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*Full Length Research Paper*

# Acute toxicity studies of the lyophilized aqueous extract of *Psychotria microphylla* leaf on *Clarias gariepinus* juveniles

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**The piscicidal activity of the lyophilized aqueous extract of *Psychotria microphylla* leaf on *Clarias gariepinus* was investigated in a semi-static bioassay to determine the median lethal concentrations ($LC_{50}$) at 96 h of exposure. Six graded concentrations of 0, 0.27, 0.33, 0.47, 0.67 and 1.33 mg/l of the lyophilized extract were applied to *C. gariepinus* juveniles (mean weight: 180 g and length: 25 cm) in plastic containers. The 24, 48, 72 and 96 h $LC_{50}$ values (with 95% confidence limits) estimated by probit analysis were 0.65 (0.57-0.77), 0.53 (0.45-0.65), 0.41 (0.33-0.49) and 0.35 (0.26-0.42) $mgl^{-1}$, respectively. During exposure, fish exhibited discolouration, gulping for air, erratic swimming, loss of reflexes, slow opercular movement and ultimately settling at the bottom motionless just before death. The toxicity of the aqueous extract of the plant against *C. gariepinus* was both time and dose dependent. It is thought that this plant extract or powder will be useful in aquaculture to eradicate predators and competing wild fish from nursery, rearing and stocking ponds prior to the stocking of commercially grown fry and fingerlings of desired species.**

**Key words:** *Psychotria microphylla, Clarias gariepinus,* toxicity, juveniles, lethal concentration.

## INTRODUCTION

In fish farming operations, it is a common management practice to eradicate predators and competing wild fish from nursery, rearing and stocking ponds prior to the stocking of commercially grown fry and fingerlings of desired species. Unwanted fish may enter fish culture ponds through water supplies, birds or along with fish seed brought into fish farm and can account for up to 40% losses in the commercial fish and shrimp harvest (Pillay and Kutty, 2001). Some producers battle this problem by using cyanide (a poison) or any other poison of similar nature that can have serious impact on other organisms in food chain, including humans. Some use tea seed cake to control predators and trash fishes, while others drain ponds, which is usually not feasible and also ineffective in controlling and eradicating unwanted fishes at commercial scale.

Sometimes inlets are screened to stop the entry of eggs or larvae of wild fish fauna if source is canal water. This too is not as effective as it is normally thought (Bardach et al., 1972). Ideally, it is advisable that ponds should be sun dried and pond bottom cracked to get rid of unwanted fish fauna. However this practice is not always possible particularly during the wet season. The best way of ensuring total obliteration of unwanted fishes is through the use of fish toxicants in pond water (Guerrero and Guerrero, 1986).

Fish toxicants (piscicides) can be herbal or synthetic. Synthetic piscicides are not degradable, and hence, pose the problems of environmental resistance, pest

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resurgence and could have detrimental effects on non-target organisms (Fafioye and Adebisi 2002). The use of plant piscicides for poisoning and stupefying fish (Olufayo, 2009) is very common globally and is most preferred to synthetic piscides. They are easily biodegradable and leave no residues in the environment and are easily reversed in fish subjected to chronic concentrations (Onusiriuka and Ufodike, 1994). Secondly plants are virtually an inexhaustible source of structurally diverse and biologically active substances (Batabyal et al., 2007). Plant poisons called botanicals are extracted from flowers, bark, pulp, seeds, roots, leaves and even the entire plant (Sirivam et al., 2004). Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties (Wang & Huffman, 1991). These can be extracted from flowers, bark, pulp, seeds, roots, leaves and even the entire plant (Sirivam et al., 2004). The rotenones, saponins and cynanide account for nearly all varieties of fish poisons; others are ichthyoethereol, triterpene and other ichthyotoxins (Béarez, 1998).

Plants which are widely used as piscicides are tea seed cake and derris root and their toxic effects have been well documented (Akinbulumo et al., 2005). However, these conventional plant piscicides are either not within the reach of the fish farmers or their use may not be cost effective, especially for farmers in developing countries such as Nigeria. There is therefore need for more information on the piscicidally useful plants that have been reported to be of great biodiversity in countries like Nigeria where aquaculture is currently recording good growth (Solbe, 1995).

