



RESEARCH ARTICLE

HISTOPATHOLOGICAL ALTERATIONS OF THE GILL, LIVER AND KIDNEY OF THE FISH *LABEO ROHITA* EXPOSED TO BIFENTHRIN THE SYNTHETIC PYRETHROID, 10% EC

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Abstract

The fish *Labeo rohita* exposed to bifenthrin, 10% EC, a synthetic pyrethroid of group I (non-cyanogroup) in the laboratory for 10 days in sublethal concentrations of $1/10^{\text{th}}$ of LC_{50} value of 96 hrs (determined by continuous flow through method). (For the first time study and report). The architectural damage of the entry point, the gill and transport of ions, gases and wastes, when histopathologically damaged impairs the respiration and metabolism too. The liver the site of metabolism because of lesions developed biochemical reactions of anabolism and catabolism are not going to be normal as result growth will be curtailed. Growth to be normal as result growth will be curtailed. Growth is the important parameter for culturing species and the resent studied one is also the as the candidate species. Kidney when damaged the aminotellic nature of the excretion and regeneration of blood cells regeneration is not the normal way of the physiological processes. All cumulatively result in the death of the organism even though, the levels of concentrations of the toxicant are not acute but chronic which in the long run that can be lethal for the fish.

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Introduction:-

Any lesions of the tissues/organs of the organisms of damaged by any pollutant toxicant, will be detrimental for the survival, sustenance and well being. Due to indiscriminate use, the pesticides in agricultural practices finding their way into the aquatic environment by any means reach and had/have effects on the ambient organisms. One such effect especially for the majority of living organisms, fish is none other than the histopathology read upon as studies. They are many investigations of this which will be comprehended by only of such examinations. Marie Xavier Bichat a French anatomist and pathologist the father of histology that had invented paving the way of explorations of the tissues/organs in the organism. Sana Ullah et al. (2019a) mentioned the biomarkers of the study in which the histopathological aspects as the indices of the toxicity especially for the synthetic pyrethroids, in general, such biomarkers of study pertaining to histopathology and others were also mentioned by Yanchova et al. (2015) and Anilava Kaviraj and Abhik Gupta (2014). Tissue deposition quantitatively pave the way for brining many qualitative changes as opined by Yanchova et al. (2019) and Javeed et al. (2017). As the contamination of aquatic bodies echoed by Tang et al. (2021); Muyaier et al. (2021), Zeshan Praveen (2020); Mohammad and nems (2020); Ankita Sarma et al. (2019); Anamika Srivastava et al. (2018); Somayyah et al. (2017) and Indira Devi et al. (2017).

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Viewing all the aspects, histopathological observations that are studied were mentioned in the review articles for the synthetic pyrethroids by Sana Ullah (2019a); Saumya Biswas (2019); Prusty et al. (2015) and Ahrar Khan (2012) and for all pesticides by Ullah and Zorriezahara (2015) and Murthy et al. (2013). Individual research study reports of Ullah et al. (2019b); Parlak (2018); Cunha et al. (2018), Lieu et al. (2018) Monir et al. (2015), Ullah et al. (2015); Singh et al. (2015); Guirdiola et al. (2014); Al. Ghanbousi et al. (2012); Kan et al. (2012); Velisek et al. (2009a&b) are for synthetic studies which are important.

By perusal of the literature available and as the farmers use only the commercial formulations that are available in the local markets, which is selected i.e., 10% EC of bifenthrin to study the vital organs such as gills, the entry point) liver (the metabolism organ – the largest gland) and kidney the (exit point) are selected and studied in this histopathology biomarker study, which due to the observations of the respective organs provide the damage to them. The fish *Labeo rohita* was studied by exposing it to 10% EC of the synthetic pyrethroid (type/group-I – a non-cyanogroup member) of Bifenthrin.

Material and Methods:-

Fresh water fish *Labeo rohita* was acclimatized to the laboratory conditions for 10 days. 50 number of fishes are exposed to 10% EC bifenthrin for 10 days by taking into consideration of LC_{50} value of 96 hours ($1/10^{th}$ of the 96h LC_{50}) value 0.011 µg/L as per the APHA guidelines (1998, 2005 & 2012 & OECD, 2019).

After the exposure period of 10 days the fish are randomly selected for histopathological examinations. The exposed fish are sacrificed and tissues viz., gill, liver and kidney of the fish are isolated and also from that fish not exposed to the toxicant which serve as control to observe so as to differentiate the tissue changes in pathological conditions.

NaCl 0.85% saline solution was used to rinse and clean the tissues that were isolated. Then were fixed in the aqueous Bouine solution and kept for 48 hr to process further through the graded series of alcohols. Then cleared with xylene and they later were embedded in the paraffin wax. The gills alone were processed by through double embedding technique (Humason, 1972).

