EFFECT OF EXPOSURE TO SUBLETHAL CONCENTRATIONS OF GAMMALIN 20 AND ACTELLIC 25 EC ON THE LIVER AND SERUM LACTATE DEHYDROGENASE ACTIVITY IN THE FISH *Clarias albopunctatus*

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ABSTRACT

One hundred and eighty adult Clarias albopunctatus (mean weight 160±2.7g) were subjected to sublethal concentrations of Gammalin 20 and Actellic 25 EC (0, 0.3;and 1.0 μg/l) in a static bioassay renewal system for 18 days. The changes in the activities of the liver and serum lactate dehydrogenase (LDH) during the period of exposure were studied. The exposure of C. albopunctatus to these pesticides evoked significant increase (P< 0.05) in both the liver and serum LDH activities. There was a progressive increase in both the liver and serum LDH activities following exposure to the pesticides. When compared with the control, the LDH activities were significantly higher (p < 0.05) in the treatment groups. The LDH activities in both the liver and the serum were higher in the fish exposed to Gammalin 20 than in the fish treated with similar concentrations of Actellic 25 EC. The serum LDH activity in the group exposed to a mixture of 0.3μg/l of Gammalin 20 and Actellic 25 EC was significantly higher the activities in the fish exposed the either 0.3 μg/l Gammalin 20 or Actellic 25 EC. These observations suggest that these pesticides affect the energy metabolism of the fish.

Keywords: *Clarias,* Liver, Serum, Lactate dehydrogenase, Gammalin 20, Actellic 25 EC

INTRODUCTION

Actellic 25 EC is an organophosphorous pesticide also known as Primiphos-methyl. It is a broad-spectrum pesticide against stored product pests (Worthing and Hance, 1991). On the other hand, Gammalin 20, a widely applied pesticide in Nigeria, is an organochloride pesticide containing 20 % lindane. The application of pesticides, either for agricultural purposes or for the control of pests of public health interests, contaminates the environment or hence endangers the non-target organisms in both terrestrial and aquatic habitats.

The value of tissue enzyme activities in the diagnosis of the effects of pollutants is one of the emerging areas of interest in aquatic toxicology, monitoring and remediation programmes. Thirugnam and Forgash (1977) studied the anticholinesterase effect of chlorpyrifos to the fish *Fundulus heteroclistis.* Increased glucose-6-phosphatase and glycogen phosphorylase activities were observed in *Cyprinus carpio* exposed to paraquat (Simon *et al.,* 1983). Also, Rashatwar and Ilyas (1983), noted that Basalin, a herbicide affected the activities of lactate dehydrogenase, alkaline phosphatase as well as glutamic pyruvate transaminase in the freshwater fish *Nemachelinus* sp. Increased activities of alanine and aspartate aminotransferases were reported in *Clarais albopunctatus* exposed to copper (Oluah and Amalu, 1998), zinc and mercury (Oluah, 1999). Verma *et al.* (1981) observed that pesticides inhibited alkaline phosphatase and glucose-6-phosphatase activities in the fish *Mytulus vittatus.*

The purpose of this study was to investigate the effect of the pesticides - Actellic 25 EC and Gammalin 20, widely used in Nigeria, on the activities of liver and serum lactate dehydrogenases in the catfish, *Clarias albopunctatus.*

MATERIALS AND METHODS

The one hundred and eighty fish (mean weight 110 ± 2.6 g) used in the study was collected from Anambra River at Otuocha, Anambra East Local Government Area of Nigeria. The fish was transported to our laboratory in a plastic container and was acclimatized for two weeks in plastic aquaria before the commencement of the study.

The fish were divided randomly into six (6) groups (1 – 6) of 30 fish per group. Each group was further divided into three replicate groups of 10 fish per replicate. The fish in group I were exposed to tap water only as the control while the fish in groups 2 and 3 were treated with 0.3 μg/l and 1.0 μg/l of Actellic 25 EC, respectively. Groups 4 and 5 were exposed to 0.3 μg/l and 1.0 μg/l Gammalin 20, respectively. The 6^{th} group was exposed to a mixture of equal concentrations (0.3 μg/l) of Actellic 25 EC and

Gammalin 20.The fish were exposed to these sublethal concentrations of the pesticide in a renewal bioassay system in which the water and the pesticides were changed every two days to maintain the toxicant concentrations. The fish were fed 30 % crude protein diet at 3% body weight daily at 8.00 h. The Experiment lasted for 18 days and the lactate dehydrogenase activity was assayed every six days.

