



Histological changes in the stomach and intestine of an air breathing teleost- *Anabas testudineus* after exposure to pesticide, an observation under scanning electron microscope.

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ABSTRACT

Pesticides, herbicides and insecticides are used abundantly to control pests, weeds and insects in the agricultural fields. *Anabas testudineus* was exposed to an environmental containing various concentration of pesticides. The effect of pesticides (Chlorpyrifos 50%+ Cypermethrin 5% EC) on the stomach and intestine of an air breathing teleost – *Anabas testudineus* were investigated in Scanning Electron Microscope. Histopathological alteration in the stomach and intestine of non-target air breathing teleost *Anabas testudineus* was studied after sub lethal exposure to combination of chlorpyrifos 50% and cypermethrin 5% EC pesticide at sublethal dose 0.2 ppm for 48 hours. Histopathological alteration included distortion of columnar epithelial cell (CEC), damage of gastric glands in stomach and rupture / damage of serosa gastric glands. In Intestine very few number of microvilli and lost of architecture of microvilli.

Keywords:- Chlorpyrifos 50%, Cypermethrin 5% EC, stomach, intestine rupture, villi

INTRODUCTION:-

Pesticides are major causes of concern for aquatic environment because of the toxicity persistency and tendency to accumulate in the organisms. Now a days various agrochemicals are widely used for better crop production. These agrochemicals enter into the aquatic environment. The use of agrochemicals in the fields has the potential to change the aquatic medium, affecting the tolerance limit of aquatic fauna and flora as well as creating danger to the ecosystem. These agrochemicals adversely affect the non-target organism, especially plankton and fish. No doubt, agrochemicals like pesticides insecticides and herbicides have brought tremendous benefits to mankind by increasing food production and controlling the vectors of man and animal diseases. But at the same time uses of these pollutants have posed potential health hazards to the life of fishes directly and human beings indirectly. Pesticides are used abundantly to control pests, weeds and insects in agricultural fields as well as aquatic systems. *Anabas testudineus* locally called Kabai and commonly called climbing perch is a common Indian fresh water fish. It can live out of water for a long period. It is predator and depends on shrimps, otopods, gastropod shells and young fishes. Male Exhibits parental care. It, very often, enters the crop fields from the adjoining water bodies, particularly during monsoon. Since reservoir fishes are

now being increasingly needed as a source of animal protein for the people, the effects of pesticides used in cultivation are to be monitored. The purpose of present study was to assess the histological changes in stomach and intestine of *Anabas testudineus* exposed to combination of cypermethrin 5% EC+chlorpyrifos 50% which is commonly used to control the insect pest of paddy fields in North Bihar of Muzaffarpur District. The chemical stability of these compounds and their high toxicity to human domestic animals and aquatic system has led government and researchers to be concerned with their presence in the environment. Studies on various organs of Fishes affected by pesticides, used to control insect pest population, are made by researchers and they found positive results regarding toxicity of these pesticides in Fishes. There are several workers who have reported on

effect of chlorpyrifos 50%+cypermethrin 5%EC on *Anabas testudineus* in stomach and intestine on the same line. Senapati et.al., (2012) studied the Ultra structural changes in the alimentary canal of *Anabas testudineus* due to Almix 20WP exposure in laboratory condition. Jabeen et.al (2008) observed biochemical and enzymological alteration in cyprinus carpio after exposure of Almix20wp herbicide. Samanta et.al., (2010) studied the digestive Enzymes activity of *Anabas testudineus* and *channa punctatus* in field condition after application of Almix20Wp herbicide.

MATERIAL AND METHODS

Sixty specimens of *Anabas testudineus* of both sexes measuring 13 ± 47 gm body weight and $7.5 \text{ cm} \pm 11 \text{ cm}$ length were collected from Market of Muzaffarpur District of North Bihar and were kept in the plastic container for acclimatization to the laboratory condition and they were treated with 0.1% Potassium Permanganate solution for 12-15 min to remove any dermal infection and then fishes were transferred to a large tank known as control tank having adequate amount of water for surviving fishes for 15-20 days in laboratory condition. They were divided into six groups of 10 (Ten) Fish, each group was maintained in various concentration of chlorpyrifos 50%+cypermethrin 5% EC solution like 0.05ppm, 0.1ppm, 0.15ppm, 0.2ppm and 0.25 ppm except the sixth group which was maintained in large water tank to serve as controlled fish. Fishes were maintained in same concentration of chlorpyrifos 50%+ cypermethrin 5% EC by changing the Water every alternate days after feeding the fish with minced goat liver, piece of soybean, piece of snail and liver of chicken etc. The average physio-chemical condition were maintained during this period. Water of this tank was renewed every day to minimize contamination as well as subjected to gut evacuation period before the experiment. In present study pesticides used as a toxicant, the stock solution, (chlorpyrifos 50% +cypermethrin 5% EC) was prepared according the method prescribed by standard method (APHA-AWWA, WEF, 1998) for Experimental Purpose. After bioassay test, stomach and intestine were removed from treated and controlled fish and prepared for Histological observation

