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Research Article

OBSERVATIONS ON THE EFFECT OF ALMIX 20 WP HERBICIDE ON ULTRA STRUCTURE (SEM) IN DIFFERENT REGIONS OF ALIMENTARY CANAL OF *ANABAS TESTUDINEUS* (CUVIER)

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ABSTRACT
Herbicide contamination in aquatic bodies may cause harmful effects on non-target aquatic organisms like fish. Almix 20WP is a widely used sulfonylurea herbicide composed of 10% metsulfuron methyl and 10% chlorimuron ethyl. In the present study rice field herbicide, ALMIX 20 WP was used to study the ultra-structural changes in different regions of alimentary canal of *Anabas testudineus* by scanning electron microscope (SEM) study. Fishes were exposed to Almix 20 WP herbicide at sub-lethal dose of 66 mg/l in the laboratory for 45 days. The most observed changes after herbicide exposure in fish, were degeneration of microridges of stratified epithelial cells in buccopharynx and oesophagus. Distorted columner epithelial cells, severe mucus secretion and damages of mucosal folds were observed in stomach and intestine.

Key Words: *Almix 20 WP, Anabas testudineus, Alimentary Canal, Ultra-Structural Changes*

INTRODUCTION
The application of herbicides is increasing in modern agricultural practices. The constant flow of agricultural runoff into the adjacent aquatic bodies can leads to herbicidal contamination. This may cause deleterious effect on non-target aquatic organisms, which ultimately contribute long-term effects on aquatic environment. The direct use of herbicides in aquatic weed control can also leads to harmful effects on the non-target organisms like fish, due to loss of habitat and food supply (Ervnest 2004). Fishes are widely accepted bioindicator to study the health of an aquatic ecosystem as they are directly or indirectly exposed to the chemicals resulting from agricultural production *via* surface runoff or through food chain of ecosystem. Almix 20 WP, a sulfonylurea herbicide is a combination of 10% Metsulfuron methyl and 10%, Chlorimuron ethyl, now popularly used in our country for controlling sedges and broad leaf weeds. Almix works through both contact *via* leaves and from soil through roots, hence it provides long term weed control (Anonymous, 1996). Reports about effects of Almix herbicide on fish are very scanty. Miron *et al., (*2005) reported the effect of metsulfuron methyl on acetylcholinesterase activity in the brain of silver catfish. Jabeen *et al.,* (2008) studied some changes in biochemical and enzymological parameters in *Cyprinus carpio* due to exposure of Almix 20 WP herbicide. Samanta *et al.,* (2010) observed effect of Almix herbicide on digestive enzyme activity of *Channa punctatus* and *Anabas testudineus.* The objective of this study is to determine the toxic effects of Almix 20WP herbicide on a non-target organism, *Anabas testudineus* with special emphasis on ultra structural changes (SEM study) in different regions of alimentary canal.

MATERIALS AND METHODS
Some live specimens of *Anabas testudineus* were collected on 30th November 2010, from local pond; these are then kept in aquarium for 7 days for acclimatization in laboratory. Live food (*Tubifex* sp) was supplied on daily basis. After acclimatization, the specimens were treated with Almix 20 WP herbicide, made by DuPont India Pvt. Ltd., at a dose of 66 mg/l for 45 days. The treatment was given every alternate day maintaining a control side by side. Water of the aquarium was changed on every alternate day. Physicochemical qualities of aquarium water such as temperature, pH, conductivity, hardness, chloride,

alkalinity, dissolved oxygen, sodium, were also monitored as per APHA, 1998. For Scanning Electron Microscope (SEM) study following procedure was followed. The sample fish, *Anabas testudineus*, was anesthetized with tricaine methonesulphonate (MS 222) and the representative portions of alimentary canal *viz*., buccopharynx, oesophagus, stomach, and intestine were removed immediately and the luminal surface was exposed, through longitudinal incision. Then the mucosal surface of the incised tissues were spread out and pinned with luminal surface uppermost on the cork sheets. The luminal surface was rinsed by heparinized saline to remove the adhering mucus. Then these were rinsed in 0.1M cacodylate buffer pH 7.5, after that tissues were infiltered with 2.5% glutaraldehyde for 24 hr at 4^0C. After fixation the tissues were rinsed in buffer, trimmed in to 8 mm squares and subjected to post fixation in 1% OsO_4 in 0.1M cacocodylate buffer, pH 7.5 for 2 hours then dehydrated through graded acetone. Subsequently, acetone was removed by amyl acetate and subjected to critical point drying (CPD) method with liquid carbon dioxide. The mucosal surface of each tissue was mounted on metal stubs, coated with gold with thickness of approximately 20 nm, and scanned in Hitachi, S-530 SEM.

