

Study of prevailing external parasitic diseases in cultured freshwater tilapia *Oreochromis niloticus* Egypt

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Abstract: The present study was carried out on 1200 specimens of cultured Nile Tilapia fishes (600 fingerlings and 600 adult fish) of different size and body weight. They were randomly collected seasonally from Kafr El- Sheikh, Fayom and Alsharkaia (Al abbassa region) Governorate cultured fish farms. The clinical signs of most examined fishes revealed some abnormalities on the external body surface of heavily naturally infested Tilapia fishes, represented as asphyxia, some aggregated on the surface, accumulated at the water inlet of the pond. The postmortem findings of investigated fish revealed the presence of excessive mucus secretions, white spots were observed in different parts of the body in some infested fishes. Marbling appearance with numerous white dots in some gill filament of infested fishes. Some external protozoa (*Trichodina fultoni*, *Chilodenella hexatia*, *Ichthyoboda necatrax*) and *Ichthyophtherious multiphilus*), monogentic trematod (*Cichlidigurus tilapia*) and crustacean parasite (*Ergasilus ovitus*) were isolated, identified and recorded. The highest infestations rate of infestations in adult fish was 35% while in fingerlings was 12%. Also, the highest infestations rate were in Kafr El- Sheikh 20% followed by Alsharkaia 15% and lowest rate were in Al Fayoum 12%. The highest infestations at adult *O. niloticus* and fingerlings prevalence possessed in summer season 17.5 %, followed by spring season 12.5 % and autumn 10 % while winter season occupied the last position 7 %. In addition, the histopathological alterations in the skin of infested fishes were recorded.

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1. Introduction

Fish become the hope for overcoming protein shortage problem all over the world. Aquaculture is necessary to increase fish production, through increasing the density of cultured fish population. In recent time, tilapias have been found to be most susceptible to parasitic diseases due to high population density predisposes of many parasitic diseases. Among of which causing higher losses in fish ponds, reducing growth rate and marketing value (Eissa *et al.*, 2010).

In Egypt, there are a long periods of optimum warm weather that enable external parasites for more production and cause bad effects on fish (Eissa *et al.*, 2013). Moreover, ectoparasitic infestation causes mass a great economic losses such mortalities due to the breakdown of epithelial integrity. Furthermore, open lesions in the dermis allow secondary infections to establish and leading to high mortality (Noga, 2010).

An external protozoal and monogentic infestation causes mass mortality and great economic losses. These cause together with anorexia are leading to high mortality (Woo, 2006). Tilapia species infected with "Ich" showed an aggregation on the apical and basal parts of the ciliated and non-ciliated endothelial lining the gill epithelium Osman *et al.* (2009). Also, parasitic crustaceans are

increasingly serious problem in cultured fish which can be seen by the naked eyes as they attach to the gills, body and fins of the host (El-Moghazy, 2008 and Alvarez-Pellitero, 2008). Water temperature has a great influence on the initiation of parasitic fish diseases. The immune system of Nile tilapia species has an optimum performance at water temperatures of about 15°C (Abbas *et al.*, 2008).

Parasites of the gills can cause irritation, leading to hyperplasia and increased mucous production, which result in decreased respiration and ion exchanging Capabilities (Noor El Deen, 2007 and Noor El Deen *et al.*, 2013).

The present study was focused on the isolation, identification and taxonomy of more prevalent external parasites with detection of the non-conventional methods of treatment.

2. Material and Methods**Fish:**

A total of 1200 specimens of *Oreochromis niloticus* (*O. niloticus*) (adult female of *O. niloticus* and fingerlings monosex *O. niloticus*) of different size and body weight were randomly collected from Kafr El- Sheikh, Al Fayom and Alsharkaia (Al abbassa region) governorate cultured fish farms. The collected fishes were obtained allover different seasons. The collected fish were 65±15 g

body weight. They were subjected to the clinical examination for detection of the prevalent infestations.

