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Acute Toxicity of Diazinon on Rotifers, Cyclops, Mosquito Larvae and Fish

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ABSTRACT: Acute toxicity tests were conducted in renewable static bioassays to determine the 48h-lc50 of Diazinon on rotifers, cyclops, mosquito larvae and fish. The 48h-lc50 values were 3.93 mg/l, 39.39,9.87 and 189.31µg/l for rotifers, cyclops, mosquito larvae and fish respectively. The rotifers had the highest value hence the least sensitive to Diazinon intoxication. The probit mortalities of the test organisms was found to be positively correlated to the log-concentration except for rotifers, which showed negative correlation at 5% level of significance. Diazinon was highly toxic to mosquito larvae and cyclops, which are targets in the control of vectors of parasitic diseases. The toxicity of Diazinon on aquatic fauna thus affects the trophic levels in aquatic biota and the productivity of water bodies. @ *JASEM*

Aquatic ecosystems could be polluted by pesticides used for crop and animal protection in agricultural practices, which enter the water bodies as a consequence of rain and leaching from soil (Kumar and Ansari, 1984). These additions of pollutants to aquatic ecosystems may be due to careless handling and disposal of empty containers of pesticides in ponds and streams; or when used in direct control of particular aquatic organisms (Mason, 1993).

Diazinon, an organophosphate insecticide, is an anticholinesterase, which causes loss of functional co-ordination that results in immobilization of the organism (Brooks, 1976). Acetlycholine is the target for poisoning (O'Brien, 1978).Diazinon get into aquatic environment through direct application to control water-inhabiting pests and disease vectors , spray drifts from normal agricultural operations and by accident and spills (Kilgore and Ming-yu,1976). It is a preferred insecticide because it has considerably less residue problem and does not accumulate in tissues (Tsumaki *et al*, 1970; Fishbein,1976).

The use of diazinon to control agricultural pests in the watersheds has a potential of finding its way either through run-offs or leaching into the water body. Cyclops species and mosquitoes are intermediate host or vector of some parasitic diseases (Ukoli,1984). Diazinon could be applied directly to aquatic ecosystems/water bodies in which these organisms have been identified to destroy them. This study is aimed at assessing the acute toxicity of diazinon on rotifers (*Brachionus* spp.), Cyclops, mosquito larvae (*Culex* spp.) and fish (*Aphyosemon gardneri*), which are representative of different trophic levels of aquatic food chain.

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MATERIALS AND METHODS

The rotifers were obtained from pure culture in the laboratory (Lubzens, 1987). The Cyclops were obtained from out-door metal tanks in which productivity was stimulated by addition of poultry droppings (Wade and Stirling,1999). The mosquito larvae were obtained by collecting their eggs from out-door metal tank and allowing them to hatch in 1-litre capacity transparent glassware. The larvae were fed Baker's yeast and allowed to grow to second instar stage before bioassay testing. The fish were collected along the banks of River Delimi behind the University of Jos students hostel. They were transported to the laboratory in 20-litre capacity plastic buckets where they were acclimated for two weeks before bioassay testing.

The acute toxicity bioassay tests to determine the 48hours lethal concentration that will result to 50% mortality of the test organisms (48h-LC_{50}) was conducted by static renewable method (OECD,1981). Each treatment was in triplicate with serial dilutions made from the stock solution. The concentrations of toxicant used ranged from 1875µg/l to 1.17µg/l.

The rotifer bioassays were conducted in 25ml glass specimen bottles. The media were inoculated with 1ml of rotifers obtained from the pure culture. The rotifer density/ml was determined by a modification of Tay *et al* (1991) method: three 1ml aliquots were collected with the aid of 1ml insulin syringe after a

thorough stirring, poured into a 1ml counting chamber, which was then mounted on a SWITF microscope. Enumeration was done with a tally counter and the mean was found to be 2,400 (±56) individuals/ml. To determine mortality in each replicate, three 1ml aliquots were collected, enumerated and the mean mortality per treatment was noted. The sub-sample taken for enumeration was returned back to the bioassay medium after counting. Mortality was assumed when heartbeat and cilia movement ceased.

The Cyclops bioassays were conducted in 100ml capacity glass beakers. Each treatment concentration was inoculated with ten Cyclops using the 1ml syringe. The Cyclops were viewed with the aid of a stereoscopic microscope and assumed dead when heartbeat stopped. All dead Cyclops were removed from the media by sucking out with the syringe.

The mosquito larvae bioassay tests were carried out using the same procedure described for Cyclops. Death was confirmed when larvae heart beat ceased.

The fish bioassay tests were conducted in 10-litre capacity glass aquaria. Ten fish (mean total length 4.5±1.2cm; mean weight 2.7±0.5g) were put into each aquarium with the appropriate toxicant concentration. Death was recorded when opercula movement and tail beat stopped. Any observed dead fish was removed from the medium.

The water quality of the bioassays were determined at the beginning and at the end of the experiments. The dissolved oxygen (DO), total alkalinity and total hardness were estimated by the methods of APHA (1985). The pH values were determined by using a Philips pH meter model PW9418. The water temperatures were taken with a mercury-in-glass thermometer.

Dose response relationships were transformed into probit mortality and plotted against log-concentration of the toxicant using regression and correlation statistics (Microsoft excel). The 48h LC_{50} values, 95% confidence interval and correlation coefficient were determined by the methods described in Wardlaw (1985).

