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Dichlorvos Induced Histological and Histochemical Alterations in the Gill of Fresh Water Fish, Channa gachua (F. Hamilton).

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Abstract: ***Indiscriminate use of pesticides has become a serious problem among human and ambient environment. Dichlorvos is highly toxic organophosphate insecticide used to control agricultural and domestic pests. Present study was aimed to assess the histopathological alterations in the gills of fresh water fish, Channa gachua after acute exposure to dichlorvos. After short term (96 hrs.) exposure degenerative changes like epithelial lifting, increased number of mucous cells, hyperplasia of epithelial cells, hypertrophy, fusion of secondary gill lamellae, degeneration of central supporting material and hemorrhage was observed in the gill of fish. The histochemical analysis of mucosubstances was carried out in the epithelial cells and mucous cells in the gills of control fish and in fishes exposed to lethal concentration of dichlorvos for 96 hrs. On the basis of histochemical staining reactivities the mucous cells could be divided in to three types such as M1, M2 and M3 mucous cells. The histochemical staining reactivities revealed fluctuations in mucosubstances in epithelial cells while neutral mucosubstances, sulfo and sialomucins was increased in mucous cells in gills of fishes. From these observations it is concluded that the pesticide dichlorvos is toxic to fish and brings significant physiological alterations.***
Keywards: Pesticide, acute exposure, histopathology, mucous cells, mucosubstances

I. INTRODUCTION

Water is a universal solvent and prime need of every living organism. But now day water pollution has become a serious problem. Everybody knows that pollution refers to the contamination of water by harmful substances. Rapid industrialization and green revolution introduced large variety of chemical in to the water bodies (Rithamma and Joseph, 2014). In modern agricultural practices pesticides has become an indispensable part to control the insect pests. Pesticides are the active chemical compounds which are used in great extent for pest control in domestic and agricultural field.

Dichlorvos is the organophosphate pesticide which is used to control pests on vegetable crops, cash crops like sugarcane, store grain pests, domestic pests etc. Dichlorvos inhibits the acetylcholine esterase in insects. Due to its low persistence and high effectiveness it is used extensively. Fish is good indicator of water pollution and has important role in food chain. Physiological alterations in fish may indicate the status of environmental pollution.

Histopathology means the pathological alterations in normal cellular architechure of tissues, which indicate presence of toxic substances in the body of organism (Rithamma and Joseph, 2014). Pathological alterations due to toxic compounds are very important tool for assessing the status of fish health and future ecological impact. The effect of pesticides on histology of gill in numerous fishes have been reported by number of workers e.g. Bhuiyan et al. (2001) in fish, Channa punctatus exposed to sumithion, by Ortiz et al. (2003) in fish, Cyprinus carpio and Barbus exposed to lindane, by Velmurugan et al. (2009) in Cirrhinus mrigala exposed to dichlorvos, by Olufayo and Alade, (2012) in Heterobranchus bidorsalis exposed to cypermethrin, by Somdare et al. (2015) in Clarius gariepinus exposed to fenthion and by Binukumari et al. (2016) in Labeo rohita exposed to monochrotophos. Histochemistry is capable tool for revealing the sensitivity and effects of toxicant on the aquatic biota. Histochemical characterization of mucosubstances in gills of numerous fishes have been studied by Sabaoia-Moraes et al. (1996) in Poecilia vivipara, Sarasquete et al. (1998) in Solea senegalensis, Diaz et al. (2001) Micropogonias furnieri, Cinar et al. (2008) in Cyprinus carpio, Mir and Channa (2011) in Schizothorax curvifrons, Reddy and Banerjee (2013) in Clarias batrachus, Senol (2014) in Tinca tinca. Mode of action of pesticides is different according to nature of pesticide, fish species, resistance capacity of fish and a type of tissue. So the present investigation was aimed to study the histopathological and histochemical alterations in gill of fish, *C. gachua* exposed to dichlorvos at acute exposure period.

II. MATERIAL AND METHODS

A. Procurement Of Test Fish

The fresh water fish, *C. gachua* were selected for the present experimentation keeping in mind their edible value and availability throughout the year. The fishes were collected from river Krishna near Karad city and brought to laboratory. Fishes were treated with 1% KMnO4 solution to eradicate any infection and acclimatized at laboratory condition for ten days. The healthy fishes having average length 15 ± 1 cm and weight about 50 ± 5 gm. were selected for experimentation.

B. Pesticide

The organophosphate pesticide, dichlorvos purchased from local agro chemist shop was used for the present study.

C. Experimental set up

For determination of LC_0 and LC_{50} well acclimatized fishes were exposed to different concentrations viz. 5, 7.5, 10, 12.5, 15, 17.5 and 20 ppm conc. of dichlorvos for 96 hrs. LC0 and LC 50 concentrations were calculated. Under the experiment healthy fishes were divided in to three groups each group containing ten fishes. Group 1^{st} was considered as control and group 2^{nd} and 3^{rd} as experimental groups. Fishes in the experimental groups were exposed to LC0 and LC50 conc. of dichlorvos for 96 hrs.

