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A Comparative Study on Effect of Agricultural Chemicals on Reptiles on Cholinesterase Activity of Pyrethroid and Organophosphate with Phytopesticide

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Abstract – Calotes versicolor, a wildlife species, was used as the subject of the study. In this investigation, two different concentrations were employed. The activity of cholinesterase in the kidney was shown to decrease following treatment with cypermethrin, malathion, and biosal in this investigation. In the current study, it was discovered that cypermethrin reduced cholinesterase activity by up to 54 percent, malathion by up to 65.09 percent, and biosal by up to 24 percent.

Keywords – Reptiles, Agricultural Chemicals, Pyrethroid, Organophosphate, Phytopesticide

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INTRODUCTION

Pesticide usage is common, which leads to bird population decreases and death in agroecosystems. Insecticides, among the numerous types of pesticides, have a greater risk of acute effects due to their greater intrinsic toxicities and increased potential for exposure. Insecticides offer an obvious danger to wild bird species, as shown by documented examples of mass death caused by poisoning and several studies documenting detrimental effects of insecticides on birds. The death of Swainson's hawks due to monocrotophos, an organophosphorus pesticide, in 1995-1996 was one incidence of mass avian mortality that drew a lot of attention in Argentina. Monocrotophos licensing was revoked in Argentina as a result of this occurrence, whereas pyrethroid insecticides grew in relevance and popularity.

Pesticide residues build up over time in the tissues of lizards exposed to pesticide contamination. Ingested as prey, whole body levels of lizards mirror levels entering the food chain, and hence the environment. The Agamidae family has more than 300 species across the globe (Rogner, 1997). Around 25% of reptiles and 20% of amphibians are categorized as vulnerable due to human activities and engagement in an endeavor to produce agricultural goods, as well as the use of indiscriminate pesticides (HiltonTaylor, 2000). When pesticides are applied in Pakistan, they may impact a variety of non-target species due to cholinesterase inhibition (Khan, 2004). Some research have looked at the levels of cholinesterase in reptiles and amphibians, as well as the consequences of enzymatic inhibition. The effects of cypermethrin and malathion on cholinesterase activity in the liver and kidney were studied in this research.

Anthropogenic activities' harmful influence on biodiversity is becoming more obvious, and amphibians are presently the most globally endangered vertebrate group (accounting for around 41% of all species). Emerging illnesses, habitat degradation, alien species introduction, and pollution of both terrestrial and aquatic environments have all been identified as major threats. Given these concerns, determining the relevance and severity of their impact on amphibian populations is crucial in order to establish effective management and conservation methods.

Direct application, runoff from agricultural and forest applications or mining, urban and industrial sewage, and atmospheric deposition are all ways that pollutants are being introduced into the ecosystem (Vitousek et. al. 1997; Linder and Grillitsch 2000; Sparling 2000; Ritter and Bergstrom 2001). In summary, the presence of pollutants is widespread and is predicted to rise in the near future (Carpenter et al. 1998; Kolpin et. al. 2002; Gilliom et. al. 2007). (Tilman et. al. 2001;

Galloway et al. 2003). What we don't have is a comprehensive study of how various contaminants harm amphibians.

Pollutants have been shown to have a variety of deadly and sublethal consequences on frogs, including lower growth and development, increased developmental anomaly frequency, illness susceptibility, and behavioral change. It's not unexpected that contaminants have varied effects on amphibians due to their wide range of effects and mechanisms of action. In natural ecosystems, pathogenic organisms and ultraviolet-B radiation, for example, are becoming more common, and these stressors may combine with chemical contaminants. As a consequence, when examining the consequences of pollution on amphibian populations, evaluating patterns in how these stressors interact with pollutants is critical.

Too far, the majority of research on the impacts of pollutants on amphibians have been undertaken in laboratories, with just a few being undertaken in more natural settings, such as outdoor mesocosms. Although laboratory studies may use ecologically relevant concentrations, the results may not be applicable to more natural conditions because actual concentrations in the environment can be affected by a variety of factors, including plant uptake, denitrification, and sediment trapping, or because of the aforementioned. As a result, frog studies in the laboratory may overstate or underestimate the impact of chemical pollutants (Boone and Bridges 2003; Gómez-Mestre and Tejedo 2003). This highlights the need of comparing the impacts of contaminants on amphibians in different experimental settings (Skelly 2002, but see Chalcraft et al. 2005).

