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ORIGINAL ARTICLE

# HISTOCHEMICAL EFFECT OF ENDOSULFAN ON DIFFERENT LAYERS OF ANTERIOR INTESTINE OF *CYPRINUS CARPIO* UNDER FIELD CONDITIONS

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**Abstract:**

To evaluate the histochemical effect of endosulfan (EDS) at concentrations 0.0015 and 0.002-ppm in different histological layers of the anterior intestine of a fresh water teleost, *Cyprinus carpio*; we estimated protein, carbohydrate and acid mucopolysaccharide contents at an interval of 10 and 20 days and compared them with one another and the control one. Our results showed that protein content after initial increase (ten days) tends to decrease (twenty days) showing recovery except longitudinal muscle and serosa where it continued to decrease; carbohydrate content increased in submucosa and circular muscle (0.0015ppm/10 & 20 days), while others showed reversion in 20 days, but at 0.002-ppm circular muscle showed extreme augmentation, while others showed slight increase; and acid mucopolysaccharide, against 0.0015ppm, showed slight increment in mucosal fold in 20 days and serosa and longitudinal muscle showed recovery (20 days) after showing decrement (10 days), while against 0.002ppm only serosa showed decrement and mucosal fold increment, while others exhibited first decrease followed by increase. Our results showed that changes in longitudinal muscle and serosa (protein), circular muscle (carbohydrate), serosa, and mucosal fold (acid mucopolysaccharide) might serve as primary biomarker and may prove as indispensable tool in diasoning the impact of the pesticide.

**Key Words:** Intestine, Protein, Carbohydrate, Mucopolysaccharide, Fish, Endosulfan

## INTRODUCTION

Endosulfan (EDS) (6,7,8,9,10,10-hexachloro-1,5,5a,6,9, 9a-hexahydro-6,9-ethano-2,4,3-benzodioxa thiepin-3- oxide) is a broad spectrum non systemic organochlorine insecticide. Technically, endosulfan contains two biologically active diastereoisomers namely α-endosulfan and β-endosulfan (Hayes and Laws 1991) in a ratio of 70 % to 30 %. With a half-life of 1 month to 6 months in water under anaerobic conditions (ATSDR 2000); in soil about four years under aerobic conditions (US EPA 2007) and even up to 6 years (GFEA-U 2007) and water solubility of log $K_{ow}$ of 3.83 for α- and 3.52 for β -isomers (ATDSR, 2000), Endosulfan is extremely toxic to fish (Naqvi and Vaishnavi, 1993) and has caused monolithic fish killing in USA, India, Benin, Sudan and Germany (EJF 2009). EDS toxicity through water has been studied in many species and its lethality was usually found at concentrations between 0.4 and 8 µg/L (EFSA Journal 2011). Succeeding EDS application to control tsetse fly in Botswana, an increased freshwater fish fatality and contaminated piscine tissue was noticed (Mathiessen*et al.* 1982). Aerial spraying of EDS against tsetse flies, in Zimbabwe, resulted in the contamination of non-target birds, reptiles, amphibians, fish, and some mammals in the Zambezi River valley (Glin *et al* 2006). In India, EDS is a commonly used pesticide, especially in rice and cotton (Jayashree & Vasudevan 2007), making rice field fish culture susceptible to contamination. Although effect and mechanism of endosulfan toxicity was comprehensively studied (Hundet et al, 2012; Scremin et al, 2011; Naqvi and Vaishnavi, 1993; Braunbeck and Appelbaum,1999; Ballesteros, 2009; and many more), but data pertaining to histochemical effect is scarce. Histochemical investigations have proved to be a sensitive tool to detect direct effects of chemical compounds at the target sites within a specific organ. This study is aimed to determine the histochemical effects of a single dose of water borne sublethal concentrations of endosulfan in various histological layers of the anterior part of intestine in a teleost fish, *Cyprinus carpio*.

