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RESEARCH ARTICLE

Role of some stressful ecological factors on cultured Nile tilapia in relation to metacercarial affections.

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Abstract

This study was carried out to investigate role of some stressful ecological factors on cultured tilapia species, 86 *Tilapia zillii* and 118 *Sarotherodon galilaeus* in relation to metacercarial affections. Physicochemical parameters of water samples were within normal level that can tilapia survive and adapte.There were no pathognomonic signs in infected tilapia species. Encysted metacercariae were identified as *Diplostomum tilapiae*, ,*Centrocestus formosanus* and *Heterophyes sp*.The whole prevalence was 28.8 % and the seasonal prevalence were recorded .We evaluated the activity of enzymes (Superoxide dismutase,Catalase,Glutathion peroxidase, Glutathion reductase,Malondialdehyde, Cytochrome oxidase and Lactate dehydrogenase) in gills,liver and musculature of both infected tilapias throughout the different seasons and the statistical analysis were done.

INTRODUCTION

Artificial contaminant and or increase of fish culture enhances the environmental changes, which may be stressful to fish [1]. This may decrease resistance by fish, activating disease spread and parasitic infection [2] .It is necessary to study the environmental factors as they affect the parasites that affect production and quality [3]. Harmful effects of parasites are aggravated when their hosts are under stress,it becomes essential to clarify the combined effects of anthropogenic stressors such as contaminants and natural stressors such as parasites.In order, water pollution and parasitism may act additively or synergistically on the health of fish [4-5].Parasites can act together with environmental pollution in diverse 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 [6].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 present study was designed to focus on some enzyme activities as biomarkers for oxidative stress produced by metacercarial affections in cultured *Tilapia zillii* and *Sarotherodon galilaeus.*

Material and Methods

Tilapia species:

A total of 186 cultured tilapia species, 86 *Tilapia zillii* and 118 *Sarotherodon galilaeus* with an average body weight (80 +10g) were gathered from El-Abbassa fishfarm, Sharkia Governorate randomly and seasonally.The tilapias were gathered from March 2014 to Febraury 2015 and were moved alive to laboratory for examination.

Parasitological examination:
We isolated encysted metacercariae from gills,liver and musculature and kept in at 4°c overnight for relaxation then,were washed with normal saline,were compressed with a small part of surrounding tissue in between 2 glass slides, were fixed and stained by semichons acetocarmin stain according to [7].

Oxidative stress analysis:
Samples from gills, liver and muscle were washed in ice-cold physiological saline (0.59% NaCl) then were homogenized by a glass-Teflon homogenizer to a 1/5 (w/v) ratio in o.25 M pH 7.4 sucrose buffer. Heidolph So 110 R2RO).They were centrifuged at 9500 xg for 30 min in a Sorvall RC2B centrifuge at 4°C and supernatant stored at-70 °C 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 methylcatechol reaction [8].**Glutathion reductase (GR)** was measured according to[9].**Malondi aldehyde (MDA)** content was determined using thiobarbituric acid (TBA) reaction according to[8].**Cytochrome oxidase** and **Lactate dehydrogenase (LDH)** were measured according to [10-11] respectively**.**

Statistical analysis:
In our study we used the one way analysis of variance (ANOVA) of SPSS according to [12].

Results

Clinical examination of naturally infected fish: No pathognomic clinical signs were detected on the external body surface of examined *Tilapia zillii* and *Sarotherodon galilaeus*,but was paleness in liver and whitish cysts in musculature of examined fishes were observed.

Parasitological examination:
The isolated encysted metacercariae were identified as ***Diplostomum tilapiae***, ***Centrocestus formosanus*** and ***Heterophyes sp*** [13-15] respectively. **Plate (1)**

Prevalence of encysted metacercariae in examined fish.
The total prevalence of encysted metacercariae of examined *Tilapia zillii* and *Sarotherodon galilaeus* was 28.8 %. The highest prevalence was found in *Sarotherodon galilaeus* 16.1 %, followed by *Tilapia zillii* 12.7% **(Table 1).**

Table (1): Prevalence of encysted metacercariae in examined fish

Tilapia species	No. of ex. Tilapia	No. of infected Tilapia	%
Tilapia zillii	86	11	12.7
Sarotherodon galilaeus	118	19	16.1
Total	204	30	28.8

