Acta Parasitologica Globalis 7 (2): 27-53, 2016
ISSN 2079-2018

DOI: 10.5829/idosi.apg.2016.7.2.103106

Effect of Processing Steps and Aqueous Extracts of Some Medicinal Plants on Controlling Fish Parasites in Egypt

Amina Ibrahim El-Sayed El-Mansy

National Institute of Oceanography and Fisheries, Aquaculture Division,
Pathology Lab., El-Qanater El-Khairia Research Station, Egypt

Abstract: A new reference includes revision on effect of processing steps and aqueous extracts of some medicinal plants on parasites vitality infected some Egyptian freshwater fishes is presented. Protozoans and helminthes from *Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus*, *Tilapia zillii* and *Clarias gariepinus* for approximately three years were isolated. According to morphology, morphometric measurements and comparison with some previous descriptions parasites were identified as *Myxobolus heterosporous*, *Henneygua suprabranchiae*, encysted metacercariae and the cestode *Monobothriodies* sp. Prevalence and intensity of infestation with each parasite were estimated. As a trial to the parasite control, *in vitro* and some *in vivo*, parasites undergone to processing steps 'salting, pickling, freezing, boiling, grilling and frying' and aqueous extracts of *Allium cepa* bulb, *Pimenta* sp. seeds, *Cinchona* sp. bark and seeds of *Nigella sativa*. Additionally, in the current work to some extent discuss activities that have been carried out by some researchers and suggestions, views for problems perhaps to be solutions.

Key words: Fishes · Parasites · Processing Steps · Medicinal Plants · Control

INTRODUCTION

There are huge numbers of parasites long recognized as causative agent of severe diseases such as Myxosporea which is pathogenic to fresh and marine water and affecting fish species [1-3]. The taxonomy of the myxosporean species that called *Myxobolus heterosporus* was revised [4] using specimens isolated from plasmodia situated in the infected cornea of tilapias from the River Nile, Egypt. Its spores had a variety of shapes and presence of five main *Myxobolus*-like spore types and tailed-spores. The light and electron microscopy supported that spores of a *Myxobolus*-like spore coexisted with the so-called tailed-spore in one plasmodium; therefore, some tailed-spores may be simply heteromorphs of *Myxobolus*. The fish's eye may be infected with other species such as *Myxobolus sclerii* from eyeball of *Catla catla* in India [5]. On the other hand, a vital staining technique was used for determination of spores' viability of *Myxobolus artus* and actinosporean stage of *M. cultus*; viable spores stained green with fluorescein diacetate and non-viable ones stained red with propidium iodide [6]. Additionally, Kallert and El-Matbouli [7] reported that two methods of determining actinospore viability were differential fluorescent staining and direct microscopic observation of morphological indicators of spore integrity.

Intestinal heterophyid infestation has been reported in man from various parts of the world, where parasitized fish is eaten raw or uncooked and several species have been reported from man, *Heterophyes heterophyes* was the first member of this group found by Bilharz in 1851 in the small intestine of a boy in Egypt, since then many other species have been reported from the intestinal tract of carnivorous mammals, birds and fishes; heterophyids in man have been reported as causing little disorder and the clinical symptoms due to their presence are often negligible [8]. Several heterophyid species, including *Haplorchis* species can cause significant pathology, often fatal, in the heart, brain and spinal cord of humans [9]. In Egypt, human infections with *H. heterophyes* are prevalent among the inhabitants of the northern part of the Nile Delta and several studies on fish metacercariae were carried out by several authors such as [10-17] and

Corresponding Author: Amina Ibrahim El-Sayed El-Mansy 'National Institute of Oceanography and Fisheries, Aquaculture division, Pathology Lab., El-Qanater El-Khairia Research Station, Egypt.
E-mail: el_mansy@hotmail.com.

also a large number of *Heterophyes* species have been reported from humans and the importance of these flukes is being increasingly recognized through recent studies from the Philippines, Thailand and Korea on several species [18-22]. In Egypt, the highest prevalence rate of encysted metacercariae was recorded in catfish; due to a lot of heterophyid species are zoonotic and have very similar transmission patterns this parasitic group is a very significant problem in food safety and quality [10, 15]. Metacercarial examination in the second intermediate host, in combination with a survey on adult worms in humans and also on larvae in the snail intermediate host, can be a useful index in the epidemiology of trematodes in a particular area [23].

Among described cestode species, *Monobothrioides woodlandi* (Cestoda: Caryophilidea) from *Clarias mellandi* Boulenger (Cypriniformes: Clariidae) in Zambia, Africa was reported by Mackiewicz and Beverley-Burton [24]. Additionally, *Polyonchobothrium clarias* that was re-described with its histopathological impact from the catfish *Clarias gariepinus* in Egypt by several investigators such as those reported by Ibrahim *et al*. and El-Mansy *et al.* [25, 26].

In this study, *in vitro* and some *in vivo* new data includes notes on previously publications such as that reported by El-Mansy [27] on the effect of storage and cooking processes on viability of *Myxobolus* species which recently described by El-Mansy [4] under name *M. heterosporus*, also that reported by El-Mansy and Mohamed [28] on the effect of aqueous extracts of some medicinal plants on some myxozoan parasites infecting fish and the study reported by El-Mansy *et al.* [29] on efficiency of cumin black seed '*Nigella sativa*' and *Cinchona* sp. 'it turned out its common name is allspice seeds' on the cestode *Polyonchobothrium clarias* and the nematode *Paracamallanus cyathopharynx* are revised.

Medications by herbs and plants in several publications and in general refer to data available in other scientific publications and websites were reported. Trimming away the belly flaps of fish and physically removing parasites are effective methods for reducing the number of parasites; however, they do not completely eliminate the hazard, nor do they minimize it to an acceptable level [30, 31]. A review summarizes human infections caused by endoparasites, including protozoa, nematodes, trematodes and cestodes which affect more than 30% of the human population and medicinal plants of potential use in their treatment was submitted by Wink [32] who also reported that the identified plants and compounds offer a chance to develop new drugs against parasitic diseases and most of them need to be tested in more detail, especially in animal models and if successful, in clinical trials.

Some fish parasites may unfortunately be able to be transmitted viable to man through consuming undercooked infected fish and parasites are a concern when human consumers eat raw or lightly preserved fish. Parasitic infections from freshwater fish are a serious problem in some parts of the world and fish that spend part of their life cycle in salt water, like salmon, can also be a problem. Parasite infection by raw fish is rare in the developed world and involves mainly three kinds of parasites: the trematode *Clonorchis sinensis*, the nematode *Anisakis* and the cestode *Diphyllobothrium* and infection by the fish tapeworm *D. latum* is seen in countries where people eat raw or undercooked fish, such as some countries in Asia, Eastern Europe, Scandinavia, Africa, North and South America [33]. Herein, additional data on effect of storage and cooking steps such as salting, pickling, freezing, boiling, grilling, frying and aqueous extracts of onion '*Allium cepa*' bulb, allspice '*Pimenta* sp.' seeds, kina '*Cinchona* sp.' bark and *Nigella sativa* seeds on vitality 'morphological characteristics' of some parasites '*Myxobolus heterosporous* [4], *Hennegya suprabranchiae* [34, 35], encysted metacercariae and the cestode *Monobothriodies* sp.' are presented.

Onion [36] is common for people and is eaten raw or cooked and it is the most popular plant cultivated in Egypt for human consumption. It was used daily internally to get rid of worms-infected children until all worms are extruded from infested intestine [37]. The onion *Allium cepa* L. (Family: Liliaceae) is used in the treatment of digestive, respiratory, urogenital, vascular and nervous disorder in humans [38]. What means onion to killer of parasites and antiseptic? [39]. Large 96 hours LC_{50} of onion value indicated that the aqueous extract of this plant is safe according to Buch *et al.* [40] who stated that, substances possessing a LC_{50} higher than 50 mg kg^{-1} body weight are considered non-toxic and thiosulfinates formed in sliced bulb of *Allium cepa* [41] may be the effective material against parasites [28], in addition to onion *Allium cepa* contains small quantities of fat, sugar and vitamins A, C and B complex; rich in magnesium, potassium and copper. So it is used as a vegetable, spice also as medicinal plant where it is an antibiotic, antiseptic, anti-infectious, antibacterial, antifungal agent, antioxidant and has anticancer properties [42-47].

In several sites on the internet, it was reported that, the herb allspice also called *Pimenta* or myrtle pepper at the market it is sold in both powdered and whole form. The fruit of allspice is a small capsule containing humorous seeds which picked when green and unripe and when dry, they are brown and resemble large brown peppercorns. Medicinal part used of allspice is fruit, particularly the shell and other parts can be used medicinally as well. Volatile oils found in this plant contain eugenol, a weak antimicrobial agent but as a precaution no use allspice without first talking to healthcare provider.

As a medicinal herb, *Cinchona* bark is stripped from the tree, dried and powdered for medicinal uses. Altaf *et al.* [48] reported that *Cinchona ledgeriana* is also known as quinine tree and belongs to family Rubiaceae. The bark of trees in this genus is the source of a variety of alkaloids such as quinine and quinidine. For centuries, the plant has been used as an antimalarial agent and remained under observation. The use of quinidine as an antiarrhythmic has been limited [49-51] and the most familiar is quinine, an anti-pyretic agent especially useful in treating malaria; but it is rarely used today as many people think it is dangerous as it can be lethal in case of large amounts and the use of the bark has been largely superseded by more effective modern medicines [52].

Several references and reports on *Nigella sativa* such as Ibn El-Qayem [53] were published; anatomically its seeds may need more clarification particularly by specialists in botany. *N. sativa* cultures in Arabic Habbet El-Baraka and in English black seed, black cumin, black caraway, nutmeg flower, Roman coriander is an annual herbaceous plant becoming to the Ranunculaceae family and identified for its Latin name based also according that reported by Heiss *et al.* [54]. It is in many Arabian, Asian and African countries and native to south-east Asia especially grown in the East Mediterranean countries for its seeds. Composition of *Nigella* seed oil from Morocco is similar to that from other Mediterranean countries known for their *Nigella* seed-oil quality which is becoming popular in and out of the Islamic world. *N. sativa* is classified as an edible plant and its seeds may potentially be an important nutritional source as the content of essential amino acids, contributes to about 30% of the total protein content while 84% of the fatty acids is composed of unsaturated fatty acids, predominantly linoleic and oleic acids [55- 57]. In addition, many components were characterized but the major ones were the essential oil; thymoquinone is the main active constitute of the volatile oil extracted from *N. sativa* [58-60]. Good quality control methods were used for quantity frying the pharmacological activities thymoquinone, dithymoquinone, thymohydroquine and thymol in both seed oils and extracts of *N. sativa* [61]. Other active principles were nigellone, which was isolated from the volatile oil fraction and was found useful in the treatment of bronchial asthma [62]. The therapeutic dose of the fixed oil of *N. sativa* has a wide margin of safety [63-66]. The acute toxicity of thymoquinone is very low; the maximum non fatal dose was 550 mg/ kg and the toxicity of *N. sativa* fixed oil extract is LD_{50}=0.542 ml/ kg [64, 65]. *N. sativa* extracts are relatively non toxic in the acute toxicity test, but the possibility of hepatic damage with its aqueous extract should be considered [67]. *N. sativa* seed oil extracts have been used in many countries for the treatment of many human illnesses and more recently the active compound found in black seed oil, thymoquinone has been tested for its efficacy against several diseases but it is recommended that patients with heart condition should take precaution [68, 69]. Thymoquinone decreases both systolic and diastolic blood pressure in a dose dependent manner by blocking serotonin, alpha-1 and endothelin receptors [48, 70 and 71]. It has positive inotropic effect and can cause beneficial cardiac hypertrophy [69]. *N. sativa* oil and its thymoquinone extract can be used as a natural dietary supplement to counteract the mutagenic effects of any environmental pollutants [72]. As it is stated in a website, Ibn Sina regarded by many as the most famous boo; in the history of medicine, East or West, refers to black seed that stimulate the body's energy and helps recovery from fatigue. Also, for man, Ji [73] reported that a lot including it enhances immune system and the seeds are characterized a very low degree of toxicity but large medicinal amount is unsafe and many of black cumin's traditionally ascribed health benefits have been thoroughly confirmed in the biomedical literature. Also, *N. sativa* seeds have wide therapeutic effects and have been reported to have significant effects against many ailments such as skin diseases, jaundice, gastrointestinal problems, anorexia, conjunctivitis, dyspepsia, rheumatism, diabetes, hypertension, intrinsic hemorrhage, paralysis, amenorrhea, anorexia, asthma, cough, bronchitis, headache, fever, influenza and eczema [74]. *N. sativa* contains many active components and possess potent hepatoprotective, anticancer, antidiabetic, antimicrobial, antiparasitic, analgesic and antihistamine etc. [75, 76]. In addition to the oil fraction of *N. sativa* contains thymoquinone, which has immune potentiating activities as well as anti-oxidative effect besides its seeds provide

relatively high amount of some essential nutrients such as carbohydrates, fats, vitamins, mineral elements and proteins including eight of the nine essential amino acids that improve natural immune system activity [77]. Moreover, *N. sativa* to be more important if it's the intended in the following talk 'as it written in a website', Narrated Abu Huraira (may Allah be pleased with him): I heard Allah's Apostle saying: "There is healing in Black cumin for all diseases except death." (Sahih Bukhari 7: 71: 592). Therefore, as a medicinal plant, other trial to study its aqueous extract affection on fish parasites '*Myxobolus heterosporous*, *Hennegyua suprabranchiae*, encysted metacercariae and cestode' is presented here.

Parasites consumed in raw or undercooked seafood can present a human health hazard; also fish raised in freshwater may have a parasite hazard [31]. A requirement that certain fishery products shall undergo a treatment sufficient to kill viable parasites that may represent a health hazard to the consumer; the larval stages of parasites representing a health hazard to the consumer are nematodes, larvae 'plerocercoids' of *Diphyllobothrium* cestodes and larvae 'metacercariae' of trematodes [78]. Several studies have reported temperatures and times needed to get rid of parasites and it is essential that raw fish potentially containing viable prevalent parasites be frozen and held in that state for a period of time that assures destruction of all viable parasites in that fish species [79]. Fish parasites are killed by freezing and heating treatments [80]. Whatever, this study to be a reference includes revision of some information reported in previous manuscripts such as those reported by El-Mansy and El-Mansy *et al.* [27, 29] with new data on effect of storage, cooking steps and water extracts of some medicinal plants on some fish parasites as additional trial to discuss fish health improvement through fish free from parasites and also to be safe for man.

MATERIALS AND METHODS

Control Parasites: For parasitological examination, a total of 70 fish belonging to families Cichlidae and Clariidae including four genera and five species of tilapias from several markets in Qalyubia and Giza Governorates were collected. *Oreochromis niloticus* Linnaeus, 1758, *O. aureus* Steindachner, 1864, *Sarotherodon galilaeus* L. and *Tilapia zillii* Gervais, 1848 with total length about 11-19.5 cm and weight 10-12 g were caught during certain times from December 2012 to April 2014. In addition to the catfish *Clarias gariepinus* Burchell, 1822 with length about 15-59 cm and weight 300-1000 g through months 3 and 6-11 in 2012-2014. As a control, fresh normal parasites by light microscopy were examined. About 24 fish with protozoans and helminthes were infected. *Myxobolus heterosporus* in eye's cornea of tilapias was noticed during winter and spring '4 December 2012, 5 February 2013 and 4 April 2014 respectively'. *Henneygua suprabranchiae* from suprabranchial organ of catfish was isolated on 15 July 2013, 6 April 2014 and 8 June 2014. *H. suprabranchiae* from intestine of catfish was isolated on 1 March 2012 and 10 February 2014. Also, encysted metacercariae type 1 on 12 May 2014 and metacercariae type 2 on 6 April 2014 from musculature of catfish were isolated. In addition, a cestode from its intestine was noticed on 5 March 2012, 10 February 2014 and 6 April 2014. For identification, the current parasites were photographed, measured and compared with previous related species. Thereafter, *in vitro* and some *in vivo,* parasitic treatments were experimentally carried out.

