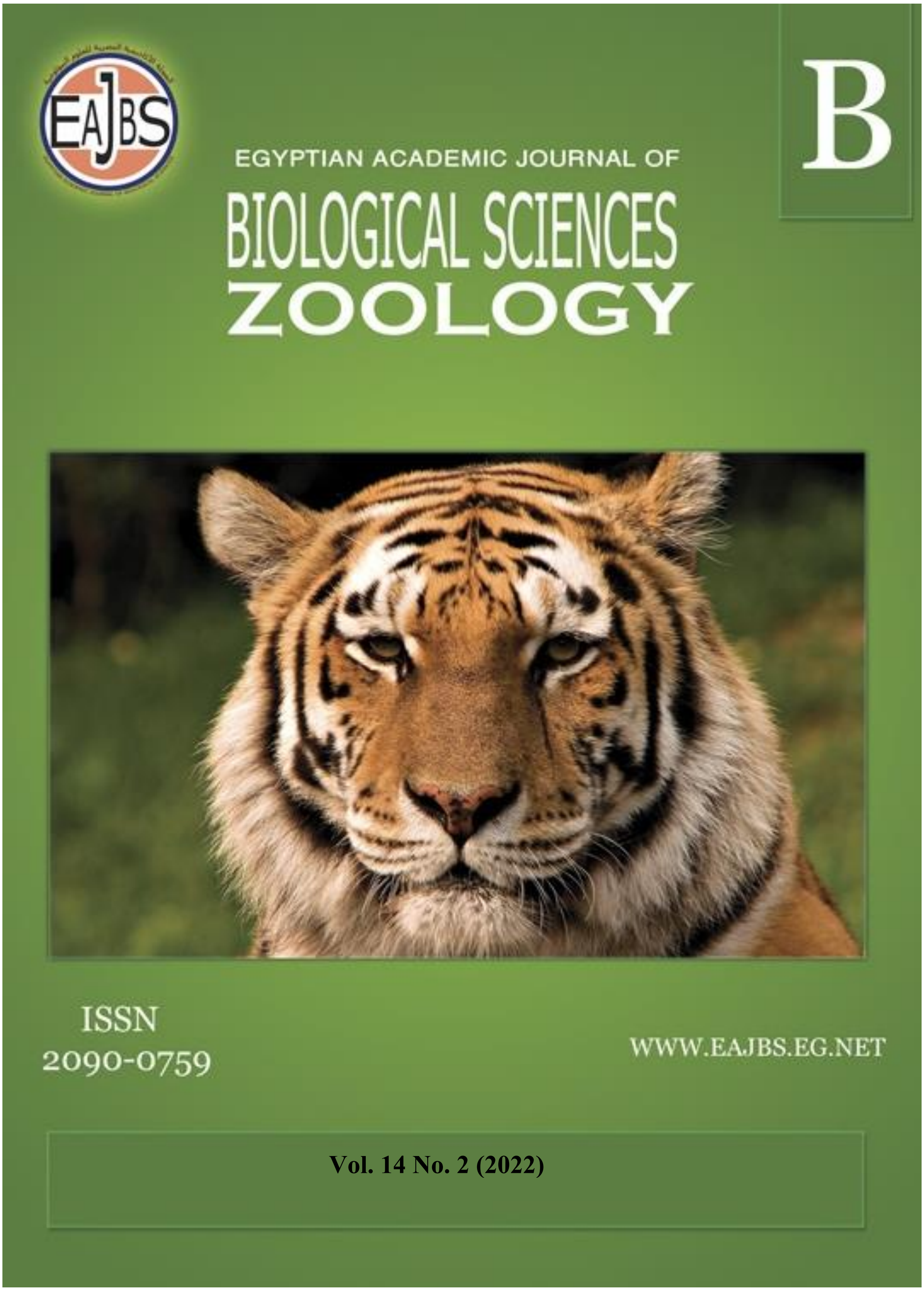

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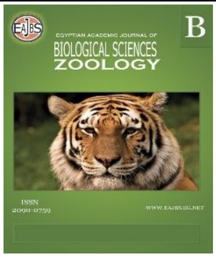

Fish Host-Intestinal Parasite Pyrethroid Accumulation, Microbial Colonization and Oxidative Stress Biomarker Response from Epe Axis of the Lekki Lagoon, Lagos, Nigeria

