International Research Journal of Biological Sciences ISSN 2278-3202
Vol. **3(4),** 18-22, April **(2014)** *Int. Res. J. Biological Sci.*

Acute Toxicity of deltamethrin and permethrin and their Sublethal effects on Growth and Feeding in *Anabas testudineus*

Sapana Devi Maisnam and Gupta Abhik
Department of Ecology and Environmental Science, Assam University, Silchar-788011, Assam, INDIA

Available online at: www.isca.in, www.isca.me
Received 22[th] October 2013, revised 8[th] December 2013, accepted 15[th] January 2014

Abstract

The 24, 48, 72 and 96 h LC_{50} values of the synthetic pyrethroid insecticides deltamethrin and permethrin for the climbing perch Anabas testudineus were 0.11, 0.09, 0.08, and 0.07 mg l^{-1}, and 2.07, 1.41, 1.02, and 0.93 mg l^{-1}, respectively. Although deltamethrin was more toxic than permethrin, the 24-96 h LC_{50} pattern of the latter displayed a steeper slope that predicted increase in its toxicity on longer exposure. The fish was also exposed to sublethal concentrations of 1% and 10 % of the 96 h LC_{50} of the two pesticides for 11 weeks. The sublethal exposure of the two pesticides affected food consumption significantly thereby resulting in inhibition of growth.

Keywords: *Anabas testudineus*, synthetic pyrethroids, acute toxicity, oxygen consumption, gill lesions, enzyme activity.

Introduction

Various freshwater ecosystems not only support biodiversity, but they also contain valuable resources essential for economic development and societal wellbeing. Hence, their contamination poses serious threats to health, livelihood and socio-economic development[1], besides causing problems such as increasing mortality of fish and other biota[2]. Water being a universal solvent plays a key role in the dispersal and transport of chemical pollutants[3]. Pesticides belonging to different major classes such as organochlorines, organophosphates, carbamates and synthetic pyrethroids are known to result in widespread contamination of freshwater ecosystems and cause harm to aquatic biota[4]. Organochlorines such as endosulfan are highly resistant to microbial bioremediation and show high persistence in agricultural soils[5]. Among the different classes of pesticides, synthetic pyrethroids have been shown to have relatively low oral toxicity to mammals with relatively high insect to mammal toxicity ratios, although they have been found to be highly toxic to non-target aquatic organisms such as fish and invertebrates[6-9]. It is, therefore, necessary to assess the toxicity of commonly used synthetic pyrethroids using various end-points, besides estimating their acute toxicity on various aquatic organisms.

Environmental and chemical stress can interfere with physiological and biochemical functions such as growth, development, reproduction and circulatory systemin fish[10]. Though synthetic pyrethroids are preferred to organochlorines, organophosphates and carbamates due to their low toxicity to birds and mammals, high potency and effectiveness, easy biodegradability, and low persistence in the environment, they are reported to be highly toxic to fish causing mortality and impairing many biochemical functions[11]. The growth rate is an index associated with stress and is generally used as a sensitive and reliable end-point in chronic toxicity investigations[12-15]. Further, permethrin, one of the compounds tested, is a type I synthetic pyrethroid without a cyanide moiety in contrast to the type II deltamethrin, which is cyano-substituted. Possible differences between them in terms of the nature and magnitude of both acute and sublethal toxicity were also investigated. The selected test fish species *Anabas testudineus* is an important food fish in India, and a *local delicacy* in its eastern and northeastern parts. It is categorized as "Data Deficient" by the IUCN[16].

Material and Methods

Procurement and Maintenance of Fish: *Anabas testudineus* weighing 12±2 g and 8-10 cm long were collected from ponds and wetlands in Cachar district, Assam, India, brought to the laboratory, and transferred to glass aquaria after dipping in 0.1% potassium permanganate solution to prevent infection and cure existing ones, if any. They were fed commercial fish food 'Tokyu' (composition: white fish meal, wheat flour, shrimp meal, dried yeast, soybean meal, wheat germ meal, dehydrated alfalfa meal, vitamin A, C, D, E, K, B_1, B_2, B_6, B_{12}, nicotinic acid, biotin, Ca- pantothenate acid, folic acid, minerals and carotenoids, NS germ and chlorophyll as special ingredients), and acclimatized for 15 days inunchlorinated tap water that was conditioned by storing for 24 h and aerated prior to the introduction of test fish. Water was regularly aerated and renewed every 48 h during acclimatization.