D. Histological and Histochemical Methods

The control as well as experimental fishes were taken out of aquaria at the end of each exposure period and sacrificed. The gills were dissected out and cut into small pieces each about 2-3 mm in size and immediately fixed in 2 % calcium acetate in 10% formalin (CAF) at 4 ^{0}C. After fixation and washing (24 hrs. each) the pieces were dehydrated through alcohol grades for 30 minutes in each grade, cleared in xylene and embedded in paraffin wax (M.P. 58- 60 ^{0}C). The sections were cut at 4-5 µ thick. The sections were stained with hematoxylin and eosin dehydrated cleared and, mounted in D.P.X. and used for histological and histopathological observations. Remaining sections were used for histochemical techniques for characterization of mucosubstances.

III. RESULTS AND DISCUSSION

On the basis of mortality study LC0 and LC50 conc. of dichlorvos for the C. gachua was obtained 5 ppm and 12.5 ppm.

A. Histology Of Gills In Control Fish

Histological architecture of the gill of *C. gachua* was found identical to most of fresh water teleosts. There were four pairs of gills covered with an operculum. Each gill was supported by gill arch (Fig.1). Each of gill arch was provided with two rows of gill filaments only on its one side called primary gill lamellae (Fig. 1). These were supported by central bony gill rays projected from gill arch. The epithelium at the tip of primary gill lamellae was multilayered. The epithelial cells were mostly oval to flat, the nucleus was centrally located and the cytoplasm was homogenous and basophilic. The number of mucous cells was abundant in the epithelial lining towards the tip of the primary gill filament (Fig. 3). The primary gill lamellae possessed two rows of thin filamentous structure called secondary gill lamellae (Fig. 2), one row on either side of their axis. At the tip of primary gill lamellae, no secondary gill lamellae were seen. The secondary gill lamellae possessed a central blood sinuses lined and spanned by large oval to rounded pillar cells (Fig. 2).

B. Histopathological Alterations in Gills

Histopathological alterations produced in the gills of *C. gachua* exposed to LC0 and LC50 concentrations of dichlorvos for 96 hrs. are shown in Fig. 4 to 7.

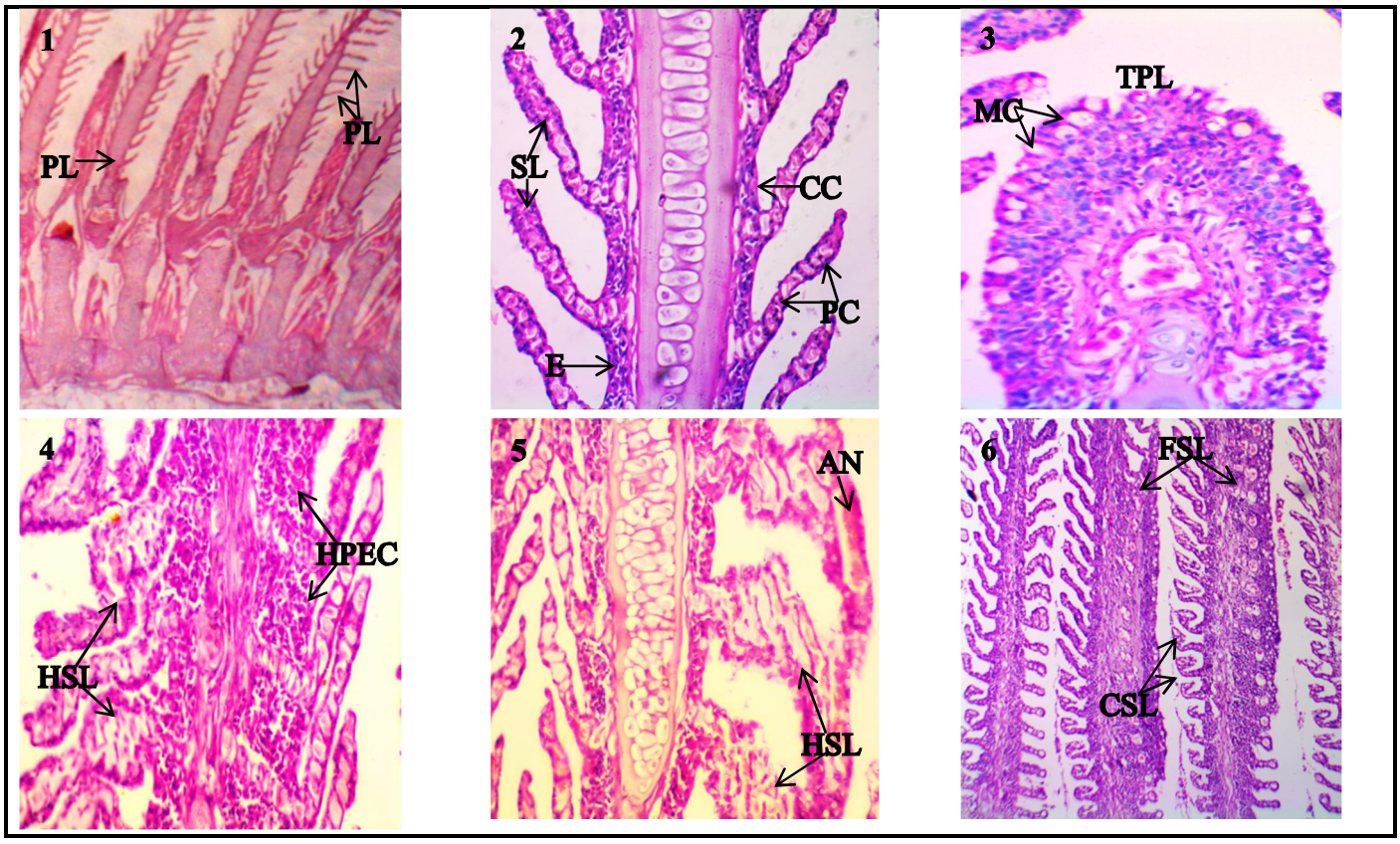
1) *5 ppm (LC0) Concentration:* At this concentration well-marked alterations were observed in gill of fish such as shortening and uniform bending of secondary gill lamellae, reduction in central supporting material at the base of primary gill lamellae (Fig. 4), hyperplasia of interlamellar epithelium of primary gill lamellae and thinning of central supporting structure of primary gill lamellae (Fig. 4), fusion of secondary gill lamellae at the bases, dilation of blood sinuses of secondary gill lamellae and aneurism observed at the tip of secondary gill lamellae (Fig.5), elongation and distortion of secondary lamellae (Fig. 4). curling and hypertrophy of secondary gill lamellae (Fig. 5), and detachment of epithelium from basement lamina (Fig. 5).
2) *12.5 ppm (LC50) Concentration:* After exposure of fishes to 12.5 ppm concentration for 96 hrs. severe degenerative changes were noticed in gill which are shown in Figs. 6 &7. Aneurism was seen at the tip of the secondary lamellae (Fig. 6). Significant swelling and hemolysis was noticed due to hypertrophy of some secondary gill lamellae with curling. Dilation of blood spaces

