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Research Article

Analysis of TNF alpha and Interlukin-1β genes in *Oreochromis niloticus*: inflammatory responses induced by *Myxobolus* spp. and *Trichodina* spp.

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Abstract: The cultured and wild specimens of the Nile tilapia (*Oreochromis niloticus*) are often infected by different hazardous as helminths; protozoa and arthropod which cause various degrees of diseases in such fish. The aim of this study was to record the most common protozoan parasites in the Nile tilapia in Egypt, and also assessment of the immunological changes through analyzing two genes (Tumor Necrosis Factor alpha, TNF-α; and Interlukin-1β, IL- 1β) in the infected fishes using qRT-PCR. Therefore, each fish individual was examined carefully, and smears were prepared from each organ; stained with Giemsa dye and examined under a light microscope. qRT-PCR was performed on infected tissues (skin and gills) by various collected parasites. Four trichodinid species (*Trichodinella heterodentata*, *T. centrostrigeata*, *T. epizootica*, Paratrichodina *Africana*,) were isolated and identified; also two *Myxobolus* spp. (*M. tilapiae* and *M. heterosporous*) were fully described. In skin, TNF-α in *M. tilapiae* and mixed groups were nearly similar and were significantly higher than *Trichodina* spp. group. IL-1β of *M. tilapiae* group was significantly lower than that of the mixed group; *Trichodina* spp. and control groups. In gills, TNF-α of the mixed group showed the significantly highest value. IL-1β was nearly same in all groups. We conclude that the skin is nearly higher in upregulation of the two studied genes comparing to the gills analysis, since it is considered as the first barrier in fish and contains many immunological defined cells.

Keywords: Nile tilapia, Tumor Necrosis Factor alpha, Interlukin-1β; Real-time Polymerase Chain Reaction.

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Introduction

The Nile tilapia, *Oreochromus niloticus* has a significant commercial value in Egypt, yet wild and cultured tilapia are facing a severe loss because of various ectoparasites; helminths and protozoa, which cause various degrees of diseases in this species (Symth 1994). Family Trichodinidae with worldwide distribution, comprises 300 species which are considered as parasites of freshwater and marine fish species, invading the skin, gills and fins. Although they do not benefit from their host, they collect bacteria from the fish body surface (Van As & Basson 1992). Presence of a large number of trichodinid species leads to different clinical signs or/ and mortality rate in the infected host especially in the young fish.

Trichodinids are commonly studied and recognized as ciliates of different fish species, and their importance are recorded in several studies as in prevalence (Ozer & Erdem 1998, 1999; Ozer 2000, 2003), pathogenicity (Van As & Basson 1987; Ozer & Erdem 1999), and the effects of the environmental aspects on the protozoan parasites (Ozer & Erdem 1999). Many Trichodinid species were isolated from the Nile tilapia (*O. niloticus*), such as *T. centrostrigeata*, *T. compacta*, *T. migala*, *T. heterodentata*, *P. africana*, *Tripartiella orthodens* (Valladao et al. 2016). There is an increasing

attention about myxozoans, since they affect wild and cultured fish populations all over the world (Feist & Longshaw 2006; Carriero et al. 2013; Kaur & Gupta 2015; Abdel Ghaffar et al. 2016). They appear to be benign but some species are pathogenic, representing environmental threat for fish cultures, leading to financial losses and decline in fish population (Gomez et al. 2014; Ben-David et al. 2016).

Myxozoans are microscopic protozoan endoparasites of freshwater and marine habitats in which their life cycles is completed with two hosts (one host is a vertebrate such as fish, while the other host is an annelid) (Gomez et al. 2014). Two myxobolus species were recorded from Egypt in the Nile tilapia; *M. tilapiae* and *M. agolus* (Abdel-Azeem et al. 2015). Different types of myxobolus spp. in *O. niloticus* and *Tilapia zilli* from the Nile River in Egypt have been reported including *M. agolus*, *M. heterosporous*, *M. clarri*, *M. tilapiae*, *M. niloticus*, *M. zilli* and *M. fahmii* (Mohammed et al. 2012).

