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Effect of Insecticidal Permethrin (NRDC-143) on the Freshwater Fish *Oreochromis niloticus:* Behavioural Responses and Glycogen Content in Selective Tissues

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Abstract: Specimens of *Oreochromis niloticus* were exposed to different concentrations (0.04-0.06 mg/l) of the insecticide Permethrin. The LC50 for 72 hours was computed as 0.054 mg/l. At sublethal concentrations (0.03 and 0.035 mg/l) some remarkable changes in behaviour were observed such as cough, yawn, finflickering, jerking movement, nudge and nip. The fish showed signs of serious stress which involved changes in the metabolic rate. Indeed, a marked decline in glycogen content in different tissues was observed after the exposure to this insecticide.

Introduction

The widespread contamination of the aquatic ecosystem with toxic substances can have many severe effects on their aquatic fauna and flora. Toxicologists who study aquatic environments [1, p.268; 2-6] have persuasively defended the utility of fish chronic toxicity tests and in particular the advantage of an early stage fish test as a relatively quick and inexpensive screening tool for testing chemicals and effluents. Responses to these chemicals can also produce a greater understanding of behavioural mechanisms and signal the limitations of fish in terms of their adapting to stressors in the environment.

Permethrin [3-phenoxybenzyl (+) cis, trans, 3-(2,2- dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (NRDC-143)] is effective insecticide with a low toxicity to birds and mammals [7;8] but is highly toxic to fish [9-13]. According to established work, fish during the period of acclimation to this insecticide show dramatically early increases in basal metabolism, presumably reflecting physiological stress, detoxication and tissue repair. Although, several researchers have demonstrated the use of behavioural toxicity tests in various freshwater fishes [3;4; 14-16; 17, pp.233-250; 18, pp.72-98;19], none have reported the effect of insecticide on fish behaviour and bioenergetics. However, the effects of Permethrin on fish behaviour have been studied by several workers [9;12;13;20;21]. This present study investigates the effect of sublethal concentrations of the insecticide on behaviour and glycogen reserves in the freshwater fish, Tilapia (*Oreochromis niloticus*). This species was selected because it is hardy and commercially used in Saudi Arabia.

Materials and Methods

Live specimens of the tilapia (length, 10-12 cm; weight, 50-55 gm) were procured from a fish culture pond at Deerab, Riyadh (23'30"N, 46'43"E). One hundred and fifty fishes were acclimatized in a large glass trough (capacity 350 liters) where they were subjected to a regime of 16 hours light and 8 hours dark using the fluorescent tubes. The water in the tank was changed on alternate days with water of the same temperature and airstone aeration was applied to the tank. During the period of acclimation, the fish were fed to satiety twice daily with a commercial fish food. The water used for acclimation of the fishes was analysed for dissolved oxygen (7.8 ± 0.4 mg/l), temperature (24.0 ± 0.5°C), water hardness (232.58 ± 1.05 mg/l as $CaCO_3$) and pH (7.5). When the acclimation period was over (judged by normal activity and feeding of the fish) ten fishes, randomly selected from the stock were transferred to a series of small tanks (capacity 25 l). A control set was run with same number of fish and same volume of water. Airstone aeration with mechanical air pumps was supplied continuously to all tanks, Following the usual practice in toxicity exposure, the fish were deprived of food during the period of investigation.

A stock solution of Permethrin was made up and the required volume was added to the experimental tanks to obtain test concentrations of 0.04 and 0.06 mg/l. These were further checked by means of a Pye-Unicam SP-190 atomic absorption spectrophotometer. The fishes were exposed for 72 hours and their mortality was registered separately for each concentration. The LC-50 for 72 hours was computed according to the method of Finney [22, p.333].

In another experiment, fifteen fishes were exposed to one of two sublethal concentrations of Permethrin (0.03 and 0.035 mg/l). A control set, having the same number of fishes was run simultaneously for comparison. The behavioural changes due to the Permethrin toxicity were observed. The observations were made for 30 minutes for each tank. The time of observation was varied from morning to evening to avoid the diurnal fluctuations in the behaviour. The behaviours recorded are described briefly in Table 1. This experiment was continued for 72 hours in two replicates. After every 24 hours, five fishes were sacrificed for analysis of glycogen content in tissues (liver, muscle, brain and heart). First the tissues were extracted by the method of Ashman & Seed [23] and glycogen estimated by the method of Montgomery [24]. The results are expressed in terms of μg glycogen/gm wet weight of tissue. The experimental data were analysed using Student's t-test.

