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Treatment of Lernaeasis in Carps With Thunder: An Organophosphate

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Abstract.- The carps; *Labeo rohita* Hamilton, 1822, *Catla catla* Hamilton, 1822, *Ctenopharyngodon idella* Valenciennes, 1844, *Hypophthalmichthys molitrix* Richardson, 1845 and *Aristichthys nobilis* Richardson, 1845 were collected from earthen ponds reared under semi-intensive conditions. The parasitic examination of these fishes showed that all fish species were infected with crustacean copepod parasite *Lernaea cyprinacea* and other *Lernaea* spp. *C. catla, L. rohita* and *C. idella* had higher prevalence (62.5%, 68.75% and 76%, respectively) and mean intensity (12.1, 8.45 and 8.84 parasite/fish, respectively) compared to *H. molitrix* (9.1% and 3.0 parasite/fish). A locally available drug "Thunder" an organophosphate (2, 2 dichlorovinyl dimethyl phosphate) was used to treat lernaeasis in these fish species. Three concentrations (0.10, 0.15 and 0.20ppm) of the Thunder were sprayed on the surface of the infected fish ponds to eradicate lernaeasis. The 0.20ppm dose proved to be the most effective in controlling the infection, without harming the fish. The water quality parameters remained in normal range and suitable for the fish after treatment indicating that 'Thunder' has no harmful effects on water quality in fish pond.

Keywords: carps, Lernaeasis, treatment, Thunder / DDVP.

INTRODUCTION

The carp fish farming in private sector in some parts of Pakistan especially in Punjab has increased in recent years. In 2010, the Directorate General of Fisheries Punjab (DGOFP) reported establishment of more than 7829 fish farms covering approximately 45650 acres in Punjab. The growth of fish culture has also highlighted many issues of fish health, such as fish diseases and their management. Horvath *et al.* (1992) discussed various factors for fish disease occurrence in ponds, such as stocking density, aquaculture technology application and pollution discharge. In addition, numerous pathogens, nutritional deficiency, sudden changes in biotic and abiotic factors are causes of diseases in pond fishes (Roberts, 1989). Disease causes mortality, low growth, poor production and loss of fecundity (Bauer *et al.*, 1973). Hoole *et al.* (2001) gave an account on carp diseases. According to Iqbal *et al.* (2000, 2001) abdominal dropsy, lernaeasis, anoxia and fungal infection are the most commonly occurring diseases in pond fishes in Punjab.

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Adult female anchor worm, *Lernaea cyprinacea* causes lernaeasis. It lacks host specificity to such an extent that it can infect all freshwater fishes (Schmidth, 1990). The red sores caused by *L. cyprinacea* on fish skin are prone to secondary bacterial and fungal infection (Putz and Bowen, 1964 and Iqbal unpublished data). The infected fish become listless and weak (Iqbal *et al.*, 2001). In Africa rapid increase in *Lernaea* burden has been associated with growing environmental stress. Oldewage (1993) reported high prevalence of *Lernaea* spp. in tilapia of Lake Victoria. Heavy infection with *Lernaea* spp. has resulted mortality in golden perch, *Macquaria ambigua* Richardson, 1845 murray cod, *Maccullochella peelii* Mitchell, 1838 and silver perch, *Bidyanus bidyanus* Mitchell, 1838, broodstock held in pond (Callinan, 1988). Low level infection in river fishes and mortality due to lernaeasis in farmed fish has been reported by Gabrielli and Orsi (2000).

A fairly comprehensive review of control measures against *Lernaea* spp has been published by Hoffman and Mayer (1974). Most control measures involve application of chemicals, sometime on a large scale in ponds. Chemicals such as Formalin (Putz and Brown, 1964), Dylox (Hoffman and Mayer, 1974), Dimilin (Burtle and Morrison, 1986) and Dipterex (Minhas *et al.*, 2001) are commonly used for the treatment of lernaeasis in

commercial fisheries. Among the existing veterinary drugs and chemicals, the organophasphate (OP) insecticide and acaricide dichlorvos have been extensively used to treat sea lice infestations by *Lepeophtheirus salmonis* and *Caligus elongates* in the Atlantic salmon *Salmo salar* culture. Organophasphate is relatively non-persistent and undergoes fast and complete hydrolysis and metabolizes in most environments (WHO, 1989). Dipterex is very expensive chemical and its availability is not certain especially in remote rural areas in Punjab. The present study was aimed to test a comparatively cheap and locally available drug, "Thunder" an organophosphate, (2, 2 dichlorovinyl dimethyl phosphate or DDVP), to treat *Lernaeasis* in five species of carp *L. rohita, C. catla, C. idella, H. molitrix* and *A. nobilis* in fish ponds.

