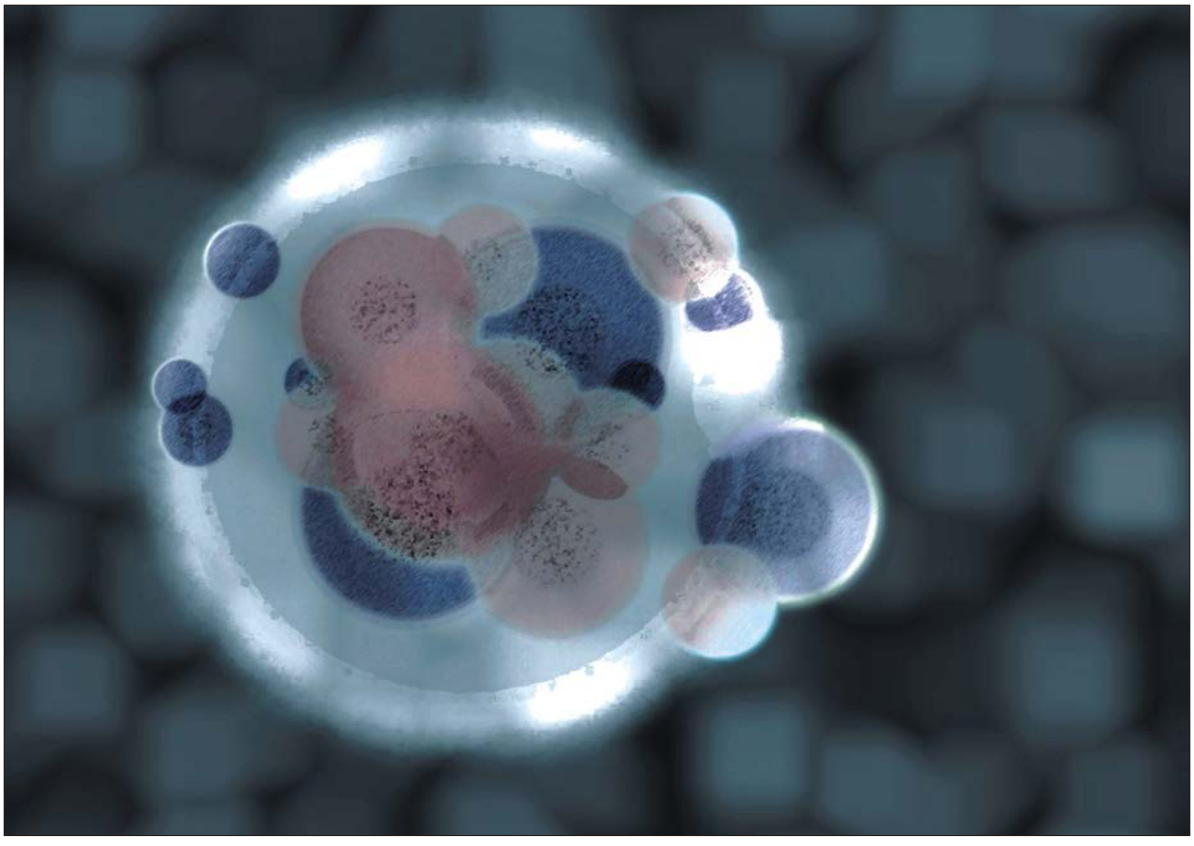

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2018.280.288

Research Article
Tail Erosion an Emerging Disease Affecting Tilapia Fry in Kafrelsheikh Hatcheries

Nadia Gabr Ali and Ibrahim Mohamed Aboyadak

National Institute of Oceanography and Fisheries, Alexandria branch, Egypt

Abstract

Background and Objective: Tilapia hatchery sector at Kafrelsheikh was suffered from increased mortality in newly hatched fry that results in severe economic losses, the present research was conducted to determine the possible causes of fry mortality affecting two *Oreochromis niloticus* hatcheries at Kafrelsheikh governorate. **Materials and Methods:** A total number of 100 tilapia fry from two affected hatcheries were randomly collected for clinical and parasitological examination, another 150 naturally infected tilapia fry was used in the treatment trial using Viroscan or formalin-malachite green combination for determination of their efficacy in controlling of such infection. Water temperature, dissolved oxygen, ammonia, pH and salinity were also determined. **Results:** The cumulative mortality was estimated by 14 and 10% during 21-day post hatch. Temperature, dissolved oxygen, pH and salinity were suitable for *Oreochromis niloticus* fry rearing while, ammonia level was on the borderline. The main clinical signs observed on diseased fry were darkening color, tail erosion and swimming near the water surface. Microscopic examination revealed infestation with *Gyrodactylus* and *Trichodina*. The total prevalence of parasitic infestation was 92 and 84%, the mean intensity was 25.97±7.28 and 17.3±3.92 parasite/fry for *Gyrodactylus*, 33.92±8.59 and 13±3.51 for *Trichodina* in first and second hatchery, respectively. *Gyrodactylus* infestation controlled with long bath treatment using formalin-malachite green combination (50+0.2 ppm)/litter for 4 h while either the recommended dose (25+0.1 ppm) or Viroscan at (100 and 50 ppm) was ineffective. *Trichodina* resist all treatments after 4 h of application. All the applied treatments were safe for the fry but the high concentration of formalin-malachite green causes limited mortality (3.33%). **Conclusion:** *Gyrodactylus* sp. and *Trichodina* sp. were the direct causes of mortality in affected hatcheries, Formalin-malachite green mixture (50+0.2 ppm) was effective in controlling *Gyrodactylus* infestation also they considered safe at this concentration.

