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Histopathological changes induced by ectoparasites on gills and skin of *Oreochromis niloticus* (Burchell 1822) in fish ponds

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ABSTRACT

The aim of this study was to investigate the effect of ectoparasites on gills and skin of (*Oreochromis niloticus*). Fish and water samples were brought to the laboratory from a tilapia fish farm in Riyadh area of Saudi Arabia. A number of (120) fish specimen were equally collected for 1 year on monthly basis. Water samples were examined in the laboratory for physicochemical characteristics (1 L month). Gills and skin tissues were prepared for histopathology and light and scanning electron microscopy. Water characteristics revealed a substantial deterioration of water quality. Mean value ± standard deviation shows low oxygen (2.18 ± 0.08–2.85 ± 0.14 mg/l), high ammonia-N (2.01 ± 0.45–2.92 ± 0.66 mg/l), and high nitrite-N. (0.82 ± 0.49–1.61 ± 0.66 mg/l). Ectoparasites including *Trichodina*, *Monogenea,* and *Ambiphyra* were observed on the infected tissues. Histopathological investigations showed severe gill alterations such as blood congestion, blood hemorrhage, a fusion of secondary lamella, and epithelial lifting. Gill erosion and mucus secretions were also reported on some fishes. The severity of the infection has worsened fish health and lead to death of many individuals. The histopathological changes induced by ectoparasites on fish gills and skin could be used to determine the severity of ectoparasite infection to take measures for better fish farm management and to produce healthy fish for human consumption.

1. INTRODUCTION

The gill is a system for bringing the blood hemoglobin into close contact with the water so that oxygen can be absorbed and carbon dioxide released [1]. The gills are delicate structures of the teleost fish. The external location of the gills and their direct contact with the water makes them liable to damage. The infected gills of tilapia is pale and covered with excessive mucus and marbling appearance of gill leaflets [2], ectoparasite infested tilapia show signs of emaciation, weakness, sluggish movements, sloughing of scales, and fading of the color [3,4].

El-Khatib [5] mentioned that the fish infested with *Trichodina* showed pale skin with slime and several spots of blood spread on the fish body, particularly at the fin base. The clinical examinations of the infested Tilapia skin with *Trichodina* sp. show an increase of mucus secretions with an overall dark gray, slimy, patchy, or mottled gray appearance [6]. Moribund fish appear uncoordinated swimming, emaciated, and abrades the skin. Heavily infested fish shows a grayish-blue coat of mucus, peeled epithelia, and frayed fins. Noga [7] found that the clinical examination of heavily infested fish with *Trichodina* sp. seemed to be anorexic, weakened condition, and usually involve low-level mortality.

Various parasite species attack the gills and skin of fish. *Trichodina* and *Monogenea* are lethal parasites in aquaculture. The reactions of the fish to these parasites include pale gill filaments or a few white spots. In serious conditions, the fish may exhibit a considerable decaying, pale colorations, a number of white spots, and severe secretion of mucus [8].

Occupation of the gills by parasites may cause proliferative alterations on the cells, a fusion of gill lamellar, gill hypertrophy, the formation of inter-lamellar sacs and edema [9-13], which is known as detectable white spots, or injuries of white mucoid on the surface of the gills [12].

Both marine and freshwater fishes are commonly infected with monogeneans on gills and skin. Monogenean parasites graze on the outer surface of the gills and skin, cause opacity of the skin or red spots resulting from the production of excessive mucus [14]. Infected fish with monogenean parasites shows epithelial hyperplasia or hemorrhage on the fish skin area [15]. Histopathological changes to fish epithelium take place due to the attachment or nourishing of monogenean parasites [16], hyperplasia with dermatitis is also common changes of the epithelium [17].

Epidermal superficial lesions may occur as a result of the attachment of the *Gyrodactylus* sp. on fish skin. The attachment and feeding

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of this parasite on fish epidermis cause the production of excessive mucus [17].

Fish skin is attacked by *Dactylogyrus* sp., while the gills are attacked by both *Gyrodactylus* sp. and *Gyrodactylus* sp. These parasites affect fish movement and cause blood spots on their body as well as falling fins [18].

