EFFECTS OF FISH BEAN (*Tephrosia vogelii*) LEAVE EXTRACT EXPOSED TO FRESHWATER CICHLID FISH – *Tilapia zilli*

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ABSTRACT

This research was designed to investigate the effect of Tephrosia vogelii leaves extract on Tilapia zilli under laboratory conditions. The concentrations of the leaves extract used were 4.00, 2.00, 1.00, 0.50 and 0.25ml/l. The 96- hour LC_{50} was 0.71ml/l with lower and upper confidence limits of 0.31ml/l and 1.63ml/l respectively. The opercular ventilation and fin counts increased with an increase in the concentrations of the leaves extract at the end of 96- hour exposure period. Respiratory distress, loss of balance, settling at the bottom motionless and erratic swimming was observed before death during the exposure period. Histopathological examination of the gill, liver and kidney of the treated fish showed oedema of gill lamella and gill hyperplasia to vacuolation and necrosis of the liver cells while the kidney cells revealed a degeneration of kidney tubules. Phytochemical analysis of the leaves extract indicated the presence of alkaloid, tannin, saponin, cardiac glycoside, rotenone, steroids, balsam, phenol and volatile oil. The result of this study calls for the need to discourage the use of toxic plants for catching fish in Nigeria water bodies.

Keywords: Leaves extract, *Tephrosia vogelii,* Phytochemical, *Tilapia zilli*, Histopathology, Toxicity

INTRODUCTION

The tremendous increase in discharge of a wide diversity of pollutants to receiving water bodies has caused undesirable effects on the different components of the aquatic environment and fisheries as reported by (Mason, 1993). Plant extracts have been reported extensively by several authors as widely available in the tropics and have been used as natural piscicides by artisanal fisherman and as medicine in curing certain diseases. Okokon *et al*. (2005) concluded that the alkaloid fractions of the leaves and stem of *C. zambesicus* is active against microorganism and that the essential oil found in the leaves also contain P-cymene, linalool and beta-caryophyllene. Most of the plants contain chemicals which are traditionally used to harvest fish in almost all part of the world (Jenness, 1967).

In recent years the use of medicinal plants as effective alternative of synthetic pesticides and fertilizers has gained importance especially to combat problems both in fish and aquatic environment, because they are highly toxic to the target organ pests (Singh and Agarwal, 1988; Dahiya and Jain, 2000; Yadav and Singh, 2001). Farida and Vander (1997) however noted that *Tephrosia vogelii* apart from being used as insecticides are useful as a poison particularly for catching fish. Agbon *et al*. (2004) documented that the leaves extract of *T. vogelii* contains rotenone which are toxic to aquatic organism including *Aphyosemion gairdneri nigerianum*. WHO (2007) reported that the higher toxicity of rotenone in fish and insects is due to the fact that the lipophilic rotenone is easily taken up through the gills or trachea, but not as easily through the skin or through the gastrointestinal tract.

Indiscriminate use of this piscicides poses a great danger to aquatic organisms, especially fishes and consequently to humans. Ekanem *et al*. (2004) showed that the use of this ichthyologic plant material (*T. vogelii*) had a significant impact on the survival of fish larvae in the field. The authors reported that the minimal concentrations of the dried extract of the leaves caused mortality on the embryo of zebra fish *Danio rerio* after 48 hour exposure period.

The objective of this research was to provide information on the acute toxicity of fish bean (*Tephrosia vogelii)* leaves extract on *Tilapia zilli.*

MATERIALS AND METHODS

Experimental Fish: Fingerlings (mixed sex) of *Tilapia zilli* mean weight (5.18 ± 0.80 g) were collected from Renaji Fish farm in Rayfield Jos, Plateau State, Nigeria. They were transported to University of Jos Fisheries Research Laboratory with the aid of oxygenated bag. The fish were held in plastic tanks and acclimated to laboratory condition for a period of two weeks.

Experimental Design: The leaves of *T. vogelii* were obtained from the Botany Garden, University of Jos, Nigeria. The identity of the plant was confirmed by a Botanist in the same University as *T. vogelii*. 20 grams of *T. vogelii* leaves was pounded, put into a plastic container and about 2 litres of distilled water was added. This was allowed to stand for 10 hours. It was stirred and filtered through Whattman Filter Paper Number 1 into a sterile beaker.