Tissue Collection and Enzyme Assay: The blood samples were collected by both the cardiac puncture method and the severance of the caudal peduncle using the disposable hypodermic syringe (Oluah, 1999). The liver was excised and washed in distilled water to remove traces of blood. The liver samples were macerated and homogenized as described by Devi *et al.* (1993). The liver was homogenized in ice-cold 0.25 M sucrose. The liver homogenate was centrifuged at 5000 rpm for 15 minutes at 4^{o}C.The blood was similarly centrifuged for 15 minutes at 1000 rpm to obtain the serum. The liver supernatant and serum were used for the lactate dehydrogenase (LDH) assay. The lactate dehydrogenase activity was calorimetrically determined at 445 nm using Sigma protocol number 500 (Sigma Chemical Company, St Louis, MO). The data from the replicate experiments were averaged and the mean (± SD) presented. The analysis of variance (ANOVA) was used to analyze the data for statistical significance ($P < 0.05$).

RESULTS AND DISCUSSION

The changes in the liver lactate dehydrogenase (LDH) activity in *Clarias albopunctatus* exposed to sublethal concentrations of Gammalin 20 and Actellic 25 EC are shown in Table 1.The results showed that exposing *C. albopunctatus* to 0.3 and 1.0 μg/l of both pesticides caused significant ($P < 0.05$) increases in the liver LDH activity when compared with the control. The liver LDH activity in the fish exposed to 0.3 μg/l Actellic 25 EC increased from 98.42 ± 1.06 μ/g on the 6^{th} day to 319.8 ± 1.12 μ/g liver on the 18^{th} day. In the fish exposed to 1.0 μg/l Actellic 25 EC, the enzyme activity increased from 246.25 ± 1.08U/g liver on the 6^{th} day to 442.89 ± 1.22 μ/g liver at the end of the study.

The liver LDH activity increased from 147.63 ± 1.15 and 246.05 ± 1.1 μ/g on the 6^{th} day to 393.68 ± 2.04 μ/g and 738.15 ± 1.14 U/g on the 18^{th} day in the fish exposed to 0.3 and 1.0 μg/l Gammalin 20, respectively. In the group exposed to the mixture of 0.3 μg/l Actellic 25 EC and Gammalin 20, the enzyme activity increased from 98.42 ± 1.6 μ/g on day 6 to 442.89 ± 1.06 μ/g on the 18^{th} day. The mean liver LDH activity in the Gammalin 20- exposed fish was significantly ($P < 0.05$) higher than in the fish exposed to Actellic 25 EC. When compared with the control, the liver LDH activity differed significantly ($P < 0.05$) in the treatment groups.

The serum LDH activity increased from 49.21 ± 1.36 U/l on day 6 to 124.61 ± 2.08 U/l on day 18 in the fish exposed to 0.3 μg/l Actellic 25 EC (Table 2). When the fish was exposed to 1.0 μg/l Actellic 25, the LDH activity increased from 123.03 ± 2.12 U/l on the 6^{th} day. When the fish was treated with Gammalin 20, the serum LDH activity increased from 113.18 ± 1.76 U/l and 147.63 ± 1.88 U/l on day 6 to 196.84 ± 2.8 U/l and 270.66 ± 1.06 U/l on the 18^{th} day in the fish exposed to 0.3 and 12.0 μg/l Gammalin 20, respectively. Similarly, the mean serum LDH activity increased from 260.81 ± 2.03 U/l on day 6 to 590 ± 1.2 U/l on the 18^{th} day in the fish treated with a mixture of equal concentrations (0.3μg/l) of Actellic25 and Gammalin20. In this group, there was a 3-fold increase in the LDH activity when compared with the activity in the groups exposed only to 0.3 μg/l of either Gammalin 20 or Actellic 25 EC. The serum LDH was significantly higher ($P < 0.05$) in the in the fish treated with Gammalin 20.When compared with the control, the serum LDH activity was significantly higher ($P< 0.05$) in the fish exposed to the pesticides.

The result showed that the increase in the tissue LDH activity was concentration-dependent and also increased with duration of exposure. The result of the study is consistent with the reports of earlier studies. Simon *et al.* (1983) reported that paraquat caused increased phosphorylase and glucose-6-phosphatase activities in *Cyprino carpio.* Similarly, Reddy *et al.* (1983) reported increased malate dehydrogenase and lactate dehydrogenase activities in the crab *Oziotelphusa senex senex* exposed to sumithion, an organophosphate insecticide. Similar increase in LDH and alkaline phosphatase activities were observed in the English sole *Parophrys vetulus* treated with carbon tetrachloride (Casillas and Ames, 1986). Also, cadmium was reported to have elicited increased muscular LDH activity in Fiddler crab, *Uca pugilator* (Devi *et al.,* 1993) and in the brook trout, *Salvelinus fontinalis* (Christensen *et al.,* 1977). On the other hand, some agrochemicals and heavy metals inhibit tissue enzymes. Thebault and De Caris (1983) reported that trichlorophenoxyacetic acid (2, 4, 5-T), an auxin herbicide, inhibited gill Ca^{2+} ATPase activity in the trout.