RESULTS AND DISCUSSION

Buccopharynx

*Control***:** In *A. testudineus* the the surface of the buccopharyngeal epithelium appeared in the form of pentagonal and/or hexagonal stratified epithelial cells (SEC). The apical surfaces of the stratified epithelial cells (SEC) are provided with labyrinth pattern microridges. The micoridges are regularly spaced. The outer most microridge of a particular cell fused with the same of neighbouring cell, forming a doubleridge structure (Fig. 1.1, 1.2). Some test buds surrounded by stratified epithelial cells and some wort like structures are located at the junction of stratified epithelial cells. This most probably represent the opening of mucous cells (Fig. 1.1).

*Treated***:** The most conspicuous change after the exposure of Almix herbicide was obliteration of microridges, which finally caused the damage of stratified epithelial cells (Fig. 1.3, 1.4, 1.5). Secretion of mucus and damages in test buds were also noticed (Fig. 1.3, 1.4, 1.5).

Oesophagus

*Control***:** In *A. testudineus*, the mucosal surface of the oesophagus is composed of regularly spaced round or oval stratified epithelial cells (Fig. 2.1, 2.2), the plasma membrane of aforesaid cell consists of thick and linearly arranged microridges (Figs 2.1 and 2.2). The microridges are regularly spaced, leaving long and deep furrow or channels in between the stratified epithelial cells.

*Treated***:** After treatment damages of stratified epithelial cells were observed in the mucosal surface of the fish (Fig. 2.3, 2.4, 2.5). Some vacuolated structures were appeared within the stratified epithelial cells (Fig. 2.4). Severe mucus secretion was observed after Almix exposure (fig. 2.4). Necrosis of test buds was also prominent in mucosal surface of Almix treated fish (Fig. 2.5).

Stomach

*Control***:** In *A.testudineus* the mucosal folds are anastomosed with each other to form deep, empty and rectangular shaped concavities. The mucosal folds are densely packed with columner epithelial cells. The mucosal surface of this region is provided with oval, round, elevations corresponding to the surface of columnar epithelial cells which are densely packed with short but stubby microvilli (Fig. 3.1, 3.2). Prominent gastric pits surrounded by the epithelial cells are also observed in this region (Fig 3.1).

*Treated***:** In the Almix treated *A.testudineus*, the columnar epithelial cells of stomach were distorted (Fig. 3.3, 3.4, 3.5). Secretion of mucus, adhered to the epithelial surface was also observed in Almix treated fish (Fig. 3.3, 3.4).

Intestine

*Control***:** In *A. testudineus* the mucosal folds are irregularly arranged and supported with regularly packed round or oval columner epithelial cells. Short and compactly arranged microvilli are present on the apical surface of the absorptive columnar epithelial cells (Fig. 4.1, 4.2).

*Treated***:** Due to Almix toxicity, the intestinal mucosal folds were damaged (Fig. 4.1). Intestinal CEC were damaged (Fig. 4.4, 4.5). The degeneration of microvilli structure on the apical part of columner epithelial cells and severe mucus secretion were also observed in the intestine of Almix treated fish (Fig. 4.4, 4.5).

