

**Studies on Lernaeosis and the efficacy of Dipterex as treatment in the Hatchery Reared Fingerlings of Cyprinids**Mohamed A. A. Abd El-Galil<sup>1</sup>, ESSA, M. A. A.<sup>2</sup>, Kornil, F. M. M.<sup>2</sup><sup>1</sup> Fish Dis. Dept., Fact. Vet. Med., Sohag Univ. Egypt<sup>2</sup> Fish Dept., Fact. Vet. Med., Beni suef Univ., Egypt[Abdelgalil1997@yahoo.com](mailto:Abdelgalil1997@yahoo.com)

**Abstract:** The lernaea infested fingerlings of cyprinids showed the attachment of worm-like grey to greenish coloured copepods distributed along the both sides of the body, peduncle region especially at the base of caudal fin, base of dorsal fin and the buccal region. The sites of attachment were hemorrhagic nodules in *C. carpio* fingerlings and reddening without swollen margins in *C. idella* and *H. molitrix* fingerlings. The prevalence was 26.5, 32 and 33.1% among *C. carpio*, *C. idella* and *H. molitrix* fingerlings respectively and the highest infestation rate was in October and May. The lernaea infestation significantly decreased (4.3 up to 71.7%) the body weight of diseased fingerlings and this decrease was directly correlated with the infestation intensity. The histopathological alterations of the lernaea infestation were studied and Dipterex could not completely eliminate the lernaea from the infested *C. carpio* fingerlings.

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**1. Introduction**

Lernaeosis has been spreading among cyprinids in different Egyptian hatcheries since 1980, (Faisal *et al.*, 1988). Lernaeosis posed a major constraint to hatchery reared cyprinids especially young fish (Abd El-Rahman, 2000 and Abd El-Galil, 2002). The main clinical signs of lernaeosis in fingerlings of cyprinids were attachment of worm-like copepods along both sides of the body, at the isthmus region and the attachment site was marked by area of inflammation around the embedded anchors of the lernaea parasites (Essa *et al.*, 1995 ; Abd El Galil, 1998 and Mancini *et al.*, 2006).

In Egypt, few studies were conducted to follow up the pattern of the disease among cyprinids fingerlings stocks reared in hatcheries. Abd El-Rahman (2000) reported that the prevalence of lernaeosis among stocks of *C. carpio*, *C. idella* and *H. molitrix* fingerlings was 23, 59.5, and 60.7% respectively and Abd El-Galil (2002) recorded that the prevalence of lernaeosis in small sized *C. carpio*, *C. idella* and *H. molitrix* reared in Beni suef fish hatchery was 54, 39.6, and 61.7 %, respectively.

Lernaeosis hinders the feeding and greatly reduces the growth of the diseased fish (Shariff and Sommerville, 1986c; Ghittino, 1987 and Faisal *et al.*, 1988)

The most common histopathological changes in adult cyprinids infested with lernaea were severe degenerative changes and necrosis in the skin and underlying muscles. The dermis and hypodermis exhibited extensive edema, hemorrhage, leucocytic infiltration and some melano-macrophages. Severe destruction, vacuolar and hyaline degeneration and

necrosis were evident in the muscles with edema, congestion, hemorrhage and leucocytic infiltration replaced the necrotic muscles. Numerous eosinophilic granular cells and melano-macrophages were also observed between muscles (Aly *et al.*, 1995 ; Essa *et al.*, 1995 and Abd El Galil, 2002).

This study was planned to study the difference in clinical signs between different cyprinid species, prevalence, effect on fingerlings body weight and histopathological alterations of Lernaeosis among fingerlings of cyprinids namely common carp (*Cyprinus carpio*, *C. carpio*), grass carp (*Ctenopharyngodon idella*, *C. idella*) and silver carp (*Hypophthalmichthys molitrix*, *H. molitrix*). The efficacy of Dipterex as a treatment of lernaeosis was investigated in the hatchery ponds.

**2. Material and Methods****2.1. Cyprinids fingerlings**

A total number of 10719 cyprinids fish were collected alive from Beni suef fish hatchery and visually examined *in situ* for parasitic copepod. The samples represented 3056, 4246 and 3417 fingerlings of *C. carpio*, *C. idella* and *H. molitrix* respectively during the time of April to October, 2007.

**2.2. Clinical examination.**

The investigated cyprinids fingerlings were individually inspected by naked eye (Stoskopf, 1993) for detection of any external gross lesions of Lernaeosis and/or visible lernaea parasite.

