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Effects of Agro Pesticides Cypermethrin and Malathion on Cholinesterase Activity in Liver and Kidney of *Calotes versicolor* Daudin (Agamidae: Reptilia)

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Abstract: Reptiles are long-lived, sedentary animals and therefore may be good biomonitors of their local habitat. Experiments were carried out to determine the effects of 2 agro-pesticides, cypermethrin and malathion, on the cholinesterase activity of *Calotes versicolor* Daudin. Two different concentrations, 0.1% and 1%, were used and cholinesterase activity was estimated in the liver and kidney. This decreased by up to 20% and 35% in the liver and 27% and 54% in the kidney under the effect of cypermethrin. In the case of malathion it decreased by up to 30.27% and 66.97% in the liver and 58.46% and 65.09% in the kidney. On the basis of the present study it is concluded that the pesticide malathion (organophosphate) is the more toxic of the pesticides tested.

Key Words: Effect, Malathion, Cypermethrin, Cholinesterase, *Calotes versicolor*

Introduction

Pesticide residues accumulate in the tissues of lizards exposed to pesticide contamination over a period of time. Whole body levels of lizards, ingested as prey, reflect levels entering the food chain, and hence the environment. There are more than 300 species belonging to the family Agamidae in the world (Rogner, 1997). Due to human activities and involvement in an effort to increase agricultural products and the use of indiscriminate pesticides, approximately 25% of reptiles and 20% of amphibians are listed as threatened (Hilton-Taylor, 2000). Organophosphate and carbamate are widely used and have a variety of lethal and sublethal effects on non-target wildlife (Parsons et al., 2000). In Pakistan a number of non-target species can be affected when pesticides are used because of their inhibition of cholinesterase activity (Khan, 2004). Some studies have reported on reptile and amphibian cholinesterase levels and the subsequent effects of enzymatic inhibition (Howe et al., 1998; Larson et al., 1998; Kegley et al., 1999; Pauli and Money, 2000; Khan, 2002; Khan et al., 2002; Khan, 2003b; Khan et al., 2003a, 2003c, 2003d). In the present study the effects of cypermethrin and malathion on liver and kidney cholinesterase activity were investigated.

Materials and Methods

Local *Calotes versicolor* lizards were collected from the field without regard to sex. They were kept in a vivarium for several days before the experiment. During captivity food and water were provided in the vivarium. Two different concentrations of cypermethrin and malathion, 0.1% and 1%, were used. The cypermethrin and malathion were injected 1 µl per lizard (single pesticide per animal). A batch of untreated (lab standard) lizards were also kept for comparison. After 24 h animals were dissected, and the, liver and kidney were removed as per Shakoori and Ahmad's (1973) methods for cholinesterase estimation. Cholinesterase activity was estimated using a Randox Kit No. CE-190. The method is based upon the hydrolysis of acetylcholine 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.

Principle:

Butyrylthiocholine + H_2O → thiocholine + butyrate

Thiocholine + DTNB → 2-nitro-5-mercaptobenzoate

DTNB = Dithiobis (nitrobenzoate)

In cholinesterase estimation 3 tubes were used for treated (i.e. liver and kidney) and 1 tube for untreated (i.e. also liver and kidney) assays. One was solution 1 (i.e. the buffer tube), the second was sample (kidney and liver) and the last was solution 2 (i.e. substrate). A buffer solution of 1.5 ml was added to the sample 1 tube, while 0.01 ml of sample and 0.05 ml of substrate were added to the sample tube and solution 2 tube, respectively. The method used was as follows: mix thoroughly, read initial absorbance and start timer simultaneously. Read again after 30, 60 and 90 s. Determine the mean absorbance change per minute.

Results

All experimental work was performed on adult lizards of the same size and almost the same weight. Pre- and post-treatment lizard body weights were also recorded, and 24 h after the treatment a very nominal weight difference was observed. Under the effect of cypermethrin, cholinesterase decreased by up to 20% and 35% in the liver (Table 1), and 27% and 54% in the kidney (Table 2). Under the effect of malathion, cholinesterase activity decreased by up to 30.27% and 66.97% in the liver (Table 3) and 58.46% and 65.09% in the kidney (Table 4).

Table 1. Activity of cholinesterase in liver of *Calotes versicolor* treated with cypermethrin.

	Time (s)	Mean (U/l)	S.D. (±)	S.E. (±)	Range at 95% confidence limit	% Inhibition
	00	00	00	00	00	
Control	30	0.484	0.022	0.0129	0.4588-0.5092	
	60	0.490	0.022	0.0127	0.4652-0.5148	00%
	90	0.486	0.032	0.0185	0.44974-0.522226	
	00	00	00	00	00	
0.1%	30	0.383	0.0401	0;0236	0.33675-0.42925	
	60	0.391	0.007	0.004	0.38316-0.39884	20%
	90	0.381	0.02	0.01156	0.35846-0.40354	
	00	00	00	00	00	
1%	30	0.314	0.033	0.0190	0.27670-0.35124	
	60	0.314	0.007	0.040	0.2356-0.3924	35%
	90	0.313	0.0122	0.006	0.30124-0.32476	

Table 2. Activity of cholinesterase in kidney of *Calotes versicolor* treated with cypermethrin.