Table (2): Seasonal prevalence of encysted metacercariae in examined fish

	Tilapia zillii					*Sarotherodon galilaeus*				
Seasons	**No.of Ex.**	**No.of inf.**	***D.tilapiae***	***C.formacenus***	***Hetrophyes sp***	**No.of Ex.**	**No. of inf.**	***D.tilapiae***	***C.formacenus***	***Hetrophyes sp***
Spring	21	4(19.04 %)	2(50%)	1(25%)	1(25%)	32	4(12.5%)	2(50%)	1(25%)	1(25%)
Summer	21	1(4.9%)	1(100%)	0(0%)	0 (0%)	36	4 (11.1%)	2(50%)	1(25%)	1(25%)
Autumn	24	1(4.1%)	1 (100 %)	0(0%)	0(0%)	22	8(36.36) %	4(50%)	2(25%)	2(25%)
Winter	20	5(25%)	3(60%)	1(20%)	1(20%)	28	3 (10.7%)	2 (66.7%)	1(33.3%)	0(0%)

The highest prevalence of *Diplostomum tilapiae* was 100 % in Summer and Autumn (*Tilapia zillii*) and lowest prevalence was 50 % in Spring while its prevalence 66.7% in Winter and 50% in other seasons in (*Sarotherodon galilaeus)* .The highest prevalence of *Centrocestus formacenus* was 33.3% in Autumn (*Sarotherodon galilaeus*) and the lowest prevalence was 0% in Summer and Autumn (*Tilapia zillii*). The highest prevalence of *Hetrophyes sp* was 25 % in Spring, Summer and Autumn in *Sarotherodon galilaeus* and in Summer in (*Tilapia zillii*).

Effect of encysted metacercariae on enzyme activities in *Tilapia zillii*

In *Tilapia zillii* infected with *Diplostomum tilapiae, Centrocestus formacenus* and *Hetrophyes sp*. the enzyme activities in the gills, liver and musculature were recorded in **Table 3, Fig.1**.Infected *Tilapia zillii* 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 encysted metacercariae on enzyme activities in *Sarotherodon galilaeus*

The enzyme activities in the gills, liver and musculature In *Sarotherodon galilaeus* infected with *Diplostomum tilapiae*, *Centrocestus formacenus* and *Hetrophyes sp*.were documented in **Table4, 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 encysted metacercariae on enzyme activities in *Tilapia zillii*

Enzymes	Infected									non Infected		
	Diplostomum tilapiae			*Centrocestus formacenus*			*Hetrophyes sp*					
	Gills	Liver	Muscul.	Gills	Liver	Muscul.	Gills	Liver	Muscul.	Gills	Liver	Muscul.
Superoxide dismutase	50.23 ±3.52^{b}	56.13 ±1.56^{b}	70.75 ±2.32^{a}	60.26 ±2.41^{a}	55.13 ±1.42^{b}	61.58 ±2.42^{b}	49.97 ±3.15^{b}	59.53 ±2.31^{a}	67.48 ±3.52^{a}	48.84 ±2.42^{b}	44.31 ±2.15^{c}	57.37 ±2.52^{b}
Catalase	39.97 ±1.42^{b}	55.2 ±1.58^{a}	61.14 ±2.41^{a}	46.91 ±2.38^{a}	54.19 ±2.62^{a}	49.63 ±1.21^{c}	38.45 ±1.62^{b}	58.54 ±3.5^{a}	54.87 ±2.41^{b}	37.87 ±1.57^{b}	48.46 ±1.98^{b}	48.72 ±2.35^{c}
Glutathione peroxidase	19.7 ±0.82^{b}	23.93 ±1.21^{b}	36.61 ±1.42^{a}	26.7 ±0.78^{a}	22.63 ±1.1^{b}	30.61 ±1.82^{bc}	18.87 ±0.52^{b}	26.65 ±1.6^{a}	32.13 ±1.72^{b}	18.35 ±0.89^{b}	20.43 ±0.48^{c}	27.78 ±2.25^{c}
Glutathione reductase	2.22 ±0.15^{b}	2.06 ±0.05^{b}	2.32 ±0.12^{b}	2.28 ±0.24^{b}	2.08 ±0.04^{b}	2.44 ±0.15^{b}	2.15 ±0.3^{b}	2.06 ±0.06^{b}	2.44 ±0.12^{b}	3.37 ±0.52^{a}	2.77 ±0.15^{a}	3.16 ±0.23^{a}
Malondi aldehye	101.12 ±3.1^{b}	154.07 ±2.58^{b}	170.23 ±4.6^{a}	131.9 ±2.4^{a}	148.22 ±3.62^{c}	146.26 ±3.52^{b}	100.1 ±3.5^{b}	161.80 ±4.51^{a}	167.93 ±7.4^{a}	97.74 ±1.26^{b}	135.74 ±2.58^{d}	140.72 ±2.53^{c}
Cytochrom Oxidase	10.02 ±1.11^{b}	13.43 ±0.78^{b}	11.07 ±1.25^{a}	13.64 ±1.58^{a}	12.72 ±0.44^{b}	7.98 ±0.42^{b}	8.98 ±1.35^{b}	14.91 ±0.62^{a}	11.23 ±1.64^{a}	8.62 ±0.32^{b}	11.52 ±0.45^{c}	7.34 ±0.35^{c}
LDH	160.19 ±7.6^{b}	236.12 ±3.6^{b}	244.17 ±4.5^{a}	197.19 ±8.4^{a}	230.82 ±1.35^{c}	226.64 ±5.2^{c}	158.61 ±6.85^{b}	244.15 ±2.84^{a}	232.69 ±6.42^{b}	150.73 ±3.68^{c}	226.15 ±2.26^{d}	219.43 ±2.45^{d}

