Acute Toxicity and Active Ingredients Concentration of *Datura innoxia* Stem on *Clarias gariepinus* Juveniles

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ABSTRACT
Despite the widespread use of Datura innoxia stem in Nigeria for demarcation, gardening and medicine, its toxicity and effectiveness to aquatic species, notably fish, has received little attention. This study was carried out to determine the toxicity, phytochemical and proximate composition of Datura innoxia stem to Clarias gariepinus. The range-finding test was carried out and repeated twice to obtain credible results that could be used in the ultimate test .The toxicant (Datura_ innoxia stem) was introduced at varying concentrations of 300mg, 330mg, 360mg, 390mg and 420mg, respectively in the initial range-finding test; nevertheless, the toxicant was added at different concentrations of 320mg, 330mg, 340mg, 350mg, and 360 milligrams per liter of water (mg/l) in the final test. The median lethal concentration of Datura innoxia stem powder to Clarias gariepinus was determined to be 334.370 mg/L based on the conclusive test. The study found that young Clarias gariepinus exhibited erratic movement at first, rapid opercular movement, skin darkening, and absence of response. Furthermore, the results show that the local composition was within acceptable limits. Among the phytochemicals discovered were steroids, tannins, saponins, phenols, cardiac glycosides, and scopolamine. The water quality data were evaluated using one way Analysis of Variance (ANOVA) with temperature range of 25.27 to 25.35^{0}C, As a result, the study suggests that Datura innoxia stem is hazardous to Clarias gariepinus juveniles.
Keywords: Datura_innoxia *stem, Acute Toxicity, Lethal Concentration, and Active* Ingredients

INTRODUCTION

Human population is growing rapidly in this period of the green revolution. Construction of homes has taken place in forests, which has negatively impacted the environmental balance (Ullah, 2014). On the other hand, we are faced with a developing and growing problem of contaminants (Klumpp *et al.*, 2002). According to Abu-Darwish *et al*. (2011), these pollutants include household garbage, partially or completely treated industrial effluents, and different substances like pesticides used in agriculture or safety measures. These pollutants are supplemented with different chemicals, pesticides, organic compounds and heavy metals (Jabeen *et al*., 2011). Many aquatic creatures live in water that has been altered by these compounds (Donohue *et al.*, 2006). These creatures are negatively impacted by the altered water quality, which can even cause their mortality in situations of extreme exposure and acute concentrations (Sabae *et al*., 2014). It is impossible to discount the possibility that deteriorating water quality is a significant cause of the drop in fish catches and aquatic productivity. The rise in agricultural operations is mostly to blame for the increased usage and use of pesticides and poisonous plants in Nigerian agriculture today, which significantly contributes to aquatic pollution; and consequently, a decline in water quality. Since fish are among the most widely spread species in aquatic environments, the degree to which environmental pollution in waters has biological effects on them may be indicated by the fish's susceptibility to environmental contamination (Ramesh *et al.*, 2009). Fish are frequently employed as indicators of environmental contamination because of the biochemical changes that they exhibit (Cavas and Ergene-Gözükara, 2005). Fish are also used to assess the health of aquatic ecosystems.