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ABSTRACT

This study was designed to determine the concentration of pyret the environmental media, fish and intestinal parasites with corresponding stress biomarker response in Epe axis of the Lekki Lagoon, Lagos. Surfa sediment, fish intestinal samples and the helminth parasite were colle analyzed for pyrethroids. A total of 125 fish samples were procured. Lengt from 15cm to 41cm and weight ranged from 36g to 883g. Intestinal samp infected and non-infected individuals were analyzed for microbial coloniza anti-oxidants; PRO, SOD, CAT, GSH, MDA and GPx. The three cong pyrethroids; Cyfluthrin, β-Cypermethrin and α-Cypermethrin were four surface water, sediment and fish but were not detected in the gut para congener with the highest concentration in surface water was β-Cypermet concentrations of 14.896±6.17ppm but lower than α-Cypermethrin in the s α-Cypermethrin was found in the sediment at the concentration 28.129±5.69 ppm, compared to Cyfluthrin and β-Cypermethrin with conce of 12.377±4.25 ppm and 14.714±6.38 ppm respectively. The fish intestine pyrethroid concentration as compared with the environmental concer Cyfluthrin (1.721±2.61 ppm), β-Cypermethrin (1.411±2.18 ppm) Cypermethrin (0.280±1.32 ppm) respectively. There were low parasitic i in the fish host with eighteen specimens recorded to be infected with gastro helminth; *Aspidogastrea africanus* (trematode) with a prevalence of 34 was however higher prevalence of gut microflora (between 30.0% to 40.0% individuals infected with the gut trematode. The infected individuals ha protein, superoxide dismutase (SOD), malondialdehyde (MDA), glutathior and glutathione peroxidase (GPx) than the non-infected individuals. Su dismutase (SOD) was the highly most induced; 394.21±48.63 min/mg while catalase was the least induced. Combined effects of parasites and p could induce stress in fish. The histopathological analysis of the infected in revealed.

INTRODUCTION

Ecosystem health is defined by Boulton, (1999) as the condition of an ecosystem void of human interference. Man over the years has impacted through his activities on the ecosystem; this is seen as rooted in societal needs and values (Steedman, 1994; Meyer, 1997; Boulton, 1999). Modification of the natural ecosystem by man as a means of satisfying his needs and meeting ever-increasing food demand, disrupt ecological balance and create new problems One of these problems is the issue of pest. Chandola et al. (2011) defined pest as an unfriendly competitor, that needs to be eradicated for maximum crop productivity. Pesticides are a chemical used in the eradication of pests, seen by men as an unwanted guest.

There are several reports on the rapid use of pesticides in agriculture (Aktar *et al.,* 2009; Chandola *et al.,* 2011; Gupta *et al.,* 2012). Due to harm caused by the indiscriminate use of pesticides, the natural water resources such as lakes, reservoirs and groundwater (Sa´nchez-Bayo 2006), wetlands and rivers (Gilliom *et al.,* 2007), and lagoons (Akinsanya *et al.,* 2015). Due to the unique physiochemical characteristics of some of these pesticides; non-biodegradability and lipophilicity, they can be persistent in the environment for a long time and get easily absorbed by the biota. Bioaccumulation and distribution of pesticides along the food chain have been reported in the Lagos lagoon (Akinsanya *et al.*, 2015a). Sa´nchez-Bayo (2006) has reported pesticide residues in surface water and groundwater due to agricultural activities.

Pyrethroids are synthetic pesticides modeled after the pyrethrin components of pyrethrum, a naturally occurring chemical found in certain chrysanthemum flowers (National Pesticide Information Center 2010). Pyrenoids have been reported to induce oxidative stress in freshwater fishes. This study used exposed *O. niloticus* and *C. carpio* 3 $\mu g\ L^{-1}$ concentration of cypermethrin for 10 days, an increase in superoxide dismutase and catalase activities and malondialdehyde levels were reported in the liver. Glutathione peroxidase activity was reported to increase in the liver of *O. niloticus* and decreased in *C. carpio*. Uner *et al.* (2001) observed changes in glutathione peroxidase, Catalase and superoxide dismutase activities in the kidney of *O. niloticus* when exposed to pyrenoids.
Parasitism in fish is noted to be responsible for a great reduction in fish production and poses health hazards to the population who are dependent on the aquatic habitat for food and livelihood. Sures (2003) reported in his study the relationship between environmental pollution and parasitism in aquatic organisms and the potential role of endoparasites, which received increasing attention in the past two decades. Oxidative stress in fish as a result of both chemical stressors and biological stressors has been reported. Pesticide contamination and parasites in freshwater are considered chemical and biological stressors respectively on the inhabiting biota.

