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J. Benrit Vimal
Department of Zoology, Scott Christian College (Autonomous), Nagercoil- 629 003, India

S. Sam Manohar Das
Department of Zoology, Scott Christian College (Autonomous), Nagercoil- 629 003, India

Correspondence
J. Benrit Vimal
Department of Zoology, Scott Christian College (Autonomous), Nagercoil- 629 003, India

Toxicity of *Euphorbia antiquorum* latex extract to fresh water fish *Poecilia reticulata*

J. Benrit Vimal and S. Sam Manohar Das

Abstract
The use of plant extract as poisons for hunting and fishing is common among fishermen of various countries. Fish poisons from plants are also known as piscicides or inchthyotoxins. Several plant species are known to possess bioactive compounds that are toxic to fish. The piscicidal activity of methanolic latex extracts of *Euphorbia antiquorum* plant belonging to Family: Euphorbiaceae against *Poecilia reticulata* (Peters) in laboratory conditions was investigated. The LC_{50} value recorded for *Euphorbia antiquorum* latex extract at 72 hr of exposure was 1.62 ml/dl. Fish larvae tested in the methanol extract of *Euphorbia antiquorum* latex showed various pathological changes such as erratic swimming, moribund behavior, depigmentation and death in various concentrations of the substance tested. No pathological changes and mortality were observed in the control group.

Keywords: *Euphorbia antiquorum*, latex extract, *Poecilia reticulata*, Acute toxicity.

1. Introduction
The use of poisons for hunting and fishing is widespread across the whole world [1]. Fish poison plants are also known as piscicides or inchthyotoxins and are particularly interesting because they are used for an area effect rather than an individual target. A multiple of plant species are known to possess chemicals that are toxic to fish and evidence suggesting that certain plant species have different effects depending on which variety of fish are targeted [2]. Fish poisons are used in various traditional remedies, not only by the indigenous populations, but also by many.
Application of synthetic pesticides is one of the methods used to control of fish population. Due to their long-term persistence, slow degradability in the water, toxicity to other organisms [3] and accumulation inside the fish body, synthetic piscicides adversely affect the aquatic environment [4, 5]. To solve this problem, studies have been carried out on the possibility of using local plants as pescicides [6, 7] and they are safe for users. A large number of compounds of various classes that have insecticidal, piscicidal and molluscicidal properties have been tested [8].
The genus Euphorbia is composed essentially of latex bearing species [9]. Many of them have been used because of its chemical and pharmacological properties of the latex [10]. A number of compounds (saponins, tannins, alkaloids, alkenylphenols, di and tri-terpenoids etc.) present in several plants belonging to different families with piscicidal activities are used to control fish [11, 12, 13]. Within the family Euphorbiaceae, the sixth largest flowering plants include the species *Euphorbia antiquorum* L. which accounts for almost a sixth of the whole group [14]. The fresh water fish *Poecilia reticulata* (Peters) was used as the test animal because it is present in almost all freshwater reservoirs in India and is suitable for toxicity monitoring
The current study deals with the piscicidal activity of methanol latex extracts of *Euphorbia antiquorum* plant to test the toxic effects *Poecilia reticulata* in laboratory conditions.

2. Materials and Methods
2.1 Plant Material
The present study was carried out in Scott Christian College, Nagercoil during the period of 2013 – 2014. The plant material used for the collection of latex was *Euphorbia antiquorum* L. belonging to Euphorbiaceae family.

2.2 Collection and preparation of *Euphorbia antiquorum* latex extract

Latex samples were collected early in the morning from plants by nipping the leaves and stem or by incision of the trunk and branches of the plant and allowing the latex to drain in clean glass tubes separately, brought to the laboratory and kept in the refrigerator till use.

The collected latex was mixed with methanol in the ratio of 1:9 (10%) and centrifuged at 3500 rpm for 5 minutes and the supernatant was collected in a glass vial and stored at 4 ºC till further use.

2.3 Toxicity of *E. antiquorum* latex to Poecilia reticulata

Toxicity of the latex extract was tested on the non-target fish *Poecilia reticulata* in plastic cups, measuring about 250 ml which was filled with 100 ml of water. The latex solutions were diluted in water in the plastic cups at the concentration of 1, 1.25, 1.50, 1.75, 2.0, 2.25, 2.50, 2.75, and 3 ml/dl of water. In each experimental cup, 10 adult *Poecilia reticulata* was released. Three replicates of the experiment and a control were kept. The control was plain water. The mortality of the fish was recorded after 12, 24, 36, 48, 60, 72, 84 and 96 hrs of exposure and the LC_{50} values were calculated.

