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ACUTE TOXICITY AND BEHAVIOURAL EFFECTS OF LAMBDA-CYHALOTHRIN PESTICIDE ON JUVENILES OF *CLARIAS GARIEPINUS*.

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ABSTRACT

Lambda-cyhalothrin was evaluated in laboratory studies to determine its 96-h acute toxicity, using juveniles of *Clarias gariepinus*. The fish was exposed in glass aquaria to 0.008mg/l, 0.009mg/l, 0.010mg/l, 0.011mg/l and 0.012mg/l. The pesticide was found to have lethal effects on fish as they changed fish behaviour resulting in death. The unique behavioural symptoms elicited by the toxicant included gill failing, excessive lateral flexure and spasms. The 96-h LC_{50} value for *C. gariepinus* was estimated as 0.008mg/l. The behavioural toxicology bioassay may be valuable in comparing and predicting the mode of action of new or unknown toxicants in this species of fish.

KEYWORDS: Lambda-cyhalothrin, acute toxicity, Behaviour, *C. gariepinus*

INTRODUCTION

Chemicals originating from agricultural activity enter the aquatic environment through atmospheric deposition, surface run-off or leaching (Kreuger *et al*, 1999) and frequently accumulate in soft-bottom sediments and aquatic organisms (Kreuger *et al*, 1999). In many parts of the world, pesticides have been found in the aquatic ecosystem and often information of how these pesticides affect other organisms in the ecosystem is scanty.

Karate 2.5EC (Syngenta Crop Protection AG, Switzerland) contains 25g/l lambda-cyhalothrin and is used for the control of insect pests, including those of cotton (bollworms, leaf rollers and stainers); cowpea, groundnut and vegetables (caterpillars and sucking pests); and maize and rice (caterpillars such as stalk borers and head borers). It may also be used in public health applications to control insects such as cockroaches, mosquitoes, ticks and flies which may act as disease vectors (EXTOXNET, 1995). Either ground or aerial application is employed. Lambda-cyhalothrin act on the axons in the peripheral and central nervous systems (Leahey, 1985). They are believed to interfere with sodium channels and the permeability of nerve cells, so affecting the transmission of nerve impulses (Leahey, 1985). Pyrethroids have been reported to modulate the release of acetylcholinesterase in the brains hippocampus region (Hossain *et al*, 2004). In addition, pyrethroids can disrupt hormone – related functions (Go *et al*, 1999). In mammals pyrethroids decrease progesterone and estradiol production (Chen *et al*, 2005), eliciting estrogenic effects in females and anti-androgenic effects in males (Kim *et al*, 2005).

Furthermore, pyrethroids have been shown to inhibit cell cycle progress (Agarwal *et al*, 1994), cause cell stress (Kale *et al.*, 1999), and have immunosuppressive effects (Clifford *et al*, 2005).

The American Chemical society database indicates that there were some 13 million chemicals identified in 1993 with some 500,000 new compounds being added annually (FAO, 2004). In 1989- 1990, world-wide annual production of pyrethroids was at least 2000 tonnes (IPCS, 1990). The aim of this study is to determine the acute toxicity and behavioural effects of lambda – cyhalothrin to juveniles of *Clarias gariepinus* which is a popular species in warm water aquaculture, indigenous to Africa and widely distributed and accepted by farmers because of its numerous good qualities among which include fast growth, hardness, large size and high market value.

MATERIALS AND METHODS

EXPERIMENTAL FISH

Healthy *Clarias gariepinus* juveniles (average weight 16.35± 1.25g and standard length 10.5± 0.05cm) were obtained from Kubani Dam, Ahmadu Bello University, Zaria. The fish were conveyed to the Fisheries Laboratory in a portable well-aerated white polythene bag containing water from the dam. They were held in large water baths of 160L capacity at 24.5-25.5^{0}C and acclimatized for two weeks in dechlorinated municipal water. A total of 180 specimens were randomly assigned to give a loading of 10 fish per tank to avoid overcrowding. During this period, the fishes were fed with pelleted diet (groundnut cake, bone meal, fish meal, maize flour, palm oil, vitamin premix, salt and warm water) containing 35% crude protein twice per day at 5% body weight. Also, the water in the glass aquaria was changed once every two days. The fishes were accepted as well as adapted to laboratory conditions when less than 5% death was recorded for the 14 days period and feeding was discontinued 24 hours before the start of the experimental run (Reish and Oshida, 1987).

