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Effect of Cypermethrin and Permethrin on Cholinesterase Activity and Protein Contents in *Rana tigrina* (Amphibia)

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Abstract: The effect of different concentrations (0.1% and 1%) of cypermethrin and permethrin were examined on the kidney and liver of *Rana tigrina*. After treatment with these pesticides, cholinesterase activity and protein contents were determined, in the kidney and liver. It was observed that protein contents and cholinesterase activities decreased in the treated animals.

Key Words: Induced effect, cypermethrin, permethrin, *Rana tigrina*, cholinesterase

Cypermethrin ve Permethrin'in *Rana tigirna*'da (Amphibia) Kolinesterazın Etkinliğine ve Protein İçeriğine Etkisi

Özet: Cypermethrin ve permethrinin farklı derişimlerinin etkisi (% 0,1 ve % 1) *Rana tigirina*'nın böbrek ve karaciğeri üzerinde araştırıldı. Bu böcek ilaçlarının uygulanmasından sonra böbrek ve karaciğerdeki kolinesteraz etkinliği ve protein içeriği belirlendi. İşlemden geçirilen hayvanlarda protein içeriğinin ve kolinesteraz etkinliğinin azaldığı gözlendi.

Anahtar Sözcükler: Tetiklenen etki, cypermethrin, permethrin, *Rana tigrina*, kolinesteraz

Introduction

Pesticides affect all members of an ecosystem, from the smallest invertebrates to birds and humans. Most toxic pesticides in both urban and agricultural settings are responsible for the deaths of many birds and fish and smaller aquatic animals that fish depend on for food. Amphibians are an important component of the food chain. They are cold-blooded animals and cannot regulate their body temperature. They live near water reservoirs or in water, so they are indirectly affected by pesticides used in agriculture for crop protection. In California many frogs and toads grow extra legs and eyes and do not survive to adulthood due to various effects of toxic chemicals.

No significant work has been done on the relationship between amphibians and pesticides, but some work on other vertebrates has been reported, such as Meeks, (1968) and Fleet et al. (1972), who worked on pesticide residues in turtles and snakes. Hutson and Casida (1978) and Shono et al. (1979) isolated cypermethrin and deltamethrin in rats and mice. Harfenist et al. (1989) studied the effect of 11 pyrethroids on frogs. Mineau, (1993), O,Hara et al. (1991), Simon et al. (1999), Bishop et al. (2000), Parsons et al. (2000) and Khan, (2000) reported the effects of pesticides on different non-target organisms. In the present research the effects of cypermethrin and permethrin on the liver and kidney and cholinesterase activity and protein contents were studied. Some previous researchers have observed the effects of different pesticides on enzymatic activities of non-target species of vertebrates i.e. Custer et al. (1985), Hill, (1989), Cooper, (1991), Mineau, (1993) and Parasons et al. (2000).

Materials and Methods

In the present research the subject organism was the adult frog *Rana tigrina*. The frogs were collected from Karachi University Campus and kept in the departmental pond. 1 ml cypermethrin and permethrin in concentrations of 0.1% and 1% (in acetone as solvent) each were injected subcutaneously in the abdominal region of the subject species with the help of an insulin syringe. After treatment with these chemicals the enzyme cholinesterase and protein contents were estimated in the kidney and liver of the frogs after 24 h post-treatment. The kidney and liver were taken as per Shakoori and Ahmad's (1973) techniques. Cholinesterase activity was estimated by a Randox Kit No. CE-190. The method is based upon the hydrolysis of acetylcholine by the action of cholinesterase, following 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 three tubes were used for treated (i.e. liver and kidney) and one tube for untreated (i.e. also liver and kidney) assays. The first was solution 1 (i.e. buffer tube), the second was sample (kidney and liver) and third was solution 2 (i.e. substrate). A buffer solution of 1.5 ml was placed in the sample 1 tube, while a 0.01 ml sample and a 0.05 ml substrate were placed in the sample tube and the solution 2 tube, respectively. These were then mixed thoroughly, the initial absorbance read and the timer started simultaneously. This was read after 30, 60 and 90 s. The mean absorbance change per min was then determined.

Protein estimation was carried out by the Biuret method after Ahmad et al. (2000) based on Khan (1998).

Results

The effects of different concentrations (0.1% and 1%) of cypermethrin and permethrin on the kidney and liver of *Rana tigrina* were examined. After treatment with these pesticides cholinesterase activity and protein contents were determined in the kidney and liver.

