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Nerocila orbignyi, Cymothoid isopoda infestation in European sea bass, *Dicentrarchus labrax*. Trials for treatment with evaluation of immune and antioxidative responses.

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ABSTRACT

Cymothoid isopods cause serious infestations in fish that may adversely affect the aquaculture. Current study targeted investigating the enormous infestation of European sea bass *Dicentrarchus labrax* with cymothoid isopod, *Nerocila* species in a marine fish farm at Kafr El Sheikh Governorate, Egypt; accompanied with trials for treatment with malathion 57% at 0.15 and 0.30 mg/L concentrations and evaluation of the immune and antioxidants responses. For this purpose, 450 *D. labrax* were randomly collected alive during April 2020 and thoroughly examined for detection of external parasites. Malathion 57% treatment trial was carried out. Blood and tissue samples were collected from infested fish at zero-day (before malathion treatment) and at 24hrs, 48hrs, 3rd day, 4th day and 5th day (after malathion treatment). Isopods collected from infested fish were identified as *N. orbigny* and fish's external body surface and gills were the predilection sites. Both malathion concentrations were effective in eradicating *N. orbigny* and decreasing the isopod prevalence from 100 to 0% at the 5th day sampling point. Infested fish revealed improved hematological parameters (Erythrocyte counts, hemoglobin, and hematocrit readings), however leucocyte and differential cells counts showed significant decrease in infested fish. Immunological parameters (Lysozyme and IgM activities) decreased, but nitric oxide increased by time after malathion application. *Nerocila* is considered serious parasite in aquaculture that negatively affects fish's immune and

antioxidative responses; and malathion denoted as a promising treatment to control *N. orbigny* in infested *D. labrax*.

Keywords: *Nerocila orbigny*; malathion; immune response; antioxidants; *Dicentrarchus labrax*.

INTRODUCTION

Dicentrarchus labrax is one of the marine species that represent the current importance of marine aquaculture to support meeting the domestic requirements **(Barfield *et al.,* 2017)**.

Parasitic infestations can greatly affect aquaculture industry and lead to great economic losses and almost 80% of fish diseases are caused by parasites **(Eissa, 2002).** Parasitic crustacean infests various fish species as seabass which faces great losses from parasitic crustacean diseases among which are Isopodiasis e.g. Cymothoid isopoda *Nerocila,* that is the most common and could be present in different parts of the fish body, including internal organs, gills and fins causing damages and inflammation of the infected tissues **(Horton and Okamura, 2003; Kayış and Ceylan, 2011).**

Genus *Nerocila is* a division from *Cymothoidae*. *Nerocila orbignyi* is a cymothoid isopod mostly distributed in Mediterranean, Red Sea, North Africa, Egypt, and New Zealand **(Trilles, 1994).** Usually infest marine fishes **(Kayis *et al.*, 2009).** *N. orbignyi* was previously reported in various fish species including *Mugil capito, Solea solea and Tilapia zillii* **(Öktener and Trilles, 2004; Alas *et al.*, 2008 and Shaheen *et al.*, 2017)**.

Isopods are expected to alter the hematological parameters of the infested fish **(Elgendy *et al.*, 2018).** Malathion is widely used for treatment of infested fish with isopods **(Scholz, 1999; Horsberg, 2004 and El-Deen *et al.,* 2013)**.

According to the available knowledge, the current investigation presents the first record to evaluate hematological, immune and antioxidative alterations produced by *N. orbignyi* in response to malathion treatment in cultured *D. labrax* in Egypt. Also, aimed to investigate the effectiveness of malathion in controlling *N. orbignyi.*

MATERIALS AND METHODS

Study area

The present study was carried out on two infested ponds (2 m depth×143 m length×43 m width) in a marine fish farm (salinity was about 28±0.1 ‰) located at FC92+5W Borg Megheizel, Metobas, Kafr El Sheikh Governorate, Egypt.

Fish sampling

A total of 450 (~90 fish/sampling point) *D. labrax* (Body weight 350±5 gm and body length 28±3 cm) were randomly collected during April, 2020 and subjected to clinical and postmortem examination according to the method described by **Noga (2010)**. Ponds were sampled daily throughout the experimental period. First sampling point was at zero-day, followed by malathion treatment, water exchange and then daily sampling until the 5th day sampling point. Sampling points included (zero day, 24hrs, 48hrs, 3rd day, 4th day and 5th day) points.

Parasitological Examination

Isopods were preserved in 70% ethanol until identification. The morphological characters were recorded following **Williams and Williams (1999) and Froese and Pauly (2013)**. Prevalence (%) of *N. orbignyi* was calculated as (number of infested hosts/number of examined hosts × 100 %) according to **Bush *et al.*, (1997).**

Malathion treatment

After the first sampling point (zero-day), malathion (57%, Elnasr chemicals, Egypt) was applied once to two infested ponds at (0.15 and 0.3 mg/L) according to **El-Deen *et al.*, (2013) and Sabullah *et al.*, (2014)** using power chemical sprayer for ~ 30 min. Then, water exchange for both ponds was carried out. Post treatment samples were taken daily starting 24hrs after treatment until the fifth day post malathion treatment (the point where complete detachment of the crustacean parasites and recovery of fish occurred).

