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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 13 (8): 405-408, 2010
ISSN 1028-8880

Investigation of Acute Toxicity of Pirimiphos-Methyl (Actellic®, 25%EC) on Guppy (*Poecilia reticulata*, Peters, 1859)

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Abstract: Pirimiphos-methyl is a broad spectrum organophosphate insecticide and potential toxic pollutant in aquatic ecosystems. The acute toxicity of Pirimiphos-methyl (Actellic®) was investigated using Guppy (*Poecilia reticulata*) in a 96 h static renewal laboratory bioassay. Based on probit analysis, the LC_{50} was 0.026, 0.024, 0.022 and 0.019 ml L^{-1} at 24, 48, 72 and 96 h, respectively. Analysis of Variance (ANOVA) showed that there was significant difference ($p<0.05$) in the quantal response (mortality) of *P. reticulata* to different concentrations of Actellic at 24, 48, 72 and 96 h of exposure. Actellic insecticide is toxic to fish; therefore its indiscriminate use in aquatic environment should be discouraged.

Key words: Toxicity, pirimiphos-methyl, *Poecilia reticulata*, Guppy, fish

INTRODUCTION

The control of insects with aquatic larval stage during the past had been dependent mainly on the use of chemicals like Paris green, larvicidal oils and organophosphorus insecticides such as temephos and fenthion (Mittal *et al*., 1999; Ansari *et al*., 2004). At present, pirimiphos-methyl (Actellic, 25EC) is used as larvicide. The use of these chemicals in water bodies in many developing nations could contribute to the decline of fishery in the aquatic environment (Ayoola, 2008). Actellic, an effective broad spectrum organophosphate insecticide is used, for the control of public health pests. The active ingredient, pirimiphos-methyl (0-2 diethylamino-6methylprimidin-4-yl 0, 0-dimethyl phosphorothioate) is applied as a mosquito larvicide directly to non-portable, stagnant, saline, brackish and polluted waters (CIPAC, 1985; Gallo and Lawryk, 1991). Because a repeated application of this pesticide is practice for the control of mosquito larva, large quantities find their ways into the water bodies. The indiscriminate use of pesticides, careless handling, accidental spillage, or discharge of untreated effluents into natural water-ways have harmful effects on the fish population, other forms of aquatic life and human health. The effects of pesticides on human health include dose-dependent DNA damage, cancer, immune system deficiencies, pulmonary and haematological morbidity (UNEP, 1993; Wang and Lin, 1995). The evaluation of the toxicity of this insecticide to fin fish is essential to understand the environmental impacts of the pesticide.

Poecilia reticulata, a small benthopelagic, non migratory fish, occupies a wide range of aquatic habitats, such as estuaries, lakes, ponds, weedy ditches and canals (Page and Burr, 1991; Skelton, 1993; Skikano and Fujio, 1997). Guppy is hardy and tolerates both well and poorly oxygenated waters. It is found in canals and ditches in Nigeria hence, its use as a biological indicator in ecotoxicological studies. According to Williams *et al*. (2003), the chronic carcinogenicity bioassays with *P. reticulata* and potentially with other small fish species are feasible and scientifically valid.

Guppy has been used as a test species for varied insecticides such as beta-cypermethrin, a synthetic pyrethroid pesticide (Polat *et al*., 2002), chlorpyrifos-methyl, a wide spectrum organophosphorus insecticide (Mahmut *et al*., 2005), alpha-cypermethrin, a synthetic pyrethroid insecticide (Mehmet *et al*., 2004), deltamethrin, a synthetic pyrethroid pesticide (Rukiye *et al*., 2003) and permethrin, a synthetic pyrethroid pesticide (Baser *et al*., 2003).

In addition, Otitoloju (2006) reported the joint action toxicity of spent lubrication oil and laundry detergent against *P. reticulata* and Saliu (2007) reported acute toxicity of premium motor spirit to the guppy. Thus, the objective of this study is to investigate the acute toxicity of actellic on guppy, *P. reticulata* with a view to discouraging indiscriminate use of the insecticide in water bodies.

