

Safety and Effectiveness of Trichlorfon in Prevention of Lernaeosis and Its Comparison with Plant Extracts in Lernaeosis Control

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ABSTRACT

This study was carried out to evaluate the safety and effectiveness of trichlorfon in prevention of lernaeosis among hatchery-reared cyprinids. Also, it compared with plant extracts (carvacrol and cymene mixture and thyme) in lernaeosis control. At the Abo-Saleh fish hatchery in BeniSuef, Egypt, the trichlorfon bioconcentrations of cyprinids fries and fingerlings, as well as soil, water and plant samples were examined for 2 years. Trichlorfon was detected in fingerlings, water and plant samples, except soil sampled 1 hr after trichlorfon was applied to the fish pond. The highest concentration of trichlorfon was in fish, followed by water and plant samples. No trichlorfon was detected in fingerlings, water, plant and soil sampled after 5 days and 5 months. Fingerlings and brooders of cyprinids were examined in situ for clinical abnormalities and prevalence of lernaeosis. In addition, the intensity of the lernaea infestation was determined. Macroscopic *Lernaea cyprinacea* females were seen attached to the skin and fins associated with swollen hyperemic nodules and open wounds. Lernaeosis appeared sporadically during examination seasons with a high intensity. Under experimental condition, trichlorfon succeeded in controlling lernaeosis among *Ctenopharyngodon idella* fingerlings, as it eliminated 100% of *Lernaea cyprinacea* in comparison with 74.28% and 50.2% in carvacrol and cymene mixture and thyme treated fish respectively.

Keywords: Trichlorfon, cyprinids, lernaeosis, carvacrol, cymene, thyme

INTRODUCTION

Hatcheries are considered very important aspect in the aquaculture industry through supplying larval and juveniles and genetic improvement by selective breeding programs which aim to improve production characteristics such as increase growth rate, survival, fecundity and diseases resistance (https://en.wikipedia.org/wiki/Fish_hatchery).

Cyprinidae is the largest family of freshwater fishes in the world (Nelson, 1994) and including the main species is reared in the hatcheries. Cyprinids have important ecological, commercial, nutritional and scientific values. Grass carp (*Ctenopharyngodon idella*, *C. idella*), silver carp (*Hypophthalmichthys molitrix*, *H. molitrix*) and common carp (*Cyprinus carpio*, *C. carpio*) are

economically important and fast growing food fish (Rashid et al., 2014). In addition, *C. idella* is used as biological control for aquatic weeds (Nelson, 2016).

The causative agent of lernaeosis, *Lernaea cyprinacea* (*L. cyprinacea*), is considered harmful ectoparasite to cyprinids wherever they cultured because of the damage and the economic losses (Noor El-Deen et al., 2013).

Lernaeosis is a dangerous disease affecting cyprinids in different parts of the world and it is endemic in Egyptian hatcheries since 1980 (Faisal et al., 1988). Several studies on the prevalence of lernaeosis among hatchery reared cyprinids in Egypt were carried out (Abd El-Galil, 2002; Saleh et al., 2009; Abd El-Galil et al., 2012; Kornı, 2014).

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Knowledge of the lernaosis prevalence gives an idea about the efficacy of the prevention and control programs. The current measures in Egyptian hatcheries for prevention and control of lernaosis rely on the use of organophosphate insecticides mainly trichlorfon. Trichlorfon is a selective organophosphate insecticide used in agriculture and veterinary to control a variety of arthropod pests through inhibition of acetylcholinesterase (Lopes et al., 2006). In Brazil, trichlorfon has not a restricted use and its products are marketed for homeowner use (EPA, 2001) and in fish cultures to control lernaosis and argulosis (Lopes et al., 2006). Also, it is used in Asia in the production of several fish species and in Europe and Chile to control epizooties in salmon and trout cultures (Lopes et al., 2006). Oppositely, in United States (USA), trichlorfon is currently registered for non-agricultural uses such as commercial animal kennels, ornamental plants and bait-fish ponds due to its toxicity to fish stocks and farm workers as well as its side effects on the environment causing water and soil pollution (Lopes et al., 2006).

