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Lethal Toxicity (LC50) Effect of *Balanites aeguptiaca* Water Root Extract on *Catla catla*

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Abstract
Lethal toxicity is a tool used in piscicide bio-safety assessment in fish farming prior to its proper application in sustainable aquaculture. Piscicides of plant origin are usually used for fish capturing. Lethal toxicity is an indication of baseline data assessment before any piscicide of plant origin could be used in sustainable aquaculture. This study assessed the lethal toxicity (LC_{50}) effect of *Balanites aeguptiaca* water root extract on fresh water fish, *Catla catla* for 96-h under renewal toxicity exposure. Lethal Concentration (LC_{50}) for *Catla catla* the mortality was obtained at 13 mg/l for 24 hrs. to 7 mg/l for 96 hrs. where fish showed abnormal behaviour such as erratic swimming, mucus secretion, loss of scales, haemorrhages, and stiff fin rays prior to death. Water root extract of *B. aeguptiaca* could serve as tool in aquaculture to assess bio-safety level of targeted and non-targeted aquatic organisms in pond
Keywords: Moringa oleifera, Acute Toxicity, Freshwater fish, Piscicide.

Introduction

Acute aquatic toxicity represents the intrinsic property of a substance to be injurious to an organism in a short-term exposure to that substance. Static acute toxicity tests provide rapidand reproducible concentration-response curves for estimating toxic effects of chemicals on aquatic organisms. With the help of these tests the relative toxicity of large number of chemicals present in the natural aquatic systems due to variety of chemical spills can be determined. There is a vigorous documentation of the use of acute toxicity tests for assessing the potential hazard of chemical contaminants to aquatic organisms **(Brack *et al.*, 2002)**.

Acute toxicity is expressed as the median lethal concentration (LC_{50}) that is the concentration in water which kills 50% of a test batch of fish within a continuous period of exposure which must be stated **(Amweg and Weston, 2005)**. The application of the LC_{50} has gained acceptance among toxicologists and is generally the most highly rated test of assessing potential adverse effects of chemical contaminants to aquatic life. The use of 96-h, LC50 has been widely recommended as a preliminary step in toxicological studies on fishes **(Parrott *et al.*, 2006; Moreira-Santos *et al.*, 2008)**. LC_{50} is customary to represent the lethality of a toxicant to a test species in terms of lethal concentration (for aquatic animals) and lethal dose (for terrestrial animal). It is always expressed in terms of g or mg/kg body weight of the animal and lethal concentrations (LC) in terms of Parts/million (ppm) or parts/billion (ppb) or milligram/liter (mg/L). The relationship between the concentration of an environmental toxicant and its lethal effects on living organisms is often a sigmoid curve.

Fish farming or aquaculture is reported to be the fastest growing food production system worldwide. This is because fishes have high feed conversion ratio coupled with their high utilization of both Agricultural and animal wastes leading to high productivity and high returns while providing the much needed employment opportunities **(Katina, 2000)**. The use of toxic plants for catching fish is a common practice worldwide. The ichtyotoxic characteristics of some of these plants make them potent tools for catching or stupefying fish all over the world. Above some forty years ago, local fishermen in Nigeria have reported used specific biocides derived from plants for fishing **(Reed *et al.*, 1967)**. Since then, this has continued unabated indifferent parts of the country. Besides, a number of laboratory studies have revealed the toxicity of plant extracts to fishes **(Ayotunde and Ofem, 2008)** being used as molluscicides **(Maikai, *et al.*, 2008)** in the aquatic environment where non-target fish species may suffer in various ways. Different species of plants employed as piscicides have different effects, depending on the species of fish targeted **(Van Andel, 2002)**. The active principles in the plant part used (leaves, seeds, kernel, bark and root) have varying potencies and modes of action depending on poisons, although plants with sufficient levels of whether it is applied directly and the forms of extracts, aqueous or alcohol used **(Sambasivam *et al.*, 2003)**. The two main groups of phytochemicals that occur in most plants used for the stunning fish, the rotenones and saponins, as well as a third group of plants which liberate cynanide in water, account for nearly all varieties of fish ichthyoethereol, triterpene and other ichthyotoxins are also used **(Béarez, 1998)**.