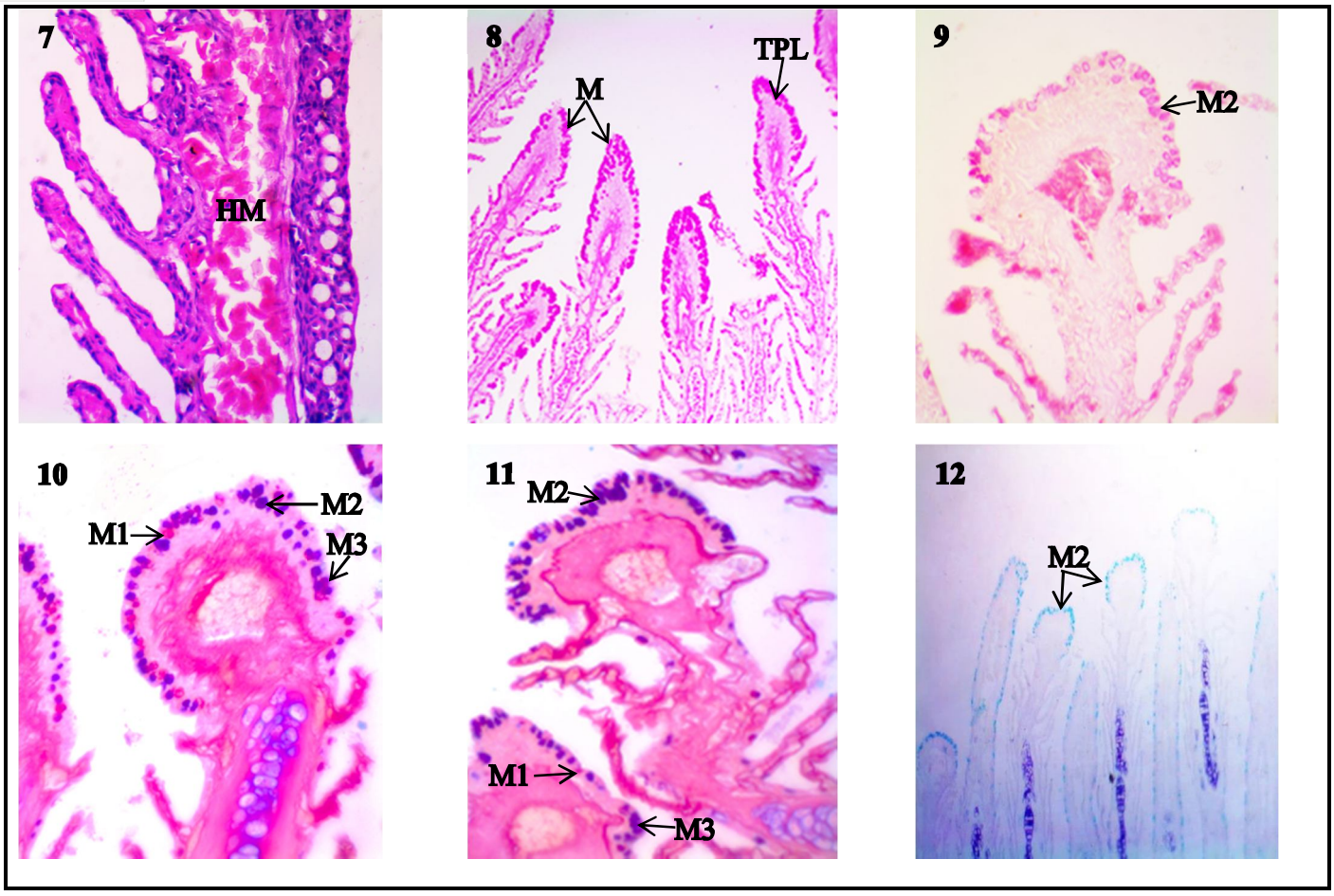
and vacuolation was also seen. Complete degeneration of central supporting material at apical region of the primary gill lamellae with hemorrhage (Fig. 7). In certain regions secondary gill lamellae were completely broken due to loss of cellular structure. Tip of primary lamellae enlarged with significant hematoma (Fig. 7). Mucous cells were significantly increased in number and size on the primary gill lamellae and excessive secretion of mucus was also seen. Mucous oozes out due to excessive secretion. Due to that fusion of adjacent secondary lamellae was seen (Fig. 6). Loss of pillar cells was observed in both elongated and shortened secondary gill lamellae and intercellular gaps filled with blood cells.

