ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Isopodiosis in Some Fishes from Egyptian Qaroun Lake: Prevalence, Identification, Pathology and In Vitro Trials to get rid of it.

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ABSTRACT

A total of 556 fish represented by 180 *Solea Solea, 203 Tilapia zilli* and 174 *Mugil capito* were collected from Qaroun llake, Fayoum province, Egypt. They were investigated for Isopods infestation during the period from June 2015 to September 2016. The total prevalence was 33.5% ,41.1% and 80.9% in *T. zilli, Solea solea* and *Mugil Capito;* respectively. Four cymothoid isopod species were encountered among the examined fish*: Anilocra physodes* was perceived from *Solea Solea, Nerocila orbignyi* from *Mugil capito, Solea solea and Tilapia zilli. Renocila thresherorum* from *Tilapia zilli. Lironeca ovalis* from both *Solea solea* and *Mugil capito.* All the recovered *Isopoda* showed seasonal variations in the rate of infestation, reaching the peak in summer. The histopathological alterations induced by isopod species were discussed. Furthermore, the efficacy of some herbal extracts as neem leaves extract (*Azadirachta indica*), chamomile flowers extract (*Matricaria chamomilla)* and shih (*Artemisia herba-alba*) was evaluated in vitro against isopod parasites and they were compared with deltamethrin bath as a chemical drug.

Keywords: Cymothoid isopods, *Tilapia zilli, Solea Solea, Mugil capito*, Medicinal plants.

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ISSN: 0975-8585

INTRODUCTION

The crustacean ectoparasites infest abundant fish species, including mostly those of commercial value in subtropical and tropical region. Many *Cymothoidae* species have been reported in Africa (12 Anilocrinae and 34 Cymothoinae) [1]. They are blood feeders that inhabit the external surfaces of fish body, the buccal cavity, the gill chamber or burrow into the fish to live in cavities [2]. In Egypt, a great attention is given to fish parasitic diseases [3], however, a diverse localities of cymothoids have been incompletely recognized. In Quaroun lake, a previous report proved the infestation of 25% of *Tilapia zilli* with *Nerocila orbignyi*, with a highest infestation rate in summer [4].

A large variety of chemical drugs has been tested against cymothoid. However, chemical treatments are not always effective, especially against the adult stage [5]. The vigorous use of some chemicals may result in damage to the fish [6]. Besides, the misuse of chemical drugs as treatments of Isopoda infecting fish cause a potential hazard, which affect both human and fish health. These substances may be toxic, allergenic, or carcinogenic, and/or may cause resistance in pathogens that affect humans. However, the active principle of some medicinal plants was proved to be of an efficient effect than other manufactured products [7].

The present study focused on Identification, determining the prevalence of isopod species infesting fish in Qaroun lake (*Solea solea , Tilapia zilli* and *Mugil capito*). Besides, an in vitro evaluation of the efficacy of some herbal extracts as neem leaves extract (*Azadirachta indica*), Chamomile flowers extract (*Matricaria chamomilla*) and shih (*Artemisia herba-alba*) against isopod parasites compared with deltamethrin bath as a chemical drug.

MATERIALS AND METHODS

Study area

The present study was carried out in Lake Qaroun, which situates about 80 km in the southwest of Cairo in Fayoum Governorate, Egypt. It locates 45 meters below sea level, its surface area is 214 Km^2, its depth is about 8 meters, and its volume capacity is 800 million cubic meters. The lake length/ width is 42 X 9 km. The lake salinity is 36-38 in summer, 32-34 in spring and autumn,28-30 in winter.

Examined Fishes

A total of 556 fish species including 180 *Solea Solea*, 203 *Tilapia zilli* and 174 *Mugil capito* were captured from the Lake at the period from June 2015 to September 2016. Fish were transferred a live to the laboratory of National Institute of Oceanography and Fisheries (NIOf), Fayoum branch, or to the Lab of Fish Diseases and Management at Fac. of Vet. Med, Benha University to be examined.

Clinical and Parasitological examination

All the collected fishes were clinically examined according to the method described by [8]. After clinical investigation, the isopods were instantly fixed in 70% ethanol, kept in well stoppered labelled tubes and conveyed to the laboratory for identification. The morphological details of isopod sp. were examined using a dissecting microscope, all the measurements were done in millimeter and photographed using a digital canon camera. The morpho-metric characters and the taxonomy of Isopods sp. were done after the key of [9,10,11].