Key words: Formalin, *Gyrodactylus*, malachite green, *Oreochromis niloticus*, *Trichodina*, Viroscan, water parameters

Received: May 06, 2018 Accepted: June 28, 2018 Published: July 15, 2018

Citation: Nadia Gabr Ali and Ibrahim Mohamed Aboyadak, 2018. Tail erosion an emerging disease affecting tilapia fry in kafrelsheikh hatcheries. J. Biol. Sci., 18: 280-288.

Corresponding Author: Nadia Gabr Ali, Laboratory of Fish Diseases, Division of Aquaculture, National Institute of Oceanography and Fisheries, Alexandria branch, Egypt Tel: 00201007167412

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Egypt is the second global tilapia producer, in 2014 the Egyptian production reached 867557 metric ton[1]. Fish fry (seeds) are the main element in aquaculture, tilapia hatcheries at Kafrelsheikh district produce about 291.300 million units that represents 88% of the total Egyptian production[2].

External parasites have a potential risk in pisciculture, they have direct effect on growth and survival of affected fish[3], it also play a role as a predisposing cause for bacterial infection through skin abrasions induced by these parasites and consider a route of entry for bacterial fish pathogens. Fry are considered more susceptible to external parasitic infestation than adult fish.

The *Gyrodactylus* species are among the monogenean flatworms parasitizing a great variety of brackish and freshwater fish, they develop directly without any intermediate hosts[4-6]. *Gyrodactylus* is provided with two large central hooks and 16 marginal booklets in their opisthaptor, these hooks cause severe irritation and damage to infested fish skin, gills and fins that adversely affect fish health[7].

Trichodinids are the most common ciliate parasites present on the skin of pond-reared fish, it can cause severe economic losses in aquaculture[8]. Heavy infection with *Trichodina* sp., particularly in small fish has caused gigantic financial losses[9,10]. Infected fish are lethargic, generate excessive mucus and become off-feed eventually which results in considerable deaths[11].

Formalin and malachite green are extensively used as biocides in the aquaculture industry, they are effective against ectoparasites.

Formalin is effective parasiticide against most of external fish parasites including monogenean trematodes and protozoa[12]. The antiparasitic effect of formalin is achieved through denaturation of the ectoparasite protein, this mechanism occurs as a result of its reaction with aromatic rings and thiol group and other active groups of the protein molecules[13].

Malachite green is a triphenylmethane dye, it has a potent antiparasitic activity and so it is widely used as bath treatment in aquaculture. The mechanism of malachite green action is achieved through destruction of respiratory chain proteins of bacteria, fungus and small parasites resulted in pathogen death[14].

Currently, Viroscan is one of the most frequently used disinfectants in tilapia farms and hatcheries at Kafrelsheikh, it used to reduce fish mortality caused by bacterial infection and is to reduce fry mortality caused by parasitic infestation at a dose of 0.5 ppm.

Many tilapia hatcheries in Kafrelsheikh suffered from increased mortality. So, the aims of the present study were to determine the direct cause of mortality and to estimate the efficacy of Viroscan in termination of such infestation in comparison with traditional method using formalin and malachite green and to study the safety of used chemicals on treated fry.

MATERIALS AND METHODS

Study area: Samples were taken from two tilapia hatcheries suffered from fry mortality in Torombat seven, Rhyead district, Kafrelsheikh governorate northern Egypt. The studied hatcheries lie between longitudes 30.9564° East and latitudes 31.3842° North, this study was conducted in April and May, 2016.

Fish: A total number of one hundred tilapia fry were randomly sampled, fifty fry was microscopically examined from each hatchery and another 150 infested fry were collected for experimental treatment trial, examined fry were ranged between 10-15 mm in the total length.

Clinical examination: Clinical examination was performed as described by Noga[12].

Parasitological examination: Parasitological examination was performed using binocular microscope, each five fry were picked up to a glass Petri dish contain pond water and methane-sulfonate (MS-222) anesthetic at a concentration of 50 mg L^{-1} as recorded by Popovic *et al.*[15] for preventing fry movement during the microscopical examination, types and number of the present parasites were recorded.

Experimental treatment trial: This trial was conducted to determine the efficacy of formalin and malachite green combination or Viroscan in controlling *Gyrodactylus* infestation.

Chemicals used in the treatment trial: Formalin 37% solution ADWIC, Co., Egypt, Malachite green Alpha chemika, India and Viroscan® disinfectant, composition: Alkyl dimethyl benzyl ammonium chloride 240 g L^{-1}, glutaraldehyde 107.25 g L^{-1}, isopropanol 146.25 g L^{-1} and pine oil 20 g L^{-1}, SLANT CHEM, Co.

Experiment design: One hundred and fifty infested *Oreochromis niloticus* (tilapia) fry were used for experimental treatment trial, fry was divided randomly into five equal groups, in triplicates, each group contain 10 infested fry, each

Table 1: Fry grouping, chemicals used in treatment trial and their concentrations

Groups	Treatments	Formalin μL L^{-1} (ppm)	Malachite green mg L^{-1} (ppm)	Viroscan μL L^{-1} (ppm)
1	Infested non-treated	-	-	-
2	Formalin-malachite green	50	0.2	-
3	Formalin-malachite green	25	0.1	-
4	Viroscan	-	-	100
5	Viroscan	-	-	50

group was kept in 20 L glass aquaria supplied with hatchery water with continuous aeration using air pump Boyo®, fry grouping and the used chemicals doses are presented in Table 1.

