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Toxicity of aqueous extract of white hoary pea, *Tephrosia candida* (Papilionoideae) on Nile tilapia, *Oreochromis niloticus* (Cichlidae) fingerlings

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Abstract

Fish poisoning using *Tephrosia candida*, which is an exotic plant to Sri Lanka is taking place in streams in the boundary of the Sinharaja forest, a tropical forest range, designated as a world heritage site by UNESCO in 1988. *T. candida* is a source of flavonoids and rotenoids including rotenone, tephrosin, and deguelin. Fishermen add large amounts of grounded plant matter to kill almost all the fishes in the stream within a short period of time. This method of unregulated fishing may have a long term negative effect on fish diversity and abundance in the area. A 96 h static renewal toxicity bioassay was carried out in the laboratory to determine the median lethal concentration (LC_{50}) of aqueous extract of *T. candida* leaves on *Oreochromis niloticus* fingerlings. Experimental fish were exposed to test water in 20 L glass aquaria with concentrations of plant extract of 5, 7.5, 10, 15 and 20 mg L^{-1}. All five treatments aquaria and the control aquaria without plant extract were triplicated. Fish exposed to plant extract showed symptoms of toxicity including, initial inactivation, agitated swimming, turning movement, air gulping, increased opercular movement followed by erratic swimming, loss of reflex, slow opercular movement, setting at the bottom motionless and knockdown before death. The gills of the dead fishes were damaged, swollen and external bleeding were observed. Lower concentrations of the extracts had sub lethal effects which manifested as zigzag movement, air gulping, increased opercular movement and some fish gathered near the air stones. The LC_{50} values at various exposure periods were 10.83 mg L^{-1} for 24 h; 8.61 mg L^{-1} for 48 h; 7.26 mg L^{-1} for 72 h and 6.43 mg L^{-1} for 96 h. It could be concluded that the application of *T. candida* extract causes lethal toxic effects on fish even at very low concentrations.

Keywords: *Tephrosia candida*; *Oreochromis niloticus*; toxicity; LC_{50}

Introduction

Many fish poisons derived from plants from different families have been used to catch fish all over the world. These poisons, also called icthyotoxins or piscicides, occur in several related plant species such as *Adenia cissampeloides, Balanites aegyptiaca, Blighia sapida,*

Derris elliptica, D. trifoliata, Kigelia Africana, Mimusops elengi, Mundulea sericea, Tetrapleura tetraptera, T. candida, T. purpurea, T. virginiana and *T. vogelii* (Guerrero and Guerrero 1989; Neuwinger 1994; Onusiriuka and Ufodike 1994; Andrei et al. 2002; Cheenpracha et al. 2007; Negi and Kanwal 2009). The active ingredients of these plants are released by mashing and grinding the appropriate plant or plant parts, which are then introduced to the aquatic environment. Fishing using white hoary pea, *T. candida* (Papilionoideae**,** Fabaceae) takes place in the freshwater streams at the boundary of Sinharaja forest. Sinharaja forest is designated as a natural world heritage site and a man and biosphere reserve by UNESCO in 1998.

T. candida, which is toxic only to cold-blooded animals (Van Andel 2000) is an erect herb, upto 3.5 m tall, with straggling branches from the base. It is native to the tropical foothills of the Himalayas in India and is now cultivated and naturalized throughout Southeast Asia. It is an exotic plant to Sri Lanka, China, Indonesia, Jamaica, Japan, Malaysia, Myanmar, New Zealand and Thailand (Orwa et al. 2009). *T. candida* was introduced to Sri Lanka as legume live mulch for weed control and to protect soil surface (Wijewardena 1984).

The genus *Tephrosia* is well-known to be a rich source of flavonoids, rotenoids, terpenoids, and sterols among their secondary metabolites (Andrei et al. 2002). Some of the ingredients isolated from *T. candida* are amorphispironone, tephrospirolactone, tephrospiroketone, deguelin, candidol (Dutt and Chibber 1983), tephrosin, amorpholone (Kole et al. 1992), candidone, rotenone and scillascillin (Stephen et al. 2003).

Lethal and sub lethal concentrations of plant poisons are known to have toxic effects on fish behaviour, heamatology, histopathology, growth, reproduction, feeding, respiration and general other physiological processes of exposed organisms. Therefore, frequent addition of large amounts of plant poisons to the streams at the boundary of Sinharaja forest raises a serious issue with regard to the conservation of aquatic biodiversity. The situation is further aggravated if the practice is extended into the Sinharaja forest and into the other areas of the country.