Although studies to yet have been ambiguous on this issue, it is nevertheless feasible to speculate that there may be significant species- or family-level disparities in sensitivity. An assessment of pollution impacts within a phylogenetic framework is required. The effects of contaminants may also differ depending on the developmental stage at which people are first exposed. Overall, it is obvious that in order to completely comprehend the impacts of pollutants on amphibians, a range of aspects must be considered, including the kind of pollutant, the presence of other stressors, the experimental venue, phylogenetic links, and ontogenetic stage.

Several evaluations on the impact of various contaminants on amphibians have been published (Cowman and Mazanti 2000; Linder and Grillitsch 2000; Sparling 2000). These evaluations summarized research using a vote counting technique (counting the number of significant vs. nonsignificant results) or simply summarizing LC50 values investigations of contaminants on amphibian survival (where LC50 is the lethal concentration that kills 50 percent of a population). Survival is the sole response variable in LC50 evaluations, which are primarily confined to single-species lab experiments (i.e., individuals taken from their natural habitat). Furthermore, because LC50 values are often much higher than actual concentrations in the field, To correctly estimate the sensitivity of amphibians in natural habitats, further information on the effects of ecologically relevant concentrations on survival and sublethal endpoints is necessary. There is always worry in vote-counting research that the findings reached may not be valid (e.g., owing to a small sample size) and that the estimates may be severely skewed owing to low statistical power (Rosenberg et al. 2000). Furthermore, vote counting is an ineffective method for determining the extent of an impact and comparing responses across established categories (Gurevitch et al. 2000).

Meta-analytic approaches may be used as an alternative to averaging LC50 experiments or vote counting. To produce test statistics of overall effect sizes, meta-analytic approaches integrate the magnitude of effects and the sample size of each research. Meta-analyses may also evaluate effect sizes between designated categories, such as phylogenetic groupings, environmental modification types, and experimental locations. These methods may also be used to investigate two-factor modifications (Gurevitch et al. 2000). Meta-analyses, for example, have recently been used to investigate the overall impact of ultraviolet-B radiation on frogs and other aquatic animals, as well as the interaction of this radiation with other environmental conditions (Bancroft et al. 2007, 2008).

The aim of our study, which utilized meta-analytical techniques, was to evaluate the overall effect on amphibian survival, mass, developmental time and abnormality of environmental-relevant concentrations of certain chemical contaminants, to evaluate the interactive effects on amphibians of the pollutants and other stressors and to assess whether significant differences are observed in etiological pollution.

MATERIAL AND METHODS

Calotes versicolor, a wildlife species, was used as the subject of the study. The concentrations were 0.1 and 1% of cypermethrin and malathion for cypermethrin and malathion, and 25 and 50 percent for biosal, respectively. One liter of cypermethrin, malathion, and biosal was administered into each lizard. For comparison, a batch of untreated (Lab standard) was retained. After 24 hours of treatment, the lizard kidney and liver were removed using Shakoori and Ahmad's (1973) cholinesterase estimate procedures. Randox Kit No. CE-190 was used to determine cholinesterase activity. Knedel and Boetteger are the foundations of this technique (1967).

RESULTS

Cholinesterase was reduced by up to 27 and 54 percent in the kidney and 20 and 35 percent in the liver when cypermethrin was used (Table 2).

Table 1: Cholinesterase activity in cypermethrin-treated calotes versicolor kidneys

	Time (sec.)	Mean (U/l)	Mean×11750	S.D. (±)	S.E. (±)	Range at 95% confidence limit	% Inhibition
	00	0.00	00.00	0.00	0.00	0.00	
Control	30	0.36	4169.72	0.00	0.00	0.36204-0.36596	
	60						00%
	90						
	00	0.00	00.00	00.00	00.00	0.00	
0.1%	30	0.28	3061.53	0.00	0.00	0.21748-0.264128	27%
	60	0.28	3120.18	0.02	0.01	0.259952-0.292068	
	90	0.25	3043.93	0.02	0.01	0.04704-0.23348	
	00	0	00.00	00.00	00.00	0.00	
1%	30	0.16	1912.99	0.00	0.00	0.154376-0.171624	34%
	60	0.16	1923.72	0.01	0.00	0.15828-0.17772	
	90	0.16	1888.51	0.27	0.15	0.15064-0.47264	

