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Behavioural Response of *Oreochromis niloticus* Fingerlings Exposed to Sub-Lethal Concentrations of Imidacloprid

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Authors' contributions

This work was carried out in collaboration among all authors. Author PEA designed the study, wrote the first draft of the manuscript, supervised and funded the study. Author SUE designed the methodology, reviewed the literature and funded the study. Author HTI collected data, analyzed data, interpreted the results, typed the initial draft and final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Imidacloprid, a neonicotinoid pesticide that was once thought to be a solution to environmental pollution caused by previous pesticides like organophosphates and organochlorines has raised global concern recently. The present study aimed at assessing the effect of Imidacloprid on the behaviour of a commercially important fish species, *Oreochromis niloticus*. The study involved a 48

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h sub-lethal toxicity test which was carried out with 0, 5, 10, 20, and 40 ppm of Imidacloprid. Fingerlings of *O. niloticus* weighing between 18 and 32g were exposed to these concentrations in a static bioassay with continuous aeration. Regular observation of the clinical signs revealed that fish exposed to higher concentrations of Imidacloprid displayed behavioural abnormalities such as erratic behaviour, respiratory stress, and irregular nervousness. The findings of this study revealed that Imidacloprid is toxic and causes behavioural distress on *O. niloticus* fingerlings. Therefore, there is urgent need for further studies to determine safe limit of Imidacloprid and other pesticides in the environment. Unregulated disposal of toxic pesticides should be avoided, to ensure human safety and environmental sustainability.

Keywords: Toxicity; freshwater habitat; neonicotinoid pesticide.

1. INTRODUCTION

The contamination of surface waters and sediments by insecticides has been reported to pose threat on the growth, reproduction and survivability of aquatic organisms [1]. This can ultimately affect fish recruitment and catch thereby affecting fisheries and man that depends on it for food. Apart from the reduction in economic value of fish, elevated levels of undesirable chemicals bio-accumulated in the tissues of fish can pose danger on the health of consumers [2].

Recently, there has been global increase in mortality among fishes in various estuaries, rivers, rivulets, streams, ponds and lakes [3]. This incidence may be reduced through the application of pesticides screening measures that are both effective and efficient. Such screening will allow knowledge of the toxic potentials of different pesticides. The strict use of only those pesticides with minimal toxicity to fish and humans could help reduce the danger of pesticide contamination. However, there is paucity of information on the safe as well as tolerance levels of fish to pesticides. This often neglected area would have helped in providing information on the effects of different pesticides on fish physiology.

Imidacloprid is a neonicotinoid pesticide widely used for the control of insect pests of crops. Although it is very toxic to insects, its effects on non-target organisms including fish have been reported to be minimal [4]. However, since no exposure of humans to Imidacloprid has been reported and the severity of effect on fishes was assumed by Lohstroh [4] to be species specific, there is need to determine the effects of the insecticide on different species of fish.

Oreochromis niloticus (Nile Tilapia) is an important aquaculture species and commercially important staple freshwater fish generally captured in rivers, ponds, canals and lakes. It is found widespread in many tropical countries including Nigeria [5]. As important food fish, it is pertinent to assess its behavioural response when exposed to chemical substances. Behavioural changes in fishes are the first and most affective indicators of water quality problem [6]. As such, they are considered as first basic parameter in preliminary bio-monitoring.

There have been several reports on the display of behavioural abnormalities among shell- and fin-fishes due to pesticide and herbicide toxicities as well as other environmental stressors [6,7]. Exposures to toxic chemicals can alter physiological functions, resulting in disruption in behavioural patterns of victims. Many such chemicals can cause damaging effects on the brain, seizures, and death of target organisms [8]. The objective of this study was therefore to evaluate the effect of different concentrations of Imidacloprid on the behaviour of a commercially important fish species, *O. niloticus*. The study defines safe limit of Imidacloprid as the concentrations with which fish exhibit normal behaviour.