Acute Toxicity Tests: Commercial grade deltamethrin ((S)-α-cyano-3- phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate) 2.8% EC (trade name Decis: Agrevo India Ltd.), and permethrin (3-phenoxybenzyl (1RS) cis, trans-3- (2,2-dichlorovinyl)-2, 2-dimethylcyclopro-panecarboxylate) 25% EC (Trade name Agniban; Devidayal Sales limited-an ISO 9001: 2000 Company, Mumbai, India) were purchased from an agrochemical dealer at Silchar, India. For determining 24-96 h LC_{50} of the two pesticides, fish were exposed for 96 h to 0.03, 0.05, 0.09, 0.16 and 0.28 mg l^{-1} deltamethrin and 0.25, 0.45, 0.8, 1.4, 2.5, 4.5, and 8 mg l^{-1} permethrin (both active ingredients) prepared in conditioned and aerated unchlorinated tap water. A control without any added pesticide was also maintained. The concentrations were selected on the basis of mortality in exploratory range finding tests in order to minimize the number of fish sacrificed. Fish were starved for 24 h before the commencement of exposure. The test media was renewed every 48 h, mortality recorded every 24 h, and dead fish immediately removed. Eighteen fishes were used in each test concentration and control. LC_{50} values were calculated using log-probitanalysis[17] with SPSS 20 software for Windows.

Water Quality: Temperature, pH and electrical conductivity of the test medium were measured with a mercury bulb thermometer, a digital pH meter, and a conductivity-TDS meter, respectively. Dissolved oxygen was estimated iodometrically by Winkler's method.

Sublethal Toxicity: For evaluating the sublethal effects of deltamethrin and permethrin in terms of growth and feeding, test fish were individually exposed to 0.007 and 0.0007 mg l^{-1} for deltamethrin, and 0.093 and 0.0093 mg l^{-1} for permethrin, respectively, which represented 10 % and 1 % of the 96 h LC_{50} of the two pesticides. Fish of similar size were acclimatized for 15 days prior to the commencement of the experiment, weighed with a top-loading balance (Shimadzu, BL-2200H),and their lengths were measured with a measuring tape. Each fish was randomly assigned to one of the pesticide treatments or to control, and individually maintained in 10 l PVC containers throughout the test period. Five replicates were used in each treatment and control. Growth was recorded weekly in control and treated fish for 11 weeks by weighing each fish after taking it out from the test solution and gently blotting excess water with a soft tissue. Being a hardy, air-breathing fish, the stress on *A. testudineus* was within reasonable limits. The fishes were fed 'Tokyu' fish food (0.14 g/fish/day), and the uneaten pellets were collected every day, dried and weighed. The difference between the initial dry weight of the pellets and that of the unconsumed pellets represented the daily food uptake of the fish. Test solutions were renewed weekly.

Data Analysis: Growth and food consumption data were first tested for normality (Kolmogorov-Smirnov test) before applying One-Way Analysis of Variance (ANOVA) to determine significant differences, if any, among control and experimental groups. Multiple comparisons were made with LSD post hoc. All statistical analysis was done with SPSS 20 software for Windows.