Parasites Treatment: It may be worthy to mention as reported by Klimpel *et al.* [81] that the anthelminthic efficacy of some differently obtained extracts of several plants was tested *in vivo* in laboratory animals and *in vitro*; the nematodes were *Angiostrongylus cantonensis*, *Trichuris muris* and *Toxocara cati*; the plants used were bulbs of onions, garlic, chives, coconut, birch tree, ananas, cistrose, banana, chicory, date palm fruit, fig, pumpkin and neem tree seeds; *in vitro* effects against *A. cantonensis* and *T. muris* were best with aqueous extracts, followed by chloroform extracts and efficacy *in vitro* systems does not guarantee as good if at all efficacy *in vivo*.

In vitro

Processing Steps 'Salting, Pickling, Freezing, Boiling, Grilling and Frying': Saltwater at different concentrations about 1%, 35%, 95% salt 'sodium chloride' and 100% 'dry salt' at room temperature was prepared. *In vitro* parasites were immersed in each previous-cited concentration. Pickling 'acetification' step was done by putting about 2-5 ml vinegar 'commercial sugarcane vinegar 5 %' in cell well plates with the defined available parasite. In freezer of home refrigerator at 2-3°C, freshly isolated parasite was put. As control, other parasitic specimens were kept with some droplets of tap water in refrigerator at about 3-5°C at same times. For boiling step, the infected fish were boiled with some water for several minutes till ripe. For grilling, the infected fish covered with some suitable cover and then allow ripening by the fire and others for frying in hot oil were prepared. Moreover,

oven tray was prepared for more ripening of fried fish. The parasite was carefully isolated, examined, photographed and compared with control after each previous-cited step. Then the results according to variations particularly in morphological characteristics of the parasite were registered.

Preparation of Aqueous Extracts of Medicinal Plants '*Allium cepa*, *Pimenta* Sp., *Cinchona* Sp., *Nigella Sativa*': In the current study, onion bulb '*Allium cepa* L.' about 70-100 g minced and boiled with some tap water nearly 55-150 ml till getting a concentrate extract. For parasites, fish were dissected and *in vitro* and some *in vivo* specimens of each detected parasite were put in about 5 ml of the previous prepared extract. Also, control of fresh isolated parasites with some water contained cell well-plates kept in refrigerator at 2-3°C. At several times they were examined and notes were registered. This method may be simple but also El-Mansy and Mohamed [28] reported other method for preparation of onion aqueous extract seems also to be suitable 'about 100 g of dried bulbs of *A. cepa* were sliced in small pieces mixed with distilled water and blended in a mixture for 5 minutes; the bulbs were digested by percolation with distilled water; thereafter filtered through two layers of muslin and the filters were placed in rotavapour to evaporate the solvents'.

About 10-20 g of allspice seeds (*Pimenta* sp.) that in Egypt store spices shops sold its seeds under name Chinese kababa 'also this that previously used in EL-Mansy *et al.* [29]' were put in about 20-150 ml water then boiled for about 15 minutes. *In vitro* about 5 ml of this extract in cell well plates or other suitable bottle with the parasite in refrigerator at about 2-3°C was placed. On the other hand, by boiling about 10 g or two pieces of dried bark of kina or quina (*Cinchona* sp.) with about 20-30 ml of tap water for about 3 minutes, cool and then *in vitro* about 5 ml of it in cell-well plates with the parasite in refrigerator at about 2-3°C was placed. Also to use later, some of this extract remained for nearly a week or a month at same conditions. Microscopically, the treated parasites in parallel with the control at certain times were investigated and compared.

Seeds of *Nigella sativa* Linnaeus, 1753 purchased from local markets. A dry seed of *N. sativa* measured length about 0.2-0.3 cm and weight 0.1-0.15 g. Preparation of aqueous extract of *N. sativa* seeds as a try to make sure it's real impact on fish parasites *in vitro* and some *in vivo* was done. Boil over some seeds of about 10-20 g or 1 small spoon in nearly 15-20 ml tap water for several minutes till getting the concentrated extract. *In vitro* the parasite was put with some amount 'about 2-5 ml' of this extract. Additional dose was directly inserted in the mouth of the fish and other mixed with water of aquaria included fish. *In vivo* effect of this extract on the fish health and parasite after certain times was macro-and microscopically investigated. Also, *in vitro* the parasite with some amount of same extract was kept in the refrigerator at 2-3°C. *In vivo* and/ or *in vitro* the treated and control parasites were at several times examined, photographed and compared.

***In vivo*:** A group of three catfish alive with some amount of aqueous extract of *A. cepa* bulb, other group of same number with aqueous extract of *N. sativa* seeds and the control fish with suitable amount of water in prepared aquaria lined by plastic black sacs to some extent at same conditions were placed. For searching parasites catfish were dissected after about 2 hours.

RESULTS

Normal Parasites: Protozoans and helminthes from tilapias and catfish during certain times for about three years according to their shape (Figs. 1-19) and size (Tables 1-3) were identified. Of protozoan parasites *M. heterosporus* (Figs. 1-4) from cornea tissue of some tilapias and *H. suprabranchiae* from the tree-like structure 'additional respiratory organ' or the so-called suprabranchial organ (Figs. 5-11) and intestine of catfish were isolated. Of helminthes encysted metacercariae type 1 (Figs. 12, 13, 16 and 17), Type 2 (Figs. 14, 15) and a cestode parasite (Figs. 18, 19) from muscular tissue and intestine of catfish were also isolated. Normal spores 'control' of *M. heterosporus* characterized by discernible vitality 'morphological characteristics' of its spores till after several days outside host (Figs 1-4). On the other hand, huge number of spores from a fresh plasmodium of *H. suprabranchiae* infected intestine of catfish was detected. Control spores appeared with the normal distinguishable shape expressing morphological vitality of the spore even after several days *in vitro* (Figs. 5-11). Macroscopically, fresh encysted metacercaria type 1 infected muscular tissue of catfish was examined. *In vitro* as control nearly 11 specimens of encysted metacercaria type 1 occupied about 2.5 cm of a flesh piece from catfish after about 3 days by naked eye were isolated. Microscopically, the parasite was noticed viable with slow movement (Fig. 12). Also, *in vitro* control encysted metacercariae types 1 and 2 after about several days from

Table 1: Measurements in µm of protozoan parasites

Parasite	Lsb	Nsm	Wsb	Nsm	Lpc	Nsm	Wpc	Nsm	Tl	Nsm	Tls	Nsm
I	12.5-13	3	7.8-11.7	2	5.2	2	2.6	1	-	-	13	-
II	13-15.6	4	7.8-10.4	2	5.2-7.8	2	2.6-5.2	2	13-57.3	4	28.6-31.2	2
II•	13	·	7.8	·	7.8	·	2.6	·	15.6-26	·	31.3	·

•One smear contained a huge number of spores may exceed about 100. Lsb (Length of spore body), Nsm (Number of spore measured), Wsb (Width of spore body), Lpc (Length of polar capsule), Wpc (Width of polar capsule), Tl (Tail length), Tls (Total length of spore).

Table 2: Measurements in µm of encysted metacercariae

Parasite	Lc	Nsm	Wc	Nsm	Lmc	Nsm	Wmc	Nsm	T	Nsm
III	312.5-500	14	250-521	12	198-354.2	5	260.4-260.4	4	156.3-364.6	2
IV	208.3-781.3	2	270.8-729.2	3	677.1	1	187.5-364.6	2	2.6	1

Lc (Length of cyst), Wc (Width of cyst), Lmc (Length of metacercarian cyst), Wmc (Width of metacercarian cyst), T (Thickness between cyst wall and the wall of the parasite), Nsm (Number of specimen measured).

Table 3: Measurements in mm of cestode based on one specimen measured

Parasite	Ls	Ws	Lht	Wht	Le	We
Cestode	0.16	0.52	0.70	0.68	0.05-0.07	0.03-0.06

Ls (Length of scolex) (anterior portion), Ws (Width of scolex) (anterior portion), Lht (Length of hind terminity) (posterior portion), Wht (Width of hind terminate). Egg based on two specimens measured; Le (Length of egg), We (Width of egg).

muscular tissue of catfish noticed viable with normal morphological characteristics (Figs. 13-17). As control, the cestode *Monobothriodies* sp. isolated from intestine of catfish; its body was elongated and anterior portion appeared with some proglottids (Fig. 18). Also, eggs of this parasite seemed vital (Fig. 19). Some morphological characteristics and measurements with previous related species to a certain degree were compared (Table 3).

Incidence of Fish Infestation with Different Parasites: In this study, 70 fish examined for parasitic infestation. The total prevalence of infestation with different parasites was about 34.3% during certain times from 2012 to 2014. Parasites included *M. heterosporus* from cornea tissue of some tilapias and *H. suprabranchiae* infected suprabranchial organ and intestine of catfish. The latter also was infected with encysted metacercariae isolated from musculature tissue which appeared with different shapes and sizes may belong to different species. In addition, from intestine of catfish a cestode parasite was isolated. Also, the prevalence of infestation with each previously mentioned parasite was estimated. About 34 of infected eye's cornea of tilapias (*O. niloticus*, *O. aureus*, *S. galilaeus* and *T. zillii*) were examined for *M. heterosporus*, 11 fish were infected with prevalence of 32.4%; of catfish 36 specimens were examined for parasites and about 13 were infected with total prevalence of infestation 36.1% and percentage of each parasite was also detected (Tables 4, 5). Prevalence of *H. suprabranchiae* from suprabranchial organ and intestine, encysted metacercariae types 1, 2 from musculature and a cestode from intestine measured 32.4, 30, 50, 33.3, 60 and 28.6% respectively.

Degree of Infection and Intensity of Parasitic Infestation: Degree of infection and/ or intensity of infestation with different parasites were also evaluated. High infection rate with *M. heterosporus* was on February 3 fish '6 eyes' were infected and high prevalence was in April this means that all fish '7' were infected with 1 cyst/ fish. High density of infection seemed with metacercaria type 1 'by naked eye about 11 cysts found in area of nearly 0.2 cm of the infected muscular tissue'. Low infection rate seemed with large size metacercaria type 2 and intensity of infestation with the cestode *Monobothriodies* sp. was about 1 worm/ fish.

Effect of Processing Steps on Morphological Characteristics of Parasites: *In vitro* and some *in vivo* effect of processing steps (storage and cooking) such as salting (Figs. 20-28), pickling (Figs. 29-32), freezing (Figs. 33-37), boiling (Figs. 38, 39), grilling (Figs. 40-42), frying (Figs. 43-45) and aqueous extract of some plants such as onion bulb (Figs. 46-53), allspice seeds (Figs. 54-57), *Cinchona* bark (Figs. 58-66) and *N. sativa* seeds (Figs. 69-78) on vitality of some detected parasites was presented and discussed (Table 6).

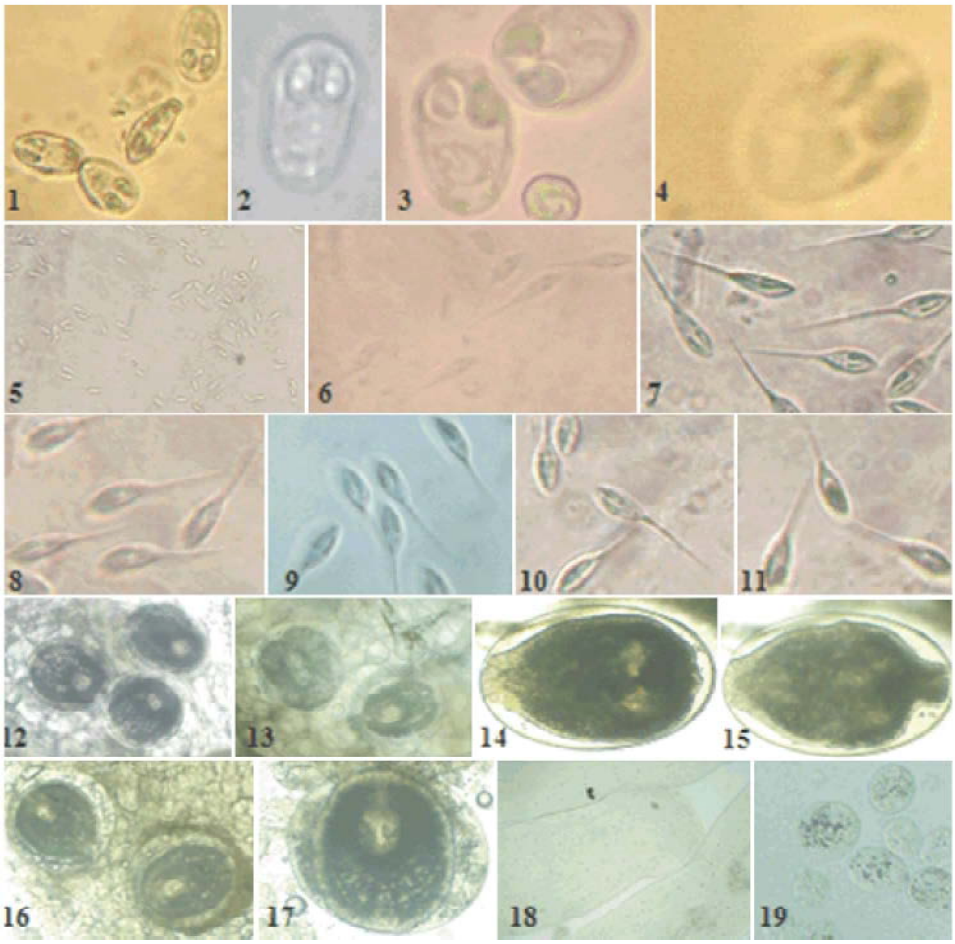

Fig. 1: Photomicrograph shows control spores of *Myxobolus heterosporus* after about 15 hours of isolation from cornea of tilapia fish, x1000

Fig. 2, 3: *M. heterosporus*, fresh control spores. Note discernible morphological characteristics of spores particularly body wall, sporoplasm and polar capsules containing polar filaments, x1000, x2500

Fig. 4: *M. heterosporus*, normal spore in some droplets of tape water in refrigerator at 2-3°C after about 1 week of its isolation, x1000

Fig. 5, 6: Fresh control spores of *Henneygua suprabranchiae* infected intestine of catfish. Note huge number of spores in a limited area, x260 and the distinguishable shape of the spores, x400.

Fig. 7: Fresh control spores of *H. suprabranchiae* infected suprabranchial organ of catfish. Note discernible morphological characteristics of spores, x1000.

Fig. 8-11: Control normal vital spores of *H. suprabranchiae* from suprabranchial organ of catfish after about 18 hours, 2, 3 and 5 days respectively, x1000.

Fig. 12: Control fresh encysted metacercariae type 1 isolated from muscular tissue of catfish noticed microscopically vital with slow move, x75.

Fig. 13: Control encysted metacercariae type 1 after about 3 days. Note normal vitality of the parasite, x82.5.

Fig. 14, 15: Control encysted metacercaria type 2 from muscular tissue of catfish after about 3 days seemed microscopically viable. Note position of the parasite terminates particularly at its posterior end, x98.

Fig. 16: Control viable encysted metacercariae type 1 after about 6 days, x85.

Fig. 17: Control vital encysted metacercaria type 1 after about 6 days. Slow move was microscopically noticed x157.5.

Fig. 18, 19: Normal cestode isolated from intestine of catfish. Note vitality of the parasite anterior portion with some proglottids, x130. Eggs of other specimen also as control are discernible, x250.

