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HISTOLOGICAL STUDIES ON THE COMBINED EFFECT OF SYNTHETIC PYRETHROID LAMBDA CYHALOTHRIN AND ORGANOPHOSPHATE CHLORPYRIFOS ON REPRODUCTIVE TISSUES IN FRESHWATER CULTIVABLE FISH *CLARIAS BATRACHUS*

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ABSTRACT

In the present study freshwater commercially cultivable catfish *Clarias batrachus* was used to evaluate the combined effect of lambda cyhalothrin, a synthetic pyrethroid and chlorpyrifos, an organophosphate insecticide widely used in agricultural fields. *Clarias batrachus* uses rice paddy slurries as its breeding and nursery grounds, inhabit sediments as they are benthic and are posed to greater threat from sediment associated toxic compounds such as pyrethroids and organophosphates mixtures which are widely used in rice fields today to control rice pests. The LC_{50} of the combined mixture was found to be 0.05 mg/L. The fishes were maintained at one-fifth sublethal concentration of 0.01mg/L for a period of 30 and 45 days. Histological examination was carried out in testis and seminal vesicle at the end of exposure period to study the extent of damage at safe concentration.

INTRODUCTION

Residues of pesticides are found in areas far off from the point of application as they are transported into aquatic environment. The presence of pesticides within aquatic environments can induce physiological and behavioural changes in aquatic organisms although, sublethal can impair the survival and biochemical fitness of the organism (Werimo *et al.,* 2009). Fishes raised in commercial ponds are also exposed to various pesticide mixtures from the nearby fields by runoff or spray-drift which often pollute the ponds and affect health of fish fauna (Wang *et al.,* 2009a). Contamination of surface waters has been well documented worldwide and constitutes a major issue at local, regional, national and global levels (Glaser, 2006). The landscape in a single river catchment may have a variety of agricultural uses where a number of different crop protection programs are employed.

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Agricultural practices can include pesticides being mixed during application. Therefore aquatic non-target organisms are exposed to multiple stressors including pesticides and their degradation products both simultaneously and consecutively (Colovic *et al*., 2011). Lambda cyhalothrin is a Type II synthetic pyrethroid and is a derivative of cyano group (Liu *et al.,* .2004; Sudakin, 2006). Monitoring studies recently in agricultural areas show pyrethroid contamination of both surface water and stream bed sediments (Amweg *et al.,* 2005). Investigations are ongoing on concern over bioavailability and effect on sediment and benthic dwelling organisms (Weston *et al*., 2004). Chlorpyrifos is a broad spectrum organophosphate insecticide which is effective in controlling insect pests (RED, 2006). Chlorpyrifos does nor partition easily from soil to water, Therefore chlorpyrifos found in runoff water is likely a result of soil bound chlorpyrifos from eroding soil rather than from dissolved chlorpyrifos (Arjmandi *et al*., 2010). It is highly toxic to freshwater fishes and potential to bioaccumulate in the tissues as metabolites 3, 5, 6 trichloro- 2-pyridinol (TCP). Sediment associated pesticides and dissolved pesticide concentration can provide a direct exposure pathway for benthic dwelling organisms and can predict a source of risk

(Solomon *et al.,* 2001). In the aquatic environment these hydrophobic chemical contaminants rapidly associate with particles in water and are deposited in sediments thus consumed by benthic organisms (Roessink *et al.,* 2005). Repeated exposures and multiple stressors such as pesticides mixtures have clear relevance to real situations, it is necessary to understand their effects on non-target organisms (Ashauer *et al*., 2006; 2007c) so that appropriate risk assessment methodology can be developed. Zhang *et al.* (2010) reported effects on the 96 hr LC50 in Zebrafish (*Brachydanio rerio*) exposed to binary combinations of pyrethroid insecticides permethrin, bifenthrin, tetramethrin and etofenprox with the organophosphorus insecticides dichlorvos or phoxim. The differences in the scale of interactions between pyrethroids and organophosphorus insecticides depends on both the strength of the interaction between the organophosphate and carboxylesterases and the importance of the carboxylesterase in the metabolic degradation of the pyrethroid (Groner and Relyea, 2011). The outcome of the present study would decipher the impact of low concentrations and synergistic effect of synthetic pyrethroid lambda cyhalothrin and organophosphate chlorpyrifos on the reproductive tissues. It would also ascertain whether these pesticides when present in combined doses in ambient water would have deleterious effect on various organs. The tissues chosen for the study was testis and seminal vesicle which are involved in reproductive process and play a critical role in fish breeding and development.

