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ACUTE TOXICITY STUDIES OF THE DRIED AQUEOUS EXTRACT OF *CESTRUM NOCTURNUM* LEAVES ON *CLARIAS BATRACHUS* JUVENILES

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ABSTRACT
The piscicidal activity of the dried aqueous extract of *Cestrum nocturnum* leaf on *Clarias batrachus* was investigated in a semi-static bioassay to determine the median lethal concentrations (LC_{50}) at 96 h of exposure. six graded concentrations of 0.00, 1.9, 2.8, 3.6, 5.8 and 10.7 mg/l of the aqueous extract were applied to *C. batrachus* juveniles (mean weight: 180 g and length: 25 cm) in glass containers. The LC_{50} values (with 95% confidence limits) estimated by probit analysis were 5.5 mg/l (24 h) to 4.4 mg/l (48 h), 3.4 mg/l (72 h) and 2.6 mg/l (96 h). During exposure, fish exhibited respiratory distress, surfacing, erratic swimming, slow opercular movement, redness in gill and finally settle at the bottom motionless and dies. The toxicity of the aqueous extract of the plant against *C. batrachus* was both time and dose dependent. It is thought that this plant extract or powder will be useful in aquaculture to eradicate predators and competing wild fish from nursery, rearing and stocking ponds prior to the stocking of commercially grown fry and fingerlings of desired species.

KEY WORDS: *Cestrum nocturnum, Clarias batrachus*, toxicity, juveniles, lethal concentration.

INTRODUCTION

In freshwater aquaculture, fish pond are periodically cleaned to remove unwanted and trash fishes. Screening and use of fish poison is standard method for this. (Bardach *et al.,* 1972). Also where water is brought from the nearby water reservoir or rivers, entry of trash and predatory fish, eggs and juvenile is very common. Therefore the best way to ensuring total eradication of unwanted fishes is through the use of fish toxicants (piscicide) in the pond water (Chakroff, 1976). Ideally, ponds should be sundried and the pond bottom cracked dried to help get rid of fish predators. However, this practice is not always possible particularly during the rainy season. (Pillay and Kutty, 2001). Presently, there is no legal registered safe fish poison is available in market except use of bleaching powder, tea leave cake, tobacco dust and rotenone. because of unavailability and cost issue, farmers tends to use unregister fish toxicant such as agro-pesticides which are fast acting and readily available and low-priced. These organophosphate and other chemicals may produce negative and harmful effect on non target animals, environment, and human health. Hence there is an urgent need to search more specific, eco-friendly and safe fish toxicant of plant origin. These indigenous plant product are focus of attention as suitable alternative to synthetic pesticides due to their easy availability, inexhaustible resource, low cost, and biodegradability in nature (Morston and Hostettmon, 1985). Already a large number of biocide of plant origin are in use in aquaculture for control of aquatic pest and harmful snails (Mohapatra and Nayak 1998; Jawale and Dama, 2010).

Diverse group of compounds like saponin, tannins, alkaloids, alkanyl phenols, esters, flavanoids, ichthyoethereol, triterpene and other ichthyotoxins (Bhatia, 1970; Béarez, 1998) have been found to be toxic to freshwater target and nontarget organisms (Singh *et al.*, 1996). Thus, there is need for generation of more information on the piscicidally useful plants that have been reported biocidal property. In India there is a great biodiversity among fish poisonous plants in various ethno botanical reports (Kulkarni *et al.*, 1990; Ramachandran *et al.,* 2009). Various authors have already demonstrated biocidal activities of plant *C. nocturnum* as mosquito larvicidal, insecticidal, molluscicidal, and antibacterial (Jawale *et al.*, 2010; Jawale and Dama 2010 a, b; Patil and Jawale, 2002, Jawale and Dama 2013). Therefore, this study was planned to assess the piscicidal activity of the dried aqueous extract of *C. nocturnum* leaf on *Clarias batrachus* juvenile, an important tropical catfish for aquaculture in India (Sinha *et al*., 2014; Dey *et al.*, 2000).

