Use of enzyme activities as biomarkers for oxidative stress induced by metacercarial affections in some cultured tilapia species

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Abstract: The present study was carried out on two different species of tilapia, *Oreochromis niloticus* and *O aureus*. They were collected randomly and seasonally from El-Abbassa fish farm, Sharkia Governorate. The clinical picture revealed no pathgnomic clinical abnormalities on the external body surface. Encysted metacercariae were identified as *Diplostomum tilapiae*, Rudolphi, 1809,*Centrocestus formosanus* Nishigori, 1924 and *Heterophyes sp*. Witenberg, 1929. The total prevalence was 13.1% and the seasonal prevalence was recorded. The enzyme activities (Superoxide dismutase, Catalase, Glutathion peroxidase, Glutathion reductase, Malondialdehyde, Cytochrome oxidase and Lactate dehydrogenase) in gills, liver and musculature of both infected tilapias were measured and discussed.
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1- Introduction

Man-made pollutant and or intensification of fish culture resulted in an increase of environmental changes, which may be stressful to fish (**Lio-Po and Lim, 2002**). This condition can result in decreased resistance by fish, causing spread of disease and parasite infection (**Eissa *et al,* 2011**).

It is imperative to investigate the relationship between the environmental factors as it affects the parasites that affect production and quality (**Mondo, 1999**).Detrimental effects of parasites are exacerbated when their hosts are stressed, it thus becomes paramount to determine the combined effects of anthropogenic stressors such as contaminants and natural stressors such as parasites. In combination, water pollution and parasitism may act additively or synergistically on the health of fish (**Eissa, 2002 and David *et.al,* 2005**).

Parasites can interact with environmental pollution in different ways. On the one hand, parasites can interfere with established bio-indication procedures owing to their effects on the physiology and behavior of the host. This could lead both to false-negative and false positive indications of pollution. On the other hand, parasites can be used as effect indicators and as accumulation indicators because of the variety of ways in which they respond to anthropogenic pollution (**Sures, 2004**).

Parasitic infection induces oxidative stress and a higher level of membrane damage in fish organs due to an imbalance between pro-oxidants and non enzymatic anti oxidants and lead to exacerbate lipid per oxidation which used as a biomarker of pathological effects caused by parasitism and stressful ecological factors

The current investigation was planned to through light on some enzyme activities as biomarkers for oxidative stress induced by metacercarial affections in cultured *Oreochromis niloticus* and *O aureus*.

2- Material and Methods

Fishes:

A total of 282 cultured tilapia species were represented as 154 *Oreochromis niloticus* and 128 *O aureus* collected randomly and seasonally with an average body weight (70 +10g) from El-Abbassa fish farm, Sharkia Governorate. They were collected from March 2011 to Febraury 2012. The tilapias were transferred alive to laboratory and were subjected to examination.

Parasitological examination:

Encysted metacercariae (E.M.S) were isolated from internal organs and musculature and left in refrigerator overnight to allow the parasites to die in a relaxed condition, then cleared by washing several times with normal saline. The collected E.M.C. were compressed with a small part of surrounding tissue in between 2 glass slides, then fixed and stained by semichon's acetocarmin stain according to **Lucky (1977)**.

Biochemical study (oxidative stress):

The gills, liver and muscle samples were washed in ice-cold physiological saline (0.59% NaCl) then homogenized to a 1/5(w/v) ratio in o.25M pH 7.4 sucrose buffer using a glass-Teflon homogenizer

(Heidolph So1 10 R2RO). They were centrifuged at 9500xg for 30 min in a Sorvall RC2B centrifuge at 4 C° and supernatant stored at-70C° until analysis. Supernatant was measured using a spectrophotometer (Shimadzu UV-mini 1240).

Superoxide dismutase (SOD) activity was measured spectrophotometrically as the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) at wavelength 560 nm. **Catalase (CAT):** was determined by H2O2 reaction. **Glutathion peroxidase (GPX)** was determined by methyl catechol reaction according to **Jin (2011)**. **Glutathion reductase (GR)** was measured using the procedure of **Goldberg and Spooner (1983)**. **Malondialdehyde (MDA)** content was determined using thiobarbituric acid (TBA) reaction according to **Jin (2011)**. **Cytochrome oxidase** and **Lactate dehydrogenase (LDH)** were measured according to **Rasmussen (2000)** and **Young (2001)** respectively**.**

Statistical analysis:

It was performed using the one way analysis of variance (ANOVA) of SPSS according to **Snedecor and Cochran (1969)**

3- Results

Clinical picture of naturally infected fishes:

The clinical signs in the naturally infected fishes (*Oreochromis niloticus* and *O aureus)* revealed no pathognomic clinical abnormalities on the external body surface. Internally, paleness in liver and whitish cysts in musculature of fishes were observed.