Histopathological examination

Samples were taken from the gills, skin and musculature of fish. The tissues were fixed in formol saline (10%) for24 hours, stained by hematoxylin and eosin staining technique and examined under alight microscope [**12**]

In vitro assessment of the efficacy of medicinal plant extracts against Isopoda

The watery extracts of neem leaves (*Azadirachta indica*), chamomile flowers (*Matricaria chamomilla*) and shih (*Artemisia herba-alba*) were prepared according to [13]. Each extract was kept in the refrigerator in a well stoppered dark bottle. The used extracts were adjusted to 1, 2 and 3% concentrations by dilution in lake water and used within thirty minutes. In order to evaluate the efficacy of the used extracts, ten isopods were used for each of the prepared concentrations (1%, 2% and3%) in three replicates. The isopods were treated in petri dishes with the selected concentration and the control groups were treated with the lake water. The viability of the parasites was examined at 6 different time intervals (5, 10, 15, 20, 30 and 60) minutes. Viability criteria were estimated by testing the reaction of the isopods after stroking them by a needle [14].

Evaluation of the effect of deltamethrin bath in vitro

A dose of 0.01 was made from a stock solution of 1 % Kafrokil (deltamethrin- pyrethroid, Kar Elziat company) using the lake water as a diluent. The diluted stock solution was applied within 30 min of prepration [14].

RESULTS AND DISCUSSION

In the present study, four cymothoid isopods (*Anilocra physodes, Renocila thresherorum, lironeca ovalis and Nerocila orbignyi*) were isolated from three fish species (*Solea Solea, Mugil capito* and *Tilapia zilli*). These results came in accordance with this finding of [10] who reported that cymothoid Isopoda showed a great tendency to infect more than one fish species and they can infest 10 orders and 20 families of fish.

The recorded species belong to Order: Isopoda, Phylum: Arthropoda, Class: Malacostraca and Family: Cymothoidae. The morphomertric characters of the identified Isopoda species in this study came in agreement with [9,10,11,23]. These were as follow:

Anilocra physodes (Linnaeus, 1758)

The body is mainly narrow 14-17 mm in length, 8-10 mm in width and it is elongated. The eyes are large, well developed, oval and containing many facets. There is a wide distance between the two eyes. Antennule are shorter than antenna, the first pair of antenna is consist of 8 segments and the second pair consists of 9 segments . The cephalon is triangular in shape and it not immersed in the 1st perionite. The anterior end of the cephalon is truncated.. The posterior margins of the 1st and 2nd perionites are rectangular, but, those of 3th, 4th ones are roundly truncated, 5th one is rounded, 6th and 7th are produced . The 6th perionite segment is considered the longest one. The pleonites are not produced posterolaterally. The pleopods are very long reaching behind the hinder aspect of pleotelson. The pleotelson is as broad as long and rounded evenly. The uropods extend behind the pleotelson. The expods are longer than endopods (Fig 2. A, B).

Renocila thresherorum (Williams & Williams, 1980)

Body is depressed, 14-32 mm length and 8-14 mm width. Cephalon is wider than long and its posterior extremity is weakly immersed in the 1st perionite. Eyes are of moderate size. There are two pairs of antenna which are sub equal in length. The first antenna has eight segments and the second antenna has seven segment. The antenna not reaching the anterior extremity of the 1st perionite. The 1st Perionite segment is the longest and 7th perionite is the shortest. The Posterolateral angles of perionite 1-5 are not produced, but that of perionite 6 and 7 are produced . The pleonites are subequal in length and width and not produced posterolaterally. The posterior extremity of the pleotelson uniformly rounded and showing nearly the same width of 5th pleonite. The uropods surpass the extremity of the pleotelson. The exopods are elongated and longer than the endopods which is oval in shape (Fig. 2. C, D).

Lironeca ovalis (Say 1818)

The body is oval in shape with 16-22mm in length and 9-11mm in width. The cepahlon is rounded anterirorly, trilobed posterirorly, not embedded in perionite1. The middle lobe of the cepahlon is the largest. Eyes are not contiguous and widely separated. The first and the second pairs of antenna are nearly equal and consist of 4 and 5 segmants respectively. First perionite segment is the longest one. Coxal plates are pointed

ISSN: 0975-8585

obtusely but not extend beyond the posterior margin of the succeeding segments. The coxal plate of the 7th perionite segment is the most pointed reaching the posterior margin of he 1st pleonite . The abdominal segments are subequal in length. The pleotelson is broad and flat. The uropods slightly extend beyond pleotelston. The exopods and endopods are subequal in length (Fig.2. E, F).