TISSUE SAMPLE PREPARATION FOR SEM

After dissection of adult fish, various organs like stomach, intestine, liver. Kidney and gonads were removed. The stomach and intestine were incised longitudinally to expose the mucosal surface, spread out on thin cork sheet with mucosal surface upper side. After rinsing them in heparinised saline water to remove excess mucous, the inner layer of the mucosa was incised with fine forceps and scissors in order to expose various cell linings the mucosa. The tissues were then fixed in 2.5 % gluteraldehyde for 24hrs.

After fixation, the tissues were removed and rinsed in buffer, trimmed thin sections, and post fixed in 1% Osmium tetra oxide (OsO_4) in 0.1m cacodylate buffer. The tissues were now rinsed with a buffer and dehydrated in kept in amylacetate. The tissues were now passed through graded amylacetate and method with liquid CO_2 . After this, they were gold plated and examined under Scanning Electron Microscope.

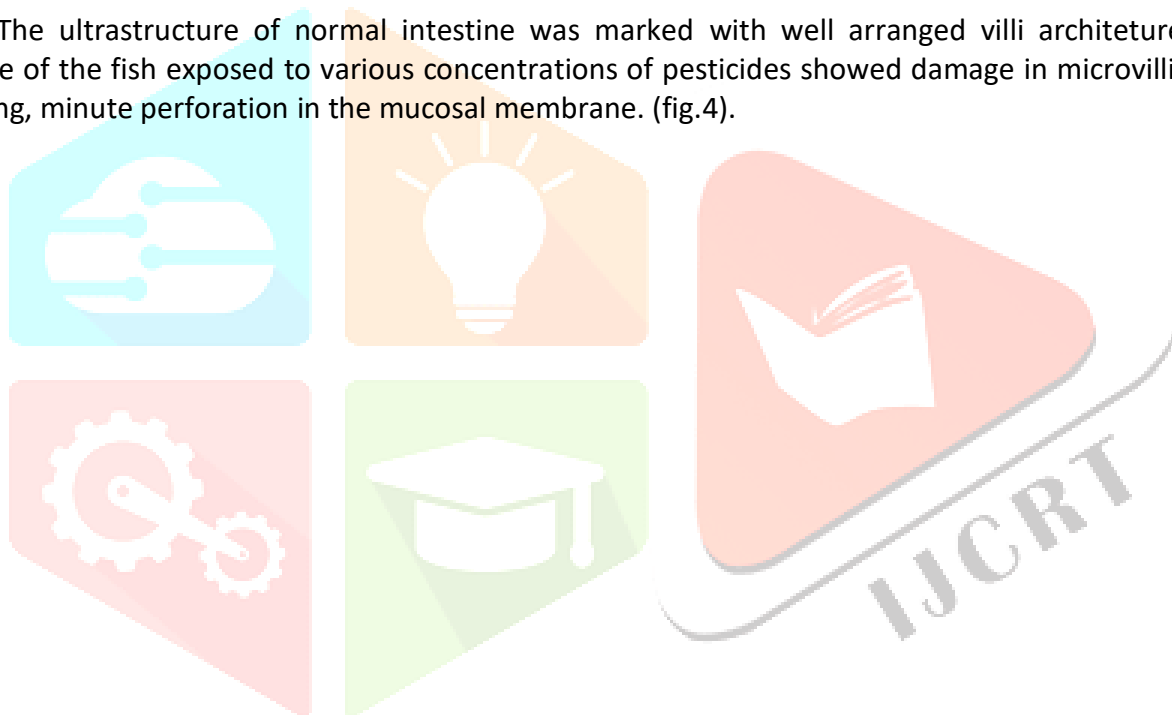
HISTOPATHOLOGICAL CHANGES IN THE STOMACH AND INTESTINE OBSERVED UNDER SCANNING ELECTRON MICROSCOPE (SEM). The stomach of *Anabas testudineus* was composed of four histological layers viz., mucosa, submucosa, muscularis and serosa. Mucosa layer was folded into varioable depths. The stomach is therefore, more sensitive organ of the fish to be affected with the degenerative effects of pesticides. Present study has revealed clear cut damage to the histological structure of the stomach of the fish exposed to the pesticide. There were marked changes in the four layers viz. serosa, muscularis, sub-mucosa of the stomach Osweiler and Van Gelder (1978) opined that the major route of lead entry is the gastrointestinal tract and it primarily affects the gastrointestinal system of various animals including intestine.