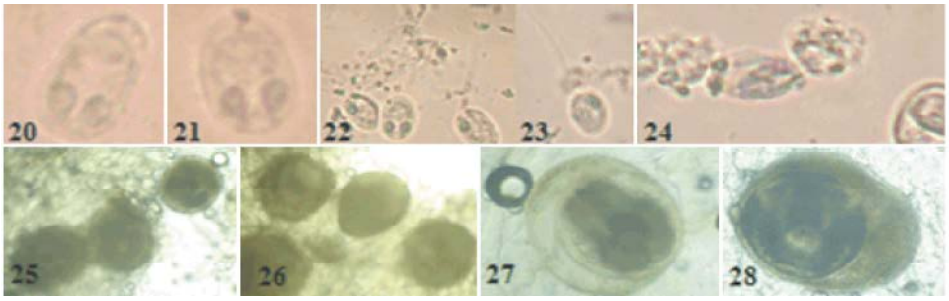

Fig. 20: Unaffected spore of *M. heterosporus* in about 95% salt after about 3 hours *in vitro*, to some extent similar to control, x2500.

Fig. 21: Unaffected spore of *M. heterosporus* in dry salt after about 3 hours *in vitro*, x2500.

Fig. 22-24: *M. heterosporus* spores in dry salt at room temperature after about 5 weeks *in vitro*. Note normal parasite, spores with extruded polar filaments and others severely deform; viability of normal spore may need sureness, x1000.

Fig. 25: Non vital encysted metacercariae type 1 in dry salt after about 17 hours *in vitro*; viability may need confirmation, x137.5.

Fig. 26: Non vital encysted metacercariae type 1 in dry salt after about 17 hours *in vitro*. Note distinguish opaque masses, x120.

Fig. 27: Encysted metacercaria type 1 in dry salt after about 4 days *in vitro*, microscopically noticed immobile, x65.

Fig. 28: Affected encysted metacercaria type 1 in dry salt after about 6 days, x75.

Table 4: Total incidence of parasitic infestation of freshwater fishes (*Oreochromis niloticus*, *O. aureus*, *Sarotherodon galilaeus*, *Tilapia zillii* and *Clarias gariepinus*) from some Egyptian markets during certain times of nearly 3 years of examination

Host	No. of examined fish	No. of infected fish	Prevalence (%)
Tilapias	34	11	32.4
Catfish	36	13	36.1
Total	70	24	34.3

Table 5: Incidence of infestation with different parasites infected fishes of the present study during certain times in 2012, 2013 and 2014

Parasite	No. of examined fish	No. of infected fish	(%)
I-*Myxobolus heterosporus*	34	11	32.4
II-*Henneygua suprabranchiae*	10	3	30
II'-*Henneygua suprabranchiae*	4	2	50
III-Encysted metacercaria type 1	3	1	33.3
IV-Encysted metacercaria type 2	5	3	60
V-Cestode	14	4	28.6

II-*Henneygua suprabranchiae* from suprabranchial organ; II*- *H. suprabranchiae* from intestine.

Table 6: *In vitro* and some *in vivo* effect of processes of storage (salting, pickling, freezing), cooking (boiling, grilling, frying) and aqueous extracts of some medicinal plants on vitality 'morphological characteristics' of parasites infected some freshwater fishes (tilapias and catfish)

Parasite	Es	Ep	Ef	Eb	Eg	Ef	Eo	Ea	Ec	En
I	[1]+++	+++	-	+++	++	+++	+++	-	+++	-
II							[1111]+	-	[1111]+	-
II'										-
III	++	++	[11]++	+++	++	[111]+++	[1111]+	-		-
IV							[1111]+			
V							[1111]+			[111111]+

E 'Effect' s 'salting' p 'pickling', f 'freezing', b 'boiling', g 'grilling', f 'frying', o 'onion bulb', a 'allspice seeds', c '*Cinchona* sp. bark, n '*Nigella sativa* seeds', Empty space means no test done, - No effect, + Moderate effect 'parasite seemed non vital but to some extent similar to control', ++ Effect 'parasite seemed non vital in comparison with control', +++ High effect 'parasite seemed non viable with severe deform'. [1] Effect of dry salt on some spores after about 5 weeks *in vitro*. [11] Effect of freezing after about 1-2 weeks in freezer of home refrigerator *in vitro*, the parasite seemed with unclear vitality; perhaps its viability needs to confirm. [111] Highly effect with more cook in oven. [1111] Effect may need sureness. [11111] Effectiveness of the extract may need longer time. [111111] Perhaps another reason affected on helminth vitality.

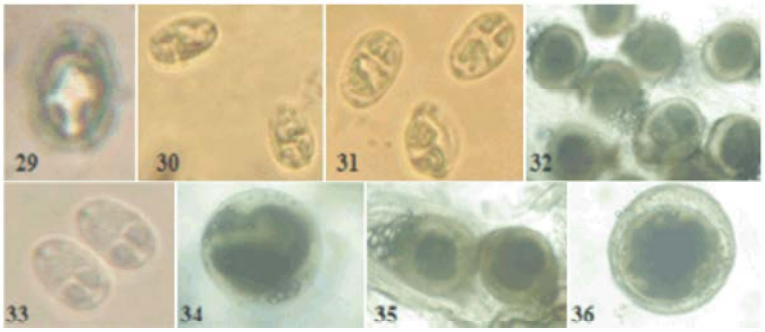

Fig. 29: Affected spore of *M. heterosporus* in vinegar at room temperature after about 3 hours *in vitro*, x1000.
Fig. 30: Deformed spores of *M. heterosporus* in vinegar after about 5 days, x1000.
Fig. 31: Non vital spores of *M. heterosporus* in vinegar after about 7 weeks, x1000.
Fig. 32: The parasite of encysted metacercariae type 1 as non vital opaque mass in vinegar after about 6 days *in vitro*, x65.
Fig. 33: Vital spores of *M. heterosporus* in freezer after about 5 days, x1000.
Fig. 34: Immobile encysted metacercaria type 1 after freezing about 21 hours, x140.
Fig. 35: As immobile opaque masses of encysted metacercariae type 1 were microscopically noticed after freezing for about 6 days, x82.5.
Fig. 36: Encysted metacercaria type 1 seems with unclear morphological characteristics after freezing for about 14 days, x130.

Morphological characteristics of *M. heterosporus* spores unaffected with salt of about 95% after nearly 3 hours *in vitro*; to some extent they were similar to control (Fig. 20). Also, no effect appeared on spores with dry salt '100%' after about 3 hours (Fig. 21) but some spores were with normal shape, other spores extruded polar filaments and others with severe deformation (Figs. 22-24) after about 5 weeks of observation. *In vitro* vitality of encysted metacercariae type 1 may be affected by dry salt after about 17 hours to several days. The parasite appeared as an opaque mass in comparison with control (Figs. 25-28). By pickling, spores of *M. heterosporus* in vinegar after about 3 hours to several weeks were severely deformed (Figs. 29-31). Also, encysted metacercariae type 1 appeared as non vital opaque masses after about 6 days (Fig. 32). By freezing in freezer of home refrigerator, spores of *M. heterosporus* seemed vital after about 5 days (Fig. 33). Microscopically, encysted metacercariae type 1 seemed immobile after about 21 hours of freezing but the parasite appeared with unclear morphological characteristics as an immobile opaque mass after about 6-14 days (Figs. 34-37). By boiling infected cornea with plasmodia of *M. heterosporus* for about 30 minutes, spores seemed non vital (Fig. 38). Also, by boiling a piece of the infected flesh of catfish infected with encysted metacercariae type 1 the parasite seemed as a non vital opaque mass (Fig. 39). By grilling fish contained cornea infected with *M. heterosporus* some spores seemed detectable and others appeared non vital (Figs. 40, 41). Grilling a piece of catfish muscle infected with encysted metacercariae type 1 the parasite showed as an immobile opaque mass microscopically (Fig. 42). Frying fish infected with *M. heterosporus* most spores were deformed and seemed non vital (Fig. 43). Frying a portion of catfish flesh infected with encysted metacercariae type 1 the parasite seemed as a non vital immobile opaque mass (Fig. 44). Moreover, most metacercariae type 1 absent after cooking more in oven the infected fried fish (Fig. 45).

Effect of Aqueous Extracts of Medicinal Plants on the Parasite Vitality: *In vitro* spores of *M. heterosporous* in aqueous extract of onion bulb were deformed and non vital after about 9 hours and 1 week (Figs. 46-48). Spores of *H. suprabranchiae* from suprabranchial organ of catfish may be affected after about 3 days (Fig. 49). Encysted metacercaria type 1 from muscular tissue of catfish noticed as an immobile opaque mass but cyst wall seemed normal after about 3 days (Fig. 50). Microscopically, encysted metacercaria type 2 appeared with slow movement after about 3 days (Fig. 51). *In vivo* the cestode *Monobothriodies* sp. isolated from fish with some aqueous extract of onion bulb seemed mobile and vital after about 4 days; also, egg seemed unaffected (Figs. 52, 53).

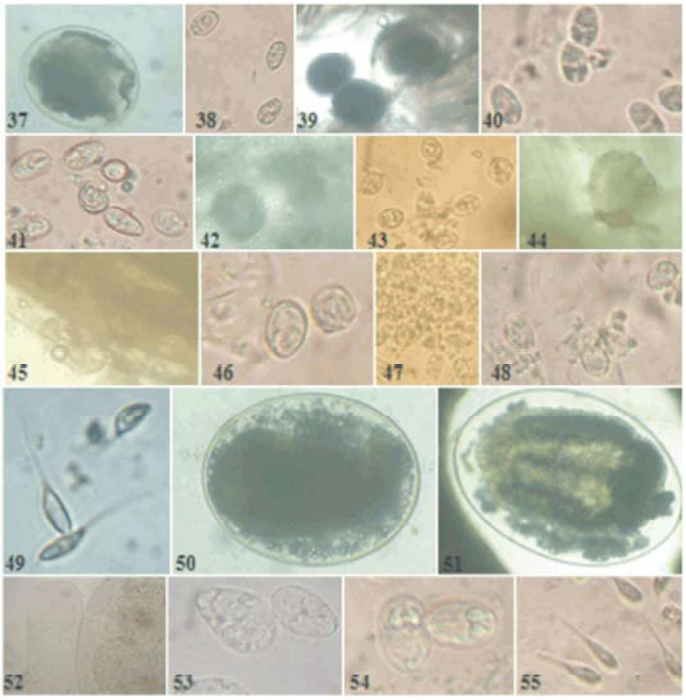

Fig. 37: Immobile encysted metacercaria type 1 after freezing for about 14 days. Note the parasite appears as opaque mass; its viability may need to sure, x142.5.

Fig. 38: Deform and apparently non vital spores after about 30 minutes of boiling cornea of tilapia fish infected with *M. heterosporus*, x1000.

Fig. 39: As non vital opaque masses of encysted metacercariae type 1 after boiling of infected flesh of the catfish are discernible x100.

Fig. 40, 41: Some spores of *M. heterosporus* to some extent still detectable, others seem deform and non vital after grilling fish with infected cornea, x1000

Fig. 42: As immobile opaque mass of encysted metacercariae type 1 after grilling portion of the infected flesh is distinguishable, x92.5.

Fig. 43: Most spores of *M. heterosporus* non vital and deform after frying of fish with infected cornea, x1000.

Fig. 44: Encysted metacercaria type 1 seems as non vital opaque immobile mass after frying portion of the infected fish flesh, x100.

Fig. 45: Fried fish infected with metacercariae type 1 undergone to more cooking in oven. Note non vital degenerate parasite, x250.

Fig. 46: Deform spores of *M. heterosporus* in aqueous extract of *Allium cepa* bulb after about 9 hours, x1000.

Fig. 47: Deform spores of *M. heterosporus* in aqueous extract of onion bulb after about 16 hours, x1000.

Fig. 48: Deform spores of *M. heterosporus* in aqueous extract of onion bulb after about 1 week *in vitro*, x1000.

Fig. 49: Affected spores of *H. suprabranchiae* from suprabranchial organ of catfish in aqueous extract of onion bulb after about 3 days *in vitro*; viability may need to sure, x1000.

Fig. 50: Metacercaria type 1 appears as an immobile opaque mass with normal cyst wall in aqueous extract of onion bulb after about 3 days; perhaps its viability needs sureness, x162.5.

Fig. 51: Encysted metacercaria type 2 was microscopically noticed with slow move in aqueous extract of onion bulb after about 3 days *in vitro*; perhaps its vitality needs to confirm, x90.

Fig. 52, 53: A vital cestode parasite in aqueous extract of onion bulb after about 4 days *in vivo*. Note distinguishable anterior portion of the parasite with some proglottids; unaffected eggs of the parasite, x320; x1000.

Fig. 54: Normal spores of *M. heterosporus* in aqueous extract of allspice seeds after about 5 days *in vitro*, x1000.

Fig. 55: Unaffected spores of *H. suprabranchial* from suprabranchial organ of catfish in aqueous extract of allspice after about 18 hours *in vitro*; vitality may need sureness, x1000.

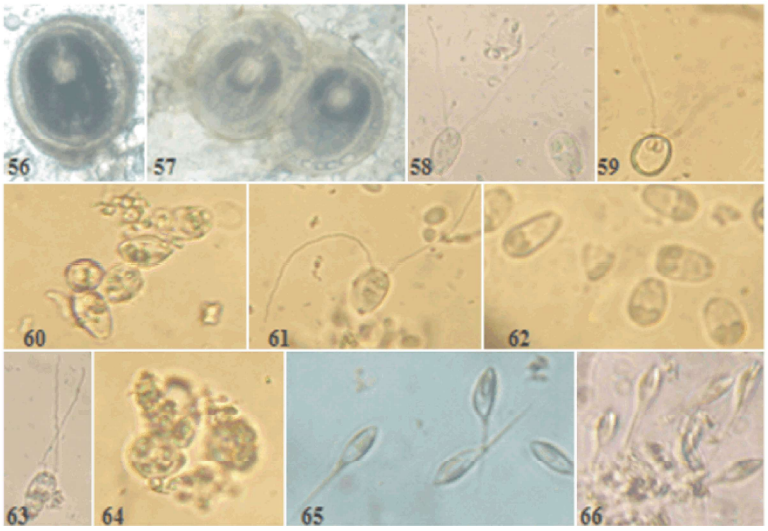

Fig. 56: Mobile vital encysted metacercaria type 1 in aqueous extract of allspice after about 3 days *in vitro*, x145.

Fig. 57: Vital encysted metacercariae type 1 in aqueous extract of allspice after about 6 days *in vitro*, x97.5.

Fig. 58, 59: *M. heterosporus*, normal spore and others extrude polar filaments in aqueous extract of *Cinchona* sp. bark after about 15 hours *in vitro*, x1000.

Fig. 60, 61: *M. heterosporus* in previous-prepared 'for about 1 month' aqueous extract of *Cinchona* sp. bark after about 5 days *in vitro*. To some extent spores affected; polar filaments extrusion; vitality may need to sure, x1000.

Fig. 62: To a certain degree normal spores of *M. heterosporus* in aqueous extract of *Cinchona* sp. bark after about 1 week; probable vitality needs confirmation, x1000.

Fig. 63: *M. heterosporus*, polar filaments extrusion and severe damage body wall of the spore in aqueous extract of *Cinchona* sp. bark after about 1 week *in vitro*, x1000.

Fig. 64: Spores of *M. heterosporus* non vital mostly with undetectable shape in aqueous extract of *Cinchona* sp. bark after about 6 weeks *in vitro*, x1000.

Fig. 65: Normal spores of *H. suprabranchiae* from suprabranchial organ of catfish in some of recently prepared aqueous extract of *Cinchona* sp. bark after about 2 days *in vitro*; vitality may need confirmation, x1000.

Fig. 66: Tangle spores of *H. suprabranchiae* from suprabranchial organ of catfish in previous-prepared aqueous extract of *Cinchona* sp. bark after about 5 days *in vitro*; perhaps viability needs sureness, x1000.