Nerocila orbignyi (Guérin-Méneville, 1832)

It is flattened dorsoventerally; measured 26 mm in length and 10mm width. Cephalon is trilobed, broadly rounded at the anterior end. Eyes are large size. The cephalon is not embedded in the 1st perionite. The 1st antenna and the 2nd antenna are short not reach the posterior border of of the 1st perionite. The three last peraeon segments are wider than the preceding ones. There is a prolongation of 4th-6th peraeon. The pleon is shorter and narrower than peraeon and consists of six segments.. The pleotelson is wider at the base . The uropods are biramus and longer than the pleotelson. The expods is longer than endopod. (Fig 2. G, H). The eggs are located in the marsupium of ovigerous females (Fig. 2.I).

Concerning the clinical signs, they were nearly similar in all the infested fish regardless their species. The fishes suffered from sluggish movement and emaciation. They were infested by large isopods measuring 14-32 mm length. One to three parasite/ fish were seen unilaterally or bilaterally on gills, occupying branchial cavity, or on the skin. The isopods were severely attached leaving a hemorrhagic necrotic ulcer or petechial hemorrhage. Body surface and branchial chamber were the common site of *Anilocra physodes. Renocila thresherorum* was mainly infesting gills. *Lironeca ovalis* were encountered from the branchial chamber. *Nericola orbigyni* was perceived from the gill arch [Table 2]. Gills showed sever hemorrhagic filament and multifocal and petechial hemorrhage in the inner side of gill operculum [Fig.1]. These findings were similar to that reported by [15,16,17]. The exerted pressure induced by the parasites on host tissues, besides, their feeding habit on host blood explain the observed inflammatory reaction and ulceration induced by cymothoid sp. at the site of attachment on fish [18].

Table 1: Total and seasonal prevalence of Isopoda sp. among examined fishes of Qaroun Lake

Season / Fish sp.	Winter			Spring			Summer			Autumn			Total		
	Exam.	Infest.	%	Exam.	Infest.	%	Exam.	Infest.	%	Exam.	Infest.	%	Exam.	Infest.	%
***Tilapia zilli* ***	-	-		80	25	31.3	18	8	44.4	105	35	33.3	203	68	33.5
Solea solea	60	12	20	60	16	26.7	40	36	90	20	6	30	180	74	41.1
Mugil capito*	-	-	-	20	10	50	153	130	85%	-	-	-	173	140	80.9
Total	60	12	20	160	51	31.9	211	174	82.5	125	41	32.8	556	282	50.7

***` Tilapia zillicannot be collected during winter season**

***** Mugil capito* cannot be collected during winter and autumn season**

Table 2: The attachment places of the encountered Isopoda sp. in fish body

Fish spp. / Isopoda spp.	*Mugil capito*	*Solea solea*	*Tilapia zilli*
Anicola physodes	ND	gills,branchial chamber , skin	ND
Renocila thresherorum	ND	ND	gills, branchial chamber
Lironeca ovalis	branchial chamber	branchial chamber	ND
Nerocila orbignyi	branchial chamber	Skin, gills	gills

ND: not detected

ISSN: 0975-8585

Table 3: Concentrations of *Azadirachta indica, Matricaria chamomilla*, *Artemisia Herba alb* extracts and deltamethrin used against Isopoda.

