Histopathological studies of selected organs of *Oreochromis mossambicus* exposed to biopesticide achook – short term and long term toxicity

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ABSTRACT

The present study is aimed at evaluating the pathological changes in various tissues of *Oreochromis mossambicus* exposed to the neem based pesticide achook. The LC_{50} value obtained after 96 hrs was 0.105 ppm and $1/5^{th}$ of LC_{50} value i.e., 0.021 ppm was taken as sublethal concentration. The fishes were exposed to this sublethal concentration for a period of 42-days. Fishes were dissected at the end of 7^{th} day and 42^{nd} day of exposure and the gill, liver and intestine were collected from exposed fishes and control group. Tissues were processed and sectioned at 4µm and stained with Haematoxylin-Eosin. The histological changes observed were mild on 7^{th} day but became severe by 42^{nd} day. The pathological changes in gills of exposed fishes include shrunken and fused gill lamellae, inflammatory cells in the primary and secondary gill lamellae. Exposed liver showed lesions consisting of vacuolar degeneration and disruption of hepatocytes. The changes found in intestine were vacuolar degeneration and disruption of epithelial cells of villi, ruptured and shapeless villi. The histology of tissues from control fishes were normal.

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INTRODUCTION

The ecosystem is currently under threat by the indiscriminate use of synthetic pesticides by human activities causing high risk to non-target organisms (Kumar *et al.,* 2010). Pesticides are the major xenobiotic compounds used extensively for pest management in agricultural fields and vector control programmes in public health; they are absorbed by soil and migrate to natural water systems through run off causing aquatic pollution, thereby resulting in negative impact on environmental quality and toxicity risk to non-target organisms especially fishes and can enter food chain when accumulated in aquatic organisms (Pant and Singh, 1983; *Grande et al.*, 1994; Madhab Prasad *et al.,* 2002; Muthukumaravel *et al.,* 2013). Contamination of water by pesticides leads to death of fish, reduced fish productivity, accumulation of undesirable chemicals in edible fish tissues which can affect the health of humans consuming these fishes (Josef Velisek *et al.,* 2011). In view of chronic environmental hazards occurring due to the heavy use of synthetic chemicals and the need of alternative methods of pest control to minimize this damage, extensive research on pest control with plant products is being conducted to overcome these problems (Wan *et al.,* 1996; Suresh *et al.,* 2013). Plants are inexhaustible sources of structurally diverse and biologically active substances (Istvan, 2000). In recent years, the use of medicinal plants as alternative for synthetic pesticides and fertilizers has gained importance especially to combat problems in fish and aquatic environment as they are highly toxic to target pests (Saravanan *et al.,* 2010).

The neem tree *(Azadirachta indica)* of the family Meliaceae is native to India, considered highly esteemed wonder tree widely spread throughout the country and was adopted to grow in other countries a few years ago (Immich *et al.,* 2009). Different parts of neem tree, *Azadirachta indica* are reported to

contain biologically active compounds including nimbin, azadirachtin, meliantroil etc., having properties ranging from insecticidal to antiviral from ancient times (Harikrishnan *et al.*, 2003). Although neeem extract is considered of low toxicity to non-target aquatic life, water extracts of the bark of neem plant caused respiratory problems in *Tilapia zilli* (Omoregie and Okpanachi, 1997), while long exposure to low concentrations of crude extracts of *A. indica* delays the growth of the cichlid fish (Omoregie and Okpanachi, 1992). Recently it is observed that some neem based formulations are toxic to the adult, embryo, fingerlings of zebrafish and also affect its reproductive ability (Sharma and Ansari, 2010; Singh and Ansari, 2010; Ahmad and Ansari, 2011).

Tilapias are next to carps as the most widely farmed fresh water fishes in the world (FAO, 2010). *Oreochromis mossambicus* exhibits various favourable characteristics that make them more suitable for fish farming which includes their general hardiness, disease resistance (Roberts and Sommerville, 1982) and ability to survive at low oxygen tension (Parez and Ma Clean, 1975). In addition it can also withstand overcrowding, tolerate adverse ecological conditions, fluctuations in salinity and still produce high quality flesh (El-Sayed, 2006).

The objective of the present study includes determination of LC_{50} value i.e., the median lethal concentration of neem based pesticide Achook at 96hrs exposure period and toxic effect of its sub-lethal concentration on histology of gill, liver and intestine of *Orechromis mossambicus* for a the period of 42-days.