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MATERIALS AND METHODS

Test animal: Four hundred specimen of *P. reticulata* with the mean weight of 0.20 g and mean total length of 2.50 cm were collected (January, 2009) with scoop net from drainage canal in Lagos Metropolis and carried to the laboratory in a plastic bucket half filled with water from source. They were kept in holding tanks (45×30×30 cm) one quarter filled with dechlorinated tap water and allowed to acclimatize to laboratory conditions (26.0±0.8°C, 78±2% RH, pH = 7.0) for two weeks before using them in bioassays. The fishes were fed with floating pellets at 10% of their body weight. Unconsumed feed and faeces were removed and water replenished twice a week to prevent accumulation of toxic waste metabolites.

Test chemical: Pirimiphos-methyl (Actellic®, 25EC) was purchased from Syngenta Nigeria, Oshodi, Lagos, Nigeria.

Acute toxicity test: A static renewal bioassay technique was adopted in which the test media was renewed at the same concentration once every 24 h (ASTM, 1991). Preliminary screening was carried out to determine the appropriate concentration range for testing chemicals as described by Solbe (1995). Depending on the test concentrations a given volume of water from the test organism environment was measured into bioassay glass tank (22×15×18 cm) and a predetermined volume of Actellic (25% EC) was added into the water to make up to 1000 mL to achieve the desired concentration.

Ten active animals were introduced into the test medium containing Actellic (25% EC). Each treatment was replicated thrice, given a total of 30 guppies per treatment, including untreated media (control). The concentrations of the test insecticide were 0.015, 0.020, 0.025, 0.030, 0.035 ml L^{-1} and control.

Assessment of quantal response (Mortality): Mortality assessment was carried out every 24 h over a 96 h experimental period. Fishes were assumed to be dead when there was no body or opercular movement, even when probed with a glass rod.

Physico-chemical parameters of the test media: Physico-chemical parameters such as Dissolved Oxygen (DO), pH and temperature of the test media were measured before and during the experimental period. The pH and temperature was measured using Hanna instrument (Model H1991301). The dissolved oxygen was determined using a Jenway DO meter (Model 9071).

Statistical analysis: The quantal response (mortality) data were analysed by probit analysis after Finney (1971). The indices of toxicity measurement derived from the analysis were:

- LC_{50} = The concentration that kills 50% of the population
- LC_{95} = The concentration that kills 95% of the population
- TF = Toxicity factor for relative potency measurements

Oneway analysis of variance, ANOVA and Student Newman-Keul's, SNK (Samuel *et al*., 2008) tests were used to test for significant difference (5% level) in the mean mortality response of *P. reticulata* to different concentrations of actellic at 24, 48, 72 and 96 h of exposure. Analysis was performed using SPSS 10.0 for windows.

RESULTS

The physico-chemical conditions of the test media during the toxicity tests were fairly constant. The pH ranged between 6.7 and 6.9, Dissolved Oxygen (DO) was between 6.5 and 7.1 mg L^{-1} while temperature ranged between 26.0 and 28.0°C over the 96 hours acute toxicity evaluations.

The results of the acute toxicity of actellic on *P. reticulata* at 24, 48, 72 and 96 h of exposures are shown in Table 1. The analysis of dose response data of actellic when tested against *P. reticulata* revealed that LC_{50} values ranged from 0.019 ml L^{-1} at 96 h LC_{50} to 0.026 ml L^{-1} at 24 h LC_{50} (Table 1). The toxicity of actellic was found to be relatively similar over the experimental period with Toxicity Factor (TF) ranging from 1.0 to

Table 1: Relative acute toxicity of Actellic® against *P. reticulata*

Time (h)	LC_{50} *CL (ml L^{-1})	Slope±SE	DF	Probit line equation	TF
24	0.026 (0.023-0.030)	4.07±0.88	3.0	Y = 11.45+4.07x	1.4
48	0.024 (0.021-0.027)	4.17±0.87	3.0	Y = 11.74+4.17x	1.3
72	0.022 (0.019-0.025)	4.79±0.85	3.0	Y = 13.01+4.79x	1.2
96	0.019 (0.016-0.021)	5.21±0.93	3.0	Y = 13.97+5.21x	1.0

*CL = 95% Confidence limit, SE: Standard error, DF: Degrees of freedom, TF: Toxicity factor

Table 2: Percentage (%) mortality of *P. reticulata* exposed to different concentrations of Actellic for 96 h

