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IMPACT OF AN INSECTICIDE ROGOR ON OVARY OF *CHANNA PUNCTATUS* (BLOCH)

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ABSTRACT

The present study deals with the toxic effects of an insecticide 'Rogor' on the ovary of *Channa punctatus*. Results give us clear data of great variation in numerical figure of different oogenic cells. It was found that in each ovarian phase, the normal development of ovary and its oocytes have been retarded due to adverse effect of Rogor. The percentage of atretic oocytes (Stage VI) was found to be greater.

INTRODUCTION

Water pollution is a worldwide phenomenon and is one of the most serious problems confronting mankind today. Contamination of water bodies takes place mostly due to discharge of effluents from industries, domestic wastes and different types of pesticides. The growth of human civilization and everincreasing population required more demand for food, comfort and infrastructure, which led to the manifold expansion of industries, agriculture and unabating process of urbanization.

A variety of chemicals as fertilizers, pesticides, herbicides and other biocides are nowadays used to control crop pests and diseases to boost agricultural production. Although the green revolution throughout the world has successfully met with the challenge of hunger, however, indiscriminate use of pesticides and fertilizers in agricultural fields has resulted in different types of pollution, especially water pollution, affecting different aquatic biota in general and fishes in particular and later causing health hazards to humans through food chains (Basak et al. 1976). Fish is an important food resource in freshwaters as well as marine waters as it is rich in protein, carbohydrate and other nutritional constituents.

In the recent past much literature has been generated about the pollutional effects of various chemicals on different organs of different fishes by different authors (Banerjee & Bhattacharya 1995, Gautam & Ritesh 1995, Nagarajan & Yuvarani 2006, Sadhu 1993, Saksena 1987), still very little attempt has been made to study the toxic effect of pesticides on ovary of fish. Keeping this in view it was thought worthwhile to trace the impact of a commonly used pesticide 'Rogor' on the ovary of *Channa punctatus*. This fish is abundantly found in different parts of Hazaribag and can be kept under laboratory condition for a long period. Further, its availability throughout the year also contributed to the selection of the fish for the present study. Experiment was conducted on the specific point of ovary such as calculation of volume of ovary and frequency percentage of different oogenic cells in different parts of Rogor treated ovary.

MATERIALS AND METHODS

Collection of fish: The live *Channa punctatus* fish were collected from local ponds and paddy fields of Hazaribag, brought to the laboratory and treated with 0.5% KMnO₄ solution for 2-3 minutes. The fish were acclimatized to laboratory conditions for ten days and kept in 100 litres cement cisterns containing pond water free from any noticeable contaminant after test. The cistern water was replaced

after every 24 hours to avoid any metabolic waste matter. Regular supply of food (powdered goat or hen lever) and additional O₂ (by aerator) were made to avoid mortality due to starvation and respiratory stress.

Determination of volume of ovary: To study the effects of the test chemical (Rogor) on ovarian activities, the experiments were carried out throughout the year in two cement cisterns of 100 L capacity marked by 'A' and 'B' containing pond water (as control) and pond water mixed with non-lethal dose of Rogor (27 ppm) (as treated) as earlier reported by Singh (2001). A batch of six fish were kept in both the cisterns. Here also the water was replaced after every 24 hours, and fish were given food.

After the end of every month the fish of both the cisterns (control and treated) were put out with the help of net and dissected ovaries were removed and volume was determined by water displacement method by following formula.

Initial volume of water in cylinder = A mL

Final volume of water in cylinder with ovary = B mL

the volume of ovary = B - A = C mL

During experimental period the water temperature was also recorded.

Calculation of frequency percentage of different oocytes: The normal and treated dissected ovaries were cut into small pieces and then fixed in Bouin's solution for 24 hrs. The tissues were then dehydrated properly through graded ethyl alcohol, cleared with xylene and embedded in paraffin. Sections were cut of 6-8 micron in thickness with the help of a microtome. The permanent slides were prepared by using graded alcohol, haematoxylin, stained with eosin and mounted in DPX.

The prepared slides were examined under compound microscope for identification of various cell parts like chromatin, nucleolus, peri-nucleolus, yolk granules, yolk vesicles, and mature and atretic oocytes in every phase of ovary. The frequency percentage of different cells were calculated from the various parts of ovary with reticulo-micrometer under the oil immersion objective of a binocular microscope.

RESULTS AND DISCUSSION

It was noticed that the volume of ovary gradually increased from the month of October to August, i.e. during post-spawning to pre-spawning period. During pre-spawning period (April-June) the volume of the ovaries was in ascending trend. The maximum volume was recorded in the month of June and July (2.2 and 2.40 mL) showing maximum activity in the ovaries during this period (Table 1, Figs. 1 & 2). In treated media the volume of ovaries was lesser than the normal every month, indicating that there are some chemical ingredients of Rogor which suppress the proper growth of ovary (Fig. 3, Table 1). These findings were similar to the result of earlier workers (Sadhu 1993, Saksena 1987).