Several trials were carried out in many parts of the world to find effective and safe methods for getting rid of lernaosis. These trials included mechanical (Faisal et al., 1988) biological (Tamuli and Shanbhogue, 1995; Xinhua, 1999) and chemical alternatives that less toxic than organophosphate for lernaosis eradication (Tamuli and Shanbhogue, 1996; Zaki, 1999; Abd El-Moula, 2001; Abd El-Galil, 2004; Noga, 2010). Recently, studies have been used plant extracts as castor oil, resin extract and thyme extract (Rosa, 2003; Sataporn, 2004; Korn, 2014) for prevention and control of lernaosis.

Depending on the aforementioned data, this study was planned to evaluate the safety and effectiveness of trichlorfon under field condition in prevention of lernaosis among hatchery reared cyprinids. Also, it was compared with plant extracts in lernaosis control among *C. idella*.

MATERIAL AND METHOD

Trichlorfon Insecticide

Trichlorfon 80% of United Company for chemicals and medical preparations (UCCMA, Egypt) is used.

Plant Extracts

Thyme, carvacrol and cymene (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) are used.

Site of Field Study (Abo-Saleh fish hatchery)

Abo-Saleh fish hatchery is located in Beni-Suef, Egypt. It supplies north Upper Egypt especially Beni-Suef and El-Fayum areas with fries and fingerling of cyprinids (General Authority for Fisheries Development of the Ministry of Agriculture, <http://www.gafdr.org>). *C. idella*, *H. molitrix* and *C. carpio* are reared in that hatchery, but the main artificially bred species is *C. idella* as it provides promising alternatives to traditional methods of weed control in Egypt. In Abo-Saleh fish hatchery, as most of Egyptian hatcheries, trichlorfon is used for lernaosis prevention through its addition to the incubated ponds (1/3 gm/m³) 72 hrs before fries stocking. There is no water changing for 5 months until the fries become fingerlings, only the evaporated water is compensated.

Determination of Trichlorfon Bioconcentration

Two trials were carried out for determination of trichlorfon bioconcentration in hatchery reared fries and fingerlings and their environments (water, plant and soil) in the field. In first one, after 5 days from spreading of field dose (1/3 gm per m³) of commercial trichlorfon in one earthen pond (16 curate and 1m water depth) having polycultured *C. idella*, *H. molitrix* and *C. carpio* fries (0.05-0.1 gm, 0.5-1 cm), water, plant and soil samples were collected. Also, after 5 months, samples of fish fingerlings (80-150 gm, 10-18 cm), water, plant and soil were taken. In second trial, after 1 h, 5 days and 5 months post spreading of the same dose of trichlorfon in similar pond having polycultured *C. idella*, *H. molitrix* and *C. carpio* fingerlings (60-120 gm, 15-18 cm), fish, water, plant and soil samples were collected.

At each sampling time, 1 kg of apparently healthy fingerlings from each fish species, 3 liters water, 1 kg of green plant and 1 kg of soil were taken (Devine 1973). Also, water temperature and pH were measured using water thermometer (UK) and pH indicator paper (USA) respectively. Each water sample was taken at 15 cm depth of water surface and at three different locations (1 liter per location). The first at water entry, the second at middle pond and the third at water exit (Lopes et al., 2006).

Each water sample was kept in dark glass bottle, while, each fish, plant and soil sample was kept in separate plastic bag, labeled and frozen at $-20 \pm 2^\circ\text{C}$ till analysis (Lopes et al., 2006). All samples were analyzed for trichlorfon qualitatively and quantitatively in the Central laboratory of pesticides (Dokki, Egypt) by using the Agilent Gas Chromatography (GC, Model 6890) and according to the method described by Hem (2010).