There is a lack in the relationship between the parasitic protozoan infection and the health condition of the fish with expression pattern on the most common relevant gene in the immunity. However, common gene expression pattern on *Ichthyophthirius multifilis* as parasitic protozoan infection has been recorded by Akbari et al. (2017). Therefore, the aim of this study is to record different ciliophoran and Myxozoan parasites in the Nile tilapia collected from Egypt, and also assessment of immunological condition changed by these parasites based on expression analysis of Tumor Necrosis Factor alpha (TNF-α) and Interlukin-1β (IL-1β) in the infected individuals (with *Trichodina* spp. and *Myxobolus* spp.) using quantitative Real-Time PCR (qRT-PCR).

Materials and Methods

Collection of fishes: A total of 360 *O. niloticus* individuals (1-year-old, 10-15 cm length) were collected between January 2018 and January 2019 from four farms (two in Kafr Elshikh, 31°06′42″N 30°56′45″E; two in El sharkia, 30.7°N 31.63°E) and from the Nile River in Giza (Fig. 1). These fishes were examined for presence of protozoan parasites infection. The fishes were transferred alive to the laboratory of Parasitology Department at Cairo University for parasitological and molecular analyses. All specimens were kept in several aerated covered glass aquaria of 30-liters capacities, until further examinations.

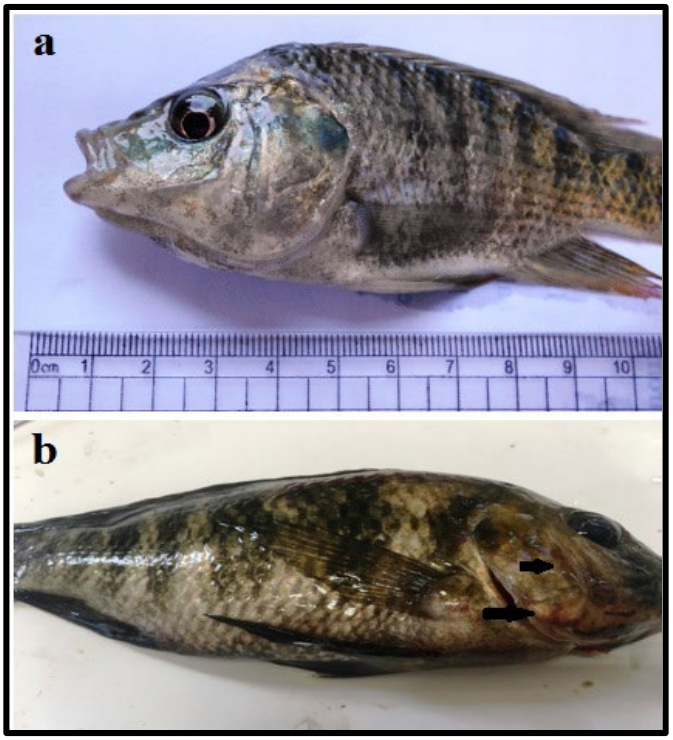

Fig.1. *Oreochromus niloticus* collected from Egypt; a: showing normal examined fish; b: mixed infection between *Trichodina* spp. and *Myxobolus* spp. indicated by different area of hemorrhage in skin.

Parasitological examination: Each fish was necropsied, and smears were made from the mucous around the skin, gills and fins. These smears were left to dry and fixed with absolute methanol; then all the smears were stained by commercial Giemsa stain. Measurements of the protozoan parasites (different types of *Trichodina* spp. and *Myxobolus* spp.) were recorded, and photographed using an Olympus CX41 microscope, Japan, following Eissa et al. (2020).

Quantitive Real-Time Polymerase Chain Reaction (qRT-PCR)

Sampling: Infected skin samples (0.5cm^2) and gills

Table 1. Forward and reverse primers used in this study.