	Time (s)	Mean (U/l)	S.D. (±)	S.E. (±)	Range at 95% confidence limit	% Inhibition
	00	00	00	00	00	
Control	30	0.364	0.00173	0.00100	0.36204-0.36596	
	60					00%
	90					
	00	00	00	00	00	
0.1%	30	0.261	0.003	0.0018	0.25748-0.264528	27%
	60	0.266	0.023	0.0133	0.239932-0.292068	
	90	0.257	0.0223	0.012	0.04704-0.23348	
	00	00	00	00	00	
1%	30	0.163	0.0077	0.0044	0.154376-0.171624	54%
	60	0.164	0.0122	0.0070	0.15028-0.17772	
	90	0.161	0.276	0.159	0.15064-0.47264	

Table 3. Activity of cholinesterase in liver of *Calotes versicolor* treated with malathion.

	Time (s)	Mean (U/l)	S.D. (±)	S.E. (±)	Range at 95% confidence limit	% Inhibition
	00	0.483	0.00208	0.00120	0.4806-0.4853	
Control	30	0.485	0.00208	0.00120	0.4826-0.4873	
	60	0.485	0.00251	0.00145	0.4821-0.4878	00%
	90	0.488	0.00152	0.00088	0.4862-0.4897	
	00	0.378	0.00208	0.00120	0.4807-0.4853	
0.1%	30	0.381	0.00251	0.00145	0.4878-0.482	
	60	0.387	0.00568	0.00328	0.478-0.491	30.27%
	90	0.391	0.00854	0.00493	0.480-0.499	
	00	0.337	0.00503	0.00290	0.331-0.343	
1%	30	0.342	0.00776	0.00448	0.333-0.3507	66.97%
	60	0.346	0.00665	0.00384	0.338-0.353	
	90	0.349	0.00608	0.00351	0.3421-0.3558	

Table 4. Activity of cholinesterase in kidney of *Calotes versicolor* treated with malathion.

	Time (s)	Mean (U/l)	S.D. (±)	S.E. (±)	Range at 95% confidence limit	% Inhibition
	00	0.361	0.00251	0.00145	0.3581-0.3638	
Control	30	0.364	0.00173	0.00100	0.3544-0.3659	
	60	0.365	0.00360	0.00208	0.3609-0.3690	00%
	90	0.367	0.00378	0.00218	0.3627-0.3712	
	00	0.286	0.00264	0.00152	0.283-0.288	
0.1%	30	0.289	0.00115	0.000667	0.287-0.290	
	60	0.295	0.00472	0.00273	0.289-0.300	58.46%
	90	0.301	0.0020	0.00115	0.298-0.303	
	00	0.270	0.00754	0.00436	0.2614-0.2785	
1%	30	0.273	0.00854	0.00493	0.2633-0.2834	
	60	0.275	0.00971	0.00561	0.2640-0.2859	65.09
	90	0.280	0.00960	0.00555	0.2691-0.2908	

Discussion

It was observed that the liver and kidney cholinesterase activity decreased after cypermethrin and malathion treatment. Mineau (1993) reported that after exposure to carbamate and organophosphate cholinesterase activity in wild birds decreased. Shakoori et al. (1995) reported 84% inhibition in *Tribolium castaneum* due to sublethal doses of cypermethrin. Gard and Hooper (1995) reported that organophosphorus and carbamate exert their effects by binding to and inhibiting the acetylcholinesterase enzyme at nerve synapses. Azmi et al. (1999) studied the effects of tetranortriterpenoids (Neem product SDS) and deltamethrin (pyrethroid) on phosphomonoesterase activity in *Cyprinus carpio* (common carp) and reported enzyme inhibition under the effect of these pesticides. Burgees et al. (1999) observed that organophosphate insecticide reduced cholinesterase activity in birds. Taylor et al. (1999) reported that a sublethal dose of field grade malathion (0.01 mg/g toad and 0.0011 mg/g toad) lowered brain cholinesterase levels by 22% and 17%, respectively. Parson et al. (2000) observed the effect of organophosphate and carbamate on non-target wild animals, and these pesticides inhibited cholinesterase activity. Khan (2002) studied the effect of permethrin and biosal in the Indian garden lizard and reported that after treatment with permethrin cholinesterase levels decreased by up to 17% and 19% in the kidney and 18% and 24% in the liver. In the case of biosal treatment, the decrease in cholinesterase was 13.6% and 18% in the kidney and 39.52% and 56.21% in the liver. Khan et al. (2002) studied the effect of permethrin in *C. versicolor* and reported that after treatment with permethrin inhibition was 17% and 19% in the kidney and 18% and 24% in the liver. We also observed that cypermethrin caused upto 35 and 54% and malathion 30% and 65.09% inhibition in the activity of cholinesterase. The present findings are generally in accordance with the previous reports on lizards and other animals. However, some differences between the present findings and previous works may be due to the different of animal species used. In the present work cypermethrin and malathion produced an inhibitory effect in the *C. versicolor* liver and kidney. It is concluded that the pesticide malathion (organophosphate) is the more toxic of the pesticides tested during this study.

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