



Acute Toxicity of *Tephrosia vogelli* on the Early Life Stages of Farmed Clarid (*Clarias gariepinus*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author PBE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UUU and ECE managed the analyses of the study. Author SEE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study examined the acute toxicity of *Tephrosia vogelli* ethanolic extract on early life stages of farmed *Clarias gariepinus*. 160 fingerlings were divided into four groups using a completely randomized design (CRD) in a factorial layout and were exposed to 25, 50 and 100 mg/L of the extract for 24, 48, 72 and 96 hours, respectively while the control animals were kept without any treatment. The percentage mortality rate and acute – lethal toxicity (LC₅₀) were determined for the different periods of exposure. Results obtained revealed that no mortality was recorded in the control group whereas a significant concentration – dependent increase in mortality was observed in the groups of animals treated with the extract of the plant. More so, duration of exposure also affected the mortality rate, as the highest percentage of mortality was observed in groups of animals exposed to the extract for 96 h. The LC₅₀ for the *T. vogelii* extract for 96 h exposure was 9.36 mg/L with lower and upper confidence limits of 4.04 and 21.68 mg/L, respectively. Conclusively, the findings of the study suggest that *T. vogelii* extract has acute toxic effect on early life stages of farmed clarid and as such, its use in fishing should be discouraged.

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1. INTRODUCTION

In the world over, plants and their derivatives have been used extensively for several purposes including curing and managing of various ailments as well as natural pesticides by artisanal fishermen to harvest fishes [1–3]. Though these plants and their derivatives are believed to be nontoxic compared to their synthetic counterparts, they may contain a number of harmful ingredients in their secondary metabolites which may have deleterious side effects including mutagenic potentials [4]. Hence, there is need for strict scientific study.

Tephrosia vogelii, commonly known as fish bean is a shrub, 1.83 - 3.05 m high, clothed with dense yellowish or rusty tomentum. The stems are more or less erect and the leaflets are five or more pairs. The flowers of the plant are 2 cm or more long and are densely crowded, conspicuous, red or purple and in dense racemes. Fruits of *T. vogelii* are large and 2-12 cm long, very densely villous or tomentose [5]. The shrub may grow as rapidly as 2-3 m in 7 months. *Tephrosia* is a Genus of legumes which belong to the family Fabaceae with about 300 *vogelii* species [6]. It was initially used by indigenous people as a mild fish poison. It is mainly found in the tropical and subtropical regions of the world [7]. It is an easy crop to grow from the seeds and manage, it remains ever green for more than four years when it is established [8]. It can be used as a cover crop, a hedge and/or for shelter while fixing nitrogen in the soils where it is planted. Physically, this plant has branches and stems with long and/or short white or rusty brown hair coat. *T. vogelii* has been known to have many uses in agriculture and human health. It is used as an abortifacient, emetic and purgative therapy for the skin diseases [9]. It has also been found to possess antimicrobial, anthelmintic activity. The dichloromethane extract from the roots and leaves was tested against *Staphylococcus aureus*, *Escherichia coli* and *Staphylococcus paratyphi* [10] and the roots decoctions are used to treat constipation. It is also used in plant protection and storage as potent natural pesticides in maize or beans storage. *Tephrosia* species contain complex mixtures of rotenoids and other flavonoids, known to be mitochondrial chain inhibitors, inhibiting cellular respiration in almost every living organism including insect and mammals [11]. These compounds block the

enzymes glutamate and succino dehydrogenase and thus H⁺ transport [12]. Rotenone is a colourless and odourless compound which is soluble in acetone and ethanol. The pharmacokinetics of rotenone is attributed to the mitochondrial electron transport destruction in the cells which hinders the utilization of oxygen in the respiration process of the organism leading into cell death [13]. Despite the toxic properties of rotenone to fish and arthropods, it is relatively safe to humans and animals when ingested, as the changes in the gut transform it into less toxic substances before it enters the blood stream where its toxicity matters [14].

2. MATERIALS AND METHODS

2.1 Experimental Fish

One hundred and sixty fingerlings of *C. gariepinus* were obtained from the University of Calabar fish farm. The fish were allowed to acclimatize to laboratory condition in an aquarium containing 100 L of de-chlorinated tap water. The water quality monitoring was carried out prior to and during the experiment. The physico-chemical parameters of the water were measured using the APHA [15] method of water quality assessment.

2.2 Collection and Preparation of Plant Material

The plant (*T. vogelii*) was collected from Idundu in Cross River State of Nigeria. The leaves were washed, air-dried for 48 h and then oven-dried for at 50°C. The dried leaves were then pulverized using an electric blender to fine powder. The powdered leaves were then subjected to Soxhlet extraction procedure using 70% ethanol as solvent. The extract was separated using rotary evaporator.

