Toxicity of Rotenone -Yielding Plant Extracts on Fish Fry and Selected Predatory Aquatic Organisms

Adebayo IA* and Akin-Obasola BJ
Department of Fisheries and Aquaculture Management, Faculty of Agricultural Sciences, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria

Abstract

Fish fry raised directly in earthen ponds grow faster and healthier but often threatened by a wide range of predatory aquatic organisms which lead to a decline in population at harvest. This study examined the toxicity of rotenone-yielding plant root extracts of Tephrosia bractiolata and Lonchocarpus sericeus on four predatory aquatic organisms namely: Water tigers (W.), Tadpoles (T.), young Crabs (Y.), and Nymph stage of Dragon fly (N.). Using the 96 hours bioassay test to determine acute toxicity for each plant extract, the lethal concentrations (LC50) for T. bractiolata and L. sericeus were 7 g and 25 g/7OL of water respectively for the test organisms. The aquatic organisms were subjected to four test concentrations (0, 6, 8 and 10 g/70L) for T. bractiolata and (0, 20, 30 and 35 g/70L) for L. sericeus respectively in three replicates. Residual effect of the rotenone extract on a week old fry of Clarias gariepinus as non -target organism was determined a week after termination of the first experiment. Results indicated that T. bractiolata at lower dose (≤ 10 g/70L) was more toxic to the predatory organisms compared to L. sericeus (≤35 g/70L). As concentrations of root extract increased, water quality parameters such as Dissolved Oxygen (DO), pH and turbidity (transparency) were negatively affected. There was no mortality of fish fry at different doses (6-10 g/70L) of T. bractiolata after a week of termination of experiment, while mortality of fish fry was recorded at higher doses (30 -35 g/70L) of L. sericeus, after a week termination of experiment, showing longer residual effect of L. sericeus on fish. In combating aquatic predators in nursery ponds, T. bractiolata has higher potency in killing predators at lower doses compared to L. sericeus with both plant extracts having minimal residual effect on fish and water quality parameters.

Keywords: Rotenone-yielding plants; Toxicity; Root extracts; Aquatic predators; Fish fry

Introduction

The effective practice of raising fish fry in earthen ponds is yet to be fully achieved. This is attributed to predatory activities of aquatic insects and amphibians arising from improper de-sitting, poor fencing and from water source(s). Predatory organism is a term used to describe organism that attack intended culture fish especially in the fry and juvenile stages. According to Ernest Hodgson [1], predatory aquatic organisms utilize fauna and flora resources available in the water for nourishment, growth, and shelter, thus inhibiting growth of the culture species. The use of rotenone - yielding plants extract aimed at curbing unnecessary aquatic organisms in a pond before stocking became necessary due to its lower residual effect on fish [2].

Rotenone is a colourless and odourless chemical used as a broad-spectrum pesticide. It occurs naturally in the stem, roots and sometimes sap of plants which mostly belongs to the legume family [3]. All aquatic organisms are sensitive to rotenone but some are more resistant than others. The study of the potency of rotenone is done by determining the median lethal concentration (LC50) values and the impact on the behavior of the aquatic organisms. The Rotenone yielding plants used in this study were Tephrosia bractiolata and Lonchocarpus sericeus. They are leguminous shrubby plants and herbs with enough toxic rotenoids that is highly unselective as it kills both target and non-target organisms. But the residual effect is harmless to fish after some weeks of application in pond bottom. The root of these plants is the source of the valuable pesticide, rotenone [4]. Control of predatory aquatic organisms is of great importance to fish breeders. Therefore, the objective of the study was to test the toxicity of the plant extracts on the test organisms as well as their effects on physico-chemical parameters of water.

Materials and Methods

Collection of the test organisms

Young Crabs (Y.), Tadpoles (T.), Water tigers (W.), and Nymph stage of dragon fly (N.) were collected from the bottom of nursery earthen ponds in the Faculty of Agricultural Sciences Fish farm. They were the endemic predatory aquatic organisms in the ponds area. Selected ponds were carefully drained in order to collect the desired test organisms alive [5-7]. The organisms were immediately sorted into equal size to avoid predation and ensured unbiased reaction to rotenone treatment. Twenty pieces (each) of these organisms were introduced into fresh water in glass tanks (70 × 45 × 40 cm) /each containing fresh water (70L of its capacity and replicated thrice. Acclimatization period was two days without drug administration.