Gill area of the fish is mostly infected by monogenean parasites. Merging of the gill secondary lamellae as a result of the attachment of monogenean opisthaptor causes epithelial proliferation of the tissues [16]. Infected individuals with monogenean parasites show fusion and disruption of secondary lamellae as well as inflammation and erosion of the epithelium of gill lamellae. This parasite reduces chloride cell number, which represents ion absorption and secretion site [19].

Kabata and Lester [15,20] studied the effects of *Gyrodactylus* on different pathological conditions in tropical fish such as epidermal hyperplasia, extensive mucus secretion, localize petechial lesions, skin, and scale sloughing may also occur. The attachment organs such as anchors and hooks create holes in the fish epidermis and enzymatic digestion that causes ulcers [21]. Secondary bacterial and fungal infections of the fish epidermis may also intensify the damage of *Gyrodactylus* pathogenicity [22].

Heavily infected fish with ectoparasites may suffer from gill lesions and skin injury. Bacteria and fungus may invade the damaged tissue parts and play a significant role in fish pathogenicity. Infested fish become sluggish, emaciated, moribund, and finally died. Histopathological changes induced by ectoparasites on *O. niloticus* gills and skin indicates the severity of *Trichodina* and *Monogenea* infestation on the farmed Nile tilapia.3.

2. MATERIALS AND METHODS

2.1. Fish Collection and Water Quality Analysis

One hundred twenty specimens of *O. niloticus* were brought to the lab of King Saud University laboratory from a freshwater fish farm in the Riyadh area of Saudi Arabia monthly in all months of the year. Water samples were collected simultaneously with fish samples from the ponds of the fish farm. Dissolved oxygen was measured by a digital oxygen meter (HANNA-HI9142). Water temperature was determined by using a mercury thermometer of (0.1°C) accuracy. The salinity of the water was measured by a refractometer (M300, Hanna, USA) in (PPT). The pH of the water was quantified by a pH meter (HANNA-HI98107). Electrical conductivity (EC) was studied by a digital conductivity meter (AD-31: EC/TDF). Nitrite–nitrogen and ammonia–nitrogen were analyzed in the laboratory using DR\2010 spectrophotometer. The outcomes of water analysis were indicated as mg/liter except for temperature and conductivity they were quantified as (°C) and millisiemens (mS/cm), respectively.

2.2. Fish Examination for Ectoparasites

Fish investigation for ectoparasites was done following the modified methodology of [15]. Scarifying the fish was done by striking the head, and instantly, skin scrapes (smears) and gills were prepared in wet mounts and seen under a low-power microscope for the analysis of the ectoparasites. Samples of the wet mount were made from each specimen to measure the intensity of *Monogenea* and *Trichodina* parasites.

2.3. Histopathological Analysis

The gills of some of the infected fish were used to study the histopathological effect of the *Trichodina* and *Monogenea* with a light microscope (LM). Gills were fixed in neutral buffered formalin solution (10%). Sections of paraffin (5-µ thick) were made and stained with hematoxylin-eosin (H & E), according to Roberts [23]. Prepared mounts were observed under the microscope. Histopathological images were captured using the software Motic Images Plus 2.0-Camera and a binocular microscope.

2.4. Scanning Electron Microscope (SEM)

Tissues of infected gills and skins were fixed with 2.5% glutaraldehyde, then fixed in 1% osmium tetroxide; next, the tissues were dehydrated with ethanol of different concentrations and critical point dried. The resulted material was mounted on aluminum stubs, sputter-coated with gold, and examined using the JSM-6380 LA SEM at 0.3–30 kV (55 steps) accelerating voltage.

2.5. Statistical Analysis

Statistical analysis was achieved by the analysis of variance (ANOVA) one factor without replications, according to Sokal and Rohlf [24], to compare the seasonal variation of *Monogenea* and *Trichodina* occurrence on gills and skin of *Oreochromis niloticus* of the studied fish farm. The results were considered significant at 5%.

