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COMPARATIVE STUDY OF TOXICITY OF CARBARYL AND ITS IMPACT ON THE BEHAVIOUR AND CARBOHYDRATE METABOLISM OF CICHLID FISH, *OREOCHROMIS NILOTICUS* (LINNAEUS , 1758) AND CATFISH *CLARIAS GARIEPINUS* (BURCHELL, 1822) FROM SAUDI ARABIA.

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ABSTRACT

Healthy fishes of *Oreochromis niloticus* and *Clarias gariepinus* were exposed to different lethal and sublethal concentrations of carbaryl (sevin) as carbamate hydrocarbon which is an acetylcholine estrase inhibitor used as insecticide. The 96 hours LC_{50} for both fish species was computed as 4.45 mg/l^{-1} for *Oreochromis niloticus* and 17.55 mg/l^{-1} for *Clarias gariepinus*. The fishes showed remarkable changes in behaviour after exposure. The behaviours observed were; cough, yawn, Fin flickering, jerks, threat, and nip etc. They showed hyperexcitation-lethargy and occasional darting movements and sometime losing balance (in catfish) due to hanging upside down and rolling on the surface of water in the tank. After 96 hours of fish exposure to sub-lethal concentrations of the insecticide, significant depletion of muscle, liver and brain tissue glycogen ($P<0.001$) was recorded. On the other hand, the level of depletion of glycogen content was more prominent in *O.*

niloticus, being 33.03% in liver and 27.3% in muscle while in *C. gariepinus* it was 15.18% in liver and 12.90% in muscles.

INTRODUCTION

Various sources of insecticides are deliberately spread over to the environment for killing some hazardous animals. But unfortunately, these insecticides are toxic to many non-target organisms including fish and other aquatic animals (Murphy, 1980; Al-Akel *et al.*, 1995 and Al-Kahem, 1996). Among the insecticides, carbamates are very much persistent in the environment which can be used as molluscicide, fungicides and herbicides including carbaryl (selvin). These are less persistent than the organophosphates and less harmful to man, however, they may cause local environmental problems if used carelessly. Carbaryl, is one of the insecticides which are very toxic to bees and other insects and are more effective on the growth of fishes (Duffus, 1980). These carbamates are acetylcholinesterase inhibitors, used as insecticides or systemic parasiticidal agents and their redicals if attached to methyl or ethyl group, produce the hydrocarbons. The alterations in the chemical composition of aquatic environment, affect the behaviour and biochemical systems of the fish (Radhaiah *et al.*, 1987 and Al-Kahem, 1996). Physiological and biochemical changes in fish and other aquatic animals due to insecticidal poisoning are well documented of (Kumaraguru and Beamish, 1986; Fernando and Mohliner, 1991 and Al-Akel *et al.*, 1995).

According to several workers (Singh and Srivastava, 1981; Murad, 1991; Gopal *et al.* 1993; Dunier and Siwicki, 1994 and Al-Akel *et al.*, 1995) fish during the period of acclimation to various insecticides show dramatically an increases in basal metabolism, presumably reflecting physiological stress, detoxication and tissue repair. Previously, some researchers (Vittozi and Dengelis, 1991; Gopal *et al.*, 1993; Johnson and Toledo, 1993; Patel and Parmer, 1993; Govindon *et al.*, 1994

and Al-Kahem *et al.*, 1999) have been used various insecticides on the behavioural toxicity tests and other physiological and biochemical tests in various freshwater fishes. As far as we know, no one yet has used carbaryl, as carbamate on fishes. So, the present investigation was designed to elucidate the acute toxic effect of that insecticide on the behaviour and some biochemical changes in *Oreochromis niloticus* and *Clarias gariepinus* exposed to lethal and sublethal concentrations. These fishes were selected because they were used as test animals and are commercially used in Saudi Arabia and their culture became substantially increased during recent years.

MATERIALS AND METHODS

The fishes, *Oreochromis niloticus* (mean weight, 62.5gm±1.0 SD and mean length 13.3 cm±1.25 SD) and *Clarias gariepinus* (mean weight, 44.4 gm±1.5 SD and mean length 11.4 cm±1.25 SD) used in the present investigation were obtained from a private hatchery at Deerab, Riyadh (23' 30''N, 46' 43'' E.). These fishes were acclimatized under laboratory conditions with oxygenated water in an aquarium for about 4 weeks. The water of the aquarium was renewed daily and the temperature, pH and dissolved oxygen were recorded.

