Studies on Trichodinosis of Some Cultured Freshwater Fishes at Sohag Governorate

[1]Fayza M. Soliman, [2]Mohamed A. A. Abd El-Galil, [1]Mohamed A. Adly and [1]Fatma El Zahraa A. A. Ahmed

[1]Department of Zoology, Faculty of Science, Sohag University, Egypt
[2]Department of Fish Dis. & Manag., Faculty of Veterinary Medicine, Sohag, University, Egypt
abdelgalil1997@yahoo.com

Abstract: Clinical signs of trichodinosis in the cultured *Oreochromis niloticus* (*O. niloticus*), *Tilapia zilli* (*T. zilli*) and *Ctenopharyngodon idella (C. idella)* revealed signs of asphyxia in the form of rapid operculum movement, surfacing and gasping; signs of irritation as swimming near borders and scratching against hard objects, dullness, detachment of scales, excessive and turbid mucus, ulcerations, frayed fins and the gills were pale in some fishes and congested in others with excessive accumulation of mucus. The highest prevalence of infection was reported during summer season and was 22.2, 15.6 & 15.6% in *O. niloticus, T. zilli* and *C. idella*, respectively. The lowest disease prevalence was recorded in the winter season and was 0% in all investigated fishes. The highest water quality deterioration was recorded during summer season where the temperature was 31.5ºC, dissolved oxygen (DO) was 6 mg/l, ammonia (NH_3) was 0.7 mg/l and pH was 7.3. The isolated *Trichodina* was identified as *T. sangwala, T. magna and T. heterodentat*a. The susceptibility or sensitivity of *Trichodina* to the steamed oil of colophony (resin of *Pinus sp.*) was tested and revealed that 5 and 10 ppm destructed 100% of the parasites within 30 minutes while 2ppm needed 120 minutes to destruct 100% of the parasites.

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1. Introduction

The ectoparasitic diseases of fishes play an effective role in the economic losses of fish farms through mortality and/or decrease growth rate of fish especially in the highly intensified systems. About 80% of fish diseases are parasitic especially in warm water fishes **(Eissa, 2002)**. External parasites constitute the largest group of pathogenic organisms in warm water fish **(El-Seify *et al.*, 2011a)** and cause severe mortalities **(Shalaby and Ibrahim, 1988 and El-Seify *et al.*, 2011a)**. Ectoparasitic protozoa are cited as the major problem in warm water fish farms where high temperature and organic content accelerate the life cycles of parasites and promote their spread **(Hassan, 1999).** Ciliates are the most identified protozoan organisms where they can easily spread among most of fish hosts **(El-Seify *et al.*, 2011a)** and trichodinids are common ciliate ectoparasites living on the skin and gills of fish, and have major importance in fish pathology **(Lyholt and Buchmann, 1995 and Jia-yun *et al.*, 2011)**.

Trichodinosis (slime disease) is one of the ectoparasitic protozoal diseases caused by *Trichodina spp.* that are a uniformly saucer shaped ciliated protozoan parasites, 50 microns diameter, with rows of cilia at both ends and macro and micronucleus **(Robert, 2013).** *Trichodina* are found in all corners of the globe and survive on and infect the external body surface (skin, fins) and/or gills of both freshwater and salt water fishes. They are regarded as a main cause of fish mortality **(Abdel-Meguid, 1989, 1995a, 1995b and 2001 and Ramadan *et al.*, 1995)**.

The majority of fish ectoparasitic protozoa are commensals which under deteriorated water quality and/or suppressed fish immune system produce serious diseases **(Hassan, 1999).** The intensification of fish in the farms creates disease problems that originate from overcrowding and/or deteriorating water quality such as unsuitable water temperature, and pH **(Kugel *et al.*, 1990)** and free ammonia concentrations **(Hassan, 1999)**. In such cases of stress, several species of *Trichodina* may become pathogenic interfering with feeding and respiration of small fishes **(Hassan, 1999)**.