MATERIALS AND METHODS

Experimental animals

Healthy adult male catfishes *Clarias batrachus* weighing 150-175 g and 30-35 cms was used as the experimental model to evaluate the combined toxicity of lambda cyhalothrin, a synthetic pyrethroid and chlorpyrifos an organophosphate widely used for agricultural applications in various agro-based sectors. The fishes were procured from Bharath fish seed farm, Budur village, Poondi, Thiruvallur district. They were bought to the laboratory and acclimatized under laboratory conditions for a period of three weeks and fed *ad libitum.* They were transported to the laboratory in oxygen filled polythene bags and acclimatized for a period of two weeks with normal photoperiod. The fishes were maintained in rectangular plastic tubs (64cm x 44cm x 29.5cm) filled with 20 litres of dechlorinated tap water. The tubs were disinfected with potassium permanganate solution and washed thoroughly prior to introduction of fishes to prevent any fungal infection. They were fed with commercially available feed one hour prior to water exchange. Water was renewed every 24 hour to maintain healthy environment of the fish and to remove the feed remains and nitrogenous waste products. Feeding was stopped 24 hours before the commencement of the toxicity test to keep the animals more or less in the same metabolic state.

Experimental Design

Formulated mixture of the two pesticide used in the study was supplied by Agriculture Division , Rallis India ,Bangalore for evaluating the toxic effect of the pesticide mixture (EC 50) synthetic pyrethriod lambda cyhalothrin (10%) and organophosphate chlorpyrifos (40%) on freshwater catfish *Clarias batrachus*. Preliminary toxicity tests were carried out to find the median lethal tolerance limit of experimental fishes. To determine the 96 hrs LC_{50} static renewable bioassay method was adopted (Sprague, 1973) and 96 hr LC_{50} was determined by probit analysis method (Finney, 1971). The LC_{50} was found to be 0.05 mg/L. The fishes were exposed for a period of 30 and 45 days at one fifth of the sublethal concentration (0.01 mg/L).The fishes were divided into 2 groups with five fishes in each group. Group I: Control fishes in toxicant-free water, Group II: Fishes maintained in 0.01 mg/L of pesticide mixture for a period of 30 days. Group III: Fishes maintained in 0.01 mg/L of pesticide mixture for a period of 45 days.

Collection of tissues for histological studies

The animals were sacrificed, testis and seminal vesicle was separated and immediately fixed in Bouin's fluid for histological studies following the method of Roberts (1978) and Humanson (1979). The sections were stained with haematoxylin and eosin.

RESULTS

Histological studies of the reproductive tissues showed marked and profound changes. The following changes were noticed in the testis and seminal vesicle of *Clarias batrachus* exposed to the pesticidal combination during 30^{th} and 45^{th} day of exposure.

Testis: Normal testis showing seminiferous tubules containing cells at different spermatogenic stages (Fig 1a). During 30 days of exposure to the pesticidal mixture extensive cytotoxic damage with large number of vacuoles were prominent. Gross condensation of spermatogenic cells. Severe degeneration and displacement of tubular elements (Fig 1b). Distortion of tubular elements increased with the number of days of exposure. Appearance of vacuoles in the tubules. Seminiferous tubules of central region show degeneration and reduction with disapperance of spermatozoa and spermatogenic cells were evident on the 45^{th} day of exposure (Fig 1c).

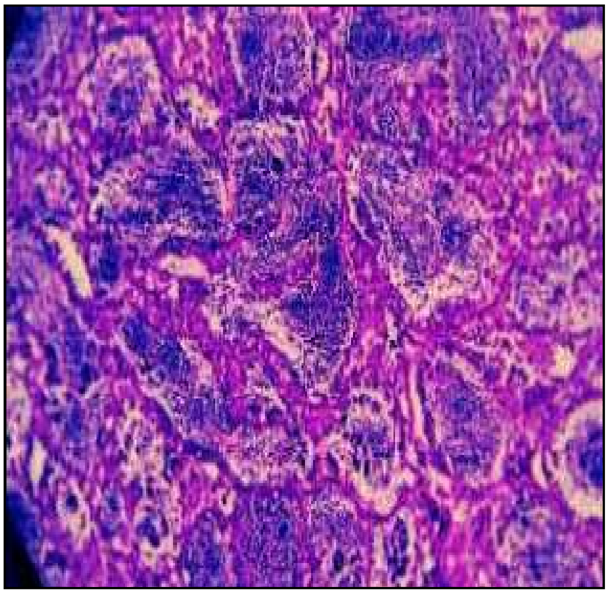

Fig. 1a. Normal Testis of *Clarias batrachus* (100x)