Short-term acute toxicity test is one of the most commonly used tests in the initial evaluation of toxicity of chemicals to organisms (Murty 1986). The acute toxicity levels of aqueous extract of *T. candida* on fish has not been investigated before. In the present study, the median lethal concentrations (LC_{50}) of the aqueous extract of *T. candida* was evaluated on *Oreochromis niloticus* fingerlings with a view to providing necessary information for law enforcement authorities and policy makers. *O. niloticus* was used as the test organism as they are widely used in toxicity studies worldwide, could easily find from the wild environment and keep under captivity.

Materials and Methods

Preparation of plant extract

Fresh leaves of *T. candida* from the apex of the plant were collected and 75 g of the plant leaves were weighed and crushed with electrically powered blender for 15 min with 500 ml of distilled water. The aqueous suspension was filtered through a muslin cloth,

centrifuged and filtrate was stored in a refrigerator for bioassay test almost immediately after extraction.

Range finding test

Healthy *O. niloticus* fingerlings with weight of 6.9±1.5 g and length of 5.1±2.0 cm were collected from the fish breeding centre of National Aquaculture Development Authority Udawalawe, Sri Lanka. They were acclimated for two weeks in a large fiberglass tank filled with aged tap water under the laboratory conditions. During the acclimation period, the fishes were fed twice daily at 5% of the body weight with a commercial fish feed and tank water was continuously aerated. Excess feed and feces were siphoned out twice a day. A prior range finding test was conducted with 0.1 – 25 mg L^{-1} of *T. candida* aqueous extract to find out the exact range of concentration of plant extract to be used for the static renewal acute toxicity test (EIFAC 1983). According to the results of range finding test the concentration of *T. candida* aqueous extract used in definitive toxicity test was between 5 – 20 mg L^{-1}.

Definitive test

Static renewal 96 h toxicity bioassay was carried out in glass aquaria filled with 20 L of aged tap water. The stock solution of aqueous extract of *T. candida* was added in required volume in order to get a concentrations of 5, 7.5, 10, 15 and 20 mg L^{-1} in the treatment aquaria. All the treatment aquaria and the control aquaria without plant extract were triplicated. The water was pre-aerated for 15 min to full oxygen saturation before the different volumes of the plant extract were added. A complete randomized design was performed in the experiment with ten fingerlings of *O. niloticus* per 20 L of aged water. Aquaria were well aerated and fish were not fed during the test period. Exposure media were renewed daily for four days.

The behavioural and morphological changes of fish were observed after introduction of fishes to the experimental aquaria. Mortalities of fish were observed and recorded at 24, 48, 72 and 96 h from stocking. A fish was considered dead when it stopped opercular movements and failed to respond to mechanical stimulation with a glass rod. At the end of the each time period, dead fingerlings in the tanks were counted and carcasses were discarded. The concentrations at which 50% mortality of fish occurred after 24, 48, 72 and 96 h were taken as the median lethal concentration (LC_{50}) for the respective times.

Temperature, pH, total dissolved solids, conductivity and dissolved oxygen (DO) in water in all the aquaria were monitored using multi parameter water quality checker (YSI incorporated 556 MPS) before and after the addition of toxicant, 24, 72 and 96 h of fish stocking thereafter. The median lethal concentration (LC_{50}) values and its corresponding 95% confidence limits were calculated by probit analysis (Agresti 1990). Physico-chemical parameters of water in treatment and control aquaria were compared by one-way ANOVA at significant level of 0.05. All the statistical tests were carried out by MINITAB software (version 14).

Results

The pH, temperature, total dissolved solids, dissolved oxygen and conductivity were not significantly different between the control and treatment aquaria ($p > 0.05$, one way ANOVA) (Table 1) during the experimental period. After exposure to aqueous extract of *T. candida*, *O. niloticus* fingerlings started to show the symptoms of toxicity including, initial inactivation, agitated swimming, turning movement, air gulping, increased opercular beat and then erratic swimming, loss of reflex followed by slow opercular movement and setting at the bottom motionless. They exhibited body imbalance and surface floating specially at higher concentrations and knockdown before death. The gills of the dead fishes were damaged, swollen and external bleedings were observed. Lower concentrations of the extracts had sub lethal effects on the fish which manifested as zigzag movement, air gulping, increased opercular movement and some fish gathered near the air stones. None of these toxic effects were observed in the fish stocked in the control tanks. The 24, 48, 72 and 96 h LC_{50} values of aqueous extract of *T. candida* on *O. niloticus* fingerlings at 95% confidence level were 10.8, 8.6, 7.3 and 6.4 mg L^{-1} respectively (Table 2).

