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Effect of Sublethal Concentrations of Endosulfan on Growth and Fecundity of Two Species of Snails

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Endosulfan (Thiodan 35 EC) is an organochlorine, cyclodiene insecticide, [6,7,8,9,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide (Rao and Murty 1982)], which is currently only registered for use on coffee in Jamaica to control the coffee pest, *Hypothenemus hampei* Ferrari (*Pesticide Control Authority, Jamaica - Personal Communication*). It is known to have entered several Jamaican rivers and streams through run-off from coffee farms (Mansingh *et al.* 1995). Endosulfan is recorded as having adverse effects on the density, growth and reproduction of aquatic organisms (IPCS 1984; Muirhead-Thomson 1987; Hassall 1990; Naqvi and Vaishnavi 1993; Raloff 1998).

Two prosobranch snails, *Melanoides tuberculata* and *Thiara granifera* (Family : Thiaridae) are common in Jamaican rivers, streams and ponds. Both species are parthenogenetic and viviparous (Berry and Kadri 1974; Dudgeon 1986), and both are introduced into Jamaica. Both species are readily available, easy to rear under laboratory conditions and can tolerate frequent handling. Very little information is available on the toxicity of endosulfan to snails (IPCS 1984; Muirhead-Thomson 1987). This study investigated the impact of sub-lethal concentrations of endosulfan on growth and fecundity of *Melanoides tuberculata* and *Thiara granifera* under laboratory and field conditions.

MATERIALS AND METHODS

Specimens of *M. tuberculata* and *T. granifera* were handpicked at random from various sections of the Mammee River (18° 04' 54" N; 76° 43' 20" W) located in the parish of St Andrew, Jamaica. This sampling site was chosen because it was least likely to be contaminated by endosulfan. In the laboratory, the snails were fed shredded lettuce, (*Lactuca sativa*) and were allowed to acclimatize for two weeks in aquaria containing de-chlorinated tap water. The aquaria were aerated by conventional air pumps with plastic tubing and standard air stones. Snails of shell length 10 – 14 mm were exposed over a four month period to treatments of 0.128 mgL^{-1} or 0.0128 mgL^{-1} of endosulfan (Thiodan 35 EC). The first concentration represented the mean 96 hr $LC_{0.1}$ which was derived from preliminary acute toxicity tests and the second, a ten fold dilution. The treatments

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and a control were prepared in glass aquaria measuring 60 cm x 30 cm x 37.5 cm, in three replicates. During the experiment the toxic solutions (18 liters) were renewed once per week. The snails were kept in a photoperiod of 12 hours light and 12 hours dark. Water temperature and pH were measured at least once per week. Mean temperature was 27 ± 1° C and pH varied from 7.9 – 8.4.

On a weekly basis, all adult snails were removed from the aquaria and shell length was measured (to the nearest 0.1 mm) from the apex of the shell to the lower tip of the aperture using Vernier Calipers. Shell length measurements were used to calculate the absolute growth rate for each group of snails. Hatchlings were removed from the aquaria on a weekly basis, counted and shell length measured. At the end of the experiment, the brood pouches of adult snails were dissected and all young snails from one shell whorl in size upwards were counted and measured. The mean number of hatchlings was calculated from the brood count data plus the hatchlings removed from the aquaria. The data were arranged into size-frequency distributions to determine whether exposure to endosulfan affected the size of hatchlings produced by the snails. The data were compared using the non-parametric Kruskal-Wallis and Mann-Whitney tests.

Three sampling sites, Rio Pedro (18° 06' 53" N; 76° 54' 08" W), Rio D'Oro (18° 07' 07" N; 76° 59' 39" W) and Great River (18° 14' 24" N; 77° 00' 58" W) were selected from the Rio Cobre River basin in the parish of St. Catherine. These sites were chosen because of their proximity to coffee farms which were known to treat their crops with endosulfan. A Surber sampler (with base measuring 30 cm x 30 cm) was used to collect three random samples of snails above and below the farm on a monthly basis from each site between February 1998 and July 1999. All live snails within the Surber sampler were collected, placed in 70 % ethanol and taken back to the laboratory. They were counted and the mean number of snails per month was calculated for each sampling site. The brood pouches of selected specimens of both species collected between February and May, 1999 were dissected. The number of hatchlings of at least one shell whorl in size was counted, measured and arranged into size classes. Class-frequency distributions were made as required and Kruskal-Wallis and Mann-Whitney tests were applied to the data.

