Punjab Univ. J. Zool., Vol.19, pp.59-66, 2004

EFFECTS OF ACUTE AND CHRONIC EXPOSURES TO DIAZINON ON THE FECUNDITY AND EGG SIZE IN *COLISA FASCIATA*

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Abstract: The fecundity and egg size of a teleost, *Colisa fasciata* following acute and chronic exposure to different concentrations of Diazinon was investigated. Adult fish were exposed to Diazinon of concentrations 16.0 mg/l (96 R-LC_{50}), 12.0 mg/l ($LC_{37.5}$) and 6.0 mg/l ($LC_{18.75}$) for 96 hours and 2.6 mg/l (30 day-LC_{50}) and 1.3 mg/l (LC_{25}) for 30 days during the preparatory phase of the ovary. The fecundity was found to decrease by 60%, 42% and 19% in acute exposure of dose concentrations of 16.0 mg/l, 12.0 mg/l and 6.0 mg/l, respectively. In chronic exposures of doses 2.6 mg/l and 1.3 mg/l the fecundity was found to decrease by 61% and 43%, respectively. The egg size was investigated to correlate directly with the increase in dose concentrations. There was 10.6% increase in egg size in 16.0 mg/l, 6.66% in 12.0 mg/l and 3.9% in 6.0 mg/l in acute exposure, whereas in chronic exposure of Diazinon it was significant in 2.6 mg/l and non-significant in 1.3 mg/l as compared to the control.

Key words: Diazinon, environmental pollution, fecundity, egg size, *Colisa fasciata*.

INTRODUCTION

Apart from the industrial run off, the indiscriminate use of pesticides has further polluted the aquatic environment resulting in a wide range of problems that need to be resolved. These involve not only the identification of possible contaminants having harmful effects on fish and other aquatic organisms but also determination of toxic levels of contaminants and their tolerance by species of different age groups.

Since chlorinated pesticides have been proven to have many deleterious effects on non-target organisms (Renata and Arnese, 1988), there has been a shift towards a greater use of organophosphorus pesticides in agriculture sector because of their biodegradable and non-cumulative properties. But unfortunately, these pesticides too have been quite harmful to the non-target organisms (Durham and Williams, 1972; Jennings *et al.*, 1975; Harbison, 1975). Organophosphorus pesticides have been shown to cause gross morphological (Henderson and Pickering, 1958; Butler *et al.*, 1969; McCann and Jasper, 1972), histopathological (Couch, 1975; Sastry and Sharma, 1981) and biochemical (Saxena *et al.*, 1988; Asztalos *et al.*, 1988; Nemcsok and Benedeczky, 1990) effects of fish. However, the effect of acute exposure to organophosphorus insecticde on the fecundity of fish is little known. In the present study, the effects of short term exposure to

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the organophosphorus insecticide. Diazinon on the fecundity and egg size in teleost fish, *Colisa fasciata* have been investigated. This acute toxicity bioassay would provide an information in establishing the water quality criteria and to estimate a safe level for fish reproduction and fecundity.

MATERIALS AND METHODS

Female fish of almost equal length ranging from 8.3-8.8 mm were selected from the acclimated fish stock. The fish were divided into seven groups of twenty individuals each and were kept in 200 l water tanks containing water of pH 7.6; total hardness 130 mg/L as $CaCO_3$; D.O. 5.0-5.5 mg/L. Experiments for the study of acute exposures three groups were exposed to Diazinon of concentrations of 16.0 mg/L (96 h-LC_{50}), 12.00 mg/L ($LC_{37.5}$) and 6.0 mg/L ($LC_{18.75}$) for 96 h while the fourth group without any treatment served as control. Fifth and sixth groups were exposed to 2.6 mg/L (30 day-LC_{50}) and 1.3 mg/L for the study of chronic exposures. The seventh group was served as control. The LC_{50s} were calculated by Litchfield and Wilcoxon method (1949). The experiment was started in the last week of February, during the preparatory phase of the ovary. During exposure period, the fish were not fed. Dead fish were removed from the tanks daily. After exposure, continuous supply of fresh water and daily feeding were maintained till the fish became fully gravid, in the last week of May. The ovaries were then removed as a whole from each gravid fish of the exposed and control groups. After weighing the ovaries were placed in separate vials containing Gilson's fluid. After 2-3 weeks of preservation in this fluid, during which vials were periodically shaken, the ovaries were broken manually and by repeated washing, the tissue debris was removed and eggs separated from one another. The eggs were then stored in vials containing 5% formaline until they were counted and measured. For counting the eggs were whirled in one litre water and at least five sub-samples of 1/30 each were counted. The counts were averaged and multiplied by appropriate conversion factor to achieve total count. The diameter of at least 20 randomly selected eggs from each fish were measured in μm by ocular micrometer. GSI was also calculated by the following formula:

$$GSI = \frac{\text{Wt. of ovary}}{\text{Wt. of fish}} \times 100$$

The statistical significance of the data was tested using student's 't' test.