Table 2: Cholinesterase activity in the liver of cypermethrin-treated calotes versicolor

	Time (sec.)	Mean (U/l)	Mean×11750	S.D. (±)	S.E. (±)	Range at 95% confidence limit	% Inhibition
	00	0.00	00.00	0.00	0.00	0.00	
Control	30	0.48	5677.12	0.02	0.01	0.4188-0.5092	
	60	0.48	5747.71	0.02	0.01	0.4852-0.5048	00%
	90	0.48	5706.78	0.03	0.01	0.44874-0.522228	
	00	0.00	00.00	0.00	0.00	0.00	
0.1%	30	0.38	4482.59	0.04	0.02	0.33675-0.42925	
	60	0.38	4586.43	0.00	0.00	0.38016-0.39884	23%
	90	0.38	4473.04	0.02	0.01	0.37846-0.40034	
	00	0.00	00.00	0.00	0.00	0.00	
1%	30	0.31	3883.23	0.01	0.01	0.27670-0.33126	
	60	0.31	3883.22	0.00	0.04	0.3156-0.3824	33%
	90	0.31	3671.48	0.01	0.00	0.30224-0.32476	

Malathion cholinesterase was 59.9% less in the kidneys and 30.27% less than in the liver and 66.97% less than in the liver (Table 3 and Table 4)

Table 3: Cholinesterase activity in the kidney of versicolor, malathion-treated calotes

	Time (Sec.)	Mean sample	Reagent blank Sample x 131.6 =	S.D.	S.E.	Range	% Inhibition
Untreated	00	0.361	13.8180	0.00251	0.00145	0.3581-0.3638	00.00%
	30	0.364	13.9496	0.00173	0.00100	0.3544-0.3659	
	60	0.365	14.0812	0.00360	0.00208	0.3609-0.3690	
	90	0.367	13.2916	0.00378	0.00218	0.3627-0.3712	
Treated (0.1%)	00	0.286	5.6588	0.00264	0.00152	0.283-0.288	58.46%
	30	0.289	7.2380	0.00115	0.00066	0.287-0.290	
	60	0.295	6.3168	0.00472	0.00273	0.289-0.300	
	90	0.301	6.9748	0.00200	0.00115	0.298-0.303	
Treated (1%)	00	0.270	4.8692	0.00754	0.00436	0.2614-0.2785	65.09%
	30	0.273	4.8692	0.00854	0.00493	0.2633-0.2834	
	60	0.275	5.0008	0.00971	0.00561	0.2640-0.2859	
	90	0.280	5.3956	0.00960	0.00555	0.2691-0.2908	

Table 4: Cholinesterase activity in the liver of the agama lizard Calotes versicolor after treatment with malathion

	Time (Sec.)	Mean sample	Reagent blank Sample x 131.6 =	S.D.	S.E.	Range	% Inhibition
Untreated	00	0.483	28.5572	0.00208	0.00120	0.4806-0.4852	00.00%
	30	0.485	28.6888	0.00208	0.00120	0.4826-0.4873	
	60	0.485	28.2940	0.00251	0.00145	0.4821-0.4878	
	90	0.488	28.4256	0.00152	0.00088	0.4862-0.4897	
Treated (0.1%)	00	0.378	20.1348	0.00208	0.00120	0.4807-0.4833	30.27%
	30	0.381	20.0032	0.00255	0.00147	0.4878-0.482	
	60	0.387	20.1296	0.00368	0.00128	0.476-0.481	
	90	0.391	20.4612	0.00854	0.00493	0.480-0.499	
Treated (1%)	00	0.337	9.0884	0.00305	0.00296	0.331-0.343	66.97%
	30	0.342	9.4752	0.00778	0.00448	0.333-0.3507	
	60	0.346	9.2120	0.00665	0.00384	0.338-0.353	
	90	0.348	9.3456	0.00608	0.00351	0.3421-0.3538	

Biosal therapy reduced cholinesterase activity by 13.06 and 18 percent in the kidney (Table 5) and 39.52 and 52.61 percent in the liver (Table 6).

