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Monocrotophos induced histopathological and biochemical Changes in gills, stomach and intestine of *Anabas testudineus* (Cuvier)

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Abstract

Monocrotophos, an organophosphate pesticide is used frequently in paddy fields of India. Although its impact of toxicity has been reported in many organisms, its effect on digestive and respiratory organs in *Anabas testudineus* is scanty. The Present investigation was conducted to evaluate the impact of histopathological and biochemical indices on freshwater fish *A.testudineus* exposed to sub-lethal concentration (45 ppm) of an organophosphorous pesticide monocrotophos (MT). Severe histoarchitectural and biochemical changes were observed in fishes exposed to monocrotophos when compared to fishes of control group. Exposure of fishes to the pesticide resulted in induction of histological abnormalities in gills, stomach and intestine. This was accompanied with reduction in total protein content and an elevation in catalase activity in gills, stomach and intestine. These structural alterations of the gills, stomach and intestine could affect respiration, digestion and absorption of nutrients which in turn could adversely affect growth and survival of the freshwater fish *A. testudineus*. The result of this investigation serves as a biomonitoring tool for the effects of organophosphorous pesticide MT on the aduatic biota.

Keywords: Catalase, Gills, Histopathology, Intestine, Monocrotophos, Protein, Stomach

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INTRODUCTION

Freshwater ecosystem has been polluted by continuous discharge of wastewater from agricultural practices. The wastewater contains various amounts of chemical substances, such as pesticides that results in potential health hazards to live stock, especially fishes. Fishes are among the group of non-targeted aquatic organism. They serve as bioindicator of water quality and the effect of pesticides can be studied by analysing the histoarchitecture and biochemical parameters of various organs (Rao and Pillai, 2001; Bartoskova et al., 2013; Faggio et al., 2014a, b; Gobi et al., 2018. A wide variety of pesticides and insecticides are used in agricultural fields (Sumithion, Lorsbon, Aluminium chloride, Endosulfan, Monocrotophos, Chlorpyrifos, Dichlorvos, Almix 20WP, Profenofos,

Diethylphthalate, Dimethoate, Phosalone). Among them monocrotophos, an organophosphorous pesticide is used by many for rice cultivation as it is cost effective.

Review of available literature on fish and environmental pollutants indicate that the sub-lethal doses of most of the pesticides cause behavioural changes, varying extent of histopathological injuries to different organs in fishes and biochemical changes; the amount of damages are usually dependent on dose, duration of exposure and type of pesticide (Tilak et al., 2005; Cengiz and Unlu, 2006; Mishra et al., 2006, 2008; Ghanbahadur and Ghanbahadur, 2012; Oguei et al., 2013; Senapati et al., 2013; Pandey et al., 2014; Ullah et al., 2014; Ullah and Zorriehzahra, 2015). Recently, Marigoudar et al. (2018), and Zahran et al. (2018) reported that chlorpyrifos induced patho-

logical lesions in gills, liver, eye and brain of *Oreochromis niloticus, Mugil cephalus* and *Chanos chanos.* It has been observed by many researchers that monocrotophos which reaches to the water bodies such as ponds and rivers affect fishes (Remia *et al.*, 2008; Velmurugan, 2007; Muthukumaravel, 2013). When it comes in contact with the internal organs, affects the histoarchitecture of the tissues and shows irreversible changes in the metabolic activities and ultimately affects the human beings consuming them as fishes are an indigenous, delicious and high quality meat species. Histological changes provide a rapid method to detect effects of irritants, especially chronic ones, in various tissues (Oruc, 2012).

Prolonged exposure of pesticides leads fishes to live in a stress condition which causes biochemical changes due to destruction of histology of the tissues. Secondary metabolites of pesticides induce severe biochemical and enzymatic changes in aquatic organisms (Rawat et al., 2002; Tiwari and Singh, 2009). Fishes exposed to different pesticides consume less oxygen as secondary metabolites reacts with oxygen present in water. Pesticide pollution alters several enzymatic pathways in organisms. Muley et al. (2007) in Labeo rohita and Prasanth et al. (2008) in Cirrhinus mrigala reported decrease in total protein and increase in free amino acids level in gill and liver in response to cypermethrin exposure and suggested high protein hydrolytic activity due to elevation of protease activity. Pesticides also affect the activity of enzymes and alter their level so they can be used as biomarkers. Reactive oxygen species (ROS) formed during biotransformation of xenobiotics damage cell structures via oxidation. To reduce the ROS effects, cell produce enzymes such as catalase involved in the antioxidant process (Van der Oost et al., 2003). Catalase is found nearly in all the organisms that catalyse the decomposition of H₂O₂. Antioxidant parameters are used as health indicators to detect the structural and functional status of fish under stress conditions (Moraes et al., 2007; Van der Oost et al., 2003).