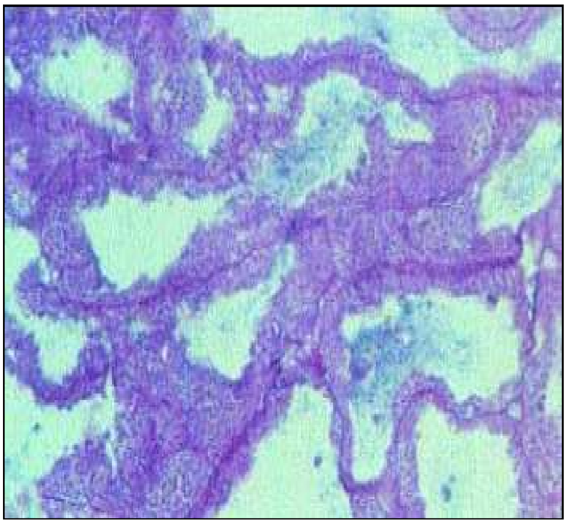

Fig. 1b. Shows vacoulization and degeneration of seminiferous tubules of testis (arrows) during 30 days of exposure (100x)

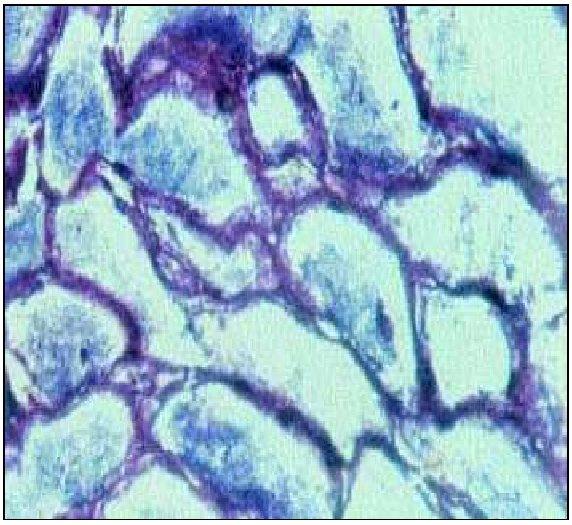

Fig. 1c. Shows vacoulization and degeneration of seminiferous tubules of testis (arrows) during 30 days of exposure (100x)

Seminal vesicle: Normal seminal vesicle showing growth buds, epithelium, new lobules and spermatozoa in the lumen (Fig 2a).

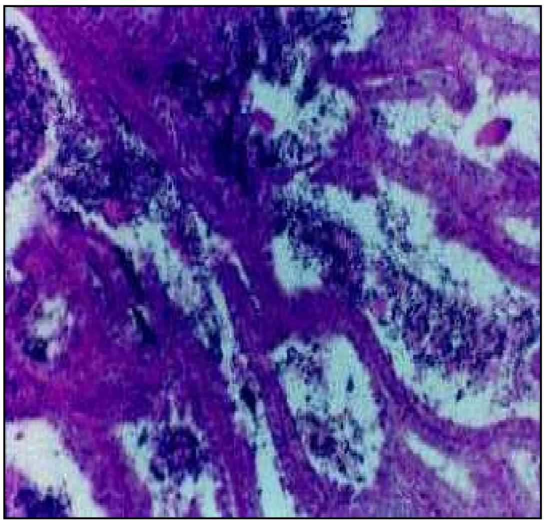

Fig. 2a. Normal Seminal vesicle of *Clarias batrachus* (100x)

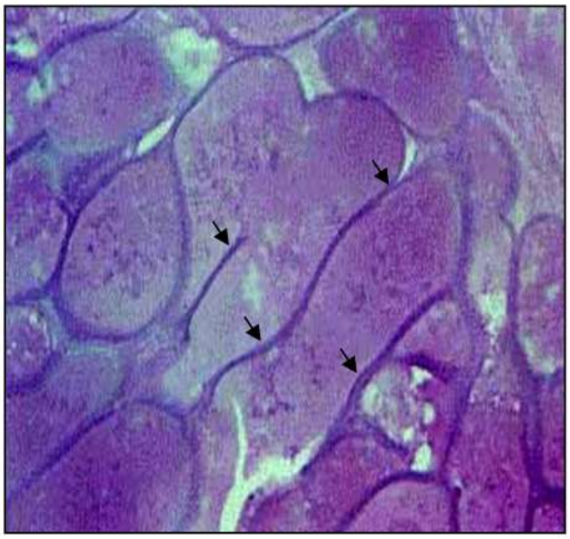

Fig. 2b. Shows epithelial cell lining damage and contents of lumen empty (arrows) during 30 days of exposure (100x)

