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Lethal Effects of the Insecticide DDVP on the Eggs and hatchlings of the Snake-head, *Channa punctatus* (Bl.) (Ophiocephliformes: Ophiocephalidae)

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Abstract Laboratory bioassays were made at 66 to 75°F to determine the effects of the organophosphorus insecticide DDVP on the viability of the sanake-head spawn and hatchlings. Sixteen different concentrations ranging from 0.10 to 20 ppm were tested against duplicate samples of eggs placed in cylindrical glass jars holding 8 litres of unchlorinated water. The eggs hatched at all test concentrations. Hatching time increased with the increase of concentration. All eggs hatched at concentrations up to 1.0 ppm within a period of 12 hours. Within the same period, about 60% eggs hatched at 1.6 to 16 ppm, and 55% eggs hatched at 20 ppm; the remainders hatched within 24 hours. Yolk-sac absorption was normal at 0.10 ppm. The yolk absorption gradually decreased at 1.6 to 4 ppm, and totally stopped at 5 ppm or more. The hatchlings were many times more sensitive to DDVP than the eggs. The median survival time of hatchlings gradually decreased with the increase of concentration. The eggs and hatchlings survived well at 0.10 ppm DDVP.

Introduction

The useof DDVP (0 : 0-dimethyl-2, 2-dichlorovinyl phosphate), an emulsifiable (8.2 lb/gal), organophosphorus insecticide (a product of Ciba Ltd., Basel, Switzerland), has been suggested for the eradication of freshwater fish predators from ponds before stocking with spawn of carps (Srivastava and Konar, 1965, 1966: Konar, in press). The effect of the insecticide is likely to persist in pond water for a few days after reclamation, when it is stocked with spawn and fry of carps. It is not known whether this residual toxic level of the insecticide affects the normal development of the spawn and fry of fishes. The effects of DDVP on the spawn of the fish, the snake-head, *Channa punctatus*, have been reported here.

Materials and Methods

Laboratory experiments were conducted at room temperature (66° to 75°F) in battery jars each holding 8 litres of unchlorinated borehole water. Sixteen different concentrations of DDVP ranging from 0.10 to 20 ppm, as listed in the table, were applied. The fertilized eggs of *C. punctatus*, collected from a local pond and acclimatized under laboratory conditions for four hours, were added into each of the test containers in two lots. Each lot contained 100 eggs. The number of eggs hatched and the number of hatchlings survived were recorded at intervals of 12 hours from the begining of the experiment up to a maximum of 168 hours after treatment and their mean percentages were calculated. Controls were maintained, in which normal growth with only negligible mortality (2%) was observed. The death of a hatchling was verified from the lack of response to mechanical stimulation and from the cessation of the heart beat. Dead hatchlings were immediately removed from test containers to avoid pollution.

The median survival times of the test hatchlings at different concentrations of the insecticide, estimated graphically by probit analysis (Bliss, 1934) from the time-mortality curves (Bliss, 1937), were plotted against test concentrations. The median survival times for test animals exposed to 13, 16 and 20 ppm DDVP could not be estimated as the cumulative mortality was either less or more than 50%.

Results and Discussion

Results are shown in Table 1 and Fig. 1. Table shows that eggs hatch at all concentrations of DDVP tested. At concentrations up to 1.0 ppm, all eggs hatched within 12 hours, and at higher concentrations, ranging from 1.6 to 16 ppm, more than 60% hatched within 12 hours, but complete hatching occurred only after 24 hours of exposure. At 20 ppm DDVP, 55% eggs hatched within 12 hours and remainders after 24 hours of exposure.

No visible symptom of the action of DDVP was observed among exposed hatchlings. In the state of rest, the hatchlings hung in water in an upside down position with the yolk sac up; in the state of motion, they rotated on their own longitudinal axis, clockwise or anti-clockwise. When the yolk sac was absorbed, the hatchlings resumed normal position with the dorsal side up, and the mode of swimming changed to the usual forward movement by to-and-fro motion of the tail. But the habit to swim in aggregation, as shown by the controlled lot, gradually disappeared in increasing concentrations of DDVP and completely abolished at 3 ppm and higher.

The rate of absorption of the yolk sac of the test hatchlings decreased with the increase of concentration of the insecticide. At 0.10 ppm, the hatchlings appeared quite normal and their yolk sacs were completely absorbed within 12 hours of exposure, similar to those in the controlled lot. Up to a concentration of 4 ppm, mixed populations of hatchlings with and without yolk sac were observed and at concentrations higher than this, hatchlings retained yolk sacs till death.