**2.3. Parasitological examination**

Collected parasitic copepods were fixed in glycerin-alcohol 70%. Permanent mounts were prepared according to Becky (2004) and identification

of the detected parasites was done as noted by Woo (1995).

#### 2.4. Histopathological examination

The skin, underlying muscles, liver and kidney of naturally infested *C. carpio* and *C. idella* fingerlings were fixed in 10% buffer formalin. These samples were histopathologically prepared then stained with Haematoxylin and Eosin (H&E) (Banchroft *et al.*, 1996).

#### 2.5. Dipterex treatment

Dipterex (trichlorfon organophosphorus pesticide – Bayer company - Elzeny tower Elmaady - Cairo ) was applied in hatchery pond at 0.3ppm for treatment of lernaecosis in two regimes. The first regime was applied during May while the second one was applied during August. Each regime included application of Dipterex once weekly for four times (4 weeks). The drug was dissolved in water and dispersed as evenly as possible over the surface of pond water. Samples of fingerlings were examined at 2 days before

the first dose and after the last dose of treatment in each regime (Stoskopf, 1993).

### 3. Results

#### 3.1. Clinical signs in different cyprinids

The infested fingerlings of cyprinids showed the attachment of worm-like grey to greenish coloured copepods with or without paired appendages. The parasite was distributed along the both sides of the body and peduncle region specially at the base of caudal fin (Photo, 1), sometimes attached to uncommon sites as eyes (Photo, 2) of the *C. carpio* and it was attached mostly to the base of the dorsal fin of the *C. idella* and along both sides of the body and the buccal region of the *H. molitrix*. The sites of attachment were characterized by macroscopic reddening and swollen margins (hemorrhagic nodules) in *C. carpio* fingerlings (Photo, 3), while in *C. idella* and *H. molitrix* it was associated with reddening without swollen margins (Photo, 4).

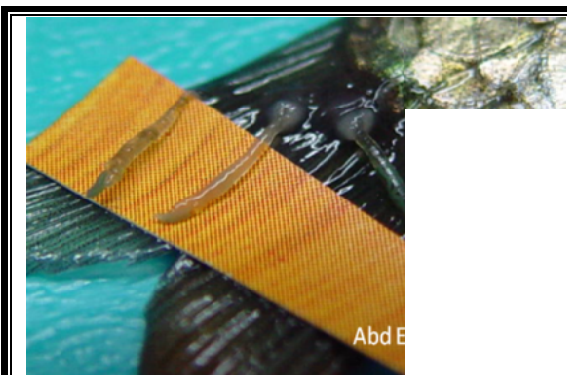


Photo (1): Showed *L. cyprinacea* at the base of caudal fin in *C. carpio*

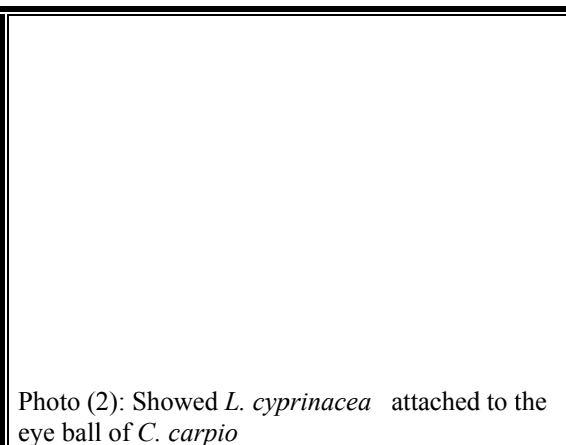


Photo (2): Showed *L. cyprinacea* attached to the eye ball of *C. carpio*

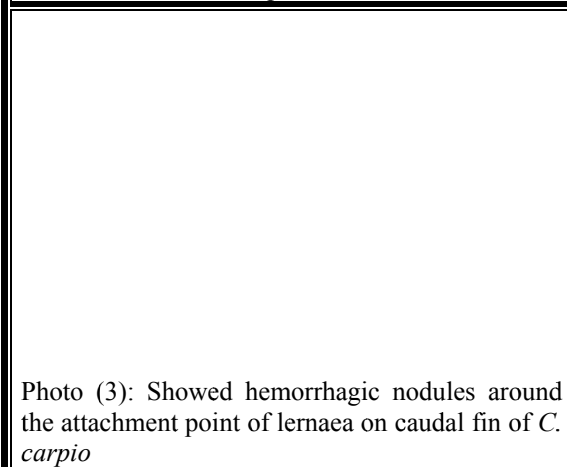


Photo (3): Showed hemorrhagic nodules around the attachment point of lernaea on caudal fin of *C. carpio*

