

SEM Study of the Structural Changes in Ovary of Fresh Water Teleost *Channa punctatus* Exposed To Chlorpyrifos 50% + Cypermethrin 5% Ec

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Abstract: The present investigation communicate with ultrastructural changes in the ovary of *Channa punctatus* after short term chronic exposure with chlorpyrifos 50% + cypermethrin 5% EC, which is a combination of two class of hybrid pesticide i.e. organophosphorous and synthetic pyrethroid. There were changes observed in the intestine of *Channa punctatus* which included less number of ovarioles damaged wall of ovariole and here an interesting micropi was also seen which is most probably seen with ovarian wall.

Keywords: Synthetic Pyrethroids, organophosphate, chlorpyrifos, cypermethrin, *channa punctatus*

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I. Introduction

Synthetic pyrethroids insecticides, combined with organophosphates are widely used for the control of insects pests in north Bihar to increase the production of crops. Physiological studies alone do not satisfy the complete understanding of pathological conditions of tissues under toxic stress. Hence it is useful to have an insight into histological analysis, as they act as biological markers to assess the toxicity conditions (Jayantharao et al 1985, tilalet al 2001, Srivastava et al 2008). Present work will be carried out on an air breathing fish *Channa punctatus* commonly found in swamps, ditches and ponds of North Bihar. This fish is chosen due to its great food value. This work has been taken with a view to find out the toxic effects of the concerned hybrid pesticide which is widely used in the agricultural practices in North Bihar. The concerned hybrid pesticide damages not only primary consumers but also the whole food chain drastically. The study of this hybrid pesticide will determine the sub-lethal effects on histopathological alterations in the body of fish.

II. Materials And Methods

Live fresh water fish *Channa punctatus* (Bloch 1793) of size ranging from 15 to 20 cm and weight 120-200 gm were collected from local fish pond of Muzaffarpur Bihar. They were transported to laboratory and transferred in to fish tank. Fishes were treated with 0.1% KMnO₄ to save them from any dermal infections and were allowed to acclimatize to the laboratory conditions for 15 days. In the period of acclimatization fishes were fed alternatively with the pieces of pila after removing alimentary canal and soyabean chunks. The average physicochemical conditions were maintained during the, acclimatization period. Water of the tank was changed every alternate days to minimize contamination as well as maintaining the physicochemical characteristic of the water. All the necessary precautions for maintaining the fish were laid down as per the recommendation of APHA, 2005.

ACUTE TOXICITY TEST

To study the toxicity, LC₅₀ of the hybrid pesticide was observed. The static renewal bioassay test method was followed. For conducting LC₅₀ experiment 5 glass Jars were set up having 10 liters of water in each jar 10 fishes were transferred in each Jar and left for 24 hrs for stability and acclimatization. The fresh water Murrel *Channa punctatus* were exposed to various concentration of the pesticide till 96 hours. No feed was given during test period. Based on the mortality observed at different concentrations during 96 hrs, LC₅₀ value was estimated for different periods i.e. 24 hrs, 48 hrs, 72 hrs and 96 hrs using straight line graphical interpolation method. After the estimation of LC₅₀ value at different hrs sub-lethal concentration was chosen for exposing the fishes for short term exposure (the sub-lethal concn as taken 1/10 of the LC₅₀ value of, 96 hrs). Sub-lethal concentration of the pesticide was taken and 10 fresh fishes were now exposed to this concentration for 30 days. After 30 day, fish from the normal as well as treated tank were dissected and their organs were

isolated, the fish organs were rinsed in physiological saline solutions (0.58% NaCl) now these organs were fixed in 2.5% glutaraldehyde for 24 hours. After 24 hours organs were washed in 50% alcohol for 5 minutes, then tissues were transferred in 70% alcohol for 30 minutes in 2 changes, after 2 changes of 70% tissues were transferred in 90% alcohol for 30 minutes in 2 changes. After 90% alcohol tissues were transferred in Abs. alcohol for 30 minutes in 2 changes. After that 3 grade of alcohol & amyl acetate were prepared which were 3:1, 2:2, 1: 3 respectively. In alcohol and amyl acetate grade tissues were kept in 30 minutes in each grade. After alcohol and amyl acetate grade tissues were kept in amyl acetate for 30 minutes. After that C.P.D. (critical point drying) was done. C.P.D. takes 1 hour & 30 minutes after C.P.D. tissues were dried then tissues were arranged on small stabs. When Tissues were fixed on stab then gold coating of tissues done. After gold coating tissues of stabs were observed under scanning electron microscope.

III. Result

OBSERVATIONS: Physicochemical characteristics of the test water: The results of physical and chemical analysis of the test water determined by using procedures as mentions APHA 2005 are as following:

1.1 Temperature	$26 \pm 2.0^{\circ} C$
1.2 Ph	7.12 ± 0.14
1.3 Dissolved O_2	7.42 ± 1.10 ppm
1.4 Total hardness as $CaCO_3$	164.76 ± 5.38 ppm
1.5 Total alkalinity as $CaCO_3$	148.64 ± 7.77 ppm
1.6 Chlorides	14.42 ± 1.05 ppm

SEM STUDY OF OVARY: SEM studies of the control ovary of the fish *Channa punctatus* showed normal development of the ova and large number of yolk calls ovarion epithelium was also seen. Sertoli cells and interstitial cells were also seen. SEM studies of the treated fish ovary showed less number of ovarioles damaged wall of ovariole and here an interesting micropi was also seen which is most probably seen with ovarian wall.