Fry was grossly observed for clinical signs and mortality they were also, microscopically examined for the survival and activity of *Gyroductylus* every 30 min during the first hour then hourly.

Tested water parameters

Water temperature: Water temperature was measured using floating thermometer according to the method number 2550 described by APHA[16].

Dissolved oxygen: Dissolved oxygen was determined according to the method number 4500 described by APHA[16] depending on the electrometric method using membrane electrodes that based on the rate of diffusion of molecular oxygen across a membrane. Dissolved oxygen was determined using portable dissolved oxygen meter for aquaculture (HANNA, Italy).

Ammonia: The NH_3 was determined using low range ammonia portable photometer (HANNA, Italy) based on Nessler method as described by the manufacturer instructions.

pH: The activity of the hydrogen ions was determined according to the method number 4500 H^+ described by APHA[16] depending on electrometric method by potentiometric measurement using a standard hydrogen electrode and a reference electrode. It was measured using pH meter (HANNA, Italy).

Salinity: Salinity was measured using refractometer as described by Zhao *et al.*[17].

Statistical analysis: One sample T-test was used to determine the mean intensity of parasitic infestation per fry expressed as mean±standard deviation using MedCalc for windows, version 17.2.2, (MedCalc Software, bvba, Ostend, Belgium, https://www.medcalc.org; 2017).

RESULTS

The recorded cumulative mortality (during first 21-day post hatch) was 14 and 10% in the first and second hatchery, respectively. Affected fry appeared dark in color (Fig. 1a), swim near the water surface, easily caught (lost escape reflex) and were smaller in size than the normal one of the same age. Microscopic examination of affected fry revealed the presence of tail erosions (Fig. 1b), *Gyrodactylus* attached to eroded fry tail (Fig. 1c), nearly all fry had mixed infestation with both *Gyrodactylus* and *Trichodina* (Fig. 1c, 2d).

There was not any evidence observed during the microscopical examination of affected fry indicating the presence of bacterial infection as skin ulceration, hemorrhage or scale desquamation.

The parasitological examination revealed the presence of mixed infestation with *Gyrodactylus* and *Trichodina* and very rarely to be alone.

Gyrodactylus was 440-510 μm in length, prohaptor has 2 cephalic lopes and was 42 μm in diameter, opisthaptor diameter was 64 μm in diameter and has one pair of central hooks (Fig. 1c, d).

Trichodina was cub shaped 40-50 μm in diameter and was 30 μm in height (lateral view), with an obvious denticulate ring and adhesive disk (Fig. 2a, b).

The total prevalence of parasitic infestation in the first hatchery was 92% (46 out of 50) the prevalence of infestation with *Gyrodactylus* and *Trichodina* was 86 and 78%, respectively, the intensity was 25.97±7.28, 33.92±8.59 and the range was 12-37 and 17-46 parasite per fry for *Gyrodactylus* and *Trichodina*, respectively (Table 2-4).

The total prevalence of parasitic infestation in the second hatchery was 84% (42 out of 50) the prevalence of infestation with *Gyrodactylus* and *Trichodina* was 80 and 70%, respectively, the intensity was 17.3±3.92, 13±3.51 and the range was 9-23 and 5-18 parasite per fry for *Gyrodactylus* and *Trichodina*, respectively (Table 2-4).

Treatment trial revealed the efficacy of formalin-malachite green at a concentration of (50+0.2 ppm) in partial control of *Gyrodactylus* infestation as it was effective in decreasing monogenean parasite population to 50% (kill 50% of