3. RESULTS AND DISCUSSION

3.1. Water Quality

Table 1 shows the results of the water quality of the studied fish farm. Mean ± standard deviation are calculated for water quality parameters. Oxygen concentration is always below 3 mg/l (2.18 ± 0.08–2.85 ± 0.14 mg/l). Water temperature varies between 18.5 ± 0.27 and 32.3 ± 1.83°C. PH of the water is given between 5.2 ± 0.2 and 7.1 ± 0.9 (tends toward the acidic side). Ammonia-N is found to be between 2.01 ± 0.45 and 2.92 ± 0.66 mg/l. Nitrite-N fluctuates between 0.82 ± 0.49 and 1.61 ± 0.66 mg/l. Nitrate-N varies from 3.41 ± 0.63 to 4.57 ± 0.47 mg/l. Salinity is shown to be between 3.87 ± 0.35 and 4.87 ± 0.33 ppt. Water conductivity is recorded between 239 ± 11.8 and 276.2 ± 10.3 mS/m.

Several water characteristics have the potential to have negative effects on the health of tilapia on the fish farm. Oxygen level is always <3 mg/l which is below the requirement of tilapia. Tran-Duy *et al*. [25] reported

Table 1: Water quality of the studied fish farm.

Parameters	Autumn ($\bar{x}$ ±sd)	Winter ($\bar{x}$±sd)	Spring ($\bar{x}$±sd)	Summer ($\bar{x}$±sd)
Dissolved oxygen (mg/l)	2.7±0.22	2.85±0.14	2.18±0.08	2.59±0.16
Temperature (°C)	26.0±0.87	18.5±0.27	27.6±0.96	32.3±1.83
PH	5.2±0.2	5.7±0.10	6.8±0.2	7.1±0.9
Ammonia-N (mg/l)	2.01±0.45	2.92±0.66	2.41±0.34	2.47±0.73
Nitrite-N (mg/l)	1.5±0.56	0.82±0.49	1.41±0.57	1.61±0.66
Nitrate-N (mg/l)	4.5±0.45	4.42±0.65	3.41±0.63	4.57±0.47
Salinity (ppt)	4.78±0.33	3.87±0.35	4.12±0.65	4.29±0.43
Conductivity (mS/m)	256.0±12.5	276.2±10.3	254±9.7	239±11.8

$\bar{x}$: Average, sd: Standard deviation, °C: Degree Celsius, mg/l: Milligram per liter, ppt: Part per thousand, mS/m: Millisiemens per meter

that Nile tilapia (*O. niloticus*) of 37 and 190 g average weight at dissolved oxygen between 2.8 mg/l and 3.2 mg/l showed a reduction in feed intake and fish growth. Wurts [26] stated that unionized ammonia increases with the increase of water pH and reduces with its reduction. He also found that high water temperature combined with the increase of the pH will increase the unionized ammonia production. The summer temperature in this study exceeds 32°C which increases unionized ammonia production, but the low pH (5.2 ± 0.2 and 7.1 ± 0.9), on the other hand, decreases its production rate to some extent. Ammonia-N was found to be as high as 2.92 ± 0.66 mg/l during winter and nitrite-N reaches 1.61 ± 0.66 mg/l in the summer. Ammonia-N and nitrite-N are the highest lethal metabolic waste products in fish ponds. Romano and Zeng [27] described that ammonia-N is much lethal to decapod crustaceans succeeded by nitrite-N and nitrate-N, which are less toxic.

Documentation of nitrite toxicity was reported by different authors, including Lewis and Morris and Jensen [28,29]. Acute toxicity of nitrite occurs at 0.2 mg/l in salmonids [30]. Studies of *Puntius gonionotus* fry showed that their growth rate was significantly lowered by nitrite concentration of 2 mg/l and 100% mortality occurred at the concentration of 4 mg/l at pH 5 after 48 h [31]. Yusoff *et al*. [32] reported that methemoglobin was formed when nitrite reacts with hemoglobin to affect the rate of oxygen-carrying capacity leading to hypoxia, cyanosis, stress, and eventually fish mortality by a disease known as brown blood syndrome. Hence, in this study, oxygen level, ammonia-N, and nitrite-N are the main responsible factors of health deterioration of *O. niloticus*, followed by a heavy infestation of ectoparasites such as *Trichodina*, *Monogenea*, and *Ambiphyra* to make it worth especially after the invasion of bacteria.

