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Acute Toxicity of Seed Aqueous Extract of *Citrullus colocynthis* to Fingerlings of the Nile Tilapia *Oreochromis niloticus*

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Abstract

This study aimed at determining the acute toxicity of the aqueous extract of the seeds of Hanzal *Citrullus colocynthis (CCT*) to fingerlings of the Nile Tilapia *Oreochromis niloticus (O. niloticus)*. Six graded concentrations of the aqueous extract were determined after a screening test for 48 hours. Concentrations of 4, 2, 1, 0.5, 0.25 and 0.125 g/l and a control 0 g/l were used in a renewable bioassay for 96 hours. At various concentrations and exposure periods intoxication symptoms has been recorded. These included excitation, jumping darting movements, odd swimming and immobility. The physiochemical namely pH, temperature and dissolved oxygen tested were within tolerable levels. The lethal concentration which kills 50 % of the population (LC_{50}) after 24 .48, 72 and 96 hours were 1.58, 0.785, 0.56, 0.50 g/L respectively. The time taken for 50% of the population to die (LT_{50}) were 0.53, 16.48, 45.7, 40.74, 120.2, and 194.98 hours at concentrations 4, 2, 1, 0.5, 0.25 and 0.125 g/l respectively. The minimum concentration harmful to Tilapia was computed as 25.2mg/l. It was concluded that *Citrullus colocynthis* aqueous extract is classified as botanical substance with low toxicity to Tilapia fingerlings. It was recommended that this extract should be used but with precautions near fish inhabiting areas.

Keywords

Citrullus colocynthis, Hanzal, Acute Toxicity, *Oreochromis niloticus*

1. Introduction

Phytochemicals are botanical substances which are naturally occurring pesticides obtained from floral resources. They are sometimes used intentionally in water bodies for fishing (Yumnamcha *et al.,* 2014) as molluscicides (Azare *et al.,* 2007 and Alsnafi, 2015) and as insecticides (Shaalan *et al*, 2005and Miresmailli, and Isman, 2014) in the aquatic environment where non-target fish species may suffer in various ways. Toxicity of plant extracts to fish have been studied by many investigators (Ayuba and Ofojekwu, 2002; Ayotunde and Ofem, 2008; Maikai, *et al.,* 2008). Different plants employed as pesticides have different effects on fish (Van den Heuvel *et al.,* 2000). The active substances in the different plant part used (leaves, seeds, kernel and bark) have varying potencies and modes of action to the fish as well depending on way of application and the forms of extracts aqueous or alcohol (Sambasivam *et al.,* 2003). *Citrullus colocynthis* (CCT), the plant used in this study is a perennial herbaceous vine (Burrows and Shaik, 2015) native to dry areas .Its local name in Sudan is Handal and common name is Bitter apple. (CCT) has been used successfully as larvicides (Edriss *et al.*, 2013). This present study aimed at examining the acute toxicity of aqueous extract of (CCT) on the juveniles of the Nile tilapia *Oreochromis niloticus.*

2. Materials and Methods

(1) Fish and Plant (citrullus colocynthis) collection:

Tilapia fish of the species *O. niloticus* were collected from Elshagara fisheries research station, 10 km south of Khartoum. Fish were caught from the ponds and transported in plastic barrels half filled with pond water early morning. 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 80 x 60 x 60 cm with continuous aeration for two weeks. Fish were left to acclimate to dechlorinated tap water, through mixing tap water and pond water Regular checks were made to remove the dead fish. (*CCT)* seeds were obtained from local markets The seeds were ground by an electric blender. The obtained powder was kept in plastic jars and left at room temperature.

(2) The set up of the Experiment

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. Total and standard lengths of the fish to the nearest 0.1 cm were measured, the weights of individual fish were first taken using an electronic balance sensitive to the nearest 0.1 gm. 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 wheat bran and groundnut cake in the ratio 3:1. Fingerlings were fed once daily at ratio of 3% of their body weight.

Prior to the acute toxicity experiments preliminary tests were carried out to determine a convenient and logarithmically spaced range of concentration to be used. These were conducted over a wide range of concentrations using only five fish at each concentration in small plastic containers. Screened (*CCT)* concentrations were: 20, 15 10, 8, 6, 4, 2, 1, 0.5, 0.125 g/L .

(3) Determination of LC50 and LT50

For preparation of the water extract, powder was dissolved in tap water, at concentration of (4, 2, 1, 0.5, 0.25, 0.125 and 0.06 g/L) of powdered seeds per litre of water, and left for 24 hours at room temperature.