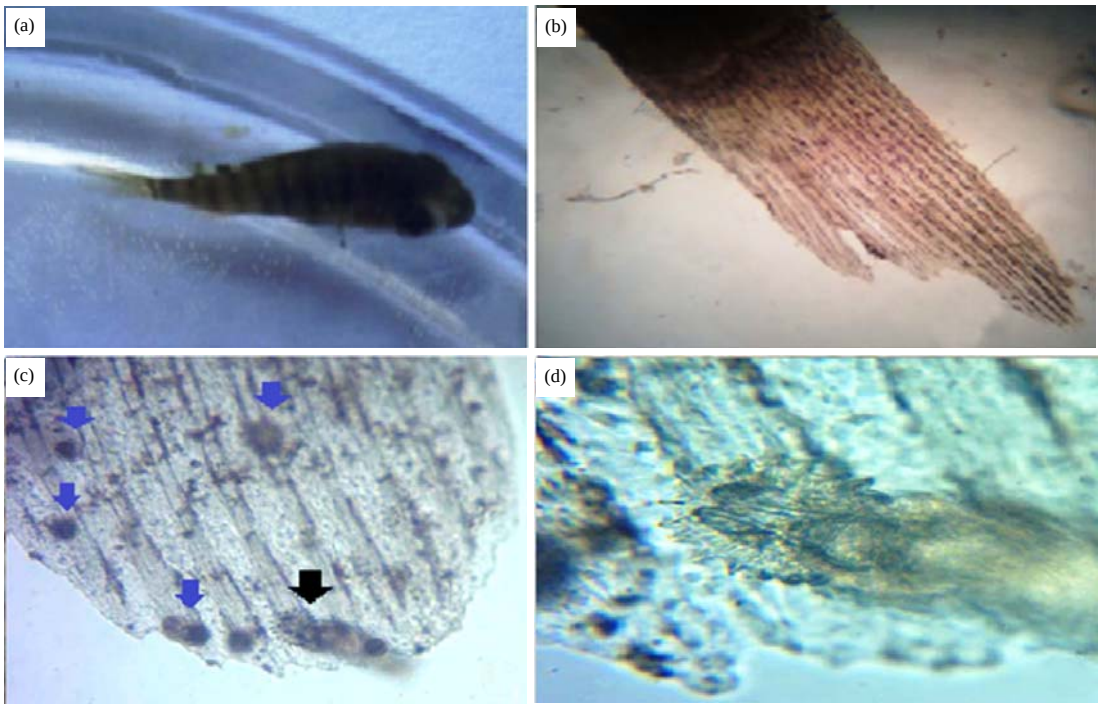

Fig. 1(a-d): (a) *Oreochromis niloticus* fry suffered from tail erosion disease with obvious dark body coloration, (b) Diseased fry tail with marked erosion, X = 40, (c) Diseased fry tail with mixed infestation, *Gyrodactylus* (black arrow) and *Trichodina* (blue arrow), X = 100 and (d) *Gyrodactylus* attached to fry tail with opisthaptor carry 16 marginal hooklets and two large central hooks, X = 400

Table 2: Total prevalence of parasitic infestation in monosex *Oreochromis niloticus* fry

Locations	Number of examined fry	Number of infested fry	Infestation (%)
Hatchery 1	50	46	92
Hatchery 2	50	42	84

Table 3: Prevalence of *Gyrodactylus* and *Trichodina* infestation in monosex *Oreochromis niloticus* fry

		Gyroductylus		*Trichodyna*	
Locations	Number of examined fry	Number of infested fry	Infestation (%)	Number of infested fry	Infestation (%)
Hatchery 1	50	43	86	39	78
Hatchery 2	50	40	80	35	70

Table 4: Intensity of *Gyrodactylus* spp. and *Trichodina* spp. infestation in monosex *Oreochromis niloticus* fry

	Gyroductylus parasite/fry		*Trichodyna* parasite/fry	
Locations	Range	Mean±SD	Range	Mean±SD
Hatchery 1	12-37	25.97±7.28	17-46	33.92±8.59
Hatchery 2	9-23	17.30±3.92	5-18	13.00±3.51

Gyrodactylus) and decrease the vitality of remaining portion after 4 h of application, while application of formalin-malachite green at a concentration of (25+0.1 ppm) have a limited effect restricted in decreasing parasite motility after 3 h of application and all parasites remain live after 4 h post application. Treatment with viroscan at a concentration of 100 or 50 ppm was also not effective in *Gyrodactylus* eradication as it is decreasing parasite motility 2 and 4 h after application but not kill any parasite (Table 5).

All treatment was ineffective in termination of *Trichodina* infestation, the protozoan remains viable, motile and attached to infested fry during treatment even after 4 h of application.

All treatments were safe to *Oreochromis niloticus* fry, they were not having any adverse effect on treated fry and so considered generally safe but formalin-malachite green (50+0.2 ppm) caused 3.33% mortality after 4 h of application (Table 6).