## MATERIAL AND METHOD

**3.1) Chemicals:** Technical grade 35% EC EDS (Acaricides; mixture of two stereoisomers — α- and ß-EDS, in a ratio of 4:1, manufactured by Excel Industries Ltd. Bombay) was obtained from the local market and its solution was prepared using acetone having negligible toxicity for fish (12000 ppm).

**3.2) Preconditioning of experimental animals and treatment:**

*Cyprinus carpio* were collected from the "Chandemari fish farm" and treated with 0.1% potassium permanganate solution for 15 minutes to get rid of dermal infections. All the fishes were kept for acclimatization in polymer fibre aquaria for 7 days.

Fishes were introduced after 24 hours of the solution preparation as EDS reacts with water and absorbs dissolved oxygen, which may be responsible for the death of fishes. So, oxygen is supplied with the help of aerator to maintain $O_2$ level before putting the fishes in experimental water. Healthy fishes, measuring 12 to 14 cm and weighing approximately 50-60 gms, were selected for experimentation. A set of 10 fishes were transferred into three differently maintained aquaria with 200 L water quantity, out of which one contained dechlorinated water and the other two contained 0.0015 and 0.002 ppm EDS concentration. Five fishes were sacrificed after 10 days and rest of the fishes was sacrificed after 20 days. The fishes were fed daily (with standard fish food) during the entire experimentation period. The intestine of the sacrificed fishes was dissected, cut into small pieces and washed in normal saline. The pieces of intestine were fixed in 10% formalin and 6 to 8 µ thick section was cut for histochemical studies of bromophenol blue test for protein, PAS tests for carbohydrate and alcian blue tests for acid mucopolysaccharides to assess the qualitative impact.

**3.3) Water quality parameter**
The water quality parameters like temperature, pH, dissolved oxygen (DO), free carbon dioxide ($CO_2$), total alkalinity, Temporary hardness, permanent hardness, chloride were checked on 1st, 5th, 10th, 15th, and 20th day by standard methods and found to be within the recommended range for carp rearing.

**Result**
**4.1) General Protein:** Bromophenol Blue Test

Table 1. INTESTINE PROTEIN

| EDS con./d → / Layers ↓ | | Control (fig.1) | 0.0015ppm/10d (fiG. 2) | 0.0015ppm/20d (fig.3) | 0.002ppm/10d (fig. 4) | 0.002ppm/20d (fig. 5) |
|---|---|---|---|---|---|---|
| Serosa | | SPR | SPR | PR | SPR | WPR |
| Longitudinal Muscle | | SPR | SPR | PR | SPR | WPR |
| Circular Muscle | | WPR | MPR | WPR | SPR | PR |
| Submucosa | | WPR | SPR | WPR | SPR | WPR |
| Mucosal Fold | Tip | MPR | PR | MPR | MPR | MPR |
| | Middle | MPR | PR | MPR | MPR | MPR |
| | Base | MPR | PR | MPR | MPR | MPR |
| Tunica Propria | | WPR | PR | PR | SPR | SPR |

