

ADVERSE EFFECTS OF ALPHAMETHRIN 10EC AN AGRICULTURAL PESTICIDE ON BLOOD CONSTITUENTS OF A FRESH WATER RAY FINNED FISH BRONZE FEATHERBACK (NOTOPTERUS NOTOPTERUS)

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Reference to this paper should be made as follows:

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“Adverse Effects of Alphamethrin 10EC an Agricultural Pesticide on Blood Constituents of a Fresh Water Ray Finned Fish Bronze Featherback (*Notopterus notopterus*)”

Voyager: Vol. XV,
Sept. 2024 (Special Issue)
Article No. 01
pp.01-07

Similarity Check 0%

Online available at:
www.anubooks.com

DOI: <https://doi.org/10.31995/voyager.2024.v15.SI.001>

ABSTRACT

The aim of this investigation is to assess the adverse effect of Alphamethrin 10EC a widely used agricultural pesticide on various blood constituents of the selected fish Bronze featherback (*Notopterus notopterus*). Continuous *in vivo* bio assays with fresh dose of pesticide containing water every six hrs was followed. The LC_{50} dose decreased sharply as the observation period was extended upto 24 hrs. Dose for Alphamethrin 10EC was 16.0 mg-1. It was reduced to 12.5 mg-1 at 48 hr duration of exposure and further decreased to 5.0 mg-1 at 72 hrs. Sampling LC_{50} 24 hrs. Alphamethrin 10EC was 16.0 mg-1 which was three fold the strength of this pesticide for LC_{50} 48 hrs. Chronic level of Alphamethrin 10EC caused marked depletion of cholesterol from 48 hrs. Acute level of Alphamethrin 10EC caused a marked globulin fall at 24 hr and produced relatively less adverse effect on serum protein. Although the globulin level continued to decrease on acute exposure from 24 through 48 to 72 hr. the amount of globulin showed some sign of recovery after 48 hr. Serum cholesterol and Serum protein were highly sensitive biochemical parameter which can be used as an index of the toxic effects of a pesticide in the ray finned fish Bronze featherback (*Notopterus notopterus*).

KEYWORDS

Serum Protein, Serum albumin, Serum cholesterol, Blood Glucose, Pesticide, Bronze featherback.

INTRODUCTION

Studies have shown that the organo-chlorine group of pesticides are more persistent in the environment, soil or water than organophosphorus but organophosphorus pesticides have been suspected to cause more serious damage to the fisheries. Mathur (1969) in his study of Thiodan toxicity to some fresh water fishes provided that fish exposed to pesticides died due to pathological changes in their organs. The Histopathology of fish exposed to pesticides have been investigated by people.

Anees (1978) made a histopathological study of the liver of the fish *Channa punctatus* on exposure to sublethal and chronic doses of organophosphorus insecticides and found important changes in liver histology. Sprague (1971) differentiated between the concept of sub-lethal and safe concentrations for a number of harmful substances.

Arora et al. (1971) studied the toxicity of Endosulfan, to *Puntius sophore*. Bhatia et al. (1972) have found a rise in serum cortisol in fish treated with organochlorine or organophosphorus pesticides. Arora et al. (1972) also worked on the gross effects of six insecticides on an Indian major carp, *Cirrhinus mrigala*. Hatfeld & Anderson (1972) found that the parr of Atlantic Salmon becomes susceptible to predatory action by brook trout if it is exposed to some insecticides. The above studies and findings provides the possibility that the pollution of water by insecticides may alter the vulnerability of some economical species of fishes which are attacked by predators a long term effect of such type of changes can be most harmful for the fishing of an area. Hatfeld and Johansen (1972) have found that a number of insecticides had affected the parr of Atlantic Salmon for learning a conditioned response. These toxicants also the retention of learning of conditioned response. From the above review it is clear that insecticides also affect the behaviour of the fishes and due to this the important aspects of behaviour are affected for example alarm reaction and escape behaviour, in this way the fish may ultimately suffer to an extent which cannot be anticipated. Toxicologists these days have developed immense interest in the studies of pesticides exposure to fish and its consequences on its blood.