The objective of the present study was to analyze and comprehend the acute toxic effect of the organophosphorous pesticide monocrotophos (MT) on histological alterations in gill, stomach and intestine and biochemical parameters in freshwater fish *Anabas testudineus*.

MATERIALS AND METHODS

A. testudineus a fresh water fish was selected for the study as it has economic value and easily available throughout the year. The fishes were collected from Central Institute of Fishery and Aquaculture (CIFA), Bhubaneswar, Odisha. The average length of fishes used for the study was 11 to 13 cm and weight 40 to 60 g. The fishes were

disinfected with 1% potassium permanganate solution. They were acclimatized to laboratory condition in large tanks (60x60x90 cm) for 20 days containing well aerated dechlorinated ground water. During acclimatization fishes were fed with fish pellets developed by CIFA. The physico-chemical characteristics of water at regular intervals were carried out in the laboratory throughout the study Table 1).

After acclimatization, fishes were divided into two groups. One group of 15 fishes i.e. control group was maintained in normal water in a tank containing 125 litres and the other group of 30 fishes i.e. experimental group was maintained in a separate tank containing 250 litres of water having 45ppm monocrotophos concentration (sublethal). The normal water and pesticide mixed water was renewed on every 2nd day in control group and experimental group tanks respectively during the period of study. Each day, the dead fishes if any were counted, recorded and removed from the tank immediately to avoid depletion of dissolved oxygen (DO) level which may adversely affect other fishes.

To study the effect of monocrotophos, the fishes of both control and experimental group were sacrificed on 10th and 20th day of the experiment. They were dissected under normal saline and gills, stomach and intestine were fixed in aqueous Bouin's fluid. Following fixation the tissues were dehydrated through graded series of ethanol, infiltrated in melted paraffin (60°C), embedded on paraffin block, and sectioned at 5um by using rotatory microtome. The tissue sections were spread on slides, rehydrated and stained with Ehrlich haematoxylin and eosin. Stained sections on slides were then mounted with Dextran Plasticizer Xylene (DPX) and covered with a cover glass. For biochemical studies, gills, stomach and intestine were taken out after dissection in normal saline on 10th and 20th day of both control and experimental groups for total protein content estimation by following standard method of Bradford (1976) and catalase activity by Aebi (1983).

RESULTS AND ANALYSIS

Histoarchitectural changes: The microscopic histoarchitecture of normal gills of *A. testudineus*

Table 1. Physico-chemical characteristics of water used for the study.

Parameters	Calculated value
Temperature	30±2°
Turbidity	6.7 silica units
pH at 30 ^o C	7.4±0.05
Total hardness (CaCO ₃)	198±6 (mg/L)
BOD	7 – 10 ppm
COD	Nil
Dissolved oxygen (DO)	5.5±0.5 mg/L

showed a row of long thin filaments, the primary lamellae projecting from the gill arch like the teeth of a comb. On its distal and ventral surface, there were the secondary lamellae which consisted of an envelope of epithelial cells, usually one layer thick, supported and separated by pillar cells arranged in rows. The gills of control group fishes had normal gill arch, primary gill lamellae and secondary gill lamellae and epithelial cells (Figs. 1 and 2). Exposure of fishes to sub-lethal dose of 45ppm concentration of monocrotophos for 10 days induced histopathological alterations like lamellar fusion, telanglectasia (marked dilation of terminal blood vessels) of the secondary lamellae, desquamation and necrosis of epithelia in the gills (Figs. 3 and 4). Prolonged exposure of fishes to pesticide (20 days) resulted in detachment of epithelium and disintegration of lamellar tissues (Fig. 5); and hyperplasia, mucous deposition and haemorrhage (Fig. 6).