*Psychotria microphylla* is one of the plants commonly used to catch fish in the South-eastern Nigeria, especially, Ebonyi State. To the best of our knowledge, no scientific work on the piscicidal activity of this plant has been reported. Therefore, this study was planned to assess the piscicidal activity of the lyophilized aqueous extract of *P. microphylla* leaf on *Clarias gariepinus* juvenile, an important tropical catfish for aquaculture in Nigeria (Hogendoorn, 1979; Olaifa et al., 2003; Rahman et al., 1992).

## MATERIALS AND METHODS

### Sample collection and preparation

As shown in Figure 1, the fresh samples of *Psychotria microphylla* were collected from the wild at Afikpo South L.G.A of Ebonyi State, Southeastern Nigeria. The plant was identified and authenticated by Mr. Ozioko of the International Bioresources and Research Centre, Nsuka, Nigeria.

### Preparation of lyophilized aqueous extract

The samples were washed and shade-dried and then ground into fine powder and sifted using 0.25 mm sieve. The leaf powder thus obtained was soaked in 1 L of double distilled water for 48 h. The stored mixture was filtered through sterile gauze and the filtrate was collected by hand pressure. Further, it was centrifuged at 3000 rpm for 10 min and the supernatant filtered through Seitz filter (0.2 nm). The filtered extract was poured into special lyophilizing flask. The flask was connected to a vacuum pump and evacuated till drying and the lyophilized powder then used for evaluating piscicidal activity of *P. microphylla.*

The test fish, *C. gariepinus* (mean weight and length, 180±g and 25±cm respectively), were obtained from Chiboy's farm in Abakaliki, Ebonyi State, Nigeria. The fish were acclimatized to laboratory conditions (25°C) for 14 days before the exposure period using plastic aquaria. During the acclimation period, the fish were fed twice daily using standard commercial fish feed.

### Experimental design and acute toxicity test:

Acute toxicity bioassays to determine the 96 h $LC_{50}$ value of the lyophilized *P. microphylla* leaf powder was conducted in semi-static system in a laboratory according to the OECD guideline NO 23 (OECD, 1992). The range finding test was determined according to the method described by Solbe (1995). From the range finding tests, six graded concentrations (0.00, 0.27, 0.33, 0.47, 0.67 and 1.33 mg/l) of the lyophilized extract was used for the definitive test. A complete randomized design was used in the experiment with three aquaria set up for each dose and each aquarium contains six fish in forty litres of de-chlorinated tap water. Performance test lasted for 96 h. The duration, however, was reduced in concentrations where death of all the fish was observed. The fish were checked for mortality at different time intervals. The dead fish if found were removed immediately to reduce pollution related effect and were counted for determination of $LC_{50}$. The toxicant and test water in each aquarium were renewed after 24 h. Physicochemical parameters of the water were monitored every 24 h using the methods described by APHA et al. (1998). The behavior and general conditions of the fish were observed before, during and after each bioassay. The 96 h $LC_{50}$ was determined as a probit analysis using the arithmetic method of percentage mortality data (Finney, 1971). Results were subjected to statistical analysis with Duncan's multiple range F-test to test for significant difference ($p< 0.05$) between various concentrations of *P. microphylla* extract and the control.

## RESULTS

### Physico-chemical parameters of the test water

#### *Water quality*

The water quality parameters in the various fish holding

**Figure 1.** The piscicidal plant: *Psychotria microphylla*.

**Table 1.** Physico-chemical properties of the test water.

| Characteristics | Unit | Mean | Range |
|---|---|---|---|
| Temperature | °C | 23.06 | 23.05-23.08 |
| Alkalinity | $mgl^{-1}$ | 29.52 | 29.0-30.45 |
| pH | - | 7.04 | 7.00-7.08 |
| Dissolved oxygen | $mgl^{-1}$ | 7.02 | 6.83-7.21 |
| Conductivity | $\mu Mcm^{-1}$ | 254 | 250-290 |
| Total hardness | $mgl^{-1}$ | 178 | 172-186 |

containers fluctuated slightly. The dissolved oxygen values decreased slightly with increase in concentrations of the plant extract (Table 1). Total alkalinity values were slightly increased at higher concentrations of the toxicants compared to the control with the highest values at concentration of 12.5 mg/l. The temperature and pH values did not show much variation. Generally, the water quality parameters determined did not show any significant differences ($P < 0.05$) between the various concentrations of *P. microphylla* and the control.