Trichodinosis manifests in the form of restlessness, loss of appetite, loss of condition, signs of irritation including swimming near borders and scratching against hard objects, excessive mucus secretion, and also respiratory function can be impaired in gill infections **(Abowei *et al.*, 2011)**. It is transmitted by direct contact with infected fish and/or contaminated water **(Robert, 2013)**. It can also be transported form pond to pond by amphibians as frogs and toads.

Chemical treatments for trichodinosis include Formalin, Malachite Green, Chloramine-T and Potassium permanganate were effective but most of them were not safe with all fish. Over dosage of

Chemical treatments are toxic to fishes; especially those already weakened by disease. However, the frequent use of these chemical drugs has resulted in serious drawbacks such as development of parasites resistance, environmental contamination, drug residue and pressure to the fish itself **(Goven *et al.*, 1980 and Klinger and Floyd, 2002)**. In the last years, the herbal treatments were developed for control of the most harmful parasitic diseases infecting humans, animals and fishes **(Abd El-Galil and Aboelhadid, 2012).** Generally, the plant-based products are more safe and non-resistible for frequent use. Moreover, these new products might be potential sources of new antiparasitic drugs **(Jia-yun *et al.*, 2011)**.

Colophony is a resin obtained from *Pinus* species and consists of about 90% resin acid (abietic acid), with the remainder consisting of neutral substances, oxidized terpentines and minor quantities of esters and anhydrides **(Thor *et al.*, 1995).** A very important aspect to be taken into consideration is the high availability of the resin, which is produced in large commercial scale **(Rosa *et al.*, 2003).** *Pine crude* resin and its steamed oil derivative were satisfactory and considered safe and cheap products for control the ectoparasitic disease as lernaeosis (**Rosa *et al.*, 2003 and Korni, 2008)**

This research aimed to study the clinical signs and seasonal prevalence of trichodinosis in some cultured freshwater fishes at Sohag Governorate, identify the species of isolated *Trichodina* and to study the susceptibility of *Trichodina* to the steamed oil of Colophony.

2. Material and Methods

Fish samples: during the period from 21-12-2011 to 20-12-2012 a total number of 180 *Oreochromis niloticus* (*O. niloticus*), 180 *Tilapia zilli* (*T. zilli*) and 180 grass carp (*C. idella*) were collected alive from El-Ahaywa fish hatchery at Sohag Governorate and transported to the fish laboratory of Zoology Dept., Faculty of Science, Sohag University in plastic containers partially filled with its local water and aerated by battery aerator **(Langdon and Jones, 2002 and Abo-Esa, 2008)**. 45 fish of each investigated species were used in each season of the year. Body weight of *O. niloticus* was ranged from 20 to 80 gm., *T. zilli* was ranged from 15 to 50gm. and *C. idella* was ranged from 40 to 95gm.

Clinical examination: of the external body surface of the investigated fishes was carried out on a live fishes in glass aquaria. Skin, fins, gills, eyes and other external features were examined according to the methods described by **Austin and Austin (1987)** and **Noga (2000).** Clinical signs and abnormalities appeared on the body surface of diseased fishes were reported and photographed.

Parasitological examination: Scraps from skin were prepared by curettage the body surface and the smears were spread on a dry clean slides with a drop of water and examined under (40X) lens; wet smears of gills were prepared by cutting the gills in petri dish then the filaments were examined under dissecting microscope in order to detect the presence of trichodinids. The *Trichodina* parasites were picked up by Pasteur pipet with amount of water and were spread on a dry clean glass slide and examined again under (40X) lens **(Bartholomew, 2003).**

Smears from infested infected fishes were air-dried and impregnated for 10 min in 2 % aqueous ($AgNo_3$) solution (Klein's silver impregnation technique) **(Klein, 1958)**, washed in distilled water, and exposed to ultraviolet light for 20 to 25 min, in order to study details of the adhesive disc and the aboral ciliary spiral **(Yemmen *et al.*, 2010/11).** The seasonal prevalence of parasites was calculated according to **Bush *et al.* (1997) and Yemmen *et al.* (2010/11).**

Parasitological identification: according to **Robert (2003) and Fahuiand Yuanjun (2011)** the species of isolated *Trichodina* could be determined from the morphology and measurements of the denticles surrounding the adhesive disc of the organism; the isolated *Trichodina* were identified by matching the morphology and number of denticles with the results of **William and Wootten (1998) and El-Tantawy and El-Sherbiny (2010)**.

