



Effect of Chlorpyrifos 50% +Cypermethrin 5% EC on the gonads of Air Breathing Fish teleost -Anabas testudineus, Observation under Scanning Electron Microscope.

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ABSTRACT

Anabas testudineus were exposed to an environmental containing various concentration of composing pesticides during the resting phase of their sexual cycle in January fish of both sexes were exposed for 24-96 hrs with pesticides (Chlorpyrifos 50% +Cypermethrin 5% EC) at different concentration. Tested of the treated fish showed deleterious changes and degeneration of germinal epithelium and post meiotic germ cells. The SEM of normal ovary of the fish has showed large number of primary oocytes as well as presence of advanced stage of oocytes. In treated ovary studies several structural changes. Similarly normal testis well developed, functional sperm were prominently seen. Treated testis show less density of sperm and several structural changes are seen.

Keywords:- Chlorpyrifos, cypermetharin, toxicity, germinal epithelium, ovarian atresia.

Introduction:-

Pesticides and related chemical produced many physiological and biological changes in fresh water organism. Pesticides are the most hazardous substance that not only affects the target organism but also the non target organism. Pesticides are useful tools in agriculture and forestry, but their contribution to the gradual degradation of the aquatic ecosystem cannot be ignored (Konar 1975; Basak and Konar 1976, 1977). The aquatic ecosystem as a greater part of the natural environment is also faced with the threat of a shrinking genetic base and biodiversity. Pesticides at higher concentrations are known to reduce the survival, growth and reproduction of fish (Mckim *et al*, 1975) and produce many visible effects on fish (Johnson

1968). Due to effect of pesticides (chlorpyrifos 50%+cypermethrin 5% EC), important organs like, ovary and testis are damaged. Until the use of pesticides in crop farming is replaced by other means of pest control such as integrated pest management, use of toxic pesticides at lowest possible doses need to be recommended. Cypermethrin produces drastic effects on both the invertebrates & vertebrate species(Gowlan *et al.*, 2002) and vertebrates (Das and Mukherjee 2003). Cypermethrin is highly toxic to fish, cypermethrin is a synthetic pyrethroid used as an insecticide in large-scale commercial agricultural application as well as in consumer products for domestic purposes. A recent study at Xuzhou Medical College in China showed that, in male rats, cypermethrin can exhibit a toxic effect on the reproductive system. After 15 days of continual dosing, both androgen receptor levels and serum testosterone levels were significantly reduced. Chlorpyrifos is a crystalline organophosphate insecticide. It was introduced in 1965 by Dow Chemical Company and is known by trade names, including Durshan and Lorsban. It acts on the nervous system of insects by inhibiting acetylcholinesterase. Chlorpyrifos is moderately toxic to human and chronic exposure has been linked to neurological effects, developmental disorders, and Specially fishes. Cypermethrin is highly toxic to fish, cypermethrin is a synthetic pyrethroid use as an insecticide in large scale commercial agriculture application as well as in consumer products for domestic purposes. A recent study at Xuzhou Medical college in china showed that, in male rate, cypermethrin can exhibit toxic effect on the reproductive system. After 15 days of continual dosing, both androgen receptor levels and serum testosterone levels were significantly reduced. Chlorpyrifos is a crystalline organophosphate insecticide. It was introduced in 1965 by Dow Chemical Company and is known by trade names, including Durshan and Lorsban. It acts on the nervous system of insects by inhibiting acetylcholinesterase. Chlorpyrifos is moderately toxic to human and chronic exposure has been linked to neurological effects, developmental disorders and autoimmune disorders. Expoure during pregnancy retards the mental development of children, and its use in homes has been banned since 2001 in the U.S. In agriculture, it is one of the most widely used Organophosphate

insecticides according to the United States Environmental Protection Agency (EPA). Toxicity of cypermethrin has also been evaluated in muscular and nervous system of fish and mammals. Pyrethroids are more toxic to fish than the organophosphate pesticides (Oros and Werner, 2005). They are potent neurotoxicants that interfere with nerve cell functions by interacting with voltage dependent sodium channel, resulting in repetitive firing of neurons and eventually causing paralysis (Shafer and meyer, 2004). The objective of this research work is to study the histopathological changes in the combined toxicity of both the classes of pesticides e.g chlorpyrifos 50%+cypermethrin 5% EC, in the ovary and testis of an airbreathing fish-Anabas testudineus, commonly called Kabbai.

