JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Toxicity of Methyl Parathion on Histology Of Climbing Perch, *Anabas testudineus* (Bloch.)

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ABSTRACT

Agricultural wastes such as pesticides are major sources of water solution is now considered to be a major problem in many regions of the world. The current investigation includes the histopathological alterations caused by chronic (21 days) exposure of the clmbing perch, Anabas testudineus to a sublethal concentrations (0.047 ppm conc.) of methyl parathion. The significant behaioural changes were found in test fishes like lost of natural colouration, mucous secretion covering body surfaces and loss of equilibrium before death. The present study revealed that significant histopathological alteration found in liver, kidney and intestine of all pesticide exposed fishes. So, it is suggested that more suitable to fish culture at water pesticide, methyl parathion concentration of < 0.047 ppm for optimum growth performance and survival rate than other water conditions.

Key words: *Anabas testudineus*, histopathology, methyl parathion and pesticides.

INTRODUCTION :

Pesticides are used worldwide in a variety of applications like pest control in agriulture and vectors control for publc health. Pesticide contamination of aquatic system has attracted the attention of researchers and has increased in the last decades due to extensive use of them in agricultural, chemical and industrial processes which are becoming threats to living organisms (Jerald & Saradhamani, 2015). It is reported that approximately three million people are poisoned and 200,000 die each year around the world from pesticide poisoning; the majority of them from developing countries (FAO, 2000).

Organochlorine pesticides have high insecticidal property and low cost production make them worldwide popular. But due to its high persistent in nature and toxicity on non target organisms it is now banned.

Anabas testudineus (Bloch.), locally known as “kawai” is a edible fish. This is an important fish of paddy field culture in wetland region of this subcontinent. This is also subjected to severe effect of pesticides on fishes during the intensive use of pesticides to the crop fields.

Hence, in this study efforts have been made to illustrate the histopathological alterations induced by pesticide, methyl parathion of air breathing climbing perch, *Anabas testudineus*. This will help in formulating guidelines for

the use of this chemical in agricultural farms without any major setback to the surrounding ecosystems and their inhabitants.

MATERIALS AND METHODS :

The climbing perch, *Anabas testudineus* procured live from the local fish market were washed with 0.1% $KMnO_4$ solution to remove dermal infection if any. Healthy fish of average length (8–10cm) and weight (30–34 g) were acclimated for 15 days to laboratory conditions. Commercial diet containing 26.58% crude protein was used throughout the experiment period with daily ration rate 3% of fish body weight in the in morning (10.00 AM). Running tap water was used in all the experiments and the fish were adjusted to natural photoperiod and ambient temperature. No aeration was done.

Static acute bioassays were performed to determine LC_{50} values of methyl parathion for 24, 48, 72 and 96 hours following the methods of APHA, AWWA & WPCF (1985). The LC_{50} values for these periods were 0.34 ppm, 0.24 ppm, 0.12 ppm and 0.095 ppm respectively. The sub-lethal concentration was determined following the formula of Hart *et al.* (1945). Twenty acclimated fish were exposed to a sub-lethal concentration (0.047 ppm) of methyl parathion for 15 and 21 days. At the end of exposure period the fish were anaesthetized with 1:4000 MS 222 (tricane, methane, sulfonate, sandoz) for two minutes. On 15^{th} and 21^{st} day fish were taken out, sacrificed and the intestine, kidney and liver were excised out and fixed in 10% Neutral Buffered Formalin for 18-24 hours fixed tissue samples were then processed and paraffin embedded tissue blocks were cut into serial sections (5-7 µ thick) by a rotary microtome and all the tissues was prepared using the standard histological methods (Luna, 1968).