Parameters	Medicinal plant extracts									Chemical
	Azadirachta indica			*Matricaria chamomilla*			Artemisia herba- alba			Deltamethrin Bath
Concentration	**1%**	**2%**	**3%**	**1%**	**2%**	**3%**	**1%**	**2%**	**3%**	**0.01ppm**
Lethal Time*	60 min	30 min	15min	30 min	15min	5 min	-	-	-	30 min

*Lethal Time at which all the treated isopods died

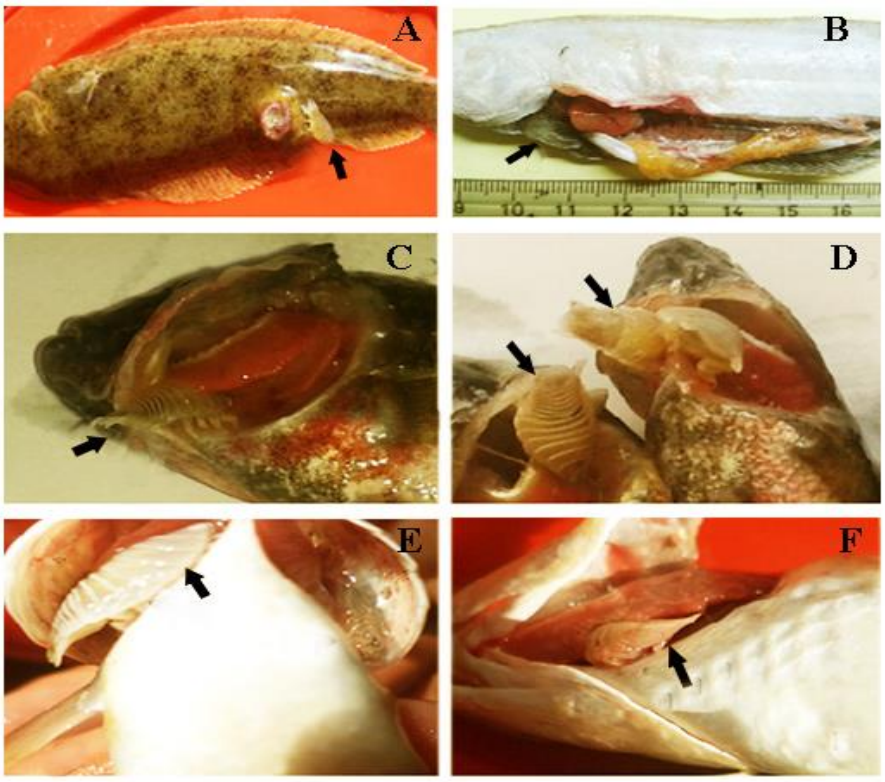

Fig 1. Showing cymothoid Isopoda infesting: A. Skin of *Solea solea* causing severe ulceration. B. Gills of *Solea solea*, C, D. Gills and Opercular cavity of *Tilapia zili*. with sever inflammatory reaction, E, H. Opercular cavity of of *Mugil capito* with ulcerative reaction .