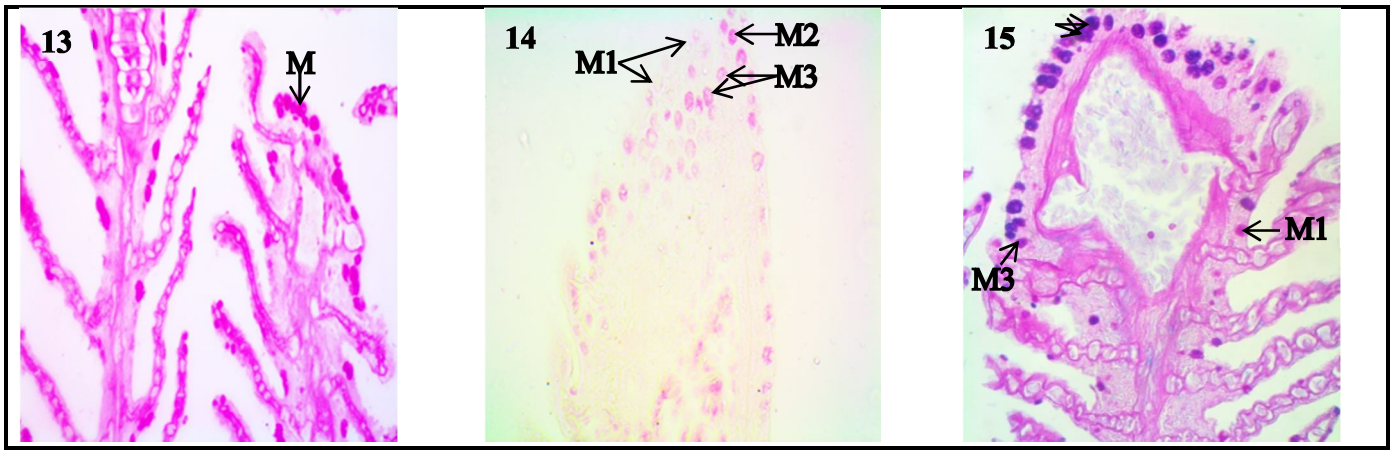
C. Histochemical Observations In The Gills

The histochemical analysis of mucosubstances was carried out in the epithelial cells and mucous cells in the gills of control fish and in fishes exposed to lethal concentration of dichlorvos for 96 hrs. The histochemical reactivities of mucosubstances in the gill epithelial cells and mucous cells of control fish and of fishes under experimentation are illustrated in microphotographs (Figs.8-21) and recorded in table No. 1 according to staining intensities (++++ - intense, +++ - moderate, ++ - weak,+ - poor, ± - trace and – - negative).