MATERIALS AND METHODS

An experiment for determination of safe concentration was done for thirty days with a dose of insecticide approximately half of the LC50 72 hr dose. Only two of the treated fish died in a period for one month in the four sets of experiments. It was therefore, decided to use an application factor equivalent to 0.1. For blood chemistry, the blood was collected in a double distilled water washed syringe. About 3 to 5 ml blood was sucked into the syringe and was poured into a heparinized tube along its wall after removing the needle from the syringe.

The heparinized blood taken from the caudal vein by cut at the tail end of the fish was left to stand for 30 minutes. After clotting, the serum was drawn by the process of centrifugation to the upper fluid phase of clotted blood in a centrifuge tube at 3000 rpm. In a corning test tube, 10 ml of 0.03% ferric chloride-acetic acid reagent (30 mg iron III chloride dissolved in 100 ml glacial acetic acid) was poured by a 10ml pipette. In this test tube, 0.1 ml of serum was added with the help of a 0.1 ml pipette.

For preparation of standard solutions, 10 ml of iron III chloride- acetic acid reagent was taken in four glass stopper test tubes. In each tube 0.1 ml of four different standard solutions of cholesterol (50 mg in 100 ml glacial acetic acid; 100 mg in 100 ml l acetic acid glacial, 150 mg in 100 ml glacial acetic acid and 200 mg in 100 ml glacial acetic acid) were added separately and were passed through all the processes as in the experimental test. Blood was drawn in a vial as described in blood cholesterol estimation and the serum separated. A 0.4 ml of serum was added in 6 ml of 23.68% sodium sulphate solution in a clean and dry hard glass test tube. As soon as serum was added the protein precipitated as yellow coloured precipitate. The solution was mixed well by repeated inversions of the test tube (not by shaking). After leaving for half an hour 2 ml of uniform suspension was pipetted out and placed in a separate test tube with the help of a 2 ml pipette. In this tube 5 ml of Biuret reagent, the composition and preparation of which is given later was added.

The remaining 4 ml solutions of sodium sulphate containing serum of the previous described protein test was incubated at 37°C for 30 minutes. Then it was filtered twice through Whatman's filter paper No. 1. Two ml filtrate was taken in a test tube and 5 ml Biuret reagent was added to it. A colour was developed. The OD was read within 10-20 minutes. The spectrophotometer was set to zero OD with the Biuret reagent as blank at 555 mu. Serum albumin of unknown sample was calculated in g% or g/ 100 ml of serum from the standard protein graph. Globulin values was calculated by deducting the albumin value from the total serum protein.

RESULT AND OBSERVATIONS

Consequences of Alphamethrin 10EC Exposure in Blood Chemistry

Serum cholesterol

The untreated fish at the start of the toxicological test recorded a level of 700.0 ±14.49 mg/100 ml of serum cholesterol. After 24 hr of exposure at 18.0 mgL⁻¹ of Alphamethrin 10EC a very significant fall ($p < 0.001$) in cholesterol level was recorded in the treated fish (456.0 ± 69.95 mg/ 100ml), There was a wide variation in the serum cholesterol level among the fish belonging to this group. Later at 48 hr the value obtained was 613.0 ± 23.36 mg/ 100 ml. This level though much lower than that of initial control was significantly ($p < 0.001$) greater than the highly depleted level recorded at 24 hr. After 72 hr serum

cholesterol content was 666.0 ± 25.17 mg/100 ml. This value although lower than the initial value, was much higher than that observed after 48 hr of exposure of the fish. There was thus a sudden extreme depletion in the serum cholesterol level within 24 hr of the exposure of the fish to Alphamethrin 10EC. Longer exposure with reduced strengths of the pesticide caused a gradual recovery.