The stomach of control group fishes showed normal histology of all four layers i.e. mucosa, sub mucosa, muscularis and serosa. Mucosa layer folded into variable depths is composed of a superficial epithelium of single layer of columnar cells, lamina propria and folded stratum compactum (Fig. 7). The submucosa layer is highly vascularized with a thick layer of connective tissue extending into the lamina propria (Fig. 8). Muscularis layer is thin and innervated by blood capillaries (Fig. 7). Fishes exposed to the sublethal concentration of monocrotophos for 10 days showed atrophy of musculature, disintegrated submucosa and mucosa epithelium with degenerated mucosal columnar epithelium (Fig. 9 and 10). Prolonged exposure (20 days) to monocrotophos induced heavy atrophy of musculature, disintegration of submucosa and mucosa (Fig. 11) and cytoplasmic vacuolization with pyknotic nuclei in mucosa epithelial cells (Fig 12).

The intestine of control group fishes showed normal four histological layers i.e. mucosa, submucosa, muscularis and serosa (Fig. 13). The mucosal layer is produced into villi lined by columnar epithelial cells with centrally and basally placed nuclei (Fig. 13 and 14). Mucous cells were present all over the intestinal mucosa. Intestinal villi were covered by a thin layer of tissue matrix. Lamina propria of submucosa was formed of loose connective tissue fibres. Muscularis layer composed of inner circular muscle fibres and outer longitudinal muscle fibres while serosa layer composed of a single layer of flat cells with blood capillaries and connective tissue fibres (Fig. 13 and 14). In case of experimental group fishes exposed to pesticide for 10 days, an alteration in the histoarchitecture of intestine was observed. There was atrophy of musculature (M), disintegration of submucosa (SM) and mucosa (MU), degeneration of columnar epithelial cells, degeneration of lamina propria and atrophy of lamina propria and disintegration of mucosa epithelium (Fig. 15 and 16). Severity of tissue damages like heavy atrophy of musculature, disintegrated sub-mucosa and mucosa (Fig. 17), cytoplasmic vacuolization and pyknotic nuclei in mucosa epithelial cells (Fig. 18) was recorded in fishes of experimental group exposed to monocrotophos for 20 days.

Biochemical changes: The result of the present study clearly showed that monocrotophos had a negative impact on total tissue protein content of gills, stomach and intestine in comparison to fishes of control group (Table 1, Fig. 19). In control group fishes, the total tissue protein content of gills, stomach and intestine after 10 days was in order of intestine >gill >stomach and after 20 days in order of intestine > stomach > gill. The variation in distribution suggests differences in metabolic calibre of various tissues. Fishes exposed to sublethal concentration of monocrotophos showed

Table 1. Percentage **c**hange in total protein content (mg of tissue/g of extract) of gill, stomach and intestine of control and fishes exposed to 45ppm of monocrotophos for 10 and 20days.

Organ and tissue	Days of exposure						
	10 th day			20 th day			
	Control	Treated	% change	Control	Treated	% change	
Gills	181.73	163.43	10.06	198.95	140.85	29.20	
Stomach	177.85	141.20	20.60	206.80	120.65	41.65	
Intestine	190.06	128.71	32.27	221.67	145.93	34.16	

Table 2. Percentage change in catalase activity (nmol H_2O_2 consumed mg protein⁻¹s⁻¹) in gill, stomach and intestine of control and fishes exposed to 45ppm of monocrotophos for 10 and 20 days.

Organ and	Days of exposure						
tissue	10 th day			20 th day			
•	Control	Treated	% change	Control	Treated	% change	
Gills	203.91	306.83	33.54	237.03	440.47	46.18	
Stomach	195.09	257.44	24.21	230.80	390.27	40.86	
Intestine	206.25	239.25	13.79	252.12	405.09	37.75	

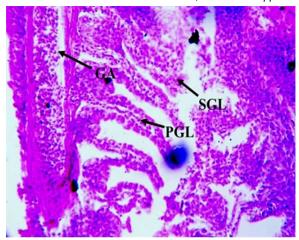


Fig. 1. Section of gills of control Anabas showing normal gill arch (GA), primary gill lamellae (PGL) and secondary gill lamellae (SGL), 400X.

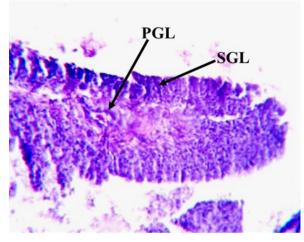


Fig. 2. Section of gills of control Anabas showing primary gill lamellae (PGL) and secondary gill lamellae (SGL), 400X..