Table 5: Cholinesterase activity in calotes versicolor kidneys treated with biosal

Hours	Treatment	Time sec	Mean (U/l)	S.D.±	S.E.±	Range at 95% confidence limit	% Inhibition
24	Untreated Control	30	2920.77	0.022	0.012	2920.74-2920.79	
		60	2897.31	0.017	0.0091	2897.29-2897.32	
		90	2875.85	0.005	0.003	2875.84-2875.85	-
24	Treated 25%	30	2616.01	0.015	0.009	2615.99-2616.02	
		60	2537.35	0.002	0.001	2537.34-2537.35	13.06
		90	2498.70	0.004	0.002	2498.69-2498.70	
24	Treated 50%	30	3460.64	0.025	0.041	3460.61-3460.66	
		60	3448.91	0.015	0.008	3448.89-3448.92	
		90	3415.43	1.001	1.001	3413.48-3427.41	18

Table 6: Cholinesterase activity in Calotes versicolor livers treated with biosal

Hours	Treatment	Time sec	Mean (U/l)	S.D.±	S.E.±	Range at 95% confidence limit	% Inhibition
24	Untreated Control	30	4704.33	0.01	0.017	4704.28-4704.35	
		60	4480.86	0.04	0.02	4480.82-4480.89	
		90	4433.94	0.05	0.03	4433.88-4433.99	-
24	Treated 25%	30	2709.86	0.316	0.298	2709.27-2710.44	
		60	2709.86	0.022	0.002	2709.85-2709.88	
		90	2698.13	0.022	0.002	2698.10-2698.15	39.52
24	Treated 50%	30	2134.86	0.025	0.004	2134.83-2134.88	
		60	2125.13	0.01	0.005	2121.12-2125.15	
		90	2111.38	0.02	0.001	2111.35-2111.40	52.61

DISCUSSION

Agricultural pesticide usage has grown in less developed nations as they cultivate more fruits and vegetables for export to more developed nations, however these pesticides have caused some damage to non-target animals such as reptiles and amphibians.

The activity of cholinesterase in the kidney was shown to decrease following treatment with cypermethrin, malathion, and biosal in this investigation. Carbamat and organophosphate exposure lowered cholinesterase activity in wild birds, according to Mineau (1993). Sublethal dosages of cypermethrin inhibited Tribolium castaneum by 84 percent, according to Shakoori et al. (1995). Organophosphorus and carbamate bind to and block the acetylcholinesterase enzyme at nerve synapses, according to Gard and Hooper (1995). Azmi and colleagues (1999) have researched and have observed that these pesticides reduced enzyme activism for Cyprinus carpio (common carp) impacts of tetranortriterpenoids (neem product SDS) and deltamethrin (pyrethroid). Burgees et al. (1999) found that cholinesterase activity in birds was lowered by organophosphate pesticides. Organophosphate and carbamate pesticides have reduced cholinesterase activity, according to Parson et al (2000). Khan (2002) investigated the effects of permethrin and biosal on cholinesterase activity in Indian Garden Lizards and found that after treatment with permethrin, cholinesterase activity was reduced by 17 and 19 percent in the kidney and 18 and 24 percent in the liver, respectively. After treatment with biosal, cholinesterase activity was reduced by 13.06 and 18 percent in the kidney and 39.52 and 56.21 percent in the liver, respectively. In the current study, it was discovered that cypermethrin reduced cholinesterase activity by up to 54 percent, malathion by up to 65.09 percent, and biosal by up to 24 percent. The current results are essentially consistent with previous results. C. versicolor kidney and liver cholinesterase activity was lowered by cypermethrin, malathion, and biosal in this study.

CONCLUSION:

Reptiles are stationary, long-lived creatures that may serve as biomonitors for their local environment. Experiments were conducted to see how agricultural chemicals like pyrethroid and organophosphate with phytopesticide affected cholinesterase activity in reptiles. Based on the current research, it has been determined that the agricultural pesticide malathion is the most harmful of the pesticides now being evaluated. If applied at lower quantities, the phytopesticide biosal might be a superior pesticide.

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