RESULTS AND DISCUSSION

The observed mean probit mortalities at different concentrations of the toxicant are presented in Fig. 1. The 48h-LC_{50} values were estimated from the regression equations shown in Fig.1 while the lower and upper confidence limits at 95% were calculated using the methods described in Wardlaw (1985) and these are presented in Table 1. The 48h-LC_{50} for the different test organisms were found to be significantly different ($p<0.05$). The mean values of the water quality parameters monitored during experiment are presented in Table 2. There was no significant difference ($p>0.05$) in the values during the course of experimentation.

Table 1. The 48h-LC_{50}, upper and lower confidence limits values of the test organisms.

Test organism	Concentration µg/l		
	Upper confidence Limit	48h-LC_{50}	Lower confidence Limit
Rotifer	4298.33	3933.69	3599.98
Cyclops	64.77	39.39	23.93
Mosquito larva	10.73	9.87	9.08
Fish	283.48	189.31	126.44

Table 2. The range and means* of water quality parameters.

Parameter	Range	Mean (S.E)
.Ph	6.65 – 6.75	6.70 (0.03)
Dissolve Oxygen (DO) mg/l	8.18 – 8.25	8.20 (0.03)
Total Hardness mg/l	75 –77	76.00 (0.58)
Total Alkalinity mg/l	153 –155	153.86 (0.38)
Temperature °C	18.0	18.0 (0.00)

*Standard errors (S.E.) in parentheses.

In the toxicity tests, mosquito larvae were found to be the most sensitive while the rotifers were the least sensitive. This agrees with Mason (1993) who reported that sensitivity to organophosphate insecticide by aquatic organisms decrease in the order from insect to crustaceans and fish. The probit mortality of the rotifers was negatively correlated to the log-concentration. The inverse relationship is not unusual in toxicology tests.

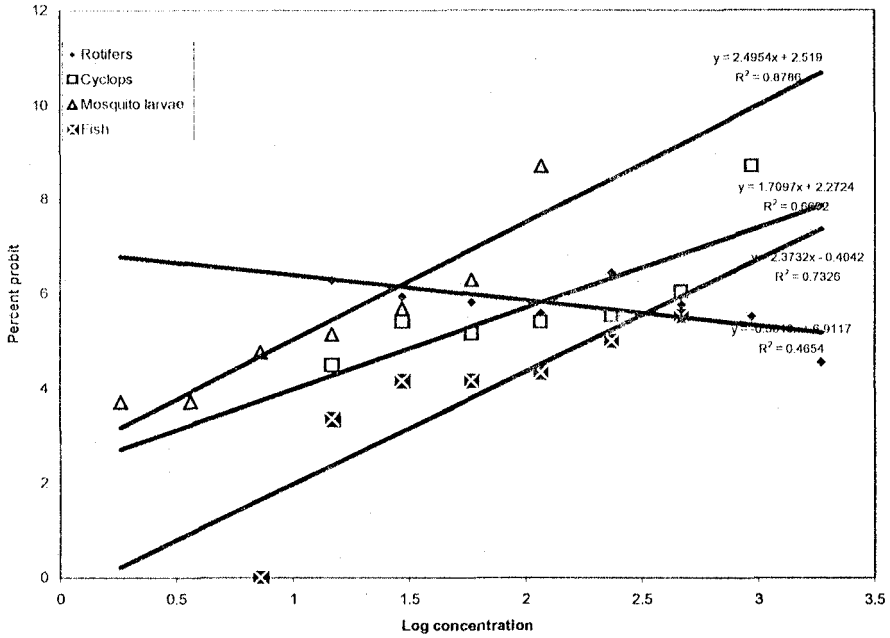

Figure 1. Probit mortalities of the different organisms exposed to Diazinon

Xiu *et al* (1989) reported that *Daphnia magna* exhibited a similar pattern on exposure to deltamethrin. Acetylcholinesterase inhibitors do not produce the same effect as would be expected (Norgrady and Alai, 1983) and diazinon being a selective poison may fail to inhibit acetylcholinesterase because the nerve sheath acts as barrier against its penetration in some organisms (Eto, 1974). Hutson and Roberts (1985) reported that metabolism of diazinon will will lead to demethylation and dearylation which may result overall in reduced toxicity. It is suspected that at higher concentration of the toxicant, the rate of bio-transformation is faster hence the negative gradient of the trend line observed in the rotifer bioassay.

The 48h-LC_{50} of diazinon in the fish bioassay was estimated to be 189.31µg/l. In sheephead minnow, *Cyprinodon variegatus*, it was reported to be 1.43mg/l (Goodman *et al*, 1979). Zebrafish, *Brachydanio rerio*, in malathion bioassay, an organophosphate, it was reported that the 48h-LC_{50} was 1.25mg/l (Kumar and Ansari, 1984). This implies that diazinon is more toxic to the test fish (*A. gardneri*) than *C. variegatus* hence a better test organism in the estimation of environmental impact of the toxicant.

The food chain of a typical aquatic ecosystem where these test organisms are present has rotifers as the primary consumers, Cyclops and mosquito larvae as secondary consumers and fish as tertiary consumer. The use of diazinon in the control of insect pests of agriculture in watersheds and in the control disease vectors in water bodies should be done with caution/restraint since this chemical will eventually end up either through run-off or leaching in the aquatic ecosystem. The implication of this on the aquatic biocenosis is that energy transfer across food chain will be greatly hampered with the final effect being a reduction in fishery production of the water body. There is also the potential risk of causing an ecological imbalance with unfathomable consequence on the environment and man.

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