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BIOLOGICAL ERADICATION OF SOME PARASITIC DISEASES IN FISHES USING *BACILLUS THURINGIENSIS* AGERIN® *PRODUCT*

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SUMMARY

In the present study, the clinical signs of naturally infested *Ctenopharyngodon idella* with *Ichthyoboda necator* and *Capillaria spp.* larval stage and naturally infested *Oreochromis niloticus* with ectoparasites of the ciliated *Trichodina* and monogenetic trematode *Gyrodactylus spp.* were observed and recorded. The prevalence of infection in *C. idella* with I. necator was 100% and Capillaria larvae was 50%. Moreover, the prevalence of infection in *O. niloticus* with mixed infestation of *Trichodina* and *Gyrodactylus spp.* were 100%. Microbial antiparasitic product (Agerin) was tested for in vivo activity against a natural infestation of *C. idella* and *O. niloticus* by the flagellated *I. necator* and larval stage of capillaria spp. and mixed infestation of *Trichodina spp.* and *Gyrodactylus spp*, respectively by bathing fishes in different concentrations and variable exposure times.

Complete elimination of *I. necator* and *capillaria* larvae in all *C. idella* fish was achieved by 1000 ppm/1 h. and 500 ppm/2 h of Agerin. *Trichodina spp.* were also completely eradicated at 500 ppm/ 1 hr, 1000 ppm/2h and indefinite use of 100 ppm with naturally infested *O. niloticus.*

The biological product Agerin was not toxic to treat ectoparasite protozoan in *C idella* (LC_{50} 6800 ppm/ 48 hrs) and *O. niloticus* (LC_{50} 6200 ppm/ 48 hrs). On the other hand, this product was not suitable for treatments of *gyrodactylus spp.*

INTRODUCTION

Many types of parasites can infest fish, either external or internal. Under unfavorable environmental condition in intensive and semi- intensive fish culture, the danger of epizootics by ectoparasites grows more and more serious striking by their rapid development and equally rapid spread by di-

rect dissemination among all living in contact (Lom, 1986).

The ectoparasitic flagellated protozoan *Icthyobo-da necator* (Henneguy, 1883) is characterized by its rapid spread among cultured fish and may reach epidemic proportions, causing heavy mortalities and drastic financial losses (Woo, 1995). The Trichodinids are ciliated ectoparasites that cause disease in stressed fish. They commonly occur in association with other ectoparasites especially monogeneans on gills and skin leading to high mortalities (Pearse, 1972). Monogeneas are Platyhelminths parasitic primarily on fishes under certain high density culture condition. Heavy infestation can occur which result in mortalities due to direct or indirect to these parasite. The clinical sings in infested fish with ectoparasites are easily detected in aquarium by their sluggish movements, swim near the water surface and cease feeding (Noga, 1996).

Nematodes are considered the most economically important helminth parasites of fishes of the world (WOO, 1995). There are few reported cases of mortality due to nematodes infection but it cause some degree of disorder to fish host like loss of appetite and emaciation (Noga, 1996). Capillarids are nematodes of fish infective to human especially in the tropics. Capillaria infection to man occur as a result of eating raw or inadequately cooked fish harbouring the infective larvae (Cross and Bhainulaya, 1983). In Egypt, lately during the period from 1989 till 2002 fourty four cases were detected in kosre -Aini University Hospital, suffer from intestinal capillariasis (El-Dib and Doss, 2002). Approaching the available literature it was found that no authors in Egypt dealt with control of capillariasis in fish.

In recent years, considerable changes have been happened in attitude towards the use of chemical parasiticide in agriculture with increasing public awareness concerning the quantities and types of chemicals used and their potential impact on the environment. Also, in last decades considerable knowledge in biotechnology has accumulated which allowed the development of new screening procedures and targetsite directed. Approaches was directed for the discovery of new biological drugs like Agerin. It is prepared from *Bacillus thuringiensis* (B.t) (Gram positive soil borne organism, spore forming) by genetic engineering and tested against numbers of biomedically significance pests. B.t. proved to be highly successful weapon for fighting some parasites infested animals like *Fasciola gigantica* (Hassanain et al. 1998). *Eimeria tenella* (Hassanain et al., 1997) and little attempts have been made to study its efficacy on nematodes (Abdel Rahman et al., 1998) eggs and larvae of *Trichostrongylidae* worms. B.t uses is still limited in developing countries specially in treating fish. Tantawy (2001) used B.t combined with *Beauveria bassiana* to treat trichodina and leeches infesting fish. The safety of B.t. is associated with low costs (Salama et al., 1993).