Water samples were collected monthly at fixed times at 12 pm of the same day of fish catching. The water samples were collected from three different sites at a depth of 15- 20 cm below water surface in dry and dark brown stoppered glass bottle **(A.P.H.A, 2005)**. Water parameters were measured monthly and the mean of each parameter is calculated for each season.

Measurement of water parameters:

Water temperature was measured in situ by using ordinary thermometer graduated in degrees Celsius (°C) the bulb of thermometer was placed under water at a depth of about 15 – 20 cm. The temperature was read without lifting the thermometer out of water after waiting a short time until the column comes to a standstill **(Coche *et al.*, 1998)**.

Dissolved oxygen was measured using oxygen-sensitive membrane electrode meter after calibration using distilled water **(A.P.H.A, 2005).**

Ammonia was measured using the Nessler method according to **(A.P.H.A, 2005)**.

Susceptibility of *Trichodina* parasite to steamed oil of colophony

The steamed oil of colophony was used at concentrations of 2, 5 and 10 ppm for 30, 60 and 120

minutes in bath treatment to evaluate its effect on *Trichodina* parasite **(Korni, 2008).**

3. Results

Clinical signs on all diseased fishes including cultured *O. niloticus, T. zilli* and *C. idella* revealed many behavioral changes as signs of asphyxia in the form of rapid operculum movement, surfacing and gasping, in addition to signs of irritation as swimming near borders and scratching against hard objects. Clinical signs appeared on some fish showed dullness and detachment of scales, Photos (A2) and (B2). Excessive and turbid mucus and ulcerations were observed, Photo (A1). Bases of some fins were congested, Photos (A3) and (B1). Gills of some fishes were pale white and congested in others with excessive accumulation of turbid mucus.

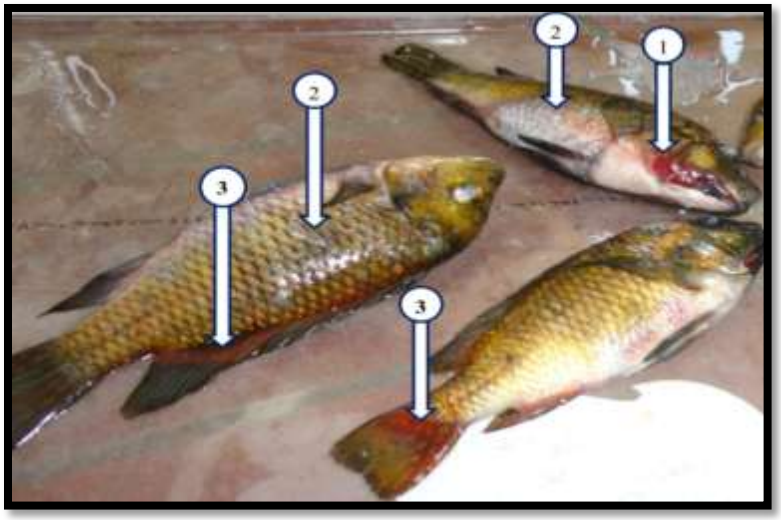


Photo (A): diseased *T. zilli* showed:
(1) - ulcerations and congestion posterior to the operculum
(2) - detachment of scales at the body sides and belly.
(3) – congestions at the bases of dorsal and caudal fins.