Table 1. Behaviour of *Oreochromis niloticus* monitered in the present study

Behaviour	Description
Cough	Rapid, repeated opening and closing of mouth and opercular covering with partial extention of fins
Yawn	Wide opening of mouth and hyperextension of fins
S. Jerk	Movement of body sequentially from head to tail
Partial-jerk	Movement of head or tail only
Fin-flickering	Repeated extension and concentration of dorsal fin
Burst swimming	Sudden and rapid movements (Forward)
Nudge	Movement of the fish towards another fish
Nip	Biting

Results

Observations made on fish exposed to sublethal concentrations of Permethrin indicate a remarkable changes in behaviour. Initially the fish seemed restless and lost schooling behaviour. This was followed by lethargy and a tendency for the fish to settle motionless on the bottom of the aquaria at both concentrations. With increasing duration of exposure to the toxic material, the symptoms observed included coughing); yawning, partial and S. Jerk activity and burst swimming, following by the fish assumes a tilting position whilst performs sudden jerks. Finally, the behaviour changed to elements including aggressiveness such as nudge and nip which were increased following exposure to the toxic materials. Toxicant exposed fish also secrete too much mucous in comparison to the controls which altered the rate of respiration in fishes as indicated by increased frequency of cough and yawn.

The changes in tissue glycogen content and the percentage depletion between treated and untreated fish are given in Fig. 1 and Table 2. The data revealed significant decreases in liver ($P < 0.001$), muscle ($P < 0.001$), brain ($P < 0.005$) and heart ($P < 0.005$) vs controls.

Table 2. Percentage of depletion of glycogen in different tissues of *O. niloticus* at different time intervals after exposure to different concentrations of permethrin

		24 Hours		48 Hours		72 Hours	
Name of tissue	Concentration mg/l	Glycogen μg/g	Depletion %	Glycogen μg/g	Depletion %	Glycogen μg/g	Depletion %
	Control	1728.00 ±93.52	–	1728.00 ±93.52	–	1664.00 ±129.98	–
Muscle	0.030	1280.00 ±50.59	4.8	1216.00 ±64.0	5.12	1024.00 ±39.19	6.40
	0.035	1152.00 ±59.86	5.76	1024.00 ±39.19	7.04	800.00 ±50.59	8.64
	Control	3008.00 ±59.86	–	3040.00 ±50.59	–	3008.00 ±59.86	–
Liver	0.030	2624.00 ±81.58	3.84	2388.00 ±93.29	6.72	2048.00 ±137.64	9.60
	0.035	2080.00 ±113.4	9.28	1824.00 ±81.58	12.16	1568.00 ±59.86	14.40
	Control	768.00 ±59.86	–	788.00 ±59.86	–	768.00 ±59.86	–
Heart	0.030	±640.00 ±25.29	1.28	500.00 ±35.78	2.08	480.00 ±50,59	2.88
	0.035	544.00 ±39.19	2.24	384.00 ±39.19	3.84	224.00 ±39.19	5.44
	Control	272.00 ±32.00	–	272.00 ±32.00	–	256.00 ±29.93	–
Brain	0.030	240.00 ±32.00	0.32	240.00 ±35.78	0.32	208.00 ±32.00	0.48
	0.035	224.00 ±39.19	0.48	192.00 ±32.00	0.80	153.60 ±3.92	1.03

Liver = Control vs 0.030 & 0.035 mg/l-($P<0.001$)
Muscle = Control vs 0.030 & 0.035 mg/l-($P<0.001$)
Heart = Control vs 0.030 & 0.035 mg/l-($P<0.005$)
Brain = Control vs 0.030 & 0.035 mg/l-($P<0.005$)