MATERIALS AND METHODS

Sample collection and preparation

As shown in Figure 1, the fresh green leaves of *Cestrum nocturnum* were collected from nearby garden at Nashik (M.S.) India. The plant was identified and authenticated by Department of Botany from same institute.

Preparation of aqueous extract

The samples were washed and shade-dried and then ground into fine powder and sifted using 0.25 mm sieve. The leaf powder thus obtained was soaked in one liter of double distilled water for 48 h. The stored mixture was filtered through sterile gauze and the filtrate was collected. Further, it was subjected to vacuum evaporate in Rota-evaporator and stored in desiccators to ensure complete dehydration of aqueous extract. Such dried powder of aqueous extract was used for evaluating piscicidal activity of *C. nocturnum.*

Figure 1. The piscicidal plant: *Cestrum nocturnum.*

The test fish, *C. batrachus* of average length (11.5 ±1.2cm) and weight (16.0 ± 0.2g) were obtained from Government fishery farm at Nashik (M.S) India. The fishes were acclimatized to laboratory conditions (25°C) for 14 days before the exposure period using large glass aquaria. During the acclimation period, the fish were fed twice daily using standard commercial fish feed.

Experimental design and acute toxicity test:

Acute toxicity bioassays to determine the 96 h LC_{50} value of the *C. nocturnum* leaf extract was conducted in semi-static system in a laboratory according to the OECD guideline NO. 23 (OECD, 2000). The range finding test was determined according to the method described by APHA *et al.,* (1998). From the range finding tests, six graded concentrations (0.00, 1.90, 2.80, 3.60, 5.80 and 1.70 mg/l) of the dried extract was used for the definitive test. A complete randomized design was used in the experiment with three aquaria set up for each dose and each aquarium contains 10 fish in forty liters of de-chlorinated tap water. Performance test lasted for 96 h. The duration, however, was reduced in concentrations where death of all the fish was observed. The fish were checked for mortality at different time intervals. The dead fish if found were removed immediately to reduce toxic waste related effect and were counted for determination of LC_{50}. The toxicant and test water in each aquarium were renewed after 24 h. The behavior and general conditions of the fish were observed before, during and after each bioassay. The 96 h LC_{50} was determined as a probit analysis using the arithmetic method of percentage mortality data (Finney, 1971). Results were subjected to statistical analysis with Duncan's multiple range F-test to test for significant difference ($p<0.05$) between various concentrations of *C. nocturnum* extract and the control.

Table 1. Fish survival Data on at different test concentrations and sampling time intervals in *C. batrachus* exposed to the dried aqueous extract of *C. nocturnum* leaf.

Exposed concentration (mgl^{-1})	Number exposed	Number of fish alive at different time intervals (hours)				% survival	% mortality
		24	48	72	96		
0.00	30	30	30	30	30	100	00
1.90	30	30	25	20	18	60	40
2.80	30	30	25	20	14	47	53
3.60	30	25	20	15	8	27	73
5.80	30	15	12	7	4	13	87
10.70	30	00	00	00	00	00	100

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RESULTS

Behavioral response of fishes to test concentrations-

When fishes were placed in toxic medium, Fish exposed to the different concentrations of aqueous extract of *C. nocturnum* leaf exhibited hyperactivity characterized by darting, breathing rapidly, held mouth wide open and kept the fins stretched laterally. After 2-3 minutes, this restlessness subsided. This brief initial period of restlessness may not be mistaken as symptoms of poisoning. Later the fish settled quietly at the bottom. The first symptom of loss of sensitivity (response) was characterized by raising of the fins. Subsequently a rigorous, spasmic, and mostly superficial movement of the fins and rapid respiration was observed. Often the mouth was partially closed and a slight twitching of the jaws were discernable. fish aggregated at the air-water interface gasping for air with their mouth permanently opened. After the laps of 10-15 hrs. there is discharge of mucus through the gills and a mucus layer was formed on eyes and all over the body. fish become discolor and remained in the state of exhaust, did not respond to external stimuli and remained diagonally suspended in the water. In the last hours of exposure there was loss of body equilibrium and fish shown turning over the back. Lastly attempts were made to avoid the toxic medium by jerky swimming, jumping out of water accelerated and arrhythmic respiration, but slowly the movements were staggered, mouth was slightly opened, fins and tail become rigid, total dullness in the eyes and body colour becomes totally lifeless. Finally fish died and body became rigid and curved. The fish floated on surface of the aquarium shortly after death These behavioral deviations were more pronounced with increasing concentrations.