Based on earlier study on *T. vogelii* leaves, the concentrations of the extract used for this study were 4.00, 2.00, 1.00, 0.50 and 0.25ml/l. Dechlorinated tap water without leaves extract (0.00ml/l) served as the control. De-chlorinated well aerated municipal tap water was used in all dilutions. For the experimental set up, eighteen circular plastic tanks (40 x 20 x 20 cm^3) were used comprising of six treatment replicated thrice in order to minimize experimental errors.

The fish were not fed for 48 hours prior to and during the exposure period. Each tank was stocked with ten fish. The tanks were examined on a daily basis and dead fish were removed and recorded immediately from the test solutions to avoid polluting the test media. Opercular ventilation rate per minute of *Tilapia zilli* exposed to varying concentrations of *T. vogelii* leaves extract was recorded at 24, 48, 72 and 96 hours post administration.

Phytochemical analysis to determine the active ingredients present in the extract was performed using the procedure of Sofowora (1982).

The physicochemical analysis of the test water vis-à-vis temperature, dissolved oxygen, alkalinity, free carbon dioxide, pH were determined using analytical methods in APHA (1995).

The 96-hour LC_{50}, lower and upper confidence limits were estimated using the methods for acute toxicity tests (UNEP, 1989).

Histopathological examinations of the gills, liver and kidney after exposure period were done using the method described by Buck and Wallington (1972).

The data obtained from this investigation were subjected to statistical analysis using two-way analysis of variance (ANOVA) to test for the level of significance between the various concentrations of *T. vogelii* leaves extract administered.

RESULTS

The result of the physicochemical parameters of the experimental media (Table 1) indicated a significant difference ($P<0.05$) in the values obtained in the level of dissolved oxygen, content free carbon dioxide and alkalinity with the control. Nevertheless, there were no significant difference ($P>0.05$) between the values of temperature and pH of test media with the control. The abnormal behavior observed in fish exposed to the extract included respiratory distress, loss of balance, gulping of air, settling at the bottom motionless, and erratic swimming. The abnormal behaviors displayed by the exposed fish increased with increasing concentrations of the leaves extract.

No mortality was observed in the group of fish in the control experiment while mortality

Table 1: Physicochemical parameters measured during 96 hour exposure of various concentrations of leaves extract of *T. vogelii* to *Tilapia zilli*

Parameters	Mean Concentrations (ml/l)					
	4.00	2.00	1.00	0.50	0.25	0.00
Temperature(^{0}C)	23.40±0.32	24.40±0.25	23.50±0.35	23.50±0.35	23.40±0.15	23.40±0.36
DO(mg/l)*	4.32±0.29	4.87±0.37	5.29±0.48	5.69±0.25	6.08±0.36	6.29±0.16
pH	7.99±1.12	6.88±1.92	6.75±142	6.67±1.30	6.63±1.29	6.36±1.25
FCD(mg/l)**	25.66±0.26	25.27±0.36	24.45±0.23	22.56±0.32	19.39±0.38	17.16±0.24
Alkalinity(mg/l)	27.78±0.42	23.17±0.32	17.78±0.63	14.53±0.79	12.35±0.85	9.18±0.65

**Dissolved oxygen ** Free Carbon dioxide*

Table 2: Mean values of opercular ventilation rate per minute of *Tilapia zilli* exposed to varying concentration of Leaves extract of *T. vogelii* for 96 hours

Concentration(ml/l)	Exposure period (h)				
	Start (0)	24	48	72	96
4.00	138±0.28	132±1.11	-	-	-
2.00	134±0.02	128±1.20	132±0.48	132±0.12	127±0.11
1.00	128±0.08	122±1.22	128±0.40	128±0.14	123±0.13
0.50	128±1.21	118±0.06	116±0.33	116±0.22	111±0.12
0.25	116±0.58	112±0.08	109±0.02	112±0.13	111±0.02
0.00	106±0.02	105±0.07	107±0.06	106±0.04	106±0.03

occurred in fish exposed to other varying concentrations of the toxicant. Mortality increased with increasing concentration of the extract showing a dose-dependent relationship. 100% mortality was observed in the group fish exposed to 4.00ml/l while 10% mortality was recorded in the group of fish exposed to 0.25ml/l. The mean value of 96-hour LC_{50} of the leaves extract of *T. vogelii* to the test fish was calculated to be 0.71ml/l with lower and upper confidence limits of $0.31ml^{-1}$ and $1.63ml^{-1}$ respectively. The exposed fish exhibited higher opercular ventilation rate per minutes compared to the values obtained for the control group (Table 2).