ACUTE BIOASSAY

Acute 96-h static bioassays were conducted in the laboratory following the methods of Sprague (1975) and APHA (1985). The nominal concentration for lambda-cyhalothrin was 0.008 mg/l, 0.009 mg/l, 0.010 mg/l, 0.011 mg/l, 0.012 mg/l. and a control with no toxicant. Each concentration was replicated three times. The desired stock solution was measured and introduced into 25 L of dechlorinated (municipal water collected in large open containers and exposed for 48-hrs before use) tap water in the glass aquaria. The mixture was allowed to stand for 30 minutes before introducing test fishes. A total of 180 fish were stocked to give a loading rate of 10 fish per tank. Survival and mortality were recorded from 1to 6, 8, 16, 24, 72 and 96 hours. Fishes were considered dead when the opercular movement ceased and there was no response to gentle probing.

BEHAVIORAL AND MORPHOLOGICAL ASSAYS

Observations of behavioral and morphological response of *C. gariepinus* juveniles exposed to lambda-cyhalothrin were conducted at 1 through 6, 8, 12, 24, 48, 72 and 96-h during the acute toxicity tests. The methods

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developed by Drummond *et al.* (1986) were used for this study .Controls without toxicant were monitored, along with the nominal concentrations, to provide a reference for assessing any behavioral and morphological changes. Responses were recorded if they differed from the controls and occurred in 10% of the fish within each test chamber. Five behavioral and morphological indicators were observed in this study: loss of equilibrium, general activity, startle response, hemorrhage, and deformity (including postural indicators). Each test chamber was observed for 5 to 10 min. Startle responses were monitored by the following procedures in sequence: passing a hand over the test chamber (overhead moving visual stimulus), rapping on the chamber (vibrational stimulus), and lightly touching the fish

STATISTICAL ANALYSIS

Graph of probit kill against log concentration was used to determine the 96-h LC_{50} GenStat – Release 4.2 programmes was used to run analysis of variance (ANOVA) and Duncan multiple range test (DMRT) was used to test for differences between different levels of treatment and to separate means respectively, were applicable (Duncan, 1955). Test of significance was at 95% probability.

RESULTS

Agitated movements accompanied with excessive lateral flexure and reduced swimming (hypoactive) occurred in the early hours (2-h to 4-h) of fish exposure to lambda-cyhalothrin and this preceded mortality (Fig 1). By 12-hrs maximum mortality had been recorded, and surviving fish gradually recovered from the symptoms. The agitated movements and lateral flexure/spasm was dose and time dependent (Fig 1). Fish mortality occurred in all the test tanks except control and the mortality was significantly dose and time dependent ($p<0.05$; Table 2 & 3). The 96-h median lethal concentration (LC_{50}) of lambda-cyhalothrin to *Clarias gariepinus* was estimated to be 0.008 mg/l (Fig 2). There was also significant difference ($p<0.05$) between the control opercular beat and that of exposed fish. The alteration was time dependent (Fig 3 and Table 3). All exposed fish except control ceased opercular beat after some minutes of introduction and resumed at about 48-hrs (Fig 3). There was significant difference ($p<0.05$) in the mean tail fin beat across time (Table 3).Tail fin beat was highest from 0 – 5 hrs and began to reduce up to 48 hrs where the least mean value was recorded (Table 3). The behavioural and morphological symptoms observed due exposure of fish to the toxicant included: Air gulping, loss of equilibrium, and under general activity, we noted agitated movements, excessive lateral flexure/spasms, gill failing for 24-hrs, mucus secretion, hyperactive to hypoactive in the caudal area (Table 1). These symptoms were preceded by lack of response to stimulus and normal swimming behaviour. No hemorrhage and deformities were observed (Table 1). All the morphological and behavioural symptoms were noted before mortality in the fish.