Parasitological examination:

Based on the morphological and parasitological examinations, the isolated EMCs were identified as *Diplostomum tilapiae* Rudolphi, 1809, *Centrocestus formosanus* Nishigori, 1924, and *Heterophyes sp*. Witenberg, 1929. **Plate (1)**

Prevalence of E.M.C. In the examined *O. niloticus* and *O. aureus*:

The total prevalence of E.M.C. of examined *O. niloticus* and *O. aureus* was 13.1%. The highest prevalence was found in *O aureus* 14.8%, followed by *O niloticus* 11.7% **(Table 1).**

Table (1):Prevalence of E.M.C. in examined *O. niloticus* and *O. auerus*

Tilapia species	No. of ex. Tilapia	No. of infected Tilapia	%
O. niloticus	154	18	11.7
O. aureus	128	19	14.8
Total	282	37	13.1

Seasonal prevalence of E.M.C. in examined *O. niloticus* and *O. aureus*:

The highest prevalence of *Diplostomum tilapiae* was 66.7% in winter (*O. aureus*) and lowest prevalence was 33.3% in autumn (*O. niloticus*). The highest prevalence of *Centrocestus formacenus* was 50% in autumn (*O. aureus*) and the lowest prevalence was 0% in summer (*O. niloticus*). The highest prevalence of *Hetrophyes sp* was 50% in summer and autumn in *O. niloticus* and *O. aureus* respectively.

Table (2): Seasonal prevalence of E.M.C. in examined *O. niloticus* and *O. aureus*

Seasons	*O. niloticus*					*O. aureus*				
	No. of Ex.	**No. of inf.**	*Diplostomum tilapiae*	*Centrocestus formacenus*	*Hetrophyes sp*	**No. of Ex.**	**No. of inf.**	*Diplostomum tilapiae*	*Centrocestus formacenus*	*Hetrophyes sp*
Spring	**41**	**5 (12.2%)**	3(60%)	1(20%)	1(20%)	**32**	**4(12.5%)**	2(50%)	1(25%)	1(25%)
Summer	**41**	**2(4.9%)**	1(50%)	0(0%)	1(50%)	**36**	**4(11.1%)**	2(50%)	1(25%)	1(25%)
Autumn	**44**	**3 (6.8%)**	1(33.3%)	1 (33.3%)	1(33.3%)	**32**	**8(25%)**	4(50%)	2(50%)	2(50%)
Winter	**28**	**8(28.6%)**	4(50%)	2(25%)	2(25%)	**28**	**3(10.7%)**	2(66.7%)	1(33.3%)	0(0%)

Effect of E.M.C. on enzyme activities in *O. niloticus*:

The enzyme activities in the gills, liver and musculature of infected *O. niloticus* with *Diplostomum tilapiae, Centrocestus formacenus* and *Hetrophyes sp.* were documented in **Table 3, Fig. 1**. Infected *O. niloticus* with *Diplostomum tilapiae* and *Hetrophyes sp*, showed significant increase in superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase GR malondialdehyde, cytochrom oxidase and LDH in liver and musculature. In case of *Centrocestus formacenus***,** a significant increase of all enzyme activities except GR in gills and liver.

Effect of E.M.C. on enzymes activities in *O. aureus*:

The enzyme activities in the gills, liver and musculature of infected *O. aureus* with *Diplostomum tilapiae*, *Centrocestus formacenus* and *Hetrophyes sp*. were documented in **Table 4 , Fig. 2**. The infected gills of with *Diplostomum tilapiae* and *Hetrophyes sp*., showed no significant increase of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, malondialdehyde, cytochrom oxidase and LDH. In infected liver with *Diplostomum tilapiae*, *Centrocestus formacenus* and *Hetrophyes sp*, showed significant increase of all enzyme activities except GR. Infected Musculature with

Diplostomum tilapiae and *Hetrophyes sp* showed significant increase in all enzyme activities.