RESULTS

Acute exposure

Fecundity estimates of control and treated groups exposed to different sub-lethal concentrations of Diazinon are given in Table I. The fecundity of the control ovary was

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Fecundity estimates of control and treated groups exposed to different sub-lethal concentrations of Diazinon are given in Table I. The fecundity of the control ovary was

2880-7320 eggs with a mean of 4654 eggs for ten fish of 8.73 ± 0.2 cm in length. In samples of fish exposed to different sub-lethal concentrations of Diazinon, fecundity decreased significantly ($P<0.001$) with the increase in dose concentration (Fig.1), particularly when treated during preparatory phase of the ovary. As compared to the control there was 19%, 42% and 60% decrease in fecundity on exposure to 6.0 mg/L, 12.0 mg/L and 16.0 mg/L of Diazinon, respectively. GSI was also found to decrease with the increase of dose concentration in lower dose groups. In the higher dose group (16 mg/L) the GSI was found to increase but the fecundity was 60% less as compared to the control (Fig.2).

Egg size was also found to be dose dependent. The diameter of the 100 control eggs measured, 10 eggs from each fish sample, ranged from 630-705 μm with the mean and SD (660 ± 19.7). In treated groups of eggs there was a significant ($P<0.001$) increase in egg size (Table I). The data collected from the treated groups of fish revealed an increase in egg size of 3.9% in 6.0 mg/L, 6.66% in 12 mg/L and 10.6% in 16 mg/L Diazinon concentration when compared with the control (Fig.3).

Chronic exposure

Fecundity estimates of chronically exposed groups of fish are also given in table. The mean egg number of fish exposed to 1.3 mg/L (30 day LC_{25}) was found to be 5659 ± 745, which was 43% less than that of control. Fecundity of fish exposed to 2.6 mg/L (30-day LC_{50}) was found to be further reduced to 1814 ± 433 which was 61% less as compared with the control.

The GSIs of both the treated groups of 1.3 mg/L and 2.6 mg/L were also found to be reduced significantly ($P<0.05$ and $P<0.001$, respectively).

As far as the egg size is concerned, the change in diameter of eggs was not significant in both the doses of acute and chronic treatment, however, in high dose group the range in diameter of eggs was almost double as compared with the control.

DISCUSSION

Fecundity studies on various species such as the European long rough dab, *Hippoglossoides platessoides* (Bagenal, 1957), Pacific herring, *Clupeaharengus pallasi* (Nagasaki, 1958), Atlantic cod, *Gadus morhua* (May, 1967) and yellow tail flounder, *Limanda ferruginea* (Pitt, 1971) have demonstrated that variations in the fecundity of these species can be adequately explained in terms of body length alone. Winters (1966) has reported that in capelin, age rather than length is the dominant factor. Previous studies (Templeman, 1948; Pitt, 1958) indicate that caplin grows rapidly up to the onset of maturity after which growth is considerably retarded and length increments become small. Hodder (1963) proposed that fish that have previously spawned have a greater fecundity than fish of the same age and size spawning for the first time. There is a clear evidence from the published data that in caplin, as the age increases the proportion of repeat spawning also increases without a significant increase in length. There are also inter- and

intra-specific differences in fecundity of fish, however, the higher or lower rate of fecundity depends upon the length, weight, GSI, age of fishes and repeated spawning (Singh *et al.*, 1982; Zirkov, 1984; Baloni, 1986) and environmental factors such as temperature or chemical stresses (Devauchelle, 1981). In present study all considerations were taken into accound in the selection of fish.

Table I: The effects of various sub-lethal doses of Diazinon on the GSI, ova number and size in *Colisa fasciata*.