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

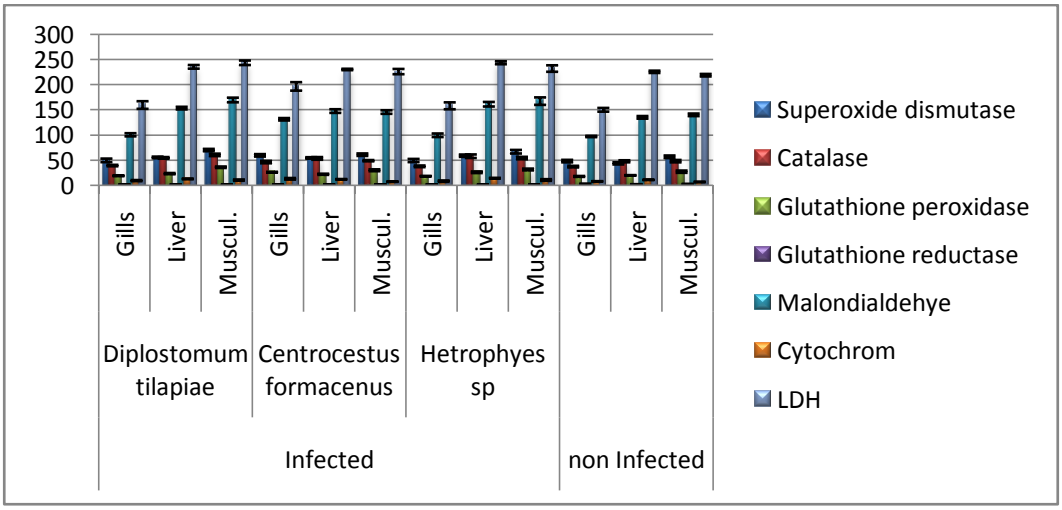

Fig. (1): Effect of encysted metacercariae on enzyme activities in *Tilapia zilli*

Table (4): Effect of encysted metacercariae on enzyme activities in *Sarotherodon galilaeus*

