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Mortality and Survival of *Procambarus clarkii* Girard, 1852 upon Exposure to Different Insecticide Products

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Procambarus clarkii (Girard, 1852) was introduced in the Guadalquivir river marshes (Spain) in 1974 (Habsburgo-Lorena, 1978; Ocete and López, 1983). Since then, crayfish increased without control, invading the whole area of rice fields and marshes. This species has become a source of wealth for an important fishing sector. In fact, the lower Guadalquivir River marshes have become the main european producer and exporter of this crustacean (Gaudé, 1984; Cano & Ocete, 1994).

In the Lower Guadalquivir River marshes (Seville, Spain) approximately 35,500 ha are dedicated to rice farming (Aguilar, 2001), and they are especially important for being close to the Doñana National Park. For this reason farmers have reduced the use of pesticides to malathion and trichlorfon. These insecticides are recommended because they have moderate persistence and relatively low toxicity (Stevens, 1991).

During the last decade the use of cleaner technologies for insect pest control has increased greatly. Plant extracts such as those from neem seeds, *Azadirachta indica*, have good potential for controlling insect pets. Where the active component is azadirachtin (Warthen, 1989), in the rice crop its use is now considered for arthropod control (Jiménez *et al.*, 1998).

A new non-ionic surfactant, Genapol OXD 080, produced by the Hoescht Company was also tested. It reduces crayfish metabolic rates (Anastácio et al., 1995) and consequently results in immobility. Genapol OXD 080 is a quimical product used in the investigation project "Integrated Management of Red Swamp Crayfish Populations in Rice Fields – An Application of Cleaner Technologies and Ecotechnology" with the main objective of controllong crayfish populations without decreasing either their production or the rice field and course looking after the environment.

The present study was carried out in order to determine the toxicity and survival of *Procambarus clarkii* to traditional products used in rice fields (malathion, triclorfon and malathion plus triclorfon mixture) and newer cleaner products (non ionic surfactant and neem seed extract).

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MATERIALS AND METHODS

Procambarus clarkii were collected (throughout the rice growing season in 1998) from the Guadalquivir river marshes (U.T.M. 29SQB534246) with fish traps, used by the local fishermen. They were transported to the laboratory in a container (50x80x50 cm) without water. They were adapted for 48 hours, in 10 cm deep aged tapwater previously aerated for 48 h to remove chlorine. Water was changed daily, and the water chemical parameters were maintained as follows: pH 7.5-8, 5.5-6 °d hardness, temperature 25 ± 1°C and (light:darkness) photoperiod 16:8.

The products used in the tests (LC_{50} 96h) were malathion, trichlorfon, malathion plus trichlorfon mixture, non-ionic surfactant and neem seed extract (Table 1).

Table 1. Products used

	% a. c.	FC/ha
Malathion [S-(1,2-bis (ethoxycarbonyl)-ethyl) 0,0-dimetil phosphorodithioate]	50	1,5 Kg
Trichlorfon [0,0-dimetyl (2,2-2 tricloro hydroxiethyl)-phosphonate]	80	2,4 Kg
Malation – Triclorfon Mixture (50 %)	50 - 80	1.5 l–1.5 Kg
Azadirachtin, Meliantriol and Nimbidin-t	0,3	0,3 mg
Genapol OX-80, Polyglicol ethers of fatty alcohols-CH_3-$(CH_2)_{12\text{-}15}$-$O(CH_2CH_2O)H$	100	2 Kg

a.c.: Active component; FC/ ha: Field concentration/ ha

Commercial size crayfish were tested (7 cm total length, measured from the distal tip of the rostrum to the distal point of the telson). The tests were made in aquaria of 35x50x35 cm and 10 cm water depth. Aeration was provided at the rate of 85 ml/min with an aquarium aerator.

A 24 h range-finding test was conducted to define the range of product dilutions to be used in the 96 hr definitive test, starting from the field concentration (Table 1). Field concentration per litre was calculated according to the average water depth in the rice basin (10 cm). The product concentrations in the definitive tests followed a geometric series between those concentrations in the range-finding test at which 0 and 100 % crayfish mortality occurred. 120 crayfish were used per test. Five replicates of controls (untreated) and five replicates of each product were tested.

During the test the crayfish were not fed. Temperature was 25 ± 1°C and pH, conductivity, oxygen and hardness were measured initially and at the end of the test using an oxygenometer, selective ion analyzer, and a colorimeter, respectively. Crayfish were considered dead if they failed to respond to antennae or leg stimuli. Dead crayfish were removed every 24 hr, and the surviving crayfish were counted. The test time for trichlorfon was 24hr, because by 48hr all the crayfish tested were dead.

Crayfish mortality data for each insecticide was pooled and analysed using a computer program incorporating probit analysis, adapted from Finney (1971) by Nogueira (1995). A computer program was used for evaluation of mixture toxicity (Rodriguez, 1997).

The toxicity of each product was compared to the other products by calculating a ratio of the lethal concentration estimated for each product. A ratio more or less than 1, suggested that an insecticide was more or less toxic than another insecticides (Mayer and Ellersieck, 1986). Survival, with time, was analysed by ANOVA (differences were considered statistically significant at 0.05) and comparisons of means by Scheffe test. Survival in rice fields was calculated by the ratio of LC_{50} 96hr value/ field concentration per ha, for each product.