Clinical Examination:

Alive fish were clinically examined for the general behaviors, changes in colour, respiratory manifestation, feeding and any clinical abnormalities on the external body surface (skin, gills, eyes and mouth), also any external growth lesions like wounds, petechial hemorrhage, ulcers, slimness, sloughing of scales from the skin or eroded fins and cysts for detection of any parasites visible by naked eye according to the methods described by Austin and Austin (1987) and Noga (2010).

Postmortem examination:

All examined fish were sacrificed by cutting the junction between the vertebral column and the head and placed on their right side. The first cut was made in front of the anus through the abdominal wall with blunt sterile scissors. The second cut was made perpendicular to the first directly behind the branchial cavity. The third cut was from anus to the head parallel to the middle line where the abdominal wall was removed and the internal organs were exposed and examined microscopically. The collected fishes were examined macroscopically using the methods described by Amlacher (1970).

Parasitological examination:

Smear scrapings from the external body surface of the collected fishes, the trunk, head, fins and gills were obtained especially the parts showing ulcers, wounds, petechial hemorrhages, slimness or sloughing of scales and any other pathological lesion (paleness, congestion or marbling appearance). Microscopic parasites were collected by a brush, special needle or dropper, Then washed for several times in warm saline solution and left in the refrigerator until the specimens has been died and completely relaxed. The smears were obtained by scraping the outer layer of gill filaments and spread with a drop of normal saline, covered with a clean cover slip and examined microscopically (Lucky, 1977).

Preparation of permanent samples for Identification of parasites.

The obtained protozoa smears by scrapings were freshly examined, then fixed in methyl alcohol and stained by Giemsa which was diluted before staining with freshly prepared distilled water (one ml of the stain was added to 9 ml of distilled water). The fixed films were immersed into the diluted stain for 20 minutes. After staining, the film was rinsed with water and left to dry. The stained preparation were immersed for a few seconds into acetone. Cedar oil was then dropped on the dried preparation and examined with an immersion objective (Lucky,

1977). Also, crustacean was refrigerated then fixed in 70% alcohol glycerin, passed through ascending grades of alcohol (70,80,90,95% and absolute) cleared in xylol, mounted in Canada balsam or by clearing in lacto phenol and mounted in glycerin gelatin (Lucky, 1977).

Identification of parasites:

The identification of the parasites was undertaken according to Paperna (1996).

Histopathological examination:

Specimens from gills were fixed in 10% formalin, prepared and stained with H & E according to Bancroft and Stevens (1996).

Statistical analysis:

Data were presented as mean \pm standard error (S.E.) and the significance of differences was estimated using ANOVA test as described by Snedecor (1964).

3. Results

1-Clinical examination:

The clinical examination of most examined fishes showed on slight and heavily naturally infested fishes (fingerlings and adult of *O. niloticus*), represented as respiratory manifestations, some aggregated on the surface, and accumulated at the water inlet of the pond. Others appeared dull with loss of escape reflex. Also, fishes showed The gills will appear swollen and be covered with thick mucus. The scale external body surface as well as sloughing (Plate, 1).

Parasitological examination:

External body surface.

Microscopic smears were taken from skin and gills of examined fish, showed a peritrichus ciliated protozoan. A denticulate ring of hollow conical structures was found with flat lateral projections. The centrifugal projections of denticles were semicircular. The macronucleus was large horseshoe shaped with a round micronucleus. Such ciliated protozoans were identified as *Trichodina heterodontata* Duncan, 1977. Plate, 3 (1) and other appeared as heart shaped. The ventral side was concave and ciliated were reduced to two longitudinal belts of cilia to body margins that arranged in parallel rows. The cytostome was distinct on the front part of ventral side with a funnel-shaped cytopharynx and two contractile vacuoles. A single oval to round macronucleus as well as round micronucleus were easily seen. Such ciliated protozoan were identified as *Chilodonella* Plate, 3(2).