ISSN: 0975-8585

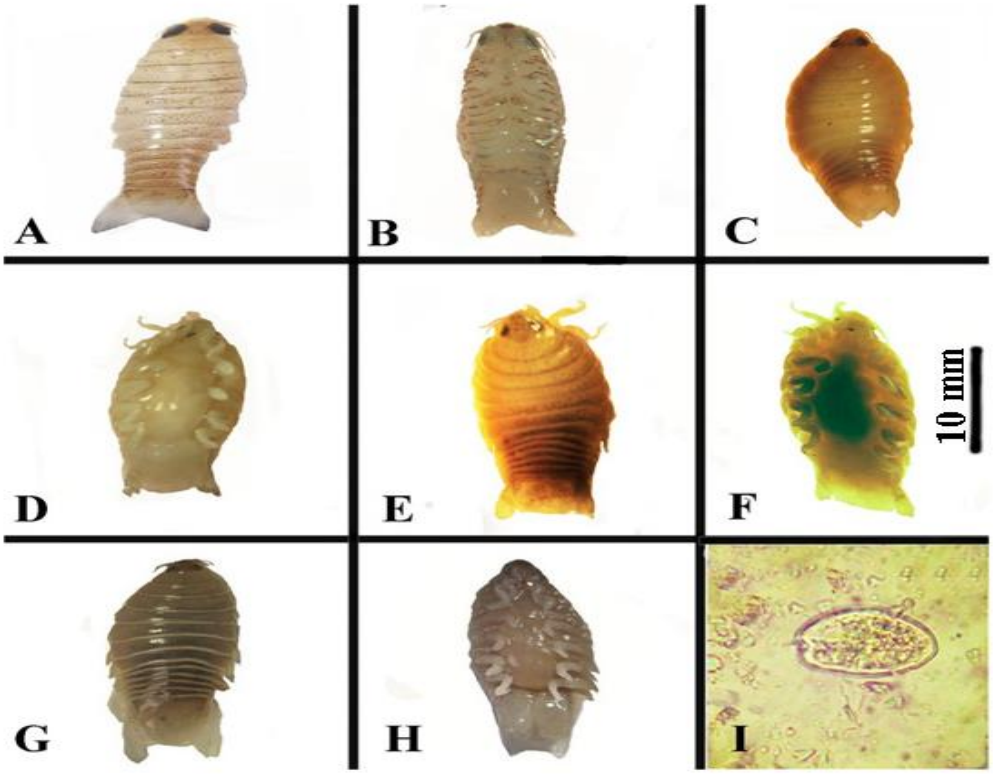

Fig 2: Cymothoid Isopoda, dorsal and ventral view. A-B *Anilocra physodes*, C-D. *Renocila thresherorum*, E-F. *Lironeca ovalis*. G-H. *Nerocila orbigyni*, I. egg of Isopoda extracted from in the marsupium of an ovigerous female.

The prevalence was assessed according to availability of the fishes in the Qaroun lake in different seasons due to the absence of *T. zilli and Mugil Capito* in winter season. Therefore, the total prevalence was 33.5 ,41.1 and 80.9 in *T. zilli, Solea solea and Mugil Capito* respectively. An earlier study revealed that 25% of the *Tilapia zilli* collected from Lake Qaroun were infested with *Nerocila orbignyi* (4).

The total infestation rate (50.7%) was higher than that of [15] who found that 22.6% of Suez Canal fish were infested by cymothoids, [21] who reported an infestation rate of (41.6%) among red sea fish and other investigators [16,17, 19,20] who reported also a lower infestation rates of 8.62%, 9%, 3.13% and 4% among Mediterranean Sea fish obtained from Port Said, Matrouh, Alexandria and Ismailia Provinces. The variability of the prevalence of cythmoids in different localities may be ascribed to the variability of the fish species examined, the disparity of environmental stress factors [22] or irregularity of the occurrence of different cythomoid sp. in natural populace [23].

Concerning the parasite seasonality, there were seasonal variations in the rate of isopods infestation. In *Solea solea*, the highest infestation rate was noticed in summer (90%), followed by autumn (33.3%), spring (26.7%) and the least infestation was perceived in winter (20%). In *T. zilli*, the highest infestation rate was noticed in summer (44.44%) followed by autumn (33.3%), spring (31.11%). In Mugil Capito, the highest infestation rate was noted in summer (85%) followed by spring (50 %) [Table1]. The lowest infestation among *Solea solea* was reported in winter season. Similar results were previously encountered by [15,20,24]. Likewise, [4] proved a high summer prevalence (65%) of *Nerocila orbignyi* among *Tilapia zilli* collected from Lake Qaroun.