Datura innoxia is a highly toxic plant that belongs to the Solanaceae family. It contains a range of active compounds, such as hyoscyamine, scopolamine, and atropine, which are known to have hallucinogenic and toxic effects (Prado *et al*., 2009). Understanding the effects of *Datura innoxia* on fish species is critical for maintaining and protecting aquatic environments and the organisms that live within them. *Datura innoxia* is a plant species with a variety of therapeutic characteristics. *Datura innoxia* has long been used in traditional medicine; however, it contains poisonous chemicals that can be harmful to both human and animal health. . *Datura innoxia* is a versatile plant found throughout the tropics; its leaves, seeds, stem, fruits, and roots are economically valuable for industrial and therapeutic purposes. Despite its widespread use in Nigeria for demarcation, gardening, and medicine, the toxicity and effectiveness of the *Datura innoxia* stem to aquatic species, notably fish, has received little study. Fish are frequently utilized as sentinel organisms in ecotoxicological research because they perform a variety of roles in the food chain, collect toxic compounds, and respond to low mutagen quantities (Cavas and Ergene-Gözükara, 2005). As a result, the use of fish biomarkers as indices of the impacts of pollution is increasingly important and can facilitate early diagnosis of aquatic environmental problems (Baser *et al.,* 2003). Acute toxicity experiments help to understand the limiting effects of various substances on organisms (Baser *et al.,* 2003; Svobodova *et al.*, 2003). Because chemical analysis cannot establish the effects of harmful substances on fish or ecological risks, mortality or bioassay trials are the preferred method for assessing their ecological impact. The objective of this study is to determine acute toxicity and active ingredients concentration of *Datura innoxia* stem on *Clarias gariepinus* juveniles.

MATERIALS AND METHODS

The Experimental Site

The experiment took place at the Fish Hatchery Laboratory, Department of Fisheries and Aquaculture, Bayero University, Kano, Nigeria. Kano is located between latitude $12^0$00N and 12.000^0N and between longitude $8^0$31’E and 8.517^0E in the Savanna, south of the Sahel. Kano is a major route of the trans-Saharan trade, having been a trade and human settlement for millennia. The State lies in North-West geopolitical zone of Nigeria and shares common borders with Katsina, Jigawa, Kaduna and Plateau State. Kano is a city in northern Nigeria and the capital of Kano State *(Encyclopedia Britannica, 2021).*

Experimental fish

One hundred and Eighty (180) species of *C. gariepinus* (mean weight, 19.56 ± 0.7g and 31.07±1.23g juveniles were used for the study, fish were purchased from a reputable fish farm from Kano, Kano State.

Source and Preparation of *Datura innoxia*

Fresh samples of *D. innoxia* plant were taken from Ankpa Local Government Area of Kogi State. The samples were separated into many components with special emphasis on the stems. The samples were allowed to air - dry to constant weight before being blended to fine powder. The resulting powder were sieved (0.2 mm sieve size)) and stored in air- tight wide mouth bottle for chemical analysis. Exactly 500g of each stored sample was dissolved in 2 litres each of distilled water at room temperature (27 ± 0.3°C) for 24 hours (Omoregie and Onuogwu, 2015). Afterwards, a vacuum pump was used to decant and filter the settled aqueous component via Whitman filter paper (No.1). To prepare them for usage, the filtrates were freeze-dried and kept in a refrigerator at 100^{o}C.

Experimental Design

The experiment was conducted using a completely randomized approach design (CRD). Eighteen (18) plastics tanks, each measuring 60cm by 40cm by 40cm, were utilized. After a thorough cleaning, 20 litres of water were added to the plastic tanks. Each plastic tank has a label on it. Ten fish were allocated each tank after each fish was weighed. A total of 180 young *C. gariepinus* fish were randomized and placed into the aquariums in triplicates with 10 fish in each tank.

Phytochemical Screening of *Datura innoxia* stem powder

Phytochemical screening for key constituents was carried out on *Datura innoxia* stem powder in the Department of Plant Biology, Bayero University, Kano. The *Datura innoxia* stem powder was analyzed for the presence of active components such alkaloid, flavonoid, tannins, saponins, steroids, glycosides and terpenoids following the techniques of Ushie *et al*. (2013).

Range finding test

Following static bioassay, a preliminary 96-hour range finding test was carried out independently for *C. gariepinus* juveniles in order to ascertain the poisonous range of *Datura innoxia* stem to *C. gariepinus* juveniles in accordance with Parrish's (1985) description. In the course of the range finding test, 10 *C. gariepinus* juveniles were individually weighed using a sensitive electronic weighing scale (Mettler Toledo FB602 model) and stocked into the eighteen tanks that were filled with twenty litres of tap water. The stems of *Datura innoxia* weighing 300 mg, 330mg, 360mg, 390mg and 420 mg, respectively were used per litre of water. The LC_{50} of *Datura innoxia* stem was found when test fish were exposed to the plant for 96 hours. The fish's reaction to mild stimuli was utilized as an index of toxicity, and their inability to react to touch was used as an index of death.