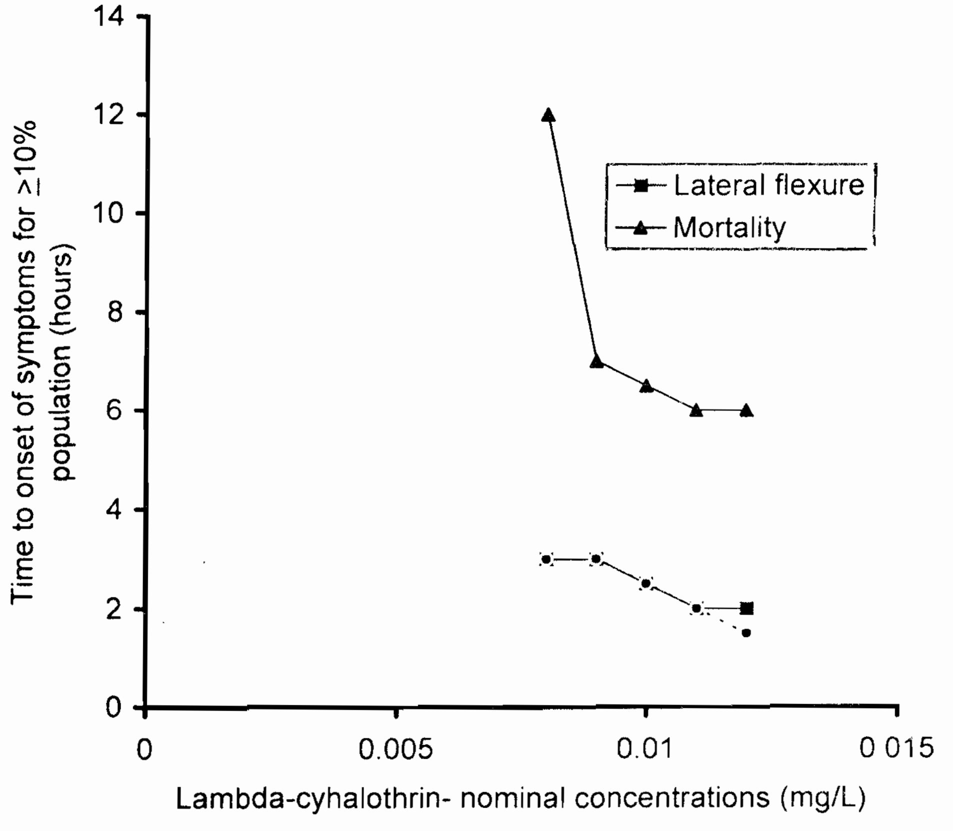

Fig. 1: Initial onset of morphological responses and mortality in juveniles of *C. gariepinus* exposed to nominal concentrations of lambda-cyhalothrin

