Original Article

Toxic Response of Mosquito *Culex Quinquefasciatus* (Diptera: Culicidae) to some Agricultural Pesticides (Butachlor and Pertilachlor)

*Aliakbar Hedayati[*1], Fatemeh Darabitabar [2], Reza Tarkhani [3]*

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ABSTRACT

Background: The expansion of herbicide used in aquatic ecosystems as well as in terrestrial if is not properly controlled may produce harmful effects on freshwater fisheries. Residue limits of these agricultural chemicals in tropical fishery waters should be established. The aim of this study was to determine the acute toxicity of butachlor and pertilachlor as potential dangerous herbicides to assess mortality effects of these chemicals to the Mosquito *Culex quinquefasciatus*.

Methods: This study was carried out in Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran at summer 2013. *Culex* samples were exposed to different concentrations of butachlor and pertilachlor (0-200ppm for butachlor and pertilachlor) for 96 h.

Results: The low toxicity of LC50s obtained for butachlor (23.81±0.04) and pertilachlor (27.97±0.05) indicate that butachlor and pertilachlor were lowly toxic to Mosquito *Cu. quinquefasciatus*.

Conclusion: Although pretilachlor and butachlor are low toxic but pretilachlor is less toxic in field conditions, these data are useful to potential ecosystem risk assessment.

Keywords: Aquatics, Mortality, Pollution, Toxicity.

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INTRODUCTION

Increasing pollution is concerned in a large scale for biology and life cycles of aquatic organisms and marine fish [1]. The use of pesticide herbicides or insecticides in modern agriculture technology is indispensable to control weeds or pests for the manufacture of more food and management of public health. Today's about 4500 pesticides are in total in the word, out of which 25 have wide toxicity efficiency to an extensive limit of fauna and flora of economic significance [2]. Most of these are not readily degradable but persists for a significant time have more effect on, aquatic biota, which are very important due to nutritive food value [3]. Atrazine herbicides are extensively consumed for control of aquatic plants and organisms in disparate section of the universe. Chemical pesticides and herbicides with persistent molecules (long half-life periods) pose a menace to aquatic life various forms and to the human population consuming the affected fish. Presence of pesticide and herbicide in level waters was reported in North America and Europe since 50 yr ago, and since then many documents have determined the toxic effects of these contaminant to aquatic ecosystems [4-7].

Culex pipiens is a common diet of freshwater fishes. Mosquitoes are flying, biting insects that develop in water during their immature stages. The maturation from egg stage to adult is in seven days, however, adults usually live in 10–60 d (about two months)**.** Butachlor is a member of the chloroacetanilide group of chemistry and is the herbicidal active ingredient MACHETER→ EC. It is used as a selective pre-emergent herbicide, and is chemically (2-chloro-2', 6'-diethyl-N-(butoxymethyl) acetanilide).

Pretilachlor chemical name is 2-chloro-2.6-diethyl-n-(2propxyethyl) acetanilide. The acute toxicity data provide useful information to realize the mode of action of a substance and help to comparison of dose-response among different chemicals. The 96-h LC_{50} tests are conducted to assess the survival potential of organisms to particular toxic chemicals. Chemicals with lower median lethal dose values are more toxic because

1. Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
2. Department of Fisheries, Khorramshahr Marine Science and Technology University, Khorramshahr, Iran.
3. Department of Fisheries, Urmia University, Urmia, Iran.
*Corresponding Author: E-mail: hedayati@gau.ac.ir

of lower concentrations of mortality in 50% of organisms, so in this study the mortality effects of some common agricultural pesticides were measured at common mosquito in freshwater ecosystems.

MATERIALS AND METHODS

This study was carried out in Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran at summer 2013. The selected *Culex* species for present study was mosquito *Cu. quinquefasciatus*. Lethal experiments were conducted using 100 *Culex*. Test chambers were petri dish. All *Culexes* were acclimated for 2 d in these aquaria before assays. Water temperature was regulated at 27 °C. Dead *Culexes* were immediately removed to avoid possible water pollution [8]. Nominal concentrations of active ingredient tested were 0, 1, 4, 10, 20, 40, 100 and 200 ppm of commercial dose (50%) for butachlor and 0, 1, 4, 10, 20, 40, 100 and 200 ppm of commercial dose (60%) for pertilachlor were used. Fourteen groups (7 for butachlor and 7 for pertilachlor) of 100 fish were exposed for 96 h in petri dish.

During acute toxicity experiment, the water in each petri dish and the temperature was 27 °C. There was not any food to the specimens during the assay. The time of mortality rates were recorded at 24, 48, 72 and 96 h. Acute toxicity experiments carried out according to Vlahos and Hotos [9]. The nominal concentration of butachlor and pretilachlor estimated to result in 50% mortality of *Culex* within 24 h (24-h LC_{50}), 48 h, 72 h, and 96 h was attained by probit analysis [10], and using the maximum-likelihood procedure (SPSS 2002, SPSS Inc., Chicago, Illinois, USA). The LC50 value was procured by fitting a regression equation arithmetically and by graphical interpolation by taking logarithms of the butachlor and pretilachlor concentrations versus probit value of percentage mortality.