When the acclimation period was over (judged by normal activity and feeding of the fish), 10 fishes were randomly selected from the stock and transferred to a series of small tanks. A stock solution of the carbaryl was prepared and diluted with the test water to produce the lethal concentrations of 2,3,4,5,6,7,8 and 9 mgl^{-1} for *Oreochromis niloticus*, and 8,10,12,14,16,18,20 and 22 mgl^{-1} for *Clarias gariepinus* separately. These two experiments in replicate were run parallel to observe the LC_{50} for each species. 10 fish of each species were exposed to a certain insecticide concentration. Fish of 16 aquaria were exposed to

the aforesaid concentrations and the remaining two were kept as control. The same procedure was also applied for *C. gariepinus*

All the fishes in both experiments were exposed for four days (96 hours) and the following was monitored: rate of mortality, behaviour such as, cough (wide opening the mouth with partial extension of fins); yawn (wide opening of mouth along with complete extension of fins); finflickering (repeated extension and contraction of dorsal fin); threat (movement of fish towards another fish); nip(bite) and nudge(resting of a fish with its head on the body and rolling on the surface of water. The water of all experimental tanks was renewed every 24hours to keep the concentration constant. The mortality of the fish was recorded and the dead fishes were immediately removed from the tank. LC_{50} for 96 hours of the insecticide was computed for both fish species according to the method of Finney (1971).

In other two experiments, 180 fishes of each species were exposed to three sublethal concentrations (0.5,1.5 and 2.5 mgl^{-1} for *O. niloticus* and 2,6, and 10 mgl^{-1} for *C. gariepinus*) for four days in three replicates. A control set having the same number of fishes with the same volume of water was run simultaneously for comparison. After 96 hours, fishes from each of the replicates of treated and untreated water were sacrificed and their blood was centrifuged at 6000 rpm for 15 minutes to separate serum from the blood for the estimation of glucose which was estimated by the method of Roe (1955). The same fishes were also used for estimation of glycogen content (liver, muscle and brain tissues) by the method of Montgomery (1957). All these parameters were subjected to one way analysis of variance (ANOVA) to assess the significance of difference between control and treated fishes.

RESULTS

A. Acute Toxicity

The results for the fishes (*O. niloticus* and C. *gariepinus*) exposed to carbaryl showed that this insecticide is moderately toxic to the fish. The 96 hour LC_{50} value in case of *O. niloticus* was found to be 4.45 mgl^{-1} while in *C. gariepinus* it was 17.55 mgl^{-1} (Table III) . This difference of LC_{50} value between the species of fish actually depends upon the tolerance and absorbance of the toxic compound in the fish.

B. Behavioural Response (Table II A & B)

Observations made on fish exposed to sub-lethal concentrations of carbaryl indicate remarkable changes in the behaviour in both fish species. In catfish, the behavioural change was followed by lethargy and tendency for the fish to settle down motionless on the bottom, but when the time of exposure was prolonged, the symptoms observed included cough, yawn, S-jerks following by the fish as hanging upside down then after a sudden jerk, the fish become aggressive (nudge and nip). In *O. niloticus*, the behavioural changes occur as uncomfortable movements like burst swimming, S- and partial jerks and darting movement then became hyperexcited and restless. After sometime, the fish became sluggish and remained at the bottom for some time. Then the frequency of other behavioural aspects like cough, yawn, fin-flickering, threat, nudge and nip were more pronounced due to the insecticidal poisoning.

C. Biochemical response

Biochemical analysis indicated that the levels of glycogen in liver, muscle and brain tissues were depleted in insecticidal exposed fish. Data presented in Table-III.A&B revealed that the glycogen level decreased significantly ($P<0.001$) for both fish species. The depletion was more at higher concentrations. In *C. gariepinus*, the reduction was low (1.77% in liver and 3.75% in muscles) at lower concentration, reaching upto 15.12% in liver and 12.96% in muscle), whilst in *O. niloticus* it was 33.63% in liver and 27.35% in muscles. The reduction in glycogen of

brain tissue was moderately recorded. Serum glucose in both the fish species was increased significantly and remained elevated throughout the investigation period at all concentrations tested.