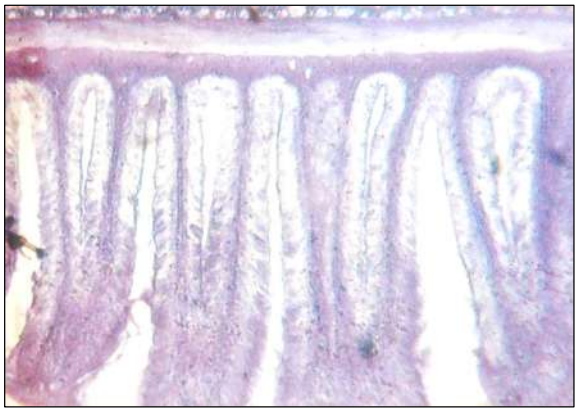

**Fig. 2 T S of control intestine of *C carpio* (Bromophenol Blue Test) X-100.**

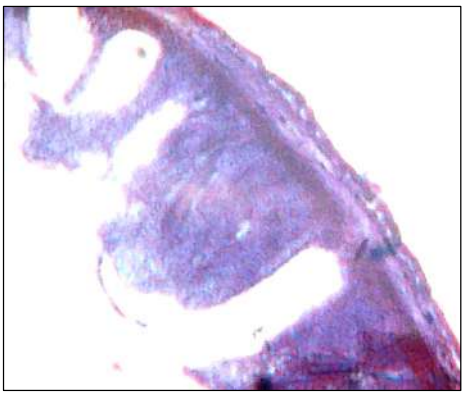

**Fig. 1 T S of intestine of *C. carpio* following 10days exposure to 0.0015 ppm EDS; X- 100.**

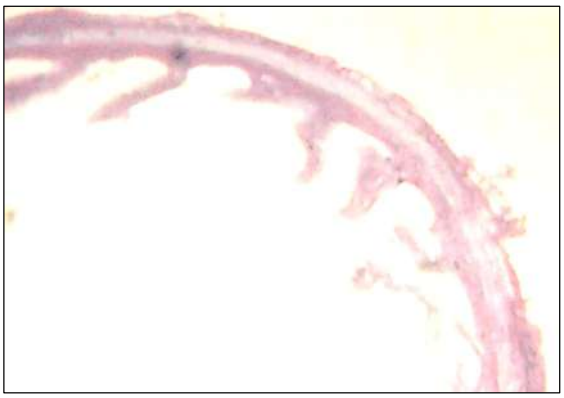

**Fig. 3 T S of intestine following 20 days exposure to 0.0015 ppm EDS(*C. carpio*); X- 100**

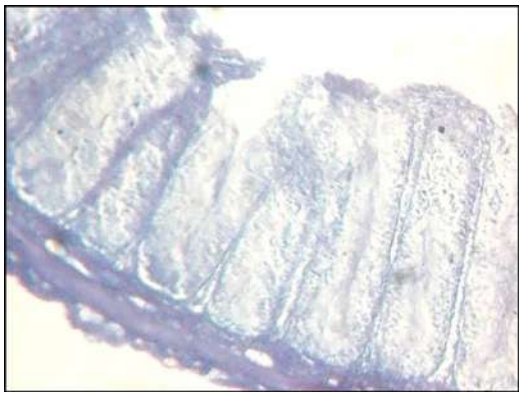

**Fig. 4 T S of intestine after 10 days exposure to 0.002 ppm EDS (*C. carpio*); X – 100.**

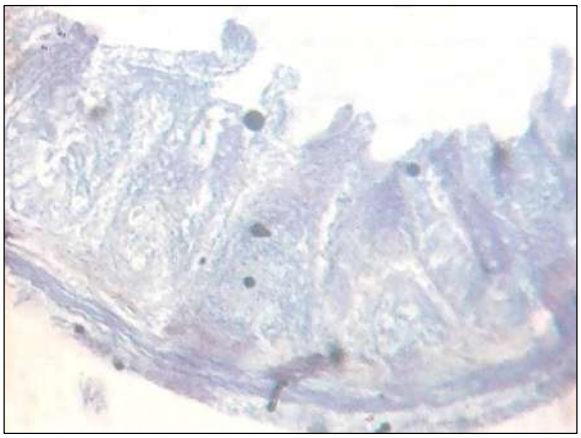

**Fig. 5 T S of intestine after 20 days exposure to 0.002 ppm EDS(*C. carpio*) X – 100.**

**4.3) General Carbohydrate:** PAS Test

| Table 1. INTESTINE CARBOHYDRATE | | | | | | |
|---|---|---|---|---|---|---|
| EDS con./d → / Layers ↓ | | Control (fig.1) | 0.0015ppm/10d (fiG. 2) | 0.0015ppm/20d (fig.3) | 0.002ppm/10d (fig. 4) | 0.002ppm/20d (fig. 5) |
| Serosa | | SPR | SPR | PR | SPR | WPR |
| Longitudinal Muscle | | SPR | SPR | PR | SPR | WPR |
| Circular Muscle | | WPR | MPR | WPR | SPR | PR |
| Submucosa | | WPR | SPR | PR | SPR | WPR |
| Mucosal Fold | Tip | SPR | PR | MPR | MPR | MPR |
| | Middle | PR | PR | MPR | MPR | MPR |
| | Base | SPR | PR | MPR | MPR | MPR |
| Tunica Propria | | WPR | PR | PR | SPR | SPR |

