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Effect of Argel Leaf Aqueous Extract (*Solenostemma argel*) on Enzyme Activity in Gills and Muscles of Juvenile Nile Tilapia (Oreochromis *niloticus)*

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All laboratory work was conducted in the wet lab of the Department of Natural Resources and Biodiversity - Environment and Natural Resources Research Institute of the National Center for Research.

Abstract: ***This study investigated the effect of acute toxicity exposure to Argel(Solenostemma argel) leaf aqueous extract on the mortality of juvenile Nile Tilapia Oreochromis niloticus (Mean length 4-7 cm and mean weight 5-6g). It also aimed at studying the effect of long term exposure to a sublethal dose(½ LC_{50}) on glutamate pyruvate transaminase(GPT) and glutamic oxaloacetic transaminase (GOT) activity levels. The acute toxicity test was conducted within 96 hours. During acute toxicity LC_{50} was calculated.The results showed that the 24,48,72 and 96 hours LC_{50} of the aqueous extract of Argel were 0.88,0.52,0.47 and 0.33 g/L respectively. After exposure to 50% 96hrs LC_{50} for 21 days gills and muscle tissues were assayed for (GOT) and (GPT).In gills GPT enzyme activities showed a significant reduction in activity level giving 89 iu/l at day one to 37 iu/l at day 21.As well in muscles GPT activity level showed a reduction from 73iu/l to 24 iu/l.GOT showed reduced activity levels in muscles from 69 iu/l at day one to 23 iu/l at day 21 of exposure. In gills GOT showed a reduction from 69 iu/l at day one of exposure to 29 iu/l at day 21. Results obtained was discussed.***

Keywords: Acute toxicity, Argel, Nile Tilapia, enzyme activity levels, GPT, GOT.

1. Introduction

Aquatic environments are continuously under threat by the use of pesticides that are intentionally formulated to be toxic to living targets. When reaching non-target organisms pesticides become pollutants and exert adverse effects. One of the ways tackling this problem is by using botanical pesticides. This includes natural chemicals derived from plant material. They are easily biodegradable, cheap and leave no residues (1). The plant Argel (*Solenostemma argel*) is a member of the family Asclepidaceae that comprises medicinal plants. It is widely distributed throughout north Africa (Egypt –Libya) North Sudan and Saudi Arabia (2).Beside its being used as a herbal medicine some studies have also shown that Argel aqueous extract has an excellent insecticidal activity against Culex *quenquifasciatus* larvae (3.4). Biochemical changes in tissues are used as stress indicator of fish health. One of these changes is enzyme activity that can be used as a sensitive biomarkers to pollution (5).

Oreochromis niloticus was selected for this study because it is available, withstands transportation and transfer hazared, enjoys consumer's preference throughout the years and has varying geographical location (6). This present study aimed at determining the lethal dose (LC^{50}) that kills 50% of the juvenile Nile tilapia *Oreochromis niloticus* population exposed to Argel leave aqueous extract as well as assessing the effects of the sub lethal dose of the aqueous extract of Argel on glutamate pyruvate transaminase(GPT) and glutamic oxaloacetic transaminase (GOT) enzyme levels in gills and muscles of juvenile *Oreochromis niloticus* on an attempt to determine the sensitivity of this extract when used as an insecticide near water bodies.

2. Materials and Methods

2.1Fish and Argel Leaves Collection

Tilapia fish of the species *Oreochromis niloticus* were collected from Elshagara fisheries research station, 10km south of Khartoum. Fish were caught from the ponds and transported in plastic barrels half filled with pond water early morning to the laboratory. The size of fingerlings ranged from 4-7 cm and 5-6gms.In the laboratory the fish were left to acclimate in indoor glass aquaria measuring 80x60x60 cm with continuous aeration for two weeks. Leaves of Argel plant was obtained from a local market at Khartoum and ground using an electric blender. The obtained powder was stored in plastic jars and left at room condition.