6 µm thickness sections were cut using the microtome which were later stained with Ehrlich haematoxylin/Eosin that was dissolved in 70% alcohol for clarity in viewing and finally mounted on the Canada balsam (Humason, 1972). The sections were observed first through the digital microscope and later the occurred histopathological lesions were examined and taken the photographs with the help of Intel Pentium Q x 3 Computer attached microscope, under 400x lens (made in China).

Observations:-

General histology of Fish Gill:- (Plate 1; Fig. A)

Teleost's have only five pairs of gill arches which are protected by a hard covering on either side of the head and is called the operculum. First four pairs are with the slender gill filaments that from the two lines facing towards the back which were are joined to each other at the base by a gill septum. The last pair of the gill arches generally transformed into a pharyngeal bone and does not play a role in the process of the respiration.

The slender gill filaments of the secondary gill that are lined along both the sides are semicircular in shape. The surface of them is covered with simple squamous epithelial cells as the connective tissue and have many capillaries that are separated by columnar cells which can also be called the pillar cells that run parallel along with the surface.

Such semicircular secondary gill lamellae of more number are also lined along both the sides of the primary gill lamellae (Plate VI.1.Fig. A). Each primary gill lamella consist of a centrally placed rod like supporting axis (PGL) with blood vessels on either sides for the copious supply of blood (EL). The secondary lamellae SGL also can be called as the respiratory lamellae that are highly by nature vascularized and also covered with a thin layer of epithelial cells (EL) of squamous nature, blood vessels (BV) which are extended into each of the secondary gill filaments. The blood cells of them have a single nucleus that is flattened. The space that lies between the two adjacent secondary gill lamellae is referred as the inter lamellar region with cells (ILC).

Pathology of the gill tissue under the exposure of the toxicant:- (Plate 1, Fig. B)

When compared with the normal gill which is with primary and secondary gill lamellae certain changes are observed in both the lamellae. Both bulging of the primary lamellae and club shaped secondary lamellae are evident. Another manifestation that was observed separation of the two lamellae that are distended delaying the process of the diffusion of ions and gases of respiratory nature. Actually the space appears to be bulged and mainly the origin points of the lamellae appears to be damaged (PLC) Erythrocytes whose primary function is to exchange the gases supposed to more in number usually but appears to be less. The epithelial cells, which are non-other than squamous cells (pavement epithelium) are not normal as of the control, gill and the whole compact structure as a compound epithelium appears to be lessioned (HGL).

General Histology of Liver:- (Plate 2, Fig. A)

The liver of the fish is bilobed structure, five lobed in (mammals) and when dissected left lobe is larger than right and gall bladder round shaped one which is well developed, hidden it appears as intestine.

The cells of liver, hepatocytes (HC) whose arrangement is systematic, colour is reddish and shape is polygonal with a prominent spherical nucleus, which is by locate on of centrally placed. The chromatin of the liver cells is at its periphery and nucleolus shows characteristic pattern of RNA synthesis for its role in metabolism. The endoplasmic reticulum both with rough (RER) and smooth (SER) to have the functions of both secretory (acinar) as well as detoxification associated with more synthesis. Mitochondria are round and elongated for energy synthesis, more associated with rough endoplasmic reticulum. The secretory part is with vacuoles and a sterna (RER) which are concentrically arranged.

The liver surface is covered with serous membrane, connective tissue that extend upto thick cords called hepatic cords (HC). It has fibers and the sinusoids, bile canilculi (BC) that are located at the centre of the hepatic cards extending upto sinusoids and each cord has glycolipid granules (GC) in large quantities.

Pathological observations of liver tissue:- (Plate VI.2)

Mild necrosis, in the liver can only be observed in the compact structure, blood is congested as the condition of pathological term congestion can be applied, infiltration (gaps) that interfere in functional continuum, lymphocytes enter into the gaps (ABS), the hapatocyte cells showed pykenotic nuclei supposed to have more functional aspect of biochemical – (transcription – translation) disturbances, the observation of the inflicted aggregation of melanomacrophages (phagocytes – Kuffer cells) (DGPP) of some component of the section that showed foamy appearance. The dialation of sinusoids are apparent apart from appearance of vacuoles (FV) and hypertrophy.

General Histology of Kidney:- (Plate 3, Fig. A)

Fish kidney, Aminotellic in secretory function, conservation of silts Agglomerular in its function apart from haemopoietic nature. It is reticulo-endothelial endocrine as well as excretory role, it has to performs. It has glomerula filtration as well as salts conservation of reabsorption have with proximal as well as distal convoluted tubules (PCT & DCT) shown as PCS & DCS. The renal corpuscle is near (PCT) and DCT ends into urinary bladder. Taller columnar cells and smaller columnar cells varied in its distribution. The glomenlar tissues consists of inner and outer layer of a single flattened epithelium (Renal Epithelium-squanous) and renal tubules too have covering. Mesangium is the space, that fills the space between loops of gloerular capillaries.