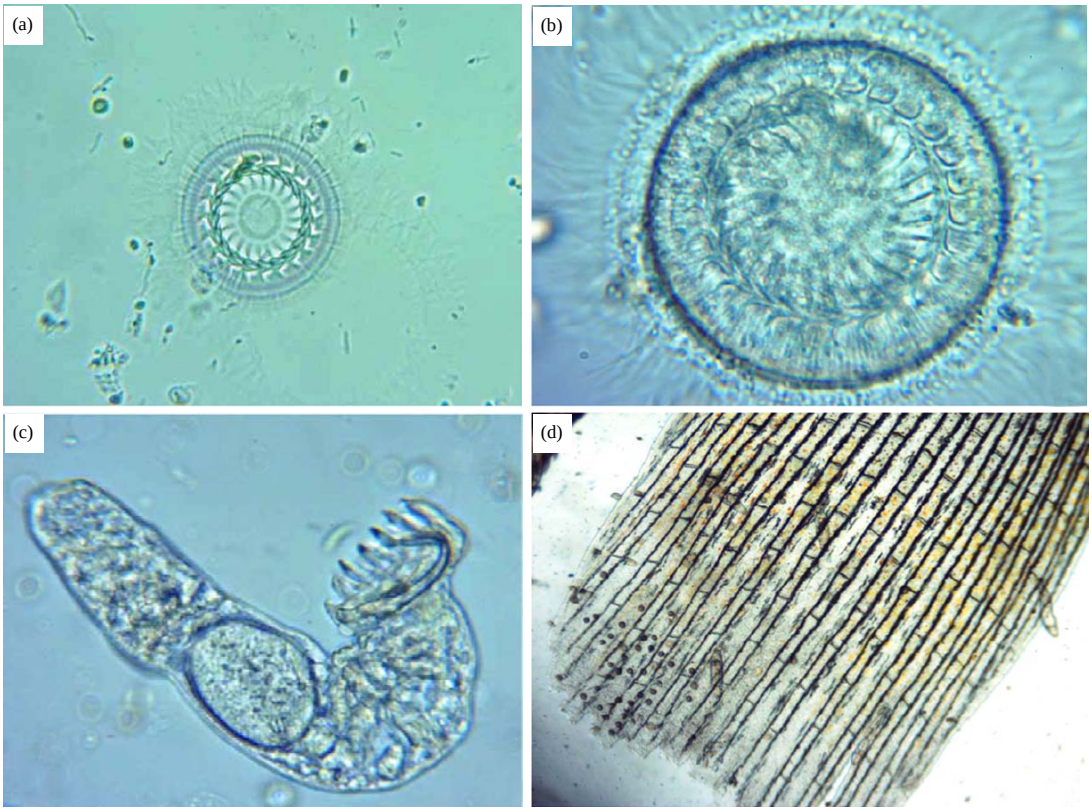

Fig. 2(a-d): (a, b) *Trichodina* aboral surface surrounded by cilia with obvious adhesive disk and denticulate ring, X = 400 in a, 1000 in b, (c) Dead detached *Gyrodactylus* after treatment, X = 400 and (d) Mixed infestation with numerous *Gyrodactylus* and *Trichodina*, X = 40

Table 5: Effect of different treatments on *Gyrodactylus* spp.

Groups	Treatments	30 min	60 min	2 h	3 h	4 h
1	Infested non-treated	-ve	-ve	-ve	-ve	-ve
2	Formalin (50 ppm)+malachite green (0.2 ppm)	-ve	+	++	+++	+++
3	Formalin (25 ppm)+malachite green (0.1 ppm)	-ve	-ve	-ve	+	+
4	Viroscan (100 ppm)	-ve	-ve	+	+	+
5	Viroscan (50 ppm)	-ve	-ve	-ve	-ve	+

-ve: Parasite viability and activity (movement or motility) not affected, +(slight): All parasite remains live but motility of 40-50% of examined parasites decreased, ++(moderate): Majority of parasite remain live but the motility of 70-80% of examined parasites decreased, +++(severe): More than 50% of examined parasites contract without any motility (die) and the rest have sluggish movement

Table 6: Effect of different treatments on monosex *Oreochromis niloticus* fry

Groups	Treatments	30 min	60 min	2 h	3 h	4 h
1	Infested non-treated	-ve	-ve	-ve	-ve	-ve
2	Formalin (50 ppm)+malachite green (0.2 ppm)	-ve	-ve	-ve	+	++
3	Formalin (25 ppm)+malachite green (0.1 ppm)	-ve	-ve	-ve	-ve	-ve
4	Viroscan (100 ppm)	-ve	-ve	-ve	-ve	-ve
5	Viroscan (50 ppm)	-ve	-ve	-ve	-ve	-ve

-ve: Fry activity not affected, +: Activity of 3 fry decreased and swim near aquarium floor, ++: One fry die (3.33%)

Table 7: Hatchery tested water parameters

Parameters	Hatchery 1	Hatchery 2
Water temperature	28.50	31.00
Dissolved oxygen (mg L^{-1})	9.10	7.30
Ammonia (mg L^{-1})	0.07	0.05
pH	7.09	7.36
Salinity (‰)	5.00	8.00

Tested hatchery water parameters were presented in Table 7, water temperature, dissolved oxygen, pH and salinity were in the acceptable rage suitable for normal growth of *Oreochromis niloticus* fry in both hatcheries. Ammonia level was slightly high in first and on borderline in the second one, generally acceptable ammonia level is less than 0.06 mg L^{-1}.

DISCUSSION

The ectoparasitic diseases play an important role in the economic losses in farmed fish through direct mortality or decreased growth rate of fry or fingerlings[18-20]. The majority of fish ectoparasitic protozoa are commensals but some of them may produce serious diseases and mortality especially in smaller fishes[21,22]. *Trichodina* spp. are a group of dorso-ventrally flattened oval ciliated protozoan parasites of marine and freshwater species of finfish. *Trichodina* spp. can cause extensive fish mortality in aquaculture systems[23]. *Gyrodactylus* is an ectoparasite of potential significance to tilapia culture, clinical outbreaks of gyrodactylosis have been recorded in pond-reared tilapia worldwide[24,25]. Protozoans and monogenean parasites are frequently infesting cultured tilapia fry affecting fish growth[26,27]. The most frequent parasites infesting masculinized *Oreochromis niloticus* fry during first 22 day post hatch were *Trichodina* and *Gyrodactylus cichlidarum* (Monogenea)[28].

The clinical signs including mortality, dark coloration and loss of condition that were observed on diseased fry could be attributed to heavy infestation with both *Gyrodactylus* and *Trichodina*, heavily infested fish do not eat well and lost their body conditions as described by Klinger and Floyd[29], Soliman *et al*.[30]. Paperna[31] recorded heavy infestation with trichodinids in cichlids fry 10-12 mm long which is similar to examined fry length, small fish and fry are especially susceptible to parasitism with *Trichodina* sp.[32]. Tail erosion is one of the most dominant clinical signs in this disease, this sign greatly affect fish swimming behavior and subsequently decrease fish speed and negatively affect fry competition for feed that subsequently resulted in underfeeding (small fry).