Spores of *M. heterosporus* in aqueous extract of *Allspice* seeds seemed normal after about 5 days *in vitro* (Fig. 54). Spores of *H. suprabranchiae* from suprabranchial organ of catfish seemed unaffected after about 18 hours (Fig. 54). *In vitro* encysted metacercaria type 1 seemed mobile and vital after about 3 and 6 days (Figs. 56, 57). *In vitro* spores of *M. heterosporus* in aqueous extract of *Cinchona* sp. bark might be affected after about 15 hours, 1, 5 days and 6 weeks. Morphologically, few spores seemed normal but most appeared non vital with severe damage (Figs. 58-64). Macroscopically, tissue of the treated cornea seemed stiff, transparent and brownish. *In vitro* spores of *H. suprabranchiae* from suprabranchial organ of catfish seemed normal and others tangle without distinct vitality after about 2 and 5 days; perhaps effectiveness of this extract needs more time (Figs. 65, 66).

Spores of *M. heterosporus* in aqueous extract of *Nigella sativa* seeds seemed unaffected, vital and similar to control after about 3 hours and 1 week *in vitro* (Figs. 67, 68). Also, spores of *H. suprabranchiae* from suprabranchial organ of catfish seemed normal and vital after about 2 and 5 days (Figs. 69, 70). *In vivo* one plasmodium contained normal spores of *H. suprabranchiae* from intestine of catfish was isolated after about 3 hours and after about 2 days through several times some of its mature spores seemed vital (Figs. 71, 72). *In vitro* encysted metacercaria type 1 in aqueous extract of *N. sativa* seeds seemed mobile and vital (Figs. 73, 74). *In vivo* catfish with some *N. sativa* seeds were alive and the isolated cestode *Monobothriodies* sp. appeared unaffected in comparison with control after about 2 hours. To some extent this helminth seemed non vital after about 3 days *in vitro* (Figs. 75, 76).

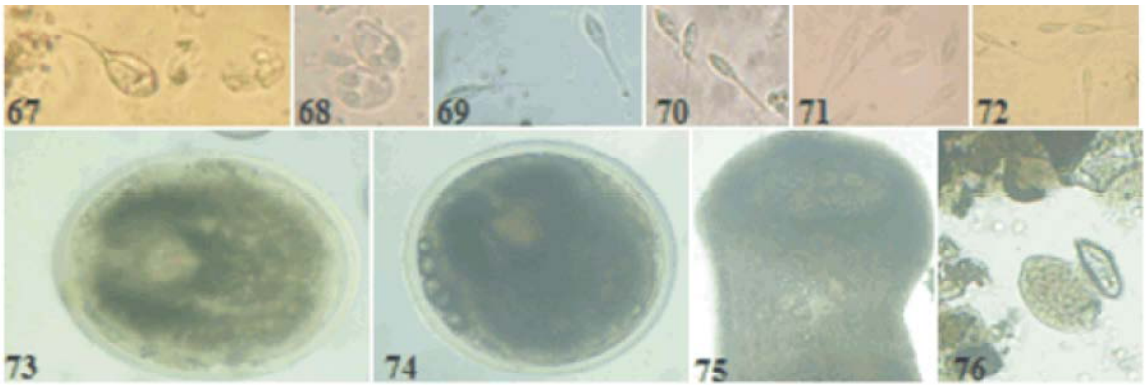

Fig. 67: Vital spores of *M. heterosporus* in aqueous extract of *Nigella sativa* seeds after about 3 hours *in vitro*, x1000.
Fig. 68: Unaffected spores of *M. heterosporus* in aqueous extract of *N. sativa* seeds after about 1 week *in vitro*, x1000.
Fig. 69: Normal vital spores of *H. suprabranchiae* from suprabranchial organ of catfish in aqueous extract of *N. sativa* seeds after about 2 days *in vitro*, x1000.
Fig. 70: Vital spore (arrow) of *H. suprabranchiae* from suprabranchial organ of catfish in aqueous extract of *N. sativa* seeds after about 5 days *in vitro*, x1000.
Fig. 71: Normal spores of *H. suprabranchiae* from intestine of catfish in aqueous extract of *N. sativa* seeds after about 3 hours *in vivo*, x1000.
Fig. 72: Vital spores of *H. suprabranchiae* from intestine of catfish in aqueous extract of *N. sativa* seeds after about 2 days *in vivo*, x650.
Fig. 73: Encysted metacercaria type 1 in aqueous extract of *N. sativa* seeds after about 3 days *in vitro* seems mobile, vital and similar to control, x160.
Fig. 74: Vital encysted metacercariae type 1 in aqueous extract of *N. sativa* seeds after about 6 days *in vitro*, x85, x170.
Fig. 75, 76: To some extent non vital cestode, anterior portion with no shrinkage of skin and egg in aqueous extract of *N. sativa* seeds after about 3 days *in vitro*, x92.5.

DISCUSSION

Parasites of Protozoa and Metazoa infected present hosts of tilapias and catfish during certain times in about three years were investigated.

El-Mansy and Bashtar [82] registered several parasites from *C. gariepinus* e.g. oocysts of coccidia, *Trypanosoma*, *H. suprabranchiae*, encysted metacercariae and histopathological effects due to some parasites such as those in sections contained the probable monogenian and nematode. Here, from protozoans *M. heterosporous* infected cornea tissue of some tilapias, *H. suprabranchiae* from suprabranchial organ and intestine of catfish seemed similar to that reported by El-Mansy and El-Mansy and Bashtar [34, 35] and from metazoan helminthes encysted metacercariae types 1 and 2 from muscular tissue of catfish and the cestode *Monobothriodies* sp. from intestine of same previous-cited host were identified based on morphology and morphometric measurements beside comparison with previous descriptions. The total prevalence of parasitic infestation seemed low about 34.3% in nearly three years.

Myxosporeans: Spores of *Myxobolus* species reported by El-Mansy [27] found in cysts or plasmodia embedded within eye's cornea of tilapias under name *M. heterosporous* taxonomically and histologically re-described by El-Mansy [4]. Here, the basic content reported by El-Mansy [27] was revised and the present spores seemed greatly similar to normal types particularly type 5 reported by El-Mansy [4]. High numbers of spores in plasmodia were observed. Although its prevalence seemed low 'a relatively small percentage of the examined fish 5% [83]' in intensive infestation it occupied wide area of the cornea tissue may affect fish vision.

Also, presence of the genus *Henneguya* Thélohan, 1892 in freshwater fishes from Chad was reported by Kostoingue *et al.* [84]. In Egypt, spores of *H. suprabranchiae* isolated from two different organs suprabranchial organ and intestine of the catfish similar to spores reported by El-Mansy [34]. A little smear from plasmodium may contain a huge number of spores, microscopically seemed exceed hundred 'about 180 spores or more'. So, a little number of mature spores of *H. suprabranchiae* may be enough for measurements

particularly those identical in shape and size. Spores from both organs were identified as one species because significantly similar in particular morphologically [34, 35] but may be due to slight difference probably are two species. Therefore, for valid definition this parasite may need other study. On the other hand, Abdel-Baki *et al.* [86] reported that the overall prevalence of *H. suprabranchiae* infecting catfish from the River Nile was 35% with maximum rate of infection in spring and minimum in summer and it is a pathogenic species as the parasite showed high intensity of infection which led to deformation of the filament structure and complete disappearance of the gill lamellae.

Encysted Metacercariae: Herein, small encysted metacercariae type 1 seemed similar to that reported by Alicata *et al.* [8] in the musculature of an infested mullet; heterophyid flukes as adults are extremely small, often only about 0.5 mm long. Walls of metacercariae were relatively thick and consisted of two layers, an inner layer of fibroblasts and collagen fibers and an outer wall of fibroblasts. Additionally, Ryang *et al.* [87] reported a case of human infection with *Heterophyes nocens* that was incidentally found in a mucosal biopsy specimen of the patient who had a history of eating raw mullets and these were presumed to be the source of infection. Several encysted metacercariae of different species were reported from different locations in Egypt. *Euclinostomum ardeolae* from the kidney of *O. niloticus* and *S. galilaeus* caught from El-Menia Governorate; metacercariae of diplostomatid and heterophyilid were isolated from the muscle of tilapias; metacercariae of acynodiplostomatid were detected from catfish muscles and a prohemistomatid was found in the muscles of all fish species. Some present encysted metacercariae seemed similar to some previous reported metacercariae [35, 88, 89, 90-95]. The infection of fish with *Clinostomum tilapiae* metacercariae may be attributed to the richness of water with the intermediate host and the presence of final host [10]. *Clinostomum* spp. metacercariae can be transmitted to human as a result of ingesting raw or improperly cooked freshwater fish, causing Halazoun-like disease leading to laryngo-pharyngitis [96].

In Egypt, a man made Abo-Zaabal Lake according to that reported by Abd-Ellah [97] consists of three Lakes and a filling phase Lake and considered as a closed aquatic ecosystem but if possible to follow nearest canal may be solution particularly to regenerate its water and turning off discharge of any waste particularly sewage if it is poured into the Lake that may also caused parasitic infestation such as those reported by El-Mansy [98] particularly metacercariae like-structure in fish gill, other parasitic stages of myxosporeans unless belong to other figure twenty-six [98] perhaps a coccidian 'Ramadan N., Personal communication' may approach to sporocysts such as of the so-called *Eimeria samehi* infected a specimen of *T. zillii* which its main description based on an oocyst in different microscopic views 'may need further study' [99] or may relate to some of those reported by El-Mansy [100]. Here, it may also be worthy to mention that, most of six lernaeid adult females were isolated from one specimen of the goldfish *Carassius auratus* [101]'. Figures thirty six-thirty nine seem to sections of fish pancreas 'Molnár K., Personal communication' unless be other in particular as that cited by El-Mansy [98]. Also other parasitic forms like the so-called unknown protistians noticed by El-Mansy [102]. In addition, cysts of metacercariae like-structure embedded in the tissue of swim bladder of tilapias from Abo-Zaabal Lake [95] seemed similar to some present ones. Incidence of the encysted metacercariae reported by El-Gohary and Samaha [103] included in the muscles of the infested fish was 72.9% in *Oreochromis* species and 68% in *Clarias lazera*; also, seasonal incidence of encysted metacercariae in *Oreochromis* species was detected maximum in summer 81.8%, minimum in spring 66.7% and in *C. lazera* maximum in winter 92.3% and minimum 33.3% in summer. In addition, El-Seify *et al.* [104] reported that prevalence of infection by examination of the muscles of 255 *Oreochromis*, 199 *C. lazera* with encysted metacercariae was 62.015%; maximum percent in *C. lazera* was 100% in February, October, November and minimum was 16.66% in July but no infection in other. El-Mansy [105] reported that about 12.6% of tilapias from the River Nile at El-Menia Province with high intensity of infection with *Clinostomum* metacercariae were recorded. Reda *et al.* [106] reported that a total of 432 *Oreochromis niloticus* of different stages obtained from River Nile tributaries in Sharqia Governorate and Abbassa fish farms and the prevalence of the encysted metacercariae was higher among wild ranging fish 84.24%. Fish act as intermediate host for larval stages of many parasites like encysted metacercariae of different species of trematodes and a possibly source of human infections and other fish eating animals with such parasites [10]. Praziquantel at a dose of 5 and 10 mg/ L for 5 days was effective in treatment of *O. niloticus* infested with encysted metacercariae [106]. To high degree, present small encysted metacercariae seem to be *Heterophyes* that caused the so-called human heterophiasis particularly which constitute a public health

problem where people eat raw, salted or other improperly prepared infected fish. In Egypt, Khalil and Martin [107, 108] described the life cycle of *H. heterophyes* and reported that *O. niloticus*, *T. zillii* and commercial size mullets from Egyptian lagoons where nearly all fish were infected by heterophiids. *Oreochromis* species and *C. lazera* a source of transmitting encysted metacercariae to man [103]. *H. heterophyes* metacercariae are relatively resistant to various heat treatments; they survived in fish temperature when kept for 9-13 day at 4-6°C or iced at 2-4°C; freezing fish at -10 or -20°C for half hour was even not sufficient to kill the parasites [107, 109 and 110]. Only prolonged cooking when all parts of the fish are well heated for several minutes will efficiently destroying all metacercariae.

Adult Helminthes: In this study, no adult digeneans were observed in the examined fish although considered as common parasites of catfish like *Orientocreadium batrachoides* and *Astiotrema reniferum* represented in figures nineteen and twenty-nine [82] those photomicrographs of such species were previously isolated from catfish caught from El-Serw experimental fish farm. The previous-cited parasites were reported by several investigators such as Matter [93] who reported *O. batrachoides* and *A. reniferum* from catfish from the River Nile and market at El-Qanater El-Khayria. Here, it worthy to mention that during the period of examination catfish were not infected with the cestode *Polyonchobothrium clarias* and the nematode *Paracamallanus cyathopharynx*. Moreover, El-Mansy *et al.* [26] reported histopathology of tissues of farmed freshwater catfish caught from El-Serw experimental fish farm infested with different helminthes such as *O. batrachoides*, *P. clarias* and *P. cyathopharynx*.

Although, the current cestode seemed similar to *Monobothrioides woodlandi* from *Clarias mellandi* in Zambia [111]; but may approach to general shape and some measurements of *Monobothriodies chalmersius* [112]. On the other side, the present cestode particularly in some dimensions seemed smaller than *Monobothriodies aegypti* from the small intestine of *C. lazera* [113]. Here, a few intensity of infestation was noticed but Hamada and El-Naggar [114] reported that living worms were firmly attached to the intestinal mucosa with heavily infected fish '50 worms/ host fish'. Also, previous-cited authors reported that all examined specimens of the Nile catfish were found unequally infected with the caryophyillid *M. chalmersius* [115, 116], either singly or in clusters in the middle section of the intestine and the majority of collected worms were immature measuring 3.6 (3-4.5) x 1 (0.9-1.2) mm, while the adult worms were able to stretch their body 2-3 times their normal length; normal unattached and extended scolex appears broad with dense like-apex. So, with comparison of morphology and measurements of the current cestode and some previous studies, it seemed similar to main characteristics particularly body width and scolex shape of *M. chalmersius* that reported by Hamada and El-Naggar [114] but it still a species of *Monobothrioides* [117]; in principal be called *Monobothrioides* sp.

Parasites Treatment: Parasites become a concern not only on fish health in aquaculture but also on human health when consumers eat raw or lightly preserved infested fish. So, the present study as an additional reference for studding effect of processing steps and aqueous extracts of medicinal plants on vitality of some Egyptian freshwater fish parasites is presented. *In vitro* and some *in vivo* parasites infected fishes (tilapias and catfish) undergone to processes of storage (salting, pickling, freezing), cooking (boiling, grilling, frying) and aqueous extracts of onion bulb, allspice seeds, *Cinchona* sp. bark and seeds of *N. sativa* as try for controlling fish parasites. Perhaps further experimental and clinical investigations may be needed to evaluate and standardize the dosage of such natural products. Herein also, a revision of some previously reported results may support for application.

Salting: After several sources on web, normal seawater is about 3.5% salt and 96.5% water by weight. The salinity of the ocean being about 35 parts per thousand or about 3.5% (35 g/ L) and also Hunt [118] reported that seawater is composed of many different ions (salts) in different concentrations but the sum of them all adds up to 3.5% and the main ions in seawater are sodium, chloride, magnesium and sulfate, forming the salts 'NaCl and $MgSO_4$'. In freshwater salinity is less than 0.21 PPT. Myxosporeans are parasites of fish in both freshwater and saltwater. They are among the most common parasites of marine fish and new families, genera and species are continually being described and many more await description [119]. Myxosporidians infecting fish are very dangerous parasites causing severe damage to a large number of economically important fishes especially in aquaculture [120]. Here, salting fish infected with *M. heterosporous* in about 95% salt did not affect on morphological characteristics of most spores after about 3 hours; to some extent still similar to control. FDA [31]

reported that brining may reduce the parasite hazard in a fish, but they do not eliminate it and nematode larvae have been shown to survive 28 days in 21% salt by weight. On the other side, it seems that myxosporeans could be tolerated high concentration of salt, in the present study this may be proved, some spores of *M. heterosporous* were deeply deformed after about 5 weeks in dry salt and also vitality of encysted metacercariae type 1 might be affected after about 17 hours to several days. Also, in figures seventeen to twenty-one reported by El-Mansy [27] it thought that spores of *Myxobolus* sp. '*M. heterosporus*' were damaged after about two hours in dry salt. Perhaps, against medicaments each spore has in time its own response. FDA [79] reported that the nematode *Anisakis simplex* seems to be sensitive to salt, the high salt concentrations and times needed for its elimination make salting an inadequate method of inactivation. Fan [121] reported that metacercariae of *Clonorchis sinensis* from freshwater fish (*Pseudorasbora parva*) were killed if kept in heavy salt. The more typical water phase salt contents of 3-3.5% in cold-smoked fish would not be sufficient to kill the organisms. Additionally, dry salting does tend to kill those parasites residing on fish surfaces, but generally does not do so for those imbedded within the tissue [121]. Anyhow, salting particularly by using dry salt may be a seemly process for fish preservation against some parasites.

