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Croton oil *Croton tiglium* fruit water extracts as piscicides under simulated farm condition

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Abstract
The study evaluated the potential of *C. tiglium* fruit water extracts (0.5, 1, 2, 4, 8 and 15 ml 1^{-1}) as piscicides for *G. affinis, O. niloticus* and *C. gariepinus* under simulated farm condition.
Based on the 24, 48 and 72 h of exposure to all concentrations, the *G. affinis, O. niloticus* and *C. gariepinus* showed no significant differences on mortality except *C. gariepinus* at 0.5 ml 1^{-1}. However, based on the 96 h of exposure to all concentrations 100 per cent mortality of *G. affinis, O. niloticus* and *C. gariepinus* was observed and showed no significant differences.
The *G. affinis* and *O. niloticus* were the most susceptible fishes among the test organisms. The toxicity tests showed that *C. tiglium* has the potential to be used as piscicides, which can be an alternate to an expensive and scarcely available imported rotenone for eradication of undesirable fish species present in fish ponds.
Signs of agitated behaviors, respiratory distress and abnormal nervous behaviors including eventual deaths were observed in exposed fish. Control fish neither died nor exhibited any unusual clinical signs.

Keywords: *C. tiglium, G. affinis, O. niloticus*, *C. gariepinus,* piscicides, fruit extracts

1. Introduction
Fish farmers and aquaculturists have long been trying a multitude of pest and predator eradication measures with varying degree of success. These unwanted fishes may be introduced into aquaculture through water supplies or along with desired fishes at stocking [5]. Pond management during pond preparation with the help of piscicides before fish stocking is an important tool [12].
To overcome the hazardous effects of these inorganic pesticides, recent emphasis is on the use of natural pesticides, which are usually of plant origin. Contradictory to synthetic fish toxicants, herbal toxicants are believed to be more environment friendly, because they are easily biodegradable and leave no residues in the environment. During the last several years, rotenone (herbal piscicide) has been used as fisheries management tool to rehabilitate lakes, ponds, streams and other waters [18].
Widespread use of pesticide on farm is now a worldwide phenomenon [23]. Pesticides currently in use are biocides that have high mammalian toxicity and necessitate considerable precautions in their application. However, these chemicals may have negative effects on the environment, farmers and health [4]. Hence, there is a need to explore other environment and health-friendly fish toxicants such as botanical plants with piscicidal activity. The study was conducted to determine the piscicidal activities of Croton oil *Croton tiglium* fruit water extracts under simulated pond condition.

2. Materials and Methods
2.1 Test plant
The study used Croton oil plant (*Croton tiglium*), locally known as "tuba". The plant was obtained at Balangbang, Mayoyao, Ifugao. Fruits of *C. tiglium* were harvested and air-dried for 3 d.

2.2 Preparation of fruit extracts
The weight of the fruit was pre-determined. The fruit was first washed and chopped before grinding. *C. tiglium* fruit was prepared separately in water extracted form. The fruit extracts were determined by preparing an equal proportion of plant material to water

(1 kg of fruit: 1L of water). Tap water was added with the measured volume then mix until obtaining the extract. The grind fruit was kept soaked in water for 24 h. After 24 h, cheesecloth was used to squeeze and strain the plant material to obtain the extract. The volume obtained after extraction was measured and recorded.

2.3 Test organisms

The test species that was used in the study includes Mosquito fish (*G. affinis*), Nile tilapia (*O. niloticus*) and African catfish (*C. gariepinus*). Size # 24 tilapia was purchased at San Antonio, Ramon, Isabela. African catfish was ordered at private hatchery at Aguinaldo, Ramon, Isabela. The test fish was cigarette size. Mosquito fish was obtained at the ponds at San Antonio, Ramon, Isabela.

Thereafter, the test organisms were transferred to plastic containers filled with aerated and de-chlorinated water. Care was observed to minimize stress incurred by the test species. The test organisms were conditioned at separate containers for 4 d prior to experiment. During acclimatization, experimental fishes were fed daily with commercial fish food which was given in the morning. Water was siphoned and replaced every 24 h after feeding in order to remove uneaten feeds and faecal matter in the conditioning tanks.

2.4 Test concentrations

Range Finding Test: The test concentrations used for the plant was predetermined using a range finding test based on the progressive bisection of intervals on logarithmic scale. The concentrations used in the range finding test were six concentrations. The concentrations were 0.5, 1, 3, 6, 12.5, and 25 ml l^{-1} in *G. affinis*, *O. niloticus* and *C. gariepinus.*

The final bioassay test also used six different concentrations (0.5, 1, 2, 4, 8 and 15 ml l^{-1} for the fruit extracts representing the different treatments. Each treatment was replicated thrice.