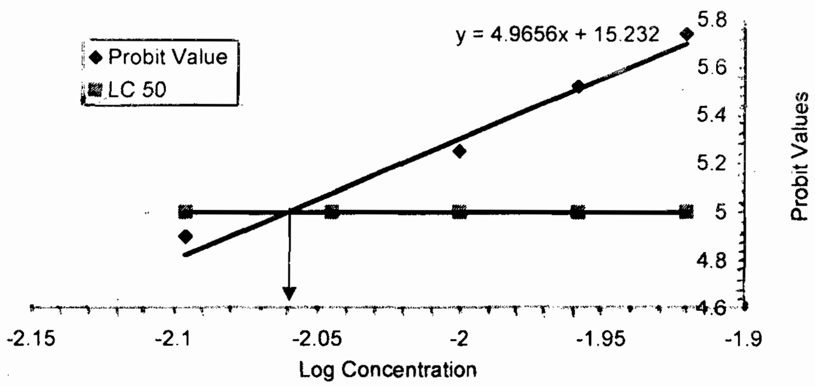

Fig 2: 96 hours LC 50 Lambda-cyhalothrin for juveniles of *Clarias gariepinus*

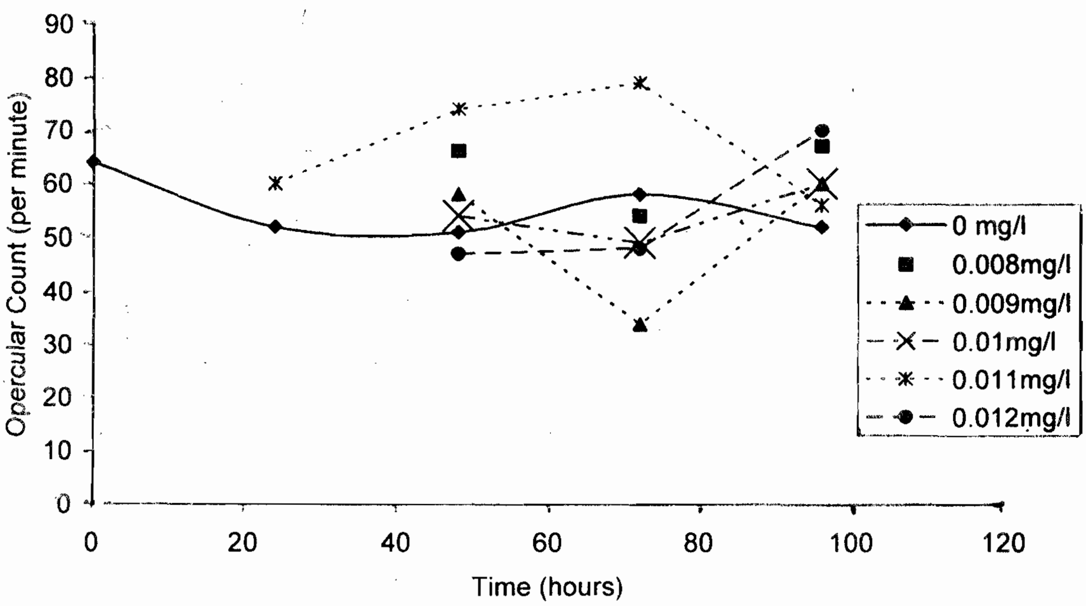

Fig 3: Onset of opercular ventilation against time at different concentrations of Lambda-cyhalothrin for juveniles of *C. gariepinus*

Table 1: Diagnostic symptoms that was used to determine the behavioural effects of lambda-cyhalothrin on juveniles of *C.gariepinus*

Behavioural and Morphological Symptoms	Diagnosis
Loss of equilibrium	Yes
General activity	Hyperactive to Hypoactive[d] "Spasm"[ac] Excessive lateral flexure[acd] Opercular ventilation after 24 hours[c] Mucus secretion[c]
Startle response	Underreactive[b]
Hemorrhage	None
Deformities	None

[a]Before death
[b]Severely intoxicated fish ceased to respond
[c]Distinguishing behaviour, morphological change or signs of stress
[d]In caudal area

Table 2: means for *c. gariepinus* behaviour exposed to acute nominal concentrations of lambda-cyhalothrin

Concentration (mg/l)	Mortality	Opercular Count	Tail fin Beat
0.00	0.00^{e}	52.67^{a}	108.20^{a}
0.008	0.93^{d}	40.00^{d}	108.40^{a}
0.009	0.93^{d}	42.67^{c}	108.20^{a}
0.010	1.20^{c}	31.93^{e}	108.40^{a}
0.011	1.40^{b}	44.53^{b}	108.00^{a}
0.012	1.53^{a}	30.40^{f}	108.40^{a}

Means with the same superscript along columns are not significantly different ($p < 0.05$)

Table 3: means for *c. gariepinus* behaviour exposed to acute nominal concentrations of lambda-cyhalothrin over time

Time Hours	Mortality	Opercular Count	Tail fin Beat
12	3.39^{a}	10.33^{d}	123.00^{a}
24	1.17^{b}	8.67^{e}	88.78^{e}
48	0.22^{bc}	59.83^{b}	111.33^{c}
72	0.22^{bc}	65.00^{a}	113.83^{b}
96	0.00^{c}	58.00^{c}	104.39

Means with the same superscript along columns are not significantly different ($p < 0.05$)

DISCUSSION

We report that lambda-cyhalothrin is highly toxic to *C. gariepinus* with 96-h LC_{50} value of 0.008 mg/l. This is consistent with the report of several other workers, Muller *et al* (1978), reported static 48-h LC_{50}'s of 0.005, 0.006 and 0.097 mg/l for adult desert pupfish (*Cyprinodon macularius*), rainbow trout (*Oncorhynchus mykiss*) and western mosquito fish (*Gambusia affinis*) respectively. Also in a comparative study of acute toxicities of insecticides, Mittal *et al* (1991) reported that the synthetic pyrethroid lambda-cyhalothrin was most toxic to the mosquito fish (*Gambusia affinis*) with LC_{50} =0.0022ppm. Bradbury and Coast (1989) had earlier stated that permethrin and other synthetic pyrethroids are highly toxic to fish with 96-h LC_{50} usually < 0.01 mg/l.

