*Turkish Journal of Fisheries and Aquatic Sciences 8: 01-05 (2008)*

# A Comparative Study on the Toxicity of a Synthetic Pyrethroid, Deltamethrin and a Neem Based Pesticide, Azadirachtin to *Poecilia reticulata* Peters 1859 (Cyprinodontiformes: Poeciliidae)

**S. Israel Stalin[1], S. Kiruba[1], S. Sam Manohar Das[1,*]**
[1] Scott Christian College (Autonomous), Department of PG studies and Research Centre in Zoology, Nagercoil 629 003, Tamil Nadu, India.

* Corresponding Author: Tel.: +91.4652 231807; Fax: +91.4652 229800;
E-mail: sambiocontrol@gmail.com

Received 12 February 2007
Accepted 25 September 2007

**Abstract**

Addition of pollutants changes the natural qualities of water. Pesticides in agricultural runoff affect fish and other aquatic organisms. Fish are common indicators of water pollution status. This study was done to check whether plant based pesticides are less toxic to fish and other non-target organisms, compared to chemical pesticides. Bioassays of a natural pesticide of plant origin (Azadirachtin) and a synthetic pyrethroid, deltamethrin were separately done on a freshwater teleost, *Poecilia reticulata* Peters 1859. Toxicity estimations were done following static bioassay and probit analyses models. The 96 h $LC_{50}$ of deltamethrin is 0.0019 and azadirachtin is 0.011 mg/L. The plant based pesticide; azadirachtin is less toxic to fish compared to deltamethrin, a synthetic pyrethroid. Plant based pesticides contain easily biodegradable molecules which are more target specific than the highly persistent broad-spectrum synthetic chemical moieties. Use of plant based pesticides is less disastrous and more ecofriendly. This study is done to compare the non-target toxicity of a natural pesticide of plant origin (Azadirachtin) with a synthetic pyrethroid, deltamethrin on a fresh water teleost, *Poecilia reticulata* Peters 1859.

*Key words:* deltamethrin, azadirachtin, *Poecilia reticulata*, non-target toxicity, 96h $LC_{50}$.

## Introduction

Increased use of chemical pesticide results in the excess inflow of toxic chemicals, mainly into the aquatic ecosystem (Baskaran *et al*., 1989; Kalavathy *et al*., 2001). The aquatic flora and fauna are affected by the toxic substances which eventually enter in to their systems or bring about external damages (Pant and Singh, 1983; Hodson, 1988; Johal and Dua, 1995). Several species of fish are susceptible to deleterious effects when exposed to heavy metals, pesticides and other environmental stressors (Khangrat *et al*., 1988; Areechon and Plump, 1990).

Recent emphasis is on the use of natural pesticides, which are usually of plant origin. Azadirachtin derived from neem (*Azadirachta indica* A. Juss) is a very effective and extensively used pesticid. Pesticides based on azadirachtin may have direct adverse effects on aquatic organisms and their toxicity depends on various factors. It has been reported that neem extracts in aquatic environments are lethal to benthic populations and drastically decrease the number of organisms in the food web and nutrient cycling process (Goktepe *et al*., 2002; El-Shazly *et al*., 2000). Even though *Azadirachtin* is not likely to accumulate or cause long-term side effects, toxicity to fish is only moderate and may not kill fish under normal use (Miller and Uetz, 1998).

Pesticides containing bioactive compounds from the neemplant, *Azadirachta indica* Juss are reported to be target specific and comparatively less toxic. Plants are virtually inexhaustible sources of structurally diverse and biologically active substances (Istvan, 2000). Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties. Unlike synthetic chemical pesticides, which leave harmful residues in the aquatic environment (Koesomadinata, 1980; Cagauan, 1990; Cagaun and Arce, 1992), botanical insecticides are believed to be more environmentally friendlier because they are easily biodegraded and leave no residues in the environment. Most of the pesticides, both plant based and chemical, applied in the various agro ecosystems reach water bodies through runoff affecting fish, the most abundant aquatic organism, and a variety of other fauna. Persistent chemical molecules with long half-life periods found in chemical pesticides pose a threat to fish and also to the human population consuming the affected fish. The purpose of this study is to establish whether azadirachtin has any non-target effect on fish and to compare its toxic impact with deltamethrin, a powerful chemical pesticide. Based on the results, the use of the less toxic pesticide could be promoted among the agriculturists. It is possible to substitute chemical pesticides with pesticides of plant origin. This needs an extensive study on the properties of the two types of pesticides, especially a study of their toxic effect on aquatic organisms (Lockwood, 1976; Woin, 1988; Glow and Godzi, 1994; Christina, 2004). Fish are considered to be indicators of water pollution (von Westernhagen, 1988). Guppies are common fresh water fish capable of tolerating wide fluctuations in water quality and hence have been selected for this work. Any impact on such hardy species is considered to be ultimate, since other susceptible species succumb at concentrations, much lower than that could be tolerated by this species. This study is designed to compare the toxicity of a synthetic pyrethroid, deltamethrin and a neem derivative, azadirachtin in the aquatic environment using *Poecilia reticulata* Peters as the test species.