2.5 Experimental set up and treatments

To study the toxicity under simulated pond condition, 18 black plastic containers (5-l capacity) and clay loam soil was put in the bottom and its four sides (one inch thick). The ten (10) test organisms were placed in the experimental containers.

2.6 Monitoring of mortality

Cumulative mortality of the test organisms was observed and recorded at 24, 48, 72 and 96 hours after stocking. The dead fishes were removed immediately after observation.

$$\text{Mortality (\%)} = \frac{\text{Number of dead fish}}{\text{Number of fish stocked}} \times 100$$

2.7 Statistical analysis

Mortality response of *G. affinis, O. niloticus* and *C. gariepinus* exposed to *C. tiglium* fruit water extracts were analyzed using 2-Factorial Analysis of Variance (ANOVA). Prior to analysis, data were transformed using the square root transformation. The General Linear Model (GLM) in Statistical Package for Social Sciences (IBM® SPSS® Statistics v. 20) was used to compare the differences among treatment means. The levels of significance were also determined using the Student-Newman-Keuls (SNK).

3. Results

3.1 Effects of *C. tiglium* fruits extracts against the experimental fish at 24 h of exposure

The cumulative mortality of *G. affinis, O. niloticus* and *C. gariepinus* exposed to various concentrations of *C. tiglium* fruits extracts were determined within 24 h of exposure is presented in Table 1.

Table 1: Cumulative mortality of *G. affinis, O. niloticus* and *C. gariepinus* exposed to various concentrations of *C. tiglium* fruits extracts within 24 h of exposure after the preparation of fruit extracts.

	Fish		
Concentration (ml l^{-1})	***Gambusia affinis***	***Oreochromis niloticus***	***Clarias gariepinus***
0.5	100±0.00	100±0.00	33.33±32.15*
1	100±0.00	100±0.00	96.67±5.77
2	100±0.00	100±0.00	80±34.64
4	100±0.00	100±0.00	100±0.00
8	100±0.00	100±0.00	100±0.00
15	100±0.00	100±0.00	100±0.00

Note: *- significant at P <0.05

When the experimental fish were exposed to the experimental units treated with various concentrations of *C. tiglium* fruits extracts. Results showed that 100 percent mortality was observed to *G. affinis* and *O. niloticus* exposed to 0.5 – 15 ml l^{-1} of *C. tiglium* fruits extract within 24 h of exposure. On the other hand, 100 percent mortality was only observed to *C. gariepinus* exposed to 4 – 15 ml l^{-1} *C. tiglium* fruit extracts. However, the cumulative mortality of the *G. affinis, O. niloticus* and *C. gariepinus* from 0.5 ml l^{-1} – 15 ml l^{-1} revealed no significant differences except at 0.5 ml l^{-1} in which *C. gariepinus* (33.33%) showed the lowest mortality.

3.2 Effects of *C. tiglium* fruits extracts against the experimental fish at 48 h of exposure

Table 2 shows the cumulative mortality of *G. affinis, O. niloticus* and *C. gariepinus* exposed to various concentrations of *C. tiglium* fruits extracts were determined within 48 h of exposure.

Table 2: Cumulative mortality of *G. affinis, O. niloticus* and *C. gariepinus* exposed to various concentrations of *C. tiglium* fruits extracts within 48 h of exposure.

	Fish		
Concentration (ml l^{-1})	***Gambusia affinis***	***Oreochromis niloticus***	***Clarias gariepinus***
0.5	100±0.00	100±0.00	66.67±41.63*
1	100±0.00	100±0.00	100±0.00
2	100±0.00	100±0.00	90±17.32
4	100±0.00	100±0.00	100±0.00
8	100±0.00	100±0.00	100±0.00
15	100±0.00	100±0.00	100±0.00

Note: *- significant at P <0.05

When the experimental fish were exposed to the experimental units treated with various concentrations of *C. tiglium* fruits extracts. Results showed that 100 percent mortality was observed to *G. affinis* and *O. niloticus* exposed to 0.5 – 15 ml l^{-1} of *C. tiglium* fruits extract within 48 h of exposure. On the other hand, 100 percent mortality was only observed to *C. gariepinus* exposed to 1 ml l^{-1} and 4 – 15 ml l^{-1} *C. tiglium* fruit extracts. However, the cumulative mortality of the *G. affinis, O. niloticus* and *C. gariepinus* from 0.5 ml 1^{-1} – 15 ml 1^{-1} revealed no significant differences except at 0.5 ml 1^{-1} *C. gariepinus* (66.67%) showed the lowest mortality occurred.