A flagellated protozoan. It has a flat, slight asymmetrical and oval, bean-shaped or kidney-shaped body, a strongly convex dorsally and slightly concave ventrally. Two flagellae are extending postero-laterally from deep flagellar pocket. They

are unequal flagella, the longer being closer to the cell margin. Longitudinal binary division begins with a doubling of the flagellar number prior to nuclear fission. Such flagellated protozoa were identified as

Ichthyobodo necator. **Plate, 3 (3)**. It is considered as the smallest external protozoan as it ranges from 3 to 18 μ in length.

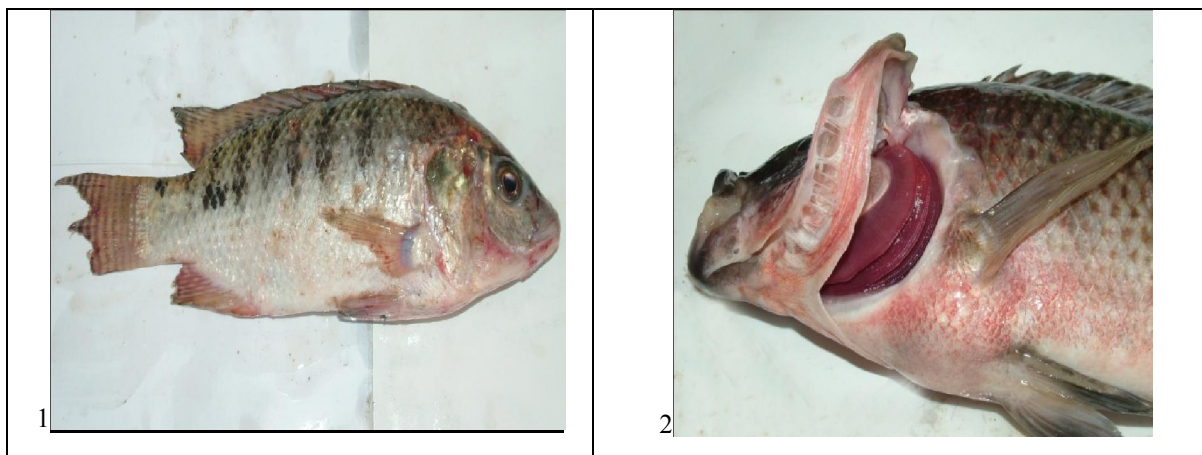


Plate 1: Showing *Oreochromis niloticus* sloughed scales with ulcerations and haemorrhages on the body surface (1), showing *Oreochromis niloticus* congestion of gills (2).

Microscopic smears were taken from skin and gills of examined fish, showed a holotrichous ciliated protozoan as the entire surface area is ciliated with a meridional row. The mature parasite (trophont) is round to oval in shape, ranging from 0.5 up to 1.5 millimeters in diameter and considered as the largest

external protozoan affecting fish. The macronucleus is embedded in the protoplasm and well characterized by a horseshoe C shape. Such flagellated protozoa were identified as *Ichthyophthirius multifiliis*. **Plate 3(4)**.

