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Acute Toxicity of Several Pesticides to Rotifer (*Brachionus calyciflorus*)

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The use of insecticides and herbicides is increasing very rapidly in agriculture and pisciculture in order to control unwanted insects and weeds. The unlimited use of these chemicals is deteriously affecting the aquatic biota. The majority of short-term acute toxicity studies under controlled conditions have used either fish or the cladoceran *Daphnia magna*, but there are limited studies using rotifers only with insecticides (Dad and Pandya 1982; Serrano *et al.* 1986), with heavy metals and with free ammonia (Snell and Persoone 1989). *Brachionus calyciflorus* is particularly useful for environmental toxicology because of its rapid reproduction, short generation time, cosmopolitan distribution and the commercial availability of its dormant eggs (cysts) (Snell and Persoone 1989; Halbach *et al.* 1982).

Our objectives in this paper are to determine the acute toxicological effects of several pesticides (endosulfan, diazinon, methylparathion, malathion and benthiocarb) on *B. calyciflorus.*

MATERIALS AND METHODS

The test pesticides used in this study were: the organochlorine endosulfan 96% (Hoechst Iberica S.A., Spain), the organophosphates diazinon 92% (Cequisa S.A., Spain), methylparathion 80% (Bayer Hispania S.A.) and malathion 95% (American Cyanamid Co.) and the carbamate benthiocarb 93% (Argos S.A., Spain)

B. calyciflorus cysts used in the following experiments were obtained from Dr. Snell (University of Tampa. U.S.A.) and they were produced in mass cultures maintained under rigorously controlled conditions. Cysts were stored in EPA medium at 6°C in the dark. Bidistilled water is the base for making EPA medium (USEPA 1985). This medium is a synthetic freshwater that is prepared from reagent grade chemicals and composed of 96 mg $NaCO_3$, 60 mg $CaSO_4 \cdot 2H_2O$, 60 mg $MgSO_4$, and 4 mg KCl per liter of water.

Cysts hatching is initiated by transferring to warmer temperatures and light (Snell and Persoone 1989). Standard environmental conditions for these bioassays were: temperature, 25°C; pH 7.4-7.8; hardness, 80-100 mg $CaCO_3$/L; alkalinity, 60-70 mg/L and darkness.

Because of the short duration of the tests (24hr), rotifers were not fed and the medium was not renewed during the bioassay. Acetona was required as a carrier for these toxicants, so a control with acetone was included. The concentration corresponded to the highest acetone concentration used in the experiments. Control survival is always 100% after 24 h.

We used Multiwell® plates in these acute toxicity experiments. A 24-well plate has six columns across and 3 rows down, so a single plate can hold all six concentrations for one acute toxicity test for a total of 30 animals per concentration (column), so the control and five concentrations of each toxic. We carried out a total of nine replicates. Bioassay plates were placed in an incubator under standard conditions, the percent dead for the pesticide treatment was recorded after 24 hr, and median lethal values (24-hr LC50s) and 95% confidence limits were calculated from survival data using "moving-average" analysis, with an IBM computer. Statistical differences between LC50 values were determined using Student's t test.

RESULTS AND DISCUSSION

We used five different concentrations for each insecticide. The highest concentrations used corresponded to the organophosphate insecticides (methylparathion, diazinon and malathion), and the lowest concentrations corresponded to the organochlorine (endosulfan) and the carbamate (benthiocarb).

The 24-hr LC50s for each pesticide were calculated from these results. Mean values and their corresponding 95% confidence intervals are given in Table 1. Comparisons of the LC50 values indicate that the organochlorine insecticide, endosulfan was the most toxic of the pesticides tested to this species, although endosulfan toxicity is similar to that exhibited by benthiocarb (carbamate). Organophosphate insecticides (diazinon, methylparathion and malathion) were the least toxic pesticides tested. Endosulfan and benthiocarb LC50 values showed statistical differences ($p< 0.005$) with organophosphate insecticides LC50 values.