Definitive Test

Result from range finding tests offered advice for the concentration level to be used in definitive test. A total of eighteen (18) plastic tanks were filled, each holding twenty litres of water, in order to conduct the final test. A concentration of *Datura innoxia* stem, previously identified through a range finding test, was used to conduct the definitive test. The amounts of *Datura innoxia* stem utilized were 320mg, 330mg, 340mg, 350mg and 360 mg respectively were per litre of water. The sensitive weighing balance was used to prepare the various concentrations. The fish's non-reaction to *Datura innoxia* stem powder was judged to be fatal at 50% of the test organism after 96 hours of exposure, whereas the fish's response to mild stimuli was employed as an indication of toxicity.

Statistical Analysis

Minitab 14 was used to analyze data on the water quality parameters. The mean lethal concentration (LC_{50}) for 96 hours was calculated using probit analysis (Finney, 1971).

Results and Discussion

Table 1 displys the result of water quality values following a 96-hour acute test in which juvenile *Clariasgariepinus* were exposed to *Datura innoxia* stems.

Table 1: Acute toxicity test criteria for experimental units' water quality of *Clarias gariepinus* exposed to *Datura innoxia* stem

S/N	Treatment Mg/L	pH	TEMP (^{0}C)	TDS (ppm)	EC (µS/cm)	DO (mg/L)
1	T0 (control)	6.57±0.01^a	25.27±0.03^{ab}	306.20±0.05^a	613.40±0.10^a	4.57±0.03^e
2	T1 (320)	6.63±0.03^a	25.30±0.06^a	314.52±0.17^b	620.03±0.35^b	4.47±0.01^d
3	T2 (330)	5.87±0.02^b	25.27±0.03^{ab}	320.00±0.05^c	635.00±0.10^c	4.42±0.01^c
4	T3 (340)	5.57±0.03^b	25.32±0.03^b	327.55±0.15^d	650.10±0.31d	4.37±0.00^c
5	T4 (350)	5.33±0.03^b	25.35±0.03^b	365.40±0.28^e	736.80±0.56^e	4.31±0.01^b
6	T5 (360)	4.67±0.02^c	25.27±0.03^{ab}	401.90±0.06^f	799.80±0.12^f	4.18±0.00^a
	P-Value	0.104	0.061	0.001	0.000	0.001

Means along the same column with different superscripts are significantly different *($p<0.05$)*

Dissolved oxygen (DO), total dissolved solids (TDS), temperature (Temp), hydrogen ion concentration (pH), and electrical conductivity (EC).

The test water's physico-chemical characteristics, as determined by the acute toxicity bioassay, fell within acceptable bounds that allowed *C. gariepinus* to survive and grow normally. Therefore, the test water's low water quality could not have caused the fish's behavioural abnormalities or eventual death. According to Badiru's (2005), the ideal pH scale for fish growth (6.67–7.02) used in this study matches the ideal range (6.5–9) for fish production. The dissolved oxygen range for this study (4.18–4.67 mg/L), however, fell within the range of reported by Badi (2005) on the dissolved oxygen scale for warm-water fishes, which indicates slow growth after prolonged

