PREVALENCE OF TRICHODINID IN MANZALA LAKE AND RIVER NILE FISHES, WITH A SPECIAL REFERENCE TO POLLUTANTS EFFECTS

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ABSTRACT

A total of 984 freshwater fish from Manzala Lake (n = 510) and River Nile (n = 438) in Egypt were examined for ectoparasitic trichodinids (Protozoa, Ciliophora) during the period extended from August 2009 to the end of July 2010. Tissue smears, stained by Phosphotungestic acid Haematoxoline and Giemsa stain techniques, were performed to examine skin, fins and gills. Trichodina species were detected in 120 (23.53%) and 90 (20.55 %) of examined fishes from Manzala Lake and River Nile, respectively. Autumn and winter had higher infection rate. Comparative description was based on the morphological features of adhesive disc and denticular ring as well as the shape and number of denticles. Six species of Tricodinids were found namely; T. californica, T. cogwheeli, T. fultoni, T. heterodentata, T. pediculus, and T. truttae. The present study concluded that there was a direct proportional relationship between water pollutants concentration and the prevalence of trichodinids infestation.

Key Words: *Trichodina, prevalence, Manzala Lake, River Nile, pollutants.*

INTRODUCTION

Trichodina species are one of the most prevalent peritrichous ciliates affecting skin, fins and gills of a wide range of fish species **(Reichenbach- Klink and Sturm, 1973).** They multiply rapidly and cause serious tissue damage due to their erratic movement. One of the important morphological features is the presence of a circular, toothed adhesive disc which increases the mucus secretion and decreases the immunity. Therefore, the infected fish become more susceptible to opportunistic bacterial pathogens. **(Dickerson and clark, 1996).**

Fish is considered as an important source of protein of high nutritional value for human and animals. Typically, heavily trichodinid infestation decrease the fitness and reproduction of the fish as well as loses of body condition so, the total productivity of fish protein was greatly affected **(Scholz, 1999).** The incidence of Trichodinids infection in fish was discussed by many authors like 100% in Oerochromis niloticus, **(Tantawy and Younis, 2003),** 17.7% in larvaeparous ornamental fish **(Rashed, 2007),** 20% in naturally collected Clarias gariepinus **(Abo-Esa, 2008)** and 56.85% in Oerochromis niloticus **(Martins, 2008).**

About 200 species of Trichodina have been described in fish **(Asmat, 2005).** In Egypt, the first valuable information concerning genus Trichodina was given by **(Abu El-Wafa, 1988)** who revealed four species of Trichodina namely T. fultoni, T. truttae, T. reticulata and T. californica from gills and external body surface of Tilapia species and C. lazera in Behera Province. Another study was carried out by **(El-khatib, 1989)** who found T. fultoni in the same fish species then, successive studies on Trichodina species were performed by many authors including **El-Gawady et al. (1992), Hassanein (2002)** and **Abou zaid (2011).** Stress factors, especially pollutants cause more propagation of T. species on its host and convert such commensals into parasites which may be attributed to reduction of the immunological capability of the hosts. Mc **Dowell et al. (1999)** and **Vera et al. (2003)**.

Therefore, this study was conducted in order to investigate pollutant effect on prevalence of Trichodinids in Manzala Lake and River Nile in Egypt as well as study the comparative morphological features of the different recorded species.

MATERIAL AND METHODS

A total number of 948 fishes were collected alive or freshly dead from Manzala Lake (n =510) and River Nile (n = 438) during the period extended from August 2009 to the end of July 2010. Fishes were collected from different localities of Manzala Lake including Dakahlia, Damietta and Port Said portions of the lake. Live or freshly dead fishes were transported to the laboratory of Parasitology Department, Fac. Vet. Med., Mansoura Univ. Samples were examined immediately just after death to avoid the disintegration of the Trichodinids. Impression smears were taken on cover slides and stained with Phosphotungestic acid Haematoxoline **(Drury and Wallington, 1980)** and Giemsa stain technique **(W.H.O., 1991)**. Water samples were collected seasonally from different regions of Manzala Lake and River Nile in clean glass bottles of one liter capacity for analysis of different chemical parameters as heavy metals. **(A.P.H.A., 1971)**. pH value was detected using pH meter. Total hardness and water salinity were estimated by titration against EDTA **(A.P.H.A., 1971)** and **(Royce, 1984)**, respectively.