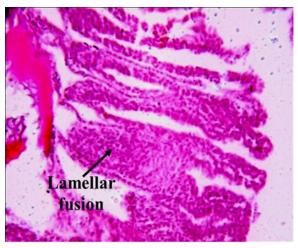


Fig. 3. Section of gills of 10th day treated Anabas showing lamellar fusion, 400X.

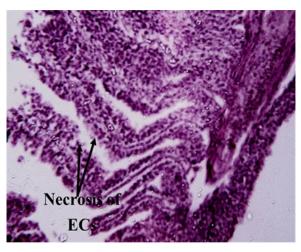


Fig. 4. Section of gills of 10th day treated Anabas showing desquamation and necrosis of epithelial cells (ECs), 400X.

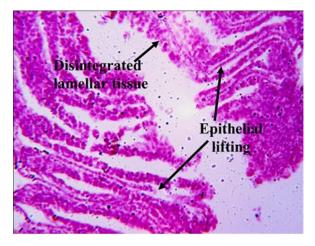


Fig. 5. Section of gills of 20th day treated Anabas showing detachment of epithelium and disintegration of lamellar tissues, 400X.

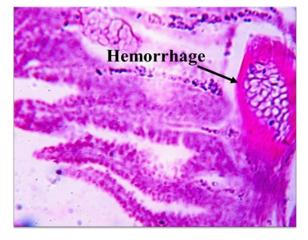


Fig. 6. Sections of gills of 20th day treated Anabas showing hyperplasia and haemorrhage, 400X.

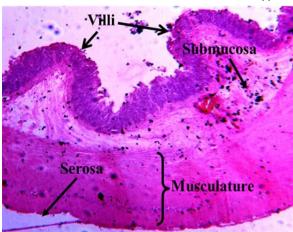


Fig. 7. Section of stomach of control Anabas showing normal histoarchitecture, 400X.

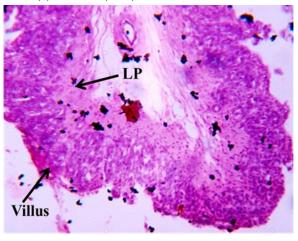


Fig. 8. Section of stomach of control Anabas showing normal villus and lamina propria (LP), 400X.

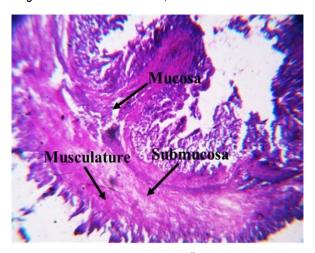


Fig. 9. Section of stomach of 10th day treated Anabas showing atrophy of musculature and disintegrated submucosa and mucosa, 400X.

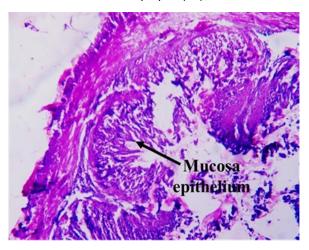


Fig. 10. Section of stomach of 10th day treated Anabas showing atrophy of musculature, disintegrated submucosa and mucosa epithelium, 400X.

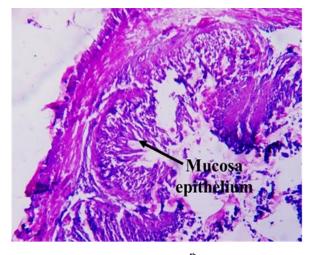


Fig.11. Section of stomach of 20th day treated Anabas showing heavy atrophy of musculature and disintegrated submucosa and mucosa, 400X.

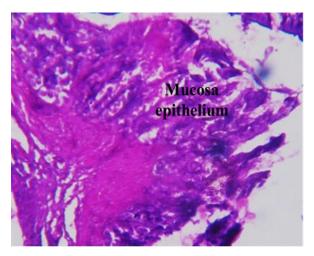


Fig. 12. Section of stomach of 20th day treated Anabas showing cytoplasmic vacuolization and pyknotic nuclei mucosa epithelial cells, 400X.

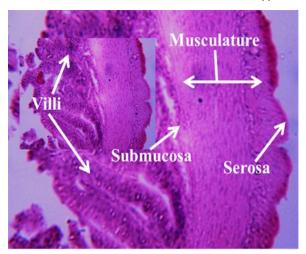


Fig. 13. Section of intestine control Anabas showing normal histoarchitecture, 400X.