The renal tubules (RT) are thin and short in the neck segment which are divided into two parts, segment I and II consisting of cubodal epithelium, densely arranged. The segment of the RT is having microvilli for absorption whereas they are absent in the segment I (Brush border).

Pathology of the Kidney – observed:- (Plate VI.3)

There are alterations related to circulatory component as hemorrhage and hyperemia, glomenlar alterations as nuclear as well as plasma alterations. Atrophy hypertrophy and hyperplasia of tissue cells (DART), in terms of pathological terms can be applied. Necrosis of tubular part, focal necrosis in the glomerulus filtering point is very conspicuous. Damage in the filtering point, increase of number of cells of the tubule capsular membrane – hyperplacia, peritubular fibrosis leucocyte related abnormality are the severe pathological alterations that are observed in the exposed fish which damage the organ in its functional aspect (DART).

Discussion:-**With Synthetic Pyrethroid I & II:-**

Sana Ullah (2022) in the fish *Ctenopharyngodon idella* reported on bifenthrin a multibiomarker study, in which due to oxidative stress resulted the damage of cells/tissues. Due to enhanced ROS (Reactive Oxygen Species) that resulted lipid peroxidation which had damage of the tissues/organs of the fish due to bifenthrin intoxication. Rapid opercular movements were resulted to in take more oxygen as a result of the damage of the epithelial tissues. The same aspect of the toxic action of bifenthrin visualized in the fish *Clarias batracus* by Saha et al. (2021) and Saha and Saha (2020) and also in the fish *Oreochromis mossambicus* (Peters, 1952).

Bano et al. (2021) in the fish *Labeo rohita*, due to DNA damage the RBC cells which are located in the blood that flows even in the gills damage due to toxic action of bifenthrin as well as chlorpyrifos.

Ullah et al. (2019b) in the fish *Hypophthalmichthys molitrix* due to deltamethrin, Parlek (2018) in the fish *Danio rerio*, Cunha et al. (2018) in the fish *Colossoma macropomum*, Liu et al. (2018) in the fish *Danio rerio*. Guardiola et al. (2014) in the fish *Sparus aurata*, Aphanis dispar by A.I. Ghanbousi et al. (2012); Khan et al. (2012) in the fish *Oreochromis niloticus* all, using the deltamethrin as toxicant and Juliant et al. (2019) in the fish *Oreochromis niloticus* using the lambda cyhalothrin (the group I toxicants of the synthetic pyrethroid) as toxicant reported that it caused the effect on the gill tissue.

Sangeetha et al. (2018) in the fish *Poecilia latipinna* using 25% EC of cypermethrin and Neelima et al. (2015) using the same toxicant in the fish *Cyprinus carpio* reported on the histopathological lesions in the gill. The destruction of the secondary lamellae, necrosis, and club shaped secondary gill lamellae all as of the present study observations were reported.

Sahoo et al. (2017) in the fish *Labeo rohita*, using deltamethrin as the toxicant reported that the branchial arch was swollen and all of it included cells that are infiltrated.

Chandrima Dey and Saha (2016) reported on the fish *Labeo rohita* by lambda cyhalothrin 5% EC as well as Marshal (carbofuran 8.5% EC). Notable changes were notice in the gill, liver and kidney as in the present study and both of them belong to same group of the synthetic pyrethroid.

Mohammad et al. (2016) in their study using Deltamethrin as the toxicant in the fish Nile tilapia too observed changes as lesions, hyperplasia of the secondary lamellae and joining of them termed as fusion which are also the same in agreement of the present study.

Neelima et al. (2015) reported in the fish *Cyprinus carpio* the exposure to cypermethrin 25% EC which caused changes in the gill tissues. They mentioned about the primary lamellae of necrosis, fusion of the secondary lamella clubbing and shortening wherein it had all the alterations that were also observed as the toxicant of the present study even though they belong to two different classes pyrethroids..

Singh et al. (2015) using lambda cyhalothrin in the fish *Clarias batracus*, Velisek et al. (2009a&b) using bifenthrin as the toxicant reported on the histopathological damages in the fish common carp *Cyprinus carpio* and *Oncorhynchus mykiss*.