The present study was carried out with the endeavour of evaluating the possible use of new commercial parasiticide Agerin against *I. necator*, Ciliate *Trichodena sp.* ,monogenatic trematode gyrodactylus and larval stage of capillaria infesting fish and hoped to shed a beam of light on the accurate optimum dose that can completely eradicate these parasites by calculating the lethal concentration 50 of Agerin and safety margin for fish.

MATERIAL AND METHODS

Fish:

Two culture fish species have been selected for the present study. *Oreochromis niloticus* (*O. niloticus)* and *Ctenopharyngodon idella* (*C. idella*). A total number of 260 fishes one hundred and thirty from each species weighted 30-100 gm, were collected alive from Abbassa fish hatchery. The fish were maintained in glass aquaria contain dechlorinated water and clinically examined for any clinical manifestation and visible lesions. Parasitological examination of skin, fin, gills and intestine were carried out according to Noga (1996).

Agerin:

Biological parasiticide from *Bacillus thuringiensis* (B.t.) waterable powder (3200 IU/mg) under license from Agricultural Genetic Engineering Research Institute. AGERI-ARC, Giza. Produced by BIOGRO-International, Egypt.

Agerin lethal concentration-5O (LC50):

A total number of 200 fishes one hundred from each species were divided into ten groups. The fishes were exposed to different concentrations of Agerin (Table, 2), put under observation for 48 hours and number of dead fish were recorded. The LC50 of Agerin were calculated for *O. niloticus* and *C. idella* by equation:

$$LC_{50} = \text{Largest dose} - \sum \frac{A \times B}{N}$$

Where A is mean of dead fish between two successive doses.

B is dose differences between two successive doses.

Measurement of dissolved oxygen concentration:

Dissolved oxygen concentrations in the aquarium containing fish and treated with different concentrations of Agerin (LC50-experiment) were measured with an oxygen meter (Cole-parmer Instrument Co., Chicago).

Agerin treatment of naturally infested *O. niloticus* with Trichodina spp. and Gyrodactylus spp:

A total number of 60 alive, naturally infested *O. niloticus* were divided into 6 groups of 10 fish

each. Three groups of them used as a control untreated. The fish in the first group were exposed to 100 ppm of the Agerin indefinite, while the second and third groups were exposed to 500 ppm for 2 hours and 1000 ppm for one hours. The infestation intensity and rates were recorded according to Tojo et al. (1994) and Goven and Amend (1982), respectively.

Agerin treatment of the naturally infested *C. idella* with *Ichthyoboda necator* and *capillaria* larval stage:-

A total number of 60 alive, naturally infested *C. idella* fish were divided into 6 groups of 10 fish each. Three groups of them used as a control. The fish in the 3 groups were exposed to 100 ppm indefinite, 500 ppm for 2 hours and 1000 ppm for 1 hour respectively. Fish were examined 24 h after the end of treatment. A treatment was considered effective when it caused a complete removal of infestation in all fish used in the assay (Tojo et al., 1994).

RESULTS

I. Incidence of the detected parasites:

In *C. idella* fish, the parasitological examination of gills, skin and fins revealed the infestation with the flagellated ectoparasites *Icthyoboda necator*. The infestation intensity was high and equal to > 50 , per microscopic filed (400 X). The internal examination of some *C. idella* fish revealed the infestation with larval stage of *capillaria* nematode in posterior part of the intestine. The infestation rate was moderate, mean number of parasites per fish between 3 and 6.

