Vol. 11(2), pp. 9-15, February 2019
DOI: 10.5897/JTEHS2018.0426
Article Number: CC6770C60069
ISSN: 2006-9820

http://www.academicjournals.org/JTEHS

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Journal of Toxicology and Environmental Health Sciences

Full Length Research Paper

Acute toxicity evaluation of water extract stem barks of *Balanites aegyptiaca* on adults of three different fish species

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Received 17 December, 2018: Accepted 7 January, 2019

A 96 h static toxicity bioassay was carried out to examine fish responses and to determine the median lethal concentration (LC_{50}) of *Balanites aegyptiaca* stem bark extract on adults of *Brycinus nurse*, *Labeobarbus bynni* and *Labeobarbus intermedius*. Experimental fish were exposed to piscicide plant extract of 0.0 (control), 15.0, 17.5, 20.0, 22.5, and 25.0 mgL^{-1}. Fish exposed to these extracts except the control showed symptoms of toxicity including darting, agitated swimming, air gulping, loss of sensitivity and knockdown before death. These responses were much frequent and faster in *L. bynni* and *L. intermedius*. The 96 h LC_{50} values for the different test fishes were 18.99, 20.72 and 20.72 mg L^{-1} for *L. bynni*, *L. intermedius* and *B. nurse*, respectively. Based on the present investigation, we can conclude that the application of *B. aegyptiaca* extract causes lethal toxic effects on different fishes even at low concentrations and hence, indiscriminate use of the plant for fishing should be discouraged and regulated in order to protect fish biodiversity lose in the Alitash National Park area.

Key words: Alitash National Park (ALNP), *Balanites aegyptiaca*, floodplain rivers, piscicide, toxicity, LC_{50}.

INTRODUCTION

The incidence of using piscicides to catch fish is part of the traditional fishing method which is not 'environmentally friendly' to the aquatic ecosystem. Plant piscicides are among the widely used fishing method which is biodegradable and less severe than synthetic piscicides (Onusiriuka and Ufodike, 1998; Neuwinger, 2004). In Africa more than 325 fishing poison plant species are commonly used to catch fish (Neuwinger, 2004). In Ethiopia, 17 plants with piscicidal properties are included in the Neuwinger's catalog.

Although prohibited, plant piscicides used to catch fish, are alternatively used throughout Africa (Ameha, 2004; Fafioye et al., 2004; Neuwinger, 2004; Fafioye, 2005). Fishermen living in areas where fishing gears are not available are very well aware that poisoning implies killing of many small fish, but often see it as an emergency method to alleviate hunger (Van Andel, 2000). Thinking the disadvantages of spending long time for fishing in the rivers and the probability of back home with no catch while using gears, fishermen are deceived to use

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piscicides for fishing in most areas of Ethiopia and the practice is now increasing alarmingly (Wudneh, 1998; Ameha and Assefa, 2002; Ameha, 2004). Fishermen of the Alitash National Park (ALNP) use different piscicides for fishing. However, plant piscicides are largely used by the fishing communities as they are easily available and cost effective in the area. This traditional and destructive fishing technique is exercised and effective during the dry season when floodplain rivers form small water sections or pools.

Studies have been conducted about responses of different fish to some piscicide plant (Ameha, 2004; Singh and Yadav, 2010; Agbor and Okoi, 2014; Ekpendu et al., 2014), but the piscicidal effect of *B. aegyptiaca* is limited (Absalom et al., 2013; Alhou et al., 2016). The acute toxicity levels of *B. aegyptiaca* on fish has not been investigated in Ethiopia but extensively used by fishermen of the ALNP. Therefore, in the present study, the median lethal concentration (LC_{50}) of the aqueous extract of *B. aegyptiaca* was evaluated on adults of different fishes and the result may be used for policy makers and authorities that enforce law otherwise its indiscriminate use could seriously affect the biodiversity.