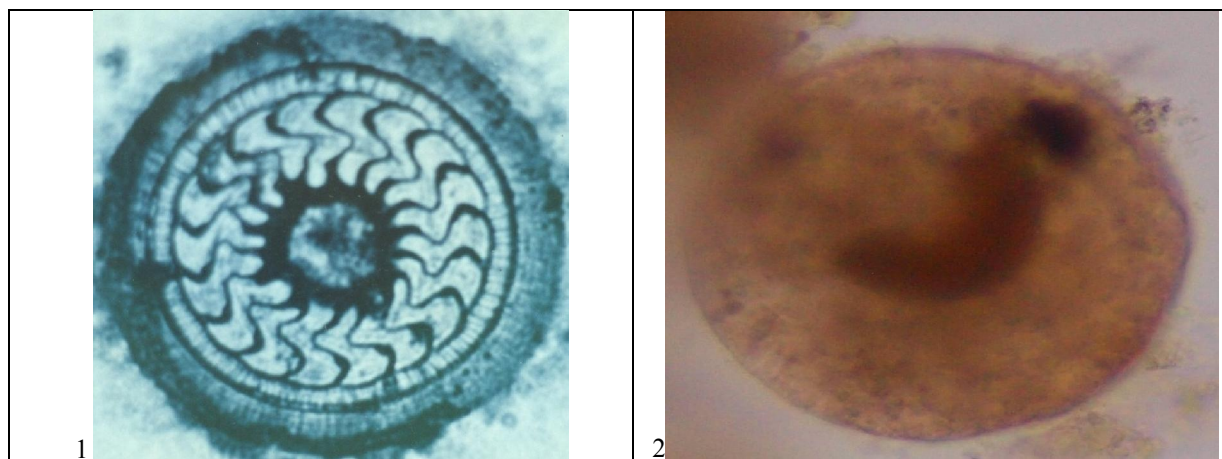


Plate 2: Magnified *Trichodina californica* (1) Stain: Gimsa stain X 400 and *Ichthyophthirius multifiliis* (wet mount) (2).

Adult worms were isolated from the gills of infested *Tilapia* sp. They were flat and elliptical worms. Their anterior end (prohaptor) were divided into four cephalic lobed heads, with sticky and adhesive organs (cephalic glands), in addition four black eye spots. The posterior end, appeared a dome shape and composed of one pairs of connecting bars (V-shaped) and seven pairs of small marginal

hooklets. The intestinal limbs were connected, the ovary located in front to testes.

Such adult worms are related to the phylum *Platyhelminthes*, class *Trematoda*, order *Mongenea* family *Dactylogyridae* and genus *Dactylogyrus* and identified as *Cichlidogyrus tilapiae*. **Plate 3(1)**.

White nodules appeared as inverted V-shaped of egg sacs on the attacked gills. The microscopic

examination revealed a dorsoventrally flattened fused cephalothorax and a single median eyespot toward the anterior end. The head has two pairs of segmented antennae of which the first one was small and setated. The second was large slender, hook-like as long as total body length, terminates with only a sickle shaped terminal powerful curved spines. The mouth parts were on the mid-ventral surface of the cephalothorax. The parasite has four pairs of segmented legs and three segmented abdomen

terminates and two elongated multiserial which egg sacs, which were attached to the swollen genital sixth segment and appeared as cigar-shaped. Such copepods are related to phylum Arthropoda, class Crustacean, subclass Copepoda, order Cyclopoidea, suborder Poecilostomatoida, and family Ergasilidae genus *Ergasilus*. the most common *Ergasilus* sp., which collected from some, infected gill fishes and identified as *Ergasilus ovatus* Plate 3(2).