Water temperature: The water temperature was recorded highest in the month of June (32.60°C) and lowest in January (18.50°C) in normal media while in treated water there was slightly increase in temperature indicating that the pesticide 'Rogor' is responsible for this. The water temperature has also been found to be closely related to the increasing and decreasing trend of volume of ovary (Figs. 1 & 2, Table 1), thus indicating that it influences directly the rebuilding and growth during post-spawning period and evacuation process during spawning period in the ovaries (Saksena 1987).

Frequency percentage of various oocytes: The female reproductive cycle of *Channa punctatus* has been divided into four phases viz., resting phase or post spawning phase (October-December), prepa-

Table 1: Volume of normal and 27 ppm, Rogor treated ovary of *Channa punctatus* and water temperature in different months of ovarian phase of a year.

Ovarian phase	Months	Water temperature, °C		Average volume of ovaries in mL	
		NM	RM	NM	RM
Resting or postspawning phase	October	23.10	23.58	0.46	0.40
	November	22.00	22.45	0.58	0.51
	December	19.25	19.36	0.70	0.62
Preparatory phase	January	18.52	18.65	0.95	0.80
	February	20.80	21.10	1.15	0.95
	March	22.60	22.90	1.50	1.00
Pre-spawning phase	April	24.70	25.50	1.75	1.50
	May	29.50	29.90	1.90	1.75
	June	32.60	32.95	2.20	2.00
Spawning	July	30.00	30.50	2.40	2.10
	August	28.40	28.90	2.50	2.15
	September	24.00	24.60	1.10	0.90

NM - Normal media (Controlled); RM - Rogor treated media (Treated)

Table 2: Comparison of frequency percentage of various oocytes in the ovary of the normal fish *C. punctatus* (Bloch.) with those of Rogor exposed fish in non-lethal dose (27 ppm).

Phase of ovary in month	Numerical percentage of different oocytes					
	S1	S2	S3	S4	S5	S6
	NM-RM	NM-RM	NM-RM	NM-RM	NM-RM	NM-RM
Resting phase (December)	74 - 88	26 - 12	-	-	-	-
Preparatory phase (March)	26 - 52	42 - 45	32 - 3	-	-	-
Pre-spawning phase (June)	3 - 15	11 - 9	9 - 6	13 - 4	53 - 19	11 - 47
Spawning (a) August (b) September	7 - 9	7 - 4	6 - 9	10 - 5	55 - 25	15 - 48
	10 - 17	8 - 6	4 -	7 -	32 - 3	39 - 74

NM - Normal media (Controlled); RM - Rogor treated media (Treated); S1 - S2 are stages I to VI

ratory phase or maturing phase (January-March), pre-spawning phase or mature phase (April-June) and spawning phase (July-September).

The numerical data of frequency percentage are shown in Table 2 of both normal and treated cases. In *Channa punctatus* various stages of oocytes were recorded from different parts of ovary:

- Chromatin nucleolus stage (stage I)
- Peri-nucleolus stage (stage II)
- Yolk granules stage (stage III)
- Yolk vesicle stage (stage IV)
- Mature stage (stage V)
- Atretic stage (stage VI)

During the investigation it was noticed that stage I and II were shown in all the phases of ovary, but stage III, i.e. yolk granules oocytes were shown only from preparatory stage. It was completely

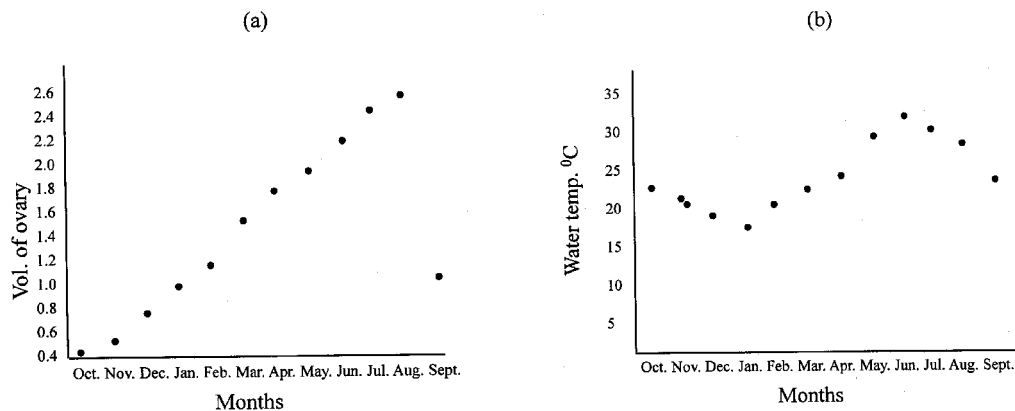


Fig. 1: Water temperature and average volume of ovary in normal media during different months of the year.