3.2. Occurrence of *Trichodina* and *Monogenea* on Tilapia Gills and Skin

Table 2 shows the prevalence, mean intensity, and mean abundance of *Monogenea* and *Trichodina* on tilapia gills. *Monogenea* data on *O. niloticus* gills revealed 81.67% mean prevalence, 495.23 mean intensity, and 405.84 mean abundance. Monogenean prevalence, mean intensity, and mean abundance on *O. niloticus* gills are significantly higher during the summer on tilapia gills with ($P < 0.05$), while *Trichodina* on tilapia gills occurs with 97.5% mean prevalence, 443.68 mean intensity, and 1790.83 mean abundance. Prevalence of *Trichodina* shows 100% occurrence in all seasons except the winter which is significantly lower with $P < 0.05$, mean intensity and mean abundance of gill *Trichodina* are significantly greater during the winter the Autumn, respectively, with $P < 0.05$.

Table 3 shows the prevalence, mean intensity, and mean abundance of *Monogenea* and *Trichodina* on tilapia skin. *Monogenea* data on *O. niloticus* skin present 66.67%, mean prevalence, 495.23 mean intensity 294.16, and mean abundance. The prevalence and mean abundance of *Monogenea* on tilapia skin are significantly higher during the summer ($P < 0.05$), while their mean intensity is higher during the autumn ($P < 0.05$). On the other hand, *Trichodina* on tilapia skin shows 97.5% mean prevalence, 1842.13 mean intensity, and 1790.83 as mean abundance, the prevalence of *Trichodina* on tilapia skin is found to be 100% in all seasons but significantly lower during the winter ($P < 0.05$) while mean intensity and mean abundance of *Trichodina* on tilapia skin are higher during the spring ($P < 0.05$).

This study showed that the occurrence of *Trichodina* and *Monogenea* on gills and skin in the fish farm was found to be throughout the year with some effects of seasonality on their prevalence, mean intensity, and mean abundance ($P < 0.01$). The same results reported by Hassan [33], he also stated some effects of seasonality on *Trichodina* prevalence in the eastern province of Saudi Arabia. Suliman and Al-Harbi [34] reported both *Trichodina* and *Monogenea* on fish ponds in all seasons, while Lizama *et al*. [35] and Jerônimo *et al*. [36] stated the presence of *Monogenea* throughout the year on farmed fish.

3.3. Histopathological Changes

The results of this study revealed the histopathological effects on the gills of *O. niloticus* cultivated in freshwater fish ponds. Histopathological studies on the parasite-infected gills showed the occurrence of *Trichodina* and *Monogenea* in the gills with secondary lamellar damage, fusion, hemorrhage, blood congestion, and mucus

Table 2: Occurrence of *Monogenea* and *Trichodina* on gills of *Oreochromis niloticus* of the studied fish farm

Season	*Trichodina*					*Monogenea*				
	IF/EF	N	*P* %	MI	MA	IF/EF	N	*P* %	MI	MA
Winter	27/30	554	90**	2051.85**	1846.67	24/30	91	80	379.17	303.33
Spring	30/30	547	100	1823.33	1823.33	23/30	89	76.67	386.96	296.67
Summer	30/30	483	100	1610	1610	30/30	173**	100**	576.67**	576.67**
Autumn	30/30	565**	100	1883.33	1883.33**	21/30	134	70	638.10	446.67
Mean	29.6/30	537.25	97.5	1842.13	1790.83	24.5	121.75	81.67	495.23	405.84

N: Number of collected parasites, IF: Infected fish, EF: Examined fish, P: Prevalence, MI: Mean intensity, MA: Mean abundance, **: High significant difference ($P<0.05$)

Table 3: Occurrence of *Monogenea* and *Trichodina* on skin of *Oreochromis niloticus* of the studied fish farm.

Season	*Trichodina*					*Monogenea*				
	IF/EF	N	*P* %	MI	MA	IF/EF	N	P %	MI	MA
Winter	27/30	189	90	700	630	19/30	76	63.33	400	253.33
Spring	30/30	302	100	1006.67	1006.67	21/30	89	70	423.81	296.66
Summer	30/30	268	100	893.33	893.33	23/30	101	76.67	439.13	336.66
Autumn	30/30	270	100	900	900	17/30	87	56.67	511.76	290
Mean	29.25/30	257.25	97.5	875	857.5	20/30	88.25	66.67	443.68	294.16

N: Number of collected parasites, IF: Infected fish, EF: Examined fish, P: Prevalence, MI: Mean intensity, MA: Mean abundance, **: High significant difference ($P<0.01$)