ISSN: 0975-8585

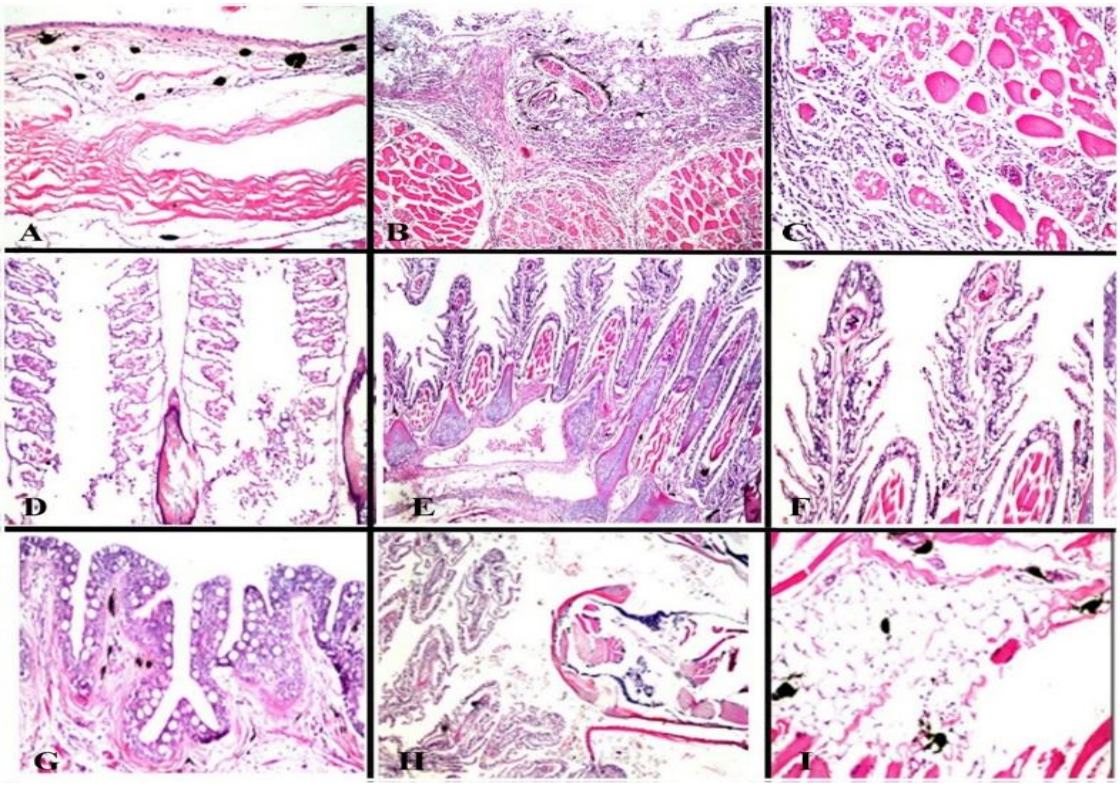

Fig. 3. Histopathological alteration induced by cymothoid isopods. A. focal melanin pigmentation of the dermis. B-C. Ulceration and necrosis of the epidermis and dermis with massive numbers of inflammatory cell infiltration. D. Necrosis in the gill filaments and lamellae E- F. Atrophy and ischemia in the gill filaments and lamellae. G . The rakers showed hyperplasia with goblet cells and polyps formation in the covering epithelium. H. Cross section in an isopod attached to the gill. I. Melanin pigmentation in the muscles.

The recorded seasonal variations of cymothoid sp. could be reliant on seasonal ecological parameters as water salinity, rainfall and temperature. Mainly, the low infestation during winter season is attributed to the weak salinity caused by the heavy rain fall which convinces an unfavorable condition for the parasite, while the increased salinity during summer facilitates the parasite infestation [25]. Moreover, the high temperature during summer enhanced the reproduction of Isopoda species and increased its abundance [26].

Concerning the histopathological investigation [Fig. 3], varying degrees of pathogenicity were detected according to the site of attachment of the parasites on the host. Mainly, the pathological changes associated with the isopods infestation begin with severe hyperemia at the site of parasite attachment and then affect the deeply situated tissues [27]. Earlier studies [28,29,30] reported deteriorative effect of the parasite on the skin and muscles of infested fish. The skin and muscle damage were induced by the isopods which used a claw like prehensile pereopods for attachment to the host skin and inserted them causing severe erosion in the dermis and the underlying muscular tissues at the site of attachment [28,31]. Histopathological inspection of the gill on which the isopods settled showed atrophy, ischemia and necrosis in the gill filament and lamellae. These findings were in accordance the report of [24]. Additionally, previous studies established the mechanical destruction of the gill lamella [32] and the impairment of the opercular respiratory movements [33] due to the Isospods. Gill rakers damage was the severe lesion observed throughout the study. This was attributed to the homophagous feeding nature of the cymothoids which may lead severe blood losses or caused by the obstruction of the circulation in the branchial chambers due to the parasite attachment [34,35].

Up to date, the pyrethroid deltamethrin is a compound that was tested for the effectiveness and toxicity against some isopods [6,14]. This compound showed moderate to substantial effect on the treatment of Copepoda, but, it had numerous drawbacks [36,37]. The chemical insecticide and formalin bath [6,14] were proved to be of a great effect against different parasitic stages of specific cymothoid species. Due to the

problems and risks accompanied the use of chemicals, there is a crucial need to use environmentally friendly, safer, and effectual alternatives that have the prospective to substitute synthetic insecticides and are appropriate to use [38].