There is no previous work on the effect of any organophosphorus insecticide on the viability of fish eggs. However, effects of a very few chlorinated hydrocarbons and some industrial wastes are known. Allison *et al* (1963) observed no effect of DDT on the development of *Salmo clarki lewisi*. The eggs of *Cyprinus carpio* and *Channa argus* are found to be more tolerant to endrin than stages after hatching

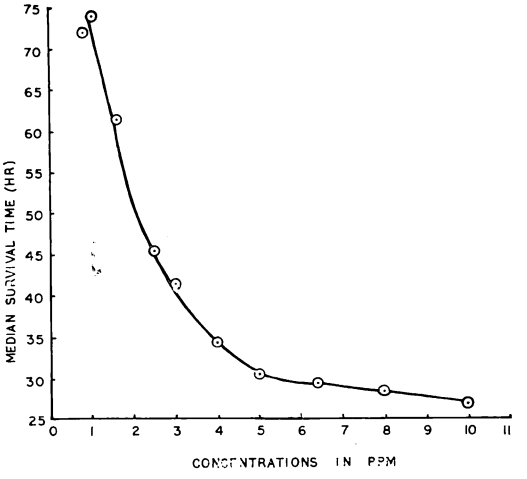

Fig. 1. Survival curve of hatchlings of the snake-head, *Channa punctatus* in different concentrations of DDVP.

(Fujiya, 1955; Iyatomi *et al.*, 1958), but a definite pattern of mortality occurred among hatchlings of *Gasterosteus aculeatus* from the fifth day (Katz and Chandwick, 1961).

The present investigation reveals that the hatchlings of *C. punctatus* are many times more susceptible to DDVP than its eggs, and their median survival time decreases gradually with the increase of concentration of the insecticide as shown by the typical hyperbola of the survival curve (Fig. 1). This high resistance of the fish eggs to toxin is particularly interesting. Obviously, the hardness and rigidity of the chorion, considerable hydrostatic pressure pressure established in egg-contents, limited water intake of the embryo and the fat-droplets of the egg-yolk make the eggs relatively impermeable to salts and other chemicals by providing protection against poison diffusion, as shown by Smith (1957). The pelagic fish-eggs in particular, as employed in this study, have an additional advantage. Since almost the upper hemisphere of each egg floats above the surface of the solution, they are only partially exposed to toxin. Apparently, the rate of toxin-absorption is comparatively slower in pelagic eggs.

Furthermore, LD_0s and LD_{100}s (minimum lethal doses at which respectively none and 100

Table 1. Viability of eggs of *Channa punctatus* in different concentrations of DDVP. Figures show the number of the eggs hatched (H) and survived (S) expressed in % of total of 200 eggs tested for each experiment.

Expt. No.	Conc. of DDVP (ppm)	Hours after treatment																Remarks
		12		24		36	48	60	72	84	96	108	120	132	144	156	168	
		H	S	H	S	S	S	S	S	S	S	S	S	S	S	S	S	
1	Control	100	100	—	100	100	98	98	98	98	98	98	98	98	98	98	98	Yolk sac absorbed at 48 h
2	0.10	100	100	—	100	100	100	98	98	98	98	98	98	98	98	98	98	-Do-
3	0.50	100	100	—	100	28	0	—	—	—	—	—	—	—	—	—	—	Yolk sac never absorbed
4	0.80	100	100	—	100	95	86	83	50	32	32	26	11	8	8	4	3	Mixed population of yolk saced and non-yolk saced at 60 h
5	1.00	100	100	—	100	99	96	86	70	10	0	—	—	—	—	—	—	-Do- at 60 h; yolk sac absorbed at 72 h
6	1.60	75	100	100	100	88	71	65	10	5	5	3	0	—	—	—	—	Yolk sac absorbed at 72 h
7	2.00	60	100	100	100	97	97	92	90	80	38	22	10	8	8	7	5	Mixed population of yolk saced and non-yolk saced at 60 h
8	2.50	65	100	100	100	84	42	10	4	1	1	1	1	0	—	—	—	Yolk sac absorbed at 84 h
9	3.00	60	100	100	97	88	20	7	3	2	1	1	1	0	—	—	—	Mixed population of yolk saced and non-yolk saced at 60 h
10	4.00	70	100	100	100	45	10	1	1	1	1	1	0	—	—	—	—	-Do-
11	5.00	65	100	100	95	15	0	—	—	—	—	—	—	—	—	—	—	Yolk sac never absorbed
12	6.40	60	100	100	96	13	0	—	—	—	—	—	—	—	—	—	—	-Do-
13	8.00	70	100	100	84	10	0	—	—	—	—	—	—	—	—	—	—	-Do-
14	10.00	65	100	100	80	10	0	—	—	—	—	—	—	—	—	—	—	-Do-
15	13.00	60	100	100	60	0	—	—	—	—	—	—	—	—	—	—	—	-Do-
15	13.00	60	100	100	60	0	—	—	—	—	—	—	—	—	—	—	—	-Do-
16	16.00	60	100	100	55	0	—	—	—	—	—	—	—	—	—	—	—	-Do-
17	20.00	55	100	100	10	0	—	—	—	—	—	—	—	—	—	—	—	-Do-

