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ACUTE TOXICITY OF *EUPHORBIA ROYLEANA* BARK EXTRACT ON FRESHWATER CATFISH, *HETEROPNEUSTES FOSSILIS*

ManiRam Prasad & Ajai K. Srivastav*
Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur 273009, India.
*Corresponding author: e-mail: ajaiksrivastav@hotmail.com

ABSTRACT
An acute toxicity test was performed by using a four-day static renewal test to determine the LC_{50} value of aqueous extract of *Euphorbia royleana* bark extract for the freshwater catfish, *Heteropneustes fossilis*. The LC_{50} values at various exposure periods are 55.163 mg L-1 for 24h; 47.600 mg L-1 for 48h; 35.431 mg L-1 for 72h and 20.713 mg L-1 for 96h. The upper confidence limits were 60.192, 52.106, 41.077 and 25.137 mg L-1 for 24, 48, 72 and 96 h and lower confidence limits were 50.587, 43.377, 29.962 and 16.105 mg L-1, respectively. The toxicity of *Euphorbia royleana* bark extract exhibit a positive correlation between fish mortality and exposure period (mortality of fish at a given concentration is increased with increased duration of exposure).

KEY WORDS: *Euphorbia royleana*, LC_{50}, Fish, Toxicity.

INTRODUCTION

During earlier half of century plant based pesticides (botanicals) such as pyrethrum and rotenone have commonly used for agricultural pest control. Now-a-day's plant derived botanical pesticides are being used in large extent for pest management. Botanical pesticides have certain advantages as they are rapidly degrade in the environment, less toxic, lack of persistence and safe for non-target animals as compared to synthetic pesticides. The toxicological impact of botanical pesticides must be considered before use, because being a natural product does not ensure that it is safe. Some botanical pesticides are photodynamic and they are toxic to all living organisms in the presence of light[1]. *Euphorbia royleana* is a common medicinal plant. Different parts of this plant have molluscicidal, piscicidal and insecticidal properties [2-5]. The chemical constituents of plants of the *Euphorbiaceae* family include phenolic compounds (phenanthrenes, coumarins, flavonoids, tannins, phenolic acids, lignans *etc*.) alkaloids, triterpenoids and related compounds (alcohol, sterols and hydrocarbons), cyanogenic glucosides and glucosinolates[6]. There exist some reports on the toxicological impact of *Euphorbia royleana* latex on fish *e.g*., histopathology of ultimobranchial gland [7] and hematological anomalies[8]. The latex of *Euphorbia royleana* produces an intense conjunctival, corneal and iritic inflammatory reaction[9,10] have shown that *Euphorbia royleana* latex has irritant to skin and eye properties. *Euphorbia royleana* have antipyretic and analgesic properties in rats and rabbits. There exist few studies which investigated acute toxicity of *Euphorbia royleana* for fish *Channa punctatus*[11], *H. fossilis*[5] but, there exist no report regarding the acute toxicity of *Euphorbia royleana* bark extract on fish *H. fossilis*. Keeping in view the wide use of *Euphorbia royleana* and its medicinal values, the present study was designed to determine the acute toxicity of *Euphorbia royleana* bark extract on the stinging catfish, *Heteropneustes fossilis*.

MATERIALS & METHODS

Stem bark of *Euphorbia royleana* were obtained from the Botanical Garden of DDU Gorakhpur University, Gorakhpur campus and dried at room temperature. Dried bark were ground into particles and then extracted with ethanol in Soxlet extraction unit. The extract solution thus obtained was evaporated at 37°C to a constant weight. The material was weighed and stock solutions were prepared in 100 % ethanol. Adult freshwater teleost *Heteropneustes fossilis* (both sexes body weight 35–45 g) were collected locally. Healthy fish showing no external signs of injury and disease were selected for experiments and were acclimatized to laboratory conditions (under natural photoperiod 11:22–12:16 and temperature 27.32 ^{0}C ± 2.31^{0}C; pH 7.15 ± 0.08; hardness 165.97 ± 4.89 mg L^{-1} as $CaCO_3$ and dissolved oxygen 7.84 ± 0.35 mg L^{-1} for 15 days in dechlorinated tap water.