Acute toxicity tests were usually conducted within 96 hours (Walker *et al.*, 2001). Within this period observations on fish mortality were recorded every 3, 6, 12, 24, 48, 72 and 96 hours. From these mortality observations two parameters were then calculated: these were the LC_{50} and LT_{50}. The former is defined as the concentration of insecticide that is lethal to 50% of the tested population, while the latter is defined as the time taken for 50% of the population to die from a single concentration.

The behaviour 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.

For determination of LC_{50}, the number of dead fish was taken as cumulative percent of the total fish under test. Then the cumulative mortality percent of fish responding for each time interval and for each size group were transformed to conventional scales. The cumulative mortality was transformed to probit scale and logarithmic scale for the concentration. Using the method described by Samara (2001).

$$SSX = \sum X^2 - \frac{\left(\sum X\right)^2}{n}$$

$$b = \frac{\dfrac{\sum XY - \left(\sum X\right)\left(\sum Y\right)}{n}}{SSX}$$

Where X is the logarithmic scale of the concentration Y is the probit scale and b is log tangent ,SSX is the sum of square of logarithmic scale of the concentration, n is the number of treatment. Knowing the value of b, the probit value of the LC_{50} was calculated using the regression equation:

$$Y = \overline{Y} - b\overline{X} + bx_1$$

For determining the LT_{50} individual response time and the cumulative percentages response were transformed into log time and probit scale respectively. In a similar way in determining the LC_{50} the LT_{50} for each concentration was calculated by applying the regression equation:

$$Y = \overline{Y} - b\overline{X} + bx$$

Where Y is the probit, $\overline{Y}$ is the mean of probits at different times, b is log tangent .and $\overline{X}$ is the mean of log times and x is log time calculated for each concentration.

(4) Physiochemical Parameters of experimental media:

Physiochemical parameters, of experimental media were recorded every 24 hours. The pH was measured using a pH meter and temperature was recorded using a mercury thermometer. Dissolved oxygen was determined according to (Arnold, 1989).

3. Results

The physiochemical properties of the experimental media (dissolved oxygen (mg/L) Temperature (°C) and pH were recorded throughout the duration of the experiments.

Fluctuations are given in table (1)

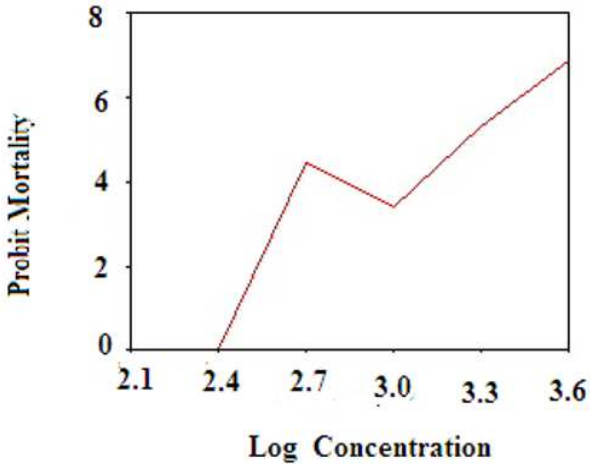
Table (1). *The physiochemical properties (O_2, Temp., pH) of the experimental media for juvenile Oreochromis niloticus exposed to Citrullus colocynthis aqueous extract for a period of 96 hours.*

Time in hrs.	**Concentrations (g/l)**								
	4.0			**2.0**			**1.0**		
	O_2	**pH**	**Temp**	**O_2**	**pH**	**Temp**	**O_2**	**pH**	**Temp**
24	8.0±0.4	7.2±0.01	23.0±0.6	8.0±0.4	7.2±0.02	21.3±0.02	7.8 ±0.2	7.1±0.01	22.0±0.1
48	-	-	-	8.0±0.0	7.0±0.0	20.7±0.6	7.8±0.2	7.1±0.03	20.3±0.6
72	-	-	-	8.5±0.0	7.2±0.0	20.0±0.0	8.4±0.5	7.1±0.05	19.0±0.0
96	-	-	-	8.2±0.1	7.1±0.05	19.8±0.2	7.9±0.1	7.2±0.02	20.0±0.0