Enzymes	Infected									non Infected		
	Diplostomum tilapiae			*Centrocestus formacenus*			*Hetrophyes sp*					
	Gills	Liver	Muscul.	Gills	Liver	Muscul.	Gills	Liver	Muscul.	Gills	Liver	Muscul.
Superoxide dismutase	50.23 ±3.52^{b}	55.13 ±1.56^{b}	69.75 ±2.32^{a}	59.26 ±2.41^{a}	54.13 ±1.42^{b}	59.58 ±2.42^{b}	48.97 ±3.15^{b}	58.53 ±2.31^{a}	66.48 ±3.52^{a}	47.84 ±2.42^{b}	43.31 ±2.15^{c}	56.37 ±2.52^{b}
Catalase	38.97 ±1.42^{b}	54.2 ±1.58^{a}	59.14 ±2.41^{a}	45.91 ±2.38^{a}	53.19 ±2.62^{a}	48.63 ±1.21^{c}	37.45 ±1.62^{b}	57.54 ±3.5^{a}	53.87 ±2.41^{b}	36.87 ±1.57^{b}	47.46 ±1.98^{b}	47.72 ±2.35^{c}
Glutathione peroxidase	19.7 ±0.82^{b}	23.93 ±1.21^{b}	36.61 ±1.42^{a}	26.7 ±0.78^{a}	22.63 ±1.1^{b}	30.61 ±1.82^{bc}	18.87 ±0.52^{b}	26.65 ±1.6^{a}	32.13 ±1.72^{b}	18.35 ±0.89^{b}	20.43 ±0.48^{c}	27.78 ±2.25^{c}
Glutathione-reductase	2.2 ±0.15^{b}	2.08 ±0.05^{b}	2.41 ±0.12^{b}	2.3 ±0.34^{b}	2.08 ±0.04^{b}	2.51 ±0.15^{b}	2.31 ±0.3^{b}	2.11 ±0.06^{b}	2.42 ±0.12^{b}	3.49 ±0.52^{a}	2.88 ±0.15^{a}	3.27 ±0.23^{a}
Malondi aldehye	101.12 ±3.1^{b}	154.07 ±2.58^{b}	170.23 ±4.6^{a}	131.9 ±2.4^{a}	146.23 ±3.62^{c}	146.26 ±3.52^{b}	100.1 ±3.5^{b}	160.81 ±4.51^{a}	167.93 ±7.4^{a}	97.74 ±1.26^{b}	136.74 ±2.58^{d}	141.72 ±2.53^{c}
Cytochrom Oxidase	10.13 ±1.11^{b}	14.33 ±0.78^{b}	12.05 ±1.25^{a}	13.54 ±1.58^{a}	13.69 ±0.44^{b}	8.88 ±0.42^{b}	9.96 ±1.35^{b}	15.81 ±0.62^{a}	11.24 ±1.64^{a}	9.62 ±0.32^{b}	12.49 ±0.45^{c}	8.35 ±0.35^{c}
LDH	161.2 ±7.6^{b}	237.14 ±3.6^{b}	245.16 ±4.5^{a}	198.29 ±8.4^{a}	231.72 ±1.35^{c}	226.64 ±5.2^{c}	158.61 ±6.85^{b}	244.15 ±2.84^{a}	232.69 ±6.42^{b}	150.73 ±3.68^{c}	226.15 ±2.26^{d}	219.43 ±2.45^{d}

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

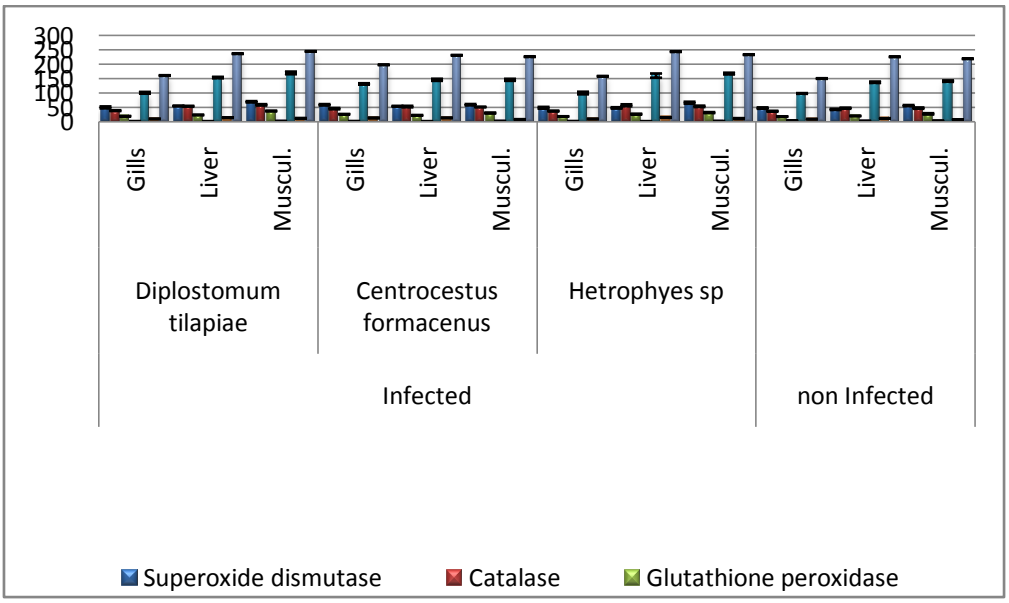

Fig. (2): Effect of encysted metacercariae on enzyme activities in *Sarotherodon galilaeus*