The parasitological examination of *O. niloticus* fish revealed the infestation of gills, skin and fins with mixed ectoparasites; the monogenetic termatodes *gyrodactylus spp.* and the ciliated *trichodina spp.*, while by internal examination they were free from any parasitic infestation. The infestation intensity with *Trichodina* revealed number of > 30 parasites per filed (100 X). Also the infestation rate with *gyrodactylus* was 20 - 30 worm/fish (Table, 2).

II. Clinical examination of natural infested fish:

The infested *C. idella* fish were anaemic and covered with grayish films on fins and body surface The fish swim near the water surface and listless.

O. niloticus fish showed slimy skin than the normal, increased breathing frequency. Signs of sever irritation with itching of body against walls of aquaria and dropping of scales with bloody spots at the base of the fins. Throughout this study many infested fish died during the period of acclimatization.

Table (1): Incidence of the detected parasites in *C. idella* and *O. niloticus*.

Fish species	Parasite	No.of examined	No.of infestation	% of infested	Infestation intensity
C.idella	Ichthyoboda	60	60	100	>50*
	Capillaria		30	50	3-6**
O.niloticus	Trichodna	60	60	100	>30*
	Gyrodactylus		60	100	20-30**

* mean number of parasite per Microscopic field (MF).

** mean number of parasite per fish.

III. Agerin lethal concentration-50 (LC_{50}):-

The results of LC_{50} of Agerin for *C. idella* appeared to be 6800 ppm and for *O. niloticus* 6200 ppm after 48 hours under observation (Table, 2).

IV. Measurements of dissolved oxygen concentration:-

The oxygen level measured at different concentration in LC_{50} test at 18 ± 1°C showed that fish suffered from signs of respiratory distress (gasping and swimming at the water surfaces) at drug concentration> 5000 ppm. Dissolved oxygen measurements showed little or no apparent difference from those of controls (6.3 - 7.2 ppm) at 1000-4000 ppm for 24 hrs and little decrease (6-6.2 ppm) for 48 hrs. Sever depletion in oxygen concentration at drug concentration > 10000 (3-6 ppm) after 24 hrs and (3.8 - 1.5 ppm) after 48 hrs.

V. Efficacy of Agerin treatment on parasites infesting *C. idella* and *O. niloticus*:-

The results of Agerin efficacy tests on the infestation of *C. idella* with mixed infestation with Ichthyoboda necator and *Capillaria spp.* revealed that short and long bath of infested fish to 1000 ppm/h and 500 ppm/ 2 hours of Agerin were sufficient to eradicate the infestation. The results of Agerin efficacy testing on the infestation of *O. niloticus* with mixed infestation with *Trichodina sp.* and *Gryodactylus sp.* revealed that short, long and indefinite exposure of infested fish were sufficient to eradicate the *Trichodina sp.* but no effect on *Gryodactylus sp.* (Table, 3).

Table (2): Determination of 48 hrs LC50 of Agerin for *C.idela* and *O.niloticus*

Exposure dose ppm	Fish No.		Difference betwwen doses	No.of dead *O.niloticus*	No.of dead *C. idella*	O_2conc.ppm	
	O.niloticus	*C.idella*				24hr	84h
1000	10	10	0	0	0	7.2	6.2
2000	10	10	1000	0	0	7.2	6.2
3000	10	10	1000	1	1	6.5	6
4000	10	10	1000	2	1	6.3	6
5000	10	10	1000	3	2	6.0	3.8
6000	10	10	1000	5	4	5.7	2.5
7000	10	10	1000	7	5	5.0	2.2
8000	10	10	1000	8	6	4.5	2.0
9000	10	10	1000	9	8	4.0	1.5
10000	10	10	1000	10	10	3.0	1.5
Control	10	10	-	-	-	7.5	7.2

48 hr LC50 for C. idella = 6800 ppm.

48 hr LC50 for O. niloticus = 6200 ppm.

Water temperature = 18 ± 1 °C.