Anitha Susan et al. (2012) studied and reported in the fish of the three major carps *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* after exposure to the synthetic pyrethroid of type II, fenvalerate. The tissues they studied, include gills also, a comparative study. The observations are in agreement of the present work even though the present tested toxicant belongs to the type I without a cyano group which imparts lesser toxicity. The Permethrin being adhered to the mud particles, (adsorption) had much effect on the bottom dwelling fish, such as *Cirrhinus mrigala*, including the *Labeo rohita* the present studied one due to water dwelling habit being teleost have five pairs of gills prone to have the damage by the toxicant tested that was reported for the fenvalerate and similar in the present work.

Velisek et al. (2011), of the fish reported on the effects of the pyrethroid and triazine pesticides mentioned also about the histopathological damages that resulted after exposure to the cypermethrin, a synthetic type II pyrethroid, Bifenthrin and also the triazines. When cypermethrin was studied in the fish rainbow trout after exposure to 96h lethal concentration, caused a pathological conditions called 'Elamgiectasia' in the secondary lamellae of the gills

and pillar /columnar cells of the compound epithelium. In the fish *Cyprinus carpio* hyperplasia of the respiratory epithelium of the chloride cells was also observed. Bifenthrin another toxicant as one of the triazines, severe Elamgiectasis was observed (dilatation of capillaries) for the same fish.

Velisek et al. (2007, 2009) observed and reported in the fish rainbow trout *Onchorynchus mykiss*, exposed to bifenthrin, type I synthetic pyrethroid, the localized tissue damage and also the weakened cell walls known by the term aneurysm in secondary lamellae, hyperplasia of the epithelium and fusion of cells in the gills. This is also similar in line of the present observation and is also of similar of other studies of type II synthetic pyrethroids, (Velisek et al. 2007 and also by Cengiz 2006a), in *Cyprinus carpio* after exposure to Deltamethrin).

Balu Velumurugan et al. (2007) reported the study of the toxicant fenvalerate another synthetic pyrethroid, to the fish *Cirrhinus mrigala*. The study revealed that epithelial hyperplasia, necrosis of the epithelial surface desquamation and fusion of lamellar fusion hindering the diffusion pathway of the gases apart from curling. The present work even though it belong to the different class of the synthetic pyrethroid is of similar line.

Diana et al. (2007) opined that the damage of the chloride cells (Hyperemia) due to the exposure of the Deltamethrin at 2 µg/L concentration that was similar as in the present study and the resultant damage preclude the chloride cells to death, that make difficulty in the process excretion of the salts through the gills.

Yildirim et al. (2006) study of the toxicant exposure of Deltamethrin to the fish *Gambusia affinis* for a long duration in sublethal concentration too revealed similar changes of fusion of the secondary lamellae of the present study. Observation as above and in addition were also reported as hyperemia and aneurysm by Cengiz, 2006b).

Organophosphates:-

Akter et al. (2020) in the stinging cat fish *Heteroyeustes fossilis* due to the exposure of the organophosphate, envoy 50 at sublethal doses which they followed a different criteria recommended dose of springing and half of the recommended dose of it for 7 days. The present study result apart from a lesion of clubbing (swelling) of gills was reported. The methodology of exposure at a concentration is different (a deviation of the recommended) and the gills showed similar alterations.

Yanchova et al. (2019) reported due to chlorpyrifos intoxication in the fish *Cyprinus carpio* gills are damaged after 72 hrs of exposure. The report that by them in terms of the alterations resulted were under the caption as circulatory disturbances (Vasodilation) regressive lesions (Epithelium-necrosis) progressive lesions (lamellar lifting, proliferation of the cells) where in the present study as of the changes as necrosis, hyperplasia, congestion, by the toxicant, tested were observed too.

Sathick et al. (2019) reported on the histopathological changes of the gill tissue of an estuarine fish *Mugil cephalus*, that resulted after exposure 10, 20 and 30 days at 10% of 96h LC₅₀ values as sublethal concentration. The changes that resulted due to more days of exposure were severe and majority of the present study results are similar, with their results of the report.

Trivany Edwin et al. (2019), reported in freshwater fish, *Oreochromis niloticus* and *Cyprinus carpio* due to the exposure of chlorpyrifos in 10, 20, 30 days of exposure at three different concentrations and gill was studied and observed histopathological changes. The results of the report that were time dependent and concentration dependent only and the severity increases even though the concentrations were at sublethal. Almost similar results in both the fish studied.

Arumugam Stalin et al. (2019) reported in the fish, *Channa punctata* exposed to chlorpyrifos in 10, 20, 30 days of exposure at sublethal concentration as (5 ppm). Except Muroid metaplasia all other results were similar of 30 days of exposure different of the present study.

Japamalai (2017) in her study, reported the changes in the gill of the fish *Labeo rohita* exposed to an organo phosphate, Dichlorovos of 76% EC. After 8 days exposure and when sections are taken the tips of primary gill lamellae were bulged, the secondary gill lamellae lost their shape, necrosis, vacuoles in the gill epithelium and fusion of disorganized cells. Even though the toxicant belongs to different class of compounds i.e. OP compound the effect is in the similar line with minor changes.