Clinical Examination of Hatchery Reared Cyprinids for Lernaosis

A total of 12600 fingerlings and 3150 brooders of cultured cyprinids were randomly collected alive from Abo-Saleh fish hatchery, Beni-Suef, Egypt. Fish samples were randomly collected 3 times per month, from April to October i.e. lernaosis season for two successive years (2015 and 2016). Each time, 100 fingerlings and 25 brooders were visually examined in situ for lernaosis according to Noga (2010). The examined *C. idella*, *H. molitrix* and *C. carpio* fingerlings and brooders were 3-150 gm (5-19 cm), 2.250-6 kg (25-32 cm), 3.5-130 gm (6-20 cm), 3-5 kg (27-35 cm), 3-160 gm (4-19 cm) and 3-7.5 kg (25-39 cm) respectively. Clinical abnormalities, prevalence of the disease and intensity of the infestation were recorded.

Water Quality Determination

During collection of fish samples for lernaosis examination, temperature and pH of pond water were measured using water thermometer (UK) and pH indicator paper (USA).

Parasitological Examination

Learnaea suspected parasite was collected from naturally infested fish and fixed in glycerin-alcohol 70%. Permanent mounts were prepared according to Becky (2004) and identification of the suspected parasites was done according to Woo (1995).

Preparation of Plant Extracts Water-Soluble Form

Each 100 ul of carvacrol and cymene was dissolved in 5 mL of Dimethyl sulfoxide (DMSO) and mixed together for using as one solution. Similarly, each 100 ul of thyme was prepared (Bernd et al., 2012).

Determination of LC50 of Trichlorfon, Carvacrol and Cymene Mixture and Thyme among *C. idella* Fingerlings

A total of 300 apparently healthy *C. idella* fingerlings with body weight ranged from 50-85 gm and total length (15-19 cm) were brought back to the wet lab. of fish diseases and management department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt. They were acclimatized for 14 days in 3 fiberglass tanks (400 L water for each) as 100 fish/tank. After that, they were re-distributed into 30 glass aquaria of 70×25×40 cm, supplied with chlorine-free tap water (water temperature 25±2°C, pH=7±0.3) and continuous aeration in a rate of 10 fish/aquaria. First ten fish groups received zero (control), 100, 200, 300, 400, 500, 600, 700, 800 and 900 ul/l of carvacrol and cymene mixture. Second ten fish groups received similar concentrations of thyme. Third ten fish groups received trichlorfon concentration of zero (control), 10, 20, 30, 40, 50, 60, 70, 80 and 90 mg/l. The LC₅₀ of each product was calculated according to Behrens and Kerber (1953) using the following formula:

24 hours LC₅₀=largest dose which killed all fish-Σ (A x B)/n

A=Mean of dead fish between 2 successive dose

B=Dose difference between 2 successive doses

Σ (AxB)=summation of (AxB) n=No. of fish/group

Lernaeosis Control among *C. idella* Fingerlings by Trichlorfon, Carvacrol and Cymene Mixture and Thyme

A total of forty eight lernaeosis naturally infested fish and equal number of apparently healthy ones with body weight ranged from 55-65 gm and total length 14-16 cm were brought back to the wet lab. of Fish Diseases and Management department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt. They were distributed into 8 groups, each one put in glass aquaria of 70×25×40 cm. First 3 groups of lernaeosis infested fish were treated with 0.33 mg/l of trichlorfon (field dose), 200 ul/l of carvacrol and cymene mixture (Korni, 2014), 200 ul/l of thyme respectively. Fourth group of infested fish was left without treatment as positive control. Fifth, sixth and seventh groups of apparently healthy fish were treated with similar doses of trichlorfon, carvacrol and cymene mixture and thyme, while eighth group of healthy fish received no treatment as negative control. All groups except controls were exposed to the various treatments for 48 hrs water bath. After bath duration, the fish was transported to clean water. The lernaea was counted just before application of treatment products and at 7th day after bath duration. The treatment products were applied in two regime, each regime included application of the products once weekly for 2 times (2 weeks).