The result concerning the tail fin beat and opercular movement suggest that the fish exposed to the toxicant tended to exhibit avoidance syndrome. The early failing of gill appears to explain why there was no significant difference in the mean tail fin beat across concentrations. Fish however resorted to only air gulping in the first 46-hrs to obtain the much needed oxygen for increased metabolic rate and energy requirement for pesticide detoxification. The decrease in the tail fin beat observed over time showed that the attempt to escape was futile, therefore fish became fatigued. The combined effects of this fatigue, decreased respiratory rate, decreased oxygen consumption and direct toxic effect of the insecticide on the body tissues, led to subsequent spasms, excessive lateral flexure and lack of response to stimulus, all of which led to death in some of the fish. The symptoms appear transient in static bioassay as mortality virtually stopped by 48-hrs and general behaviour approached control pattern. The implication of this is that the toxic effect of lambda-cyhalothrin is almost completely neutralized after 48-hrs, thereby enabling fish to resume opercular beat and other behaviours observed in control.

Behavioural monitoring is a promising diagnostic tool for screening and differentiating chemicals according to their mode of action (Drummond *et al*, 1986). They proposed that chemicals with different modes of action will evoke a distinct behaviour pattern. Some of the distinguishing behavioural symptoms observed in *C. gariepinus* exposed the toxicant when compared with control were similar to the effects described in other fish species. Rice *et al* (1997) reported that juveniles *O. latipes* exposed to > 0.009 mg/l of permethrin swam hyperactively with an excessive lateral flexure in the caudal area, and sheepshead minnows exposed to < 22 ppb permethrin developed abnormal lateral flexure (Hansen *et al*, 1983). Also Edwards *et al* (1986), reported that rainbow trout exposed to 10µg/l *cis*-cypermethrin exhibited toxic signs of gill failing and hyperactivity, followed by loss of buoyancy and trim control.

The observed hyperactivity, loss of equilibrium, spasms, excessive lateral flexure and lack of response to stimuli are all indications of metabolic and nervous system failure due lambda-cyhalothrin acute poisoning. And the high toxicity noted in this investigation can be attributed to accumulation of the toxicant in the body tissues and poor ability of the fish to metabolize and eliminate them. Matsumura (1975) had earlier reported that hyperactivity is a primary and principal sign of nervous system failure due pesticide poisoning, which affects physiological and biochemical activities. Pal and Konar (1987) also reported that disruption of the functioning of nervous system of fish might be the cause of slow and lethargic swimming, erratic movement and loss of equilibrium. Also Bradbury and Coast (1989), explained that fish metabolise and eliminate pyrethroids more slowly than mammals or birds, perhaps explaining its higher toxicity to fish compared to other organisms. Due to the lipophilicity of pyrethroids, they have a high rate of gill absorption, which in turn would contribute in the sensitivity of fish to aqueous pyrethroid exposures. Fish seem to be deficient in the enzyme system that hydrolyzes pyrethroids. The main reaction involved in the metabolism of pyrethroids in mice and rats is ester cleavage mainly due to the action of carboxyesterase. Metabolism in fish in largely oxidative (Demoute, 1989; Rukiye *et al*, 2003).

CONCLUSION

Synthetic pyrethroids to which lambda-cyhalothrin belongs are widely used in agriculture in Nigeria. They are increasingly being used in veterinary applications on farm and pet animals, for the protection of stored foodstuffs, for the control of endemics and parasites in public health programmes as well as for household applications in kitchens and bedrooms. Acute exposures of aquatic biota and individuals may result through run-off and adsorption of pyrethroids to small dust particles and various other surfaces respectively. The results of our study suggest that acute exposure of *C. garipinus* to lambda-cyhalothrin elicited gill failing, excessive lateral flexure and spasms, when fish were severely intoxicated, they became hypoactive, underreactive to startle stimuli, and mortality eventually resulted. This is in agreement with earlier report by Rice *et al* (1997), on *O. latipes* exposed to >0.009 mg/l permethrin. These behavioural symptoms are unique for pyrethroids and may be useful for monitoring effluents, and for testing water from wastewater treatment facilities before its discharge. In the light of the above observations, it is also recommended that lambda-cyhalothrin should be used with caution and in a sustainable manner, as it could be hazardous to aquatic biota, domestic animals and human beings as well.

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