Hematological examination

Blood samples were collected from 9 randomly selected *D. labrax* from each pond at each sampling point, using 3 ml sterile syringe coated with EDTA anticoagulant for measurement of red blood cells (RBCs) and white blood cells (WBCs); hemoglobin (Hb) and corpuscular hemoglobin concentrations; packed cell volume (PCV) and mean corpuscular volume (MCV); mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) following methods described by **Elgendy *et al.*, (2018)** and **Elabd *et al.*, (2019).**

Immunological parameters

Plasma was separated from blood samples by cool centrifugation at 1500 rpm at 4°C for 20 min for assaying immune parameters. lysozyme activity was assayed fluorometrically at absorption ~494 nm and emission ~518 nm according to the company protocol (EnzChek lysozyme assay kit E-22013, Molecular probes, USA). Nitric oxide was measured at 450 nm

according to **Miranda *et al.*, (2001)** and IgM was also assayed at 450 nm following the commercial kit (CUSABIO, China).

Antioxidants and biochemical parameters

Liver samples were collected in phosphate-buffered saline (PBS), pH 7.4 for assaying SOD, GPx and MDA activities following McCord and **Fridovich (1969)** and **Paglia and Valentine (1967).** ALT and AST were assayed in liver samples at 340 nm using commercial kit **(**SPINREACT, Spain) and following protocols described by **Murray (1984a and b)**.

Glucose and cortisol concentrations in plasma were measured at 340 nm using commercial kit (SPINREACT, Spain) following schemes and formulas described by **Trinder (1969).**

Statistical analysis

Data of the current study was statistically investigated using one-way ANOVA, results were presented as Means ± Standard Error (M ± SE) with Duncan's multiple range tests for estimation of significant differences between different groups through Social Sciences (SPSS) software (version 22.0). A value of $P < 0.05$ was regarded significant.

RESULTS

Clinical and postmortem examination

Macroscopic isopods were recorded on skin, fins, gills and inside the buccal cavity. Infested fish showed external hemorrhages, inflammation, scales detachment and ulcers (Fig. 1). Internally, gills were pale in color with mucoid secretion; and liver was either pale or congested and hemorrhagic in investigated samples (Fig. 2).

Parasitological examination

Sixty-nine (69%) of randomly examined fish were found to be infested with isopods. The collected isopods were recognized as *Nerocila orbignyi* (28 mm length, 14 mm width) mainly from gills and external surface, the adult crustacean parasite body shape was somewhat cylindrical and broad at the center of the body. The head appeared to be divided by a tightening from the rest of the body. The mandible had 7 denticles. exopod had 5 short distal spines and the caudal rami are short, while the endopod had slender bristled seta and carried a stretched distal spine. A tiny papilla-like process is located at the base of the endopod (Fig. 3, A-D).

Gravid females were found to be carrying eggs in the brood pouch on its ventral surface (Fig. 3, D). Few (5-6) Manca (post-larvae) was collected from randomly examined fish. They appeared to be lighter in body color

and characterized by presence of six pairs of legs instead of seven in adults (Fig. 3, E-F).

Malathion treatment trial

Results revealed that the prevalence of isopods in randomly investigated fish from malathion treated groups was better for 0.30 mg/L group. Complete detachment of *N. orbignyi* was observed and the prevalence decreased from 100 to 0% at the 5th day sampling point after malathion treatment (Table1).

Hematological examination

Tables 2 and 3 shows hematological parameters of infested groups before (zero-day) and after 0.15 and 0.30 mg/L malathion treatment (24hrs, 48hrs, 3rd day, 4th day and 5th day). The treated groups showed ($P < 0.05$) markedly increased RBCs count, hemoglobin (Hb), hematocrit (Ht) and platelets count, while there was a significant decrease in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) over the experimental period. WBCs also ($P < 0.05$) significantly decreased after malathion treatment accompanied with lymphopenia and improved monocytes and neutrophils' percentage over the sampling points.

Immunological parameters

0.15 mg/L malathion treated group showed nearly similar IgM and lysozyme activities with no ($P < 0.05$) significant decrease than the zero-day sampling point (before treatment). NO levels showed a slight ($P < 0.05$) non-significant increase than the non-treated group (zero-day) (Figure 4). While 0.30 mg/L malathion treated group revealed a ($P < 0.05$) significant decrease in both IgM and lysozyme activities with ($P < 0.05$) marked increased NO levels over the time (Figure 4).

Antioxidants and biochemical parameters

Antioxidants SOD, GPXAS and MDA showed ($P < 0.05$) significant increased levels for 0.15 mg/L malathion than the zero-day; and reached its highest level at the 5th day after treatment (Figure 5 A, B and C). Also, 0.30 mg/L malathion group revealed ($P < 0.05$) significant increased SOD and GPXAS and levels over the experimental period, while MDA readings ($P < 0.05$) significantly decreased (Figure 5 C).

