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Comparative Acute Toxicity of Ichthyotoxic Plants (*Tephrosia vogelii*, *Adenia cissampeloides* and *Asystasia vogeliana*) on Farmed African Catfish (*Clarias gariepinus*)

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Authors' contributions

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

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ABSTRACT

This study was carried out to evaluate the effect of concentration and duration of ichthyotoxic plants (*Tephrosia vogelii*, *Adenia cissampeloides and Asystasia vogeliana*) on the mortality rate of catfish (*Clarias gariepinus*). The total of 370 catfish were distributed into different aquaria containing the different concentrations (0, 25, 50, 75, 100 mg/l) of *Tephrosia vogelii*, *Adenia cissampeloides and Asystasia vogeliana* extracts, respectively with 10 animals in each aquarium using a completely randomized design (CRD) in a factorial layout. They were exposed to the extracts for 24, 48, 72 and 96 hours, respectively. The control group was also set under the same experimental conditions without the extracts. The result revealed that the extracts concentration had a significant effect ($p<0.05$) on the mortality rate of the fish. Also, the effect of the different durations was significant ($p<0.05$) on mortality rates of the exposed animals. Mortality rate was increased as the duration

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increased. The interaction effect between the concentrations and durations showed that the toxic effect of the extracts on the fish was more pronounced at the highest concentration and duration of exposure. Thus, this study has shown the potency of *Tephrosia vogelii*, *Adenia cissampeloides and Asystasia vogeliana* leaves extract as a fish poisoning plant suggesting that the use of the plants as a fishing stock should be regulated.

Keywords: T. vogelii; acute toxicity; Clarias gariepinus; Adenia cissampeloides; Asystasia vogeliana.

1. INTRODUCTION

The use of plant has long been part of local cultures and traditions as source of food, medicines and other derivable products [1-2]. The role of indigenous knowledge in the identification, conservation and utilization of plant species cannot be overemphasized. Though these plants and their derivatives are believed to be nontoxic compared to their synthetic counterparts, they may contain a number of harmful ingredients on their secondary metabolites which may have deleterious side effects including mutagenic potentials [3].

Tephrosia vogelii, commonly known as fish bean is a shrub, 1.83 - 3.05 m high, clothed with dense yellowish or rusty tomentum. *Tephrosia* is a genus of legumes which belong to the family Fabaceae with about 300 species [4]. 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 [5]. 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 [6]. 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. It has also been found to possess antimicrobial, anthelminthic activity [7].

Adenia cissampeloides is a climber that thrives in humid areas of West Africa [8]. It is one of the fish poison plants used in West African countries like Ghana and Nigeria to obtain fish by local fishermen [9]. The leaves are roasted, pounded and thrown into a demarcated portion of the river or stream that is known to contain large population of fishes and within fifteen minutes small fishes are found floating while it takes about an hour for the larger ones to float [10]. Ekanem et al. [11] reported on acute toxicity of ethanol extracts from two ichthyotoxic plants (*Adenia cissampeloides* and *Blighia sipidia*) on *Heterobranchus longifilis* juveniles. They reported that the extract of the two poison plants showed abnormal behavioural changes, organ deformity, mortality, depigmentation, moribund swimming behaviour and spine break on the juveniles in a dose-dependent manner. The understanding here is that the exposure of water bodies to these poisonous plants could cause lethal or sub-lethal effects on the fish depending on the concentration, duration and the toxicity of the poisoning plant. *Asystasia vogeliana* belongs to the family *Acanthaceae*. It is an herb/shrub with leaves that are simple, ovate and decussate without stipules. The flowers are bisexual, zygomorphic and usually with coloured bracts [12]. There is paucity of literature on the toxic effect of *Aystasia vogeliana.* This study hopes to fill this gap.

The African catfish (*Clarias gariepinus*) is a species of catfish of the family Clariidae and comprise the most cultivated fishes in Nigeria. It is the most and highly demanded freshwater fish the world over due to its resistant to stress, ability to tolerate a wide range of environmental conditions and high stocking densities under culture conditions and relatively fast growth [13]. They are found throughout Africa and the Middle East, and live in freshwater lakes, rivers, and swamps, as well as human-made habitats, such as oxidation ponds or even urban sewage systems. The African catfish can never be failed to mention when talking about traditional fish capture. In the present system of aquaculture practices, they are similar to tilapia in terms of culture [14].

2. MATERIALS AND METHODS

2.1 Experimental Fish

Three hundred and seventy 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, during and after the experiment. The physico – chemical parameters of the water were measured using the APHA [13] method of water quality assessment.

