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Pesticide-Induced Atresia in Ovary of a Fresh Water Fish, *Colisa lalia* (Hamilton–Buchanan)

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In paddy culture carbofuran, the systemic insecticide, was consistently administered in view of the recent outbreak of the brown plant hopper, Nilaparvata lugens, Stal. (Jalees and Vemuri 1980), without knowing the consequences. However, it has been realised that pesticide, undoubtedly, is one of the major sources of water pollution, as it eradicates the beneficial species either indirectly through breaking the biological chains or directly producing toxic stress and chemical changes. Predominantly, as a result of extensive application of pesticide, large scale mortalities of fish occurred widely (Singh and Yadew 1978). Therefore, a considerable recent interest has been directed towards the possible hazards to aquatic species and to associated human population posed by insecticides in aquatic environments.

Few investigations reported the toxic and histopathological effects of carbofuran on certain fish (Carter and Groves 1973; Kabir and Ahmed 1979; Karpagaganapathy and Sukumar 1988; Ram and Singh 1988; Sukumar and Karpagaganapathy 1986). But its impact on the ovary, which the pesticide may deteriorate, is less explored. The experiments presented here display the histological effects of carbofuran on a single stage of ovary, when the fish, Colisa lalia were exposed to median lethal (LC_{50} for 24 hr) and sublethal (LC_0 for 20 d) concentrations of carbofuran.

MATERIALS AND METHODS

The experimental fish, C. lalia belonging to the family, Anabantidae and order, Acanthopterygii, were collected from the fresh water ponds around Annamalainagar (11° 29' N, Long 79° 49' E). The females measuring in

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length from 40 to 50 mm and in weight from 1.0 to 1.8 g were reared and fed with boiled eggs. They were acclimated to the laboratory conditions, until there was less than 10% mortality in four days prior to the commencement of the toxicity test.

The toxicity tests were conducted in the well water at different concentrations prepared from the stock solution of the (99% pure) technical grade carbofuran (M/S. Rallis India Ltd., India) which was made up in the carrier solvent, acetone. The water criteria which were maintained during the toxicity studies, consisted of dissolved oxygen 72 mg/L, salinity 450‰, alkalinity 246 mg/L as $CaCO_3$, hardness 370 mg/L as $CaCO_3$, pH 7.3 and temperature 27 ± 1°C. The 24 hr median lethal (1.7 mg/L) and 20-day sublethal (0.7 mg/L) concentrations of carbofuran were estimated following the renewal techniques of static toxicity tests in which the fish were periodically exposed to the test concentration of the same composition, usually once every 24 hr by transferring the fish from one experimental tank to another (US EPA 1975).

The fish with average size 45 ± 0.1 mm and weight 1.5 ± 0.02 g were divided into four batches each containing 10 female C. lalia. The first two batches were administered with sublethal (0.7 mg/L for 20 d) and median lethal (1.7 mg/L for 24 hr) concentrations of carbofuran and the third and fourth treated as controls (for 24 hr and 20 d) in the pesticide free well water containing the maximum aliquot of acetone (0.1 mL/L). Ovaries of treated and control fish particularly, which were alive at the end of each exposure, were dissected and immediately fixed in Boun's fixative. The heavily yolked eggs were treated with 5% phenol in 70% alcohol for a period of 24 hr following the method of Silfer and King (1933) to soften the yolk while sectioning. Then, the tissues were processed, embedded in the paraffin wax, sectioned into 6-µm thick sections, stained with Heidenhain's Haematoxylin and counterstained with Eosin adopting the standard histological method (Gurr 1962).

RESULTS AND DISCUSSION

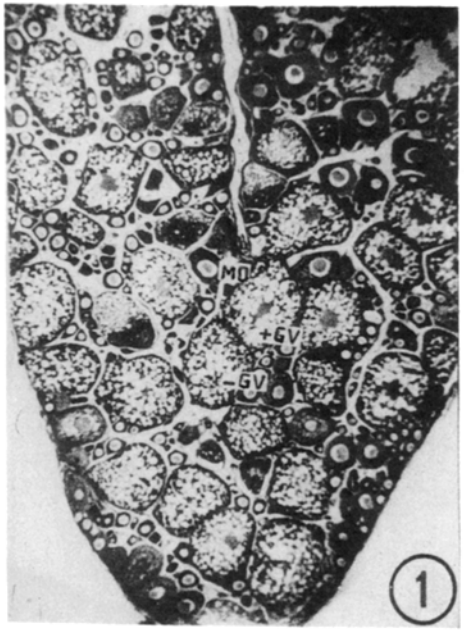
In the control fish of C. lalia the predominantly perceived oocytes were 1) the oocytes with the germinal vesicles (stage VII oocytes), 2) oocytes without the germinal vesicles and 3) the developing oocytes of the smaller size below 70 µm. The cytoplasm of such oocytes was fully distended with a heavy amount of yolk tending to coalesce. The ground cytoplasm was sparsely distributed between the large and small yolk globules. The germinal vesicle was still oval and lobulated and showed a tendency to break up. The nucleoli showed a

