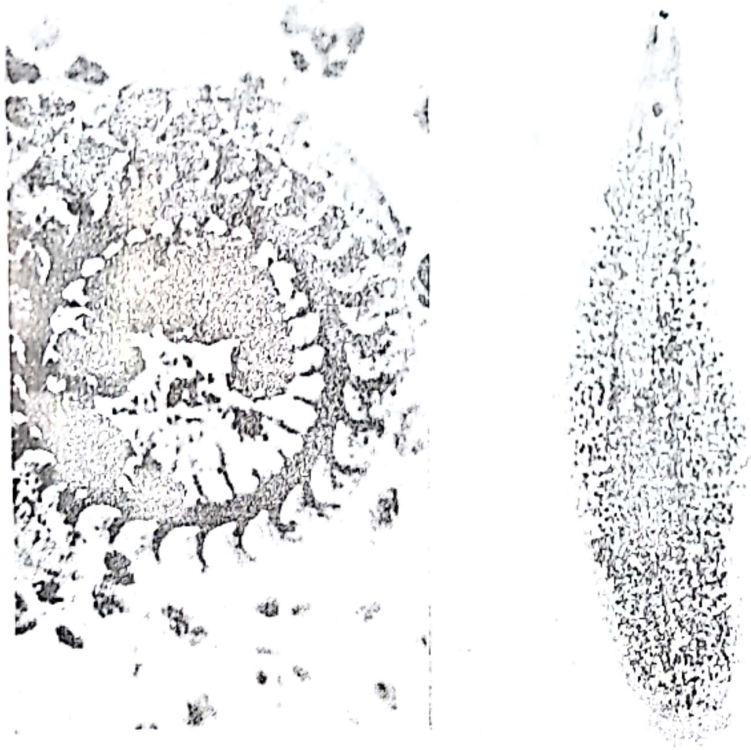

Fig. (3 A): *Trichodina spp.* (X 100) (C: Cilia, A.d. : Adhesive disc, H.S.N. : Horse-shoe shaped nucleus B: *Glossiphonia spp.* (Flat leeches) (X5) (O.S.: Oral sucker, E. : Eyes, P.: Probosis, B.S.: Body Segments, A.d. : Adhesive disc).

Fig.(4) :Sever inflammation of the skin and infilteration with inflammatory cells in dermal layer (X 100)

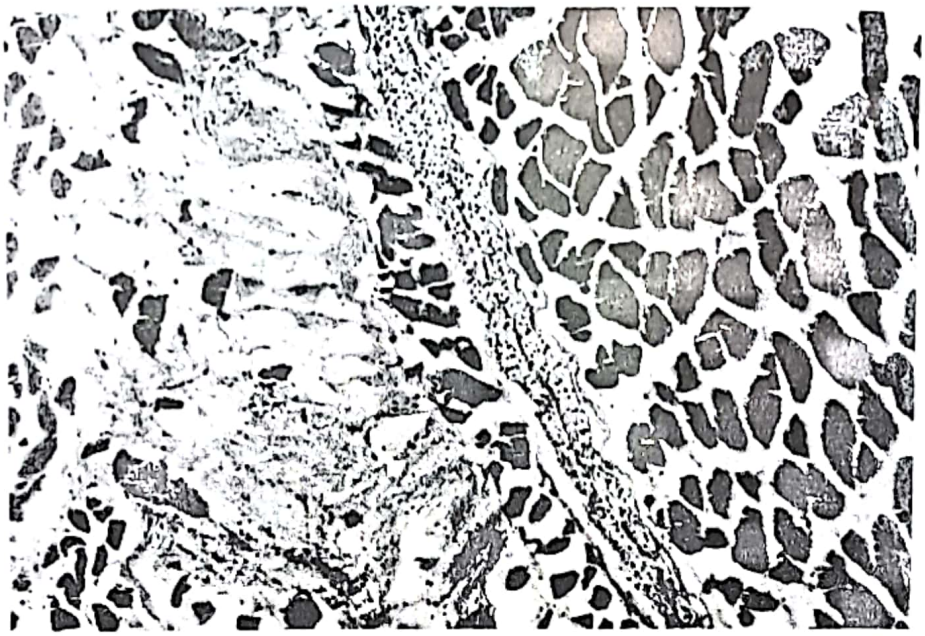

Fig. (5) :Muscle showing coagulative necrosis (Zinkers) of most of the muscle bundles associated with oedema and interstitial round cells infilteration (H & E, X 25).