## Materials and methods

### Test Animals

Healthy, unsexed *P.reticulata* (Guppy) (1.12±0.5 g) were brought to the laboratory from the freshwater pond of the Zoology Research Laboratory, Scott Christian College (Autonomous), Nagercoil. The fish were acclimatized to the laboratory environment for about 5 days. They were fed with commercial food pellets and only healthy fish were used in the experiments. Toxicant was prepared in wide ranging concentrations and the fish were exposed in one litre glass aquaria. This range finding test gave an idea as to the effective concentration of the toxicant required to bring about mortality in the test species and 15 different concentrations were set up. The test water used for dilution was drawn from the campus well.

### Experimental Design

Two different types of toxicants were used; deltamethrin and juerken (Azadirachtin). Stock solutions were prepared by adding 0.5 ml insecticide with 999.5 ml water and stored in glass bottles. Working stock solution (0.1%) of the toxicant was prepared and stored in glass bottles. Proper dilutions of the stock solutions were made to make a series of test solutions of varying concentrations. *P. reticulata* is exposed to different concentrations of deltamethrin ranging from 0.0014 to 0.0028 mg/L and azadirachtin, ranging from 0.002 to 0.030 mg/L.

To each concentration, 10 healthy fish were introduced. The mortality was recorded after 6, 12, 18, 24, 36, 48, 72, 96 and 120 h of exposure. The mortality of a single fish was recorded as 10 percent mortality. The toxicity studies were conducted following the static bioassay method (Sprague, 1973). The toxicant was renewed everyday. The dead fish were carefully removed to avoid contamination. The mortality of exposed fish was observed at different intervals.

### Calculation of $LC_{50}$ Values

The results of the static bioassay were analysed using linear regression probit analysis (Finney, 1971) using the statistical package, (POLO-PC, LeOra software, 1987) The $LC_{50}$ values were calculated for 24, 48, 72, 96, and 120 h of exposure to deltamethrin and azadirachtin separately. The upper and lower fiducial limits were also calculated.

## Results

The $LC_{50}$ values for 24, 48, 72, 96 and 120 h of exposure to deltamethrin are 0.0024, 0.0021, 0.0020, 0.0019 and 0.0018 and to azadirachtin 0.0020, 0.017, 0.014, 0.011 and 0.010 mg/L, respectively (Table 1). The toxicity curves (Figure 1 A and B) follow the regular pattern with asymptotes close to 72 hours. Based on probit analysis, regression equation for delta methrin (96 h exposure) is $Y = 17.72 x – 17.33$ (Table 2) and azadirachtin (96 h exposure) is $Y = 4.24 x +0.43$ (Table 3).

## Discussion

Newer biological pesticides are developed to replace deleterious chemical pesticides. Even though chemical pesticides are target specific and effective, their impact on the environment is mostly deleterious. Plant based pesticides contain active principles with low half-life period and their effects on the environment are not too detrimental (Sharma *et al*., 1995). In the present study, the pesticide containing azadirachtin is less toxic to fish compared to deltamethrin. The 96 h $LC_{50}$ of deltamethrin is 0.0019 mg/L. whereas azadirachtin is much higher 0.011 mg/L indicating the less toxic nature of the plant based pesticide. Svobodova (2003) reported that the 96 h $LC_{50}$ of deltamethrin to *Cyprinus carpio* juveniles was 0.00145 mg/L. Boran *et al*. (2007)

**Table 1.** $LC_{50}$ values (mg/L) with their fiducial limits

| | Deltamethrin | | | Azadirachtin | | |
|---|---|---|---|---|---|---|
| Hours | LCL | $LC_{50}$ | UCL | LCL | $LC_{50}$ | UCL |
| 24 | 0.0022 | 0.0024 | 0.0025 | 0.018 | 0.02 | 0.023 |
| 48 | 0.002 | 0.0021 | 0.0022 | 0.015 | 0.017 | 0.019 |
| 72 | 0.0019 | 0.002 | 0.0021 | 0.012 | 0.014 | 0.016 |
| 96 | 0.0018 | 0.0019 | 0.002 | 0.009 | 0.011 | 0.014 |
| 120 | 0.0017 | 0.0018 | 0.0019 | 0.008 | 0.010 | 0.012 |