Mandeep Kaur and Rajendra Jendal (2016), reported a study of the branchial architecture and its changes in the fish *Ctenopharyngodon idella*, after exposure to chlorpyrifos an organophosphate. After exposure to the toxicant at sub lethal concentrations for 15, 30 and 60 days duration which differs to the present work in methodology and the direct contact point, of the entry of the toxicant and the thin surfaces with larger surface area had shown damage. The effect is duration dependent mucous secretion and also the disorted secondary lamellae. They too observed changes of the primary lamellae which resulted denudation as the toxicant of different chemical exposure. Hyperplasia, damage of the squamous epithelium, of the compound tissue were also the observations that were reported.

With others

Manjit Singh et al. (2020) in the fish *Labeo rohita* due to the exposure of the bleaching powder (calcium hypochlorite) and reported the histopathological changes in the gills, liver and kidney. Lifting of the secondary gill lamellae and its curling rupture and pillar cells got broken apart from odema as well as the epithelial cells too ruptured were all observed (In the liver cells, Nuclear degeneration and cellular necrosis both were also reported).

Pradip Kumar Maurya et al. (2019) reported on the histological changes in the fish *Heteropneustes fossilis* due to industrial washes exposure in the natural environment due to contamination, located in Muzaffarnagar (M.P., India) area. Due to pesticides defilement hyperplasia curling of the secondary lamellae aneurism fusion of lamellae epithelial lifting dialation and congestion were also observed.

Omkar Singh and Kaur (2017) reported on the histopathological alterations of *Labeo rohita* due to the lead toxic stress action. The cellular hypertrophy, hyperplasia vacuolation epithelial lifting shortening and curling edema, necrosis aneurysm were all observed, due to the metal toxicity.

Tauseef Ullah et al. (2017) in the milk fish, *Hypophthalmichthys molitrix* and *Labeo rohita* due to the toxic stress at the sublethal exposure of effluents from Textile Industry, Fasailabad, Pakistan. The degeneration of the gill filament, fusion of the secondary lamellae, Haemorrhage in both primary and secondary lamellae, necrosis, Atrophy and congestion of the blood vessels were noticed. (In the liver tissue severe hemorrhage and congestion too were observed).

Tamil Selvi and Ilavazhahan (2012) reported on the sublethal concentrations of heavy metals which caused histopathological changes in the gills of the fish *Catla catla*. The bulging of the tips of the primary gill filaments, fusion and also the disorganized secondary gill lamellae.

Discussion – Liver:-

Liver with synthetic pyrethroids:-

Karim et al., (2016) too reported histological and biochemical study of the liver of silver carp *Hypophthalmichthys molitrix* after acute exposure to pyrethroid deltamethrin. The study revealed that necrosis, nuclear pyknosis hypertrophy of hepatocytes vacuolization nuclear atrophy and congestion of blood vessels, were noted in their study.

Suvetha et al. (2015) reported that any biochemical alterations in the organs of the fish *Labeo rohita*, liver metabolically active and kidney for excretion were due to intoxicification of a synthetic pyrethroid deltamethrin the type II wherein histopathological changes finally manifested impact in the biochemical enzymes of tissues/organs.

Neelima et al. (2015) reported in the *Cyprinus carpio* after cypermethrin exposure and the result was of the similar lines of the present study. Andem et al., (2017), Velisek et al., (2006a) too reported on the toxicant cypermethrin in the fish *Clarias gariepinus* and *Onchorynchus mykiss* respectively and their observations are also of similar nature of the present study.

Anitha Susan et al. (2012) too reported damage to the liver tissue when fenvalerate toxicant is tested on the three Indian major carps and it also coincides results of the present work.

Velisek et al. (2007) reported in the fish *Onchorynchus mykiss*, due to exposure to deltamethrin that showed pathological variations in the largest gland of the body, the liver and is also in the organ of ammanotellic excretory component.

Cengiz and Unlu (2006b) also reported that in liver tissue of the mosquito fish *Gambusia affinis* after exposure to commercial deltamethrin (EC), hypertrophy of hepatocytes the kupffer – (immune) cells of the disturbance in circulation necrosis and also narrowing of the sinusoids. Some of the present studied, changes are also observed in the fish experimented.

Sree Vani and Veeraiah (2014) reported the effect of cypermethrin on the histopathology of the freshwater fish *Cirrhinus mrigala*. The toxicant induced changes, such as the cytoplasm of the hepatocytes degeneration, formation of the vacuoles and the rupture in the blood vessels and the disappearance of the hepatocytic cell walls and also the disposition of the hepatic cords.