exposure (1–5 mg/L). Similarly, Howerton (2001) indicated that the temperature range for this study (25.27°–25.32°C) fells within the typical tropical temperature range to which fish get acclimated (25° – 32°C). It was noted that both the dissolved oxygen content and pH dropped. The impact of *Datura innoxia* stem powder on the quality of the water may be the cause of this. This is comparable to the *Datura innoxia* root extract research by Ayuba and Ofojekwu (2002); Ayuba *et al.* (2012). Total dissolved solids (TDS) and electrical conductivity (EC) levels were found to be within permissible limits, even though they differed from the control values significantly ($p<0.05$) (Ayuba *et al.*, 2012).
The lack of a statistically significant difference in temperature between the groups may have resulted from *Datura innoxia* not reacting as it would have in an exothermic or endothermic environment. Nevertheless, the concentration of *Datura innoxia* stem decreased with increased concentration, and in general, toxicity increased with reduced oxygen concentration. These findings are consistent with reports from Adigun (2003) and (Ayuba *et al.*, 2012) who reported on other toxicants. The aforementioned statement is consistent with the findings of Warren (1997), who reported that the introduction of a toxicant into an aquatic system may result in a decrease in dissolved oxygen concentration, which will impair respiration and cause asphyxiation. Raised temperature and other physiological states of the fish can be linked to acute fish death, according to Rahman *et al.* (2002), Mekkawy *et al*. (2013), and Isiyaku *et al.* (2015).

Table 2 shows the screening test for the presence of atropine, scopolamine, hyosyamine, alkaloids, saponins, and tannins.

Table 2: Phytochemicals in *Datura innoxia* stem

Parameters	Qualitative Analysis
Scopolamine	+
Steroid	-
Saponins	+++
Atropine	++
Flavonoid	+
Carboxylic acids	-
Terpenoids	++
Tannin	-
Coumarins	-
Essential Oils	+
Phenol	+
Valepotriates	-

Cardiac Glycoside	+

+ = Present
- = Absent

The active principles of many drugs found in plants are secondary metabolites (Okeke, (1998). Therefore, basic phytochemical investigation of these extracts for their major phyto constituents is also vital. The presence of saponin, flavonoids, tannin, steroid, cardiac glycosides, total phenol and terpenoids were detected in *Datura innoxia* stem. The presence of saponin which has sedative, anaesthetic as well as medicinal potency as evidenced in the various uses have been reported by Okeke (1998) and Ayuba and Ofojekwu (2005). Isiyaku *et al.* (2022) also reported the presence of some of these substances in the leaves and root bark of tamarind plant. The observed signs of toxicity, including the ultimate mortality in some of the exposed fish might have been due to these substances. This is because of the toxic nature of some of them (Bent, 2004). The observed mortality in some of the exposed fish might have been due to the saponins and tannin content of *Datura innoxia* stem.

Table 3 shows the behavioural changes in *Clarias gariepinus* juveniles after 96-hours exposure at different concentrations of *Datura innoxia* stem.

Table 3: **Behavioural changes in *Clarias gariepinus* juveniles after 96-hours exposure at different concentrations of *Datura innoxia* stem**

Behaviours	Concentration (mg/L)					
	0.00	320	330	340	350	360
Breathing in deeply	-	-	+	+	+	+
Deformation of Barbel	-	-	-	-	-	-
Discoloration	-	-	+	+	+	+
Swimming erratically	-	-	+	+	+	+
Rubbing against plastic tank	-	-	-	+	+	+
Leaping	-	-	+	+	+	+

Resting at bottom	-	+	+	+	+	+
Suspended vertically in water column	-	+	+	+	+	+
Fin deformation	-	-	+	+	+	+

