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Effect of Diazinon 60 EC on *Anabas testudineus, Channa punctatus* and *Barbodes gonionotus*

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Abstract

Anabas testudineus, Channa punctatus and *Barbodes gonionotus* were exposed to 5.62, 6.25, 6.87, 7.50, 8.12 and 8.75 ppm; 1.13, 2.26, 3.39, 4.52, 5.65 and 6.78 ppm; and 2.00, 2.50, 3.00, 3.50, 4.00 and 4.50 ppm of Diazinon 60 EC, respectively. The median lethal concentration (LC_{50}) values of Diazinon 60 EC on *A. testudineus, C. punctatus* and *B. gonionotus* were 6.55, 3.09 and 2.72 ppm for 96 hrs of exposure. The fish species showed several abnormal behaviors which included restlessness, arena movements, loss of equilibrium, increased opercular activities, strong spasm, paralysis and sudden quick movements during the exposure. For histopathological studies, *A. testudineus, C. punctatus* and *B. gonionotus* were exposed for 7 days to sublethal concentrations of 1.13 and 3.75 ppm; 1.13 and 2.26 ppm; and 1.13 and 2.26 ppm of Diazinon 60 EC, respectively. Hypertrophy, necrosis and pyknosis of hepatocytes, pyknosis and degenerative changes such as necrosis of tubular and haematopoietic cells of kidney were the major histopathological effects.

Introduction

Pesticides are useful tools in agriculture and forestry, but their contribution to the gradual degradation of the aquatic ecosystem cannot be ignored (Konar 1975, Basak and Konar 1976, 1977). The aquatic ecosystem as a greater part of the natural environment is also faced with the threat of a shrinking genetic base and biodiversity. *Anabas testudineus, Channa punctatus* and other indigenous small fishes use paddy fields as breeding and nursery grounds. *Barbodes gonionotus* is an important species for integrated rice-fish farming. Pesticides at high concentrations are known to reduce the survival, growth and reproduction of fish (Mckim et al. 1975) and produce many visible effects on fish (Johnson 1968).

Due to the residual effects of pesticides, important organs like the kidney, liver, gills, stomach, brain, muscles and genital organs are damaged. Until the use of pesticides in crop farming is replaced by other means of pest control such as integrated pest management, less toxic pesticides at lowest possible doses need to be recommended. Organophosphorus pesticides vary in their toxicity to different species. Very limited work has been done on the histo-pathological effects of pesticides on fishes. This study determines the median lethal concentration (LC_{50}) values and behavioral impacts of the pesticide Diazinon 60 EC on the three fish species mentioned. The histo-pathological state of the liver and kidney of tested fish species at sublethal concentration for 7 days exposure time was also studied.

Materials and Methods

A. testudineus, averaging 6.12 ± 0.258 cm and 4.369 ± 0.452 g; *C. punctatus*, averaging 10.20 ± 0.506 cm and 11.722 ± 1.208 g and *B. gonionotus* averaging 2.82 ± 0.166 cm and 0.240 ± 0.046 g were used in the study.

Twenty-one glass aquaria of size 60 cm x 30 cm x 30 cm with 50 liters of tap water were used in the experiments. Initially, range finding tests were performed. It was observed that none of the *A. testudineus*, *C. punctatus* and *B. gonionotus* died when exposed to concentrations below 5.625, 1.13 and 2.00 ppm and complete mortality was observed at concentrations of 8.750, 6.78 and 4.50 ppm, respectively. Based on this, six concentrations of the pesticide were tested for each of the species. The

different concentrations of Diazinon 60 EC for *A. testudineus, C. punctatus* and *B. gonionotus* were 5.62, 6.25, 6.87, 7.50, 8.12 and 8.75 ppm; 1.13, 2.26, 3.39, 4.52, 5.65 and 6.78 ppm; and 2.00, 2.50, 3.00, 3.50, 4.00 and 4.50 ppm, respectively, with three replications including a control. Ten acclimated fish were released in each aquarium containing different concentrations of pesticide as well as in the control. All tests were done at room temperature. The behavior and other external changes in the body of fishes were observed. Dead fishes were removed and mortality was recorded at 6, 12, 24, 48, 72 and 96 hrs of exposure time. Temperature, dissolved oxygen and pH were recorded daily. The LC_{50} values for different fish species were calculated for 96 hours of exposure time by probit analysis.

For histopathological investigations, the fish were exposed to various sublethal concentrations: 1.13 and 3.75 ppm for *A. testudineus*, 1.13 and 2.26 ppm for *C. punctatus* and 1.13 and 2.26 ppm for *B. gonionotus* for a period of 7 days with three replications for each.