Gene	Gene Sequence [5-3]	Accession number	Reference
TNFα-1. F	GGTTAGTTGAGAAGAAATCACCTGCA	NM_001279533	Praveen et al. (2006)
TNFα-1. R	GTCGTCGCTATTCCCGCAGATCA		
IL-1β-F	TGCACTGTCACTGACAGCCAA	DQ061114	Heinecke & Buchmann (2013)
IL-1β-R	ATGTTCAGGTGCACTATGCGG		
β-Actin. F	CAGCAAGCAGGAGTACGATGAG	XM_003455949	Akbari et al. (2017)
β-Actin. R	TGTGTGGTGTGTGGTTGTTTTG		

(with the parasites; mixed *Trichodina* spp. and *M. tilapiae*) were aseptically dissected. Samples from three uninfected fishes were collected in a similar manner (these fishes were kept in laboratory aquarium and examined carefully for presence of any parasites), which were used as negative controls; samples classified as skin with *Trichodina* spp.; skin with *M. tilapiae*; skin with mixed infection of *Trichodina* spp. and *M. tilapiae*; gills with *M. tilapiae*; gills with *Trichodina* spp.; gills with mixed infection of *Trichodina* spp. and *M. tilapiae*. Samples were collected from 10 heavily infected fishes with high parasitic infection: at least 10-20 parasites in 1cm^2 of skin or gills.

RNA isolation: The selected fishes for gene expression analysis were those only infected with the two parasites under investigation (*M. tilapiae* and *Trichodina* spp.). Therefore, all fishes were examined carefully for any intestinal and kidney parasites, as well as encysted metacercaria in muscles. All fish organs were examined carefully for bacterial and fungal infection and only the organs with no bacteria and fungi were selected.

Total RNA from 100mg of skin and gills were isolated using the total RNA isolation kit (Ambion, Applied Biosystems), following the manufacturer's instructions. Homogenization of the tissues was performed in a homogenizer (MP Biomedicals, 3 cycles of 30s). The RNA purity and quantity were measured using Nanodrop (Thermo Scientific). A 500ng of RNA was handled with DNaseI amplification grade (Invitrogen) following the manufacturer's instruction. The treated RNA was transcribed using the High-Capacity cDNA Archive Kit (Applied Biosystems) following the manufacturer's protocol (Picard-Sanchez et al. 2019).

Quantitive real-time PCR protocol (qRT-PCR): The PCR primer sets specific for TNFα-1 and interluckin1β specific of *Oreochromus niloticus* were designed based on previously published sequences which are deposited in GeneBank (Table 1). We used β-actin as the normalized reference gene (Younis et al. 2020). The expression of the two tested genes in this study were evaluated on a separate pool of cDNA, generated from three non-infected control fish (which were examined carefully for presence of any parasites).

For cDNA synthesis, the extracted RNA (2μL) was added to a hexamer primer. Then, the mixture was incubated in a thermal cycler at 65°C for 5min, then it was immediately sited on ice for at least 1min. Then, 10μL of 2X first standard reaction (10mM MgCl2, 1mM dntp) and reverse transcriptase (2μL) were incubated at 25°C for 10min, followed by a reaction of 50 min at 40°C, and finally, at 80°C for 5 min. The threshold value (Ct), calculated according to Akbari et al. (2017). Quantitative Real-Time PCRs were performed in a PCR System (Step One™; Applied Biosystems, USA). A mixture of SYBR® Premix (Ex Taq™; Tli RNase H Plus) was added as 10μl, 1μL of cDNA and 0.5μL of primers (forward and reverse) were mixed in a reaction, to have a final volume to 20μL with ultra-pure water. The used PCR cycling conditions were recorded in Table 2. The ΔCT value was calculated according to the equation recorded by Akbari et al. (2017); as subtraction of the β-actin ΔCT as an internal control, from the ΔCT which detectable at the achieved signals; the qPCR

Table 2. PCR cycling conditions.

The cycle	Time	Temperature
One incubation cycle	10 min	95 °C
45 amplification cycles	30 s	94°C
	60 s	60°C
One cycle for SYBER channel	30 s	55 -99 °C

Table 3. Morphological parameters of the collected *Trichodina* spp. in *Oreochromis niloticus* collected from Egypt.