Pickling: *In vitro* by using vinegar, spores of *M. heterosporous* appeared severely damaged and deformed after about 3 hours to several weeks and also encysted metacercariae type 1 seemed non vital as opaque masses after about 6 days. In figure sixteen reported by El-Mansy [27] it thought that polar filaments of some spores of this species might be protruded. Pickling may reduce the parasite hazard in a fish [31] but herein it deserves to conclude that, against such parasites perhaps vinegar to be utilized as an antiseptic but unlikely be used as a preservative material particularly for long time because it may lead to softness of the treated fish tissue to be unmarketable.

Freezing: The effectiveness of freezing to kill parasites depends on several factors including the temperature of the freezing process, the length of time the fish is held frozen, the species of the fish and the type of parasite [31]. CDC [122] reported that there are two reliable techniques to kill fish parasites, freezing fish or fish products to an internal temperature of -35°C for 15 hours, -20°C for at least 7 days, or to -35°C until frozen and held at -20°C for a minimum of 24 hours; the critical factor is to ensure that the centre of the fish is solidly frozen and not all home freezers can freeze to these temperatures. Freezer of home refrigerator may not be cold enough to kill current spores of *M. heterosporus*, which its vitality seemed unaffected after about 5 days. El-Matbouli and Hoffman [123] reported that *Myxobolus cerebralis* myxospores that caused whirling disease in rainbow trout *Oncorhynchus mykiss* can tolerate freezing at -20°C for at least three months and aging in mud at 13°C for at least 5 months and can be transferred to an oligochaete intermediate host in the later triactinospores were developed. Moreover, the long-term viability of *M. cerebralis* held at 5°C is less than 1 year [124]. For fish parasites other than flatworms or flukes 'trematodes' freezing treatments must be equivalent to -20°C for not less than 24 hours [80]. On the other side, by freezing some of fish flesh infected with the present encysted metacercariae type 1 seemed affected. The parasite appeared as an immobile non vital opaque mass after about 21 hours and 6-14 days; perhaps its viability needs sureness. Freezing of tilapia spp. at -4°C for 10 and 12 days failed to produce infection to experimental animals [11]. Deep freezing of fish lead to prevent the danger of fish parasites which have a public health importance and freezing freshwater fishes for 72-96 hours at -10°C was sufficient to destroy the encysted metacercariae [10, 12]. Additionally, Mahmoud [125] reported that freezing of tilapia spp. muscles at -2°C for a period not less than 9 days may be considered enough to kill the contained metacercariae.

Boiling: Spores of *M. heterosporus* and the parasite of encysted metacercariae type 1 seemed deform and non vital after boiling infected fish tissue for about 30 minutes. Control the hazards associated with fish consumption can be done by efficient heat processing 80°C of the fish before consumption for period not less than 15-20 minutes [10]. FDA [31] reported a step for parasite control that the process of heating raw fish is sufficient to kill parasites. Also, fish parasites can be killed by cooking fish to an internal temperature of 63°C. So, fish boiling for at least about half an hour may be enough to kill such fish parasites [122]. By boiling *Anisakis* and mackerel infections can be avoided [33]. For some fish parasites heating treatments need to be more than 60°C for at least 1 minute [80].

Grilling: In this study, grilling infected fish was not sufficient to destroy all spores of *M. heterosporus*; some still detectable, others appeared non vital but by grilling a piece of infected flesh with encysted metacercariae type

1, most metacercariae seemed as immobile opaque masses. Perhaps temperature of grilling did not strongly affect particularly on some spores of *M. heterosporus* due to the cover material and may need more time. The proper timing and temperature of grilling should be emphasized because superficial grilling doesn't affect deep muscles which of course contain encysted metacercariae are not affected by the fire. Grilling of infected *O. niloticus* fish with encysted metacercariae for 15-20 minutes at 60-80°C was sufficient to destroy the encysted metacercariae [10, 126 and 127].

Frying: Most present spores of *M. heterosporous* and encysted metacercariae type 1 isolated from infected fried fish were non vital but the highest effect was occurred by additive cooking in oven. Good frying for 5 minutes was sufficient to destroy all encysted metacercariae [10, 126].

Aqueous Extracts of Medicinal Plants: Modern antibiotics are used against various types of infectious diseases caused by micro-organisms but where microbial resistance to antibiotics has developed in rural areas different medicinal plants have been used successfully by traditional practitioners. In recent years, medicinal plants have increased significantly to cure humans and animals [77, 128]. So, for parasitic control several of them were studied.

***Allium cepa* Bulb:** *In vitro* spores of *M. heterosporous* seemed non vital with severe deform in aqueous extract of onion bulb after about 9 hours and 1 week. Also, *in vitro* unless another reason it was noticed destruction of *Myxobolus* species from eyes treated with aqueous extract of onion kept in refrigerator for about 24 hours; some appeared with normal shape in comparison with control [28]. Also, *H. suprabranchiae* spores might be affected after about 3 days. On the other hand, encysted metacercaria type 1 was observed as an opaque immobile mass although its cyst wall seemed normal after about 3 days and microscopically encysted metacercaria type 2 seemed mobile after about 3 days; after about 4 days cestode parasite seemed vital and probable the noticeable egg was not affected, so with the exception of former-cited spores viability may still need to sure. On another host, Mehlhorn *et al.* [129] reported that sheep with gastrointestinal nematodes and cestodes were fed on three farms with a combination of specially prepared extracts of onion and coconut (*Cocos nucifera*) for 8 days containing each 60 g coconut and onion extract, combined with milk powder and/ or polyethylene glycol. In all cases, the worm stages were not found 9 and 20 days after the end of the feeding with this plant combination and all treated animals increased their body weight considerably when compared to untreated animals. Also, Jatzlau *et al.* [130] reported that in the case of the horse treatment, the worm load decreased that mostly only single eggs or larvae were found in those horses that had accepted the onion-coconut food addition. Abdel-Hafeez *et al.* [131] reported that *Blastocystis hominis* inhabits the gastrointestinal tract of humans and many animals; metronidazole is the main therapy for blastocystosis; frequent reports of treatment failure suggesting isolates resistance to metronidazole; *in vitro* onion treatment insignificantly lowered the number of the parasite after 48 hours.

Although few studies have considered the effects of supplemental *A. cepa* on some farmed fish, growth enhancing properties of an onion bulb based diet showed an increase in body weight gain through different mechanisms either separately or synergistically [45-47, 132 and 133]. In addition, aqueous extract of onion bulb seems to be efficient against vitality of certain parasites. In conclusion, onion bulb may prepared to be of additive fish food and its aqueous extract as a treatment bath for fish infested with some parasites 'particularly in cases of high prevalence and intensity of infestation' may be used as well.

Allspice Seeds: *In vitro* parasites of *M. heterosporus*, *H. suprabranchiae* and encysted metacercariae type 1 appeared vital in aqueous extract of allspice '*Pimenta* sp.' seeds after about 18 hours-6 days; perhaps viability needs to sure. Also, encysted metacercariae type 1 seemed mobile and vital after about 3 and 6 days. In this study, a try to revise some results reported by El-Mansy *et al.* [29]; here no found the cestode *Polyonchobothrium clarias* and the nematode *Paracamallanus cyathopharynx* infected catfish but *in vitro* other cestode called *Monobothriodies* sp. seemed unaffected by aqueous extract of allspice seeds although *in vitro* some larvae of previous-cited *P. cyathopharynx* by same extract might be affected [29]; perhaps due to other reason such as long period outside host. Thus, it can be concluded that, this extract may be ineffective and no preferable its use against such parasites.

***Cinchona* sp. Bark:** *In vitro* most spores of *M. heterosporus* in aqueous extract of *Cinchona* sp. bark appeared non vital with severe damage after about 15 hours, 5 days, 1 week and 6 weeks. Morphologically, few spores appeared normal but mostly seemed non vital. Spores of *H. suprabranchiae* from suprabranchial organ seemed normal and others tangle with no distinct vitality

after about 2 and 5 days; probable effectiveness of this extract may need more time. As reported on websites, *Cinchona* is the only economically practical source of quinine, a drug that is still recommended for the treatment of malaria and also it is used as a treatment for *Cryptocaryon irritans* infection of marine aquarium fish [52, 134 and 135]. In addition, the present extract is probably efficient against some current fish parasites but perhaps needs more tests. On the other hand, malaria is one of the most severe public health worldwide, 90% of which were reported from Sub-Saharan African countries and it is a severe disease in Ethiopia, traditional malaria preventing techniques are effective for temporarily reducing the severity of the disease [136]. Furthermore, internal migration brought malaria to many regions in Brazil where, given suitable *Anopheles* mosquito vectors, it thrived; multiple regional and national malaria control efforts have been attempted with varying success [137]. Larvicidal potentiality of the bandotan (*Ageratum conyzoides*) leaves for controlling the three important species of mosquitoes was reported by Massuod *et al.* [138]; the results uncover the possibility for further investigations of the efficacy of larvicidal properties of natural product extracts; perhaps against mosquito vectors the aqueous extract of kina bark for controlling malaria may also be a suggestion.

***Nigella sativa* Seeds:** In the current study, spores of *M. heterosporus*, *H. suprabranchiae* and encysted metacercaria type 1 in aqueous extract of *N. sativa* seeds seemed vital similar to control after about 3 hours-6 days *in vitro*. Also, spores of *H. suprabranchiae* appeared vital after about 2 days *in vivo*. The effect of aqueous extract of *N. sativa* seeds on adult helminthes of the cestode *Polyonchobothrium clarias* and the nematode *Paracamallanus cyathopharynx* isolated from catfish [29] was tried to revise because dead parasites *in vitro* might be due to other cause; perhaps some parasites could not survive long time outside host. Herein, no found former-cited helminthes but other tape worm called *Monobothriodes* sp. with aqueous extract of *N. sativa* seeds to some degree could be investigated. *In vivo* this cestode seemed similar to control after about 2 hours. *In vitro* it appeared non vital after about 3 days may also due to another factor; perhaps its viability needs sureness. On the other side, *N. sativa* has been reported to possess potent antiparasitic; it among medicinal plants that could serve as safe effective treatment and is a promising adjuvant with anti-helminthic drugs in the treatment of schistosomiasis [139, 140].

The effectiveness of *N. sativa* seeds and thymoquinone is variable and depends on species of target microorganisms [74]. *N. sativa* seeds did not inhibit avian infectious bronchitis virus infection [141]. *N. sativa* oil was found to be effective against *Salmonella* species; its seed has been reported to have many biological properties including antibacterial and anti-parasitic [77, 142-144]. 5% *N. sativa* seeds offered the most protection against columnaris disease in channel catfish [145]. In addition to parsley that was found to be safe and successful in protection of *O. niloticus* from aflatoxicosis particularly at the low level [146] oil of *N. sativa* at concentration of 1-3% was also completely inhibited aflatoxin production [147, 148].

Anyhow, seeds of *N. sativa* are widely used in traditional Islamic medicine for culinary purposes worldwide and it is generally safe when taken by mouth; about 1 g twice daily may activate the human immune system [48, 55 and 149]. In conclusion, seemingly, *N. sativa* seeds did not die fish parasites but it is likely with more time, safe dose and appropriate management may lead to an indirect effect against such pathogens through stimulation the innate immunity of the host. In it what be necessary for strengthen of the immune system and then general health. Therefore, agree with Zaki and Fawzi [77] that seeds of *N. sativa* may be used as feed additives.

CONCLUSION

For farmed fishery products, fish must be fed their whole life on a diet that cannot contain viable parasites and they have been reared in an environment that is free from parasites to ensure that fishery products are not infected with parasites that may represent a health hazard [122]. For some myxozoans drying was effectiveness as sporicidal treatment [6]; storage at lower temperatures yielded higher viability in others [7]. It is undesirable that the manufacturer fish be infected with parasites, which differs according to the parasite species, intensity and temperature of storage or cooking [150]. Parasites do not present in thoroughly cooked fish [151]. Infections with parasite such *Anisakis* can generally be avoided by boiling, burning, preserving in salt, or freezing overnight [33]. Freezing and cooking may kill *Anisakis simplex*, but may not protect consumers against allergic reactions to ingest *A. simplex* antigens [152]. It can be concluded that, for safe storage the recommended critical points should be followed. Good cooking may be enough to get rid of fish parasites, but also necessary to avoid toxic byproducts of the parasite.

It may be worth to mention that, parasites such as *Trichodina* infestations are associated with high stocking densities and feeding rates, which lead to high ammonia concentrations [153, 154]. Although length and weight of the farmed catfish in agricultural drain to some extent were lower than those from the River Nile [35] values of the total muscle protein of catfish from both locations still within normal range of total protein of fishes. In any case, it is necessary that agricultural drains to be assembled with safe treatment then to take independent way and its water may be used for irrigation of certain types of plants in public parks; in particular specific woody trees in desert. Moreover, El-Mansy and Abdel-Ghaffar [155] reported the so-called proliferative kidney disease in tilapias caught from Rosetta branch of the River Nile at El-Rahawy drain. In addition, fish deformities [93, 156] may be due to pollution, so it should find a general diplomatic solution [157]. Drains that carries wastewater 'domestic and industrial waste' should be rerouted away out of all aquatic habitats for several factors including water quality, aquatic fauna, humans, general health and the environment especially through pathogens prevention like infectious parasitic stages. Therefore, it is essential particularly to sewage and industrial waste should be away from any uses and be collected from the sewerage network with other residues to a general waste plant which can be designed as a controlled furnace of very high temperature for cleansing by incineration, preferably be in the desert apart from population residence; or perhaps uses of melt process to convert such effluents to a pure new synthetic product may structurally be like raw petroleum and/ or other of energy sources.

Lake Qarun is a closed inland Lake, saline, located at the Western Desert and constitutes a very important sector in the Egyptian fisheries [158]. According to studies reported via web, its water quality is polluted [159] and it is from the most heavily-polluted Lakes in Egypt. Lake Qarun is a reservoir for agriculture drainage water of Fayoum Province; extensive evaporation of its water increases concentration of salts, heavy metals, pesticides and other pollutants which changes the quality of water with a profound impact on its fauna and flora [160-163]. The environmental contamination of Lake Qarun induced several histopathological alterations in fish tissues, subsequently may affect fish production and human health [164, 165]. Heavy metals are recognized as cumulative toxic substances causing serious health hazards to man depending on their concentration [166]. Moreover, microbes, protozoans and helminth parasites in fishes from Lake Qarun were recorded by several authors such as [167-171]; more than fifty percent of the total fishes collected from Lake Qarun had parasitic infection [172]. Through this study it can be deduced that for parasites in May 2006, 27 samples of *Solea* species was presented from market at El-Anfoshy, Alexandria, Egypt; measured about 14-16.5 cm in length and 25-45 g in weight. Later, other specimens of same genus from Lake Qarun were macroscopically noticed 'El-Mansy, unpublished', which ostensibly may agree with Shalloof [163]. *Solea* fish from Lake Qarun somewhat seemed more emaciated with dark skin color compared to those from Alexandria, mostly skin with minor dark spots was discernible. In addition, it was thought that fishes in open Egyptian lagoons such as those might be caught from Manzala and Burullus Lakes were also infested with parasites such as [100, 102, 173 and 174]. It seems that some organisms including man can have parasites and are common in fish [150]. At all events, pollution is detestable and leads to great problems. Finally, to be normal life in all aquatic habitats including Oceans, Seas, Rivers, Fish farms and Lakes; particularly the closed Lakes such as the previous-cited Abo-Zaabal and Qarun Lakes. More integrated studies are needed to separate anthropogenic and natural causes of Lake Qarun ecosystem changes [175]; but Lake Qarun needs practical root solutions to be more fruitful. Pollution should be prevented through stopping access of any waste not only to this Lake but also to all aquatic ecosystems then to be redirected away 'as previous-cited proposals' and try to renew its water, through digging a new canal follows the Mediterranean Sea; that can provide a lot of necessary resources for healthy environment free from pollution may lead to beautiful landscape and perhaps, other benefits in future 'God willing'.