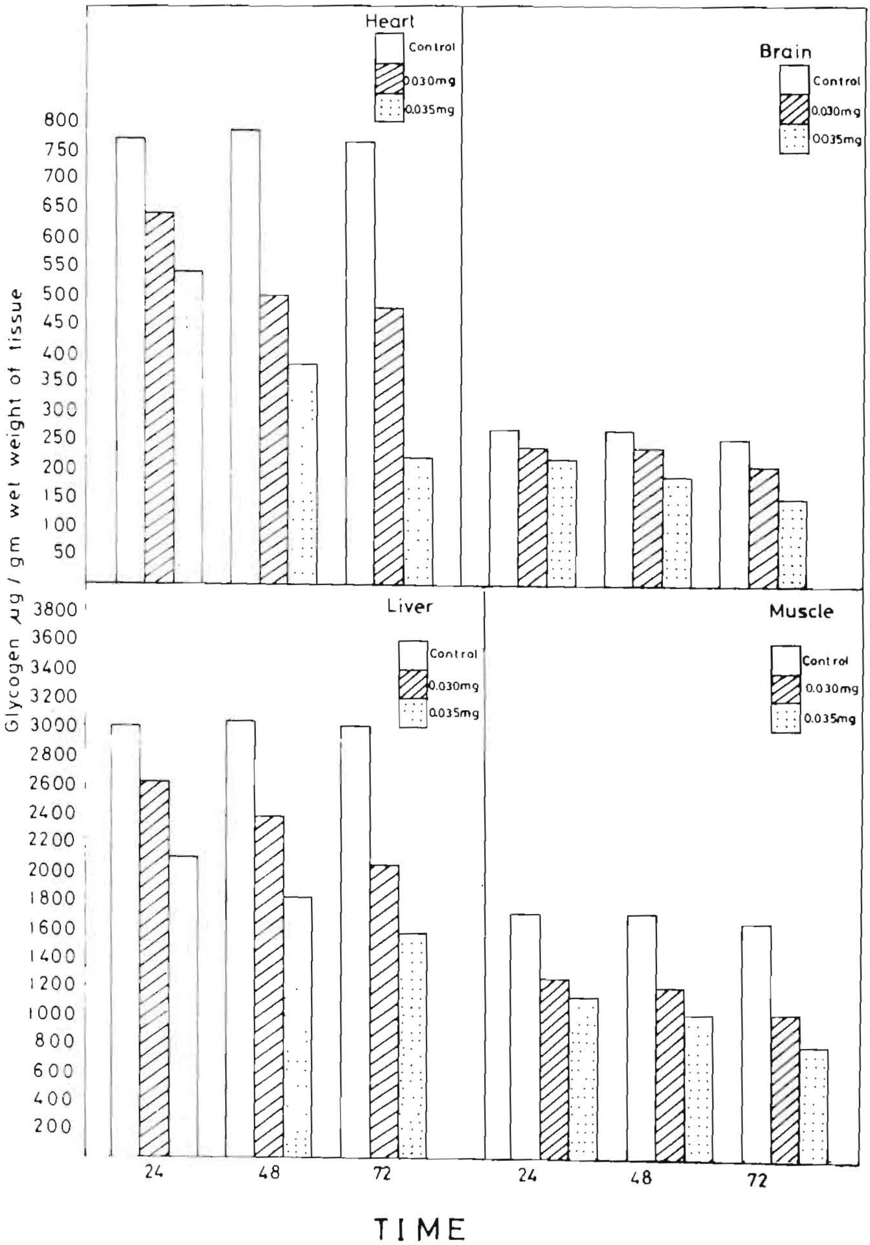

Fig. 1. Changes in Glycogen content (μg/g wet weight) of different tissues in – *Oreochromis niloticus* at different concentrations of insecticidal permethrin

Discussion

It was clear in the present investigation that exposure to permethrin alters the behaviour of *O. niloticus*. These changes are attributed to physiological disturbances and may be caused by the increase of opercular movement which increases the oxygen demand [2;3]. The fish kept in hypoxic condition perform more muscular activity which requires more oxygen in comparison to normal behaviour of the fish, hence the marked changes may takes place in the circulatory system. The most crucial circulatory function during the activity is oxygen transport because, if the muscular activity increased, the available oxygen is soon exhausted and the anaerobic energy system activated causes the fish to become fatigued [25]. Fish exposed to the toxic substance also profusely secreted mucous to protect the gills. The retention of mucous on the gill filaments may also reduce the interfilamental space, probably reducing the gas exchange rate [5;26]. This stress may damage the gill epithelium on initial exposure and be manifested by an elevation of basal metabolism affecting the metabolic rate and oxygen consumption, resulting in an increase in energy demand and a change in fish behaviour. This is consistent with findings for rainbow trout [27]. This consumption of oxygen for balancing the metabolic rate causes the hyperexcitability, hence the fish become stressed and may exhibit a severe hypoxia that will later trigger biochemical changes in different tissues of the body. It has been reported [4;28, pp. 22-30] that hypoxia increases carbohydrate demand which is met by body glycogen stores. This tends to result in anaerobic stress which may cause in the breakdown of tissue glycogen, utilized possibly through a process of anaerobic glycolysis to meet the energy demand. This finding is supported by earlier observations [2;3;5;29] showing a significant decline in glycogen content in the exposed fish to different toxicants for many species of freshwater teleosts. Iwama, *et al.* [30] and Chaudhry *et al.* [31] have also reported that environmental stress depletes energy reserves as indicated by decreased liver and muscle glycogen accompanied by hyperglycemia. Thus, it can be concluded that all stress conditions invariably retard growth and alter the physiology of fish [4].

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تأثير المبيد الحشري بيرميثرين Permethrin (NRDC-143) على سلوكية سمك البلطي Oreochromis niloticus وكذلك على نسبة النشاء الحيواني في بعض أنسجته

علي بن سليمان العقل ومحمد جاويد كمال شمسي وعلي مشبب آل ـ حيافه
قسم علم الحيوان ـ كلية العلوم ـ جامعة الملك سعود،
ص.ب ٢٤٥٥ ، الرياض ١١٤٥١ ، المملكة العربية السعودية
(سُلِّمَ في ١٢/١١/١٤١٤هـ، وقُبل للنشر في ١٠/٦/١٤١٥هـ).

ملخص البحث. تمّ دراسة تأثير نسب مختلفة من المبيد الحشري بيرميثرين (٠,٠٤٠ ـ ٠,٠٦٠ ملجم/لتر) على الأنـماط السلوكية لسمك البلطي، وقد كان معدّل التركيز القاتل لنصف المجموعة هو ٠,٠٥٤ ملجم/لتر في تجربة ٧٢ ساعة.

وعنـد تعـريض الحيوانات لتركيزات أقل (٠,٠٣٠، ٠,٠٣٥ ملجم/لتر) شوهد أنماط سلوكية عديدة وكذلك انخفاض في نسبة النشاء الحيواني في الأنسجة المدروسة (المخ والكبد والعضلات والقلب).