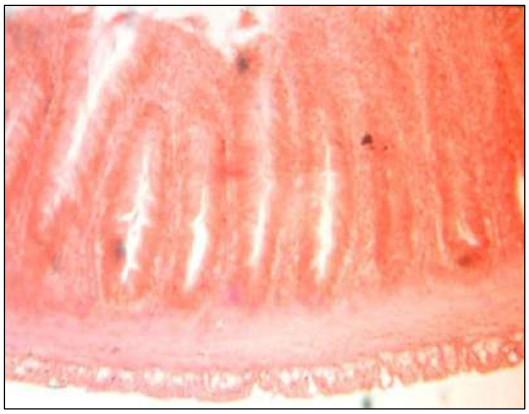

**Figure 6 T S of intestine of control *C. carpio* PAS test X – 100.**

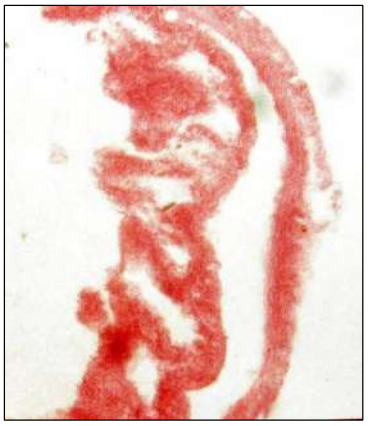

**Figure 7 T S of intestine (PAS) after 20 days EDS exposure to 0.0015 ppm X- 100**

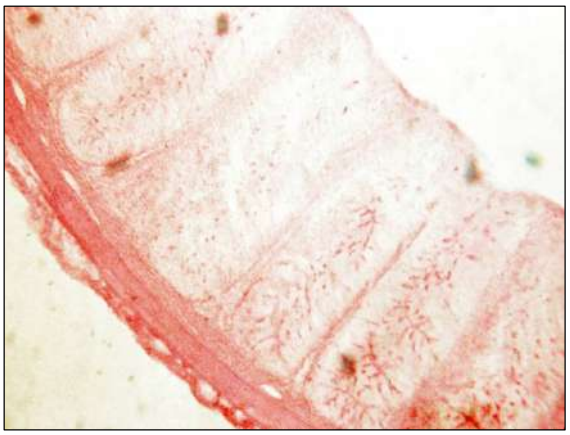

**Fig. 8 T S of intestine of *C. carpio* (PAS) after 10 days EDS exposure to 0.002 ppm X- 100.**

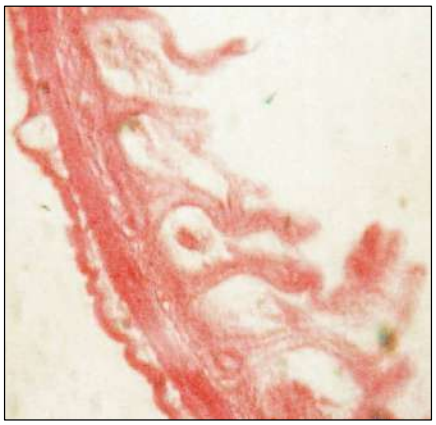

**Fig. 9 T S of intestineof *C. carpio* (PAS) after20 days EDS exposure to 0.002 ppm. X- 100.**

**4.4) Acid mucopolysaccharide: A**lcian **B**lue **T**est

Table 3. INESTINE ACID MUCOPOLYSACCHARIDE

| EDS con./days → / Layers ↓ | Control (fig. 10) | 0.0015ppm/10d (fig.11) | 0.0015ppm/20d (fig. 12) | 0.002ppm/10d (fig. 13) | 0.002ppm/20d (fig. 14) |
|---|---|---|---|---|---|
| Serosa | PR | WPR | PR | WPR | WPR |
| Longitudinal Muscle | PR | MPR | PR | WPR | PR |
| Circular Muscle | WPR | WPR | WPR | NEGATIVE | WPR |
| Submucosa | SPR | SPR | SPR | PR | SPR |
| Mucosal Fold | PR | PR | SPR | PR | SPR |