Ecto-parasitic Protozoa :

Order : Ciliophora (Ciliates) :

Family : Trichodinidae (Raabe, 1959)

Genus : Trichdina.

Trichodina spp. was isolated from skin, fin and gills of naturally infested fish. It was cup-shaped, 20-100 µm in diameter, provided with several rows of cilia at the circular periphery and a circle of more centrally laying hooklets. The nuclear apparatus consists of the horse-shoe shaped nucleus and a small oval micro-nucleus which is difficult to be seen (Fig. 3A).

B. Aquatic Leeches :

Order : Rhnchobdellidae (Chard, 1897)

Family : Glossiphoniidae (Flat leeches)

Genus : Glossiphonia

Leeches of *Glossiphonia spp.* are infesting fish skin. Most of leeches were sufficiently large up to 25 mm long and 6 mm width with green to pale yellow colour. Their body is not divided into trachelosome and urosome, usually flattened and much wider than the head. They have sucker or sucker-like depression at the anterior end of the body and a well-developed sucker at the posterior end. There were two pairs of eyes on the head region (Fig. 3B).

3. Histopathological examination :

The histopathological alterations of *O. niloticus* with a mixed infestation of Trichodina spp. and Glossiphonia spp. were manifested microscopically as follows :

Table (2):Determination of 48 hours LC_{50} of Bio-Clean for *O. niloticus*

Group number	Fish Number	Doses (mg/L)	No.of Dead Fish	A	B	A X B
1	10	25	0	0	0	0
2	10	40	2	1	15	15
3	10	55	3	2.5	15	37.5
4	10	70	4	3.5	15	52.5
5	10	85	5	4.5	15	67.5
6	10	100	6	5.5	15	82.5
7	10	115	7	6.5	15	97.5
8	10	130	9	8	15	120
9	10	145	9	9	15	135
10	10	160	10	9.5	15	142.5

A : Mean of dead fish between 2 successive doses.
B : Dose differences between 2 successive doses.
A x B : Summation of (A x B).

48 hour LC_{50} :
Largest dose (which kill all fish) - $\frac{\text{"A x B}}{N}$

$$160 - \frac{750}{10} = 85 \text{ mg/L.}$$

Table (3):Efficacy of Bio-Clean bath on ectoparasites infesting *O. niloticus*

Bio-Clean dose (mg/dl)	Trichodina spp. (10/field)	Trichodina spp. (10/field)
10*	5	5
10**	3	5
25*	2	4
25**	-	3
40*	-	2
40*	-	1

* Long bath for 1 hour.
** long bath for 2 hours.

Skin :

Sever inflammation and infiltration with mononuclear inflammatory cells in the dermal connective tissue layer (Fig. 4).

Muscles :

Muscles of infested fish showed coagulative necrosis (Zenker;s necrosis) of most of the muscle bundles associated with fibrinous oedema. Also, there is interstitial round cells infiltration (Fig. 5).

4. Bio-Clean Lethal Concentration- 50 (LC_{50}) :

The results of LC_{50} of Bio-Clean for *O. niloticus* appeared to be 85 mg/L / 48 hours (Table 2).

5. Efficacy of Bio-Clean bath on the external parasites infesting *O. niloticus* :

The results of Bio-Clean efficacy testing on the infestation of *O. niloticus* with a mixed infestation with Trichodina spp. and Glossiphonia spp. revealed that long exposure of infested fish for 2 hours (long bath) for Bio-Clean at a concentration of 25 mg/L was sufficient to eradicate Trichodina spp. in infested fish. Neither 25 mg/L nor 40 mg/L were sufficient to eradicate the leeches (Table 3).