ACKNOWLEDGEMENT

Gratitude to Allah, prayer and peace on messenger of Allah the prophet Mohammad, his family and companions. Thank each one offered any help. Allah knows best, praise be to Allah Lord of the worlds.

REFERENCES

1. Scholz, T., 1999. Parasites in cultured and feral fish. Veterinary Parasitology, 84: 317–335, (www.webpages.uidaho.edu/).

2. Kent, M.L., K.B. Andree, J.L. Bartholomew, M. El-Matbouli, S.S. Desser, R.H. Devlin, S.W. Feist, R.P. Hedrick, R.W. Hoffmann, J. Khattra, S.L. Hallett, R.J.G. Lester, M. Longshaw, O. Palenzeula, M.E. Siddall and C. Xiao, 2001. Recent advances in our knowledge of the Myxozoa. J. Eu. Mi., 48(4): 395-413.
3. Abdel-Ghaffar, F., A. El-Toukhy, S. Al-Quraishy, K. Al-Rasheid, A.S. Abdel-Baki, A. Hegazy and A.R. Bashtar, 2008. Five new myxosporean species (Myxozoa: Myxosporea) infecting the Nile tilapia *Oreochromis niloticus* in Bahr Shebin, Nile Tributary, Nile Delta, Egypt. Parasitology Research, 103: 1197-1205, (http://www.ncbi.nlm.nih.gov/pubmed/18688646).
4. El-Mansy, A., 2005. Revision of *Myxobolus heterosporus* Baker, 1963 (syn. *Myxosoma heterospora*) (Myxozoa: Myxosporea) in African records. Diseases of Aquatic Organisms, 63: 205-214.
5. Kaur, H. and R. Singh, 2010. A new myxosporean species Myxobolus sclerii sp. nov. and one known species M. stomum Ali *et al.* 2003 from two Indian major carp fishes. J. Parasit. Dis., 34(1): 33-39, (http://www.ncbi.nlm.nih.gov/pmc/).
6. Yokoyama, H., T. Danjo, K. Ogawa and H. Wakabayashi, 1997. A vital staining technique with fluorescein diacetate (FDA) and propidium iodide (PI) for the determination of viability of myxosporean and actinosporean spores. Journal of Fish Diseases, 20(4): 281-286, (http://onlinelibrary.wiley.com/).
7. Kallert, D.M. and M. El-Matbouli, 2008: Differences in viability and reactivity of actinospores of three myxozoan species upon ageing. Folia Parasitologica 55(2): 105-110, (www.ncbi.nlm.nih.gov/pubmed/18666413).
8. Alicata, J.E., O.L. Schattenberg and T.H. Hoxolulu, 1938. A case of intestinal heterophyidiasis of man in Hawaii. The Journal of the American Medical Association, 110: 1100-1, (https://books.google.com.eg/).
9. WHO, 1995. Control of food-borne trematode infections. WHO Tech. Rep. Ser. No., 849: 1-157.
10. Abou-Eisha, A.M., R.E. Saleh, H.M. Fadel, E.M. Youssef and Y.A. Helmy, 2008. Role of freshwater fishes in the epidemiology of some zoonotic trematodes in Ismailia province. SCVMJ, XIII (2): 653-676, (vet.scuegypt.edu.).
11. Yousef, M., N.S. Mansour, N.A. Hommouda, I.N. Awadalla and L.M. Boulos, 1981. Effect of freezing and grilling on *Pygidiopsis genata* metacercariae in tilapia. J. Egypt. Soc. Parasitol., 11: 425-428.
12. Raid, R.M., 1992. Helminth parasites of freshwater fish and their public health importance. M. V. Sc. The., Fish hygiene, Fac. Vet. Med., Assuit Univ., Egypt.
13. Abo-Esa, J.F.K., 1993. The role of fish in transmitting some parasites to man. M. V. Sc. Thesis, Zoonosis, Fac. Med., Alexandria University, Egypt.
14. Ramadan, R.A.M., 1994. Studies on internal parasites of some freshwater fishes. M. V. Sc. Thesis, Parasitology, Fac. Vet. Med., Suez Canal Univ., Egypt.
15. Eissa, I.A.M., M.A. Mona, M.E. Hala and S.M. Aly, 2001: Yellow grub disease among *Oreochromis niloticus* in relation to food quality and pathological alterations. SCVMJ, IV (2): 179-194.
16. Bazh, E.K.A., 2003. Epidemiological studies on some fish-borne parasites. M. V. Sc. Thesis, Parasitology, Tanta University, Egypt.
17. Saba, S.E.R., 2004. Some studies on parasites encystations of freshwater fishes. Ph. D. Thesis, Parasitology, Fac. Vet. Med., Zagazig University, Egypt.
18. Waikagul, J., 1991: Intestinal fluke infections in Southeast Asia. Southeast Asia J. Trop. Med. Public Health, 22: 158-162.
19. Yu, S.H. and K.E. Mott, 1994. Epidemiology and morbidity of food-borne intestinal trematode infections. Tropical Diseases Bulletin, 91: 125-152, (www.ncbi.nlm.nih.gov/pmc/articles/PMC2876759/).
20. Belizario, V.Y.Jr., M.J. Bersabe, W.U. de Leon, V.Y. Hilomen, G.V. Paller, A.D. de Guzman and M.G. Bugayon, 2001. Intestinal heterophyidiasis: an emerging (fish?)-borne parasitic zoonosis in southern Philippines. Southeast Asian J. Trop. Med. Public Health, 32(2): 36-42, (www.ncbi.nlm.nih.gov/).
21. Sukontason, K.L., K. Sukontason, S. Piangjai, S. Pungpak and P. Radomyos, 2001. Prevalence of *Opisthorchis viverrini* infection among villagers harboring Opisthorchis-like eggs. Southeast Asian J. Trop. Med. Pub. Heal., 32(2): 23-26.
22. Chai, J.Y. and S.H. Lee, 2002. Food borne intestinal trematode infections in the Republic of Korea. Parasitol. Int., 51: 129-154.

23. Cho, S.H., I.S. Kim, E.J. Hwang, T.S. Kim, B.K. Na and W.M. Sohn, 2012. Infection status of estuarine fish and oysters with intestinal fluke metacercariae in muan-gun, jeollanam-do, Korea. Korean J. Parasitol., 50(3): 215-220, (http://parasitol.kr/upload/pdf/kjp-50-215.pdf).
24. Mackiewicz, J.S. and M. Beverley-Burton, 1967. *Monobothrioides woodlandi* sp. nov. (Cestoda: Caryophilidea) from *Clarias mellandi* Boulenger (Cypriniformes: Clariidae) in Zambia, Africa. The Helminthological Society of Washington, 34(2): 125-128, (http://bionames.org/bionames-archive/issn/).
25. Ibrahim A.M., H.A. Taha and M.M. El-Naggar, 2008. Re-description of the cestode *Polyonchobothrium clarias* and its histopathological impact on the stomach of *Clarias gariepinus*. Egypt. J. Aquat. Biol. & Fish., 12(4): 165-174.
26. El-Mansy, A., Sh. Hamada, S. Hasan and D. El-Sarnagawy, 2011. Histopathology of tissues of farmed freshwater fish infested with different helminthes. Egypt. J. Aquat. Biol. & Fish., 15(1): 1-13.
27. El-Mansy, A., 2001. Effect of cooking and storage processes on the viability of *Myxobolus* sp. (Myxosporea, Myxobolidae) infesting fishes in the River Nile, Egypt. 2nd Conf. Exhibition for Life & Environ., Alexandria, Egypt, pp: 405-421.
28. El-Mansy, A. and E. Mohamed, 2003. Effect of *Allium cepa* (onion) and *Ambrosia maritima* (damsisa) extracts on some myxozoan parasites infecting fish. J. Egypt. Vet. Medical Association, 63(5): 325-338.
29. El-Mansy, A., K. Shalloof and S. Mekkawy, 2008. Efficiency of cumin black seed (*Nigella sativa*) and *Cinchona* sp. on *Polyonchobothrium clarias* (Cestode) *Paracamallanus cyathopharynx* (Nematode) *in vitro*. Egyptian Journal of Aquatic Research, 34(1): 202-208.
30. FDA (Food and Drug Administration), 1998. Fish & Fisheries Products Hazards & Controls Guide. 2nd ed. Washington, D.C.: FDA, Office of Seafood, P.276, (http://www.fda.gov/).
31. FDA, 2011. Parasites. Chapter 5: 91-98, (www.fda.gov/).
32. Wink, M., 2012. Medicinal plants: a source of anti-parasitic secondary metabolites molecules, 17:12771-12791, (Users/new/Downloads/molecules-).
33. Wikipedia, F., 2015. Fish diseases and parasites, (https://en.wikipedia.org/).
34. El-Mansy, A., 2002. Immature stages and re-description of *Henneguya suprabranchiae* (Myxosporea: Myxobolidae), an intestinal parasite of the catfish *Clarias gariepinus* in the River Nile, Egypt. Dis. of Aquat. Orga., 51(3): 179-186.
35. El-Mansy, A. and A.R. Bashtar, 2002. Histopathological and ultrastructural studies on *Henneguya suprabranchiae* Landsberg, 1987 (Myxosporea: Myxobolidae) parasitizing suprabranchial organ of the freshwater catfish *Clarias gariepinus* Burchell, 1822 in Egypt. Parasitol. Res., 88: 617-626.
36. Ba'albaki, M., 1985. Al-Mawrid. A modern English-Arabic Dictionary. Dar El-Elm Ll-Malayen, pp: 1118.
37. Wahid, L., 1990. Pharmacy under the demand. Medicaments by herbs and plants. Modern Office of University-Alexandria-Jeddah 'In Arabic'.
38. Wagnar, H. and N.R. Farnsworth, 1994. Economic and medical plant research. Academic press, London, U. K., 6: 329.
39. Abdel-Aziz, N., 2000. Medicaments by herbs. Scientific revision and comment by Prof. Dr. Ismaeil Owis Amin. El-Safa Library "In Arabic", pp: 31.
40. Buch, W.B., G.D. Osweiler and A.G. Van Gelder, 1982. Concepts and basic toxicology in clinical and diagnostic veterinary toxicology. Edited by Van Gelder AG, Kendall S.1.
41. Singh, S., V.K. Singh and D.K. Singh, 1997. Molluscicidal activity of some common spice plants. Biolological agriculture and Horticulture, 14: 237-249.
42. Benkeblia, N., 2004. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). LWT Food Sci. Technol., 37(2): 263-268, (http://dx.doi.org/10.1016/j.lwt.2003.09.001).
43. Ramos, F.A., Y. Takaishi, M. Shirotori, Y. Kawaguchi, K. Tsuchiya, H. Shibata, T. Higuti, T. Tadokoro and M. Takeuchi, 2006. Antibacterial and antioxidant activities of quercetin oxidation products from yellow onion (*Allium cepa*) skin. J. Agric. Food Chem., 54: 3551-3557, (http://dx.doi.org/).
44. Gabor, E.F., A. Sara and A. Barbu, 2010. The effects of some phytoadditives on growth, health and meat quality on different species of fish. Animal Science Biotechnology, 43(1): 61-65, (www.usab-tm.ro/).

45. Bello, O.S., B.O. Emikpe and F.E. Olaifa, 2012. The body weight changes and gut morphology of *Clarias gariepinus* juveniles in feeds supplemented with walnut (*Tetracarpidium concphorum*) leaf and onion (*Allium cepa*) Bulbs Resiues. Int. J. Morphol., 30(1): 253-257, (dx.doi.org/).
46. Bello, O.S., F.E. Olaifa, B.O. Emikpe, Emikpe and S.T. Ogunbanwo, 2012.The effect of walnut (*Tetracarpidium conophorum*) leaf and onion (*Allium cepa*) bulb residues on the tissue bacteriological changes of *Clarias gariepinus* juveniles. Bull. Animal Health Produ. Africa, 60(2): 205-212, (www.ajol.info/).
47. Saleh, N.E., F.R. Michael and M.M. Toutou, 2015. Evaluation of garlic and onion powder as phyto-additives in the diet of sea bass (*Dicentrarcus labrax*). Egyptian Journal of Aquatic Research, 41: 211-217, (http://www. sciencedirect.com/ science/article/pii/S1687428515000278, ac.els-cdn.com/).
48. Altaf, A., M.Z. Asmawi, A. Dewa and M.I. Umar, 2012. Sources and possible mechanisms of action of important phytoconstituents with cardiovascular properties. African Journal of Pharmacy and Pharmacology, 6(9): 563-580, (http://www.academicjournals.org/).
49. Abdulrahman, S., M.E. Harrison, K.J. Welham, M.A. Baldwin, J.D. Phillipson and M.F. Roberts, 1991. High-performance liquid chromatographic-mass spectrometric assay of high value compounds for pharmaceutical use from plant cell tissue culture: *Cinchona* alkaloids. J. Chromatogr. B Biomed. Sci. Appl., 562: 713-721, (http://www.academicjournals.org/).
50. Dreifus, L.S. and J. Morganroth, 1980. Antiarrhythmic agents and their use in therapy. Pharmacol. Ther., 9: 75-106, (http://www.academicjournals.org/).
51. Cooper, M.J., 2007. Assessment of efficacy of pharmacotherapy for ventricular tachycardia. Heart Lung Circ., 16: 170-175, (www.academicjournals.org/).
52. WHO, 2010. Guidelines for the treatment of malaria. Second edition, (https://en. wikipedia.org/wiki/Cinchona).
53. Ibn El-Qayem El-Gozy-Mohamad Ibn Aby Bakr Ibn Aiob, 2006. El-Teb El-Nabawy, El-Rehab Liberary "In Arabic" pp: 161.
54. Heiss, A., C. Krof, M. Soutag and A. Weber, 2011. Seed morphology of *Nigella* S. I. (Raunculaceae): Identification, diagnostic traits and their potential phylogenetic relevance. Int. J. Plant. Sci., 172(2): 267-284, (de.academic.ru/).
55. Gharby, S., H. Harhar, D. Guillaume, A. Roudani, S. Oulbaroud, M. Ibrahimi, M. Ahmad, Sh. Sultana, T. Ben Hadda, I. Chafchaouni-Moussaoui and Z. Charrouf, 2013. Chemical investigation of Nigella sativa L. seed oil produced in Morocco. J. of the Saudi Soci. of Agricul. Sciences, 14(2):172-177, (www.sciencedirect.com/).
56. Al-Jassir, M.S., 1992. Chemical composition and microflora of black cumin (*Nigella sativa*) seeds growing in Saudi Arabia, 45: 239-242, (globinmed.com/).
57. Nergiz, C. and S. Ötles, 1993. Chemical composition of *Nigella sativa* L. Food Chem., 48: 259-261, (sciencedirect.com/).
58. Hosseinzdeh, H. and S. Parvardeh, 2004. Anticonvulsant effects of thymoquinone, the major constituent of *Nigella sativa* seeds, in mice. Phytomedicine, 11(1): 56-64, (www.ncbi.nlm.nih.gov/pubmed/14971722).
59. El-Gazzar, M.E., R.E. Mezayen, J.C. Marecki, M.R. Nicolls, A. Canastar and S.C. Dreskin, 2006.Anti-inflammatory effects of thymoquinon in a mouse model of allergic lung inflammation. International Imunopharmacology J. of Pharmaceutical and Biomed. Analysis, 6: 1135-1142, (www.globinmed.com/).
60. D'Antuono L.F., A. Moretti and A.F. Lovato, 2002. Seed yield, yield components, oil content and essential oil content and composition of *Nigella sativa* L. and *Nigella damascena* L. Industrial crops and products, 15(1): 59-69, (nara.ac.rs/).
61. Sayed, M.D., 2002. Traditional medicine in health care. Journal of Ethnopharmacology, 2:19-22, (www.globinmed.com/).
62. Atta-ur-Rahman, S. Malik, S.S. Hasan, M.I. Choudhary, C-Z. Ni and J. Clardy, 1995. Nigellidine. A new indazole alkaloid from the seeds of *Nigella sativa*. Tetrahedron Letters. Pub.: Elsevier, 36(12): 1993-1996, (www.sciencedirect.com).
63. Badary, O.A., O.A. Al-Shabanah, M.N. Nagi, A.M. Al-Bekairi and M.A. Elmazar, 1998. Acute and sub-chronic toxicity of thymoquinone in mice. Drug Dev. Res., 44: 56-61, (http://onlinelibrary.wiley.com/doi/).
64. Steinmann, A., M. Schatzle, M. Agathos and R. Beit, 1997. Allergic contact dermatitis from black cumin (*Nigella sativa*) oil after tropical use. Contact dermat., 36: 268-269, (file:///C:/Users/new/Downloads/3177-11859-1-PB.pdf).