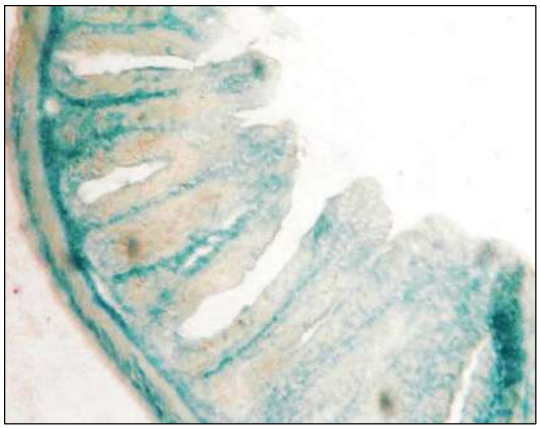

**Fig. 10 T.S. of intestine of untreated *C.carpio* Alcian Blue Test X-100.**

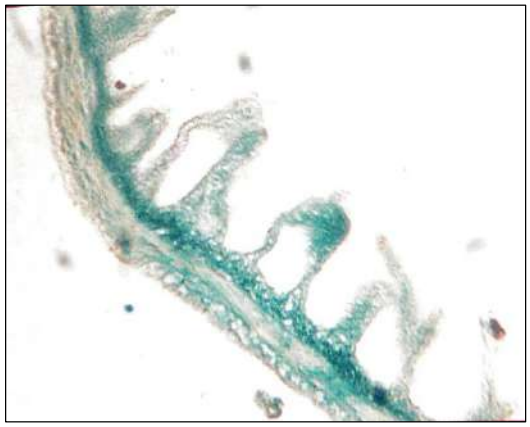

**Fig. 11 T.S. of intestine of *C.carpio* after 10 days exposure to 0.0015 ppm EDS (X-100).**

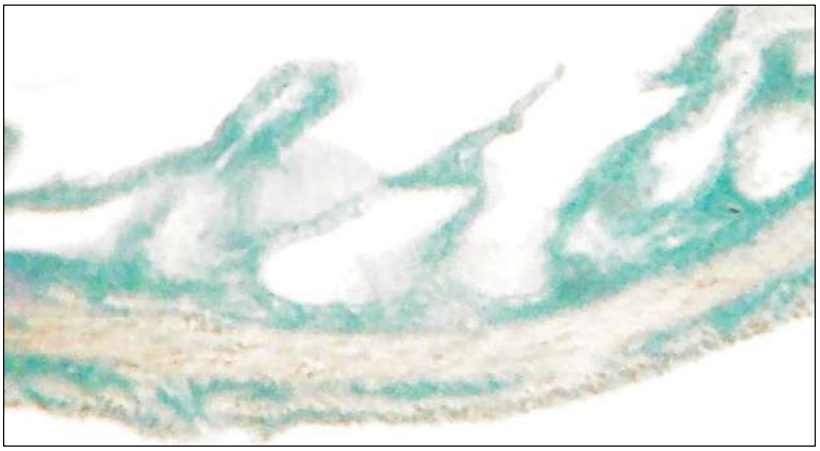

**Fig. 12 T.S. of intestine of *C. carpio* following 20 days exposure to 0.0015ppm EDS (Alcian Blue Test) X-100.**

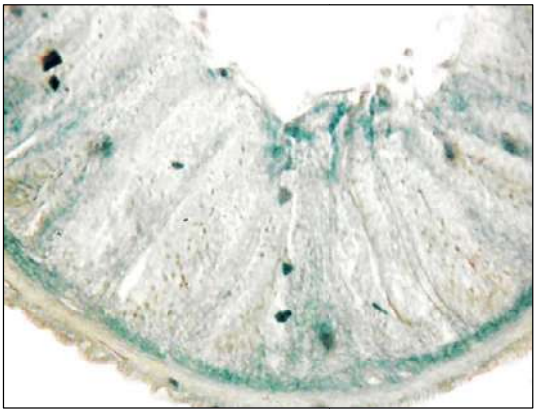

**Fig. 13 T.S. of intestine of *C.carpio* following 10 days exposure to 0.002 ppm EDS (Alcian Blue Test) X-100**

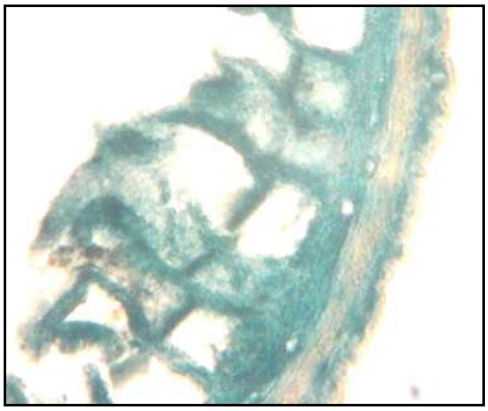

**Fig. 14 T.S. of intestine of *C.carpio* following 20 days exposure to 0.002 ppm EDS (Alcian Blue Test) X-100.**

**Abbreviations used:**

SPR : STRONG POSITIVE TEACTION
MPR: MODERATE POSITIVE REACTION
d : day
SPR □ PR □ MPR □ WPR
PR : POSITIVE REACTION
WPR: WEAK POSITIVE REACTION
ppm : pars per million

**DISCUSSION**

Protein contents in circular muscle, submucosa and tunica propria suggest that these layers are most sensitive to endosulfan at 0.002-ppm in comparison to 0.0015-ppm as it shows highest augmentation in 10 days and greatest deterioration in 20 days exposure towards recovery, whereas mucosal folds indicate least sensitivity and serosa indicates its most vulnerability (table 1 and fig. 1-5) at higher concentration. Dubale & Awasthi (1982) reported diminution of protein content in *Heteropneustes fossilis* following exposure to sublethal dimethol and recuperation of protein after 3 to 4 weeks of contact, whereas we have found that protein content enhanced slightly in most of the layers in 10 days, and reduced to that of control in 20 days EDS exposure, except in longitudinal and serosa where further decrement is recorded showing more sensitivity. Reduction in