Table (3): Effect of E.M.C. on enzyme activities in *O. niloticus*

Enzymes	Infected									non Infected		
	Diplostomum tilapiae			*Centrocestus formacenus*			*Hetrophyes sp*					
	Gills	Liver	Muscul.	Gills	Liver	Muscul.	Gills	Liver	Muscul.	Gills	Liver	Muscul.
Superoxide dismutase	45.13 ±2.46^{b}	52.75 ±2.11^{b}	55.43 ±0.82^{a}	60.57 ±3.55^{a}	50.43 ±3.65^{b}	48.61 ±2.12^{c}	44.32 ±3.64^{b}	58.84 ±2.78^{a}	51.03 ±0.74^{b}	41.48 ±1.6^{b}	45.32 ±1.36^{c}	46.35 ±0.92^{c}
Catalase	35.4 ±2.72^{b}	39.69 ±1.72^{b}	41.6 ±1.35^{a}	45.23 ±1.62^{a}	40.13 ±1.61^{b}	30.43 ±1.68^{c}	34.8 ±2.41^{b}	46.47 ±2.72^{a}	36.6 ±2.14^{b}	30.02 ±2.61^{c}	35.24 ±1.46^{c}	28.42 ±0.98^{c}
Glutathione peroxidase	12.82 ±1.48^{b}	23.71 ±1.58^{a}	19.72 ±1.35^{a}	17.86 ±0.62^{a}	22.63 ±1.8^{a}	15.13 ±0.74^{b}	11.31 ±0.61^{b}	25.93 ±1.41^{a}	18.32 ±0.68^{a}	10.2 ±1.3^{b}	18.69 ±1.83^{b}	13.82 ±0.79^{b}
Glutathione reductase	2.65 ±0.24^{c}	3.03 ±0.21^{b}	3.08 ±0.12^{b}	2.89 ±0.26^{b}	3.01 ±0.24^{b}	3.23 ±0.15^{b}	2.64 ±0.15^{c}	3.15 ±0.17^{b}	3.18 ±0.14^{b}	4.23 ±0.21^{a}	4.63 ±0.6^{a}	4.69 ±0.86^{a}
Malondialdehye	150.01 ±3.8^{b}	171.9 ±3.52^{b}	188.71 ±4.62^{a}	168.87 ±6.8^{a}	167.08 ±3.68^{b}	168.03 ±3.57^{c}	147.1 ±4.5^{b}	180.32 ±4.52^{a}	178.06 ±4.21^{b}	143.24 ±3.62^{b}	150.97 ±2.41^{c}	162.58 ±2.66^{c}
Cytochrom Oxidase	9.15 ±1.52^{b}	8.85 ±1.22^{b}	13.39 ±0.58^{b}	11.12 ±1.15^{a}	8.34 ±0.82^{b}	12.23 ±0.46^{c}	9.63 ±1.47^{b}	10.24 ±0.74^{a}	17.14 ±0.64^{a}	8.15 ±0.82^{b}	6.58 ±0.45^{c}	11.74 ±0.36^{d}
LDH	146.14 ±4.25^{b}	180.42 ±4.6^{a}	250.66 ±4.62^{a}	161.23 ±2.42^{a}	179.98 ±3.57^{a}	209.13 ±3.62^{c}	144.41 ±3.27^{b}	183.38 ±2.53^{a}	238.03 ±4.78^{b}	141.64 ±2.71^{b}	144.96 ±3.82^{b}	203.19 ±5.21^{c}

Mean have different letters in same row for same organ are significantly different (P<0.05)