Statistical Analysis

Statistical analysis was performed using the GRAPHPAD IN-STAT (software, Philadelphia, USA). Data were expressed as means±stander error (SE).

Ethics

The present study was approved by the BSU-IACUC (Beni-Suef Institutional Animal Care and Use Committee).

RESULT AND DISCUSSION

Trichlorfon Bioconcentration

In first trial, trichlorfon was not detected in water, soil and plant samples post 5 days from its application in the pond. In second one, trichlorfon was detected in water, plant and fish fingerlings except soil sampled after 1 hr from its application (Figure 1) and its concentrations were 0.039 ppm, 0.004 ppm and 1.076 ppm respectively. On contrarily, it was not detected after 5 days and 5 months in all samples. In first trail, the temperatures and pH at 5 days and 5 months post trichlorfon application were 22°C, 27°C, 7 and 8 respectively. In the second trial, the temperature and pH at 1 h, 5 days and 5 months post trichlorfon application were 23°C, 21, 25°C, 7, 8 and 7 respectively.

Clinical Abnormalities of Lernaeosis Infested Cyprinids

The infested fingerlings and brooders of cyprinids showed the attachment of worm- like parasite that was focally distributed along both fish sides. Also, there were swollen hyperemic nodules and open wounds at the site of attachment (Figure 2). Based on the morphological characters and the structure of two pairs of horn-shaped cephalic chitinous appendages, the parasite was identified as *L. cyprinacea*.

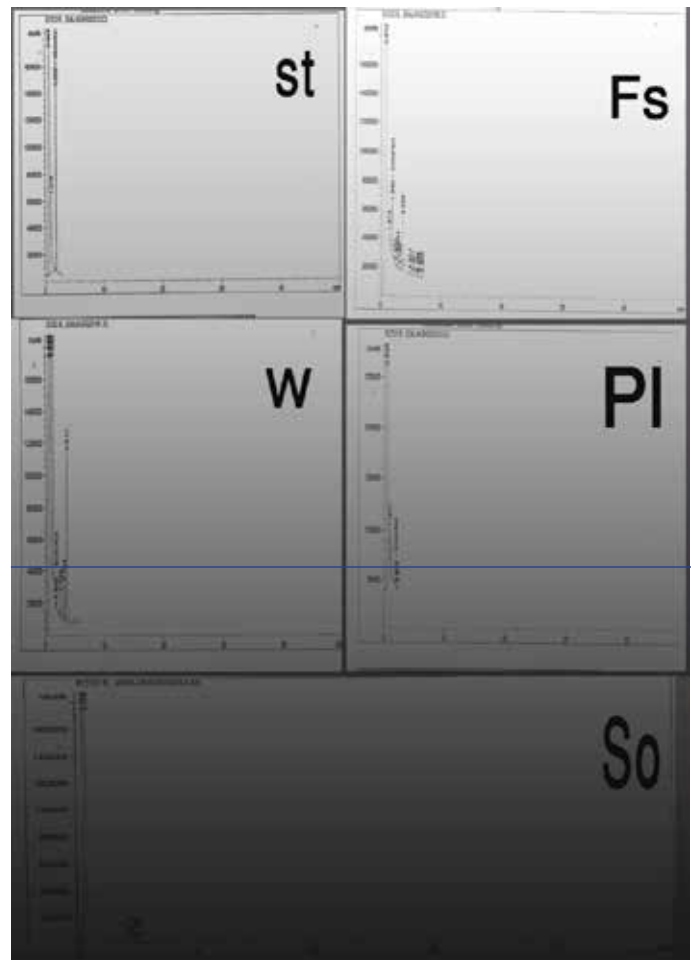


Figure 1. Trichlorfon standard (St) and its level in fish fingerlings (Fs), water (W), plant (PI) and soil (So)