2.2 The Experiment Setup

The experiment setup consisted of glass aquaria with vigorously aerated water kept at room temperature and diffused day- light. The test media were changed every twenty four hours to avoid chemical degradation, volatization, adsorption to the container or reaction with fish excreta. Measured and weighed fish were then introduced to

the experimental system 30 minutes after introduction of the prepared aqueous extracts. Experiments were carried out in triplicates with regular feeding for both experimental and control fish. Fish were fed with a commercial feed consisting of 30 % protein obtained from the Ministry of agriculture.

2.3Acute toxicity test

Prior to the acute toxicity experiments preliminary tests were carried out to determine a convenient and logarithmically spaced range of concentration to be used. To determine the half lethal dose (LC_{50}) fish were divided into six equal groups. One served as a control and five others were exposed to concentrations of 2, 1.5,1, 0.5, 0.25 g /L aqueous leaf extract of Argel. Acute toxicity tests were usually conducted within 96 hours (10). Within this period observations on fish mortality were recorded every 3, 6, 12, 24, 48, 72 and 96 hours. The half lethal dose was then calculated according to Samara (7). Physiochemical parameters, namely pH (using pH meter), temperature (using thermometer), and dissolved O_2 (using oxygen meter) of experimental media were recorded every 24 hours. The behavior of the fish exposed in the extracts was observed during the whole experimental period. Fish were considered dead only when they failed to react to stimuli.

2.4 Determination of Enzyme activity

Fish were divided into 2 groups with the one group as control the other as experimental group. The later was exposed to 50% of the 96 hrs LC_{50} of Argel leaf aqueous extract daily for 21 days. The fish were sacrificed after 1, 7 and 21 days. The gills and muscle tissues were taken for enzyme assays. They were blotted dry with filter paper. Then 100mg was homogenized with 0.25 m sucrose solution in ice cold condition. The homognates were centrifuged for 20 min at 6000rpm (ice cold condition) and the clear supernatant fluid was collected. Using a commercial Kit the level of GOT and GPT were determined according to manufacturer instructions. Activities were measured at 505nm against distilled water using a UV - spectrophotometer.

3. Results

3.1Acute toxicity test

The concentrations used to determine acute toxicity t of Argel to the Nile tilapia *Oreochromis niloticus* ranged between 0.25 to 2.0 g/L. Exposure to these concentrations the fish showed different rates of mortalities at different concentrations and at different exposure times. Results showed that no mortalities occurred in all controls throughout the 96 hours of the experiment. The physiochemical properties of the experimental media (dissolved oxygen (mg/L) Temperature (^oC) and pH were recorded throughout the duration of the experiments. Fluctuations are given in table (1).

The water quality monitored during the experimental period did not differ within the various concentrations of the aqueous extracts as with the control. These parameters exhibited minimal variation. The LC_{50} of Argel aqueous extract showed regular decrease with increase in time for the juvenile tilapia fish. The 24, 48, 72 and 96 hrs LC_{50} were found to be 0.88, 0.52, 0.47 and 0.33 g/L respectively.

3.3 Enzyme activity tests

The activities of both glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) were determined in Gills and Muscles samples of juvenile tilapia (*Oreochromis niloticus*) after 21 days of exposure to 50 % 96 hrs LC50 (0.17g/L).

3.3.1 GPT Activity

GPT activity in muscles showed a significant ($p<0.05$) reduction from control (99 iu/l g) after 24 hours of exposure to Argel aqueous extracts that recorded 73 iu/l. Likewise after 7 days of exposure to 50 % LC_{50} sublethal dose of Argel GPT activity level showed a significant ($p<0.05$) reduction till 61 iu/l.At 21 days of exposure GPT in muscles reduced to 24 iu/l, although not significant from the control. Table (2). Results revealed that GPT activity in gills showed a non significant reduction throughout the experimental period. Although GPT showed a non significant elevation after 24 hours exposure yet the activity level dropped from 89 iu/l after 1 day of exposure to 37 iu/l after 21 days of exposure.(Table 3)

3.3.2. GOT activity

GOT activity in muscles of fish exposed to Argel leaf aqueous extract showed a significant reduction when compared to control. After exposure to a sublethal dose GOT activity level reduced from 69 iu/l at day 1 to 23 iu/l at day 21(Table (4).Detecting GOT activity level in gills revealed no change after 1 day of exposure to Argel. After which the enzyme activity showed a continuous reduction till day 21, reaching 29 iu/l(Table 5). Comparing the effect of exposing the fish to Argel aqueous extract for 21 days, results showed that both GPT and GOT activities showed irregular but significant reduction as shown in Figure 1(a,b)

4. Discussion

This work was conducted under laboratory conditions, to study the magnitude of acute and chronic toxicity of a botanical insecticide, Argel, to juvenile tilapia fish of the species *Oreochromis niloticus*.