RESULTS AND DISCUSSION

Exposure to sub-lethal concentrations of endosulfan resulted in significantly lower growth rates for *Thiara* between treatments and between both treatments and control ($p < 0.05$, Kruskal-Wallis tests), but not for *Melanoides* ($p > 0.05$). The growth rates for *Melanoides* were significantly greater than those for *Thiara* ($p < 0.05$, Kruskal-Wallis tests) under all laboratory conditions (Table 1).

Melanoides grew at a faster rate than *Thiara* even in the absence of endosulfan. Endosulfan is known to reduce feeding rates (Naqvi and Vaishnavi 1993), to interfere with neurological functions (Hassall 1990; Gill *et al.* 1991; Naqvi and Newton 1991) and to slow metabolic activities (Matthiessen 1981; Rao and Murty

Table 1. Mean absolute growth rate (mm/month) and mean number of hatchlings (in brackets) of *Thiara granifera* and *Melanoides tuberculata*

Treatment (mgL^{-1})	*Thiara*		*Melanoides*	
0.128	0.18 ± 0.01	(91 ± 44)	0.59 ± 0.06	(88 ± 19)
0.0128	0.26 ± 0.02	(188 ± 82)	0.63 ± 0.06	(104 ± 31)
0 (control)	0.39 ± 0.08	(199 ± 47)	0.71 ± 0.04	(174 ± 41)

mean ± SD, n = 90

1982) and would therefore be expected to have an adverse effect on growth and reproduction. The overall laboratory growth rates reported by Berry and Kadri (1974) for *Melanoides* (in Malaysia) of shell length 12 – 14 mm was 12 – 15 mm in 3.5 years. Assuming a constant growth rate, this is roughly 0.29 – 0.36 mm per month which is much lower than 0.59 – 0.63 mm per month for *Melanoides* in this study. Studies done in Puerto Rico by Chaniotis *et al.* (1980) indicated that shell growth in *Thiara* was a relatively slow process which varied with the age (initial length) of the snail.

Laboratory studies showed that a lower mean number of hatchlings was produced by both snail species exposed to 0.128 mgL^{-1} as compared to 0.0128 mgL^{-1} of endosulfan (Table 1). The difference between treatments was significant for both species ($p < 0.05$, Kruskal-Wallis tests). This may indicate that endosulfan induces changes in the reproductive capacities of the snails perhaps by inhibiting the development of the hatchlings. Hassall (1990) points out that organochlorine insecticides may affect calcium ion levels in nerve cells, as well as the ATPases which are associated with muscular action. Endosulfan may have interfered with the snails' Ca^{2+} metabolism which is important for shell development and for muscular action which in turn, may affect the release of hatchlings from the brood pouch.

Significantly higher percentages of hatchlings in the 1.6 – 3.0 mm and 3.1 – 4.5 mm size classes were produced by both species of snails exposed to the higher concentration of endosulfan as compared to the control ($p < 0.05$, Mann-Whitney tests), (Table 2). For both species and for all size classes, the percentage of hatchlings produced in the 0.0128 mgL^{-1} treatment was not significantly different from that produced in the control ($p > 0.05$, Mann-Whitney tests), (Table 2).

Table 2. Class / Frequency (percentage) distribution of hatchlings of *Melanoides tuberculata* and *Thiara granifera* produced February – May, 1999 under laboratory conditions.

	0.1 – 1.5 mm		1.6 – 3.0 mm		3.1 – 4.5 mm		4.6 – 6.0 mm	
	t	m	t	m	t	m	t	m
Treatment								
0.128 mgL^{-1}	35.9	20.8	63.0	66.7	1.1	12.5	0	0
0.0128 mgL^{-1}	48.7	31.4	50.5	59.9	0.7	8.3	0	0.3
0 (control)	47.6	33.3	51.2	59.4	0.8	7.3	0.3	0

(t = *Thiara*, m = *Melanoides*).

At all sites and for both species, the early stage (0.1 – 1.5 mm) was the dominant size class in the brood pouches. More hatchlings of this size class were found above as compared to below the farm. A significantly higher percentage of the 1.6 - 3.0 mm and 3.1 – 4.5 mm size hatchlings was found in the brood pouches of snails below the coffee farms compared to that above ($p < 0.05$, Mann- Whitney tests) (Table 3). At Great River, hatchlings in the largest size class (4.6 – 6.0 mm) could only be found in the brood pouches of snails below the farm (Table 3).