Collection of fish samples

Twenty pieces of a week old fry of Clarias gariepinus hatched within the Faculty of Agricultural Sciences fish hatchery were stocked in glass tanks (70 × 45 × 40 cm) /each containing fresh water (70L of its capacity) in three replicates. This second experiment on non –target organism (fish) was a continuation of first experiment after a week

*Corresponding author: Adebayo IA, Department of Fisheries and Aquaculture Management, Faculty of Agricultural Sciences, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria, Tel: +234 803 583 5; E-mail: dayadeisrael@yahoo.com; isreal.adebayo@eksu.edu.ng

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termination with predatory aquatic organisms, to establish the residual effect of rotenone-yielding root extracts on the young fish [8-11]. The fish fry were allowed to acclimatize for two days without food.

**Source and collection of experimental plant extracts**

The plant root extracts used were *Tephrosia bractiolata* and *Lonchocarpus sericeus*. They were collected from different locations in Ikere-Ekiti, Ekiti state, Nigeria where these plants were endemic. The roots of the plants were collected, and after washing, crushed and further processed by sun-drying and made into powdery state. The analysis procedures involved more sample preparation at performance liquid chromatography (HPLC) of [13-15] was employed. The analysis procedures involved more sample preparation at performance liquid chromatography (HPLC). The physico-chemical parameters of the experimental media such as pH, temperature, and dissolved oxygen were determined before and after the experiment using appropriate methods.

**Determination of rotenone from the root extracts**

Method of quantifying rotenone concentrations using high performance liquid chromatography (HPLC) of [13-15] was employed. The analysis procedures involved more sample preparation at concentration as low as 5 µg/L.

**Range finding test**

After acclimatization of the test organisms, lethal concentrations (LC50) for concentration as low as 5 µg/L. The analysis procedures involved more sample preparation at performance liquid chromatography (HPLC) of [13-15] was employed. The roots of the plants were collected, and after washing, crushed and further processed by sun-drying and made into powdery state. The analysis procedures involved more sample preparation at performance liquid chromatography (HPLC).

| Trts pH | Before | During | After | Before | During | After | Before | During | After |
|---------|--------|--------|-------|--------|--------|-------|--------|--------|-------|
| 0 g/70L | 7.20 ± 0.01a | 7.20 ± 0.02a | 7.20 ± 0.00a | 27.20 ± 0.02a | 26.20 ± 0.01a | 25.91 ± 0.00a | 5.40 ± 0.00a | 5.40 ± 0.02a | 5.40 ± 0.02a |
| 6 g/70L | 7.30 ± 0.03a | 6.75 ± 0.01a | 6.42 ± 0.02a | 27.22 ± 0.01a | 24.75 ± 0.02a | 25.07 ± 0.01a | 5.40 ± 0.00a | 4.25 ± 0.01a | 4.20 ± 0.01a |
| 8 g/70L | 7.20 ± 0.02a | 6.53 ± 0.03a | 6.50 ± 0.01a | 27.30 ± 0.02a | 24.33 ± 0.03a | 25.03 ± 0.02a | 4.42 ± 0.01a | 3.50 ± 0.01a | 3.20 ± 0.01a |
| 10 g/70L | 7.25 ± 0.01a | 6.42 ± 0.02a | 6.60 ± 0.03a | 27.20 ± 0.02a | 24.11 ± 0.02a | 30.00 ± 0.01a | 4.40 ± 0.02a | 3.70 ± 0.02a | 3.20 ± 0.02a |

Means with the same superscript along the vertical columns are not significantly different (P>0.05).

**Experimental setup**

Twenty four Glass Aquaria Tanks of size (70 × 45 × 40 cm)/each that contained 70L of freshwater were arranged in three replicates in completely randomized design for both *T. bractiolata* and *L. sericeus*. They were collected from different locations in Ikere-Ekiti, Ekiti state, Nigeria where these plants were endemic. The roots of the plants were collected, and after washing, crushed and further processed by sun-drying and made into powdery state. The potency of the root extracts were tested in the Laboratory using the methods of Organization for Economic Cooperation and Development (OECD) standard guidelines for testing chemicals [12] before usage.