There has been no report on the bioaccumulation of pyrenoids in parasites and oxidative stress on the fish. This study aims to determine the oxidative stress response and histopathological alterations in *Chrysichthys nigrodigitatus* and its intestinal helminth parasite to pyrenoids in Epe axis of the Lekki Lagoon, Lagos.

MATERIALS AND METHODS

Study Area:

The study site is Lekki lagoon located in Lagos state, Nigeria. The lagoon is between longitudes $4^0$0’00’’ -$4^0$15’0’’E and latitudes $6^0$25’0’’N and $6^0$37’0’’ N. The lagoon supports a major fishery in Nigeria. (Fig 1).

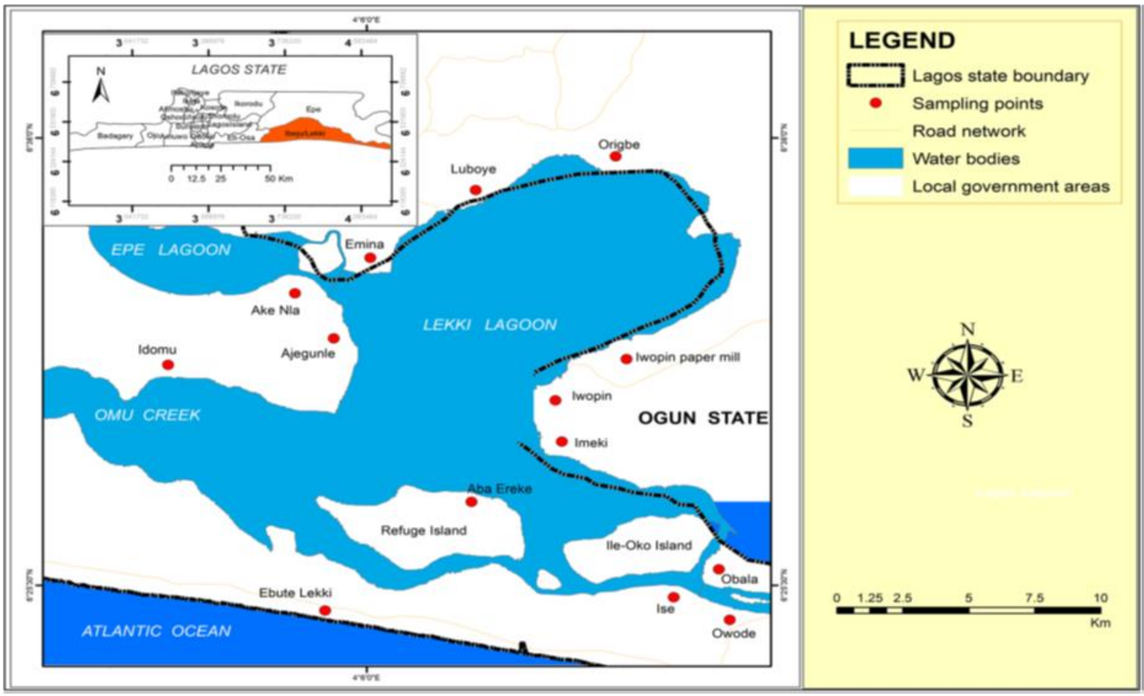

Fig. 1: Map of Epe-Lekki Lagoon**.**

Collection of Surface water and Sediment Samples:

From the sampling stations, water and sediment samples were collected monthly during the field trips. Water samples were collected using a five litres container which was firstly rinsed with the surface water at the site. The collected samples were sent for analysis of pyrenoids.

Collection of Fish Samples and Parasite:

Fishes from the wild were randomly caught by fishermen at the sample location. A total of ninety-five (95) fish were collected. The length ranged from 15cm to 41cm while the weight ranged from 36g to 883g. The fishes were procured between June and November 2019 and were dissected for intestinal helminth parasites while the recovered parasites and the infected intestines were preserved at 4ºC prior to analysis.