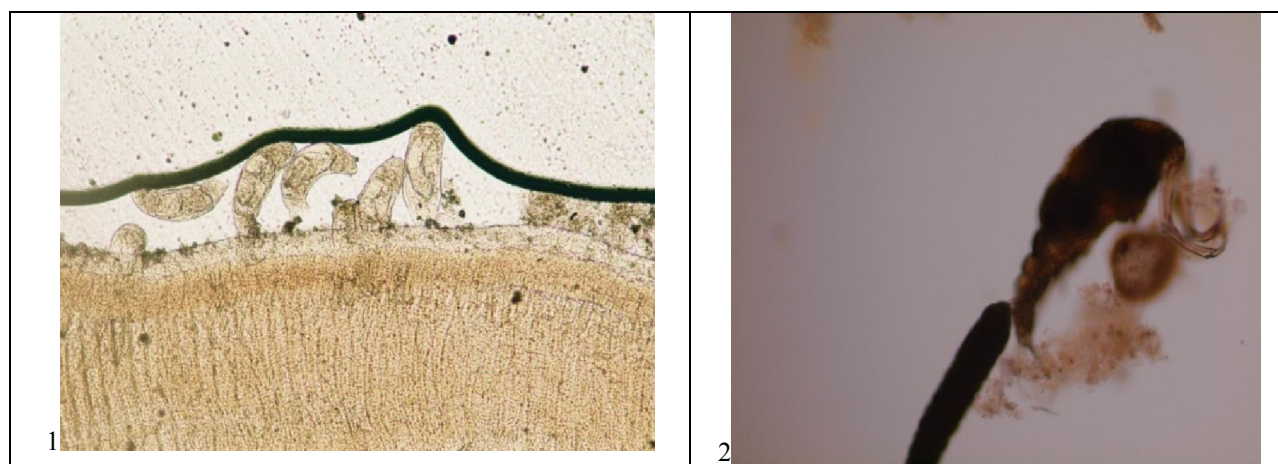


Plate 3: Magnified *Cichlidogyrustilapiae* (1), *Ergasilus* sp (2) (wet mount).

Prevalence of infested *Tilapia* sp.

In the present study, 1200 various stages of cultured fish showed a great variation in the

infestation percentage in (fingerlings and adult *O. niloticus*) from 3 districts (Kafr El Sheikh, Alfayoum and Alsharkia Governorate as in tables, 2 & 3.

Table (2): Incidence of parasitic infestations in different districts fish farms in relation to different age.

Locations	Total No. of Examined Tilapia	Total No. of infested Tilapia		Age			
				Fingerlings		Adults	
		No.	%	No.	%	No.	%
Alshakaya fish farms	400	180	15	60	5	120	10
Kafr El Sheikh fish farms.	400	240	20	60	5	180	15
Alfayoum fish farms	400	144	12	24	2	120	10
Total	1200	564	47	144	12	420	35

Table (3): Seasonal dynamics of Protozoa among investigated *O. niloticus* in different localities:

Locality Season	Kafr El sheikh			Alabasa			Alfayom			Total		
	No.Ex.	No. Inf.	%	No. Ex	No.Inf	%	No. Ex.	No.Inf.	%	No.Ex.	No.Inf.	%
Autumn	100	60	5	100	30	2.5	100	30	2.5	300	120	10
Winter	100	30	2.5	100	30	2.5	100	24	2	300	84	7
Spring	100	60	5	100	60	5	100	30	2.5	300	150	12.5
Summer	100	90	7.5	100	60	5	100	60	5	300	210	17.5
Total	400	240	20	400	180	15	400	144	12	1200	564	47

Histopathological studies.

The histopathological alterations in the gills of tilapia fish from Kafr El Sheikh, Alabasa and

Alfayoum region during summer season includes hyperplasia in the epithelium of gill filaments and secondary lamellae, resulting in fusion of secondary

lamellae, severe degenerative and necrotic changes in gill filaments, curling of secondary lamellae and mucous cells proliferation. Edematous changes, characterized by epithelial detachment, were observed in gill filaments and secondary lamellae. Moreover, aggregations of inflammatory cells were noticed in gill filaments. Also, dilation and congestion in blood vessel of gill filaments were observed. Parasitic sections were observed in between the respiratory epithelium also in between the secondary lamellae.

The histopathological changes in the skin revealed hypertrophy and hyperactivation of mucous secreting cells. Vacuolar degeneration and necrotic changes in the epidermal cells also detected associated with lymphocytic and leucocytic infiltration and accumulation of oedematous fluid subepidermal leading to splitting of the subepidermal connective tissue. Parasitic sections also detected in between the muscle bundles.

