



Effects of organophosphate pesticide chlorpyrifos on fresh water Snail

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Abstract

The indiscriminate use of pesticides in the agricultural land and affects the aquatic ecosystem adjacent to it. Aquatic Snails are cosmopolitan in distribution and found in the entire fresh water ecosystem like pond, swamp, and ditches. In Kanyakumari district most of the aquatic ecosystem is adjacent to the agricultural land. Chlorpyrifos is very highly toxic to freshwater snail and it accumulates in the tissues and also causes significant biochemical alteration in the living system. In the present study shows exposure period 24, 48, 72, 96, and 108h LC₅₀ value of chlorpyrifos of snail sp. were increased with increase in mortality. After that the last (108h) in the animal acclimatized in experimental water because in the snail has the capacity to live in any water oxygen low or high concentration turbid water. So the mortality rates decrease in 0.6% concentration of exposure 108h. The present study reveals since very minimum contact toxicity were observed when the animals are exposed to short term. There is mild change in the protein, carbohydrate and fat analysed in the liver and muscle of the snail.

Keywords: chlorpyrifos, snail, protein, fat, carbohydrate

Introduction

In India pesticides are widely used in agriculture. Today, around 81,000 metric tons of technical grade pesticides are used based on around 144 products registered under insecticide Act 1968 (Bami 1996) [2]. Among the pesticide usage organophosphate pesticides superseded the organochlorine, owing to their rapid biodegradability, high degree of activity and shorter persistence in the environment (Singh *et al.* 1989; Jagadish and Dhingra 1991) [18, 8].

Many organisms colonized fresh water secondarily via terrestrial ancestors. Among fresh water Snails, Prosobranchs (eg Limpets) have gills and colonized directly from the sea, while pulmonates eg. Pond Snails (Mike Dodson and Chris Frid 1998) [11]. In aquatic communities a great majority of pesticide impacts are determined from single species experiments conducted under laboratory conditions (Racke 1992) [16].

The toxic substance may enter the ecosystem, both aquatic and terrestrial, by urbanization or by indiscriminate use of pesticides in agriculture. The pesticides in agriculture. The pesticides not only affect target organism, but also affect the innocent organism like snail, crab, fish etc. which are found in the agriculture land (Mike Dodson and Chris Frid 1998) [11].

In fact literature shows that of the pesticides applied by farmers only 50% gets to the target organisms (Pimentel, D. and Burgess, M. 2012) and the rest end up on nontarget organisms where they usually cause deleterious effects on these organisms. Literature reports show that metals and pesticides induce oxidative stress in cells of living organisms and this result from an imbalance between the generation and elimination of reactive oxygen species (Patra, R.C., *et al.*, 2011) [15].

One of the organophosphate pesticide chlorpyrifos pollution of water sources, water supply and ground water through run-off and other means of transpiration. These aquatic pesticide pollutants tend to accumulate in the body of aquatic organisms (Granmo and Kolberg 1976; Konar

1979) [7, 9] and produce biological or pathological changes and also cause significant biochemical alterations in the living system (Eister and Edmunds 1966; Singh H. and Singh TP 1980) [3, 17].

In the present study, we carry out an experiment on the friendly organism snail. We studied the toxic effect of the organophosphate pesticide chlorpyrifos and found out the LC₅₀ value. We also studied how the chlorpyrifos affect the liver, muscle tissue of the aquatic snail.

Materials and Methods

Study area and subjects

Live and healthy adult snail was procured from kodukulam pond located in the Kulappuram village in vilavancode Taluk in Kanyakumari Dist. They were collected by and picking during the early morning hours and transported to the laboratory in plastic containers filled with water. Pila were acclimatized for one week in laboratory in a glass tank (46 x 23 x 23cm). Healthy and active snail of 2.5gm – 3.5gm were taken. The experiment was carried out in a 9 troughs with 3 litre of fresh water. 9 different concentrations were taken in each trough. 10 experimental animals were introduced in each trough. The toxicity study was carried out in triplate.