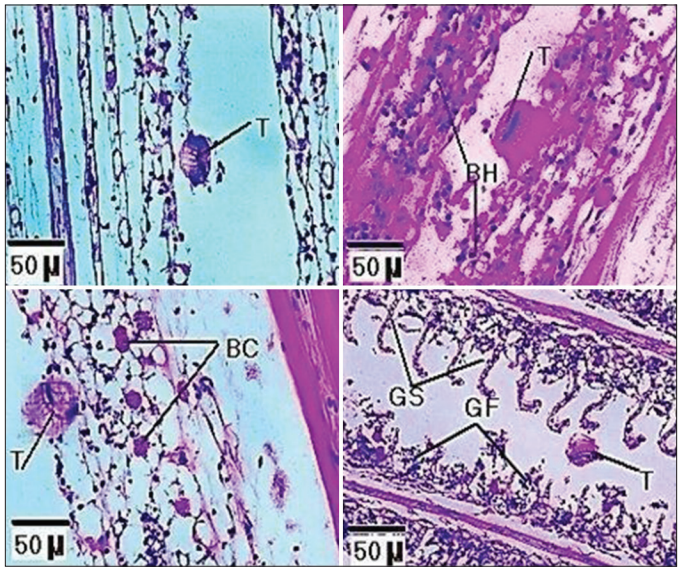

Figure 1: Photomicrographs of *Oreochromis niloticus* gills infected with *Trichodina*. T=*Trichodina*, BH=Blood hemorrhage, BC=Blood congestion, GF=Gill fusion, and GS=Gill sloughing

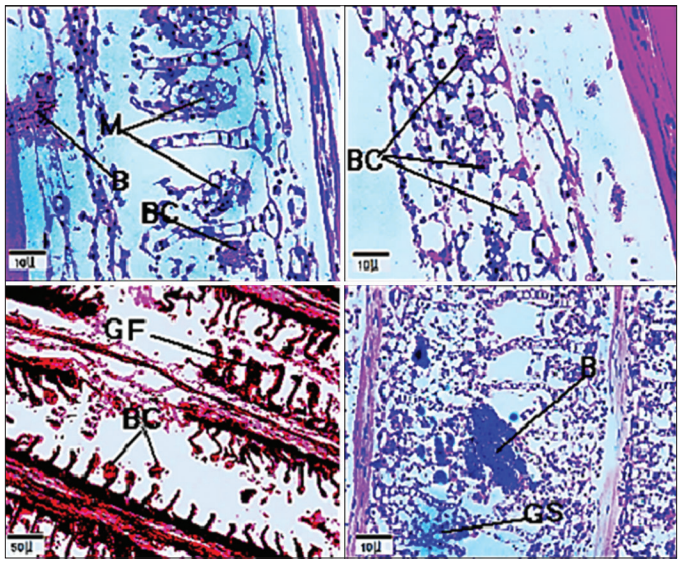

Figure 2: Photomicrographs of the gill of *Oreochromis niloticus* infected with ectoparasites. B=Bleeding, BC=Blood congestion, GF=Gill lamella fusion and GS=Gill lamella sloughing, M=Mucus

secretion [Figures 1-3]. More severely affected gill tissue showed gill sloughing and epithelial lifting and secondary lamellar fusion.

Degeneration of gill secondary lamellae displays erythrocytes in the attachment position of *Trichodina* and *Monogenea*. The continuous dislocation of gill epithelial cells results in gill filament destruction. The fish response was in the form of the extensive secretion of mucus and hyperplasia of gill epithelium [Figures 1-3].

