ORIGINAL RESEARCH PAPER

Zoology

KEY WORDS: Freshwater crab, Ovary, Reproductive organs, Histopathology, pesticides, Malathion

HISTOPATOLOGICAL IMPACT OF MALATHION ON THE OVARY OF FRESHWATER CRABS BARYTELHUSA CUNICULARIS (Westwood, 1836).

Shipra Sinha* Department of Zoology, Kalyan Post Graduate Autonomus College, Bhilai (C.G) INDIA. *Corresponding Author

Gajendra Yadav Department of Zoology, Kalyan Post Graduate Autonomus College, Bhilai (C.G) INDIA.

Geetanjalee Shandilya Department of Zoology, Kalyan Post Graduate Autonomus College, Bhilai (C.G) INDIA.

Mithleshvari Sahu Department of Zoology, Kalyan Post Graduate Autonomus College, Bhilai (C.G) INDIA.

Chandrakala Dewangan Department of Zoology, Kalyan Post Graduate Autonomus College, Bhilai (C.G) INDIA.

Yogita Netam Department of Zoology, Kalyan Post Graduate Autonomus College, Bhilai (C.G) INDIA.

Bharti Ganjir Department of Zoology, Kalyan Post Graduate Autonomus College, Bhilai (C.G) INDIA.

ABSTRACT

Crabs are particularly useful in aquatic environment and play a very important role in the ecosystem processes and good indicator for polluted condition. The terrestrial crab plays a significant modal in the study of population and also beneficial for human activities. The present study deals with the histochemical changes of organophosphate, malathion in the freshwater crab *Barytelphusa cunicularis.* Live specimens of *Barytelphusa cunicularis* were collected from local wet areas of Maroda-sector, Bhilai, (C.G). The crabs were sexed on the basis of the shape of the abdomen. Average weight of crabs varies from (45-50 gms), the carapace length and carapace width varies from 3.20-4.50mm and 4.25-5.80mm respectively. The crabs were acclimatized in the laboratory condition, and kept in two groups the control group set free from Malathion and the experimental group was exposed to malathion for LC_{50} at different concentration 0.45ppm, 0.30ppm, 0.26ppm and 0.25ppm for 24hrs, 48hrs, 72hrs and 96hrs respectively. The sections of 5µm were taken for the histopathological examination of ovary; it exhibited irregular arrangement of nucleus, epithelial layer destruction, degeneration of oocytes, Shrinkage in ooplasm, vacuolization and reduction in cytoplasmaic material. The result suggests that rapid utilization to meet the energy demands under the impact of malathion.

INTRODUCTION

Crabs are particularly useful in aquatic environmental studies for several reasons and play important role in ecosystem processes and good indicator for polluted condition. The terrestrial crab plays a significant and useful modal in the study of population and also beneficial for human activities. It also contains concentrated forms of proteins, and oil that protect against heart disease. As the crabs are abundantly available locally and used in food by as diet by some people with great nutritive value, there is a potential to project it as **poor man's protein**. The wide spread use of chlorinated pesticides to control pest species creates ecological disturbances which in turn affects the non target organisms. A good amount of information in toxicities of pesticide pollution on aquatic animals are available, **(Dalela et al. (1979), Dubale and Shah (1984), Rashatwar and Ilays (1984), Das et al. (2013), Guise et al. (2004), Dutta et al. (1993), Deka and Mahanta (2012), Dode et al. (2012).** particularly useful in aquatic environmental studies for several reasons highly social animal. These days pollution of the environment by pesticides is a great problem. Pesticides constitute major agriculture chemical groups which though play an important role in agriculture productivity but have posed potential hazard to non target species. Histochemical studies have been useful in evaluating such effect of an organism. Since, the trace amounts of their chemical which don't bring mortality over a period are capable of producing considerable organ damage. Analysis of histological changes in target organ provides a valuable tool in understanding the role of specific cells & organ. However histochemical investigation to determine the effect of pesticides on B. cunicularis has received comparatively little attention.

Malathion is very widely used organophosphate insecticide. It used in agriculture and houses for the control of disease vectors it is also a major source of experimental poisoning widely used in the developing countries. Once Malathion is introduced in the environment, usually from spraying on crop or in wide urban or residential areas, droplets of Malathion in the air fall on soil, plants, water or man –made surfaces. Malathion breaks down quickly by the action of water and bacteria present in it. After reacting with other chemicals formed naturally in the air, malathion broke down quickly by the action of water and bacteria in to more toxic substance called Malaxon, **Magar and bias (2013).** Once the Malathion is introduced into the environment it may cause serious intimation to aquatic organisms and is notorious to cause severe metabolic disturbance in non target species like crabs, fishes and other aquatic animals like fresh water mussels etc. **Pugazhvendan et al. (2009)** exposed *ophiocephalus punctatus* for 7 days to Malathion and different concentrations and reported severe histochemical changes in brain, liver, ovary and tissues.