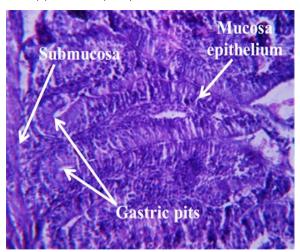


Fig. 14. Section of intestine of control Anabas showing normal villus (V) and lamina propria (LP), 400X.

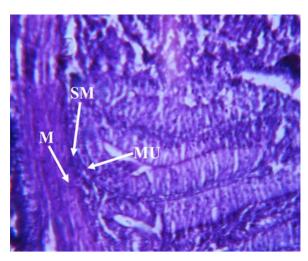


Fig. 15. Section of intestine of 10th day treated Anabas showing atrophy of musculature (M) and disintegrated submucosa (SM) and mucosa (MU), 400X.

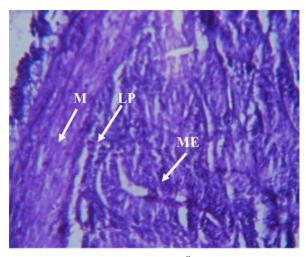


Fig. 16. Section of intestine of 10th day treated Anabas showing atrophy of lamina propria, disintegrated mucosa epithelium (ME), 400X.

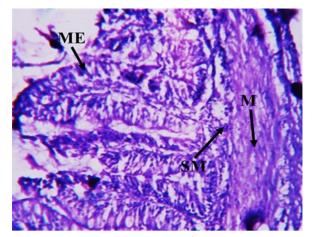


Fig. 17. Section of intestine of 20th day treated Anabas showing heavy atrophy of musculature and disintegrated submucosa and mucosa, 400X.

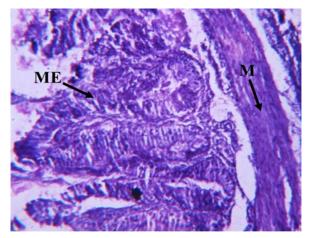


Fig. 18. Section of intestine of 20th day treated Anabas showing cytoplasmic vacuolization and pyknotic nuclei mucosa epithelial cells, 400X.

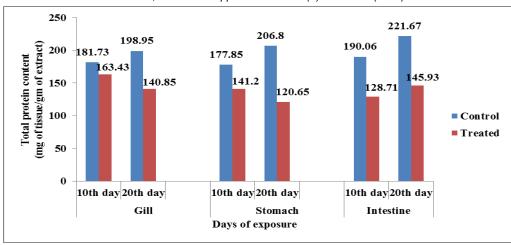


Fig. 19. Total protein content of gill, stomach and intestine of control and experimental fishes (exposed to 45ppm of monocrotophos) for 10 and 20 days.

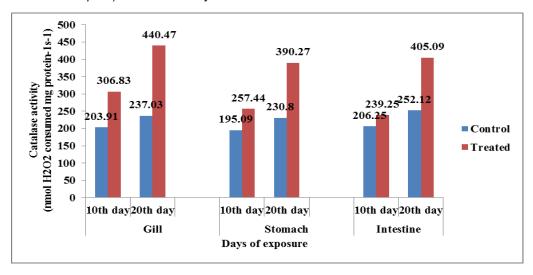


Fig. 20. Comparison between the catalase activity of gills, stomach and intestine in control and fishes exposed to 45ppm of monocrotophos solution for 10 and 20 days.

significant depletion of total tissue protein content in gills, stomach and intestine. After 10 days of exposure it was in order of Intestine < Stomach < Gill and after 20 days it was Stomach < gill < intestine. The severity of monocrotophos on total protein content after 10 days of exposure was maximum in intestine (32.27%) and minimum ingills (10.06%). In case of prolonged exposure of fishes to monocrotophos for 20 days, the maximum decrease in total protein content was observed in stomach (41.65%) and minimum in Gills (29.20%) when compared with their respective controls.

A significant increase in catalase activity was observed in fishes exposed to sub-lethal concentration of monocrotophos (Table 2, Fig. 20). This increase can be explained by the stimulation of antioxidant defence system in all tissues under study. In control group fishes, the catalase activity after 10th and 20th day was in order of intestine >

gill > stomach. Fishes exposed to monocrotophos pesticide showed enhanced activity of catalase in tissues after 10 days was in order of gill < stomach < intestine and after 20 days it was in order of gill < stomach < intestine. Maximum percentage of enhancement of catalase activity after 10 days of exposure to pesticide was observed in gills (33.54%) and minimum in intestine (13.79%). When fishes were exposed for a prolonged period (20 days), a further enhancement in catalase activity was recorded maximum in gills (46.18%) and minimum in intestine (37.75%).