protein content is also observed by; Nammalwar (1984) in mullet *Liza macrolepis,* where a decrease in protein content due to DDVP and BHC; Najmi (1986) in different layers of the digestive tract of *Clarius batrachus* due to EDS intoxication; Mishra (1988) in the intestine of *Glossogobius guiris* due to increase in duration and intensity of pesticide and Somnath (1991) in the intestine of *Labeo rohita* after tannic acid exposure. The reduction in protein content showed variations following changes in dose and duration of exposure to the toxicants. Analogous results were also obtained by Medioros *et al*. (1970) in the digestive tract of *Pimitodus maculates*; Sharma & Seth (1980) in the digestive tract of *Channa motitius* ; Kasotia (1988) in the digestive tract and its associated glands in *Channa gachua*; Shah (1992) in the intestine of *Glossogobius giuris* and Rai (1993) in *Cyprinus carpio.* Hundet and Prabhat (2013) had also reported concomitant loss of protein, carbohydrate and acid mucopolysaccharide contents with dose and time along with a good recuperation in carbohydrate content in the liver of *Cyprinus carpio*. Decline in protein content in intestine is conceivably due to increased proteolytic activity to reimburse extra demand of energy in response to toxins or decreased protein synthesis as EDS weakens cortisol production (Bisson & Hontela 2002; Dorval et al 2003) or due to starvation caused by augmented mucous secretions, which results into rapid degradation of cytosolic protein (Anders Rills Kristensen et al.2008). Proliferation of lysosomes and their accumulation in supranuclear portions of enterocytes has been detected by T. Braunbeck *et al*.(1999) in cytological studies of intestine of *Cyprinus carpio* may also be attributed to protein degradation.