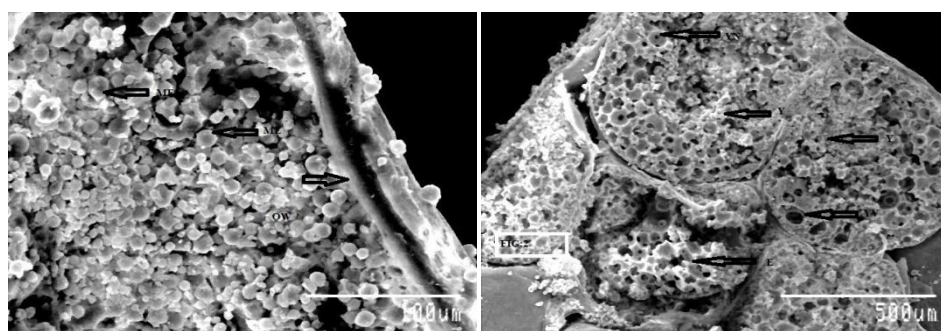


Fig1: ovary of normal fish showing mature eggs(ME) And ovarian wall (OW) 4(00X) Fig2: ovary of normal fish showing yolk nucleus(YN), yolk(y) and eggs (80X)

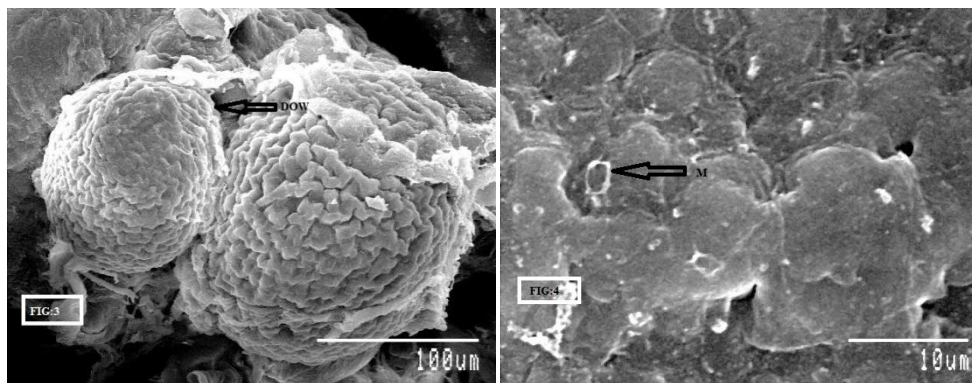


Fig3: treated ovary section showing outer wall Of ovariole. With micropi.

Fig4: section of treated ovary shows outer wall

IV. Discussion

Histological studies on ovary of the fish *channa punctatus* shows several spherical structure formed by the fragmentation of nucleus lying randomly in the chromatin network. Yolk vesicles are arranged at the periphery, vacuolization in the nucleus, stromal hemorrhage and damage of germinal epithelium. Histoanatomical abnormalities in ovary may be caused by viz., ionizing radiations, electric current, parasitic infection mechanical injuries, xenobiotic toxicants (Sarojni and Victor 1985) and by a variety of effluents and aquatic pollutants (Shukla et al., 1984; Mc comic et al., 1989, Davis and Cook, 1998 farmer et al., 1995 Kumar et al., 2000. Almost similar histopathological Changes were reported by Hussain et al., 2002) in the ovaries of *Anabas testudineus* and *C. punctatus* after the exposure 0.5 and 5.0 ppm concentration of pesticide, dimecron 100 scw.

In 2000 Giri et al., find and reported the effects of insecticide basathrin induced histoanatomical insult of ovarian tissue of cat fish, *H. Fossillish*. They reported marked damage in germinal epithelium atresia of oocyte stromal hemorrhage vacuolization of oocytes and general inflammation.

In the present study also, Reduction in the ovarian weight as well as retarded growth of the provitellogenic oocytes have been also reported in fish, *Glossogobins giuris* (Ham) exposed to lower dosage of malathion (Rama Chandra M.M., 2000).

Degeneration of the follicular walls, connective tissues and vacuolation in the ooplasm was also apparent (Dutta et al., 1994) has reported microscopic changes in ovigerous lamellae clumping of cytoplasm, degeneration of follicular cells increased atretic oocytes and ruptured follicular epithelium in the ovary of breathing cat fish, *Heteropneustus fossilis* exposed to a sublethal (1.2 mg/l) concentrations of organophosphate, Malathion.

V. Conclusion

The histological changes seen in ovary of fish shows adverse effect of chlorpyriphos 50% + cypermethrin 5% EC. Point to the fact that the hybrid pesticide used now a days as an ecofriendly substitute for organic insecticide may not be panacea for all the ills. their unrestrained used may affect the biodiversity and productivity of fish.

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