Table 3. Class / Frequency (percentage) distribution of hatchlings of *Thiara granifera* and *Melanoides tuberculata* dissected from the brood pouches of snails from Rio Pedro, Rio D'Oro and Great River, February – May, 1999.

	0.1 – 1.5 mm		1.6 – 3.0 mm		3.1 – 4.5 mm		4.6 – 6.0 mm	
	t	m	t	m	t	m	t	m
Sampling site								
Rio Pedro-a	82.9	75.0	16.3	25.0	0.8	0	0	0
Rio Pedro-b	70.2	66.6	27.3	33.4	2.5	0	0	0
Rio D'Oro-a	83.4	-	16.1	-	0.5	-	0	-
Rio D'Oro –b	76.0	-	22.9	-	1.1	-	0	-
Great River-a	-	55.5	-	33.7	-	10.8	-	0
Great River-b	-	44.0	-	37.6	-	17.7	-	0.7

(a = above coffee farm, b = below coffee farm, t = *Thiara*, m = *Melanoides*).

Evidence from both field and laboratory studies (Tables 2 & 3) suggested that the presence of endosulfan delayed the release of hatchlings from the brood pouch of thiarids. Endosulfan caused parent snails to retain the hatchlings in the brood pouch, resulting in larger sized hatchlings being released, perhaps, increasing the chance of survival. Similar effects have been recorded by Naqvi and Newton (1991) and IPCS (1984) for *Procambarus clarkii* and *Mytilus edulis*, respectively. The retention of hatchlings as a survival strategy by the thiarids is supported by Dudgeon (1986) and Berry and Kadri (1974). There were indications that the Jamaican thiarids bred throughout the year and had at least two periods of juvenile recruitment. This supports observations made by Berry and Kadri (1974) in Malaysia, however in Hong Kong, the release of hatchlings by *Melanoides* and another thiarid, *Brotia hianensis* was seasonal with one peak of juvenile recruitment (Dudgeon 1986).

In total, 15099 snails were sampled from all sites. These comprised 812 *Melanoides* and 14287 *Thiara*. In general, the number of snails was lower at "below" as compared to "above farm" locations (Table 4). Mann-Whitney tests undertaken on the number of snails collected per month reveal that the values above and below the farm were significantly different at Great River for *Melanoides* and at Rio Pedro and Rio D'Oro for *Thiara* ($p < 0.05$), (Table 4). This indicates that endosulfan from coffee cultivation may have contributed to decreased density of snails in the rivers. This was supported by the laboratory studies which revealed that endosulfan reduced the fecundity and lowered the growth rate of at least one species. Reduced fecundity and growth rates usually

Table 4. Total snails collected from each site (February 1998 – July 1999).

Sampling site	*Melanoides*	*Thiara*
Rio Pedro – above farm	23	3072
Rio Pedro – below farm	24	1752
Rio D'Oro – above farm	-	5664
Rio D'Oro – below farm	-	3799
Great River – above farm	489	-
Great River – below farm	276	-
Total	812	14287

have negative consequences on density. Muirhead-Thomson (1987) reports that organochlorine insecticides reduce the number of organisms living in contaminated rivers. The decline in snail numbers downstream of the coffee farms may therefore be due to insecticide induced mortality and / or dispersal.

Between May to July each year, there was a decline in snail number at all sites. This reflects changes that may be at least partly connected with the spraying of nearby farms with endosulfan between April to June. At Rio Pedro where both species co-existed, *Thiara* was clearly more abundant (Table 4). In Jamaica, *Thiara* is omnipresent so at locations where *Melanoides* only is found, a possible explanation is that endosulfan has eliminated the more susceptible *Thiara*. Therefore, the present study demonstrates that thiarid snails may serve as indicators of the level of pesticide pollution in rivers and streams. In Jamaica, most of the coffee farms are located very near to rivers and streams hence judicious use of endosulfan should be promoted amongst coffee farmers. The phasing out of the use of endosulfan should also be encouraged as there is a strong case for believing that this insecticide is contributing to a decline in the number of organisms in rivers and streams.

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