Blood congestion, hemorrhage, a fusion of secondary lamellae, epithelial lifting, and gill erosion were seen on tilapia gills in this study. Ectoparasites such as *Trichodina*, *Monogenea*, and *Ambiphrya* have infested gills and skin of *O. niloticus*. Ectoparasite infections

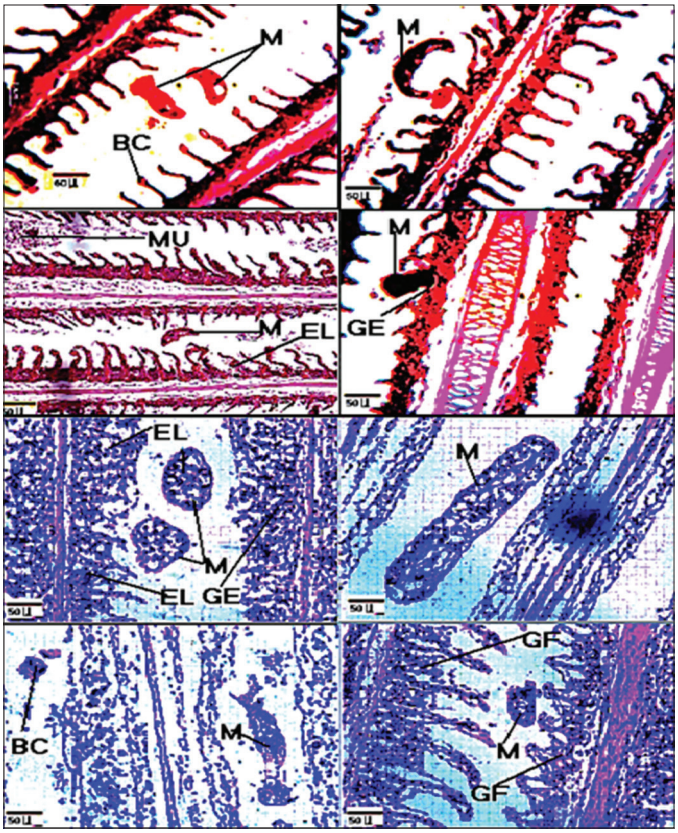

Figure 3: Photomicrographs of the gill of *Oreochromis niloticus* infected with monogenean worms. M=*Monogenea*; BC=Blood congestion; GF=Gill lamella fusion and GE=Gill erosion; EL=Epithelial lifting; MU=Mucus

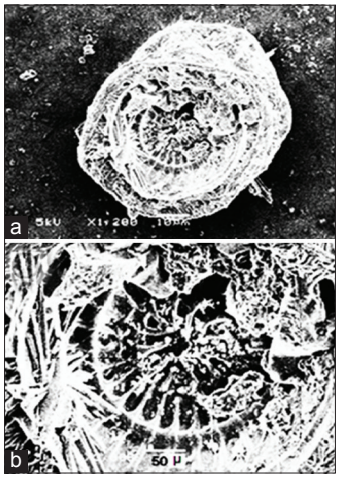

Figure 4: Scanning electron micrograph of *Trichodina* collected from the skin *Oreochromis niloticus* showing the concave side and the denticular ring of *Trichodinid* sp. (a) low magnification; (b) high magnification

cause many problems to *O. niloticus* in fish farms, which varies from simple irritation to full mortality of the fish.

Roberts [37] stated that aquaculture in tropical and subtropical countries suffer greatly from the parasitic infestation of cultured fish due to direct or indirect economic losses.

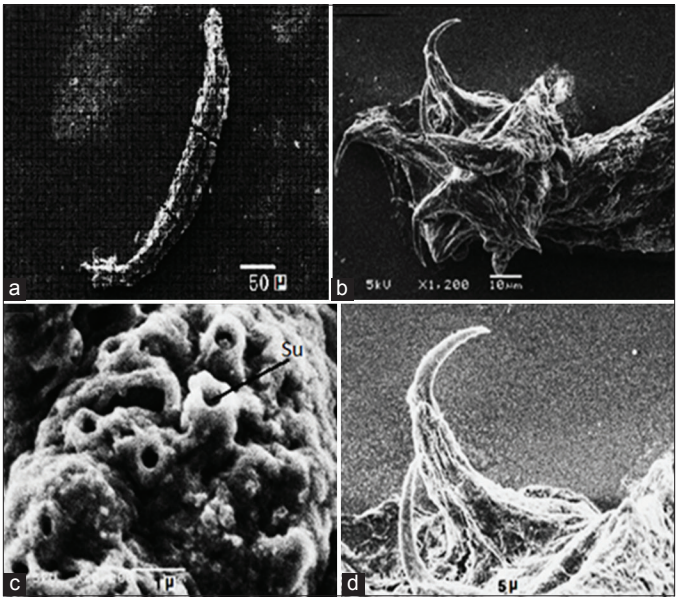

Figure 5: Scanning electron micrograph of *Monogenea* collected from the skin of *Oreochromis niloticus* showing: (a) Full size of the worm. (b) The anchors and marginal hooks. (c) The suckers (Su) at the head region. And (d) the anchors with higher magnification