Results and Discussion

Acute Toxicity: The physico-chemical properties of the test water were: temperature: 22.1-22.3 °C; pH 6.54-6.73; conductivity: 80.9-82.6 µS cm^{-1}; and dissolved oxygen: 6.72-7.09 mg l^{-1}, respectively. There was no control mortality. LC_{50} values of deltamethrin at 24, 48, 72 and 96 h were 0.11, 0.09, 0.08 and 0.07mg l^{-1} (95% confidence limits: 0.09-0.12; 0.08-0.11; 0.07-0.09; and 0.06-0.08 mg l^{-1}, respectively); and those of permethrin 2.07, 1.41, 1.02, and 0.93 mg l^{-1}(95 % confidence limits:1.4-3.1; 1.1-1.8; 0.82-1.2; and 0.75-1.1 mg l^{-1}, respectively) (figure 1), indicating that the acute toxicity of deltamethrin was more than one order of magnitude higher than that of permethrin. The cyano-substituted synthetic pyrethroids such as deltamethrin are known to have higher toxicity when compared to the type I permethrin that lacks a cyanide moiety[18-19]. Comparison of the acute toxicity of deltamethrin and permethrin to *A. testudineus* with available data for other species reveal that the 48 h LC_{50} of deltamethrin was 5.13 µg l^{-1} for guppy (*Poeciliareticulata*) and 1.215 µg l^{-1} for fingerlings of European catfish *Silurusglanis*[20-21]. The 96 h LC_{50} of deltamethrin for *Salmogairdneri, Cyprinuscarpio* and *Sarotherodonmossambica* were 0.39, 1.84 and 3.80 µg l^{-1}, respectively[22] and 14.6 µg l^{-1} for monosex Nile tilapia (*Oreochromisniloticus*)[23]. Therefore, deltamethrin was more toxic to all these species than to *A. testudineus*. Permethrin was also less toxic to *A. testudineus* than to *Poeciliareticulata* (48 h LC_{50} 245.7 µg l^{-1}), and to brook trout and rainbow trout (96 h LC_{50} 3.2 µg l^{-1} and 0.69 µg l^{-1}, respectively)[7,24-25]. Thus *A. testudineus* appears to be fairly tolerant to both these synthetic pyrethroids, although inter-species comparisons should be considered with caution, as body size, weight or life stage could greatly influence toxicity[25]. When the 24-96 h LC_{50} trends of the two pesticides were compared (figure 1), that of permethrin had a steeper slope than that of deltamethrin, revealing that although permethrin had to be applied at higher concentrations to affect 50 % mortality at 24 h, this was followed by a sharp decline in concentration, and consequently, an increase in toxicity as time elapsed. Some of the other effects observed during acute pesticide exposure included increased mucus secretion in exposed fish. Defecation increased and more fecal matter was found at the bottom of the exposed test containers than those in the control. This was probably due to the stimulation of muscarinic receptors in the smooth muscles of the end organs such as gastrointestinal tract and secretory glands[26]. Profuse mucus secretion accompanied by depigmentation could be attributed to dysfunction of the endocrine system, especially the pituitary gland under toxic stress, causing changes in the number and area of mucus glands and chromatophores[27]. Increased mucus secretion probably constituted an adaptive response to counter the irritating effect of pesticides on body surface and mucus membrane as well.

Sublethal effects on growth and food consumption: In the 11 weeksublethal exposure of *A. testudineus* to both the pesticides, there was significant reduction in wet weight in fish exposed to both deltamethrin and permethrin. Length was significantly reduced in permethrin treatment only, while deltamethrin exposure caused statistically insignificant increase in length. Food consumption was significantly reduced in permethrin treatment, but increased indeltamethrin treatment (table 1). Thus all the three endpoints were significantly affected by permethrin, suggesting that despite its considerably lower short-term acute toxicity, permethrin was found to have persistent toxicity on long-term, sublethal exposure. Acute toxicity tests for the two pesticides revealed a sharp reduction in LC_{50} values (and hence increased toxicity) of permethrin from 24 to 96 h (figure 1). It appears that this trend continues through long-term exposure to render permethrin more toxic with time. Permethrin was found to have the highest persistence in estuarine environment among three synthetic pyrethroid pesticides[28]. Exposure to synthetic pyrethroid pesticides is known to decrease growth and impair swimming performance[29]. Esfenvalerate caused reduction in "young-of-the-year" growth in bluegill sunfish[30]. In the present study, exposed fish exhibited erratic swimming behavior that is likely to have impaired their ability to feed, thereby affecting growth. However, Barry et al.[31] found mortality to be a more sensitive indicator of esfenvalerate toxicity than growth. Growth decreased when copper-exposed common carp spent more energy sustaining their normal metabolism, leaving less energy available for growth. Further, the cessation of feeding, accompanied by the catabolic effects of the catecholamines and corticosteroids on the energy reserves stored in the body tissues, resulted in reduced growth in stressed fish.[12]. However, the effects of deltamethrin resulted in significant increase in food consumption along with insignificant increase in length, but significant reduction in wet weight (table 1). Because of this imbalance, the deltamethrin-treated fishes had slender body and werein highly emaciated condition. Thus the two pesticides exhibited some differences in their mode of action and effects.