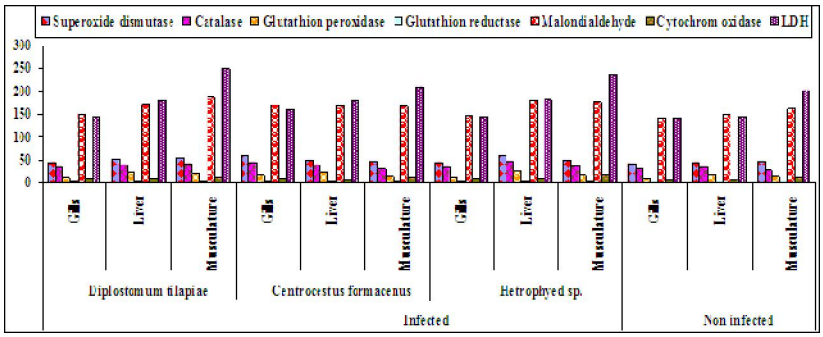

Fig. (1): Effect of E.M.C. on enzyme activities in *O. niloticus*

Table (4): Effect of E.M.C. on enzymes activities in *O. aureus*

Enzymes	Infected									Non Infected		
	Diplostomum tilapiae			*Centrocestus formacenus*			*Hetrophyes sp*					
	Gills	Liver	Muscul.	Gills	Liver	Muscul.	Gills	Liver	Muscul.	Gills	Liver	Muscul.
Superoxide dismutase	51.23 ±3.52^{b}	57.13 ±1.56^{b}	70.75 ±2.32^{a}	61.26 ±2.41^{a}	56.13 ±1.42^{b}	61.58 ±2.42^{b}	50.97 ±3.15^{b}	60.53 ±2.31^{a}	68.48 ±3.52^{a}	49.84 ±2.42^{b}	45.31 ±2.15^{c}	58.37 ±2.52^{b}
Catalase	40.97 ±1.42^{b}	56.2 ±1.58^{a}	61.14 ±2.41^{a}	47.91 ±2.38^{a}	55.19 ±2.62^{a}	50.63 ±1.21^{c}	39.45 ±1.62^{b}	59.54 ±3.5^{a}	55.87 ±2.41^{b}	38.87 ±1.57^{b}	49.46 ±1.98^{b}	49.72 ±2.35^{c}
Glutathione peroxidase	20.7 ±0.82^{b}	24.93 ±1.21^{b}	37.61 ±1.42^{a}	27.7 ±0.78^{a}	23.63 ±1.1^{b}	31.61 ±1.82^{bc}	19.87 ±0.52^{b}	27.65 ±1.6^{a}	33.13 ±1.72^{b}	19.35 ±0.89^{b}	21.43 ±0.48^{c}	28.78 ±2.25^{c}
Glutathione reductase	2.32 ±0.15^{b}	2.07 ±0.05^{b}	2.42 ±0.12^{b}	2.28 ±0.34^{b}	2.08 ±0.04^{b}	2.54 ±0.15^{b}	2.25 ±0.3^{b}	2.07 ±0.06^{b}	2.44 ±0.12^{b}	3.47 ±0.52^{a}	2.87 ±0.15^{a}	3.26 ±0.23^{a}
Malondialdehye	102.12 ±3.1^{b}	155.07 ±2.58^{b}	171.23 ±4.6^{a}	132.9 ±2.4^{a}	148.23 ±3.62^{c}	147.26 ±3.52^{b}	101.1 ±3.5^{b}	161.81 ±4.51^{a}	168.93 ±7.4^{a}	98.74 ±1.26^{b}	137.74 ±2.58^{d}	142.72 ±2.53^{c}
Cytochrom Oxidase	10.12 ±1.11^{b}	14.43 ±0.78^{b}	12.07 ±1.25^{a}	13.64 ±1.58^{a}	13.72 ±0.44^{b}	8.98 ±0.42^{b}	9.98 ±1.35^{b}	15.91 ±0.62^{a}	11.23 ±1.64^{a}	9.62 ±0.32^{b}	12.52 ±0.45^{c}	8.34 ±0.35^{c}
LDH	161.19 ±7.6^{b}	237.12 ±3.6^{b}	245.17 ±4.5^{a}	198.19 ±8.4^{a}	231.82 ±1.35^{c}	227.64 ±5.2^{c}	159.61 ±6.85^{b}	245.15 ±2.84^{a}	233.69 ±6.42^{b}	151.73 ±3.68^{c}	227.15 ±2.26^{d}	220.43 ±2.45^{d}

Mean have different letters in same row for same organ are significantly different (P<0.05)