\+ = Present
 \- = Absent

The behavioral changes found in this study are consistent with the findings of other authors. Fish in exposed groups exhibited hyperactivity for 12 to 24 hours, which may have been an attempt to flee the poisonous environment. When fish are exposed to an unfavorable environment, hyperactivity has been proposed as the key indicator of nervous system failure brought about by *Datura innoxia* poisoning, which impacts physiological and metabolic functions. According to Isiyaku *et al*. (2022), catfish exposed to tamarind exhibited similar behavioural responses, such as increased opercular movement, mucous secretion, jerky movement, floating on the sides, and hypersensitivity exhibiting violent, erratic, and fast swimming. The authors concluded that the fish's abnormal behavior is indicative of the tamarind's toxic effects on the cardiovascular and central nervous systems. The hyperactivity of *C. gariepinus* exposed to datura was also reported by Ayuba *et al*. (2007). This hyperactivity was characterized by rapid and erratic swimming or darting, partial loss of equilibrium, rapid movement of the pectoral fins and opercular, a decrease in feeding activity, bleeding from the fins, and loss of some skin parts. Similar clinical signs, including rapid breathing, were reported by Velisek *et al*. (2010) in rainbow trout poisoning cases involving metribuzin. Table 4 shows the juvenile *Clarias gariepinus's* mortality following 96 hours of exposure to various *Datura innoxia* stem concentrations during the acute test.

Table 4: Mortality of *Clarias gariepinus* juveniles exposed to *Datura innoxia* stem over the 96 hour period

Treatments/ Concentration (mg/L)	Log Concentration	Number Stocked	Mortality							Probit
			12 hrs	24 hrs	48 hrs	72 hrs	96 hrs	Total	%	
T0 (Control)	0	30	0	0	0	0	0	0	0	0
T1 (320)	2.51	30	0	0	3	3	2	8	26.67	4.39

T2 (330)	2.52	30	0	3	5	3	2	13	43.33	4.82
T3 (340)	2.53	30	3	4	5	3	3	18	60	5.25
T4 (350)	2.54	30	6	5	5	2	4	22	73.33	5.61
T5 (360)	2.56	30	9	7	4	3	3	26	86.67	6.13

Result showed that when the concentration of *Datura s*tem increased, the mortality rates also became severe in this study. The first mortality was reported 178 minutes after the toxicant was added to the bowl with the greatest amount of mercuric chloride (360 mg/l). This is in line with the findings of Ayuba *et al*. (2007), who observed the first mortality occurring 30 minutes after a toxicant was introduced to *Clarias gariepinus* in an acute concentration of several *Datura innoxia* components. Three hours after the injection of the toxicant, Olaifa *et al*. (2004) reported the first mortality in *Clarias gariepinus* exposed to lethal and sub-lethal concentrations of copper. Datta and Kaviraj (2002); Fafioye *et al*. (2004) and Okomoda *et al.* (2010) reported the first fatality 36 hours after the acute toxicity treatment of *Clarias gariepinus* with synthetic pyrethyroid, Deltamethrin, *Raphia vinifera* extracts and formalin. Guedenon *et al.* (2011) reported the first mortality of *Clarias gariepinus* following 30 hours of treatment with 120 mg/l cadmium sulfate. In comparison to previous studies, the current study appears to have the shortest period of *Clarias gariepinus* resistance. *Clarias gariepinus* has shown high resistance to a number of toxicants (Datta and Kaviraj, 2002; Okomoda *et al*., 2010; Guedenon *et al*., 2011; Isiyaku *et al.*, 2015), although; it has extremely low resistance to mercuric chloride. The LC_{50} determined in this study was 334.37mg/l (Fig. 1), which is similar to the value reported by Isiyaku *et al*. (2011) when *Oreochromis niloticus* was exposed to an acute concentration of *Tamarindus indica* seed husk.

