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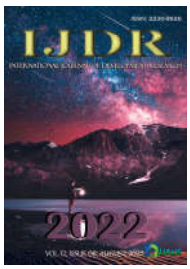

RESEARCH ARTICLE OPEN ACCESS

IMPACT OF DELTAMETHRIN (2.8% EC) ON SERUM BIOCHEMISTRY AND HISTOPATHOLOGY OF FISH, CHANNA PUNCTATUS (BLOCH 1793)

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ABSTRACT

A Pedagogia da Alternância apresenta princípio pedagógico desenvolvido nas Escolas Famílias Agrícolas (EFAs). Trata-se uma metodologia de ensino que faz parte dos pilares que rege esse modelo de escola, que contribui para o desenvolvimento socioambiental das comunidades rurais onde atuam, de modo a evitar o êxodo dos jovens para a cidade, incentivando a elaboração de propostas concretas voltadas para o trabalho no campo e valorização da cultura e modo de vida inerentes a estas comunidades. Este artigo possui como objetivo geral analisar os impactos da metodologia da pedagogia da alternância na EFAC, na aprendizagem dos ex-alunos do 3º ano do ensino médio. Realizou-se uma abordagem quanti-qualitativa, por meio de estudo de caso. A maior contribuição para escolas do campo é quanto ao sentido do saber e do fazer, aproximando a escola do meio e o aluno da comunidade.

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INTRODUCTION

Pollution by agricultural run-off has negatively affects the environment (Desai and Parikh, 2014). The aquatic ecosystem is now facing a threat of biodiversity loss due to the indiscriminate use of pesticides (Haloi *et al*., 2013). Pesticides are mainly used for controlling pest, weeds and diseases in plants in various agronomic practices (Nayak and Solanki, 2021). The synthetic pyrethroid derived from pyrethrin, naturally occurring substance obtained from pyrethrum of dried *Chrysanthemum cineraiaefolium* flowers. Deltamethrin is a type II synthetic pyrethroid that was synthesized in 1974 and has been used since then to kill insects through different exposure routes. Pyrethroids have been reported to be extremely toxic to fish and some beneficial aquatic arthropods, such as lobster and shrimp (Selvi *et al*., 2009). Sub-lethal concentration of pesticides in aquatic medium is likely to cause the altered biochemical induced structural and functional damages in fish. Deltamethrin may even lead to histopathological damage in various organs. Therefore, the present study was conducted to evaluate the sub-lethal toxicological effects of deltamethrin on biochemical and histopathological parameters of commercially important fish, *Channa punctatus*.

MATERIALS AND METHODS

Acclimatization of fish model: Fish specimen, *Channa punctatus*, a fresh water teleost, collected from a local water body. The collected fish were of 23±2 g and 11±13 cm. Fish were treated with 2% $KMnO_4$ in aquarium of 80 L capacity. Fishes were acclimatized for 10 days and were fed twice daily with commercial food. The tap water in aquarium was aerated with an air pump and was changed daily.

Determination of 96 h-LC_{50} of Deltamethrin (2.8% EC): Deltamethrin (2.8% EC) was procured from the local agro-market. Its 96 h-LC_{50} was estimated by following Trimmed Spearman-Karber method (Hamilton *et al*., 1977). For this, 10 fishes were stocked in 10 separate aquaria, separately. Fishes in each aquaria was exposed with speculated definitive concentration of deltamethrin and their percentage mortality was recorded after a regular intervals of 24, 48, 72 and 96 h. The 96 h-LC_{50} was determined 0.26 ppm.

Experimental design: The acclimatized fishes in three aquaria were subjected to sub-lethal concentration of deltamethrin ($1/10^{th}$ of LC_{50}-96 hr) for the duration of 7, 14, 28 days and one aquaria was kept as control. During experimentation, the various physicochemical parameters such as dissolved oxygen, alkalinity, hardness and chloride was approximately maintained at constant level. The temperature and pH were also stabilized and all these parameters were recorded daily.