Dose (mg/L)	Av. Length of fish (cm)	Av. Wt. of fish (g)	Av. Wt. of ovary (g)	GSI	Ova No.		Ova Size	
					Range	Mean ± S.D	Range	Mean ± S.D
Control	8.73± 0.2	11.19± 1.3	1.43± 0.37	12.66± 2.25	2880-7320	4654± 1112	630-717	660± 19.7
Acute treatment								
6.0	8.78± 0.20	11.168± 0.95	1.02± 0.41	9.9± 2.3	2016-5538	3763± 1230	637-714	686± 18
12.0	8.73± 0.2	11.27± 0.74	0.82± 0.30	7.31± 2.8	1160-5200	2699± 1556	660-756	704± 35
16.0	8.71± 0.22	10.89± 0.97	1.04± 0.30	9.58± 2.8	1056-2800	1857± 640	699-766	730± 25
Chronic treatment								
1.3	9.06± 0.32	10.69± 0.76	0.921± 0.30	9.109± 2.3				
2.6	9.15± 0.34	10.87± 0.77	0.643± 0.36	5.874± 3.2				

Little information is available on the possible lethal, sub-lethal and chronic effects of insecticides and other pollutants on fish fecundity (Trojnar, 1977; Barron and Adleman, 1984). However, the effects of radiation such as X-rays, metal toxicants and Dieldrin on early life stages of fishes have been investigated.

Foster *et al.* (1949) found significant decrease in fecundity of rainbow trout from parents subjected to 50 and 100 rhontgen (R) of X-rays, 3 months before spawning. The growth in weight of rainbow trout fry was impeded by X-rays of doses as low as 100 R (Kobayashi and Mogami, 1958). Till (1978) reported that ^{238}Pu (Plutonium) was uniformly distributed throughout the perivitelline fluid and yolk in the eggs of carp and fat-head minnow when exposed chronically to ^{238}Pu. Lead (Holcombe *et al.*, 1976) was shown to penetrate the chorion only slightly and hardly to accumulate in the embryos of brook trout although the exposure was at the egg stage. In experiments with Cobalt, Kunze *et al.* (1978) showed that ^{57}Co accumulated rapidly during the first 24 h, but almost all the accumulated Cobalt was reversibly bound by mucopolysaccharide in the chorion. Preferential accumulation in the chorion was also found for Cadmium (Mitchibata, 1981) and Zinc (Wedemeyer, 1968) in the embryo of the teleost, *Oryzias latipes*. Therefore, the chorion appears to 'protect' the embryo from the uptake of toxicants not by completely

preventing but by slowing down the intrusion. Aromatic hydrocarbon (Korn and Rice, 1981) and tetra-chlorobiphenyl (Guiney *et al.*, 1980) are also known to accumulate primarily in the yolk. In experiments with Dieldrin, Van Leeuwen *et al.* (1985) investigated its accumulation in the rainbow trout egg-yolk after short term exposure during egg-stage. With the resorption of yolk, these chemicals redistribute thus causing the death of juveniles of rainbow trout (Seinen *et al.*, 1981). It can thus be speculated that yolk acts as a temporary 'toxicant sink'. This is in support of the hypothesis proposed by Longwell (1977) who postulated that pollutants enter the egg with imbedded water during the process of perivitelline fluid formation. This holds true with the present study that increase in concentration of Diazinon possibly increases the accessibility of the pollutant in the egg and, therefore, the toxicity. This appears in the form of decrease in fecundity and GSI, and increase in the size of the egg.