2. MATERIALS AND METHODS

Fig. 1 is the flow chart of how the testing process was carried out. The test pesticide, Imidacloprid was obtained from a local stall in Calabar, Cross River State, Nigeria. The choice of this pesticide was due to its wide patronage in Nigeria after many previous pesticides like organophosphates and organochlorines were reported to be toxic to target and non-target organisms.

Fingerlings of *O. niloticus* which ranged in weight between 18 and 32g were obtained from the University of Calabar Fish Farm, Cross River State, Nigeria in the morning and transferred alive to the laboratory. In the laboratory, the fish specimens were acclimatized for 7 days in a 300 L tank made of fiberglass. Continuous aeration of the fiberglass tank and daily feeding of the specimens with Coppens feed was ensured throughout the acclimatization period.

Before the end of this period, a range finding test (RFT) was conducted to determine the range of concentration of the test pesticide [9]. The preliminary test took place for 24 h when the test organisms were exposed to several dilutions of Imidacloprid concentrations (5, 10, 15, 20, 25, 30, 35, 40, 45, 50 ppm). The RFT revealed a range between 5 and 40 which was used for the sub-lethal test.

After acclimatization, healthy fish specimens were randomly selected for the static bioassay. Twenty-four hours (24 h) prior to and during the bioassay, specimens were starved to ensure that food constituents do not inflate the end-results [10]. The sub-lethal test conducted on the test organisms was done in accordance with the Organization for Economic Co-operation and Development Guideline Test No. 203 [11].

Static bioassay with continuous aeration lasted for 48 h as provided in the standard procedure of APHA [12]. One hundred milliliter (100 mL) of Imidacloprid was made to dissolve in 900 ml distilled water to form 1000 ml stock solution. The stock solution was therefore diluted to different concentrations such as 5ppm, 10ppm, 20ppm and 40ppm following the formula C1V1= C2V2 [13].