RESULT :

BEHAVIOURAL RESPONSE :

The control fish shows a tendency to remain at the bottom of the aquarium with little disturbance. However, mortalities were removed immediately, and behavioural abnormalities were assessed at these regular intervals using a modified behavioural protocol checklist (Klesius *et al*. 2000). Scores were assigned daily to individual fish in the experiment and were based on the following scoring system: 0, no observed changes in behaviour; 1, swimming abnormally, lethargic or unresponsive, changes in skin coloration; 2, hyperactive or excitable, rapid operculation; 3, death. Mean behaviour scores were calculated per replicate treatment.

Just after introduction to test solution fishes showed increased swimming, surfacing and hyperactivity. Restlessness, rapid surfacing, peeling of skin and colour fading were prominent after 24 hrs exposure. After 48hr exposure the fishes showed slightly reduced activity and gradual increase in colour fading. Gill adhesion and a thin film of mucous were noticed on gills, operculum and general body surface at this stage. After 72h exposure increased surfacing and gulping of air was observed. At this stage fishes showed loss of balance and jerky movements during swimming. The school formation, a characteristic of this fish, was found weakened in test animals as compared to controls at this stage. After 96h ulceration on trunk, base of caudal and pectoral fins were prominent in 95% of the animals. A thick film of mucous on whole body and gills was observed in almost all test fishes. Test fishes lost their natural colouration. Loss of equilibrium before death is a symptom shown all the test fish.

HISTOPATHOLOGY:

Tissue samples liver, kidney and intestine of *Anabas testudineus* were treated with sublethal methyl parathion concentration 0.047 mg/l at 15 day and 21 day after sacrificed and processed by conventional method, sectioned at 5-7 µm and stained with Haematoxylin and Eosin (Luna 1968).

LIVER:

Liver is the major metabolic center and any damage to this organ would subsequently do, so many physiological disturbances leading to subsequent mortality of fish (Ojolo *et al* 2005; Saxena *et al*. 2008; and Ogamba *et al*. 2016). In histopathological examination, the tissue samples taken from control groups were in normal structure. The liver is composed of hepatic lobule in which the central vein obscure. The parenchyma of the hepatic lobule is formed from hepatocytes which are arranged around the blood sinusoid in cord-like structure known as hepatic cell cord. There are bile ductile in between the cord of hepatic cells which are directed toward the periphery of the lobule to open in the bile duct (Figure:- A1). Liver lesions consisted of hydropic degenerations and cloudy swelling in the hepatocytes with focal aggregation of melanomacrophage cells in between the hepatocytes (Figure:-A2). Focal areas of necrosis, mononuclear inflammatory cells and hyperplasia in the wall of the bile duct were also detected (Figure:-A3). In the liver tissue of the group treated with fertilizers, vacuolar degeneration, necrosis, hyperemia and mononuclear cells filtration in portal regions were observed (Figure:- A2, A3).

KIDNEY:

The fish kidney consists of head and body kidneys. The head kidney is the anterior portion of the kidney and consists of lymphoid tissue. The body kidney is composed of nephron and renal tubules. The nephron is formed of renal corpuscle and Bowman's capsule. The capsular epithelium is continuous with the renal epithelium. The renal tubules begins with :- a) short neck portion lined by low cuboidal epithelium with long cilia, b) proximal convoluted tubule which has divided into segment I lined with acidophilic cuboidal to columnar epithelium with distinct brush border. The epithelial cells of the segment II are columnar and taller than those of segment I. The epithelium becomes lower and more cuboidal in the intermediate segment. The distal convoluted tubules have epithelium with lightly eosinophilia and have no brush border (Figure:- B1) and kidneys displayed glomerulonephritis (Figure:- B2), vacuolar degenerative changes in the tubular epithelium and slight congestion (Figure:- B3).

INTESTINE:

The intestinal wall of *Anabas testudineus* comprised of four distinct layers, viz. mucosa, submucosa, muscularis and serosa. The mucosal layer being thrown into finger like villi, which is made up of simple, long columnar cells and numerous goblet cells (mucous cells) with centrally placed nuclei. Sub-mucosa is thin and projected into mucosal folds constituting the lamina propria. This layer is composed of loose connective tissue with numerous collagen fibres and blood cells. Muscularis consists of inner, thick, circular, and outer, thin, longitudinal muscular layers. Serosa is formed of peritoneal layer and blood capillaries (Figure:- C1).