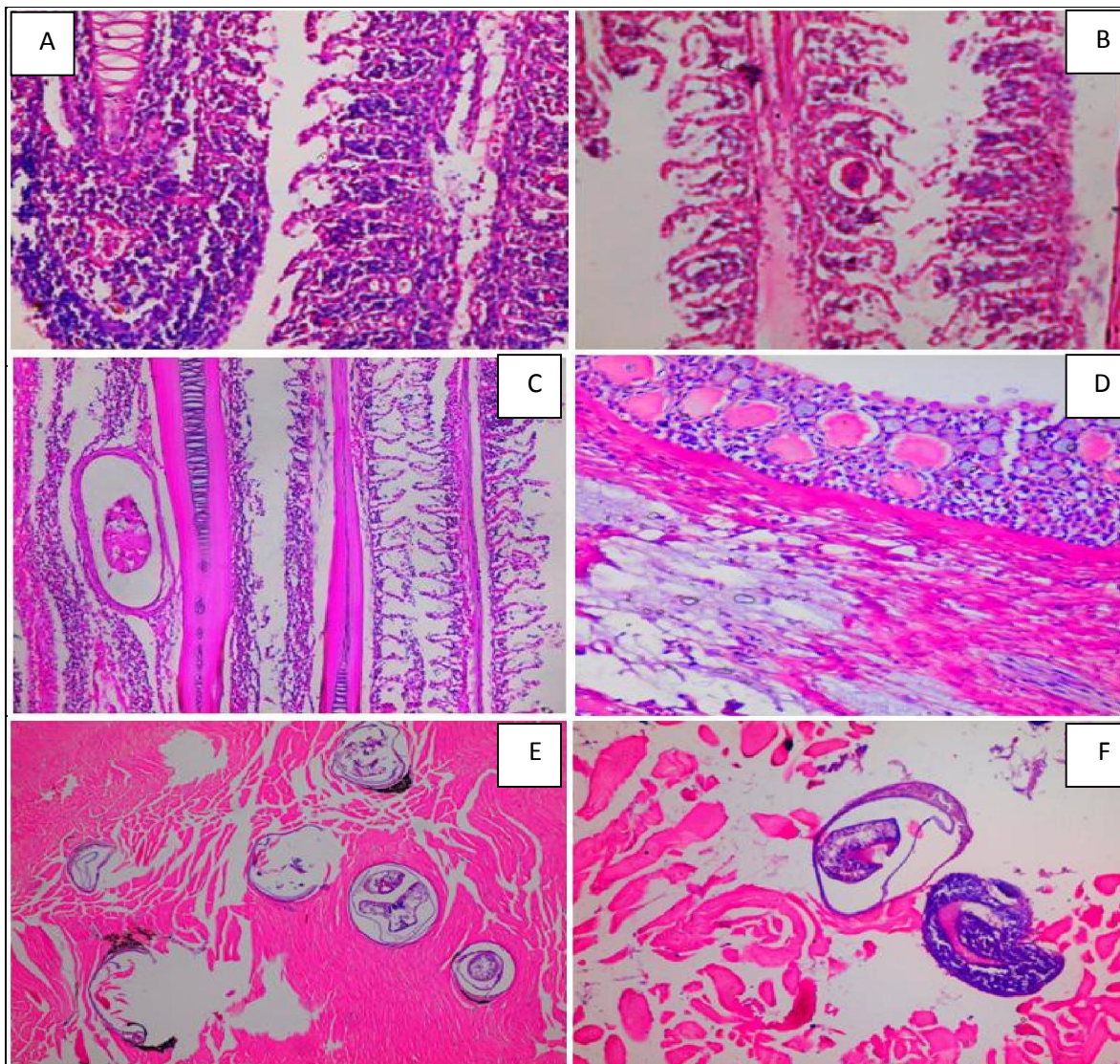


Plate 1, A,: Showed hyperplasia in the epithelium of gill filaments and secondary lamellae, fusion of secondary lamellae, severe degenerative and necrotic changes (H&E, X400). **B,** Showed curling of secondary lamellae and mucous cells proliferation. Edematous changes, characterized by epithelial detachment, aggregations of inflammatory cells and **C,** Parasitic sections were observed in between the respiratory epithelium (H&E, X400&X200).