MATERIALS AND METHODS

Fish

Oreochromis mossambicus (Length 8-10 ±0.5 cms and weight 6-7±0.25 grams) were collected from Madinaguda tank, Hyderabad, Telangana, India. The fishes were transported to the laboratory in aerated containers and were stocked in 500 L capacity tank provided with de-chlorinated tap water. Fishes were acclimatized to lab conditions for 20-day prior to experimentations. During the acclimatization period the fishes were fed twice daily with commercially available fish feed pellets and the water was renewed daily after 24 hrs.

Pesticide

The pesticide used in current study is biopesticide named Achook (Godrej Achook) which is a commercial formulation purchased from local market of Hyderabad, Telangana, India. It's a neem oil based Emulsifiable Concentrate (EC) containing biologically active substance Azadirachtin.

Determination of 96 hrs LC_{50} and sub-lethal toxicity testing

Fishes were divided into 10 groups; each group comprising 10 fishes maintained in glass aquaria containing 15 L of dechlorinated tap water. The fishes were stopped feeding 24 hrs prior to the commencement of LC_{50} testing. The concentration of biopesticide Achook given to these groups is 0.101 ppm, 0.102 ppm, 0.103 ppm, 0.104 ppm, 0.105 ppm, 0.106 ppm, 0.107 ppm, 0.108 ppm, 0.109 ppm respectively and the 10^{th} group did not receive any concentration which served as control. The aquaria were observed for 96 hrs to obtain the LC_{50} value. During this 96 hrs LC_{50} testing the fishes were observed for clinical signs like skin pigmentation, response to stimuli, swimming pattern and mortality. The 96hr LC_{50} value was recorded and tested by probit analysis as described by Finney (1971).

$1/5^{th}$ of the LC_{50} value was taken as sub-lethal concentration i.e., 0.021 ppm. 15 fishes were exposed to the sub-lethal concentration for a period of 42-day. Throughout this exposure period the fishes were fed twice daily with commercial pellet feed and water was renewed every 24 hrs along with the su-blethal concentration of biopesticide, simultaneously one group of fishes was maintained as control group. After the expiry of exposure periods i.e., at the end of 7^{th} day and 42^{nd} day the fishes from control group and exposed group were dissected and the gill, liver and intestine were collected and processed for histopathological studies. The tissues were carefully removed and washed in 0.9% saline and fixed in 10% formalin for 24 hrs. The

tissues were then dehydrated in graded series of ethanol, embedded in paraffin wax and sectioned at 4µm and stained with Haematoxylin-Eosin stain. The slides were observed under light microscope at 40x magnification and photographed with Olympus digital camera attached to the microscope.

RESULTS AND DISCUSSION

Table 1. Achook concentration and fish mortality at 96 hrs, in LC_{50} experiment.

Concentration of Achook (ppm)	No. of fishes exposed	No. of fishes dead	Mortality (%)
0	10	0	0
0.101	10	0	0
0.102	10	1	10
0.103	10	3	30
0.104	10	4	40
0.105	10	5	50
0.106	10	6	60
0.107	10	8	80
0.108	10	10	100
0.109	10	10	100

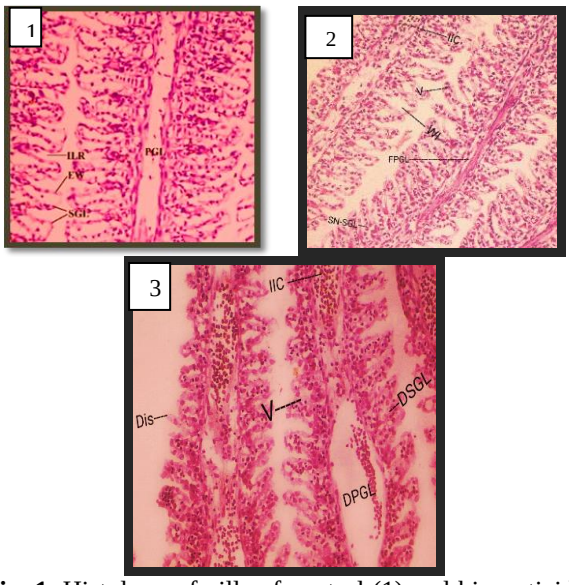

Fig. 1. Histology of gills of control (1) and biopesticide Achook exposed *Oreochromis mossambicus* on 7th day (2) and 42nd day (3). Normal gill- PGL (primary gill lamellae), SGL (secondary gill lamellae), ILR (inter lamellar region), EW (epithelial wall), shrunken and narrow secondary gill lamellae (SN-SGL), V (Vacuolation), IIC (infiltration of inflammatory cells), FPGL (Fibrosis of Primary gill lamellae), WI (Widened Intersticium); V (Vacuolation), Dis (distortion), DPGL (dilation of primay gill lamellae), IIC (infiltration of inflammatory cells), DSGL (dilation of SGL).