In the present study, marked histopathological changes in the intestine of *Anabas testudineus* have been observed in intestinal tissue, hydropic degeneration, necrosis and desquamation in epithelium cells at the apex of the villi were determined and mononuclear cell infiltration in the lamina propria was slightly observed (Figure:- C2, C3).

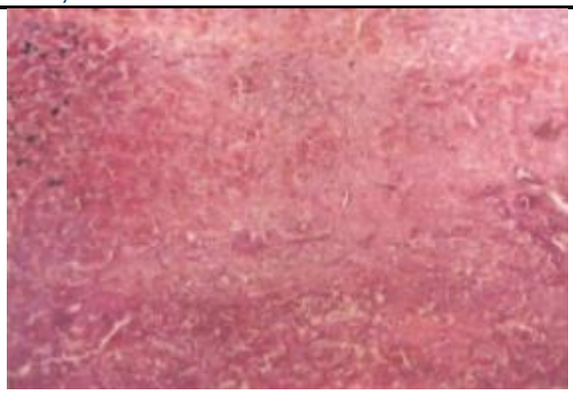

Figure:- A1. Photomicrograph of liver section from control *Anabas testudineus* at days 21 showing the central vein (V) and normal place of hepatocytes . H&E (mag. ×100).

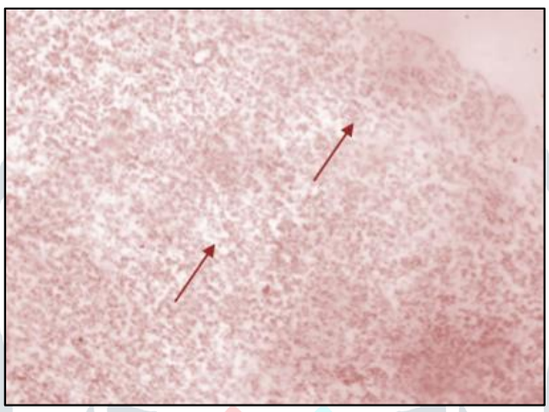

Figure:- A2. Histologic section of liver of *Anabas testudineus* treated with 0.047 mg/L methyl parathion at 15 days showing multifocal areas of hepatocyte degeneration. (H&E) mag ×100.

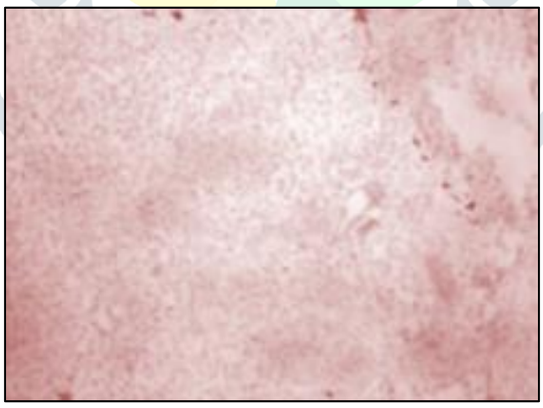

Figure:- A3. Photomicrograph of liver of *Anabas testudineus* treated with 0.047 mg/l methyl parathion at day 21 showing centrilobular vacuolation of hepatocytes. H&E (mag ×100).

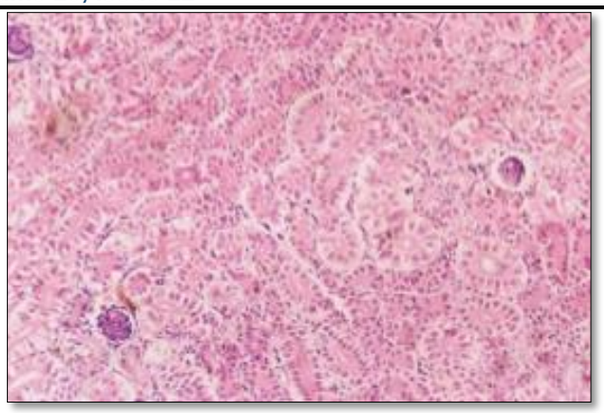

Figure:- B1. Photomicrograph of kidney of *Anabas testudineus* from control group showing normal. H&E, X 250.