Glucose level in 0.15 and 0.30 mg/L malathion groups showed ($P < 0.05$) marked increase, while Cortisol level revealed ($P < 0.05$) significant decrease over the time compared to the zero-day sampling point (Table 4).

AST also showed ($P < 0.05$) marked decrease over time compared to the zero-day sampling point for both 0.15 and 0.30 mg/L malathion groups (Table 4), while ALT showed only an increase at the first sampling point (24 hrs.) after 0.15 mg/L malathion treatment followed with a ($P < 0.05$) marked decrease and return to the zero-day levels and showed an increase at the first two sampling points (24 and 48 hrs.) after 0.30 mg/L malathion treatment then a ($P < 0.05$) marked decrease through the following sampling points (Table 4).

DISCUSSION

Cymothoids isopods infestations are well known in aquaculture and have been reported in various fish species **(Rajkumar *et al.*, 2005; Hadfield *et al.*, 2013; Shaheen *et al.*, 2017)**. In the present study, *N. orbignyi* was collected from branchial cavity, buccal cavity, lateral body surface of sea bass suggesting these locations as the predilection sites on sea bass. This comes in accordance with **Elgendy *et al.*, (2018),** who isolated *Nerocila bivittate* from similar sites on *Tilapia zilli*. Presence of isolated *N. orbignyi* inside buccal and branchial cavities, may be because these sites provide a good protection for isopods. *N. orbignyi* caused mechanical irritation, damage, and paleness of gills structure of the infested fish. Similarly, **Shaheen *et al.*, (2017)** and **Elgendy *et al.*, (2018)** reported same finding and damage of gill structure with complete absence of gill rackers. This can be attributed to mechanical irritation caused by cymothoids to the infested fish, interruption of blood circulation and sucking behavior of isopods.

To the best of our knowledge, there are few studies addressing *N. orbignyi* in fish and there is a great variation within *Nerocila* that is not yet fully studied, with extreme variations within some species. Those species icludes: *Nerocila orbignyi* **(Rameshkumar *et al.*, 2005).** Presence of male or female is greatly variable and differ among fish species. It usually difficult to differentiate both sexes, however usually male is narrower than female **(Rohde, 2005)**. In the current study, collected isopods were recognized as *Nerocila orbignyi* (28 mm length, 14 mm width). The adult crustacean parasite, gravid females carrying eggs in the brood pouch and few (5-6) Manca (post-larvae) was collected from randomly examined fish. These results come in accordance with **(El-Deen *et al*., 2013; Kazuya and Sho, 2017).**

Fig.1. *Dicentrarchus labrax* showing *N. orbignyi* on peduncle region and ulcers at site of attachment.

Fig.2. *Dicentrarchus labrax* showing internally pale liver with focal hemorrhage.

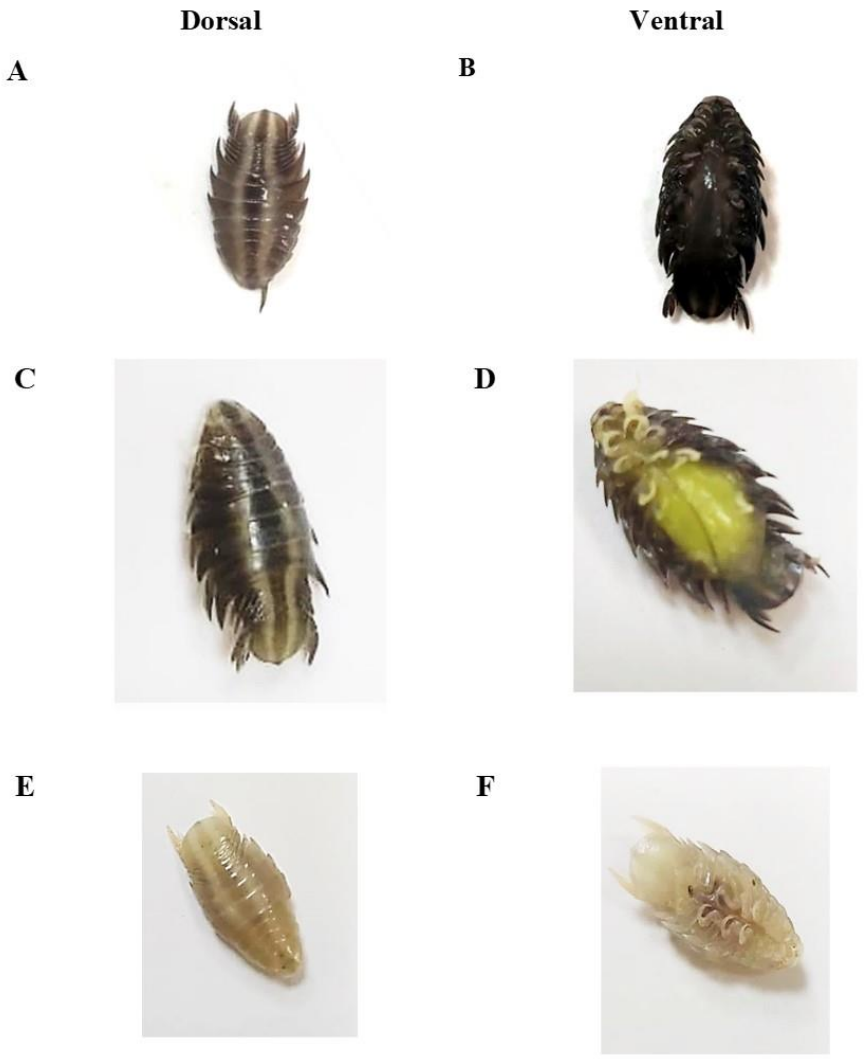

Fig.3. *Nerocila orbignyi* adult (A-C), gravid female carrying eggs in marsupium (D) and manca (post-larvae) (E and F) retrieved from *Dicentrarchus labrax* (Dorsal and ventral view).