The comparative studies observed in the organs of the fish as revealed by light microscopy was further confirmed by the observation made through the scanning electron microscope.

The Scanning electron micrograph of normal stomach of the fish revealed well arrange serosa and submucosa layers (fig.1).

The fish *Anabas testudineus* exposed to the pesticides (chlorpyrifos 50% and cypermethrin 5% EC) showed remarkable degenerative altration in serosa and submucosa layers. (fig.3). Irregular ruptures in serosa were also detected at many regions.

The ultrastructure of normal intestine was marked with well arranged villi architerture. (fig.2). The intestine of the fish exposed to various concentrations of pesticides showed damage in microvilli architecture, sloughing, minute perforation in the mucosal membrane. (fig.4).



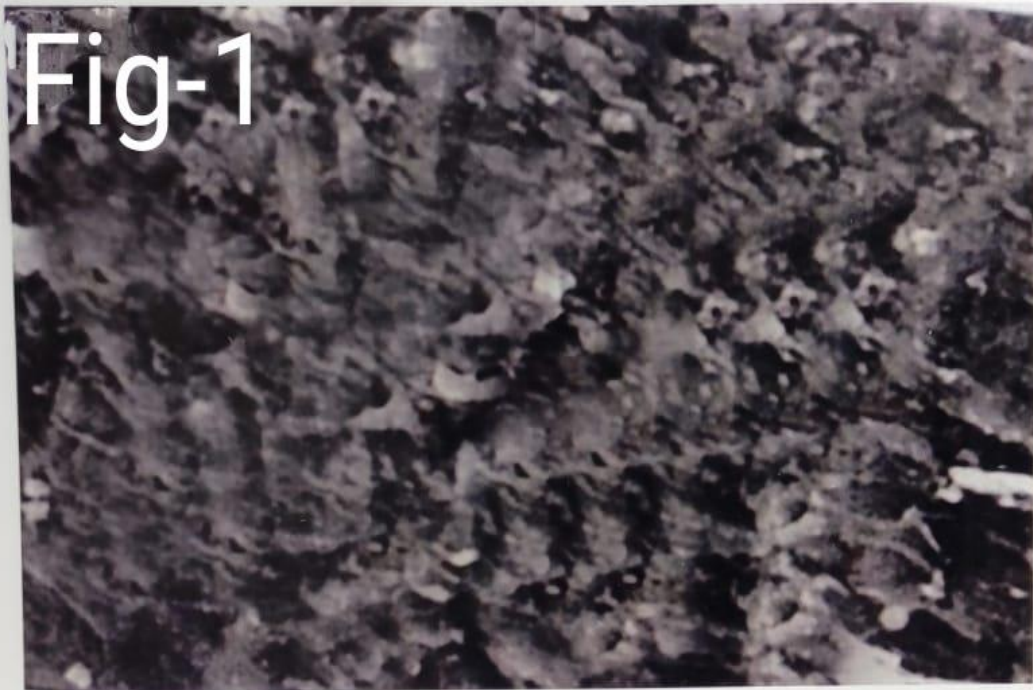


Fig-(1) Scanning Electron Micrograph(SEM) of the normal stomach of the fish showing well arranged serosa and sub mucosa layers. (1000X).