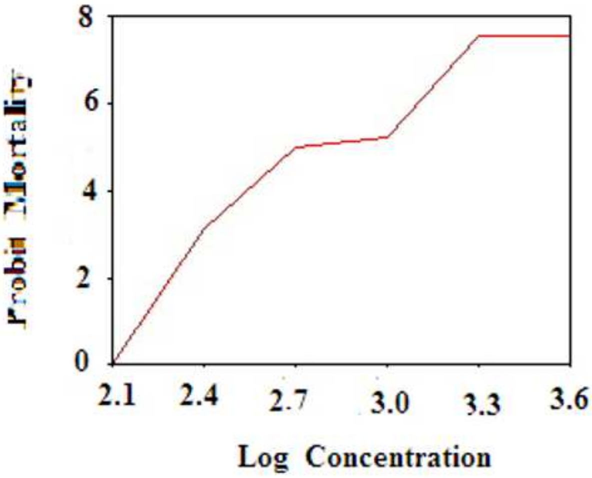
Table (1). *Continue.*

Time in hrs.	**Concentrations (g/l)**								
	0. 5			**0.25**			**0.06**		
	O_2	**pH**	**Temp**	**O_2**	**pH**	**Temp**	**O_2**	**pH**	**Temp**
24	7.9±0.5	7.0±0.03	21.0±0.0	7.8±0.3	7.1±0.06	20±0.6	8.0±0.1	7.1±0.02	22.0±0.1
48	8.0±0.0	7.2±0.03	19.8±0.3	8.0±0.7	7.2±0.01	20.0±0.1	8.0±±0.1	7.2±0.02	20.0±0.3
72	8.3±0.7	7.2±0.1	19.6±0.6	8.2±0.2	7.2±0.09	20.0±0.0	8.2±0.4	7.1±0.02	20.0±0.4
96	8.1±0.4	7.3±0.2	20.0±0.5	8.1±0.1	7.1±0.05	19.8±0.7	7.9±0.6	7.3±0.2	21.0±0.1

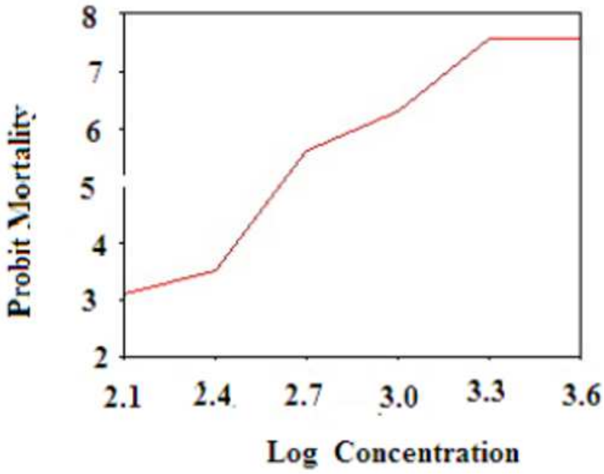
The concentrations used to determine acute toxicity of (CCT) to the Nile tilapia *O. niloticus* ranged between (4.0 to 0.06 g/L). When exposed to these concentrations the fish showed different rates of mortalities at different concentrations and at different exposure times as in Figure (1a-d).



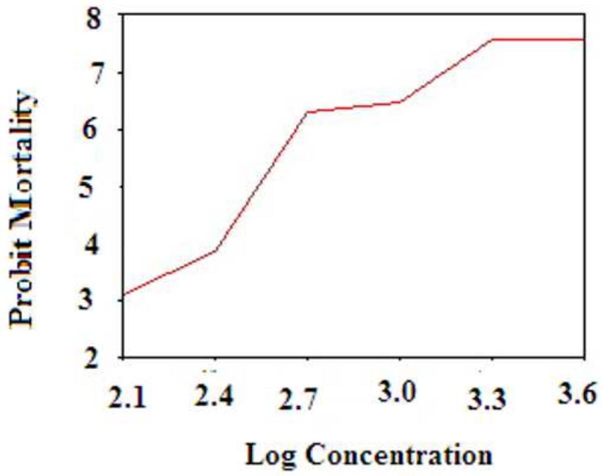
(a)



(b)



(c)



(d)

Fig. (1 a-d). *Dose-mortality regression line of aqueous extract of Citrullus colocynthis against Oreochromis niloticus fingerlings exposed for 24 hours (a), 48 hours (b), 72 hours (c) and 96 hours (d).*

Results showed that there was a significant ($p < 0.05$) irregular but progressive increment in rates of mortalities at exposure times of 24, 48, 72 and 96 hours. Thus in all concentrations the highest cumulative mortalities were recorded after the 96 hours exposure as shown in Figures (1a-d).