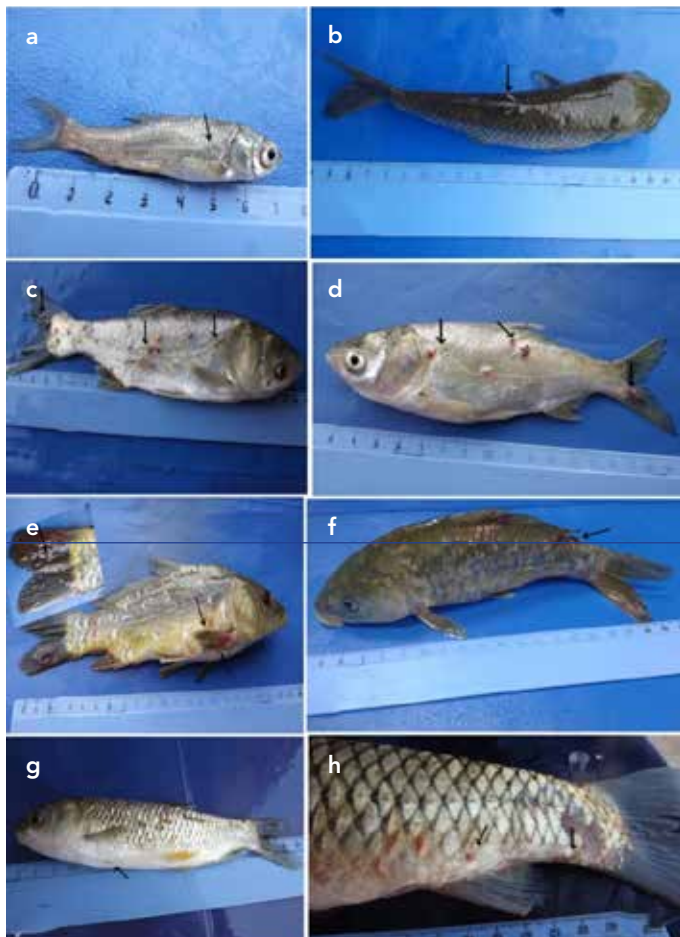


Figure 2. A-H. Clinical abnormalities of lernaeosis naturally affected cyprinids fingerlings and brooders. The *L. cyprinacea* was attached to pectoral (a) and dorsal fins (b) of *C. idella* fingerlings. *L. cyprinacea* penetrated the body sides of *H. molitrix* fingerlings showing their egg sacs with hyperemic nodules and open wounds (c, d). Macroscopic reddening and hemorrhagic nodules around the attachment point of *L. cyprinacea* around pectoral fin, tail fin and dorsal fin of *C. carpio* fingerlings (e, f). *C. idella* brooders showed the attachment of *L. cyprinacea* at the ventral side of the body (g) and caudal peduncle (h)

Lernaeosis Prevalence and Intensity

The prevalence among fingerlings and brooders of cyprinids varied according to species and seasons. In the first season of study (April to October, 2015), the prevalence of lernaeosis among fingerlings of *C. idella*, *H. molitrix* and *C. carpio* was 0.047%, 0% and 0% respectively, while, its prevalence among *C. idella*, *H. molitrix* and *C. carpio* brooders were 1.71%, 0% and 0% respectively. In the second season of study (April to October, 2016), the prevalence of lernaeosis among fingerlings of *C. idella*, *H. molitrix* and *C. carpio* was 3.33%, 1.57% and 1.42% respectively, while, its prevalence among *C. idella*, *H. molitrix* and *C. carpio* brooders were 0.19%, 0% and 0% respectively. The infestation intensity in the infested fingerlings of *C. idella*, *H. molitrix* and *C. carpio* were 1-15, 1-19 and 1-13 parasite/fish respectively.