DISCUSSION

External parasites were described as one of the most important affections among cultured freshwater fishes that usually reflect on their productivity and reproductivity. In this study most of the infested *O. niloticus* have excess mucus which appeared on the skin accompanied with detached scales (Fig. 1). This proved the irritation which caused by adhesive discs of trichodina and proboscis of infesting leeches. The skin of the infested fish was often pale (Fig. 1&2) which may be attributed to reduction in haemoglobin content as a results of blood sucking activity of infesting leeches and the consequent anaemia (Woo, 1995). Some fish had petechial haemorrhages and skin ulceration (Fig. 2). This was related to the infestation with Trichodina spp. that usually damage the epidermal cell layer to feed on the disrupted cells. Moreover, Glossiphonia spp. leeches fed on host blood producing localized petechial haemorrhages in several cases with the result of sever skin damage manifested by erosion and ulceration which in turn was invaded and complicated by secondary opportunistic bacterial and fungal agents.

In Table (1), the prevalence of *Trichodina spp.* was relatively high (70%). Its wide distribution among different fishes may be due to their low specificity of most parasite species (Lom, 1995). Moreover, trichodoniosis accompanied with low temperature stress which plays an important role in the epizootic outbreaks in Cichilid fish (Paperna, 1996). On the other hand, the prevalence and intensity of leeches infestation was relatively low (30%) with 5 parasites / microscopic field. This could be attributed to the biological properties of this parasites that have a temporal of semi-

permanent attachment to the infested fish (Burreson, 1995). In this study, the morphological characters of Trichodina spp. and Glossiphonia spp. infesting *O. niloticus* were coincide with those of Lome (1995) and Burreson (1995) respectively.

The histopathological investigation indicated sever inflammation of skin with infiltration with mononuclear inflammatory cells in the dermal connective tissue layer. This could be due to the mechanical irritation induced by the attachment of trichodina adhesive discs and leeches proboscis. Also, the coagulative Zenker's necrosis of muscle bundles associated with fibrinous oedema may be attributed to the extension of the cell damage and the fiberoplasia to the underlaying muscular layer. These results supported those of observed by Burreson (1995) and Paperna (1996).

Difficulties associated with chemical treatment of parasitic diseases as well as the undesirable side effects and the public health hazards, stimulate the efforts for production of biological materials to be used in the control program of such parasites. In this study, the Bio-Clean biological product was screened for its efficacy in treating *O. niloticus* infested with a mixed infestation of Trichodina spp. and Glossiphonia spp. For estimating the toxicity of such product to the infested fish, the LC-50 test was carried out. The value of 85 mg/L /48 hours appeared to be toxic to 50% of the exposed fish (Table 2). This dose was relatively higher than those of the other chemicals used for treating such affections. Previous studies proved the safety of Bacillus thuringiensis and Beauveria bassiana organisms to fish, birds, live stock and human (Hinz and Wright, 1997) and Johnson and Bishop (1997).

The efficacy of Bio-Clean bath on the external parasites of *O. niloticus* (Table 3), showed that the most suitable dose for Trichodina spp. infestation was 25 mg/L, where the mode of action of Bacillus thuringiensis is through the activity of this bacteria to separate the protozoal cilia from the underlying fish tissues and destruction of the internal structure of the parasite. Moreover, Beauveria bassiana cause adverse effects and abnormalities in the parasites. These results supported those of Davies (1991) and Middaugh and Genthner (1994). Also, in this study, the complete eradication of Trichodina spp. at a dose of 25 mg/L proved the effectiveness of such biological product against ciliated Trichodina spp infesting *O. niloticus* with a wide safety margin as the recorded fish LC-50 dose was 85 mg/L., wherethe safety margin was calculated as LC-50 dose / Effective dose (Hardman et al., 1996). On the other hand, the doses of 25 mg/L and 40 mg/L were not sufficient to treat Leeches, the matter of which this biological product was unsuitable for treating such Glossiphonia spp. parasites at these given doses.

In conclusion, the present study Bio-Clean is recommended to be used in fish farms ponds due to

the suitability of Bacillus thuringiensis and Beauveria bassiana organisms as effective for treatment of trichodina infestation in freshwater fish. Also, as it has undesirable side effects when compared with other chemicals, cheap and easy to produce locally.

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