DISCUSSION

Histopathological changes have been widely used as biomarkers for monitoring the effects of pollutants on specific target organs like gills, liver, kidney and gut of fishes that are responsible for vital functions (Schwaiger, *et al.*, 1997; Teh *et al.*, 1997; Gernhofer *et al.*, 2001 and Joseph and Raj,

2011). The alterations found in these organs on exposure to chemicals like pesticides are normally easier to identify than the functional ones and serve as markers of damage to animal health (Ceingiz and Unlu, 2003).

Gills in fishes are not only involved in respiration but also play an important role in osmoregulation and elimination of nitrogenous wastes as gills are directly exposed to external environment. Various dissolved toxicants from water constantly enter through the surface of gill and get accumulated in the tissues. Several histological alterations were recorded in the present study of gills of A. testudiens after 10 days of exposure to sublethal concentration of monocrotophos (45ppm) such as hypertrophy of secondary gill lamellae, telanglectasia, fusion of secondary lamellae and vacuolization. The damage was more severe and progressive after 20 days of exposure to pesticide. The gill epithelium surrounding the axis of primary and secondary lamellae were damaged to great extent. There was desquamation and necrosis of epithelial lining, disintegration of lamellar tissues and haemorrhage. The results of the present study are consistent with the histopathological work carried out by Katuli et al. (2014) in Rutilus rutilus, Rosety-Rodriguez et al. (2002) in Scophthalmus maximus, Maharajan and Parurukmani (2012) in Catla catla and Ghasemzadeh et al. (2015) in Scatophagus argus. They all reported severe necrotic lamellae in gill tissues, edema, epithelial lifting, lamellar fusion and shortening up of secondary lamellae on exposure to different pesticides. Similarly, Zahran et al., (2018) in Oreochromis niloticus and Devi and Mishra (2013) in Channa punctatus observed histopathological changes in gills on exposure to Chlorpyrifos. They noticed damages such as fused tips of secondary lamellae, decreased inter-lamellar space, mucous accumulation and hyperplasia of epithelium. The results of the present study confirms the above findings i.e mucous accumulation inside secondary lamellae and decreased inter-lamellar cell mass which has also been reported by Karmakar et al. (2015) in Labeo rohita on exposure to malathion. Earlier workers like De Silva and Samayawardhena (2002) and Al-Ghanin et al. (2008) have reported that reduced gaseous exchange in fishes exposed to toxicants was due to increased mucous deposition on gills and damaged gill lamellae. In the present study, death of fishes that occurred during experimental period might be due to degeneration of respiratory epithelium and the damage of gill tissues leading to tissue hypoxia and consequent decrease in enzyme metabolism. Histopathological alterations in the alimentary canal of A. testudineus in the present study showed the toxicity of monocrotophos. Exposure to monocrotophos showed pathological lesion in stomach with atrophy of musculature, disintegrated submucosa and mucosa epithelium, degeneration of columnar epithelial cells of mucosa. Prolonged exposure showed heavy atrophy of musculature and disintegrated submucosa and mucosa and cytoplasmic vacuolization and pyknotic nuclei in mucosa epithelial cells. Senapati et al. (2013) in Anabas testudineus upon exposure to Almix 20WP herbicide reported degeneration and vacuolation of the gastric epithelium, columnar epithelial cells and damaged gastric glands. Ghanbahadur and Ghanbahadur (2012) observed sub-mucosal vacuolization and shrinkage of mucosal folds in Rasbora daniconius on exposure to endosulfan. The gastric glands present in submucosa secrete gastric juice contains enzymes for digestion of proteins. The mucosal cells secrete mucous that protect gastric epithelium from gastric acidity and other chemical reaction. The distortion of columnar epithelium as observed in the present study might be due to impaired secretion of mucous that protects it from gastric acidity and chemical reac-