In the present work the recorded mortality was 14 and 10% in the first and second hatchery this is mainly due to increased prevalence (86 and 80%) for *Gyrodactylus* and (78 and 70%) for *Trichodina*. The incidence of parasitic infestation also followed the same trend (25.97±7.28 and 17.3±3.92) for *Gyrodactylus* and (33.92±8.59 and 13±3.51) for *Trichodina* in first and second hatchery, respectively. This trend is mainly due to the direct life cycle of both parasites that transmitted through direct contact and via water so increased mortality, prevalence and incidence follow the same manner (directly proportional to each other). These observations supported by Thoney and Hargis[33], who reported, rapid propagation and direct transfer of monogenean parasites between fish.

Microscopic examination showed tail erosions, it could result from destruction induced by the attachment organs of infesting parasites i.e., the two central hooks and the marginal hooklets of opisthaptor in *Gyrodactylus* and denticulate ring that carry many sharp denticles in *Trichodynia*. These attachment organs mainly responsible for irritation and tearing of fry tail and fins.

Chemical treatments are the most common method used in controlling fish diseases in aquaculture[34]. It is the most successful way in termination of parasitic infection[35], it provides short-term control and is effective against attached parasite stages[36]. In the current research formalin-malachite green combination at the recommended concentration (25+0.1 ppm) appear to be ineffective or have a limited activity restricted only in decreasing the parasite motility, while duplicated concentration i.e. (50+0.2 ppm) is more effective against *Gyrodactylus*, Rowland *et al*.[37] recorded the ineffective effect of formalin at 25 ppm against *Gyrodactylus*, while they observed a significant decrease in parasite numbers occurs at 40 ppm. Srivastava *et al*.[38] and Schelkle *et al*.[39] also recorded the effectiveness of formalin-malachite green combination against *Gyrodactylus*. Anti-parasitic effect of formalin is induced through denaturation of parasite protein together with irreversible uncoupling of oxidative phosphorylation achieved by malachite green.

Trichodina was resistant to all treatment that may be due to insufficient concentration of formalin used in current study, Smith[23] recorded that effective concentration of formalin against *Trichodina* is 170-250 ppm for 1 h, Hassan[21] also, found that formalin long path (250 ppm) for 24 h is effective in termination of *Trichodina* infestation in *Oreochromis niloticus*.

Formalin-malachite green combination appears to be safe for fry, as only one fry dies after 4 h of application, Perera and Pathiratne [40] indicated the safety of formalin when used for therapeutic purposes at a dose of 50 ppm in tilapia fingerlings without any adverse effect on treated fish. Omoregie *et al*.[41] determined the 96 h LC_{50} of malachite green in *Oreochromis niloticus* fingerlings by 0.427 ppm, that indicated its safety when used at 0.2 ppm.

Viroscan and structurally related compounds are the most commonly used disinfectant in controlling mortality at *Oreochromis niloticus* hatchery in Kafrelsheikh governorate so it was important to determine their efficacy against *Gyrodactylus* infestation. Both Viroscan concentrations (50 and 100 ppm) were ineffective in such disease termination even it not only appears safe but also well tolerated by *Oreochromis niloticus* fry. Viroscan (quaternary Ammonium Compounds) are active against bacteria, molds and some viruses by bind irreversibly to the negatively charged phospholipids and proteins of the cell membranes of microorganisms, impairing membrane permeability[42]. This

explain ineffectiveness of Viroscan in controlling the parasitic infestation, despite this product contain glutaraldehyde which works similarly to formalin but its concentration, when calculated alone, was 10 ppm (when viroscan used at 100 ppm) which consider insufficient to affect *Gyrodactylus*.

Measured water parameters were within the acceptable limit for optimum fry rearing as mentioned by El-Sayed [27], so they are not incorporated in this disease condition. Ammonia level was slightly elevated or on the borderline which consider a stress factor predisposing to parasitic infestation, this coincides with the observations recorded by Jimenez-Garcia *et al.*[28]. Stressful environmental conditions decrease the resistance of fish to monogenean infections[43].

The current research results regarding the clinical signs and microscopical examination indicating the absence of any evidence for bacterial infection together with acceptable hatchery water quality indicating the responsibility of parasitic infestation for the occurrence of the erosion disease affecting *Oreochromis niloticus* fry at examined hatcheries.

CONCLUSION

In conclusion *Gyrodactylus* sp. and *Trichodina* sp. were the direct causes of mortality in affected tilapia hatcheries, the mortality percent is directly proportional to the incidence of infestation. Formalin-malachite green mixture at (50+0.2 ppm) was effective in controlling *Gyrodactylus* infestation also they considered safe at this concentration while the lower concentration does not affect the parasite survival. Viroscan fails to kill *Gyrodactylus* after 4 h of exposure at the tested concatenations (100 and 50 ppm). The low concentration of Formalin-malachite green mixture and Viroscan should not be used at the tested concentration in controlling *Gyrodactylus* infestation in *Oreochromis niloticus* fry.

SIGNIFICANT STATEMENT

This article identified *Gyrodactylus* and *Trichodina* as the actual causes of the huge economic losses in tilapia hatcheries, it also, proves the inefficacy of the most commonly used drug in tilapia hatcheries (Viroscan) together with inefficacy of formalin-malachite green mixture at (25+0.1 ppm) while when it used at (50+0.2 ppm) it was effective and safe in controlling this disease.