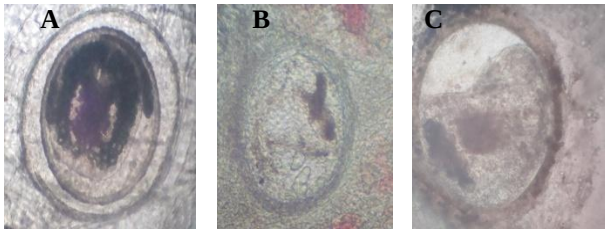

Plate (1):
(A)-*Diplostomum tilapiae* encysted metacercariae in musculature.(B)-*Centrocestus formosanus* encysted metacercariae from gills. (C)- *Heterophyes sp* encysted metacercariae.in liver (X100).

4- Discussion

In the present study,parasitism due to stressful ecological factors may affect the health of fish and brought higher oxygen and energy consumption in fish [16]. Our goal is to investigate the metacercarial affections role on tilapia species, to find out their seasonal prevalence and impact on activity of enzymes. The total prevalence of encysted metacerca in tilapia species was 28.8%.The highest prevalence was found in *Sarotherodon galilaeus* 16.1% followed by *Tilapia zillii* 12.7 %.Such outcome conflict with [17] who revealed 77.37% infestation rate of encysted metacercariae in tilapia species .These variants might be approved to the immunological status of the fishes and factors affecting cercarial penetrations, site and time of sampling.

The isolated encysted metacercariae were identified as *Diplostomum tilapiae* [13], *Centrocestusformosanus* [14], and *Heterophyes sp* [15]. 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 [5]. Concerning the results of the our work, we exposed 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.In addition, a significant increase of GR in gills, liver, and musculature of non-infected fishes. Also, the present findings agree with [18-19].who registered increased catalase activity in musculature with the increase of *Diplostomum sp* numbers**.** However, [20**]** measured enzyme activities and recorded unstable reduction significant levels in treatment groups compared with the infected tilapia with saprolegnia.The role of some heavy metals in the flesh of some catfishes and tilapias in Nigeria discussed by [21] and he revealed that the antioxidant enzymes alteration and lipid peroxidation induction imitates the presence of heavy metals producing oxidative stress in fishes. As well, In Turkey, [22**]**collected *Cyprinus carpio* from polluted area by untreated waste waters and examined the presence of certain pro oxidative compounds which can progress to oxidative stress in the fish and oxidative stress biomarkers may be important to assess the effects of untreated wastewaters on living organisms. Our results oppose to [23] who studied *Clinostomum detruncatum* metacercariae effect on the activities of superoxidedismutase and catalase in musculature of the fresh water fishes which were similar in infected and uninfected fishes. Conversely, our results concur with [24-25] and [18] who presented decreased activity of glutathione reductase in gill tissues. the present study proposed 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 ones.we concluded that the enzyme activities be used as biomarkers for oxidative stress induced by metacercarial affections in cultured *Tilapia zilli and Sarotherodon galilaeus.*

References

1-Lio-po G. D. and Lime L.H. S. (2002): Infectious diseases of warm water fish in fresh water. PP. 231-281. In: Woo P.T. K; Bruno D.W; Lim L.H.S. (Eds.). Diseases and disorders offin fish in cage culture. CAB publishing ,Walling Ford. UK.

2-Eissa I.A. M; Mona S. Z ; Noor El Deen A. I .E;Ibrahim A. Z. and Abdel Hady O. K. (2011) : Field Study on Cadmium pollution in relation to internal parasitic diseases in cultured Nile Tilapia at Kafr El-Sheikh Governorate. J of American Science.Vol.7, No. 4, pages1. Bell1 A. R. R; Fortes E; Klein A. B; Bellóo A. A; Llesuy S. B; Robaldo R. B.and BianchiniA.

3-Mondo U.B. (1999): Protozoan, crustacean and ciliated diseases of fish. In fish pathology term paper. Vet. Parasitology and Pathology A.B.U;Zaria.