For the determination of LC_{50} value of stem bark extract of *Euphorbia royleana* on *H. fossilis* the four-day static renewal acute toxicity test was used[12]. Five replicates, each containing 10 fish (kept in glass aquaria in 30L tap water, stocking density was modified for the experiment keeping in view the air breathing nature of this fish species) were exposed to each concentration (10, 20, 30, 40, 50, 60, 70 and 80 mg/L) of stem bark extract of *Euphorbia royleana*. The dried extract powder of *Euphorbia royleana* was weighed and a stock solution (5 mg/0.1ml) was prepared in ethanol. The required volume of stock solution was mixed in tap water to obtain the above mentioned concentrations. A control group with five replicates each containing ten fish kept in 30L tap water (having equal volume of ethanol) was also run. The study was approved by the Animal Research Ethical Committee of DDU Gorakhpur University.

The media (the control and test solutions) in the aquaria were renewed daily. The fish were not fed 24 h before and during the experiment. Dead fish were removed immediately. At different exposure periods (24, 48, 72 and 96 h), the mortality of the fish was recorded and subjected to Probit- log analysis with the POLO-PC software (LeOra Software) to calculate the LC_{10}, LC_{50} and LC_{90} values, their slope functions and confidence limits. Regression analysis was performed between the LC values and exposure periods.

RESULTS

The percent mortality of *Heteropneustes fossilis* after exposure to various concentrations of stem bark extract of *Euphorbia royleana* for 24, 48, 72 and 96 h has been depicted in Fig. 1 to 4. The LC_{50} values at the different exposure periods were 55.16 mg/L at 24 h, 47.60 mg/L at 48 h, 35.43 mg/L at 72 h, and 20.71 mg/L at 96 h. The LC_{10}, LC_{50} and LC_{90} values, their upper and lower confidence limits and slope functions are given in Table 1. In this study, the toxicity of stem bark extract of *Euphorbia royleana* was noticed to be time and dose-dependent. The regression coefficient showed that there was a significant negative correlation between exposure time and the different LC values.

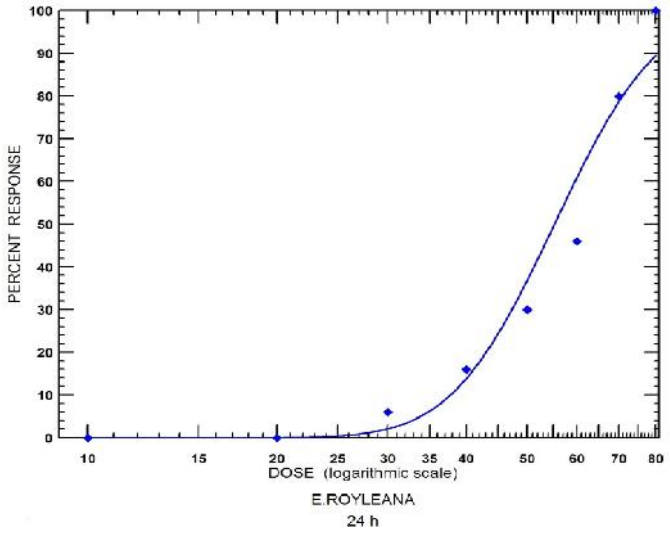

FIGURE 1: Percent mortality of the fish *Heteropneustes fossilis* after 24 h exposure to different concentrations of *Euphorbia royleana* bark extract (mg/L).

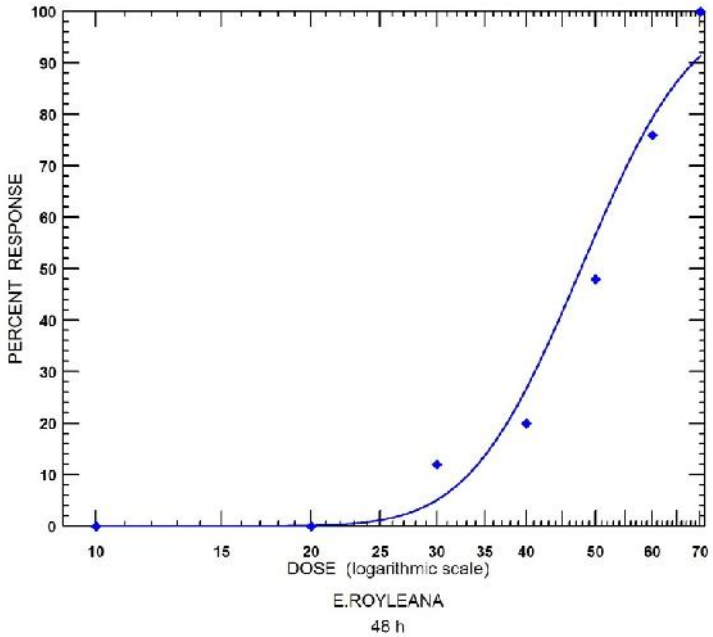

FIGURE 2: Percent mortality of the fish *Heteropneustes fossilis* after 48 h exposure to different concentrations of *Euphorbia royleana* bark extract (mg/L)