65. Ozbek, H., M. Ozturk, E. Ceylan and Z. Yener, 2004. Determination of lethal dose of volatile and fixed oils of several plants. East. J. of Medicine, 9(1): 4-6.
66. Mohamadin, A.M., B. Sheikh, A.A.A. El-Aal, A.A. Elberry and F.A. Al-Abbasi, 2010. Protective effects of *Nigella sativa* oil on propoxur-induced toxicity and oxidative stress in rat brain regions. Pesticide biochemistry and physiology, 98(1): 128-134, (www.ijppsjournal.com/Vol5Suppl3/7604.pdf).
67. Vahdati-Mashhadian, N., H. Rakhshandeh and A. Omidi, 2005. An investigation on LD_{50} and subacute hepatic toxicity of *Nigella sativa* seed extracts in mice. Pharmazie, 60(7): 544-7, (http://www.ncbi.nlm.nih.gov/).
68. Padhye, S., S. Banerjee, A. Ahmad, R. Mohammad and F. Sarkar, 2008. From here to eternity-the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond. Cancer Ther., 6(b): 495-510, (ncbi.nlm.nih.gov/).
69. El-Bahai, M.N., M.T. Al-Hariri, T. Yar and A.O. Bamosa, 2009. Cardiac inotropic and hypertrophic effects of *Nigella sativa* supplementation in rats. International J. of Cardiology, 131: 115-117, (www.academicjournals.org/).
70. Dehkordi, F.R. and A.F. Kamkhah, 2008. Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension. Fundamental and Clinical Pharmacology, 22: 447-452, (www.academicjournals.org/).
71. Suddek, G.M., 2010. Thymoquinone-induced relaxation of isolated rat pulmonary artery. J. Ethnopharm., 127: 210-214, (www.academicjournals.org/).
72. Khalil, W.K.B., F.K.N. Abd El-Gawad, N.B. Abd El-Rahman and M.A. Abd El-Wahab, 2011. Global Veterinaria, 7(3): 283-293.
73. Ji, S., 2013. Black Seed is 'The Remedy for Everything but Death', (http://www.gre enmedinfo.com, www.iamthesoulman.com/).
74. Forouzanfar, F., B.S.F. Bazzaz and H. Hosseinzadeh, 2014. Black cumin (*Nigella sativa*) and its constituent (thymoquinone): a review on antimicrobial effects. Iran. J. Basic Med. Sci., 17(12): 929-938, (www.ncbi.nlm.nih.gov/).
75. Tembhurne, S.V., S. Feroz, B.H. More and D.M. Sakarkar, 2014. A review on therapeutic potential of *Nigella sativa* (kalonji) seeds. Journal of Medicinal Plants Research 8(3): 167-177, (http://www.academicjournals.org/).
76. Aljabre, S.H.M., O.M. Alakloby, M.A. Randhawa, 2015: Dermatological effects of *Nigella sativa*. Journal of Dermatology & Dermatologic Surgery, 19(2): 92-98, (http://www.sciencedirect.com/science/article/).
77. Zaki, M.S. and O. Fawzi, 2015. Immunological control of fish diseases. Life Science Journal, 12(2): 112-114, (http://www.lifesciencesite.com/).
78. SCFCAH (The Standing Committee on the Food Chain and Animal Health), 2011. Guidance on viable parasites in fishery products that may represent a risk to the health of the consumer. Section Biological Safety of the Food Chain Point D of Chapter 3 of Annex III to Regulation (EC) No 853/2004, (http://ec.europa.eu/).
79. FDA, 2013. Processing parameters needed to control pathogens in cold smoked fish. Chapter V. Potential hazards in cold-smoked fish: parasites, (http://www.fda.gov/Food/FoodScienceResearch/ucm092182.htm).
80. FSAI (Food Safety Authority of Ireland), 2015. Fish Parasites, (www.fsai.ie/faq/fish_ parasites.html).
81. Klimpel, S., F. Abdel-Ghaffar, K.A. Al-Rasheid, G. Aksu, K. Fischer, B. Strassen and H. Mehlhorn, 2011. The effects of different plant extracts on nematodes. Parasitol. Res., 108(4): 1047-54, (www.ncbi.nlm.nih.gov/).
82. El-Mansy, A. and A.R. Bashtar, 2002. Effect of parasitism on the total muscle protein of the catfish *Clarias gariepinus* Burchell, 1822 collected from agricultural drains in Qalioubia province and the River Nile at Giza. 9th Conf., Egypt. J. Zool., 38: 327-348.
83. Eissa, A.E., I.M.K. Abu Mourad and T. Borhan, 2006. A contribution on Myxosoma infection in cultured *Oreochromis niloticus* in Lower Egypt. Nature and Science, 4(4): 40-46, (www.sciencepub.net/nature/).
84. Kostoingue, B., C. Diebakate, N. Faye, B.S. Tonguebaye, 2001. Presence of Myxosporidea (Myxozoa: Myxosporea) of the genus *Henneguya* Thélohan, 1892 in freshwater fishes from Chad (Central Africa). Acta Protozoologica, 40:117-123, (http://www1.nencki.gov.pl/pdf/).
85. Tossavi, N.D., A. Gbankoto, A. Yessoufou, M.L. Escande, G. Dimitri, A.C. Ribou, K. Moutaïrou and G.N. Sakiti, 2015. Histopathological and ultrastructural studies of a *Henneguya* species (Myxozoa: Myxosporea) infesting the intestine of *Clarias gariepinus* from Benin (West Africa). Parasitology Research, 114(3): 861-72, (www.researchgate.net/, PubMed).

86. Abdel-Baki, A.S., T.h. Sakran and E. Zayed, 2011. Validity, impacts and seasonal prevalence of *Henneguya* species infecting the catfish *Clarias gariepinus* from River Nile, Egypt. Parasitol. Research, 109(1): 119-23, (www.ncbi.nlm.nih.gov/).
87. Ryang, Y.S., C.Y. Lee, K.J. Lee, S.H. Lee and J.Y. Chai, 1999. An incidental case of human *Heterophyes nocens* infection diagnosed by sectional morphology in a biopsy specimen of the small intestine. The Korean Journal of Parasitol., 37(3): 189-194, (http://parasitol.kr/journal/).
88. El-Shahawi, G.A.M., 1983. Studies on some fishes as intermediate hosts of some birds and animals. M. Sc. Thesis Fac. Sci. El-Minia Univ., Egypt.
89. Mahdy, O.A., M.A. Essa and M. El-Easa, 1995. Parasitological and pathological studies on the heterophyid infection in tilapia species from Manzala Lake, Egypt. Egypt. J. of Comp. Pathol. & Clin. Pathol., 8(2): 131-145, (https://scholar.google.com/citations?user=gRyOmJgAAAAJ&hl=en).
90. El-Garhy, M.A., 2003. A survey on the parasites of some freshwater fishes from the River Nile. J. Egypt. Ger. Soc. Zool., 40D: 115-137.
91. Elnemaki, F.A., 2003. Intensity and density of *Pyigidiopsis summa* and *geneta* and their effect on some of the serum constituents of tilapia sp. Egypt. J. Aquat. Biol. & Fish., 7(4): 109-124.
92. Abdel-Meguid, M.A., 2004. The intensity of the encysted metacercariae of *Posthodiplostomum minimum* and their lesions on the liver of bluegill sunfish *Lepomis macrochirus*. Egypt. J. Aquat. Biol. & Fish., 8(2): 115-126.
93. Matter, S.A.I., 2005. Effect of the parasitic infestation on the reproductive process of the River Nile fishes in Egypt. M. Sc. The., Fac. of Science (Girls) Al-Azhar Univ., Egypt.
94. Diab, A.S., Z.M. El-Bouhy, S.F. Sakr and Y.M. Abdel-Hadi, 2006. Prevalence of some parasitic agents affecting the gills of some cultured fishes in Sharkia, Damietta and Fayium governorates. Zagazig Univ. Fac. of Vet. Med., CLAR Abbassa, Egypt, (www.slidefinder.net/).
95. El-Mansy, A., 2009. A report on parasitic infestation of fishes caught from Abo-Zaabal Lake, northeastern of Cairo, Egypt, within the project of the environmental evaluation of Abo-Zaabal Lake and Uses it as Fish Farms, pp: 21.
96. Chung, D.I., C.H. Moon and H.H. Kong, 1995. The first human case of *Clinostomum complanatum* (Trematoda: Clinostomatidae) infection in Korea. Korean J. Parasitol., 33(3): 219-223.
97. Abd-Ellah R.G., 2003. On physical limnology of Abu Za'bal Lakes, Egypt. Bull. Nat. Inst. Oceanogr. & Fish., A.R.E., 29: 461-471.
98. El-Mansy, A., 2005. Pathological conditions in *Tilapia zillii* Gervais, 1848 caught from Abo-Zaabal Lake, northeastern of Cairo, Egypt. Journal of Egyptian Academic Society for Environmental Development, 6(2): 57-88.
99. El-Mansy, A., 2008. A new coccidian *Eimeria samehi* n. sp. (Protozoa: Eimeriidae) isolated from the swim bladder of *Tilapia zillii* Gervais, 1848 from the River Nile, Egypt. Egyptian Journal of Zoology, 51: 53-61.
100. El-Mansy, A., 2008. New exogenous stages of oocysts, sporocysts and sporozoites of *Goussia cichlidarum* Landsberg and Paperna, 1985 (Sporozoa: Coccidia) and impact of endogenous stages on the swim bladder of tilapias in Egypt. Parasitology Research, 202: 233-241.
101. El-Mansy, A., 2009. On the occurrence of adult females of *Lernaea* species (Crustacea: Copepoda) parasitic on goldfish *Carassius auratus* Linnaeus in some commercial aquaria in Egypt. Egypt. J. Aquat. Biol. & Fish., 13(1): 7-36.
102. El-Mansy, A., 2007. New parasitic forms described as a characteristic group in the kingdom Protista found in fishes from different sites in Egypt. African Journal for Biological Sciences, 3(3): 23-30.
103. El-Gohary, A.H. and I.A. Samaha, 1997. *Oreochromis* spp. and *Clarias lazera* as a source of transmitting encysted metacercariae to man. AJAS 10(4): 439-443, (www.ajas.info/).
104. El-Seify, M.A.O., A.Y. Desouky and E.K. Bazh, 2005. Epidemiological studies on some fish-borne parasites. Department of Parasitology, Faculty of Vet. Med., Kafr El-Sheikh, Tanta Univ., Egypt. Vet. Med. Soc. Paras. J., II (3): 570-592, (http://ag.arizona.edu/azaqua/ista/ista6/ista6web/pdf/199.pdf).
105. El-Mansy, A., 2009. A report on *Clinostomum metacercariae* infested tilapias from the River Nile at El-Menia Province, Egypt, pp: 7.

106. Reda, R.M., G.A. El-Nobi, M.E. Hassanin, M.A. El-Hady and Z.M. El-Bouhy, 2010. Study on some encysted metacercaria "digenetic trematodes" affecting gills of *Oreochromis niloticus*. 10th Sci. Vet. Med. Zagazig Conference, Luxor, Egypt, (www.academia.edu/).
107. Khalil, M., 1937. The life history of human treated parasite *Heterophyes heterophyes*. C. R. Congress. Int. 2001. Lispon., pp: 1889-1899.
108. Martin, W.E., 1959. Egyptian heterophyid trematodes. Trans. Am. Microsc. Soc., 78: 78-181.
109. Esa, M., 1988. Water reuse. Acad. Scient. Res., pp: 145.
110. Hamed, M.G.E. and A.N. Elias, 1970. Effect of food processing methods up on survival of trematode *Heterophyes* spp. in flesh of mullets caught from brackish Egyptian waters. J. Food Sci., 35(4): 386-388, (www.ajas.info).
111. Mackiewicz, J.S. and M. Beverley-Burton, 1967. *Monobothrioides woodlandi* sp. nov. (Cestoda: Caryophilidea) from *Clarias mellandi* Boulenger (Cypriniformes: Clariidae) in Zambia, Africa. The Helminth. Soci. of Washington, 34(2): 125-128, (http:bionames.org/bionames-archive/).
112. Nassef, T.M.N., 1988. Morphological studies on some gastrointestinal parasites of freshwater fishes. M. Sc. Thesis, Fac. Vet. Med. Cairo Univ., Egypt.
113. El-Ganiny, S., 1995. Studies on the parasites of the Nile fishes at El-Minia Governorate. Ph. D. Thesis Fac. of Sci. El-Minia Univ., Egypt.
114. Hamada, Sh.F. and El-Naggar, M.M. 2003. Surface topography, mode of attachment and histopathology of *Monobothriodes chalmersius* (Cestoidea: Caryophyllidea) from *Clarias gariepinus* in Egypt. 10th conference Feb. 2003. Egypt. J. Zool., 40: 185-201.
115. Woodland, W.N.F., 1924. On a new species of the cestodearian genus *Caryophyllaeus* from an Egyptian siluroid. Proc. Zool. Soc. London, 2: 529-532 (source: Hamada and El-Naggar, 2003).
116. Hunter, G.W.III., 1930. Studies on the Caryophyllaeidae of North America. III Biol. Monog., 11: 1-186, (source: Hamada and El-Naggar, 2003).
117. Fuhrmann, O. and J.C. Baer, 1925. Zoological results of the third Tanganyika expedition Report on Cestoda. Proc. Zool. Soc. London, pp: 79-100.
118. Hunt, P., 2013. Marine Aquarium Basics, Part 1: Water and Salt (Full Article). (http://www.tfhmagazine.com/details/articles/marine-aquarium-).
119. MacKenzie, K. and C. Kalavati, 2014. Myxosporean parasites of marine fishes: their distribution in the world's Oceans. Parasitology, 141(13): 1709-17, (http://www.ncbi.nlm.nih.gov/pubmed/).
120. Abdel-Ghaffar, F., K. Morsy, S. El-Ganainy, M. Ahmed, Ahmed, S. Gamal, A.R. Bashtar, S. Al-Quraishy and H. Mehlhorn, 2015. Twelve myxosporean species of the family Myxobolidae infecting freshwater fishes of the River Nile, Egypt, with the description of four novel species. Parasitology Research, 114(8): 2985-98, (www.ncbi.nlm.nih.gov/pubmed/).
121. Fan, P.C., 1998. Viability of metacercariae of Clonorchis sinensis in frozen or salted freshwater fish. Int. J. Parasitol., 28: 603-5.
122. CDC (Center for Disease Control), 2013. Illness-Causing Fish Parasites (Worms), (www.bccdc.ca/NR/rdonlyres/).
123. El-Matbouli, M. and R.W. Hoffman, 1991. Effects of freezing, aging and passage through the alimentary canal of predatory animals on the viability of *Myxobolus cerebralis* spores. J. Aquatic Animal Health, 3: 260-262.
124. Nehring, R.B., G. Schisler, L. Chiaramonte, A. Horton and B. Poole, 2015. Assessment of the Long-Term viability of the myxospores of *Myxobolus cerebralis* as determined by production of the actinospores by *Tubifex tubifex*. J. Aquat. Anim. Health, 27(1): 50-6, (www.ncbi.nlm.nih.gov/pubmed/25706032).
125. Mahmoud, A.F., 1983. Parasitic infections. In: Nelson textbook of pediatrics. WB Saunders Company, Philadelphia. WB Saunders Company, Philadelphia, (http://www.sciencepub.net/academia/).
126. EL-Leathy, A.M., 1997. Prevalence of parasites of public health importance in some freshwater fishes. M. V. Sc. The., Fac. of Vet. Med. Zagazig Univ, Egypt.
127. EL-Sherbiny, M.M.T., 1988. Parasites in market fish and then-public health importance. M. V. Sc. Thesis, Fac. of Vet. Med. Cairo Univ, Egypt.
128. Ara, N., S.A.R. Choudhury and R. Amin, 2005. *In vitro* antimicrobial activity of the volatile oil of *Nigella sativa* Linn. seeds. The J. of Teachers Association, 18(2): 109-112, (file:///C:/Users/new/Downloads/3177-11859-1-PB.pdf).