MATERIALS AND METHODS

Experimental design

The experimental setup consisted of seven geo-membrane lined ponds with 1 × 0.5 m and with a depth of 0.5 m; each filled with 100 L river water. Among the seven ponds one was left empty during toxicity commencement and used as a control. In order to reduce the rate of evaporation and to eliminate direct sunlight exposure, the ponds were covered with geo-membrane, fresh plant leafs and dried grasses. The water was pre-aerated through stirring for some time to full oxygen saturation before addition of the different concentration grades of the plant extract.

Collection and preparation of plant extract

The stem barks of *B. aegyptiaca* were collected from the Derhassen Village following the Gelegu River course near the ALNP. Stem barks obtained from the wild, sliced into smaller sizes using a knife and an axe and then ground to a fine powder using a wooden mortar with pistil. The plant extract was used immediately after extraction to ensure its freshness. The different test concentrations prepared from aqueous extracts of *B. aegyptiaca* and used for the experiment are summarized in Table 2.

Test fish collection and acclimatization

Beach seine, mosquito nets, traps and castnets were used to collect alive and active fishes in the Gelegu River. The candidate specimens were acclimated for 4 days prior to toxicity commencement in a pond (2.5 m × 2.5 m with a depth of 0.5 m) having water diverted from the river for this purpose. Test fishes were selected based on the prerequisite observation made at fishing grounds from April, 2015 to December, 2017 by the investigators. Therefore, based on the reconnaissance study for the experiment, the most affected and easily stupefied groups, economically viable species, readily available for test individual and species with important ecological role (that is, in feeding relation) were selected. In order to reduce the effect of size, all test specimens of a species had comparable size. The individuals of *B. nurse* (n=77) with a mean of 165±0.18 g and 17.5±0.25 cm total weight and total length, respectively; *L. intermedius* (n=77) 245±2.30 g total weight and 22.3±1.26 cm total length and *L. bynni* (n=55) 226±3.8 g and 21.2±2.6 cm total weight and total length, respectively were used for the experiment. Seven test individuals per extract were used but 5 in *L. bynni* due to a limited sample.

The acclimated individuals were collected using scoop nets and then distributed to ponds with different level of concentration: C1=0.00 (control), C2=15.00, C3=17.50, C4=20.00, C5=22.50 and C6=25.00 mgL^{-1} of the pounded and powdered *B. aegyptiaca* poison extract based on the OECD guideline No. 203 (1992) in duplicates. Pilot tests were carried out to establish appropriate range finding concentration grades used in the actual experiment along with the control as described by EPA (1996).

Fish responses and LC_{50} estimation

A fish is considered affected by the plant toxicant when it manifests erratic swimming, hyperactivity, hyperventilation and pronounced ataxia coinciding with decreased capacity to respond to visual stimuli. As an end point of toxicity action, fish mortalities were observed and recorded at every 8 h throughout the 96 h toxicity test as recommended by OECD (1992). When a fish did not respond to any mechanical prodding it is proven to be dead. The LC_{50} of the extract determined using dose response mechanisms of the piscicide and test animal over the 96 h period was calculated using the method of Karber as adapted by Dede (1992) as follows:

$$LC50 = LC100 - \frac{\Sigma \text{ probit}}{\text{No. of fish per extract concentration}} \quad (1)$$

where LC_{100}= extract concentration with 100% fish mortality occurred.

Ethical statements

Experimental procedures involve handling of experimental animal in accordance with the standard practice as specified in the OECD and EPA guidelines (OECD, 1992; EPA, 1996). Prior to the experiment, letter of permission to conduct the experiment was issued by the Quara Woreda Agriculture and Rural Development Office. The procedures and all tasks of the experiments were approved by the extension core process committee of the office declared and assigned as an ethical committee by stakeholders to evaluate the test procedure.

Data analysis

The mortality response of different fishes exposed to *B. aegyptiaca* was subjected to probit which is a specialized regression analysis model of binomial response variables used to analyze dose-response experiments in toxicology to determine the relative toxicity of chemicals to the test organisms for a linear regression method (Finney, 1971) to calculate the LC_{50} of the extract for the exposed fish using Microsoft Excel (Agresti, 1992). Physico-chemical parameters of water in each treatment and control pond were compared by one-way analysis of variance (ANOVA). All the statistical tests were carried out by SPSS version 24.0 and Microsoft Office Excel 2007.