Endosulfan LC50 value was 5.15 mg/L with a coefficient of variation of 14.75%. Our results on *Brachionus calyciflorus* agree with the 24-hr LC50 value of 5.6 mg/L reported for *Brachionus plicatilis* to endosulfan by Serrano *et al.* (1986). The medial lethal concentrations (LC50) for this species are much higher than those registered in the literature for the same organochlorine insecticide in other aquatic invertebrates and fish. Nebeker *et al.* (1983) determined a 48-hr LC50 of 0.343 mg/L for *Daphnia magna* exposed to endosulfan. Cebrian *et al.* (1988) conducted tests on *Procambarus clarkii* and found the 96-hr LC50 of 0.12 mg/L for this toxicant. Johnson and Finley (1980) determined 96h endosulfan LC50 values of 5.8μg/L in *Gammarus lacustris.*

The 24-hr LC50 value for diazinon in the present study was 29.22 mg/L for *B.calyciflorus* with a coefficient of variation of 2.54%. There are no data about the toxicity of this insecticide on rotifers. However, there is information available in the literature for the same organophosphorus insecticide in other aquatic invertebrates and fishes. Johnson and Finley (1980) determinated a 24-hr LC50 of 0.8 mg/L for *Daphnia pulex* exposed to diazinon and 0.2 mg/L in *Gammarus fasciatus* and Meier *et al.* (1976) found a 96-hr LC50 of 0.002 mg/L for *Daphnia magna*. With respect to fishes, the data are very different: Meier *et al.* (1976) conducted tests on *Salmo gairdneri* and found 96-hr LC50 of 0.11 mg/L; Anon (1972) established the 96-hr LC50 for *Carassius auratus* as 9 mg/L.
Thus, diazinon was found to be less toxic to *B.calyciflorus* than to other fresh water invertebrates and fishes.

Methylparathion 24-hr LC50 value was 29.19 mg/L with a coefficient of variation of 12.84%. Johnson and Finley (1980) also tested this organophosphorus insecticide with *Daphnia magna* (first instar) and they found a 48-hr LC50 of 0.14 μg/L and for *Gammarus fasciatus* a 96-hr LC50 of 3.8 μg/L. Rotifers are less sensitive to methylparathion than are fishes. The reported 48-hr LC50 value for *Gambusia affinis* is 17.48 mg/L (Chambers and Yarbrough 1974), and Johnson and Finley (1980) established the 96-hr LC50 value for this pesticide in *Salmo trutta* as 4.7 mg/L.

Table 1. Mean *B.calyciflorus* 24-hr LC50 (mg/L) values (X) for five pesticides, 95% confidence limits and variation coefficient (C.V.).
* p< 0.005

Pesticide	24-hr LC50			
	X	C.V.(%)	95% C.L.	N
Endosulfan	5.15 *	14.75	4.38-5.92	9
Diazinon	29.22	2.54	28.47-29.96	9
Methylparathion	29.19	12.84	25.44-32.94	9
Malathion	33.72	14.62	28.79-38.65	9
Benthiocarb	6.50 *	10.15	5.84-7.16	9

Malathion 24-hr LC50 value was 33.72 mg/L with a coefficient of variation of 14.62%. Our results agree with the LC50 value of 35.3 mg/L reported by Snell and Persoone (1989b) on *Brachionus rubens* exposed to this insecticide. If we compare our results with others from the literature, we find that rotifers are less sensitive to malathion than are other species of aquatic invertebrates . The 48-hr LC50 for this toxicant was 1.0 µg/L for *Daphnia magna* (first instar) and 0.76 µg/L for *Gammarus fasciatus* (Johnson and Finley 1980).The resistence of *B.calyciflorus* was also higher than most of the bioassayed fish species. Johnson and Finley (1980) found 96-hr LC50 of 0.20 mg/L for *Salmo gairdneri* and 10.7 mg/L for *Carassius auratus* exposed to this organophosphorus insecticide.

The 24-hr LC50 for benthiocarb in the present study was 6.50 mg/L with a coefficient of variation of 10.15%. Benthiocarb 96-hr LC50 data generated by Johnson and Finley (1980) for *Salmo gairdneri* and *Lepomis macrochirus* were 1.2 and 2.5 mg/L, respectively.

The results of the present study indicate that the osganophosphorus insecticides (malathion, diazinon and methylparathion) were the least toxic compounds for *B.calyciflorus*, and the carbamate (benthiocarb) and the organochlorine (endosulfan) were the most. On the other hand, our data demonstrated that *B.calyciflorus* is a more resistent species than other aquatic invertebrates and fishes.

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