RESULTS & DISCUSSION

I- Prevalence and intensity of Trichodinid infestation:

Examination of the collected specimens revealed that 210 (22.15%) out of 948 examined fish were infested with T. species. The infection rate in Manzala Lake was 120 (23.53%) higher than that of River Nile 90 (20.55%). By studding the comparative incidence between different regions of Manzala Lake, it is found that the higher incidence was in Port Said region (24.83%) followed by Damietta region (23.28%) and finally, Dakahlia region (22.73%) Table (1). Regarding the seasonal prevalence of Trichodinid, spring and summer seasons had the lowest prevalence in both Manzala Lake and River Nile (20.45%, 19.7% and 20%, 18.42%, respectively). While, the highest prevalence was inversed in both regions, in Manzala Lake, it was high in autumn (27.43%) followed by winter (27.08%) and in River Nile, it was high in winter (22.92%) followed by autumn (21.24%) Table (2). On the other hand, the biochemical pa-

rameters and heavy metals of water sample obtained from Manzala Lake and River Nile were estimated. It was found that pH, salinity, hardness and heavy metals were generally higher in Manzala Lake than River Nile. There was a reverse relationship between pH and prevalence of parasite (the decrease of pH as in winter, the increase of percent of parasite) and in contrast there was a proportional relationship between heavy metals, salinity and hardness and prevalence of parasite (the increase of heavy metals, salinity and hardness the increase of percent of parasite). Table (3).

II- Morphological description of Trichodinid:

Six species of Trichodina parasitizing the body surface, gills and fins were detected. Their classification was based on the morphological features of adhesive disc and denticular ring as well as the shape and number of denticles. T. species had asymmetrically body shape, the dorsal side was convex and ventral one was concave which form adhesive disc. Adhesive disc was composed of complicated structures arranged in the form of three connected rings, the inner ring (denticular ring), the second one (a circular ribbon like structure) overlapping the hooks of denticles and the last one which was composed of radial pins.

Trichodina californica **(Davis, 1947)**: It is reported in the skin, gills and fins of Tilapia species, Clarias species and common carp. The parasite was a moderate trichodinid (43-49 µm in diameter) with disc to bell- shaped body. The adhesive disc was saucer shaped measuring 36 - 43 µm in diameter and surrounded by finely striated border membrane. The denticular ring measured 21 - 25 µm in diameter with 21- 28 denticles. Denticles were stout and straight with sharp tips. The blade had a bluntly, rounded tip and attached to the central part of denticle at same level of rays. Number of radial pins per denticle was 8-10 (Fig. 1).

Trichodina cogwheeli **(Abu El-wafa et al, 1999)** is found inhabiting the skin and fins of Tilapia species and Clarias species. It was a disc-shaped body in ventral view measuring (42- 71 µm in diameter). The adhesive disc ranged between 35 - 60 µm in diameter and bounded by finely striated border membrane (2 - 5 µm in width). The denticular ring measured 21- 34 µm in diameter and carrying 18-20 denticles with 8- 10 radial pins per each. The denticles measured 8 - 16 µm in length and have cup-shaped blades with broad ends and narrow bases, measured 4- 9 µm giving the denticular ring a general appearance of a cogwheel. The ray of denticle was short, stout, slightly curved posteriorly and tapered rapidly to form sharp point and measured 4- 8 µm in length. (Fig. 2).

Trichodina fultoni **(Davis, 1947)**: parasitized on skin, gills and fins of Clarias species. It is a large trichodinid (51- 76 µm in diameter) with disc shape body. The adhesive disc (38 - 61 µm in diameter) was surrounded by a finely striated membrane. The denticular ring (32 - 38 µm in diameter) had 20- 26 denticles which had 8- 12 radial pins per each. The ray of the denticle was stout, curved posteriorly with pointed tip and attached to the central part of the denticle slightly posterior to the aperture. The blade was curved posteriorly with rounded tip and not attached to the cen-

tral part of the denticle at the same level as the ray (Fig. 3).

Trichodina heterodentata **(Duncan, 1977)**: The parasite was isolated from skin and fins of Tilapia species. It was a large trichodinid (73 - 91 µm in diameter). The diameter of the adhesive disc varied between 51 - 65 µm and the denticular ring was 34 - 40 µm in diameter with 20- 24 denticles and 8- 12 radial pins per denticle. The border membrane was finely striated, surrounding the denticular ring and of 5 - 7 µm in width. The denticle (7 - 11 µm in length) had sickle- shaped blades (5 - 9 µm in length) and the central part was triangular in shape with mean width of 3 - 4 µm. The thorn (7- 10 µm in length) was wide, straight, directed to the center and had a sharp pointed tip (Fig. 4).