The 95% confidence limits for LC50 are commute by using the formula:

$$LC50\ (95\%\ CL) = LC_{50} \pm 1.96\ [SE\ (LC_{50})]$$

The SE of LC_{50} is calculated from the formula:

$$SE(LC50) = 1/b\sqrt{pnw}$$

Line: p=the number of chemical used

w = the average weight of the observations

Where: b=the slope of the chemical/probit response (regression)

n = the number of animals in each group.

After the acute toxicity experiment, the NOEC= No Observed Effect Concentration and LOEC= Lowest Observed Effect Concentration, were determined for each measured endpoint.

RESULTS

There was not any mortality during the acclimation period before exposure. Besides, there was not any control died *Culex* during acute toxicity tests. Mortality of mosquito for both butachlor and pertilachlor doses 0, 1, 4, 10, 20, 100 and 200 ppm were examined during the exposure times at 24, 48, 72 and 96 h (Table 1, 2). Mortality of mosquito was significantly increased (P<0.05) with increasing concentrations from 1 ppm to higher concentrations for butachlor and 4 ppm to higher concentration for pertilachlor. However, for both of butachlor and pertilachlor there were 100% mortality and higher concentrations within the 96 h after exposure, and no observed 100% mortality within the 24 h (Table 1, 2).

Table 1. Mortality rate of mosquito (n=100 each concentration) exposed to acute butachlor.

Concentration (ppm)	No. of mortality 24h	48h	72h	96h
0	0	0	0	0
1	0	3	7	13
4	2	9	14	23
10	5	11	28	39
20	15	21	42	57
40	29	41	53	62
100	53	62	84	100
200	74	93	100	100

Table 2. The mortality rate of Mosquito (n=100, each concentration) exposed to acute pertilachlor.

Concentration (ppm)	No. of mortality 24h	48h	72h	96h
0	0	0	0	0
1	0	0	0	0
4	0	0	3	9
10	0	3	10	17
20	3	13	27	41
40	17	31	53	71
100	41	62	91	100
200	71	93	100	100

Median lethal concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 99% experiment are presented in Table 3, 4. Because mortality (or survival) data are collected for each exposure concentration in a toxicity test at various exposure durations 24-96h, information can be sketched in other ways, the direct line of best suitable was then drawn through the points. These were time fatality lines. The median lethal survival time (LT_{50}) can be evaluated for each concentration.

Toxicity Testing Statistical Endpoints are in two parts: 1- Point Estimates: what toxicant concentration will cause a specific effect on the test population? The median Lethal Concentration or LC50. Our result for toxicity experiment is shown in Fig 1 and 2.

Table 3. Lethal Concentrations (LC_{1-99}) of butachlor (mean ± Standard Error) depending on time (24-96h) for Mosquito *Culex quinquefasciatus.*

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_1	-	-	-	-
LC_{10}	14.14 ± 0.01	-	-	-
LC_{20}	51.06 ± 0.01	26.03 ± 0.01	4.68± 0.02	-
LC_{30}	77.68 ± 0.01	47.38 ± 0.01	19.50 ± 0.02	8.63 ± 0.04
LC_{40}	100.43±0.01	65.62 ± 0.01	32.17 ± 0.02	16.48 ± 0.04
LC_{50}	**121.69±0.01**	**82.68 ± 0.01**	**53.79 ± 0.02**	**23.81 ± 0.04**
LC_{60}	142.95±0.01	99.73 ± 0.01	44.02 ± 0.02	31.15 ± 0.04
LC_{70}	165.70±0.01	117.97±0.01	68.53 ± 0.02	38.99 ± 0.04
LC_{80}	192.32±0.01	139.32±0.01	83.36 ± 0.02	48.18 ± 0.04
LC_{90}	229.24±0.01	168.93±0.01	103.92±0.02	60.91 ± 0.04
LC_{99}	316.92±0.01	239.53±0.01	152.76±0.02	91.16±0.04

Table 4. Lethal Concentrations (LC_{1-99}) of pretilachlor (mean ± Standard Error) depending on time (24-96h) for *Mosquito Culex quinquefasciatus.*

Point	Concentration (ppm) (95 % of confidence limits)			
	24h	48h	72h	96h
LC_1	-	-	-	-
LC_{10}	47.97 ± 0.01	20.88 ± 0.01	8.28±0.02	5.98±0.05
LC_{20}	80.24 ± 0.01	45.47± 0.01	21.73± 0.02	13.53 ± 0.05
LC_{30}	103.50±0.01	63.20 ± 0.01	31.43 ± 0.02	18.97± 0.05
LC_{40}	123.39±0.01	78.35 ± 0.01	39.72 ± 0.02	23.62± 0.05
LC_{50}	**130.01±0.01**	**92.50 ± 0.01**	**47.47 ± 0.02**	**27.97± 0.05**
LC_{60}	160.55±0.01	106.66±0.01	55.22 ± 0.02	32.32± 0.05
LC_{70}	180.43±0.01	121.81±0.01	63.50 ± 0.02	36.97± 0.05
LC_{80}	203.69±0.01	139.54±0.01	73.20 ± 0.02	42.41 ± 0.05
LC_{90}	31.84 ± 0.01	164.13±0.01	86.66 ± 0.02	49.96±0.05
LC_{99}	235.96±0.01	222.52±0.01	118.61±0.02	67.89±0.05