MATERIALS AND METHODS

The diseased fishes were collected from four ponds, at the University of the Punjab Research Fish farms during the spring of 2005. Each fish was wrapped in plastic bag laid in crushed ice and brought to laboratory. The fishes were weighed and measured. All fishes were examined thoroughly for the presence of any parasites or lesions visible to naked eye and also with the help of magnifying glass. The parasites were examined under microscope at x10 magnification. *Lernaea* spp were collected and preserved in specimen bottles in 70% alcohol for subsequent examination.

"Thunder" is extensively used insecticide in Pakistan. Its active ingredient is dichlorvos, which contains DDVP (2, 2 dichlorovinyl dimethyl phosphate) and other ingredients (58.33% w/w). Thunder is supplied in 500 ml packing in plastic bottles by Welgreen Chemicals (Pvt.) Ltd. Lahore.

Three fish ponds (1, 2, 3; stocking density of 350 fishes in each pond) having infected fishes (infection level in these ponds before treatment is given in Table I) were selected for treatment of lernaeasis. The area of the ponds and volume of pond water was calculated according to Minhas *et al*. (2001). The dimension of infected ponds were 175 x 85 x 4.5 feet (ponds 1-3) and 175 x 100 x 4.5 feet (pond 4). The desired quantity of "Thunder" was dissolved in 15-litre clean water in hand sprayer and sprayed on the surface of each pond (0.10ppm in pond 1; 0.15ppm in pond 2 and 0.20ppm in pond 3, respectively, according to Burtle and Morrison (1987). The pond 4 was used as control and no treatment was applied. Treatment in these ponds was repeated in seven days interval and continued for three weeks. One dose was given at the start of each week. Dose 1 on first week, dose II on second week, and dose III on the last week respectively, was repeated after every seven days interval and continued for 21 days. The fishes were examined after each treatment. Pond water temperature, dissolved oxygen and pH were measured on site with DO meter YSI (Model 57) and digital pH meter on sampling day before and after the treatment in these ponds. Water samples were taken in sterilized one liter glass bottles for subsequent analysis at water quality management laboratory, Fisheries Research and Training Institute, Lahore.

RESULTS

Infection of fish with Lernaea cyprinacea

In total 71 fish were examined to assess the level of infection in the stock. These fishes were heavily infected with *Lernaea cyprinacea* and other *Lernaea* spp. The parasites were attached to skin, fins and abdomen of the infected fish. The attachment of parasite on fish caused lesions on the skin. The infected fish showed discoloration of skin, darkening of the scales and poor growth and health. The detail of the fish examined and infected is given in Table I.

Table I indicates that all four ponds were infected and had high infection of *Lernaea, viz.*, pond 1, 62.5%; pond 2, 42.1%; pond 3, 76.5% and pond 4, 63.2 %.

Table I also indicates 69% prevalence in *L. rohita;* 62.5% in *C. catla*; 76% in *C. idella*; 9% in *H. molitrix* and 67% in *A. nobilis*. The mean intensity was 8.45 in *L.rohita*; 12.1 in *C.catla* and 8.84 in *C.idella*. The number of parasites per infected fish varied from 4 to 20 in *L.rohita*; 3 to 32 in *C.catla* and 1 to 26 in *C.idella* in these ponds. Overall infection was about 61% and mean intensity 9.0.

Treatment of lernaeasis

The treatment of lernaeasis in these infected

Table I.- Prevalence and mean intensity of *Lernaea* spp. in different ponds.

Fish	Weight (g)	Pond 1 No. of fish		Pond 2 No. of fish		Pond 3 No. of fish		Pond 4 No. of fish		Total		Prevalence %	Mean intensity
		Exa.	Infec.	Exa.	Infec.	Exa.	Infec.	Exa.	Infec.	Exa.	Infec.		
L. rohita	243-1015	4	2	5	3	3	3	4	3	16	11	69	8.45
C. catla	135-235	2	1	2	1	7	5	5	3	16	10	625	12.1
C. idella	237-786	6	6	8	3	5	4	6	6	25	19	76.0	8.84
H. molitrix	218-324	4	1	3	-	1	-	3	-	11	1	9	3
A. nobilis	422-781	-	-	1	1	1	1	1	-	3	2	67	1
Prevalence		62.5%		42.1%		76.2%		63.2%		60.56%			
Mean intensity		8.5		8.12		9.40		9.5		9.0			