FIGURE 1

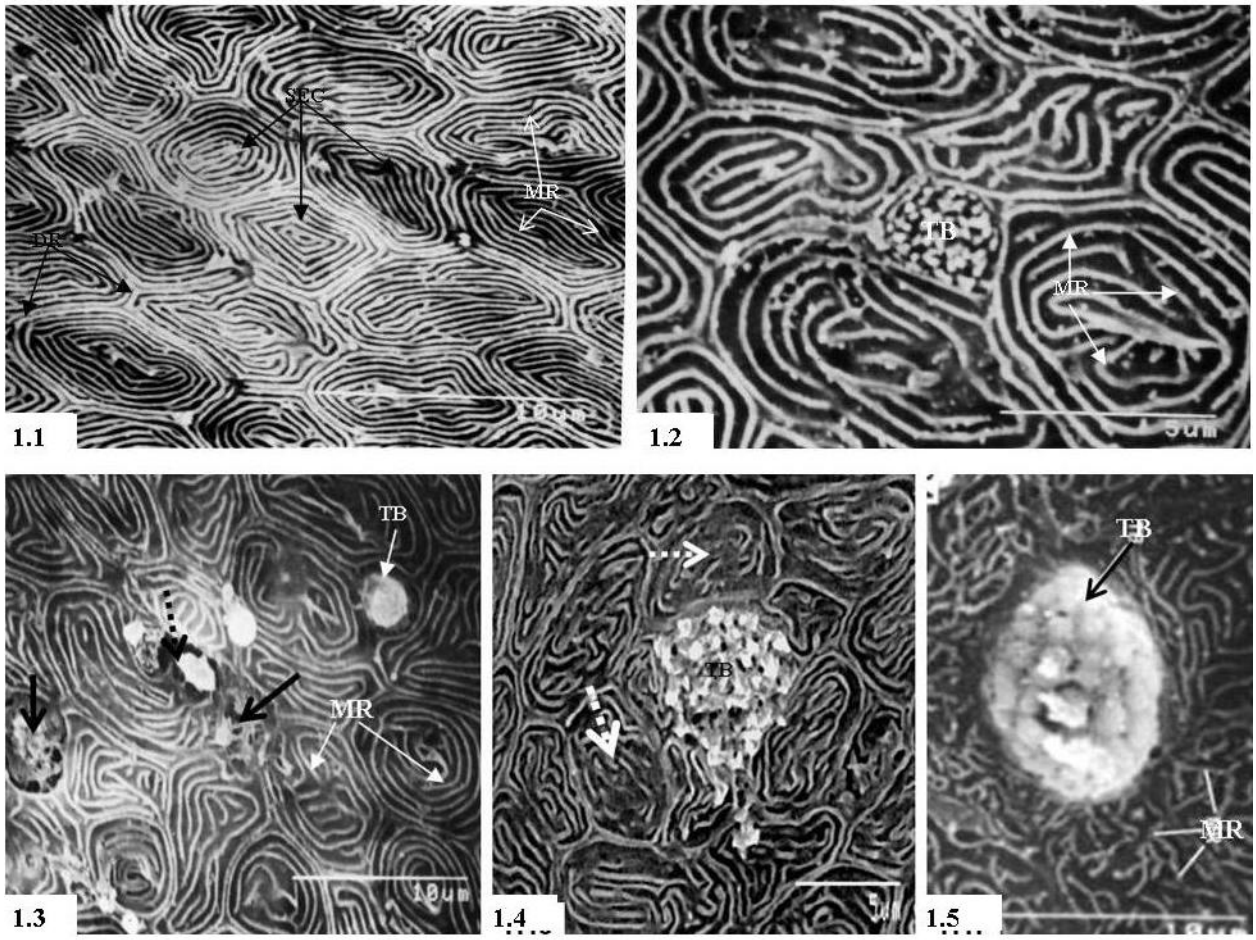

Figure 1: Scanning electron micrographs of buccopharynx of control and Almix treated *A. testudineus*

Fig.1.1-1.2. Buccopharynx of *A. testudineus* (Control)

Fig.1.3. Buccopharynx (Treated). Note opening of mucous cells with mucus (dotted arrow), Damage of test buds (black arrows)

Fig.1.4. Buccopharynx (Treated). Note obliteration of microridges(MR) (white dotted arrow)

Fig.1.4. Buccopharynx(Treated). Note loss of pentagonal structure of stratified epithelial cells (SEC)