MATERIAL AND METHODS

Healthy specimen of Anabas testudineus of uniform size (7.5 cm + 11 cm) and weight (13 + 47 gm) were collected from local pond at Muzaffarpur District of North Bihar. The healthy fishes were acclimatised in laboratory aquaria for one month. Fish were disinfected by subjecting them to a bath of 0.1% aqueous potassium permagnate (K₂MnO₄) solution for 12-15 min to remove any dermal infection and fish were transferred to a large tank known as control tank, having adequate amount of water. During the period of acclimatization fishes were feed alternatively different type of food like, bread, piece of soya bean, piece of snail and sliced goat and chicken liver, No mortality was recorded among the fish during this period. The average Physiochemical condition were

maintained during this period Water of the tank was renewed every day or alternate to minimize contamination as well as subjected to a gut evacuation period before the experiment. In present study pesticides used as a toxicant, the stock solution of chlorpyrifos 50% and cypermethrin 5% EC was prepared according to the method prescribed in the standard method (APHA – AWWA, WFF, 1998) for experimental purpose. It is filtered first through cotton cloth and later through filter paper. The filtrate was a saturated solution of mixture of chlorpyrifos 50% + cypermethrin 5% EC, and sub-dilute solutions were prepared from this stock solution. The different concentration of cypermethrin 5% EC +chlorpyrifos 50% for *Anabas testudineus* were 0.05, 0.1, 0.2 and 0.25 ppm respectively with their control condition. Ten acclimated fish were released in each aquarium containing different concentrations of pesticide. All tests were done at room temperature and physiochemical conditions were maintained. The behaviour and other external changes in the body of fishes were observed. Dead fishes were removed and mortality was recorded at 24, 48, 72 and 96 hrs of exposure time, temperature, dissolved oxygen and PH were recorded daily. The LC50 Value of *Anabas testudineus* recorded time to time. For histopathological investigation the fish were exposed to various sublethal concentrations. At the end of 96 hrs exposure period testis and ovary samples were collected in small plastic vials with ten (10%) percentage neutral buffer formalin and Bouin's solution fixatives. The numbers of section of samples were prepared using a microtom, stained and studied under a microscope.

RESULTS

Physico-chemical characteristics of test water

The physico-chemical characteristics of the water were observed throughout during the present study, using procedures described by APHA (2005). Recorded parameters as below – Temperature 25.0 ± 0.20 C, PH 7.10 ± 0.15 Dissolved O 27.50 ± 1.50 ppm. Total hardness of CaCO_3 165 ± 5 ppm, total alkalinity of CaCO_3 150.20 ± 7.77 ppm and 3 chlorides 14.42 ± 1.05 ppm. All the above mentioned values are average values with standard error after observations. After exposure to pesticides (Chlorpyrifos 50% + Cypermethrin 5% EC) fishes showed abnormal behavior such as restlessness sudden quick movements, rolling movements swimming on the back because cypermethrin is very toxic in nature.

Effects of Chloropyrifos 50% +Cypermethrin 5% EC on Ovary:

The scanning electron microscope (SEM) of normal ovary of the fish was showed large number of primary oocytes as well as presence of advanced stages of oocytes. (fig.1).

In ovary, studies under scanning electron microscope also revealed damage to the ovary of the fish exposed to the pesticide. Ultra structure of ovary exposed to pesticide of the treated fish revealed breakaged in germinal epithelium, irregular oocytes and damaged ovum. The atretic follicles were also detected in ovary which revealed the detrimental effects of toxicant in ovary. The presence of less number of advanced staged of oocytes was marked observations which minimised the reproductive cycle of fish and finally noticeable outcome of pesticide for survival of the fish generation. (fig.2).

Effects of Chloropyrifos 50% +Cypermethrin 5% EC on Testis:

In observation of SEM of normal testis, well developed functional sperm were prominently seen. The seminiferous tubules were clearly observed under scanning electron micro scope (SEM) with functional primary spermatocytes, spermatids and fully develop sperms. (fig.3).

Extensive cytoanatomical damage was observed in seminiferous tubules and wall of seminal vessicles when the fish treated with different concentration of pesticides. The sperm density significantly reduced during exposure period which quite prominent histological abnormalities found (fig.4).



Fig-1: Scanning Electron Microscope (SEM) of the normal ovary of fish showing large number of primary Oocytes and presence of advanced stage of oocytes are also seen.



Fig-2: Scanning Electron Microscope (SEM) of the normal ovary of fish exposed to pesticides showing irregular and damaged ovum.