### Behavioral response of fishes to test concentrations

Fish exposed to the different concentrations of aqueous extract of *P. microphylla* leaf exhibited hyperactivity characterized by surfacing and jumping outside of water, loss of balance, erratic swimming, respiratory distress, rapid opercula movement, incessant gulping of air, spiral movement, discolouration of the whole body and excessive mucus secretion within 15 min of exposure as shown in Figure 2. In acute concentrations prior to death, fish aggregated at the air-water interface gasping for air with their mouth permanently opened. These behavioral deviations were more pronounced with increasing concentrations. The fish floated on surface of the fish holding container shortly after death as shown in Figure 3.

### Toxic effects of acute concentrations

The results of *C. gariepinus* juveniles exposed to the lyophilized aqueous extract of *P. microphylla* leaf are presented in Tables 2 and 3. Toxicity experiment was performed by the method of OECD NO 23. Results obtained from this study were computed using SPSS computer program version 20. With this program, lethal concentrations (LC values) were calculated through

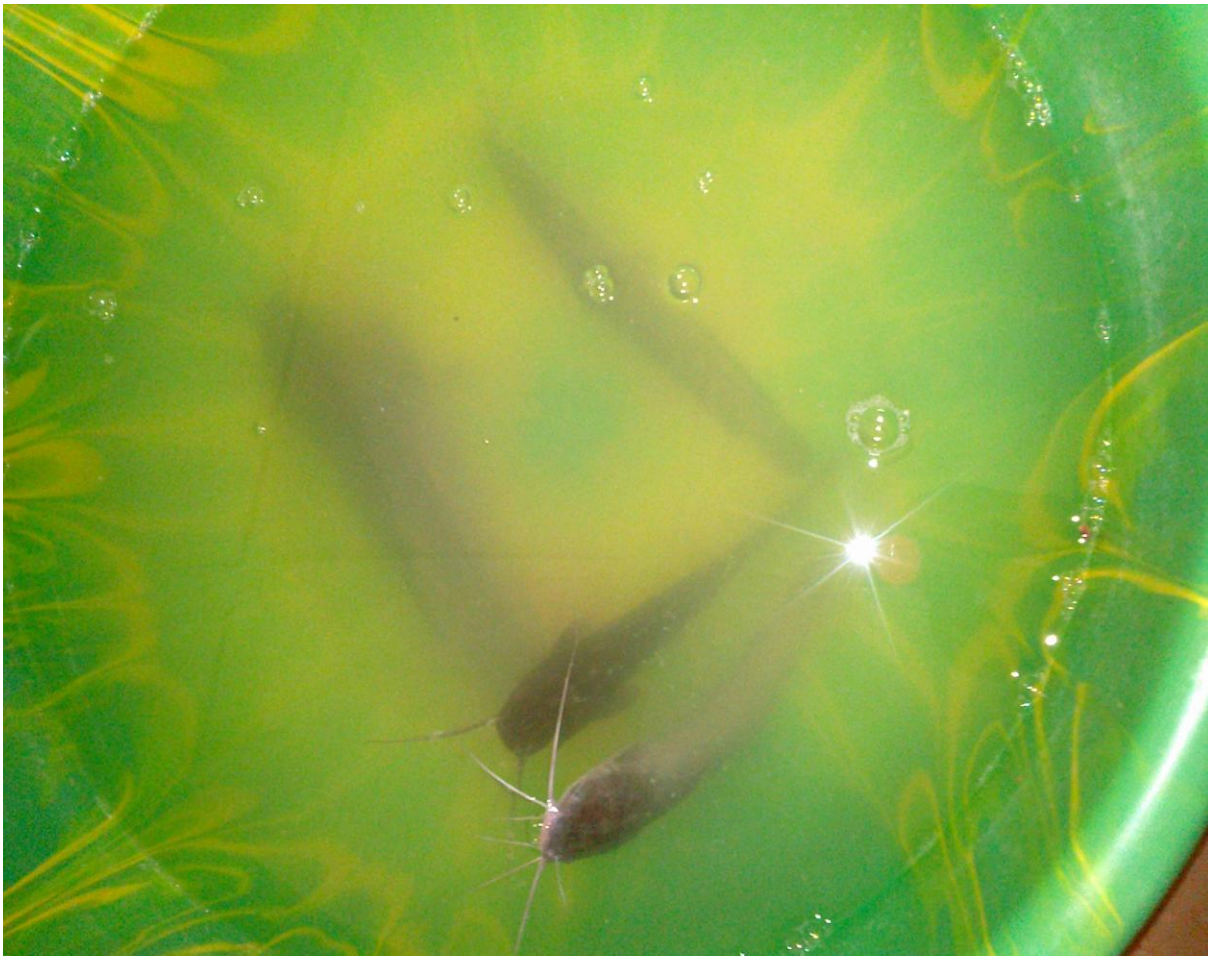

**Figure 2.** Behavioral responses of the fish to the plant extract.

probit analysis method. Table 3 shows the effective doses ($LC_{10,\ 50,\ 90}$ values) of the lyophilized aqueous extract of *P. microphylla leaf. C. gariepinus* juveniles (mean weight and length, 180 g and 25 cm respectively) were generally very sensitive to *P. microphylla* leaf aqueous extract. At a concentration of 1.33 mg/l, there was 100% mortality within one hour. Mean mortalities of 33, 50, 67, 83, and 100% were recorded in the experimental tanks containing 0.27, 0.33, 0.47, 0.67, and 1.33 mg/l of the extract, respectively. No mortality was recorded in the control (0 mg/l). From the results, a 96 h $LC_{50}$ value for *C. gariepinus* juveniles was calculated to be 0.35 mg/l. There was a significant negative correlation between $LC_{50}$ values and exposure time. Thus $LC_{50}$ of the lyophilized aqueous extract of *P. microphylla* decreased from 0.65 mg/l (24 h) to 0.53 mg/l (48 h), 0.41 mg/l (72 h) and 0.35 mg/l (96 h).

## DISCUSSION

The mortality of the fish was chosen as the measurable effect to determine the toxicity of the crude aqueous extract of the plant. Our studies revealed that *C. gariepinus* juveniles exposed to the lyophilized aqueous extract of *P. microphylla* leaf exhibited marked behavioural changes like swift opercular movement, sudden jerky swimming body movements, loss of balance, respiratory distress, which demonstrated