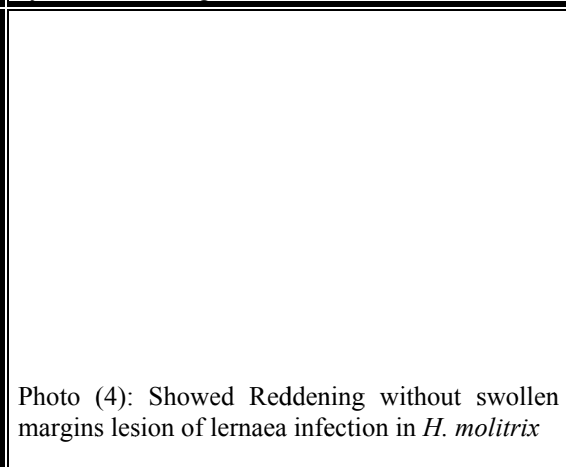


Photo (4): Showed Reddening without swollen margins lesion of lernaea infection in *H. molitrix*

#### 3.2. Prevalence of lernaecosis among fingerlings:

The prevalence of Lernaecosis among *C. carpio*, *C. idella* and *H. molitrix* fingerlings was 26.5, 32 and 33.1% respectively (Table, 1). The highest

infestation rates of the disease in *C. carpio* and *H. molitrix* fingerlings were detected in October (37.9 & 41.8%) and in *C. idella* it was detected in May (39.8%).

**Table (1): Prevalence of lernaecosis among fingerlings of examined hatchery reared cyprinids**

Fish species	No. of examined fingerlings	Fingerlings	
		No.	%
<i>C. carpio</i>	3056	810	26.5
<i>C. idella</i>	4246	1357	32
<i>H. molitrix</i>	3417	1130	33.1

### 3.3. Effects of lernaecosis on the body weight of the infested fingerlings:

The body weight of the lernaecia infested *C. carpio* fingerlings was significantly decreased in comparison to the body weight of lernaecia free fingerlings (Tables 2). The body weight of the infested

fingerlings was in indirect correlation with the infestation intensity where the light, moderate and heavy infestation decreased the body weight of infested *C. carpio* fingerlings about 19.6 %, 63.6% & 70.7% respectively (Tables 3).

**Table (2): Effect of Lernaecosis on the body weight of naturally infested *C. carpio* fingerlings**

Fish species	Infested fingerlings			Body weight of control fish (g)	Decrease in the body weight %
	No. of fish	No. of lernaecia/fish	Body weight (g)		
<i>C. carpio</i>	20	1	12.6 – 20 (17.6±0.9)	13 – 20 (18.4±0.6)	4.3
	20	2	7 – 20(12.3**±1.5)		33.2
	20	3	3.5 – 12(7.7***±0.7)		58.2
	20	4	3.5 – 12 (6.2***±0.8)		66.3
	20	5	4 – 7 (5.5***±0.5)		70.1
	20	6	3.5 – 9 (5.2***±0.8)		71.7
	20	≥ 7	3.5 – 7.5(5.5***±0.5)		70.1

\*\*  $P < 0.01$ \*\*\*  $P < 0.001$ **Table (3): Effect of light, moderate and heavy lernaecia infestation on the body weight of naturally infested *C. carpio* fingerlings**

Infestation Degree	Infested fingerlings			Body weight of control fish (g)	decrease in the body weight
	No. of fish	No. of lernaecia/fish	Body weight (g)		
Light	40	≤ 2	7 – 20(14.8 **± 1.1)	13 – 20 (18.4±0.6)	19.6
Moderate	60	3-5	3.5 – 12(6.7***±0.4)		63.6
Heavy	40	≥ 6	3.5 – 9(5.4***±0.4)		70.7

\*\*  $P < 0.01$ \*\*\*  $P < 0.001$ 

### 3.4. Histopathological alterations

#### 3.4.1. Skin

The epidermis, dermis and hypodermis of *C. carpio* infested with lernaecia were infiltrated with massive number of inflammatory cells associated with edema and congested blood vessels as well as melanin pigmented cells in the hypodermis (Photo, 5). On the other hand, the skin of infested *C. idella* showed complete destruction of the epidermal layer with massive number of inflammatory cells infiltrating the dermal and subcutaneous tissue (hypodermis) (Photo, 6).