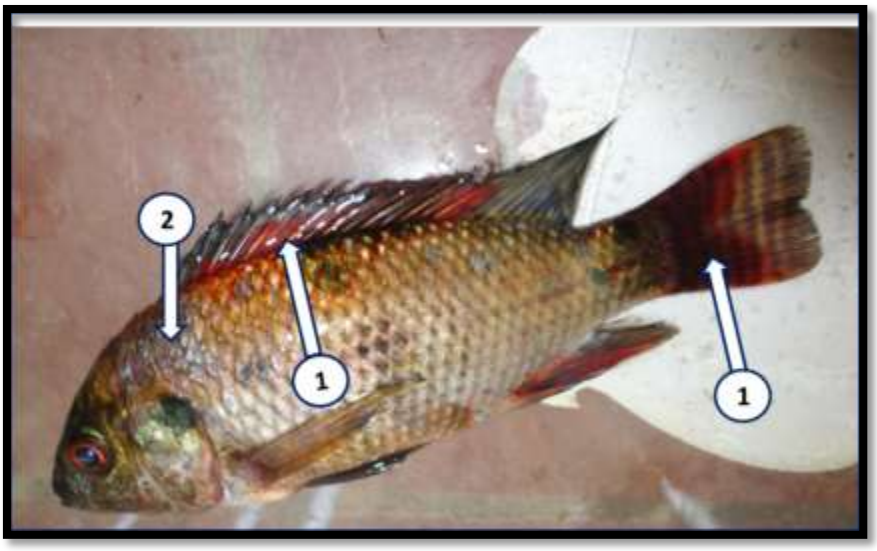


Photo (B): diseased *O. niloticus* showed :
1– Congestion at the bases of dorsal and caudal fins.
2 – Detachment of scales.

Prevalence of trichodinosis in cultured fish species:

Trichodinosis in the cultured fishes was prevalent throughout the warm seasons of the year, where diseased cases were reported during summer, autumn and spring seasons of the year in all examined fish species. The highest prevalence of trichodinosis was recorded in *O. niloticus* (12.2%) and the lowest infection was recorded in *T. zilli* (8.9%) while it was 10% in *C. idella*, Table (1) and Fig. (1). The seasonal prevalence of trichodinosis in the cultured fishes revealed the highest level during summer season and recorded 22.2, 15.6& 15.6% in *O. niloticus, T. zilli* and *C. idella*, respectively and its lowest prevalence (0%) was recorded during winter in all examined fishes where no diseased cases were recorded. The trichodinosis prevalence during autumn was 15.6, 8.9 and 13.3% in *O. niloticus, T. zilli* and *C. idella*, respectively and in spring season was 11.1% in all examined fish species, Table (1) and Fig. (1).

Table (1): Prevalence of trichodinosis in the cultured freshwater fishes ***O. niloticus, T. zilli*** and ***C. idella***

Season	Cultured Fish species						
	No of examined fish	*O. niloticus*		*T. zilli*		*C. idella*	
		No of diseased fish	%	No of diseased fish	%	No of diseased fish	%
Winter	45	0	0	0	0	0	0
Spring	45	5	11.1	5	11.1	5	11.1
Summer	45	10	22.2	7	15.6	7	15.6
Autumn	45	7	15.6	4	8.9	6	13.3
Total	180	22	12.2	16	8.9	18	10