The 96hr acute toxicity test, also called short- term toxicity test is one of the most commonly used tests in evaluating the of toxicity of pesticides (8). This study showed that under laboratory conditions the LC50 of Argel leaf aqueous extract to *Oreochromis niloticus* was found to range form0.88g/L after 24hours of exposure to 0.33g/l after 96 hours of exposure. GPT and GOT activities detected in this study showed significant variation in relation to control in both muscles and gills. They exhibited inhibition in their activities in response to exposure to Argel aqueous extract for 21 days. These findings agreed with several investigators who observed that toxicants in different fish species strongly reduces GPT and GOT activities.(9,10,11).

The enzyme inhibition is attributed to the disruption of cell membrane permeability which replaces the electro-chemical important elements in the cell causing functional failure(12). Likewise inhibition of GOT and GPT may be due to a reduction in metabolic activities and blockage of protein metabolism(15). Low activity could also be due to defective or inactive enzymes, which were unable to catalyze their reactions (10,13). At the 7th day of exposure to Argel, gills showed an increment in GPT activity. This increase is attributed to damage in the gills, which results in the liberation of this intercellular enzyme raising its levels in plasma. This observation support earlier findings (14,15). Based on the results obtained in this piece of work and within the confines of the conditions under which the different experiments were carried out it can be concluded that Argel is considered as less toxic when compared to chemical pesticides, but precautions must be taken when it is used in fish inhabiting areas since the excess application can affect the life of juvenile fish.

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Table 1: The physiochemical properties (Oxygen, Temp, and pH) of the experimental media for juvenile *Oreochromis niloticus* exposed to aqueous Hargel extract for a period of 96 hours

Time of exposure	*Concentrations*														
	0			0.25			0.5			1.0			2.0		
	O_2	pH	Temp	O_2	pH	Temp	O_2	pH	Temp	O_2	pH	Temp	O_2	pH	Temp
24	5.93 ± 0.67	7.98 ± 0.26	31.00 ± 0.00	6.10 ± 0.36	8.o9 ± 0.05	30.00 ± 0.00	5.63 ± 0.25	8.08 ± 0.00	30.33 ± 0.56	6.03 ± 0.25	8.01 ± 0.05	30.33 ± 0.56	5.83 ± 0.21	8.05 ± 0.07	30.33 ± 0.56
48	5.77 ± 0.25	8.00 ± 0.08	31.00 ± 0.00	6.17 ± 0.64	8.65 ± 1.14	30.67 ± 0.58	5.63 ± 0.32	8.02 ± 0.11	31.00 ± 0.00	5.80 ± 0.35	8.02 ± 0.11	30.67 ± 0.58	5.97 ± 0.06	8.20 ± 0.13	31.00 ± 0.00
72	6.23 ± 0.49	8.03 ± 0.15	31.00 ± 0.00	6.73 ± 0.29	8.02 ± 0.07	30.67 ± 0.58	6.40 ± 0.40	8.27 ± 0.23	31.00 ± 0.00	6.40 ± 0.35	7.98 ± 0.09	30.67 ± 0.58	6.10 ± 0.26	8.07 ± 0.06	31.00 ± 0.00
96	6.00 ± 0.50	8.11 ± 0.14	30.00 ± 0.00	6.23 ± 0.29	8.04 ± 0.09	30.00 ± 0.00	6.00 ± 0.40	8.00 ± 0.08	31.00 ± 0.56	6.03 ± 0.38	8.01 ± 0.13	30.33 ± 0.56	6.10 ± 0.26	8.06 ± 1.14	31.00 ± 0.00