#### 3.4.2. Skeletal muscle

Inflammatory cells infiltration and extra-vascular red blood cells in association with congested blood

capillaries were detected in the musculature adjacent to the hypodermal layer of the skin of *C. carpio* infested with lernaecia (Photo, 7). While the skeletal muscles and subcutaneous tissue were infiltrated by massive number of leucocytic inflammatory cells and granular acidophilic cells in addition to fine capsule of fibroblast was noticed around the embedded part of the lernaecia parasite in *C. idella*, (photo, 8).

#### 3.4.3. Kidney

Severe congestion and dilatation of the blood vessels were observed between the renal glomeruli and tubules of the infested *C. carpio*, (photo, 9) and no histopathological findings were observed in kidney of *C. idella* fingerlings.



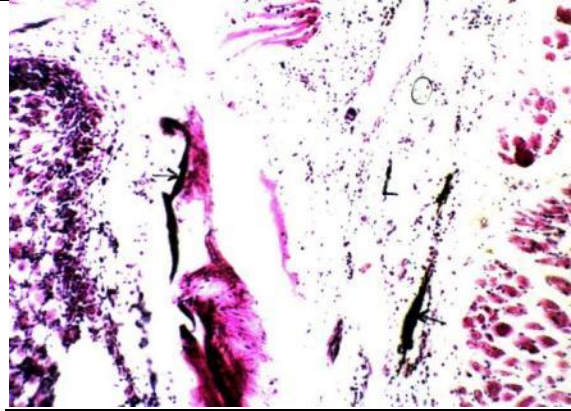


Photo (5): Showed skin of *C. carpio* had lernaea with massive number of inflammatory cells in the epidermis, dermis and hypodermis associated with edema and congested blood vessels as well as melanin pigmented cells (H&E x40)

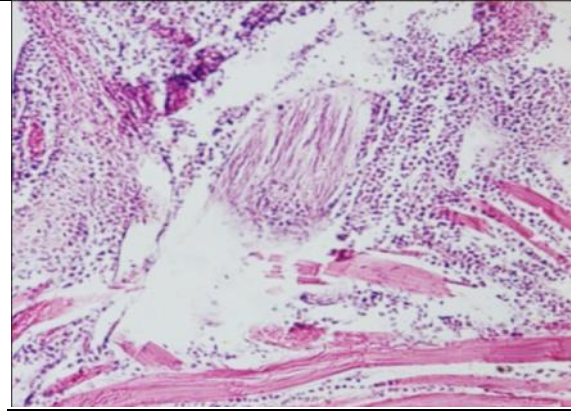


Photo (6): Showed skin of lernaea infested *C. idella* showed complete destruction of the epidermal layer with massive number of inflammatory cells infiltrating the dermal and subcutaneous tissue (H&E x40)

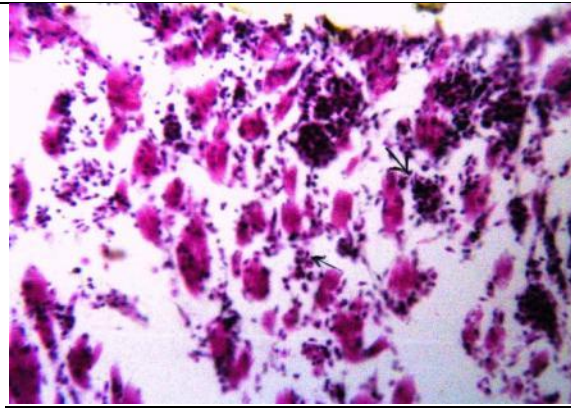


Photo (7): Showed skeletal muscles of *C. carpio* had massive number of inflammatory cells, extravasated red blood cells and congested capillaries in the muscle layer adjacent to the hypodermis (H&E x64)