Table 1. The temperature and pH of pond water during fish sampling for lernaeosis examination (2015 & 2016)

Year	2015		2016	
	Temperature (°C)	pH	Temperature (°C)	pH
April	21.66±0.3	6.3±0.3	22.57±0.5	6.6±0.6
May	29±0.5	6.3±0.3	28±1.1	6.3±0.3
June	29±0.5	7.6±0.3	29±0.5	7.6±0.3
July	32±0.5	7±0.5	29.6±0.3	7±0.5
August	29±0.3	7±0.5	27±1.4	7.3±0.3
September	29±0.5	7.3±0.3	23.3±0.8	8.1±0.1
October	27±0.3	7.3±0.3	23.66±0.8	7.6±0.3

Table 2. 24 hours median lethal concentration of trichlorfon in *C. idella* fingerlings

Fish group	No. of fish/ group	Dose (mg/l)	No. of dead fish	A	B	(AxB)
1 (control)	10	0	0	0	0	0
2	10	10	0	0	0	0
3	10	20	0	0	10	0
4	10	30	0	0	10	0
5	10	40	2	1	10	10
6	10	50	4	3	10	30
7	10	60	5	4.5	10	45
8	10	70	7	6	10	60
9	10	80	9	8	10	80
10	10	90	10	9.5	10	95

24 hours LC₅₀ = largest dose which killed all fish - Σ (AxB)/n
 24 hours LC₅₀ of trichlorfon = 90-320/10 = 58 mg/l

Regarding, the intensity in infested *C. idella* brooders was 1-10 parasite/fish.

Water Quality

In the first season of study (April to October, 2015), the temperature and pH of pond water during lernaeosis examination ranged from 21.66 to 32°C and 6.3 to 7.6 respectively. On the other hand, the temperature and pH of pond water throughout the second season of study (April to October, 2016) ranged from 22.57 to 29.6°C and 6.3 to 8.1 respectively (Table 1).

The LC50 of Trichlorfon, Carvacrol and Cymene Mixture and Thyme

The LC₅₀ of trichlorfon, carvacrol and cymene mixture and thyme was 58 mg/l, 540 ul/l and 660 ul/l (Table 2, 3).

Table 3. 24 hours median lethal concentration of carvacrol and cymene mixture and thyme in *C. idella* fingerlings

Group	No. of fish/ group		Dose (ul/l)		No. of dead fish		A		B		(AxB)	
	Fish group plant extracts	Carv. and cym. mix. Thyme	Carv. and cym. mix. Thyme	Carv. and cym. mix. Thyme	Carv. and cym. mix. Thyme	Carv. and cym. mix. Thyme	Carv. and cym. mix. Thyme	Carv. and cym. mix. Thyme	Carv. and cym. mix. Thyme	Carv. and cym. mix. Thyme	Carv. and cym. mix. Thyme	
1 (control)	10		0		0	0	0	0	0	0	0	0
2	10		100		0	0	0	0	0	0	0	0
3	10		200		0	0	0	0	100	100	0	0
4	10		300		1	0	0.5	0	100	100	50	0
5	10		400		2	1	1.5	0.5	100	100	150	50
6	10		500		5	2	3.5	1.5	100	100	350	150
7	10		600		6	3	5.5	2.5	100	100	550	250
8	10		700		8	5	7	4	100	100	700	400
9	10		800		9	8	8.5	6.5	100	100	850	650
10	10		900		10	10	9.5	9	100	100	950	900

24 hours LC₅₀ = largest dose which killed all fish - Σ (AxB)/n
 24 hours LC₅₀ of carvacrol = 900 - 3600/10 = 540 ul/l
 24 hours LC₅₀ of thyme = 900 - 2400/10 = 660 ul/l

Table 4. The efficiency of trichlorfon and carvacro and cymene mixture and thyme in lernaeosis control