Histopathological examinations of the test fish showed some pathological disruptions. The liver cells revealed necrosis and vacuolation of the liver cell, the gill showed oedema of the gill lamella while the kidney revealed degeneration of the kidney tubules. The damage done to these organs as the result of the toxicant correlates with the concentrations of the toxicant in each experimental tank. The phytochemical analysis of the leaves extract revealed the presence of alkaloid, tannin, saponin, cardiac glycoside, rotenone, steroids, balsam, phenol and volatile oil.

DISCUSSION

The result obtained from this research from water quality of the test media is sufficient for the survival of living organism however; the mortality of the test fish could be attributed to the direct toxicity of the leaves extract of *T. vogelii* on *Tilapia zilli*. The behavioral changes which were characterized by respiratory distress, loss of balance, air-gulping, settling at the bottom motionless and erratic swimming as reported in this investigation compared favorably with the observation of (Pascual *et. al.,* 1994; Svecevicius, 2006; Absalom *et al.* 2009) when they exposed some species of fish to different toxicants.

Respiratory distress noticed in exposed fish could be caused by mucous precipitation and neurological dysfunction of gill epithelia in response to the toxicant which resulted in high respiratory rate as reported by (Lin and Lin, 1990; Banerjee, 2007). The opercular ventilation count which declined after each 24

hours could be as a result of the inhibitory action of the toxicant on respiration as well as malfunctioning of some vital organs which may reduce the available energy for respiration. Increased ventilation rates could be as the result of the toxicant in the test media as it reduced the amount of oxygen present in the media. The fish could have increased ventilation rates in an attempt to make up for the loss in oxygen content in the gill. High opercular ventilation has been reported by (Sprague, 1973) as an index of stress when fish come in contact with an unfavorable environmental condition. The 96-hour LC_{50} which was estimated as 0.7ml/l means that the reduction of fish ability to maintain body equilibrium was recorded in concentration close to and in excess of the LC_{50} value (Figure 1).

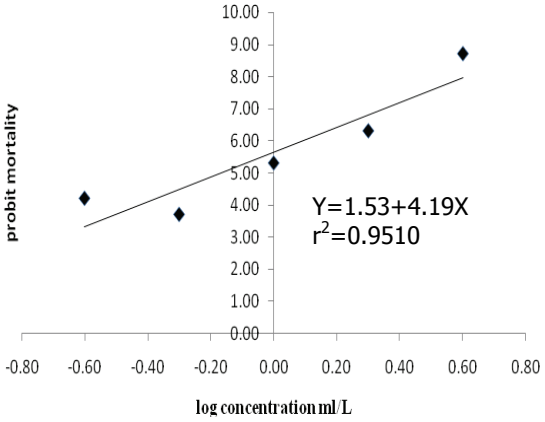

Figure 1: Linear relationship between probit mortality of *Tilapia zilli* exposed to various concentrations of *Tephrosia vogelii*

High mortality occurred in fish exposed to the leaves extract show severe gill epithelial hyperplasia, separation of the gill epithelial layers from supportive tissues. These according to (Omoregie and Ufodike, 1991; Banerjee and Chandra, 2005) can lead to brachial malfunction of which may affect physiology or causes death to fish. The vacuolated cells and necrosis of the liver as observed in the exposed fish are the liver lesions associated with the *T. vogelii* toxicity which could be the result of the excessive work required by the fish to get rid of the extract from its body during the process of detoxification by the liver. Similar investigation was reported by Wade *et al*. (2002) and Akpa *et al.* (2009) when cassava effluent and crude bark extract of Indian siris were exposed to *Oreochromis niloticus* respectively. The degeneration of the kidney tubule may be as a result of the toxicity effects of the extract. Rotenone as one of the active ingredients of the *T. vogelii* as observed in the phytochemical analysis is one of the contributing elements in fish mortality. This agreed with WHO (2007) that reported rotenone to be lethal to fish because it readily enters the blood stream of the fish through the gills. Robertson and Smith-vaniz (2008) documented that the specific site of action of rotenone is in the electron transport system where it blocks a mitochondrial enzyme called NADH ubiquinone reductase, consequently the blood oxygen content of the fish will increase because oxygen is now unavailable for respiration.

From the result obtained from this study, it is evident that *T. vogelii* is toxic to *Tilapia zilli* and could possibly affect other aquatic animals. However, fishing methods which include the use of plants that contain toxic substances as active ingredients should be discouraged so to conserve the biodiversity of fish and other aquatic organisms in Nigeria water bodies.

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