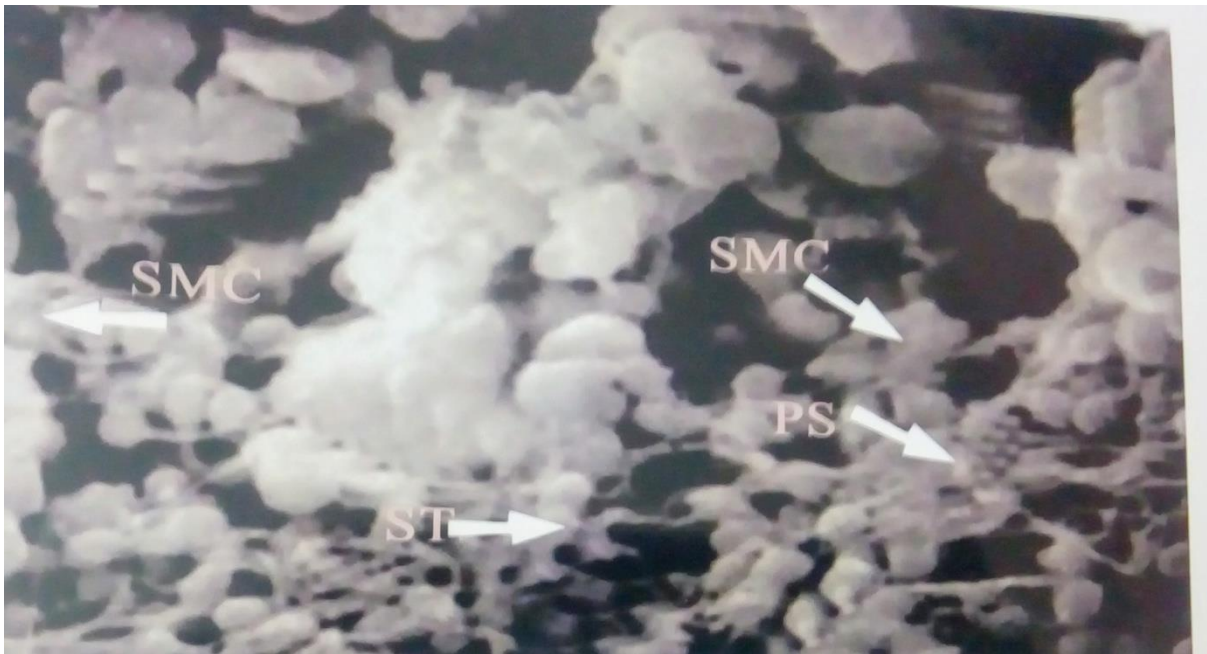
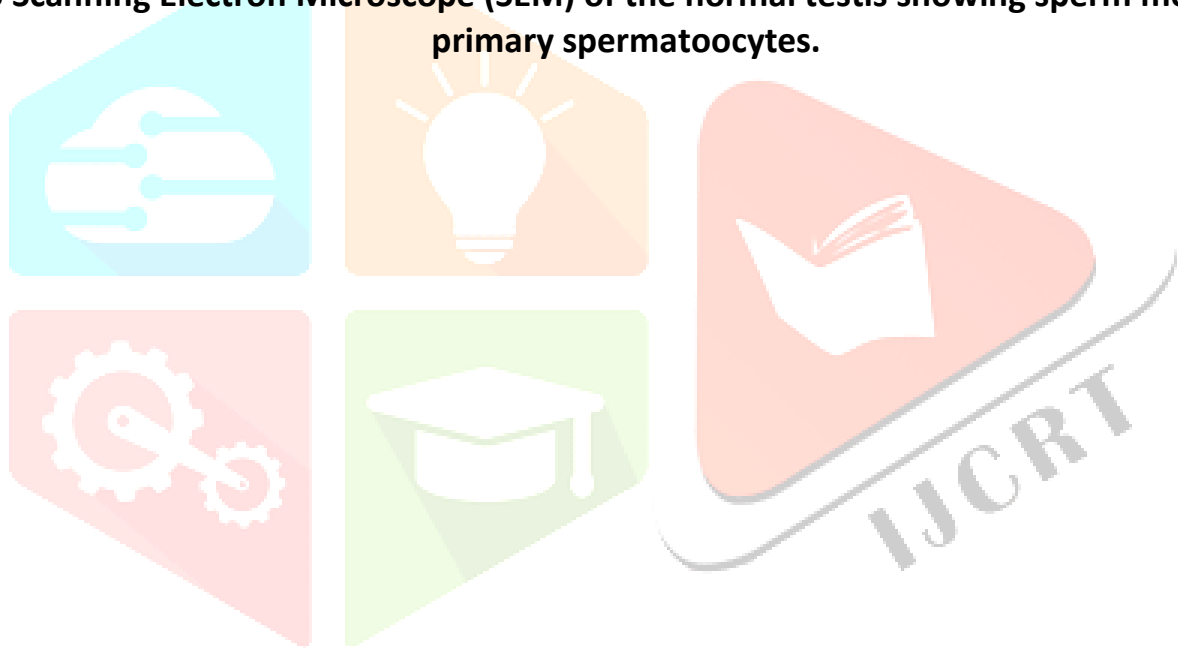


Fig-3 Scanning Electron Microscope (SEM) of the normal testis showing sperm mother cell, primary spermatocytes.