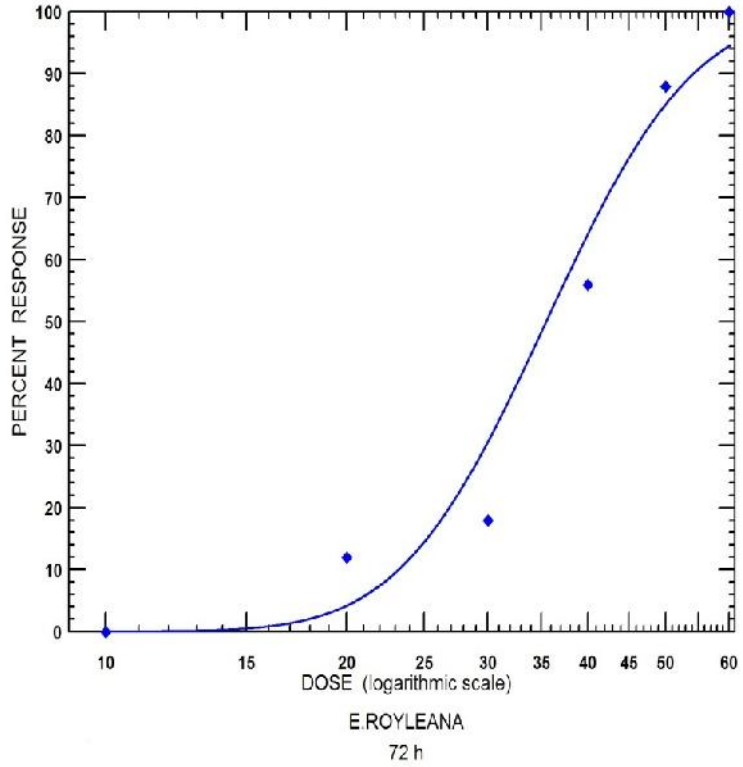

FIGURE 3: Percent mortality of the fish *Heteropneustes fossilis* after 72 h exposure to different concentrations of *Euphorbia royleana* bark extract (mg/L)

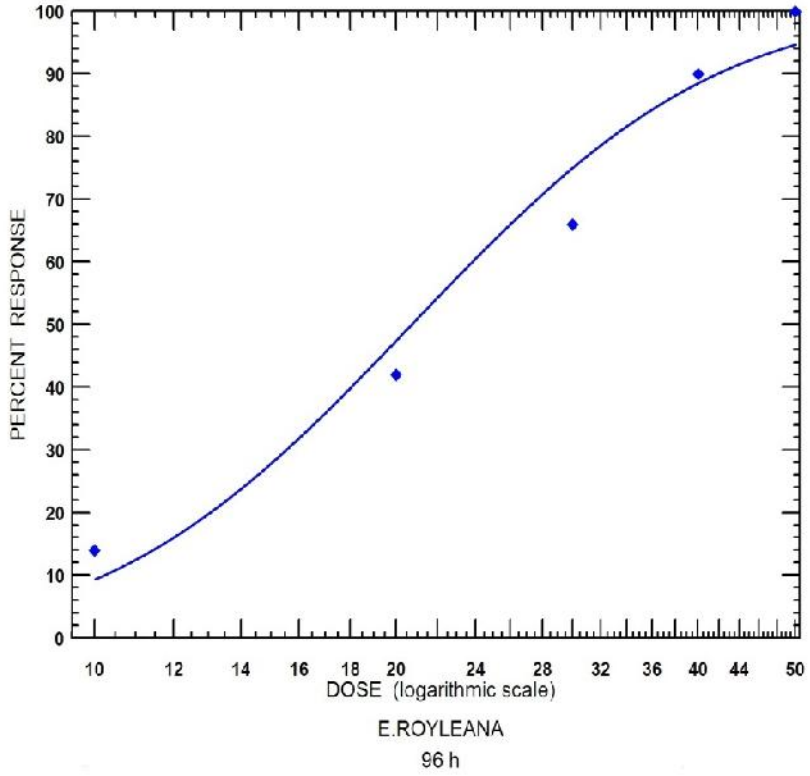

FIGURE 4: Percent mortality of the fish *Heteropneustes fossilis* after 96 h exposure to different concentrations of *Euphorbia royleana* bark extract (mg/L)

