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Application of Some Herbal Extracts and Calcium as an Antidote to Counteract the Toxic Effects of Cypermethrin and Carbofuran in Indian Major Carp, *Labeo Rohita*

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1. Introduction

Pesticides that are transported to the aquatic environment are primarily of agricultural origin. Sometimes, pesticides are applied to the fish ponds to control fish diseases. In the process, the residues that reach the hydrosphere are concentrated in certain parts of the aquatic ecosystem or remain in solution for extended periods or adsorbed to the particulate matter and thereby deposited in the sediments. Thus, pesticides could be accumulated in the body of the aquatic animals. Most of the pesticides act on the respiratory process and cholinergic nervous system and hamper the cell metabolism in addition to other disturbances. Thus, a formulation of antidotes to counteract pesticides is an important aspect of pollution research and work in this direction is in the initial stages. Zamfir (1979) worked on the possibilities of removal of pesticide polluted water in treatment stations and described some methodologies, i.e., flocculation and filtration that can partially removed DOT, 2,4,5-T, Endrin, Parathion and Lindane. Chlorine oxidation can remove parathion; diuron etc., ozone and potassium permanganate appear to extract effects similar to those of chlorination. Activated charcoal has positive effects in the removal of absorption of most pesticides and U-V rays also can remove a certain amount of pesticides.

Some indirect approaches have also been employed by some scientists and their methods were environmental or nutritional manipulation. Sado et. al. (1992) reported that increased temperature and optimum levels of dissolved oxygen (by aerator) can decrease the pesticidal action. The application of lime to increase the pH for counteraction of the toxic effects of pesticides is also documented. Ghazaly (1994) and Mukherjee (1996) evaluated efficacy of ascorbic acid (vitamin C) for the intoxication of different pollutants including pesticides. Application of different herbal extracts for this purpose could play a very important role to mitigate the toxic effect of pesticides.

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2. Aim of the study

To investigate some herbal extracts and water calcium as antidotes to counteract the pesticidal effects on Indian Major Carp, *Labeo rohita* (rohu).

3. Materials and methods

Three herbal extracts and calcium as possible antidotes were tried for their efficacy to counteract Cypermethrin, a synthetic pyrethroid (trade name-Cypermethrin, chemical name- Cyano methyl,2,2-dimethyl,cyclopropane-carboxylate) and Carbofuran, a carbamate pesticide (trade name-Furadan 3G, chemical name- 2,3 dihydro,2-2 dimethyl,7 benzo furymethyl carbamate) toxicosis in fish. Antidotes study was conducted in 2 steps (1) Preparation of Crude extracts and (2) Experimental trials.

Datura (*Datura ripens*), *Kalka* (*Nerium indicum*) and *Neem* (*Azadiricta indica*) were the plants selected for antidote study and fruits of *datura* and *kalka*, and leaves of *neem* were the specific materials. These plant materials were minced, then grounded and finally extracted using acetone (50%) solution. The ratio of plant material and acetone solution was maintained at 1:10. The paste was collected in a fine meshed cloth and the filtrate was collected for the study. Everytime fresh extracts were prepared before trials. The doses of *datura* extract were 2.5 and 5.0 ml/l, for both the pesticides. The doses of *kalka* extract were 2.5 and 5.0 ml/l, for the Cypermethrin experiment while the same were 0.75 and 1.5 ml/l, for the Carbofuran experiment. The doses of *neem* extract were 10 and 25 ml/l for the Cypermethrin experiment while the same were 5.0 and 10 ml/l for the Carbofuran study.

For the application of calcium solution in water, calcium chloride was chosen as reagent. Fresh solutions were prepared before experiment and applied with different doses (50, 100, 200, 300 and 400 mg/l for both the pesticides under study) to the experiments.

Rohu fingerlings (2.25 ± 0.16 g) were used for the antidote study. The fishes were collected from the institute's pond and acclimatized at laboratory condition for 15 days after treating the fish with 0.1% potassium permanganate solution. Probable lethal doses for both cypermethrin and carbofuran were detected by error and trial method in 40 litre plastic tubs containing 20 litres of water. The antidote study was also performed for both the pesticides in the same type of plastic tubs with 20 L of freshwater. The water used for this study was 7.5 pH and 80 mg/l total alkalinity as CaCO_3 . Four treatments, viz., control (only fish, no pesticides, no antidote), fishes treated with only pesticide (lethal dose), fishes treated with only antidote and fishes treated with pesticide (lethal dose) and antidote solution, were maintained. In every case, antidotes were applied half an hour after the pesticide treatment. Two different doses of each antidote were tried for each of the pesticides. Three replications were maintained for all the treatments. For each case, sampling was done after 1, 2, 4, 6, 12, 24, 48, 72 and 96 hours, respectively (if any mortality occurs), and observations were made and final data were calculated on the basis of comparison between the different experimental observations. The results were expressed as percentage of fish survivability.