Determination of Pyrethroid Concentration:

The fish samples treatment was adapted from Feo *et al.* (2012). The extraction procedure was carried out with 20mL of hexane: dichloromethane 2:1 and assisted by ultrasound for 15min. This extraction was repeated twice and all solvents were dried by an N_2 stream. The following tandem SPE (basic alumina and C18 cartridges, 30 mL acetonitrile as eluent) was cleaned up. The eluent was evaporated under N_2 and the sample reconstituted 100µL of ethyl acetate.

Analyses were performed on an Agilent Technologies 7890A coupled to a 7000A GC–MS Triple Quad. The columns chosen were a DB5-ms (15 m× 0.25 mm × 0.1 µm) for the quantitative analysis and a BGB-172 (BGB Analytik, Switzerland) (30m× 0.25mm× 0.25 µm) for the enantiomeric determination. Details of chromatographic conditions to both achiral and chiral analyses are found in Corcellas et al. (in press). The selected mass spectrometry (MS) mode was negative chemical ionization with ammonium as a reagent gas. All MS parameters are found in Feo *et al.* (2011).

In parallel, 1 g of sample was extracted with an equivalent extraction in order to determine the lipid content gravimetrically. After quantitative analysis, representative samples of each river and species were selected in order to be analyzed with the chiral column. This method allowed discerning the isomeric proportion of Cyfluthrin, Baythroid (beta-Cypermethrin) and alpha-Cypermethrin.

Antioxidant Enzyme Assessment:

Oxidative enzymes were assayed in the gastrointestinal tract of the fish samples. The fish caught were immediately dissected, and the intestines were collected into labelled sampling bottles and preserved at 4 °C prior to analysis. Superoxide dismutase (SOD) activity was determined as described by Sun and Zigma (1978). Catalase was determined as described by Aebi (1974). The reduced glutathione content was determined as described by Sedlak and Lindsay (1969). Malondialdehyde (MDA), an index of lipid peroxidation, was determined using the method of Buege and Aust (1978).

Histopathology:

The histology of the intestine was studied using the method by Akinsanyar *et al.*, (2015b). Cut tissues were fixed in 10% formalin, dehydrated in graded ethanol (Akinsanya et al, 2015b), cleared in xylene, embedded in paraffin wax and sectioned at 5 µm on a rotary microtome. Slides were stained using the haematoxylin and eosin technique for light microscopy.

RESULTS

Table 1 shows the concentrations of the congeners of pyrethroids in the surface water, sediment, fish and the gut parasite. The three congeners of pyrethroids were found in the surface water, sediment and in fish but were not detected in the gut parasite. The congener with the highest concentration in surface water was β-Cypermethrin with concentrations of 14.896±6.17ppm (Mean±Standard Deviation) but lower than α-Cypermethrin in the sediment. α-Cypermethrin was found in the sediment at the concentration level of 28.129±5.69 ppm, compared to Cyfluthrin and β-Cypermethrin with concentrations of 12.377±4.25 ppm and 14.714±6.38 ppm respectively. The fish intestine had low pyrenoid concentration as compared with the environmental concentrations; Cyfluthrin (1.721±2.61 ppm), β-Cypermethrin (1.411±2.18 ppm) and α-Cypermethrin (0.280±1.32 ppm) respectively. These congeners were not detected in the gut parasite, *Aspidogastrea africanus.*

Table 1: Concentrations of congeners of pyrethroids in fish intestine and parasites.

Pyrethroids	Surface water Mean±SD (ppm)	Sediment Mean±SD (ppm)	Fish Intestine Mean±SD (ppm)	Gut Parasite ppm
Cyfluthrin	7.623±3.61	12.377±4.25	1.721±2.61	BDL
β-Cypermethrin	14.896±6.17	14.714±6.38	1.411±2.18	BDL
α- Cypermethrin	8.603±2.61	28.129±5.69	0.280±1.32	BDL
Total Pyrethroid	31.127	55.220	3.95	BDL

Ninety-five (95) fishes were caught, weighing 34.00g to 159g with a mean value of 69.06g and the standard length ranging, from 11.00cm to 24.00cm with an average length of 17.70cm (Mean ± SD) significant at 0.01 level. Table 2 shows the prevalence of intestinal parasites of *C. nigrodigitatus* in the Lagoon. The prevalence of *Aspidogastrea africanus* (trematode) of *C. nigrodigitatus* in the Lagoon is 37.89% (combined sex), meaning 36 fishes were infected out of 95 fishes. Among the infected fishes, 15 (15.79%) were females while 21 (22.11%) were males. There were more males infected with the parasite. The distribution of parasitic infection among the fish population was significant at 0.05 level, Chi-Square $\chi^2 = 17.72$.