In this study therefore, we have made a preliminary trial to investigate the activity of some medicinal plants against Isopoda. Where, the efficacy of neem (*A. indica*), chamomile (*M. chamomilla*) and shih (*A. herba- alba*) were estimated in vitro against fish isopods compared with deltamethrin . *A. indica*, and *M. chamomilla* were effective at concentration of 3%, where they inhibited the activity of all the tested Isopoda within 15, and 5 min respectively. Concentration of 1% inhibits the isopods activity in much longer time (60 and 30 min. respectively). On the other hand, *A. herba- alba* had no effect on the isopods at any of the used concentrations. Deltamethrin (pyrethroid) solutions at 0.01 ppm concentration was also effective against Isopoda, where they inhibit the activity of all the tested isopods within 30 min [Table 3].

The activity of the neem and chamomile flowers' extract against cymothoid isopods, had not been formerly reported. There are some reports on the insecticidal and acaricidal activities of both plants [39]. Terprnoids azarocide component of neem [40,41] and the flavonoid components of the dry flowers of chamomile [42] contribute to their insecticidal effect and they could be used in many medical assests. Moreover, neem and chamomile are considered safe and it has a relative low toxicity for man and animals [43,44,45]. Thus, they can be used as alternative methods for the isopods without a dramatic effect on the environment.

CONCLUSIONS

Cymothoid isopods lead to economic losses among fish species in Qaroun Lake. Some herbal plants showed high efficacy against Isopoda. Further studies are needed to investigate the strategy of control Isopoda in the Lake.

ACKNOWLEDGMENT

The authors wish to thank Prof. Dr. Adel Bakeer (Department of pathology, Faculty of Veterinary Medicine, Cairo University, Egypt) for his help in histopathological study. Also, Dr. Attalla and Dr. Yassin National Institute of Oceanography and Fisheries (NIOf), Fayoum branch for their advices and help.

REFERENCES

[1] Trilles J.P. 1986. Bull. Mus. natn. Hist. nat., Paris, section A. 3: 617-636.
[2] Horton T. ,Okamura B. Journal of the Marine Biological Association of the United Kingdom. 2002; 82, 415-417.
[3] Eissa I. A. M. 2002: Parasitic Fish Diseases in Egypt, Dar El-Nahda EL-Arabia publishing 32 Abd EL Khalek Tharwat st. Cairo Egypt.
[4] Abdel-Latif H. M. R. International Journal of Innovative Studies in Aquatic Biology and Fisheries 2001;Volume 2, Issue 3, 2016, PP 1-9.
[5] Papapanagiotou E.P., Trilles, J.P. Mar. Ecol. Prog. Ser. 2001; 45, 237–239.
[6] Sievers G., Palacios P., Inostroza R., Dölz H. Aquaculture 1995;.134, 9–16.
[7] Fayad N.K. , AL- Obaidi HOS. , Al-Noor H T., Ezzat M.O. Innovative Systems Design and Engineering. 2013;Vol.4, No.1. 2222-2871
[8] Noga E.J. 1996. Fish Diseases diagnosis and treatment. Mosby Electronic Publishing USA, 163.
[9] Williams L. B., Williams, E. H. 1981. Proceedings of the Biological Society of Washington 94 (4): 1005-1047.
[10] Williams E.H., Williams, L.B. 1999. Parasites of North American freshwater fishes. Order Isopoda. J. Hoffman, ed. Cornell Uni. Ithaca, New York Pages 310.
[11] Froese R, and Pauly D. 2013. Fish stocks. In: Levin S, Waltham MA (eds) Encyclopedia of biodiversity, 2nd edn. Academic Press/Elsevier, Amsterdam, pp 477–487
[12] Bancroft J.D., Stevens, A. 1996. The haematoxylin and eosin. Theory and practice of histological techniques. Churchill Livingstone, London, New York & Tokyo, pp.99–112.
[13] Shetty. S.A., Prakash H.S. , Shetty H.S. Can. J. of Bot. 1989; 67(7): 1956-1958.
[14] Athanassopoulou F., Bouboulis D., Martinsen B. L. Bull. Eur. Assoc. Fish Pathol. 2001; 21, 26–29.