For significance of antidote study, one way ANOVA (Duncan Multiple Range Test) were done (Zar, 1974). Test of significance was examined at 5% level.

4. Results and discussion

Among the four doses of calcium, both at 100 and 200 mg/l of water calcium levels, 67 percent of fish survived up to 96 hours compared to 100 percent fish mortality at 50,300 and 400 mg/l concentration of water calcium against the lethal concentration of cypermethrin.

There was no significant difference in fish survivability between 100 and 200 mg/l levels of water calcium (Table 1). Fifty percent of fish survivability after 96 hours of exposure with lethal concentration of carbofuran was recorded both at 100 and 200 mg/l levels of water calcium while 100 percent of fish mortality was obtained with 50,300 and 400 mg/l levels of calcium against lethal concentration of carbofuran up to 96 hours (Table 3).

Observation at different time intervals (hrs.)	Survivability percentage of control	Survivability percentage in Cypermethrin (37 ml a.i./ha/m)	Survivability percentage in cypermethrin and different doses of antidote calcium(mg/l)*				
			50	100	200	300	400
1	100	100	100a	100a	100a	100a	100a
2	100	100	100a	100a	100a	100a	100a
4	100	100	100a	100a	100a	100a	100a
6	100	25	50b	100a	100a	100a	100a
12	100	0	50b	100a	100a	50b	25b
24	100	0	25c	67b	67b	50b	25b
48	100	0	0	67b	67b	25c	0
72	100	0	0	67b	67b	0	0
96	100	0	0	67b	67b	0	0

*Values followed by the same superscript were not significantly different at the 0.05 level.

Table 1. Effect of water calcium on the toxicity of cypermethrin to *Labeo rohita* under laboratory conditions

The effects of neem, datura and kalka extracts as probable antidotes against the cypermethrin toxicity using fish survivability as indicator have shown in Table 2. Datura extract (10%w/v) at the levels of 2.5 and 5.0 ml/l exhibited 37 and 50% fish survivability after 96 hours of exposure with lethal concentration of cypermethrin. The fish survivability between these two levels of datura extract showed a significant (P<0.05) difference. Hundred percent of fish mortality recorded both at neem and kalka extract after 96 hours of exposure with lethal level of cypermethrin. It may be mentioned here that at neem and kalka extracts, the fish died within 12 hours.

Observation at different time intervals (hrs.)	Survivability percentage of control	Survivability percentage in cypermethrin (37 ml a.i./ha/m	Survivability percentage in cypermethrin and an		
			Neem (10%w/v)@ 10 ml/l	Neem (10% w/v)@ 25 ml/l	Datura@ 2.5 ml/l
1	100	100	100 ^a	100 ^a	100 ^a
2	100	100	100 ^a	100 ^a	100 ^a
4	100	100	100 ^a	100 ^a	100 ^a
6	100	25	100 ^a	100 ^a	100 ^a
12	100	0	0	12 ^b	62 ^b
24	100	0	0	0	37 ^c
48	100	0	0	0	37 ^c
72	100	0	0	0	37 ^c
96	100	0	0	0	37 ^c

*In control experiment of Kalka (i.e. only kalka), the fish died within 12 hours.

*Values followed by the superscript were not significantly different at the 0.05 level.

Table 2. Efficacy of some antidotes to cypermethrin on survivability (%) of Labeo rohita under laboratory conditions.

Observation at different time intervals (hrs.)	Survivability percentage of control	Survivability percentage in Carbofuran (15 mg a.i./l)	Survivability percentage in carbofuran and different doses of antidote calcium(mg/l)*				
			50	100	200	300	400
1	100	37	100a	100a	100a	100a	100a
2	100	37	100a	100a	100a	100a	100a
4	100	37	100a	100a	100a	100a	100a
6	100	25	50b	50b	50b	100a	100a
12	100	0	50b	50b	50b	50b	50b
24	100	0	25c	50b	50b	0	0
48	100	0	0	50b	50b	0	0
72	100	0	0	50b	50b	0	0
96	100	0	0	50b	50b	0	0

*Values followed by the same superscript were not significantly different at the 0.05 level.