2.3 Experimental Design and Procedure

The 160 fingerlings were randomly divided into four groups using a completely randomized design (CRD) in a 4x4 (concentration x duration of exposure) factorial layout. The fish were exposed to three different concentrations of the of *T. vogelii* (25, 50 and 100 mg/L) for 24, 48, 72 and 96 hours, respectively. The aquaria were set up for each concentration containing 10 fish. Control animals were kept under similar

conditions without any treatment. Mortality was recorded every 24 hours throughout the 96 h exposure period. Fish were considered dead if they failed to respond to vigorous poking with a glass rod [16]. Dead fish were removed from the aquaria as soon as possible in order to prevent their bodies from decomposing.

2.4 Statistical Analysis

Data obtained was analyzed using analysis of variance (ANOVA) test. Significant means were separated using least significant difference (LSD) test. Mortality data were used to obtain the LC₅₀ using the Probit Software (2000).

3. RESULTS

Results obtained from the study showed that no mortality was observed in the control group while mortalities were recorded in groups of fish exposed to different concentrations of the treatment (Table 1). Results revealed a significant ($P = .05$) concentration – dependent increase in the mortality of fish with the lowest percentage mortality (43.13%) observed in group of fish treated with 25 mg/L of the extract while fish exposed to 100 mg/L had the highest percentage of mortality rate of 78.13% as shown in Table 1.

Table 1. Percentage mortality of fish treated with the extract of *T. vogelii*

Concentration (mg/L)	Percentage mortality
Control	0.00 ^d
25	43.13 ^c
50	68.75 ^d
100	78.13 ^a

Values along the table with similar superscripts are not significantly different at 5% based on ANOVA

Results obtained also indicated that the duration of exposure to the toxicant also had significant effect ($P = .05$) on the mortality rate of the fish exposed to the toxicant. The groups of fish exposed to 24, 48, 72 and 96h recorded 50.00, 62.50, 75.00 and 87.50 percentage mortality when compared to the control group that recorded no mortality. The LC₅₀ for 24, 48, 72 and 96 hours are 50.00, 25.32, 15.20 and 9.36 mg/L, respectively (Table 2).

More so, some abnormal behaviours were observed in groups of fish exposed to the graded concentrations of the extract such as respiratory distress, erratic swimming, loss of balance,

gulping of air and remaining still at the bottom of the aquarium. The degree of the various abnormal behaviours increased with increase in the concentration of the extract.

Table 2. Percentage mortality of fish based on the duration of exposure to the treatment

Concentration (mg/L)	Percentage mortality	LC ₅₀ (mg/L)
Control	0.00 ^e	0.00
24	50.00 ^d	50.00 (18.62 – 134.24)
48	62.50 ^c	25.32 (9.77 – 65.64)
72	75.00 ^b	15.20 (6.61 – 36.46)
96	87.50 ^a	9.36 (4.04 – 21.68)

Values along the table with similar superscripts are not significantly different at 5% based on ANOVA

4. DISCUSSION

Results obtained from the present study revealed that *T. vogelii* extract significantly increased the mortality of the exposed fish in a concentration – dependent manner which indicates acute toxic effect of the extract on the fish and agrees with the findings of Epel [17], Tiwari and Singh [18], who reported that *T. vogelii* has negative impact on fish. The fish exhibited a range of abnormal behaviours at higher concentrations of the extract in line with the observations of Svecericus [19] and Absalom et al. [20]. Studies have shown that fish exhibit different behavioural changes when exposed to toxicants to adapt to the toxins, but at higher concentrations and duration of exposure, the toxic reactions intensify and later result in the death [21–22], as observed in this study.

Respiratory distress, erratic swimming and loss of balance observed in exposed fish could be due to mucous precipitation and/or neurotoxicity in response to the treatment which resulted in higher respiratory rate, affected the physiology and caused death of the fish. This assertion is corroborated by Banerjee [23]. It is worthy of note that animal behaviour is a neurotrophically regulated phenomenon which is mediated by neurotransmitter substances [24–25]. The stressful breathing behaviour exhibited by the fish might also be attributed to respiratory impairment due to the effect of the extract on the gills. Results also showed that the LC₅₀ values lie within the 95% confidence limit with the longest duration of exposure (96 h) having LC₅₀ of 9.36 mg/L which implies that the toxic effect was

observed in concentration close to and in excess of the LC₅₀ value.

5. CONCLUSION

The findings of the study indicate that *T. vogelii* extract possesses acute toxic effect on early life stages of farmed clarid (*C. gariepinus*). Therefore, its use in fishing should be discouraged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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