D: hypertrophy and hyperactivation of mucous secreting cells. Vacuolar degeneration and necrotic changes in the epidermal cells, lymphocytic and leucocytic infiltration and accumulation of oedematous fluid (H&E, X400)

E&F: Parasitic sections also detected in between the muscle bundles (H&E, X400).

4. Discussion

In Egypt, more attention is being focused nowadays to the improvement of fish aquaculture to solve the shortage of animal protein.

The present study deals with different external parasitic diseases among naturally infested the cultured *Tilapia sp* in relation to the seasonal prevalence and water parameters in Kafr El- Sheikh, Alsharkia and Alfayoum fish farms with treatment trials.

The parasitic external ciliated protozoan parasite that may infest almost all freshwater fish species caused significant economic damage to the aquaculture industry specially *Ichthyophthirius multifiliis* (Ich).

The present study revealed the clinical picture of infested freshwater fishes as abnormal, swimming, flashing, rubbing the body against the sides of aquaria to get rid of the irritation induced by the parasites on the skin, fins and gills. Fish gathered at the water surface (surface breathing) with gulping the atmospheric air. They appeared asphyxiated and exhausted this may be due to low respired oxygen of destructed gill epith, which caused by feeding activity, attachment, fixation and locomotion of trichodina and monogenea causing massive destruction of the respiratory epithelial cells. This result may be similar to that recorded by **Eissa et al. (2010)**.

Concerning the hemorrhagic areas with small wounds, abrasions or ulcers on the body surface with frayed and ragged appearing fins with darkening of skin of some fishes. This may be attributed to *Gyrodactylus* (skin fluke) are provided with a pair of too long and strong anchors in the opisthaptor and 7 pairs of small strong hook lets used for fixation firmly on the external body surface of its host to resist the external water currents as well as continuous regularly locomotion and relocation from side to side and around the fin margin and frequently cross over the body surface to another fin, the caudal, pectoral and pelvic ones. These results nearly agree with that met by **Osman (2005)**.

Regard's the congested or pale gills (*Marbling appearance*) it may be caused due to destruction of the efferent vessels by monogenea and *Ergasilus* where the blood pressure is low and extensive hemorrhage are caused the very hard clotting of blood brings about rapid occlusion of the vessel, thrombus is formed resulting in ischemia which in turn leads to necrosis in some areas occurred due to the inflammation and congestion of some areas with progressive degeneration of the other parts of the gill filaments giving the appearance of such phenomenon (**Eissa, 2006**). All stages of inflammation,

degeneration and necrosis of the epith. Cells of the gill filament and gills become pale, anemic and the tips become grayish in colour. The over mucous secretion by fish may be to dilute the irritation as defense mechanism against these pathogens.

Concerning the clinical signs in tilapia sp infested with ciliated protozoa Ich was causes white nodules covering the skin. Also showed signs of skin irritation manifested by rapid swimming behavior and aggregation of fishes around the water inlet. Sloughing of scales was noticed. In addition erosions and ulcerations on trunk area of the skin and sloughing of scales. The same clinical signs were previously obtained by **Noor El Deen (2010)**.

The ciliated protozoans, *Ichthyophtheriuis multifilis*, *Trichodina fultoni* and *Chilodenella hexastica* were ectoparasitological were morphologically and parasitologically identified. Such results were nearly similar to the descriptions given by **Kabata (1992)**.

Concerning the results of parasitological examinations of Tilapia fishes, isolation of Ich sp. was provided with **Xu (2010)**. The morphological characters of these parasites were nearly similar to descriptions given by **Eissa et al. (2010)**.