Trichodina pediculus. **(Muller, 1786)**: parasitized on the skin of adult fish of Clarias species. It was a large trichodinid (73 - 96 µm in diameter) disc-shaped in ventral view and surrounded by a finely striated border membrane (6 - 7 µm in width). The denticles had pointed needle-shaped thorn. Its adhesive disc ranged from 46 to 68 µm. The denticular ring (28 to 38µm in diameter) had 20 to 32 denticles which had 8- 10 radial pin per each (Fig. 5).

Trichodina truttae **(Mueller, 1937)**: It was detected in the gills and skin of Tilapia species. The parasite was characterized by its large size (102 - 103 µm in diameter). The adhesive disc was saucer shaped and also large measured up to 77 µm. The denticular ring was 38- 39 µm in diameter and carrying 27-28 denticles. The ray of the denticle was long, thin, slightly curved and had a sharp pointed tip. The blade was narrow, slightly curved posteriorly and not attached to the central part of the denticles at same level as the ray and posterior to the denticle aperture (Fig. 6).

Concerning Trichodinids infecting freshwater fish, the prevalence of T. species in Manzala Lake (23.53%) was relatively higher than that of River Nile (20.55%). This is due to that the water of Manzala Lake is exposed to industrial and agricultural effluents which constitutes the primary source of chemical pollutants and have synergistic effects upon parasitic infestation. These results are found agreed with **Dabrowska (1974)**, **Pascoe** and **Cram (1977)**, **Jana** and **Ghosh (1987)**, **Brown** and **Pascoe (1989)**, **Khan and Thulin (1991)** and **Svobodova, et al. (1993)**. Our findings showed that the prevalence of T. species in Port Said region was the highest (24.83%) followed by Damietta (23.28%) then Dakahlia region (22.73%). This variation appears to be related to the distance from marine water as Port Said region has higher incidence, followed by Damietta and finally Dakahlia region. In the present investigation, it is noticed that the increase of heavy metals, salinity and hardness, the increase is the prevalence of parasite. These parameters may decrease immunity of the fish and become more susceptible to infection **(Dickerson and clark, 1996)**.

Regarding the seasonal dynamics of T. species, the highest infection rate was detected in winter and autumn seasons in different localities while the lowest was in summer season that because the trichodinids had enhanced reproduction in the low temperature. These

results were coincided with (**Abu El-Wafa, 1988**) who found that maximum rate of infection in O. niloticus was in spring and autumn and disagreed with **(Abd El- Hady, 1998)** who noted that the infection was higher in summer.

Six species of Trichodina were isolated in the present study from the skin, gills and fins of the investigated fish species. Identification of different species was based on the morphological characters of the adhesive disc and denticular ring as well as shape and number of denticles according to **Wellborn (1967)**. The light microscopic description of the revealed T. species was in agreement with that reported by **Abu El-Wafa (1988)**, **El-khatib (1989)**, **Woo (1995)** and **Hassanein (2002)**. The ventral side of T. species had adhesive disc which is composed of complicated structures arranged in the form of three concentric rings, the inner denticular ring, followed by circular ribbon like structure overlapping the hooks of denticles and the last one composed of radial pins. It was agreed with **(Hassanien, 2002)**. T. cogwheeli had a unique morphological character that it was easily distinguished from the cup-shaped blades with broad ends and narrow bases, giving the denticular ring a general appearance of a cogwheel. The shape of the blade plays an important role in characterization of different T. species. In this regard, T. californica **(Davis, 1947)** was identified by its characteristic shape of the denticles where the blade and the ray were attached to the central part of the denticle at the same level while the blades of T. fultoni **(Davis, 1947)** had a rounded tip, curved posteriorly and not attached to the central part of the denticle at the same level as the ray. Moreover, T. heterodentata **(Duncan, 1977)** was charactrized by wide, straight thorn directed to the center with sharp pointed tip. Some rays appeared shorter and asymmetrically distributed within others. T. pediculus. **(Mueller, 1786)** had extremely long, needle-pointed thorn. On the other hands, T. truttae. **(Mueller, 1937)** had characteristic large size 102- 103 µm in diameter.