Evaluation of serum biochemical parameters: For the estimation biochemical parameters, blood sample was collected from fish in

EDTA containing tubes. Blood was collected from caudal using syringe. The total protein content of the fish blood was estimated by following the method of Lowry *et al*. (1951) and serum albumin was determined with bromcresol green (Doumas *et al*., 1971). Globulin and A: G ratio were calculated using the measured total protein and measured albumin.

Analysis of histopathological alterations of heart of fish: For the histological parameters, heart tissue samples were collected from both the control and exposed fish and kept in saline water. The tissues were thoroughly washed, cleaned and dehydrated using a series of graded alcohol solutions, cleared in xylene, embedded in paraffin wax and sectioned 3 µm thickness using a rotatory microtome. After that, the staining was done with haematoxylin and eosin. The prepared slides were examined using a light microscope and photographed.

Statistical analyses

The data was calculated as mean ± standard error mean (S.E.M.). The values were tested at the significant ($p < 0.05$) level using one-way analysis of variance (ANOVA) with Tukey's post hoc test by Statistical Package for the Social Sciences (SPSS) software (version 20.0, Chicago, IL, USA).

RESULTS

Physicochemical parameters: The parameters of test medium were expressed in Table 1, as compared to control and their values were found within the prescribed limits (APHA, 2017).

Biochemical measurements: The sub-lethal exposure of deltamethrin significantly ($p < 0.05$) decreased the total protein content by 15.87%, 34.8%, 57.5% after 7, 14, 28 days, respectively, as compared to control (Fig. 1A). The albumin level showed a gradual and significant ($p< 0.05$) decrement as 22.76%, 44.45% and 54.23% after 7, 14 and 28 days, relative to control (Fig. 1B). The globulin content in exposed fish blood revealed a gradual and significant ($p < 0.05$) decline by 5.12% after 7 day, 23.25% after 14 and 62.79% after 28 days of exposure intervals, as compared to unexposed group (Fig. 1C). Further, the A:G ratio declined by 20.67% after 7 day, followed by a significant ($p < 0.05$) reduction 26.97% after 14 days. After 28 day exposure period, its ratio significantly ($p < 0.05$) increased by 23.77%, as compared to control (Fig. 1D). These altered biochemical parameters of fish blood were displayed in Table 2.

Histopathological alterations in heart of deltamethrin exposed fish: In the control group (Fig. 2A), normal histology was observed. The wall of the heart consists of three layers innermost endocardium lined by endothelium on a basil lamina. The presence of sub endothelial layers of collagenous elastic fibres, fibroblasts, smooth muscle layers along with blood vessels and nerve vessels were also observed. Presence of myocardium and epicardium was also distinct.The exposed heart tissue samples showed vascular congestion, necrosis, vacuolisation in cells, inflammatory cell infiltration, and fragmentation of muscles as a result of sub-lethal exposure of deltamethrin. Myocardial infarction noticed in fish of exposed group after 28 days (Fig. 2D) whereas degeneration and separation of epicardium in exposed fishes after 7 days (Fig. 2B) which also increased in fishes after 14 days of exposure period (Fig. 2C).

Table 1. Physicochemical parameters of test water constantly maintained after 7, 14, 28 days, as compared to control

Parameters	Control	7 Days	14 Days	28 Days
Temperature (°C)	19	22	24	27
pH	7.1	6.8	7.2	6.9
Dissolved oxygen (mgl^{-1})	5.2 ± 0.002	5.7 ± 0.012	6.1 ± 0.023	5.7 ± 0.112
Hardness (mgl^{-1})	127.3 ± 0.002	122.6 ± 0.012	117.3 ± 0.002	124 ± 0.123
Alkalinity (mgl^{-1})	106.6 ± 0.012	105.3 ± 0.023	107.3 ± 0.032	106.5 ± 0.142
Chloride (mgl^{-1})	43 ± 0.012	40.91 ± 0.034	41.39 ± 0.192	41.24 ± 0.112

(Values were given as mean ± S.E.M.)