FIGURE 2

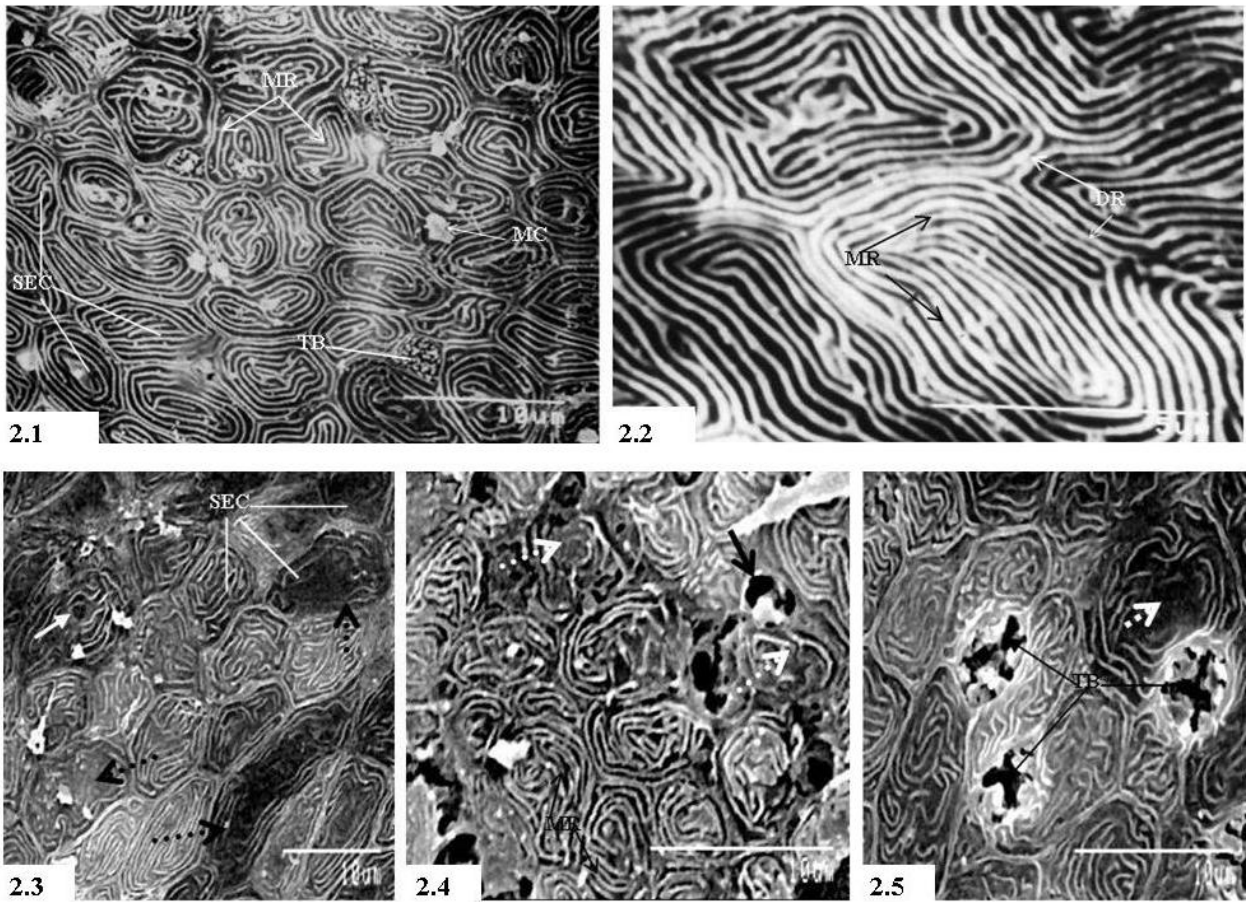

Figure 2: Scanning electron micrographs of oesophagus of control and Almix treated A. testudineus

Fig. 2.1. Oesophagus (Control) Note SEC with labyringth pattern MR, double ridge structure with neighbouring SEC, MC with mucusin between SEC

Fig. 2.2. Oesophagus (Control) Note Microridges and double ridge structure at higher magnification