Note:
LCL = Lower Confidence Limit
UCL = Upper Confidence Limit
$LC_{50}$ = Lethal Concentration for 50 percent of the exposed fish

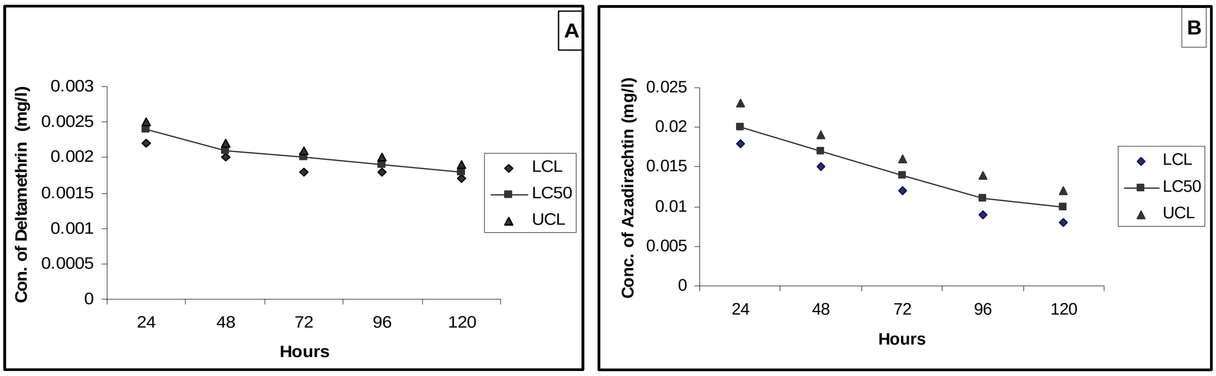


**Figure 1.** Toxicity curve showing the response of *P. reticulata* **A.** Deltamethrin **B.** Azadirachtin.

**Table 2.** Log-dose/ probit regression line analysis of the response of *Poecilia reticulata* exposed to deltamethrin for 96 h

| S. No | Dose (mg/L) | No | Mor % | Log dose | Emp. Pro | Exp. Pro | Work Pro | Wt. Coef | Weight w | Wx | Wy | Y |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.0016 | 10 | 10 | 1.2 | 3.72 | 3.66 | 3.72 | 0.34 | 3.36 | 4.05 | 12.51 | 3.62 |
| 2 | 0.0017 | 10 | 20 | 1.23 | 4.16 | 4.13 | 4.16 | 0.47 | 4.71 | 5.8 | 19.6 | 4.09 |
| 3 | 0.0018 | 10 | 40 | 1.26 | 4.75 | 4.57 | 4.75 | 0.6 | 6.01 | 7.55 | 28.54 | 4.53 |
| 4 | 0.0019 | 10 | 50 | 1.28 | 5 | 4.99 | 5.01 | 0.64 | 6.37 | 8.15 | 31.88 | 4.94 |
| 5 | 0.002 | 10 | 60 | 1.3 | 5.25 | 5.38 | 5.25 | 0.6 | 6.01 | 7.82 | 31.56 | 5.34 |
| 6 | 0.0021 | 10 | 70 | 1.32 | 5.52 | 5.76 | 5.5 | 0.5 | 5.03 | 6.65 | 27.64 | 5.71 |
| 7 | 0.0022 | 10 | 80 | 1.34 | 5.84 | 6.12 | 5.8 | 0.14 | 4.05 | 5.44 | 23.3 | 6.07 |
| 8 | 0.0023 | 10 | 90 | 1.36 | 6.28 | 6.46 | 6.24 | 0.27 | 3.69 | 3.66 | 16.78 | 6.41 |
| 9 | 0.0024 | 10 | 100 | 1.38 | 7.33 | 6.78 | 7.13 | 0.18 | 1.8 | 2.49 | 20.84 | 6.74 |

STATISTICS
SW=40.030 SWX=51.605 X Bar=1.389 SWY=204.856 Y Bar=5.118
SWX*X=66.622 SWY*=1073.500 SWXY=265.604 b Value=17.724
Regression equation y=17.724x -17.73
If y=5.0 then x=1.283 this corresponds to dose of 0.0019
Variance=0.0001 Chi-square=1.02 (with 7 Deg. of freedom p)
Lower Limit 1.2629 log dose 1.2825 Upper Limit 1.3021

