

Biological de-activation of granular formulation of a carbamate insecticide Cartap in water under laboratory conditions

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Abstract. Cartap 4% (Padan 4G™) at different concentrations was evaluated for its biological deactivation in laboratory-conditions. The insecticide at 1.88 ppm or above concentrations showed significant increase in percent of dead fingerlings up to 46 days insecticide aging as compared to the control, while 0.8 ppm and below proved to be sub-lethal concentrations. The LD₅₀ at 0-day (fresh solution) was 0.997 ppm, which gradually rose to 2.074 ppm up to the day 46. The chemical attained half-life in 44.89 days. Being a very slowly degrading insecticide, Cartap is not desirable in rice-fish culture and a threat to aquatic fauna.

Key words: half life, LD₅₀, Cartap 4%, *Labeo rohita*, de-activation.

Resumen. El Cartap 4% (Padan 4G™) en diferentes concentraciones fue testado, en laboratorio, para la evaluación de su desactivación biológica. El insecticida en concentración de 1.88 ppm o más demostró su nocividad. Hasta la "edad" de 46 días del insecticida, la mortalidad de peces incremento significativamente en comparación con el lote testigo. Las concentraciones de 0.8 ppm o menos se han mostrado submortales. El LD₅₀ en el 0 día (solución fresca) era de 0.997 ppm, valor que incremento gradualmente a 2.074 ppm hasta el día 46. Las sustancias químicas alcanzaron el medio período de vida en 44.89 días. Cartap, un insecticida lentamente degradable, no es indicado para los cultivos mixtos arroz-pescado, siendo nocivo para la fauna acuática.

Palabras clave: half-life, LD₅₀, Cartap 4%, *Labeo rohita*, desactivación.

Rezumat. Cartap 4% (Padan 4G™), în diferite concentrații, a fost testat în condiții de laborator în vederea evaluării dezactivării lui biologice. Insecticidul și-a demonstrat nocivitatea la concentrații de 1.88 ppm sau mai mari, crescând semnificativ mortalitatea puietului de pești, comparativ cu lotul martor, până la "vârsta" de 46 zile a insecticidului, pe când concentrațiile de 0.8 ppm sau mai mici decât aceasta s-au dovedit subletale. La ziua 0 (soluția proaspătă), LD₅₀ a fost de 0.997 ppm, care a crescut treptat la 2.074 ppm până în ziua a 46-a. Compusul chimic a atins o durată de înjumătățire de 44.89 zile. Fiind un insecticid lent degradabil, Cartap nu este indicat în culturile mixte orez-pește, fiind un factor nociv pentru fauna acvatică.

Cuvinte cheie: durată de înjumătățire, LD₅₀, Cartap 4%, *Labeo rohita*, dezactivare.

Introduction. Cartap is one of the most commonly applied insecticides in soils for cultivation of rice and sugarcane. It belongs to the carbamate group of agrochemicals with systemic properties. Pesticides used in rice fields indiscriminately kill beneficial and/or adversely affect aquatic life either by direct hitting or by consuming a poisoned food. The dissolved insecticide is not only taken up by the plant with water and other nutrients, but also leach into the ground water posing threat to aquatic fauna and human. Even the sub-lethal concentration (0.01-0.05 ppm) of insecticide could affect the respiration, swimming ability, reproduction and over all growth of fish (Sprague 1971). Fish that survived the sub-lethal dose of insecticides like Hytox, Hopcin and Baycarb are not safe for human consumption (Magisa & Dela-Cruz 1978). Contamination by pesticides with long residual toxicity in water may eventually cause high levels of fish mortality (Kyaw 2001). Cartap was shown to produce acute toxicity to white rabbits (Liao et al 2003) and to fish (Lakota et al 1981). However, Kegley et al (2007) consider the available ecotoxicity data for this insecticide insufficient. Rice growers can increase their

farm income by culturing fish in rice field (Khayhuat & Tan 1980). However, in Pakistan this is not being practiced because farmers use pesticides, even if otherwise it would be feasible (Baloch et al 1997). The study in hand was designed with objective to elucidate the deactivation index of Cartap using bioassay techniques.