Exa, examined; Infec, infected.

ponds was carried by spraying "Thunder" on the surface of the ponds. The first dose of "Thunder" was applied to pond 1, 2, and 3 at a concentration of 0.10ppm, 0.15ppm and 0.20ppm, respectively. The treatment continued for 3 weeks. Table II indicates that fishes were still infected after dose-1 and dose-11 of "Thunder" application. The infection was 85.7% in pond 1; 52.6% in pond 2; 50% in pond 3 after dose-1. All fish species showed infection even after application of dose-11 which was 64.2% in pond 1; 32.5% in pond 2 and 18.7% in pond 3.

There was no infection after the application of dose-III in pond 2 and pond 3. However, 5.5% infection was recorded in Pond 1. The average number of parasites ranged from 8-19 (pond 1); 6-8 parasites (Pond 2) and 6 parasites per infected fish (Pond 3) after dose I. Table III, shows that infection was present in ponds 1, 2 and 3 even after application of dose II. However, average number of parasites reduced to 6, 5 and 4 in these ponds, respectively. In case of Pond 3, infection reduced after dose II application, only three fish specimens (two *C. idella* and one *C. catla*) were found with less infection. The fishes were examined after dose-III application and all the fishes showed no infection. Whereas in control pond, fish infection persisted even mortality of one fish specimen occurred due to lernaeasis.

Water quality parameters

The water analysis of the ponds before and after treatment (Table III) shows that "Thunder" does not have adverse effect on water quality of the ponds after treatment.

DISCUSSION

Treatment of *L. cyprinacea* involving baths and dips in a container is a prolonged and time-consuming process. Direct application of chemicals in a pond is a convenient and effective method and also avoids fish from handling stress. This study showed that "Thunder" in various concentrations is an effective drug to treat and eradicate lernaeasis in five species of carp. However, 0.20ppm is considered as the most effective concentration and has no harmful effect on fish. The infective stages of *Lernaea* in the ponds could not survive after repeated application of "Thunder". Also, it minimized fresh infection and re-infection in the fishes in each pond. The fishes observed after treatment appeared to be healthy. Abidi *et al.* (2000) reported that Thunder at 0.5ppm is not harmful to fish life, flesh quality or aquatic environment. At 0.5 and 1.5ppm Thunder has no adverse effect on growth of micro-algae in freshwater (Ayub *et al.*, 2000).

Table II.- Prevalence and mean intensity of *Lernaea* spp. after treatment with "Thunder" in three fish ponds.

Pond	Fish	Dose I (0.10 ppm)		Dose II (0.15 ppm)		Dose III (0.20 ppm)	
		Examined	Infected	Examined	Infected	Examined	Infected
1	*L. rohita*	4	4	3	2	4	-
	C. catla	1	1	2	2	6	-
	C. idella	6	5	6	5	5	1
	H. molitrix	2	1	3	0	2	-
	A. nobilis	1	1	-	-	1	-
	Total	14	12	14	9	18	1
	Prevalence (%)		85.7		64.2		5.5
	Mean intensity		7.0		3.5		2
2	*L. rohita*	5	4	4	0	2	-
	C. catla	3	3	4	2	4	-
	C. idella	9	7	6	4	6	-
	H. molitrix	2	1	2	0	3	-
	A. nobilis	-	-	-	-	-	-
	Total	19	15	16	6	15	0
	Prevalence (%)		52.6		32.5		0
	Mean intensity		5.0		3		0
3	*L. rohita*	2	1	5	0	6	-
	C. catla	7	5	1	1	3	-
	C. idella	4	2	6	2	5	-
	H. molitrix	3	0	4	0	3	-
	A. nobilis	-	-	-	-	1	-
	Total	16	8	16	3	18	0
	Prevalence (%)		50		18.7		0
	Mean intensity		3.5		2.0		0