REFERENCES

1. FAO., 2016. The State of World Fisheries and Aquaculture: Contributing to Food Security and Nutrition for All. Food and Agriculture Organization, Rome, Italy, pp: 17.
2. GAFRD., 2016. Fish Statistics Yearbook 2014. General Authority for Fish Resources, Cairo, Egypt, pp: 79.
3. Ali, N.G. and I.M. Aboyadak, 2018. Histopathological alterations and condition factor deterioration accompanied by isopod infestation in *Tilapia zilli*, *Mugil capito* and *Solea aegyptiaca* from Lake Qaroun. Egypt. J. Aquat. Res., 44: 57-63.
4. Nemeth, S., Z. Horvath, Z. Felfoldi, G. Beliczky and K. Demeter, 2013. The use of permited ectopar asite disinfection methods on young pike perch (*Sander lucioperca*) after transition from over win tering lake to RAS. AACL Bioflux, Vol. 6.
5. Kvach, Y., M. Janac, S. Nehring, M. Ondrackova and P. Jurajda, 2017. Parasite communities and infection levels of the invasive Chinese sleeper *Perccottus glenii* (Actinopterygii: Odontobutidae) from the Naab river basin, Germany. J. Helminthol., 91: 703-710.
6. Ozer, A., Y. Kornyychuk, T. Ozturk and V. Yurakhno, 2016. Levels of infection and seasonal occurrence of *Gyrodactylus alviga* (Monogenea: Gyrodactylidae) on the whiting *Merlangius merlangus* off the Turkish and Russian coasts of the Black sea. J. Helminthol., 90: 347-352.
7. Tekmedash, F.S., M. Hemmatzadeh and H. Khara, 2016. Stress indices of grass carp, *Ceteopharyngodon idella*, (*Cuvier* and *Valenciennes*, 1884) change in response to Monogenean parasites pollution, *Gyrodactylus* spp. and *Dactylogyrus* spp. J. Parasitic Dis., 40: 1044-1046.
8. Yao, J.Y., X.L. Li, J.Y. Shen, X.Y. Pan and G.J. Hao *et al*., 2011. Isolation of bioactive components from *Chelidonium majus* L. with activity against *Trichodina* sp. Aquaculture, 318: 235-238.
9. Saha, M. and P.K. Bandyopadhyay, 2017. Seasonal incidence of protozoan parasitic infestation in ornamental fishes of West Bengal, India. J. Parasitic Dis., 41: 523-526.
10. Tavares-Dias, M. and M.L. Martins, 2017. An overall estimation of losses caused by diseases in the Brazilian fish farms. J. Parasitic Dis., 41: 913-918.
11. Chitmanat, C., K. Tongdonmuan and W. Nunsong, 2005. The use of crude extracts from traditional medicinal plants to eliminate *Trichodina* sp. in tilapia (*Oreochromis niloticus*) fingerlings. Songklanakarin J. Sci. Technol., 27: 359-364.
12. Noga, E.J., 2010. Fish Disease Diagnosis and Treatment. 2nd Edn., Blackwell, Iowa State, USA., pp: 13.
13. Van Ham, E.H. and M.R. Hall, 1998. The effects of prophylactic formalin bath treatment on blood glucose in the giant tiger prawn *Penaeus monodon*. J. World Aquacult. Soc., 29: 357-364.
14. Alderman, D.J., 1985. Malachite green: A review. J. Fish Dis., 8: 289-298.
15. Popovic, N.T., I. Strunjak Perovic, R. Coz Rakovac, J. Barisic, M. Jadan, A.P. Berakovic and R.S. Klobucar, 2012. Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. J. Applied Ichthyol., 28: 553-564.