Table (1) Prevalence of *Trichodina species* in Manzala Lake in different locations and River Nile.

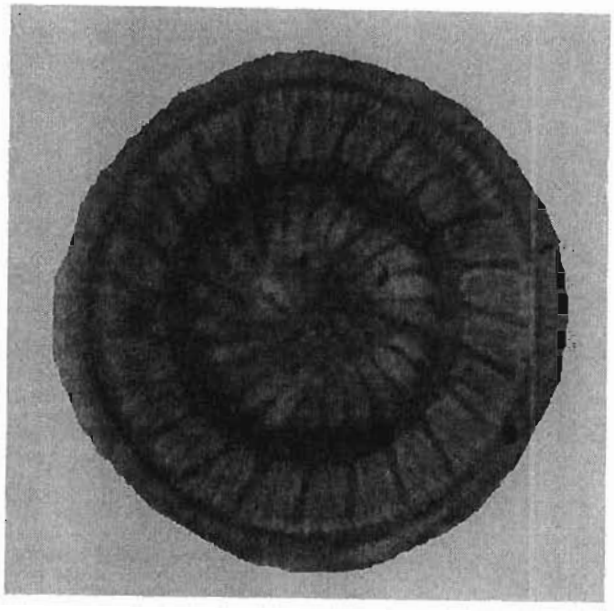
Manzala lake												River Nile			Total		
Total			DK			DT			PS								
Total No. of examined fish	Total No. of infected fish	%	No. of examined fish	No. of infected fish	%	No. of examined fish	No. of infected fish	%	No. of examined fish	No. of infected fish	%	No. of examined fish	No. of infected fish	%	No. of examined fish	No. of infected fish	%
510	120	23.53	176	40	22.73	189	44	23.28	145	36	24.83	438	90	20.55	948	210	22.15

Table (2) Seasonal prevalence of *Trichodina species* recovered from Manzala Lake in different location and River Nile

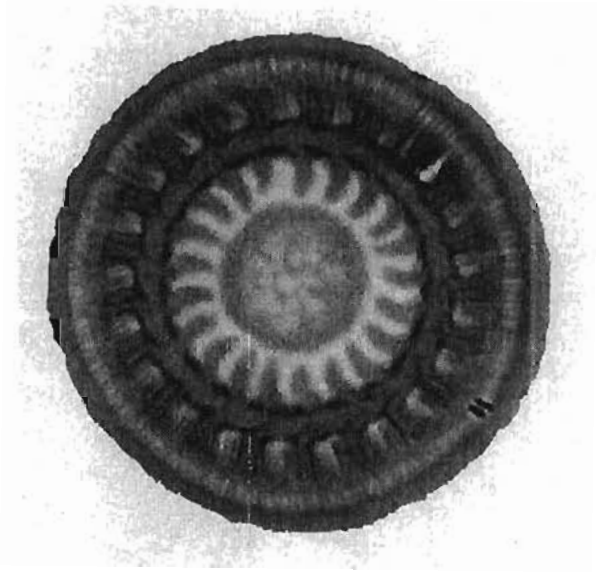
Location		Seasons - Total			Winter			Spring			Summer			Autumn		
		Total Number of examined fish	Total Number of infected fish	%	Number of examined fish	Number of infected fish	%	Number of examined fish	Number of infected fish	%	Number of examined fish	Number of infected fish	%	Number of examined fish	Number of infected fish	%
MANZALA	Total	510	120	23.53	133	36	27.08	132	27	20.45	132	26	19.7	113	31	27.43
	DK	176	40	22.73	44	12	27.27	44	9	20.45	44	8	18.18	44	11	25
	DT	189	44	23.28	45	11	24.44	45	10	22.22	45	9	20	54	14	25.93
	PS	145	36	24.83	44	14	31.82	43	11	25.58	43	2	4.65	15	3	20
NIle		438	90	20.55	96	22	22.92	115	23	20	114	21	18.42	113	24	21.24

Table (3) Biochemical parameters and heavy metals of water samples obtained from Manzala Lake and River Nile.