Mortalities recorded for juvenile *Oreochromis niloticus* being exposed to different concentrations of (CCT) aqueous extract (4g/l, 2g/l, 1 g/l 0.5 g/l , 0.2 g/l and 0.1g /L) at various times(24hours, 48 hours, 72hours, and 96 hours) were used to compute the half lethal concentration (LC_{50}) and half lethal time (LT_{50}). Using the least square method of transferred variables of probit, Log concentration and Log time as described by Samara (2001).

Variations of the LC_{50} and LT_{50} at various time intervals and different concentrations were illustrated in the histograms in Figs (2) and (3) respectively. Fig. (2) showed that decrease in LC_{50} was almost regular with increase in time. The variation of LT_{50} in tilapia exposed to different concentrations of the aqueous extract of (CCT) was a sharp decrease with increase in the concentration, as shown in fig (3).

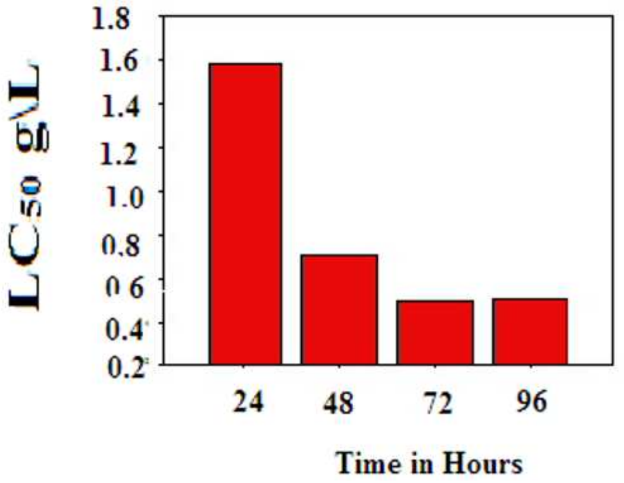


Fig. (2). *Variation of LC_{50} among fingerlings of Oreochromis niloticus within each Time interval.*

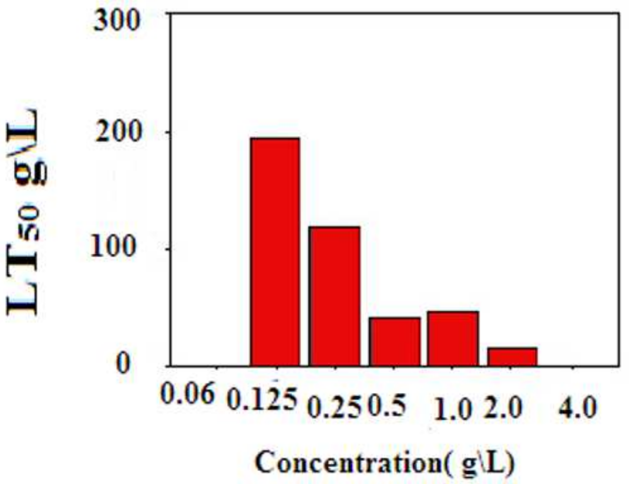


Fig. (3). *Variation of LT_{50} Showed by Oreochromis niloticus exposed to various concentrations of Citrullus colocynthis aqueous extract.*

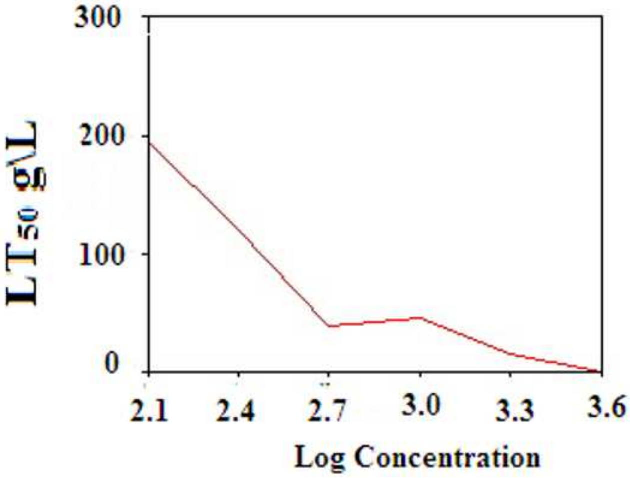


Fig. (4). *The concentration curve for Oreochromis niloticus by plotting the half lethal time (LT50) in hours against log concentrations of the aqueous extract of Citrullus colocynthis.*