Dilution procedure

Since commercial pesticides were used in the study their percentage composition was noted from the label of the container. This pesticide is water soluble. A convenient concentration of stock solution was prepared for the pesticide and required quantity of the stock solution was by dissolving stock solution was prepared 1ml of Decis in 100ml of water. From this stock solution 1ml was dissolved in 100ml of water. This was considered as the 0.1% solution similarly different concentrations of decis were prepared. Different concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 percentages were chosen to find out the wide range of tolerance limit. Mortalities were recorded at each

of the concentrations for an exposure period at 108 hours. A minimum of 10 pila were tested for one particular concentration and noted the percent of mortality. The concentration of the pollutant which will cause mortality in a particular proportion of the experimental animals. For example, LC₅₀ is the median lethal concentration which will 50% treats individuals. The exposure to me can be specified is LC₅₀ 24 hr, LC₅₀ hr, LC₅₀ 72 hr, LC₅₀ 96 hr and so on. The concentrations above these values are lethal and those below are sublethal. The dose of the pollutant which will cause mortality in a particular proportion for the experimental animals. The exposure time can also be incorporated as in lethal concentration (LD₅₀ 24 hr, LD₅₀ 48 hr, LD₅₀ 72 hr, LD₅₀ 96 hr and so on. The probit analysis method (Finney, 1991) [5] was used in evaluating acute toxicity.

Biochemical analysis

The present study biochemical analysis was carried out in the liver, and muscle of pila to determine the protein, Fat and carbohydrate content. Standard methods are use for the estimations of protein (Lowry’s method 1951) fat (Folch *et al.* 1957) [6] and carbohydrate (Anthrone Method).

Result

Table 1. Shows the mortality of snail exposed to different concentration chioropyrifos. At 0.1 %, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.7%, 0.8%, 0.9% concentration the mortality occured at 96h, 72h, 72h, 48h, 24h, 24h, 24h at the percentage of mortality was 5.2 %, 2.2%, 5.6%, 4.6%, 4.6%, 4.6%4.6%, 6.3%, 8%, 10.6%, 17% and increased with increase in exposure periods.

Table 1: Shows mortality (%) of snail exposed to different concentration of chloropyrifos. (Population from Kodukulam)

Concentration of pesticide	Duration of exposure (hrs)				
	24	48	72	96	108
Control	0	0	0	0	0
0.1%	0	0	0	5.25	83
0.2%	0	0	2.25	3.66	8.33
0.3%	0	0	5.6	7	9
0.4%	0	4.66	8	11.33	17.33
0.5%	4.66	10	18.33	23.33	31
0.6%	6.32	13.65	20.30	25	29.66
0.7%	8	13.33	20.33	28.0	26.0
0.8%	10.66	15.33	20.66	27.66	20.66
0.9%	17	20.66	25.60	30.75	-

Table 2. Indicates the calculated 24, 48, 72, 76 and 108 h LC₅₀ value of chloropyrifos for pila adult were 2.537, 2.081, 1.686, 1.956 and 2.31 respectively. The calculated 24,48,72,96 and 108 h LC₅₀ value of chloropyrifos for pila sp.were increased with increase in exposure period. At the 108h the Deci’s increased.

The regression equation fitted for log concentration and probit mortality pila the (y intercept) values ranged between 2.00 (24 h) and 3.46 (108 h) and the ‘b’ (regression coefficient) the rate at which y (% morality) changes with a change in X (concentration) valued from 1.074 (108 h) to 2.255 (48h). The calculated variance for chloropyrifos on snail ranged between 0.695 (72h) and 0.2788 (108h) respectively. The variance is increased at the first and last day but decreased at the 48, 72, 96h. The Chi – square space test at different exposure periods calculated 24, 18, 77, 96 and 108 h, X2 Value of chloropyrifos for pila sp.were increased with increased in concentration. The empirical value is compared with the expected value and the calculated x2 is insignificant.