Yildirim et al. (2005) reported in the fingerlings of *Oreochromis niloticus* L., popularly known as Nile tilapia after exposing them to type II synthetic pyrethroid, deltamethrin wherein liver damage of hydrophobic nature as being observed..

Cengiz and Unlu (2002 and Cengiz 2003) reported on the histopathology of liver after exposure to endosulphan, an organochlorine and malathion, an organo phosphate respectively in the fish *Gambusia affinis*, mosquito fish and the reports of them as lesions that are observed also the same of the present study.

The present study reveal that the xenobiotic compounds effect the fish organs like liver and kidney and the fish in pesticides in the environment, when our target is for monitoring of pollution and this type of biomarker study of histopathology as a tool of approach is a good one. Metabolically active liver organ and excretory function of kidney is impaired even in sub lethal concentrations on the survival of the non-target organism. The formulations of the pesticides used in then applications of both agri & aqua products must be viewed seriously.

Liver – Discussion:-

With organophosphates:-

Sandhya and Rasool (2021) while due to Malathion exposure to the fish *Channa punctatus* and the dialation of the sinusoids, hypertrophied hepatocytes and also the erythrocytes along the central vein were all reported.

Akter et al. (2020) in the fish *Heteropneustes fossilis* due to Envoy 50 SC organophosphate toxic action, resulted pathological alterations which was time and concentration dependent. Vacuolation in the hepatocyte cytoplasm, hypertrophy all were reported.

Arumugam Stalin et al. (2019) in the fish *Channa punctata* due to the toxic action of chlorpyrifos necrotic hepatocytes were reported as in the present study. The method of toxicity determination and duration of the exposure were different from the present study.

Somayyah et al. (2017) in their report of organophosphorous pesticides effect on liver, opined that histomorphological alterations in the organ/tissue, apart from biochemical alterations as a consequence the metabolism is impaired.

Golam Mohammad et al. (2015), in the fish *Barbonymus gonionotus* (silver barb) reported alteration in the liver due to the toxic action of Quinolphos an organophosphate. Necrosis and damage of liver sinusoids were all reported as in the present study.

Ragadi et al. (2015) in the fish *Channa punctatus*, due to Monocrotophos toxic action the cytoplasm damage, hepatocytes atrophy, rupture of the blood vessels (liver canaliculi) were all reported.

Yogita Devi and Abha Mishra (2013) in the fish *Channa punctata* were treated with Hilban (chlorpyrifos) resulted changes in the morphological and anatomical nature that the hepatic organ. Fibrosis, infiltration of leukocytes as in the present study apart from necrosis and hypertrophy were all reported.

Clarias gariepinus due to paraquat Dichloride an herbicide too in the liver was reported by Ladipi et al. (2011) that in the organ, damage and histopathological changes too were very prominent. Tilak et al. (2005) reported on the histopathological changes in the fish *Catla catla* due to chlorpyrifos as toxicant and the present study reiterate the same observations.

Kidney – Discussion:-

Sandhya and Rasool (2021) reported on the fish *Channa punctata* due to Malathion exposure. The kidneys had shrinkage, basement membrane detachment, vacuoles were developed and also the volumes of cells damaged.

Shalini Anand and Harender (2016) reported the pathological changes in the kidney fish of the fish *Clarias batracus*. Shrinkage of the glomerulus and degeneration were observed due to toxic action.

Neelima et al (2015) also reported in the fish *Cyprinus carpio* after exposure to the cypermethrin a commercial formulation in the kidney and present work coincides with their report.

Velumurugan et al (2009) also reported that the kidney of the fish was effected with Cypermethrin as the toxicant in the fish *Clarias gariepinus*. Much of the observations were similar to the present study. Anitha et al (2012) in the three major carps using fenvalerate a synthetic pyrethroid of type II and Sree Veni and Veeraiah (2014) in the fish *Cirrhinus mrigala* after the exposure to the cypermethrin of 10% reported the degenerative changes in the kidney.

Velisek et al. (2009a&b) reported on histopathological changes that are observed in the fish *Onchorhynchus mykiss*, exposed to bifenthrin the present tested toxicant degenerative changes were observed.

In the fish, *Cirrhinus mrigala*, on exposure to two sublethal concentrations of lambda cyhalothrin of 0.3 and 0.6 ppb by Velumurugan et al. (2007) reported necrosis, pyconotic nature of the nuclei in the haemopoietic tissue, the hypertrophy of the cells in the epithelium of tubules, the its lumen was narrowed and the space in the Bowman's capsule and glomerulus was expanded. Except the severity of the situation was not that much in the present study as described by them.