Total protein contents

Total protein contents were estimated in the kidney and liver of *Rana tigrina* at 24 h post-treatment with the selected chemicals. A batch of untreated (control) sample was also kept for comparison purposes.

In cypermethrin-treated samples the reduction in protein content was 79% and 69% whereas in the permethrin-treated samples 61% and 16% decreases in the kidney were observed. In the liver the reduction was 75% and 44% in the cypermethrin-treated samples at concentrations of 0.1 and 1%, respectively.

Cholinesterase activity

Cholinesterase activity was estimated in the kidney and liver of treated organisms. After 0.1% and 1% of cypermethrin and permethrin treatment, the inhibition was found to be 36% and 35% in the cypermethrin- and 35% and 6.76% in the permethrin-treated kidney, respectively. In the liver of cypermethrin-treated frogs the inhibition was 52% and 47%, whereas in permethrin, treated animals the inhibition was 29% and 23%, respectively.

Discussion

Cholinesterase activity and the protein contents of kidney and liver decreased after cypermethrin and permethrin treatment (0.1% and 1%). Custer et al. (1985) described how after parathion spraying brain cholinesterase activity reduced up to 7%, 40%, 54% and 57% in blackbirds, pheasants, mice and coots respectively. Fairbrother et al. (1989) reported that 12 h after the second application of azinphos-methyl, plasma ChE levels in adult tree swallows were significantly inhibited, by 41%, as compared to the controls. ChE activity in another group of adult swallows dropped by 21% and 19% following the first and second sprayings.

Wilson et al. (1991) reported that hawks trapped near orchards recently treated with organophosphates diazinon and chlorpyrifos had lower levels of cholinesterase than the control group. Burgees et al. (1999) observed that organophosphate insecticides decreased cholinesterase activity in birds, and that these insecticides also produced tremors, loss of muscular control and paralysis. Flickinger et al. (1991) described how after parathion spraying geese collected from wheat fields exhibited up to 77% depression of their brain ChE activities 77%. Mineau (1993) reported that after

exposure to carbamate and organophosphate, cholinesterase activity in wild birds decreased. Taylor et al. (1999) observed the effects of sublethal doses (0.01 mg/g toad) and (0.0011 mg/g toad) of field grade malathion. The brain cholinesterase levels, decreased by 22% and 17% respectively. Parson et al. (2000) studied the effects of organophosphate and carbamate on non-target wildlife. These pesticides inhibited cholinesterase activity. In the present study cypermethrin and permethrin produced an inhibitory effect in the kidney and liver cholinesterase activity of the frog (*Rana tigrina*). The present findings are therefore in line with earlier results with reference to all the used compounds (organophosphate, organochlorine, carbamate and pyrethroids) applied directly or indirectly. In the present research cypermethrin and permethrin were applied to the frog body directly (0.1% and 1% conc.), and both compounds reduced cholinesterase activity in the kidney up to 36% and 35% after cypermethrin and 35% and 6.76% after permethrin treatment, whereas in the liver the percentage inhibition was 52% and 47% after cypermethrin treatment while in the case of permethrin 29% and 23% decreases were noted. These results appear to be in agreement with previous observations.

Total protein content also decreased in non-target vertebrate fauna after pesticide treatment, indicating pesticide-produced changes in the biochemical systems of non-target organisms. Proteins play an important role in the life of all living organisms. Pesticides disturb protein synthesis. In the present case the total protein contents of the liver and kidney in the frog (*Rana tigrina*) decreased after cypermethrin and permethrin (0.1% and 1% concentration at 1 ml dose) treatment. The reduction of protein content in the kidney was up to 79% and 69% after cypermethrin treatment and 61% and 16% after permethrin treatment. In the liver the reduction was 76% and 44% after cypermethrin treatment and 89% and 87% in permethrin-treated samples. Naqvi et al. (1986), Javid (1989), Nizam (1993), Saleem et al. (1998), Ahmad et al. (2000) and Tabassum and Naqvi (2001) also observed a reduction in total protein contents after pesticide application in different insects. Khan (2000) and Fatima (2001) also reported a reduction in the liver and kidney of reptiles. The present investigations also appear to be in line with the earlier findings. The present results therefore confirm the Khan, (2000) and Fatima, (2001) findings in this respect.

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