4- Eissa I.A. M (2002): Parasitic fish diseases in Egypt. Dar El Nahda El-Arabia publishing, 32Abd El-Khalik tharwat St. Cairo,Egypt.
5- David J. M; Lila G.B; François G. and Andrée D. G. (2005): Joint effects of parasitism andpollution on oxidative stress biomarkers inyellow perch *Perca flavescens*. Dis Aquat Org.Vol. 63: 77–84.
6-Sures B. (2004): Environmental parasitology: relevancy of parasites in monitoring environmental pollution. Trends in Parasitology.Vol. 20,No.4.
7. Lucky Z. (1977): Methods for the diagnosis of fish diseases. American publishing Co; Pvt. Ltd;New Delhi, Bombay Calcutta, New York
8. Jin J; Xiangjun C; Jinhua W; Jianying W. and Guoze W. (2011): Physiological and biochemical responses of halophyte *Kalidiumfoliatum* to salt stress. African Journal of Biotechnology Vol. 10 (55): 11468-11476.
9-.Goldberg D. M. and Spooner R.J. (1983): Methods of enzymatic analysis (Bargemen,H.V. Ed.) 3rd. Verlog Chem, Vol.3, pp:258-265.
10- Rasmussen U.F. and Rasmussen H.N. (2000): Determination of cytochrome C oxidase activity in soluble and membrane bound mitochondrial samples. Mol. Cell. Biochem. Vol.208: 37-44
11-Young D.S. (2001): Effects of disease onclinical lab.tests. 4th ed AACC press 9. Eman Zahran and Engy Risha (2013): Protectiverole of adjuvant and potassium permanganate onoxidative stress response of Nile tilapia (*Oreochromis niloticus)* challenged with *Saprolegnia ferax***.** Springerplus; 2: 94.
12-Snedecor G. and Cochran W. (1969): Statistical methods 6th.ed.Iowa State Univ. Press. Atms.Iowa, USA. 12. Karadag, H and Fırat, Oand Fırat, OZ(2014):Use of Oxidative Stress Biomarkers in *Cyprinuscarpio* L. for the Evaluation of Water Pollutionin Ataturk Dam Lake (Adiyaman, Turkey).Bulletin of Environmental Contamination and Toxicology.
15- Witenberg G. (1929): Studies on the trematode family Heterophyidae, Ani. Trop. Med. And Parasitol. Vol. 23:131-240.
16- Lemly D. and Esch M.J. (1984): Platyhlminta .In: de Almeida A (ed) Elementos de Ictioparasitologia. Fundaçao Eng Porto, p 158
17-Shaapan R. M. (1997): Parasites of fishes and its effect on public health. M. V. Sc. Thesis, Fac.Vet. Med. Cairo Univ.
18- David J. M; Claire D; Andree D.G. and Michel F. (2010): Interaction between parasites and pollutants in yellow perch (Perca flavescens) in the St. Lawrence River, Canada: implication for resistance and tolerance to parasites. Canadian J.of Zoology, vol. 88(3): 247- 258.
19- Eissa I A M, Derwa H I, Mona Ismail, Ramadan R A, Mona Zaki and Nashwa Mohamed.(2014): Use of enzyme activities as biomarkers for oxidative stress induced by metacercarial affections in some cultured tilapia species. Life Science Journal 2014;11(3)

20-Eman Zahran and Engy Risha (2013): Protective role of adjuvant and potassium permang-anate on oxidative stress response of Nile tilapia (*Oreochromis niloticus)* challenged with *Saprolegnia ferax***.** Springerplus; 2: 94.
21-Doherty V.F. 1, Ogunkuade1O.O and 1.U.C.Kanife (2010): Biomarkers of Oxidative Stressand Heavy Metal Levels as Indicators of Environmental Pollution in some selected fishesin Lagos, Nigeria, American-Eurasian J. Agric & Environ. Sci., 7 (3): 359-365
22-Karadag, H and Fırat, O and Fırat, OZ (2014):Use of Oxidative Stress Biomarkers in *Cyprinus carpio* L. for the Evaluation of Water Pollution in Ataturk Dam Lake (Adiyaman, Turkey).
23-Bell A. R. R; Fortes E; Klein A. B; Bell A. A; Llesuy S. B; Robaldo R. B.and Bianchini A.(2000): Lipid peroxidation induced by *Clinostomum detruncatum* in muscle of the freshwater fish *Rhamdia quelen***.** Dis Aquat Org.Vol. 42: 233–236.
24- Dickinson D.A. and Forman H.J. (2002): Cellular glutathione and thiols metabolism.Biochem Pharmacol. Vol. 64:1019–1026.
25- Dautremepuits C; Betoulle S. and Vernet G.(2003): Stimulation of antioxidant enzymelevels in carp *(Cyprinus carpio* L.) infected by*Ptychobothrium* sp. (Cestoda). Fish Shellfish Immunol. Vol. 15:467–471.