The mortality of *O. niloticus* fingerlings increased with the increase in concentration of plant extract according to the mortality concentration curves obtained from probit analysis for 24, 48, 72 and 96 h exposure of different concentrations (Figure 1). Furthermore, LC_{50} values decreased with exposure time and 95% confidence limits for LC_{50} values overlapped between 48 h and 72 h and between 72 h and 96 h LC_{50} values.

Table 1. Mean±SD (range in parenthesis) of physico-chemical parameters of water in the aquaria treated with aqueous extract of *T. candida* and the control.

Parameter	Plant extract					
	Control	5 mg L^{-1}	7.5 mg L^{-1}	10 mg L^{-1}	15 mg L^{-1}	20 mg L^{-1}
pH	7.84±0.15 (7.4-8.0)	7.88±0.09 (7.6-8.0)	7.89±0.10 (7.6-8.1)	7.98±0.04 (7.9-8.1)	8.02±0.01 (8.0-8.1)	8.02±0.01 (8.0-8.1
Temperature °C	27.2±0.2 (26.9-27.6)	27.2±0.2 (27.0-27.7)	27.2±0.1 (27.0-27.5)	27.3±0.1 (27.1-27.6)	27.4±0.1 (27.2-27.7)	27.4±0.1 (27.2-27.7)
Dissolved Oxygen (mg L^{-1})	5.40±0.12 (5.10-5.61)	5.35±0.75 (5.20-5.55)	5.44±0.09 (5.20-5.60)	5.54±0.12 (5.18-5.70)	5.56±0.10 (5.30-5.80)	5.51±0.08 (5.30-5.70)
Conductivity (µS cm^{-1})	95±0.5 (94-96)	95±0.5 (94-96)	95±0.5 (94-96)	95±0.5 (94-96)	95±0.5 (94-96)	95±0.3 (94-95)
TDS (mg L^{-1})	0.059±0.0 (0.059-0.0)	0.058±0.0 (0.058-0.06)	0.058±0.0 (0.058-0.06)	0.059±0.0 (0.059-0.01)	0.059±0.0 (0.059-0.01)	0.059±0.0 (0.059-0.01

Values in rows are not significantly different ($p < 0.05$) as indicated by one way ANOVA.

Table 2. Median lethal concentrations (LC_{50}) of aqueous extract of *T. candida* for 24, 48, 72 and 96 h exposure of *O. niloticus*.

Exposure Period	LC_{50} (mg L^{-1})	Standard Deviation	95% Confidence Limits	Slope
24h	10.83	0.51	(9.85 – 11.93)	1.41
48h	8.61	0.38	(7.87 – 9.41)	1.35
72h	7.26	0.32	(6.61 – 7.92)	1.32