TABLE 1. LC_{10}, LC_{50} and LC_{90} values, slope functions and confidence limits for *Euphorbia royleana* bark extract at different time intervals for the fish *Heteropneustes fossilis.*

Exposure period	Effective dose (mg/L)	Limits (mg/L)		Slope function	t- ratio	Heterogeneity
		LCL	UCL			
24 h	LC_{10} = 37.705	30.956	42.343	7.755 ± 0.538	14.415	5.345
	LC_{50} = 55.163	50.587	60.192			
	LC_{90} = 80.704	71.784	98.540			
48 h	LC_{10} = 33.145	26.796	37.380	8.153 ± 0.593	13.378	5.136
	LC_{50} = 47.600	43.377	52.106			
	LC_{90} = 68.361	60.839	83.833			
72 h	LC_{10} = 23.194	15.453	27.939	6.964 ± 0.502	13.883	8.102
	LC_{50} = 35.431	29.962	41.077			
	LC_{90} = 54.126	45.731	70.721			
96 h	LC_{10} = 10.247	05.605	13.758	4.193 ± 0.319	13.161	4.728
	LC_{50} =20.713	16.105	25.137			
	LC_{90} = 41.869	33.325	63.778			

DISCUSSION

In the present study, ethanol extracted stem bark of *Euphorbia royleana* toxicity exhibit a positive correlation between exposure period and fish mortality (at a given concentration the mortality of fish increased with increased exposure period.

Toxic effects of botanical pesticides on fish, *Heteropneustes fossilis* have been studied [13, 5, 14, 15]. Few studies exist regarding the LC_{50} values of *Euphorbia royleana* on fish species-- *Channa punctatus* [11, 16, 17], *H. fossilis* [5]. [16] reported the 24 h LC_{50} value of chloroform, diethyl ether, acetone and methanol extracted *Euphorbia royleana* latex on the fish *Channa punctatus* as 13.34 mg/L, 16.19 mg/L, 12.88 mg/L, 11.76 mg/L, respectively. [11] have reported the LC_{50} value as 0.05 g/L at 24 h for fish, *Channa punctatus* for aqueous extract of *Euphorbia royleana*. [17] observed the 96 h LC_{50} value of chloroform, diethyl ether, acetone and methanol extracted *Euphorbia royleana* stem bark on the fish *Channa punctatus* as 56.26 mg dried weight (DW)/L, 31.76 mg DW/L, 65.77 mg DW/L, 56.80 mg DW/L, respectively. For *H. fossilis* the 96 h LC_{50} values for organophosphates are 2.20 mg/L for chlorpyrifos[18] and 6.60 mg/L for metacid-50[19]. Considering the LC50 values for *Channa punctatus*, it seems that *H. fossilis* is very sensitive to ethanol extracted stem bark of *Euphorbia royleana* since the concentration to produce toxicity is lower than that reported for *Channa punctatus* (as can be compared from the above mentioned toxicities for both fish species, *H. fossilis* and *Channa punctatus*). In the present study the 96 h LC_{50} value for ethanol extracted stem bark of *E. royleana* for *H. fossilis* has been estimated to be 20.71 mg/L. This is comparable to the reported LC_{50} values for the lyophilized latex of *E. royleana* on fish *H. fossilis* which is 3.06 mg/L [5]. However, the 96 h LC_{50} value for latex of *E. royleana* on *H. fossilis* is higher as compared to the toxicity of ethanol extracted stem bark of *Euphorbia royleana* for *H. fossilis*.

It is concluded from present study that *Euphorbia royleana* exhibit non-target toxicity to fish. It is suggested that application of plant based pesticides should be encouraged in place of synthetic pesticides as they (synthetic pesticides) are more toxic at low doses than botanical pesticides. Use of plant derived pesticides would eventually help in keeping the environment free from hazardous chemicals. Also, care should be taken to use plant based pesticides at moderate levels as these are also harmful to fish species.

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