Table 2: Prevalence of Intestinal Parasites of *Malapterurus electricus* in Epe axis, lekki lagoon, lagos

Sex	Infected Individuals	Non-infected Individuals	Total Population
Female	15 (15.79%)	21 (22.11%)	36 (37.89%)
Male	21(22.11%)	38 (40.00%)	59 (62.11%)
Combined Sex	36 (37.89%)	59 (62.11%)	95(100.00%)
Chi-Square $\chi^2 = 17.70$, df = 1, $p<0.05$			

Table 3 shows the prevalence of microbes in the gut of the infected and non-infected individuals of *Chrysichthys nigrodigitatus* in the Epe axis of the Lekki Lagoon. There was a higher prevalence of gut microbes (between 30.0% to 40.0%); *Salmonella sp, Escherichia coli*, *Pseudomonas sp* and *Bacillus sp* among individuals infected with gut trematode, *Aspidogastrea africanus* compared with non-infected individuals. The non-infected individuals had higher gut *Staphylococcus sp, Klebsiella sp*, and *Proteus sp* (between 10.0% to 40.0%).

Table 3: Prevalence of microflora in the infected and non-infected intestines of *Chrysichthys nigrodigitatus* in Epe Axis Of The Lekki Lagoon.

Identified Microbial Cultures	Infected fish (Intestine) (%)	Non-Infected fish (Intestine) (%)
Salmonella	40.0	30.0
Staphylococcus sp	30.0	40.0
Klebsiella sp	20.0	30.0
Proteus sp	0.0	10.0
Escherichia coli	40.0	10.0
Pseudomonas sp	40.0	30.0
Bacillus sp	30.0	20.0

Table 4 shows the antioxidative responses in the intestines of infected and non-infected individuals of *Chrysichthys nigrodigitatus* in Epe Axis of the Lekki Lagoon. The infected individuals had higher protein, superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (GPx) than the non-infected individuals. Superoxide dismutase (SOD) was the highly most induced; 394.21±48.63 min/mg protein, while catalase was the least induced.

Table 4: Antioxidative enzyme responses in the intestines infected and non-infected individuals of *Chrysichthys nigrodigitatus* in epe axis of the lekki lagoon.

Parameter	Infected fish (Intestine) Mean±SD	Non-Infected fish (Intestine) Mean±SD
Protein (PRO)(g/l)	29.70±2.26	32.12±1.45
Superoxide dismutase (SOD) (min/mg protein)	394.21±48.63	145.89±12.78
Catalase (CAT) (min/mg protein)	1.58±0.17	2.70±8.70
Malondialdehyde (MDA) (nmol/ml)	24.84±0.58	21.40±5.06
Glutathione (GSH) (µmol/ml)	7.44±0.30	5.65±0.45
Glutathione peroxidase (GPX) (µmol/ml)	5.15±0.87	3.60±2.89

Histopathological Alterations Of Infected And Non- Infected Tissues:

Plates 1-2 show the histopathological alterations in the intestines of infected and non-infected *Chrysichthys nigrodigitatus* in Epe Axis of the Lekki Lagoon. The intestines of the infected individuals revealed moderate to severe degeneration of the epithelial layer and congestion of the mucosa and submucosa compared to the non-infected individuals with no significant lesion observed.