Table 1 Effect of malathion on prevalence (%) of *Nerocila orbignyi* infection in randomly examined sea bass *Dicentrarchus labrax*.

Malathion mg/L	Zero day	24 hrs	48hrs	3rd day	4th day	5th day
0.15	100	88	50	20	8	0*
0.30	100	85	45	10	3	0*

*Statistical difference at $P < 0.05$.

Table 2 Hematological picture of infested Sea bass (*Dicentrarchus labrax*) with *Nerocila orbignyi* before (zero-day) and after 0.15 mg/L malathion treatment (24hrs, 48hrs, 3 days, 4 days and 5 days).

Parameters	Zero day	24 hrs	48hrs	3rdday	4th day	5th day
Hemoglobin (g dL^{-1})	6.33 ± 0.05^{d}	7.14 ± 0.00^{c}	9.51 ± 0.00^{b}	9.88 ± 0.00^{b}	10.15 ± 0.00^{a}	10.21 ± 0.00^{a}
R.B.Cs ($\times10^{6}$ μL^{-1})	1.51 ± 0.00^{c}	2.23 ± 0.05^{b}	2.4 ± 0.00^{ab}	2.40 ± 0.00^{ab}	2.47 ± 0.00^{a}	2.757 ± 0.0^{a}
Hematocrit (%)	21.16 ± 0.10^{d}	22 ± 0.05^{c}	23.23 ± 0.05^{c}	24 ± 0.02^{b}	28.63 ± 0.02^{b}	36.03 ± 0.00^{a}
Platelets ($\times10^{3}$ μL^{-1})	72.66 ± 0.00^{e}	101 ± 0.02^{d}	122 ± 0.05^{c}	128 ± 0.02^{c}	145 ± 0.02^{b}	153.33 ± 0.02^{a}
W.BCs ($\times10^{3}$ μL^{-1})	14.14 ± 0.00^{a}	12.43 ± 0.05^{b}	8.15 ± 0.00^{d}	8.63 ± 0.02^{d}	8.31 ± 0.02^{d}	10.17 ± 0.02^{c}
M.C.V (fL)	139.03 ± 0.10^{a}	130 ± 0.10^{b}	116.7 ± 0.00^{c}	109.9 ± 0.05^{d}	100.3 ± 0.00^{e}	99.43 ± 0.00^{e}
M.C.H (pg)	41.46 ± 0.00^{ab}	32.6 ± 0.20^{d}	40.1 ± 0.02^{b}	42.3 ± 0.05^{a}	40.7 ± 0.00^{b}	36.33 ± 0.02^{c}
M.C.H.C (g(dl)	29.86 ± 0.03^{c}	24.96 ± 0.00^{d}	40.3 ± 0.02^{ab}	41.2 ± 0.00^{a}	35.43 ± 0.05^{b}	28 ± 0.02^{c}
Basophils %	2.66 ± 0.02^{a}	2.33 ± 0.00^{a}	1 ± 0.05^{b}	1 ± 0.00^{b}	1 ± 0.00^{b}	1 ± 0.20^{b}
Eosinophils %	10 ± 0.00^{b}	11 ± 0.00^{a}	8 ± 0.05^{c}	6 ± 0.00^{c}	5.66 ± 0.02^{d}	5.33 ± 0.20^{d}
Neutrophils %	19.66 ± 0.00^{d}	15 ± 0.02^{d}	26 ± 0.00^{c}	32 ± 0.00^{b}	32.33 ± 0.02^{b}	36.66 ± 0.05^{a}
Lymphocytes %	70 ± 0.20^{a}	60 ± 0.02^{b}	62 ± 0.00^{b}	58 ± 0.02^{c}	55 ± 0.05^{c}	53.66 ± 0.05^{d}
Monocytes %	2 ± 0.00^{b}	2 ± 0.02^{b}	3 ± 0.00^{a}	3 ± 0.02^{a}	3 ± 0.05^{a}	3 ± 0.05^{a}

Values with different letters superscripts are significantly different ($P < 0.05$).

Table 3 Hematological picture of infested Sea bass (*Dicentrarchus labrax*) with *Nerocila orbignyi* before (Zero day) and after 0.3 mg/L malathion treatment (24hrs, 48hrs, 3 days, 4 days and 5 days).