Table 3. Effect of water calcium on the toxicity of carbofuran to *Labeo rohita* under laboratory conditions

The effects of datura, neem and kalka extracts as probable antidotes against carbofuran toxicity using fish survivability as indicator are presented in Table 4. Datura extract (10% w/v) at the concentrations of 2.5 and 5.0 ml/l showed 50 and 37 percent fish survivability after 96 hours of exposure with lethal concentration of carbofuran. The fish survivability at these two levels of datura extract exhibited a significant ($P<0.05$) difference. Hundred per cent of fish mortality was observed at all the concentrations of neem and kalka extracts with the exposure of lethal level of carbofuran. It is important to note that the fish died within an hour at neem extracts while at kalka extracts, the fish died within 4 hours against the lethal level of carbofuran.

The toxic effects of both cypermethrin and carbofuran decreased at medium level (100-200 mg/l) of calcium of water. The results indicate that the degradation of these insecticides take place at medium calcium level of water. The decreased toxicity at this level of calcium could be associated with accumulation of insecticides in excess amount which may be metabolized and stored in different tissues. The accumulated insecticides are eliminated through urine of faeces or both with the help of liver, intestine and kidney (Subbiah et al., 1985). Metz and

Observation at different time intervals (hrs.)	Survivability percentage of control	Survivability percentage in carbofuran (15 mg a.i./l)	Survivability percentage in carbofuran and Datura		
			Neem (10% w/v)@ 5ml/l	Neem (10% w/v)@ 10ml/l	Datura@ 2.5ml/l
1	100	100	0	0	75 ^a
2	100	100	0	0	75 ^a
4	100	100	0	0	75 ^a
6	100	25	0	0	62 ^a
12	100	0	0	0	50 ^b
24	100	0	0	0	50 ^b
48	100	0	0	0	50 ^b
72	100	0	0	0	50 ^b
96	100	0	0	0	50 ^b

*Values followed by the superscript were not significantly different at the 0.05 level.

Table 4. Efficacy of some antidotes to carbofuran on survivability (%) of *Labeo rohita* under laboratory conditions.

Branacin (1975) reported that the degradation of endosulfan took place at high pH and hardness of water but in case of triazophos, its toxicity was increased in similar conditions

while Khalid (1985) reported that water hardness had no significant affect on endosulfan toxicity to four species of fish. Synthetic pyrethroids and natural pyrethrums were more toxic in hard water (Mauck et al., 1976).

From the present study, it is clear that both the neem and kalka extracts had no antitoxic effects against cypermethrin and carbofuran poisoning, while acetone extracts of datura served as anti-toxicant against cypermethrin and carbofuran poisoning. The medicinal and therapeutic roles of datura fruit and its seeds have already been established. The young fruits are sedative and slightly intoxicating. The seeds are antispasmodic, narcotic, febrifuge, anthelmintic, works well in inflammation, alexiteric, emetic and useful in leucoderma, ulcers, itching, etc. The seeds contain both hysocyamine and scopolamine (Kirtikar and Basu, 1935). These medicinal qualities have a positive role for the survivability of pesticide intoxicated fish but the specific causes behind it is unknown and its need further investigation. However, it may be due to narcotic and intoxicated properties of datura which may have a neutralizing action against pesticide contamination.

It can be mentioned here that vitamins are also having antitoxic effects against insecticide poisoning. For example, vitamins like Macraberin forte (a mixture of vitamins) served as antitoxicant against malathion poisoning to *C. punctatus* (Vaid and Mishra, 1977). When Channel catfish were exposed to various amounts of toxaphene (an organochlorine insecticide) in the diet for 150 days, a dose dependent depression of backbone collagen and spiral deformities were occurred but it recovered effectively when ascorbate (vitamin C) was added to the diet. Vitamin C supplements also reduced the whole body residues of toxaphene (Mayer et al., 1978).

5. Conclusion

From the present investigation, it is evident that though, pesticides induced changes of the fish could be improved/protected to a great extent by the application of acetone extract of datura and liming (calcium) up to a certain extent, further investigations are necessary in this regard. Therefore, judicious application of carbofuran in the paddy field to control rice pest and cypermethrin in the pond to control mosquito eggs and Argulus of fish are highly essential for sustainable growth of aquaculture.

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This book is a compilation of 29 chapters focused on: pesticides and food production, environmental effects of pesticides, and pesticides mobility, transport and fate. The first book section addresses the benefits of the pest control for crop protection and food supply increasing, and the associated risks of food contamination. The second book section is dedicated to the effects of pesticides on the non-target organisms and the environment such as: effects involving pollinators, effects on nutrient cycling in ecosystems, effects on soil erosion, structure and fertility, effects on water quality, and pesticides resistance development. The third book section furnishes numerous data contributing to the better understanding of the pesticides mobility, transport and fate. The addressed in this book issues should attract the public concern to support rational decisions to pesticides use.

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