Items	Trichlorfon	Carvacrol and cymene mixture	Thyme	Control
The first application				
Dose	0.33 mg/l	200 ul/l	200 ul/l	0
No. of fish	12	12	12	12
Initial no. of <i>L.cyprinacea</i>	59	70	62	64
Intensity (parasite/fish)	2-15	2-14	1-12	1-14
No. of dropped <i>L.cyprinacea</i> at 7 th day post application	44	42	26	4
% of reduction of lernaea no. at 7 th day post application	74.5	60	41.9	6.2
The 2nd application				
Dose	0.33 mg/l	200 ul/l	200 ul/l	0
Initial no. of <i>L.cyprinacea</i>	15	28	36	60
No. of dropped <i>L.cyprinacea</i> at 7 th day post application	15	4	3	2
% of reduction of lernaea no. at 7 th day post application	100	14.28	8.3	3.3
Total % of lernaea reduction after 2 applications	100	74.28	50.2	9.5

The efficiency of Trichlorfon, Carvacrol and Cymene Mixture and Thyme in Lernaeosis Control

The percentage of *L. cyprinacea* reduction at 7th day post first application of trichlorfon, carvacrol and cymene mixture and thyme was 74.5, 60 and 41.9 respectively in comparison with 6.2 reduction in control group. Regarding, the total percentage of *L. cyprinacea* reduction at 7th day post second applications of trichlorfon, carvacrol and cymene mixture and thyme was 100, 74.28 and 50.2 respectively in comparison with 9.5 reduction in control group (Table 4).

Trichlorfon is an organophosphate insecticide used to control a variety of arthropod pests in agriculture and veterinary (Lopes et al., 2006). Safety of trichlorfon is questionable as it has not a restricted use in various countries including Egypt and it prohibited for food fish in others. Also, several trials in the world were conducted to find effective safe alternatives to trichlorfon for lernaeosis control. Consequently this study was planned to evaluate the safety and effectiveness of trichlorfon in lernaeosis prevention among cyprinids as they are economically important food fish under field condition. In addition, it compared with plant extracts (carvacrol and cymene mixture and thyme) as safe materials for lernaeosis control.

To evaluate the trichlorfon safety, its bioconcentration in cyprinids fries and fingerlings and their environments (water, plants and soil) was determined after 1 hr, 5 days and 5 months from application of its field dose. Concerning the fries, post 5 days, no fish sample could be examined as they were very small in size and all environmental samples were negative. Similarly, all samples including fish that collected after 5 months were free from the trichlorfon. On the other hands on exposure of the fingerlings to the field dose, trichlorfon was detected in all samples except soil after 1 hr, but it disappeared from them including soil when tested after 5 days and 5 months. Highest trichlorfon concentration appeared in fish fingerlings (1.076 ppm) then water (0.039 ppm) and plants (0.004 ppm) (Figure 1). Undetection of trichlorfon in soil may be due to its high water solubility and low octanol-water partition coefficient, so, it remains in water rather than partition to organic matter (Canadian Council of Ministers of the Environment 2012). This is supported by HSDB (1999) who mentioned that trichlorfon has a low soil adsorption coefficient which indicating that it does not have an affinity for sediment or suspended solids. Additionally, trichlorfon was not detected in other samples after 5 days or 5 months may be due to its degradation into dichlorvos (Lopes et al., 2006) especially at pH levels greater than 5.5 (IPCS, 1992). Dichlorvos is the main metabolic form of trichlorfon and it does not significantly bioaccumulate in fish or other aquatic life due to its high solubility in water (Howard, 1991). Once it becomes in contact with water, hydrolysis reactions is the predominant mechanisms for degradation, and hydrolysis proceeding more rapidly with increasing pH and temperatures (Faust and Suffet, 1966; Lamoreaux and Newland, 1978). These findings were supported by (Lamoreaux and Newland, 1978) who confirmed that degradation of dichlorvos is due to its abiotic degradation via hydrolysis which is the major transformation process (70%) and biodegradation by some microorganisms as *Escherichia coli* and *Serratia plymuthica* (30%). So, dichlorvos shows little tendency to sorb to soil particles or bioconcentrate in living tissues (PIP-Dichlorvos, 1993).