129. Mehlhorn, H., S. Al-Quraishy, K.A.S. Al-Rasheid, A. Jatzlau and F. Abdel-Ghaffar, 2011. Addition of a combination of onion (*Allium cepa*) and coconut (*Cocos nucifera*) to food of sheep stops gastrointestinal helminthic infections. Parasitol. Res., 108(4): 1041-6, (www.ncbi.nlm.nih.gov/ pubmed/21120531).
130. Jatzlau, A., F. Abdel-Ghaffar, G. Gliem and H. Mehlhorn, 2014. Nature helps: food addition of micronized coconut and onion reduced worm load in horses and sheep and increased body weight in sheep. Parasitol. Res., 113(1): 305-10, (http://www.ncbi.nlm.nih.gov/pubmed/24271156).
131. SAbdel-Hafeez, E.H., A.K. Ahmad, N.H. Abdelgelil, M.Z. Abdellatif, A.M. Kamal and R.M. Mohamed, 2015: *In vitro* effect of some Egyptian herbal extracts against *Blastocystis hominis*. J. Egypt. Soc. Parasitol. (JESP), 45(1): 93-100, (http://www.ncbi.nlm.nih.gov/pubmed/26012223).
132. Apines-Amar, M.J.S., E.C. Amar, J.P. Faisan, V. Rolando, R.V. Pakingking and S. Satoh, 2012. Dietary onion and ginger enhance growth, hemato-immunological responses and disease resistance in brown-marbled grouper, *Epinephelus fuscoguttatus*. AACL Bioflux, 5(4): 231-239.
133. Cho, S.H. and S.M. Lee, 2012. Onion powder in the diet of the olive flounder, *Paralichthys olivaceus*: effects on the growth, body composition and lysozyme activity. J. World Aquaculture Society, 43: 30-38, (http://dx.doi.org/).
134. Porritt, M., 2009. *Cryptocaryon irritans*. Reef Culture Magazine, (https://en.w ikipedia.org/wiki/ Quinine#cite_note-33).
135. Wikipedia, F., 2015a. Quinine, (https://en.wikipedia.org/wiki/Quinin).
136. Aschalew, A. and D. Tadesse, 2016. Current status of malaria in Ethiopia: evaluation of the burden, factors for transmission and prevention methods. Acta Parasitologica Globalis, 7(1): 01-06, (http://www.idosi.org/apg/7(1)16/1.pdf).
137. Griffing, S.M., P.L. Tauil, V. Udhayakumar and L. Silva-Flannery, 2015. A historical perspective on malaria control in Brazil. Mem Inst Oswaldo Cruz, Rio de Janeiro, 110(6): 701-718, (online | memorias.ioc.fiocruz.br).
138. Massuod, W.A.M., S.M. Raqib, A. Budiharjo and E. Mahajoena, 2014. Larvicidal potentiality of the bandotan (*Ageratum conyzoides*) leaves for controlling the three important species of mosquitoes (*Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles maculatus*). IJCBS, 1(6): 33-43, (http://www.ijcbs.org/Document/September/ijcbs.org%2027.pdf).
139. Sheir, S.K., O.A.M. Maghraby, A.H. Mohamed, G.Y. Osman and S.A. Al-Qormuti, 2015. Immunomodulatory and ameliorative role of *Nigella sativa* oil on *Schistosoma mansoni* infected mice. Canadian Journal of Pure and Applied Sciences, 9(2): 3345-3355, (http://www.cjpas.net/wp-content/uploads/pdfs).
140. Tembhurne, S.V., S. Feroz, B.H. More and D.M. Sakarkar, 2014. A review on therapeutic potential of *Nigella sativa* (kalonji) seeds. Journal of Medicinal Plants Research, 8(3): 167-177, (http://www.academicjournals.org/).
141. Chen, C., D.M. Zuckerman, S. Brantley, M. Sharpe, K. Childress, E. Hoiczyk and A. R. Pendleton, 2014. *Sambucus nigra* extracts inhibit infectious bronchitis virus at an early point during replication. BMC. Vet. Res., 10: 24, (www.ncbi. nlm.nih.ngov/), Posted on June 1, 2015 by Dr. Gary Pack, (www.rainbow.coop).
142. Shalaby, H.A., N.M.T. Abu El-Ezz, T.K. Farag and H.A.A. Abou-Zeina, 2012. *In vitro* efficacy of a combination of Ivermectin and *Nigella sativa* oil against helminth parasites. Global Vet., 9(4): 465-473, (www.lifesciencesite.com).
143. Arslan, S. and L. Zakia, 2015. GC-MS characterisation and antibacterial activity evaluation of *Nigella sativa* oil against diverse strains of *Salmonella*. Nat. Prod. Res., 29(5): 447-51, (www.ncbi.nlm.nih.gov/ pubmed/25147934).
144. Mohammed, H.H. and C.R. Arias, 2015. Protective efficacy of *Nigella sativa* seeds and oil against columnaris disease in fishes. Journal of Fish Diseases (http://onlinelibrary.wiley.com/doi/10.1111/jfd.12402/pdf).
145. Ebru, U., U. Burak, S. Yusuf, B. Reyhan, K. Arif, T.H. Faruk, M. Emin, K. Aydin, II. Atilla, S. Semsettin and E. Kemal, 2008. Cardioprotective effects of *Nigella sativa* oil on cyclosporine a-induced cardiotoxicity in rats. Basic & Clinical Pharmacology and Toxicology, 103 (6): 574-580, (http://onlinelibrary.wiley.com/, www.academicjournals.org/).

146. El-Barbary, M.I. and A.I. Mehrim, 2009. Protective effect of antioxidant medicinal herbs, rosemary and parsley, on subacute aflatoxicosis in *Oreochromis niloticus*. Journal of Fisheries and Aquatic Science, 4(4): 178-190, (http://scialert.net/abstract/?doi=jfas.2009.178.190).
147. Maraqa, A., N.F. Alsharoa, H. Farah, W.M. Albjeirami, A.K. Shakya and A.J. Sallal, 2007. Effect of *Nigella sativa* extract and oil on aflatoxin production by *Aspergillus flavus*. Turk. J. Biol., 31: 155-159, (source: El-Nagerabi *et al*., 2013).
148. El-Nagerabi, S.A.F., A.E. Elshafie, S.S. AlKhanjari, S.N. Al-Bahry and M.R. Elamin, 2013. The potential of baobab (*Adansonia digitata* L.) extracts as biocontrol on the growth and aflatoxin production by *Aspergillus flavus* and *A. parasiticus*. J. of Food Res., Published by Canadian Center of Science and Education, 2(3): 93-103, (file:///C:/Users/new/Downloads/).
149. Salgueiro, M., M. Zubillaga, A. Lysionek, M.I. Sarabia, R. Caro, T. De Paoli, A. Hager, R. Weill and J. Boccio, 2000. Zinc as an essential micronutrient: a review. Nutrition Research, 20(5): 737-755, (http://www.lifesciencesite.com).
150. El-Mansy, A., 2003. A prepared report in Arabic. Egypt. El-Elm Journal, 318: 44-45.
151. Van Muiswinkel, W.B., G.F. Wiegertjes and R.J.M. Stet, 1999. The influence of environmental and genetic factors on the disease resistance of fish. Aquaculture, 172: 63-110.
152. Audicana, L., M.T. Audicana, L. Fernández de Corres and M.W. Kennedy, 1997. Cooking and freezing may not protect against allergenic reactions to ingested *Anisakis simplex* antigens in humans. Veterinary Record, (http://seafood.oregonstate.edu/.pdf).
153. Mahmoud, A.M., S.M. Aly, A.S. Diab and G. John, 2009. The role of ornamental goldfish *Carassius auratus* in transfer of some viruses and ectoparasites to cultured fish in Egypt: comparative ultra-pathological studies. African Journal of Aquatic Science, 34(2): 111-121, (www.academia.edu/).
154. Durborow, R.M., 2003. Protozoan parasites. South Regional Aquaculture Center Publication No. 4701, (http://www.academia.edu/).
155. El-Mansy, A. and F. Abdel-Ghaffar, 2003. Tilapian proliferative kidney disease (TPKD) and a diagnostic evidence for the presence of myxosporean parasites. J. Egypt. Ger. Soc. Zool., 40D: 139-159.
156. Abou El-Gheit, E.N., M.S. Zaki, A.S. Abo El-Ezz and H.A. El-Sherei, 2001. Vertebral column curvature syndrome in common carp (*Cyprinus Carpio* L.) fish. J. Egypt. Vet. Med. Asso., 61(5): 57-69, 'source: Zaki *et al*., 2009'
157. El-Mansy, A.I.E. and K.A.Sh. Shalloof, 2015. A case of deformation in a fish from Lake Manzala, Egypt. Global Veterinaria, 14(5): 679-685, (http://www.idosi.org/gv/gv14(5)15/8.pdf, http://www.researchgate.net).
158. Abdel-Satar, A.M., M.E. Gohar and M.F. Sayed, 2010. Recent Environmental changes in water and sediment quality of Lake Qarun, Egypt. J. of Fisheries and Aquatic Science, 1-14, (http://docsdrive.com/pdfs/academic journals/jfas/).
159. Khalaf-Allah, H.M.M., 2014. Seasonal distribution and abundance of small fish in the south coast of Lake Qarun, Egypt. World Journal of Fish and Marine Sciences, 6(1): 109-118, (http://www.idosi.org/wjfms/wjfms6(1)14/14.pdf).
160. Ali, F.Kh., S.A. El-Shafai, F.A. Samhan and W.K.B. Khalil, 2008. Effect of water pollution on expression of immune response genes of *Solea aegyptiaca* in Lake Qarun. African J. Biotechnol., 7(10): 1418-1425, (www.ajol.info/).
161. Sabae, S.Z. and S.A. Rabeh, 2000. Bacterial indices of sewage pollution in Lake Qarun, Fayoum, Egypt. Egypt. J. Aquat. Biol. Fish., 4(3): 103-116, 'source: Ali, *et al*., 2008'.
162. Ali, M.H.H. and M.R.A. Fishar, 2005. Accumulation of trace metals in some benthic invertebrate and fish species relevant to their concentration in water and sediment of Lake Qarun, Egypt. Egypt. J. Aquat. Res., 31(1): 289-301, (http://www.oceandocs.org/bitstream/handle/1834/1215/TEXT.PDF).
163. Shalloof, K.A. Sh, 2009. Some observations on fisheries biology of *Tilapia zillii* (Gervais, 1848) and *Solea vulgaris* (Quensel, 1806) in Lake Qarun, Egypt. World J. Fish and Marine Sci., 1(1): 20-28, (http://citeseerx.ist.psu.edu/).
164. Mohamed, F.A.S., 2009. Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. World Journal of Fish and Marine Sciences, 1(1): 29-39, (http://idosi.org/wjfms/wjfms1(1)09/4.pdf).
165. Tayel, S.I., S.A. Ibrahim and S.A. Mahmoud, 2013. Histopathological and muscle composition studies on *Tilapia zillii* in relation to water quality of Lake Qarun, Egypt. Journal of Applied Sciences Research, 9(6): 3857-3872, (http://www.aensiweb.com/old/jasr/jasr/2013/3857-3872.pdf).

166. Zaki, M.S., S.O. Mostafa, O.M. Fawzi, M. Khafagy and F. S. Bayumi, 2009. Clinicopathological, biochemical and microbiological change on grey mullet exposed to cadmium chloride. American-Eurasian J. Agric. & Environ. Sci., 5(1): 20-23, (http://www.idosi.org/aejaes/jaes5(1)/4.pdf).
167. Moustafa, M., L.A. Mohamed, M.A. Mahmoud and M.Y. El-Gendy, W.S. Soliman, 2010. Bacterial infections affecting marine fishes in Egypt. Journal of American Science, 6(11): 603-612, (http://scholar.cu.edu.eg/).
168. Abou El-Gheit, E.N., M.H. Abdo and S.A. Mahmoud, 2012. Impacts of blooming phenomenon on water quality and fishes in Qarun Lake, Egypt. International Journal of Environmental Science and Engineering (IJESE), 3: 11- 23, (http://www.pvamu.edu/texged).
169. Ali, M.A., 2009. Light and scanning electron microscopy of *Dipartiella simplex* (Raabe, 1959) Stein, 1961 infesting common sole (*Solea vul*garis) and goby fish (*Gobius* sp.) in Lake Qarun, Egypt with, a new character to the genus. Acta Protozool., 48: 265-270, (https://www.wuj.pl/UserFiles/ File/acta%20 protozool ogica/Acra%20 Protozoologica%2048/protozoologica_48_3_6.pdf).
170. Al-Bassel, D.A., A.S. Abdel-Baki and M.S. Atwa, 2007. Trichodinid ectoparasites (Ciliophora: Peritrichia) of *Mugil cephalus* Linnaeus, 1758 from Lake Qarun, Egypt. Egypt. J. Aquat. Biol. & Fish., 11(4): 13-26, 'source: Ali, 2009'.
171. Saoud, M.F.A., A.A. Ashour, M.M. Ramadan and D.A.M. Lamloom, 1990. Helminth parasites of fishes from two inland lakes in Egypt. Jpn. J. Parasitol., 39: 267-276, 'source: Ali, 2009'.
172. Kotb, H.L., 2015. Pathological study on parasitic infection in fish from Fayoum governorate. M. V. Sc., Department of Pathology, Faculty of Veterinary Medicine, Cairo Univ., Egypt. (http://eulc.edu.eg/eulcv5/Libraries/Thesis/).
173. El-Mansy, A., 2008. Myxosporidia (Myxozoa: Myxosporea) infecting fishes from different habitats in Egypt. Egyptian German Union of Life Sciences, Invertebrate Zoology and Parasitology, 55D: 127-135.
174. El-Mansy, A., 2008. A new finding of *Dermocystidium*-like spores in the gut of cultured *Oreochromis niloticus*. Global Veterinaria, 2(6): 369-371.
175. El-Shabrawy, G., M. Gohar, M. Germoush, E. Anufriieva and N. Shadrin, 2014. Long-term changes in the saline Lake Qarun (Egypt). Acta Geologica Sinica (English Edition), 88(supp. 1): 180-182, (http://www.geojournals.cn/dzxben/ch/ index.aspx).