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Effect of Lambda Cyhalothrin (Pyrethroid) and Monocrotophos (Organophosphate) on Cholinesterase Activity in Liver, Kidney and Brain of *Rana cyanophlyctis*

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Key Words:

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The present studies investigate the induced effects of two pesticides lambda cyhalothrin (pyrethroid) and monocrotophos (organophosphate) on *Rana cyanophlyctis* (common skittering frog). Two different concentrations i.e. 0.1 and 1% were used and cholinesterase activity was observed in liver, kidney and brain of these frogs. It was decreased upto 34.6 and 46.3% in liver, 25.08 and 57.1% in kidney and 31.64 and 50.7% in brain under the effect of lambda cyhalothrin. In the case of monocrotophos treatment, cholinesterase decreased upto 37.7 and 57.7% in liver, 57.5 and 67.5% in kidney and 47.6 and 65.9% in brain, respectively.

Amphibians are important for ecological studies, giving information on the ecological impact of global change. Global decline in amphibian populations has caused great concern in the scientific community. As environmen-tal contaminants including using of pesticides residues have been implicated as possible cause of some declines. Many species of amphibians provide a direct benefit to Humans. They provide food for fish, birds and mammals. Frog legs are widely considered to be a delicacy. Many countries in Europe and USA import large quantities of frog legs, mostly from Asia (Martin, 2000). In Pakistan pesticides are reduced enzyme activity of cholinesterase in Frog *Rana tigrina* (Khan et al., 2002b; Khan et al., 2002c.). A number of non-target species can be affected when pesticides are used because of their reduction of cholinesterase activity. This enzyme occurs in a number of species, and reduction can result in sub-lethal toxicity and death (Cooper 1991). Researches on amphibian and reptiles and other species of wildlife have been reported. Bradbury and Coats (1982), Hill and Fleming (1982), Balasundaram and Selvarajan (1990), Berrill et al. (1993), Clark et al. (1990, 1995), Cooper (1991), Mineau (1993), Carey and Bryant (1995), Burn and Leighton (1996), Garcelon and Thomas (1997), Kegley et al. (1999), O'Hara et al. (1999), Bishop et al. (2000), Khan (2000), Pauli et al. (2000), Khan and Fatima (2002), Khan et al (2002a), Bridges and Boone (2003), Gilbertson et al. (2003) and Khan et al. (2003a, b) reported effect of pesticides on different non-target species of wildlife. In the present study the effect of lambda cyhalothrin and monocrotophos on liver, kidney and brain cholinesterase activity was studied.

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Material And Methods

The experimental work was carried out on the adults of frog *Rana cyanophlyctis*, collected from Karachi region and Thatta city (Pakistan) kept in glass aquarium in the Wildlife Lab, Department of Zoology, University of Karachi, Karachi. Two concentrations were applied, i.e. 0.1% and 1% of lambda cyhalothrin and monocrotophos were applied for cholinesterase estimation. For this purpose 1 µl pesticide was injected in the sub coutanously in the abdominal region of the frog using insulin syringe. The cholinesterase activity was estimated after 24 hours of treatment. Each batch of treated animals consisted of four Frogs. A batch of untreated frogs was also kept for comparison. For this purpose liver, kidney and brain were taken by Shakoori and Ahmad's (1973) methods.

Cholinesterase activity was estimated by Randox Kit No. CE-190. The method is based upon hydrolysis of acetyl-choline by the action of cholinesterase, (Knedel and Boetteger, 1967). The reaction between thiocholine and dithiobis (nitrobenzoate) gives 2-nitro-5-mercaptobenzoate, a yellow compound which can be measured at 405 nm.

Results

After the treatment of lambda cyhalothrin and monocrotophos effects were observed on cholinesterase activity. It was decreased upto 34.6 and 46.3% in liver (Table 1), 25.08 and 57.1% in kidney (Table 2) and 31.64 and 50.7% in brain (Table 3) under the effect of Lambda cyhalothrin. In the case of Monocrotophos treatment, cholinesterase activity decreased upto 37.7 and 57.7%

Table 1. Activity of cholinesterase in liver of *Rana cyanophyctis* treated with lambda cyhalothrin

	Time Sec.	Mean (U/l)	S.E (+ –)	Range at 95% confidence	Inhibition %
Control	00	00	00	00	00
	30	1.227	0.0560	1.117-1.336	
	60	1.084	0.1510	0.788-1.379	
	90	1.088	0.0196	1.049-1.126	
0.1%	30	1.0802	0.016	1.04-1.112	34.6
	60	1.0842	0.016	1.05-1.115	
	90	1.0880	0.017	1.05-1.121	
1%	30	0.6586	0.1734	0.318-0.997	46.3
	60	0.6564	0.1592	0.33-0.968	
	90	0.6558	0.1587	0.34-0.966	

Table 2. Activity of cholinesterase in kidney of *Rana cyanophyctis* treated with lambda cyhalothrin

	Time Sec.	Mean (U/l)	S.E (+ –)	Range at 95% confidence	Inhibition %
Control	00	00	00	00	00
	30	1.148	0.0054	1.137-1.158	
	60	1.152	0.0057	1.140-1.163	
	90	1.154	0.0100	1.134-1.173	
0.1%	30	0.8608	0.0224	0.816-1.299	25.08
	60	0.8280	0.0181	0.792-0.863	
	90	0.8812	0.0199	0.842-0.920	
1%	30	0.4922	0.163	0.172-0.811	57.1
	60	0.4918	0.163	0.171-0.811	
	90	0.4926	0.161	0.177-0.800	

in liver (Table 4), 57.5 and 67.5% in kidney (Table 5) and 47.6 and 65.9% in brain (Table 6), respectively.