Table 2. Biochemical parameters altered in serum of deltamethrin exposed fish *C. punctatus* after 7, 14, 28 days, as compared to control

Parameters	Control	7 Days	14 Days	28 Days
Total Protein (g/dl)	4.91 ± 0.03	4.13 ± 0.02	3.20 ± 0.09	2.11 ± 0.01
Albumin (g/dl)	2.79 ± 0.09	2.16 ± 0.04	1.55 ± 0.04	1.28 ± 0.02
Globulin (g/dl)	2.15 ± 0.08	2.04 ± 0.05	1.65 ± 0.04	0.83 ± 0.01
A:G Ratio	1.29 ± 0.11	1.06 ± 0.04	0.94 ± 0.04	1.54 ± 0.05

(Values were given as mean ± S.E.M.)

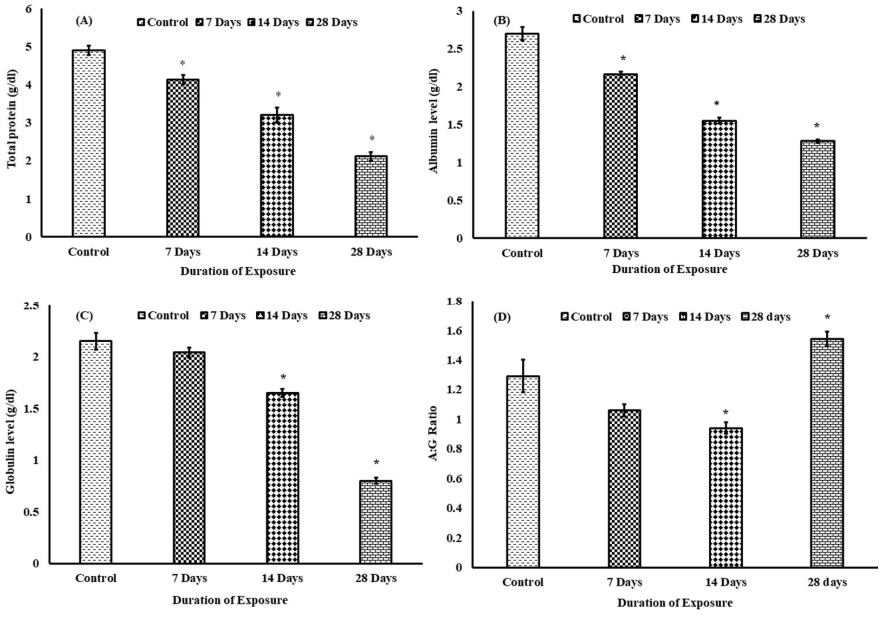

Fig. 1. Graphs showing alterations in total protein (A), albumin (B), globulin (C) and A:G ratio (D) in the blood of fish of control and exposed group after 7, 14 and 28 days of exposure periods (The values expressed as mean ± S.E.M., n = 9 fish from each group, *denotes the significant ($p < 0.05$) values in comparison to control)