**Water quality**

The physico-chemical parameters of the experimental media such as pH, temperature, and dissolved oxygen were determined before and after the experiment using appropriate methods.

**Statistical analysis**

Data collected from the experiment were subjected to a one-way analysis of variance (ANOVA) and the statistical differences were identified using the Duncan multiple range test [22].

**Results**

**General effects**

Prior to the introduction of varying concentrations of rotenone-extracting root extracts...
on aquatic organisms, normal behavior was observed in the control treatment. The effect of varying concentrations and exposure time of the two rotenone-yielding plant extracts on test organisms is presented in Tables 5 and 6 below. All the aquatic organisms showed sensitivity

### Table 3: Mortality and survival of aquatic predatory organisms at different concentrations of L. sericeus.

| Trts     | pH Before | Temp (°C) Before | DO (mg/L) Before | pH After | Temp (°C) After | DO (mg/L) After |
|----------|-----------|------------------|------------------|----------|-----------------|-----------------|
| 0 g/70L  | 7.20 ± 0.02^a | 27.10 ± 0.02^a | 5.40 ± 0.01^a   | 7.20 ± 0.00^b | 27.10 ± 0.02^a | 5.20 ± 0.02^b   |
| 20 g/70L | 7.10 ± 0.00^c | 25.10 ± 0.02^c | 5.40 ± 0.02^c   | 7.30 ± 0.01^d | 25.07 ± 0.01^d | 3.20 ± 0.01^d   |
| 30 g/70L | 7.20 ± 0.02^e | 25.30 ± 0.01^e | 5.42 ± 0.01^e   | 7.30 ± 0.03^f | 25.30 ± 0.03^f | 3.50 ± 0.01^f   |
| 35 g/70L | 7.30 ± 0.02^g | 25.30 ± 0.05^g | 5.40 ± 0.03^g   | 7.30 ± 0.00^h | 25.30 ± 0.00^h | 2.90 ± 0.03^h   |

Means with the same superscript along the vertical columns are not significantly different (P>0.05).

### Table 4: Mean values of water quality parameters before, during and after the introduction of L. sericeus in experimental tanks.

| Plant Extracts | Diff. Conc. | 24 hrs | 48 hrs | 72 hrs | 96 hrs |
|----------------|-------------|--------|--------|--------|--------|
| T. bractiulata | 0 g/70L     | 0      | 60     | 0      | 60     |
|                | 6 g/70L     | 6      | 54     | 3      | 51     |
|                | 8 g/70L     | 6      | 54     | 0      | 54     |
|                | 10 g/70L    | 15     | 45     | 3      | 42     |
| L. sericeus    | 20 g/70L    | 6      | 54     | 0      | 54     |
|                | 30 g/70L    | 15     | 45     | 0      | 45     |
|                | 35 g/70L    | 24     | 36     | 0      | 36     |

Key: M= Mortality, S= Survival, Wt = Water tiger, Tp = Tadpoles, Yp = Young crabs, Np = Nymph; T1 - T4 (Treatments)

### Table 5: Residual effects of T. bractiulata and L. sericeus on fish fry after a week of application in water.

| Root Extracts | Trts     | pH        | Temp (°C) | DO (mg/L) |
|---------------|----------|-----------|-----------|-----------|
| T. bractiulata| 0 g/70L  | 7.2 ± 0.02^a | 27.2 ± 0.02^a | 5.4 ± 0.01^a |
|               | 6 g/70L  | 7.2 ± 0.02^c | 27.2 ± 0.02^c | 5.4 ± 0.02^c |
|               | 8 g/70L  | 7.2 ± 0.02^g | 27.2 ± 0.02^g | 5.4 ± 0.03^g |
|               | 10 g/70L | 7.2 ± 0.03^h | 27.2 ± 0.02^h | 5.4 ± 0.04^h |
| L. sericeus   | 20 g/70L | 7.2 ± 0.06^a | 27.3 ± 0.00^a | 3.37 ± 0.02^a |
|               | 30 g/70L | 7.2 ± 0.06^b | 27.3 ± 0.00^b | 3.37 ± 0.02^b |
|               | 35 g/70L | 7.2 ± 0.04^c | 27.3 ± 0.00^c | 3.37 ± 0.02^c |