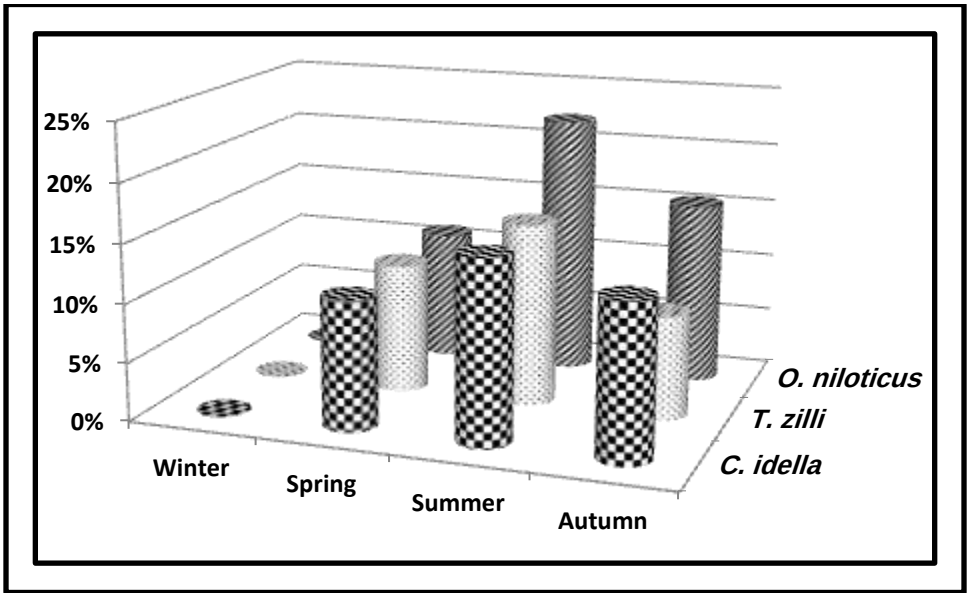


Figure (1): Histogram showing the seasonal prevalence of trichodinosis between cultured fishes *O. niloticus, T. zilli* and *C. idella*

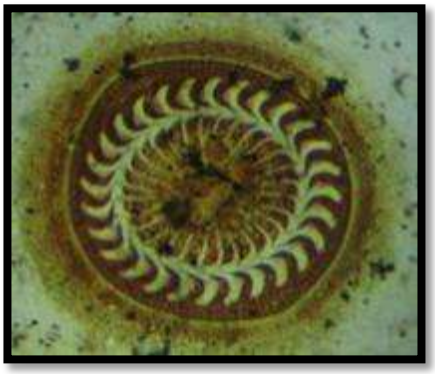
Parasitological isolation and identification:

1- In fresh wet mount preparations, the parasite is very motile and appears as a circular or bell-shaped ciliated organism.

2- In the silver impregnated slides, the adhesive disc (saucer-shaped) could be seen, the organism surrounded with several circular rows of cilia and supported with a circle of more centrally lying hooklets. The isolated trichodinids were identified as *T. Sangwala, T. magna and T. Heterodentat*a, Photos C (1, 2, and 3).

Photos (C):

1- *Trichodina sangwala*



2- *Trichodina magna*

3- *Trichodina heterodentata*

Water quality of fish farm pond:

1- In spring season, mean water temperature was 25.6ºC, DO was 7.3mg/l, unionized ammonia (NH_3) was 0.4 mg/l and pH was 7.7, Table (2).
2- In summer season, mean of water temperatures was 31.5ºC, DO was 6 mg/l, unionized ammonia (NH_3) was 0.7 mg/l and of pH was 6.9, Table (2).
3- In autumn season, mean water temperatures was 24.9ºC, DO was 7.2 mg/l, unionized ammonia (NH_3) was 0.3 mg/l and of pH was 7.6, Table (2).
4- In winter season, mean water temperatures was 15.5ºC, DO was 7.9 mg/l, unionized ammonia (NH_3) was 0.06 mg/l and of pH was 7.9, Table (2).

Table (2): water quality criteria

No. of water samples	Seasons	Water Parameters			
		Temp. (°C)	DO. (mg/l)	Unionized ammonia (mg/l)	pH
1	Spring	23.1	8.0	0.3	7.9
2		25.9	7.3	0.4	7.6
3		27.9	6.7	0.4	7.5
Mean		**25.6**	**7.3**	**0.4**	**7.7**
1	Summer	31.0	6.1	0.7	7
2		33.0	6.2	0.8	6.8
3		30.4	5.7	0.6	6.9
Mean		**31.5**	**6.0**	**0.7**	**6.9**
1	Autumn	28.0	7.5	0.2	7.3
2		25.2	6.9	0.3	7.7
3		21.5	7.3	0.4	7.8
Mean		**24.9**	**7.2**	**0.3**	**7.6**
1	Winter	18.3	7.8	0.1	8
2		13.3	8.2	0.04	7.8
3		15.0	7.6	0.03	7.9
Mean		**15.5**	**7.9**	**0.06**	**7.9**

Susceptibility of *Trichodina* to steamed oil of colophony:

Using steamed oil of colophony at concentrations 5 and 10ppm eradicates the *Trichodina* completely within 30 minutes; while 2ppm needed 120 minutes to destruct 100 % of the parasite, Table (3).