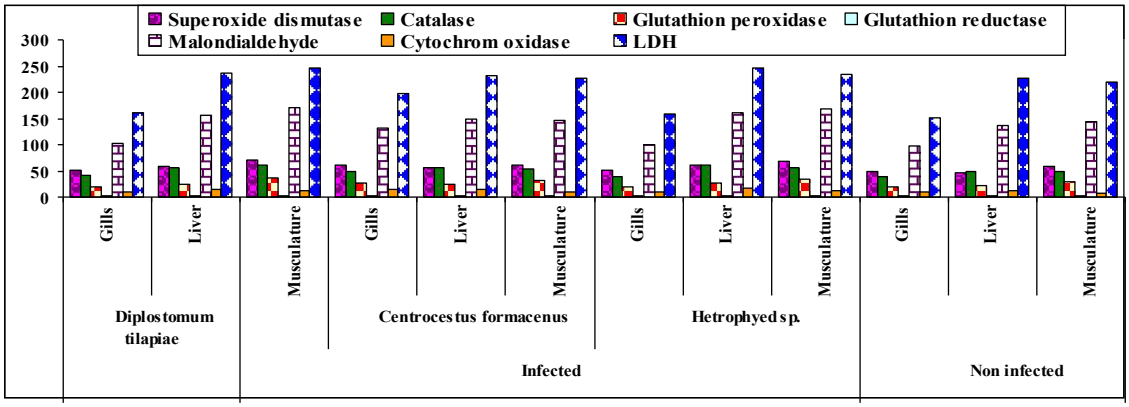

Fig. (2): Effect of E.M.C. on enzyme activities in *O. aureus*

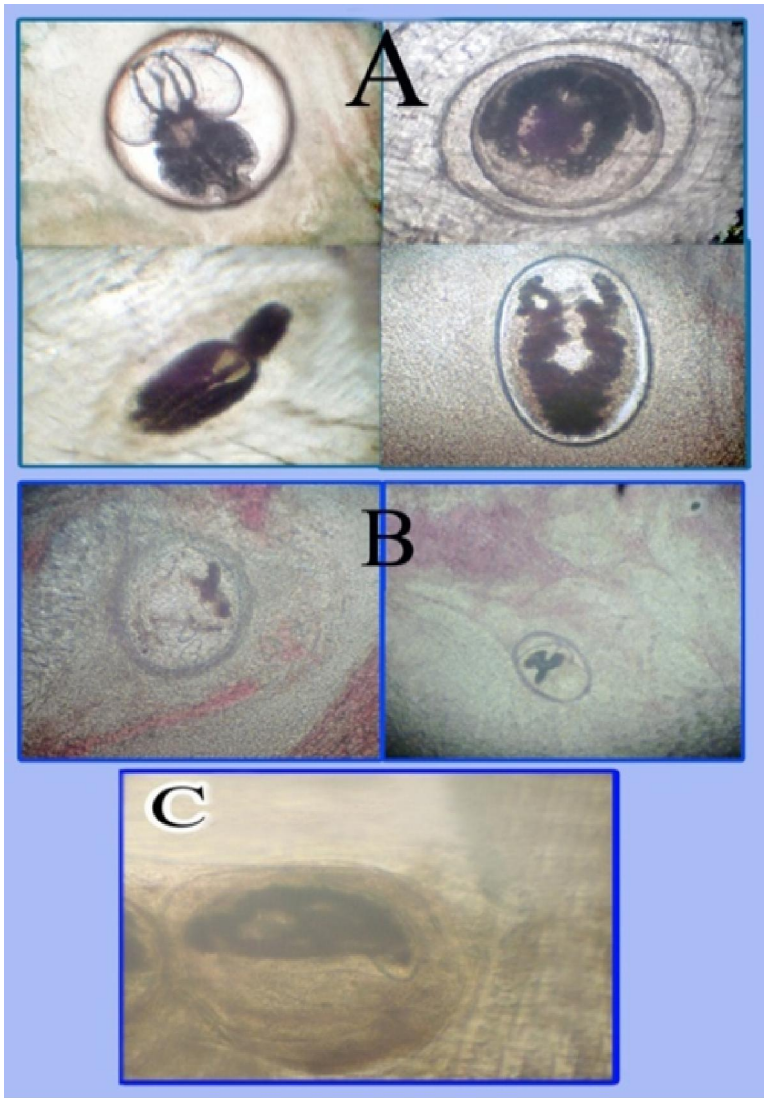

Plate (1):
A-Encysted metacercariae of ***Diplostomum tilapiae*** in musculature of *Oreochromis niloticus* .
B- Encysted metacercariae of ***Centrocestus formosanus*** isolated from gills of *Oreochromis niloticus*.
C- Encysted metacercariae of ***Heterophyes sp***.in liver of *Oreochromis aureus*. **(X100)**