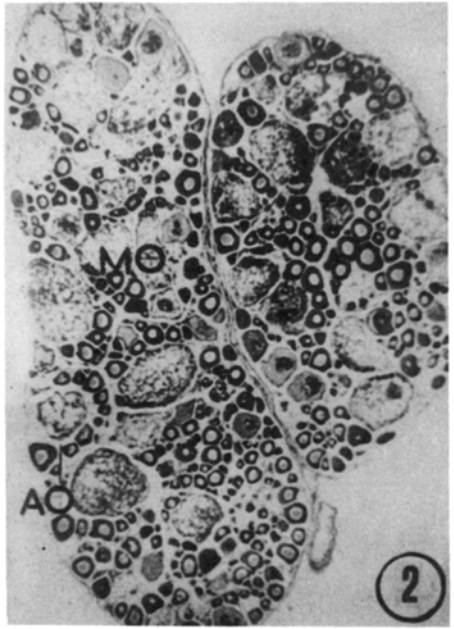
reduction in their number. The existing nucleoli appeared large perhaps due to fusion of the smaller ones. During the breakdown of the germinal vesicle, the nuclear membrane exhibited a wavy contour and the nucleoli were released in the cytoplasm. The breakdown of the germinal vesicle, which appeared simultaneously in all advanced oocytes, heralded the onset of maturation stage (Fig. 1). Thus, the stage of ovary of the fish exposed to control medium for 20 d was indentified as early maturation stage. Since there was no marked variation between the ovaries of fish exposed to control medium for 24 hr and 20 d, the latter was treated as control for both the exposures.

C. lalia, when subjected to sublethal concentration of carbofuran, showed a characteristically retrogressive type of ovaries. In such ovaries the developing oocytes ranged from 50 to 100 µm (stage I and II); the oocytes without the germinal vesicle measured from 220 to 300 µm and atretic oocytes were from 80 to 180 µm. Though some oocytes indicated the early maturation stage of oocytes having the cytoplasm fully expanded with a heavy accumulation of yolk granules, the presence of more atretic oocytes and lesser mature oocytes revealed the atresia of ovarian follicle (Fig. 2). The quantity of yolk granules in the oocytes of C. lalia exposed to sublethal concentration of carbofuran was significantly less compared with that of the ovary of control fish (Table 1).

When C. lalia were subjected to median lethal concentration of carbofuran, the hypertrophic and more wrinkled oocytes, reduced size of oocytes and disintegrated lamigerous lamellae were the important atrophies observed. Such immediate changes were comparable to those observed in the ovary of fish treated with sublethal concentration of carbofuran. But the resorption of yolk leading to the atretic condition was not evident within the short period of 24-hr median lethal exposure (Fig. 3). Similar observations in the ovaries of fathead minnows, Anabas testudineus and Heteropneutes fossilis exposed respectively to carbaryl, thiourea and sumithion were reported (Carlson 1972; Kabir and Ahmed 1979; Rao and Kukhar 1972).

Thus, it was discerned that there was large number of mature oocytes in the early maturation stage of ovaries of control C. lalia and that there were fewer mature oocytes and the remaining mature oocytes became atretic in the ovaries of fish exposed to sublethal concentration of carbofuran. Kling (1981) reported that self assimilation of yolk granules during the exposure and simultaneous arrest of vitellogenesis caused the reduction in size of oocytes, resulting in a total





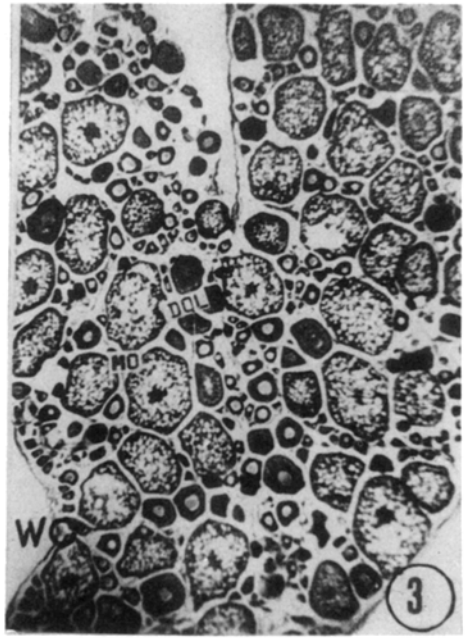


Figure 1. Longitudinal section of (Early maturation stage) ovary of control fish, Colisa lalia, showing a larger number of mature oocytes (MO) with germinal vesicle (+GV) and without germinal vesicle (-GV) (X 80).
Figure 2. Longitudinal section of (Early maturation stage) ovary of the fish, C. lalia subjected to sublethal concentration of carbofuran, displaying a larger number of atretic oocytes (AO) and mature oocytes (MO) (X 80).
Figure 3. Longitudinal section of (Early maturation stage) ovary of the fish, C. lalia treated with the median lethal concentration of carbofuran, representing the mature oocytes (MO), wrinkled oocytes (WO) and disturbed ovigerous lamellae (DOL) (X 80).

Table 1. Effect of carbofuran on the number and size of oocytes in *Colisa lalia*

The Stage of Oocytes	Control		Sublethal		Lethal	
	Number mean ± S.D.	Size in Diameter µm	Number Mean ± S.D.	Size in Diameter µm	Number Mean ± S.D.	Size in Diameter µm
Oocytes with Germinal Vesicle	14 ± 1.85	350-430	0	0	16 ± 1.5	200-360
Oocytes without Germinal Vesicle	24 ± 1.11	330-420	20 ± 0.5	220-300	21 ± 3.0	240-320
Atretic Oocytes	4 ± 1.11	180-240	35 ± 2.1	80-180	6 ± 1.0	180-200

Note: Mean is for 10 observations, each in 10 middle sections of ovary.

atresia of ovaries of Tilapia leucosticta exposed to insecticide, lebaycid for 14 d. Further, Pawar and Katdare (1983) reported that at sublethal exposure inhibiting action of sumithion on stereoidogenesis caused lack of sufficient endogenous gonadotrophin consequently resulting in atretic condition of ovaries of fish, Garrya mullya.

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