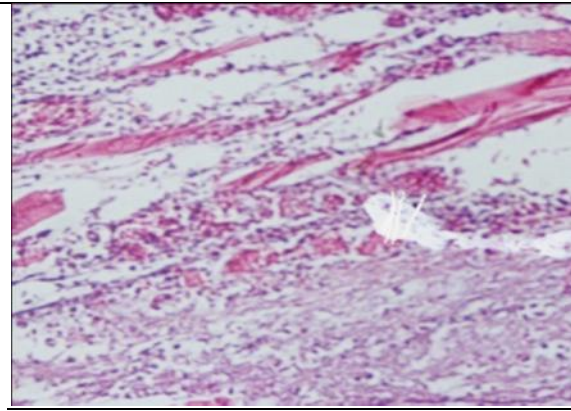
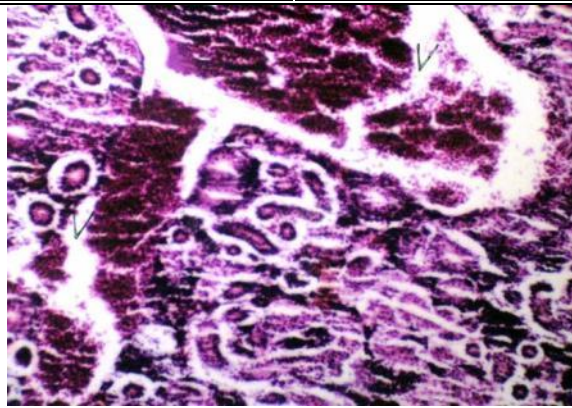


Photo (8): showed Skeletal muscles and subcutaneous tissue were infiltrated by massive number of leucocytic inflammatory cells and granular acidophilic cells. (H&E x40)



Photo(9): Showed severe congestion in the blood vessels of kidney of *C. carpio* infested with lernaea (H&E x40)

### 3.5. Dipterex treatment of lernaecosis

Dipterex could not completely eliminate the lernaea from the infested *C. carpio* fingerlings and the best result obtained was 76.6% reduction in the

infestation rate after the 1<sup>st</sup> treatment, the lowest lernaea infestation rates among the treated *C. carpio* fingerlings were 10.6% post the 1<sup>st</sup> treatment and 19.4% post the 2<sup>nd</sup> treatment regime, table (4).

**Table (4): Treatment of Lernaeosis in *C. carpio* fingerlings by Dipterex**

Fish stock	Fingerlings stocks ( <i>C. carpio</i> )		
	No. of treated fish	No. of exam. fish	Infested fish No.      %
Time of examination			
May (2 days before treatment)		210	77      36.7
June (2 days post treatment)		170	18      10.6
The 1 <sup>st</sup> day of July		224	45      20.1
August (2 days before treatment)	35-40 x 10 <sup>3</sup>	203	70      34.5
September (2 days post treatment)		310	60      19.4
The 1 <sup>st</sup> of October		294	105     35.7

#### 4. Discussion

Infectious epidemic diseases are considered as the main limiting factor facing the commercial fish production especially in intensive systems and the parasitic agents are taking a superior position as infectious agents. Lernaea parasite is the most commonly injurious parasite of warm freshwater fish in different parts of the world. Frequent appearance of lernaeosis among cultured cyprinids fish in local fish farms raises the possibility that hatcheries are responsible for the spreading of the disease via purchase of infested fingerlings. Little studies concerned with the incidence of lernaeosis and its deleterious effects in the hatchery reared cyprinid fingerlings

The parasitic copepods were identified as *I. cyprinacea* (Woo, 1995) and all members of family cyprinidae (*C. carpio*, *C. idella*, and *H. molitrix*) in Beni suif fish hatchery were susceptible to Lernaeosis and these results confirmed by Abd El- Rahman (2000) and Abd El-Galil (2002) who recorded the susceptibility of *C. idella*, *C. carpio* and *H. molitrix* to lernaea infestation

The prevalence of lernaeosis among *C. carpio*, *C. idella* and *H. molitrix* fingerlings was 26.5, 32 and 33.1 % respectively, higher infestation rates (54, 39.6 and 61.7% respectively) were reported by Abd El-Galil (2002) and this difference may be attributed to the difference in stocking density and the regular use of organophosphorous compounds treatment. The infestation rate indicated that the most susceptible species was *H. molitrix* fingerlings (33.1%) and this agreed with the results of Abd El-Galil (2002) who reported highest infestation rates in *H. molitrix* fingerlings.

The infested fingerlings of cyprinids showed attachment of worm-like grey to greenish coloured copepods which was distributed along both sides of the body and peduncle region especially at the base of caudal fin in *C. carpio*, at the base of the dorsal fin in *C. idella* and along both sides of the body and the buccal region in *H. molitrix*. Similar findings were recorded by Essa *et al.* (1995); Dorovskikh (1996);

Noga (1996); Tamuli and Shanbhogue (1996); Carlos (1997); El-Nobi (1998) and Abd El -Rahman (2000), these findings mean that the lernaea parasite preferred the scaleless areas of the fish body.