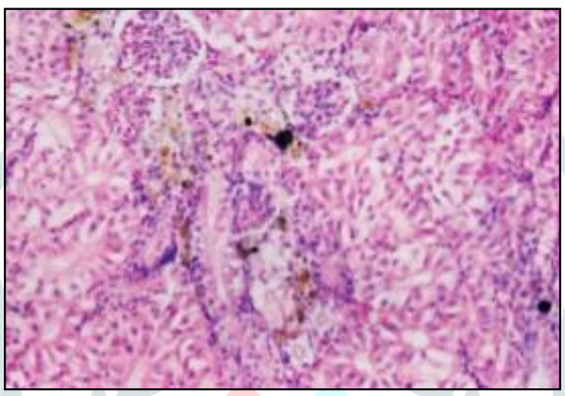

Figure:- B2. Photomicrograph of kidney of *Anabas testudineus* treated with 0.047 mg/l methyl parathion at day 15. Degeneration of renal tubular epithelium (a), vacuolation and necrosis of renal tubules (b) along with necrosis of melanomacrophage center (arrow). H&E, X 10.

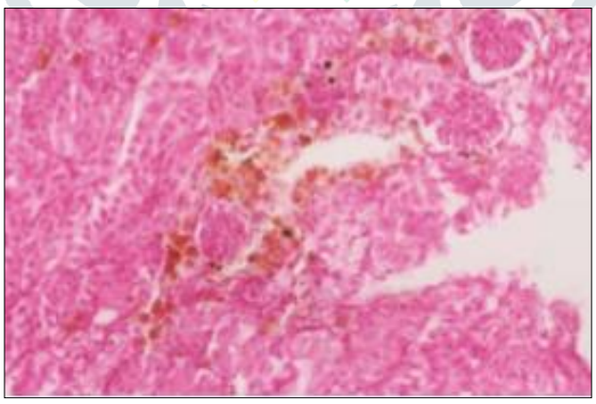

Figure:-B3. Photomicrograph of kidney of *Anabas testudineus* treated with 0.047 mg/l methyl parathion at day 21 Infiltration of melanomacrophage center between the renal tubules. H&E, Mag. X 200.

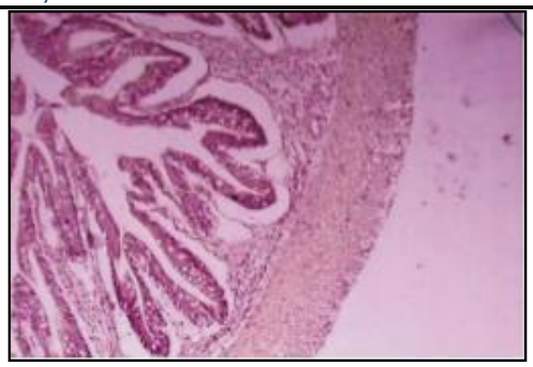

Figure:- C1. Photomicrograph of Intestine tissue of *Anabas testudineus* in control groupshowing normal appearance of circular muscles, longitudinal muscles, serosa and villi. H&E 40X.