Materials and Methods

Experimental Procedures. The insecticide, Cartap [1,3-di(carbamoylthio)-2-dimethylaminopropane] is commercially available Padan (Granular insecticides Pvt. Ltd.), was used in these experiments. The fish species, *Labeo rohita* (Hamilton) - 'Rohu' - was obtained from the seed nursery of the Fisheries Department, Dera Ismail Khan, of average size of 1.27 cm [SE=0.65] and 4.69 g [SE=0.73]. A stock solution of 50 ppm strength was prepared by adding 25 g of formulated insecticide (4%) to 20 L of filtered tap water and stored light proof plastic container, at ambient temperature ($\approx 33^{\circ}\text{C}$, 65%RH). The tests were run at 0, 3, 6, 9, 18, 21, 27, 30, and 46 day aged-solutions. The concentrations tested were 1.34 ppm, 1.06 ppm, 0.8 ppm, 0.54 ppm, 0.26 ppm and control (tap water). Three replicates of each treatment was carried out, and each lasted for up to 15 days. Since the mortality at day 15 in the highest strength tested (1.34 ppm) was very low and the deactivation index (DI) wasn't reached to 2, the test strength was raised to 2.15, 1.88, 1.61, 1.34 and 1.06 ppm from day-18 up to 46 days. Plastic container (33 cm dia) of 5-liter capacity was used. The known quantity of stock solution was added to tap water making a net volume of 4 liters, used in this experiment. The fish of possible uniform, age, length and weight (1.27 cm [SE=0.65] and 4.69 g [SE=0.73]) was collected from the hatching ponds and acclimatized in the 40-liter tub (59 cm dia) for 3-4 hours. Ten active fingerlings were used in each treatment. After 24 hours, dead fish fingerlings were counted, and percent mortality was calculated. The weight and the length of the dead fingerlings were also recorded. Extra ordinary mortality due to power breakdown or jumping-out of fish was excluded from the analysis.

In a separate experiment, fish fingerlings were released following the protocol mentioned above. The fish mortality was observed after every hour till all the fingerlings were dead. The experiment was replicated five times. The dead fish were converted into percent mortality and plotted against the time (hrs).

Statistical Analysis. All the data recorded were subjected to statistical analysis using MSTATC software. The out flyer mortality data (after 15, 24, 36 and 39 days) was excluded from the analysis. Percent mortality of each test was analyzed using Completely Randomized Design (CRD) to demonstrate any concentration effect. Probit analysis was performed to calculate Lethal Concentration (LC_{50}) for each test /day. All generated LC_{50} of different aged stock solution was plotted. The deactivation index (DI) for each day was calculated by dividing the LC_{50} of the fresh insecticide solution to the LC_{50} of aged solution. The LC_{50} test run were continued till deactivation index reached to 2 or above, in order to find the half life of Cartap in water. Lethal Time (LT_{50}) test was also analyzed by Probit procedure.

Results. Fresh solution of Cartap 4% caused 100% mortality at 1.34 ppm after 24 hours, followed by 44.25 and 6.25% in 1.06 and 0.8 ppm strengths (see Table 1). Aged solution tested (3, 6, 9 and 12 day old) resulted in significantly highest mortality with 1.34 ppm solution followed by 1.06 ppm. However, no mortality was observed in solutions weaker than 1.06 ppm. No fish mortality was observed in untreated control treatments except in fresh solution which was attributed to the mishandling during experimentation. Probit analysis showed effective dose for 50% mortality (ED_{50}) 0.99 ppm for fresh solution and 1.10 and 1.13 for 3 and 6 day old solution, respectively. Similarly deactivation index showed a slow and gradual deactivation of 1.14 after 6 days.

Table 1

Biological deactivation of granular formulation of Cartap 4% in water on fish seedlings (*Labeo rohita*) at 0, 3, 6, 9, and 12 days old-solution.

Age of the solution (days)	Concentration of 4% Cartap (ppm)						SE	LSD _{0.05}	ED ₅₀	Deactivation Index (DI)
	Control (n=60)	1.34 (n=60)	1.06 (n=60)	0.8 (n=60)	0.54 (n=60)	0.26 (n=60)				
0	10.00 c	100 a	44.25 b	6.25 c	0.00 c	0.00 c	9.29	27.5	0.99	1.00
3	0.00 c	100 a	25.00 b	0.00 c	0.00 c	0.00 c	8.88	3.631	1.10	1.10
6	0.00 c	80 a	10.00 b	0.00 c	0.00 c	0.00 c	7.15	7.263	1.13	1.14
9	0.00 c	100 a	50.00 b	0.00 c	0.00 c	0.00 c	9.40	14.53	--	--
12	0.00 c	100 a	83.00 b	0.00 c	0.00 c	0.00 c	10.63	12.13	--	--

Each value is a mean of 3 replications.