At the end of 7-day exposure period, liver and kidney samples (1 cm^3) were collected from the three fish species from each concentration and preserved in small plastic vials with ten times 10% neutral buffer formalin fixatives. The numbers at section of samples was prepared using a microtome, stained and studied under a microscope.

Results

General effects

The effects of different concentrations and exposure time of Diazinon 60 EC on the three species are presented in Tables 1, 2 and 3. The LC_{50} values based on probit analysis were found to be 6.55, 3.09 and 2.72 ppm for *A. testudineus, C. punctatus* and *B. gonionotus* for 96 hrs of exposure, respectively.

Table 1. Cumulative mortality of **Anabas testudineus** *at Diazinon 60 EC treatments during the experimental period.*

Concentration (ppm)	Cumulative mortality %					
	6 h	12 h	24 h	48 h	72 h	96 h
Control	00	00	00	00	00	00
5.625	00	00	00	03	13	17
6.250	00	03	17	23	33	37
6.875	00	13	30	40	53	63
7.500	00	13	23	43	63	77
8.125	10	17	27	47	73	93
8.750	43	53	60	70	83	100

Table 2. Cumulative mortality of **Channa punctatus** *at Diazinon 60 EC treatments during the experimental period.*

Concentration (ppm)	Cumulative mortality %					
	6 h	12 h	24 h	48 h	72 h	96 h
Control	00	00	00	00	00	00
1.13	00	00	00	00	00	00
2.26	00	00	00	00	17	27
3.39	00	00	00	03	23	57
4.52	00	03	03	20	53	80
5.65	00	10	13	33	67	93
6.78	07	20	23	50	70	97

Table 3. Cumulative mortality of **Barbodes gonionotus** *to Diazinon 60 EC treatments during the experimental period.*

Concentration (ppm)	Cumulative mortality %					
	6 h	12 h	24 h	48 h	72 h	96 h
Control	00	00	00	00	00	00
2.00	00	00	00	00	03	17
2.50	00	00	03	10	23	37
3.00	00	03	07	23	43	63
3.50	00	03	10	17	47	77
4.00	03	10	27	50	70	90
4.50	10	10	40	73	80	100

Abnormal behavior such as restlessness, sudden quick movement, rolling movements, swimming on the back (at higher doses) were observed when the media started to act on test species. The affected fish became very weak, settled at the bottom and died in increasing numbers at the higher doses. Normal color and behavior were observed in the control groups. However, the color became pale progressively with higher doses at the end of 96 hours of exposure time.

The average dissolved oxygen was higher at lower concentrations. However, the water quality parameters of temperature, dissolved oxygen and pH varied little in different treatments.

Histopathological effects

Liver

The hepatocytes and other cells of the liver in control groups were normal and systematically arranged.

Hypertrophy of hepatocytes, mild necrosis and minor vacuolation were found at the dose of 1.13 ppm for *A. testudineus*. At the dose of 3.75 ppm, severe necrotic hepatocytes, pyknosis, hypertrophy, haemorrhage and vacuolation were observed for the same fish (Fig. 1).

Hypertrophy, minor vacuolation and haemorrhage were recorded at the dose of 1.13 ppm for *C. punctatus* while at a dose of 2.26 ppm severe necrosis and hypertrophy occurred resulting in disappearance of liver cells and vacuolation. Haemorrhagic areas were also noticed (Fig. 2).

Liver tissue from *B. gonionotus*

treated with 1.13 ppm showed necrosis, hypertrophy and vacuolation. Marked hypertrophy, necrotic hepatocytes and pyknotic cells resulting in empty spaces at the dose of 2.26 ppm were observed for the mentioned fish species (Fig. 3).

Kidney

Kidney tubules and haematopoietic cells were normal and systematically arranged in the control treatment for all the three tested fish species.

Kidney tissue from *A. testudineus* exposed to 1.13 ppm showed necrosis, degenerated kidney tubules and pyknosis having haemorrhagic areas. Degeneration of kidney tubules and haematopoietic cells, necrosis, pyknosis and haemorrhage were also recorded at the dose of 3.75 ppm for the fish species (Fig. 4).

Kidney tissue from *C. punctatus* exposed to 1.13 ppm showed mild necrosis, pyknosis and tubular degeneration (Fig. 5). Severe necrosis, vacuolation and tubular degeneration were recorded at the dose of 2.26 ppm.

At the dose of 1.13 ppm, mild necrosis, pyknosis and melanogenesis having inflammatory cells were recorded for *B. gonionotus*. Severe degenerative changes such as necrosis of tubular and haematopoietic cells, pyknosis and vacuolation were observed at the dose of 2.26 ppm (Fig. 6).