Blood Glucose

Blood glucose in the untreated fish at the commencement of toxicity tests stood at 107.0 ± 6.13 mg/100 ml. On exposure to LC₅₀ 24 hr of Alphamethrin 10EC, a significant hyperglycemia in the treated fish was found 177.50 ± 13.74 mg/100 ml ($p < 0.001$). Alphamethrin 10EC treatment in the initial stage evoked a hyperglycemic response in the fish. After 48 hr exposure to 10.5 mg/l of Alphamethrin 10EC corresponding to LC₅₀ blood glucose showed a slight fall (97.6 ± 8.30 mg/100 ml) compared to the untreated initial value ($p > 0.05$). At LC₅₀ 72 hr dose blood glucose again registered a marked rise to 224.80 ± 8.80 mg/100 ml in fish exposed to Alphamethrin 10EC. Alphamethrin 10EC treatment 16.0 mg/l caused a marked hyperglycaemia at 24 hr, whereas 10.5 mg/l of Alphamethrin 10EC after 48 hr actually produced a slight fall in blood glucose, Extended exposure (72 hr) at a reduced strength (5.0 mg/l) however, again caused a massive hyperglycaemia.

Table: Blood glucose in mg/100 ml in untreated and pesticide exposed Bronze featherback (*Notopterus notopterus*)

Control	24hr at LC ₅₀ dose	48hr at LC ₅₀ dose	72hr at LC ₅₀ dose
110.72 ± 4.40	70.0 ± 4.60	94.90 ± 5.80	122.40 ± 13.76

Total Serum Protein

At LC₅₀ 24 hr of Alphamethrin 10EC there was a decrease ($p < 0.001$) in the amount of serum protein from 9.21 ± 0.29 g/100 ml in the initial control to 3.53 ± 0.53 g/100ml in the treated fish when the exposure was continued to 48 hr with lowered strength (12.5 mg/l) of the pesticide, total serum protein improved to 6.25 ± 0.52 g/100 ml. At the completion of LC₅₀ 72 hr dose the total serum protein in the untreated fish increased further to 6.67 ± 0.66 g/100 ml. There was thus initially a sharp decline in total serum protein but continued exposure with decreasing strength of the pesticide elicited a tendency towards recovery. However, at the end of all the three total serum protein level was significantly lower than the control.

Serum Albumin

The amount of albumin in the control group at the beginning of the toxicological tests with Alphamethrin 10EC was 2.21 ± 0.14 g/ 100 ml. At LC₅₀ 24 hr with 18.0 mg/l of Alphamethrin 10EC a very marked decrease to 0.59 ± 0.22 g/ 100ml in serum albumin content was noted ($p < 0.001$). After 48 hours of treatment with 10.5 mg/l of Alphamethrin 10EC, equivalent to LC50 45 hr, the albumin level of the serum rose to 1.75 ± 0.20 g/ 100 ml but this was still lower than the control value ($p < 0.001$). At LC50 72 hr a slight rise compared to that of 48 hr was noted. This level (1.85 ± 0.16 g/ 100 ml) was also significantly less than the initial control level ($p < 0.05$). In short, after a massive depletion in albumin content after 24 hours of exposure, there was a distinct tendency towards recovery in fish exposed to longer duration at gradually reduced strength.

Serum Globulin

The globulin content of blood at the end of 24 hr exposure fell to 2.94 ± 0.16 g/ 100 ml from 7.0 ± 0.20 g/ 100 ml in the control. Later, at LC 50 48 hr dose globulin content recovered to 4.50 ± 0.22 g/ 100 ml and at LC50 72 hr dose it further increased to 4.82 ± 0.14 g/ 100 ml. The trend i.e. initial marked decrease followed by a gradual recovery in fluctuation of globulin content was the same as recorded for total serum protein. All these depletion were statistically important as compared to initial control ($p < 0.001$).

Table: 1 Serum Protein, Serum Globulin and serum protein in g/100 ml in untreated and pesticide exposed Bronze featherback (*Notopterus notopterus*).