Regarding the effectiveness of trichlorfon in lernaeosis prevention in hatchery reared cyprinids, it was found that prevalence of lernaeosis was low during 2 successive years. In 2015, 1 and 9 infested *C. idella* fingerlings and brooders respectively were recorded. In 2016, the overall prevalence among fingerlings of *C. idella*, *H. molitrix* and *C. carpio* was 3.33%, 1.57% and 1.42% respectively, while in brooders, there was only one infested *C. idella* with a prevalence of 0.19%.

The intensity of infestation was still higher especially in *H. molitrix* fingerlings as it reached to 19 parasite/fish our results were agreed with Abd El-Galil et al. (2012).

Lernaeosis was endemic in Egyptian hatcheries till 2014. Consequently, Hatcheries are responsible for frequent appearance of lernaeosis among cultured cyprinids in fish farms due to their role in spreading of the disease via purchase of infested fingerlings. Several studies were conducted on the prevalence of lernaeosis among hatchery reared cyprinids and proved that the prevalence till 2014 was high and reached to 72% in fingerlings and 44% among brooders (Abd El-Rahman, 2000; Abd El-Galil, 2004; Saleh et al., 2009; Abd El-Galil et al., 2012; Korn, 2014).

These changes in lernaeosis prevalence may be due to climate changes associated with high unsuitable water temperature as it reached to 32°C in 2015 (Table 1), whereas, environmental temperature plays a significant role in survival and development of lernaeid copepods and water temperature of 26-27°C is ideal for complete the life cycle (Raissy et al., 2013; Mirzaei et al., 2014). Additionally, these prevalence variations may be due to periodic using of trichlorfon in the hatchery for prevention and control of lernaeosis. In 2016, the prevalence was relatively higher due to better climate conditions (Table 1) with suitable temperature in this year, but, the prevalence was still low in comparison with the previous years.

In this study, lernaeosis affected fish showed swollen hyperemic nodules and open wounds at the site of attachment of macroscopic lernaea parasitic females on the body and fins (Figure 2). Similar clinical abnormalities of affected cyprinids were reported by Abd El-Rahman (2000); Abd El-Galil (2002); Mancini et al. (2006); Abd El-Galil et al. (2012); Noor El-Deen et al. (2013) and Korn (2014).

Also, in this investigation the safety and effectiveness of trichlorfon in lernaeosis control in *C. idella* fingerlings was compared with carvacrol and cymene mixture and thyme which are the major components of thyme extract (Rattanachaiakunsopon and Phumkhachorn, 2010) as safe natural by-products. First, on determination of the LC₅₀ of trichlorfon and previous plant extract, that of the LC₅₀ of trichlorfon was 58 mg/l (Table 2), 540 ul/l and 660 ul/l for carvacrol and cymene mixture and thyme respectively (Table 3). However, trichlorfon still safe as treatment dose is 0.33 mg/l. About their efficiency in lernaeosis control, trichlorfon succeeded in complete removal of lernaea from infested *C. idella* fingerlings after 2 applications, whereas carvacrol and cymene mixture and thyme eliminated 74.28% and 50.2% of the lernaea respectively (Table 4). These results were supported by Korn (2014) who proved that carvacrol and cymene mixture succeeded in eliminating 88.8% of lernaea at 96 hrs after 48 hrs water bath treatment of *C. carpio* fries. Also, Troncoso et al. (2011) and Bernd et al. (2012) reported that carvacrol had a high potential to reduce sea lice infestation by bath treatment as it killed 100% of the parasite within 48 h of in vitro application.

CONCLUSIONS

Trichlorfon may be safe and effective in lernaeosis prevention and control. It has a low persistence in water and had no residue in fish tissues. Also, with periodic using of trichlorfon, there was only sporadic infested fish in the hatchery which has no impact on the economy. Moreover, it succeeded in elimination of 100% of *L. cyprinaea* comparing with 74.28% and 50.2% in carvacrol and cymene mixture and thyme treated fish respectively. So, trichlorfon can be used in food fish for lernaeosis prevention and control.

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