DISCUSSION

Carbaryl, an acetylcholineestrase inhibitor used as insecticide was found to be comparatively less toxic to *C. graiepinus* than *O. niloticus*. The 96 hours LC_{50} value for C. *gariepinus* (17.55 mgl^{-1}) and for *O. niloticus* (4.45 mgl^{-1}) recorded in the present investigation show variable tolerance for the toxicity by both fish species. This tolerance can also be attributed to difference in susceptibility related to its accumulation and exertion (Johnson and Toledo, 1993; Al-Kahem *et al.*, 1994 and Al-Akel and Shamsi (1999). The effect of such insecticide on aforesaid fish species was found to be dose and time dependent. Data indicate that carbaryl exposure affect respiratory behaviour (cough and yawn). It has been noticed that initially the exposed fish were less active and most of the time remained calm, motionless and settled down at the bottom (Berge *et al.*, 1983; Thomas *et al.*, 1987 and Al-Kahem, 1995). This reduction in activity of the fish can be ascribed to anaesthetic effect of hydrocarbons present in the insecticide. These hydrocarbons might alter the physiological process in which the physical activity increased and finally the breathing problems might affect the fish (Waiwood and Johnson, 1974; Srivastava, *et al.*, 1977 and Al-Kahem *et al.*, 1998). Al-Kahem *et al.*, 1994 pointed out that exposure of C. *carpio* to sublethal concentration of urea resulted in hyper-excitability, fast swimming activity, increase of frequency of cough and yawn. The hyperexcitation and abnormal behaviour like fin-flickering observed in the insecticidal exposed fish may be due to the irritating effect of the toxicant while the fish making an attempt to get relieved from such stress. The secretion of mucus which is more obvious in *C. gariepinus* is probably a mean to the reduction of the body contact with toxic media in order to minimize its

irritating effect or to eliminate it through the epidermal mucus. This might be a factor for the higher value of LC_{50} in *C. gariepinus*. (Varansi and Marky, 1978; Murad and Mustafa, 1989; Al-Kahem, 1994 and 1996). Mucus deposition on the gills most probably interferes with gaseous exchange, thus the fish experience more coughing and yawning to improve the gas exchange by clearing the gills. Cough and yawn have the clearing effect on gills and thus increase the diffusion of oxygen across them. (Al-Kahem *et al.*, 1990 and Al-Kahem 1996). The fish would require more oxygen for the oxidation of fuel molecules to meet the energy requirement for carrying out the increased physical activity, thus, a hypoxic condition produced at tissue level due to high demand and reduced supply of oxygen. This can be correlated with the findings of Gopal *et al.*, 1993 and Al-Akel *et al.*, 1995).

Data presented in Table III, demonstrate that the level of glycogen in tissues of the insecticide exposed fish was depleted significantly ($P<0.001$). This depletion was more pronounced in *O. niloticus* than in C. *gariepinus*. The pattern of depletion was the same but higher in muscles of *O. niloticus*. Previous studies have shown that due to the physical activities of fish, (Nakano and Tomlinson, 1967) and stress of acute hypoxia due to insecticidal effect, (Kumaraguru and Beamish, 1986; Shamsi and Al-Akel 1986 and Al-Akel, 1994), the glycogen level of liver and muscles of fish become depleted rapidly. The hyperglycemia and depletion in the glycogen level in the tissue indicate that insecticidal exposed fish produces more glucose probably by the process of glycogenolysis. Similar to the above finding, hyperglycemia was also observed in fishes after the exposure of oil and hydrocarbons by Wardle (1972); Perrier *et al.* (1977) and DiMichele and Tylor, (1978). Some investigators such as Hoar *et al.*,(1979) and Al-Akel *et al.*, (1988) have reported that hypoxia increases carbohydrate demand causing the anaerobic stress in which the tissue glycogen was broken down possibly

brain tissue was moderately recorded. Serum glucose in both the fish species was increased significantly and remained elevated throughout the investigation period at all concentrations tested.

DISCUSSION

Carbaryl, an acetylcholineestrase inhibitor used as insecticide was found to be comparatively less toxic to *C. graiepinus* than *O. niloticus*. The 96 hours LC_{50} value for C. *gariepinus* (17.55 mgl^{-1}) and for *O. niloticus* (4.45 mgl^{-1}) recorded in the present investigation show variable tolerance for the toxicity by both fish species. This tolerance can also be attributed to difference in susceptibility related to its accumulation and exertion (Johnson and Toledo, 1993; Al-Kahem *et al.*, 1994 and Al-Akel and Shamsi (1999). The effect of such insecticide on aforesaid fish species was found to be dose and time dependent. Data indicate that carbaryl exposure affect respiratory behaviour (cough and yawn). It has been noticed that initially the exposed fish were less active and most of the time remained calm, motionless and settled down at the bottom (Berge *et al.*, 1983; Thomas *et al.*, 1987 and Al-Kahem, 1995). This reduction in activity of the fish can be ascribed to anaesthetic effect of hydrocarbons present in the insecticide. These hydrocarbons might alter the physiological process in which the physical activity increased and finally the breathing problems might affect the fish (Waiwood and Johnson, 1974; Srivastava, *et al.*, 1977 and Al-Kahem *et al.*, 1998). Al-Kahem *et al.*, 1994 pointed out that exposure of C. *carpio* to sublethal concentration of urea resulted in hyper-excitability, fast swimming activity, increase of frequency of cough and yawn. The hyperexcitation and abnormal behaviour like fin-flickering observed in the insecticidal exposed fish may be due to the irritating effect of the toxicant while the fish making an attempt to get relieved from such stress. The secretion of mucus which is more obvious in *C. gariepinus* is probably a mean to the reduction of the body contact with toxic media in order to minimize its

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Table I-A. Behaviour of *Oreochromis niloticus* monitered after exposure with Carbaryl insecticide.