[15] El-Lamie M.M. and Abdel-Mawla I.H. Egy. J. Aquac. 2015; Vol 5, No. (2):13-26
[16] Abd El Aal A.M.I. , El Ashrum A. M.M. Egyptian J. Aquaculture 2011 ; V. (1) 17-26
[17] Youssef M. E., Nahla, H. Salam, Eissa I .A. M., Mona S. Zaki . Life Science Journal 2014;11(1) : 227
[18] Moles A. Trans Am Fish Soc.1983; 112: 201-204.
[19] Badawy G. A. 1994. Some studies on ectoparasites infecting marine fish in Egypt. Ph. D. Thesis, ParasitoL. Dept. Vac. Vet. Med. Zag. Uni.
[20] Samn M. A.A., Metwally M.K., Zeina F. A,, Khalaf Allah M. H. Journal of American Science2014;10(7).
[21] El-Halfawy M.M., Abdel-Mawla I. Heba and Ramadan M. A. Egy. J. of Aqua. Res. 2011; 37(3): 275-282.
[22] Matthiessen P., Thain J.E., Law RJ, Fileman T.W. Mar Pollut Bull;1993 ; 26: 90-95.
[23] Brusca R.C. 1981. Zool. J. Linn. Soc. 73, 117–199.
[24] Noor El-Deen A. E., Mona Zaki S. , Shalaby I.S. Belg. J. Zool.2013; 137 (1): 67-74.
[25] Aneesh P.T., Sudha K., Arshad K., Anilkumar G., Trilles J.P. Acta Parasitologica, 2013; 58(1), 80-90; ISSN 1230-2821.
[26] Owens L. , Rothlisberg P.E. Mar. Ecol. Prog. Ser. 1995; 122: 159-164.
[27] Ravichandran S. , Ajith KumarT.T. , Ronold RossP . MuthulingamM. Research journal of parasitology.2007; 2(1); 68-71
[28] Rand TG. J. Fish Dis. 1986 ; 9(2): 143-146.
[29] Yambot A.V. , Lpez E.A.1977 Gill parasite Lamproglina monody capart, infecting the Nile tilapia, Oreochromis niloticus L., cultured in philipines.As. Fish. Soc. Manila.
[30] Lester R.J.G. , Hayward C.J. 2006. Fish Diseases and Disorders Volume 1: Protozoan and Metazoan Infections, 2nd edition. CAB international, London, pp. 466–565
[31] Rameshkumar G., Ravichandran S. Asian Pac J Trop Biomed. 2013;3(2): 118-122.
[32] Paperna I. , Zwerner D.E. J Fish Biol. 1976; 9:267–287.
[33] Kabata Z. (1985). Parasites and diseases of fish cultured in the tropics.Isopoda: Tayler and Francis.
[34] Romestand B., Janicot M., Trilles J.P. Annales de parasitologie Humaine et compare. 1977 ;52:171–180.
[35] Romestand B. Ann de parasitol Paris. 1979;54:423–448.
[36] Csillik B., Fazakas J., Nemcsok J., Knyikar-Csillik E. Neurotoxicology 2002;21 (3), 343–352
[37] Varanka Z., Deer K.A., Rojik I., Varanka, I., Laszlo K., BartokT.,NemcsokJ., Abraham, M. Acta Biol. Hung. 2002;53 (3), 351–365.
[38] Tapondjou C., Adler D.A., Fontem H., Bouda C. , Reichmut Journal of Stored Products Research2005; 41, pp. 91–102
[39] Pirali-Kheirabadi K. , Mehdi Razzaghi-Abyaneh.J Zhejiang Univ Sci B2007.; 8(9): 693–696.
[40] Carvalho S. M. , Trevisan D. Santa-bárbara contra a vaquinha. Ciência Hoje, 1990; 11, 65-67.
[41] Schmutterer H. (1990). Properties and potential of natural pesticides from the neem tree, Azadiracta indica. Annu. Rev. Entomol., 35, 271-297.
[42] Srivastava K. J., Eswar Shankar, Sanjay Gupta.Chamomile: A herbal medicine of the past with bright future. Mol Med Report. 2010 Nov 1; 3(6): 895–901.
[43] Boeke I.S ., Boersma G M., Alink M G., vanLoon J. A. J., Huis VA Dickea M., Rietjens M. Journal of Ethnopharmacology . 2004;94 . 25–41
[44] Bradley P. (1993). The British Herbal Compendium: Vol. 1: A Hand book of Scientific Information on Widely Used Plant Drugs. British Herbal Medicine Association, London,UK.
[45] Newall C.A., Anderson L.A. , Phillipson, J.D. (1996). Herbal Medicines: a guide for healthcare professionals. Pharmaceutical Press, London.