Parameters	**Zero day**	**24 hrs**	**48hrs**	**3rd day**	**4th day**	**5th day**
Hemoglobin (g dL^{-1})	6.33 ± 0.05^{d}	7.69 ± 0.05^{c}	8.19 ± 0.07^{ab}	9.06 ± 0.15^{b}	10.43 ± 0.00^{a}	10.55 ± 0.05^{a}
R.B.Cs ($\times10^{6}$ μL^{-1})	1.51 ± 0.15^{c}	0.84 ± 0.35^{d}	1.46 ± 0.50^{c}	1.87 ± 0.05^{b}	2.12 ± 0.05^{ab}	2.757 ± 0.00^{a}
Hematocrit (%)	21.16 ± 0.05^{c}	13.2 ± 0.15^{e}	19.73 ± 0.00^{e}	26.06 ± 0.15^{d}	33.73 ± 0.05^{b}	36.03 ± 0.25^{a}
Platelets ($\times10^{3}$ μL^{-1})	72.66 ± 0.05^{d}	75 ± 0.05^{d}	83.66 ± 0.07^{c}	138.33 ± 0.05^{ab}	132.33 ± 0.05^{b}	153.33 ± 0.05^{a}
W.BCs ($\times10^{3}$ μL^{-1})	14.14 ± 0.00^{a}	13.43 ± 0.15^{ab}	11.97 ± 0.00^{b}	9.29 ± 0.05^{c}	7.79 ± 0.35^{d}	6.87 ± 0.15^{e}
M.C.V (fL)	139.03 ± 0.10^{d}	152 ± 0.10^{b}	133.43 ± 0.00^{e}	147.1 ± 0.07^{c}	162.26 ± 0.15^{a}	99.43 ± 0.10^{e}
M.C.H (pg)	41.46 ± 0.10^{d}	102.86 ± 0.00^{a}	51.16 ± 0.10^{b}	48.8 ± 0.10^{c}	46.16 ± 0.05^{c}	36.33 ± 0.15^{e}
M.C.H.C (g(dl)	29.86 ± 0.10^{d}	65.56 ± 0.05^{a}	38.36 ± 0.15^{b}	35.26 ± 0.05^{c}	31.56 ± 0.15^{c}	28 ± 0.10^{d}
Basophils %	2.66 ± 0.05^{b}	1 ± 0.10^{d}	3 ± 0.05^{a}	1.66 ± 0.15^{c}	1.33 ± 0.05^{c}	1 ± 0.05^{d}
Eosinophils %	10 ± 0.15^{a}	7 ± 0.10^{b}	7.33 ± 0.15^{ab}	7 ± 0.07^{b}	6 ± 0.15^{c}	5.33 ± 0.10^{d}
Neutrophils %	19.66 ± 0.05^{d}	37.33 ± 0.07^{c}	36.33 ± 0.07^{c}	48.33 ± 0.15^{a}	46.66 ± 0.15^{b}	36.66 ± 0.05^{c}
Lymphocytes %	70 ± 0.15^{a}	51.33 ± 0.15^{c}	51.66 ± 0.10^{c}	37 ± 0.10^{e}	41.33 ± 0.10^{d}	53.66 ± 0.15^{b}
Monocytes %	2 ± 0.07^{c}	2.33 ± 0.07^{c}	2 ± 0.07^{c}	3.66 ± 0.07^{a}	3 ± 0.15^{b}	3 ± 0.10^{b}

Values with different letters superscripts are significantly different ($P < 0.05$).