Behaviour	***O. niloticus***
Cough	Rapid, repeated opening and closing mouth and opercular covering with partial extension 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.
Fin-flickering	Repeated extension and contraction of dorsal fin.
Threat	Movement of fish towards another fish
Nip	Bite to another fish

Table I-B. Behaviour of *Clarius gariepinus* monitored after exposure with carbaryl Insecticide.

Behaviour	***C. gariepinus***
Cough	Rapid, repeated opening and closing mouth and opercular covering with partial extension of fins.
Yawn	Wide opening of mouth.
S.jerk	Movement of body partially from head to tail.
Threat	Aggressive and the movement of fish towards another fish.
Hanging	Horizontally the fish hanging upside down.
Rolling	Rapid movement on the surface of water in circular direction.
Nip	Bite to another fish.

Table II-A. Effect of various sublethal conc. of carbaryl on glycogen content in muscle, liver, brain and serum glucose after 96 hrs of exposure in *O. niloticus*.

Carbaryl Concentration	Muscle glycogen µg/g w.wt.		Liver glycogen µg/g w.wt.		Brain tissue glycogen µg/g w.wt.		Serum glucose mg/100ml
	Glycogen	% Depletion	Glycogen	% Depletion	Glycogen	% Depletion	
Control	5452.32 ±23.36	-	6875.34 ±30.21	-	1951.68 ±9.62	-	71.06 ±0.28
0.5mgl^{-1}	4568.76 ±20.73	8.83	5805.30 ±18.03	10.70	1487.40 ±14.04	1.64	72.96 ±6.09
1.5mgl^{-1}	3525.36 ±9.21	19.26	5361.30 ±19.35	15.14	1141.08 ±11.49	5.10	76.83 ±0.08
2.5mgl^{-1}	2717.28 ±33.73	23.75	3571.98 ±19.20	33.03	668.22 ±22.06	9.83	84.62 ±0.35

± Standard Error: Given data is the mean of three replicates.

Table II-B. Effect of various sublethal conc. of carbaryl on glycogen content in muscle, liver, brain and serum glucose after 96 hrs of exposure in C. *gariepinus*.

Carbaryl Concentration	Muscle glycogen µg/g. w.wt		Liver glycogen µg/g. w.wt		Brain tissue glycogen µg/g. w.wt		Serum glucose mg/100ml
	Glycogen	% Depletion	Glycogen	% Depletion	Glycogen	% Depletion	
Control	2977.02 ±23.15	-	4594.34 ±17.64	-	1149.96 ±9.21	-	76.83 ±0.13
2.0mgl^{-1}	2601.84 ±20.96	3.75	4366.74 ±17.92	1.77	994.56 ±20.96	1.5	80.90 ±0.15
6.0mgl^{-1}	2255.52 ±21.61	7.21	3552.00 ±26.08	9.92	763.68 ±17.59	3.86	86.48 ±0.14
10.0mgl^{-1}	1680.54 ±33.75	12.96	3032.52 ±17.59	15.28	421.800 ±21.00	7.28	92.82 ±0.12

± Standard Error: Given data is the mean of three replicates.

Table III . Toxicity test of carbaryl exposed for 96 hours.

O. niloticus

Carbaryl Concentration (mg/l^{-1})	No. of fish used		No. of dead fish		Percent imorality		
	Replicate I	Replicate II	Replicate I	Replicate II	Replicate I	Replicate II	Mean
2	10	10	-	-	-	-	-
3	10	10	2	2	20	20	20
4	10	10	4	4	40	40	40
5	10	10	6	5	60	50	55
6	10	10	7	6	70	60	65
7	10	10	8	7	80	70	75
8	10	10	8	8	80	80	80
9	10	10	8	9	80	90	85

C. gariepinus

Carbaryl Concentration	No. of fish used		No. of dead fish		Percent morality		
	Replicate	Replicate	Replicate	Replicate	Replicate	Replicate	Mean
8	10	10	-	-	-	-	-
10	10	10	-	-	-	-	-
12	10	10	1	2	10	20	15
14	10	10	3	3	30	30	30
16	10	10	4	4	40	40	40
18	10	10	5	6	50	60	55
20	10	10	7	8	70	80	75
22	10	10	8	8	80	8	80