The 96 hrs LC_{50} value of Achook obtained for the fish *O. mossambicus* is 0.105 ppm and 1/5th of the LC_{50} value i.e., 0.021 ppm has been taken as sub lethal concentration for observing the histopathological changes in gill, liver and intestine after the exposure period of 7-day and 42-day and the results were compared with control.

The normal gill of control fish shows the following features. Below the operculum four branchial arches are present, each branchial arch bears two hemi-branches consisting of two rows of tapered and flattened primary gill lamellae (PGL), which lies parallel to one another and perpendicular to the arch. On the upper and lower surfaces of each PGL are a series of flattened leaf like structures called secondary gill lamellae (SGL) which form the respiratory surface.

The epithelial wall (EW) of SGL is held apart and supported by pillar cells between the two adjacent SGL lies the inter-lamellar region (ILR). After 7-day exposure to the biopesticide Achook, the following changes were observed in the gills, shrunken and narrow secondary gill lamellae (SGL), intersticium (space between two secondary gill lamellae) is widened, mild fibrosis of primary gill lamellae. Vacuolation of SGL epithelial cells, nucleus of epithelial cells were normal with little shrinkage. The 42-day exposure to sub-lethal concentration of achook showed the following pathological changes in gills. Infiltration of numerous inflammatory cells in PGL, moderate to severe dilation of central portion of PGL, mild vacuolar degeneration, distortion and infiltration of inflammatory cells at the tip of SGL and dilated SGL.

The Normal Liver form the control fish shows the presence of hepatic cells arranged in cords. Hepatocytes are polygonal in shape with distinct round and centrally located nucleus. Vacuolar areas are predominant with fatty mass which is common in fish liver and the blood Sinusoids are present between the hepatic cords. After 42-day exposure the liver shows swollen nuclei, central vein is dilated and filled with blood cells (congestion) and hydrobic degeneration of hepatocytes.

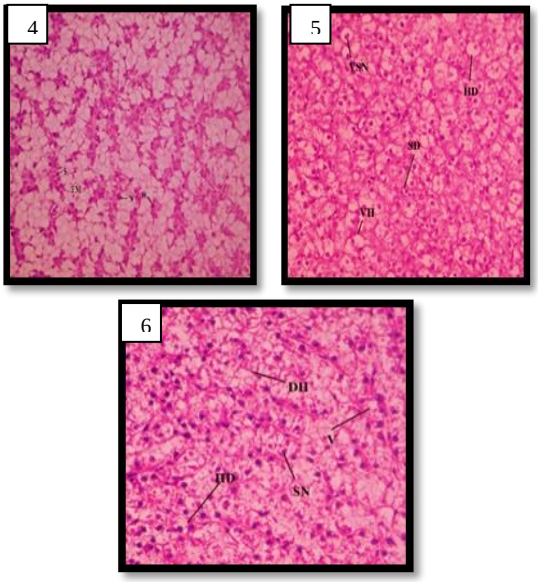

Fig. 2. Histology of liver of control (4) and biopesticide Achook exposed *Oreochromis mossambicus* on 7th day (5) and 42nd day (6). Normal liver- H (hepatocytes), N (nucleus), S (sinusoids), FM (fatty mass). VH (vacuolated hepatocytes), SDN (swollen and disrupted nuclei), DH (disrupted hepatic-locule), SD (sinusoid dilation), SN (swollen nuclei), HD (hydrobic degeneration), DH (disruption of hepatocytes).

Normal Intestine of control fish shows presence of Outermost layer, serosa (S1) consisting of single layer of epithelial cells. Sub serosa or Muscularis layer (S2) made of smooth muscle fibres arranged in definite pattern, the outer being longitudinal and inner circular. Sub-mucosa (S3) consisting of connective tissue fibres blood vessels and nerve endings. Gastric mucosa (M) is folded into a number of finger like processes- the intestinal villi, made of columnar epithelium. Histological changes in the intestine of the exposed fish after 7-day includes the vacuolar degeneration of epithelial cells, mild connective tissue proliferation in central portion of villi prominent, narrow villi and thin muscularis. After 42-day of exposure severe changes observed are massive infiltration of inflammatory cells throughout the base of villi, prominent thin muscularis, vacuolated and ruptured epithelial cells. The purpose of the study was to evaluate the structural damage induced by sub-lethal concentration of neem based biopesticide Achook to the gill, liver and intestine of *O. mossambicus*. Fishes in the control group did not exhibit any pathological alterations in the examined tissues.