Table (3) :Efficacy of Agerin bath on parasites infesting *C. idella* and *O. niloticus*

Parasite	Fish sp.	Agerin dose (ppm)	Exposure time	Infestation density	
				Before	After
Ichthyoboda	*C.idella*	1000	1hour	>50	0
Capillaria				3-6	0
Trichodina	*O.niloticus*			>30	0
Gyrodactylus				20-30	20-30
Ichthyoboda	*C.idella*	500	2hour	>50	0
Capillaria				3-6	0
Trichodina	*O.niloticus*			>30	0
Gtrodactylus				20-30	25
Ichthyoboda	*C.idella*	100	Indefinite	>50	0
Capillaria				3-6	3-6
Trichodina	*O.niloticus*			>30	0
Gyrodactylus				20-30	15

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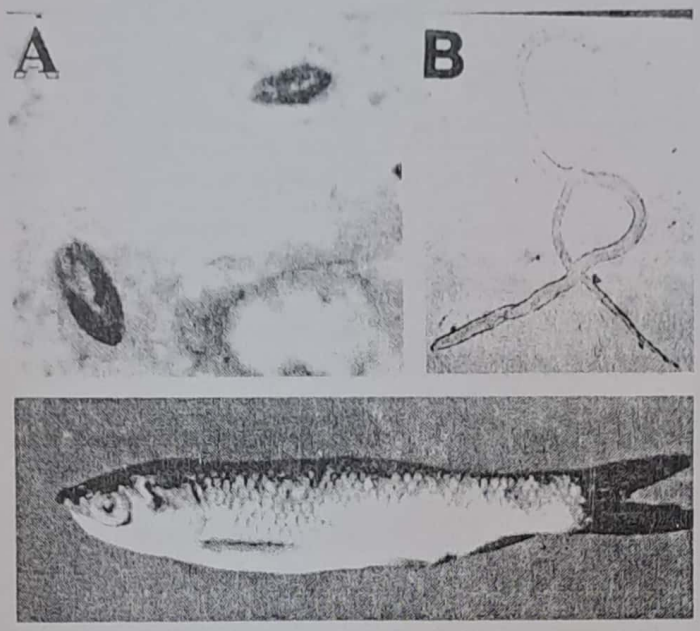

Fig. (1): C. idella showing graish film of mucus
(A) Ichthyoboda necator (X 100).
(B) Capillaria larvae (X 10).

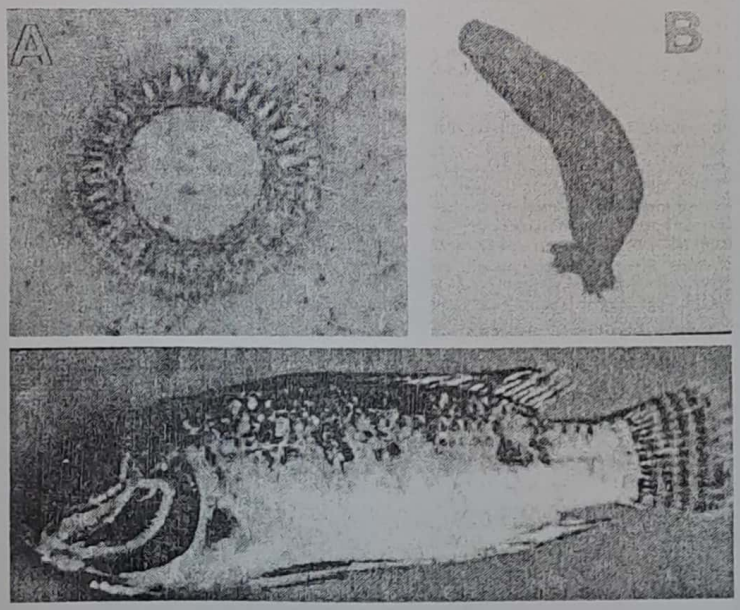

Fig. (2): O. niloticus showing eroded fins, blood spots and congested gills.
(A) Trichodina spp. (X 100).
(B) Gyrodactylus spp. (X 10).