1) *Control fish:* The mucous cells were found distributed in the epithelium of primary gill lamellae and secondary gill lamellae. These were more numerous at the tip of primary gill lamellae (Fig.8). On the basis of results obtained they could be divided in to M1, M2 and M3 mucous cells (Figs. 10&11). The obtained results are given in the table 1. In present study histochemical reactivities revealed the absence of glycogen in both the epithelial cells and mucous cells in control fish. The present histochemical study demonstrated presence of neutral mucosubstances in trace amount and only sulfomucins (acidic mucosubstances) in poor amount in the gill epithelial cells of control fish. However M1 mucous cells contained only neutral mucosubstances in moderate amount, M2 mucous cells contained sulfomucins (predominant) and carboxymucins (poor) and M3 mucous







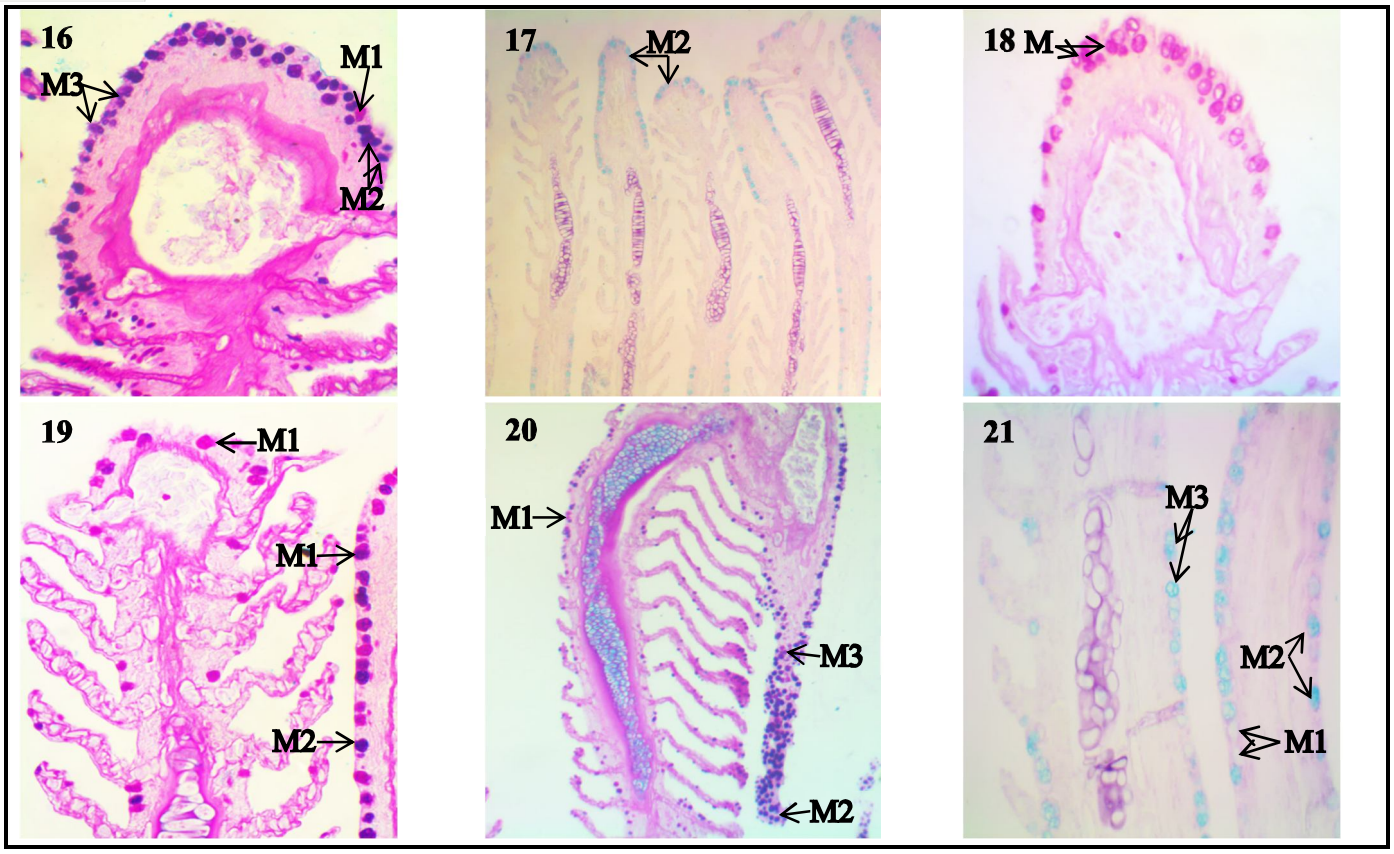


Fig.1,**2 &3**. L.S. of gills in control fish. HE (100X, 400X), **Fig.4 &5**. L.S. of gills in fish exposed to 5 ppm. HE (400X), **Fig.6&7**. L.S. of gills in fish exposed to 12.5 ppm. HE (400X), **Fig.8**. L.S. of gills in control fish. PAS (100X), **Fig.9**. L.S. of gills in control fish. P-PAS (400X), **Fig.10**. L.S. of gills in control fish. AB pH 1.0-PAS (400X), **Fig.11**. L.S. of gills in control fish. AB pH 2.5-PAS (400X), **Fig.12**. L.S. of gills in control fish. AF-AB pH2.5 (100X), **Fig.13**. L.S. of gills in fish exposed to 5 ppm. PAS (100X), **Fig.14**. L.S. of gills in fish exposed to 5 ppm. P-PAS (400X), **Fig.15**. L.S. of gills in fish exposed to 5 ppm. AB pH 1.0-PAS (400X), **Fig.16**. L.S. of gills in fish exposed to 5 ppm. AB pH 2.5-PAS (400X), **Fig.17**. L.S. of gills in control fish. AF-AB pH2.5 (100X), **Fig.18**. L.S. of gills in fish exposed to 12.5 ppm. PAS (400X), **Fig.19**. L.S. of gills in fish exposed to 5 ppm. AB pH 1.0-PAS (400X), **Fig.20.