The mean water temperature, dissolved oxygen and pH of different treatments including controls during the experimental period for histological study were $27.85 \pm 0.76^{\circ}C$, 4.98 ± 0.88 ppm and 7.82 ± 0.68, respectively.

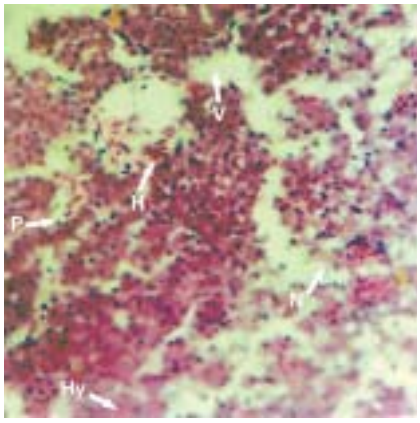

Fig. 1. Photomicrography of liver of* A. testudineus *after 7 days exposure to 3.75 ppm Diazinon 60EC. Severe necrosis (N) of hepatocytes, pyknosis (P), hypertrophy (Hy), haemorrhage (H) and vacuolation (V) were observed.

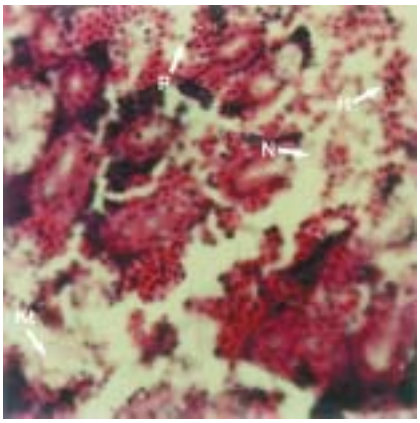

Fig. 4. Photomicrography of kidney of* A. testudineus *after 7 days exposure to 3.75 ppm Diazinon 60EC. Degenerated kidney tubules (Kt), necrosis (N), pyknosis (P) and haemorrhage (H) were observed.

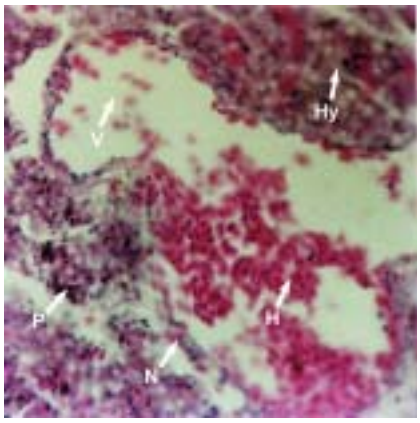

Fig. 2. Photomicrography of liver of* C. punctatus *after 7 days exposure to 2.26 ppm Diazinon 60EC. Hypertrophy (Hy), severe necrosis (N) of hepatocytes, pyknosis (P), hypertrophy (Hy) and haemorrhagic (H) areas were observed.

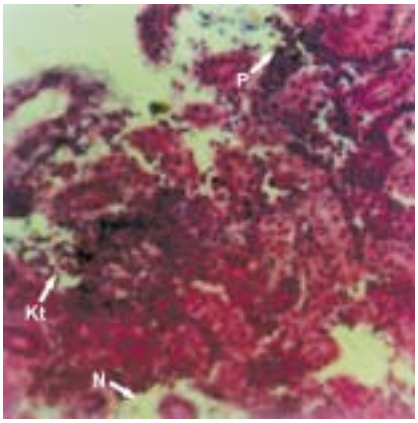

Fig. 5. Photomicrography of kidney of* C. punctatus *after 7 days exposure to 1.13 ppm Diazinon 60EC. Mild necrosis (N), pyknosis (P) and tubular degeneration (Kt) were observed.

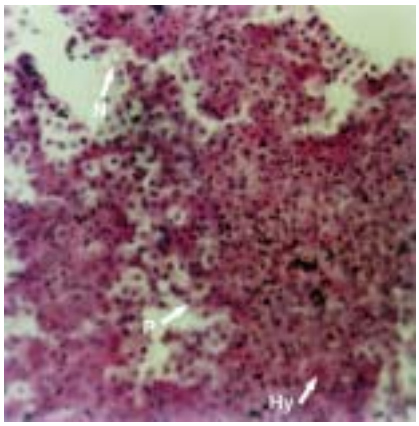

Fig. 3. Photomicrography of liver of* B. gonionotus *after 7 days exposure to 2.26 ppm Diazinon 60EC. Marked hypertrophy (Hy), necrotic hepatocytes and pyknotic cells (P) resulted some empty spaces.