The Indian major carps *Catla catla* (Hamilton) was used as the test animal because it is present in almost all freshwater reservoirs in India and is suitable for toxicity monitoring **(Nair, Sherief, 1998)**and pose a potential direct threat to freshwater organism, particularly to sensitive animals, such as fishes **(Sarvanan *et al.*, 2003)**.

Material and Methods

Collection Preparation and Extraction of plant materials by maceration method

The roots of *Balanites aegyptiaca* were collected from local area. After shade drying the plant material was grounded into powder using pestle and mortar. Exposure to sunlight was avoided to prevent the loss of active components. One liter of distilled water was mixed with 200 g of powdered plant material. The mixtures were kept for 2 days in tightly sealed vessels at room temperature and stirred several times daily with a sterile glass rod. This mixture was filtered through muslin cloth. Further extraction of the residue was repeated 3-5 times until a clear colorless supernatant extraction liquid was obtained indicating that no more extraction from the plant material was possible. The extracted liquid was subjected to water bath evaporation to remove the solvent. The water bath temperature was adjusted to 40^0 C. The semi-solid extract produced was kept under a ceiling fan to dry. The extract was weighed and portion of it used for phytochemical screening while the rest was use for the susceptibility test.

Experimental animals

One hundred samples of *Catla catla* were brought to the laboratory from the local market of daryapur area and acclimatized for 14 days in de-chlorinated water, under the following conditions; water temperature 23 –25 0C, 12-h light-dark cycle; pH 7.0 –7.2. Fishes were fed twice a day with commercial floating feed containing 30 % of protein and fed at 3 % of their body weight as maintenance ration. Faeces and food debris were siphoned out on a daily basis and water replaced every 24 hours to avoid contamination of the fishes before the beginning of the experiment.

Exposure of test fish to water extract of root of *Balanites aegyptiaca*

A total number of ten (10) plastic tanks each with a capacity of 35 litres were used for the bioassay experiment. Different acute concentrations were prepared in triplicate. Each of the tanks was stocked with 10 fishes of mean weight (400gms) and mean length (16cm). The 96-hour LC_{50} (lethal concentration) was determined. The water quality parameters were monitored at every 24 hours. Mortality and observed behaviours of the fish were recorded daily. Dead fish were immediately removed from each test tank to avoid polluting the tanks. The root extract concentrations in the various test tanks were renewed daily after changing the water in the test tanks to maintain their potency.

Lethal toxicity test

Lethal concentration of 13.00 mg/l was selected for this experiment. Ten fishes were exposed to each concentration. Along with this, appropriate control was maintained for each test. The mortality did not exceed 5% during the test period in control. Survival and mortality percentage were tabulated after 24, 48, 72 and 96 hrs.

For the lethal toxicity test, the fresh water fishes were divided in two groups as follows.

Group I: - Control group of *Catla catla*

Group II: - Fishes *Catla catla* were exposed to lethal concentration of root water extract.

Result and discussion

The fresh water fish ***Catla catla***when exposed to different concentration of root waterextract of ***B. aegyptiaca***at different time intervals, from percent survival and mortality rate, LC_{50} values were calculated from direct observation as follows.

At 1.00 mg/l conc. of root extract ***B.aegyptiaca*** the survival rate are 100% at 24hrs, 48hrs, 72hrs and 96hrs. No mortality obtained. At 3.00 mg/l conc. of root extract ***B.aegyptiaca*** the survival rate are 100% at 24hrs, 48hrs, and 72hrs but at 96hrs. survival rate is 90% and mortality 10%.At 5.00 mg/l conc. of root extract ***B.aegyptiaca*** the survival rate at 24hrs.-100%, 48hrs-90% and 10%, 72hrs-80% and 20%, 96hrs.-80% and 20%.At 7.00 mg/l conc. of root extract ***B.aegyptiaca*** the survival rate at 24hrs. - 80% and 20%, for 48hrs-70% and 30%, 72hrs-60% and 40%, 96hrs.-50% and 50%.At 9.00 mg/l conc. of root extract ***B.aegyptiaca*** the survival rate at 24hrs. - 70% and mortality 30%, for 48hrs-60% and 40%, 72hrs-50% and 50%, 96hrs.-40% and 60%.At 11.00 mg/l conc. of root extract ***B.aegyptiaca*** the survival rate at 24hrs. - 60% and mortality 40%, for 48hrs-50% and 50%, 72hrs-40% and 60%, 96hrs.-30% and 70%.At 13.00 mg/l conc. of root extract ***B.aegyptiaca*** the survival rate at 24hrs. - 50% and mortality 50%, for 48hrs-30% and 70%, 72hrs-20% and 80%, 96hrs.-zero% and 100%.LC_{50} values obtained were 13.00 mg/l, 11.00 mg/l, 9.00 mg/l, and 7.00 mg/l, 5.00 mg/l, 3.00 mg/l and 1.00 mg/l for 24, 48, 72 and 96 hrs. respectively (Table 1.1).