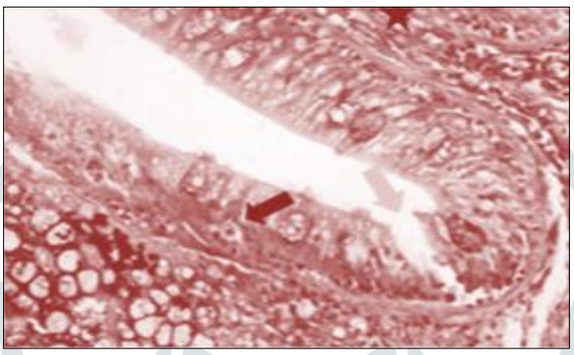

Figure:- C2. Photomicrograph of Intestine tissue of *Anabas testudineus* exposed to 0.047 mg/L methyl parathion at 15 days. Desquamation (orange arrow), mononucleear cell infiltration (MHI) in connective tissue.H.E. 40X

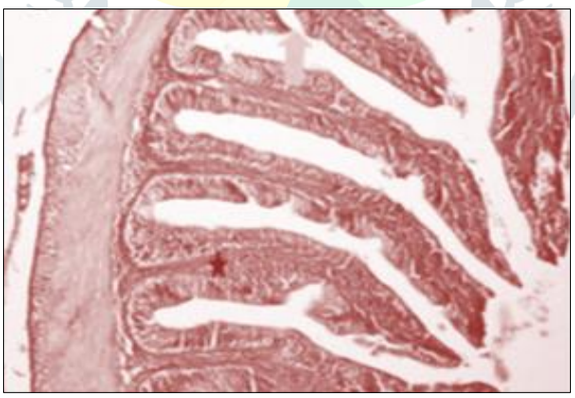

Figure:- C3. Photomicrograph of Intestine tissue of *Anabas testudineus* exposed to 0.047 mg/L methyl parathion at 21 days. Desquamation (orange arrow) and mononuclear cell infiltration (MHI) (arrow). H-E. 40X

DISCUSSION :

In the present study, certain deformities and unusual swimming patterns were found in fish exposed to 0.095 mg/L and above concentrations. The results of the present study also indicate that the fish exposed to this fertilizer recover quickly when they were moved to freshwater. It is concluded that the fertilizers may have toxic potentials in the shallow water and therefore it should be carefully used in the areas closed to waterside. The responses recorded for the fish in this study are similar to those reported by other authors under various stress conditions (Paul

and Banerjee, 1996; Rani *et al.*, 1997; Palanivelu *et al.*, 2005; Ufodike and Onusiriuka, 2008; Lata *et al*., 2008). Behavioural responses of fish to most toxicants are the most sensitive indicators of potential toxic effects (EIFAC, 1983). Acute toxic effect mercuric chloride was observed on zebrafish by Vutukuru SS, Basani K. (2013). The toxic effects of surfactant, dodecyl dimethyl benzyl ammonium chloride (1227) on larval locomotors of zebrafish was observed by Yanan, W. *et al.* (2015). It is, therefore, conclude that the toxicity of the pesticide, methyl parathion depend upon a number of physical, chemical and biological factors. Each of which may be used as a tool for pesticide toxicity to fish.

Histopathological studies on fish are a noteworthy and promising field to understand the structural organization that occurs in the organs due to pollutants in the environment. These structural changes vary with the body parts, nature of the pollutant, medium and duration of exposure. Water quality characteristics also influence histopathological manifestations of toxic effects (Galat *et al.,* 1985). The structural changes in the organs at microscopic cellular and organ level leads to alterations of the function systems (Bhatkar, N.V., 2011).