RESULTS AND DISCUSSION

The environmental physico-chemical factors are important to the survival of animals. Water quality during toxicity testing was within the normal range for the optimal survival of crayfish (Cano, 1994). During toxicity tests, the water quality (Table 2) was within the range of optimal survival of crayfish (Cano, 1994). Dissolved oxygen was > 5 mg/l.

Table 2. Mean conductivity (μS), pH and total hardness (°d) of exposure water during LC_{50} determinations with *Procambarus clarkii*, in the different products.

	Conductivity		pH		Total Hardness °d	
	0hr	End Test	0hr	End Test	0hr	End Test
Malathion	254	287	7.5	7.8	9.9	10.9
Malathion+Trichlorfon	257	265	7.6	8	5.7	5.6
Trichlorfon	308	315	7.5	8	8	8.5
Azaridachtin	264	337	7.5	7.1	5.5	6
Genapol OX 80	273	322	8	7.7	7.8	8
Control	321	552	7.5	8	5.5	5.5

The LC_{50} values obtained for the different insecticides in each test are shown in Table 3. We observed that the LC_{50} values for every insecticide decrease with time. Comparisons of means study show that there are three groups in which the means are not significantly different from one another in malathion, azadirach and genapol, and two groups by trichlorfon and malathion mixture.

The degradation period and the toxicity of different insecticides are variable depending on various environmental physico-chemical factors (in general 90 hr of mean life) (Chapman and Cole, 1982; Sorgorb *et al.*, 1986; Beyers and Myers, 1996; Cano *et al.*, 1999a).

In general the authors used the LC_{50} 96hr value, however we take into account the LC_{50} 24hr, 48hr, 72hr or 96hr value, because the sensibility to these products eventually varies as we can see on table 3 and it is demostrates with the media comparation study, being *P. clarkii* more sensitive to all products after 96 hr. It is important to have in mind the LC_{50} values at different hours, as in the rice field systems the time the water is help up (without rechange), varies depending on the areas, therefore the concentration of the product should be chosen to apply depending on the time the water could be held up.

Table 3. $LC_{50} \pm$ SD values for the insecticides (mg/l) through time and the ratios obtained for each.

Insecticides	24h	48h	72h	96h	P	Ratio
Malathion	2.4 ± 0.75	2.135 ± 0.475	1.95 ± 0.5	1.75 ± 0.45	0.0001	1.16
Trichlorfon	5.155 ± 0.48	------	------	------	0.0001	0.024
Azaridachtin	0.057 ± 0.0075	0.021 ± 0.0024	0.016 ± 0.002	0.014 ± 0.0016	0.0001	4.315
Genapol	8.4 ± 2.3	8.15 ± 2.5	7.89 ± 2.5	6.88 ± 1.26	0.0001	1.31
Mal+tri mixture	1.329 ± 0.13	1.150 ± 0.132	0.78 ± 0.119	0.669 ± 0.111	0.0001	0.297

The mixture of malathion+trichlorfon is showing synergy compared to malathion alone or trichlorfon alone. The ratio for the LC_{50} 96hr value/ field concentration per ha (Table 3), was less than 1 for trichlorfon and for the malathion+trichlorfon mixture. The ratios calculated for the estimated lethal concentration of all products to *Procambarus clarkii* were less than 1 for azadirachtin with malathion and trichlorfon, and malathion+trichlorfon mixture with malathion, trichlorfon and genapol (Table 4).

Azaridachtin is a product of natural original, but it was the most toxic for *Procambarus clarkii* (Table 4), compared to the other insecticides tested.

Table 4. Ratio of the lethal concentrations estimated for each product.

	Malathion	Trichorfon	Azadirachtin	Genapol	Mal+Tri
Malation	1	2.946	0.0082	3.931	0.382
Trichorfon	-	1	0.0027	1.334	0.129
Azadirachtin	-	-	1	478.775	46.5
Genapol OXD	-	-	-	1	0.097
Mal+Tri	-	-	-	-	1

Therefore, the use of azaridachtin would not be advise for this crustacean.

In general, the LC_{50} values are similar to other reports (Table 5), except for malathion. This difference may be due to the crayfish stree condition prior to applications of pesticides to the rice fields (Cano *et al.*, 1999a).

Table 5. LC_{50} values cited in the literature.

Products	LC_{50}	Time Test	References
Malathion	1.34 mg/l	96h	Cano *et al.*, 1999a
	50 mg/l	96h	Cheah *et al.*, 1980
Trichorfon	5 µg/l	96h	Repetto *et al.*, 1988
	5.15 mg/l	24h	Jiménez *et al.*, 1998
Mal + Tric. Mixture	0.143µl/l+1.431 10^{-4}g/l (0.8581 mg/l)	96h	Cano *et al.*, 1999b
Genapol OX 80	6.95 mg/l	96h	Cano *et al.*, 1999a
Azadirachtin	0.057 mg/l	24h	Jiménez *et al.*, 1998

Apart from damaging rice fields, *Procambarus clarkii* is a commercialized species for human consumption, therefore it is interesting to know the relation between the concentration in the field for the different tested insecticides and LC_{50} value obtained from this paper (Table 3). We can see the insecticides influence on the crayfish survival in the rice fields of the Guadalquivir river marshes. The ratio LC_{50} 96h value/ Field concentration per ha values, were superior to 1 for malathion, genapol and azadirachtin, therefore are not damaging to the *Procambarus clarkii* survival. From all this value of products the lesser harmful is the azadirachtin as its values for superior to 1, it could therefore be a good product for the insect pests control in the rice fields without damaging the production of crayfish in these areas.

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