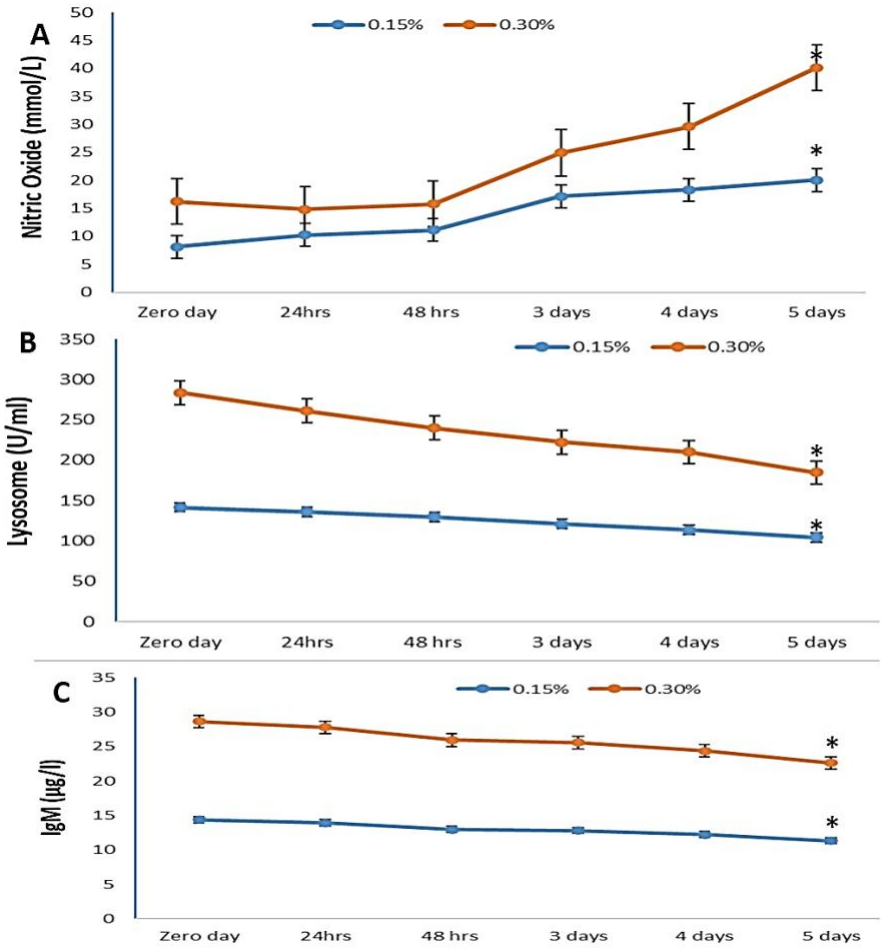

Fig.4. Immunological parameters [NO (A), lysozyme (B) and IgM (C)] of *D. labrax* infested with *N. orbignyi* before (zero-day) and after 0.15 and 0.30 mg/L malathion treatment (24hrs, 48hrs, 3 days, 4 days and 5 days). *Statistical difference at $P < 0.05$.

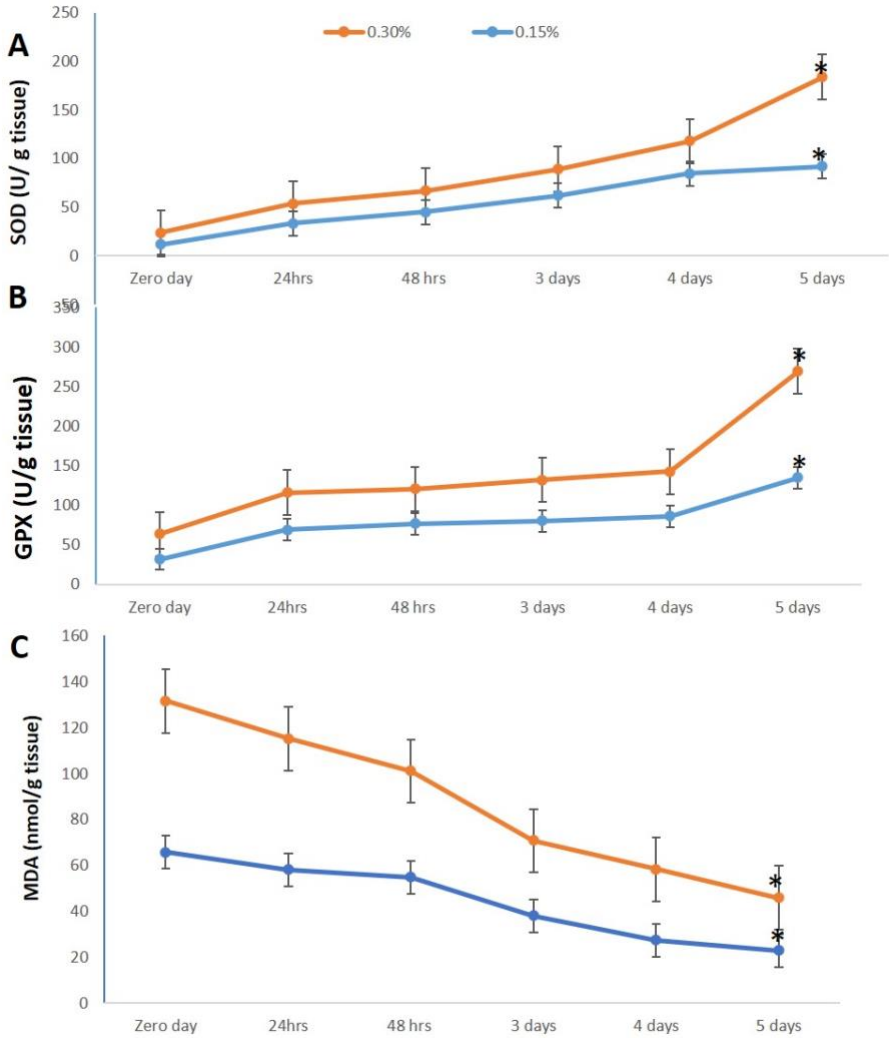

Fig.5. Antioxidants SOD (A), GPXAS (B) and MDA (C) of *D. labrax* infested with *N. orbignyi* before (zero-day) and after 0.15 and 0.30 mg/L malathion treatment (24hrs, 48hrs, 3 days, 4 days and 5 days). *Statistical difference at $P < 0.05$.

Table 4 Glucose, cortisol, AST and ALT of infested Sea bass (*Dicentrarchus labrax*) with *Nerocila orbignyi* before (zero-day) and after 0.15 and 0.30 mg/L malathion treatment (24hrs, 48hrs, 3 days, 4 days and 5 days).