Parasite spp. / Parameters	*T. heterodentata*	*T. centrostrigeata*	*P. africana*	*T. epizootica*
Body diameter	40-60 (57±3.0)	37-57 (47±1.5)	18-25 (22 ±1.3)	25-30 (27 ±1.0)
Adhesive disc	38-52 (43±2.1)	33-48 (40±2.1)	14-20 (19±1.5)	16-28 (20±2.0)
Border membrane	3-5 (4.3±0.5)	2.5-5.2 (4.3 ±1.2)	1.0-2.0 1.5±0.5)	1.8-2.9(1.8 ±0.1)
Denticulate ring	18-32 (23±1.0)	19-30 (25 ±1.7)	7-10 (9.5±1.0)	10-17 (13.4
Number of	20-29 (25± 1.3)	25-30 (27 ±1.0)	20-28 (25±1.5)	20 –25 (22 ±1.0)
Denticle length	6-10 (9 ±1.5)	3.0-6.5 (5.5 ±1.0)	2.0-.5(3.0±0.5)	1.5–2.5(2.2 ±0.5)
Blade length	3-6 (4.5 ±0.7)	3.5- 6 (4.5 ±1.0)	2.5-.0(2.8±0.7)	2.3–3.8(2.8 ±0.3)
Ray length	4.5-8 (6.5± 1.0)	3.6–5.5(4.9 ±0.7)	1.5-.5(1.9±0.5)	0.8-1.7 (1.0±0.5)

assay was repeated 3 times in each sample according to Attia et al. (2020).

Statistical analysis: Groups means were compared using one-way-ANOVA. Values with $P<0.05$ were considered as statistically significant. All statistical analyses were performed in IBM SPSS 18.0 (SPSS Inc., Chicago, IL, USA). All measurements of the parasitic protozoa are expressed as means and standard errors.

Results

Prevalence of the collected protozoan parasites: A total of 100 fish individuals (27.78%) were infected with different *Trichodina* spp., while 60 fish individuals (16.67%) were infected with *Myxobolus* spp. (56 indiiduals (93.3%) with *M. tilapiae* and four individuals (6.67%) with *M. heterosporous*). Mixed infection of *M. tilapiae* and different species of *Trichodina* were observed in 20 individuals (5.5%).

Identification of the collected protozoan parasites:

***Trichodina* spp.:** Four species of *Trichodina* were collected from gills, skin and fins, including 2 medium size (*T. heterodentata* and *T. centrostrigeata*) and two small size (*Paratrichodina africana* and *Trichodinella epizootica*). Mixed infection with different species of *Trichodina* were recorded in several fishes. Taxonomic identification of the four protozoan parasites (Fig. 2) was performed based on numerous morphological parameters such as body diameter, adhesive disc, border membrane, denticulate ring diameter, number of denticles, denticles length, blade length and ray length; all of this parameters are presented in Table 3. The largest *Trichodina* spp. was *T. heterodentata* and the smallest one was *Paratrichodina africana*.

***Myxobolus* spp.**

Two species of *Myxobolus* were collected: *M. tilapiae* and *M. heterosporous.*

***Myxobolus tilapiae*:** Spores are medium size, ovoid in shape with two smalls polar capsules generally located at anterior end, polar capsules ovoid, and equal in size. Sporoplasm contains one rounded iodinophilous vacuole in the upper part of it, between and below the two polar capsules in the middle of the spore (Fig. 3). All measurements of the spores such as length and width of the spores, length and width of larger polar capsules, length and width of smaller polar capsules, number of polar coils, and diameters of iodinophilous vacuole are shown in Table 4.

Table 4. Morphological parameters of the collected *Myxobolus* spp. in *Oreochromus niloticus* collected from Egypt.

Parameters \ Parasite spp.	*M. tilapiae* Min.-Max (mean± SD)	*M. heterosporus* Min.-Max (mean± SD)
Length of the spore	11.1 -19.1(14.7 ±0.51)	12-15 (14.6 ± 0.5)
Width of the spore	5.61-6.12 (6.36± 0.53)	4.5- 5.5 (5.1± 0.7)
Length of Larger Polar Capsule	8.2 -14.2 (10.64±0.4)	6.7- 7.9 (7.5 ± 1)
width of Larger Polar Capsule	2.3 - 3.9 (2.99±0.14)	2- 2.8 (2.5 ± 0.6)
Length of Smaller Polar Capsule	7.1- 12.9 (9.55±0.42)	5- 7 (6.7 ± 0.8)
Width of Smaller Polar Capsule	2.2 - 3.6(2.79±0.13)	1.5- 2.5 (2.3 ± 0.9)
Number of coils	8–9 (8±0.3)	7-8 (7.5 ± 0.5)
Diameter of iodonophilious vacuole	2-3.1 (2.7±0.3)	1.9- 2.3 (2 ± 0.3)