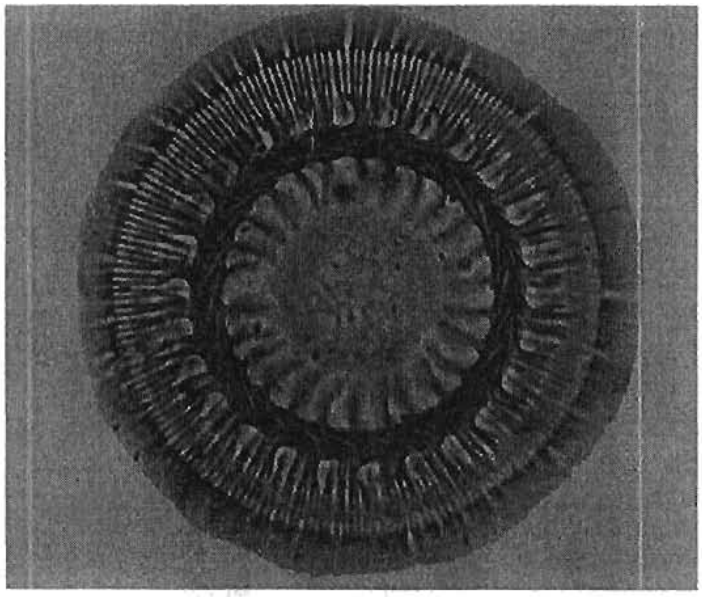
Season	Manzala Lake					River NIle				
	Water parameters			Heavy metals (ppm)			Water parameters			Heavy metals (ppm)		
	pH value	Salinity g/L	Total hardness g/L	Lead ppm	Cadmium ppm	Mercury ppm	pH value	Salinity g/L	Total hardness g/L	Lead ppm	Cadmium ppm	Mercury ppm
Winter	7.6	1.91	7.67	0.00431	0.00215	0.039	7.3	0.487	2.13	0.00422	0.00222	0.021
Spring	8.6	1.721	7.44	0.00266	0.00104	0.034	7.1	0.481	2.16	0.00398	0.00092	0.009
Summer	8.98	1.452	5.21	0.00239	0.00091	0.017	7.9	0.501	3.01	0.00221	0.00082	0.001
Autumn	7.2	3.01	8.11	0.00475	0.00245	0.052	8.8	0.511	2.92	0.00231	0.00198	0.011
Permissible limit of WHO (ppm).				0.050	0.005	0.001				0.050	0.005	0.001



(Fig. 1): *Trichodina californica* (x 100) stained with Giemsa stain.

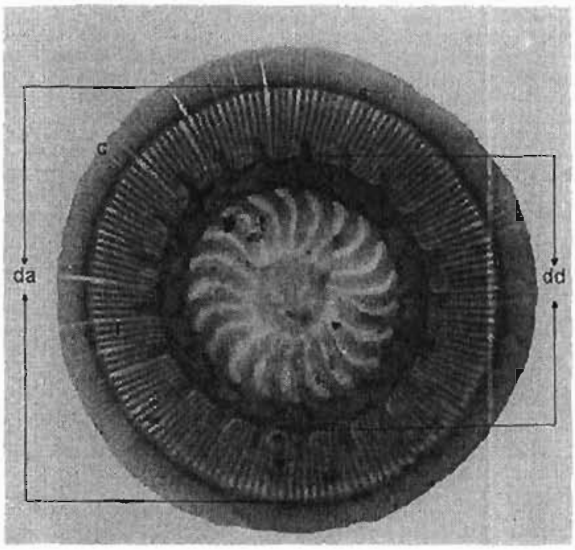


(Fig. 2): *Trichodina cogwheeli* (x 100) stained with Giemsa stain

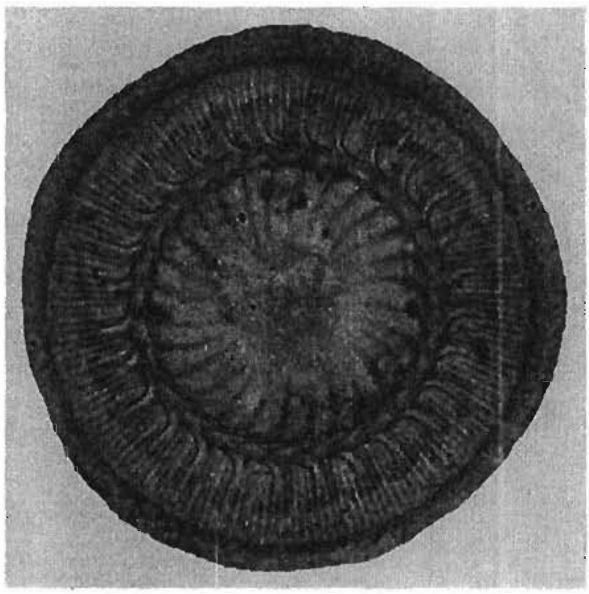


(Fig. 3): *Trichodina fultoni* (x 100) stained with Phosphotungestic acid haematoxoline stain.