** L.S. of gills in fish exposed to 5 ppm. AB pH 2.5-PAS (400X), **Fig.21**. L.S. of gills in control fish. AF-AB pH2.5 (100X). CC-Chloride cell, CSC-Central supporting material E–Epithelium, AN-Aneurism, MC-Mucous cell, PC-Pillar cell, PL-Primary gill lamellae, SL-Secondary gill lamellae, TPL- Tip of primary lamellae, CSL-Curling of secondary lamellae, FSL-Fusion of secondary lamellae, HM-Hemorrhage, HPEC-Hyperplasia of epithelial cells, M-Mucous cell, M1-Mucus cell type 1, M2-Mucus cell type 2, M3-Mucus cell type 3.

Table 1. Comparative histochemical reactivities of mucosubstances in epithelial cells and mucous cells in the gill of control fish and fish exposed to lethal concentrations of dichlorvos for 96 hrs.

Histochemic-al Reactions	Control				Fish exposed to lethal concentrations of dichlorvos for 96 hrs.							
					5 ppm (LC0)				12.5 ppm (LC50)			
	Epi. cells	**M1**	**M2**	**M3**	**Epi. cells**	**M1**	**M2**	**M3**	**Epi. cells**	**M1**	**M2**	**M3**
PAS	+±P	+++P	+++P	+++P	++P	++++± P	++++P	+++±P	+±P	++++ P	+++P	+++P

P-PAS	+P	-	+++P	++P	±P	-	++++P	++±P	+P	-	+++P	++P
D-PAS	++±P	+++P	+++P	+++P	++P	+++±P	++++P	+++±P	++±P	++++P	+++P	+++P
AB pH 1.0	+B	-	++P	++B	±B	-	+++B	++±B	+B	-	++P	++B
AB pH 1.0 - PAS	++±PB	+++P	+++B	+++PB	++PB	+++±P	++++PB	+++±PB	++±PB	++++P	+++B	+++PB
AB pH 2.5	+B	-	+++B	+++B	±B	-	++++B	++±B	+B	-	+++B	+++B
AB pH 2.5 - PAS	++±PB	+++P	+++B	+++PB	++PB	+++±P	++++PB	+++±P	++±PB	++++P	+++B	+++PB
C. I.	+B	-	+++B	++B	±B	-	++++B	++±B	+B	-	+++B	++B
C.I.- PAS	++±PB	+++P	+++B	+++PB	++PB	+++±P	++++PB	+++±P	++±PB	++++P	+++B	+++PB
AF	+P	-	++P	++P	±P	-	+++P	++±P	+P	-	++P	++P
AF - AB pH 2.5	+P	-	+++BP	++P	±P	-	++++BP	++±P	+P	-	+++BP	++P
Acid hydrolysis	+B	-	++B	+++B	±B	-	+++B	++±P	+B	-	++B	+++B
Sialidase - AB pH 2.5	+B	-	++B	+++B	±B	-	+++B	++±P	+B	-	++B	+++B
Hyaluronidase-AB pH2.5	+B	-	+++B	+++B	±B	-	++++B	++±P	+B	-	+++B	+++B
Pepsin - AB pH 2.5	+B	-	+++B	+++B	±B	-	++++B	++±P	+B	-	+++B	+++B

M1-M1 mucous cells, M2-M2 mucous cells and M3-M3 mucous cells. Staining intensities ++++ = intense, +++ = moderate, ++ = weak,+ = poor, ± = trace and – = negative.

cells elaborated both neutral mucins (poor) and sulfomucins (weak) but absence of glycogen.