Table 2. Toxicity ($LC_{10, 50, 90}$ values) of the dried aqueous extract of *C. nocturnum* leaf at 24 h., 48 h., 72 h. and 96 h. time intervals to the fish, *C. batrachus.*

Exposure period (Hours)	Effective dose (mg/l)	Limits (mg/l)	
		LCL	UCL
24	LC_{10} =3.8	3.4	4.9
	LC_{50} =5.5	5.0	6.2
	LC_{90} =10.6	8.6	12.9
48	LC_{10} =2.6	1.9	3.5
	LC_{50} =4.4	3.8	5.2
	LC_{90} =11.2	8.2	15.3
72	LC_{10} =1.7	1.0	2.2
	LC_{50} =3.4	1.4	4.0
	LC_{90} =10.8	6.8	12.3
96	LC_{10} =1.3	0.9	2.4
	LC_{50} =2.6	2.0	4.2
	LC_{90} =9.8	7.5	10.2

LCL (Lower confidence limit and UCL (upper confidence limit) at 95% confidence limits.

Toxic effects of acute concentrations

The results of *C. batrachus* juveniles exposed to the dried aqueous extract of *C. nocturnum* leaf are presented in Tables 1 and 2. Toxicity experiment was performed by the method of OECD NO 23. Results obtained from this study were computed using SPSS computer program version 20. With this program, lethal concentrations (LC values) were calculated through probit analysis method. Table 2 shows the effective doses ($LC_{10, 50, 90}$ values) of the dried aqueous extract of *C. nocturnum leaf*. At a concentration of 10.7 mg/l, there was 100% mortality within one hour. Mean mortalities of 40, 53, 73, 87, and 100% were recorded in the experimental tanks containing 1.9, 2.8, 3.6, 5.8, and 10.7 mg/l of the extract, respectively. No mortality was recorded in the control (0.0 mg/l). From the results, a 96 h LC_{50} value for *C. batrachus* juveniles was calculated to be 2.6 mg/l. There was a significant negative correlation between LC_{50} values and exposure time. Thus LC_{50} of the dried aqueous extract of *C. nocturnum* decreased from 5.5 mg/l (24 h) to 4.4 mg/l (48 h), 3.4 mg/l (72 h) and 2.6 mg/l (96 h).