Histopathological studies in cell & tissue of organism provide useful data about effect of different chemical & pesticides on particular organism. It is very simple and common tool for determining the effect of various toxic substances in animal body. Pollution makes our environment more & more virulent. When the relation among the element of nature like air and water are disturbed, ecological balance is harmed. Our environment is polluted by different types of pollution Including water pollution, air pollution, sound pollution, soil pollution etc. Water pollution is one of those which concern with the undesirable changes in medium which effect hardly to aquatics as well as terrestrial body. Sewage waste, agriculture waste, industrial effluents etc. are various means by which water get polluted.

Aquatic medium is highly contaminated with heavy metal that's all are release from industries, man-made activity by different process. Pollution decreases the floral & fauna diversity on earth. Majority of animals sensitive towards change in the chemical as well as parametric quantity of aquatic medium. In animal kingdom arthropods have largest diversity they are much susceptible towards contamination with water pollutants. They were also taken into consideration for various reasons such as environmental pollution, remedial traits & tourist attraction. Biochemical constitutes like glycogen, protein and lipids are considered as sensitive indicators of pollution effect in crabs. The present

investigation is being proposed was to determine the histopathological changes in the ovary of fresh water crab, B. cunicularis after expose with Malathion.

MATERIALS AND METHODS

Live specimens of *Barytelphusa cunicularis* was collected from local wet areas, fresh water ponds and garden area of Kalyan P.G. College at Bhilai **(Lat: 21° 13N; long.: 81° 26E),** Chhattisgarh. Samples of the specimens were collected by hand, forceps and trapping nets. After capture prior to the experiment all the specimens were kept in the glass aquarium (80cm×45cm×30cm) under constant aeration and the temperature was maintained approx 27°C for a week. **(FIG-1-b).** The crabs were sexed on the basis of the shape of the abdomen. Females have oval or rounded abdomen; in contrast, the males have triangular or inverted **"T" shaped** abdomen. Only healthy crabs were collected and fed with wheat grains. Average weight of crabs varies from (40-50gms), the carapace length and carapace width varies from 3.20-4.50mm and 4.25-5.80mm respectively. The morphometric measurement of the crab was taken through Vernier Caliper and the weight of the crabs was measured through the single pan balance.

After acclimatization in the laboratory condition, female crabs were kept in two groups-: the control group set free from malathion and the experimental group was exposed to malathion for LC_{50} at different concentration 0.45ppm, 0.30ppm, 0.26ppm and 0.25ppm for 24hrs, 48hrs, 72hrs and 96hrs respectively. All the crabs were cold anesthetized and the ovary was dissected from both the control and experimental crabs and fixed in Bouin's solution for 12-15 hours. The tissues were dehydrated in increasing concentration of ethanol, cleaned in xylol and soaked in paraffin in order to make the sections of 5µm thick cut with digital rotatory microtome. For histopathological analysis the tissues were fixed in Bouin's solution for 12-15 hours. The sections were taken in slides and stained with Haemotoxylin-Eosin through different grades of alcohol. Stained slides of both control and experimental crabs were studied and compared by using microscope and were photographed (10x, 40x, 100x).

RESULT AND DISCUSSION

Morphology

Female crab

The reproductive system of the female crab is bilaterally symmetrical, in the antero-lateral portion of the cephalothorax (H-shaped Structure) consists of Ovaries, oviduct and female gonophores. **(FIG-1a-b)**

Ovary: The color of the ovary varies from milky white to deep orange in color according to their maturity. The shape of each Ovary is Filliform, cylindrical to oval and transparent in appearance depending upon the maturity of ova and visibility of the oocyte. The ovary is bilobed and extends from behind the antero-lateral carapace along the anterior edge of carapace medially to the stomach. The width and diameter of right and left ovary varies and is not uniform along the length. (**FIG-2a,b)**

Oviduct: The distal ends of both the ovary are joined the anterior ends of both the oviduct together the ovary and oviduct from a commissure or isthmus so as to give 'H' like structure. Behind the stomach, the oviduct then dips ventrally under the pericardial sac. The oviduct then extend posterior into extensively dense structure called bursa, each bursa is deep brown in color and the distial end of the oviduct ultimately opens into thin female gonopores.