2.2 Collection and Preparation of Plant Material

The plants (*T. vogelii*, *A. cissampeloides and A. vogeliana*) were obtained from Idundu in Cross River State of Nigeria. The leaves were washed, air – dried for 48 h and then oven – dried in hot air oven (Model: AVI560) at 60ºC for 24 hours. The dried leaves were then milled into powder using heavy duty miller (Christison 37 BLIB, model 204C BC). The powdered leaves were then subjected to Soxhlet extraction procedure using 70% ethanol as solvent. The extracts were separated using rotary evaporator.

2.3 Experimental Design and Procedure

The 370 fingerlings were randomly divided into 10 groups using a completely randomized design (CRD) in a factorial layout. The fish were exposed to three different concentrations of the *T. vogelii*, *A. cissampeloides and A. vogeliana* (25, 50 and 100 mg/L) for 24, 48, 72 and 96 hours, respectively. The aquaria was set up for each concentration containing 10 fish. Control animals were kept under similar conditions without any treatment. Mortality was recorded 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 [14]. Dead fish were removed from the aquaria as soon as possible in order to prevent their bodies from decomposing.

2.4 Statistical Analysis

All data collected on the mortality were subjected to analysis of variance (ANOVA) using predictive analysis software (PASW), version 18.0. Significant means were separated using the least significant difference at 5% probability level.

3. RESULTS

3.1 Effect of the Extracts Concentration on Mortality of *C. gariepinus*

The effect of the extracts concentration on mortality of *C. gariepinus* is shown in Table 1. The result revealed the effect of the extracts was dose – dependent. The highest percentage mortality was observed in groups of fish treated with 100 mg/l of the different extracts (62.50, 78.13 and 100% for *A. cissampeloides*, *T. vogelii* and *A. vogeliana*, respectively). Comparatively, *A vogeliana* had the highest mortality rate (84.38, 100.00, 100.00% for 25, 50 and 100 mg/l concentration of the extract, respectively) followed by *T. vogelii* (45.13. 68.75 and 78.13% respectively for 25, 50 and 100 mg/l) while the lowest mortality rate was recorded in group treated with *A. cissampeloides* extract which showed 25.00, 45.83 and 62.50% mortality for 25, 50 and 100 mg/l concentration of the extract, respectively.

3.2 Effect of Duration of Treatment on Mortality of *C. gariepinus*

Table 2 shows the effect of duration of treatment on the mortality of *C gariepinus*. Result indicates that duration of the different treatments had significant effect (p= .05) on the mortality of the animals. The highest percentage of mortality was observed in groups of fish treated for 96 h (38.80, 87.50 and 100% for *A. cissampeloides*, *T. vogelii* and *A. vogeliana*, respectively) while the lowest was obtained in groups exposed for 24 h (0.00, 50.00 and 91.67% for *A. cissampeloides*, *T. vogelii* and *A. vogeliana*, respectively). Also, animals treated with *A. vogeliana* had the highest mortality rate in all the durations of exposure (91.67, 91.67, 100.00, and 100.00% for 24, 48, 72 and 96 h, respectively) when compared to the other extracts. It was followed by *T. vogelii* (50.00, 62.50, 75.50 and 87.50% for 24, 48, 72 and 96 h, respectively)

Table 1. Effect of the different extracts concentrations on percentage of mortality of *C. gariepinus*

Extracts		Concentration	(mg/l)	
	0	**25**	**50**	**100**
***T. vogelii* (%)**	0.00^{a}	45.13^{b}	68.75^{c}	78.13^{d}
A. cissampeloides	0.00^{a}	25.00^{e}	45.83^{f}	62.50^{g}
A. vogeliana	0.00^{a}	84.38^{h}	100.00^{j}	100.00^{j}

Values followed with different superscript along the same vertical and horizontal array indicate significant difference (p<0.05)

while the lowest mortality was observed in group of animals treated with *A. cissampeloides* being 0.00, 33.33, 37.50 and 38.00% for 24, 48, 72 and 96 h, respectively.

3.3 Effect of Interaction between Extracts Concentration and Duration on Mortality of *C. gariepinus*

As presented in Table 3, results revealed significant difference in the mortality rate of the fish as a result of the combination of concentration and duration of the exposure. Interactively, fish exposed to 100 mg/l of the extracts for 96 h recorded the highest mortality rate (84.43, 74.00 and 82.82% for *T. vogelii*, *A. cissampeloides* and *A. vogeliana*, respectively). Comparatively, animals treated with graded doses of *T. vogelii* for the different durations of treatment had the highest mean mortality rate (79.56%) followed by *A. vogeliana* (73.45%) while the lowest was observed in group of fish treated with *A. cissampeloides* (48.71%). More, some abnormal behaviours were observed in the different groups of fish exposed to the graded concentrations of the extracts for various durations of the treatments. There include respiratory distress, desultory swimming, imbalance, gulping of air and immobility at the bottom of the aquarium. The degree of the various abnormal behaviours increased with increase in the concentration of the extracts and duration of exposure.