Fig.2.3. Oesophagus (Treated). Note Damaged SEC with distorted MR (black dotted arrow)

Fig.2.4.Oesophagus (Treated) Note Distorted SEC(white dotted arrow), Opening of MC with mucus(black arrow), vacuolated structure within SEC

Fig.2.5. Oesophagus (Treated) Note distorted SEC with loss of MR (white dotted arrow), damage of test buds (TB)

FIGURE 3

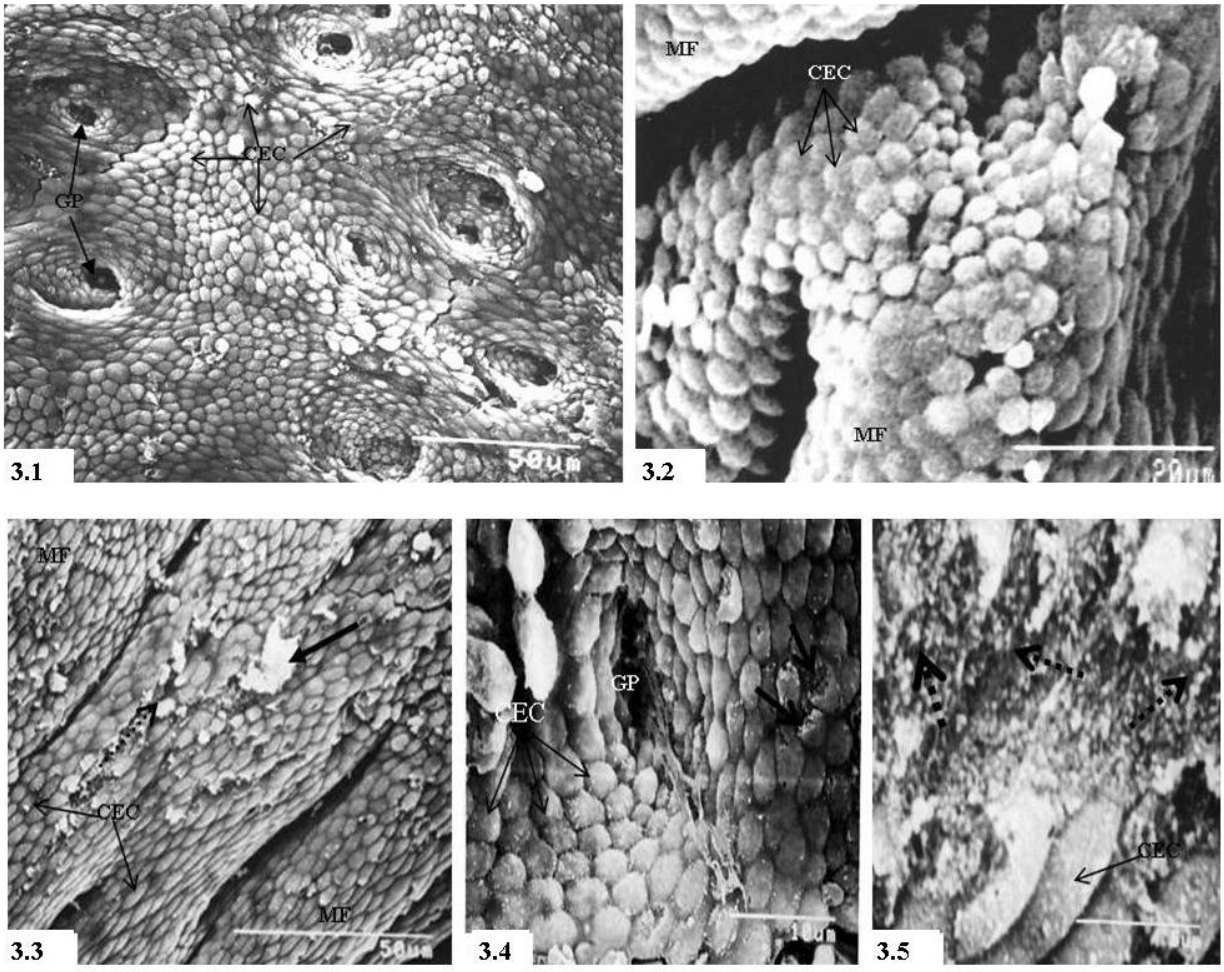

Figure 3: Scanning electron micrographs of stomach of control and Almix treated A. testudineus