Table 2: Reveals lethal concentration, regression equation, variance and Chi – square test (X2) to adult pila sp. (population from Kodukulam)

Experimental Period	LC50 ppm	Regression Equation Y = (a + bx)	Variance	Chi – square test
24	2.537	Y = 2.00 + 2.135x	0.2758	0.05
48	2.081	Y = 2.03 + 2.255x	0.1866	0.19
72	1.686	Y = 2.41 + 2.113x	0.0695	0.40
76	1.956	Y = 3.08 + 1.488x	0.0729	0.91
108	2.731	Y = 3.46 + 1.074x	0.2788	1.51

Biochemical Analysis

Carbohydrate, fat and protein in snail muscle

The carbohydrate content present in the snail muscle of control animal was 11.2 mg/dl and the carbohydrate content of the experimental animal was 11.52 mg/dl. The fat present in the snail muscle of control animal was 18.92 mg/dl and the fat of the experimental animal was 19.15mg/dl. The protein present in the snail muscle of control animal was 0.10 g/dl and the protein of the experimental animal was 0.11g/dl.

Carbohydrate, fat & protein in snail liver

The carbohydrate present in the snail liver and control animal was 15.10 mg/dl and the protein of the experimental animal was 15.50 mg/dl. The fat present in the snail in the liver of control animal was 20.10 mg/dl and the cholesterol of the experimental animal was 20.89 mg/dl. The protein present in the snail liver of control animal was 0.10 g/dl and the protein of the experimental was 0.9 g/dl.

Discussion

LC50 values of molluscicide chioropyrifos were determined for a snail *Lymnaea acuminata*, with concomitant estimation of levels of temperature, PH, dissolved oxygen and carbon-dioxide and electrical conductivity both in control and test water. It was noted that LC50 value of 10.39, 10.90 and 11.19 mg/l during the months of May, June, and July respectively were most effect in killing the snails while the molluscicide was least effective in the month of January. When its 24h LC50 was 65.84 mg/l. There was a significant positive correlation between LC50 of chloropyrifos and levels of dissolved oxygen/pH of water in corresponding month. (Singh V and Singh DK. 2008).

Toxicity testing is an essential tool for assessing the effect and fate of toxicants in aquatic ecosystems and has been widely used as a tool to identify suitable organisms as a bioindicator and to derive water quality standards for chemicals. There are many different ways in which toxicity can be measured, and most commonly the measure (end

point) is death. (Walker CH., *et al.*, 2006, Adams WJ and Rowland CD 2003, Luoma SN and Rainbow PS 2008) ^[19, 1, 12].

In the present study reveals that increase in concentration increase the exposure period increases the mortality. And alter that the last (108h) in the animal acclimatized in experimental water because in the snail has the capacity to live in any water oxygen low or high concentration and turbid water, so the mortality rate decrease in 0.6% concentration of exposure 108hrs. Low degradability and bioaccumulation at different trophic levels through aquatic food webs make them major stress factors to aquatic organisms (Nunes, B. 2011) ^[13].

Histopathological alterations induced by Thiodan in three tissues, namely digestive gland, foot and mantle of the fresh water snail *Lymnaea* study showed that degenerative change the muscle fibre of the food protein and pigment cells of the food and the connective tissue element of the mantle were recovered after 30 days of recovery of the snail in pesticide-free fresh water. (Erhan Unlu, *et al.*, 2005) ^[4]

The protein, carbohydrate and fat present in the muscle of control snail is 0.10g/dl, 11.2mg/dl, 18.92mg/dl respectively. The protein, carbohydrate and fat present in the muscle of experimental snail is 0.11g/dl, 11.52mg/dl, 19.52mg/dl respectively. The protein, carbohydrate and fat present in the liver of control snail are 0.10g/dl, 15.10mg/dl, 20.10mg/dl respectively.

The protein, carbohydrate and fat present in the liver of experimental snail is 0.9g/dl, 15.50mg/dl, 19.15mg/dl respectively. The present study reveals since very minimum contact toxicity observed when the animals exposed to short term. There is mild change in the protein, carbohydrate and fat analysed in the liver and muscle of the snail.

Conclusion

The present study reveals that increase chloropyrifos concentration increase the exposure period increases the mortality. The use of chloropyrifos minimized to save the life of snail and also control the biochemical alterations in the liver and muscle of snail.

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