Parameters	**zero day**	**24 hrs**	**48hrs**	**3rdday**	**4th day**	**5th day**
0.15 mg/L malathion						
Glucose (mg/dl)	45.48±0.05^{e}	66.03±0.05^{e}	112.29±0.00^{d}	166.63±0.05^{c}	178.17±0.02^{b}	180.35±0.05^{a}
Cortisol (mg/dl)	62.44±0.05^{a}	40.98±0.00^{b}	26.32±0.02^{c}	24.32±0.12^{c}	20.2±0.02^{d}	18.8±0.02^{d}
AST (U/g tissue)	38.2±0.00^{a}	31.67±0.00^{b}	28.75±0.05^{c}	23.58±0.05^{c}	20.76±0.02^{d}	20.22±0.03^{d}
ALT (U/g tissue)	74.63±0.05^{c}	88.36±0.05^{a}	81.1±0.00^{b}	72.77±0.03^{d}	75.36±0.05^{c}	67.17±0.10^{d}
0.30 mg/L malathion						
Glucose (mg/dl)	45.48±0.05^{e}	92.02±0.05^{e}	104.36±0.00^{d}	140.26±0.05^{c}	144.33±0.02^{b}	180.35±0.05^{a}
Cortisol (mg/dl)	62.44±0.05^{c}	102.15±0.00^{a}	85.76±0.00^{b}	69.22±0.00^{d}	65.80±0.00^{d}	46.18±0.05^{e}
AST (U/g tissue)	38.27±0.00^{cd}	63.45±0.00^{a}	48.43±0.05^{c}	56.11±0.00^{b}	51.28±0.00^{b}	20.65±0.05^{d}
ALT (U/g tissue)	74.63±0.00^{b}	96.89±0.05^{ab}	98.43±0.00^{a}	62.61±0.05^{c}	57.47±0.02^{d}	51.48±0.00^{d}

Values with different letters superscripts are significantly different ($P < 0.05$).

Malathion treatment was effective in treating *N. orbignyi* infestation with the best result for 0.30 mg/L malathion that showed decrease in its prevalence from 100 to 0% at the 5th day sampling point after malathion treatment. The prevalence of parasites decreased gradually in both concentrations of malathion and reached its half percent at 48_{hrs} sampling point. The better result was recorded for 0.30 mg/L malathion that showed a better decrease in *N. orbignyi* prevalence than 0.15 mg/L concentration. Similarly, 0.15 mg/liter malathion treatment for 20 minutes of Caligus infested Cultured sea bass and Mullet **(El-Deen *et al.,* 2013)**. This effect can be caused by the ability of malathion to inhibit several enzymes, mainly acetylcholinesterase that results in spastic paralysis of the parasite through blockage of cholinergic nerve transmission.

Parasites' infestations are usually accompanied with changes in hematological picture of host fish **(El-Deen *et al.,* 2013).** In our study, hematological analysis revealed that malathion 0.15 and 0.30 mg/L treated groups had increased RBCs count, hemoglobin (Hb), hematocrit (Ht) and platelets count, while there was a decrease in mean corpuscular hemoglobin concentration mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). This increase may be because of the effectiveness of malathion to treat the isopod infestation and thus enhancing the hematological picture. WBCs also decreased after malathion treatment, which may be because the ability of isopods to decrease the blood cell count **(Witeska *et al.,* 2016)**; accompanied with lymphopenia and improved monocytes and neutrophils' percentage, this may be attributed to fish's defense mechanism against the isopod **(Lockhart *et al.,* 1984).** Similarly, **Elgendy *et al.* (2018)** reported decreased WBCs with lymphopenia, enhanced monocytes, and neutrophils levels in infested *Tilapia zillii* with *N. bivittate*.

Immunological parameters (IgM, lysozyme and NO activities) showed nearly no significant decrease than the zero-day sampling point for the 0.15 mg/L malathion treated group. While 0.30 mg/L malathion treated group revealed a decrease in both IgM and lysozyme activities with increased NO levels over the time. This non-significant decrease may be because of the malathion treatment that kept the fish's immune system from breakdown in response to the isopod infestation and then the decrease for 0.30 mg/L malathion may be related to the exhaustion of the system and ability of isopods to decrease the blood cell count and thus negatively affecting the immune response of fish. Similarly, **Yingdong *et al.*, (2020)** reported stable lysozyme activity in *Macrobrachium nipponense* infested

with isopod *Tachaea chinensis*, which may be attributed to that the immune system stimulated in response to the infestation. Also, **Yin *et al.*, (2015)** recorded that ectoparasite *Cryptocaryon irritans* promoted lysozyme activity in large yellow croaker *Pseudosciaena crocea*.

Antioxidants could be affected with parasitic infestations **(Li *et al.*, 2019)**. Studies reporting changes in oxidative response and lipid peroxidation accompanying parasitic infections in aquaculture are extremely scarce **(Marcogliese *et al.*, 2005 and Stumbo *et al.*, 2012)** and in current study SOD, GPXAS and MDA showed significant increased levels in malathion treatment with the highest level at the 5th day after treatment and there was a decrease in MDA level for 0.30 mg/L malathion group. Similary, SOD and GPx activities were increased, followed by subsequent suppression in oriental river prawn *Macrobrachium nipponense* infested with isopod *T. chinensis* **(Yingdong *et al.*, 2020).** This indicates that malathion treatment was able to avoid this decrease caused by the oxidative activity of the isopod **(Li *et al.*, 2019)** and effectively kept the antioxidants in elevated levels.