a sensitive indicator of physiological stress in fish. Davis (1973) observed similar behaviors when fish were subjected to sub-lethal concentrations of pollutants. The behavioural responses observed in the current studies are in tandem with the reports of Muhammad et al. (2010), Ajani and Ayoola (2010) and Ayuba and Ofojekwu (2002) when they exposed fish to acute concentrations of different plant extracts. Similarly, Nwani et al. (2012) reported abnormal movement and high respiration rate in tilapia exposed to glyphosate herbicide. The erratic behaviour prior to death in the present and past studies can be conveniently associated with the impact of toxicants on fish. In this investigation also, mucus accumulation was observed on body surfaces and gill filaments of dead fish. According to Annune (1994), mucus accumulation results from increase in the activity

**Figure 3.** Dead fish by the plant extract.

**Table 2.** Data on fish survival at different test concentrations and sampling time intervals in *C. gariepinus* exposed to the lyophilized aqueous extract of *P. microphylla* leaf.

| Exposed concentration ($mgl^{-1}$) | Number exposed | Number of fish alive at different time intervals (hours) | | | | % survival | % mortality |
|---|---|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 | | |
| 0.00 | 18 | 18 | 18 | 18 | 18 | 100 | 00 |
| 0.27 | 18 | 18 | 15 | 12 | 12 | 67 | 37 |
| 0.33 | 18 | 18 | 15 | 12 | 09 | 50 | 50 |
| 0.47 | 18 | 15 | 12 | 09 | 06 | 33 | 67 |
| 0.67 | 18 | 09 | 07 | 04 | 03 | 17 | 83 |
| 1.33 | 18 | 00 | 00 | 00 | 00 | 00 | 100 |

of mucus cells subsequent to pollutants exposure, which results in an increase in the production of mucus over the body of the fish. Our observation here of excessive mucus secretions in exposed fish agrees with the report of Jothivel and Paul (2008), and Abalaka and Auta (2010). Excessive mucus secretions are natural defense mechanisms by exposed fish to coat their body surfaces in order to prevent and/or reduce the absorption of the offending toxicant (Cagauan et al., 2004). However, such excessive mucus secretions are reported to reduce respiratory activity in fishes (Konar, 1975). which together with decreasing oxygen content of reconstituted extracts results into hypoxic states in exposed fishes (Usman et al, 2005) leading to subsequent respiratory distress and deaths in exposed fishes (Omitoyin et al, 1999). Abnormal nervous behaviours are associated with the impacts of the toxicants on fishes (Fafioye, 2005). This may be due to nervous system involvement or failure (Ufodike and Omoregie, 1994; Oti and Ukpai, 2000) or may be due to biochemical body derangement including