Similarly, Cengiz (2006) reported in the fish *Cyprinus carpio* on exposure to deltamethrin of synthetic pyrethroid deltamethrin of 50% and 70% of 96h LC₅₀ value. As observed by them that showed a regional damage and degeneration of the cells of the renal tubules, pyconotic nuclei in the haemopoietic tissue, dilation of glomerular capillaries and intra cytoplasmic vacuoles in the epithelial cells apart from hypertrophoid and narrowing of the part and the results were not observed in the present study.

Yildirim et al (2006), studied histopathological effects of deltamethrin and also apart from Staicu et al. 2007 too reported in the fish *Carassu sauradus* using the same toxicant.

Akter et al. (2020) due to envoy 50% SC an organophate, in the kidney of the fish *Heteropneustes fossilis* reported that the kidney tissue degeneration, glomerular expansion that increased the diameter of the renal tubule necrosis, pyknosis, vacuolation and hemorrhage. Majority of the reported observations reiterate the results of the present study.

Golam Mohammad et al. (2015), in the fish *Barbonymus gonionotus* (silver barb), by using Quinolphos an organophate reported the alterations in the kidney due to toxic stress. Renal lesions, defect in Proximal Convoluted Tubule (PCT) and the damage was extended from glomerulus to distal convoluted tubule (DCT) and all such were time and concentration dependent.

Ragada et al. (2015) in the fish *Channa punctatus* using monocrotophos as atoxicant reported necrosis, cellular hypertrophy and also granules in the cytoplasm which are similar even in the present study also.

Tilak et al. (2005) using chlorpyrifos as toxicant reported in the fish *Catla catla*, reported kidney degenerative changes and the observations also support to reiterate the present findings.

Conclusion:-

The important tissues gills, liver and kidney of the fish, can be termed as vital when exposed to the toxicants like bifenthrin 10% EC that showed pathological degenerative changes which culminate the biochemical changes and the very survival of the fish in questionable even in the sub-lethal concentrations and apprehend that sub lethal concentrations are really lethal to the fish. If any, representation of the toxicant is going to be given for its usage, the sublethal, chronic devastations that are going to manifested be thoroughly studied and the actual mechanisms of

toxicant and such lesions needs to be understood. It is evident the work on bifenthrin on histopathological studies are very few.

PLATE-1

Histopathological Changes -GILL- LABEO ROHITA . Exposed to BIFENTHRIN 10% EC

FIG-A
CONTROL GILL

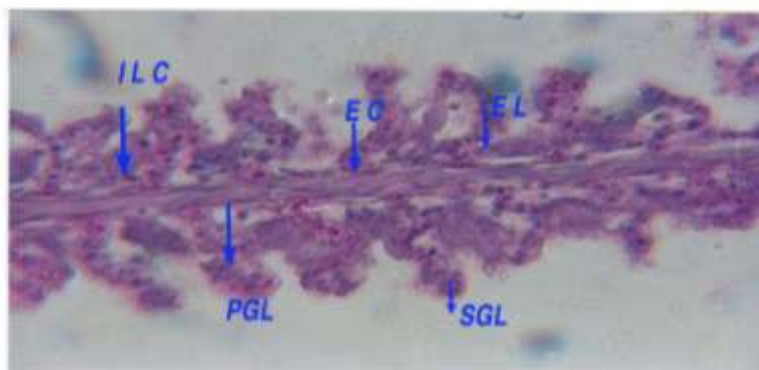


FIG-B
EXPOSED GILL

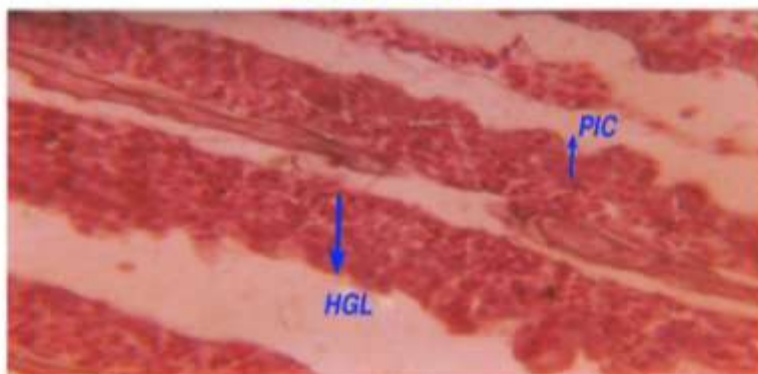


FIG.A Normal Gill Lamella of Labeo rohita,Haematoxylin/Eosin Stain (HE),X400
PGL:Primar Gill Lamella SGL: Secondary Gill Lamella EL:Epithelial Cells
EC:Erythrocyte ILC: Inter Lamellar Cells