4. DISCUSSION

Ichthyotoxic plants have been widely used in harvesting of fishes. Local and artisanal fishermen still prefer to use these ichthyotoxic plants to obtain fish from small bodies of water since they are presumably cheaper and more accessible. A decline in fish stock population was reported in South America due to indiscriminate use of these ichthyotoxic plants for fishing [15].

The administration of the three extracts studied caused variable effects on the mortality rate of the animals. The concentration of the extracts had a significant dose – dependent effect on the percentage of mortality when compared to the control that had no mortality. This might be due to the inherent phytochemicals in the extracts. The effect increased with increase in the concentration of the test extracts. This may probably result from the fact that when the concentration increased, the amount of individual bioactive components in the extracts is also increased which could then contribute adversely to hinder and disrupt biosynthetic and physiological processes in the animals. This findings is corroborated by Ekpo et al. [16-17] Ibiam et al. [18] and Svecevicus [19].

Comparatively, results revealed that *A. Vogeliana* had the highest adverse effect on the mortality rate of the animals when compared to the other extracts. This might be as a result of the number and/or concentrations of the phytochemical constituents of the plant extract when compared to other extracts. Certain ichthytoxic plants are reported to certain bioactive substance that disrupt development in fishes leading to subsequent death [20].

The duration of the various treatments also significantly affected the mortality rate of the fish which agrees with the findings of Ekpo et al. [16-17]. More so, according to Udolisa and Lebo [21] fish poison plants are used to obtain encircling and loss of balance of fishes within 10 minutes and subsequent death of fishes within 1 to 2 hours later. The various durations used in this study showed related results to this report. This may suggests that the effect of this plants on the animals could be more detrimental when the duration is increased. Relatively, the effect of the extract duration was also dose-dependent and more pronounced as the duration of exposure was increased. More fish died at 96 hours compare to 24 hours. This may also not be unconnected with some inherent toxic bioactive compounds in the extract.

The respiratory distress, desultory swimming, imbalance, gulping of air and immobility at the bottom observed in exposed fish can be attributed to neurotoxicity and/or mucous coagulation in response to the various extracts giving rise in higher breathing rate with a concomitant effect on the physiology and eventually caused death of the fish. This assertion is supported by Banerjee [22] and Ekpo et al. [17] and more so, these abnormal behaviours have been shown to be influenced by neurotropic regulation mechanism which is mediated by neurotransmitter substances [23–24].

At this juncture, it is pertinent to mention that the effect of these toxic plant extracts on the fish could even be more when different

Table 2. Effect of extracts duration on percentage of mortality of *C. gariepinus*

Extracts	Duration (hours)			
	24	**48**	**72**	**96**
T. vogelii	50.00[a]	62.50[b]	75.50[c]	87.50[d]
A. cissampeloides	0.00[d]	33.33[e]	37.50[f]	38.00[f]
A. vogeliana	91.67[g]	91.67[g]	100.00[h]	100.00[h]

Values followed with different superscript along the same vertical and horizontal array indicate significant difference (p<0.05)

Table 3. Interaction effect between concentration (mg/l) and duration (hours) of the different extracts on percentage of mortality of *C. gariepinus*

Concentration Mg/l	Leaf extracts/Duration of exposure (hours)											
	T. vogelii				***A. cissampeloides***				***A. vogeliana***			
	24	**48**	**72**	**96**	**24**	**48**	**72**	**96**	**24**	**48**	**72**	**96**
Control	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
25	63.89[b]	72.22[c]	75.70[c]	76.08[c]	00[a]	45.83[d]	58.33[b]	60.03[b]	46.57[d]	52.82[b]	59.07[b]	65.32[b]
50	70.14[e]	78.47[f]	81.95[f]	82.35[f]	00[a]	54.17[b]	66.67[b]	72.38[e]	59.38[b]	65.63[b]	71.88[b]	78.13[b]
100	71.13[a]	79.56[f]	83.10[g]	84.43[a]	00[a]	54.17[b]	66.67[b]	74.00[e]	64.07[b]	70.32[e]	76.57[a]	82.82[g]
Mean ±SE	79.56				48.71				73.45			

Values followed with different superscripts along the same horizontal and vertical arrays indicate significant difference (p<0.05)

concentrations interact with individual duration (Table 3). In the mortality, the poisoning effect of the extract was more pronounced at the highest concentration and duration of exposure. For instance, from concentrations of 50 mg/l to 100 mg/l under 96 hours, the highest percentage mortality was observed.

5. CONCLUSION

Implicitly, our findings suggest that *Tephrosia vogelii*, *Adenia cissampeloides and Asystasia vogeliana* leaves extract have acute toxic effect on farmed African catfish (*C. gariepinus*) and could prove to be potent fishing stocks. However, holistic measures should always be taken considering the effect that it could exert on other aquatic inhabitants and systems.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTEREST

Authors have declared that no competing interests exist.

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