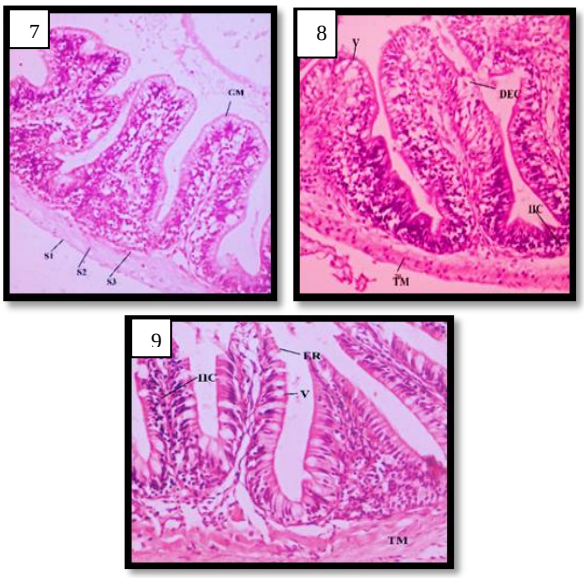

Fig. 3. Histology of intestine of control (7) and biopesticide Achook exposed *Oreochromis mossambicus* on 7th day (8) and 42nd day (9). S1 (serosa), S2 (sub mucosa), S3 (mucosa), GM (gastric mucosa), V (Vacuolation), DEC (disruption of epithelial cells), TM (thin muscularis), IIC (infiltration of inflammatory cells), V (Vacuolation), TM (thin muscularis), ER (epithelial rupture).

Gill is the main target organ which gets easily affected when the organism is exposed to pesticides. Fish gills have many important functions involving gaseous exchange, ion transport, nitrogenous waste excretion, uptake and excretion of xenobiotics (Zayed and Mohamed 2004; Evans *et al.*, 2005). Pathological alterations like dilation of secondary gill lamellae and primary gill lamellae, infiltration of inflammatory cells and vacuolation of SGL epithelium became more severe by 42-day of exposure to Achook in the present study. The similar transformation in the gills were also observed by Pandey *et al.* (1993) in DDT treated *Liza parsia*, biopesticide Azardirachtin in *Clarius gariepinus* (Tennyson *et al.*, 2008) and endosulfan treated mosquito fish *Gambusia affinis* (Cengiz and Unlu, 2002). The secondary gill lamellae became shapeless and distorted and these observations were in agreement with fenvalerate treated

Ctenopharyngodon idellus, the results of Tilak and Yacobu (2002).

Liver is an important detoxifying organ which breaks down toxic substances and metabolites of administered substances. This process is carried out by hepatocytes. Due to this reason the hepatic cells are severely damaged on exposure to toxicants. In the present study exposed liver showed dilation of sinusoids, vacuolation and hydrobic degeneration of hepatocytes, swollen and variously shaped nuclei and necrosis. These alterations of histology were seen to less prominent in 7-day exposure but were highly prominent in 42-day exposure. These changes were also reported in the liver of estuarine mullet, *Liza parsia* exposed to DDT by Pandey *et al*. (1996). Several pathological changes in hepatic cells in liver of *Channa punctatus* induced by malathion toxicity was observed by Magar and Afsar Shaikh (2013).

Several pathological changes in the intestinal tissues were noticed as a result of the toxicant exposures in fishes. The present study showed vacuolation, inflammation and disruption of epithelial cells, damaged villi and necrosis of achook exposed intestine. The changes became more severe after 42-day of exposure. Similar pathological changes in the intestine of Malathion treated *Esomus danricus* observed by Suchismita and Abhik Gupta (2013). The above changes in the intestine were also in agreement with the observations of Wagh and Khalid (1985) and Banaee *et al*. (2013).

In the present study the toxicity tests showed that the biopesticides are toxic to fresh water fish species which constitute the non-target organism. The sensitivity of *O. mossambicus* to the neem based biopesticide Achook is evaluated for short and long term duration in this study. The sub-lethal concentrations caused considerable deterioration to fish health effecting the structure and function of gill, liver and intestine. Thus the present study suggests that because of its toxicity the biopesticide usage must be well monitored in aquaculture farms and agricultural fields.

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