Female Gonophores: The size and color of receptacles varies and may be small, hard and creamy in color with a jelly plug on the external pore.

Histology

Ovary

The ovary of the control crab consists of outer epithelium and inner germinal epithelial layer. The oocytes are covered with the follicles cells. And the follicles are filled with different types of maturing oocytes and responsible for supplying nutrients. The ooplasm consists of thick yolk granules. In mature oocytes there is no visualization of connective tissues, the ovarian capsule is very thin, breaks and easily releases mature oocytes. Behind the stomach, the oviduct then dips ventrally under the pericardial sac. The oviduct then extend posterior into extensively dense structure called bursa, each bursa is deep brown in color and the distal end of the oviduct ultimately opens into thin female gonophores. **(FIG-3a)**

When exposed to Malathion ovaries of Barytelphusa cunicularis exhibited irregular arrangement of nucleus, epithelial layer destruction, degeneration of oocytes, Shrinkage in ooplasm, vacuolization and reduction in cytoplasmaic material. **(FIG-3b)**

DISCUSSION

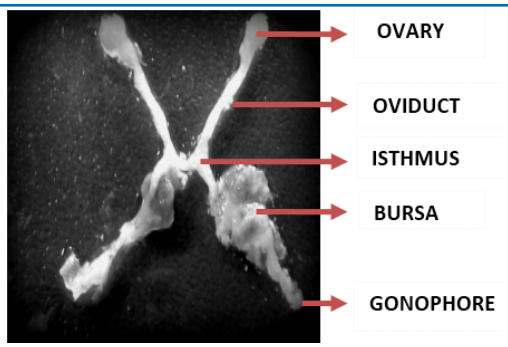
Treatment of malathion also changes the biological parameter in some reproductive organ (**Bhatnagar et al. 1996),** a fall in the glycogen level maybe due to the interference of glycogenolysis. Kharat et al., 2011 reported that the histology change in the tissue of fresh water prawn. *Macrobrachium kistensis* exposed to TBTCL. The reproductive system of *Barytelphusa cunicularis* a general layout was similar to those found in other decapods i.e. paired testis and vas deferens (**Krol et al 1992; Cumberlidge, 1999; Gracia and Silva 2006; Castilho et al 2008**). In *Barytelphusa cunicularis* and some decapods the first two pleopods are modified to serve as gonopods in the insemination of female. Histology or histochemical studies of gonopods was not reported in the present study, however in some crustacean species the role of gonopods in the transfer of spermatophore during copulation. **Cumberlidge, 1999; Berg and Sandifer, 1984**, describe the dired role of the gonopods in transferring sperm to the female. **Indira et al. (1989)** noticed effect in the developing oocytes inter viewing the enzyme system in metabolism under stress conditions**. Machle et al. (1990)** studied that cuprous oxide exposure induced significant alterations in the ovary of the crab *Barytelphusa* querini**,** arrangement of oocyte and disappearance of nucleus were observed in fresh water crab *Barytelphusa cunicularis*. Similaraly the level of lipid and protein also affected by the impact of Malathion on the experimental crabs.

Freshwater femaleCrab- *Barytelphusa cunicularis* (FIG-1a)

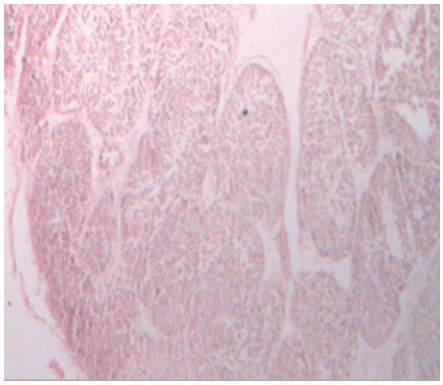
Crab Burrows (FIG-1b)

(A) **(B)**

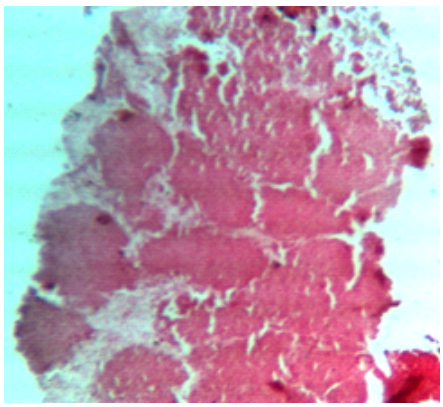
Female Crab Dissected Ovary (FIG-2a)



Female reproductive organs (FIG-2b)



T.S of Ovary (Control) (FIG-3a)



T.S. of Ovary (Effected) (FIG-3b)

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