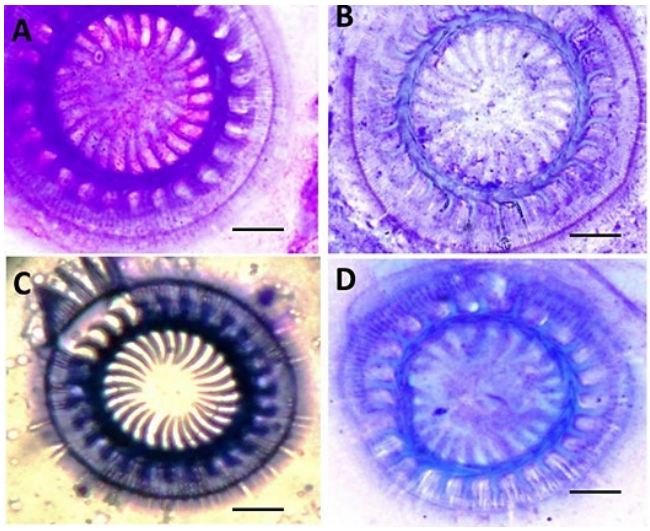

Fig.2. Light microscopic photograph of the collected *Trichodina* spp. in *Oreochromus niloticus* collected from Egypt: a: *T. heterodentata; b: T. centrostrigeata; c: P. africana*; d: *T. epizootica*; scale bar 10µm; magnification length X100.

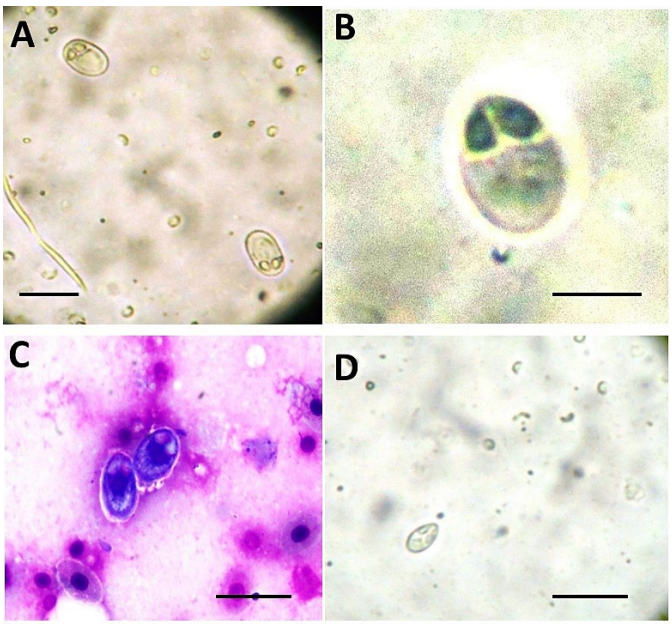

Fig.3. Light microscopic photograph of the collected *Myxobolus* spp. in *Oreochromus niloticus* collected from Egypt: a, b, c: *M. tilapiae*; d: *M. heterosporus;* magnification length 100X; scale bar 10µm.

***Myxobolus heterosporous*:** Spores are elongated, ellipsoidal, anterior end more pointed, polar capsules pyriform and very elongate reaching 2/3 of spore length; the anterior end of the spores is pointed (Table 4, Fig. 3).

TNF-α and IL-1β expression in infected tissues with *M. tilapiae* and *Trichodina* spp. (Tables 5-6; Figs. 4-5): In skin (Table 5), TNF-α means in *M. tilapiae* and mixed groups were nearly similar (15.00±0.58 and 14.67±0.88; respectively) and were significantly higher than the *Trichodina* spp. group (10.00±0.58). TNF-α means of *M. tilapiae* mixed and *Trichodina* spp. group were significantly higher than the control group (3.00±0.00, *P*=0.0001).

IL-1β mean of the *M. tilapiae* group (10.00±0.58) was significantly lower than that of the mixed (20.00 ±1.15), *Trichodina* spp. (8.00±0.58) and control (3.00±0.00) groups (Table 5, Fig. 4). All groups showed significantly higher IL-1β means comparing to the control group (*P*=0.0001).