Means followed by same letters in respective columns are not significantly different at $\alpha = 0.05$.

Statistically non-significant mortality was observed in 2.15, 1.88, 1.61 and 1.34 ppm solution of 18-day old, whereas significantly less mortality (15%) was observed in 1.06 ppm solution (see Table 2). After 21 and 27 days, fish mortality was 100% in 2.15 and 1.88 ppm solutions whereas, other solution strengths has less than that fish mortality. Test solution of 30 days age, had 100% mortality in only 2.15 ppm strength followed by 90% in 1.88 ppm (statistically non-significant) and significantly less mortality in rest of the solutions. After 46 days, highest mortality of 60% was observed in 2.15 ppm solution and all other solutions had significantly less mortality.

The ED₅₀, was 1.19 after 18 days gradually increased to a level of 2.07 after 46 days. Similarly, DI was 1.14 after 18 days rose 1.20 after 21 days, 1.39 after 27 days, 1.67 after 30 days and 2.09 after 46 days (Figure 1). The calculated half-life of Cartap 4% was 44.89 days.

Table 2

Biological deactivation of granular formulation of Cartap 4% in water on fish seedlings (*Labeo rohita*) at 18, 21, 27, 30, and 46 days old-solution

Age of the solution (days)	Concentration of 4% Cartap (ppm)						SE	LSD _{0.05}	ED ₅₀	Deactivation Index (DI)
	Control (n=60)	2.15 (n=60)	1.88 (n=60)	1.61 (n=60)	1.34 (n=60)	1.06 (n=60)				
18	0.00 c	100 a	100 a	95.0 a	85.0 a	15.0 b	9.40	15.83	1.19	1.14
21	0.00 c	100 a	100 a	95.0 a	50.00 c	5.00 c	10.63	6.29	1.45	1.20
27	0.00 d	100 a	100 a	85.0 b	25.0 c	5.00 d	10.96	12.04	1.38	1.39
30	0.00 c	100 a	90.0 a	40.0 b	10.0 c	0.00 c	10.51	29.05	1.66	1.67
46	0.00 c	60.0 a	42.0 b	10.0 c	0.00 c	0.00 c	6.00	14.64	2.07	2.09

Each value is a mean of 3 replications.

Means followed by same letters in respective columns are not significantly different at $\alpha = 0.05$.