The damage as more severe and progressive after 15 and 20 days exposure. Histological changes in the liver of fishes have been extensively reported. The results of the present observations in *Anabas testudineus* exposed to methyl parathion were in agreement with those of the earlier workers especially in the vacuolization and necrosis in hepatic tissue. In the present study, cloudy swelling and hydropic degenerations on the liver were observed where liver being the main organ of various key metabolic pathways, toxic effects of chemicals usually appear primarily in the liver. The most frequently encountered types of degenerative changes are those of hydropic degeneration, cloudy swelling, vacuolization and focal necrosis on fish exposed to different kinds of contaminants (Hinton and Lauren, 1990). Wajsbrot *et al.* (1993) observed clear signs of liver pathology in gilthead sea bream (*Sparus auratus*) after 20 days of exposure to 13 mg l_1 TA-N (0.7 mg l_1 NH3–N). Hematopoietic tissues had occurred necrosis and vacuolar degeneration on proximal tubules of the kidney (Çapkın, E., *et. al.*, 2009). An another study, *Oncorhynchus mykiss* applied to 0.1 mg/L NH_3 for 2h, filament and lamella epithelium have superficially folded, the same concentration after 24 hours, telangiectasia in the filament on the 2 or 3 lamellae has been observed (Kirk, R. S., Lewis, J. W.,1993). In Ontario (Canada), in the *Oncorhynchus mykiss* farm in April-May as result of come to toxic levels of ammonia in water was indicated that death of 4000 fish within 48 hours, in pathological examination of fish was reported that telangiectasia in gill lamellae and kidney congestion (Speare, D. and Backman, S., 1988). *Osman et al.,* (2009) recorded congestion and hemorrhage in the hepatic sinusoids with dilation of hepatic vessels, vacuolization and degeneration of hepatic cells with fatty changes with atrophy of pancreatic acini; in liver of the *Oreochromis neloticus* exposed to the polluted water containing heavy metal salts.

In our study, kidney tissues displayed glomerulonephritis and hyperemia after being exposed to different concentrations of sublethal methyl parathion concentrations where the kidney is a one of the major organs of the toxic effects. Thurston *et al.* (1978) observed hydropic degeneration in the kidney of trout after exposure to 0.34 mg /1 NH_3–N. Intracellular vacuolation, necrosis and shrinkage of nuclei were also apparent in the present study in methyl parathion treated *Anabas testudineus*. Smith and Piper (1975) and Thurston *et al.* (1984), reported that degeneration of renal tubule epithelia, hyaline droplet degeneration and in some instances, partially occluded tubule lumens invariably result in impaired glomerular blood flow and filtrations, and eventually may induce renal failure. Hyaline droplets in kidney tubule epithelium suggest re absorption of excessive amounts of proteins from glomerular filtrate (Robert and Rosemarie, 1983).

The intestine is the most important organs in digestion and absorption of nutrients from food, and therefore, monitoring of these organs is considered necessary (Takashima, F. *et al.*1982). Histological analysis of the digestive system is considered a good indicator of the nutritional status and toxicant ingestion of fish (Caballero *et al*. 2003). In the present study, marked histopathological changes in the intestine of *Anabas testudineus* have been observed in intestinal tissue, hydropic degeneration, necrosis and desquamation in epithelium cells at the apex of the villi, mononuclear cell infiltration in the lamina propria was slightly observed (Fig. C1.). All the pathological alterations showed a relationship with prevalence increasing with increasing methyl parathion concentration and exposure time. Desquamation mononuclear cell infiltration (MHI) in connective tissue was observed in treated fish at 20 days. Similar observations made by earlier workers relating to histopathological changes in intestine in response to various toxicants are being enumerated here. The proliferation, necrosis of serora and mucosa and rupture of villi have been reported by Konar (1970) and in *Labeo rohita*; Wong *et al*. (1977) in *Cyprinus carpio* and *Ctenopharyngodon idellus*; Sastri and Gupta (1978) in *Channa punctatus*; Kumar and Pant (1984) in *Barbus conchonius*; against exposure to heptachlor, zinc and copper salt mercuric chloride, dimecron, aldicarb and furadan, respectively.

CONCLUSION:

It could be concluded that *Anabas testudineus* with average weight 30.0± 4.0 g, were more suitable to culture at water pesticide, methyl parathion concentration of < 0.047 ppm for optimum growth performance and survival rate than other water conditions. Therefore, it can be recommended to be carried out under the similar experimental conditions.

ACKNOWLEDGEMENT:

The authors are thankful to the P.G. Department of Zoology, LNM University, Darbhanga, for the provision of laboratory facilities used in this study.

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