In gills (Table 6), TNF-α mean of the mixed group showed the significantly highest value (15.67±0.88). TNF-α means of the *M. tilapiae* and *Trichodina* spp. groups were (13.00±0.58 and 7.33±0.67, respectively) and significantly higher than that of the

Table 5. e expression analysis of TNF and IL-1β from skin of *Oreochromus niloticus*.

Groups	TNF	IL-1β
Mixed	15.00 ±0.58[a]	20.00 ±0.58 [a]
Trichodina spp.	10.00 ±0.58[b]	8.00 ±0.58 [c]
Myxobolus spp.	14.67 ±0.88[a]	10.00 ±1.15 [b]
Control	3.00 ±0.00[c]	3.00 ±0.00 [d]
P- value	0.0001*	0.0001*

* SE: Standard error; [a,b,c,d] Different superscripts indicate significant difference at $P< 0.05$.
Mixed: Mixed infection of *Trichodina* spp. and *Myxobolus* spp.

Table 6. Gene expression analysis of TNF and IL-1β from the gills of *Oreochromus niloticus*

Groups	TNF	IL-1β
Mixed	15.67±0.88[a]	13.67±0.88[a]
Trichodina spp.	7.33±0.67[b]	5.33±0.33[c]
Myxobolus spp.	13.00±0.58[b]	16.00±0.58[b]
Control	3.00 ±0.00[c]	3.00 ±0.00[d]
P- value	0.0001	0.0001

* SE: Standard error; [a,b,c,d] Different superscripts indicate significant difference at $P<0.05$.
Mixed: Mixed infection of *Trichodina* spp. and *Myxobolus* spp.

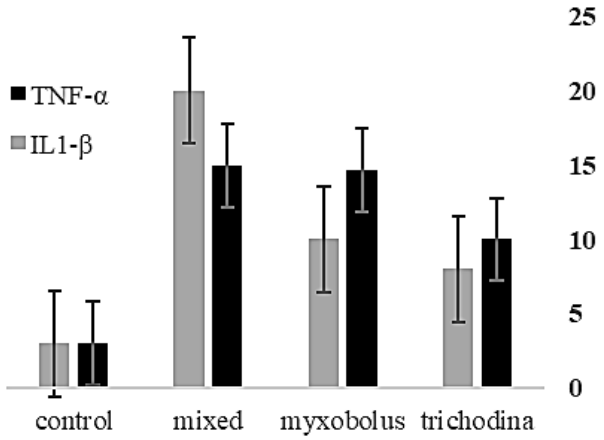

Fig.4. Gene expression analysis of TNF and IL-1β from the skin of *Oreochromus niloticus*.

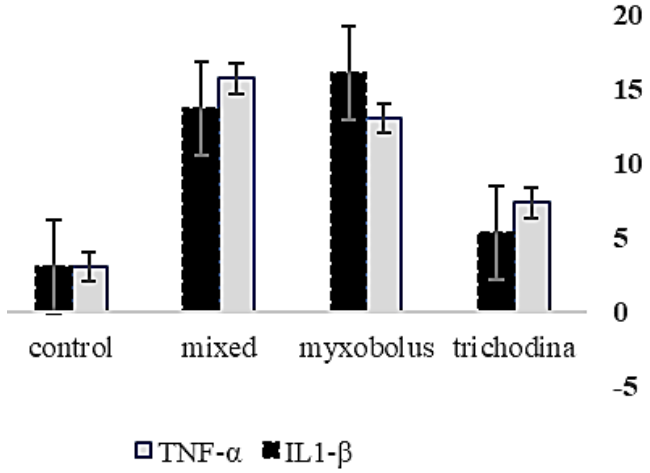

Table 5. Gene expression analysis of TNF and IL-1β from the skin of *Oreochromus niloticus.*

control group (3.00±0.00). TNF-α means of *M. tilapiae*, mixed and *Trichodina* spp. means were significantly higher than that of control group (P=0.0001). IL-1β means of the *M. tilapiae*, mixed and *Trichodina* groups (Table 6, Fig. 5) were significantly different from each other (16.00 ± 0.58, 5.33±0.33, and 13.67±0.88, respectively) and significantly higher than that of the control group (3.00±0.00, P=0.0001).