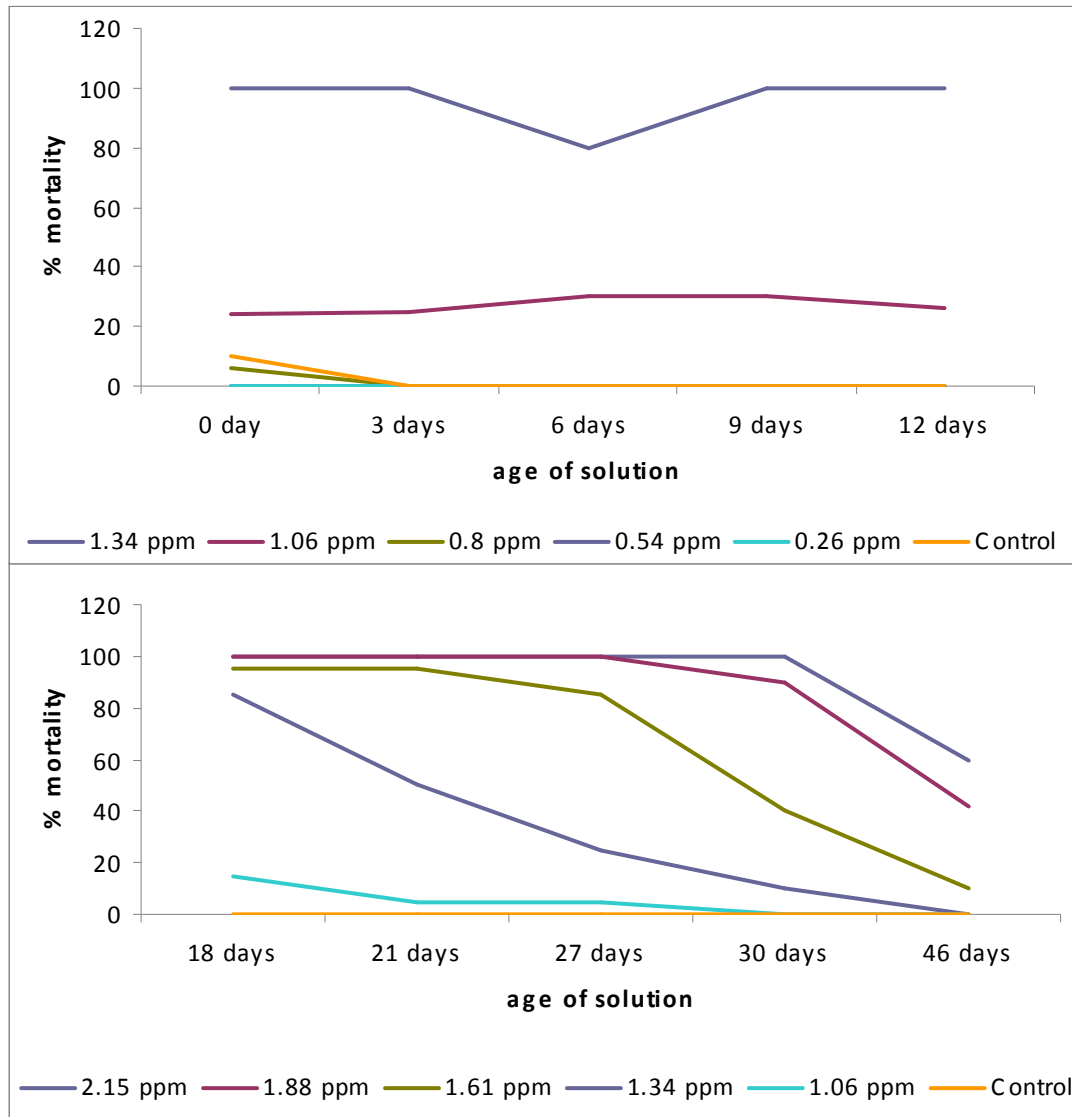


Figure 1. Percent mortality of fish seedlings (*Labeo rohita*) as affected by various concentration of Cartap 4% and solution aging

Deactivation Index and Half Life. The deactivation is the depletion of toxicity of an insecticide over time. The deactivation index was 1 of the fresh solutions, and it showed a linear decrease as the age of the solution increased. In the first set of tests, the deactivation index reached to 1.14 after 6 days. Similarly, the pesticide gradually deactivated about 1.2, 1.45, 1.67, and 2.1 after 18, 21, 30, and 46 days, respectively (Figure 2 and Table 2).

The insecticide reached to its half life or deactivated 50% of its toxicity to biological organisms in 44.89 days. The correlation between deactivation and age of solution proved to be positive and significant ($r^2 = 0.913$).

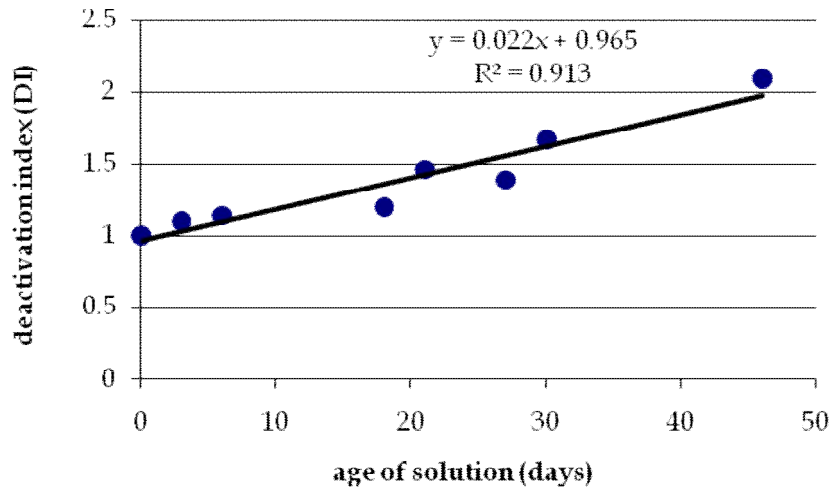


Figure 2. Deactivation index of Cartap 4% over a period of time (days)

Lethal Time (LT₅₀). The data generated in LT₅₀ revealed that insecticide (1.06 ppm) took 10.228 hours to kill 50% of the test population (see Figure 3). Hour 8 to 14 showed the highest pesticide activity by causing mortality of about 50% of the population, while exposure for first 4 hours also caused < 30% mortality of the test organism. None of the fish could survive up to 24 hours of pesticide exposure.