Kashara (1962) used organophosphate trichlorophan for effective eradication of anchor worm larva at 0.20ppm in two days and in one day at 0.5ppm. It destroyed free floating larvae and adult parasites. Putz and Bowen (1964) used formalin at 250ppm to treat larval stages of *L. cyprinacea*. However, this concentration is considered unsafe for fish health. *Lernaea* spp. was killed by weekly application of 0.27ppm of Lexone (Gopalkrishan, 1964). Hoffman and Mayer (1974) listed Benzene Hexachloride as effective against *Lernaea* parasites on *Carassius auratus*. Routine application of Masoten by spraying on ponds (0.2-0.5ppm) twice monthly during summer has also been recommended but more as a precautionary measure in Japan (Kabata, 1985). In Indonesia, Dipterex was used successfully at 0.5ppm for three days to treat lenaeasis (Kabata 1985). Chinese aquaculturists eradicated *Lernaea* after Trichlorophon was used at rate of 2.0ppm for 24 hours (Kabata, 1985). Burtle and Morison (1987) concluded that Dimilin applied at 10ppb in ponds was effective to kill *Lernaea* and other copepods in golden shiner, *Notemigonus crysoleucas* Mitchell, 1814.

Minhas *et al.* (2001) applied two doses of Dipterex at 0.2ppm in 2 weeks intervals. This dose was found effective to eliminate *L. cyprinacea* in culturable carps. The application of 'Thunder' at 0.20ppm in the present study is comparable to Minhas *et al*. (2001) and is evident from low infection in pond 3 after dose-II and no infection after dose-III. The effect of "Thunder" at 0.20ppm to *H. molitrix* seems to be very quick as compared to other fish species as observed in this study (Table II). Hemaprasanth *et al.* (2008) suggested a single intramuscular administration of the drug Doramectin at 200 ppb/kg body weight for treatment of heavily infected adults and brood stock of carps with *L. cyprinacea.*

Although *Lernaea* induced fish kill are not common (Shariff and Roberts, 1989), however, presence of *Lernaea* has been observed in case of

Table III.- Water analysis of fish pond water before and after treatment.

Pond	Temp. (°C)	pH	DO (mg/ L)	Bicarbonate as $CaCO_3$ (mg/L)	Total alkalinity as $CaCO_3$ (mg/L)	Calcium as $CaCO_3$ (mg/L)	Total hardness as $CaCO_3$ (mg/L)	Chloride (mg/L)	TDS (mg/L)
P-1 *	24	7.6	5.6	483	483	108	211	55	715
**	30.5	7.8	4.9	457	491	115	204	79	618
P-2*	24	7.4	5.5	470	470	100	200	68	650
**	30	7.5	5.1	486	520	111	204	94	618
P-3*	23	7.6	5.6	474	474	104	207	78	715
**	30	7.5	5.0	441	499	108	200	69	618
P4*	23	7.5	5.5	337	337	92	180	72	650
***	30.5	7.7	4.9	436	470	123	230	64	585

*Before treatment, **After treatment, *** Analysis after three weeks in P-4 (control pond).

culturable carps in Punjab (Iqbal *et al*., 2001) and in the present study. To maximize fish production in an intensive and semi-intensive culture system the relationship between fish species cultured and crustacean copepod causing infection and diseases must be understood (Piasecki *et al*., 2004).

"Thunder" is ecologically safe due to its biodegradability. It remains in water for 36h before it disintegrates and completely disappears from water (Abidi *et al*., 2000). "Thunder" at a dose of 0.5 and 1.5ppm has no adverse effects on growth of an important micro-algae *Scenedesmus quardicauda*, natural food of filter feeding fish (Ayub *et al*., 2000). All water quality parameters in our study were suitable for fish culture and within the normal range for warm water fish culture as defined by Boyd and Tucker (1998).

The treatment of lernaeasis with "Thunder' at 0.20 ppm (dose-III) has shown the promising results in three weeks. There was no indication that fish were likely to be adversely affected by the use of "Thunder" at this level. Further trial with higher doses may probably reduce the treatment time, however, this remains to be tested. Since, 'Thunder" has been reported to be safe for aquaculture industry at a dose of 0.5ppm (Abidi *et al*., 2000) we suggest that precautionary measures be taken at this level of application during fish rearing to minimize infection and further, this treatment level may increase stress to fish in pond. In our study, crustacean copepod parasites *L. cyprinacea* and other *Lernaea* spp. are more sensitive to "Thunder" than fish and differences in sensitivity impact the characterization of risk. The actual extent of mortality would depend on the rate at which "Thunder" is applied, its concentration, the degree of mixing that occurs in the water and the rate of breakdown and dissipation. These processes suggest that adverse effects in sensitive species are plausible. No effects are likely in less sensitive species. As discussed earlier the hydrolysis of Thunder in water is rapid and it is likely that the estimates of adverse effects in some fish would apply only to a limited area rather than to the larger area of the body of water that is contaminated.

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