Fig. 3.1-3.2. Stomach (Control) Note columnar epithelial cells (CEC) with prominent gastric pits (GP)
Fig.3.3. Stomach (Treated). Note Damaged CEC (dotted arrow), mucus secretion (black arrow)
Fig.3.4. Stomach (Treated) Note Distorted CEC (black arrow), mucus secretion
Fig.3.5. Stomach (Treated) Note distorted CEC (black dotted arrow)

FIGURE 4

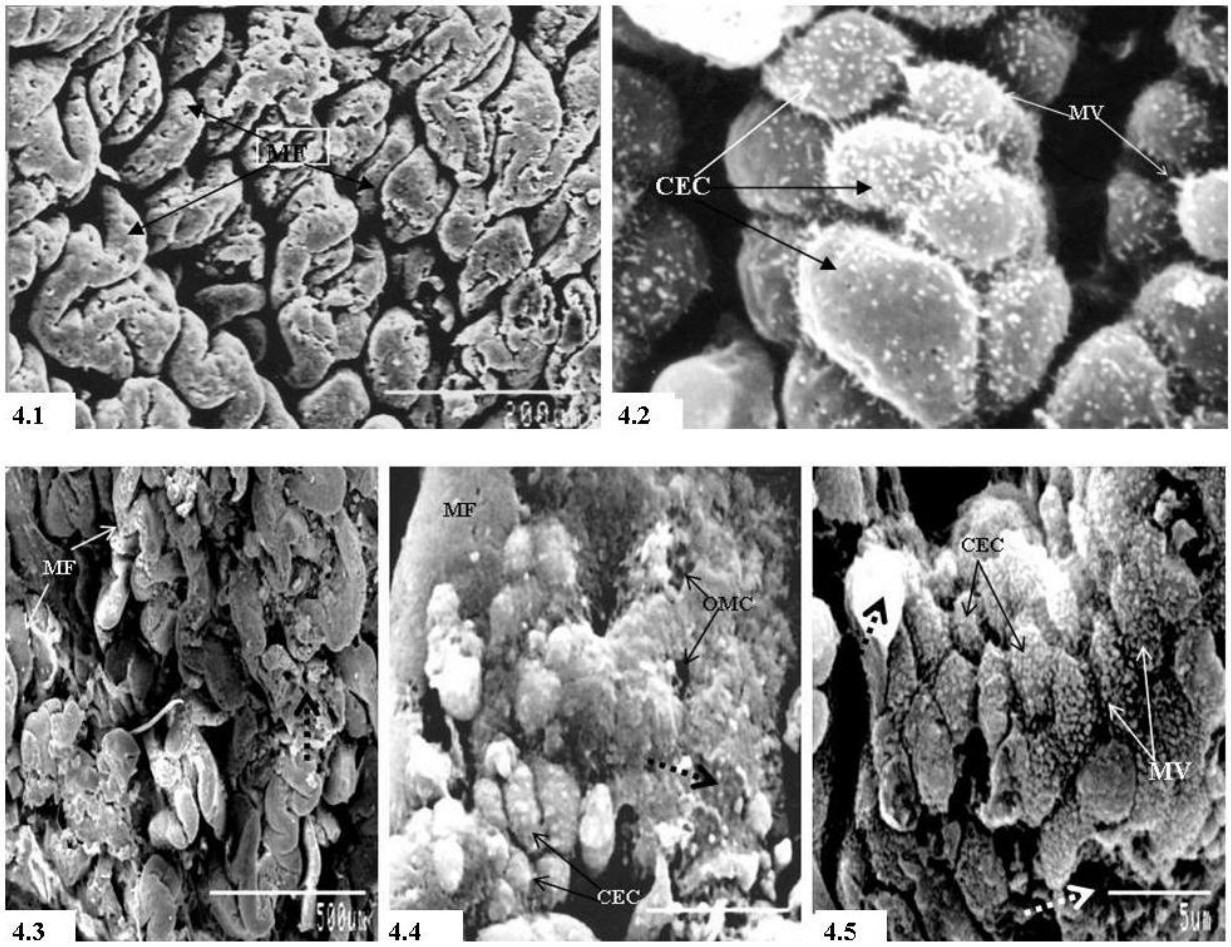

Figure 4: Scanning electron micrographs of intestine of control and Almix treated *A. testudineus*