		Percentage mortality time			
Concentration (ml L^{-1})	No. of tested organism	24 h	48 h	72 h	96 h
Control	30	0.0^{A}	0.0^{A}	0.0^{A}	0.0^{A}
0.015	30	16.7^{B}	20.0^{B}	26.7^{B}	30.0^{B}
0.020	30	30.0^{B}	33.3^{B}	43.3^{C}	56.7^{C}
0.025	30	50.0^{C}	56.7^{C}	60.0^{D}	73.3^{D}
0.030	30	60.0^{CD}	66.7^{C}	70.0^{DE}	83.3^{DE}
0.035	30	70.0^{D}	73.3^{C}	80.0^{E}	93.3^{E}

Means with the same superscript letter(s) in a column are not significantly different in the SNK test at p = 0.05

1.4 between 24 h LC_{50} to 96 h LC_{50} data (Table 1). Analysis of Variance (ANOVA) showed that there was significant difference ($p<0.05$) in the quantal response (mortality) of *P. reticulata* to different treatments (concentrations) of actellic at 24, 48, 72 and 96 h of exposure. Further analysis using Student Newman Keul's (SNK) test (p = 0.05) revealed that the percentage mortality response of *P. reticulata* to 0.035 ml L^{-1} of actellic at 24, 48, 72 and 96 h of exposure was significantly difference ($p<0.05$) from its response to all other concentrations (Table 2). There was no significant difference ($p>0.05$) in the percentage mortality of *P. reticulata* when exposed to 0.015 and 0.020 ml L^{-1} of actellic at 24 and 48 h except at 72 and 96 h while there was significant difference ($p<0.05$) in the percentage mortality response of *P. reticulata* to 0.025 and 0.030 ml L^{-1} of actellic at 24, 48, 72 and 96 h exposure (Table 2).

No adverse behavioral changes or any mortality was recorded in the control fish throughout the period of bioassay. Symptoms of toxicosis observed in the behavior of fish exposed to pirimiphos-methyl before the eventual death include lack of balance, erratic swimming and restlessness.

DISCUSSION

This study showed that the 96h LC_{50} value of Actellic insecticide was 0.019 ml L^{-1} while its toxicity is shown to increase with increasing concentration. The observation is in consonance with Ayoola (2008). According to Aguigwo (2002), the level of toxicity of any pesticide depends on its bioaccumulation, the different chemical composition of the compound forming the pesticide and the reactions of the organisms receiving the toxicant.

The highest concentration of the toxicant resulted in the highest mortality rate which is in agreement with the study of FAO (1977) and Ayoola (2008), who reported that in all toxicants, threshold is reached above which there is no drastic survival of animal and below the threshold, animal is in a tolerance zone while below the tolerance zone is the zone of resistance.

The exposure of *P. reticulata* to Actellic resulted in the exhibition of aggressive behavior, rapid gulping of water, increased opercular movement and abnormal swimming movements. Mehmet *et al.* (2004) and Mahmut *et al.* (2005) reported similar behavioral responses in *P. reticulata* when exposed to alpha-cypermethrin, a synthetic pyrethroid insecticide and chlorpyrifos-methyl, a wide spectrum organophosphorus insecticide, respectively. *Poecilia reticulata* was stressed progressively with time before death. The stressful behavior of respiratory impairment due to the toxic effect of actellic on the gill was similar with the report of Aguigwo (2002) and Rahman *et al.* (2002) that pesticide impair respiratory organ.

In this study, the acute toxicity level based on the 96 h LC_{50} value of actellic was found to be 0.019 ml L^{-1} when tested against the guppy, *P. reticulata*. Analysis of Variance (ANOVA) showed that there was significant difference ($p<0.05$) in the quantal response at 24, 48, 72 and 96 h of exposure. Further analysis using Student Newman Keul's (SNK) test (p = 0.05) revealed that the percentage mortality response of *P. reticulata* to 0.035 ml L^{-1} of actellic at 24, 48, 72 and 96 h of exposure was significantly different ($p<0.05$) from its response to all other concentrations.

The findings in this study has revealed that pirimiphos-methyl is toxic to fish and considering the public health implication of the pesticides in aquatic environments, the indiscriminate use of this insecticide on/near fish farm or in water bodies should be discouraged.

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