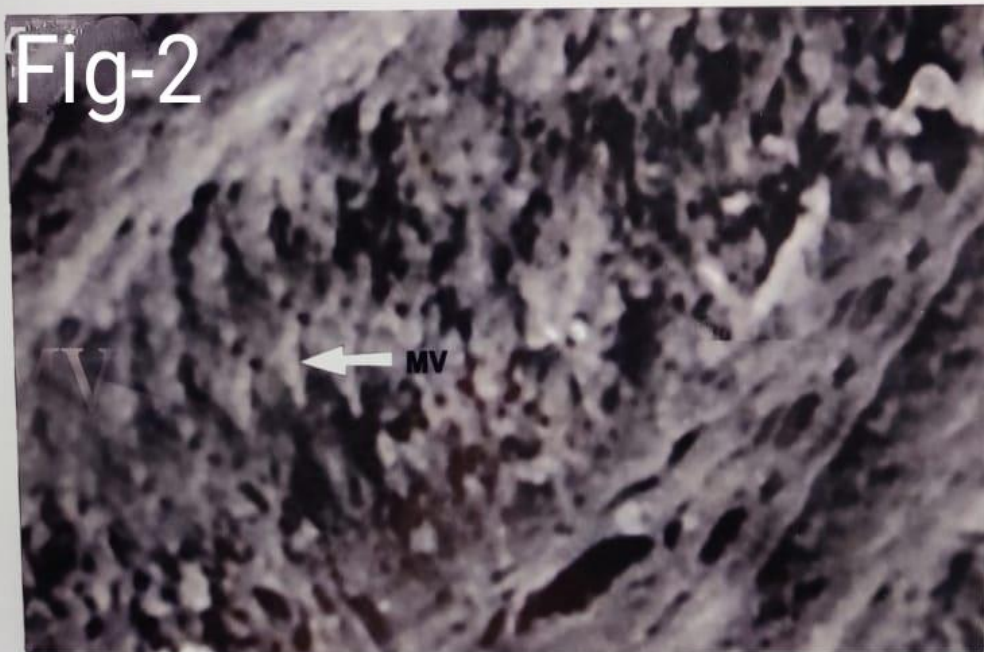
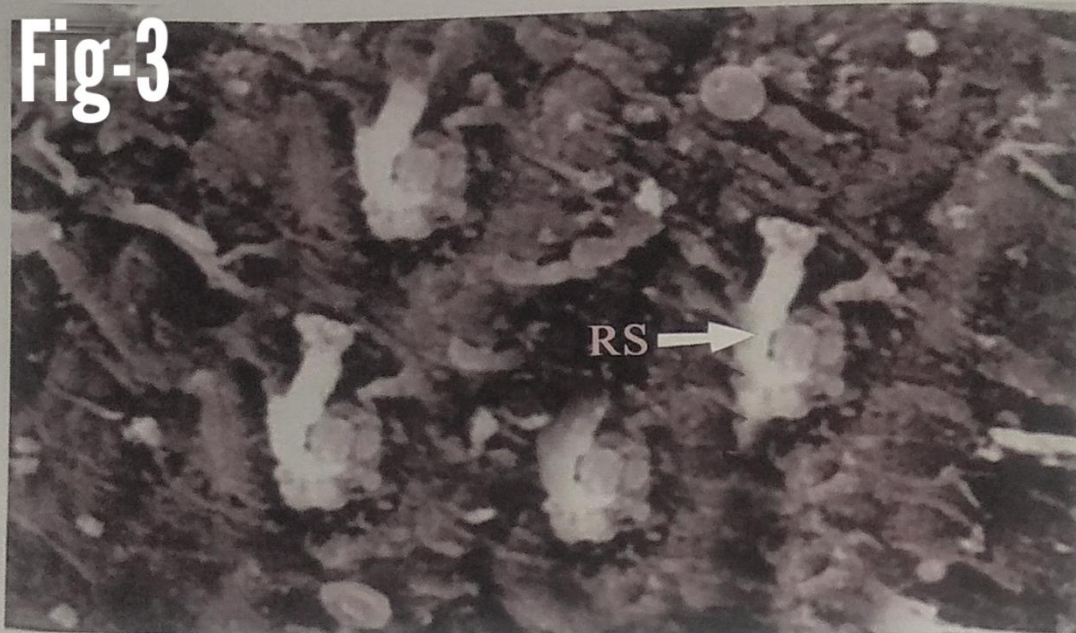


Fig-(2) Scanning Electron Micrograph(SEM) of the normal intestine of the fish showing well marked villi architecture. (1000X).



Fig(3) Scanning Electron Micrograph(SEM) of the stomach of fish exposed to pesticide showing damaged/ruptures serosa. (1000X).



Fig(4) Scanning Electron Micrograph(SEM) of the intestine of fish exposed to pesticide showing very few number of cells in the villi and architecture of the villi section is lost. (2000X).

DISCUSSION

The present Study revealed that administration of a sub lethal dose of various concentration of pesticide (Chlorpyrifos 50% + Cypermethrin 5% EC) on the systemic organs of an *Anabas testudineus*. Pesticides are the most hazardous substance that not only affects the target organism but also the non-target organisms. This can be said that the toxic chemicals enter the food chain and causes bio-magnification in different strata of food chain. Although the pesticides are frequently used in the paddy fields to yield a higher production of crops, perhaps it acts as a silent killer that have a detrimental effect on environmental, damaging and causing that to non-target organism.

Thus it can be concluded that the organ system of fish- *Anabas testudineus* under go severe histopathological and histochemical alteration exposed to the pesticides, If this process will be continue, it ultimately produce a negative impact on population and production of fish which might to be face danger of extinction.

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