Similarly, cholinesterase activity was inhibited in the fish *Callchthys cacllichtys* treated with methyl parathion (Da Silva *et al.,* 1993) and in *Funulus heteroclitus* due to chorphyrifos intoxication (Thirugnanam and Forgash, 1977). Inhibition of Acetylcholinesterase activity was also reported in the Walleye following exposure to chlorphyrifos (Philips *et al.,* 2002). Hilmy *et al.*

Table 1: Changes in the concentrations of liver lactate dehydrogenase of *C. albopunctatus* exposed to varying concentrations of Actellic 25 EC and Gammalin 20

Pesticide	Concentration (μg/l)	Duration of Exposure (days)		
		6	12	18
		Enzyme concentration (U/mg)		
Control	0	49.21 ± 2.0	98.42 ± 1.6	98.42 ± 1.6
Actellic 25 EC	0.3	98.42 ± 1.06	196.84 ± 2.0	319.87 ± 1.12
Actellic 25 EC	1.0	246.05 ± 1.08	275.58 ± 1.1	442.89 ± 1.22
Gammalin 20	0.3	147.63 ± 1.15	275.58 ± 1.3	393.68 ± 2.04
Gammalin 20	1.0	246.06 ± 1.10	344.47 ± 1.7	738.15 ± 1.44
Actellic 25 EC and Gammalin 20	0.3/0.3	98.42 ± 1.6	113.18 ± 1.1	442.89 ± 1.06

Values= (mean ± sd) of LDH activity in each group for 3 determinations

Table 2: Changes in the concentrations of the serum lactate dehydrogenase of *C. albopunctatus* exposed to varying concentrations of Actellic 25 EC and Gammalin 20

Pesticide	Concentration (μg/l)	Duration of Exposure (days)		
		6	12	18
		Enzyme Concentration (U/l)		
Control	0.0	26.61 ± 2.4	24.6 ± 1.8	24.61 ± 2.8
Actellic 25 EC	0.3	49.21 ± 1.36	96.42 ± 2.6	124.61 ± 1.8
Actellic 25 EC	1.0	123.03 ± 2.12	196.84 ± 1.5	246.05 ± 3.04
Gammalin 20	0.3	113.18 ± 1.76	147.63 ± 1.2	196.84 ± 2.8
Gammalin 20	1.0	147.63 ± 1.8	240.05 ± 2.0	270.66 ± 1.06
Actellic 25 EC and Gammalin 20	0.3/0.3	260.81 ± 2.03	344.47 ± 1.8	590.52 ± 1.2

Values= (mean ± sd) LDH activity in each group for 3 determinations

(1985) reported that cadmium inhibited LDH activity in the heart, liver and gills but not in the serum of the fish *Mugil cephalus*. Reduced LDH activity was also observed in the hepatopancreas of the crab *Uca pugilator* (Devi *et al.,* 1993). The result of this study is consistent with the reports of the effect of agrochemicals on LDH activity in mammals.

Parathion was found to elicit increased LDH activity in rats (GalloandLawryk, 1991). Similarly, Junge *et al.* (2001) reported a 2-fold increase in liver myelopreoxidase activity in the rat exposed to lindane.

Lactate dehydrogenase is known to catalyse the biochemical process of converting pyruvate to lactate with the attendant oxidation of NADPH. Thus, the increased LDH activity in both the liver and serum are indications of a shift in the carbohydrate metabolism from the glucose and glycogen catabolism to lactate synthesis. This shift in carbohydrate metabolism reflects the possible dependence of *C. albopunctatus* on anaerobic pathway during exposure to sublethal Actellic 25 EC and Gammalin 20. This goes to confirm the report of Omoregie *et al.* (1990) that these insecticides induce increased plasma lactate concentration in *Oreochromis niloticus.* This situation would predispose the fish to lactic acidosis, which may impact adversely on the health of the fish.

In conclusion, Actellic25 EC and Gammalin 20 were found to elicit increased lactate dehydrogenase activity in the fish *C. albopunctatus* with its attendant physiological stress.

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