Table 1. Mean±SD (range in parenthesis; measured 12 times in the 96 h) of water physico-chemical parameters in the pond exposed for aqueous extract stem bark *B. aegyptiaca* and the control.

Parameter	After/Before piscicide addition	Concentration level (mgL^{-1})					
		0.00 (Control)	15.00	17.50	20.00	22.50	25.00
pH	Before	7.2	-	-	-	-	-
	After	-	7.9±0.44 (7.7-8.8)	6.2±0.47 (7.2-8.7)	7.41±0.37 (7-8.1)	7.23±0.48 (6.9-7.3)	7.08±0.6 (7-7.6)
Temp. (°C)	Before	27.4	-	-	-	-	-
	After	-	27.80±0.89 (27.2-30.2)	28.65±0.89 (27.5-30)	28.09±0.47 (27.8-29.1)	28.38±0.74 (27.8-31.6)	28.9±0.8 (28.6-31)
DO (mgL^{-1})	Before	7.60	-	-	-	-	-
	After	-	6.80±0.27 (6.6-7.2)	6.9±0.26 (6.8-7.4)	6.3±0.27 (6.0-6.6)	6.0±0.34 (5.6-6.8)	6.0±0.74 (5.6-6.5)
Cond. (µS/cm)	Before	270	-	-	-	-	-
	After	-	280.17±11.13 (279-293)	285.83±9.46 (280-300)	289.42±7.1 (276-293)	284.83±9.98 (270-300)	291.46±12.31(276-298)

Temp=temperature, Cond=conductivity, DO=dissolved oxygen.

RESULTS

Pond water physico-chemical parameters

The physico-chemical parameters were measured and recorded prior to fish introduction and taken every 8 h throughout the experiment (Table 1). Mean values of the parameters during acute exposure of different fish species to *B. aegyptiaca* stem bark have shown slight fluctuations (Table 1). In all the treatment ponds, DO values decreased slightly but insignificant ($p>0.05$) with increasing extract concentration. Electrical conductivity values were increased at higher concentrations of the toxicants compared to the control with the highest values at C6. Temperature values did not show a significant variation ($p>0.05$). pH values showed changes and increased at higher concentration with significant difference from the control ($p<0.05$).

Observed behavioral responses of fishes to test concentrations

Fish exposed to the different concentration levels of aqueous extract of *B. aegyptiaca* exhibited hyperactivity characterized by darting, rapid opercular movement, held mouth wide open and kept the fins stretched laterally. These responses were species specific and on average, after 5 to 10 min, this restlessness subsided in all species. The first symptom of loss of sensitivity (response) was characterized by rising of the fins and was highly pronounced in the *L. intermedius* and *B. nurse*. Subsequently, a rigorous and mostly superficial movement of the fins and rapid respiration was observed. Fish aggregated at the air-water interface gasping for air with their mouth permanently opened and this response was frequent in *B. nurse* and *L. bynni*.

On average after 6 to 10 h, there was discharge of mucus through the gills and a mucus layer was formed on eyes and all over the body. As time goes, normal colors of the fish were changed. *L. bynni* and *B. nurse* were discolored and remained in the state of exhaustion, which did not respond to external stimuli and remained diagonally suspended in the water. Subsequently, attempts were made to avoid the toxic medium by bumpy swimming, jumping out of water accelerated and arrhythmic respiration, but slowly the movements were staggered, mouth was partly opened, and fins and tail become rigid. These behavioral deviations were more pronounced with increasing concentration and it was species specific and extreme on *L. bynni* followed by *B. nurse* and

Table 2. Percentage mortality of fish species exposed to various acute concentrations of the stem bark of *B. aegyptiaca*.