3.3 Effects of *C. tiglium* fruits extracts against the experimental fish at 72 h of exposure

The cumulative mortality of test organisms exposed to various concentrations of *C. tiglium* fruit extracts within 72 h of exposure is shown in Table 3.

Table 3: Cumulative mortality of *G. affinis, O. niloticus* and *C. gariepinus* exposed to various concentrations of *C. tiglium* fruits extracts within 72 h of exposure.

	Fish		
Concentration (ml l^{-1})	***Gambusia affinis***	***Oreochromis niloticus***	***Clarias gariepinus***
0.5	100±0.00	100±0.00	70±36.06*
1	100±0.00	100±0.00	100±0.00
2	100±0.00	100±0.00	93.33±11.55
4	100±0.00	100±0.00	100±0.00
8	100±0.00	100±0.00	100±0.00
15	100±0.00	100±0.00	100±0.00

Note: *- significant at P <0.05

When the experimental fish were exposed to the experimental units treated with various concentrations of *C. tiglium* fruits extracts. Results showed that 100 percent mortality was observed to *G. affinis* and *O. niloticus* exposed to 0.5 – 15 ml l^{-1} of *C. tiglium* fruits extract within 72 h of exposure. On the other hand, 100 percent mortality was only observed to *C. gariepinus* exposed to 1 ml l^{-1} and 4 – 15 ml l^{-1} *C. tiglium* fruit extracts. However, the cumulative mortality of the *G. affinis, O. niloticus* and *C. gariepinus* from 0.5 ml 1^{-1} – 15 ml 1^{-1} revealed no significant differences except at 0.5 ml 1^{-1} *C. gariepinus* (70 %) in which the lowest mortality was observed.

3.4 Effects of *C. tiglium* fruits extracts against the experimental fish at 96 h of exposure

The cumulative mortality of *G. affinis, O. niloticus* and *C. gariepinus* exposed to various concentrations of *C. tiglium* fruits extracts within 96 h of exposure is presented in Table 4.

Table 4: Cumulative mortality of *G. affinis, O. niloticus* and *C. gariepinus* exposed to various concentrations of *C.tiglium* fruits extracts within 96 h of exposure.

	Fish		
Concentration (ml l^{-1})	***Gambusia affinis***	***Oreochromis niloticus***	***Clarias gariepinus***
0.5	100±0.00	100±0.00	76.67±40.41
1	100±0.00	100±0.00	100±0.00
2	100±0.00	100±0.00	93.33±11.55
4	100±0.00	100±0.00	100±0.00
8	100±0.00	100±0.00	100±0.00
15	100±0.00	100±0.00	100±0.00

When the experimental fish were exposed to the experimental units treated with various concentrations of *C. tiglium* fruits extracts. Results showed that 100 percent mortality was observed to *G. affinis* and *O. niloticus* exposed to 0.5 – 15 ml l^{-1} of *C. tiglium* fruits extract within 96 h of exposure. On the other hand, 100 percent mortality was only observed to *C. gariepinus* exposed to 1 ml l^{-1} and 4 – 15 ml l^{-1} *C. tiglium* fruit extracts. However, the cumulative mortality of the *G. affinis, O. niloticus* and *C. gariepinus* from 0.5 ml 1^{-1} – 15 ml 1^{-1} revealed no significant differences.

3.5 Observation of fish behavior

Test organisms stocked in various concentrations of *C. tiglium* fruit extracts exhibited zigzag movement, jumping out of water, and occasional jerking of tail, sprawling and rolling movement, reddening of head. Later the test organisms loss their balance after which death occurred.

4. Discussions

Fish toxicants (i.e., ichthyocides, piscicides, or fish poisons) are the primary method for eradicating invasive fishes, with more than 40 different chemicals used worldwide [16]. Most products have not been fully developed or tested, many are not approved for fish management and only a few are widely and consistently used [6, 8, 7, 16]. The most commonly used and commercially available ichthyocides are rotenone and Antimycin-A (Fintrol®).

The behavior of negative control test species sets were well balanced, showing normal behavior and morphology throughout the experiments. They remained healthy, active and alive with vigorous movement within the 96 hours of the experiment. Unlike the positive control it shows jerky movements and leads to death also there was a slime secretion observed.