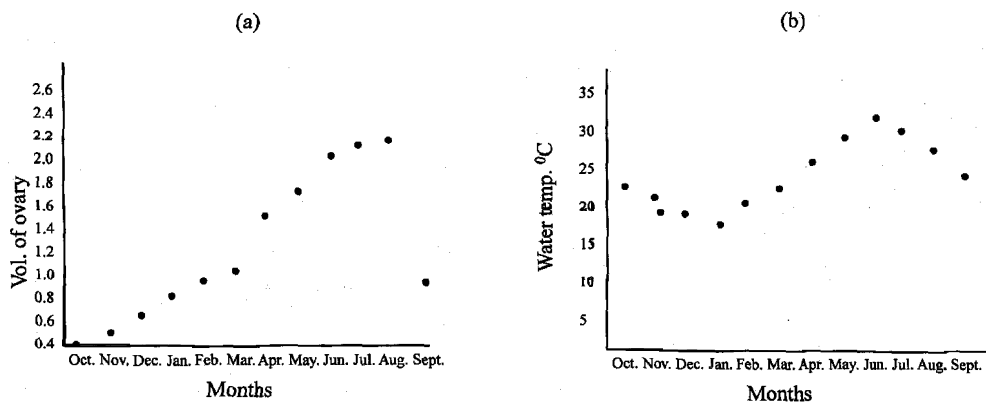


Fig. 2: Water temperature, average volume of ovary in treated media during different months of the year.

absent in resting phase of the ovary. The oocytes of stage IV, V and VI were visible from pre-spawning to spawning phase of the ovary in normal case. It was also marked that the stage I oocytes were greater in number during resting phase and low during pre-spawning phase (June), 74% and 3% respectively (Table 2).

From August the percentage of stage I oocytes again showed increasing trend (7%). The percentage of atretic oocytes (stage VI) were 11% and 39% in pre-spawning and spawning phase respectively (Table 2).

In treated fish, it was noted that the percentage of stage I oocytes, chromatin-nucleolus from resting to spawning phase were increased as compare to normal one (Table 2) while rest type of cells decreased, except atretic ones (stage VI). These oocytes (stage VI) showed upward percentage (from 47 to 74%) like earlier reporters (Das & Sengupta 1993, Marandi 2000, Singh & Sadhu 2001).

The higher percentage of atretic oocytes may be because of either improper growth of ovary due to which the oocytes were unable to grow into mature ones due to toxic effect of Rogor or diminished gonadotropin supply from hypophysis or direct pollutional impact on the intra-ovarian scene (Singh & Singh 1981). Das & Sengupta (1993) also showed similar results where malathion demonstrated a

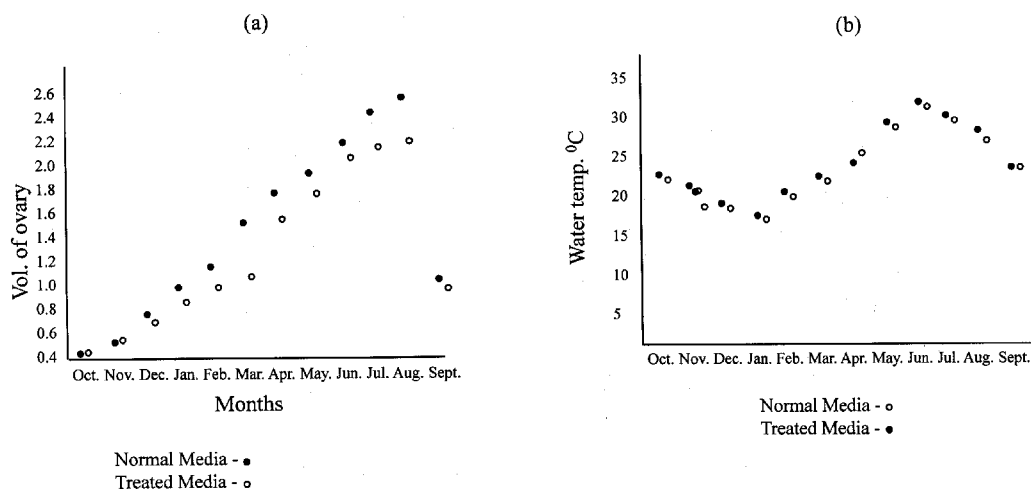


Fig. 3: Comparison of volume of *C. punctatus* in controlled and treated media in different months of the year.

dose dependent inhibition of brain acetyl cholinesterase activity in *Clarius batrachus* resulting in loss of stage I and II oocytes of ovarian cycle.

In the present study it was revealed that in the month of September (end of spawning phase) the oocytes of stage IV and V were lacking in treated ovary unlike the result of Marandi (2006). It was noticed that the normal ovarian development and gain in weight and size of the ovaries have been hampered due to impact of the pesticide 'Rogor'. The volume as well as frequency percentage of all the types of oocytes were on downward trend as compare to the normal. Temperature also plays an important role in growth and development. These changes were of great physiological significance and were attributable to the nature of the chemical ingredients of the pesticide 'Rogor'.

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