Table (3): Susceptibility of *Trichodina* to steamed oil of colophony

Steamed oil of colophony (ppm)	Number of *Trichodina* parasites /MF			
	Before treatment	Bath treatment		
		30 minutes	60 minutes	120 minutes
Zero	**15**	15	14	14
2		10	2	0
5		0	0	0
10		0	0	0

4. Discussion

Trichodinids are among one of the most common ectoparasites in wild and cultivated fish **(Basson and Van As, 1994 and Martins and Ghiraldelli, 2008)**. This parasite lives normally in a few numbers in the mucous surface of skin and gills. When host/parasite/environment relationship is broken by nutritional deficiency, poor water quality and infectious and/or parasitic diseases, trichodinids may proliferate rapidly and become responsible for severe epidermal lesions and disease outbreaks **(Madsen *et al.*, 2000; Martins *et al.*, 2002; Khan, 2004 and Huh *et al.*, 2005)**. Trichodinids may cause serious damage to the epithelial or epidermal cells by their constant attachment and also by their movement **(El-Tantawy and El-Sherbiny, 2010)**.

Trichodina spp. are ecto-commensal parasites on fish and have been reported in cichlids **(Hassan, 1992 and 1999; Younis *et al.*, 2009 and Abd El-Galil and Aboelhadid, 2012)**. *Trichodina* is an opportunistic parasite which becomes pathogenic under stressful conditions **(Eisa *et al.*, 1985)**. On the other hand, **Abdel-Meguid (1989)** reported that *Trichodina sp*. is a true and permanent parasite which was noticed to bring about marked mortalities among newly-hatched grass carp.

The recorded clinical signs of trichodinosis in the cultured *O. niloticus*, *T. zilli* and *C. idella* were summarized in signs of irritation as erratic swimming, swimming near borders and scratching against hard objects, detached scales, excessive and turbid mucus, ulcerations and frayed fins. Most of these clinical

signs were observed before by **Schaperclaus (1992), Noga (1996), Dey and Chandra (1998), Yousef (2008) and Younis *et al.* (2009).** These lesions may be attributed to movement and adhesive disc of *Trichodina* **(Schaperclaus, 1992 and Reed *et al.*, 2003).** Also signs of asphyxia in the form of rapid operculum movement, surfacing (aggregation of fish near the water surface), Piping or gasping may be observed; and these signs may be attributed to the feeding behavior of *Trichodina* on the disrupted cells and host's gills in addition to the penetration of the parasite deeply into the gill tissue **(Lom and Dykovà, 1992 and El-Tantawy and El-Sherbiny, 2010)**. Excessive mucus was observed on the skin and gills of infected fish and this massive production of mucus is regarded as a defense mechanism to eliminate the parasite or dilute its irritating effects **(Hasssan, 1999)**.

Regarding the prevalence of trichodinosis in the examined fishes, trichodinosis was prevalent throughout the warm seasons of the year; the highest level was 22.2, 15.6 & 15.6 % in *O. niloticus; T. zilli* and *C. idella*, respectively and recorded during summer season. The lowest level of trichodinosis (0%) was recorded during winter season in all examined fish species. This finding disagreed with the findings of **Hassan (1999)** who reported that the highest infection of *Trichodina* sp. was found in spring season (62.9%) and th**e** lowest rate was in summer season (35%), **Yemmen *et al.* (2010/11)** who stated that the prevalence of trichodinosis reached its maximum in winter season (84.4%) and its minimum in summer season (12.03%) and **Huh *et al.* (2005)** reported that the highest rate of infection with *Trichodina spp.* was in late winter to early spring 2002.