(Fig. 4): *Trichodina heterodentata* (x 100) stained with Giemsa stain.



(Fig. 5): *Trichodina pediculus* (x 100) stained with Phosphotungestic acid haematoxoline stain.



(Fig. 6): *Trichodina truttae* (x 100) stained with Giemsa stain.

dd= diameter of denticular ring. da= diameter of adhesive disc. s= striation of border membrane. b= blade of denticles.	r= radial pins. c= cilia. t= thorn of denticles.

REFERENCES

Abd El-Hady, O. K. (1998): Comparison studies in some parasitic infection of fishes in fresh and pollutant water sources. Ph. D. Thesis. Fac. Vet. Med., Cairo Univ.

Abo-Esa, J. F. K. (2008): Study on some ectoparasitic diseases of catfish Clarias gariepinus with their control by Ginger Zingiber officiale. Mediterranean Aquaculture J., (1): 8-18.

Abou zaid, A. A. (2011) : The effect of external parasites on clinicopathological changes of freshwater fish exposed to environmental pollutants. M.V.Sc. Thesis, Kafr El-Sheikh Univ.

Abu El-Wafa, S. A. (1988) : Protozoal parasites of some freshwater fishes in Behera Governorate, Egypt. M.V.Sc. Thesis, Alexandria Univ.

Abu El-wafa, S. A.; Ezz El-Dien, N. M.; Loutfy, H. S. and Abd El-Khalek, H. M. (1999) : Description of Trichodina cogweeli n.sp. and other newly recorded ectoparasites from Egyptian freshwater fish. Beni-Suef Vet. Med. J., 9 (2): 216- 203.

Asmat, G. S. M. (2005): Trichodinid ectoparasites (Ciliophora : Trichodinidae) of fishes in India. Res. J. Agric. Biol. Sci., 1(1): 3-37.

A. P. H. A. (American Society for testing and materials) (1971): Standard methods of examination of water and waste water. 13th Ed., Washington.

Brown, A. F. and Pascoe, D. (1989): Parasitism and host sensitivity to cadmium and Acanthocephalan infection of the freshwater. J. of Appli. Ecol., 26: 473.

Dabrowska, H. (1974): An attempt to evaluate the state of health of fish from the Lyna and Walsza River in concentration to their pollution. Fish health news, 6: 156.

Davis, H. S. (1947): Studies of the protozoan parasites of freshwater fishes. U.S. Dept. Interior fishery Bull., 41: 1-29.

Dickerson, H. W. and Clark, T. G. (1998): Immune response of fishes to Ciliates. Annual Review of Fish Diseases, 6 : 107-120.

Drury, R. A. B. and Wallington, E. A. (1980): Carleton's histological technique. 5th Ed., Oxford Univ. Press.

Duncan, B. L. (1977): Urceolariid ciliates, including three new species from cultured Philippine fishes. Trans. Am. Microsc. Sco., 96: 76 - 81.

El-Gawady, H. M.; Eissa, I. A. and Bahran, A. F. (1992): The prevalent ectoparasitic diseases of Oerochromis niloticus fish in Ismailia city and their control. Zag. Vet. J., 20 (2): 277-285.

El-Khatib, N. R. H. (1989): Some studies on ectoparasites in freshwater fishes M.V.Sc. Thesis, Fac. Vet. Med., Cairo Univ.

Hassanein, M. E. (2002): Studies on some problems facing intensive fish cultured in A.R.E. M.V. Sc. Thesis, Fac. Vet. Med., Zagazig Univ.

Khan, R. A. and Thulin, J. (1991) : Influence of pollution on parasites of aquatic animals Advances in Parasitol., 30 : 201.

Jana, S and Ghosh, K. (1987): Effect of heavy metals on population growth of a fish nematode Spinicauda spinicauda in aquatic environment. Enviro. and Eco., 5: 811.

Martins, M. L. and Ghiraldelli, L. (2008): Trichodina magna (Ciliophora: Peritrichia) from cultured Nile Tilapia in the state of Santa Catarina, Brazil, Braz. J. Biol., 68(1): 169 - 172.