96h	6.43	0.24	(5.95 – 6.92)	1.21

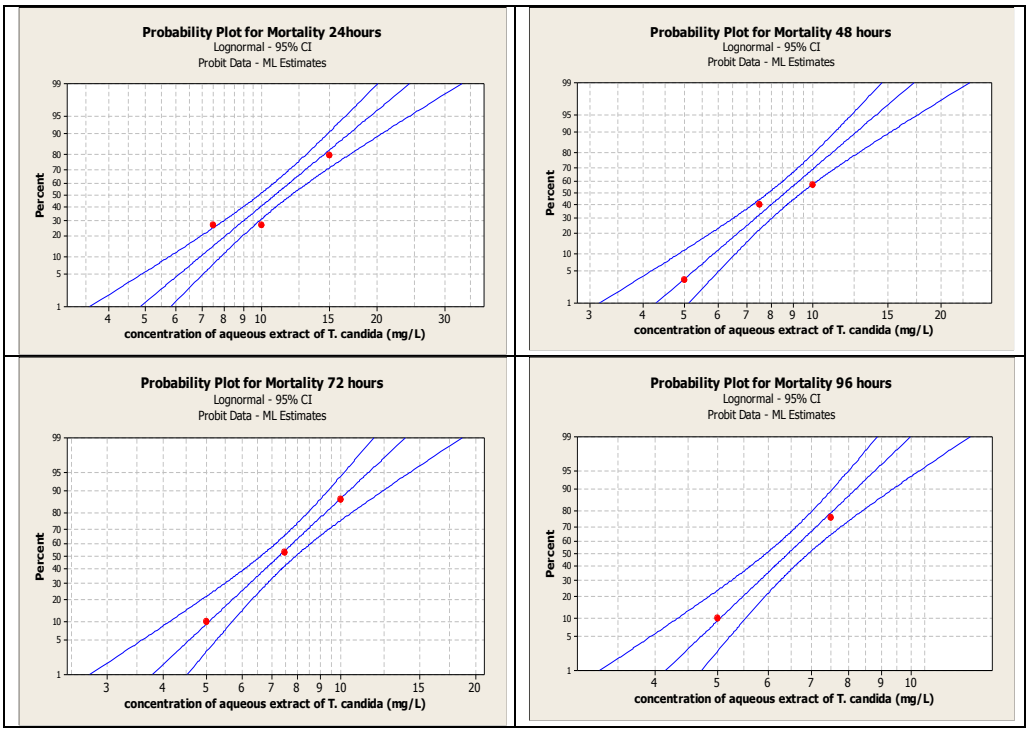

Figure 1. Mortality concentration curves obtained from probit analysis for 24 h, 48 hour, 72h and 96h exposure of *O. niloticus* (n=30) to different concentrations of aqueous extract of *T. candida*.

Discussion

It was observed that different concentrations of *T. candida* had an inversely proportional relationship with time of exposure of *O. niloticus* as highest concentrations had the shortest duration of action and the lowest concentration when fish survived, the highest period of exposure. The 24, 48, 72 and 96 h LC_{50} values of aqueous extract of *T. candida* on *O. niloticus* fingerlings at the 95% confidence level were 10.8, 8.6, 7.2 and 6.4 mg L^{-1} respectively. The 24 h LC_{50} value of *Derris elliptica* was 186 mg L^{-1} (Akinbulumo et al. 2004) and 96 h LC_{50} to *Derris* root powder was 10 – 20 mg L^{-1} for *O. niloticus* fingerlings (Guerrero and Guerrero 1986). According to the results of the present study aqueous extract of *T. candida* showed comparatively higher toxicity on *O. niloticus* fingerlings even with very low concentrations.

Similar toxicity symptoms, shown by fingerlings of *O. niloticus* after the exposure to aqueous extract of *T. candida*, have also been shown by fingerlings of *Claris gariepinus* exposed to aqueous extracts of *Blighia sapida* and *Kigelia africana* (Onusiriuka and Ufodika 1994). The stressful behaviour exhibited by fish fingerlings in the present study may be the result of respiratory impairment due to the effect of the components of the

extracts such as rotenone and tephrosine on the gills and general metabolism of the exposed fishes (Oberg 1967; Reed et al. 1967). Rotenone stuns fish by impairing their oxygen consumption (Lindahl and Öberg 1961) and its acute toxicity to fish is attributable to inhibition of nicotinamide adenine dinucleotide hydrate (NADH): ubiquinone oxidoreductase activity as the primary target (Robert et al. 1994). The specialized structure of the gills favours entrance of rotenone and other toxic compounds into the blood stream, where upon the toxicants are transported to vital organs for inhibition of respiration (Oberg 1967).

The exposed fish were motionless before death, possibly due to the loss of muscular contraction as a result of the interference of the poison with the normal functioning of the nervous system and consequently the coordination of muscular activities (Gbem et al. 1990). The damaged, swollen and bleeding gills of fish fingerlings may have caused due to the gill lesions caused by the higher concentrations of plant toxins (Onusiriuka and Ufodika 1994). *O. niloticus* fingerlings exposed to lower concentrations of plant extract showed loss of balance, air gulping, increased opercular movement and settling at the bottom. Similar sub lethal effects were observed on *O. niloticus* exposed to *Derris* powder extracts (Akinbulumo et al. 2004) and on *Cyprinus carpio* exposed to a concentration of pure rotenone (0.1 mg L^{-1}) (Fajta and Grizzlea 1998).

It could be concluded that the application of *T. candida* aqueous extract causes lethal and sub lethal effects on fish even at very low concentrations hence, indiscriminate use of the *T. candida* as a toxicant to catch fish should be discouraged and regulated in order to protect fish biodiversity in the Sinharaja forest area. Moreover, exotic cichlid species (*O. mossambicus* and *O. niloticus*) support productive fisheries in inland reservoirs of Sri Lanka (Amarasinghe et al. 1989; Amarasinghe 2002) and as such, stern action should be in place to prevent spread of this harmful fishing practice to inland reservoirs. Further research is warranted to study the potential use of *T. candida* to remove invasive fish species from natural environment and to remove unwanted fish from aquaculture facilities.

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