The concentration response curves of *Oreochromis niloticus* were obtained by plotting the LT_{50} against the logarithm of concentrations (fig.4). The curve was similar to curve B given by Lloyd (1979). According to the explanation given for such curve, the fish can detoxify the pollutant but at a slow rate.The minimum concentration of pollutant (M.C.P) harmful to the fish could be estimated according to Lloyd (1979) as:

$$\text{M.C.P}=\frac{\text{96 hours } LC_{50}}{\text{Application factor} \times \text{species factor}}$$

Where application factor is equal to 20 in case of curve type B and species factor is equal to one for *Oreochromis niloticus* (Lloyd, 1979). The M.C.P was then calculated:

MCP = 25.2 mg/l

4. Discussion

Botanical pesticides are developed to replace chemical synthesized pesticides. Even though the later being very effective, their impact on the environment is mostly deleterious. During the last three decades, pest control methods were directed to the use of insecticides of plant origin. This trend appeared as a result of the accumulated side effects and environmental contamination from a long term extensive application of toxic synthetic insecticides. This work was conducted under laboratory conditions, trying to throw some light on the magnitude of acute toxicity of the aqueous extract of *Citrullus colocynthis* to tilapia fish of the species *Oreochromis niloticus*. Water temperature is most important factor affecting different aspects of fish life and its environment. In acute toxicity experiments the temperature values between replicates did not vary much throughout the duration of the test. The dissolved oxygen content of the water is another limiting factor beside temperature as a significant factor in aquatic environments. According to (Olufayo, 2009) Nile tilapia can survive at dissolved oxygen concentration of below 0.5mg/l and can live for about 6 hours. In this study the oxygen content of experimental media was within the tolerable levels of tilapia fish. Likewise the pH was within the tolerable level. Therefore the selected parameters were within tolerable levels. Consequently it could be said that (CCT) aqueous extract might have acted alone in toxicating the fish without interference from the environment.

The 96 hours acute toxicity test, also called short- term toxicity test is one of the most commonly used tests in evaluating the toxicity of pesticides (Alim, 2008). This study showed that (CCT) has some toxicity at different concentrations giving different rates of mortalities. The 24, 48,72 and 96 hours LC50 was 1584 ,785.05 , 558.47 and 503.5 mg/l respectively. The toxicity and lethality of the seed-extract may be due to any one or more of the phytochemicals present in the aqueous extract, some of which have been isolated and identified (Abdel-Hassan *et al.,* 2000; Adam *et al.,* 2001; Chen *et al.,* 2005; Yoshikawa *et al.,* 2007).To the best of our knowledge little or almost non is done in toxicity of (CCT) to aquatic organisms. Many investigators worked in acute toxicity of (CCT). Diwan et al., (2000) reported 200mg/kg Ld50 in mice, while Soufane *et al.* (2013) stated that the LD_{50} in male rats is 1311.45 mg/ kg. They concluded that (CCT) is moderately toxic .In this study the 96 hours LC_{50} of the seed aqueous extract of (CCT) was found to be 503.5 mg/l. Thus according to the OECD (1992) Classification Categories for hazardous to the aquatic environment, (CCT) with a 503.5mg/L LC_{50} is classified as moderate category i.e has no toxicity but may cause long lasting harmful effects to aquatic life . This also confirms the findings of the concentration response curve of *Oreochromis niloticus* According to the explanation given by Lloyd (1979) for such curve, the fish can detoxify the pollutant but at a slow rate.

The LT50 is the time taken by a concentration to kill 50 % of the population. In this study the LT50 was found to increase with decrease in (CCT) concentration giving 0.53 hours in concentration 4g/l to 194.98 hours when fish exposed to a concentration of 0.125 g/L of the aqueous extract. The low value of LT50 (0.53 hour) at concentration 4g/L and the minimum harmful concentration assures that (CCT) may threaten survival of juvenile *Oreochromis niloticus*, which is considered as an important stage for the survival of this species.

5. Conclusion

Based on the results obtained in this study it can be concluded that aqueous extracts of *Citrullus colocynthis* seeds have some toxicity to juveniles of *Oreochromis niloticus.* Thus such biocide should be used with precaution near water bodies since the excess application can affect life of fish.

Moreover the aqueous extract an be recommended to be used in controlling unwanted organisms in fish ponds as an environmentally friendly biocide. However further investigations should be conducted to provide information for its safe use in aquaculture.

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