*Catla catla*were stressed progressivelywith time before death. The pattern of mortality wassimilar for various concentrations of the root water extract of *B.aegyptiaca*. Athigher concentration in lethal effect (7.00, 9.00, 11.00 and 13.00mg/l), rate ofmortality significantly (P< 0.05) increased from 20% at 24hrs to 100% at 96hrs (Tables 1.2). There was nomortality in the control (0.00mg/l) experiment.The piscicidal potential and phytotoxic properties of plant extracts have been reported by several researchers such as **(Akinwande *et al.*, 2007; Ayoola *et al.*, 2011)**. The estimated lethal toxicity for 96hrs LC_{50} (07.00 mg/l) of*B.aegyptiaca*water root extract for the fish *Catla catla* (Table 1.3)in the present study is far higher than 2.44 mg/l reported by **(Fafioye, 2012)**, when white Tilapia fingerlings were exposed to water extract of Almond *Terminalia catappa* estimates vary in different fish species and in the same species under different conditions **(Omitoyin *et al.* 2006)**.

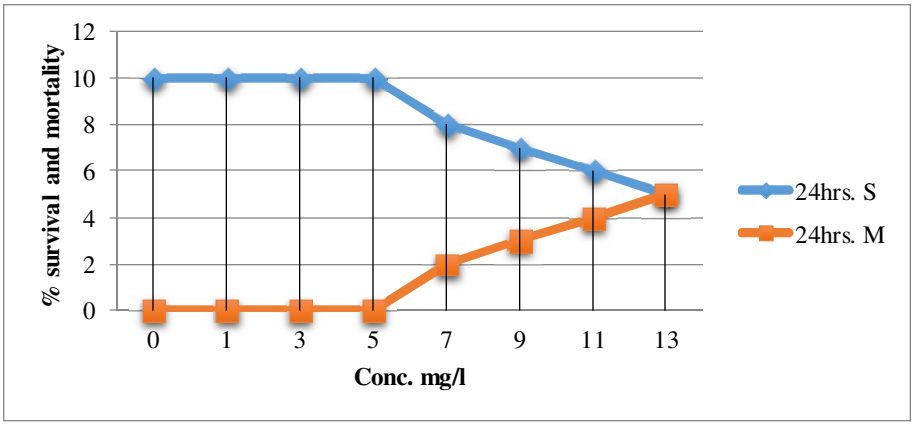
Table 1.1: % Survival and mortality rate of fish***Catla catla*** exposed to root waterextract of ***B.aegyptiaca***at 24, 48, 72, 96 hrs. respectively.

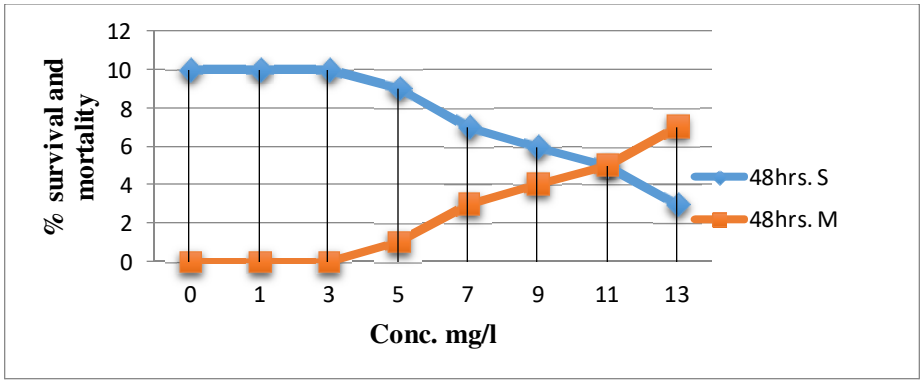
Sr. No.	Conc. Mg/l	24hrs.		48hrs.		72hrs.		96hrs	
		S	M	S	M	S	M	S	M
1	0.00	10	00	10	00	10	00	10	00
2	1.00	10	00	10	00	10	00	10	00
3	3.00	10	00	10	00	10	00	09	01
4	5.00	10	00	09	01	08	02	08	02
5	7.00	08	02	07	03	06	04	**05**	**05**
6	9.00	07	03	06	04	**05**	**05**	04	06
7	11.00	06	04	**05**	**05**	04	06	03	07
8	13.00	**05**	**05**	03	07	02	08	00	10