Discussion

The present study recorded four species of ciliophoran parasites and two species of myxozoan parasites in the Nile tilapia (*O. niloticus*) collected from Egypt. The ciliophoran species were *T. heterodentata*, *T. centrostrigeata*, *Paratrichodina Africana*, *Trichodinella epizootica*, and the two *Myxobolus* spp. were *M. tilapiae* and *M. heterosporous.* The collected protozoan parasites were measured and photographed.

***Trichodina* spp.**

Around the world, there are more than 112 species of *Trichodina* spp. recorded in the freshwater fishes. Of

these, seven species have been recorded in the Nile tilapia by Valladao et al. (2016) including *T. centrostrigeata, T. compacta, Tripartiella orthodens*, *T. migala, T. heterodentata* and *Paratrichodina africana.* According to Tantry et al. (2016) trichodinids lack the host specificity. The presented data of this study on *Trichodina* spp. Are almost in agreement with the other studies by Al-Rasheid et al. (2000), Valladao et al. (2013) and Valladao et al. (2016).

Trichodina spp. moves along the surface of skin, fins and gills. It feeds on old mucous and sloughed cells on surface of fish using denticles which scrape debris from host surface to mouth of parasite. When abundant, the scraping and movement of *Trichodina* spp. irritate skin and gills of infested fish. Fish parasitized with trichodina often have white patches Valladao et al. (2016) and sometimes mottling of skin and fins with production of excessive bluish mucous are observed. Fish exhibit flashing behavior by scraping their bodies against hard objects with exposure of their abdomen.

Myxobolus

Myxobolus tilapiae which was identified here, has been collected from *O. niloticus*, *Tilapia zilli* and *Tilapia mossambicus* in Africa (Fomena and Bouix 1997), and from *O. niloticus* in Cameroon by Fall et al. (2000).

Myxobolus tilapiae was full described as it had spherical polar capsules. While *M. heterosporou*s was more pyriform (see Abdel-Azeem et al. 2015) and it is found in the kidney of Nile tilapia in the River Nile in Egypt. The general characteristics of identified species here, was almost similar to the other studies. *Myxobolus heterosporous* are more pyriform than *M. tilapiae* which was confirmed by the description given by Mohammed et al. (2012) in Nile tilapia.

Different studies on the immune mechanism which monitored using different genes in *Ichthyophthirius multifiliis* including i) Akbari et al. (2017) which evaluated TNF-α; ii) Gonzalez et al. (2007) which evaluated different genes of TNF-α, CXCa, CXCb and IL-1β in common carp; iii) Wang et al. (2019) who described the immune response against the ICH as model for protozoan parasites and use the Toll- like receptor and IgM as examples for genes expression and iv) Heinecke and Buchmann 2013 who expressed the TNF-α, IL-1β, IL-8, IL-6, IL-10, IL-22, IgM, IgT, iNOS, SAA, Hepcidin and Cathelicidin-2.

In this study we, evaluated two genes of TNF-α and IL-1β in two protozoan parasites (*M. tilapiae*; and *Trichodina* spp.) either with single infection or in mixed infection in *O. niloticus* in gills and skin of fishes. Cultured *O. niloticus* can respond immunologically and upward regulate the expression patterns of different genes in response to parasitic protozoan infection. The skin and gills of fishes are the common sites of parasitic infection which carry the barrier against different pests by secreting mucus. This mucus limits the parasitic load (Simon Jones 2001). Simon Jones (2001) explained the mechanism of innate immunity against different protozoan parasites as well as explain the roles of mucus as the 1st barrier of the body against different infection by secreting different immunoglobulin; lectins lysozymes complement C-reactive protein.

Tumor Necrosis Factor alpha (TNF-α) upward regulation in the skin and gills with nearly the same in mixed group; *Trichodina* spp. group and *M. tilapiae* group, while IL-1β was expressed upregulated in the skin of mixed infection than gills with the same infection. IL-1β was expressed high in the gills than skin while in *Trichodina* spp. group it was expressed high in the skin than in gills. These results reveal that the upregulation was higher in the skin than in gills due to presence of mucous and macrophages as reported by Zhu et al. (2013). It explains production of interferons, interleukins and tumor necrosis factor from the mast cells, macrophages and lymphocytes.