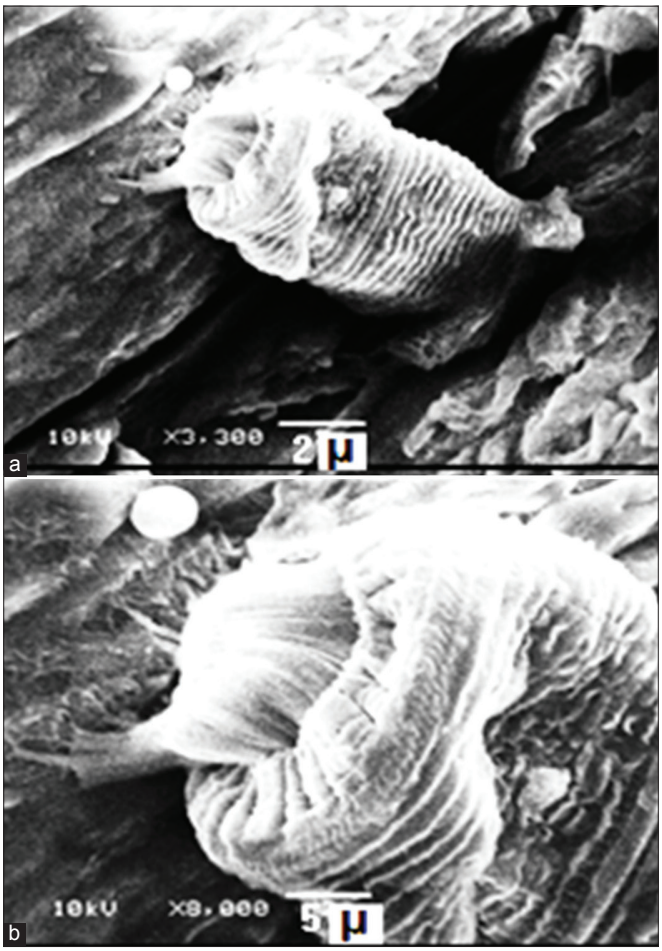

Figure 6: Scanning electron micrograph showing general morphology of the *Ambiphyra* spp. on the skin of *Oreochromis niloticus*. (a) Low magnification; (b) high magnification