Cortisol level is indicative of stress and disease conditions **(Triki *et al.*, 2016).** Glucose level in the current study showed marked increase in malathion groups, while cortisol level revealed significant decrease over the time compared to the zero-day. The increase in glucose level is mostly cause by the presence of the isopod which may activate the release of glucose**,** while the decrease in cortisol level may be due the ability of malathion treatment to overcome that effect of *N. orbignyi* that can increase cortisol level through activation of the hypothalamus pituitary inernal axis leading to release of the cortisol hormone **(Galhardo and Oliveira, 2009)**. Other studies confirm our studies as that of **Triki *et al.*, (2016)**, who reported higher cortisol levels in *Scolopsis bilineatu*'s parasite (ectoparasite *Gnathia aureamaculosa*) group compared with the control treatment.

AST and ALT showed marked decrease over time compared to the zero-day sampling point for both 0.15 and 0.30 mg/L malathion groups, which indicates the effectiveness of malathion treatment to improve the elevated levels of AST and ALT and lower them. On this regard, infection with external parasites (*Dactylogyrus* spp. and *Gyrodactylus* spp.) caused liver dysfunctions and elevations in AST and ALT levels in Common carp, *Cyprinus carpio* **(Rastiannasab *et al.*, 2016).** Also, **Younis (1999)** reported same elevated levels of both AST and ALT in Nile tilapia, *Oreochromis niloticus*. This elevation could be because of hepatic cells

injury or increased hepatic synthesis of AST and ALT **(Yang and Chen, 2003).**

Conclusively, to the best of our knowledge the current work presents the first record to evaluate hematological, immune and anti-oxidative alterations produced by *N. orbignyi* in response to malathion treatment in cultured *D. labrax* in Egypt. Our results indicated that malathion was effective in eradicating *N. orbignyi* and preventing its negative effects on immune and anti-oxidative responses of *D. labrax.* However, for better immune response results we recommend using an immunostimulant supplement and this will be the core of further investigations.

Conflict of interest

The authors declare no conflict of interests.

Ethical approval

The authors followed all institutional guidelines for the care and use of animals.

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إصابه الإيزوبود السيموثويد *Nerocila Orbigny* لاسماك قاروص البحر الأوروبي ، *Dicentrarchus labrax*. تجربه للعلاج مع تقييم الاستجابه المناعية ومضادات الأكسدة

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الملخص العربى

تتسبب الايزوبودا السيموثويدية في حدوث إصابات خطيرة في الأسماك و التى قد تؤثر سلبًا على تربية الأحياء المائية. هدفت الدراسة الحالية إلى بحث الإصابة فى قاروص البحر الأوروبي *Dicentrarchus labrax* بأنواع الإيزوبود السيموثويد وأنواع النيروسيلا *Nerocila Orbigny* في مزرعة أسماك بحرية بمحافظة كفر الشيخ بمصر. مصحوبة بتجارب للعلاج بالملاثيون 57٪ بتركيزات 0.15 و 0.30 مجم / لتر وتقييم الاستجابة المناعية ومضادات الأكسدة. لهذا الغرض ، تم جمع 450 *D. labrax* بشكل عشوائي على قيد الحياة خلال أبريل 2020 و تم فحصها بدقة للكشف عن الطفيليات الخارجية. تم إجراء تجربة علاج الملاثيون بنسبة 57٪. تم جمع عينات الدم والأنسجة من الأسماك المصابة في يوم الصفر (قبل العلاج بالملاثيون) وبعد 24 ساعة ، 48 ساعة ، اليوم الثالث ، اليوم الرابع والخامس (بعد علاج الملاثيون). تم التعرف على الايزوبودا التي تم جمعها من الأسماك المصابة على أنها أوربيجني من سطح الجسم الخارجي للأسماك و الخياشيم. كلا التركيزين للملاثيون كانا فعالين في القضاء على النيروسيلا أوربيني وتقليل انتشار الأيزوبود من 100 إلى 0٪ عند اليوم الخامس. كشفت الأسماك المصابة عن تحسن في قراءات صورة الدم (تعداد كريات الدم الحمراء ، الهيموغلوبين ، وقراءات الهيماتوكريت) ، لكن تعداد الكريات البيضاء أظهر انخفاضًا كبيرًا في الأسماك المصابة. انخفضت المعاملات المناعية (أنشطة الليزوزيم و IgM) ، ولكن زاد أكسيد النيتريك بمرور الوقت بعد العلاج بالملاثيون. تعتبر النيروسيلا طفيلا خطيرًا في تربية الأحياء المائية يؤثر سلبًا على استجابات الأسماك المناعية والمضادة للأكسدة ؛ والملاثيون يعد كعلاج واعد للسيطرة على لنيروسيلا أوربيني فى اسماك قاروص البحر الأوروبي المصابه.