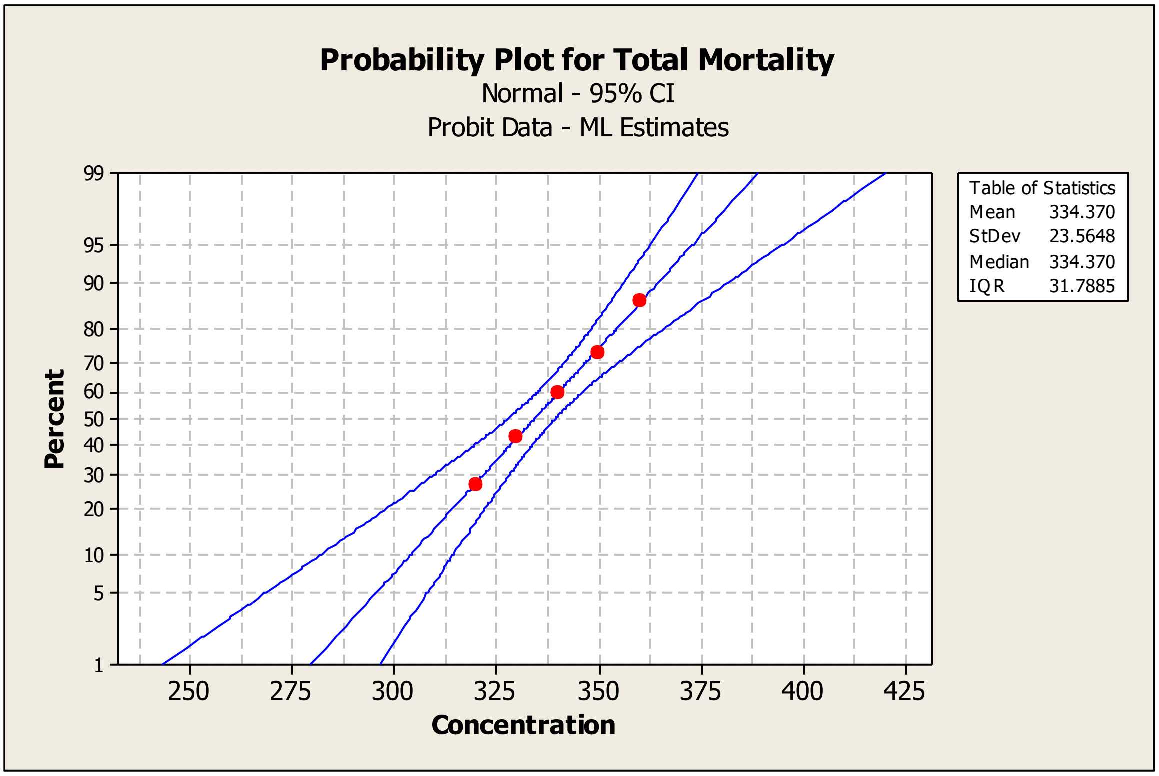

FIG. 1: A linear association exists between mean probit mortality and log concentration of Clarias gariepinus treated to various concentrations of Datura innoxia stem over 96 hours.

Ishikawa *et al.* (2007) determined that the LC_{50} for treating *Oreochromis niloticus* acute mercury toxicity was 0.22 mg/l. This study had the greatest median lethal concentration documented among those reported by earlier workers. Considering, that the chemical product is the same, the variation in the species employed may have contributed to the different outcomes. The LC_{50} discovered in the study, however, was significantly lower than those reported with *Clarias gariepinus* by Ayuba and Ofojikwu (2002); Ezike and Ufodike (2008); Lawson *et al.* (2011); Guedenon *et al*. (2011). These studies reported 204.17 mg/l for *Datura innoxia*, 334 mg/l for gasoline, 129 mg/l for Lindane (Gamma-Hexachloro-cyclohexane), and 46.11 mg/l for cadmium sulphate. The varied compounds and substances employed in the separate studies, as well as the unique ambient conditions, could be the cause of the variations.

CONCLUSION

i. Datura stem powder significantly induced anaemia, leukocytosis and lymphocytosis.
ii. Datura also inhibited the growth and nutrient utilization of *Clarias gariepinus* juveniles.
iii. Aquatic species experience increased stress when their environment is contaminated by toxic plants, whether as a result of acute or long-term events.

RECOMMENDATION

i. The use of Datura stem in agricultural fields should be controlled to prevent possible contamination by leaching into the aquatic environments. In this way aquatic organisms could be protected from these kinds of herbicides.

ii. The indiscriminate use of Datura as well as their use near water bodies should be discouraged. Instead, more environmentally friendly approaches to pest control (such as bio control) should be explored.

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