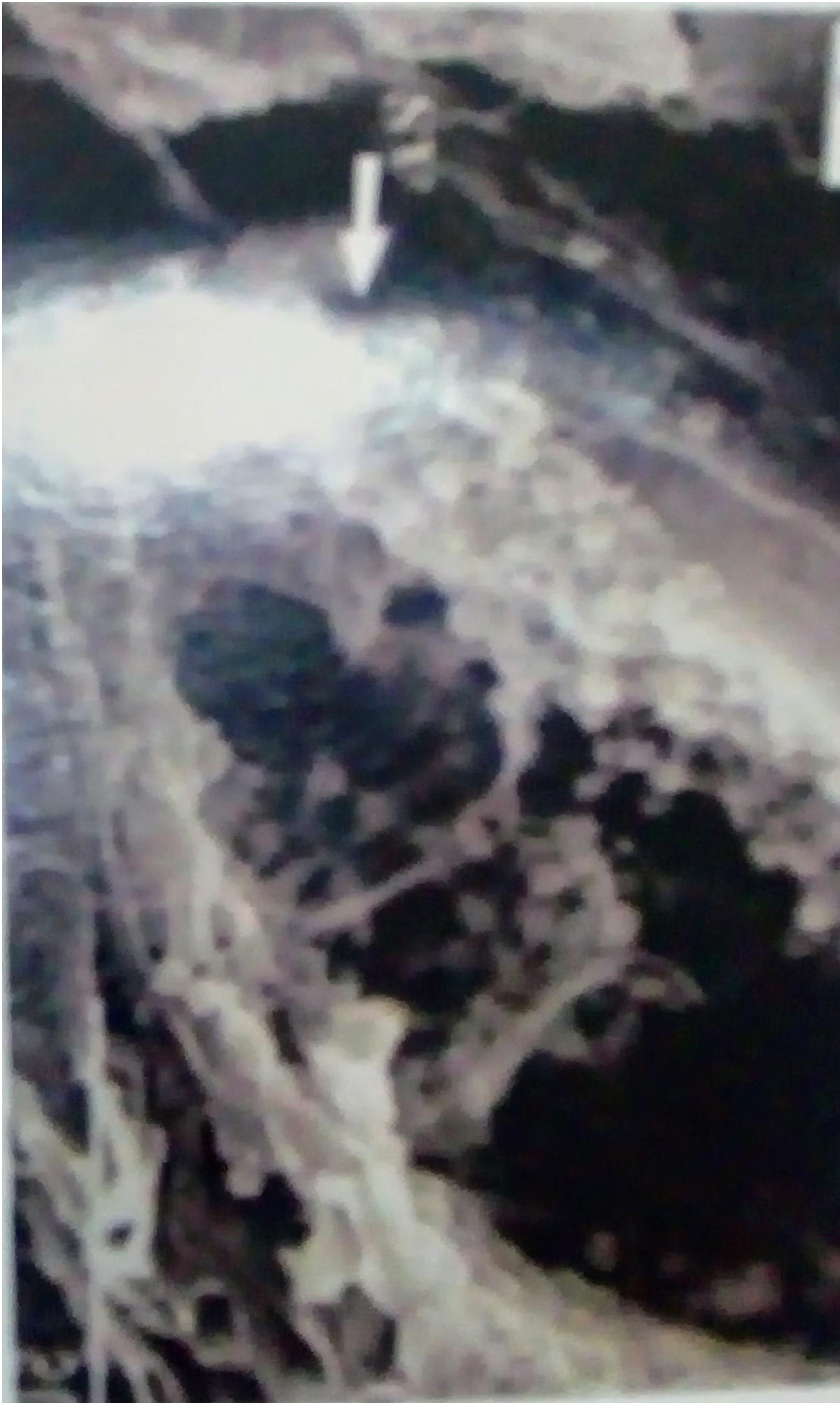


Fig-4 Scanning Electron Micrograph(SEM) of the treated testis showing damaged wall of the seminal vesicles.

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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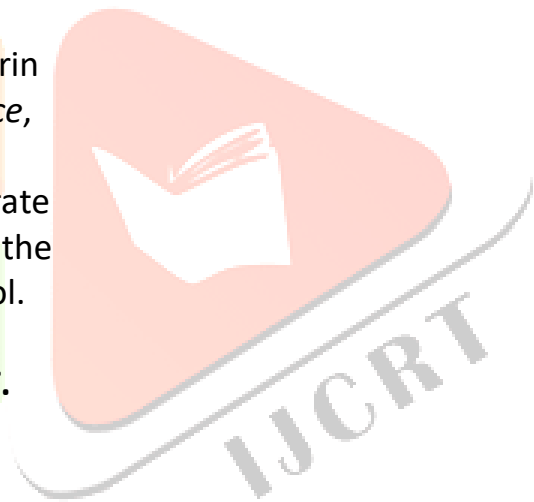
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