Means with the same superscript along the vertical columns are not significantly different (P>0.05).
to rotenone extracts, but some were more resistant to the chemical than others, especially at higher dosage [23,24]. Low swimming performance and early mortality were observed in aquaria tanks containing Tadpoles as they clustered together at the surface of water for the two extracts. Young crabs and Water tigers proved to be more resistant to the two plant extracts. Though, *T. bractiolata* achieved total eradication of Crabs and water tigers at 96 hours of 10 g/L concentration. As concentrations of *T. bractiolata* increased, Nymph of dragon fly recorded progressive number of mortality. On the other hand, *L. sericeus* produced higher mortality tendency towards the 96th hour for all the organisms. The rate of mortality of Water tiger and Nymph of dragon fly was highest in test concentrations of 30 -35 g/L for *L. sericeus* between 72-96 hours, while resistance of young crabs reduced drastically [25,26], a result similar to *T. bractiolata* concentration at 10 g/L between 72-96 hours exposure.

### Discussion

It was observed that the higher the concentration of the toxicant (rotenone extracts), the higher the mortality rate. This was in line with the observation of Vlawing [3] that in all toxicant, a threshold reach above which there is no drastic survival of animal. Below the threshold, animal is in a tolerance zone. Consequently, it was duly observed from test results that the potency of each test concentrations reduced drastically with the time of exposure. Hence, the peak of potency of *Lonchocarpus sericeus* was between 48 hours to 72 hours during which a high number of test organisms were lost. The peak of potency in *Tephrosia bractiolata* was achieved at 10g, though it produced effects at lower doses of concentrations. Although high, mortality was recorded between 24 to 72 hours in *Lonchocarpus sericeus*, it was achieved at much higher concentrations [27]. Thus, since *Tephrosia bractiolata* achieved nearly the same effect as observed in *Lonchocarpus sericeus* but at lower doses, *Tephrosia bractiolata* is considered to be more potent.

Furthermore, increase in the test concentrations of both extracts shows that the extracts are insoluble in water. Since the concentrations of *Lonchocarpus sericeus* were increased to have desired effects on test organisms, the insolubility also increased.

Nevertheless, this study has shown that Rotenone extracts are also toxic to fish fry. The range doses that result in toxic effects varies widely with concentration and with the individual species being exposed [28]. Introduction of test concentrations brought about changes in behavior. The effect of the rotenone extracts showed that rotenone causes disruption in gaseous exchange efficiency of the gills and this is similar to the observation of Rahman et al. [29] and Omitoyin [30] change in chemical properties thereof may be reflected in the organisms’ respiratory activity particularly if the environmental factors affect respiratory gaseous exchange. In the study of the dissolve oxygen, the treatments did not only show a dose-dependent decline in survival of test organisms but rapid decline in the depletion of oxygen with time. Holden [31] had earlier reported that the introduction of toxicant (pesticide) into aquatic system might decrease dissolved oxygen.

This study has shown that rotenone is toxic to fish as well as other test organisms. It has also proved that the toxicity of the plant extracts varies with the duration of exposure. Prior investigations have shown that pesticide at higher doses in the aquatic environment can accumulate in the tissues of fish as well as other aquatic organisms, thereby disrupting physiological processes. It is however incorrect to deduce that many organisms are unstable in water [31]. Since the crabs are more resistant in water, the concentration can be increased in practical evaluation in earthen fish ponds but *Tephrosia bractiolata* should be employed [32]. However, the adoption of either of the extracts used in this study in practical terms depends on the resistance of the organisms to be evaluated. Mild doses of the less potent can be used as determined by the fish farmer especially where production cannot be delayed further. Hence, understanding mechanisms of body defense system against Rotenone extracts would enable the farmer in determining the extract and dose to be used [33]. In cases where *Lonchocarpus sericeus* is used, consistent flow through should be done for a period of weeks before stocking. Fish farmers should use more of *Tephrosia bractiolata* in eradicating predators as it tends to yield desired results with less residual effects on fish fry.

### Conclusion

Plant root extracts have been widely used to kill predatory aquatic organisms under laboratory rather than natural water bodies. Detailed knowledge of the residual effects on non- target organisms such as fish have not been seriously documented under natural water conditions. Therefore, Fisheries managers must apply rotenone yielding plant extracts in natural water bodies in such a measure that only the target organisms are eliminated with no residual effect on fish.

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