2) Exposed fishes

a) 5 ppm (LC0) Concentration: The histochemical reactivities in the epithelial cells of gill of fish exposed to this concentration of dichlorvos are illustrated in microphotographs (Figs.13-17). The histochemical results obtained in these cells showed only trace amount of sulfomucins and poor to weak amount of neutral mucosubstances. M1 mucous cells in the gill of this fish, elaborated only moderate to intense amount of neutral mucosubstances, M2 mucous cell also elaborated moderate amount of sulfomucins and poor amount of sialomucins and M3 mucous cells contained neutral mucosubstances in weak amount and sulfomucins in poor to weak amount.

b) 12.5 ppm (LC50) Concentration: The histochemical staining reactivities revealed (Figs. 18-21) poor amount of sulfomucins (Acidic mucosubstances) and trace amount of neutral mucosubstances in the gill epithelial cells but no glycogen. However, M1 mucous cells in this fish contained intense amount of neutral mucosubstances, M2 mucous cells contained weak amount of sulfomucins and poor amount of sialomucins and M3 mucous cells contained acidic mucosubstances in weak amount in the form of sulfomucins and neutral mucosubstances (poor amount).

IV. DISCUSSION

The histopathological study gives information about toxicant severity, extent of tissue injuries, damages, and subsequent physiological disfunctioning because of the toxicant stress. Gill is one of the prime organs in the body of fish. Histological structure of gills and their functions has become a matter of great interest (Patil et al., 2012). The gills in fishes plays an important function such as acid-base balance, gaseous exchange, osmoregulation and ionic regulation (Fosket et al., 1983 and Laurent et al., 1994) and remain in close contact with external environment therefore considered as primary target organ. The primary lamellae through which gaseous exchange takes place between blood and external medium. The chloride cells are responsible for ionic exchanges which are usually distributed on the secondary gill lamellae (Deore 1993). In present study severe histopathological alterations were observed in the gills due to the action of lethal concentrations of dichlorvos.

Number of earlier workers also reported that the harmful toxicants could affect the histological structure of gills at cellular and subcellular levels in many fishes exposed to different toxicants even at low concentration. Jiraungkoorskul et al. (2002) in Nile tilapia (Oreochromis niloticus) exposed to glyphosate, Das and Mukharjee (2002) in Labeo rohita exposed to

hexachlorocyclohexane, Khan et al. (2011) in catfish, Clarias batrachus exposed to Lead nitrate, Doaa and Hanan (2013) in Oreochromis niloticus exposed to lead acetate, Sharma and Sharma (2016) in Labeo rohita exposed to endosulphan and lindane. In most studies histological alterations were characterized by damage in the epithelial cells, epithelial hyperplasia, lamellar swelling, and fusion of secondary lamellae, necrosis, aneurysm and excessive mucous secretion. According to Machado and Fanta (2003) and Kumar et al. (2010) epithelial hypertrophy may increases the distance between the toxicant and blood stream there by causing impaired oxygen uptake. The hyperplasia was characterized by cellular proliferation in the inter lamellae region leads in to lamellar fusion decreasing the surface area for gaseous exchange. Increase in number of mucous cells and excessive mucous secretion might be the compensatory response against the pesticide toxicity that develops a defense mechanism against pesticides. In present study significant hemorrhage was seen due to the loss of pillar cells, the blood pressure in fish is controlled by pillar cells (Evans et al., 2005) and due to loss of pillar cells the flow of blood exerted on the epithelial cells it may results into the distribution of blood cells or hemorrhage into the lamellae. Curling and distortion of secondary gill lamellae might be due to the degeneration of pillar cells and epithelial lining due to O_2 deficiency. Deshpande (2000) stated that histopathological changes may be the result of partially metabolized pesticides or their active metabolites reaching to the gills through the circulatory pathway. The present findings are well in accordance with the findings.

The studies on histochemistry assess the quantity of mucosubstances at the cellular level. This technique helps in analysis and localizing the biochemical constituents and mucosubstances as well as molecular changes at cellular level (Pathan, 2009) in normal and also in toxicant stressed organism. Different types of mucosubstances have been described by Carmiganani and Zaccone (1974) in fish, T. mormorata and T. ocellata, Yamada and Yokote (1975) in A. japonica, Ingale (1981) in Katarna, shingati, kharpa and sheengati and reported that the harmful toxicants interferes with the quantity of mucosubstances. In present investigation epithelial cells exhibited neutral mucosubstances and sulfomucins while absences of glycogen. However three types of mucous cells were found in the gills and histochemical reactivities revealed the presence of neutral mucosubstances, sulfo and sialomucins but absence of glycogen. After exposure to pesticide the production of mucosubstances was reduced in epithelial cells while increased in mucous cells. Similar findings also reported by Patil et al. (2012) in fish, Channa punctatus exposed to malathion. Vigario and Saboia-Morais (2014) reported four types of mucous cells in Poecilia vivipara and increment in mucosubstances after exposure of 2,4-D herbicide. Some controversy is also reported by Bagale et al., (2014) reported reduction in neutral mucosubstances while increased acidic mucosubstances in the mucous cells of the gill of fish, Oreochromis niloticus exposed to sodium fluoride. Mucins are glycoproteins, which protects the gill in fishes. According to Vigario and Saboia-Morais (2014) viscosity of mucous is closely related to osmotic balance and gas exchange. So increased mucous production may leads into formation of plaques that could inhibit gaseous exchange and osmoregulation from toxic medium. Mucus forms the barrier between toxic medium and protects delicate organs like gill. Therefore, it is assumed that the fishes may create protective phenomenon against dangerous effects of the pesticide by secreting excessive mucus.