The sites of attachment in *C. carpio* were characterized by macroscopic reddening and swollen margins (hemorrhagic nodules). On the other hand, these sites in *C. idella* and *H. molitrix* were associated with reddening without swollen margins and sometimes no visible inflammatory signs were detected at these sites especially in *C. idella*. Similar macroscopic signs on the infested cyprinids were reported by Faisal *et al.* (1988); Aly *et al.* (1995); Essa *et al.* (1995); Woo (1995); Noga (1996); Abd El-Rahman (2000); Abd El-Galil (2002) and Mancini *et al.* (2006)

Regarding the effect of Lernaeosis on the body weight of *C. carpio* fingerlings, it was found that the lernaea significantly decreased the body weight of diseased fingerlings which were 4.3 to 71.7% lighter than lernaea free fingerlings, the decrease in the fish' body weight was directly correlated with the intensity of lernaea infestation and this result supported by Shariff and Sommerville (1986c) who found that the lernaea infested bighead carp were up to 35% lighter than non infested fish. The decrease in the body weight of the infested fingerlings may be attributed to the reduction in the fish appetite and/or the parasite consumes the fish body nutrients (Ghittino, 1987; Faisal *et al.*, 1988 & Woo, 1995) in addition to the elevation of cortisol level which has catabolic effects on the fish body (Poole *et al.* 2000; Abd El-Galil, 2002 and Korn, 2008). Also, lernaea has an anemic effect on diseased fish (Mahfouz, 1997 and Abd El-Galil, 2002) which caused drop in the fish body weight.

The present investigation studied the histopathological alterations of lernaeosis in *C. carpio* and *C. idella* fingerlings which revealed that the epidermis, dermis and hypodermis of the infected *C. carpio* were infiltrated with massive number of inflammatory cells associated with edema and congested blood vessels and this strong tissue reactions

may be responsible for the appearance of red, hemorrhagic and swollen nodules at the site of infestation. Egusa (1992); Aly *et al.* (1995); Essa *et al.* (1995); Daskalov *et al.* (1999) and Abd El-Rahman (2000) reported similar histopathological lesions in *C. carpio* infested with lernaea. Severe congestion with blood vessels dilatation was observed between the renal glomeruli and tubules and this may be attributed to absorption of the lernaea metabolic end products (Shariff and Roberts, 1989) from the site of infestation to the fish body and reached to the internal organs especially kidney.

On the other hand, the *C. idella*' tissue reactions against lernaea infestation were reported as infiltration of massive number of the inflammatory cells in the dermis and hypodermis, acidophilic granular cells in the skeletal muscles as well as subcutaneous tissue and formation of fibrous connective tissue capsule around the embedded part of the parasite in addition to no histopathological findings were observed in the internal organs, Abd El-Galil (2002) recorded similar histopathological picture in *C. idella* fish infested with lernaea parasite. The inflammatory cells and the acidophilic granular cells have phagocytic functions and defense mechanism (Stoskopf, 1993) in addition to the fibrous connective tissue capsule may greatly reduced the absorption of lernaea metabolic end products (secretory-excretory products) from the site of infestation to the fish body so minimized their bad effects on the internal organs especially kidney and reduce the gross lesions where no hemorrhagic nodules were recorded at the site of infestation.

Concerning the efficacy of Dipterex as a treatment of Lernaeosis, the Egyptian hatcheries regularly used organophosphate pesticides Dipterex to control lernaea infestation (Essa *et al.*, 1995 and Abd El Rahman, 2000) with the attendant problems of the development of parasite resistance (Hoole *et al.*, 2001 and Sandra, 2004), toxicity to both fish stocks and farm workers, their release to the environment causes water and soil pollution and disturbed the normal feeding behavior of the fish where the fish went off their food and remained fasting for as long as the effects of Dipterex persisted (Shariff *et al.*, 1986). In this investigation, Dipterex did not completely eliminate lernaea infestation from the infested *C. carpio* fingerlings and the best result obtained was 76.6% reduction in the infestation rate. Although the relatively high dose used of Dipterex, the non promising effect of its use as a treatment may be attributed to the development of parasite resistance (Hoole *et al.*, 2001 and Sandra, 2004) as a result of its recurrent and disordered application in the investigated hatchery for many previous years in addition to the efficacy of Dipterex decreased at high temperature (Kabata, 1985 and Egusa, 1992).

In conclusion, *C. carpio*, *C. idella*, and *H. molitrix* fingerlings were highly susceptible to Lernaeosis which significantly decreased the body weight of the infested fish fingerlings, the lesions at the attachment point differ among cyprinid species. Dipterex did not completely eliminate lernaea infestation from the infested *C. carpio* fingerlings.

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