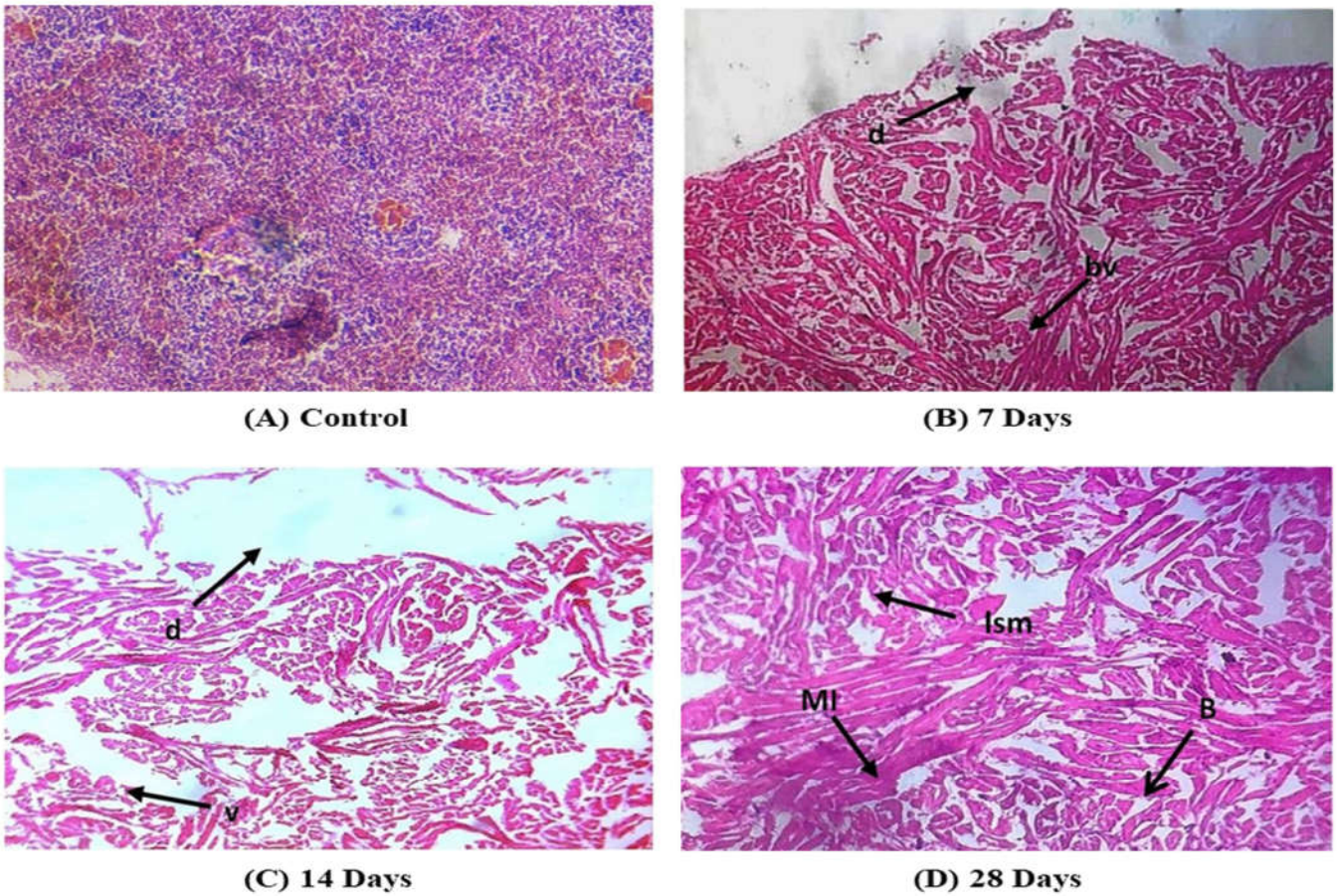

Fig. 2. Histopathological microphotographs of heart of *C. punctatus* A. Section of heart of control fish showing no observable damage B.Section of heart of fish, exposed to 10% of 96 h-$LC_{50}$of deltamethrin for 7 days, showing degenerated (d) epicardium, narrowing of blood vessels (bv), disruption of epicardium, cellular infiltration and inflammation (In) C. Section of heart of fish,exposed to 10% of 96 h-LC_{50} of deltamethrinfor 14 days,illustrating vacuolisation (v), degeneration and separation of cardiac muscles D. Section of heart of fish, exposed to 10% of 96 h-LC_{50} of deltamethrin for 28 days, displaying loss of striation of muscles (lsm), myocardial infarction (MI) with necrosis and fragmentation of muscle fibres (B) by using Haematoxylin (H) and Eosin (E) staining with 10 X magnification of objective lenses