Mc Dowell, J. E.; Lancaster, B. A.; Leavitt, D. F. and Ripley, B. (1999): The effects of lipophilic organic contaminants on reproductive physiology and disease process in marine bivalve mollusks. Limnol. Oceanogr., 44: 903- 909.

Mueller, J. F. (1937): Some species of Trichodina (Ciliate) from freshwater fishes. Ttans. Amm. Micr. Soc., 56: 117-184.

Mueller, O. F. (1786): Animalcula Infusoria fluviatilia et marina. Copenhagen. Havniae et Lipsiae, 367.

Pascoe, D. and Cram, P. (1977): The effect of parasitism on the toxicity of cadmium to the three spined stickleback, Gastrosteus aculeutus L.J. Fish Biol., 10: 467.

Rashed, M. A. (2007): Studies on some parasitic diseases affecting viviparous ornamented fishes. M. V. Sc. Thesis, Fac. Vet. Med., Kafr El-Sheikh Univ.

Reichenbach - Klinke, H. and Sturm, G. (1973): Myxidium - Arten and andere sporozoan in fish fletsch. Munich seminar of biology of fish. Fisch and Umwelt, 1: 51-57.

Royes, W.F. (1984): Introduction to the practice of fishery science. Academic Press, Inc London, United Kingdom Ed. Printed in U.S.

A-Scholz, T. (1999): Parasites in cultured and feral fish. Vet. Parasitol., 84: 317- 335.

Svobodova, Z.; Lloyed, R.; Marchova, J. and Vykusova, B. (1993): Water quality and fish health. Eifac. Tech. Pap., F.A.O. Rome. Italy. 53.

Tantawy, E. A. and Younis, A. A. (2003): comparative study on the effect of formalin and Garlic (Allium sativum) on ectoparasitic infesting Oreochromis niloticus fish with reference to their effect on blood picture and serum constituent. Egypt. J. Agric. Res., 81 (1).

Vera, N.; Simonovic, P. and Paleksic, V. (2003): Preferances on Trichodinides (Ciliata: Peritrichia) occurring on fish-pond crap for particular organs and morphological implications. Acta., 135: 41- 46.

Wellborn, T.L. (1967): Trichodina (ciliate: Urceolariidae) of fresh water fishes of the southestern united states. J. Protozool., 14: 399- 412.

W.H.O. (World health organization) (1991): Basic laboratory methods in medical parasitology. Parasitol. Lab Manuals.

Woo, P. T. K. (1995) : Fish disease and disorders. Dep. Zool. Guelph Univ. Canada.

الملخص العربى

الانتشار والوصف الظاهري المقارن و تاثير نسبة الملوثات على طفيل التريكودينا في أسماك بحيرة المنزلة ونهر النيل

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أجريت هذه الدراسة على بعض أنواع أسماك المياه العذبة الموجودة فى نهر النيل والأماكن المختلفة في بحيرة المنزلة بمصر؛ لعمل مسح شامل لطفيل التريكودينا، وذلك لمعرفة الأنواع الموجودة من هذا الطفيل، ووصفه ظاهريا، وتحديد نسبة الإصابة منه في الاماكن موضع الدراسة، كما تم دراسة نسبة الانتشار الموسمية للطفيل. وكذلك تعيين بعض المعايير الكيميائية والمعادن الثقيلة في الماء لمعرفة مدى تأثيرها على نسبة إنتشار هذا الطفيل. وقد وجد سبع أنواع وهم تريكودينا كاليفورنيكا، تريكودينا كوجويلي، تريكودينا فولتوني، تريكودينا هتيرودنتاتا، تريكودينا بليوسيداس، تريكودينا رتيكيولاتا و تريكودينا تروتي . وقد تم وصفها ظاهريا. ووجد أيضا ان نسبة الاصابه في بحيرة المنزله اعلي من نهر النيل، ونسبتها اعلى في المناطق ذات الملوحه العاليه عن غيرها. أما عن النسبه الموسميه فقد وجد ان الخريف والشتاء هما أعلى نسبة 27.43% و 27.08% بالترتيب في المنزلة و 21.24% و 22.92% بالترتيب في النيل. وان الصيف اقل نسبة 19.7% في المنزلة و 18.42% في النيل. ووجد ان للملوثات علاقه طرديه مع نسبة الإصابه و كلما قل الأس الهيدروجيني زادت نسبة الاصابة، وكلما زادت الملوحة وعسر الماء والمعادن الثقيلة زادت نسبة الإصابة.