FIG.B Gill Lamella of Labeo rohita exposed for 10 days to sublethal concentration of Bifenthrin 10% EC Haematoxylin/Eosin Stain (HE),X400
PIC: Proliferation of Inter Lamellar Cells filling the spaces between secondary gil lamellae.HGL: Hyperplasia in Gill Lamella

PLATE-2

Histopathological Changes -LIVER- LABEO ROHITA . Exposed to BIFENTHRIN 10% EC

FIG-A
CONTROL LIVER

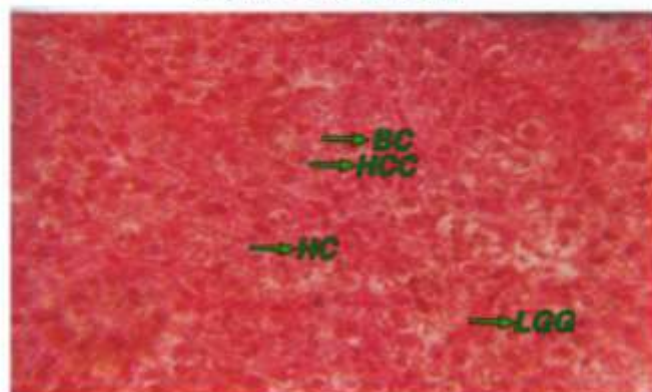


FIG-B
EXPOSED LIVER

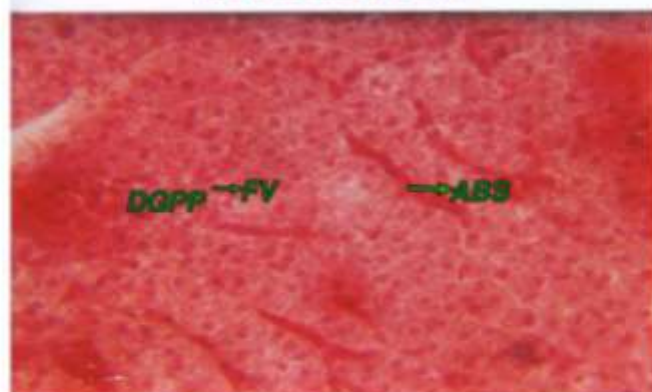


FIG.A Normal structure of Liver in Labeo rohita, Haematoxy/Eosin Stain (HE),X400
HC: Hepatic Cells HCC: Hepatic Cell Cords BC: Bile Canaliculus
LGG: Lipid and Glycogen Granules

FIG.B Liver of Labeo rohita exposed for 10 days sublethal concentration of
Bifenthrin 10% EC Haematoxylin/Eosin Stain (HE),X400
FV: Formation of Vacuoles ; intercellular empty spaces DGPP: Degenerative
Changes in peripancreatic tissue ABS: Appearance of Blood streaks among hepatocytes

PLATE-3

Histopathological Changes -KIDNEY -LABEO ROHITA . Exposed to BIFENTHRIN 10% EC

FIG-A
CONTROL KIDNEY

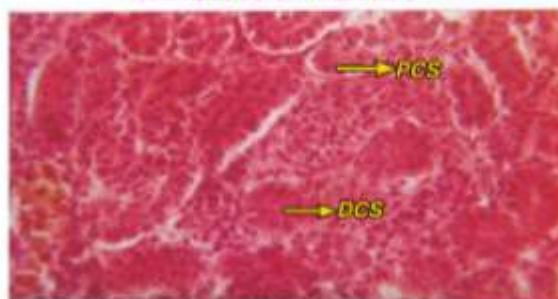


FIG-B
EXPOSED KIDNEY

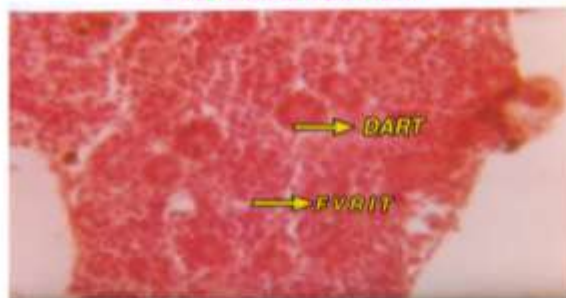


FIG.A Normal Kidney Structure in *Labeo rohita*, Haematoxylin/Eosin Stain (HE), X400
PCS: Proximal Convoluted Segment DCS: Distal Convoluted Segment

FIG.B Kidney of *Labeo rohita* exposed for 10 days to sublethal concentration of Bifenthrin 10% EC Haematoxylin/Eosin Stain (HE), X400
FVRIT: Formation of Vacuoles in the Renal Interstitial Tissue
DART: Degeneration and Atrophy in Renal Tubules

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