Test fish	Conc. (mgL^{-1})	Log of conc.	Treatment	Exposure time (h)				Total mortality	% Mortality
				24	48	72	96		
B. nurse	25.00 (C6)	1.39	R1	3	4	0	0	7	100
			R2	4	2	1	0	7	
	22.50 (C5)	1.35	R1	1	3	2	0	6	85.71
			R2	1	0	3	2	6	
	20.00 (C4)	1.30	R1	2	1	4	0	7	71.43
			R2	0	2	1	0	3	
	17.50 (C3)	1.24	R1	0	1	2	1	4	57.14
			R2	1	1	1	1	4	
	15.00 (C2)	1.18	R1	0	0	1	1	2	28.57
			R2	0	1	0	1	2	
	0.00 (C1)	0.00	R1	0	0	0	0	0	0
			R2	0	0	0	0	0	
L. bynni	25.00 (C6)	1.39	R1	2	3	2	0	7	100
			R2	3	3	1	0	7	
	22.50 (C5)	1.35	R1	1	2	2	1	6	85.72
			R2	2	0	2	2	6	
	20.00 (C4)	1.30	R1	0	1	2	2	5	85.72
			R2	1	1	2	3	7	
	17.50 (C3)	1.24	R1	0	1	0	2	3	42.86
			R2	1	1	0	1	3	
	15.00 (C2)	1.18	R1	0	1	0	1	2	28.57
			R2	0	0	0	2	2	
	0.00 (C1)	0.00	R1	0	0	0	0	0	0
			R2	0	0	0	0	0	
L. intermedius	25.00 (C6)	1.39	R1	2	4	1	0	7	100
			R2	2	2	3	0	7	
	22.50 (C5)	1.35	R1	2	1	3	0	6	92.86
			R2	3	3	1	0	7	
	20.00 (C4)	1.30	R1	2	0	1	2	5	64.29
			R2	1	2	0	1	4	
	17.50 (C3)	1.24	R1	0	0	1	2	3	42.86
			R2	0	1	1	1	3	
	15.00 (C2)	1.18	R1	0	0	0	2	2	35.72
			R2	0	0	2	1	3	
	0.00 (C)	0.00	R1	0	0	0	0	0	
			R2	0	0	0	0	0	0

Results of two replicate over 96 h.
R1 and R2: replicates of the experiment.

finally *L . intermedius*. Finally, fish died and mortality increased with increasing concentration (Table 2). Any

Table 3. Lethal median concentration (LC_{50}) of *B. aegyptiaca* on a 96 hr acute toxicity exposure of the different test fishes.

Test fish	Conc. levels (mgL^{-1})	Log of conc.	% Mortality	Probit	LC_{50} (mgL^{-1})
B. nurse	C1	0.00	0	0	20.724
	C2	1.18	28.57	4.43	
	C3	1.24	57.14	5.18	
	C4	1.30	71.43	5.55	
	C5	1.35	85.72	6.05	
	C6	1.39	100	8.72	
L. intermedius	C1	0.00	0	0	20. 720
	C2	1.18	35.72	4.62	
	C3	1.24	42.86	4.81	
	C4	1.30	64.29	5.37	
	C5	1.35	92.86	6.44	
	C6	1.39	100	8.72	
L. bynni	C1	0.00	0	0	18.988
	C2	1.18	28.57	4.43	
	C3	1.24	42.86	4.81	
	C4	1.30	85.72	6.05	
	C5	1.35	85.72	6.05	
	C6	1.39	100	8.72	

strange behavioral responses were not observed on individuals kept in the control.

Average mortality and LC_{50} of test individuals

There was complete (100%) mortality in the fish exposed to the highest concentrations of 25.00 mgL^{-1} in the first 24 h, while the least mortality of 28 to 35% (species specific) was recorded in the fish exposed to the lowest extract concentration of 15.00 mgL^{-1} (Table 2). The acute lethal concentration after 96 h showed that 29, 43, 86, 86 and 100% of *L. bynni* mortality occurred at concentrations of C2, C3, C4, C5 and C6 respectively.