Piscicidal plants contain different active ingredients known as alkaloids such as nicotine, pyrethrum, ryania, rotenone, coumerin, resin, akuammine, tannins, saponins and diosgenin [31, 21]. The toxicity of *Euphorbia antiquorum* latex extract to fresh water fish *Poecilia reticulata* and they noted that the mortality of fish occurred steadily with increase in time and concentration of the latex extract [30]. In addition, the acute toxicity of lyophilized aqueous extract of *Psychotria microphylla* leaf on *Clarias gariepinus* by Orji *et al*., (2014) they found out that the fish exhibited hyperactivity characterized by surfacing and jumping out the 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 minutes of exposure. In acute concentrations prior to death, fish aggregated at the air-water interface gasping for air with their mouth permanently opened which confirmed to the present findings.

The erratic behaviour prior to death in the present and past studies can convincingly associated with the impact of toxicants on fish. The excessive mucus secretions observed in the exposed fish agrees with the report of [14, 1, 24]. 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 [5]. In addition, the excessive mucus secretions reduce respiratory activity in fishes [17]. In the study of Heath (1989) collaborates this observation by stating that death of fish under toxicant action is usually due to the failure of the gills [13].

Oreochromis niloticus exhibited erratic movement and aggressiveness when placed in the bioassay tanks. Some attempted to jump out of the tanks. This behavior continued for a few hours after which their movement becomes normal and calm. This agreed with the findings of Omitoyin *et al*., (2006) on *Sarotherodon galilaeus* (Tilapia) fingerlings exposed to piscicidal plant extracts of *Tetrapleura tetraptera* [23]. Other studies, reported that the introduction of a toxicant into an aquatic system might decrease the dissolve oxygen concentration, which will impair respiration leading to asphyxiation [32]. Based on the other study, under cemented pond conditions the toxicity of tested plants was reduced [9]. The reason for this reduced toxicity could be a sand particle adsorption or acceleration of the toxicant degradation process by temperature. The effective concentration must be determined against the predatory air breathing fish, such as *Clarias* sp. *Ophiocephalus striatus* and *Anabas testudineus* that are generally more tolerant to toxicants than other fishes [25].

The present study revealed that fish exposed to toxicants exhibit marked behavioral changes like swift opercular movement, sudden jerky swimming body movements, which demonstrated a sensitive indicator of physiological stress to fish. The exhibited clinical signs and eventual deaths of exposed fish may be due to direct poisoning leading to pathological alterations in their tissues and organs [11, 20] or may indirectly be due to changes in the physicochemical conditions of their immediate external environment [3, 22]. The observed respiratory distress in our study may be due to ensuing hypoxic states of exposed fish brought about by both decreasing dissolved oxygen contents of reconstituted extracts vis-à-vis decreasing ability of exposed fish to respire [2]. Decreasing dissolved oxygen content of reconstituted extracts may be due to the continuous oxidative bio-degradation of the constituents of both extracts which may cause oxygen tension in such reconstituted extracts by diverting the much needed dissolved oxygen for this bio-degradation process [10]. Pharmacological action of *Croton tiglium* is due to the presence of alkaloids and terpenoids [27] irritant co-carcinogenic phorbol esthers [26]. It has been reported that *C. tiglium* seeds include 30-50% oil, 10% proteins and small amount of albumin [15]. In addition, the phytochemical analysis of seeds confirmed that seeds contain various secondary metabolites, such as, alkaloids, flavonoids and saponins and Gas Chromatography (GC) analysis of hexane soluble extract of seeds identified about eight fatty acids such as linoleic acid, oleic acid, myristic acid etc. [28].

Agrochemicals such as pesticides especially chlorinated hydrocarbons are routinely employed as part of the integrated farming practice to protect crops and animals from insects, weeds and diseases. Widespread use of pesticides on farm is now a worldwide phenomenon [19, 29].

5. Conclusions

The fruit extract of *C. tiglium* plant demonstrated its efficacy to piscicide under simulated pond condition. The fruit extract of croton oil plant showed high piscicidal activity against the test organisms within 24 h of exposure.

Based on observations after exposure to *C. tiglium* fruit extracts, *G. affinis* and *O. niloticus* are the most susceptible organisms since 100% was observed immediately after exposure even to the lowest concentration of *C. tiglium* fruit extract. On the other hand, *C. gariepinus* is the most resistant organism to the lowest concentration of *C. tiglium* fruit extract.

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