Interleukin-1 releases in the early stage of infection as pro-inflammatory cytokines that regulates both types of immunity (innate and acquired stage). IL-1β is the most potent and fastest

gene in humoral immunity that stimulates the inflammation and triggers the immunity (Zhu et al. 2013). IL-1β has its origin from the epidermis, dermis and underlying tissues (Lindenstrom et al. 2003; Gonzalez et al. 2007).

Hutson (1993) and Zhu et al. (2013) reported that macrophages are responsible for secreting a different cytokine such as TNF-α. The expression pattern of this gene was relevant in immune response for recruitment of the infection with the protozoan parasites. Moreover, immunity against different protozoan parasites includes Th1 cell-responses, with TNF-α as proinflammatory cytokines (Ovington et al. 1995; Ovington & Smith 1992; Dickerson 2012; Sigh et al 2004a, b).

Akbari et al. (2017) studied the immune mechanisms of TNF-α1 which could control the infection with *I. multifiliis* infection in rainbow trout. In this study that evaluate the immune response of two relevant genes, TNF-α and IL-1β which are secreted from the macrophages, were nearly similar in upregulation in infection especially in mixed infection than the single infection of *M. tilapiae* and these genes were lower in *Trichodina* spp. infection than *Myxobolus* infection. The skin was acted nearly higher in upregulation in two genes under investigation than gills. It was due to the 1st barrier and its immunological defined cells as recorded by Zhu et al. (2013) and Gonzalez et al. (2007). In conclusion, it is possible to measure the immune response of the fish against the infection with the two parasites using transcription of the RNA using the two genes (TNF-α and IL-1β). It shows that two genes transcripts in the infections especially in the fish skin.

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مقاله پژوهشی

تجزیه و تحلیل ژن TNF-α و IL- 1β در گونه *Oreochromis niloticus*: پاسخ‌های التهابی ناشی از *Myxobolus* spp. و *Trichodina* spp.

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چکیده: نمونه‌های پرورشی و وحشی تیلاپیای نیل اغلب به کرم‌های انگلی، تک یاخته‌ای‌ها و بندپایان آلوده هستند که سبب سطوح متفاوتی از بیماری‌ها در این ماهیان می‌شوند. هدف از این مطالعه ثبت معمول‌ترین انگل‌های تک یاخته‌ای در تیلاپیای نیل در مصر و همچنین ارزیابی تغییرات ایمونولوژیک از طریق تجزیه و تحلیل دو ژن (TNF-α و IL-1β) در ماهیان آلوده با استفاده از تکنیک qRT-PCR بود. بنابراین، هر ماهی با دقت مورد بررسی قرار گرفت، از هر اندام لام‌هایی تهیه شد و با رنگ گیمسا رنگ‌آمیزی و با استفاده از میکروسکوپ نوری بررسی شدند. چهار گونه (*T. heterodentata, Trichodinella centrostrigeata, T. epizootica, Paratrichodina Africana,*) جدا و شناسایی شد و به‌علاوه، دو گونه (*M. tilapiae* و *M. heterosporous*) نیز به‌طور کامل توصیف شد. در پوست، TNF-α در *M. tilapiae* و گروه‌های ترکیبی تقریباً مشابه بود و به‌طور قابل توجهی بالاتر از گروه *Trichodina* spp. بود. IL-1β مربوط به *M. tilapiae* به‌طور قابل توجهی پایین‌تر از گروه ترکیب، *Trichodina* spp. و گروه کنترل بود. در آبشش، TNF-α از گروه ترکیب به‌طور قابل توجهی بالاترین مقدار را نشان داد. IL-1β در همه گروها تقریباً یکسان بود. نتیجه می‌گیریم که پوست در تنظیم مجدد دو ژن مورد مطالعه در مقایسه با تجزیه و تحلیل آبشش تقریباً دارای جایگاهی بالاتر است، چرا که به عنوان اولین مانع در ماهی در نظر گرفته می‌شود و شامل بسیاری از سلول‌های شناخته شده از نظر ایمنی است.

کلمات‌کلیدی: تیلاپیای نیل، فاکتور نکروز تومور آلفا، اینترلوکین-1β، واکنش‌های زنجیره‌ای پلیمراز در زمان واقعی.