Discussion

In Pakistan there is no information available on hazards of agricultural chemicals to amphibians as non-target wildlife. In the present study cholinesterase activity of frog liver, kidney and brain were decreased after treatment of lambda cyhalothrin (pyrethroid) and mono-crotophos (Organophphate). Balasundaram and Selvarajan (1990) reported the. inhibition of acetylcho-linesterase in the central nervous system of *Rana tigrina* under the effect of organophosphate. Flickinger et al. (1991) studied the poisoning of Canadian geese in Texas by parathion sprayed for control of Russian wheat aphid and reported that brain cholinesterase activity was depressed 62% and 77% below normal in the two geese collected at the mortality site compared to that in controls. In the present finding also, it was observed that cyhalothrin caused upto 20% and 57.1% inhibition in activity of cholinesterase. The present findings are generally in accordance with the previous reports on other wildlife species. However, some difference between the present findings and previous works may be due to the difference of animal class as previous reports are on avian fauna whereas presently subject species was a amphibian. Nizam (1993) reported 49% and 57% inhibition of AchE after Nfb and nimocinol treatment in subject animal. Burgees et al. (1999) observed that organophosphate decreased cholinesterase activity in birds and these insecticide also produced tremors loss of

Table 3. Activity of cholinesterase in brain of *Rana cyanophyctis* treated with lambda cyhalothrin

	Time Sec.	Mean(U/l)	S.E (+ –)	Range at 95% confidence	Inhibition %
Control	00	00	00	00	00
	30	1.147	0.0062	1.457-1.159	
	60	1.159	0.0063	1.146-1.171	
	90	1.154	0.0062	1.141-1.166	
0.1%	30	0.784	0.151	0.488-1.079	31.64
	60	0.777	0.149	0.481-1.073	
	90	0.782	0.150	0.487-1.076	
1%	30	0.565	0.1793	0.213-0.916	50.7
	60	0.564	0.1789	0.214-0.914	
	90	0.566	0.1794	0.214-0.917	

Table 4. Activity of cholinesterase in liver of *Rana cyanophyctis* treated with monocrotophos

	Time Sec.	Mean (U/l)	S.E (+ –)	Range at 95% confidence	Inhibition %
Control	00	00	00	00	00
	30	1.2270	0.0560	1.117-1.336	
	60	1.0840	0.1510	0.788-1.379	
	90	1.0880	0.0196	1.049-1.126	
0.1%	30	1.0440	0.00116	1.041-1.046	37.7
	60	1.0454	0.00120	1.042-1.047	
	90	1.0452	0.00214	1.040-1.049	
1%	30	0.5212	0.0244	0.473-0.569	57.7
	60	0.5178	0.0241	0.470-0.565	
	90	0.5254	0.0348	0.457-0.593	

Table 5. Activity of cholinesterase in kidney of *Rana cyanophyctis* treated with monocrotophos

	Time Sec.	Mean (U/l)	S.E (+ –)	Range at 95% confidence	Inhibition %
Control	00	00	00	00	00
	30	1.148	0.0054	1.137-1.158	
	60	1.152	0.0057	1.140-1.163	
	90	1.154	0.0100	1.134-1.174	
0.1%	30	0.487	0.0063	0.472-0.499	57.5
	60	0.481	0.0069	0.467-0.494	
	90	0.478	0.0080	0.462-0.493	
1%	30	0.3844	0.039	0.307-0.460	67.5
	60	0.3784	0.038	0.030-0.452	
	90	0.3714	0.033	0.306-0.971	

Table 6. Activity of cholinesterase in brain of *Rana cyanoohyctis* treated with monocrotophos

	Time Sec.	Mean (U/l)	S.E (+ –)	Range at 95% confidence	Inhibition %
Control	00	00	00	00	00
	30	1.147	0.0062	1.457-1.159	
	60	1.159	0.0063	1.146-1.171	
	90	1.154	0.0062	1.141-1.166	
0.1%	30	0.391	0.0415	0.309-0.472	47.6
	60	0.387	0.0433	0.302-0.471	
	90	0.404	0.0170	0.370-0.437	
1%	30	0.6026	0.0307	0.541-0.661	65.9
	60	0.6191	0.0183	0.583-0.799	
	90	0.7490	0.0690	0.613-0.884	

muscular control and paralysis. Mineau (1993) reported that post exposure of carbamat and organophosphate cholinesterase activity reduced in wild birds. Taylor et al. (1999) reported the effect of field grade malathion sublethal dose (0.01 mg/g toad) and 0.0011 mg/g toad). The brain cholinesterase level decreased 22% and 17%, respectively, Parson et al. (2000) observed the effect of organophosphate and carbamate on non-target wildlife species, these pesticides inhibited cholin-esterase activity. Khan (2002) studied the effect of Permethrin on cholin-esterase activity against lizard, *Calotes versicolor* and reported that cholinesterase inhibited upto 17 and 19% in kidney and 18 and 24% in liver. In the present work lambda cyhalothrin and monocrotophos were produced inhibitory effect in *Rana cyanophlyctis* liver, kidney and brain, and so the present findings are in the line with previous reports. In the present study pesticides lambda cyhalothrin and monocrotophos were applied directly by injection method in frogs body which reduced cholin-esterase activity. It was decreased upto 34.6 and 46.3% in liver, 25.08 and 57.1% in kidney and 31.64 and 50.7% in brain under the effect of lambda cyhalothrin. In the case of monocrotophos treatment, cholinesterase activity de-creased upto 37.7 and 57.7% in liver, 57.5 and 67.5% in kidney and 47.6 and 65.9% in brain, respectively. These results are in agreement with previous research. On the basis of the present studies it is concluded that both pesticides are toxic to frogs and reduce the choline-sterase activities.

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