DISCUSSION

Fish mortality bioassay and behavioral changes during toxicity were chosen as the computable effect to determine the toxicity of the crude dried aqueous extract of plant *C. nocturnum* leaves. Our experiments revealed that *C. batrachus* juveniles when exposed to dried aqueous extract of *C. nocturnum* leaves for short term exposure to lethal dose (1.07mg/l) for 24 hours showed that the extract hampered the normal and quite behaviour of the fish. As the exposure time laps, fish showed vigorous spasmic and rapid movements with open mouth (Jawale and Dama 2012). After laps of 10-15 hours there was discharge of mucus through body surface and gills, later body equilibrium was lost, body colour changed, fins and tails became rigid. These observations inferred that piscicidal compound disturbs respiration, initiate restlessness and discharges abundant of mucus as well as loss of equilibrium with paralysis of body muscles. Bhuyan (1967), observed behavioural changes in weed fishes, *Ambossis nama; Barbus sophore; Amblypharyngodom mala* and *Esomus danricus* with plant *Mallitia pachycarpa*. They found that roots and fruit extract affected the fishes within 1-3 hrs. of exposure and higher doses proved lethal. This toxic compound did not show any adverse changes in physio-chemical condition of water. The toxic effect of Mangrove plants was tested with fish *Tilapia nilotica* and the behavioural changes were observed. The most toxic effect was shown by *Derris trifoliata* and *Excoecaria agallocha* resulted into frenzied and spasmodic movement in the fish and eventually fish settled at the bottom of tank and died (Gomez *et al*., 1986). Similar behavioural effect was observed on guppy after exposure to extract of *Trichonstychnu nuxvnmica* (Datta *et al*., 1991). Ray and Datta (1986), Bhatt and Dhyani (1990) Rooj (1994) and Jawale (2013), studied the behaviour of fishes, when exposed to plant extract. Most of these recorded results showed synonymous toxic effect on the fish, what are shown by *C. nocturnum* in the current research, only the magnitude of the toxic effect changes with exposure time and the compound used for the studies. Recently, Sinha *et al.,* (1999) revealed the effect of alkaloids on Chromatophore movement in the fish *Labeo bata*. Alkaloids cause decrease in the number of chromatophore during early hours of exposure, however chromatophore increase during late hour of exposure. An interesting observation was also made by these researcher was an increase in area of mucus gland with increase in exposure period. This may be due to defense mechanism, hypertrophy of mucus gland, which lead to tremendous discharge of mucus. According to Annune (1994), mucus accumulation results from increase in the activity of mucus cells subsequent to pollutants exposure, which results in an increase in the production of mucus over the body of the fish. Our observation here of excessive mucus secretions in exposed fish agrees with the report of Jothivel and Paul (2008), and Abalaka and Auta (2010). Excessive mucus secretions are natural defense mechanisms by exposed fish to coat their body surfaces in order to prevent and/or reduce the absorption of the offending toxicant (Cagauan *et al.,* 2004). However, such excessive mucus secretions are reported to reduce respiratory activity in fishes (Konar, 1975). which together with decreasing oxygen content of reconstituted extracts results into hypoxic states in exposed fishes (Usman *et al.,* 2005) leading to subsequent respiratory distress and deaths in exposed fishes (Omitoyin *et al.,* 1999). Similarly, others also reported abnormal movement and high respiration rate in different fishes when exposed to plant products (Muhammad *et al.,* 2010; Ajani and Ayoola, 2010; Ayuba and Ofojekwu, 2002). Abnormal nervous behaviours are associated with the impacts of the toxicants on fishes (Fafioye, 2005). This may be due to nervous system involvement or failure (Ufodike and Omoregie, 1994) or may be due to biochemical body derangement including hepatic compromise (Jawale 2013). From the observation made by other researcher and results obtained in the current research conclude that under toxic stress the fish tries to acclimate with surrounding, if toxic dose is lethal then the death of the fishes is ultimate.

Results obtained from this research revealed that the 96 h LC_{50} for catfish exposed to *C. nocturnum* was 2.6 mg/l. The 96 h LC_{50} had earlier been reported for the fish, *Channa punctuates* by Jawale *et al.,* (2012) to be 46.44, 40.32, and 30.13 mg/l for 24hr, 48, and 72 hrs. respectively of alcoholic extract of *C. nocturnum* leaves in acute static bioassay. Comparatively, the 96 h LC_{50} value of 2.6 mg/l observed in this study was much lower than most of the reported literature. The variations observed in these studies can be attributed to the type of fish species used, its size, environmental factors, food, or water parameters and selective mode of action of toxicants.

Statistical analysis of the experimental data on toxicity brings out several important findings. The X^2 test for goodness of fit established that the mortality counts were not observed to be significantly heterogeneous. Other variables like resistance do not significantly affect the LC_{50} values, as these were observed to lie within the 95% confidence limits. The dose concentration verses percent mortality graphs exhibit steep slope. The steepness of the slope line indicates that there is a large increase in the mortality of fish with a relatively small increase in the concentration of the toxicant. The slope is an index of the susceptibility of the target fish to the biocidal material used. A steep slope also indicate rapid absorption and initiation of effects (Yadav and Singh, 2006; Jawale *et al.,* 2012). The authors Dama and Jadhav 1997; Dama *et al.,* 1998; 1999; Dama, 2002 studied the activity of plant extract on various animals. In conclusion, it is clear from the results that *C. nocturnum* crude extract is very toxic against the freshwater fish, *C. batrachus*, thus the plant is piscicidal. It is thought that this plant extract or powder will be useful in aquaculture to eradicate predators and competing wild fish from nursery, rearing and stocking ponds prior to the stocking of commercially grown fry and fingerlings of desired species.

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