These concentration levels in the order brought mortality in *L. intermedius* by amounts of 36, 43, 64, 93, and 100%. The 29, 57, 71, 86 and 100% deaths that took place in *B. nurse* were also due to these concentration levels. However, no mortality was recorded in the control (C1) of all test fishes during the 96 h test period (Table 2). Mean mortality increased significantly ($p<0.05$) with extract concentrations but changes were insignificant with exposure period. The highest mortality was recorded for all fish species in the 25.00 mgL^{-1} concentration within the first two recording hours. The LC_{50} of the aqueous extract of *B. aegyptiaca* stem bark for the treatment fish species over the 96 h exposure period was calculated to be: 18.99, 20.72, and 20.72 mgL^{-1} for *L. bynni*, *L. intermedius* and *B. nurse*, respectively (Table 3).

DISCUSSION

Pond water quality

The analysis showed that pH and conductivity differ significantly from the control. The pH of the water samples varied from concentration to concentration and the values obtained for the different treatments, however, it was within the standard given by WHO (2004) for fish survival. The decline in pH with time may be due to the production of acidic products of metabolism by the plant material in water. Electrical conductivity increased across the different concentration level, this may be due to the chemical composition of *B. aegyptiaca* responsible for conductivity (Abalaka et al., 2014).

In the present study, there was insignificant ($p>0.05$) decline in DO within 96 h after stocking. Treatments with higher concentration of the plant extract had lower DO which can be attributed to the chemical oxygen demand of the extract. Further drop in DO concentrations was attributed to the oxygen consumption of the test fishes (Fafioye, 2005). Edafe and Vincent-Akpu (2016) reported that the reduction in DO content in a bioassay media as toxicant concentration increase may be due to antioxidant property of the toxicant. The physico-chemical parameters monitored in this study tend to have contributed little or none to the toxicity of *B. aegyptiaca*. This implies that, it was only the piscicidal natures of the plant independent of test condition which brought strange behavioral responses and deaths on test fishes.

Behavioral response of fishes to test concentrations

All test fishes used for this experiment showed different responses against the acute concentrations of *B. aegyptiaca* stem bark extract. *L. bynni*, *L. intermedius* and *B. nurse* exposed to toxic extract of *B. aegyptiaca* showed some behavioral responses and death that confirmed the plant hampered fish normal physiology. All fishes subjected for higher concentration levels appeared in a more distressed condition at first, jumping over the surface and gasping air then they became inactive followed by loss of balance and finally sank to the bottom. The behavioral responses against acute concentration of *B. aegyptiaca* observed in the current studies are in tandem with earlier findings of Ufodike and Omoregie (1994), Absalom et al. (2013), Abalaka et al. (2014) and Alhou et al. (2016). However, at lower concentrations (that is 15.0 and 17.5 mgL^{-1}), such physical distress was recorded only for *B. nurse* within a short time of exposure and the other test fishes showed such behavioral actions after long exposure. The initial increase in opercular movement can be taken as index of the suffocation stress felt by the fishes exposed to poisons (Ferdous et al., 2018) and attempted to increase ventilation rates (Fernandes and Mazon, 2003). This is in addition to the engagement of aerial mode of respiration so as to disengage gill respiration and by implication, prevent continuous gill contact with the toxicant (Absalom et al., 2013). Fishes in the control were free from such types of behavioral changes and therefore only the plant extract responsible for the altered behavior and mortality. In addition to respiratory distress, the test fishes exhibited increasing states of motionlessness, adoption of different postures, sudden darts, slow sluggish movements and ataxia. These might be due to the acetyl cholinesterase inhibition property of the piscicide plant as similar hyperactivity, uncoordinated movements and acetyl cholinesterase inhibition was reported in *Oreochromis niloticus* exposed to *B. aegyptiaca* stem bark extract (Alhou et al., 2016).