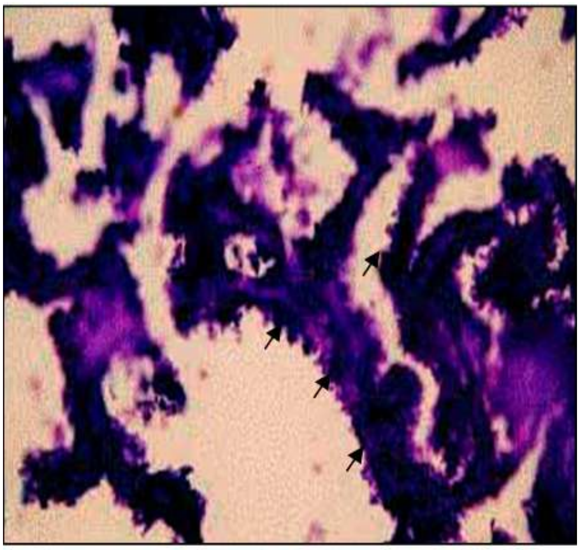

Fig. 2c. Shows epithelial cells desquamation and lumen lobules degeneration (arrows) during 45 days of exposure (100x)

Lobulated lumen empty, collapsed epithelium of lumen during 30 days of exposure (Fig 2b), epithelium lining damaged, absence of vesicular contents and lobes of seminal vesicle could be observed with disorientation during 45^{th} day of exposure (Fig 2c).

DISCUSSION

In the fishes exposed to lambda cyhalothrin and chlorpyrifos mixture seminiferous tubule vacoulization was observed with decreased number of developing spermatocyte of different stages in the testis. Colloidal mass was observed in the vacuoles. After 45 days of exposure there was central region degeneration of testis, shrinkage of interstitial cells with reduced number of developing germ cells. The effect of lambda cyhalothrin could have brought about retardation of testicular functions and degeneration of testis with seminiferous tubules losing their shape. The spermatogonia and spermatocytes lose their characteristics and are greatly reduced in number. Kinneberg *et al.* (2000) have also documented concentration dependent effects on nonylphenol on testicular structure of the fish *Xiphophorus maculatus*. *Glossogobius giuris* exposed to fenthion showed reduction in size of testis, degenerating sperms and spermatids (Zutschi, 2003). Direct toxic effects of diazinon was noticed in cyprinid fish *Rutilus kutum* with degeneration of seminiferous tubules and low quality of sperm (Fadakar Masouleh, 2011), testis of blue gill fish (Duttaa and Meijer, 2003; Maxwell and Dutta, 2005) and common carp (Banaee *et al.,* 2009). Disorganized testicular architecture and disrupted germinal epithelium was reported in air- breathing catfish *Heteropneustes fossilis* exposed to cadmium (Shivani Sharma *et al.,* 2013). Impairment in reproduction have been observed with a number of pollutants in freshwater and marine fishes (Govind and Madhuri, 2014). The seminal vesicle fluid is secreted by the epithelium lining the tubules.

The tubules in the proximal part of the lobes are predominantly lined by a simple cylindrical and those of the distal part by a simple squamous epithelium. These epithelial cells contain enzymes involved in energy-liberating processes, the enzyme activities being proportional to the height of the cells (Fishelson *et al.,* 1994). The lobulated lumen showed less luminal contents on the 30th day with epithelial lining damage. The complete epithelium was disoriented and lobule damage of the seminal vesicle was evident in the fishes exposed to the pesticidal mixture for 45 days.

The study suggest that reproductive tissues are susceptible to high degree of damage even at very low toxicant of different combinations present in ambient water. Moreover, considering the commercial importance of this freshwater fish, the present study has implication for the aquaculture industry which may lead to reduction in maturation associated growth, reproductive vigour and deterioration in flesh quality leading to heavy loss in fish production.

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