Fig. 4.1 Intestine (Control) Note mucosal folds (MF)

Fig. 4.2. Intestine (control) Note columnar epithelial cells (CEC) with microvilli (MV)

Fig. 4. 3. Intestine (Treated). Note Damaged MF (dotted arrow)

Fig. 4. 4. Intestine (Treated) Note Distorted CEC (dotted black arrow), mucus secretion

Fig.5. Intestine (Treated) Note distorted CEC, mucus secretion (black dotted arrow), loss of microvilli (MV)

The study of herbicide toxicosis is now getting more importance because of it's further cumulative effects and biomagnifications quality. Llyod (1960); McCarty *et al.*, (1978); Datta and Sinha (1989); Ghosh and Chakrabarty (1990); Senapati *et al.*, (2009), studied the effect of different xenobiotics on fish alimentary canal. But study on effect of herbicide in fish alimentary canal is very rare. In the present study severe pathological lesions, secretion of mucus were observed in different regions of alimentary canal of *A. testudineus* due to the exposure of almix in the laboratory. Ghosh and Chakrabarty (2001) observed similar pathological lesions through Scanning electron microscope (SEM) investigation on gut of *Notopterus notopterus* after arsenic exposure. Bose (2005) reported damages in different regions of alimentary canal of *A. testudineus* by scanning electron microscope (SEM) study after lead and cadmium

treatment. Senapati *et al.,* (2009) also reported pathological changes in alimentary canal of *Channa punctatus* due to toxicity of glyphosate herbicide in a similar type of investigations. The mucosa of buccopharynx and oesophagus are intimately connected with stratified epithelial cells, supported with microridges in carnivorous fishes. Similar pattern of microridges in the aforesaid regions was also reported in other carnivorous fish (Sinha and Chakrabarti, 1986; Chakrabarti and Sinha, 1987). These microridges on the epithelial cells play a significant role on the anchorage of thin mucus film over the soft mucous membrane and this mucus film lubricates the ingested food in these regions. Thus it helps the ingested food to pass into the stomach and also helps to overcome the trauma resulting from ingested material. In the present study, in *A.testudineus* disruption of microridges in the stratified epithelial cells of the buccopharynx and oesophagus has been found to be affected due to this herbicide toxicity. The microridge structure of the epithelial cells of the aforesaid regions retains mucus film and plays a significant role for the lubrication of the food and protects the mucosa layer from mechanical rubbing (Sinha and Chakrabarti, 1986; Chakrabarti and Sinha, 1987). So, the disruption of microridges of the epithelial cells in the aforesaid regions can reduce the retention ability of mucus film. This may have negative effect in the ingestion of foodstuffs and transmission of the food to the next regions in the concerned fishes which ultimately resulting in to deterioration of fish health with consequent production. In the present study damages columner epithelial cells and severe mucus secretion was found in epithelial surface of stomach of *A. testudineus*. This may be due to herbicidal action on gastric epithelium which ultimately reduces the protection ability of gastric epithelium from chemical injuries and cell lysis. Excessive secretion of mucus has also been found from the gastric epithelium, which indicates that Almix herbicide exposure triggers the activity of the aforesaid cells. In present study, disruption of mucosal folds and damages of microvilli structure in the intestinal portion of *A. testudineus,* were caused by Almix toxicity. This might hamper the storage and digestive capacity of the concerned fish. In the present study, the secretion of mucus by exocytosis from the different parts of the alimentary canal was another effect of Almix toxicity in *A. testudineus*. It may be due to the entering of the aforesaid herbicide into the alimentary tract of fish, and may cause the changes of the luminal environment which induces the mucus secretion throughout the length of the alimentary tract.

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