In the present study, histopathological lesions were detected in intestine of A. testudineus due to monocrotophos toxicity. The concomitant changes in the intestine of fishes exposed to pesticide in the present study were atrophy of musculature, disintegration of submucosa and mucosa, degeneration of lamina propria and distortion of columnar epithelial cells. Senapati et al. (2013) reported similar type of histopathological changes in the intestine of Anabas testudineus like degeneration of columnar epithelial cells, lamina propria and prominent luminal mucous secretion on exposure to herbicide Almi 20WP. Ghanbahadur and Ghanbahadur (2012) in Rasbora daniconius and Sharma et al. (2001) in Cirrhina mrigala also showed similar histological alterations in the intestine due to toxicological effects of different pesticides. The results of the present study showed severity of tissue damages like heavy atrophy of musculature, distortion of villi, cytoplasmic vacuolization and pyknotic nuclei in mucosal epithelial cells due to prolonged exposure of fishes to monocrotophos. These observations are consistent with the result of Ravanaiah and Narasimha (2010) carried out in Tilapia mossambica.

Proteins are important organic substances required by organism as an alternative source of energy and tissue building (Yeragi *et al.*, 2003). The level of total tissue protein used as a biochemical parameter to evaluate the toxicity of pollutants. The result of the present study showed significant decrease in total tissue protein content in gills, stomach and intestine in fishes exposed to monocrotophos pesticide after 10 days in comparison to fishes of control group. Several workers have also observed decline in tissue protein under toxic stress of various chemicals (Susan *et al.* (1999); Jha and Verma (2002); Tilak and Yacob

(2002); Hazarika et al. (2003), Tilak et al. (2003); Vishal (2004); Kalender et al. (2005); Venkataramana et al. (2006); Rohankar et al. (2012) and Nagarajun and Rathnaman (2013). Remia et al. (2008) suggested that reduction of total tissue protein may be due to proteolysis and increased metabolism under toxicant stress. A progressive hyperproteinemia was observed when fishes were exposed to monocrotophos for a prolonged period (20 days). Similar trend of severity of pesticides on tissue protein when exposed for a prolonged period was reported by Al-Kahtani (2011); Tripathi and Yadav (2015); Sulfath et al. (2013); Muley et al. (2007): Jenkins et al. (2003). The decline in tissue protein in gills, stomach and intestine might be due to enhanced breakdown of proteins into amino acids for various metabolic activities. There are two possible reasons to degrade tissue protein under stress condition due to toxicity of pesticides i.e. synthesis of membrane lipoproteins for repairing cell damages and increased energy requirement. The decline in total tissue protein might be due to reduced or inhibition of protein synthesis due to tissue necrosis (Jyothirmayee et al., 2006).

Catalase is an important enzyme that plays an important role in removing H₂O₂ by decomposing it in to H₂O and molecular O₂. Sulfath *et al.* (2013) showed decreased catalase activity in Oreochromis mossambicus on exposure to lindane. They suggested that it might be due to reduction in NADPH concentration in relation to high energy requirement or immense production of free radicals on exposure to lindane. Pesticide induced inhibition of catalase activity has also been reported in Chana punctatus by Sharma et al. (2001) on exposure to endosulfan, Sayeed et al. (2003) on exposure to delta methrin and Tripathi and Singh (2013) on exposure to alphamethrin and in Danio rerio by Ansari and Ansari (2014) on exposure to Alphamethrin. However, the results of the present study showed significant augmented catalase activity in gills, stomach and intestine of monocrotophos exposed fishes in comparison to tissues of control group fishes. Similar results have been reported by Moraes et al. (2007) in Leporinus obtusidens and Karamati et al. (2010) in Rutilus rutilus. The increase in catalase activity as observed in the present study indicated the excess production of H₂O₂ due to metabolism of monocrotophos as a defence of the organism against oxidative damage as catalase acts on H₂O₂.

Conclusion

Pesticides are often considered a quick, easy, and inexpensive solution for controlling weeds and insect pests in agriculture. It has contaminated almost every part of our environment and residues are found in soil and air and in surface and ground water across the country. Present study showed

toxicity of monocrotophos in *Anabas testudineus*. It induced histological abnormalities in gills; stomach and intestine together with reduction in total tissue protein content and an elevation in catalase activity. Adverse effects of pesticide could affect growth and survival of the fish due to structural alterations of the gills, stomach. If pesticides are selected wisely, used in combination with other pest control measure, and applied safely, the pollution of our surface waters and contamination of aquatic life can be avoided. Besides for safe use of pesticides more experimental work should be performed to determine the concentration and time of exposure that don't induce significant sublethal effects on fish.

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