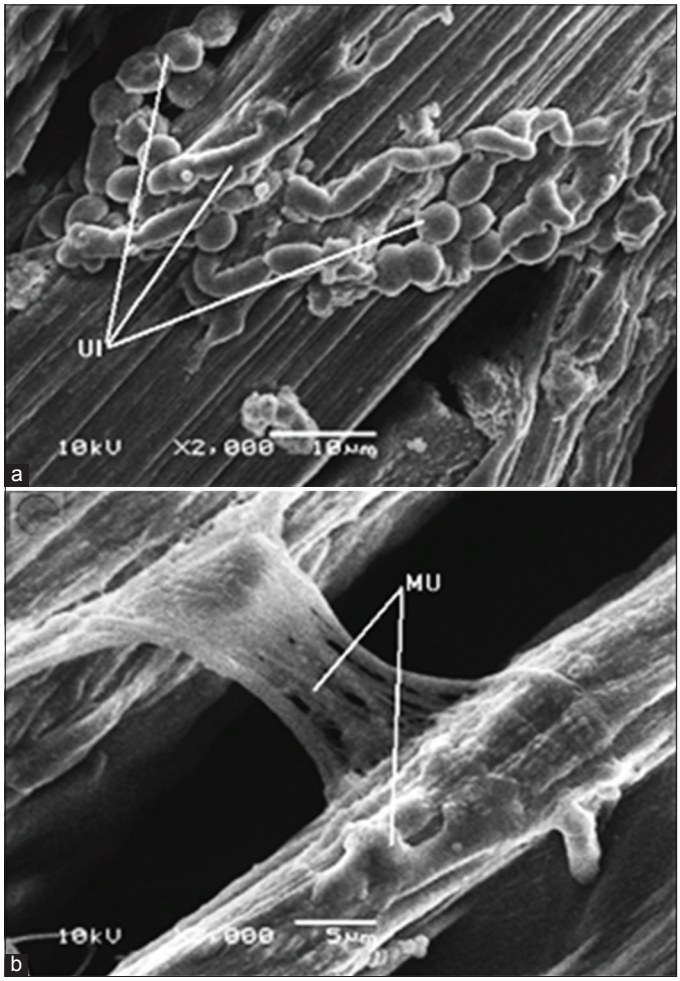

Figure 7: *Oreochromis niloticus* skin shows excessive secretion of mucus on fins

In general, slight numbers of parasites on gills and skin of the fish do not induce serious problems on fish health, but large numbers, on the other hand, can cause more severe problems and eventually death of fish [8]. Grazing by *Trichodina* sp. on the epithelial layer of the gill surface and fish skin will cause hyperplasia (proliferation) of the epithelial cells, gill destruction, and filamentous merging. The capability of gills to keep ideal respiratory and execratory functions and the skin to perform optimal osmoregulation activities will be affected by ectoparasite infestation. A severe manifestation of ectoparasites can also lead to ulcerative skin lesions, which permit for secondary bacterial and fungal infection [38]. *Trichodina* sp. can cause considerable fish mortality in the aquaculture systems.

Monogeneans (Platyhelminthes) are external parasites of the fish. They firmly attach themselves to fish tissues by a posterior organ named opisthaptor with specialized attachment parts [39]. The larger the fluke body, the higher the number of clamps that could attach to the epithelium. The site of attachment causes lamellar clubbing, which could lead to disruption of the gill epithelium and blood vessels. When they disconnected from the filament, lamellar clubbing was even more obvious. The consequences of monogenean infestation on gill lamellae are the proliferation of epithelial tissues and secondary lamellae fusion. The resulting images of histopathological and SEM confirm manifestation of *O. niloticus* with *Monogenea* and *Trichodina* and their significant alterations on gills and skins of the farmed *O. niloticus* in this study.

3.4. SEM

The result of the SEM shows the *Trichodina* parasite at low magnification and high magnification [Figure 4a and b], respectively. The denticular ring is seen in both structures. The *Trichodina* is found to be 72.21 µ in diameter and 26.12 µ in width. SEM shows the whole body of the monogenean ectoparasite [Figure 5a]. The total length of the *Monogenea* is 534 µ and its width is 64.7 µ. The anchor which is used for attachment to fish tissues [Figure 5b] is found to be 53.6 µ and the sucker on the head region [Figure 5c] is 0.345 µ in diameter, and Figure 5d shows the anchor at a higher magnification.

SEM of *Ambiphrya* in Figure 6a and b shows the parasite attached to the fin of *O. niloticus* with its broad scapula at low and high magnifications, respectively. The *Ambiphrya* is found to be 25.6 µ in height and 13.8 µ in width.

SEM of fins of *O. niloticus* infected with ectoparasites showed unidentified materials and excessive mucus secretion on the fins [Figure 7a and b], respectively.

Many dead fishes were seen floating on the ponds during water and fish sampling. Investigated fish were found to be emaciated, starved, lethargic, and sick.

4. CONCLUSION

It can be concluded that histopathological investigations showed severe ectoparasite infestation occurred on *O. niloticus* farmed in freshwater ponds with serious alterations on gill lamellae and skin epithelium. Water characteristic was found to be extremely deteriorated with low oxygen, high temperature in the summer, high ammonia-N, and nitrite-N. Fish health was negatively affected by poor water quality which leads to heavy infestation with ectoparasites such as *Trichodina*, *Monogenea*, and *Ambiphrya*. They infect the gills and skin of *O. niloticus*. Fish mortality occurs as a result of parasitic infestation and poor water quality of the ponds. Although ectoparasites occur in all seasons, the severity of infection occurs during the summer as a result of low oxygen, which is always below 3 mg/l, high ammonia-N, equal to (1.61 ± 0.66 mg/l), and high nitrite-N, equal to1.61 ± 0.66 mg/l. Their effect increases during the summer with the increasing temperature.

5. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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7. CONFLICTS OF INTEREST

The authors report no conflicts of interest in this work.

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