The disagreement may be attributed to the difference in locality and water quality which was deteriorated during warm seasons especially summer season where the high temperature and organic content accelerate the life cycles of parasites and promote their spread **(Hassan, 1999)** and this explanation was confirmed by **Ogut and Palm (2005), Kristmundsson *et al.* (2006)** and **Yemmen *et al.* (2010/11**) who reported that the fluctuation in the prevalence of trichodinosis may related to the temperature, ammonia (NH_3), dissolved oxygen and salinity levels.

The identification of the isolated *Trichodina* species was done on silver impregnated slides and based on the denticle shape, orientation of rays, cut-like notches in the convex side of denticle of blade, non-staining granule at the base of the blades and rays. The isolated *Trichodina* species were very similar to *T. sangwala, T. magna* **(Van As and Basson, 1989)** and *T. Heterodentata* **(El-Tantawy and El-Sherbiny, 2010).**

The water quality of the investigated fish farm was near the acceptable level during winter season and deteriorated during the warm seasons of the year. The deterioration of water quality increased during the autumn and spring and reached its peak during summer season. In summer water temperature was 31.5ºC, DO was 6 mg/l, unionized ammonia (NH_3) was 0.7 mg/l and pH was 6.9. This deterioration may be correlated with the fish intensification and the increase in water temperature that accelerates the rate of fish metabolism, feeding and excretion. This explanation supported by **Houlihan *et al.* (1993), Britz *et al.* (1997) and Azevedo *et al.* (1998)** who stated that the fish is affected by the temperature of the surrounding water which influences the body temperature, growth rate, food consumption, feed conversion and other body function. Also, High temperature and organic content accelerate the life cycles of parasites and promote their spread **(Hassan, 1999).** Moreover, **Steffensen (2002)** reported that fishes are less tolerant to thermal stresses where at higher water temperature, more energy has to be spent for basic metabolism and the metabolic rate of fish exponentially increases.

Regarding the correlation between water quality and prevalence of trichodinosis among the examined fish species, water pollution can affect the immune system of the fish, reduce the fish immunity **(Poulin, 1992)** and accelerate the life cycle of the parasites promoting their spreading **(Hassan, 1999; Noor El-Din, 1997 and El-Seify *et al.*, 2011b).** This investigation revealed that there is a direct correlation between the degree of water quality deterioration and the prevalence of trichodinosis, the highest level of trichodinosis was recorded during the worse degree of water quality during summer season where the highest level of ammonia (NH_3) (0.7 mg/l) and water temperature (31.5ºC) and the lowest level of DO (6 mg/l) were recorded. On the other hand, the lowest level of trichodinosis was recorded during winter season with good water quality where ammonia (NH_3) was 0.06 mg/l; water temperature was 15.5ºC and DO was 7.9 mg/l and these results were come in contact with finding of **Kugel *et al.* (1990) and Hassan (1999)**.

Dealing with the susceptibility or sensitivity of *Trichodina* to the steamed oil of colophony, 5 and 10 ppm of this steamed oil destructed 100% of *Trichodina* parasite within 30 minutes while 2ppm needed 120 minutes to destruct 100% of the *Trichodina*, where this dose reduced the parasite number from15 parasites/MF to 4 parasites/MF within 90 minutes. The effect of steamed oil of colophony may be attributed to its direct effect on the parasite **(Rosa *et al.*, 2003**) and the steamed oil was highly safe to be applied as water bath for fish treatment

because up to 100ppm steamed oil caused no mortality in the fish (**Korni, 2008**).

In conclusion, trichodinosis is a warm season disease and the highest prevalence of the disease was recorded in summer season, *O. niloticus* is the highest susceptible fish of the investigated fishes to trichodinosis, the deteriorated water quality enhances the disease spreading and increases its prevalence in the cultured fishes. 5 ppm of steamed oil of colophony destructed 100% of *Trichodina* parasite within 30 minutes.

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Corresponding Author:
Mohamed A. A. Abd El-Galil, Dept. of Fish Dis. & Manag., Faculty of Veterinary Medicine, Sohag University, Egypt.
abdelgalil1997@yahoo.com

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