V. CONCLUSION

The histopathological and histochemical alterations produced in the gill of fishes indicate the toxic nature of dichlorvos at short term exposure. Thus, from the present study it is concluded that the pesticide intoxication disturbs the functional activity of organ system and cells with consequential changes in physiology of fish, which affect the nutritional value of fishes and deteriorating the food value of fish. It will also be a great danger to human being due to continuous consumption of such fish from contaminated water bodies.

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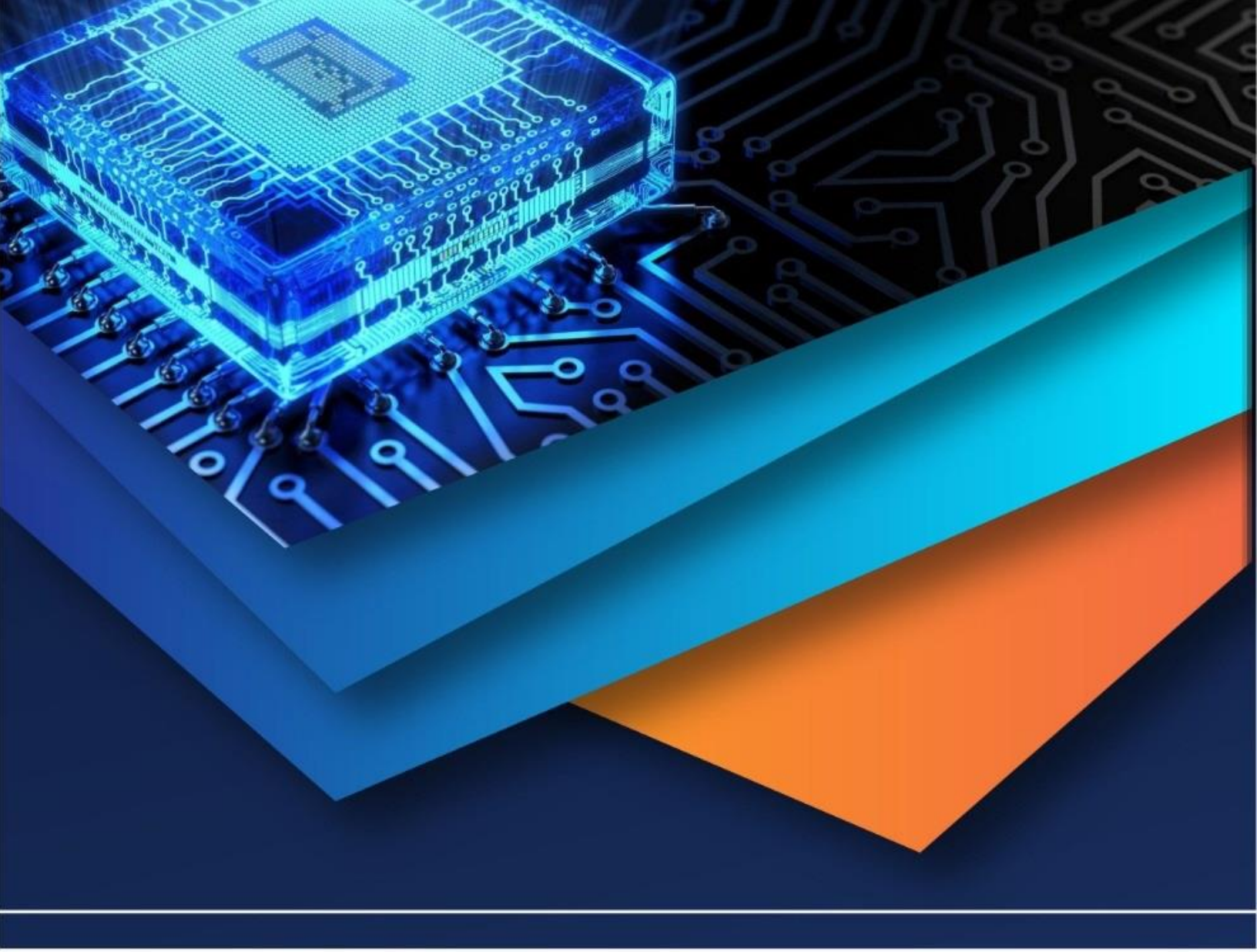

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