per cent died) of DDVP for fry, fingerling and adult of *C. punctatus* are respectively 0.3 and 2.5 ppm, 0.8 and 3 ppm, and, 1.3 and 5 ppm (Srivastava and Konar, 1966; Konar, 1966). Table shows that the tolerance limit of hatchlings is only 0.1 ppm DDVP, and all died at 1 ppm. Evidently, except for the eggs, the resistance to DDVP increases with the increase of age of the fish. This increase in resistance with age may be attributed to the gradual development of efficient excretory organs, increasing non-interference in carbohydrate metabolism, and, controlled and limited intake of water as the fish grows older.

Lastly, the body and gills of the fry, fingerlings and adults of this fish exposed to DDVP solutions became covered with mucus (Konar, 1966), whereas the eggs and hatchlings remained uncovered with it, as revealed from the present investigation. This secretion of mucus acts not only as defence mechanism in the excretion of the toxin (Neuhold and Sigler, 1960) but also protects the fish from further penetration of the poison.

However, DDVP tends to suppress the development of the embryos and hatchlings of *C. punctatus*, possibly by disrupting oxidative phosphorylation (Ishida, 1944). Consequently, and more probably, due to inhibition of proteolytic hatching enzyme (Gray, 1926; Hayes, 1942), the time of hatching is increased at higher concentrations. But the normal development of the embryos occurs only at 0.10 ppm DDVP. This means that a treated pond should not retain more than 0.10 ppm level of DDVP at the time of stocking. But since 20 ppm DDVP detoxifies within three weeks after treatment (Srivastava and Konar,

1966), the treated pond should be left unstocked with spawn at least for this period.

It is concluded that: (i) DDVP inhibits the development of fertilized eggs of *C. punctatus*; (ii) hatching time increases in more than 1 ppm DDVP; (iii) yolk sac absorption decreases in 1.6 ppm DDVP and more, until in 5 ppm it ceases; (iv) eggs are more tolerant than post-embryonic stages; and (v) the spawn can well tolerate 0.1 ppm DDVP.

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Summary

The laboratory experiments show that eggs are the most resistant to DDVP and hatch at concentrations 0.10 to 20 ppm tested. Except up to 1.0 ppm, the hatching time increases and save for 0.10 ppm, absorption rate of yolk sac decreases, with the increase of concentration. The spawn develops normally at 0.10 ppm DDVP.

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殺虫剤 DDVP の *Channa punctatus* (タイワンドジョウ科) の卵，ふ化仔魚に与える致死効果 S.K. コナール

有機燐性殺虫剤の DDVP インド産タイワンドジュウ (の 1 種) に及ぼす致死効果を生物学的定量法によって実験した．8 l のガラス円筒，無塩素水を用い，温度 19～24°C で，16 段階の濃度 (0.10～20 ppm) を 2 組の材料で験した．卵は全ての濃度でふ化したが，ふ化時間は濃度に比例して増加した，そして，濃度 1.0 ppm に達するまでは 12 時間であった．この同じ時間内では，1.6～16 ppm では約 60%，20 ppm では 55% の卵がふ化した．他の卵は全て 24 時間内にふ化した．卵黄の吸収は 0.10 ppm では正常であったが，1.6～4 ppm ではその作用は低下し，5 ppm およびそれ以上の濃度では全く停止した．DDVP の影響は卵よりもふ化仔魚に強く現われ，後者の中央生残時間は濃度の増加とともに除々に減小した．卵もふ化仔も 0.10 ppm の DDVP ではよく生残した．

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