Total Serum Protein				Serum Albumin				Serum Globulin			
Control	LC ₅₀ does at			Control	LC ₅₀ does at			Control	LC ₅₀ does at		
	24 hr	48 hr	72 hr		24 hr	48 hr	72 hr		24 hr	48 hr	72 hr
8.17	0.46	0.12	0.42	2.31	0.09	0.03	0.03	5.49	0.37	0.09	0.34
±	±	±	±	±	±	±	±	±	±	±	±
0.08	0.16	0.06	0.14	0.20	0.04	0.02	0.00	0.16	0.05	0.02	0.01

Albumin/Globulin Ratio

Albumin/Globulin ratio in the untreated fish was 0.31:1. Exposure to Alphamethrin 10EC for 24 hr with 16.0 mg/l caused the ratio to decrease to 0.20 : 1, due to a greater reduction in the amount of albumin than that of globulin ($p < 0.05$). At LC50 48 hr this ratio increased to 0.34 : 1 owing to a far greater recovery in albumin at 48 hr than that in globulin.

After 72 hr of exposure to 5.0 mg/l of Alphamethrin 10EC equivalent to LC50 72 hr the albumin / globulin ratio was the same as that recorded after 48 hr exposure. In short the A/G ratio initially decreased considerable, but later on recovered to rise more than the ratio recorded in the control fish.

DISCUSSION AND CONCLUSION

Fish exposed to Alphamethrin showed greater agitated swimming. In the present study Alphamethrin 10EC an agricultural pesticide, also adversely affected the equilibrium centers of the brain but was different from the organophosphorus pesticide in causing inhibition of mucous cells.

Normal blood cholesterol levels have been studied in fishes by a number of workers (Love, 1970). The blood cholesterol content is usually high in fishes, often much higher than mammals. Cholesterol is utilized for conversion into bile salts, for synthesis into steroid hormones for conjugation to degradation products of foreign chemicals and is often eliminated from the body with the bile. Although a simultaneous estimation of cholesterol in liver was not made in this study, it can be assumed that depletion in serum cholesterol which was a regular feature in Bronze featherback (*Notopterus notopterus*) exposed to the pesticides was probably caused by its transport to liver in response to the presence of pesticides in the body. Bano, (1982) observed a reverse condition in *C. batrachus* exposed to Thiodan. Serum cholesterol increased and cholesterol in liver decreased. In view of these, it is suggested that the pesticides specially organophosphorus and organochlorine, caused a transfer of cholesterol from blood to liver where they were utilized to produce increasing amount of bile salts to eliminate the insecticides. It has been suggested in the past that the high cholesterol level found in the blood of salmon at the end of the spawning migration provide energy for the fish by gluconeogenesis after exhaustion of their lipid reserves. In the present study in between, at 48 hr. there was a slight fall in blood glucose level. In Bronze featherback (*Notopterus notopterus*) Alphamethrin 10EC produced less pronounced effect on serum protein. The albumin level continued to fall on acute exposure from 24 hr through 48 – 72 hr, the amount of globulin showed clear sign of recovery after 48 hrs. With Alphamethrin 10EC the recovery was observed from 24 hr onwards. A late marginal recovery in the level of globulin in the treated fish may be due to the feeble resistance probably developing in the fish against the toxicants through formation of anti-toxicants which are also proteinaceous in nature. Significant decrease in organ protein levels on pesticide exposure have been found by a number of workers in fishes.

FUTURE SCOPE

The present study highlights the insights on the lethal toxicity of various levels of Alphamethrin 10EC in the blood chemistry of Bronze featherback (*Notopterus notopterus*),

whereas a concrete conclusion cannot be determined based on only a certain type of agropesticide. Hence, it is proposed that a detailed study using several other agropesticide can be investigated on several Haematological aspects of various Indian fishes residing in water bodies close to agricultural fields where the practice of such pesticides are critically high.

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