2.4 Data analysis

The mortality response of mosquito larvae exposed to *E. antiquorum* latex extract was subjected to probit analysis [15].

3. Results

The 24 hrs LC_{50} value recorded for *P. reticulata* was 2.27, LCL 2.10 and UCL 2.45 ml/dl and the 48 hrs LC_{50} value recorded was 1.82 ml/dl, LCL 1.67 and UCL 1.99 ml/dl. The 72 hrs LC_{50} value recorded for *P. reticulata* was 1.62, LCL 1.48 and UCL 1.78 ml/dl (Table 1).

Table 1: LC_{50} and confidence intervals for *Ae. aegypti* larvae exposed to *E. antiquorum* latex extract

S. No.	Hours of exposure	LCL	LC_{50} (ml/dl)	UCL
1	12	2.33	2.74	3.2
2	24	2.10	2.27	2.45
3	36	1.95	2.12	2.30
4	48	1.67	1.82	1.99
5	60	1.67	1.82	1.99
6	72	1.48	1.62	1.78
7	84	1.25	1.41	1.59
8	96	1.18	1.32	1.48

LCL – Lower Confident Limit
UCL – Upper Confident Limit

4. Discussion

For toxicity studies, fishes which are hardly in nature are selected. In the present study *Poecilia reticulata* Peters, a fresh water fish was selected to test the toxicity of *Euphorbia antiquorum* latex in laboratory conditions. Guppies (*P. reticulata)* have the capacity of tolerating wide fluctuations in water [16]. Applications of chemical synthetic pesticides are used to control fish population, which can adversely affect the aquatic environment, slow degradability in water and accumulation in fish's tissue [17]. To solve this problem, studies have been carried out by using plants as piscides which degrade easily.

In the present study the LC_{50} value of *Euphorbia antiquorum* latex to *P. reticulata* exposed for different time intervals (24, 48, 72 and 96 hr.) were 2.27, 1.82, 1.62 and 1.32 ml/dl (respectively). Singh and Singh [18] recorded the Lc50 value of 5.25 mg/l (24 h) and 4.94 mg/l (48 h) in the latex of *Euphorbia pulcherrima* against *Labeo rohita.* The Lc50 value of latex was 0.1 ml/5l for 96 hrs of exposure against *Anabas testudineus.* Tiwari and Singh [19] observed the 96h Lc50 value of diethyl ether, chloroform and acetone extracted *Euphorbia royleana* stem bark on the fish *Channa punctatus* was 31.76 mg dried weight (DW/L), 56.26 mg DW/L, 56.80 mg Dw/L, 65.77 mg DW/L, respectively.

In the present study the mortality of fish occurred steadily with the passage of time and concentration of the latex extract. Ayotunde *et al* [20] observed that the mortality rates in the study had a clear relationship between dose, mortality and exposure period. The concentration of the toxicant is directly proportional to the mortality rate.

The fish in the present study died due to certain physiological and biochemical changes. Tiwari *et al* [21]. attributed that the cycloart-24-en-3β isolated from *Euphorbia royleana* latex altered the oxidative metabolism and lead to the toxicity of fresh water predatory fish *Channa punctatus.* Single and Agarwal [22] *reported* that the latex of *Euphorbia royleana* and *Euphorbia antisyphilitica* inhibit the activity of acetyl cholinesterase, which eventually results in the death of the fish.

Studies revealed that fish exposed to toxicants usually exhibited changes in the opercular movement rate, erratic jumping movements, irregular swimming activity and jerky movements. Almost all these changes were noticed in the present study when the fishes were exposed to different concentrations of the latex. Cagauan *et al* [23] observed erratic swimming behaviour and rapid opercular movement when the fishes like Nile tilapia were exposed to higher concentrations of plant extracts. Yadav *et al* [24] reported that the fish *Channa punctatus* when exposed to *Croton tiglium* plant extract exhibited drastic movement and irregular swimming activity.

Cagauan *et al* [23] reported that fish farmers should use piscides of botanical origin that are not hazardous to the environment and have shorter residual effects rather that chemical pesticides that prove to be very dangerous to the environment and to humans. Latex is also reported to be a hunter's tool applied in local farming and arrow poisoning in tropical Africa [25] The results of the study showed that the latex of plants have the potential to be used as piscicide which can be an alternative to harmful chemical piscides that are widely used today to eradicate fishes in the ponds.

5. Conclusion

The findings of the present study suggest that the *Euphorbia antiquorum* latex extract may be explored as potential piscicidal agent.

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