**Table 3.** Log-dose/ probit regression line analysis of the response of *Poecilia reticulata* exposed to Azadirachtin for 96 h

| S.No | Dose (mg/L) | No | Mor. % | Log dose | Emp. Pro | Exp. Pro | Work Pro. | Wt. Coef. | Weight. w | Wx | Wy | Y |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.006 | 10 | 20 | 0.78 | 4.16 | 3.83 | 4.24 | 0.37 | 3.7 | 2.88 | 15.69 | 3.74 |
| 2 | 0.008 | 10 | 30 | 0.9 | 4.48 | 4.36 | 4.48 | 0.56 | 5.58 | 5.04 | 25 | 4.27 |
| 3 | 0.01 | 10 | 40 | 1 | 4.75 | 4.77 | 4.74 | 0.63 | 6.27 | 6.27 | 29.74 | 4.68 |
| 4 | 0.012 | 10 | 50 | 1.08 | 5 | 5.11 | 5 | 0.63 | 6.34 | 6.84 | 31.7 | 5.01 |
| 5 | 0.014 | 10 | 50 | 1.15 | 5 | 5.39 | 4.98 | 0.6 | 6.01 | 6.89 | 29.93 | 5.3 |
| 6 | 0.016 | 10 | 50 | 1.2 | 5 | 5.38 | 5.98 | 0.56 | 5.58 | 6.72 | 29.13 | 5.54 |
| 7 | 0.018 | 10 | 70 | 1.26 | 5.52 | 5.85 | 5.46 | 0.47 | 4.71 | 5.91 | 25.73 | 5.76 |
| 8 | 0.02 | 10 | 80 | 1.3 | 5.84 | 6.05 | 5.87 | 0.44 | 4.39 | 5.71 | 25.57 | 5.95 |
| 9 | 0.022 | 10 | 90 | 1.34 | 6.28 | 6.22 | 6.28 | 0.37 | 3.7 | 4.97 | 23.22 | 6.13 |
| 10 | 0.024 | 10 | 100 | 1.38 | 7.33 | 6.39 | 6.87 | 0.3 | 3.02 | 4.17 | 20.76 | 6.29 |

STATISTICS
SW=49.300 SWX=55.413 X Bar=1.124 SWY=256.457 Y Bar=5.202
SWX*X=63.741 SWY*Y=1356.993 SWXY=293.575 b Value=4.246
Regression equation y=4.246x+0.43
If y=5.0 then x=1.076 this corresponds to dose of 0.011
Variance=0.0016 Chi-square=3.50 (with 8 deg.of freedom p)
Lower Limit 0.9971 log Dose 1.0764 Upper Limit 1.1557

reported that 96 h $LC_{50}$ of carbosulphan to guppies was 0.122 mg/L, methiocarb 1.256 mg/L and carbaryl 1.383 mg/L. Cagauan *et al*. (2004) showed that the lethal concentration of neem to Nile tilapia *Oreochromis niloticus* L. was 12.4 ml/L and mosquito fish *Gambusia affinis* Baird and Girard was 8.31 ml/L and the corresponding 96 h $LC_{50}$ values were 2.57 and 3.0 ml/L.

Both deltamethrin and azadirachtin, two established insecticides exhibit non-target toxicity to fish. Comparison of the different $LC_{50}$ values clearly indicates that the plant based pesticide is less toxic compared to the chemical one. To reduce the chemical load on the environment, it is suggested that use of plant based pesticides should be encouraged (Schmutterer, 1990). However, care should be taken to use even the plant based pesticide at moderate levels. Furthermore, plant based pesticides disintegrate easily into constituent elements without leaving any indelible impression in different regions of the environment (Khan and Ahmed, 2000).

The test fish in the present study is a hardy ornamental fish. Kharat *et al*. (2003) rated *P. reticulata* as a tolerant species along with *Oreochromis mossamibicus* and *G. affinis*. *P. reticulata* is affected by the non-target action of both the chemical and biological pesticides. The impact of the biological pesticide is much less compared to the chemical one, (deltamethrin 96 h $LC_{50}$ – 0.0019) (Table 2), and (azadirachtin 96 h $LC_{50}$ – 0.011 mg/L) (Table 3). It is advocated that more and more plant products should be developed with proper and targeted action and this eventually helps in keeping the environment free from hazardous chemicals.

## Acknowledgement

The authors wish to thank Dr. S. Prasanna Kumar, Dean of science and HOD of Zoology and Dr. E. James R Daniel, Principal, Scott Christian College (Autonomous), Nagercoil, for the facilities provided.

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