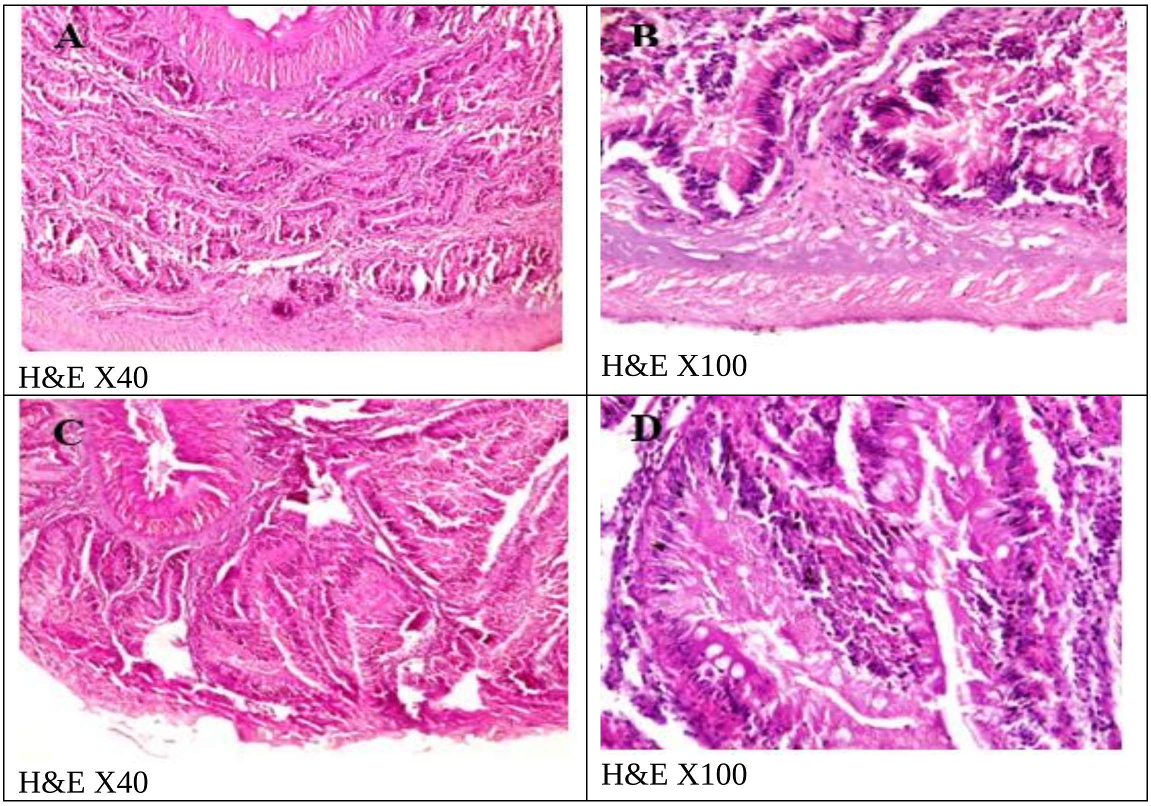

Plate 1: Section through the intestine of non-infected individuals of *Chrysichthys nigrodigitatus*

Photomicrographs of intestinal tissue show normal epithelial mucosa, submucosa, muscularis and serosa. No significant lesion was seen.