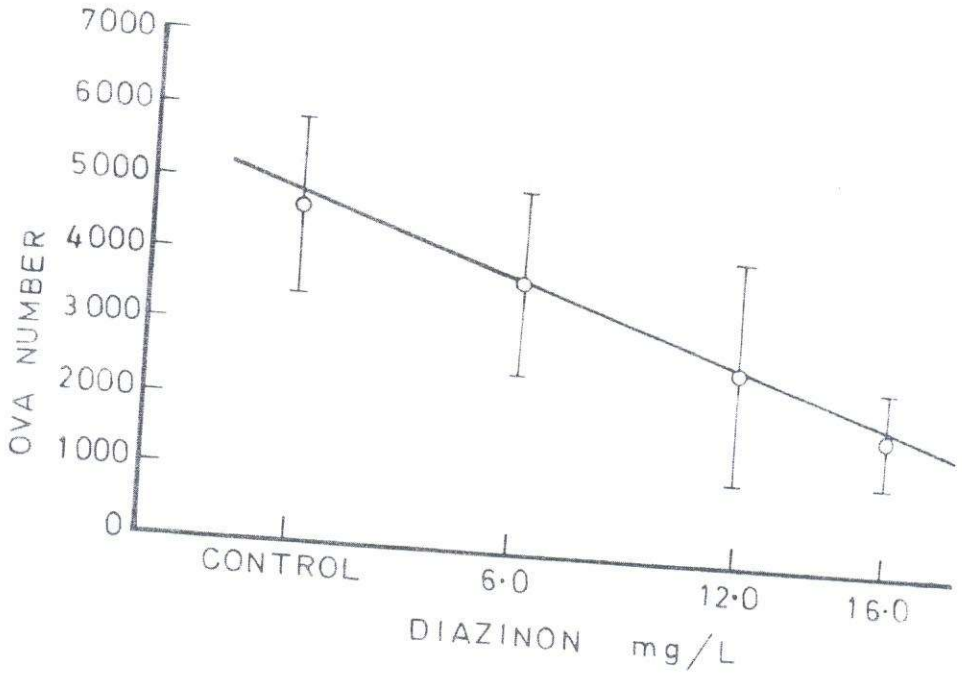

Fig. 1: Eggs (mean ± S.D) produced by *Colisa fasciata* after exposure to various concentrations of Diazinon.

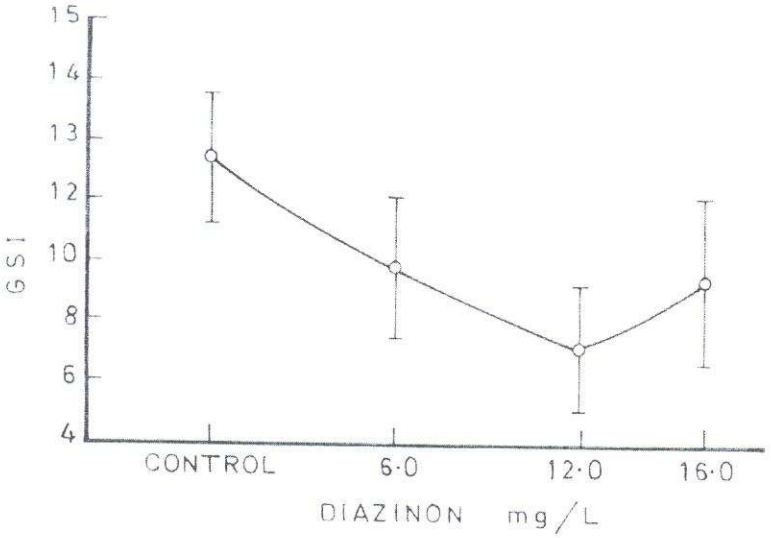

Fig. 2: **GSIs (mean ± S.D) of *Colisa fasciata* following the treatment with various concentrations of Diazinon.**

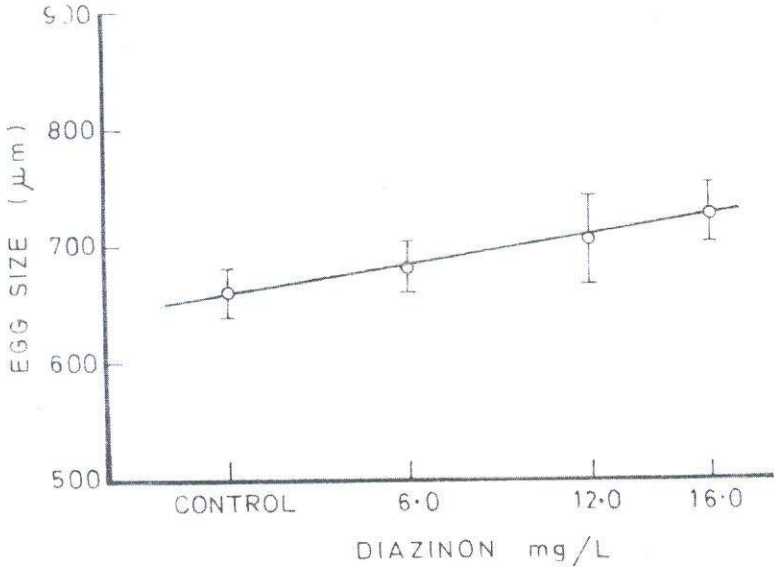

Fig. 3: **Size of the eggs (mean ± S.D) produced by *Colisa fasciata* after the treatment of various concentrations of Diazinon.**

The decreased fecundity and GSI in treated fish may be attributed to either reduced vitellogenin or other proteins, glycogen and other carbohydrates and lipids to be accumulated in the oocyte cytoplasm. Insufficient production of ganadotrophins in the Diazinon treated fish may also be one of the causes of low fecundity, since, it is well established that in teleosts, oocyte growth and maturation is accomplished by the precise production and regulation of ganotrophin and gonadal hormones.

This study thus adds new evidence to support that Diazinon has inhibitory effect on the ovarian growth and development because of its accumulative properties.

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(Received: January, 2004)