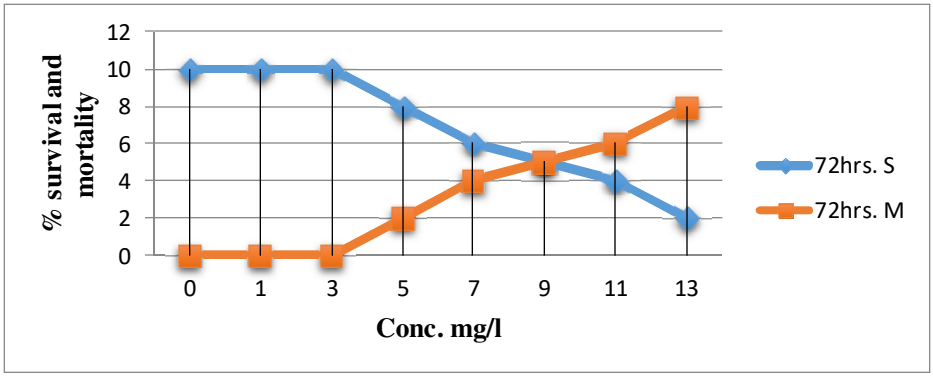
Table 1.2: % Survival and mortality rate of fish ***Catla catla*** exposed to root water extract of ***B.aegyptiaca***at 24, 48, 72, 96 hrs. respectively.

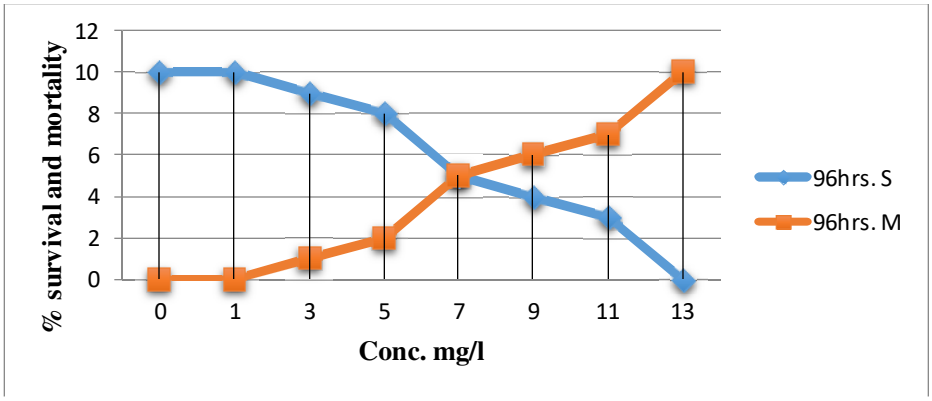
Sr. No.	Exposure Period	LC_{50}
1	24hrs.	13 mg/l
2	48hrs.	11 mg/l
3	72hrs.	09 mg/l
4	96hrs.	07 mg/l

Figure.1.3: Different concentration of root water extract of ***B.aegyptiaca*** at 24,48,72,96 hrs. Survival and mortality rate, LC_{50} values were shown.









Conclusion

Lethal and sub-lethal toxicity studies clearly indicates that through effective mitigation steps and proper management of our ecosystem the negative impact of piscicides toxicity on fishes could be reduced to certain levels. It is also suggestive that these types of toxicological studies are highly required to monitor the aquatic system and to assess the toxic effect of piscicides on aquatic organisms particularly fishes.

A brief review of fish poisons derived from plants used throughout the world, not only as piscicides, but for a rangeofother uses, including insecticidal and in folk medicineshas been presented in the hope of providing a useful background for students interested in natural products. As more research is carried out on the bio-active compounds producedby these plants the potential for discovering new medicaldrugs increases. Much work is still to be done on the field of these interesting plants.

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