Toxicity evaluation of *B. aegyptiaca*

Plant piscicides as an easy method of fishing has been extensively used from time immemorial perceptions in Ethiopia (Wudneh, 1998; Neuwinger, 2004). In Ethiopia, *B. aegyptiaca* and *Millettia ferruginea* are among the extensively used potentially active plant piscicides for fishing. These two plants have been used for fishing in the ALNP and *B. aegyptiaca* is grown near the river sides of the area and largely used as an effective fishing method during the dry season. This is in agreement with the study of Neuwinger (2004) and Abalaka et al. (2014) in which *B. aegyptiaca* was in the first listing order among the piscicide plants widely used in Africa. Therefore, the efficacy of this commonly used plant piscicide was evaluated and found to be more toxic than other plant piscicides evaluated on different fish species (Neuwinger, 2004; Absalom et al., 2013; Alhou et al., 2016). In an experiment done for comparing the lethal toxicity effect of *B. aegyptiaca* and *Kigelia africana* on *O. niloticus*, the former was found more toxic and opercular ventilation and tail fin beat increased with concentration and then declined as the fish became fatigued (Ufodike and Omoregie, 1994).

Dose responses of the aqueous extract of *B. aegyptiaca* depend on concentration levels and the type of test individual. For example, in the present study, the LC_{50} was relatively smaller in *L. bynni* (18.99 mgL^{-1}), showed that the extract was toxic to this fish as higher LC_{50} values signify less toxicity (Eisler and Gardener, 1993). *O. niloticus* exposed for an aqueous extract of *B. aegyptiaca* showed signs of toxicosis and the LC_{50} of the extract for this fish was 26.22 mgL^{-1} (Alhou et al., 2016). In another experiment done on juvenile *Clarias gariepinus* exposed for aqueous *B. aegyptiaca* fruit extract confirmed the 96 h LC_{50} value of 12.59 gL^{-1} (Absalom et al., 2013). The 96 h LC_{50} recorded for all species in the present study were lower than those reported previously. This implies that *B. aegyptiaca* is more toxic for sensitive cyprinid fishes than cichlids and clariid. It is, however, imperative to note that the differences in the present study from those of the aforementioned reports could be attributed to the differences in type of test species, age and experimental conditions. This was a real situation of what happened in the actual river when this plant was used for fishing by which the first species that comes out and dazed easily were Cyprinidae and Alestidae, Distichodontidae, Schilbeidae, Bagridae but finally Mochokidae, Clariidae and very rarely Arapaimidae in the ALNP area. This relative difference for piscicide tolerance may be due to the presence of some elaborative breathing organs in the catfish families and an intricate physiology in some fishes that is able to detoxify ichthyotoxins to a level of insignificant toxicity (Abalaka et al., 2014).

Conclusion

The main objective of this study was to evaluate the potential toxicity of *B. aegyptiaca* in fishes sampled from the wild from the Gelegu River along the ALNP area in northwestern Ethiopia. As a result, *B. aegyptiaca* is found to be toxic to fishes of some groups and even our personal observation showed that the plant is even toxic to the resilient catfish. It was much toxic for *L. bynni* since smaller values of LC_{50} was calculated at 18.988 mgL^{-1} followed by *L. intermedius* with LC_{50} values of 20.720 mgL^{-1} but less toxic for *B. nurse* with relatively high LC_{50} values. The use of *B. aegyptiaca* by local fishermen in rivers, streams and lakes, is ill advised, as the resultant deleterious effects will subsequently lead to death of not only target fish but other aquatic organism, hence reduce the biodiversity. Therefore, the indiscriminate use of *B. aegyptiaca* as a toxicant to catch fish should be discouraged and regulated in order to protect fish bio-diversity in the ALNP. If the present rate at which these piscicide plants are being used by fishermen of the ALNP is not checked, the continuous existence of the aquatic fauna, including biologically important fish species, will be in serious jeopardy. Thus, the Amhara regional environmental authority that is responsible for conserving the ALNP biodiversity is in need to ban the use of this plant extract for fishing in any natural environment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank Ashagrie Fetene (fisherman) for his help during the field work. They also acknowledge Addis Ababa and Debre Berhan Universities for the financial support.

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