Carbohydrate content seems to be increasing at 0.0015ppm/10days exposure but revert back to control level against 20 days exposure except circular muscle and submucosa where instead of showing recovery it maintained its increased level; with higher dose of 0.0021ppm EDS, it increased in serosa, longitudinal muscle and circular muscle heavily and in submucosa slightly in 10 days exposure and keep it in 20 days treatment showing strong inhibitory metabolic effect, and mucosal fold showed first decrease and then increase in some portions towards recovery showing its less vulnerability to EDS (Table 2, and fig. 6-9). Thus, our result showed that carbohydrate metabolism is severely affectd with higher dose (0.002ppm) of endosulfan concentration. Diminution in mucosal fold may be attributed to increased anaerobic respirationin in response to endosulfan which may be analogous to sevin intoxication in *Channa punctatus* (Sastry & Siddiqui, 1982a). Slight qualitative boost in carbohydrate content in some intestinal sublayers (serosa, longitudinal and circular muscle) in *Cyprinus carpio* may be the result of reduced carbohydrate utilization or due to damages in intestine. Sastry et al. 1982b have reported reduction in intestinal glucose transport due to endosulfan. Our result does not support the data of Rajan (1990) who reported significant decrease in carbohydrate content of the *C. carpio* with increasing concentration of textile mill effluent and Shah (1992) who reported decrease of carbohydrate content with higher dose of dichlorvous in longer exposure as we have noted increase in carbohydrate content (table 2). Jebakumar et al (1990) studied the carbohydrate level of *L. thermalis* exposed to sublethal levels of Cypermethrin and found very little difference from control up to 9 days and thereafter they observed decreasing trend in the whole of the body. Ambrose et al. (1994) reported depletion in carbohydrate content in the liver and intestine of *C. carpio* after tannery intoxication.

In response to 0.0015ppm endosulfan treatment acid mucopolysaccharide showed recovery after decrease in serosa and longitudinal muscle, while submucosa and circular muscle exhibited no change and mucosal fold showed increase in twenty days exposure (table 3 figure 10-12); but with higher dose (0.002ppm) serosa showed decrease and longitudinal and circular muscle after showing diminution (10 days) regained to the control level and serosa exhibited moderate decrement in 10 - 20 days exposure and mucosal fold slight increase in twenty days exposure (table 3 and figure 10, 13 & 14). Increase in acid mucopolysaccharide content in mucosal folds of intestine may be a protective measure against the pesticide. Raised mucous can hinder the absorption of nutrients from intestine which ultimately leads to starvation and finally depletion of protein in enterocytes. Deposition of acid mucopolysaccharides is reported in *Clarias batrachus*, Shafi (1974), in *Channa striatus* Kulshreshtha & Jauhar (1984), in *Glossogobius giurus* by Mishra (1988), in *Channa gachua*, Kasotia (1988), Shah (1992) in *Glossogobius giuris* and Rai (1993) in *Cyprinus carpio.* Field studies in India (Tamilnadu and Punjab) and Australia (New South Wales) points to continual build-up of EDS in soil (Jayashree & Vasudevan 2007; Vig *et al* 2008; Kennedy *et al* 2001) which is alarming and requires immediate attention.

## CONCLUSION

Our studies clearly show that metabolism is altered in both ten and twenty days exposure that will affect piscine health and reduce their nutrient value. Several studies point to bioaccumulative, biomagnifying, carcinogenic and long distance travelling characteristics of endosulfan that is terrifying and indicates towards a grave ensuing danger for both target as well as nontarget organisms. Therefore, we should opt ecofriendly, nontransferable pesticide.

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