The flagellated protozoon, (*Ichthyoboda necatrix*) was identified with nearly similar morphometric characters to that described by **Eissa et al. (2010)**.

Regarding monogenean trematodes (*Gyrodactylus sp* and *Cichlidogyrus tilapiae*) were morphologically and parasitologically described and were nearly similar to the descriptions given by **Yamaguti (1963)**.

Concerning the copepods crustacean (*Ergasilus ovatus*) was identified parasitologically and morphologically. These results are nearly similar that recorded by and **Paperna (1996)**.

Regarding the seasonal dynamics of external protozoa, the highest infection rate was in summer. This result was in agreement with **Noor El Deen (2007)** stated that the seasonal incidence of protozoal infection was high in spring.

Concerning the seasonal variation on the prevalence of Monogenetic trematodes in the present study, the highest rate of infection was during summer. This result agreed with **Osman (2005)**.

Concerning the seasonal dynamics of crustacean's infection the maximum rate of infection was during spring. This result agreed **El-Moghazy (2008)** mentioned that the highest incidence was recorded during summer. These differences in the rates and seasonal dynamics of infection between the different localities may be attributed to the differences in environmental conditions and the

differences in the degree of infestation as well as number of examined samples.

Histopathological alterations of gills of *Tilapia* sp ingested with parasitic revealed multiple forms of deformities due to hyperplasia of epithelial cells of secondary lamellae accompanied with hyperplastic of epithelial cells proliferation oedema was found in between the epithelial cells of secondary gill lamellae degenerative changes as well as necrosis were recorded in both primary and secondary gill lamellae. Numerous inflammatory cells mainly of eosinophils, lymphocytes and mononuclear leukocytes. The histopathological examination of *Tilapia* sp infested with *Trichodina* sp showed congestion and numerous eosinophilic granular leukocytes in the gill arch and gill lamellae. Also, hyperplastic proliferation.

This results to gill damage by protozoan parasites which caused by feeding activity, attachment, fixation and locomotion causing massive destruction of the respiratory epithelial cells. These results agreed with **Abd EL- Hady (1998)** who recorded that the histopathological examination of *Tilapia* sp for *Trichodina* affections showed hemorrhage, congestion, oedema and mononuclear leukocyte infiltration in the gill arch and lamellae in both *Tilapia* ages. *Chilodnella* spp revealed oedema and congestion in the central venous sinus of primary lamellae. The secondary lamellae cells were hyperplastic fused sloughing of epithelial cells with marked oedema of gill lamellae. This results was similar to that recorded by **Roberts (2012)** who reported that the most common response of the gill to damage by protozoan parasites is hyperplasia and hypertrophy of epithelial cells. On the other hand the presence of monogenetic Trematodes on the gill filament in some cases or the marbled gills in other cases gill damage was manifested clinically by signs of asphyxia this may be attributed to poor quality which minimize the defense and the immunogenic status of *Tilapia* species these results were agree with results recorded by **Noor El Deen (2007)**. *Tilapia* sp. infested with monogenetic Trematodes the gill lamellae and suprabranchial organs were severely congested also the pathological lesions started in the free ends of the gill lamellae in the form of hypertrophy and hyperplasia and diffuse lymphocytic infiltration oedema noticed in the middle of lamellae while the distal parts as well the gill arch still having apparently normal in case of heavy monogenetic infestation the epithelial hyperplasia was access to the distal parts of the gill lamellae. This agreed with the finding recorded by **Noor El Deen et al. (2013)**. These may be attributed to the mechanical injury induced the central hooks and marginal hooks insert into the gill epithelial tissues that leads to swelling, fusion and deformation of gill lamellae with collapse

of capillaries. The present findings indicated that Fingerlings *O. niloticus* are less exposed external and internal parasites than adult *O. niloticus*, the highest infested district was Kafr El Sheikh followed by Alsharkia district and Alfayoum and overcrowded play the important role of external parasitic infestations.

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