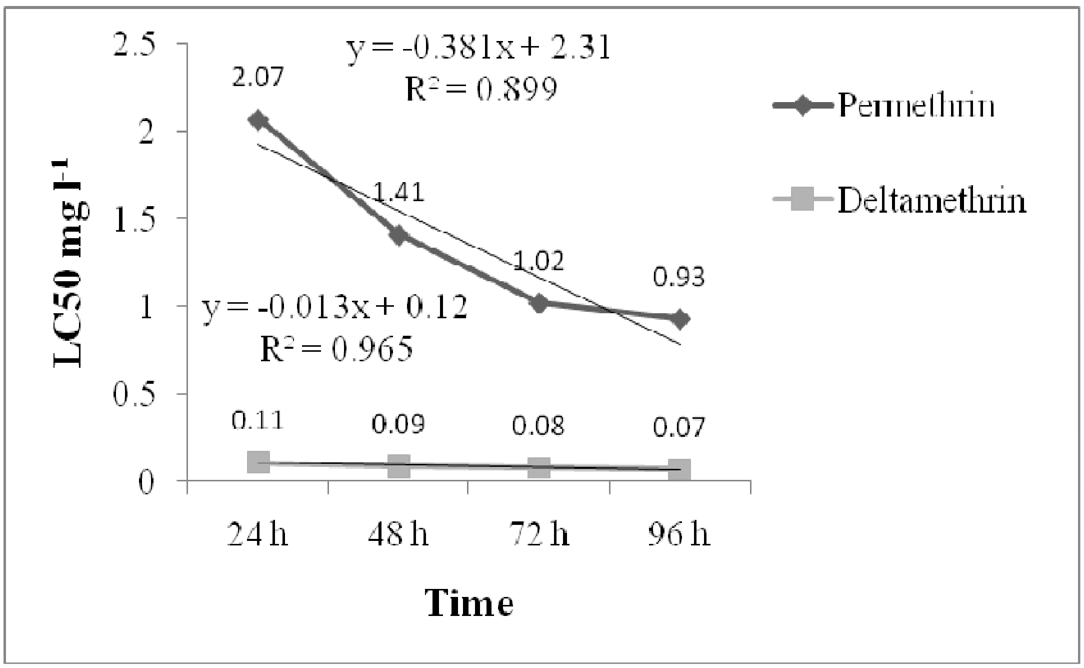

Figure-1
24-96 h LC_{50} values of deltamethrin and permethrin for *Anabas testudineus*

Table-1
Alterations in body weight, length and food consumption rate (mean ± SD) in *Anabas testudineus* exposed to sublethal concentrations of deltamethrin and permethrin for 11 weeks

Concentrations (mg l^{-1})	Change in weight (gm)	Change in length (cm)	Food consumption mg/g/h
Control	0.252±0.133	0.251±0.052	0.50±0.06
Deltamethrin (DM) - 10 % of 96 h LC_{50} (0.007)	-0.199±0.114*	0.291±0.018	0.74±0.04*
DM – 1 % 96 h LC_{50} (0.0007)	-0.234±0.118*	0.294±0.034	0.85±0.14*
Permethrin (PM) - 10 % 96 h LC_{50} (0.093)	-1.527±0.356*	0.133±0.034*	0.23±0.05*
PM – 1 % 96 h LC_{50} (0.0093)	-1.614±0.481*	0.133±0.039*	0.27±0.17*

*indicates significant difference from control at $p \leq 0.05$

Conclusion

The cyano-substituted deltamethrin had over an order of magnitude higher acute toxicity to *Anabas testudineus* than that of the non-cyanopermethrin. However, a narrowing down of this difference with longer duration of sublethal exposure could be predicted from the steeper slope of permethrin toxicity. This trend was reflected in the food consumption and growth of *A. testudineus* exposed to both the pesticides, when both length and weight increments were affected by permethrin, while deltamethrin suppressed weight increase only.

Acknowledgements

MSD is grateful to the University Grants Commission, India, for the award of a UGC-AUS research fellowship.

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