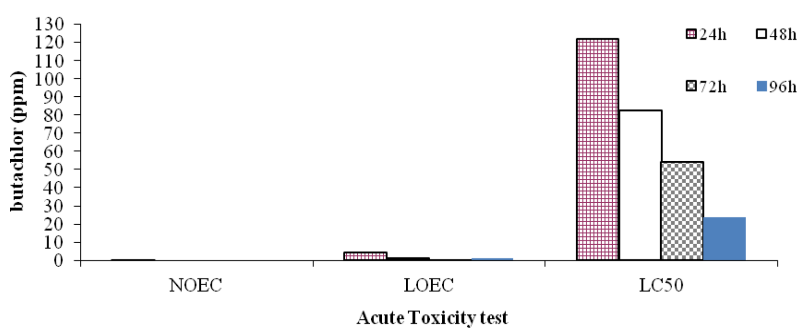

Figure 1. Acute toxicity experiment statistical endpoints in Mosquito exposed to crude butachlor in different times (24-96h).

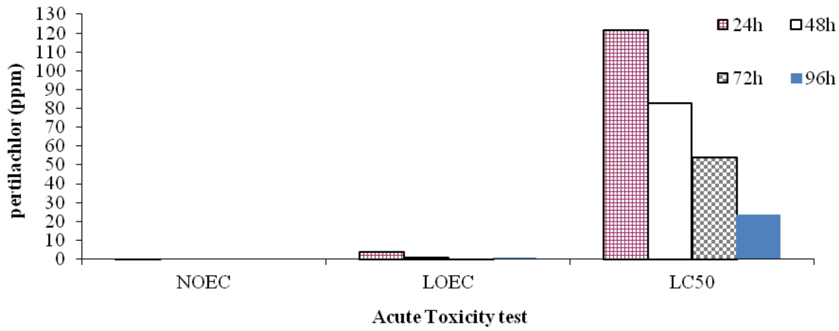

Figure 2. Acute toxicity experiment statistical endpoints in Mosquito exposed to pretilachlor in different times (24-96h).

DISCUSSION

Both chemicals butachlor and pertilachlor varied in their acute toxicity to Mosquito. The toxicity of butachlor and pertilachlor on Mosquito *Culex quinquefasciatus* increased with increasing concentration and exposure time.

Incidence of pesticides in high concentrations in agricultural wastewater and their acute toxicity to water organisms especially fish species have been reported by many researchist [5, 7, 11]. Contamination of aquatic ecosystem with pesticides via rainfall runoff is very possible [12]. The level of German cockroach sensitivity was determined, 4 organophosphate insecticides, including malathion group, Chlorpyrifos, primfos methyl, diazinon and 2 carbamate insecticides from the group consisting of pyrethroid insecticide, propoxur and bendiocarb and 4 of including Syprmtryn, deltamethrin, lambda cyhalothrin and cyfluthrin, on 5 strains of German cockroach collected from field applied. The high levels of resistance to diazinon and moderate to high levels of resistance to primfos methyl was observed. In addition, the sensitivity of strains collected from field to Chlorpyrifos pesticides, propoxur and cyfluthrin were found and only a low level of resistance to malathion and chlorpyrifos strain, showed [13]. Differences between male and female sensitivity to toxins may be due to differences in size and weight, body fat and differences in detoxification of toxins in them [14]. The amount of toxic pesticides and formulations may be by various physiological resistance, insect age, reproductive status and temperature affected [15].

In addition, we found that both butachlor and pretilachlor are low toxicity substrates to Mosquito. The 96h LC_{50} was calculated to be 23.81 ± 0.04 ppm for butachlor and 27.97± 0.05 ppm for pretilachlor.

The LC50 values of Aqueous and organic solvent extracts of plants/plant parts on Mosquito species reported from tenths to several tens of mg l^{-1} [16]. The fifty percent of Lethal Concentration (LC50) amount and stress behavior in the Mosquito larvae and aquatic organism under exposure to three widely used xenobiotic combination like butachlor and pentachlorophenol [17]. Mosquito larvae were generally more resistant than the aquatic vertebrates. The toxic effects of butachlor were reported not only in fish but also in other aquatic organisms include daphnia and they concluded that it is toxic for biota [18]. The remainder of butachlor in a paddy field was studied, even though lower than the safe concentration, caused a poisonous effect to common carp, *Cyprinus carpio* [19].

CONCLUSION

$LC50_{96h}$ of Mosquito has calculated 23.81 ± 0.04 ppm for butachlor and 27.97± 0.05 ppm for pretilachlor. Maximum allowable concentration for pretilachlor butachlor was 2.79 and 2.38 mg/l, respectively. Although pretilachlor and butachlor are low toxic at this species, but pretilachlor is less toxic in field conditions, these data are useful to potential ecosystem risk assessment.

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