**Table 3.** Toxicity (LC 10, 50, 90 values) of the lyophilized aqueous extract of *P. microphylla* leaf at different time intervals to the fish, *C. gariepinus*.

| Exposure period (hour) | Effective dose (mg/l) | Limits (mg/l) | |
|---|---|---|---|
| | | **LCL** | **UCL** |
| | $LC_{10}$ = 0.45 | 0.35 | 0.51 |
| 24 | **$LC_{50}$ = 0.65** | 0.57 | 0.78 |
| | $LC_{90}$ = 0.93 | 0.77 | 1.42 |
| | $LC_{10}$ = 0.27 | 0.18 | 0.33 |
| 48 | **$LC_{50}$ = 0.53** | 0.45 | 0.65 |
| | $LC_{90}$ = 1.05 | 0.81 | 1.75 |
| | $LC_{10}$ = 0.19 | 0.09 | 0.24 |
| 72 | **$LC_{50}$ = 0.41** | 0.33 | 0.50 |
| | $LC_{90}$ = 0.93 | 0.69 | 2.59 |
| | $LC_{10}$ = 0.16 | 0.07 | 0.22 |
| 96 | **$LC_{50}$ = 0.35** | 0.26 | 0.42 |
| | $LC_{90}$ = 0.75 | 0.59 | 1.37 |

- LCL (Lower confidence limit) and UCL (Upper confidence limit) at 95% confidence limits.

hepatic compromise (Fadina et al., 1991).

Results obtained from this research revealed that the 96 h $LC_{50}$ for African catfish exposed to *P. microphylla* was 0.35 mg/l. The 96 h $LC_{50}$ had earlier been reported for the fish, *C. gariepinus* by Onusiruka and Ufodike (1991) to be 25.71, 26.92, and 8.3 mg/l for the floral parts of Akee apple (Blighia sapida), the bark of Sausaje plant (Kigelia Africana) and the bark of *B. sapida*, respectively Onusiriuka and Ufodike, (1998) reported that Blighia sapida bark extract is more toxic to C. gariepinus than Kigelia Africana bark extract. In addition, the 96 h $LC_{50}$ of 0.36, 13.18, 204.17 and 296.14 mg/l have also been reported by Omotoyin et al. (1999), Ayuba and Ofojekwu (2010) and Abalaka and Auta (2010) respectively working with different plants. Comparatively, the 96 h $LC_{50}$ value of 0.35 mg/l obtained from this study was much lower than most of the reported literature. The variations observed in these studies can be attributed to the type of plants and part of the plants used, size of fish, environmental factors, food, or water parameters and selective action of toxicants.

Statistical analysis of the data on toxicity brings out several important points. The $X^2$ test for goodness of fit (heterogeneity) demonstrated that the mortality counts were not found to be significantly heterogeneous. Other variables (for example, resistance) do not significantly affect the $LC_{50}$ values, as these were found to lie within the 95% confidence limits. The dose mortality graphs exhibit steep slope values. The steepness of the slope line indicates that there is a large increase in the mortality of fish with a relatively small increase in the concentration of the toxicant. The slope is, thus, an index of the susceptibility of the target animal to the tested material used. A steep slope is also indicative of rapid absorption and onset of effects (Yadav and Singh, 2006). The physico-chemical parameters of the test solutions fluctuated slightly during the bioassays but were not thought to have affected fish mortality since they were within the suggested tolerance range (Mackereth, 1963).

In conclusion, it is clear from the results that *P. microphylla* crude extract is very toxic against the freshwater fish, *C. gariepinus*, thus the plant is piscicidal. It is thought that this plant extract or powder will be useful in aquaculture to eradicate predators and competing wild fish from nursery, rearing and stocking ponds prior to the stocking of commercially grown fry and fingerlings of desired species.

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