DISCUSSION

Synthetic pyrethroid, deltamethrin is known to cause excessive toxicity in aquatic organisms including fish.Water serve as a medium and is essential for proper growth and survival of the aquatic dwellers. The quality of water approximately maintain at a constant level during the analysis of perturbations in fishes of aquatic ecosystem. The physicochemical parameters such as temperature of water and dissolved oxygen are insignificantly altered. Usually, it is stated that the decline in dissolved oxygen due to an increase in temperature of water. Growth rate and feed intake are also reported to be significantly influenced by temperature (Eriegha and Ekokotu2017; Handeland *et al*., 2008). The prolonged exposure of deltamethrin significantly ($p < 0.05$) decreased the serum biochemical parameters such as, total protein, albumin, globulin and A:G ratio in time-dependent manner. In the present study, reduction in the levels of serum total protein was observed under the sub-lethal influence of deltamethrin which may indicates hepatic insufficiency and probable malnutrition (Srivastava *et al*., 2016). Protein is in indispensable constituent required in tissue building and is important source of energy during chronic condition of stress (Remia *et al*., 2008, Shah and Parveen, 2020). Eventually, these biochemical alterations engender the histopathological alterations in fish tissues. The histopathological parameters, a useful biomarker used to determine the toxicological effect of environmental contaminants and generally helps in the estimation of organism health (Hossain, 2012). Notably, the heart of *Channa punctatus* is a vital circulatory organ. The fish heart is located ventrally in front of the pectoral fins and peritoneal cavity but behind the gill structures.

Normally, the heart is positioned within a membrane sac, the pericardial cavity. The heart of *Channa punctatus* is two chambered and venous that contains only deoxygenated blood. The differentiated chambers are auricle and ventricle. Specifically, all cardiac chambers are lined with endothelial cells, the endocardium. The outer most layer of epithelial cells and connective tissue, referred to as epicardium. Remarkably, a sub-epicardial space of loose connective tissue contains collagen and elastic fibres, and a smooth muscle cell layer with lymphatic vessels is also present. The deltamethrin exposure has been reported to be fatal to fresh water fish, *Channa punctatus*. The insecticide enters the fish body via gill and heart serves as an intermediate organ that circulates the insecticide contaminated blood throughout the body which may lead to structural defects in heart and in turn reduce the cardiac output and swimming ability of the exposed fish (Haverinen and Vornanen, 2016). In the present experiment, the histological study of the deltamethrin exposed heart tissue reported the inflammatory cells filled in pericardial spaces surrounding the heart which may indicate pericarditis. The vascular degeneration as a result of cloudy swellings of cells was also reported along with separation of epicardium, narrowing of blood vessels in the cardiac tissue and fragmented muscle fibres. The increment in the fish heart lesions were reported in a time-dependent manner. The frequency and intensity of tissue lesions of a fish depend on the concentrations of pesticides and the length of exposure period (Fanta *et al*., 2003; Qadir and Iqbal, 2016). Similarly, Islam *et al*. (2019) have also recorded the destruction of blood vessels and fragmentations of muscle fibres of the heart of *Channa punctatus* induced by Furadan. Further, the similar types of tissues abnormalities were observed in different fishes *Heteropneustes fossilis* and *Anabas testudineus* (Islam *et al*., 2019).

CONCLUSION

This study describes the impacts of deltamethrinon fishes in terms of serum biochemical changes and histopathological anomalies in their heart. The serum biochemical parameters like total protein, albumin, globulin and A: G ratio significantly ($p < 0.05$) declined and the conspicuous histopathological changes such as vacuolization, inflammation, degeneration, necrosis, fragmentation, etc., in heart of fish, *Channa punctatus* observed in time-dependent manner. These stated aforementioned perturbations effectively used as molecular indicators for biomonitoring of deltamethrin contamination in aquatic ecosystem. Therefore, this study also valuable for the conservation of biodiversity of aquatic fauna including fishes.

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