4- Discussion

It appears that parasitism in the presence of stressful ecological factors may further compromise the health of the host, even at low intensities. Taking into account that parasitism induced higher oxygen and energy consumption in fish (**Lemly and Esch, 1984**). The main intension of the current work is studying role of metacercarial affections on tilapia species, determination their prevalence and their impact on activity of enzymes throughout different seasons. The main clinical signs observed in infected tilapia species with E.M.C. revealed no characteristic clinical signs. The total prevalence of E.M.C. in tilapia species was 13.1%.The highest prevalence was found in *Oreochromis aureus* 14.8%, followed by *Oreochromis niloticus* 11.7%. These results disagree with **Shaapan (1997)** who showed infestation rate of encysted metacercariae in tilapia species was 77.37%. These variations might be attributed to the factors affecting cercarial penetrations, site and time of sampling and the immunological status of the fishes. Based on the morphological and parasitological examinations, the isolated EMCs were identified as *Diplostomum tilapiae* Rudolphi, 1809, *Centrocestus formosanus* Nishigori, 1924, and *Heterophyes sp.* Witenberg.

Parasite infection induces oxidative stress (an imbalance between pro-oxidants and non-enzymatic antioxidants) and a higher level of membrane damage in the fish (lipid peroxidation used as biomarker of water contamination and pathological effects caused by parasitism, these scientific facts are supported by **David *et al.* (2005)**. Regarding the results of the present work, it was revealed a significant increase of enzyme activities (SOD, CAT, GPX, MDA, cytochrome oxidase and LDH) in gills, liver, and musculature of both infected tilapia species by different parasitic infections with encysted metacercariae. Moreover, A significant increase of GR in gills, liver, and musculature of non infected fishes. Also, the present findings agree with **David *et al.* (2010)** who recorded that catalase activity in musculature increased with the increase of numbers of *Diplostomum sp.* However, **Eman Zahran and Engy Risha (2013)** found that almost of the measured enzyme activities showed varying reduction significant levels in treatment groups compared with the infected tilapia with saprolegnia. In this respect, **Doherty *et al*. (2010)** discussed the role played by some heavy metals in the flesh of some catfishes and tilapias in Nigeria and demonstrated that alteration in the antioxidant enzymes and induction of lipid peroxidation reflects the presence of heavy metals which may cause oxidative stress in fishes provide a rational use of biomarkers of oxidative stress in biomonitoring of aquatic pollution. Also, In Turkey, **Karadag *et al.* (2014)** used several oxidative stress biomarkers in *Cyprinus carpio* taken from polluted area by untreated wastewaters .They measured that the presence of certain prooxidative compounds that can lead to oxidative stress in the fish and oxidative stress biomarkers may be important in order to evaluate the effects of untreated wastewaters on living organisms.

The present findings disagree with **Bello *et al.* (2000)** who analyzed effect of *Clinostomum detruncatum* metacercariae infestation on the activities of the antioxidant enzymes superoxide dismutase and catalase in musculature of the freshwater fish *Rhamdia quelen* and as enzyme activities were similar in infected and uninfected fishes. However, the present results agree with **Dickinson and Forman (2002)**, **Dautremepuits *et al.* (2003) and David *et al.* (2010)** who showed that activity of glutathione reductase in gill tissues decreased with increasing numbers of *A. brevis*. In these results, high correlation between temperatures, the parasite number and enzyme activities in the same tilapia species, agrees with **Rudneva *et al.* (2004)**. Also, the fluctuation of water temperature and other environmental conditions as well as metacercarial infestation. In addition, the present study suggested that a significant increase in infected both tilapias with lipid peroxidation can be used in a comparative manner to measure the degree of pathogenicity exerted by different parasites especially metacercarial . From the present investigation, it was concluded that the enzyme activities act as biomarkers for oxidative stress induced by metacercarial affections in cultured *Oreochromis niloticus* and *O aureus*.

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