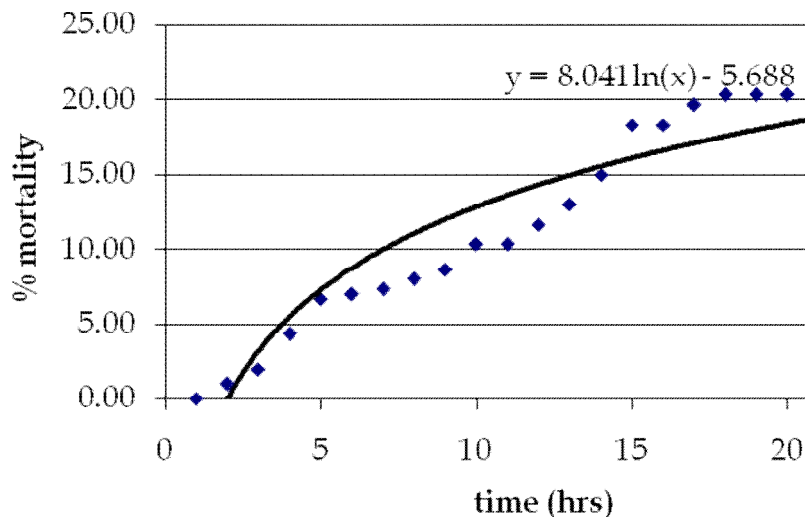


Figure 3. Mean percent fish mortality over time (hrs)

Discussion. The results of the present studies are comparable with the results of Dela-Cruz & Circa (1981) who observed that Cymbush - 2.5ml/2.5 lt water - applied in 50 square meters was tolerable to *Tilapia nilotica* in rice-fish culture system but was toxic at higher concentration. Dela-Cruz & Circa (1981), Cauguan & Arce (1992) reported that all insecticides and their groups have different toxicity levels. The aging and the concentration also have strong relation to the toxicity. Besides, concentration levels, the toxicity of insecticides are also affected by temperature, resistant level of the test

organism, exposure time, body weight of organism, and impure water etc. The findings of the current studies are quite in conformity with the results of afore-mentioned workers.

Kathiresan et al (2001), Sivakumar & Balasubramaniam (2001), Dela-Cruz & Circa (1981), calculated the LD₅₀ or ED₅₀ of different pesticides using different fish species. The variation among the values of the previously reported work in comparison to the present studies is mainly due to the difference in the insecticide used, fish species, methods of data recording and the environmental conditions.

The insecticide tested took 44.89 days to reduce its biological activity to half. Kathiresan et al (2001) reported 12 days after herbicides application is safe to grass carp fish in rice fish culture. However, Parkash & Devi (2000) calculated half life of Butachlor 52.40 to 59.37 days. The differences in findings might be mainly due to the reason that they used different chemicals (herbicides, emulsifiable concentrate) and the test organisms.

Magisa & Dela-Cruz (1978) stated that Hytox, Baycarb and Hopcin had LT₅₀ lower than 24 hours. The results of the present studies coincide with the findings of afore mentioned authors, although they used different insecticides.

The present studies confirm that Cartap deactivated with the aging of dissolved solution, the deactivation pattern seems to be very linear although it was quite long. As the test insecticide took 44.89 days to deactivate its insecticidal properties by 50%. These findings bring concerns in culturing fish in rice fields previously applied Cartap in and also the runoff of field irrigation or rain water to water bodies. Even if the pesticide is non lethal to aquatic fauna, it might affect their physiology and development.

Using bioassay for determination of toxic properties of agrochemicals is to be considered more authentic than of analytical techniques where only active ingredient (*a.i*) is detected and not the alkaloids (most of the time toxic ones) it breaks into after going in to ecosystem.

The present studies showed Cartap used in the field crops should be given extra care for the polluted water to contaminate the main water bodies and its application restricts the rice-fish culture, which has great future prospects in terms of uplifting of farmer's socio-economic condition and to meet the protein requirement of the country.

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