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DISCUSSION

The outbreak of an ectoparasitic infestation always indicates that the defence capabilities of the fish are compromised due to adverse environmental conditions. Control of parasites need less expensive, water soluble, quick acting, short-lived and harmless chemicals to fish (Bailey, 1983). In the present study, the infested *C. idella* have grayish film of mucus on fins and body surface in case of *Ichthyoboda* necator infection, while accompanied with anaemic appearance in mixed infested fish with larval nematode of *cappillaria spp.* (Fig., 1). Most of these clinical signs agreed with the findings of Tojo et al. (1994) who considered *Ichthyoboda* necator to be one of the most dangerous external protozoa causing heavy mortalities. Noga (1996) found that nematodes infection to fish causing loss of appetite and emaciation. Moreover, Mansour (1996) revealed that Cappillaria philippinensis infection may be introduced into Egypt in the imported living fish *Ctenopharyngodon idella* (grass carp) which introduced for weed control in irrigation canals.

The infested *O. niloticus* fish with ectoparasites showed excess mucus with severe irritation, dropping of scales and increased breathing frequency (Fig., 2). Trichodinids commonly occur in association with other ectoparasites and heavily infected fish have excessive mucus (Lom, 1995).

In Table (1) the incidence of *I. necator* infestation of *C. idella* was high (100%). Its wide distribution among fish due to direct dissemination among all living in contact and rapid development and its low specificity (Tojo et al., 1994). On the other hand, infestation of *C. idella* with *cappillaria spp* larval stage was moderate (50%) with a mean infestation rate of 3-6 parasites/fish. The maintenance of infection and the wide distribution of the parasite depend upon the migrating fish eating-birds acting as reservoir hosts (Cross and Basaca-Sevilla, 1987). The incidence of infestation of *O. niloticus* with ciliated *Trichodina spp.* and *Gryodoctylus spp.* was high (100% and 100%) low specifity of most species among fishes was the reason of widely distribution and its direct transmission from one host to another (Woo, 1995).

In the context of the growing awareness of parasitic diseases and intensive use of parasiticides which led to sever resistance in parasites of veterinary importance, this has created a public demand for effective and safe control agents. The results represented in this study offers the possibility for using a new microbial parasiticide (Agerin) for treating protozoa, nematode and monogenatic trematodes infesting two fish species (*C. idella* and *O. niloticus*).

Since the toxicity of Agerin to *C. idella* and *O. niloticus* has not been accurately quantified, so

LC50 test was carried out for *C. idella* and *O. niloticus*. The values of 6800 ppn 6200 ppm/48 hours appeared to be toxic to fifty percentage of C. idella and O. niloticus respectively (Table, 2). The observed mortality during exposure to drug concentrations (5000- 10000 ppm) is believed to be indirect effect due to sever dissolved oxygen depletion by components of the formation of drug rather than specific of the B.t. protein. This result agreed with Snarsk (1990) who used commercial formula of B.t. (Vectobac-G) in fathead minnows and recorded fish displayed signs of respiratory distress and dissolved oxygen in the water declined from a mean 7.0 mg/L to severe depletion 0.5 mg/L at 100% fish mortalities. The effectiveness of treatment with Agerin tested on *I. necator* and *cappillaria spp.* larval stage showed that the most suitable dose 1000 ppm/l h and 500 ppm/2 hrs where the mode of action of B.t. is through the destruction of the internal structure of parasite. These results supported those of Gill et al. (1992) who said that B.t. protein binds to the parasite cells lining the mid gut membrane and creates pores in it and then parasite stop feeding and starve to death. The efficacy of Agerin on the external parasites of *O. niloticus* (Table, 3) showed that complete eradication of *Trichodina spp.* was occurred at doses 500 ppm/2 hrs, 1000 ppm/1 hr and indefinite use of 100 ppm. On the other hand, no effect on *Gryodoctylus sp.* was suggested that no increase that dose of drug to reach the suitable dose for eradication of *Gryodoctylus sp.* because the increase lead to narrow safety margin especially, little effect on parasite till 1000 ppm and LC50 of Agerin for *O. niloticus* was 6200 ppm (Hardmen et al., 1996).

In conclusion, the present study concluded that Agerin is recommended to be used in *C. idella* farms and *O. niloticus* due to its safetness and effectiveness. Also, it has the advantage namely cheap, water soluble, quick acting and locally produce.

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