16. APHA., 2012. Standard Methods for Examination of Water and Wastewater. 22nd Edn., American Public Health Association, Washington, DC., USA.
17. Zhao, Y., B. Zhang and Y. Liao, 2003. Experimental research and analysis of salinity measurement based on optical techniques. Sensors Actuat. B: Chem., 92: 331-336.
18. Hagen, A.G., S. Hytterod and K. Olstad, 2014. Low concentrations of sodium hypochlorite affect population dynamics in *Gyrodactylus salaries* (Malmberg, 1957): Practical guidelines for the treatment of the Atlantic salmon, *Salmo salar* L. parasite. J. Fish Dis., 37: 1003-1011.
19. Hoai, T.D. and K.V. Van, 2014. Efficacy of praziquantel against external parasites infecting freshwater fish. J. Sci. Dev., 12: 711-719.
20. Paredes-Trujillo, A., I. Velazquez-Abunader, E. Torres-Irineo, D. Romero and V.M. Vidal-Martinez, 2016. Geographical distribution of protozoan and metazoan parasites of farmed Nile tilapia *Oreochromis niloticus* (L.) (Perciformes: Cichlidae) in Yucatan, Mexico. Parasites Vectors, Vol. 9. 10.1186/s13071-016-1332-9.
21. Hassan, M.A.E.A.H., 1999. Trichodiniasis in farmed freshwater tilapia in Eastern Saudi Arabia. Mar. Sci., 11: 3-17.
22. Dobberstein, R.C. and H.W. Palm, 2000. *Trichodinid ciliates* (Peritrichia: Trichodinidae) from the Bay of Kiel, with description of *Trichodina claviformis* sp. n. Folia Parasitol., 47: 81-90.
23. Smith, S. and M. Schwarz, 2009. Dealing with *Trichodina* and *Trichodina*-like species, publication 600-205. Virginia Cooperative Extension Publication 600-205, Virginia State University, Virginia, USA. https://pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/600/600-205/600-205_pdf.pdf
24. Garcia-Vasquez, A., H. Hansen and A.P. Shinn, 2007. A revised description of *Gyrodactylus cichlidarum* Paperna, 1968 (Gyrodactylidae) from the Nile tilapia, *Oreochromis niloticus niloticus* (Cichlidae) and its synonymy with *G. niloticus* Cone, Arthur et Bondad-Reantaso, 1995. Folia Parasitol., 54: 129-140.
25. Amoako, M., 2006. Infestation of ectoparasites on Nile tilapia (*Oreochromis niloticus*) in aquaculture production in the Ashanti region, Ghana. Master's Thesis, Department of Marine and Freshwater Biology Norwegian College of Fishery Science University of Tromso, Norway.
26. Barker, D.E., D.K. Cone and M.D.B. Burt, 2002. *Trichodina murmanica* (Ciliophora) and *Gyrodactylus pleuronecti* (Monogenea) parasitizing hatchery-reared winter flounder, *Pseudopleuronectes americanus* (Walbaum): Effects on host growth and assessment of parasite interaction. J. Fish Dis., 25: 81-89.
27. El-Sayed, A.F.M., 2006. Tilapia Culture. CABI Publishing International, Wallingford, UK., pp: 34-43.
28. Jimenez-Garcia, I., C.R. Rojas-Garcia, C.N. Castro-Jose, S. Pavon-Suriano, F. Lango-Reynoso and M.D.R. Castaneda-Chavez, 2012. Growth enhancement, survival and decrease of ectoparasitic infections in masculinized Nile tilapia fry in a recirculating aquaculture system. Trop. Subtrop. Agroecosyst., 15: S51-S56.
29. Klinger, R.E. and R.F. Floyd, 2013. Introduction to freshwater fish parasites. Institute of Food and Agricultural Sciences (IFAS) University of Florida, Florida, Fla, USA. https://www.sdstate.edu/sites/default/files/nrm/outreach/pond/upload/Fish-Parasites.pdf
30. Soliman, F.M., M.A.A. Abd El-Galil, M.A. Adly and A.A. Fatma El Zahraa, 2013. Studies on trichodinosis of some cultured freshwater fishes at Sohag governorate. Life Sci. J., 10: 1400-1409.
31. Paperna, I., 1996. Parasites, infections and diseases of fish in Africa: An update. CIFA technical paper. FAO., Rome, pp: 52-57.
32. Lom, J., 1995. Protozoan and Metazoan Infections. In: Fish Diseases and Disorders, Woo P.T.K. (Ed.)., CABI Publishing International, New York, USA.
33. Thoney, D.A. and W.J. Hargis, Jr., 1991. Monogenea (Platyhelminthes) as hazards for fish in confinement. Ann. Rev. Fish Dis., 1: 133-153.
34. Aboyadak, I.M., M.A.A. Mohamed, M.S. Gado, K.A. El-Shazly and N.G. Ali, 2016. Role of some antibacterial drugs in control *Streptococcus iniae* Infection in *Oreochromis niloticus*. J. Pharmacol. Clin. Res., Vol. 1. 10.19080/JPCR.2016.01.555573.
35. Eissa, I.A.M., V.H. Zaki, N.G. Ali, M. Zaki and I.M. Aboyadak, 2013. Gut digeneaiasis in African catfish *Clarias gariepinus* with estimating the efficacy of some anthelmintics. Life Sci. J., 10: 1605-1609.
36. Ernst, I., I.D. Whittington, S. Corneillie and C. Talbot, 2005. Effects of temperature, salinity, desiccation and chemical treatments on egg embryonation and hatching success of *Benedenia seriolae* (Monogenea: Capsalidae), a parasite of farmed *Seriola* spp. J. Fish Dis., 28: 157-164.
37. Rowland, S.J., M. Nixon, M. Landos, C. Mifsud, P. Read and P. Boyd, 2006. Effects of formalin on water quality and parasitic monogeneans on silver perch (*Bidyanus bidyanus* Mitchell) in earthen ponds. Aquacult. Res., 37: 869-876.
38. Srivastava, S., R. Sinha and D. Roya, 2004. Toxicological effects of malachite green. Aquat. Toxicol., 66: 319-329.
39. Schelkle, B., A.P. Shinn, E. Peeler and J. Cable, 2009. Treatment of gyrodactylid infections in fish. Dis. Aquat. Org., 86: 65-75.
40. Perera, H.A.C.C. and A. Pathiratne, 2005. Effects of short term exposure to therapeutic levels of formalin on health status of Nile Tilapia, *Oreochromis niloticus*. J. Nat. Sci. Found. Sri Lanka, 33: 239-245.

41. Omoregie, E., P.C. Ofojekwu, J.C. Anosike and A.O. Adeleye, 1998. Acute toxicity of malachite green to the Nile Tilapia, *Oreochromis niloticus* (L). J. Aquacult. Tropics, 13: 233-237.
42. Boothe, H.W., 1998. Antiseptics and Disinfectants. In: Clinical Pharmacology and Therapeutics, Boothe, D. (Ed.)., 2nd Edn., Harcourt Brace Jovanovich, Inc., Philadelphia, PA., pp: 233-248.
43. Buchmann, K. and J. Bresciani, 2006. Monogenea (Phylum Platyhelminthes). In: Fish Diseases and Disorders, Woo, P.T.K. (Ed.)., 2nd Edn., Vol. 1, CAB International, Nosworthy Way, Walling Ford, Oxford Shire, USA., pp: 297.