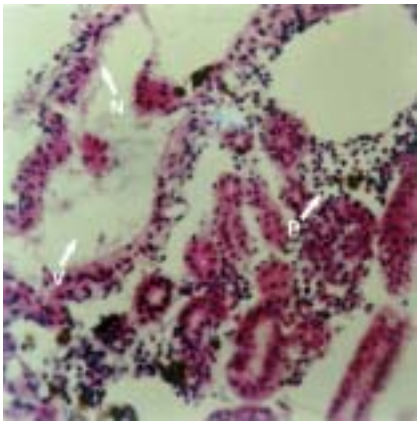

Fig. 6. Photomicrography of kidney of* B. gonionotus *after 7 days exposure to 2.26 ppm Diazinon 60EC. Severe necrosis (N), pyknosis (P) and vacuolation were marked.

Discussion

The LC_{50} values for 96 hours were 6.55, 3.09 and 2.72 ppm for *A. testudineus, C. punctatus* and *B. gonionotus*, respectively. The LC_{50} value in the present study for *C. punctatus* is similar to the findings of Haider and Inbaraj (1986), who used formulations of Malathion. Rao et al. (1985) reported the LC_{50} value of Elson for *C. punctatus* to be 0.43 ppm for 48 hrs of exposure. Hoque et al. (1993) observed the LC_{50} value to be 3.67 ppm at 96 hours when *B. gonionotus* of 12.7 cm in length were exposed to Diazinon. By taking into account the size of the present experimental fish (2.82 ± 0.166 cm), the LC_{50} value is close to the result obtained by Hoque et al. (1993). Al-Arabi et al. (1992) reported the LC_{50} value for 96 hours of exposure to Diazinon for the fingerlings of *Labeo calbasu* to be 1.54 ppm which is almost close to present findings, while the LC_{50} value of 1.002 ppm reported by Alam et al. (1995) was not comparable with that of the present work. In both cases, however the test fishes were of different sizes.

Several abnormal behaviors such as restlessness, loss of equilibrium, increased opercular activities, surface to bottom movement, sudden quick movement, resting at the bottom, etc. were similar to the observations of Hoque et al. (1993) and Lovely (1998). However, swelling in the abdominal region and gas-filled stomach were not observed, which is contrary to the findings of Kabir and Begum (1978) and Lovely (1998). It is an indication that the effect of pesticides is species-specific.

Temperature, hardness, pH, alkalinity, sex, age and other physiological status of the test animals have profound effects on the toxicity of agro-chemicals (Boyd 1979, Rand and Petrocelli 1985). Meletev et al. (1971) reported that pesticides affect the gas exchange of fish and other aquatic organisms. This might be one of the probable reasons for the decline of oxygen concentrations in the lower to higher concentration test media during the present work. As the recorded parameters had little variation, it is evident that physical and chemical properties of aquarium water were within the desirable range of fish culture (Boyd 1979).

At the agricultural dose of 1.13 ppm, mild pyknotic and necrotic hepatocytes, hypertophy and in a few cases vacuolation were recorded for all the fish species. At the highest doses (3.75 ppm for *A. testudineus*, 2.26 ppm for both *C. punctatus* and *B. gonionotus*), degenerative changes of hepatocytes and in some samples severe degenerative changes like necrosis, pyknosis, vacuolation, rupture of blood vessel causing haemorrhage were recorded for all the fish species. Kabir and Begum (1978) reported cytoplasmic degeneration, pyknotic nuclei in liver tissues; vacuolation in hepatic cells and rupture of blood vessels; degenerative hepatic cells and necrotic nuclei when *Heteroneutes fossilis* was exposed for 25 days to 5, 10 and 20 ppm Diazinon, respectively. Shastry and Sharma (1979) exposed *C. punctatus* to a sublethal concentration (0.01 mg/l) of Endrin and observed hypertrophy of hepatic cells and liver cord disarray, vacuolation of cytoplasm and necrosis, rupture of hepatic cell membrane and necrotic centrolobular area.

At the agricultural dose of 1.13 ppm, mild tubular degeneration, necrosis, pyknosis and haemorrhage were observed in cells of kidney. At the highest dose (3.75 ppm for *A. testudineus* and 2.26 for both *C. punctatus* and *B. gonionotus*), degeneration of kidney tubules and haematopoietic cells, necrosis, pyknosis and haemorrhage were recorded. Rand and Petrocelli (1985) reported necrosis of tubular and surrounding haematopoietic cells, nuclear changes of pyknosis and karyorrhexis of kidney tissue when coho salmon was exposed to 100 ppm Amitrole for 144 hours.

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