Table 2: Rate of Change of GPT activity level(iu/l) in Muscle of *O.niloticus* after exposure to Argel leaf aqueous extract for 21 days

Exposure time	*Control*	*Argel treated*	*P value*	*Percent change*
1	99	73*	0.05	-26.3
7	120	61*	0.001	-49.2
21	31	24*	0.003	-22.6

*(-) denotes percent reduction from control, * Values are significant at p < 0.05, (based on t test)*

Table 3: Rate of Change of GPT activity level(iu/l) in Gills of O.niloticus after exposure to Argel leaf aqueous extracts for 21 days

Exposure time	*control*	*Argel*	*P value*	*Percent change*
1	99	89	0.17	-10.1
7	82	98	0.3	+19.5
21	36	37	0.5	+2.7

*(-) denotes percent reduction from control,(+)denotes percent increase over control * Values are significant at p < 0.05 (based on t test)*

Table 4: Rate of Change of GOT activity level(iu/l) in Muscle of O.niloticus after exposure to Argel leaf aqueous extract for 21 days

Exposure time	*control*	*Argel*	*P value*	*Percent change*
1	75	69*	0.004	-8
7	71	49*	0.004	-31
21	31	23*	0.003	-26.8

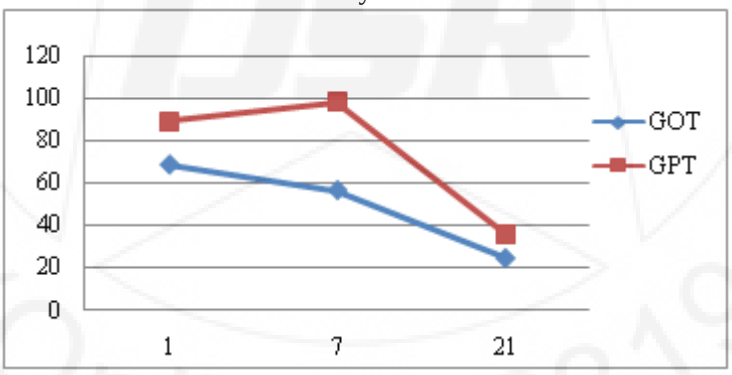
*(-) denotes percent reduction from control * Values are significant at p < 0.05, (based on t test*

Table 5: Rate of Change of GOT activity level(iu/l) in gills of O.niloticus after exposure to Argel leaf aqueous extracts for 21 days.

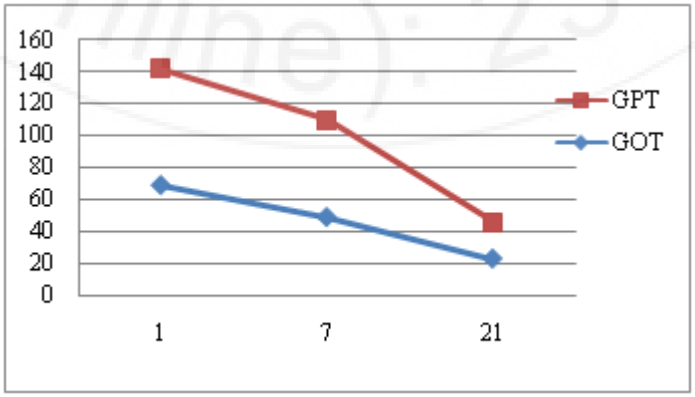
Exposure time	*control*	*Argel*	*P -value*	*Percent change*
1	69	69	0.5	0
7	67	57*	0.012	-14.9
21	36	29	0.19	-10.44

*(-) denotes percent reduction from control * Values are significant at p < 0.05, (based on t test)*

Figure 1 (a,b) : Changes in Glutamate Pyruvate Transaminase (GPT) and Glutamate Oxaloacetate Transaminase (GOT) activity levels (iu/l) in gills (a) and muscles (b) of the Nile tilapia (*O. niloticus*) exposed to Argel leaf aqueous extract for 21 days.



(a)



(b)