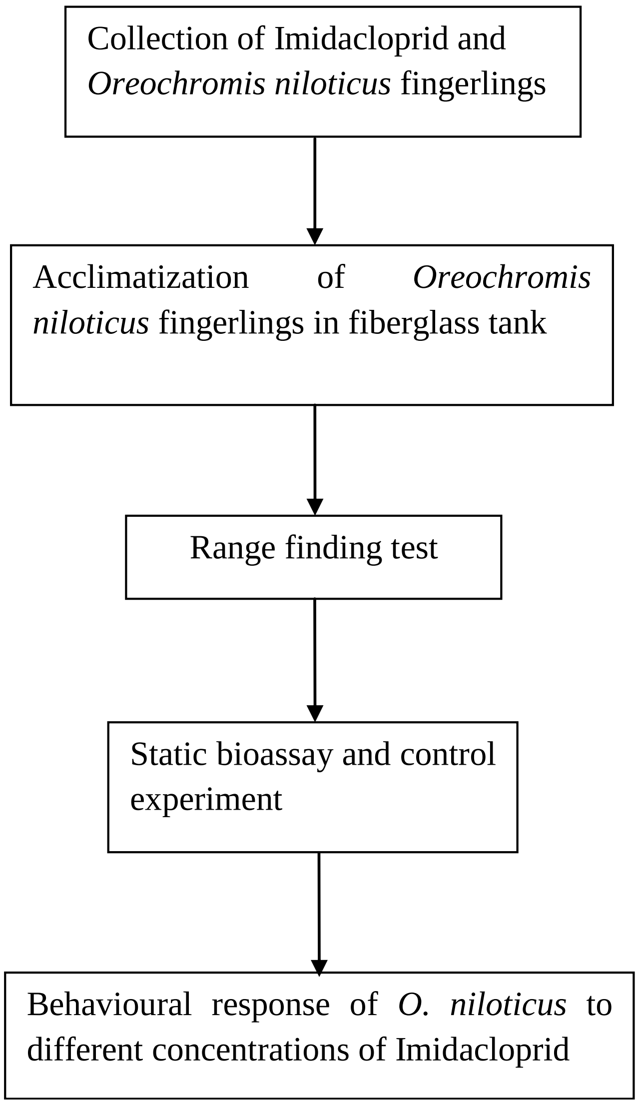

Fig. 1. Flow chart showing the testing process

The concentrations and a control (freshwater only) were arranged in triplicates per concentration. The derived concentrations were carefully poured into 25 L of freshwater in each of the test aquaria and made to homogenize by gently stirring with a clean glass rod. The study adopted a completely randomized design with stocking density of 5 fish specimens per 50 L of freshwater.

The laboratory was maintained with 12 h of light as well as 12 h of darkness to mimic normal day and night by nature. Basic room and water quality variables such as temperature, pH, and total hardness were monitored daily till the end of the experiment. Surface water temperature was measured using thermometer, pH with pH meter, while total hardness was measured following the method described by APHA [12].

Fish were observed regularly for behavioural changes in the different concentrations of Imidacloprid and the control. Each clinical sign exhibited by the test organisms were classified according to Hassan et al. [13] and Norhan et al. [6]. The flow chart showing the summary of the testing process is depicted in Fig. 1.

3. RESULTS AND DISCUSSIONS

The results of the physicochemical test for water quality during the bioassay are presented in Table 1. The study revealed that there was no significant difference (P>0.05) in all the tested physicochemical variables between control and treatments. Although the present study showed no significant difference in levels of temperature, pH and total hardness, mean values were higher in treated group than control. Also, other water quality parameters not observed in the present study may be responsible for observed behavioural abnormality in *O. niloticus* exposed to Imidacloprid.

Fish and other animals are exposed to contaminated water through three main routes including skin, gills and mouth. The aquatic environment in Nigeria and other tropical countries of the world is constantly faced with incessant pollution due to uncontrolled use of pesticides. Agricultural runoff aggravates the burden of contaminants in coastal areas leading to fish kills and poisoning to man and other higher trophic level organisms that depend on fish for food [14]. Although exposure to contaminated substances like Imidacloprid may not cause instant fish kills, behavioural distressed and reproductive abnormality are likely to be observed. This can impact negatively on survivability and fish recruitment thereby affecting fisheries and the economy of the coastal communities [15].

Table 2 is a record of the distressed behaviour of *O. niloticus* exposed to different concentration of Imidacloprid within 96 h. The distressed behaviour was observed to increase with increased concentration of the Imidacloprid. The agitated behaviour that was recorded during the 48 h of experiment was aggression, stunned posture, erratic swimming and regular movement from surface to bottom of the aquaria. At higher concentration 20 ppm, *O. niloticus* showed more movement from surface to bottoms, and moderate changes in erratic swimming. The fish appeared weak in stunned position and generally exhibited weak distressed behaviour at the highest concentration.

Table 1. Ranges of physicochemical variables during the bioassay on the toxicity of Imidacloprid to test organisms

Variables	Control	Imidacloprid
Temperature (°C)	27.8 – 28.2	27.8 – 29.2
pH	8.2 – 8.7	8.4 – 9.1
Total hardness (ppm)	200 – 233	219 – 255

Table 2. Distressed behaviour of *O. niloticus* subjected to Imidacloprid within 48 h

Clinical signs	Imidacloprid concentration (ppm)				
	0	5	10	20	40
Aggressiveness	-	-	+++	++	+
Erratic swimming	-	-	+	++	+
Stunned position	-	-	-	-	+
Frequent surface to bottom movement	-	+	++	+++	+

None (-), Weak (+), Moderate (++), Strong (+++)

Table 3. Respiratory stress of *O. niloticus* subjected to Imidacloprid within 48 h