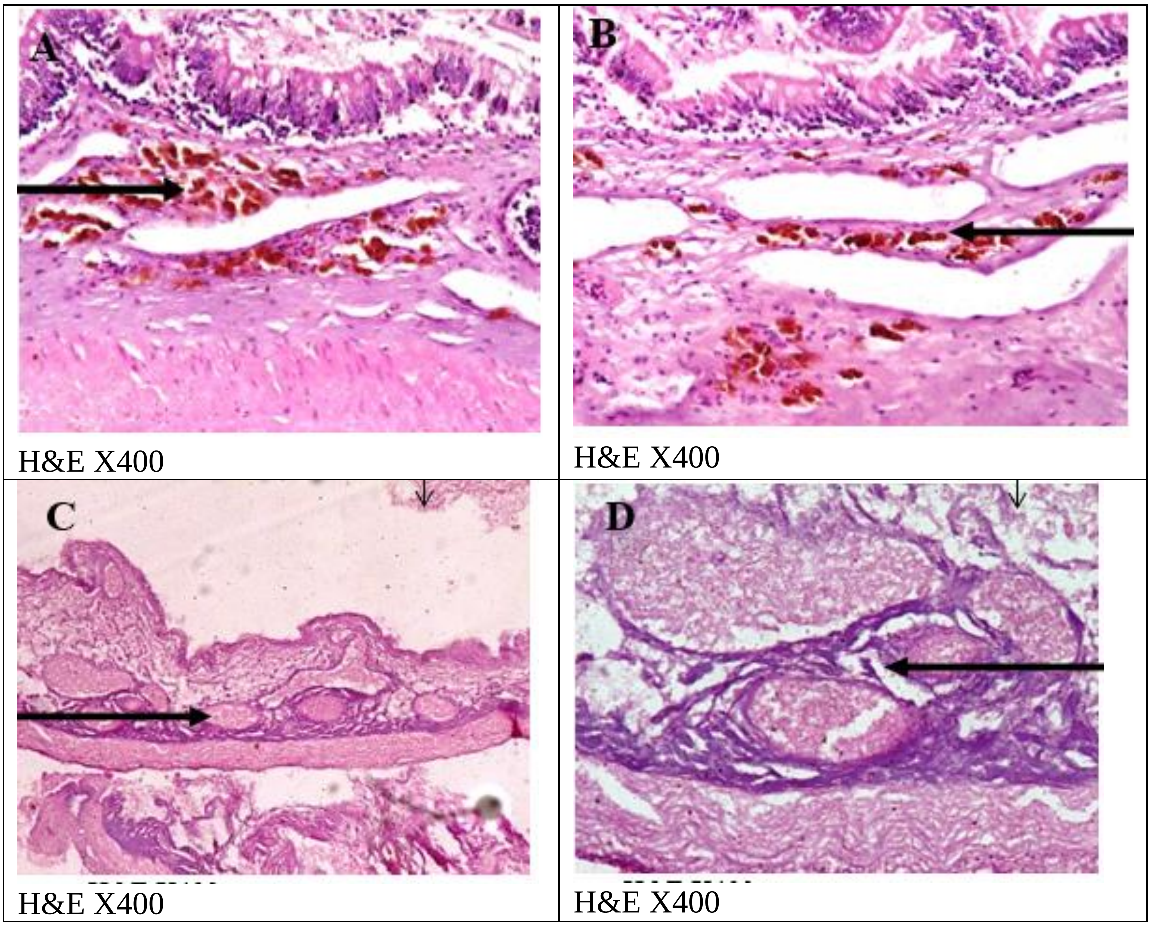

Plate 2: Section through the intestine of infected individual of *Chrysichthys nigrodigitatus* showing different pathological conditions

A & B: Photomicrographs of intestinal tissue show moderate congestion of the mucosa (Thick arrow) and submucosa (thin arrow) **C & D:** Photomicrographs of intestinal tissue show severe degeneration of the epithelial layer (thin arrow) of the mucosa and moderate congestion of the mucosa (thick arrow).

DISCUSSION

The prevalence of intestinal helminth parasites in fish varies between sex and is independent of pesticide contamination. In this study, the prevalence of *Aspidogastrea africanus* (trematode) of *C. nigrodigitatus* in the Lagoon is 37.89% (combined sex). The parasite infected more of the male fish than females, in spite of the pesticide contamination of the Lagoon. The three congeners of pyrethroids were found in the surface water, sediment and in fish in the Epe axis of the Lekki Lagoon. Surface water and sediment concentrations of pyrethroids were 31.127ppm and 55.22ppm respectively. β-Cypermethrin, Cyfluthrin and α-Cypermethrin sediment concentrations were 14.896±6.17ppm, 12.377±4.25 ppm and 28.129±5.69 ppm respectively.

Microbes play several key roles in pesticide biotransformation and metabolism; they could also be linked to pathological tendencies in the host fish. In this study, there was a higher prevalence of gut microbes (between 30.0% to 40.0%); *Salmonella sp, Escherichia coli, Pseudomonas sp* and *Bacillus sp* among individuals infected with gut trematode, *Aspidogastrea africanus* compared with non-infected individuals. The non-infected individuals had higher gut *Staphylococcus sp, Klebsiella sp,* and *Proteus sp* (between 10.0% to 40.0%).

The fish intestine had low pyrenoid concentration as compared with these environmental concentrations. These concentrations are quite high in reference to Bradbury and Coats (1989) review on the toxicology of pyrethroids in freshwater fish and aquatic invertebrates.

The author gave acute exposure concentrations for cypermethrin for *Tilapia nilotica*, *Cyprinus carpio, Salmo trutta*, *Salmo gairdneri,* Scardinius *erythropthalmus*. One of the toxic effects of pyrethroids is oxidative stress, which could lead to the production of antioxidants (Uner *et al.*, 2001). These antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) protect the cells and tissues against reactive oxygen species (Uner et al, 2001). In this study, the infected individuals had higher protein, superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (GPx) than the non-infected individuals.

Parasites could also induce pathological alteration in the host fish as shown in this study, the intestines of the infected individuals had moderate to severe degeneration of the epithelial layer and congestion of the mucosa and submucosa compared to the non-infected individuals. This is in agreement with that reported by Akinsanya *et al.*, (2015b), Saliu *et al.*, (2015) and Ukwa *et al.*, (2018). This study shows that the combined effects of parasites and pesticides could induce stress in fish.

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