Clinical signs	Imidacloprid concentration (ppm)				
	0	**5**	**10**	**20**	**40**
Rapid opercula movement	-	+	++	++	+++
Air gulping	-	-	+	++	+++
Ventral position with snout exposed	-	++	+++	+++	+++
Secretion of mucus	-	-	++	+++	+++

None (-), Weak (+), Moderate (++), Strong (+++)

Table 4. Abnormal nervous behaviour of *O. niloticus* subjected to Imidacloprid within 48 h

Clinical signs	Imidacloprid concentration (ppm)				
	0	**5**	**10**	**20**	**40**
Slow and twirling movement	-	-	++	++	+++
Motionlessness	-	-	+	++	+++
Sudden darting	-	++	+++	+++	+++
Found in different positions at a time	-	-	++	+++	+++
Moribund condition	-	-	-	+	++
Death	-	-	-	-	-

None (-), Weak (+), Moderate (++), Strong (+++)

These distressed behaviours exhibited by *O. niloticus* could be as a result of coordination imbalance as fish responded to Imidacloprid toxicity. The erratic swimming and irregular surface to bottom movement could be prompted by bioaccumulation of toxic substances in the synaptic muscle and neuromuscular junction with attendant failure in proper coordination of the muscles [16].

Table 3 shows changes in respiratory behaviour of *O. niloticus* at different concentrations of Imidacloprid. The abnormal respiratory behaviour increased as the concentration of Imidacloprid increased. At concentration level of 5 ppm and above, *O. niloticus* began to show mild to strong changes in respiratory behaviour such as frequent opercula movement, air gulping, ventral position with snout exposed and secretion of excess mucus. Air gulping by *O. niloticus* could have been a tactic to avoid breathing in the contaminated water. The mucus film observed on the gill of the test fish could act as a barrier to further entry of the toxicant into the fish. Similarly, mucus secretion all over the body of the fish could reduce the amount of toxicant contacting the skin of the fish [17].

Table 4 gives an account of nervous behaviour of the test organism. The fish samples were observed to show sudden darting behaviour in 5 ppm and began to show slow and twirling movement as the concentration of Imidacloprid increased. Other behaviourial changes such as motionlessness and moribund condition were observed in highest concentrations. There was no record of fish death in all concentrations of Imidacloprid throughout the study. The control experiment did not show any unusual behaviour from the test organisms.

The cause for the abnormal nervous behaviour of fish exposed to toxicant could be as a result of nervous and muscular discoordination triggered by the bioaccumulation of chemical substances from Imidacloprid in nerves and muscles of fish [18]. The slow and twirling movement as well as sudden darting behaviour could be as a result of the chemical imbalance of the aquaria water. The moribund condition of the fish could be due to undue stress imposed by the toxicant on the test organisms.

Mortality was not observed throughout the 48h exposure period. This could be because the Imidacloprid concentrations used in the present study did not reach the lethal dosage. This may be an indication of low effect of toxicant on the fish. Another reason could be that the liver of the exposed fish was able to detoxify and excrete the toxicant from the body [19]. At lethal dosage, the toxicant can disrupt the detoxification potency of the liver with attendant death of the exposed fish. However, the toxicant residue in the sublethal condition could not interrupt the normal physiological function of the fish, thereby unable to cause death [6].

4. CONCLUSIONS

The study revealed that *O. niloticus* exposed to concentrations of Imidacloprid between 10 and

40 ppm exhibited different behavioural abnormalities. The clinical signs ranged from restlessness to moribund condition as the concentration increased. There is therefore need for further investigation on the safe limit of Imidacloprid in the environment. Proper disposal of the pesticide without impairing the aquatic environment and compromising human safety is advised.

ETHICAL APPROVAL

We hereby declare that "Guide for the Care and Use of Laboratory Animals" (US National Research Council Committee 8th edition) were followed, as well as national laws where applicable. All experiments have been examined and approved by appropriate committee on animal use and care.

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COMPETING INTERESTS

We declare that there is no competing interest in terms of finance or social relationships or whatever that could bias this research.

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